Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

Estimation of genetic variation of performance traits in common carp to predict potential of selective breeding under pond management conditions

Odhad genetické variance užitkových vlastností kapra obecného s cílem předpovědět potenciál selekčního šlechtění v rybničních podmínkách chovu

Martin Prchal

Czech Republic, Vodňany, 2018

I, Martin Prchal, thereby declare that I wrote the Ph.D. thesis by myself using results of my own work or collaborative work of me and colleagues and with help of other publication resources which are properly cited.

I hereby declare that, in accordance with the § 47b Act No. 111/1998 Coll., as amended, I agree with publicizing of my Ph.D. thesis in full version electronically in a publicly accessible part of the STAG database operated by the University of South Bohemia in České Budějovice on its web sites, with keeping my copyright to the submitted text of this Ph.D. thesis. I also agree so that the same electronic way, in accordance with above mentioned provision of the Act No. 111/1998 Coll., was used for publicizing reviews of supervisor and reviewers of the thesis as well as record about the progress and result of the thesis defence. I also agree with compering the text of my Ph.D. thesis with a database of theses "Theses.cz" operated by National Register of university theses and system for detecting of plagiarisms.

In Vodňany 11th June, 2018

Supervisor:
Assoc. Prof. Martin Kocour
University of South Bohemia in České Budějovice (USB)
Faculty of Fisheries and Protection of Waters (FFPW)
Research Institute of Fish Culture and Hydrobiology (RIFCH)
South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses (CENAKVA)
Zátiší 728/II, 389 25 Vodňany, Czech Republic

Consultants:
Miloš Havelka, Ph.D.
University of South Bohemia in České Budějovice (USB)
Faculty of Fisheries and Protection of Waters (FFPW)
Research Institute of Fish Culture and Hydrobiology (RIFCH)
South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses (CENAKVA)
Zátiší 728/II, 389 25 Vodňany, Czech Republic

Girish Kumar, Ph.D.
University of South Bohemia in České Budějovice (USB)
Faculty of Fisheries and Protection of Waters (FFPW)
Research Institute of Fish Culture and Hydrobiology (RIFCH)
South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses (CENAKVA)
Zátiší 728/II, 389 25 Vodňany, Czech Republic

Head of Laboratory of Molecular, Cellular and Quantitative Genetics
Prof. Martin Flajšhans

Dean of Faculty of Fisheries and Protection of Waters:
Prof. Pavel Kozák

Board of doctorate study defence with reviewers:
Assoc. Prof. Josef Matěna – head of the board
Prof. Petr Ráb – board member
Prof. Otomar Linhart – board member
Assoc. Prof. Tomáš Policar – board member
Prof. Lukáš Kalous – board member
Assoc. Prof. Ondřej Slavík – board member
Assoc. Prof. Zdeněk Adámek – board member

Mathilde Dupont-Nivet, Ph.D., GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France – thesis reviewer
Matti Janhunen, Ph.D., Natural Resources Institute Finland (Luke), Aquatic production systems, Jyväskylä, Finland – thesis reviewer

Date, hour and place of Ph.D. defence:
20th September 2018 in USB, at 10:00, FFPW, RIFCH, Vodňany, Czech Republic
Name: Martin Prchal

Title of thesis:
Estimation of genetic variation of performance traits in common carp to predict potential of selective breeding under pond management conditions
Odhad genetické variance užitkových vlastností kapra obecného s cílem předpovědět potenciál selekčního šlechtění v rybničních podmínkách chovu

---

Ph.D. thesis, USB FFPW, RIFCH, Vodňany, Czech Republic, 2018, 130 pages, with the summary in English and Czech.

Graphic design & technical realisation: JENA Šumperk, www.jenasumperk.cz

ISBN 978-80-7514-076-0

CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1. HISTORY OF CULTURE AND BREEDING IN COMMON CARP

Common carp is a highly important freshwater fish species for world aquaculture, with an annual production exceeding 4,000,000 tons (4th place on the world fish production; FAO, 2016), and farmed in a wide variety of environments and production systems (Horváth et al., 1992; Balon, 1995). Carp culture started in China in approximately 2000 B.C., but domestication itself began much later (probably between the 13th and 16th centuries). The first mentions of carp culture suggest that wild carp fry was caught in rivers and subsequently cultured in artificial lagoons/ponds on rice fields until market size, of which a portion of the matured fish were kept further for natural spawning (Janssen et al., 2015). In Europe the first articles about carp reproduction appeared in the 13th century. However, the culture of carp may have already occurred in Roman times, also practised within monasteries from the early Middle Ages onwards (Balon, 1995, 2006). Controlled spawning techniques in separate ponds were developed and became more sophisticated during the 19th century, resulting also in the development of many different, often highly inbred European carp strains (Kohlmann and Kersten, 1999; Kohlmann et al., 2003, 2005).

Common carp has, according to current taxonomy, two genetically distinct branches that are recognized as separate species; European carp *Cyprinus carpio* and Asian carp *Cyprinus rubrofuscus* (Kottelat, 2001, 2013; Huckstorf, 2012; Dyldin and Orlov, 2016; Froese and Pauly, 2018). However, other authors who have conducted genetically orientated studies on common carp rather distinguish common carp, *Cyprinus carpio,* as having two or three subspecies as follows: *Cyprinus c. carpio, Cyprinus c. haematopterus* and *Cyprinus c. rubrofuscus* (Zhou et al., 2004; Thai et al., 2005; Chistiakov and Voronova, 2009; Xu et al., 2014a). The European carp strains are mostly derived from wild carp from the River Danube (Flajšhans and Hulata 2007; Bogeruk, 2008). Nevertheless, a few intercross breeds between European and Asian carp exist e.g. Ropsha scaly carp and Amur mirror carp (Bogeruk, 2008; Flajšhans et al., 2015).

The breeds differ in qualitative traits such as general appearance, scaliness, morphology, and in quantitative traits related to growth, survival, disease resistance, or fat content implying the possibility of carp breeding focused on specific production traits (Gorda et al., 1995; Vandeputte, 2003). Breeding is the most important tool used in genetic improvement of a given trait of interest (Vandeputte, 2003; Gjedrem, 2005). In other words, genetic improvement is focused on a genetic component of phenotype. However, breeding should be understood as a complex strategy of which environmental conditions (environmental component of phenotype) play an important role that might limit the potential from genetic breeding. The main breeding goal is to shift the average value of traits with continuous variability (e.g. growth, slaughter yields, fat content) or increase frequency of required classes in the population within the traits of clearly defined classes (e.g. survival, flesh colour) (Gjedrem, 2005). Recently, great attention has been paid to the study of the potential of breeding methods in the genetic improvement of common carp, especially of genome manipulations, crossbreeding and selective breeding.

1.1. Genome manipulations

Genome manipulations have been focused on induced polyploidy, especially triploidy (3n) and tetraploidy (4n), and the production of monosex (all-female) carp stocks. The results of triploid (Cherfas et al., 1993; Basavaraju et al., 2002) and tetraploid carp (Recoubratsky et al., 1989; Linhart et al., 1991; Cherfas et al., 1993) showed that those fish achieved lower growth and survival than normal diploid carp stock. The low effectivity of artificial polyploidization might have been caused due to the fact that common carp itself is considered as an evolutionary allotetraploid species (Larhammar and Risinger, 1994; Xu et al., 2014a). Therefore,

induced polyploidy has not been used in commercial carp breeding until now. Conversely, a suitable method seems to be the production of all-female stocks. An all-female population may be achieved by crossing neomales, produced by combination of meiotic gynogenesis and sex reverse of XX gynogenetic females to males, and normal females (Cherfas et al,. 1996; Gomelsky et al., 1994, 2003). All-female stock released to commercial farms resulted in 10–15% yield improvement over existing commercial stocks (Cherfas et al., 1996). Better performance of all-female carp stock compare to the mixed-sex stock was also observed by Kocour et al. (2005b). On the other hand, the economic benefits of all-female stock are reduced due to higher initial costs for their establishment. Additionally, under Central European conditions the females grow better and have better slaughtering value only during the first three years. Therefore, the differences in performance in older market size fish (4-year old) might be negligible as both sexes are mature (Kocour et al., 2005b; Flajšhans and Hulata, 2007). Hence, unlike other European fish species, all-female carp have not found such broad application in the production of commercial stocks.

1.2. Crossbreeding

Nowadays, genetic improvement in common carp is largely based on intra / interspecific crossbreeding of various breeds / strains / populations (Vandeputte, 2003; Nielsen et al., 2010). Crossbreeding uses a non–additive component of genetic variance that may lead to a heterosis effect or so-called hybrid vigour. Heterosis is a superiority observed in the performance of the crossbred when compared to the mean of the purebreds that typically results in increased growth, survival, slaughter yields, fertility, resistance to adverse environmental conditions, and resistance to diseases (Gjedrem, 2005). Hybrids arisen from crossbreeding are usually tested before their commercial utilization compared to purebreds and mutually (Wohlfarth, 1993; Bakos and Gorda, 1995; Hulata, 1995; Gela and Linhart, 2000; Gela et al., 2003; Vandeputte, 2003; Kocour et al., 2005a; Nielsen et al., 2010; Piačková et al., 2013). Previous studies have shown that on average some performance traits (especially growth and survival) increased up to 35% due to heterosis. However, heterosis is a common but not universal phenomenon and it is limited especially to the first filial generations (F_1) (Wohlfarth, 1993; Hulata, 1995). Thus, F1 crossbreds are used only as commercial stocks and not for further breeding. Additionally, observed heterosis is most likely caused by the fact that the parental strains used are inbred (Kohlmann and Kersten, 1999, Kohlmann et al., 2003, 2005). Therefore, crossbreeding is not a suitable method for long term genetic improvement, where genetic gain is cumulated over multiple generations (Nielsen et al., 2010).

Likewise, interspecific hybridization has been performed in the past between common carp and the Indian major carp rohu; *Labeo rohita*, mrigal; *Cirrhinus mrigala*, and catla; *Gibelion catla* (Khan et al., 1990). Such hybrids are functional triploids – sterile fish (Reddy et al., 1990). All hybrids exhibited a faster growth rate than the maternal parent under monoculture, although the juveniles had lower survival caused by the occurrence of a relatively large number of malformations (Khan et al., 1990).

1.3. Selective breeding

Selective breeding holds high potential for the genetic improvement of fish and shellfish. In contrast to crossbreeding, additive genetic variation is utilized in genetic improvement. The main benefit of selective breeding is that the genetic gain of improved traits is cumulative and permanent over multiple generations, and thus suitable for a long-term breeding program (Gjedrem and Baranski, 2009; Gjedrem et al., 2012; Gjedrem and Rye, 2016). However,

unlike other important European fish species, selective breeding in common carp is still at the beginning and plays only a minor role in carp breeding (Vandeputte, 2003; Wang, 2009; Chavanne et al., 2016; Janssen et al., 2015, 2017a). However, several recent studies have confirmed that the additive genetic variation in several performance traits and resistance to Koi herpesvirus (KHV) is considerable and reasonable for additional research (Vandeputte et al., 2004, 2008; Kocour et al., 2007; Nielsen et al., 2010; Ødegård et al., 2010; Ninh et al., 2011; Dong et al., 2015; Nguyen, 2016; Hu et al., 2017; Tadmor-Levi et al., 2017). More recently, new genomic approaches might contribute towards genetic improvement through genomic selection (GS) using genome-wide based genetic markers, or in marker-assisted selection (MAS) using enough informative quantitative traits locis (QTLs) or candidate genes (Yáñez et al., 2015; Lv et al., 2016; Peng et al., 2016; Zheng et al. 2016; Lu et al., 2017; Robledo et al., 2017; Wang et al., 2018). Nonetheless, there is still much space for studying key problems that could contribute to anchor selective breeding as a common breeding method in common carp production. As a result, the topic of the present thesis is focused on unexplored fields in order to spread the knowledge about the potential of selective breeding for the genetic improvement of common carp under pond management conditions.

2. SELECTIVE BREEDING IN AQUACULTURE

Unlike terrestrial livestock, the potential benefits of selective breeding in aquaculture species have not been implemented until recently. The first selection program started on Atlantic salmon (*Salmo salar*) in the 1970s and has played a deciding role in the success of selective breeding for other aquaculture species (Gjedrem, 1979; Gjedrem, 2010; Gjedrem, 2012). Since that time, selective breeding has become one of the most useful breeding methods in several European aquaculture species (Gjedrem and Baranski, 2009; Gjedrem and Rye, 2016). Of the total European aquaculture production 80–83% originates from the 37 different selective breeding programs. The majority of them concern Atlantic salmon and are based on family selection (Gjedrem et al., 2012; Janssen et al., 2017a). On the other hand, only 8.2% of the world's total aquaculture production is based on material achieved in selective breeding programs. Thus, implementation of selective breeding in fish without any program gives a strong potential for increase in fish production (Gjedrem and Rye, 2016). Moreover, selective breeding in aquatic species provides even higher genetic gain compared to terrestrial livestock. The reason might be given due to the higher fecundity of fish enabling higher selection intensities and larger phenotypic and genetic variation of quantitative traits, suggesting a strong potential for positive response to selection (Gjedrem, 2005; Gjedrem and Baranski, 2009). Recent reviews of the topic have reported that the average genetic gain per generation for harvest weight in fish and shellfish is around 12% (Gjedrem and Rye, 2016; Janssen et al., 2017a). Taking into account an average generation interval of four years, this would lead to an increase of 3% per year (Janssen et al., 2017a), in comparison with terrestrial livestock where annual genetic gain of body weight is around 1% in cows (Hill, 2010) and up to 2% in poultry (Hill and Bünger, 2004).

Despite significant assumptions for successful selective breeding in aquaculture species, several selection experiments have failed in the past. These were caused most likely by inbreeding and loss of genetic variation (Hulata, 2001; Vandeputte, 2003; Gjedrem and Baranski, 2009). The hypothetical scenario was likely thus: "Because of high fish fecundity, only small broodstock sizes had been used in the past and the practice after generations led to close relationships (inbreeding) and rapid loss of genetic variation (Allendorf, 1986; Hedrick, 2005; Vandeputte and Haffray, 2014). This resulted in slowing growth and higher mortality with no response to selection and selective breeding of some fish species, including

common carp, seemed to be ineffective". Thus, control of the level of inbreeding (sufficient effective population size) is an essential part of the unbiased estimation of genetic potential in aquaculture species (Falconer and Mackey, 1996; Vandeputte, 2003; Gjedrem, 2005). Additionally, recent investigation of genetic markers and pedigree data have clearly confirmed that the control mating design used in the selection for better growth in European seabass (*Dicentrarchus labrax*) over three generations would not lead to any significant reduction of genetic variation. However, due to the selection of specific (better performed) genotypes, a decreasing trend in allelic richness (loss of low frequency alleles) was observed (Hillen et al., 2017). Similarly, five generations of selection for growth performance traits in rainbow trout resulted in only modest inbreeding accumulation of 0.86% per generation (Leeds et al., 2016). Thus, sufficient effective population size and a suitable mating design are the main assumptions for a successful estimation and exploitation of genetic variation in long-term selective breeding. The most frequent mating designs used are partial and full-factorial schemes. Such matings provide an appropriate number of full sib as well as half sib families for each sire and dam and this enables a solid estimation of genetic parameters and separation of non-genetic (maternal, common environment) and genetic (additive, dominance) effects (Gjedrem, 2005).

Both partial and full factorial mating designs were used for establishment of experimental stock related to studies concerning the topic of this thesis.

2.1. Heritability and genetic correlations

Another important point for potentially successful selective breeding is that the given trait of interest needs to have a medium to high coefficient of heritability – h^2 (Gjedrem, 1983). Thus, the total phenotypic variation of a trait must be explained with a significant share of genetic variation that predicts the rate of genetic gain (Falconer and Mackay, 1996). Likewise, genetic correlation (r_g) between the traits of interest is also a key genetic predictor for a selection program. Genetic correlation is explained as the proportion of variance that two traits share due to genetic causes (Falconer and Mackay, 1996). Therefore, it is necessary to know the genetic correlations between the potentially selected traits before the selection as existing correlations might often complicate any selection program. For instance, selection on increased desired trait can indirectly lead to an increase in undesired trait. Oppositely, selection on one trait might beneficially influence another trait under interest. This is very important in multitrait selection when several selection indices are included in a selection program. Therefore, complex knowledge of genetic parameters in traits of interest should be always taken into account before the selection itself (Falconer and Mackay, 1996; Dunham, 2011).

2.2. Estimation of genetic parameters

The genetic parameters are statistically estimated using various software (e.g. VCE – Groeneveld et al., 2008; ASReml – Gilmour et al., 2009; DMU – Madsen and Jensen, 2013) that uses multivariate genetic mixed models. However, such estimates may vary over studies and may differ due to differences within populations and environment. However, there are some options to make the estimates more unbiased e.g. making the environment more uniform, using communal rearing of families to eliminate a common environmental effect, or measuring the traits more accurately (Bourdon, 2000). The present research of genetic parameters in quantitative traits may be divided into two main experimental approaches.

The first approach is based on the separate rearing of families until the fish are big enough to be physically tagged and consequently mixed into a communal stock. Although this is an effective design in family-based selective breeding programs of major aquaculture species such as salmonids, tilapia, oysters, or shrimps (reviewed by Vandeputte and Haffray, 2014), main limitations can be seen in: i) the families are reared separately and therefore, environmental effects common to full sibs may inflate heritability estimates ii) the evaluation of genetic variation with separate rearing of families requires the existence of a high number of family rearing units iii) the number of families is limited to the number of family rearing units that, in some cases, result in a low number of families established by single mating or nested design which do not allow separating non-genetic and genetic effects (Gjedrem, 2005).

The second approach, when all families are kept in communal stocks post hatching (or even post fertilization) and then, at an appropriate size, physically tagged for solid pedigree evidence, might overcome all the previous constraints. However, in this case the pedigree must be constructed using molecular markers, making the approach more costly. On the other hand, generally higher genetic gains have been achieved using communal family rearing in comparison to separate family rearing (Vandeputte et al., 2009; Ninh et al., 2013). Efficient parentage assignment in fish started in the 1990s with the availability of microsatellite markers (Herbinger et al., 1995; Estoup et al., 1998). However, the future development of single nucleotide polymorphisms (SNPs) could replace traditional microsatellite-based parentage assignment (Vandeputte and Haffray, 2014). Currently, SNPs are searched for using genome-wide association studies (GWAS) or whole genome sequencing that have become financially available (Kumar and Kocour, 2017; Robledo et al., 2017). Complex genomic data utilization has improved QTL mapping as well as selection accuracy using genomic prediction of breeding values (Meuwissen et al., 2001, 2013; Hickey et al., 2017; Robledo et al., 2017).

Studies included in this thesis utilized both microsatellites as well as SNPs for parentage assignment and GWAS were used for estimation of genomic-based genetic variation and for QTL searching. Furthermore, all experimental stocks were physically tagged by PIT (passive integrated transponder) at the fish age of one-year in order to record the individual phenotypes for the given period or in a link to the subsequent period.

2.3. Methods used in selective breeding

The simplest individual/mass selection is solely applied on the best performing candidates that are crossed in a control mating design to create the next generation, which is then expected to be genetically improved in comparison with the base (unselected) population. Furthermore, more complex selection methods (e.g. family-based, sib selection, BLUP – best linear unbiased prediction based selection, or a combination of all) are commonly used in genetic improvement of aquaculture species (Lind et al., 2012; Vandeputte and Haffray, 2014). Traditional pedigree-based selective breeding, especially through application of BLUP methodology (Henderson, 1975) has greatly benefitted animal husbandry. On the other hand, the utilization of just the between-family component of genetic variation in pedigree-based selective breeding imposes limitations to selection accuracy and thus genetic gain (Meuwissen et al., 2013) As a result, selective breeding can be significantly enhanced by the application of genomic tools via improvement of selection accuracy (use whole family variation) and potentially also by identification of causative factors impacting key performance traits (Meuwissen et al., 2001, 2013). Therefore, modern selective breeding is going to be focused on marker-assisted selection, and especially on genomic selection. Marker-assisted selection is an indirect selection process in which a trait of interest is selected based on a genetic marker (candidate gene or QTLs) that significantly explains its total phenotypic (or

genetic) variation (Ribaut and Hoisington, 1998). However, most production traits are highly polygenic and only a few of them may be determined by QTLs large enough to be used in MAS (Daetwyler et al., 2013). Conversely, genomic selection may overcome such limitation. GS is a new approach for improving quantitative traits in aquaculture species that takes into consideration all genomic variation that is related to specific traits (Meuwissen et al., 2001; Dunham et al., 2014). Restriction-site-associated DNA sequencing (RAD-seq) is a fractional genotyping by sequencing (GBS) strategy (Baird et al., 2008) widely used for the concurrent detection and genotyping of SNP markers that generate medium to high-density linkage maps usable for QTLs mapping and genomic selection in aquaculture species (Campbell et al., 2014, Palaiokostas et al., 2013ab, 2016; Robledo et al., 2017). In general, genomic prediction combines generated linkage maps with phenotypic and pedigree data (when available) in an attempt to increase the accuracy of the prediction of breeding and genotypic values utilizing both within and across family variation. Besides, newly developed SNP array platforms (based on high density linkage maps) may be used to predict genomic breeding values for economically important traits more precisely (Meuwissen et al., 2001, 2013; Yáñez et al., 2015 Hickey et al., 2017; Robledo et al., 2017). In addition, MAS, as well as GS, could resolve the main issue of traits that cannot be recorded on live breeding candidates, e.g. disease resistance and slaughter yields (Gjedrem and Rye, 2016). Besides, GWAS conducted on production traits, such as growth-related traits, fat content or generally traits with limited heritability, are also under scientific interest (Nolasco-Alzaga et al., 2018).

3. SELECTIVE BREEDING IN COMMON CARP

3.1. The past, present and the future prospect

It has been said that selective breeding is still not significantly implemented in the genetic improvement of common carp stocks. This may be due to the findings of Moav and Wohlfarth (1976), who observed no improvement in growth after five generations of bidirectional selection. Accordingly, this study has often been recalled as proof that selective breeding in common carp is not effective. However, many limitations (e.g. lack of genetic variation within the strain, inbreeding depression, poor experimental and statistical design, a strong genotype by environment interactions) have been described in detail by Vandeputte (2003) and Wang (2009) in most of the past selective breeding experiments, including the above-mentioned study. On the contrary, better designed studies have recently shown mostly moderate to high heritability of production traits or resistance to KHV (Vandeputte et al., 2004, 2008; Kocour et al., 2007; Nielsen et al., 2010; Ødegård et al., 2010; Ninh et al., 2011; Dong et al., 2015; Nguyen, 2016; Hu et al., 2017; Tadmor-Levi et al. 2017) and also a reasonable selection response after the applied selection experiment (Vandeputte et al., 2008; Ninh et al., 2013; Dong et al., 2015; Nguyen, 2016). Therefore, selective breeding could be a promising breeding method in the genetic improvement of common carp similarly as in other European fish species (Chavanne et al., 2016; Janssen et al., 2017a). Furthermore, rapid development of genetic markers point to the fact that genetic improvement in common carp could be alternatively applied by using marker-assisted (MAS) or genomic selection (GS) (Yáñez et al., 2015; Lv et al., 2016; Peng et al., 2016; Zheng et al. 2016; Lu et al., 2017; Robledo et al., 2017; Wang et al., 2018).

3.2. Carp strains used in selective breeding studies

One of the main limitations of previous selective breeding studies was the lack of genetic variation within carp strains used. Hence, the experimental stocks were established from a few pairs of highly inbred, long-term domesticated fish (Kohlmann and Kersten, 1999, Kohlmann et al., 2003, 2005), leading to loss of within-breed genetic variation and potential failure of the selection itself (Vandeputte, 2003; Nielsen et al., 2010; Janssen et al., 2015). To eliminate such a problem, Vandeputte (2003) and Nielsen et al. (2010) suggested establishing so-called synthetic strains with a high genetic variability, which may be good material for starting selective breeding programs in common carp, or alternatively use of domesticated purebreds with acceptable genetic variation together with an appropriate mating design in order to eliminate prospective inbreeding.

In studies within this dissertation, Hungarian synthetic mirror (HSM; Bogeruk, 2008) or Amur mirror carp (AM; Flajšhans et al., 2015) were used for establishing experimental stocks that displayed sufficient within-breed genetic variation.

3.3. The potential of genetic improvement of economically important performance traits

3.3.4. Growth

Growth, commonly measured by body weight at a given age, is one of the most important economic traits in farmed fish species (Gjedrem and Baranski, 2009; Gjedrem et al., 2012; Gjedrem and Rye, 2016). Moreover, growth is easy to record in the field and may be measured either as weight or as standard length due to very high phenotypic and genetic correlation between the two traits (Gjedrem, 2005; $r_g \geq 0.90$). In addition, growth rate over studied periods is also often quantified as specific growth rate (SGR), or as thermal growth coefficient (TGC). Although both traits express the growing potential of fish, the recent bio-economic model of profitability in production traits has suggested TGC as a more valuable trait in a long-term selection program (Janssen et al., 2017b). From the genetic point of view, body weight is a moderately to highly heritable trait ($h^2 = 0.17 - 0.70$; Kocour et al., 2007; Vandeputte et al., 2008; Nielsen et al., 2010; Ninh et al., 2011; Dong et al., 2015; Hu et al., 2017). Likewise, high coefficient of variation (typically 20 – 30%) implies body weight as a good predictor for expected positive response to selection. Accordingly, applied selection on growth resulted in genetic progress per generation of 21.4% (two generation; Ninh et al., 2013), 12% (one generation; Vandeputte et al., 2008) and 7% (four generation; Dong et al., 2015). Taking all these facts into account, selection on growth may lead to significant genetic progress in future generations.

3.3.5. Muscle fat and its quality

Muscle fat content is also often included as a trait to be under control in selective breeding of aquaculture species (Powell et al., 2008; Saillant et al., 2009; Kause et al., 2011; García-Celdrán et al., 2015; Kause et al., 2016; Janhunen et al., 2017). Kocour et al. (2007) observed muscle fat content in common carp as a highly heritable trait ($h^2 = 0.58$). Selection for muscle fat content can be performed directly on live breeding candidates using a fat meter. Muscle fat content can be affected also indirectly through correlated traits (e.g. body weight, slaughter yields). However, uncontrolled increasing of fat content could negatively affect both sensory properties and the quality of flesh (Oberle et al., 1997; Nguyen et al., 2010; Janhunen et al., 2017). On the other hand, a certain lipid level is needed as an emergent energy reserve

during overwintering and its uncontrolled decrease may negatively affect winter survival (Steffens, 1996). Overwintering is a bottleneck for European carp production that every year causes significant economic losses to producers and reduces animal welfare (Bauer and Schlott, 2004; Horváth et al., 1992). When the water temperature drops below 8°C, common carp significantly reduce their metabolism, decrease feed intake and lose weight (Bauer and Schlott, 2004). Despite reduced metabolism, low feed intake results in utilizing lipid reserves firstly that become essential for winter survival (Hurst, 2007). However, only little is yet known about the genetic background of muscle fat content and its impact on other traits including carp winter survival. As a result, the study focusing on this issue was performed with the aim of revealing the phenotypic and genetic variation of muscle fat content and its dynamics (in absolute and relative values) in relation to important performance traits (growth, slaughter yields, survival) during overwintering and the successive growing period in which fish reached the market size (Chapter 2).

Fish fat is also an important source of essential fatty acids (FAs), mainly omega-3 polyunsaturated fatty acids (n-3 PUFAs), represented especially by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are very beneficial for human health (Calder and Yaqoob, 2009; Tocher, 2015; Steffens, 2016). It is well known that environmental and nutritional factors can significantly impact the fish fatty acid profile (Mráz and Pickova 2011; Marković et al. 2016; Trbović et al. 2017). The situation in common carp is in fact much more complicated in comparison with other fish species as rearing of common carp is practised mainly in earthen ponds where nutritional requirements are covered both by natural food (zooplankton, zoobenthos) and by supplemental feeding (plant-based pellets altered later with wheat grain). However, the genetic background of flesh FA composition also plays an important role as has been found in other fish species (Nguyen et al., 2010; Leaver et al., 2011; Overturf et al., 2013). However, knowledge about the genetic variation of fatty acids in common carp has been poor. In addition, little has been known about the effect of selective breeding on flesh quality. So far, only three studies have reported the genetic parameters in fish flesh fatty acids and their potential in a selection program (Nguyen et al., 2010, Nile tilapia, *Oreochromis niloticus*; Leaver et al., 2011, Atlantic salmon, *Salmo salar*; Overturf et al., 2013, rainbow trout, *Oncorhynchus mykiss*). To fill these gaps in common carp, the study on the genetic variation of FA composition and its relation to performance traits was performed (Chapter 4).

3.3.6. Slaughter yields

Processing traits such as fillet yield (fillet weight relative to body weight) and headless carcass yield are economically valuable traits especially for fish species sold processed. However, direct evaluation of slaughter yields is time-consuming work and especially lethal for breeding candidates. This is usually overcome by sib-selection in which live candidates are ranked according to the average performance of their slaughtered sibs or by indirect selection on correlated traits recorded *in vivo* (Kause et al., 2007). However, this limits the genetic progress by using only genetic variation occurring between-families without exploiting within-family variation (Haffray et al., 2013). Such limitation could be overcome by using whole genome genetic markers (SNPs – single nucleotide polymorphisms) that would allow within-family genomic selection (GS) (Gjedrem and Rye, 2016). Nevertheless, no genomic information related to carp slaughter yields is available so far. Therefore, indirect (non-invasive) selection criteria that can be measured in vivo looks like a more efficient alternative.

From the genetic point of view, heritability of headless carcass and fillet yields was found moderate (h^2 = 0.20 – 0.38), making enough space for their genetic improvement (Kocour

et al., 2007). Likewise, significant negative correlation between relative head length (RelHL) and slaughter yields was estimated. Thus, selection against relative head length may result in an increase in slaughter yields in carp (Kocour et al., 2007). However, there are gills in the head, the main respiratory organ of fish, so selection for lower RelHL in a long-term selection program may lead to functional damage of respiration, adaptation or osmoregulation capacities (Fraslin et al., 2018). As an alternative, previous studies reported the application of indirect measurements used for the prediction of processing yields on live fish (Bosworth et al., 2001; Rutten et al., 2004; Van Sang et al., 2009). Recently, Haffray et al. (2013) reported in rainbow trout (*Oncorhynchus mykiss*) that using external and internal measures combined with a linear regression could solidly predict slaughter yields and such measurements could be used as indirect selection criteria for the genetic improvement in yields of edible parts. A similar study was performed on European seabass, but in this case no effective morphological predictor was found for both carcass and fillet yield (Vandeputte at al., 2017).

Searching for non-invasive selection criteria and their application in genetic improvement of carp slaughter yields was also the goal of this thesis (Chapter 3).

3.3.7. Disease resistance, overall survival

Disease resistance represents one of the main research issues in the genetic improvement of aquaculture species (Ødegård et al., 2011; Houston, 2017). In common carp culture, a serious threat is the viral disease named Koi herpesvirus (KHV; CyHV-3). The first major outbreaks of KHV were reported in Israel and the USA in 1998 (Hedrick et al., 2000). Subsequently, KHV has spread almost worldwide (Haenen et al., 2004). Moreover, the seriousness of the threat of KHV is highlighted by its being listed as a notifiable disease in the European Union (Taylor et al. 2010). Research has focused on identification of purebreds and crossbreds of common carp which could be more resistant to the virus. In these studies special challenge tests were performed and positive outcomes in some carp strains/crossbreds were found (Shapira et al., 2005; Ødegård et al., 2010; Piačková et al., 2013; Tadmor-Levi et al., 2017). In addition, it was observed that the genetic variation of resistance to KHV is considerable unlike resistance to *Aeromonas hydrophila* (Ødegård et al., 2010; Tadmor-Levi et al., 2017). Therefore, KHV resistance could be increased using more resistant carps and additionally improved by selection. This suggestion could be supported by results of selection experiments conducted in the past where positive responses to dropsy (spring viremia, SVC) resistance have been observed (Schäperclaus, 1962; Kirpichnikov et al., 1993). However, direct selection for improved disease resistance is problematic due to the ethical issue (fish must be directly exposed to disease), potentially low selection intensity (when mortality decreases below 80%) and practically inapplicable for diseases (e.g. KHV) in which local directives suppose the elimination of all stock in the case of their outbreak. As a result, utilization of genomic data for marker-assisted selection or genomic selection may be effective alternatives. However, deeper genomic information about resistance to KHV are still unknown. Hence, the first GWAS of resistance to KHV was conducted (Chapter 6).

Regarding overall survival, Nielsen et al. (2010) reported on the same batch of fish as used by Ødegård et al. (2010) moderate heritability of pond survival ($h^2 = 0.34$). Oppositely, Dong et al. (2015) observed only low heritability of survival over four carp generations selected for faster growth ($h^2 = 0.17$).

3.4. Use of molecular tools

3.4.1. Microsatellite markers

Microsatellites are often used as genetic markers for numerous applications in aquaculture research. As one option, they are empirically used for pedigree reconstruction of mixed families used in genetic studies and/or selection programs. Such a tool, allowing communal rearing of families from the beginning, eliminates the impact of common environmental effect, and makes the selection experiments more unbiased (Ninh et al., 2011; Vandeputte and Haffray 2014). The disadvantage of this tool is the still relatively high price and therefore, genetic parentage assignment is mostly used in research (Ninh et al., 2011). However, parental assignment is becoming more and more interesting with the continuous drop in genotyping costs (Gjedrem and Robinson, 2014).

Microsatellite markers in common carp have been developed for their usage in population genetics, breeding and evolutionary studies (Crooijmans et al., 1997; Aliah et al., 1999; David et al., 2001; Kohlmann et al., 2003, 2005). The first experiment estimating heritability using molecular pedigree in common carp was conducted in 2004 (Vandeputte et al., 2004). In this experiment, 95% of 550 offspring originating from a full factorial cross of 10 dams and 24 sires were exactly assigned to a single parental pair using eight microsatellite markers. Kocour et al. (2007) genotyped progenies using five to eleven microsatellites and 75.7% of 812 G_2 offspring were assigned to single parental pairs in a full factorial cross of 8 dams and 147 sires. Vandeputte et al. (2008) assigned 1451 (78.3%) out of 1852 fish to a parental pair. The lower parental assignment accuracy in the two experiments by Kocour et al. (2007) and Vandeputte et al. (2008) was likely a result of insufficient resolution of the microsatellite loci used in crosses with the high number of potential parental combinations (Vandeputte et al. 2008) together with the low allelic richness of carp strains (Kohlmann et al., 2005). In another study, seven microsatellite loci were used for parentage allocation in order to test the differences between heritability estimates in early communal and separate rearing of common carp (Ninh et al., 2011). Out of the 1327 genotyped offspring in G_1 and 1332 in G_2, 96.8% and 96.2% of individuals, respectively, were assigned to single families. In a recent study, 97.6% individuals from a nested mating design (37 dams and 14 sires) were unambiguously assigned to a single parental pair using nine pairs of primers (Hu et al., 2017). Summing up, microsatellites are reliable genetic markers for successful parentage assignment used for post-hatching communal rearing of families, and thus contribute to more unbiased estimation of genetic parameters.

3.4.2. Marker-assisted and genomic selection

It is often claimed that breeding programs in aquaculture species in the future will utilize marker-assisted and genomic selection based on GBS techniques or SNP array platforms (Robledo et al., 2017). The main advantage of those approaches is speeding up the genetic improvement in fish species exploiting overall genetic variation (Sonesson, 2007; Yáñez et al., 2015; Robledo et al., 2017).

The genome of common carp has been recently completely sequenced and the functional genes have been annotated (Xu et al., 2014a). Accordingly, a 250K SNP array platform was constructed (Xu et al., 2014b). Both findings were milestones for genetic and population biologic studies in common carp, opening new possibilities for the improvement of economically important traits in common carp (Xu et al., 2016).

Several GWAS focused on detection of QTLs for various economic traits such as growth-related traits (Lv et al., 2016; Peng et al., 2016 Wang et al., 2018), feed conversion efficiency (Lu et al., 2017) and muscle fat content (Zheng et al. 2016) have been previously reported. The above-mentioned studies detected several usable QTLs for application of MAS. However, all of them explain only a part of the phenotypic/genetic variation of a given trait. Moreover, it was verified that most of the quantitative traits under interest in common carp, as well as in other aquaculture species, are under polygenic control (Robledo et al., 2017). For such traits, GS is a more appropriate method in which genomic breeding values are calculated and used for genetic improvement.

Until now, no study has attempted investigating GS usability in common carp and comparing traditional pedigree-based (PBLUP) and genomic best linear unbiased predictions (GBLUP). As a consequence, prediction of GS efficiency, QTL and candidate gene mapping related to growth and KHV resistance in one-year old common carp were performed (Chapters 5 and 6).

THE AIMS OF THE THESIS

The overall aim of this thesis was to estimate genetic and genomic parameters of the most important performance traits in common carp and to predict the potential of targeted selection-based breeding for typical pond management conditions.

The specific objectives were to:

1) Estimate genetic parameters of overwintering performance traits in two-year-old common carp and their relation to performance traits until and at market size.
2) Define morphological predictors and their potential for genetic improvement of the main slaughter yields in common carp.
3) Estimate genetic parameters of the composition of fatty acids in the flesh of market size common carp and their relation to performance traits.
4) Assess the accuracy of genomic evaluations of growth in juvenile common carp using RAD-sequencing.
5) Search for quantitative trait loci affecting resistance to Koi herpesvirus in common carp using RAD-sequencing.

REFERENCES

Aliah, R.S., Takagi, M., Dong, S., Teoh, C.T., Taniguchi, N., 1999. Isolation and inheritance of microsatellite markers in the common carp *Cyprinus carpio*. Fish. Sci. 65, 235–239.

Allendorf, F.W., 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biol. 5, 181–190.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE 3, e3376.

Bakos, J., Gorda, S., 1995. Genetic improvement of common carp strains using intraspecific hybridization. Aquaculture 129, 183–186.

Balon, E.K., 1995. Origin and domestication of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. Aquaculture 129, 3–48.

Balon, E.K., 2006. The oldest domesticated fishes, and the consequences of an epigenetic dichotomy in fish culture. J. Ichthyol. Aquat. Biol. 11, 47–86.

Basavaraju, Y., Mair, G.C., Kumar, H.M., Kumar, S.P., Keshavappa, G., Penman, D.J., 2002. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp, *Cyprinus carpio*, in Karnataka, India. Aquaculture 204, 407–418.

Bauer, C., Schlott, G., 2004. Overwintering of farmed common carp (*Cyprinus carpio* L.) in the ponds of a central European aquaculture facility — measurement of activity by radio telemetry. Aquaculture 241, 301–317.

Bogeruk, A.K., 2008. Catalogue of Carp Breeds (*Cyprinus carpio* L.) of the Countries of the Central and Eastern Europe, Ministry of Agriculture of the Russian Federation, Moscow, 160pp.

Bosworth, B., Holland, M., Brazil, B., 2001. Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. J. Anim. Sci. 79, 1483–1490.

Bourdon, R.M., 2000. Understanding Animal Breeding. Second edition. Prentice–Hall, London, pp. 538.

Calder, P.C., Yaqoob, P., 2009. Omega-3 polyunsaturated fatty acids and human health outcomes. BioFactors 35, 266–272.

Campbell, N.R., LaPatra, S.E., Overturf, K., Towner, R., Narum, S.R., 2014. Association mapping of disease resistance traits in rainbow trout using restriction site associated DNA sequencing. G3: Genes. Genom. Genet. 4, 2473–2481.

Chavanne, H., Janssen, K., Hofherr, J., Contini, F., Haffray, P., Komen, H., Nielsen, E.E., Bargelloni, L., 2016. A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry. Aquacult. Int. 24, 1287–1307.

Cherfas, N., Gomelsky, B., Peretz, Y., Bendom, N., Hulata, G., Moav, B., 1993. Induced gynogenesis and polyploidy in the Israeli common carp line Dor-70. Isr. J. Aquac. 45, 59–72.

Cherfas, N., Gomelsky, B., BenDom, N., Joseph, D., Cohen, S., Israel, I., Kabessa, M., Zohar, G., Peretz, Y., Mires, D., 1996. Assessment of all-female common carp progenies for fish culture. Isr. J. Aquac. 48, 149–157.

Chistiakov, D.A., Voronova, N.V., 2009. Genetic evolution and diversity of common carp *Cyprinus carpio* L. Cent. Eur. J. Biol. 4, 304-312.

Crooijmans, R., Van der Poel, J., Groenen, M., Bierbooms, V., Komen, J., 1997. Microsatellite markers in common carp (*Cyprinus carpio* L.). Anim. Genet. 28, 129-134.

Daetwyler, H.D., Calus, M.P., Pong-Wong, R., de los Campos, G., Hickey, J.M., 2013. Genomic prediction in animals and plants: simulation of data, validation, reporting, and benchmarking. Genetics 193, 347–365.

David, L., Rajasekaran, P., Fang, J., Hillel, J., Lavi, U., 2001. Polymorphism in ornamental and common carp strains (*Cyprinus carpio* L.) as revealed by AFLP analysis and a new set of microsatellite markers. Mol. Genet. Genomics 266, 353–362.

Dong, Z., Nguyen, N.H., Zhu, W., 2015. Genetic evaluation of a selective breeding program for common carp Cyprinus carpio conducted from 2004 to 2014. BMC Genet. 16, 94.

Dunham, R.A., 2011. Aquaculture and Fisheries Biotechnology: Genetic Approaches. 2nd Edition, CABI Publishing, Oxfordshire, UK. pp. 495.

Dunham, R.A., Taylor, J.F., Rise, M.L., Liu, Z., 2014. Development of strategies for integrated breeding, genetics and applied genomics for genetic improvement of aquatic organisms. Aquaculture 420–421, 21–23.

Dyldin, Y.V., Orlov, A.M., 2016. Ichthyofauna of fresh and brackish waters of Sakhalin Island: An annotated list with taxonomic comments: 2. Cyprinidae–Salmonidae families. J. Ichthyol. 56, 656-693.

Estoup, A., Gharbi, K., SanCristobal, M., Chevalet, C., Haffray, P., Guyomard, R., 1998. Parentage assignment using microsatellites in turbot (*Scophthalmus maximus*) and rainbow trout (*Oncorhynchus mykiss*) hatchery populations. Can. J. Fish. Aquat. Sci. 55, 715-723.

Falconer, D.S., MacKay, T.F.C., 1996. Introduction to Quantitative Genetics. fourth ed.Longman Scientific & Technical, Harlow, UK, pp. 464.

FAO, 2016. Fishery and aquaculture statistics [aquaculture production (Quantities and values) 1950-2014] (FishStatJ). In: FAO fisheries and aquaculture department. http://www.fao.org/fishery/statistics/software/FishStatJ/en. (Accessed on 17 October 2017).

Flajšhans, M., Gela, D., Kocour, M., Rodina, M., Kašpar, V., Linhart, O., Ošanec, J., Němec, R., Chytka, R., 2015. Amur mirror carp, a recently certified breed of common carp in the Czech Republic. In: Book of abstracts: 3rd International Conference on Common Carp, Vodňany, September 3.–4., 2015, pp. 21–23.

Flajšhans, M., Hulata, G., 2007. Common carp - *Cyprinus carpio*. In: Genimpact - Evaluation of genetic impact of aquaculture activities on native populations. (eds Svaasand, T., Crossetti, D., García-Vásquez, E., Verspoor, E.,). pp. 32–39.

Fraslin, C., Dupont-Nivet, M., Haffray, P., Bestin, A., Vandeputte, M., 2018. How to genetically increase fillet yield in fish: New insights from simulations based on field data. Aquaculture 486, 175–183.

Froese, R., Pauly. D., 2018. FishBase. *Cyprinus rubrofuscus* Lacepède, 1803. Available at: http://www.fishbase.org/summary/59920.

García-Celdrán, M., Ramis, G., Manchado, M., Estévez, A., Navarro, A., Armero, E., 2015. Estimates of heritabilities and genetic correlations of raw flesh quality traits in a reared gilthead sea bream (*Sparus aurata* L.) population sourced from broodstocks along the Spanish coasts. Aquaculture 446, 181–186.

Gela, D., Linhart, O., 2000. Evaluation of slaughter value of common carp from diallel crossings. Czech J Anim. Sci. 45, 53–58.

Gela, D., Rodina, M., Linhart, O., 2003. Top-crossing with evaluation of slaughtering value in common carp (*Cyprinus carpio* L.) offspring. Aquacult. Int. 11, 379–387.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Thompson, R., 2009. ASReml user guide release 3.0. VSN International Ltd., Hemel Hempstead, HP1 1ES, UK.

Gjedrem, T., 1979. Selection for growth rate and domestication in Atlantic salmon. J. Anim. Breed. Genet. 96, 56–59.

Gjedrem, T., 1983. Genetic variation in quantitative traits and selective breeding in fish and shellfish. Aquaculture 33, 51–72.

Gjedrem, T., 2005. Selection and breeding programs in aquaculture. Springer, Dordrecht, The Netherlands, 364 pp.

Gjedrem, T., Baranski, M., 2009. Selective Breeding in Aquaculture: an Introduction. Springer, Dordrecht, The Netherlands, pp. 221.

Gjedrem, T., 2010. The first family-based breeding program in aquaculture. Reviews in Aquaculture 2, 2–15.

Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. Aquaculture 350, 117–129.

Gjedrem, T., 2012. Genetic improvement for the development of efficient global aquaculture: a personal opinion review. Aquaculture 344, 12–22.

Gjedrem, T., Robinson, N., 2014. Advances by selective breeding for aquatic species: a review. Agricult. Sci. 5, 1152.

Gjedrem, T., Rye, M., 2016. Selection response in fish and shellfish: a review Reviews in Aquaculture 0, 1–12.

Gomelsky, B., 2003. Chromosome set manipulation and sex control in common carp: a review. Aquat. Living Resour. 16, 408–415.

Gomelsky, B., Cherfas, N., Peretz, Y., Ben-Dom, N., Hulata, G., 1994. Hormonal sex inversion in the common carp (*Cyprinus carpio* L.). Aquaculture 126, 265–270.

Gorda, S., Bakos, J., Liska, J., Kakuk, C., 1995. Live gene bank of common carp strains at the Fish Culture Research Institute, Szarvas. Aquaculture 129, 199–202.

Groeneveld, E., Kovač, M., Mielenz, N., 2008. VCE - User's Guide and Reference Manual - Version 6.0. Institute of Farm Animal Genetics, Neustadt, Germany., Neustadt, Germany.

Haenen, O., Way, K., Bergmann, S., Ariel, E., 2004. The emergence of koi herpesvirus and its significance to European aquaculture. Bull. Eur. Assoc. Fish Pathol. 24, 293–307.

Haffray, P., Bugeon, J., Rivard, Q., Quittet, B., Puyo, S., Allamelou, J.M., Vandeputte, M., Dupont-Nivet, M., 2013. Genetic parameters of in-vivo prediction of carcass, head and fillet yields by internal ultrasound and 2D external imagery in large rainbow trout (*Oncorhynchus mykiss*). Aquaculture 410–411, 236–244.

Hedrick, P.W., 2005. Genetics of Populations. 3rd ed. Jones and Barlett Publishers, Sudbury, MA, pp. 737.

Henderson, C.R., 1975. Best linear unbiased estimation and prediction under a selection model. Biometrics 31, 423–447.

Herbinger, C.M., Doyle, R.W., Pitman, E.R., Paquet, D., Mesa, K.A., Morris, D.B., Wright, J.M., Cook, D., 1995. DNA fingerprint based analysis of paternal and maternal effects on offspring growth and survival in communally reared rainbow trout. Aquaculture 137, 245–256.

Hickey, J.M., Chiurugwi, T., Mackay, I., Powell, W., Eggen, A., Kilian, A., Jones, C., Canales, C., Grattapaglia, D., Bassi, F., 2017. Genomic prediction unifies animal and plant breeding programs to form platforms for biological discovery. Nat. Genet. 49, 1297.

Hill, W.G., 2010. Understanding and using quantitative genetic variation. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 365, 73–85.

Hill, W.G., Bünger, L., 2004. Inferences on the genetics of quantitative traits from long-term selection in laboratory and domestic animals. Plant Breed. Rev. 24, 169–210.

Hillen, J., Coscia, I., Vandeputte, M., Herten, K., Hellemans, B., Maroso, F., Vergnet, A., Allal, F., Maes, G., Volckaert, F., 2017. Estimates of genetic variability and inbreeding in experimentally selected populations of European sea bass. Aquaculture 479, 742-749.

Horváth, L., Tamás, G., Seagrave, C., 1992. Carp and pond fish culture including Chinese herbivorous species, pike, tench, zander, wels catfish and goldfish. Oxford, Fishing News Books Ltd, pp. 170.

Houston, R.D., 2017. Future directions in breeding for disease resistance in aquaculture species. Rev. Bras. Zootec. 46, 545–551.

Hu, X., Li, C., Shang, M., Ge, Y., Jia, Z., Wang, S., Zhang, Q., Shi, L., 2017. Inheritance of growth traits in Songpu mirror carp (*Cyprinus carpio* L.) cultured in Northeast China. Aquaculture 477, 1–5.

Huckstorf, V., 2012. *Cyprinus rubrofuscus*. The IUCN Red List of Threatened Species 2012, e.T166052A1108337.

Hulata, G., 1995. A review of genetic improvement of the common carp (*Cyprinus carpio* L.) and other cyprinids by crossbreeding, hybridization and selection. Aquaculture 129, 143–155.

Hulata, G., 2001. Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica 111, 155–173.

Hurst, T.P., 2007. Causes and consequences of winter mortality in fishes. J Fish Biol. 71, 315–345.

Janhunen, M., Nousiainen, A., Koskinen, H., Vehviläinen, H., Kause, A., 2017. Selection strategies for controlling muscle lipid content recorded with a non-destructive method in European whitefish, *Coregonus lavaretus*. Aquaculture 481**,** 229–238.

Janssen, K., Prchal, M., Kocour, M., Berentsen, P.B.M., Komen, H., 2015. Common Carp — Current Status of Selective Breeding in Europe. http://www.fishboost.eu/uploads/2/5/8/8/25888062/common_carp_-_current_status_of_selective_breeding_in_europe.pdf (Accessed on 14 September 2015).

Janssen, K., Chavanne, H., Berentsen, P., Komen, H., 2017a. Impact of selective breeding on European aquaculture. Aquaculture 472, 8–16.

Janssen, K., Berentsen, P., Besson, M., Komen, H., 2017b. Derivation of economic values for production traits in aquaculture species. Genet. Sel. Evol. 49, 5.

Kause, A., Paananen, T., Ritola, O., Koskinen, H., 2007. Direct and indirect selection of visceral lipid weight, fillet weight, and fillet percentage in a rainbow trout breeding program. J. Anim. Sci. 85, 3218–3227.

Kause, A., Quinton, C., Airaksinen, S., Ruohonen, K., Koskela, J., 2011. Quality and production trait genetics of farmed European whitefish. J. Anim. Sci. 89, 959–971.

Kause, A., Kiessling, A., Martin, S.A., Houlihan, D., Ruohonen, K., 2016. Genetic improvement of feed conversion ratio via indirect selection against lipid deposition in farmed rainbow trout (*Oncorhynchus mykiss* Walbaum). Br. J.Nutr. 116, 1656–1665.

Khan, H., Gupta, S., Reddy, P., Tantia, M., Kowtal, G., 1990. Production of sterile intergeneric hybrids and their utility in aquaculture and reservoir stocking. Carp Seed Production Technology, edited by P. Keshavanath & KV Radhakrishnan. Special Publication Asian Fisheries Society, Indian Branch, 41–48.

Kirpichnikov, V., Ilyasov, I., Shart, L., Vikhman, A., Ganchenko, M., Ostashevsky, A., Simonov, V., Tikhonov, G., Tjurin, V., 1993. Selection of Krasnodar common carp (*Cyprinus carpio* L.) for resistance to dropsy: principal results and prospects Aquaculture 11, 7–20.

Kocour, M., Gela, D., Rodina, M., Linhart, O., 2005a. Testing of performance in common carp *Cyprinus carpio* L. under pond husbandry conditions I: top-crossing with Northern mirror carp. Aquacult. Res. 36, 1207–1215.

Kocour, M., Linhart, O., Gela, D., Rodina, M., 2005b. Growth Performance of All-Female and Mixed-Sex Common Carp *Cyprinus Carpio* L. Populations in the Central Europe Climatic Conditions. J. World Aquacult. Soc. 36, 103–113.

Kocour, M., Mauger, S., Rodina, M., Gela, D., Linhart, O., Vandeputte, M., 2007. Heritability estimates for processing and quality traits in common carp (*Cyprinus carpio* L.) using a molecular pedigree. Aquaculture 270, 43–50.

Kohlmann, K., Kersten, P., 1999. Genetic variability of German and foreign common carp (*Cyprinus carpio* L.) populations. Aquaculture 173, 435–445.

Kohlmann, K., Gross, R., Murakaeva, A., Kersten, P., 2003. Genetic variability and structure of common carp (*Cyprinus carpio*) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. Aquat. Living Resour. 16, 421–431.

Kohlmann, K., Kersten, P., Flajšhans, M., 2005. Microsatellite-based genetic variability and differentiation of domesticated, wild and feral common carp (*Cyprinus carpio* L.) populations. Aquaculture 247, 253–266.

Kottelat, M., 2001. Fishes of Laos. WHT Publications Ltd., Colombo 5, Sri Lanka. pp. 198.

Kottelat, M., 2013. The fishes of the inland waters of southeast Asia: a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. Raffles Bull. Zool. 27, 1–663.

Kumar, G., Kocour, M., 2017. Applications of next-generation sequencing in fisheries research: A review. Fish. Res. 186, 11–22.

Larhammar, D., Risinger, C., 1994. Molecular genetic aspects of tetraploidy in the common carp *Cyprinus carpio*. Mol. Phylogenet. Evol. 3, 59–68.

Leaver, M.J., Taggart, J.B., Villeneuve, L., Bron, J.E., Guy, D.R., Bishop, S.C., Houston, R.D., Matika, O., Tocher, D.R., 2011. Heritability and mechanisms of n–3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. Comp. Biochem. Physiol. Part D Genomics Proteomics 6, 62–69.

Leeds, T.D., Vallejo, R.L., Weber, G.M., Gonzalez-Pena, D., Silverstein, J.T., 2016. Response to five generations of selection for growth performance traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 465, 341–351.

Lind, C., Ponzoni, R., Nguyen, N., Khaw, H., 2012. Selective breeding in fish and conservation of genetic resources for aquaculture. Reprod. Domest. Anim. 47, 255–263.

Linhart, O., Flajšhans, M., Kvasnička, P., 1991. Induced triploidy in the common carp (*Cyprinus carpio* L.): a comparison of two methods. Aquat. Living Resour. 4, 139–145.

Lu, C., Laghari, M.Y., Zheng, X., Cao, D., Zhang, X., Kuang, Y., Li, C., Cheng, L., Mahboob, S., Al-Ghanim, K.A., 2017. Mapping quantitative trait loci and identifying candidate genes affecting feed conversion ratio based onto two linkage maps in common carp (*Cyprinus carpio* L). Aquaculture 468, 585–596.

Lv, W., Zheng, X., Kuang, Y., Cao, D., Yan, Y., Sun, X., 2016. QTL variations for growth-related traits in eight distinct families of common carp (*Cyprinus carpio*). BMC Genet. 17, 65.

Madsen, P., Jensen, J., 2013. DMU version 6, http://dmu.agrsci.dk/DMU/Doc/Current/dmuv6_guide.5.2.pdf (Accessed on 1 December 2017).

Marković, Z., Stanković, M., Rašković, B., Dulić, Z., Živić, I., Poleksić, V., 2016. Comparative analysis of using cereal grains and compound feed in semi-intensive common carp pond production. Aquacult. Int. 24, 1699–1723.

Mráz, J., Pickova, J., 2011. Factors influencing fatty acid composition of common carp (*Cyprinus carpio*) muscle. Neuroendocrinol. Lett. 32, 3-8.

Meuwissen, T., Hayes, B., Goddard, M., 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819–1829.

Meuwissen, T., Hayes, B., Goddard, M., 2013. Accelerating improvement of livestock with genomic selection. Annu. Rev. Anim. Biosci. 1, 221–237.

Moav, R., Wohlfarth, G., 1976. Two-way selection for growth rate in the common carp (*Cyprinus carpio* L.). Genetics 82, 83–101.

Nguyen, N.H., Ponzoni, R.W., Yee, H.Y., Abu-Bakar, K.R., Hamzah, A., Khaw, H.L., 2010. Quantitative genetic basis of fatty acid composition in the GIFT strain of Nile tilapia (*Oreochromis niloticus*) selected for high growth. Aquaculture 309, 66–74.

Nguyen, N.H., 2016. Genetic improvement for important farmed aquaculture species with a reference to carp, tilapia and prawns in Asia: achievements, lessons and challenges. Fish Fish. 17, 483–506.

Nielsen, H.M., Ødegård, J., Olesen, I., Gjerde, B., Ardo, L., Jeney, G., Jeney, Z., 2010. Genetic analysis of common carp (*Cyprinus carpio*) strains. I: Genetic parameters and heterosis for growth traits and survival. Aquaculture 304, 14–21.

Ninh, N.H., Ponzoni, R.W., Nguyen, N.H., Woolliams, J.A., Taggart, J.B., McAndrew, B.J., Penman, D.J., 2011. A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): estimation of genetic parameters. Aquaculture 322-323, 39–46.

Ninh, N.H., Ponzoni, R.W., Nguyen, N.H., Woolliams, J.A., Taggart, J.B., McAndrew, B.J., Penman, D.J., 2013. A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): Responses to selection. Aquaculture 408–409, 152–159.

Nolasco-Alzaga, H.R., Perez-Enriquez, R., Enez, F., Bestin, A., Palacios-Mechetnov, E., Haffray, P., 2018. Quantitative genetic parameters of growth and fatty acid content in the hemolymph of the Whiteleg shrimp Litopenaeus vannamei. Aquaculture 482, 17–23.

Oberle, M., Schwarz, F., Kirchgessner, M., 1997. Growth and carcass quality of carp (Cyprinus carpio L.) fed different cereals, lupin seed or zooplankton. Arch. Tierenahr. 50, 75–86.

Ødegård, J., Olesen, I., Dixon, P., Jeney, Z., Nielsen, H.M., Way, K., Joiner, C., Jeney, G., Ardó, L., Rónyai, A., Gjerde, B., 2010. Genetic analysis of common carp (*Cyprinus carpio*) strains. II: Resistance to koi herpesvirus and Aeromonas hydrophila and their relationship with pond survival. Aquaculture 304, 7–13.

Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011. Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. Aquacult. Res. 42, 103–114.

Overturf, K., Welker, T., Barrows, F., Towner, R., Schneider, R., LaPatra, S., 2013. Variation in rainbow trout, Oncorhynchus mykiss, to biosynthesize eicosapentaenoic acid and docosahexaenoic acid when reared on plant oil replacement feeds. J. World Aquacult. Soc. 44, 326–337.

Palaiokostas, C., Bekaert, M., Davie, A., Cowan, M.E., Oral, M., Taggart, J.B., Gharbi, K., McAndrew, B.J., Penman, D.J., Migaud, H., 2013a. Mapping the sex determination locus in the Atlantic halibut (*Hippoglossus hippoglossus*) using RAD sequencing. BMC Genom. 14, 566.

Palaiokostas, C., Bekaert, M., Khan, M.G., Taggart, J.B., Gharbi, K., McAndrew, B.J., Penman, D.J., 2013b. Mapping and validation of the major sex-determining region in Nile tilapia (*Oreochromis niloticus* L.) using RAD sequencing. PLoS ONE 8, e68389.

Palaiokostas, C., Ferraresso, S., Franch, R., Houston, R.D., Bargelloni, L., 2016. Genomic prediction of resistance to pasteurellosis in gilthead sea bream (*Sparus aurata*) using 2b-RAD sequencing. G3: Genes. Genom. Genet. 6, 3693–3700.

Peng, W., Xu, J., Zhang, Y., Feng, J., Dong, C., Jiang, L., Feng, J., Chen, B., Gong, Y., Chen, L., 2016. An ultra-high density linkage map and QTL mapping for sex and growth-related traits of common carp (*Cyprinus carpio*). Sci. Rep. 6, 26693.

Piačková, V., Flajšhans, M., Pokorová, D., Reschová, S., Gela, D., Čížek, A., Veselý, T., 2013. Sensitivity of common carp, *Cyprinus carpio* L., strains and crossbreeds reared in the Czech Republic to infection by cyprinid herpesvirus 3 (CyHV-3; KHV). J. Fish Dis. 36, 75–80.

Powell, J., White, I., Guy, D., Brotherstone, S., 2008. Genetic parameters of production traits in Atlantic salmon (*Salmo salar*). Aquaculture 274, 225–231.

Recoubratsky, A., Gomelsky, B., Emelyanova, O., Pankratyeva, E., 1989. Obtaining triploid and tetraploid carp progeny with heat shock. Proc. Res. Inst. Pond Fish 58, 54–60.

Reddy, P., Khan, H., Gupta, S., Tantia, M., Kowtal, G., 1990. On the ploidy of three intergeneric hybrids between common carp (*Cyprinus carpio communis* L.) and Indian major carps. Aquacult. Hungarica 6, 5–11.

Ribaut, J.-M., Hoisington, D., 1998. Marker-assisted selection: new tools and strategies. Trends Plant Sci. 3, 236–239.

Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P., Houston, R., 2017. Applications of genotyping by sequencing in aquaculture breeding and genetics. Reviews in Aquaculture 0, 1–13.

Rutten, M.J., Bovenhuis, H., Komen, H., 2004. Modeling fillet traits based on body measurements in three Nile tilapia strains (*Oreochromis niloticus* L.). Aquaculture 231, 113–122.

Saillant, E., Dupont-nivet, M., Sabourault, M., Ha, P., Laureau, S., Vidal, M.-O., Chatain, B., 2009. Genetic variation for carcass quality traits in cultured sea bass (*Dicentrarchus labrax*). 22, 105–112.

Shapira, Y., Magen, Y., Zak, T., Kotler, M., Hulata, G., Levavi-Sivan, B., 2005. Differential resistance to koi herpes virus (KHV)/carp interstitial nephritis and gill necrosis virus (CNGV) among common carp (*Cyprinus carpio* L.) strains and crossbreds. Aquaculture 245, 1–11.

Schäperclaus, W., 1962. Trate de Pisciculture en Etang. Vigot Freres, Paris 208, 208–227.

Sonesson, A.K., 2007. Within-family marker-assisted selection for aquaculture species. Genet. Sel. Evol. 39, 301.

Steffens, W., 1996. Protein sparing effect and nutritive significance of lipid supplementation in carp diets. Arch. Tierenahr. 49, 93–98.

Steffens, W., 2016. Aquaculture produces wholesome food: cultured fish as a valuable source of n-3 fatty acids. Aquacult. Int. 24, 787–802.

Tadmor-Levi, R., Asoulin, E., Hulata, G., David, L., 2017. Studying the genetics of resistance to CyHV-3 disease using introgression from feral to cultured common carp strains. Front. Genet. 8, 24.

Thai, B., Burridge, C., Pham, T., Austin, C., 2005. Using mitochondrial nucleotide sequences to investigate diversity and genealogical relationships within common carp (*Cyprinus carpio* L.). Anim. Genet. 36, 23-28.

Tocher, D.R., 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture 449, 94–107.

Trbović, D., Živić, I., Stanković, M., Živić, M., Dulić, Z., Petronijević, R., Marković, Z., 2017. Dependence of the common carp (*Cyprinus carpio* L.) fatty acid profile on diet composition in a semi-intensive farming system: tissue and time variability. Aquacult. Res 48, 3121–3133.

Van Sang, N., Thomassen, M., Klemetsdal, G., Gjøen, H.M., 2009. Prediction of fillet weight, fillet yield, and fillet fat for live river catfish (*Pangasianodon hypophthalmus*). Aquaculture 288, 166–171.

Vandeputte, M., 2003. Selective breeding of quantitative traits in the common carp (Cyprinus carpio): a review. Aquat. Living Resour. 16, 399–407.

Vandeputte, M., Dupont-Nivet, M., Haffray, P., Chavanne, H., Cenadelli, S., Parati, K., Vidal, M.-O., Vergnet, A., Chatain, B., 2009. Response to domestication and selection for growth in the European sea bass (*Dicentrarchus labrax*) in separate and mixed tanks. Aquaculture 286, 20–27.

Vandeputte, M., Haffray, P., 2014. Parentage assignment with genomic markers: A major advance for understanding and exploiting genetic variation of quantitative traits in farmed aquatic animals. Front. Genet. 5, 1–8.

Vandeputte, M., Kocour, M., Mauger, S., Dupont-Nivet, M., De Guerry, D., Rodina, M., Gela, D., Vallod, D., Chevassus, B., Linhart, O., 2004. Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). Aquaculture 235, 223–236.

Vandeputte, M., Kocour, M., Mauger, S., Rodina, M., Launay, A., Gela, D., Dupont-Nivet, M., Hulak, M., Linhart, O., 2008. Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio* L.): Heritability estimates and response to selection. Aquaculture 277, 7–13.

Vandeputte, M., Puledda, A., Tyran, A.S., Bestin, A., Coulombet, C., Bajek, A., Baldit, G., Vergnet, A., Allal, F., Bugeon, J., Haffray, P., 2017. Investigation of morphological predictors of fillet and carcass yield in European sea bass (*Dicentrarchus labrax*) for application in selective breeding. Aquaculture 470, 40–49.

Wang, C., 2009. Quantitative genetic estimates of growth-related traits in the common carp (*Cyprinus carpio* L.): A review. Frontiers of Biology in China 4, 298–304.

Wang, X., Fu, B., Yu, X., Qu, C., Zhang, Q., Tong, J., 2018. Fine mapping of growth-related quantitative trait loci in Yellow River carp (*Cyprinus carpio haematoperus*). Aquaculture 484, 277–285.

Wohlfarth, G.W., 1993. Heterosis for growth rate in common carp. Aquaculture 113, 31-46.

Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y., Xu, J., Zheng, X., Ren, L., Wang, G., 2014a. Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. Nat. Genet. 46, 1212–1219.

Xu, J., Zhao, Z., Zhang, X., Zheng, X., Li, J., Jiang, Y., Kuang, Y., Zhang, Y., Feng, J., Li, C., 2014b. Development and evaluation of the first high-throughput SNP array for common carp (*Cyprinus carpio*). BMC Genomics 15, 307.

Xu, P., Jiang, Y., Xu, J., Li, J., Sun, X., 2016. Genomics in the common carp, Genomics in Aquaculture. Elsevier, pp. 247–274.

Yáñez, J.M., Newman, S., Houston, R.D., 2015. Genomics in aquaculture to better understand species biology and accelerate genetic progress. Front. Genet. 6, 128.

Zheng, X., Kuang, Y., Lv, W., Cao, D., Sun, Z., Sun, X., 2016. Genome-wide association study for muscle fat content and abdominal fat traits in common carp (*Cyprinus carpio*). PLoS ONE 11, e0169127.

Zhou, J., Wu, Q., Wang, Z., Ye, Y., 2004. Molecular phylogeny of three subspecies of common carp Cyprinus carpio, based on sequence analysis of cytochrome b and control region of mtDNA. J. Zool. Syst. Evol. Res. 42, 266–269.

CHAPTER 2

THE GENETICS OF OVERWINTERING PERFORMANCE IN TWO-YEAR OLD COMMON CARP AND ITS RELATION TO PERFORMANCE UNTIL MARKET SIZE

Prchal, M., Kause, A., Vandeputte, M., Gela, D., Allamelou, J.M., Girish, K., Bestin, A., Bugeon, J., Zhao, J., Kocour, M., 2018. The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. PLoS ONE 13, e0191624.

My share on this work was about 40%.

PLOS ONE

RESEARCH ARTICLE

The genetics of overwintering performance in two-year old common carp and its relation to performance until market size

Martin Prchal[1]*, Antti Kause[2], Marc Vandeputte[3,4], David Gela[1], Jean-Michel Allamellou[5], Girish Kumar[1], Anastasia Bestin[6], Jérôme Bugeon[3], Jinfeng Zhao[1], Martin Kocour[1]

1 University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodňany, Czech Republic, **2** Natural Resources Institute Finland, Jokioinen, Finland, **3** GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France, **4** Ifremer, Palavas-les-Flots, France, **5** LABOGENA-DNA, Jouy-en-Josas, France, **6** SYSAAF, Rennes, France

* mprchal@frov.jcu.cz

Citation: Prchal M, Kause A, Vandeputte M, Gela D, Allamellou J-M, Kumar G, et al. (2018) The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. PLoS ONE 13(1): e0191624. https://doi.org/10.1371/journal.pone.0191624

Editor: Peng Xu, Xiamen University, CHINA

Received: April 20, 2017

Accepted: December 30, 2017

Published: January 25, 2018

Data Availability Statement: The data are fully available in the public repository, Open Scientific Framework (OSF: osf.io/5py7k).

Funding: This study was supported by the Ministry of Education, Youth, and Sports of the Czech Republic (projects CENAKVA – CZ.1.05/2.1.00/01.0024 and CENAKVA II – LO1205 under the NPU I program), European Union's Seventh Framework Programme (KBBE.2013.1.2-10) under grant agreement no. 613611 FISHBOOST (http://www.fishboost.eu/), the Grant Agency of the University of South Bohemia (project no. 125/2016/Z) and

Abstract

Using farmed common carp, we investigated the genetic background of the second year overwintering performance and its relation to the performance during the third growing season and at market size. The experimental stock was established by partial factorial design with a series of 4 factorial matings of 5 dams and 10 sires each. The families were reared communally and pedigree was re-constructed with 93.6% success using 12 microsatellites on 2008 offspring. Three successive recordings (second autumn, third spring, and third autumn—market size) covering two periods (second overwintering, third growing season) were included. Body weight, Fulton's condition factor and percent muscle fat content were recorded at all times and headless carcass yield and fillet yield were recorded at market size. Specific growth rate, absolute and relative fat change and overall survival were calculated for each period. Heritability estimates were significantly different from zero and almost all traits were moderately to highly heritable (h^2 = 0.36–1.00), except survival in both periods and fat change (both patterns) during overwintering (h^2 = 0.12–0.15). Genetic and phenotypic correlations imply that selection against weight loss and fat loss during overwintering is expected to lead to a better winter survival, together with a positive effect on growth in the third growing season. Interestingly, higher muscle fat content was genetically correlated to lower survival in the following period (r_g = -0.59; -0.53, respectively for winter and the third summer). On the other hand, higher muscle fat was also genetically linked to better slaughter yields. Moreover, selection for higher condition factor would lead to better performance during winter, growing season and at market size.

Introduction

The aquaculture sector is among the fastest growing agricultural industries, as an increasing demand for fish cannot be supplied with stagnating capture fisheries. Genetic improvement of cultured stocks is playing an important role in optimizing and increasing aquaculture production [1].

project of the Czech NAAR (NAZV) no. QK1710310. The projects/grants provided financial support for people working on the study, data collection and analysis but had no role in decision to publish and to prepare the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Common carp (*Cyprinus carpio* and *Cyprinus rubrofuscus*) is one of the most cultured fish species in the world. Its aquaculture production is still increasing and reached over 4 million tons worldwide and 145 thousand tons in Europe in 2014 [2]. In the recent past, many studies concerning genetic improvement in common carp have been carried out [3–8]. However, most studies have been focused on production traits such as growth and yields. Yet, little is known about the genetic background of traits related to overwintering performance in common carp and about the impact of overwintering performance on the performance in the subsequent growing season.

Overwintering of common carp in temperate climate is considered a critical period with the risk of heavy loss of valuable fish. When water temperature is below 8˚C, common carp significantly reduce their metabolism, decrease feed intake and lose weight [9,10]. Despite reduced metabolism, low feed intake results in utilizing of energetic reserves that become essential for winter survival [11]. In common carp lipid reserves are mobilized first [12], followed by glycogen and protein depletion [13,14] and as a result fish lose weight [9,13,15–17]. High losses of body weight and fast depletion of energetic reserves caused by a severe winter, suboptimal environmental conditions, predation, diseases, parasite assault, or combination of these factors lead to higher winter mortality [10]. The quantification of the impact of individual factors on winter mortality in a pond system is however hardly possible, and the impact of factors influencing fish survival varies over different conditions. Still, the survival of fish in a pond is a result of genetic and environmental factors which operate in combination. Good physical condition of fish before winter is thought to be important for survival, and may be represented with various traits. Therefore, the estimation of genetic parameters of such traits can help to understand their importance for winter survival.

After the overwintering period and the associated feed deprivation, re-feeding results in rapid recovery with catching-up growth and an increase in body fat [18,19]. Therefore, the impact of re-feeding and its genetic and/or phenotypic relation to traits at market size could also be of importance.

Survival is also a major economic trait related to performance over all rearing periods in common carp cultured under pond conditions. The overall survival of a stock may be seen as an indicator showing the ability of organisms to deal with environmental conditions. Until recently, nothing was known about genetic variance of survival in common carp. However, two studies confirmed that the heritability of survival is low to medium but significant [6,8]. Other important traits such as body weight, fat content, condition factor and slaughter yields showed moderate to high heritability [3–8], and thus selection seems to be a valuable tool for their improvement. However, data about genetic variance in dynamics of growth, muscle fat, survival and their correlations with other traits are scarce.

The aim of this study was to estimate the phenotypic and genetic parameters related to the second overwintering in common carp and to the third growing period at the end of which the fish reached market size. The intent was to i) reveal relationships among various traits which may affect the winter performance, ii) see how the winter performance is associated with the performance of fish during the third growing season and at market size and iii) estimate how breeding programs focused on various traits could affect winter survival or performance at market size in a carp breed.

Materials and methods

Ethics statement

The methodological protocol of the current study was approved by the expert committee of the Institutional Animal Care and Use Committee (IACUC) of the University of South

Bohemia (USB) in České Budějovice, Faculty of Fisheries and Protection of Waters (FFPW) in Vodňany according to the law on the protection of animals against cruelty (Act no. 246/1992 Coll., ref. number 16OZ15759/2013-17214). The study did not involve endangered or protected species. The experimental stock was reared under the common semi-intensive pond management with regular checks (three times a week) of fish health and behavior. The experiment, from individual tagging to market size, ran from April 2015 to November 2016 (second and third growing seasons—GS and second winter period—W). At the end the fish were euthanized (humane endpoint) for evaluation of slaughter yields. The standard survival rates in common carp cultured in ponds are 60–80% during 2nd GS, 80–95% during 2nd W and 85–95% during 3rd GS [20]. As we needed to have at least 1500 fish at the end of the experiment to ensure reliability of genetic parameters, 3000 fish were taken initially. At the end of the experiment, 1622 fish assigned to a single parental pair were euthanized for data collection. The observed survival rates were 67% during 2nd GS, 98% during 2nd W and 89% during the 3rd GS. The total mortality for the whole period was lower than expected by Horváth et al. [20] and statistics of the Klatovy Fish Farm. The causes of mortality are likely multiple, including natural stress effects (fasting due to low temperatures, naturally occurring parasites) and predation, typical in the traditional ponds. To enhance animal welfare and decrease suffering during all fish handling, the fish were anaesthetized using 2-phenoxyethanol for each live trait recording, and humanely euthanized for final processing. The main author of study owns the certificate (CZ 01704) giving capacity to conduct and manage experiments involving animals according to section 15d paragraph 3 of Act no. 246/1992 Coll.

Establishment of experimental stock

Artificial spawning of common carp was carried out at the hatchery of USB, FFPW, in Vodňany, Czech Republic. The broodstock fish were Amur mirror carp (Vodňany line), recently accepted as a new Czech common carp breed [21]. The Amur mirror carp are derived from the 2nd generation of intercross of European mirror carp, *Cyprinus carpio*, and Amur wild (scaled) carp, *Cyprinus rubrofuscus*, selected to fix the homozygous "mirror" scale cover while incorporating genetic variation from the Amur wild carp. Presently, this new breed is used in crossbreeding programs and the commercial crossbreds perform 20–40% better in survival and growth compared to others [22]. Moreover, the breed, and even its crossbreds, displayed higher resistance to Koi herpes virus (KHV) infection than standard European carp breeds [23].

In March 2014, the pond with Amur mirror carp broodstock was drained and the available mature fish were transferred and kept in two ponds of 0.2 ha, one for each sex. In May 2014, the broodstock was checked again and fish in a good pre-spawning condition (evaluated by eye and hand inspection) were transferred to the hatchery and kept in tanks separated by sex at water temperature of 18˚C. Fin tissue from caudal fin (approx. 1 cm^2) was collected from each potential parent and stored in Eppendorf tube filled in with 98% ethanol at room temperature.

Artificial spawning of the broodstock fish was performed using the same protocol as described by Vandeputte et al. [3] at a water temperature of 21˚C. Fish gametes were individually stored for a short time until mating. Sperm from 40 males was stored on ice in 200 ml cell culture containers, while stripped eggs from 20 females were stored at the hatchery temperature in dishes covered with foil.

A partial factorial design with a 4 series of mating designs of 5 dams and 10 sires each were used to produce experimental families. For each series, 100 grams of eggs from each dam were taken and pooled into one dish. The pooled eggs were then divided into 10 equal batches of 50

g of eggs and transferred into 10 cups of 200 ml. The cups with eggs were placed on an orbital agitator and inseminated individually by the sperm of a male. Fertilization was done by adding 50 ml of hatchery water while mixing the sperm and eggs with constant 200 rpm rotation speed with deflection 10 mm. One minute later, all the cups within each series were pooled to one dish and egg stickiness was eliminated with a milk solution. The process of fertilization was repeated four times, one per series. The duration between the first and the fourth series was less than two hours. At the end of all four mating series, the eggs from each mating were incubated in separate Zuger jars. After hatching, the yolk-sac fry from each Zuger jar were transferred and nursed in separate post-hatching incubators until swimming stage.

Rearing of experimental stock and phenotypic recordings

At swimming stage the experimental stock was created by pooling equal quantities (estimated volumetrically) of larvae from all four post-hatching incubators. Larvae were transferred in plastic bags under oxygen atmosphere into prepared nursery ponds at USB FFPW and Klatovy Fish Farm (size of ponds 0.2–1 ha, stocking density 150,000 larvae. ha^{-1}). The progenies were reared under semi-intensive pond conditions and the fish were feeding on natural food (plankton, benthos) and on additional pelleted feed [3] through the first growing season and first wintering until March 2015. Then, ponds were harvested and the pond with the best fish survival (50% survival, mean weight 15.8 ± 4.7 g) was taken for the next steps of the experiment. The fish were transferred to two tanks at USB FFPW facility in Vodňany. A random sample of 3000 fish was anesthetized with 2-phenoxyethanol (dose of 0.5 ml per 1 l of water) and then individually PIT-tagged and fin-clipped for further DNA extraction and genotyping in order to assign the fish to their parents. Each tagged individual was weighed (to the nearest 0.01 g) and measured for standard length (to the nearest mm).

In April 2015, all the tagged fish together with a reserve group of untagged fish from the same stock were transferred into an 1 ha pond (stocking density 6000 fish. ha^{-1}) at Klatovy Fish Farm and reared for the second growing season the same way as during the first growing season.

In October 2015, the fish were harvested again. All survived fish (including a total of 2008 PIT tagged individuals) were transferred to tanks in the USB FFPW facility to be measured. Each tagged individual was identified with a tag reader and recorded for body weight (BW_1) and standard length (SL_1). Because of high genetic and phenotypic correlations between SL and BW at that stage, only BW was included in the further analysis. Fulton's condition factor (FC) was calculated as $FC_1 = 10^5 * BW_1 / SL_1^3$. Fat content in muscle (% Fat_1) was measured using a Fish Fatmeter FM 692 (Distell Ltd., UK), using calibration CARP– 1. The fat percentage for each individual was calculated as the mean of four repeated measurements on the left side of the fish performed according to the manufacturer's guidelines. The fat content measurements were performed by the same person during the whole experiment. To validate the accuracy of the muscle fat measurements, the values from Distell Fatmeter were compared with those analysed with sulpho-phospho-vanillin method [24] in 100 randomly sampled market-size fish. For the chemical fat analysis, the whole fillet with skin from each fish were homogenized using a mixer. The correlation between the Distell Fatmeter values and the chemically analysed values was 0.85.

After measuring of the PIT tagged individuals, they were stocked for overwintering in a 0.2 ha pond at USB FFPW pond facility in Vodňany. During winter, when water temperature is below 8˚C, the fish radically reduce their metabolism, movement, and feed intake, and thus they are not fed with any additional food. However, during the experiment the winter conditions were mild. Average water temperature between November and March was 4.3˚C,

significant ice cover stayed for two weeks only and water temperature even increased above 8˚C in November (two weeks) and February (four days). That is why altogether 75 kg of pelleted food was distributed to the pond during wintering in order to help the fish to stay in a good condition.

In March 2016, the pond was harvested and all survivors (n = 1976) were transferred into indoor tanks for data recording. The same traits as before the overwintering were recorded, BW_2, SL_2, FC_2, and % Fat_2. Furthermore, overwintering performance traits were calculated as follows: i) body weight change during wintering expressed as specific growth rate, $SGR_{1\text{-}2} = (\ln w_t - \ln w_0)/t^{-1} * 100$, where w_t is the final body weight (g), w_0 is the initial body weight (g) and t is the duration of growth period in days, ii) absolute fat change, $FatCh_{1\text{-}2} = Fat_2 - Fat_1$, where Fat_2 is the percent fat content after wintering and Fat_1 is the percent fat content before wintering, iii) relative fat change, % $FatCh_{1\text{-}2}$ = (Fat2 –Fat_1) / Fat_1 * 100, and iv) survival, during overwintering, $Surv_{1\text{-}2}$, with 1 given for survived fish and 0 to fish not found during the trait recording.

In April 2016, the tagged fish were stocked in a 4-ha pond at Klatovy Fish Farm for the third growing season. Market-size fish were harvested in October 2016 and transferred into a storage pond in Vodňany and kept there for three weeks. This reflects the common commercial practice to empty the intestines and to refresh the odour and taste of the flesh [17,25]. Final recording was performed at the fish slaughter house of USB FFPW in České Budějovice, Czech Republic. In total 1622 fish with a single parental assignment were dressed out in November 2016. The fish were killed by a hit on the head and bled by cutting the gills according to the local rules. Standard length (SL_3) was measured to the nearest 0.1 mm with an in-house electronic ruler. Fish were weighed (BW_3) to the nearest 0.5 g, and muscle fat content (% Fat_3) was recorded using the Distell Fish Fat Meter as described above. Subsequently, the fish were gutted, one fillet detached, sexed by visual inspection of gonads (females, males, immature) and each part of the processed body (head, fillet, viscera, gonads, skin, half carcass, ribs, fins, scales) was weighed to the nearest 0.5 g. Percentage of processed body [5] or so-called headless carcass yield (% hl-Carss) and fillet yield (% Fill) were calculated as the most important slaughter traits: % hl-Carss = (fillet + skin + trimmings + ribs + half carcass) / body weight * 100); % Fill = (fillet + skin) * 2 / body weight * 100. In addition, similar to the overwintering period FC_3, $SGR_{2\text{-}3}$, $FatCh_{2\text{-}3}$, % $FatCh_{2\text{-}3}$, and $Surv_{2\text{-}3}$ were calculated for the growing period from spring to autumn. The fish with a visible deformity (n = 35) were excluded from further analysis.

Parentage assignment

The fins tissue of parents and the experimental progeny (0.2 cm^2) were placed into 96 well plates and sent to LABOGENA-DNA, the French laboratory for livestock genotyping (ISO 170025 accredited, Jouy-en-Josas, France). The parentage assignment was based on the analysis of 12 microsatellite loci (CCE46, HLJE265, HLJ2241, HLJ2346, HLJ2382, HLJ24657, HLJ2544, HLJ334, HLJ526, HLJ534, J58, and KOI 57–58). The parentage allocation was performed using AccurAssign software, applying a maximum-likelihood method [26]. The individuals without assignment to a single parental pair were discarded from further analysis (n = 129 fish).

Quantitative genetic analysis

All trait values were checked for outliers that might indicate errors during measurements and recordings. Phenotypic (V_P) and genetic variances (V_A) and correlations ($r_{p,}$ and r_g, respectively) were estimated using DMU software [27]. The phenotypic variance (V_P) was taken as

the sum of all of the variance components as follows: $V_P = V_A + V_D + V_R$, where V_D is the non-genetic maternal (dam) variance, and V_R is the residual variance. The software analyses data in multivariate mixed models using the restricted maximum likelihood method [28]. The genetic parameters were estimated using the following animal model:

$$Y_{ijkl} = \mu_i + sex_j + dam_{ik} + anim_{il} + e_{ijkl}$$

Where Y_{ijkl} is the vector of observations (for all analysed traits), μ_i is the overall mean for trait i, sex_j is the fixed effect for sex (j = female, male, unidentified), dam_{ik} is the random maternal effect of dam k for trait i, $anim_{il}$ is the random genetic effect of an animal l (l = 1, 2, etc.–no. of individual) for trait i, and e_{ijkl} is the random residual. Models with and without the maternal effect were used to specifically test the effect of the model on heritability estimates. Genetic correlations were estimated without the dam effect because in most cases the maternal effect was negligible. Heritability estimated without the maternal effect was calculated as $h^2 = V_A / (V_A + V_R)$ and with the maternal effect as $h^2 = V_A / (V_A + V_D + V_R)$. The maternal effect was calculated as $m^2 = V_D / (V_A + V_D + V_R)$. Heritability for survival was estimated on the observed binary scale and subsequently transformed to the underlying normally distributed liability scale using the formula by Dempster and Lerner [29]. Furthermore, residual covariance between a survival trait and the traits recorded at or after the survival trait recording was set to zero. Therefore, phenotypic correlations between these traits were not applicable.

Results

Parentage assignment

From the total of 2008 genotyped fish, 1879 fish (93.6%) were successfully assigned to a single parental pair, 96 (4.8%) were assigned to multiple parent pairs, and 33 (1.6%) were not assigned to any parental pair. Assigned fish (single parental pair) belonged to 199 out of the possible 200 full-sib families. The number of progeny per sire varied from 18 to 98, the average was 47. The number of progeny per dam varied from 27 to 160, the average was 94. The parentage assignment results were solid and similar to those achieved in other studies on common carp [3–5,7].

Descriptive statistics of traits

Three successive recordings (before second wintering, after second wintering = before third growing season, and after third growing season) covering two periods (second overwintering, third growing season) were studied (Table 1). During overwintering, only a slight decrease in muscle fat content ($FatCh_{1-2}$, % $FatCh_{1-2}$), and surprisingly, a slight increase in weight were observed. The overwintering survival was high (98%). The growing period was expressed with rapid (recovery) growth (SGR_{2-3}) and increasing fat content ($FatCh_{2-3}$, % $FatCh_{2-3}$). At market size, the *CV* for body weight was lower compared to the previous periods (14.6% vs 19.2 and 19.7%). The yields of hl-carcass (66%) and fillets (50%) were higher than usual in common carp, probably due to the specific dress out process which was different from the commercial one but reflected better the biological values of the traits. The level of $Surv_{2-3}$ (89%) was typical for this age category and climatic conditions.

Heritability estimates

All the estimated heritabilities were significantly different from zero and almost all traits were medium to highly heritable (0.36–0.68) (Table 1). Surprisingly, the Fulton's condition factors (FC1 –FC_3) achieved heritability estimates from high to close to unity (0.73–1.0). Low

Table 1. Number of observations (*n*), traits means (mean ± S.D.), *CV* (coefficient of variation), V_P (phenotypic variance), V_A (genetic variance), h^2 (heritability estimates ± S.E.), m^2 (maternal effect ± S.E.) for traits within each studied period ($_1$ before winter period, $_2$ after winter period, $_3$ at harvest) and for traits changes during $_{1–2}$ overwintering period and $_{2–3}$ growing period.

Trait	*N*	Mean ± S.D.	*CV*	V_P	V_A	h^2 ± S.E.	m^2 ± S.E.
BW_1	1847	336.1 ± 64.6	19.2	4124.5	2022.1	0.49 ± 0.08	0.05 ± 0.07
BW_2	1814	340 ± 67.1	19.7	4508.5	2307.4	0.51 ± 0.08	0.04 ± .0.07
FC_1	1847	2.85 ± 0.25	8.8	0.0644	0.0468	0.73 ± 0.10	0.03 ± 0.09
FC_2	1813	2.84 ± 0.24	8.5	0.0632	0.0598	0.93 ± 0.10	0.03 ± 0.11
% Fat1	1847	4.94 ± 1.26	25.5	1.62	1.01	0.62 ± 0.12	0.00 ± 0.08
% Fat2	1814	4.35 ± 1.11	25.5	1.26	0.45	0.64 ± 0.14	0.00 ± 0.08
SGR_{1-2}	1814	0.004 ± 0.02	N/A	0.34	0.16	0.47 ± 0.11	0.00 ± 0.06
$FatCh_{1-2}$	1813	-0.59 ± 0.64	108.5	0.41	0.052	0.12 ± 0.05	0.00 ± 0.02
% $FatCh_{1-2}$	1814	-11 ± 12.8	116.4	164.64	21.42	0.13 ± 0.05	0.00 ± 0.02
$Surv_{1-2(Obs)}$	1814	0.98 ± 0.16	N/A	0.0250	0.0004	0.02 ± 0.01	0.00 ± 0.03
$Surv_{1-2\ (Lia)}$	1814	N/A	N/A	N/A	N/A	0.13 ± 0.06[1]	0.00 ± 0.03
BW_3	1559	1910 ± 279	14.6	80835.7	50873.1	0.63 ± 0.12	0.00 ± 0.08
FC_3	1558	3.40 ± 0.32	9.4	0.0990	0.0986	1.00 ± 0.09	0.05 ± 0.12
% Fat_3	1559	11.56 ± 2.96	25.6	8.40	5.67	0.67 ± 0.13	0.00 ± 0.09
SGR_{2-3}	1559	0.89 ± 0.07	7.9	0.48	0.24	0.49 ± 0.10	0.00 ± 0.06
$FatCh_{2-3}$	1557	7.24 ± 2.56	35.4	6.18	3.45	0.56 ± 0.10	0.00 ± 0.06
% $FatCh_{2-3}$	1557	175 ± 72	41.1	5156.29	2398.84	0.47 ± 0.10	0.00 ± 0.06
$Surv_{2-3\ (Obs)}$	1622	0.89 ± 0.35	N/A	0.12	0.007	0.06 ± 0.02	0.00 ± 0.02
$Surv_{2-3\ (Lia)}$	1622	N/A	N/A	N/A	N/A	0.15 ± 0.05[1]	0.00 ± 0.02
% hl-Carss	1559	66.21 ± 2.19	3.3	3.83	1.36	0.36 ± 0.08	0.00 ± 0.05
% Fill	1559	49.75 ± 1.95	3.9	3.43	1.23	0.36 ± 0.08	0.00 ± 0.05

BW_1 –BW_3 = body weight, FC_1 –FC_3 = Fulton's condition factor, % Fat_1 –% Fat_3 = muscle fat percent, % hl-Carss = headless-carcass yield, % Fill = fillet yield. Indices: 1 = the trait was recorded before wintering, 2 = the trait was recorded after wintering (before the third growing season), 3 = the trait was recorded after the third growing season (at market size). Overwintering period: SGR_{1-2} specific growth rate, $FatCh_{1-2}$ = absolute fat change, % $FatCh_{1-2}$ = relative fat change %, $Surv_{1-2(Obs)}$ = overall survival on observed scale, $Surv_{1-2\ (Lia)}$ = overall survival on liability scale. Growing period: SGR_{2-3} specific growth rate, $FatCh_{2-3}$ = absolute fat change, % $FatCh_{2-3}$ = relative fat change %, $Surv_{2-3\ (Obs)}$ = overall survival on observed scale, $Surv_{2-3\ (Lia)}$ = overall survival on liability scale. N/A = not applicable.

[1] = h^2 ± S.E. was transformed to the liability scale using the formula by Dempster and Lerner [29].

https://doi.org/10.1371/journal.pone.0191624.t001

heritability estimates were observed for $FatCh_{1-2}$ and % $FatCh_{1-2}$ (0.12–0.13). The heritabilities for survival were low for both periods (overwintering and growing season) and for both kind of estimations, on the observed scale ($Surv_{1-2obs}$ = 0.02, $Surv_{2-3obs}$ = 0.06) and on the underlying liability scale ($Surv_{1-2lia}$ = 0.13, $Surv_{2-3lia}$ = 0.15). The maternal effects m^2 for all traits were insignificant and close to zero (<0.05).

Genetic and phenotypic correlations

Correlations of traits related to overwintering. Genetic and phenotypic correlations among traits during overwintering are presented in Table 2. High (values in range 0.51–0.80) to strong (values > 0.80) phenotypic (0.68–0.98) and genetic correlations (0.98 for all traits) were observed for the same trait recorded before and after overwintering (i.e. BW1 –BW_2, % Fat_1 - % Fat_2 and FC_1 –FC_2; S1 Table).

Low positive phenotypic correlations were observed between BW_1 and % Fat_1 (r_p = 0.28) and between SGR_{1-2} and % $FatCh_{1-2}$ (r_p = 0.29). A low negative correlation was estimated between % Fat_1 and % $FatCh_{1-2}$ (r_p = -0.29) and a moderate one was found between % Fat_1 and $FatCh_{1-2}$ (r_p = -0.46). A high positive phenotypic correlation was observed between $FatCh_{1-2}$ and % $FatCh_{1-2}$ (r_p = 0.93).

Table 2. Genetic (above the diagonal; ± S.E.) and phenotypic correlations (below the diagonal) of traits before wintering and traits changes during overwintering.

	BW_1	SGR_{1-2}	FC_1	% Fat_1	$FatCh_{1-2}$	% $FatCh_{1-2}$	$Surv_{1-2}$
BW_1	x	0.27 ± 0.14	0.08 ± 0.14	0.32 ± 0.13	-0.10 ± 0.18	0.05 ± 0.18	0.19 ± 0.31
SGR_{1-2}	0.11	x	0.50 ± 0.11	-0.39 ± 0.16[1]	0.63 ± 0.12	0.67 ± 0.15	0.47 ± 0.29
FC_1	0.06	0.14	x	-0.25 ± 0.14	0.21 ± 0.17	0.22 ± 0.16	0.26 ± 0.28
% Fat_1	0.28	-0.14	0.00	x	-0.51 ± 0.14	-0.16 ± 0.17	-0.59 ± 0.26[2]
$FatCh_{1-2}$	-0.05	0.16	-0.01	-0.46	x	0.92 ± 0.04	0.68 ± 0.30
% $FatCh_{1-2}$	-0.01	0.29	0.00	-0.29	0.93	x	0.46 ± 0.33
$Surv_{1-2}$	0.02	N/A	0.03	-0.01	N/A	N/A	x

See Table 1 for trait abbreviations. When covariate of body weight to % muscle fat content was used

[1] r_g = -0.47 ± 0.12

[2] r_g = -0.68 ± 0.28. N/A = not applicable.

https://doi.org/10.1371/journal.pone.0191624.t002

Regarding the genetic correlations between different traits, a strong positive genetic correlation was estimated between $FatCh_{1-2}$ and % $FatCh_{1-2}$ (r_g = 0.92 ± 0.04). High positive genetic correlations were estimated between SGR_{1-2} and the fat change traits, i.e. $FatCh_{1-2}$ (r_g = 0.63 ± 0.12) and % $FatCh_{1-2}$ (r_g = 0.67 ± 0.15), and also between $FatCh_{1-2}$ and $Surv_{1-2}$ (r_g = 0.68 ± 0.30), showing that several overwintering traits are related to each other. High negative genetic correlations were observed between % Fat_1 and $FatCh_{1-2}$ (r_g = -0.51 ± 0.14) and interestingly also between % Fat_1 and $Surv_{1-2}$ (r_g = -0.59 ± 0.26), indicating a link between reduced winter survival and high fat before winter. A moderate positive genetic correlation was estimated between BW_1 and % Fat_1 (r_g = 0.32 ± 0.13) and between SGR_{1-2} and FC_1 (r_g = 0.50 ± 0.11). A moderate negative genetic correlation was observed between % Fat_1 and SGR_{1-2} (r_g = -0.39 ± 0.16). To ensure that the negative relationships of % Fat_1 with $Surv_{1-2}$, $FatCh_{1-2}$, % $FatCh_{1-2}$ and SGR_{1-2} were not generated by the relation of % Fat_1 with BW_1, the analysis was also run using BW_1 as a covariate for % Fat_1. With such a model, the genetic correlations become either more negative (SGR_{1-2} and $Surv_{1-2}$) (Table 2.), or remain the same ($FatCh_{1-2}$ and % $FatCh_{1-2}$). Among the other pairs of traits, no significant genetic correlations were observed.

Correlations between traits related to overwintering and traits related to the third growing season. Estimated correlations among overwintering traits and traits related to the third growing season are listed in Table 3. When looking at the same traits between periods (before the third growing season and after the growing season) strong positive correlations (phenotypic as well as genetic) were observed for body weight, FC and muscle fat content (r_g = 0.70–0.94, r_p = 0.52–0.73). For the other traits, the correlations were insignificant (SGR) or with negative pattern for phenotypic and genetic correlations (FatCh, % FatCh and Surv).

Generally, phenotypic correlations were in most cases lower than genetic correlations. Only 11 phenotypic correlations out of 63 investigated were higher than 0.20 of which only four were higher than 0.50. For genetic correlations, 24 values out of 63 were significant of which 12 were higher than 0.50, and three were 0.70 or higher.

When looking at correlations between different traits, body weight and muscle fat content before the third growing season (BW_2; % Fat_2) were negatively correlated with specific growth rate (SGR_{2-3}) during the third growing season (r_g = -0.59, -0.54; r_p = -0.62; -0.33, respectively). So, the leaner and smaller fish were performing better and catching up their larger counterparts. However, positive genetic and phenotypic correlations between BW_2 and BW_3 (r_g = 0.74; r_p = 0.72) also indicate that selection for body weight before the third growing season may increase market weight.

Table 3. Genetic (first line; ± S.E.) and phenotypic correlations (second line) of traits changes during overwintering and traits after overwintering (left hand side) related to traits changes during growing period and traits at market size (upper heading).

	BW_3	SGR_{2-3}	FC_3	% Fat_3	$FatCh_{2-3}$	% $FatCh_{2-3}$	$Surv_{2-3}$	% hl-Carss	% Fill
BW_2	0.74 ± 0.07,	-0.59 ± 0.10,	0.15 ± 0.14,	0.22 ± 0.14,	0.13 ± 0.15,	-0.14 ± 0.15,	-0.29 ± 0.22,	-0.19 ± 0.15,	0.03 ± 0.16,
	0.72	-0.62	0.10	0.24	0.15	-0.07	-0.05	0.06	0.19
SGR_{1-2}	0.62 ± 0.10,	0.11 ± 0.16,	0.54 ± 0.11,	-0.35 ± 0.13,	-0.33 ± 0.14,	-0.19 ± 0.15,	0.31 ± 0.24,	-0.37 ± 0.14,	-0.29 ± 0.15,
	0.34	-0.06	0.22	-0.10	-0.09	-0.05	0.03	-0.05	-0.05
FC_2	0.55 ± 0.10,	0.34 ± 0.13,	0.94 ± 0.02,	-0.27 ± 0.13,	-0.22 ± 0.14,	-0.05 ± 0.15,	0.45 ± 0.21,	-0.14 ± 0.15,	-0.16 ± 0.15,
	0.29	0.08	0.73	-0.10	-0.11	-0.08	0.04	-0.06	-0.04
% Fat_2	-0.15 ± 0.15,	-0.54 ± 0.11,	-0.27 ± 0.13,	0.70 ± 0.08,	-0.42 ± 0.13,	-0.36 ± 0.13,	-0.53 ± 0.19,	0.15 ± 0.15,	0.23 ± 0.15,
	0.04	-0.33	-0.04	0.52	0.16	-0.46	-0.02	0.05	0.14
$FatCh_{1-2}$	0.17 ± 0.18,	0.17 ± 0.18,	0.19 ± 0.17,	-0.50 ± 0.14,	-0.50 ± 0.14,	-0.26 ± 0.17,	0.32 ± 0.26,	-0.45 ± 0.16,	-0.38 ± 0.16,
	0.03	0.06	0.06	-0.11	-0.16	-0.17	-0.02	-0.08	-0.08
% $FatCh_{1-2}$	0.24 ± 0.17,	0.05 ± 0.18,	0.18 ± 0.17,	-0.25 ± 0.17,	-0.34 ± 0.16,	-0.37 ± 0.16,	0.10 ± 0.27,	-0.38 ± 0.17,	-0.26 ± 0.18,
	0.04	0.01	0.06	0.03	-0.12	-0.25	-0.02	-0.08	-0.06
$Surv_{1-2}$	0.56 ± 0.31,	0.18 ± 0.30,	0.40 ± 0.27,	-0.18 ± 0.30,	-0.02 ± 0.30,	0.18 ± 0.33,	0.58 ± 0.35,	0.34 ± 0.30,	0.27 ± 0.30,
	-0.07	-0.04	0.00	0.08	-0.03	-0.11	0.24	0.002	0.001

See Table 1 for trait abbreviations.

https://doi.org/10.1371/journal.pone.0191624.t003

SGR_{1-2} showed high genetic correlations with BW_3 and FC_3 (r_g = 0.62 ± 0.10 and 0.54 ± 0.11, respectively) but phenotypic correlations were twice lower (0.34, 0.22, respectively). Significant but moderate negative genetic correlations were observed for SGR_{1-2} with % Fat_3, $FatCh_{2-3}$, and % hl-Carss and at the edge of significance with % Fill.

FC_2 was positively genetically correlated to BW_3 and $Surv_{2-3}$ (high relationship) and SGR_{2-3} (moderate relationship) and negatively weakly correlated to % Fat_3. In all cases, the phenotypic correlations were much lower. The condition factor after winter period thus seems to be a good indicator of the genetic merit of fish for several production traits in the third growing season.

Muscle fat content before the third growing season (% Fat_2), while significantly correlated, was only in negative relationships with the traits of the following growing season. The genetic correlation with $Surv_{2-3}$ (-0.53 ± 0.19) was highly negative indicating that higher muscle lipid level after winter is related to lower survival in the third growing season. Moreover, there were moderate genetic correlations with $FatCh_{2-3}$ and % $FatCh_{2-3}$. The phenotypic correlations were low to moderate or not existing.

The genetic correlations of fat change traits ($FatCh_{1-2}$ and % $FatCh_{1-2}$) both showed similar negative patterns with traits of the next period ($FatCh_{2-3}$, % $FatCh_{2-3}$ and % hl-Carss). For $FatCh_{1-2}$, the correlations were moderate, for % $FatCh_{1-2}$ low or moderate, but always lower than for $FatCh_{1-2}$. Moreover, $FatCh_{1-2}$ was significantly correlated with % Fat_3 (r_g = -0.50 ± 0.14) and with % Fill (r_g = -0.38 ± 0.16). Oppositely, % $FatCh_{1-2}$ was significantly correlated with % $FatCh_{2-3}$ (r_g = -0.37 ± 0.16). The phenotypic correlations were low or negligible.

Genetic and phenotypic correlation between $Surv_{1-2}$ and the traits after the third growing season were not significant.

Discussion

The present study focused on the genetic variance of the second winter performance and on the effect of overwintering traits on traits of the third growing season, at the end of which the fish reached the market size. The most important fish characteristics for winter performance

are survival, but also a condition that ensures good recovery and performance of fish in the next rearing period. A trait that has often been mentioned to be important for winter survival is muscle fat content [12]. Complementary traits that may indicate either relationship to overwintering performance or recovery after the winter period, are weight change and fat change, which were in our study expressed as specific growth rate [30] and as absolute and relative fat change. Furthermore, Fulton's condition coefficient is also often used in common carp culture as a trait indicating actual condition [9]. So, all these traits were evaluated for their importance for winter survival and performance until market size.

Genetic and maternal variance

The heritability of traits in this study was estimated using two models: either including or excluding the random non-genetic maternal effect m^2. The maternal effect for all the estimates (after second and third growing season) was negligible, similar to the studies by Vandeputte et al. [3] and Ninh et al. [7].

The heritability estimates for BW, % Fat, SGR, FatCh, % hl-Carss and % Fill were mostly moderate to high and tended to increase with the age of the fish. This observation was in accordance with other recent studies on common carp [3–8] in different breeds/strains and under various pond management conditions. Thus, common carp has sufficient genetic variance in most important performance traits (growth, fat and yield) for selective breeding programs. The results also show that in Central European climatic conditions, the selection of fish should be done optimally after the second wintering (S2 and S3 Tables). At this period the fish are still small enough for easy handling and short-term storage and there is a reasonably high genetic correlation (0.74) between the weight at this age and market-size weight.

Low but significant heritability estimates were found for survival during wintering as well as third growing season. Dong et al. [8] observed similar estimates for overall survival during four generations, while Nielsen et al. [6] observed heritability of 0.34 for survival during the last growing season. Similar variability was also reported in other fish species [31–37]. This is not surprising as reasons for fish mortality and the range of mortality differed across studies. Generally, survival has low h^2 when low mortality rates are observed. This was also our case. However, the existing genetic variation for overall survival indicates that there is a potential for improving general robustness against various mortality factors [34].

Fulton's condition factor was very highly heritable in all periods (from yearling to market size) and even close to unity at harvest ($h^2 = 0.997$). Vandeputte et al. [3] estimated a much lower heritability for FC in juvenile common carp (0.37). Thus, in our study the FC variation was mostly genetic variance and environmental variance became negligible. Moreover, we found that genetic and phenotypic correlations among FC and biometrical indices (relative body height, relative body width) were strong (> 0.9; S4 Table). So, FC indicated also the shape of fish–fish with higher FC had higher and wider body. Similarly, a strong relationship between body shape and FC was observed in European whitefish, *Coregonus lavaretus* [36]. In our study, this phenomenon might be partly due to the fact that the great-grand parents of the experimental stock were very different in body shape (oblong-like body shape in Amur wild carp and square-like body shape in the maternal strain [21]). This generates high genetic variance in the F3 generation used in the present study.

Overwintering performance

No significant genetic correlations were observed between winter survival ($Surv_{1\text{-}2}$) and fish weight (BW_1). So, selection for higher body weight before winter would not lead to any favorable response in overwintering survival. Interestingly, despite generally high survival (98%), a

significant negative genetic correlation between % Fat_1 and $Surv_{1-2}$ (-0.59) was observed. Hence, selecting for higher muscle fat could lead to lower winter survival. However, this observation might be specific only for the mild winter conditions that were experienced here. Such observations contradict the general assumption concerning size-selective mortality in fish–smaller fish tend to have lower energy reserves and use those reserves more rapidly due to the allometry of metabolic rate, which results in lower survival [12,38,39]. However, Biro et al. [39] observed in rainbow trout (*Oncorhynchus mykiss*) that the larger/fatter individuals unlike the smaller/leaner ones consumed more of their lipid reserves than predicted by standard metabolic allometry. We observed that heavier fish before winter were slightly fatter (r_p = 0.28) and that selection for heavier fish would lead to a slight increase in muscle fat content (r_g = 0.32). However, phenotypic correlations between % Fat_1 and $FatCh_{1-2}$ as well as % Fat_1 and % $FatCh_{1-2}$ were negative. So, it also shows that fatter fish mobilized their lipid reserves more than the leaner ones.

We assume that due to the mild winter conditions, fish were more active than normally, needed more energy and were looking for food. Otherwise, fish could not increase or keep their weight (decreased BW in 713 fish, no BW change in 67 fish, increased BW in 1034 fish). Similar foraging behavior in three-year old carp was observed by Bauer and Schlott [9] but in their study, all fish except one lost weight during winter, probably because of colder winter and absence of winter feeding. However, due to climate change, mild winter conditions might be more often expected in Europe in the future [40]. Former recommendations and our observation suggest that importance of parameters for survival might depend on e.g. the age of fish, food availability during winter and the climatic conditions [10].

Other results supporting a disadvantage of having too high muscle fat content prior to overwintering might be seen from the correlations among % Fat_1, SGR_{1-2}, $FatCh_{1-2}$, % $FatCh_{1-2}$ and $Surv_{1-2}$. Selecting fish for higher muscle fat content before winter would lead to spending more muscle fat during winter in absolute value (moderate correlation), and to having lower SGR_{1-2}. Moreover, if a selection on lower weight loss during winter was done, the fish would tend to be initially leaner (decreasing of % Fat_1) and to have lower fat decrease during winter in absolute ($FatCh_{1-2}$) as well as relative values (% $FatCh_{1-2}$). Summing up, selection for lower decreases in weight and muscle fat content is expected to result in better winter performance. This observation is in accordance with assumptions by Schäperclaus [41], Bernard and Fox [42], and Pratt and Fox [43]. However, such selection would likely lead also to a decreasing of muscle fat content before wintering. This is contradictory to studies on European sea bass (*Dicentrarchus labrax*), where fish that lost less weight when fasting were also those that exhibited higher muscle fat content after starvation [44–46]. On the other hand, those fish were completely feed-deprived and this fact might be a reason for this opposite result, while in our case fish had a chance to forage. It might happen that during mild winter, natural selection would privilege leaner fish to perform better e.g. due to compulsion to ingest more feed and thus maintain their weight and lipid stores more effectively. The same strategy of leaner fish was also observed in Atlantic salmon (*Salmo salar* L.) by Johansen et al. [47]. Oppositely, fatter fish, not being forced to forage, would be handicapped during mild winter and due to higher metabolic activity they would lose more lipid stores and weight which would affect their survival. Thus, fish may be able to recognize their lipid reserves status, a capacity termed lipostatic regulation that was also reported in other fish species [18,47–52].

The condition factor before winter (FC_1) was not phenotypically correlated to any trait, but selection for this trait should result in lower weight loss (or weight gain) during winter (r_g for FC_1: SGR_{1-2} = 0.50) that might be advantageous for winter performance. However, due to mild winter, FC decreased only slightly during winter, even survival was high, and this could be reason why this trait was only slightly related to overwintering performance. Higher

mortality during winter in fish with FC decrease exceeding 15–20% was observed in a previous study [53] and accordingly, FC was of interest when assessing winter survival in common carp [9].

Impact of overwintering performance on the next growing season until market size

The conditions during the third growing season were optimal as seen from standard-level survival (89%), average water temperature from April to October (17.5˚C), from the mean body weight gain of 1570 g, from the total pond production of 665 kg. ha^{-1}, and from a considerable increase in muscle fat (abs: 7.2%), which were higher than usual.

Similar to overwintering period, we found that the muscle fat content after overwintering (% Fat_2) was negatively genetically correlated (-0.53) to survival during the third growing season ($Surv_{2-3}$). Conversely, there was no correlation between % Fat_2 and BW_3. Thus, selective breeding for restricted fat content in spring may increase survival without affecting final body weight. The negative correlation between muscle fat content and survival has no straightforward explanation. Kause et al. [36] observed in European whitefish a rather positive genetic correlation between higher fillet lipid at harvest and survival. However, in that case larger fish were also fatter than the smaller ones. While a certain level of muscle fat is essential for various biological functions of fish [54,55], our observations suggest that an excess might have disadvantageous effects.

The negative moderate to high phenotypic and high genetic correlations of BW_2 and % Fat_2 with SGR_{2-3} indicated that initially smaller and leaner fish grew faster during the third growing season. On the other hand, smaller fish did not catch in weight the larger ones at market size. Still, it looks that worse performing genotypes were supported after the period of growth depression by good rearing conditions, as described above, to catch up the other genotypes. This fact also decreased *CV* of body weight. Likewise, Mas-Muñoz et al. [56] observed negative phenotypic correlation between initial body weight and SGR in sole (*Solea solea*) grown in ponds.

The change of weight during overwintering (SGR_{1-2}) was favorably genetically and phenotypically correlated to BW_3 and FC_3 (r_g = 0.62, 0.54; r_p = 0.34, 0.22, respectively). So, the fish which grew or lost less weight during overwintering achieved also higher body weight and condition at market size. Accordingly, selection for higher SGR_{1-2} (the lowest negative or positive value) could positively affect the body weight and condition at market size. On the other hand, selection for higher SGR_{1-2} would likely lead to a decrease of muscle fat content (% Fat_3) and slaughter yields at market size (r_g = -0.29 and -0.35, respectively). The same situation regarding final muscle fat content and edible parts yield would happen when selecting on higher $FatCh_{1-2}$ (lower decrease or slight increase in muscle fat content during winter). Thus, the correlations indicate that genotypes which tended to lose more muscle fat during winter compensated the muscle fat during growing season and the higher muscle fat content very likely increased hl-carcass and fillet yields in such fish (as estimated based on positive correlations between % Fat_3 related to % hl-Carss and % Fill; S4 Table). Similarly, positive correlations between muscle fat content and slaughter yields were observed in rainbow trout [57] and previous study in common carp [5]. Hence, the possible decrease in slaughter yields makes the selection on SGR_{1-2} or $FatCh_{1-2}$ less appealing. Nevertheless, the negative correlation between SGR_{1-2} or $FatCh_{1-2}$ and yields might be overcome with a multitrait selection method, e.g. looking for predictors for better slaughter yields similarly as in rainbow trout [58,59] and European seabass [60]. However, in case that flesh quality were more profitable for fish farmers than increased dress-out yields, decreasing the fat in muscle would most likely lead to increasing

relative rate of polyunsaturated fatty acids (PUFAs) and improving the omega 3: omega 6 fatty acids profile [61,62]. Then SGR_{1-2} or $FatCh_{1-2}$ would become interesting traits for a selection program.

Selection for higher FC_2 would lead to increasing final weight (BW_3), SGR_{2-3} and $Surv_{2-3}$ (r_g = 0.34–0.55) with a slight decrease in a final muscle fat content (r_g = -0.27) with no effect on slaughter yields. A high positive genetic correlation between FC and BW was found e.g. in rainbow trout by Haffray et al. [63], but oppositely Sae-Lim et al. [37] found no genetic correlation between fingerling FC and BW or survival at harvest. Similarly, correlations between FC and muscle fat content differ among studies [36]. In our study, FC_2 after overwintering seemed to be quite a valuable trait for a potential selective breeding program in Amur mirror carp.

Conclusions

Muscle fat content is a trait playing an important role in biological functions of common carp. In this study it was found that selection for i) lower fat content before and after winter, ii) lower decrease in muscle fat content and/or body weight during winter, may both lead to better survival and growth during the subsequent growing period. On the other hand, edible parts yield may slightly decrease. We also showed that selection for higher condition factor might result in better performance during the winter, and mainly during the third growing season and at market size. However, this would also lead to a change of fish conformations to a less favourable square-like body shape.

Supporting information

S1 Table. Genetic and phenotypic correlations of traits before and after second overwintering.
(DOCX)

S2 Table. Genetic correlations of body weight and Fulton's condition factor in one-year old common carp related to traits (BW, FC, % Fat) during all recorded periods.
(DOCX)

S3 Table. Phenotypic correlations of body weight and Fulton's condition factor in-one year old common carp related to traits (BW, FC, % Fat) during all recorded periods.
(DOCX)

S4 Table. Genetic and phenotypic correlations of selected traits at market size.
(DOCX)

Acknowledgments

We thank to Jaroslav Záviška for editing the data, as well as staff of Faculty of Fisheries and Protection of Waters, especially to Marie Pečená and Ivana Samková for help with collecting the data and to Klatovy Fish Farm for providing the sources for on-growing the experimental stock during the studied periods.

Author Contributions

Conceptualization: Antti Kause, Marc Vandeputte, Martin Kocour.

Data curation: Martin Prchal, Antti Kause.

Formal analysis: Martin Prchal, Antti Kause.

Funding acquisition: Antti Kause, Marc Vandeputte, Martin Kocour.

Investigation: Martin Prchal, Marc Vandeputte, David Gela, Jean-Michel Allamellou, Girish Kumar, Anastasia Bestin, Jérôme Bugeon, Jinfeng Zhao, Martin Kocour.

Methodology: Antti Kause, Marc Vandeputte, Jean-Michel Allamellou, Martin Kocour.

Project administration: Antti Kause, Marc Vandeputte, Martin Kocour.

Resources: Marc Vandeputte, David Gela, Jérôme Bugeon, Martin Kocour.

Software: Antti Kause, Jean-Michel Allamellou.

Supervision: Antti Kause, Marc Vandeputte, Martin Kocour.

Validation: Martin Prchal, Antti Kause, Marc Vandeputte, Martin Kocour.

Visualization: Martin Prchal, Antti Kause, Martin Kocour.

Writing – original draft: Martin Prchal, Marc Vandeputte, Martin Kocour.

Writing – review & editing: Martin Prchal, Antti Kause, Marc Vandeputte, David Gela, Jérôme Bugeon, Jinfeng Zhao, Martin Kocour.

References

1. Gjedrem T, Robinson N, Rye M. The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. Aquaculture. 2012; 350–353: 117–129.
2. FAO: Food and Agriculture Organization of the United Nations, Fisheries and Aquaculture department. 2015; Available from: http://www.fao.org/fishery/species/2957/en.
3. Vandeputte M, Kocour M, Mauger S, Dupont-Nivet M, De Guerry D, Rodina M, et al. Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). Aquaculture. 2004; 235: 223–236.
4. Vandeputte M, Kocour M, Mauger S, Rodina M, Launay A, Gela D, et al. Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio* L.): Heritability estimates and response to selection. Aquaculture. 2008; 277: 7–13.
5. Kocour M, Mauger S, Rodina M, Gela D, Linhart O, Vandeputte M. Heritability estimates for processing and quality traits in common carp (*Cyprinus carpio* L.) using a molecular pedigree. Aquaculture. 2007; 270: 43–50.
6. Nielsen HM, Ødegård J, Olesen I, Gjerde B, Ardo L, Jeney G, et al. Genetic analysis of common carp (*Cyprinus carpio*) strains. I: Genetic parameters and heterosis for growth traits and survival. Aquaculture. 2010; 304: 14–21.
7. Ninh NH, Ponzoni RW, Nguyen NH, Woolliams JA, Taggart JB, McAndrew BJ, et al. A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): Estimation of genetic parameters. Aquaculture. 2011; 322–323: 39–46.
8. Dong Z, Nguyen NH, Zhu W. Genetic evaluation of a selective breeding program for common carp *Cyprinus carpio* conducted from 2004 to 2014. BMC Genet. 2015; 16(94).
9. Bauer C, Schlott G. Overwintering of farmed common carp (*Cyprinus carpio* L.) in the ponds of a central European aquaculture facility—measurement of activity by radio telemetry. Aquaculture. 2004; 241: 301–317.
10. Hurst TP. Causes and consequences of winter mortality in fishes. J Fish Biol. 2007; 71: 315–345.
11. Crespel A, Bernatchez L, Garant D, Audet C. Genetically based population divergence in overwintering energy mobilization in brook charr (*Salvelinus fontinalis*). Genetica. 2013; 141: 51–64. https://doi.org/10.1007/s10709-013-9705-x PMID: 23412995
12. Steffens W. Protein sparing effect and nutritive significance of lipid supplementation in carp diets. Arch Anim Nutr. 1996; 49: 93–98.
13. Blasco J, Fernández J, Gutierréz J. Fasting and refeeding in carp, *Cyprinus carpio* L.: the mobilization of reserves and plasma metabolite and hormone variations. J Comp Physiol B. 1992; 162: 539–546.
14. Urbánek M, Hartvich P, Vácha F, Rost M. Investigation of fat content in market size common carp (*Cyprinus carpio*) flesh during the growing season. Aquacult Nutr. 2010; 16: 511–519.
15. Geldhauser F, Gerstner P. Der Teichwirt. Stuttgart: Ulmer Eugen Verlag; 2003.

16. Reichle G. Die Karfenwinterung. Fisch. Teichwirt. 1998; 49: 439–440.
17. Zajic T, Mraz J, Sampels S, Pickova J. Fillet quality changes as a result of purging of common carp (*Cyprinus carpio* L.) with special regard to weight loss and lipid profile. Aquaculture. 2013; 400–401: 111–119.
18. Ali M, Nicieza A, Wootton RJ. Compensatory growth in fishes: a response to growth depression. Fish Fish. 2003; 4: 147–190.
19. Jobling M. Are compensatory growth and catch-up growth two sides of the same coin? Aquacult Int. 2010; 18: 501–510.
20. Horváth L, Tamás G, Seagrave C. Carp and pond fish culture, 2nd ed. Oxford: Fishing News Books. Blackwell Scientific Publications Ltd; 1992.
21. Flajšhans M, Gela D, Kocour M, Rodina M, Kašpar V, Linhart O, et al. Amur mirror carp, a recently certified breed of common carp in the Czech Republic. In: Book of abstracts: 3rd International Conference on Common Carp; 2015. pp. 21–23.
22. Kocour M, Piačková V, Veselý T, Gela D, Pokorová D, Flajšhans M. Perspectives for utilization of Amur mirror carp strains in crossbreeding program of common carp, Cyprinus carpio L., in the Central Europe. In: Abstract Book of AQUA 2012 conference, Global Aquaculture: Securing our future, September 1–5, Prague, Czech Republic; 1992. p. 356.
23. Piačková V, Flajšhans M, Pokorová D, Reschová S, Gela D, Čížek A, et al. Sensitivity of common carp, *Cyprinus carpio* L., strains and crossbreeds reared in the Czech Republic to infection by cyprinid herpesvirus 3 (CyHV-3; KHV). J Fish Dis. 2013; 36: 75–80. https://doi.org/10.1111/jfd.12007 PMID: 23009156
24. Zoellner N, Kirsch K. Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion. Res Exp Med. 1962; 135: 545–561.
25. Einen O, Waagan B, Thomassen MS. Starvation prior to slaughter in Atlantic salmon (*Salmo salar*): I. Effects on weight loss, body shape, slaughter- and fillet- yield, proximate and fatty acid composition. Aquaculture. 1998; 166: 85–104.
26. Boichard D, Barbotte L, Genestout L. AccurAssign, software for accurate maximum-likelihood parentage assignment. Presented at 10th WCGALP. 2014; Available from: https://asas.org/docs/default-source/wcgalp-posters/397_paper_9157_manuscript_448_0.pdf?sfvrsn=2
27. Madsen P, Jensen J. DMU version 6. 2013; Available from: http://dmu.agrsci.dk/DMU/Doc/Current/dmuv6_guide.5.2.pdf.
28. Jensen J, Mäntysaari EA, Madsen P, Thompson R. Residual maximum likelihood estimation of (co)variance components in multivariate mixed linear models using average information. Jour Ind Soc Ag Statistics. 1997; 49: 215–236.
29. Dempster ER, Lerner IM. Heritability of threshold characters. Genetics. 1950; 35: 212–236. PMID: 17247344
30. Virk P, Saxena PK. Potential of amaranthus seeds in supplementary feed and its impact on growth in some carps. Bioresource Technol. 2003; 86: 25–27.
31. Rye M, Lillevik KM, Gjerde B. Survival in early life of Atlantic salmon and rainbow trout: estimates of heritabilities and genetic correlations. Aquaculture. 1990; 89: 209–216.
32. Gjerde B, Terjesen BF, Barr Y, Lein I, Thorland I. Genetic variation for juvenile growth and survival in Atlantic cod (*Gadus morhua*). Aquaculture. 2004; 236: 167–177.
33. Charo-Karisa H, Komen H, Rezk MA, Ponzoni RW, Van Arendonk JAM, Bovenhuis H. Heritability estimates and response to selection for growth of Nile tilapia (*Oreochromis niloticus*) in low-input earthen ponds. Aquaculture. 2006; 26: 479–486.
34. Vehviläinen H, Kause A, Quinton C, Koskinen H, Paananen T. Survival of the currently fittest: Genetics of rainbow trout survival across time and space. Genetics. 2008; 180: 507–516. https://doi.org/10.1534/genetics.108.089896 PMID: 18757927
35. Vehviläinen H, Kause A, Kuukka-Anttila H, Koskinen H, Paananen T. Untangling the positive genetic correlation between rainbow trout growth and survival. Evol Appl. 2012; 5: 732–745. https://doi.org/10.1111/j.1752-4571.2012.00251.x PMID: 23144659
36. Kause A, Quinton C, Airaksinen S, Ruohonen K, Koskela J. Quality and production trait genetics of farmed European whitefish, *Coregonus lavaretus*. J Anim Sci. 2011; 89: 959–971. https://doi.org/10.2527/jas.2010-2981 PMID: 21097679
37. Sae-Lim P, Komen H, Kause A, Martin KE, Crooijmans R, Van Arendonk JAM, et al. Enhancing selective breeding for growth, slaughter traits and overall survival in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2013; 372–375: 89–96.

38. Sogard SM, Olla BL. Endurance of simulated winter conditions by age-0 walleye Pollock: effects of body size, water temperature and energy stores. J Fish Biol. 2000; 56: 1–21.

39. Biro PA, Morton AE, Post JR, Parkinson EA. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Onchorhynchus mykiss*). Can J Fish Aquat Sci. 2004; 61: 1513–1519.

40. IPCC: Climate Change: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (Eds.)]. IPCC, Geneva, Switzerland; 2014.

41. Schäperclaus W. Lehrbuch der Teichwirtschaft. Parey, Berlin; 1961.

42. Bernard G, Fox MG. Effects of body size and population density on overwinter survival of age-0 pumpkinseeds. N Am J Fish Manage. 1997; 17: 581–590.

43. Pratt TC, Fox MG. Influence of predation risk on the overwinter mortality and energetic relationships of young-of-year walleyes. T Am Fish Soc. 2002; 131: 885–898.

44. Grima L, Vandeputte M, Ruelle F, Vergnet A, Mambrini M, Chatain B. In search for indirect criteria to improve residual feed intake in sea bass (*Dicentrarchus labrax*). Part I: phenotypic relationship between residual feed intake and body weight variations during feed deprivation and re-feeding periods. Aquaculture. 2010; 300: 50–58.

45. Grima L, Chatain B, Ruelle F, Vergnet A, Launay A, Mambrini M, et al. In search for indirect criteria to improve feed utilization efficiency in sea bass (*Dicentrarchus labrax*) Part II: Heritability of weight loss during feed deprivation and weight gain during re-feeding periods. Aquaculture. 2010; 302: 169–174.

46. Daulé S, Vandeputte M, Vergnet A, Guinand B, Grima L, Chatain B. Effect of selection for fasting tolerance on feed intake, growth and feed efficiency in the European sea bass *Dicentrarchus labrax*. Aquaculture. 2014; 420–421: 42–49.

47. Johansen SJS, Ekli M, Jobling M. Is there lipostatic regulation of feed intake in Atlantic salmon *Salmo salar* L.? Aquac Res. 2002; 33: 515–524.

48. Jobling M, Johansen SJS. The lipostat, hyperphagia and catch-up growth. Aquac Res. 1999; 30: 473–478.

49. Thompson JM, Bergersen EP, Carlson CA, Kaeding LR. Role of size, condition, and lipid content in the overwinter survival of age-0 Colorado squawfish. T Am Fish Soc. 1991; 120: 346–353.

50. Brodersen J, Chapman BB, Nilsson PA, Skov C, Hansson LA, Brönmark C. Fixed and flexible: Coexistence of obligate and facultative migratory strategies in a freshwater fish. PLoS ONE. 2014; 9: e90294 https://doi.org/10.1371/journal.pone.0090294 PMID: 24594698

51. Bell RJ. Winter Feeding as an overwintering survival strategy in young-of-the-year winter flounder. T Am Fish Soc. 2012; 141: 855–871.

52. Eckmann R. Overwinter changes in mass and lipid content of *Perca fluviatilis* and *Gymnocephalus cernuus*. J Fish Biol. 2004; 65: 1498–1511.

53. Lukowicz M, Gerstner P. Hältern und Wintern. In: Schäperclaus W., Lukowicz M. (Eds.), Lehrbuch der Teichwirtschaft. Berlin: Parey; 1998. pp. 495–503.

54. Tocher DR. Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci. 2003; 11: 107–184.

55. Kause A, Kiessling A, Martin SAM, Houlihan D, Ruohonen K. Genetic improvement of feed conversion ratio via indirect selection against lipid deposition in farmed rainbow trout (*Oncorhynchus mykiss* Walbaum). Brit J Nutr. 2016; 116: 1656–1665. https://doi.org/10.1017/S0007114516003603 PMID: 27813470

56. Mas-Muñoz J, Blonk R, Schrama JW, van Arendonk J, Komen H. Genotype by environment interaction for growth of sole (*Solea solea*) reared in an intensive aquaculture system and in a semi-natural environment. Aquaculture. 2013; 410–411: 230–235.

57. Bugeon J, Lefevre F, Cardinal M, Uyanik A, Davenel A, Haffray P. Flesh quality in large rainbow trout with high or low fillet yield. J Muscle Foods. 2010; 21: 702–721.

58. Kause A, Paananen T, Ritola O, Koskinen H. Direct and indirect selection of visceral lipid weight, fillet weight and fillet percent in a rainbow trout breeding program. J Anim Sci. 2007; 85: 3218–3227. https://doi.org/10.2527/jas.2007-0332 PMID: 17709780

59. Haffray P, Bugeon J, Rivard Q, Quittet B, Puyo S, Allamelou JM, et al. Genetic parameters of in-vivo prediction of carcass, head and fillet yields by internal ultrasound and 2D external imagery in large rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2013; 410–411: 236–244.

60. Vandeputte M, Puledda A, Tyran AS, Bestin A, Coulombet C, Bajek A, et al. Investigation of morphological predictors of fillet and carcass yield in European sea bass (*Dicentrarchus labrax*) for application in selective breeding. Aquaculture. 2017; 470: 40–49.

61. Leaver MJ, Taggart JB, Villeneuve L, Bron JE, Guy DR, Bishop SC, et al. Heritability and mechanisms of n−3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. Comp Biochem Phys D. 2011; 6: 62–69.

62. Mráz J, Máchová J, Kozák P, Pickova J. Lipid content and composition in common carp-optimization of n-3 fatty acids in different pond production systems. J Appl Ichthyol. 2012; 28: 238–244.

63. Haffray P, Bugeon J, Pincent C, Chapuis H, Mazeiraud E, Rossignol MN, et al. Negative genetic correlations between production traits and head or bony tissues in large all-female rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2012; 368–369: 145–152.

CHAPTER 3

POTENTIAL FOR GENETIC IMPROVEMENT OF THE MAIN SLAUGHTER YIELDS IN COMMON CARP WITH *IN VIVO* MORPHOLOGICAL PREDICTORS

Prchal, M., Bugeon, J., Vandeputte, M., Kause, A., Vergnet, A., Zhao, J., Gela, D., Genestout, L., Bestin, A., Haffray, P., Kocour, M., 2018. Potential for genetic improvement of the main slaughter yields in common carp with *in vivo* morphological predictors. Front. Genet. 9, 283.

My share on this work was about 40%.

frontiers in Genetics

ORIGINAL RESEARCH
published: 30 July 2018
doi: 10.3389/fgene.2018.00283

Potential for Genetic Improvement of the Main Slaughter Yields in Common Carp With *in vivo* Morphological Predictors

Martin Prchal[1]*, Jérôme Bugeon[2], Marc Vandeputte[3,4], Antti Kause[5], Alain Vergnet[4], Jinfeng Zhao[1], David Gela[1], Lucie Genestout[6], Anastasia Bestin[7], Pierrick Haffray[7] and Martin Kocour[1]

[1] Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, Vodňany, Czechia, [2] LPGP, INRA, Rennes, France, [3] GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France, [4] Ifremer, Palavas-les-Flots, France, [5] Biometrical Genetics, Natural Resources Institute Finland (Luke), Jokioinen, Finland, [6] LABOGENA-DNA, Jouy-en-Josas, France, [7] SYSAAF Section Aquacole, Rennes, France

OPEN ACCESS

Edited by:
Ross Houston,
The University of Edinburgh,
United Kingdom

Reviewed by:
Fabyano Fonseca Silva,
Universidade Federal de Viçosa, Brazil
Chuanju Dong,
Henan Normal University, China

****Correspondence:***
Martin Prchal
mprchal@frov.jcu.cz

Specialty section:
This article was submitted to Livestock Genomics, a section of the journal Frontiers in Genetics

Received: *27 April 2018*
Accepted: *09 July 2018*
Published: *30 July 2018*

Citation:
Prchal M, Bugeon J, Vandeputte M, Kause A, Vergnet A, Zhao J, Gela D, Genestout L, Bestin A, Haffray P and Kocour M (2018) Potential for Genetic Improvement of the Main Slaughter Yields in Common Carp With in vivo Morphological Predictors. Front. Genet. 9:283. doi: 10.3389/fgene.2018.00283

Common carp is a major aquaculture species worldwide, commonly sold alive but also as processed headless carcass or filets. However, recording of processing yields is impossible on live breeding candidates, and alternatives for genetic improvement are either sib selection based on slaughtered fish, or indirect selection on correlated traits recorded *in vivo*. Morphological predictors that can be measured on live fish and that correlate with real slaughter yields hence remain a possible alternative. To quantify the power of morphological predictors for genetic improvement of yields, we estimated genetic parameters of slaughter yields and various predictors in 3-year-old common carp reared communally under semi-intensive pond conditions. The experimental stock was established by a partial factorial design of 20 dams and 40 sires, and 1553 progenies were assigned to their parents using 12 microsatellites. Slaughter yields were highly heritable (h^2 = 0.46 for headless carcass yield, 0.50 for filet yield) and strongly genetically correlated with each other (r_g = 0.96). To create morphological predictors, external (phenotypes, 2D digitization) and internal measurements (ultrasound imagery) were recorded and combined by multiple linear regression to predict slaughter yields. The accuracy of the phenotypic prediction was high for headless carcass yield (R^2 = 0.63) and intermediate for filet yield (R^2 = 0.49). Interestingly, heritability of predicted slaughter yields (0.48–0.63) was higher than that of the real yields to predict, and had high genetic correlations with the real yields (r_g = 0.84–0.88). In addition, both predicted yields were highly phenotypically and genetically correlated with each other (0.95 for both), suggesting that using predicted headless carcass yield in a breeding program would be a good way to also improve filet yield. Besides, two individual predictors (P_1 and P_2) included in the prediction models and two simple internal measurements (E4 and E23)

exhibited intermediate to high heritability estimates (h^2 = 0.34 – 0.72) and significant genetic correlations to the slaughter yields (r_g = |0.39 – 0.83|). The results show that there is a solid potential for genetic improvement of slaughter yields by selecting for predictor traits recorded on live breeding candidates of common carp.

Keywords: heritability estimates, genetic correlations, indirect selection, morphological landmarks, slaughter yields, ultrasound imagery

INTRODUCTION

Common carp (*Cyprinus carpio* and *C. rubrofuscus*) is highly important freshwater fish species for world aquaculture, with an annual production exceeding 4,000,000 tons (FAO, 2016). Yet, selective breeding programs of carp are less developed than in other aquaculture species (Hulata, 1995; Vandeputte, 2003; Janssen et al., 2017). Crossbreeding of notably inbred strains (Kohlmann et al., 2003, 2005) remains the most used method for genetic improvement of common carp stocks in Europe (Vandeputte, 2003; Nielsen et al., 2010; Janssen et al., 2017). However, the genetic progress is limited only to the first generation, and crossbreeding is not relevant to achieve long term cumulative gains (Nielsen et al., 2010). Selective breeding is more valuable because then the genetic gain is cumulative over multiple generations and a change in the breeding goal is possible over generations (Gjedrem and Baranski, 2009). Nevertheless, selective breeding in common carp is still only emerging and plays a minor role in carp aquaculture (Vandeputte, 2003; Chavanne et al., 2016; Janssen et al., 2017).

Several recent studies have shown a significant additive genetic variation of several performance traits in common carp (Vandeputte et al., 2004, 2008; Kocour et al., 2007; Nielsen et al., 2010; Ninh et al., 2011; Dong et al., 2015; Hu et al., 2017; Prchal et al., 2018) suggesting that important production traits, such as body weight and processing traits, could be genetically improved through selective breeding. Processing traits such as filet yield (filet weight relative to body weight) and carcass yield are more economically valuable traits than body weight itself for species sold processed (Bauer and Schlott, 2009; Kankainen et al., 2016). Processed carp are commonly sold as processed body (headless carcass) or as trimmed filets (Gela et al., 2003; Kocour et al., 2005a, 2007; Bauer and Schlott, 2009).

The use of filet yield in selection programs of fish has been criticized by several authors (Powell et al., 2008; Nguyen et al., 2010a; Gjerde et al., 2012; Van Sang et al., 2012) as in their studies low heritability of filet yield or insignificant response to selection were observed. The conclusion has been that it would be challenging to improve filet weight independently of body weight. A recent simulation study based on field data from three fish species (European sea bass; *Dicentrarchus labrax*, gilthead sea bream; *Sparus aurata* and rainbow trout; *Oncorhynchus mykiss*) indicated that filet yield can be specifically improved in a selection program (Fraslin et al., 2018). Nevertheless, mass selection is not possible in practice as slaughter yields can be only recorded destructively from slaughtered fish. As an alternative, such traits are mostly selected through sib selection or indirect selection on correlated traits recorded *in vivo* (Kause et al., 2007). However, sib selection, where breeding candidates are ranked according to the average performance of their slaughtered sibs, limits the genetic progress by using only genetic variation occurring between-families without exploiting within-family variation (Gjedrem, 2010; Haffray et al., 2013). Such limitation could be overcome by using indirect (non-invasive) selection criteria that can be measured on live breeding candidates, and that would allow exploiting the whole genetic variation related to slaughter yields (Vandeputte et al., 2017). Several studies have reported a possible application of external and internal (ultrasound imagery) morphological measurements predicting filet weight (Cibert et al., 1999; Bosworth et al., 2001; Rutten et al., 2004; Van Sang et al., 2009) or filet yield (Kause et al., 2007; Van Sang et al., 2009; Haffray et al., 2013; Vandeputte et al., 2017), and even their utilization in selective breeding (Kause et al., 2007; Van Sang et al., 2012; Haffray et al., 2013; Vandeputte et al., 2017).

The aim of this study was to (i) determine morphological predictors by external (phenotyping, 2D imaging) and internal measurements (ultrasound imagery) that can be combined by linear regression to predict slaughter yields (headless carcass and filet yields) in common carp, (ii) estimate genetic parameters of slaughter yields and their predictors, (iii) predict and compare the potential genetic gain based on hypothetical mass selection, sib selection and indirect selection based on the predictors of slaughter yields.

MATERIALS AND METHODS

Ethics Statement

The methodological protocol of the current study was approved by the expert committee of the Institutional Animal Care and Use Committee (IACUC) of the University of South Bohemia in České Budějovice (USB), Faculty of Fisheries and Protection of Waters (FFPW) in Vodňany according to the law on the protection of animals against cruelty (Act no. 246/1992 Coll., ref. number 16OZ19179/2016-17214). To enhance animal welfare and decrease suffering during all fish handling, the fish were anesthetized using 2-phenoxyethanol for each live trait recording, and humanely euthanized (humane endpoint) for final recording of slaughter traits. The main author of study owns the certificate (CZ 01704) giving capacity to conduct and manage experiments involving animals according to section 15d paragraph 3 of Act no. 246/1992 Coll.

Establishment and Rearing of Experimental Stock

In May 2014, the experimental stock was produced at the Genetic Fishery Center of University of South Bohemia (USB) in České Budějovice, Faculty of Fisheries and Protection of

Waters (FFPW) in Vodňany, Czech Republic. Amur mirror carp (AM), Vodňany line, recently certified as a new Czech common carp breed (Flajšhans et al., 2015), was chosen as the base population. The AM was used due to its higher genetic diversity (non-published data) compared to other carp breeds available in the Czech Republic that was given by the history of AM establishment. During 1 day, gametes from 20 dams and 40 sires were collected and a partial factorial design with four series of 5 dams and 10 sires in each was used. After fertilization, the eggs from each series were incubated in four separate Zuger jars. Each parental fish was fin-clipped for parentage assignment of the offspring fish. After hatching, the yolk-sac fry from each Zuger jar were transferred and nursed in four separate post-hatching incubators until swimming stage, when the experimental stock was created by pooling equal quantities (estimated volumetrically) of larvae from all four post-hatching incubators. These larvae were released (150,000 larvae. ha^{-1}) to the prepared nursery ponds at the Klatovy fish farm. Since then, the families were reared communally in ponds. The families were reared in various pond sizes depending on age of fish and annual period (0.2–4 ha) under semi-intensive pond management based on natural food and supplementary feeding (plant-based pellets altered later with wheat grain) served three times a week. At fish age of 1-year, a random sample of 3000 fish from the best pond (50% survival, mean weight ± SD = 15.8 ± 4.7 g) was anesthetized with 2-phenoxyethanol (0.5 ml per 1 l of water) and individually PIT-tagged and fin-clipped for parentage assignment. After the second growing period and the second overwintering, the fish were harvested and the data were collected for a related study about the genetic potential of overwintering performance in common carp (Prchal et al., 2018). At market size (third growing season) of 1910 g mean weight (October 2016), the fish were harvested and transferred to a storage pond in Vodňany for 3 week-fasting, before final traits recording. This was done to mimic the practice in commercial production, where fasting is used to empty the intestines and to improve the taste and quality of the flesh (Zajic et al., 2013).

Fish Processing and Final Traits Recording

In November 2016, the fish were transferred to the fish slaughter house of USB FFPW in České Budějovice, Czechia. A total of 1622 individuals were humanely sacrificed by a hit on the head and bled by cutting the gills according to the local rules. These were the fish that had been assigned to a single parental pair based on the microsatellite analysis (see section "Parentage Assignment"). Total length (TL), standard length (SL), body length (BL), head length (HL), body height (BH), and body width (BWI) were measured to the nearest 0.1 mm with an in-house electronic ruler, and body weight (BW) was weighed to the nearest 0.1 g with an electronic scale. For external phenotypic measurements, fish were photographed (left side) using a digital camera (CANON EOS 1000D). Internal measurements were recorded by ultrasound tomography (Hospimedi LC100, 7.5 MHz). The total muscle fat content (% Fat) was recorded using a Fish Fatmeter FM 692 (Distell Ltd., United Kingdom), using calibration option 'CARP – 1.' The phenotype for % Fat is expressed as the mean of four repeated measurements (three just above the lateral line from anterior to posterior and one close to the back line in the intermediate part of body) taken on the left side of the fish performed as guided by the manufacturer's guideline. In addition, selected yield-related biometric indicators were calculated as follows: Fulton's condition factor: FC = 10^5 * BW/SL, relative body height: RelBH = BH/SL, and relative head length: RelHL = HL/SL. After biometric recordings, each fish was processed and the following body portions were weighed (to nearest 0.5 g): head, left filet, viscera, gonads (sexed by visual inspection), left filet skin, half carcass, left filet ribs + trimmings, fins, and scales. The weight of slaughter body parts and vertebral axis was created by combining the previous body portions: headless carcass weight [hl-CarssW = left filet + left skin + left ribs and trimmings + half carcass], filet weight with skin [FiletW = (left filet + left filet skin) * 2] and vertebral axis weight: [AxisW = half carcass − (left filet + left skin + left ribs and trimmings)]. The percent slaughter yields were calculated as follows: headless carcass yield % [% hl-Carss = (hl-CarssW/BW) *100], and filet yield [% Fil = (left filet + left skin) * 2/BW * 100]. In addition, sex effect of analyzed traits was calculated using one-way ANOVA and HSD Tukey test at $p = 0.05$. Finally, to obtain alternative trait definitions to ratio-based traits, the natural logarithm was calculated for the weight of each slaughter body part and regressed on the logarithm of body weight to obtain growth-independent allometry residuals that fix the bias of ratio traits (Gunsett, 1984) and problems connected with estimating of genetic parameters (Gunsett, 1987; Haffray et al., 2013; Vandeputte et al., 2014). Thus, for % headless carcass and % filet yield, the surrogate traits defined as log–log residuals (Logr) are termed as Logr_hl-Carss and Logr_Fil, respectively. To visualize body allometry, logarithm of weight of all body portions mentioned above was regressed on the logarithm of body weight (see Supplementary Figure 1).

Digitization of 2D Morphometric Landmarks and Ultrasound Tomography

To quantify the shape of body, head and lateral line, a total of 20 coordinates of morphological points were digitized using ImageJ with the Point Picker plugin (Rueden et al., 2017) that allows storage and retrieval of a collection of landmarks (**Figure 1**). Furthermore, vertical blue lines were added into each image in order to facilitate the manual positioning of landmarks on the surface of the fish. We also checked if the magnification of camera between each working day was unchanged (the difference of pixels of each calibration line was not more/less than 3 pixels out of 2019 pixels. The distances between two landmarks were characterized as A (x_A, y_A) and B (x_B, y_B) and calculated with the formula:$d = \sqrt{(x_B - x_A)^2} + \sqrt{(y_B - y_A)^2}$. These distances were then used to calculate lengths and heights and areas using the Geometry R packages.

The internal measurements were collected using ultrasound imagery (Hospimedi LC1000, 7.5 MHz). Four muscular thicknesses from anterior (E4), intermediate (E5, E8), and

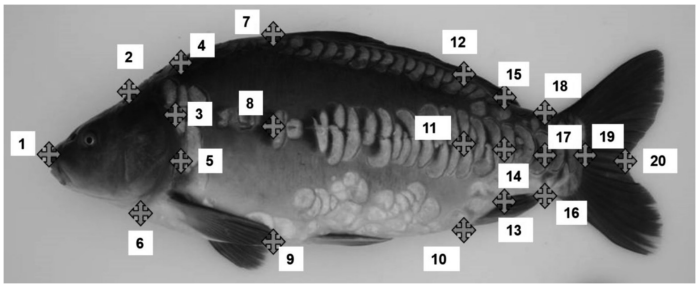

FIGURE 1 | Landmarks placed on each common carp photo. **(1)** Head extremity; **(2)** end of the head beginning of the filet on the back; **(3)** intersection between the back and the vertical of point 4; **(4)** intersection between opercula and lateral line; **(5)** opercula at the maximum length from the landmark 1; **(6)** end of the head beginning of the filet on the ventral part; **(7)** beginning of the dorsal fin; **(8)** intersection between the lateral line and the vertical of landmark 6; **(9)** intersection of the ventral part and the vertical of point; **(10)** beginning of the anal fin; **(11)** intersection between lateral line and vertical of point 9 toward the carp back; **(12)** vertical of point 10 on the back; **(13)** end of anal fin; **(14)** intersection of lateral line and vertical of 12; **(15)** vertical of point 12 on the carp back; **(16)** narrowest point on the caudal peduncle on the back; **(17)** intersection of the lateral line and vertical of 15; **(18)** vertical of point 16 on the ventral part (normally the narrowest point of the caudal peduncle; **(19)** end of the filet (on the skin) on the lateral line; **(20)** end of the caudal fin at the fork.

posterior (E6) muscles and one internal depth of the body cavity (E23) were measured, at the same position described by Haffray et al. (2013) and Vandeputte et al. (2017).

2D Morphology and Prediction Models of Slaughter Yields

The association of the variation in carp morphology to the variation in real processing yields was analyzed using the MorphoJ software (Klingenberg, 2011). This method consists of a Procrustes superimposition of the recorded landmarks used to describe body shape. The log-log residuals slaughter traits, Logr_hl-Carss and Logr_Fil, were used as a surrogate for traditional percent yields and introduced in MorphoJ as a covariates. To quantify the shape variation associated to yield, a regression analysis between Procrustes coordinates and the covariates was performed. The shape changes associated to the covariates were visualized with a wireframe graph. This visualization contributes to identifying relevant morphologic variables to include in a multiple linear regression, for example head area and ventral height.

A multiple linear regression using the reg.best function of the FactoMineR of R software package was performed using the external morphology descriptors, ultrasound measurements and fat meter value as independent variables and the Logr_hl-Carss and Logr_Fil as dependent variables. The best prediction model identification corresponds to those with the highest R^2 and F-value. The models were used to calculate the predicted yield values for each fish that are termed as Mod_hl-Carss for headless carcass yield and Mod_Fil for filet yield.

Models were validated by cross validation method using the crossval function of the bootstrap package in R software (Efron and Tibshirani, 1993). Such analysis shows how predictive the equations are on other individuals than the ones that were used to generate the equations. First, the dataset was divided into K subsets (here K = 20), the analysis is performed on the data of the $K-1$ subsets (training sets) and validated on the data of the remainder of the dataset (validation set). Then the coefficient of determination of the cross validation (R^2CV) was calculated.

Parentage Assignment

The fin tissues of the 60 parents and 2035 offspring (sampled after the second growing period) were placed into 96 well plates and sent to LABOGENA-DNA, the French laboratory for livestock genotyping (ISO 170025 accredited, Jouy-en-Josas, France). Parentage assignment was based on the analysis of 12 microsatellite loci and performed using the AccurAssign software, applying a maximum-likelihood method (Boichard et al., 2014). The parental pairs retained were chosen using the default thresholds of AccurAssign, i.e., they combined both (i) a difference in log-likelihood between the chosen pair and the second best which was >3 (20 times more likely), and (ii) an average Mendelian transmission probability higher than the highest 99% of 5.000 simulated incorrect trios (dam, sire and offspring).

Estimation of Genetic Parameters and Expected Genetic Gains

Before genetic analysis, the data quality was checked. The fish for which the total sum of all body portions was greater, or 3% lower than the total body weight were considered as recording errors and excluded from the final analysis. Likewise, a few individuals were also excluded due to aberrant values of external and internal measurements. As a result, 69 fish were excluded and 1553 individuals with a complete set of variables remained in the final analysis. Heritability (h^2), phenotypic and genetic correlations (r_p and r_g, respectively) were estimated using DMU statistical software (Madsen and Jensen, 2013), with animal mixed model fitted with the restricted maximum likelihood method:

$$y = X\beta + Z\alpha + \varepsilon$$

where y is the vector of observed phenotypes, X and Z are appropriate incidence matrices relating phenotypes to vectors β and α. β is the vector of fixed effects (sex with three levels – female, male, unidentified sex) and α is the vector of random additive genetic effects (1613 levels corresponding to all animals – parents and offspring- in the pedigree), and ε is the vector of random residual effects. The additive (animal) genetic effects were assumed to follow $N(0, G \otimes A)$, with G the genetic (co) variance matrix between traits and A the numerator relationship matrix relating all animals in the pedigree, while the residual effects were assumed to follow $N(0, R \otimes I)$, R the residual (co) variance matrix between traits and I an appropriate identity matrix. In the first step, an additional random effect common to dams (non-genetic maternal effect) was included in the model. However, this effect was negligible for all traits, and thus it was not included in the final model.

Heritabilities were estimated using a univariate model, and were calculated as the ratio of additive genetic variance (V_A) divided by the total phenotypic variance (V_P), $h^2 = V_A/V_P$. A model with maximum three traits at a time was used to obtain convergence for genetic correlations. However, when condition factor (FC) was calculated, convergence could not be obtained, and thus the genetic correlations between FC and other traits were obtained from a bivariate analysis of such traits. The likelihood ratio test (LRT) was used for comparing the goodness of fit of two models (including vs. excluding the animal genetic effect). The animal additive genetic effect (and thus the associated heritability estimate) was considered significant when the difference in −2Log-likelihood was higher than the threshold value for $p < 0.05$ of a χ^2 distribution with 1 degree of freedom (Pinheiro and Bates, 2000). Genetic correlation was considered significant if $|r_g| - |1.96 \times \text{S.E.}|$ was higher than zero (two-tailed hypothesis).

Expected genetic gains (ΔG) per generation for filet yield were calculated using the equations of Falconer and MacKay (1996) under a mass (MS), full-sib (FSS) and indirect (IS) selection. The genetic gain under theoretical mass selection based on the lethal criteria was calculated by $\Delta G_{MS} = i\ h^2\ \sigma_P$, where i is the selection intensity and h^2 and σ_P are the heritability and phenotypic standard deviation of the trait under selection, respectively. The genetic progress of FSS was estimated by $\Delta G_{FSS} = \frac{i \times \sigma_P \times h^2 \times n \times r}{\sqrt{n(1+(n-1)\,t)}}$, where n is the number of slaughtered sibs sampled per family (n = 10 sibs), r is the genetic correlation between sibs (r = 0.5 for full sibs) and t is the phenotypic intra class correlation ($t = rh^2$). The predicted genetic gain through indirect selection criteria was calculated by $\Delta G_{IS} = i \times h_1 \times h_2 \times r_g \times \sigma_{P2}$, where ΔG_{IS} is the estimated genetic gain on the target trait, h_1 and h_2 are the square roots of heritability of the indirect selection trait (on which selection is applied) and of the target trait, respectively, r_g is the genetic correlation estimated between the indirect trait and the target trait and σ_{P2} is the phenotypic standard deviation of the target trait. As genetic gains for filets were calculated in log units, the real genetic progress was scaled back to the percent body weight units by multiplying ΔG by the real mean filet yield in the present experimental stock. The selection intensities were set up of 10 and 30%, with 10 sibs per family in FSS as the most reasonable values related to a potential carp selection program.

RESULTS

Distribution of Families

Out of the 2035 offspring genotyped at the end of the second summer, 1901 (93.4%) could be assigned to a single parental pair, 84 (4.1%) had two possible parent pairs and were considered unassigned, 28 (1.4%) could not be assigned to any parent pair and 23 (1.1%) had DNA quality problems and thus no exploitable genotype. Out of the 1901 uniquely assigned fish, 1622 were still alive at the time of final sampling, and of those 1553 had adequate phenotypes after removal of outliers.

The 1553 fish used in the analysis originated from 197 out of the possible 200 full-sib families. The number of progeny per sire varied from 14 to 79, the average was 39. The number of progeny per dam varied from 25 to 128, the average was 78. The sexes were distributed equally (males – 754, females – 751, unidentified sex – 48).

Descriptive Statistics of Traits

Mean, standard deviation, differences between sexes (males, females, and unidentified sex) and minimum and maximum values of yield-related traits and slaughter yields are listed in **Table 1**. Sex effect was significant and % Fat, RelBH and both yields were higher for females than for males. Yields of headless carcass (66%) and filets (50%) were higher than usual in common carp, probably due to the specific experimental processing which

TABLE 1 | Mean and standard deviation (*SD*) for yield-related traits and percent slaughter yields in males, females and unidentified individuals of common carp.

Trait	Mean ± *SD*	Males*	Females*	Unidentified*	Minimum	Maximum
BW	1910.5 ± 278.9	1899.5^{a} ± 289.4	1923.9^{a} ± 269.8	1873.8^{a} ± 232.2	890.6	2859.5
% Fat	11.56 ± 2.97	10.88^{a} ± 3.06	12.21^{b} ± 2.70	12.10^{ab} ± 3.06	4.10	22.60
FC	3.40 ± 0.32	3.42^{a} ± 0.32	3.38^{a} ± 0.33	3.39^{a} ± 0.25	2.51	5.18
RelBH	0.365 ± 0.023	0.366^{a} ± 0.024	0.364^{a} ± 0.024	0.368^{a} ± 0.020	0.303	0.484
RelHL	0.295 ± 0.013	0.292^{a} ± 0.012	0.297^{b} ± 0.012	0.298^{b} ± 0.013	0.263	0.366
% hl-Carss	66.21 ± 2.19	65.12^{a} ± 2.02	67.27^{b} ± 1.71	67.06^{b} ± 2.90	55.18	72.32
% Fil	49.75 ± 1.95	49.06^{a} ± 1.94	50.41^{b} ± 1.70	50.23^{b} ± 1.92	39.72	55.39

**Groups with identical alphabetic marker are not significantly different at $p < 0.05$. BW – body weight, % Fat – percent muscle fat, FC – Fulton's condition factor, RelBH – relative body height, RelHL – relative head length, % hl-Carss – headless carcass yield, % Fil – filet yield.*

was different from the commercial one but reflected better the biological characteristics of traits.

Body Allometry of Different Body Parts

A positive allometry (regression coefficient >1 in log-log plots) was observed for filet weight, viscera weight and skin weight, showing that heavier fish have proportionally heavier filet, viscera and skin than smaller fish (Supplementary Figure 1). On the contrary, negative allometry was seen for head, vertebral axis, left ribs and trimmings and fins, showing that these parts proportionally decrease in heavier fish. Weights of scales and gonads were hardly linked to body weight. For gonads, there was a clear bimodal distribution, with larger gonads in males than in females (Supplementary Figure 1).

2D Morphology and Prediction Models of Slaughter Yields

A graphical visualization of body morphology associated to low and high yield for Logr_hl-Carss and Logr_Fil is given in **Figure 2**. The main shape differences were observed on the ventral part of the fish and the head. Carp with a high Logr_hl-Carss and Logr_Fil present a lower ventral area especially a lower ventral height under the dorsal fin. Carp with a higher Logr_Fil also have a lower head area with a shorter length between the nose and the operculum. Carp with higher Logr_hl-Carss and Logr_Fil present also a more developed caudal part with a larger caudal peduncle.

The set of best morphological predictors (P_{1-5}) included into two prediction equations, and their R^2 and Fisher test values (F) are shown in **Table 2**. Logr_hl-Carss could be predicted with a model combining three individual predictors (P_1, P_2, P_3): the ratio of head area to total body area (P_1), the ratio of abdominal filet thickness to height between the lateral line and the aligned ventral point (P_2), and the ratio of caudal part area to ventral part area (P_3). Mod_hl-Carss explains 63% (R^2CV = 0.624) of total phenotypic variance in Logr_hl-Carss. Logr_Fil was best predicted by the model using the same predictors as for Logr_hl-Carss (P_1, P_2, P_3) and in addition body weight (P_4) and % Fat (P_5). Mod_Fil explains 49% (R^2CV = 0.489) of total phenotypic variance of Logr_Fil.

Heritability Estimates

Heritability estimates of yield-related phenotypes, slaughter yields (Logr) and model-predicted (Mod) slaughter yields are given in **Table 3**. Heritabilities were high for BW and % Fat (0.63 and 0.68, respectively) and maximal (1.00) for traits associated to body shape (FC, RelHL, RelBH).

Logr slaughter yields had higher heritability than the commonly used percentage yields (Logr_hl-Carss, Logr_Fil = 0.46 and 0.50, respectively, vs. 0.36 for percent yields: % hl-Carss, % Fil – Supplementary Table 1). Model yields had a higher heritability than predicted slaughter yields (Mod_hl-Carss, Mod_Fil = 0.48 and 0.63, respectively; **Table 3**).

Heritability estimates of the single predictors used in the models ranged from 0.34 to 0.68 (**Table 4**). Heritabilities of internal measurements were moderate to high (0.34 – 0.72; Supplementary Table 2).

Heritabilities obtained for allometric log-log residuals of the weights of different body portions to body weight were low for vertebral axis (0.04) and ribs (0.18), which are very prone to measurement errors, and moderate to high (0.31 – 0.62) for the other body parts (Supplementary Table 3). All heritability estimates shown in this study were significantly different from zero ($p < 0.05$).

Genetic Correlations

Genetic relationship between Logr yields and percent yields was high ($r_g > 0.91$; Supplementary Table 1).

The genetic correlations between yield-related phenotypes, Logr and Mod slaughter yields are listed in **Table 3**. Body weight was slightly negatively correlated to both Logr slaughter yields ($r_g = -0.35$) and to both predicted slaughter yields ($r_g = -0.15$ for Mod_hl-Carss, −0.29 for Mod_Fil). Oppositely, % Fat was positively associated to Logr and Mod slaughter yields (range 0.25–0.56). To ensure that the positive relationships of % Fat with Logr and Mod slaughter yields were not generated by the relation of % Fat with BW, the analysis was also run using BW as a covariate for % Fat. With such a model, the genetic correlations become more positive (r_g = 0.40–0.68). Body shape traits (FC, RelBH, RelHL) were highly correlated to each other (0.78–0.96) but differed in their relation to slaughter yields. Both FC and RelBH were only slightly negatively and mostly insignificantly correlated to yield traits. Oppositely, RelHL was intermediately negatively associated to Logr and Mod slaughter yields ($r_g = -0.47 - -0.64$).

Logr slaughter yields (Logr_hl-Carss and Logr_hl-Carss) were highly correlated to each other (r_g = 0.96), similarly as in case of predicted slaughter yields (r_g = 0.95).

Interestingly real and predicted slaughter yields were highly associated (r_g = 0.84–0.88), suggesting a good possibility of using predicted yields as indirect selection criterion.

The genetic correlations of the predictors that composed the predictive models with Logr slaughter yields are presented in **Table 4**. P_1 and P_4 showed low to moderate negative genetic associations to Logr slaughter yields (−0.35 – −0.57). Oppositely, P_2 (0.76 – 0.83), P_3 (0.29 – 0.34), and P_5 (0.25 – 0.27) were positively correlated to Logr slaughter yields. Hence, individual predictors might be also used in non-invasive genetic improvement of slaughter yields.

The internal measurements E23, E4, E5, E6, E8 achieved negative relationship to the Logr slaughter yields (−0.03 – −0.61), only E8 was in slightly positive relation to Logr yields (Supplementary Table 2). Consequently, simple internal measurements may be useful alternatives for *in vivo* selection for improved yields.

The correlations between Logr body portion yields, BW and % Fat are presented in Supplementary Table 3. The genetic correlation between head (Logr_Head) and left filet (Logr_LFil) was negative (−0.61), showing that fish with smaller head have more filet yield. There was also a positive genetic correlation of % Fat with viscera yield (Logr_Viscera = 0.63), showing that filet fat and viscera (consisting largely of fat) share some

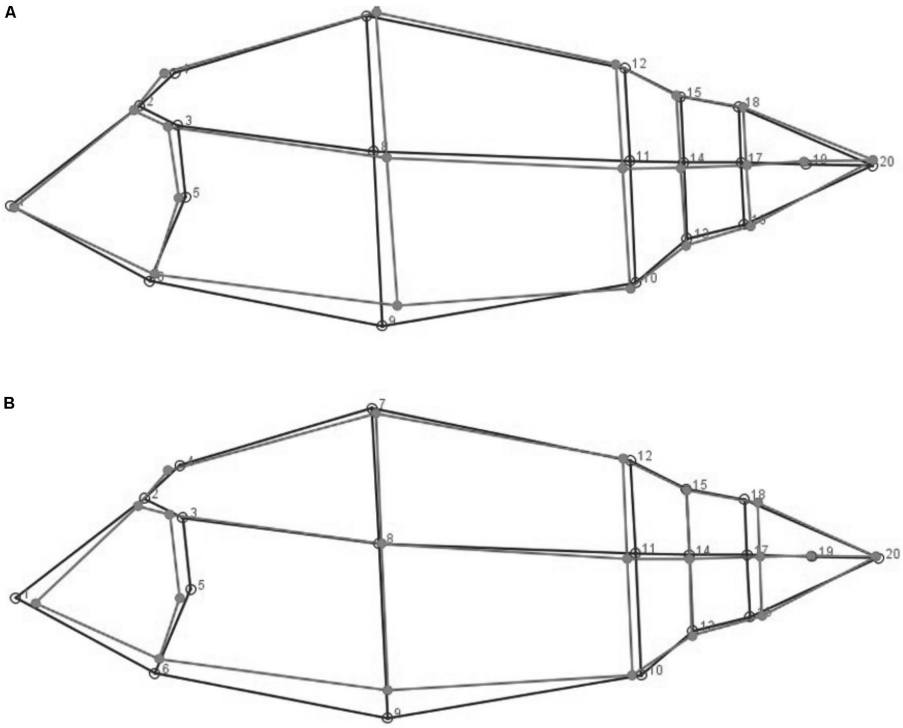

FIGURE 2 | Graphical representation of body morphology for highest values (red lines) and lowest values (blue lines) of Logr_hl-Carss **(A)** and Logr_Fil **(B)**.

TABLE 2 | Multiple linear regression models to predict headless carcass (Mod_hl-Carss) and filet yields (Mod_Fil) in common carp including predictors description, R^2, F – Fisher test value and prediction equation.

Predicted yield	Predictors	Predictor description	Regression characteristics
Logr_hl-Carss	P_1	Head area (1-2-4-5-6-1)/total body area (1-2-4-7-12-15-18-19-16-13-10-9-6-1)	$R^2 = 0.626$, $F = 866.6$, $p < 0.001$, R^2CV = 0.624
	P_2	Ultrasound E8/height between points 8-9	
	P_3	Caudal part area (12-15-14-13-10-11-12)/ventral part area (3-8-11-10-9-6-5-3)	Mod_hl-Carss = −0.06–0.37 P_1 + 6.12 P_2 + 0.06 P_3
Logr_Fil	P_1	Head area (1-2-4-5-6-1)/total body area (1-2-4-7-12-15-18-19-16-13-10-9-6-1)	
	P_2	Ultrasound E8/height between points 8–9	$R^2 = 0.492$, $F = 300.9$, $p < 0.001$, R^2CV = 0.489
	P_3	Caudal part area (12-15-14-13-10-11-12)/ventral part area (3-8-11-10-9-6-5-3)	
	P_4	Body weight	Mod_Fil = −0.02 – 0.63 P_1 + 5.30 P_2 + 0.06 P_3 −7.84E-06 P_4 + 0.0007 P_5
	P_5	% fat content	

common genetic basis. Gonad yield was negatively correlated with left filet yield (r_g Logr_LFil = −0.49), viscera yield (r_g Logr_Viscera = −0.40), % Fat (r_g = −0.46), ribs yield (r_g Logr_Ribs = −0.65) and fins yield (r_g Logr_Fins = −0.50) implying a tradeoff of investing in reproduction compared to somatic growth and reserves.

TABLE 3 | Heritability (± standard error) estimates (diagonal) in bold, phenotypic (below the diagonal) and genetic correlations ± standard error (above the diagonal) in common carp for yield-related traits and log-log residuals (Logr) of slaughter yields and models (Mod) to predict slaughter yields.

	BW	% Fat	FC	RelBH	RelHL	Logr_hl-Carss	Logr_Fil	Mod_hl-Carss	Mod_Fil
BW	**0.63 ± 0.09**	0.13 ± 0.14	0.45 ± 0.11	0.52 ± 0.10	0.53 ± 0.10	−0.35 ± 0.13	−0.35 ± 0.13	−0.15 ± 0.15	−0.29 ± 0.13
% Fat	0.21	**0.68 ± 0.10**	−0.09 ± 0.13	−0.15 ± 0.13	−0.33 ± 0.12	0.25 ± 0.14[1]	0.27 ± 0.14[2]	0.41 ± 0.13[3]	0.56 ± 0.10[4]
FC	0.34	0.03	**1.00 ± 0.09**	0.96 ± 0.01	0.78 ± 0.05	−0.10 ± 0.13	−0.17 ± 0.13	−0.15 ± 0.13	−0.25 ± 0.13
RelBH	0.40	−0.03	0.88	**1.00 ± 0.09**	0.83 ± 0.04	−0.18 ± 0.13	−0.26 ± 0.13	−0.25 ± 0.13	−0.36 ± 0.12
RelHL	0.16	−0.24	0.61	0.64	**1.00 ± 0.10**	−0.47 ± 0.10	−0.53 ± 0.10	−0.47 ± 0.11	−0.64 ± 0.08
Logr_hl-Carss	−0.03	0.20	−0.03	−0.04	−0.20	**0.46 ± 0.08**	0.96 ± 0.02	0.88 ± 0.04	0.87 ± 0.04
Logr_Fil	−0.02	0.27	−0.02	−0.10	−0.33	0.76	**0.50 ± 0.08**	0.83 ± 0.05	0.84 ± 0.05
Mod_hl-carss	0.10	0.27	−0.05	−0.11	−0.27	0.73	0.61	**0.48 ± 0.08**	0.95 ± 0.01
Mod_Fil	−0.03	0.43	−0.11	−0.19	−0.42	0.72	0.65	0.95	**0.63 ± 0.09**

When covariate of body weight to % muscle fat content was used.
[1] r_g = 0.40 ± 0.13
[2] r_g = 0.42 ± 0.13
[3] r_g = 0.55 ± 0.11
[4] r_g = 0.68 ± 0.08.

TABLE 4 | Heritability (h^2 ± standard error) of individual predictors (P_1–P_5) included in models to predict headless carcass and filet yields and their genetic correlations (r_g) ± standard error with Logr slaughter yields.

	P_1	P_2	P_3	P_4	P_5
h^2	**0.34 ± 0.07**	**0.48 ± 0.07**	**0.46 ± 0.08**	**0.63 ± 0.09**	**0.68 ± 0.10**
r_g Logr_hl-Carss	−0.52 ± 0.12	0.83 ± 0.13	0.29 ± 0.14	−0.35 ± 0.14	0.25 ± 0.14
r_g Logr_Fil	−0.57 ± 0.11	0.76 ± 0.16	0.34 ± 0.14	−0.35 ± 0.13	0.27 ± 0.14

Expected Genetic Gains

Expected genetic gains using various selection schemes are listed in **Table 5**. Absolute genetic gains for the hypothetical mass selection on real filet yield were 0.70 and 0.46% per generation when 10 and 30% selection intensities were applied, respectively. Genetic gain for FSS with 10 sibs selected per family with the 10 and 30% selection pressure was slightly lower (0.61 and 0.40%) than for mass selection. Estimated genetic gains achieved by indirect selection on the predictor Mod_Fil were 0.66% for 10% selection intensity and 0.43% for 30% which is better than FSS and only slightly lower than direct mass selection on filet yield (which is not possible in practice). Genetic gains ranged from 0.15 to 0.52% for the single predictors used in the models, and from 0.21 to 0.51% for best two internal measurements.

DISCUSSION

The present study provided important results relative to the possibility to genetically improve processing yields in common carp: (i) we found high heritability estimates of real and predicted slaughter yields showing a solid potential for their genetic improvement; (ii) high positive genetic correlations were observed between the real and the predicted yields, showing that the latter might be used as non-invasive selection criteria; (iii) expected genetic gain achieved by indirect selection on the predicted yields were higher than those obtained by sib selection that is traditionally applied for improvement of traits needing destructive recording. Thus, we showed that selection of common carp for improved slaughter yields should be feasible, even in a simple breeding program using indirect selection criteria.

Sex Effect

In this study, sex of the fish had a significant effect on some traits, including slaughter yields. Conversely, BW was found to be independent of sex. However, females had significantly greater relative head length, muscular fat and both yields. This is in accordance with the previous studies performed on common carp in Central European conditions (Kocour et al., 2005a,b, 2007). The explanation is that at market size after the third growing season, female gonads are in younger developmental stage, whereas males are practically mature with fully developed gonads, and thus females have higher slaughter yields. Accordingly, the sex effect was included as a fixed effect in the final genetic model used for estimation of genetic parameters. In later ages, the differences between sexes decrease (Kocour et al., 2005a).

Genetic Parameters of Yield-Related Traits and Slaughter Yields

Heritability estimates of yield-related traits and body morphology were high and in the upper range when compared to previous studies done on the different batches of common carp (Ankorion et al., 1992; Vandeputte et al., 2004, 2008; Kocour et al., 2007; Nielsen et al., 2010; Dong et al., 2015; Hu et al., 2017) showing a solid potential for genetic improvement of such traits.

The slaughter yields in fish are commonly calculated as a ratio between the given processed body part weight and body weight.

TABLE 5 | Expected genetic gain – E.G.G. (in percent body weight units) per generation with two selection intensities (% selected – 10%, 30%) using mass (MS), full sib (FSS), and indirect (IS) selection for filet yield improvement.

Trait selected	Target trait	Type of selection	E.G.G. with 10%	E.G.G. with 30%
Logr_Fil	% Fil	MS	0.70%	0.46%
Logr_Fil	% Fil	FSS	0.61%	0.40%
Mod_Fil	% Fil	IS	0.66%	0.43%
P_1	% Fil	IS	0.33%	0.22%
P_2	% Fil	IS	0.52%	0.34%
P_3	% Fil	IS	0.23%	0.15%
P_4	% Fil	IS	0.27%	0.18%
P_5	% Fil	IS	0.22%	0.15%
E23	% Fil	IS	0.51%	0.34%
E4	% Fil	IS	0.31%	0.21%

However, ratio traits are often biased by growth allometry that is common between body portions and body weight (Gunsett, 1984), and ratios also cause problems when genetic parameters and expected genetic responses are estimated (Gunsett, 1987; Haffray et al., 2013; Vandeputte et al., 2014). On the other hand, such problems might be overcome by calculation of simple residuals (or log-log residuals) between the component traits of a ratio (feed efficiency, slaughter yields) as proposed and applied by Haffray et al. (2012) and Vandeputte et al. (2014, 2017), and in this study. In the present study, heritabilities of slaughter yields expressed as log–log residuals (Logr) were higher (0.46–0.50) than the heritabilities for percent slaughter yields (0.36 for both slaughter traits). The latter are more in line with the previous study using also percent slaughter yields (h^2 = 0.28–0.36; Kocour et al., 2007). However, yields as residuals and percent yields were highly genetically correlated showing that the both variables explain the same trait similarly as described by Vandeputte et al. (2017). Therefore, residuals are more valuable surrogates for slaughter yields both due to their higher inheritance and the potential biases of ratio traits mentioned above.

We observed a strong genetic correlation between Logr_hl-Carss and Logr_Fil (0.96 ± 0.02). Likewise, Kocour et al. (2007) estimated high but lower genetic relationship between slaughter yields in common carp (0.79 ± 0.13). A similar genetic association between yields was found in rainbow trout (0.97 ± 0.01; Haffray et al., 2013), and European sea bass (0.79 ± 0.20; Vandeputte et al., 2017). Our study confirms that, similar to rainbow trout and sea bass, headless carcass yield (faster processing, less technical errors) might be proposed as a reliable surrogate for filet yield, especially when sib selection (evaluated on slaughtered sibs) is applied for genetic improvement of carp yields.

Harvest body weight and Logr slaughter yields were slightly negatively genetically correlated (r_g = −0.35). On the contrary, a high positive genetic correlations of body weight and slaughter yields (0.73–0.74) were found previously in common carp (Kocour et al., 2007). However, in this case slaughter yields were expressed as percent ratios and might have been effected by positive growth allometry. Therefore, comparison of these two studies is not relevant. On the other hand, even when Logr type of traits are used, such correlations are not consistent among other fish species, with zero genetic correlations observed in European sea bass (Vandeputte et al., 2017) and slightly negative correlations observed in rainbow trout (Haffray et al., 2012). This points to the fact that such correlations are probably breed and species specific and modified by biological and/or genetic phenomena between growth and slaughter yields across fish species. In our scenario, body weight should be integrated in a selection index with slaughter yields, to avoid a negative impact on growth when selecting for slaughter yields.

Positive genetic correlations were observed between % Fat and Logr slaughter yields (r_g = 0.25–0.27). A strong genetic relationship of % Fat to percent yields (0.66–0.76) was reported earlier in common carp (Kocour et al., 2007). So, selection for improved yields would indirectly lead to a slight increase of fat in the muscle. However, an excessive increase of muscle fat level without a change in the feeding strategy might lead to an unfavorable decrease of beneficial omega-3 polyunsaturated fatty acids (n-3 PUFAs) in the muscle (Nguyen et al., 2010b). Thus, selection program for increased percent yield may worsen the quality of final product. A selection program focused on increased edible parts yields should minimize risk of this phenomena using appropriate measures, e.g., by simultaneously controlling lipid deposition (Bugeon et al., 2010; Nguyen et al., 2010b; Janhunen et al., 2017).

The traits related to body shape, FC and RelBH, were slightly negatively related to slaughter yields implying that selection for improved yields in long term may change the general body shape. This is visible also in **Figure 2** where body morphology for the fish with the highest slaughter yields is represented by a more prolonged body shape. A similar relationship of body shape to % yields was observed in common carp (Kocour et al., 2007) and other fishes (Navarro et al., 2009; Haffray et al., 2012; Van Sang et al., 2012). On the other hand, due to its very high heritability, body shape itself might be changed quite simply in common carp by direct selection, as reported by Prchal et al. (2018) and proved in a selection experiment by Ankorion et al. (1992).

The relative head length (RelHL) was moderately negatively correlated to both yields (−0.47 – −0.53) implying that selection for lower RelHL could be an indirect selection criterion for increased yields in common carp. This is in agreement with Kocour et al. (2007), where even stronger negative genetic

correlations were observed. Moreover, both percent or Logr head yield were also negatively associated to slaughter yields in other fish species (Rutten et al., 2005; Kause et al., 2007; Saillant et al., 2009; Haffray et al., 2012; Vandeputte et al., 2017). However, there are gills in head, main respiratory organ of fish, so selection for lower RelHL in a long term selection program might lead to functional damage of respiration, adaptation or osmoregulation capacities (Haffray et al., 2012; Fraslin et al., 2018) and this could affect general fish performance and fitness. Moreover, selection for lower RelHL has to be considered with caution and in any case integrated in a global selection index due to a high positive genetic correlation between RelHL and BW (r_g = 0.53) as well as RelBH (r_g = 0.83). Uncontrolled selection for a smaller relative head length may thus lead to a limitation of gains in growth and faster change to an oblong-like body shape that may be less favorable for some carp consumer buying whole fish on the traditional market.

Genetic and Phenotypic Parameters of Predicted Slaughter Yields

Phenotypic correlations between Logr and Mod yields were moderately high (0.73 for headless carcass, 0.65 for filet yields). The accuracy of phenotypic prediction was high for Logr_hl-Carss (R^2 = 0.63), and intermediate for Logr_Fil (R^2 = 0.49). Such prediction of slaughter yields, combining external and internal measurements, was recently performed on rainbow trout (Haffray et al., 2013) and European seabass (Vandeputte et al., 2017). Our phenotypic predictions of yields were more accurate compared to these studies (R^2 = 0.38 for headless carcass yield in Haffray et al., 2013, R^2 = 0.02 – 0.18 for filet and 0.27 – 0.41 for carcass yield in Vandeputte et al., 2017). Hence, slaughter yields can be effectively predicted on live breeding candidates in common carp. Remarkably, Mod_hl-Carss is easier to construct in comparison with Mod_Fil (3 predictors vs. 5 predictors), and it has higher phenotypic prediction accuracy and strong phenotypic and genetic correlations (0.95 for both) to Mod_Fil. Thus, Logr headless carcass is recommended as a trait to be predicted to select for improved filet yields. This is also supported by its favorably lower negative genetic relation to the body weight and lower positive association to % Fat. In addition, Mod yields achieved high heritability (0.48–0.63), higher than Logr yields (h^2 = 0.46–0.50), and also higher when compared to other studies in which inheritance of predicted yields were estimated (Van Sang et al., 2012; Haffray et al., 2013; Vandeputte et al., 2017). This is important as it shows a good possibility of using Mod yields as an indirect selection criterion, further supported by high genetic correlations between Logr and Mod yields (0.84–0.88). It must be stressed that our results were obtained from data recorded on Amur mirror carp in semi-intensive pond conditions and at fish market size specific to Central and Eastern Europe. Validation of the predictors would be necessary before their utilization on other carp breeds, strains, lines, and size categories. Still, many of our conclusions are in line with those drawn from the previous studies in rainbow trout (Kause et al., 2007; Haffray et al., 2012, 2013), European sea bass (Vandeputte et al., 2017) and a previous small-scale study on common carp (Kocour et al., 2007), and thus our results are expected to have a reasonable level of generality.

Heritability estimates of individual predictors, that were included in the prediction models, were moderate for ratio predictors (0.34–0.48) and high for BW (P_4) and % Fat (P_5) (0.63–0.68). In the recent studies (Haffray et al., 2013; Vandeputte et al., 2017), h^2 for predictors from which the models were constructed ranged from 0.06 to 0.54 for various simple and combined predictors. Besides, P_1 and P_2 predictors were moderately to highly genetically correlated with the Logr yields. P_1 was defined as a ratio between head area to total body area (2D measurements) with negative association to yields. So, selection on lower value of P_1 would lead to higher yields as smaller head is related to higher yields (r_g = −0.52–0.57) similar to RelHL discussed above. P_2 was the ratio between ultrasound measurement of abdominal thickness (E8) and external belly height measured between landmarks 8 and 9 in 2D, and was highly positively associated to yields (r_g = 0.76–0.83). A similar relation occurs in rainbow trout (Haffray et al., 2013) with the ultrasound measurements ratio of E8 to E23. Thus, P_2 could be an even more suitable indirect selection criterion in genetic improvement of slaughter yields.

Although the added value of external morphology combined with internal measurements is interesting, the time needed for trait recording and the accuracy of prediction are more in favor of simple ultrasound measurements. Accordingly, rapid internal measurements (especially E4 and E23) might be used as alternative indirect criteria in accordance to their high heritability and intermediately high genetic correlations to yields (see Supplementary Table 2). Furthermore, 3D collection of external body landmarks could accelerate digitization of potentially relevant morphological predictors as proposed by Haffray et al. (2013). Thus, 2D and 3D collection of morphological landmarks and their power to predict yields should be under further research.

Expected Genetic Gain

Based on the expected genetic gain calculations, full-sib selection (FSS) would produce slightly lower genetic improvement than hypothetical mass selection (MS) applied on filet yields in both selection intensities. Still, sib selection might be effectively applied in genetic improvement of common carp yields. In addition, FSS method could be practically performed on real headless carcass yield, which is easier to be measured and less prone to measurement errors than filet yield, but has a simultaneous favorable effect on filet yields due to the high genetic correlation between both (0.96). On the other hand, sib selection utilizes only between-family genetic variation without exploiting genetic variation within families (Gjedrem, 2010; Haffray et al., 2013).

On the other hand, indirect selection using Mod filet yields (or the simpler Mod_hl-Carss) recorded *in vivo* could overcome limitations from sib selection mentioned above and give an even better response compared to FSS (expected genetic gain was 0.43–0.66% for indirect filet yield improvement). Alternatively, simple

internal measurements (E4 and E23) or individual predictors P_1 and P_2 might be used as traits for indirect genetic improvement of yields (0.21–0.52%) as it was also suggested by Haffray et al. (2013) and Vandeputte et al. (2017).

Nevertheless, it must be stressed that using Logr yields values that were used for derivation of best predictors and Mod yields might slightly overestimate the potential genetic gains. This was visible when simulation selection analysis was run in accordance with Fraslin et al. (2018) (data not shown). Such bias could be eliminated by linear index theory developed to improve selection gain on ratio traits (Lin, 1980; Lin and Aggrey, 2013), and optimized to improve filet weight/waste weight ratio or filet weight/body weight ratio in fish species (Fraslin et al., 2018). However, it is unclear how linear index theory could be connected to external predictors of yields, as the theory uses absolute values of body portions (weights) and not relative values (yields). Hence, this issue should be under further research.

CONCLUSION

In the present study, model-predicted slaughter yields in common carp were highly heritable and strongly genetically associated to highly hereditary real yields, expressed as log-log residuals. The results show potential for genetic improvement of processing yields through selective breeding, also by using *in vivo* morphological predictors. In addition, both real and predicted headless carcass yield might be used as an efficient surrogate (faster processing, easier to predict) for filet yield improvement through sib or indirect selection. Besides, two internal ultrasound measurements and two individual predictors could be also alternatively used as traits for indirect selection in genetic improvement of slaughter yields in common carp. As predictors are combining several sources of information, further information on the resulting breeding accuracies and realized genetic gains would be valuable in the future to quantify the expected progress. Furthermore, validation of best predictors would be necessary before their transfer to other carp breeds, strains, lines, and size categories.

DATA ACCESSIBILITY

The dataset underlying our findings is fully available in the public data repository (OSF: https://osf.io/vfnbs/).

AUTHOR CONTRIBUTIONS

MP, DG, and MK shared on establishing and on-growing the experimental stock, PIT tagging, and fin clipping the fish. PH and MP provided the methodology and equipment. MP, JB, MV, AV, JZ, DG, AB, and MK shared on final trait recordings. JZ digitized the morphological points. AK introduced MP to the quantitative genetic analysis. JB carried out the phenotypic prediction of slaughter yields. LG performed the DNA extractions and parentage assignment. MP and MV estimated the genetic parameters. All authors contributed to drafting the manuscript and approved the final version.

FUNDING

This study was supported by European Union's Seventh Framework Program (KBBE.2013.1.2-10) under grant agreement no. 613611 FISHBOOST (http://www.fishboost.eu/), Ministry of Education, Youth and Sports of the Czechia - projects, CENAKVA (No. CZ.1.05/2.1.00/01.0024), CENAKVA II (No. LO1205 under the NPU I program), Biodiverzita (CZ.02.1.01/0.0/0.0/16_025/0007370), the Grant Agency of the University of South Bohemia in České Budějovice (project no. 125/2016/Z) and Ministry of Agriculture – project of the Czech NAAR (NAZV) no. QK1710310.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2018.00283/full#supplementary-material

REFERENCES

Ankorion, Y., Moav, R., and Wohlfarth, G. (1992). Bidirectional mass selection for body shape in common carp. *Genet. Sel. Evol.* 24, 43–52. doi: 10.1186/1297-9686-24-1-43

Bauer, B. C., and Schlott, G. (2009). Fillet yield and fat content in common carp (*Cyprinus carpio*) produced in three Austrian carp farms with different culture methodologies. *J. Appl. Ichthyol.* 25, 591–594. doi: 10.1111/j.1439-0426.2009.01282.x

Boichard, D., Barbotte, L., and Genestout, L. (2014). "AccurAssign, software for accurate maximum-likelihood parentage assignment," in *Proceedings of the Tenth World Congress on Genetics Applied to Livestock Production*, Vancouver.

Bosworth, B., Holland, M., and Brazil, B. (2001). Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. *J. Anim. Sci.* 79, 1483–1490. doi: 10.2527/2001.7961483x

Bugeon, J., Lefevre, F., Cardinal, M., Uyanik, A., Davenel, A., and Haffray, P. (2010). Flesh quality in large rainbow trout with high or low fillet yield. *J. Muscle Foods* 21, 702–721. doi: 10.1111/j.1745-4573.2010.00214.x

Chavanne, H., Janssen, K., Hofherr, J., Contini, F., Haffray, P., Komen, H., et al. (2016). A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry. *Aquacult. Int.* 24, 1287–1307. doi: 10.1007/s10499-016-9985-0

Cibert, C., Fermon, Y., Vallod, D., and Meunier, F. J. (1999). Morphological screening of carp *Cyprinus carpio*: relationship between morphology and fillet yield. *Aquat. Living Resour.* 12, 1–10. doi: 10.1016/S0990-7440(99)80009-6

Dong, Z., Nguyen, N. H., and Zhu, W. (2015). Genetic evaluation of a selective breeding program for common carp *Cyprinus carpio* conducted from 2004 to 2014. *BMC Genet.* 16:94. doi: 10.1186/s12863-015-0256-2

Efron, B., and Tibshirani, R. (1993). *An Introduction to the Bootstrap*. New York, NY: CRC Press. doi: 10.1007/978-1-4899-4541-9

Falconer, D. S., and MacKay, T. F. C. (1996). *Introduction to Quantitative Genetics*, 4th Edn. Harlow: Longman Scientific & Technical.

FAO (2016). *FishStat Database*. Available at: http://faostat.fao.org/site/629/default.Aspx

Flajšhans, M., Gela, D., Kocour, M., Rodina, M., Kašpar, V., Linhart, O., et al. (2015). "Amur mirror carp, a recently certified breed of common carp in

the Czech Republic," in *Book of abstracts: Third International Conference on Common Carp*, Vodňany.

Fraslin, C., Dupont-Nivet, M., Haffray, P., Bestin, A., and Vandeputte, M. (2018). How to genetically increase fillet yield in fish: new insights from simulations based on field data. *Aquaculture* 486, 175–183. doi: 10.1016/j.aquaculture.2017.12.012

Gela, D., Rodina, M., and Linhart, O. (2003). Top-crossing with evaluation of slaughtering value in common carp (*Cyprinus carpio* L.) offspring. *Aquacult. Int.* 11, 379–387. doi: 10.1023/A:1025721723369

Gjedrem, T. (2010). The first family-based breeding program in aquaculture. *Rev. Aquacult.* 2, 2–15. doi: 10.1111/j.1753-5131.2010.01011.x

Gjedrem, T., and Baranski, M. (2009). *Selective Breeding in Aquaculture: an Introduction*. Dordrecht: Springer Netherlands. doi: 10.1007/978-90-481-2773-3

Gjerde, B., Mengistu, S. B., Ødegård, J., Johansen, H., and Altamirano, D. S. (2012). Quantitative genetics of body weight, fillet weight and fillet yield in *Nile tilapia* (*Oreochromis niloticus*). *Aquaculture* 342, 117–124. doi: 10.1016/j.aquaculture.2012.02.015

Gunsett, F. (1984). Linear index selection to improve traits defined as ratios. *J. Anim. Sci.* 59, 1185–1193. doi: 10.2527/jas1984.5951185x

Gunsett, F. (1987). Merit of utilizing the heritability of a ratio to predict the genetic change of a ratio. *J. Anim. Sci.* 65, 936–942. doi: 10.2527/jas1987.654936x

Haffray, P., Bugeon, J., Pincent, C., Chapuis, H., Mazeiraud, E., Rossignol, M. N., et al. (2012). Negative genetic correlations between production traits and head or bony tissues in large all-female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 368, 145–152. doi: 10.1016/j.aquaculture.2012.09.023

Haffray, P., Bugeon, J., Rivard, Q., Quittet, B., Puyo, S., Allamelou, J. M., et al. (2013). Genetic parameters of in-vivo prediction of carcass, head and fillet yields by internal ultrasound and 2D external imagery in large rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 41, 236–244. doi: 10.1016/j.aquaculture.2013.06.016

Hu, X., Li, C., Shang, M., Ge, Y., Jia, Z., Wang, S., et al. (2017). Inheritance of growth traits in Songpu mirror carp (*Cyprinus carpio* L.) cultured in Northeast China. *Aquaculture* 477, 1–5. doi: 10.1016/j.aquaculture.2017.04.031

Hulata, G. (1995). A review of genetic improvement of the common carp (*Cyprinus carpio* L.) and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture* 129, 143–155. doi: 10.1016/0044-8486(94)00244-I

Janhunen, M., Nousiainen, A., Koskinen, H., Vehviläinen, H., and Kause, A. (2017). Selection strategies for controlling muscle lipid content recorded with a non-destructive method in European whitefish, *Coregonus lavaretus*. *Aquaculture* 481, 229–238. doi: 10.1016/j.aquaculture.2017.09.016

Janssen, K., Chavanne, H., Berentsen, P., and Komen, H. (2017). Impact of selective breeding on European aquaculture. *Aquaculture* 472, 8–16. doi: 10.1016/j.aquaculture.2016.03.012

Kankainen, M., Setälä, J., Kause, A., Quinton, C., Airaksinen, S., and Koskela, J. (2016). Economic values of supply chain productivity and quality traits calculated for a farmed European whitefish breeding program. *Aquacult. Econ. Manag.* 20, 131–164. doi: 10.1080/13657305.2016.1155961

Kause, A., Paananen, T., Ritola, O., and Koskinen, H. (2007). Direct and indirect selection of visceral lipid weight, fillet weight, and fillet percentage in a rainbow trout breeding program. *J. Anim. Sci.* 85, 3218–3227. doi: 10.2527/jas.2007-0332

Klingenberg, C. P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* 11, 353–357. doi: 10.1111/j.1755-0998.2010.02924.x

Kocour, M., Gela, D., Rodina, M., and Linhart, O. (2005a). Testing of performance in common carp *Cyprinus carpio* L. under pond husbandry conditions I: top-crossing with Northern mirror carp. *Aquacult. Res.* 36, 1207–1215. doi: 10.1111/j.1365-2109.2005.01340.x

Kocour, M., Linhart, O., Gela, D., and Rodina, M. (2005b). Growth performance of all-female and mixed-sex common carp *Cyprinus Carpio* L. populations in the Central Europe climatic conditions. *J. World Aquacult. Soc.* 36, 103–113. doi: 10.1111/j.1749-7345.2005.tb00136.x

Kocour, M., Mauger, S., Rodina, M., Gela, D., Linhart, O., and Vandeputte, M. (2007). Heritability estimates for processing and quality traits in common carp (*Cyprinus carpio* L.) using a molecular pedigree. *Aquaculture* 270, 43–50. doi: 10.1016/j.aquaculture.2007.03.001

Kohlmann, K., Gross, R., Murakaeva, A., and Kersten, P. (2003). Genetic variability and structure of common carp (*Cyprinus carpio*) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. *Aquat. Living Resour.* 16, 421–431. doi: 10.1016/S0990-7440(03)00082-2

Kohlmann, K., Kersten, P., and Flajšhans, M. (2005). Microsatellite-based genetic variability and differentiation of domesticated, wild and feral common carp (*Cyprinus carpio* L.) populations. *Aquaculture* 247, 253–266. doi: 10.1016/j.aquaculture.2005.02.024

Lin, C. (1980). Relative efficiency of selection methods for improvement of feed efficiency. *J. Dairy Sci.* 63, 491–494. doi: 10.3168/jds.S0022-0302(80)82960-2

Lin, C., and Aggrey, S. (2013). Incorporation of economic values into the component traits of a ratio: feed efficiency. *Poultry Sci.* 92, 916–922. doi: 10.3382/ps.2012-02688

Madsen, P., and Jensen, J. (2013). *DMU version 6*. Available at: http://dmu.agrsci.dk/DMU/Doc/Current/dmuv6_guide.5.2.pdf

Navarro, A., Zamorano, M. J., Hildebrandt, S., Ginés, R., Aguilera, C., and Afonso, J. M. (2009). Estimates of heritabilities and genetic correlations for growth and carcass traits in gilthead seabream (*Sparus aurata* L.), under industrial conditions. *Aquaculture* 289, 225–230. doi: 10.1016/j.aquaculture.2008.12.024

Nguyen, N. H., Ponzoni, R. W., Abu-Bakar, K. R., Hamzah, A., Khaw, H. L., and Yee, H. Y. (2010a). Correlated response in fillet weight and yield to selection for increased harvest weight in genetically improved farmed tilapia (GIFT strain), *Oreochromis niloticus*. *Aquaculture* 305, 1–5. doi: 10.1016/j.aquaculture.2010.04.007

Nguyen, N. H., Ponzoni, R. W., Yee, H. Y., Abu-Bakar, K. R., Hamzah, A., and Khaw, H. L. (2010b). Quantitative genetic basis of fatty acid composition in the GIFT strain of *Nile tilapia* (*Oreochromis niloticus*) selected for high growth. *Aquaculture* 309, 66–74. doi: 10.1016/j.aquaculture.2010.08.034

Nielsen, H. M., Ødegård, J., Olesen, I., Gjerde, B., Ardo, L., Jeney, G., et al. (2010). Genetic analysis of common carp (*Cyprinus carpio*) strains. I: genetic parameters and heterosis for growth traits and survival. *Aquaculture* 304, 14–21. doi: 10.1016/j.aquaculture.2010.03.016

Ninh, N. H., Ponzoni, R. W., Nguyen, N. H., Woolliams, J. A., Taggart, J. B., McAndrew, B. J., et al. (2011). A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): estimation of genetic parameters. *Aquaculture* 32, 39–46. doi: 10.1016/j.aquaculture.2011.09.031

Pinheiro, J. C., and Bates, D. M. (2000). *Mixed-Effects Models in S and S-PLUS*. New York, NY: Springer-Verlag. doi: 10.1007/978-1-4419-0318-1

Powell, J., White, I., Guy, D., and Brotherstone, S. (2008). Genetic parameters of production traits in Atlantic salmon (*Salmo salar*). *Aquaculture* 274, 225–231. doi: 10.1016/j.aquaculture.2007.11.036

Prchal, M., Kause, A., Vandeputte, M., Gela, D., Allamelou, J. M., Girish, K., et al. (2018). The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. *PLoS One* 13:e0191624. doi: 10.1371/journal.pone.0191624

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., et al. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 18:529. doi: 10.1186/s12859-017-1934-z

Rutten, M. J., Bovenhuis, H., and Komen, H. (2004). Modeling fillet traits based on body measurements in three *Nile tilapia* strains (*Oreochromis niloticus* L.). *Aquaculture* 231, 113–122. doi: 10.1016/j.aquaculture.2003.11.002

Rutten, M. J., Bovenhuis, H., and Komen, H. (2005). Genetic parameters for fillet traits and body measurements in *Nile tilapia* (*Oreochromis niloticus* L.). *Aquaculture* 246, 125–132. doi: 10.1016/j.aquaculture.2005.01.006

Saillant, E., Dupont-Nivet, M., Sabourault, M., Ha, P., Laureau, S., Vidal, M.-O., et al. (2009). Genetic variation for carcass quality traits in cultured sea bass (*Dicentrarchus labrax*). *Aquat. Living Resour.* 22, 105–112. doi: 10.1051/alr/2009010

Van Sang, N., Klemetsdal, G., Ødegård, J., and Gjøen, H. M. (2012). Genetic parameters of economically important traits recorded at a given age in striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture* 34, 82–89. doi: 10.1016/j.aquaculture.2012.03.013

Van Sang, N., Thomassen, M., Klemetsdal, G., and Gjøen, H. M. (2009). Prediction of fillet weight, fillet yield, and fillet fat for live river catfish (*Pangasianodon hypophthalmus*). *Aquaculture* 288, 166–171. doi: 10.1016/j.aquaculture.2008.11.030

Vandeputte, M. (2003). Selective breeding of quantitative traits in the common carp (*Cyprinus carpio*): a review. *Aquat. Living Resour.* 16, 399–407. doi: 10.1016/S0990-7440(03)00056-1

Vandeputte, M., Garouste, R., Dupont-Nivet, M., Haffray, P., Vergnet, A., Chavanne, H., et al. (2014). Multi-site evaluation of the rearing performances of 5 wild populations of European sea bass (*Dicentrarchus labrax*). *Aquaculture* 42, 239–248. doi: 10.1016/j.aquaculture.2014.01.005

Vandeputte, M., Kocour, M., Mauger, S., Dupont-Nivet, M., De Guerry, D., Rodina, M., et al. (2004). Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). *Aquaculture* 235, 223–236. doi: 10.1016/j.aquaculture.2003.12.019

Vandeputte, M., Kocour, M., Mauger, S., Rodina, M., Launay, A., Gela, D., et al. (2008). Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio* L.): heritability estimates and response to selection. *Aquaculture* 277, 7–13. doi: 10.1016/j.aquaculture.2008.02.009

Vandeputte, M., Puledda, A., Tyran, A. S., Bestin, A., Coulombet, C., Bajek, A., et al. (2017). Investigation of morphological predictors of fillet and carcass yield in European sea bass (*Dicentrarchus labrax*) for application in selective breeding. *Aquaculture* 470, 40–49. doi: 10.1016/j.aquaculture.2016.12.014

Zajic, T., Mraz, J., Sampels, S., and Pickova, J. (2013). Fillet quality changes as a result of purging of common carp (*Cyprinus carpio* L.) with special regard to weight loss and lipid profile. *Aquaculture* 40, 111–119. doi: 10.1016/j.aquaculture.2013.03.004

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling Editor declared a past co-authorship with several of the authors MP, MV, AB, PH, and MK.

CHAPTER 4

ESTIMATION OF GENETIC PARAMETERS OF FATTY ACIDS COMPOSITION IN FLESH OF MARKET SIZE COMMON CARP (*CYPRINUS CARPIO* L.) AND THEIR RELATION TO PERFORMANCE TRAITS REVEALED THAT SELECTIVE BREEDING CAN INDIRECTLY AFFECT THE FLESH QUALITY

Prchal, M., Vandeputte, M., Gela, D., Doležal, M., Buchtová, H., Rodina, M., Flajšhans, M., Kocour, M., 2018. Estimation of genetic parameters of fatty acids composition in flesh of market size common carp (*Cyprinus carpio* L.) and their relation to performance traits revealed that selective breeding can indirectly affect the flesh quality. Czech J. Anim. Sci. 63, 280–291.

My share on this work was about 30%.

Original Paper *Czech J. Anim. Sci., 63, 2018 (7): 280–291*

https://doi.org/10.17221/30/2018-CJAS

Estimation of Genetic Parameters of Fatty Acids Composition in Flesh of Market Size Common Carp (*Cyprinus carpio* L.) and Their Relation to Performance Traits Revealed that Selective Breeding Can Indirectly Affect Flesh Quality

Martin Prchal[1], Marc Vandeputte[2,3], David Gela[1], Marek Doležal[4], Hana Buchtová[5], Marek Rodina[1], Martin Flajšhans[1], Martin Kocour[1]*

[1]South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Vodňany, Czech Republic

[2]GABI, INRA, AgroParisTech, University of Paris-Saclay, Jouy-en-Josas, France

[3]Ifremer, Palavas-les-Flots, France

[4]Department of Food Chemistry and Analysis, Institute of Chemical Technology in Prague, Prague, Czech Republic

[5]Department of Meat Hygiene and Technology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

**Corresponding author: mprchal@frov.jcu.cz*

ABSTRACT

Prchal M., Vandeputte M., Gela D., Doležal M., Buchtová H., Rodina M., Flajšhans M., Kocour M. (2018): **Estimation of genetic parameters of fatty acids composition in flesh of market size common carp (*Cyprinus carpio* L.) and their relation to performance traits revealed that selective breeding can indirectly affect flesh quality**. Czech J. Anim. Sci., 63, 280–291.

Fish are a rich source of omega-3 polyunsaturated fatty acids (n-3 PUFAs) and thus, they should be an integral part of human diet at least twice a week. As a result, high attention has been devoted to the improvement of fatty acids (FA) content in the flesh of farmed fish through nutrition. Conversely, there are very few data on the potential of selective breeding to improve FA composition in fish. We estimated genetic parameters of fillet fatty acid content and performance traits in market size common carp cultured under semi-intensive pond conditions. The experimental stock arose through factorial mating of 7 dams and 36 sires. All families were reared communally. Pedigree was reconstructed with microsatellite markers, and 158 individuals were dressed out and selected for flesh FA composition analysis. Heritability estimates of total muscle fat, FA composition in total fat (TF) (n-3 PUFA-TF, PUFA-TF, EPA-TF – eicosapentaenoic acid, n-6/n-3 – omega6/omega3 PUFA ratio), and most performance traits were moderately heritable (h^2 = 0.23–0.41), and body weight was highly heritable (h^2 = 0.62 ± 0.20). Genetic correlations show that selection for faster growth would indirectly lead

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Projects "CENAKVA" No. CZ.1.05/2.1.00/01.0024 and "CENAKVA II" No. LO1205 under the NPU I program, and Project Biodiverzita No. CZ.02.1.01/0.0/0.0/16_025/0007370) and by the Grant Agency of the University of South Bohemia in České Budějovice (Project No. 125/2016/Z).

Czech J. Anim. Sci., 63, 2018 (7): 280–291 *Original Paper*

https://doi.org/10.17221/30/2018-CJAS

to fillet yield improvement (r_g = 0.50–0.62) while having little impact on muscle fat (r_g = 0.21). However, lipid quality in flesh would be affected: n-3 PUFA-TF would decrease and the n-6/n-3 PUFA ratio would increase. A likely interpretation is that faster growing genotypes consume more supplemental feed, which was poor in the beneficial FAs. For sustainable selective breeding, supplemental feed composition should be modified, so that faster growing carps would maintain an appropriate flesh quality.

Keywords: genetic correlations; genetic improvement; growth; heritability; slaughtering traits, supplemental feeding

Fish are an important source of omega-3 polyunsaturated fatty acids (n-3 PUFAs, mainly eicosapentaenoic acid – EPA and docosahexaenoic acid – DHA) with a favourable ratio of omega-6/omega3 PUFA ratio (n-6/n-3) and thus, they should be an integral part of human diet at least twice a week (Mraz et al. 2012a; Rodriguez et al. 2017). In general, n-3 PUFAs are higher in marine fish species in comparison to freshwater fish species and phenotypic variation of fatty acids (FA) composition is considerable among fish species (Fontagne-Dicharry and Medale 2010). Environmental and nutritional factors can significantly impact the FA profile (Mraz and Pickova 2011; Markovic et al. 2016; Trbovic et al. 2017). Besides, genetic background in FA composition also plays a certain role as has recently been observed in Nile tilapia (*Oreochromis niloticus*) (Nguyen et al. 2010), Atlantic salmon (*Salmo salar*) (Leaver et al. 2011), and rainbow trout (*Oncorhynchus mykiss*) (Overturf et al. 2013).

Common carp is one of the most important freshwater fish species for world aquaculture and its annual production is continuously increasing (http://www.fao.org/fishery/statistics/software/FishStatJ/en). In the recent past, several studies confirmed the possibility to favourably improve common carp FA composition in the flesh by increasing n-3 PUFAs using special diets (Mraz et al. 2012a, b; Steffens 2016). Likewise, it was observed that the muscle fat content is a highly heritable trait (Kocour et al. 2007). Yet, information about genetic variation of FA composition in common carp is still missing. While recent studies in common carp confirmed potential for genetic improvement of growth (Vandeputte et al. 2004, 2008; Kocour et al. 2007; Prchal et al. 2018) and slaughtering yields (Kocour et al. 2007; Prchal et al. 2018) by systematic selection, nothing is known about how those traits are correlated to flesh FA composition.

The aim of this study was to quantify genetic and phenotypic variation related to FA composition in flesh and performance traits in 3-year-old common carp. The intent was (*i*) to estimate genetic variation of the most important FA groups and performance traits, (*ii*) to assess genetic and phenotypic correlations among FA groups and between FA groups and performance traits, (*iii*) to evaluate prospects for selective breeding programs targeting on flesh quality improvement in common carp.

MATERIAL AND METHODS

Experimental stock. The study was performed on the Hungarian synthetic mirror carp strain (HSM) bred at the University of South Bohemia (USB), Research Institute of Fish Culture and Hydrobiology (RIFCH) in Vodňany, Czech Republic (Vandeputte et al. 2004). In the period 2002–2007, the HSM was an object of studies focused on genetic variation of various performance traits (Vandeputte et al. 2004, 2008; Kocour et al. 2007). The G3 stock used for the present study was established by artificial spawning of 8 G0 females and 96 G2 males with individual collection of gametes applying a full-factorial mating design in May 2005 at the fish hatchery of the USB RIFCH. More details about reproduction and mating design are described by Kocour et al. (2007). Before mating, fin tissue from caudal fin (approximately 1 cm^2) was collected from each broodstock fish used (n = 104) and stored in 98% ethanol at room temperature until genotyping.

Rearing of experimental stock until market size. During the first growing season and first wintering, the experimental stock was reared under common semi-intensive pond conditions in two 0.16 ha nursery earthen ponds (stocking fish density was 125 000 larvae per ha) (Vandeputte et al. 2008). The nutritional requirements were covered by natural food (zooplankton and zoobenthos) and supplemental feeding using plant-

Original Paper *Czech J. Anim. Sci., 63, 2018 (7): 280–291*

https://doi.org/10.17221/30/2018-CJAS

based pellets (ZZN Strakonice, Czech Republic); feed was distributed 3 times a week from eight weeks of age until the end of September in doses of 5–10% of the fish stock biomass per feeding day adjusted according to the abundance of zooplankton, oxygen level, and water temperature. In the second spring (April 2006) 750 randomly selected fish from each pond were PIT-tagged, fin-clipped for future genotyping and parentage assignment. Subsequently, all fish were communally reared in one pond throughout the second growing season, the second wintering, and the third growing season when fish reached the market size (Vandeputte et al. 2008). Details on stocking densities and other fish handling are described by Kocour et al. (2007). During the second and the third growing season carp were fed with natural food (zooplankton, zoobenthos) developing in ponds and plant-based pellets altered later with wheat grain without any treatment. The supplemental feed was served three times a week in doses of 1.5–3% of the fish stock biomass per feeding day adjusted according to the abundance of zooplankton, level of dissolved oxygen, water temperature, and required harvest weight. The natural food and the additional feed contribute approximately 1 : 1 to the weight gain of fish (Horvath et al. 1992). The natural food is an important source of proteins, fat, and other bioactive compounds (nutritional profile in % of dry matter: crude protein (CP) 54.8–69.8%, carbohydrates (CH) 3.0–4.8%, total fat (TF) 5.7–13.2%, FA composition in % of total fat: saturated FAs (SFAs) 22.6–28.4%, monounsaturated FAs (MUFAs) 18.2–25.8%, omega-3 polyunsaturated FAs (n-3 PUFAs) 33–59.2%, omega-6 polyunsaturated FAs (n-6 PUFAs) 6.95–13.6%; dry matter (DM) 10–20%) (Mraz and Pickova 2009). The additional food serves mostly as a source of energy in carbohydrates that are well utilized by common carp (nutritional profile of plant-based pellets: DM 88.8%, CP 17.9%, CH 58.9%, TF 3.7%, FA composition in % of total fat: SFAs 13.6–16.5%, MUFAs 17.5–37.9%, n-3 PUFAs 4.5–5.6%, n-6 PUFAs 42.9–61.0%; nutritional profile of wheat grain: DM 88.3%, CP 10.4%, CH 72%, TF 2.4%, FA composition in % of total fat: SFAs 18.3%, MUFAs 16.3%, n-3 PUFAs 4.2%, n-6 PUFAs 61.3%) (Mraz et al. 2012b). Unutilized carbohydrates are stored as glycogen or they are changed to FAs (mostly MUFAs) and stored in muscle and hepatopancreas (Mraz et al. 2012b). After pond harvest at the end of the third growing season, all survivors (n = 336) were kept in a storage pond for three weeks to empty the intestines and to refresh the odour and taste of flesh, a practice commonly known as purging (Zajic et al. 2013).

Phenotypic recordings and parental allocation. Final recordings were performed at the facility of the USB RIFCH in Vodňany, Czech Republic in October 2007. The fish were killed by a blow to the head, then bled by cutting the gills according to local rules. Immediately after bleeding, standard length (SL in mm) and body weight (BW in g) were recorded. Subsequently, the fish were gutted, filleted, sexed by visual inspection of gonads (females, males), and each part of the processed body (head, fillets, viscera, gonads, skin, skeleton with remnants, fins, scales) was weighed to the nearest 0.5 g. Each fillet without skin was labelled, packed in aluminum foil, kept on ice until the end of the day when deeply frozen and stored at –80°C until fat and fatty acids analysis. Percentage of processed body (Kocour et al. 2007) or so-called headless carcass yield (% hl-Carss) and fillet yield with skin (% Fill) and without skin (% Fill DS) were calculated as the most important slaughtering traits:

$$\%\ \text{hl-Carss} = (\text{fillet weight} + \text{skin weight} + \text{weight of skeleton with remnants})/\text{body weight} \times 100$$

$$\%\ \text{Fill} = (\text{fillet weight} + \text{skin weight})/\text{body weight} \times 100$$

$$\%\ \text{Fill DS} = (\text{fillet weight}/\text{body weight}) \times 100.$$

In addition, we calculated Fulton's condition factor (FC) and gonadosomatic index (GSI):

$$\text{FC} = 10^5 \times \text{body weight}/\text{standard length}^3$$

$$\text{GSI} = \text{gonadal weight}/\text{body weight} \times 100$$

The parentage assignment was based on the analysis of 11 microsatellite loci: MFW7, MFW9, MFW11, MFW16, MFW18, and MFW26 for all fish, MFW3, MFW12, MFW20, MFW29, and MFW40 for some fish only. The parental allocation was performed by exclusion with one or two mismatches tolerated, using the VITASSIGN software (Vandeputte et al. 2006).

Lipid and fatty acids analysis. Based on parentage assignment, 158 individuals, the progeny of

Czech J. Anim. Sci., 63, 2018 (7): 280–291 *Original Paper*

https://doi.org/10.17221/30/2018-CJAS

7 females and 36 males, belonging to 115 full-sibs families, were selected for the FA composition analysis. The reduction of samples for the FA analysis was done due to the costs of analysis. For a given sample size in such a factorial design, the precision of heritability estimates mostly depends on a combination of a minimum number of sires and a minimum number of offspring per sire (Dupont-Nivet et al. 2002). Thus, the progeny of all sires that were represented in the whole set of 336 slaughtered fish with less than 3 progeny were not considered as suitable for this study. Fillets of selected fish were homogenized using a flesh-suitable mixer and for fat and FA analysis an appropriate aliquot was taken.

The total fat content in wet muscle tissue was determined gravimetrically by the Soxhlet method according to Application note 390/revision 2.8/2007 (FOSS Analytical AB 2003) by extraction in solvent petroleum ether using Soxtec 2055 (FOSS Tecator AB, Sweden) after the acid hydrolysis of samples using SoxCap 2047 (FOSS Tecator AB).

The composition of fatty acids was determined from total lipids in wet muscle tissue which were extracted with chloroform-methanol (2 : 1 v/v) according to the method of Folch et al. (1957). Derivatization of fatty acids was based on the base-catalysed reaction using NaOH-methanol as reagent. Fatty acid methyl esters (FAMEs) were then extracted to hexane. FAMEs were analysed by gas-liquid chromatography using a SP-2560 fused silica capillary column (100 m × 0.25 mm i.d., 20 µm film thickness) (Supelco, USA) in an Agilent 6890 gas chromatograph (Agilent Technologies, USA) equipped with flame ionization detector (FID). The oven temperature was 175°C for 30 min, then it was increased by 1°C/min to 210°C where it was maintained for 40 min. Detector and injection port temperatures were 220°C and the nitrogen carrier gas flow was 1 ml/min. For the identification of FAME, standard FAME mixtures were analysed. To confirm the identification of some FAMEs, the gas chromatography–mass spectrometry (GC/MS) analysis was carried out in the GC/MSD system Agilent 5975 (Agilent Technologies) with the same column and temperature conditions as above, except for the helium flow, which was 0.6 ml/min and the detector temperature was 250°C.

Quantitative genetic analysis. The final dataset comprised percentage of total muscle fat in wet muscle tissue (Fat-M), relative values of FAs presented as % of total fat (SFA-TF, MUFA-TF, PUFA-TF, n-6 PUFA-TF, n-3 PUFA-TF, EPA-TF, DHA-TF), absolute values of FA in % of wet muscle tissue (SFA-M, MUFA-M, PUFA-M, n-6 PUFA-M, n-3 PUFA-M, EPA-M, DHA-M), ratio of n-6/n-3 PUFAs and performance traits (BW, FC, GSI, % hl-Carss, % Fill DS, % Fill) (for trait abbreviations see Table 1). Variance (phenotypic: V_P, genetic: V_A) and covariance (phenotypic: r_p, genetic: r_g) components were estimated in multivariate mixed models using the restricted maximum likelihood method in VCE (Groeneveld et al. 2008) and DMU softwares (Madsen and Jensen 2013). The statistical model to estimate (co)variance components for the traits recorded was:

$$Y_{ijk} = \mu_i + sex_{ij} + (\beta_i \times \text{body weight}_k) + anim_{ik} + e_{ijk}$$

where:

Y_{ijk} = vector of observations (for all analysed traits)

μ_i = overall mean for a trait i

sex_{ij} = fixed effect of sex (j = female, male, unidentified) for trait i

β_i = regression coefficient between the weight of body part i and the covariate body weight, so that the genetic parameters estimated were those of the residual of the regression of the weight of a given body part on body weight, which was used as a surrogate for the yield of this body part, as proposed by Vandeputte et al. (2014); this regression on body weight was used only for weight of body parts (yield traits)

$anim_{ik}$ = random genetic effect of an animal k (k = 1, 2, etc. – no. of individual) for a trait i

e_{ijk} = random residual.

Likewise, a random maternal effect was calculated. However, this effect was negligible for all traits, and thus it was not included in the final model. Moreover, no other significant covariates (including body weight in relation to FAs) or fixed effects (including pond effect during the first growing season) associated to analyzed traits were found. Heritability estimates were calculated as the ratio of genetic variance (V_A) divided by the total phenotypic variance (V_P), where V_P is the sum of genetic (V_A) and residual variance (V_R), $h^2 = V_A/(V_A + V_R)$. The likelihood ratio test (LRT) was used for comparing the goodness of fit of two models (including vs excluding the animal genetic effect). The animal additive genetic effect (and thus the associated heritability estimate)

Original Paper *Czech J. Anim. Sci., 63, 2018 (7): 280–291*

https://doi.org/10.17221/30/2018-CJAS

was considered significant when the difference in –2Log-likelihood was higher than the threshold value for $P < 0.05$ of a χ^2 distribution with 1 degree of freedom (Pinheiro and Bates 2000). Genetic correlation was considered significant if $|r_g| - |1.96 \times$ standard error (SE)$|$ was higher than zero (two-tailed hypothesis) (Kause et al. 2016).

RESULTS

Fatty acids composition. The basic statistics of FA composition in 3-year-old common carp flesh are listed in Table 1. The mean fat content presented as % in wet muscle tissue was 3.23%. Among the main FA groups, MUFA-TF (59.41%) and MUFA-M (1.92%) represented the largest fraction, followed by SFA-TF (26.77%) and SFA-M (0.86%) and PUFAs (PUFA-TF 13.82%, PUFA-M 0.43%). The amount of omega-6 FAs (n-6) was 10.13% for n-6 PUFA-TF and 0.32% for n-6 PUFA-M. Omega-3 FAs (n-3) were 3.14% for n-3 PUFA-TF and 0.10% for n-3 PUFA-M. The ratio of n-6/n-3 was 3.29. The most beneficial groups of n-3 PUFAs, EPA and DHA, achieved in relative values: EPA-TF 0.29%, DHA-TF 0.26%, and in absolute values: EPA-M 0.009%, DHA-M 0.007%.

Performance traits. The phenotypic values of performance traits are presented within Table 1. The mean body weight of carps used for this study was 1395 g with condition factor of 3.18 and GSI of 2.36. The mean yields of headless carcass (63%), fillets with skin (40%) and without skin (31%) were similar to values observed in other studies done on the same breed and conditions (Kocour et al. 2007). Sex had a significant effect on BW and GSI only.

Parentage assignment. The parental allocation followed by sample reduction (see Material and Methods) resulted in 158 pedigreed animals with fatty acids phenotype, from 115 full-sibs families produced from 7 dams and 36 sires, with an average full-sibs family size of 1.37 (range 1–4), an average paternal half-sibs family size of 4.39 (range 3–8), and an average maternal half-sibs family size of 26.17 (range 17–42).

Heritability estimates. The heritability estimates are reported as values ± standard errors (SE) in diagonal (bold) within Table 2 (FA composition) and Table 5 (performance traits). Only five heritability estimates out of 16 analyzed FA traits were significantly different from zero (Fat-M, PUFA-TF, n-3 PUFA-TF, EPA-TF, n-6/n-3). The heritability of total fat and FA composition was moderate (0.24–0.37). Performance traits also had moderate heritability, (only GSI was not significant) except body weight (BW) which was highly heritable ($h^2 = 0.62 \pm 0.20$).

Correlations among fat and fatty acids. Genetic and phenotypic correlations among total fat and

Table 1. Mean ± standard deviation (SD) and coefficient of variation (CV) of fatty acid composition and performance traits in 3-year-old common carp ($n = 158$)

Trait	Mean ± SD	CV
Fat-M	3.23 ± 1.92	59.4
SFA-TF	26.77 ± 1.62	6.1
MUFA-TF	59.41 ± 1.94	3.3
PUFA-TF	13.82 ± 1.51	10.9
n-6 PUFA-TF	10.13 ± 1.07	10.5
n-3 PUFA-TF	3.14 ± 0.57	18.1
EPA-TF	0.29 ± 0.12	41.4
DHA-TF	0.26 ± 0.21	80.8
SFA-M	0.86 ± 0.52	60.4
MUFA-M	1.92 ± 1.18	61.5
PUFA-M	0.43 ± 0.24	55.8
n-6 PUFA-M	0.32 ± 0.17	53.1
n-3 PUFA-M	0.10 ± 0.06	60.0
EPA-M	0.009 ± 0.005	55.5
DHA-M	0.007 ± 0.006	85.7
n-6/n-3	3.29 ± 0.47	14.2
BW*	1395 ± 273	19.6
FC	3.18 ± 0.32	10.1
GSI*	2.36 ± 1.47	62.3
% hl-Carss	62.7 ± 2.35	3.7
% Fill DS	30.76 ± 1.96	6.3
% Fill	39.83 ± 2.09	5.2

M = absolute value (in % of wet muscle tissue), TF = relative value (in % of total fat), Fat = total fat content, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, n-6/n-3 = ratio between omega-6 and omega-3 polyunsaturated fatty acids, BW = body weight, FC = Fulton's condition factor, GSI = gonadosomatic index, % hl-Carss = headless carcass yield, % Fill DS = deskinned fillet yield, % Fill = fillet yield

*significant sex effect

Czech J. Anim. Sci., 63, 2018 (7): 280–291 Original Paper
https://doi.org/10.17221/30/2018-CJAS

Table 2. Heritability estimates (bold, diagonal, ± standard error (SE)), genetic (above diagonal, ± SE) and phenotypic (below diagonal) correlations in fatty acids composition of 3-year-old common carp

Traits	Fat-M	SFA-TF	MUFA-TF	PUFA-TF	n-6 PUFA-TF	n-3 PUFA-TF	EPA-TF	DHA-TF	SFA-M	MUFA-M	PUFA-M	n-6 PUFA-M	n-3 PUFA-M	EPA-M	DHA-M	n-6/n-3
Fat-M	**0.24 ± 0.12***	0.59 ± 0.16	0.50 ± 0.13	−0.93 ± 0.14	−0.95 ± 0.14	−0.46 ± 0.13	−0.39 ± 0.15	−0.27 ± 0.14	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.01	0.96 ± 0.01	0.93 ± 0.10	0.71 ± 0.06	0.78 ± 0.12	0.07 ± 0.16
SFA-TF	0.05	**0.24 ± 0.19**	−0.32 ± 0.08	−0.72 ± 0.17	−0.73 ± 0.18	−0.10 ± 0.14	0.06 ± 0.17	−0.41 ± 0.14	0.67 ± 0.16	0.57 ± 0.16	0.53 ± 0.15	0.55 ± 0.16	0.73 ± 0.16	0.61 ± 0.16	0.43 ± 0.15	−0.55 ± 0.16
MUFA-TF	0.33	−0.63	**0.10 ± 0.15**	−0.57 ± 0.10	−0.06 ± 0.10	−0.62 ± 0.12	−0.70 ± 0.11	−0.10 ± 0.20	0.65 ± 0.14	0.72 ± 0.13	0.70 ± 0.13	0.13 ± 0.13	−0.14 ± 0.13	−0.05 ± 0.14	0.23 ± 0.13	0.77 ± 0.17
PUFA-TF	−0.47	−0.24	−0.58	**0.29 ± 0.17***	0.96 ± 0.01	0.67 ± 0.05	0.36 ± 0.10	0.48 ± 0.10	−0.92 ± 0.16	−0.88 ± 0.15	−0.81 ± 0.16	−0.86 ± 0.14	−0.81 ± 0.15	−0.54 ± 0.17	−0.59 ± 0.15	−0.18 ± 0.16
n-6 PUFA-TF	−0.47	−0.16	−0.61	0.95	**0.25 ± 0.21**	0.74 ± 0.13	0.18 ± 0.13	−0.09 ± 0.10	−0.97 ± 0.14	−0.95 ± 0.13	−0.87 ± 0.15	−0.85 ± 0.15	−0.78 ± 0.15	−0.67 ± 0.17	−0.79 ± 0.15	−0.23 ± 0.18
n-3 PUFA-TF	−0.32	−0.31	−0.36	0.79	0.60	**0.37 ± 0.22***	0.79 ± 0.10	0.64 ± 0.06	−0.43 ± 0.14	−0.50 ± 0.13	−0.39 ± 0.14	−0.61 ± 0.15	−0.38 ± 0.15	0.08 ± 0.16	−0.28 ± 0.14	−0.85 ± 0.08
EPA-TF	−0.39	0.02	0.04	0.65	0.50	0.75	**0.34 ± 0.20***	0.71 ± 0.10	−0.27 ± 0.14	−0.36 ± 0.14	−0.41 ± 0.15	−0.40 ± 0.15	−0.12 ± 0.16	0.27 ± 0.17	−0.1 ± 0.15	−0.75 ± 0.15
DHA-TF	−0.26	−0.06	−0.04	0.72	0.58	0.75	0.69	**0.03 ± 0.10**	−0.25 ± 0.14	−0.26 ± 0.13	−0.13 ± 0.14	−0.03 ± 0.14	0.22 ± 0.13	0.12 ± 0.14	0.08 ± 0.10	−0.36 ± 0.13
SFA-M	0.99	0.17	0.25	−0.49	−0.49	−0.34	−0.37	−0.25	**0.21 ± 0.19**	0.99 ± 0.00	0.95 ± 0.01	0.94 ± 0.01	0.94 ± 0.02	0.76 ± 0.10	0.79 ± 0.12	−0.09 ± 0.18
MUFA-M	1.00	0.01	0.38	−0.49	−0.49	−0.32	−0.40	−0.27	0.98	**0.23 ± 0.18**	0.96 ± 0.00	0.95 ± 0.01	0.90 ± 0.02	0.70 ± 0.10	0.79 ± 0.12	0.04 ± 0.17
PUFA-M	0.98	0.01	0.26	−0.33	−0.32	−0.21	−0.30	−0.17	0.96	0.97	**0.12 ± 0.18**	0.99 ± 0.00	0.97 ± 0.01	0.74 ± 0.04	0.90 ± 0.1	−0.03 ± 0.14
n-6 PUFA-M	0.97	0.02	0.23	−0.33	−0.33	−0.26	−0.32	−0.19	0.95	0.96	0.95	**0.16 ± 0.19**	0.95 ± 0.01	0.68 ± 0.05	0.87 ± 0.10	0.04 ± 0.16
n-3 PUFA-M	0.94	−0.05	0.26	−0.27	−0.03	−0.08	−0.21	−0.10	0.92	0.94	0.79	0.95	**0.16 ± 0.17**	0.86 ± 0.04	0.94 ± 0.09	−0.20 ± 0.16
EPA-M	0.76	0.04	0.05	−0.04	−0.10	0.10	0.18	0.14	0.75	0.74	0.83	0.81	0.86	**0.22 ± 0.19**	0.85 ± 0.10	−0.59 ± 0.17
DHA-M	0.46	0.03	0.03	0.22	0.15	0.27	0.27	0.52	0.46	0.44	0.58	0.56	0.60	0.80	**0.17 ± 0.17**	−0.21 ± 0.15
n-6/n-3	0.03	−0.30	−0.04	−0.27	−0.03	−0.77	−0.48	−0.37	−0.09	0.05	0.01	0.07	−0.17	−0.21	−0.21	**0.28 ± 0.20***

M = absolute value (in % of wet muscle tissue), TF = relative value (in % of total fat), Fat = total fat content, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, n-6/n-3 = ratio between omega-6 and omega-3 polyunsaturated fatty acids

*heritability estimates significantly different from zero ($P < 0.05$)

Original Paper *Czech J. Anim. Sci., 63, 2018 (7): 280–291*

https://doi.org/10.17221/30/2018-CJAS

FAs are presented in Table 2. Medium negative phenotypic correlations were observed between Fat-M and all beneficial FA groups in relative values (PUFA-TF, n-6 PUFA-TF, n-3 PUFA-TF, EPA-TF, DHA-TF; r_p = –0.26 to –0.47). Similarly, absolute values of SFA-M, MUFA-M, and PUFA-M were negatively correlated to relative values of beneficial FA groups as described before (r_p = –0.17 to –0.49). Conversely, medium to close to one positive correlations were found between Fat-M and absolute values of FA groups (r_p = 0.46–1.00). Likewise, among other absolute values of FA groups a general positive trend of phenotypic correlations was observed (0.44–0.98). Moreover, relative values of SFA-TF and MUFA-TF were negatively related to each other and to relative values of PUFA-TF, n-6 PUFA-TF, and n-3 PUFA-TF (r_p = –0.16 to –0.63). The ratio of n-6/n-3 exhibited negative phenotypic associations with beneficial FA groups in relative values: PUFA-TF, n-3 PUFA-TF, EPA-TF, DHA-TF.

In most cases, genetic correlations had a pattern similar to phenotypic correlations. Fat-M was negatively genetically related to beneficial FA groups (–0.27 to –0.93). Oppositely, positive genetic correlations were observed between Fat-M and SFA-TF (0.59) and MUFA-TF (0.50). The absolute values of SFA-M, MUFA-M, and PUFA-M were negatively correlated to several favourable FA groups in relative values (PUFA-TF, n-6-TF, n-3 PUFA-TF, EPA-TF; r_g = –0.27 to –0.97). Strong positive genetic correlations were observed between Fat-M and other absolute values of FAs and among all FA groups in absolute values. The relative values of SFA-TF and MUFA-TF were negatively genetically correlated to each other and to relative values of PUFA-TF, n-6 PUFA-TF, and n-3 PUFA-TF. However, not all estimates were significantly different from zero. Regarding the n-6/n-3 ratio, medium to high negative genetic correlations were observed with SFA-TF, n-3 PUFA-TF, EPA-TF, DHA-TF, and EPA-M (r_g = –0.36 to –0.85. Oppositely, MUFA-TF was positively correlated to n-6/n-3.

Correlations between fatty acids and performance traits. Table 3 presents phenotypic correlations between FA groups and performance traits. Generally, phenotypic correlations were in most cases low or negligible. The highest negative and

Table 3. Phenotypic correlations between fatty acid composition and performance traits

Traits	Phenotypic					
	BW	FC	GSI	% hl-Carss	% Fill DS	% Fill
Fat-M	–0.02	0.21	–0.10	0.05	–0.10	–0.10
SFA-TF	0.03	–0.17	0.11	0.02	0.10	0.03
MUFA-TF	0.01	0.35	–0.13	0.04	–0.07	–0.01
PUFA-TF	–0.04	–0.25	0.05	–0.07	–0.02	–0.02
n-6 PUFA-TF	–0.01	–0.29	0.04	–0.06	0.02	0.00
n-3 PUFA-TF	–0.10	–0.11	0.02	0.01	–0.10	–0.06
EPA-TF	–0.14	–0.27	0.10	–0.01	–0.01	–0.10
DHA-TF	–0.03	–0.18	0.10	–0.07	–0.08	–0.10
SFA-M	–0.01	0.20	–0.08	0.08	–0.08	–0.10
MUFA-M	–0.01	0.24	–0.12	0.07	–0.10	–0.10
PUFA-M	–0.02	0.17	–0.08	0.04	–0.10	–0.11
n-6 PUFA-M	–0.04	0.16	–0.08	0.03	–0.10	–0.13
n-3 PUFA-M	–0.07	0.17	–0.10	0.04	–0.13	–0.13
EPA-M	–0.10	0.00	0.02	0.03	–0.11	–0.17
DHA-M	–0.08	–0.04	0.09	–0.03	–0.12	–0.19
n-6/n-3	0.15	–0.10	0.01	–0.03	0.10	0.03

M = absolute value (in % of wet muscle tissue), TF = relative value (in % of total fat), Fat = total fat content, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, n-6/n-3 = ratio between omega-6 and omega-3 polyunsaturated fatty acids, BW = body weight, FC = Fulton's condition factor, GSI = gonadosomatic index, % hl-Carss = headless carcass yield, % Fill DS = deskinned fillet yield, % Fill = fillet yield

Czech J. Anim. Sci., 63, 2018 (7): 280–291 *Original Paper*

https://doi.org/10.17221/30/2018-CJAS

Table 4. Genetic correlations (± standard error) between fatty acid composition and performance traits

Traits	Genetic					
	BW	FC	GSI	% hl-Carss	% Fill DS	% Fill
Fat-M	0.21 ± 0.23	0.28 ± 0.12	0.80 ± 0.14	–0.01 ± 0.15	0.16 ± 0.16	–0.22 ± 0.16
SFA-TF	0.26 ± 0.24	0.63 ± 0.15	0.21 ± 0.16	0.74 ± 0.18	0.46 ± 0.17	0.52 ± 0.17
MUFA-TF	0.22 ± 0.21	0.15 ± 0.12	0.43 ± 0.14	–0.05 ± 0.16	–0.15 ± 0.16	–0.38 ± 0.17
PUFA-TF	–0.41 ± 0.26	–0.65 ± 0.12	–0.46 ± 0.19	–0.58 ± 0.22	–0.53 ± 0.20	–0.18 ± 0.20
n-6 PUFA-TF	–0.15 ± 0.23	–0.45 ± 0.14	–0.79 ± 0.17	–0.36 ± 0.18	–0.34 ± 0.18	–0.10 ± 0.19
n-3 PUFA-TF	–0.59 ± 0.29	–0.76 ± 0.17	–0.58 ± 0.18	–0.44 ± 0.22	–0.77 ± 0.22	–0.38 ± 0.21
EPA-TF	–0.45 ± 0.28	–0.79 ± 0.17	–0.35 ± 0.17	–0.02 ± 0.20	–0.34 ± 0.20	–0.20 ± 0.20
DHA-TF	–0.85 ± 0.24	–0.65 ± 0.14	–0.55 ± 0.17	–0.55 ± 0.18	–0.75 ± 0.18	–0.40 ± 0.18
SFA-M	0.23 ± 0.23	0.46 ± 0.15	0.42 ± 0.17	0.34 ± 0.18	0.26 ± 0.17	–0.08 ± 0.16
MUFA-M	0.19 ± 0.22	0.41 ± 0.14	0.42 ± 0.16	0.27 ± 0.18	0.18 ± 0.17	–0.15 ± 0.17
PUFA-M	0.20 ± 0.22	0.32 ± 0.14	0.57 ± 0.15	–0.09 ± 0.17	–0.08 ± 0.16	–0.26 ± 0.16
n-6 PUFA-M	–0.08 ± 0.21	0.08 ± 0.14	0.68 ± 0.15	–0.05 ± 0.17	–0.04 ± 0.16	–0.39 ± 0.17
n-3 PUFA-M	–0.24 ± 0.21	–0.07 ± 0.14	0.64 ± 0.14	–0.15 ± 0.16	–0.24 ± 0.16	–0.45 ± 0.17
EPA-M	–0.04 ± 0.24	–0.30 ± 0.14	0.61 ± 0.17	–0.06 ± 0.17	–0.21 ± 0.16	–0.44 ± 0.16
DHA-M	–0.19 ± 0.23	–0.10 ± 0.14	0.48 ± 0.17	–0.4 ± 0.17	–0.34 ± 0.16	–0.48 ± 0.15
n-6/n-3	0.66 ± 0.24	0.73 ± 0.16	0.09 ± 0.16	0.34 ± 0.21	0.77 ± 0.16	0.44 ± 0.20

M = absolute value (in % of wet muscle tissue), TF = relative value (in % of total fat), Fat = total fat content, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, n-6/n-3 = ratio between omega-6 and omega-3 polyunsaturated fatty acids, BW = body weight, FC = Fulton's condition factor, GSI = gonadosomatic index, % hl-Carss = headless carcass yield, % Fill DS = deskinned fillet yield, % Fill = fillet yield

positive phenotypic correlations were observed especially between FC and most FA groups.

Genetic correlations between FA groups and performance traits are reported in Table 4. In contrast to phenotypic correlations, the effect of genetic correlations was more visible. Negative correlations were observed between BW and n-3 PUFA-TF and BW and DHA-TF (r_g = –0.59; –0.85, respectively). Furthermore, BW was positively correlated to n-6/n-3 ratio (0.66). An intermediately high positive genetic correlation was observed between FC and SFA-TF (r_g = 0.63 ± 0.15) and between FC and absolute values of main FA groups (SFA, MUFA, PUFA) including the n-6/n-3 ratio (r_g = 0.32–0.73). FC also exhibited medium to high negative genetic correlations to all beneficial FA groups in relative values (r_g = –0.45 to –0.79). Similarly, GSI was negatively associated with the

Table 5. Heritability estimates (bold, diagonal, ± standard error (SE)), genetic (above diagonal, ± SE) and phenotypic (below diagonal) correlations among performance traits of 3-year-old common carp

	BW	FC	GSI	% hl-Carss	% Fill DS	% Fill
BW	**0.62 ± 0.20***	0.50 ± 0.23	0.22 ± 0.22	0.13 ± 0.23	0.62 ± 0.22	0.50 ± 0.22
FC	0.04	**0.23 ± 0.15***	0.21 ± 0.15	0.68 ± 0.17	0.91 ± 0.17	0.82 ± 0.18
GSI	–0.02	–0.10	**0.27 ± 0.17**	0.003 ± 0.17	0.07 ± 0.17	–0.05 ± 0.17
% hl-Carss	0.26	0.09	–0.18	**0.41 ± 0.19***	0.78 ± 0.17	0.77 ± 0.16
% Fill DS	0.39	–0.09	–0.01	0.45	**0.33 ± 0.19***	0.79 ± 0.15
% Fill	0.31	–0.02	–0.11	0.50	0.84	**0.36 ± 0.20***

BW = body weight, FC = Fulton's condition factor, GSI = gonadosomatic index, % hl-Carss = headless carcass yield, % Fill DS = deskinned fillet yield, % Fill = fillet yield

*heritability estimates significantly different from zero ($P < 0.05$)

Original Paper *Czech J. Anim. Sci., 63, 2018 (7): 280–291*

https://doi.org/10.17221/30/2018-CJAS

same desirable FA groups as BW and FC (r_g = –0.35 to –0.79). Furthermore, GSI was positively correlated with all FAs in absolute values (r_g = 0.42–0.68) and as the only trait also with Fat-M (0.80). When looking at genetic correlations between FA groups and slaughtering yields, positive correlations with SFA-TF as well as the n-6/n-3 ratio (r_g = 0.34–0.77) were observed. Importantly, negative genetic associations were estimated with some beneficial FA groups in relative values. Oppositely, significant association with Fat-M was not observed.

Correlations among performance traits. Genetic and phenotypic correlations among performance traits are listed in Table 5. Low positive phenotypic correlations were observed between BW and slaughtering yields (0.26–0.39), while they were medium to high among slaughtering yields.

A medium positive genetic correlation was observed between BW and FC (0.50 ± 0.23), and FC had strongly positive genetic correlations with all slaughtering yields (0.68–0.91). Oppositely, BW was only related to fillet yields traits (0.50–0.62). Besides, the expected positive genetic correlations were found out among slaughtering yields (r_g = 0.45–0.84).

DISCUSSION

The present study is a first insight into the genetic variation of FA composition in flesh of market-size common carp. Furthermore, genetic and phenotypic correlations among the main FA groups and relationships between FA groups and performance traits were studied. It should be stressed that the present results were obtained on a relatively small sample size (158 offspring from 36 sire half-sib families), and thus they should be considered with caution. However, existing data on genetic variability of FA composition in fish generally rely on relatively small datasets, due to the high cost of FA analyses (220 fish from 44 families in Overturf et al. 2013, 514 fish from 154 families in Nguyen et al. 2010, and 416 fish from 48 families in Leaver et al. 2011). Still, we can observe that (1) several heritability estimates of FA composition significantly differ from zero ($P < 0.05$) and (2) our estimates of heritability for production traits are in the same range as previous results obtained on larger datasets of common carp (Vandeputte et al. 2004, 2008; Kocour et al. 2007; Prchal et al. 2018). Taking this into account, we may assume that our heritability estimates for some FA profile traits, even if done on a small sample size, are meaningful enough, especially when heritability and genetic correlations estimates are significantly different from zero.

Heritability of traits. In this study, we found significant genetic variation of several FA groups in common carp flesh. Studies on genetic variation of FA profile in fish are scarce, and so far limited to Nile tilapia (Nguyen et al. 2010), Atlantic salmon (Leaver et al. 2011), and rainbow trout (Overturf et al. 2013). Nguyen et al. (2010) did not observe any significant heritability in the main FA groups (SFA, MUFA, PUFA) presented as relative values. Significant heritabilities were observed only for 7 (out of 22) individual fatty acids (e.g. behenic acid: h^2 = 0.39, eicosenic acid: h^2 = 0.48). Conversely, in Atlantic salmon, Leaver et al. (2011) observed muscle n-3 PUFA (in percentage of total FAs) as a highly heritable trait (h^2 = 0.77). Likewise, heritability of total muscle fat was high (0.69). Similarly, Overturf et al. (2013) observed high heritability estimates of EPA and DHA in rainbow trout flesh. We observed significant heritability for n-3 PUFA-TF, EPA-TF, and Fat-M, but lower than those reported above in salmonids. Concerning heritability estimates of total muscle fat content, our observation was in accordance with results by Saillant et al. (2009), Garcia-Celdran et al. (2015), Kause et al. (2016) in other fish species, but lower compared to results by Kocour et al. (2007) and Prchal et al. (2018) in common carp.

Effect of selective breeding on FA profile under traditional rearing conditions. Selective breeding in common carp is at the beginning and plays only a minor role in common carp breeding (Janssen et al. 2017). However, the main focus of selective breeding is going to be devoted to faster growth, improved edible parts yield, and resistance to suboptimal conditions and diseases (Chavanne et al. 2016). The genetic correlations from the present study show that selection for faster growth could lead indirectly also to fillet yield improvement while keeping fat content in the muscle stable. However, the quality of fat would tend to get worse. The relative amount of n-3 PUFAs would decrease and the n-6/n-3 PUFA ratio would increase. In the case of an additional selection on improved edible parts yield, the change in fat flesh quality

Czech J. Anim. Sci., 63, 2018 (7): 280–291 *Original Paper*

https://doi.org/10.17221/30/2018-CJAS

would be even more evident. Similar unfavourable genetic correlations of performance traits (growth traits, fillet weight and yield) with EPA and n-3/n-6 ratio were observed in Nile tilapia (Nguyen et al. 2010). Likewise, Leaver et al. (2011) found a negative phenotypic correlation between relative n-3 PUFAs and final mass in 48 families of Atlantic salmon. Besides, we could also expect a decrease of PUFAs-TF. The quality of flesh regarding lipids would also worsen when selecting for increased muscle fat content or for higher FC. FC seems to be a simple and suitable selection criterion for increased BW as well as edible parts yields, despite the expected side effect of slightly increased muscle fat. Similarly, Saillant et al. (2009) observed in European seabass (*Dicentrarchus labrax*) strong positive genetic correlations of FC with BW and fillet yields. However, in this latter species, FC was only slightly heritable.

An important question is why selection for faster growth, higher edible parts yield or better condition (expressed as FC) may lead to worse flesh quality. There might be one logical explanation – fish with better performance tend to feed more on supplemental feed, probably due to higher appetite. As the supplemental feed in pond culture is based mainly on grain (wheat, barley, triticale) rich in carbohydrates, the unutilized energy is stored in fish in the form of MUFAs and this makes the FA profile in carp flesh less favourable (Mraz et al. 2012b). Also PUFA n-6/n-3 ratio would get higher than required due to FA composition of the grain (Mraz and Pickova 2011; Markovic et al. 2016). It is well known that the fatty acids profile in the feed significantly affects the composition of fish flesh lipids (Mraz and Pickova 2011; Markovic et al. 2016; Trbovic et al. 2017). Thus, the n-6/n-3 PUFA ratio in flesh being good in this study for human health as lower than 4 (3.29) (Rodriguez et al. 2017) could increase over 4 and this would make the carp flesh of lower quality.

In the traditional rearing conditions of Central Europe, the grain contributes about 50% to the total weight gain of common carp (Horvath et al. 1992; Kocour et al. 2007). The second half of the weight gain comes from natural food (zooplankton and zoobenthos) (Horvath et al. 1992). Ponds under typical Central European management must be looked at as complex ecosystem units in which produced carps compete among each other and with the other animals about the natural food (Anton-Pardo and Adamek 2015). The question is whether the carp stocks improved by selective breeding would utilize the natural food more effectively than the common stocks in order to keep the components contributing to their weight gain in the ratio 1 : 1 (natural food : supplemental food). Otherwise the fish would require more supplemental feeding and that would lead to above described effects. In the case that the consumers have required the same flesh quality, the present pond management would have had to be modified.

Possible effect of selective breeding on flesh FA profile under modified rearing conditions. Natural food developing in ponds has a better (more n-3 PUFAs) FA profile compared to grain (Markovic et al. 2016; Trbovic et al. 2017). The problem is that the ponds have limited natural food production capacity for stocking densities that are used in common carp pond management, even after fertilizing the ponds with organic material. More intensive fertilization is not practically feasible due to regulations on surface water quality and its protection (Hlavac et al. 2014). So, if we want to keep at least the present flesh meat quality of common carp selected for faster growth and higher fillet yield, produced under semi-intensive pond management conditions, we would have to either decrease the stocking densities (Anton-Pardo and Adamek 2015) or change the strategy of supplemental feeding (Mraz et al. 2012a, b; Markovic et al. 2016; Trbovic et al. 2017). Decreasing the stocking densities, however, would not bring the producers the expected economic benefit from selective breeding. Thus, the only way is to alter the technology of supplemental feeding and to look for alternative plant components with better FA profiles that would keep the flesh quality without significant increasing production costs.

Recently, several studies confirmed that carp FA profile can be improved by special diets based on precursors of EPA and DHA (Mraz and Pickova 2011; Mraz et al. 2012a, b; Steffens 2016). It was found that freshwater fish species, including common carp, and contrary to marine fish, have the ability to synthesise EPA and DHA from its precursor alpha-linolenic acid (ALA) (Zheng et al. 2004; Tocher 2010). Therefore, we also studied a hypothetic genetic relationships of ALA with EPA and DHA which could support biosynthesis of EPA and DHA in carp flesh. We observed that the relative value of ALA is a heritable trait (h^2 = 0.43

Original Paper *Czech J. Anim. Sci., 63, 2018 (7): 280–291*

https://doi.org/10.17221/30/2018-CJAS

± 0.22). However, no significant genetic associations were found (in the relative values) between ALA and EPA (r_g = 0.36 ± 0.21) or ALA and DHA (r_g = 0.13 ± 0.18). Still, supplemental feeding rich in ALA, e.g. rapeseed, linseed or hempseed, leads to an improved FA profile in carp flesh (Mraz et al. 2012a, b). Such modified diet (or so called finishing feeding) can be used just during the last (or only part of the last) growing season before reaching the market size to keep the FA profile in a favourable range (Mraz et al. 2012a, b). Thus, the above mentioned feeding strategy should not dramatically change the FA profile in the flesh of common carp even when the contribution of supplemental food on weight gain goes beyond 50%. Still, as the contribution of supplemental food will increase and the supplemental food does not contain all required nutrients, it seems that for sustainable selective breeding program the feeding strategy in pond culture may have to change from supplemental food to a complete compound food (Markovic et al. 2016). Then, the carp stocks could positively respond to selection for faster growth while maintaining an appropriate flesh quality.

CONCLUSION

Results in this study point to the fact that selective breeding for faster growth and/or higher edible part yields under the current Central European carp pond management would very likely negatively affect carp flesh quality with respect to FA composition. Therefore, together with the selective breeding programme, the feeding strategy should be modified in order to enable a positive response to selection while keeping the carp meat as a valuable healthy diet for humans.

REFERENCES

Anton-Pardo M., Adamek Z. (2015): The role of zooplankton as food in carp pond farming: a review. Journal of Applied Ichthyology, 31, 7–14.

Chavanne H., Janssen K., Hofherr J., Contini F., Haffray P., Komen H., Nielsen E.E., Bargelloni L. (2016): A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry. Aquaculture International, 24, 1287–1307.

Dupont-Nivet M., Vandeputte M., Chevassus B. (2002): Optimization of factorial mating designs for inference on heritability in fish species. Aquaculture, 204, 361–370.

Folch J., Lees M., Stanley G.H.S. (1957): A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226, 497–509.

Fontagne-Dicharry S., Medale F. (2010): Lipids of aquaculture fish species and their variation factors. OCL – Oilseeds and fats, Crops and Lipids, 17, 209–213. (in French)

FOSS Analytical AB (2003): Application note 390/revision 2.8/2007: Total Fat Determination Using SoxCap™ 2047 in Combination with Soxtec Extraction Systems. FOSS Analytical AB, Höganäs, Sweden.

Garcia-Celdran M., Ramis G., Manchado M., Estevez A., Afonso J.M., Armero E. (2015): Estimates of heritabilities and genetic correlations of carcass quality traits in a reared gilthead sea bream (Sparus aurata L.) population sourced from three broodstocks along the Spanish coasts. Aquaculture, 446, 175–180.

Groeneveld E., Kovac M., Mielenz N. (2008): VCE – User's Guide and Reference Manual – Version 6.0. Institute of Farm Animal Genetics, Neustadt, Germany. Available at ftp://ftp.tzv.fal.de/pub/vce6/doc/vce6-manual-3.1-A4.pdf (accessed Aug 1, 2010).

Hlavac D., Adamek Z., Hartman P., Masilko J. (2014): Effects of supplementary feeding in carp ponds on discharge water quality: a review. Aquaculture International, 22, 299–320.

Horvath L., Tamas G., Seagrave C. (1992): Carp and Pond Fish Culture including Chinese Herbivorous Species, Pike, Tench, Zander, Wels Catfish and Goldfish. Fishing News Books Ltd., Oxford, UK.

Janssen K., Chavanne H., Berentsen P., Komen H. (2017): Impact of selective breeding on European aquaculture. Aquaculture, 472, 8–16.

Kause A., Kiessling A., Martin S.A.M., Houlihan D., Ruohonen K. (2016): Genetic improvement of feed conversion ratio via indirect selection against lipid deposition in farmed rainbow trout (Oncorhynchus mykiss Walbaum). British Journal of Nutrition, 116, 1656–1665.

Kocour M., Mauger S., Rodina M., Gela D., Linhart O., Vandeputte M. (2007): Heritability estimates for processing and quality traits in common carp (Cyprinus carpio L.) using a molecular pedigree. Aquaculture, 270, 43–50.

Leaver M.J., Taggart J.B., Villeneuve L., Bron J.E., Guy D.R., Bishop S.C., Houston R.D., Matika O., Tocher D.R. (2011): Heritability and mechanisms of n-3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 6, 62–69.

Czech J. Anim. Sci., 63, 2018 (7): 280–291 *Original Paper*

https://doi.org/10.17221/30/2018-CJAS

Madsen P., Jensen J. (2013): DMU Version 6. Available at http://dmu.agrsci.dk/DMU/Doc/Current/dmuv6_guide.5.2.pdf (accessed Dec 1, 2013).

Markovic Z., Stankovic M., Raskovic B., Dulic Z., Zivic I., Poleksic V. (2016): Comparative analysis of using cereal grains and compound feed in semi-intensive common carp pond production. Aquaculture International, 24, 1699–1723.

Mraz J., Pickova J. (2009): Differences between lipid content and composition of different parts of fillets from cross-bred farmed carp (Cyprinus carpio). Fish Physiology and Biochemistry, 35, 615–623.

Mraz J., Pickova J. (2011): Factors influencing fatty acid composition of common carp (Cyprinus carpio) muscle. Neuroendocrinology Letters, 32, 3–8.

Mraz J., Zajic T., Pickova J. (2012a): Culture of common carp (Cyprinus carpio) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy. Neuroendocrinology Letters, 33, 60–67.

Mraz J., Machova J., Kozak P., Pickova J. (2012b): Lipid content and composition in common carp – optimization of n-3 fatty acids in different pond production systems. Journal of Applied Ichthyology, 28, 238–244.

Nguyen N.H., Ponzoni R.W., Yee H.Y., Abu-Bakar K.R., Hamzah A., Khaw H.L. (2010): Quantitative genetic basis of fatty acid composition in the GIFT strain of Nile tilapia (Oreochromis niloticus) selected for high growth. Aquaculture, 309, 66–74.

Overturf K., Welker T., Barrows F., Towner R., Schneider R., LaPatra S. (2013): Variation in rainbow trout, Oncorhynchus mykiss, to biosynthesize eicosapentaenoic acid and docosahexaenoic acid when reared on plant oil replacement feeds. Journal of the World Aquaculture Society, 44, 326–337.

Pinheiro J.C., Bates D.M. (2000): Mixed-Effects Models in S and S-PLUS. Springer-Verlag, New York, USA.

Prchal M., Kause A., Vandeputte M., Gela D., Allamelou J.M., Girish K., Bestin A., Bugeon J., Zhao J., Kocour M. (2018): The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. PLoS ONE, 13**,** e0191624.

Rodrigues B.L., da Cruz Silva A.C.V., da Costa M.P., da Silva F.A., Marsico E.T., Conte-Junior C.A. (2017): Fatty acid profiles of five farmed Brazilian freshwater fish species from different families. PLoS ONE, 12, e0178898.

Saillant E., Dupont-Nivet M., Sabourault M., Ha P., Laureau S., Vidal M.-O., Chatain B. (2009): Genetic variation for carcass quality traits in cultured sea bass (Dicentrarchus labrax). Aquatic Living Resources, 22, 105–112.

Steffens W. (2016): Aquaculture produces wholesome food: cultured fish as a valuable source of n-3 fatty acids. Aquaculture International, 24, 787–802.

Tocher D.R. (2010): Fatty acid requirements in ontogeny of marine and freshwater fish. Aquaculture Research, 41, 717–732.

Trbovic D., Zivic I., Stankovic M., Zivic M., Dulic Z., Petronijevic R., Markovic Z. (2017): Dependence of the common carp (Cyprinus carpio L.) fatty acid profile on diet composition in a semi-intensive farming system: tissue and time variability. Aquaculture Research, 48, 3121–3133.

Vandeputte M., Kocour M., Mauger S., Dupont-Nivet M., De Guerry D., Rodina M., Gela D., Vallod D., Chevassus B., Linhart O. (2004): Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (Cyprinus carpio L.). Aquaculture, 235, 223–236.

Vandeputte M., Mauger S., Dupont-Nivet M. (2006): An evaluation of allowing for mismatches as a way to manage genotyping errors in parentage assignment by exclusion. Molecular Ecology Resources, 6, 265–267.

Vandeputte M., Kocour M., Mauger S., Rodina M., Launay A., Gela D., Dupont-Nivet M., Hulak M., Linhart O. (2008): Genetic variation for growth at one and two summers of age in the common carp (Cyprinus carpio L.): heritability estimates and response to selection. Aquaculture, 277, 7–13.

Vandeputte M., Garouste R., Dupont-Nivet M., Haffray P., Vergnet A., Chavanne H., Laureau S., Ron T.B., Pagelson G., Mazorra C. (2014): Multi-site evaluation of the rearing performances of 5 wild populations of European sea bass (Dicentrarchus labrax). Aquaculture, 424–425, 239–248.

Zajic T., Mraz J., Sampels S., Pickova J. (2013): Fillet quality changes as a result of purging of common carp (Cyprinus carpio L.) with special regard to weight loss and lipid profile. Aquaculture, 400–401, 111–119.

Zheng X., Seiliez I., Hastings N., Tocher D.R., Panserat S., Dickson C., Bergot P., Teale A. (2004): Characterization and comparison of fatty acyl Δ6 desaturase cDNAs from freshwater and marine teleost fish species. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 139, 269–279.

Received: 2018–03–09
Accepted after corrections: 2018–05–16

CHAPTER 5

ACCURACY OF GENOMIC EVALUATIONS OF JUVENILE GROWTH RATE IN COMMON CARP (*CYPRINUS CARPIO*) USING GENOTYPING BY SEQUENCING

Palaiokostas, Ch., Kocour, M., Prchal, M., Houston, R. D., 2018. Accuracy of genomic evaluations of juvenile growth rate in common carp (*Cyprinus carpio*) using genotyping by sequencing. Front. Genet. 9, 82.

My share on this work was about 10%.

frontiers in Genetics

ORIGINAL RESEARCH
published: 13 March 2018
doi: 10.3389/fgene.2018.00082

Accuracy of Genomic Evaluations of Juvenile Growth Rate in Common Carp (*Cyprinus carpio*) Using Genotyping by Sequencing

Christos Palaiokostas[1], Martin Kocour[2], Martin Prchal[2] and Ross D. Houston[1]*

[1] The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom, [2] Faculty of Fisheries and Protection of Waters, South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, Vodňany, Czechia

Cyprinids are the most important group of farmed fish globally in terms of production volume, with common carp (*Cyprinus carpio*) being one of the most valuable species of the group. The use of modern selective breeding methods in carp is at a formative stage, implying a large scope for genetic improvement of key production traits. In the current study, a population of 1,425 carp juveniles, originating from a partial factorial cross between 40 sires and 20 dams, was used for investigating the potential of genomic selection (GS) for juvenile growth, an exemplar polygenic production trait. RAD sequencing was used to identify and genotype SNP markers for subsequent parentage assignment, construction of a medium density genetic map (12,311 SNPs), genome-wide association study (GWAS), and testing of GS. A moderate heritability was estimated for body length of carp at 120 days (as a proxy of juvenile growth) of 0.33 (s.e. 0.05). No genome-wide significant QTL was identified using a single marker GWAS approach. Genomic prediction of breeding values outperformed pedigree-based prediction, resulting in 18% improvement in prediction accuracy. The impact of reduced SNP densities on prediction accuracy was tested by varying minor allele frequency (MAF) thresholds, with no drop in prediction accuracy until the MAF threshold is set <0.3 (2,744 SNPs). These results point to the potential for GS to improve economically important traits in common carp breeding programs.

Keywords: aquaculture breeding, carps, high-throughput sequencing, RAD-seq, genomic prediction

OPEN ACCESS

Edited by:
Riccardo Negrini, Università Cattolica del Sacro Cuore, Italy

Reviewed by:
Nicholas Andrew Robinson, Nofima, Norway
Mario Calus, Wageningen University & Research, Netherlands

***Correspondence:**
Ross D. Houston
ross.houston@roslin.ed.ac.uk

Specialty section:
This article was submitted to Livestock Genomics, a section of the journal Frontiers in Genetics

Received: *13 January 2018*
Accepted: *26 February 2018*
Published: *13 March 2018*

Citation:
Palaiokostas C, Kocour M, Prchal M and Houston RD (2018) Accuracy of Genomic Evaluations of Juvenile Growth Rate in Common Carp (Cyprinus carpio) Using Genotyping by Sequencing. Front. Genet. 9:82. doi: 10.3389/fgene.2018.00082

INTRODUCTION

Carps have the highest global production volume of all aquaculture fish (FAO, 2015) and are farmed in a wide variety of environments and production systems (Balon, 1995). In common with most aquaculture species, only a minority of farmed common carp are derived from family-based selective breeding programs, and crossbreeding of partially inbred strains is commonly applied to benefit from heterosis (Hulata, 1995; Vandeputte, 2003; Janssen et al., 2017). Family-based programs have the advantage of enabling cumulative increases in economic traits, and maintaining a high degree of control of the level of inbreeding of stocks. Empirical data relating to selective breeding of family-based programs in several fish species show an increase in genetic gain of up to 15% per generation (Gjedrem, 2000). While initial studies suggested that within breed selection

is ineffective in common carp (Moav and Wohlfarth, 1976), recent studies focusing on growth and survival traits highlighted the potential of applying selective breeding to enhance production (Kocour et al., 2007; Vandeputte et al., 2008; Nielsen et al., 2010; Ninh et al., 2013; Dong et al., 2015).

Traditional pedigree-based selective breeding incorporating best linear unbiased predictor (BLUP) methodology (Henderson, 1975) has greatly benefited both animal and plant agriculture. Nevertheless, the utilization of just the between-family component of genetic variation imposes limitations to selection accuracy and therefore genetic gain (Meuwissen et al., 2013). Selective breeding can be significantly enhanced by the application of genomic tools, via improvement of selection accuracy and potentially also by identification of causative factors impacting on key production traits (Meuwissen et al., 2001, 2013; Hickey et al., 2017). Genomic selection (GS) involves the simultaneous use of genome-wide genetic markers to estimate breeding values for selection candidates utilizing both within and across family variation (Meuwissen et al., 2001). By using all markers in the calculation of breeding values, GS overcomes the limitations of marker-assisted selection for such traits, where typically only a small percentage of genetic variation can be utilized for polygenic traits (Daetwyler et al., 2013). In aquaculture species, GS has been enabled by the increased technical feasibility and reduced cost of generation of genome-wide marker data in non-model organisms via SNP arrays or genotyping by sequencing (Davey et al., 2011; Robledo et al., 2017).

The effectiveness of GS at deriving more accurate breeding values than traditional pedigree-based selection has been demonstrated using simulated and empirical data in both livestock and aquaculture (Goddard and Hayes, 2009; Sonesson and Meuwissen, 2009). Empirical data collected to date suggest that the majority of traits of interest for animal production (e.g., growth and disease resistance) are underpinned by a polygenic genetic architecture (de los Campos et al., 2013). In aquaculture species, where large full-sibling family sizes are typically available, the advantages of genomic prediction of breeding values in aquaculture species are clear from several studies of such polygenic traits (e.g., Odegård et al., 2014; Tsai et al., 2015, 2016; Vallejo et al., 2017), albeit genomic prediction is less effective when only distant relatives are used in deriving the prediction equation (Tsai et al., 2016).

Restriction-site-associated DNA sequencing (RAD-seq) is a reduced representation of high-throughput sequencing technique for the concurrent detection and genotyping of SNP markers (Baird et al., 2008). RAD-seq and similar genotyping by sequencing techniques rely on digestion of the genomic DNA with a restriction enzyme, and subsequent high-depth sequencing of the flanking regions. These techniques have been widely applied due to their cost-efficiency in a wide range of aquaculture species (Robledo et al., 2017), both in genome-wide association studies (GWAS) (e.g., Campbell et al., 2014; Palti et al., 2015; Barria et al., 2017) and GS studies (e.g., Palaiokostas et al., 2016; Vallejo et al., 2016). The main aim of this study was to investigate the potential of genomic prediction of an exemplar polygenic trait in common carp (juvenile growth) using genome-wide SNP markers generated by RAD-seq. To achieve this, samples of 1,425 carp measured for body weight and length at approximately 4 months of age were used. RAD-seq was used to genotype genome-wide SNP markers, parentage assignment was performed, and heritability (of body weight and length) was estimated. The obtained SNPs were utilized for construction of a medium density linkage map, followed by a GWAS to test the association between individual loci and growth. Finally, GS was tested to evaluate the potential of incorporating genomic data for selective breeding compared to pedigree-based selection using this exemplar polygenic trait.

MATERIALS AND METHODS

Sample Collection

A population of Amur Mirror Carp was created at the University of South Bohemia, Czech Republic in May 2014 using artificial insemination (Vandeputte et al., 2004) involving four factorial crosses each comprising 5 dams × 10 sires (20 dams and 40 sires in total). Incubation of eggs was performed in 9 lt Zugar jars at 20°C. At the swimming stage, randomly sampled progeny of the same total volume from each mating was pooled and reared under semi-intensive pond conditions throughout the growing season (from May to September). In September, a sample of 1,425 fish was fin-clipped, passive integrated transponder (PIT)-tagged, weighed to the nearest 0.01 g, and measured for standard length (SL) (from the tip of the nose to the end of the caudal peduncle) to the nearest millimeter. All working procedures complied with the European Union Directive 2010/63/EU for the protection and welfare of animals used for scientific purposes.

RAD Library Preparation and Sequencing

Genomic DNA was extracted from fin samples using the REALPure genomic DNA extraction kit (Durviz S.L.) and treated with RNase. Each sample was quantified by spectrophotometry (Nanodrop), and its quality was assessed by agarose gel electrophoresis, before being diluted to a concentration of 20 ng/μL [measured by Qubit Fluorometer (Invitrogen)] in 5 mmol/L Tris, pH 8.5.

The RAD-specific P1 and P2 paired-end adapters and library amplification PCR primer sequences used in this study are detailed in Baxter et al. (2011). Briefly, each sample (0.72 μg parental DNA/0.24 μg offspring DNA) was digested at 37°C for 60 min with *Sbf*I (recognizing the CCTGCA| GG motif) high fidelity restriction enzyme (New England Biolabs, NEB). The reactions (12 μL final volumes) were then heat inactivated at 65°C for 20 min. Individual-specific P1 adapters, each with a unique 5 bp barcode, were ligated to the *Sbf*I-digested DNA at 20°C for 60 min by adding 1.8/0.6 μL 100 nmol/L P1 adapter, 0.45/0.15 μL 100 mmol/L rATP (Promega), 0.75/0.25 μL 10× Reaction Buffer 2 (NEB), 0.36/0.12 μL T4 ligase (NEB, 2 M U/mL), and reaction volumes made up to 45/15 μL

with nuclease-free water for each parental/offspring sample. Following heat inactivation at 65°C for 20 min, the ligation reactions were slowly cooled to room temperature (over 1 h) then combined in appropriate multiplex pools. Shearing and initial size selection (300–600 bp) by agarose gel separation were followed by gel purification, end repair, dA overhang addition, P2 (individual-specific adapters) paired-end adapter ligation, library amplification, as described in the original RAD protocol (Baird et al., 2008; Etter et al., 2011). A total of 150 μL of each amplified library (14–17 PCR cycles, library dependent) was size selected (ca. 400–700 bp) by gel electrophoresis as described in Houston et al. (2012). Following a final gel elution step into 20 μL EB buffer (MinElute Gel Purification Kit, Qiagen), 66 libraries (24 animals each) were sent to BMR Genomics (Italy), for quality control and high-throughput sequencing. Libraries were run in 14 lanes of an Illumina NextSeq 500, using 75 base paired-end reads (v2 chemistry). The sequence reads were deposited at the NCBI Sequence Read Archive (SRA) under the accession PRJNA414021.

Genotyping RAD Alleles – SNP Identification

Reads missing the restriction site, with ambiguous barcodes and PCR duplicates, were identified and discarded using the Stacks software 1.4 (Catchen et al., 2011). The remaining reads were aligned to the common carp reference genome assembly version *GCA_000951615.2* (Xu et al., 2014) using bowtie2 (Langmead and Salzberg, 2012). The aligned reads were sorted into loci and genotypes using the Stacks software 1.4 (Catchen et al., 2011). A minimum stack depth of at least 10 or 5 was required for the parental and offspring samples, respectively. Only loci containing one or two SNPs were considered for downstream analysis. SNPs with minor allele frequency (MAF) <0.01, >25% missing data, and deviating from expected Hardy–Weinberg equilibrium in the parental samples ($P < 1e{-}06$) were discarded.

Parentage Assignment

Due to the partial factorial crosses performed in this experiment, the family structure of the offspring was unknown at the time of sampling. Parentage assignment was performed using the RAD-seq-derived SNP data and the R/hsphase (Ferdosi et al., 2014) software allowing for a maximum overall genotyping error of 4%. The pedigree obtained was further validated for possible erroneous assignments using FImpute (Sargolzaei et al., 2014).

Linkage Map Construction

Linkage map construction was performed using Lep-Map v2 (Rastas et al., 2013). SNPs with MAF <0.05 in individual families and those deviating from expected Mendelian segregation ($P < 0.001$) were excluded. Linkage groups were formed using a minimum LOD threshold value of 18 in the "*SeparateChromosomes*" module, allowing a maximum distance between consecutive SNPs of 50 cM. Marker order within each linkage group was performed using the "*OrderMarkers*" module using the *SexAverage* option. Map distances were calculated in centiMorgans (cM) using the Kosambi mapping function.

Heritability Estimation

Heritability estimates of weight and length were performed using both the pedigree-based relationship matrix and the genomic relationship matrix. Variance components was estimated using AIREMLF90 (Misztal et al., 2002) with the following animal model:

$$y = Xb + Zu + e, \quad (1)$$

where y is the vector of recorded phenotypes, b vector of the fixed effects (the four-level factorial cross), X the incidence matrix relating phenotypes with the fixed effects, Z the incidence matrix relating phenotypes with the random animal effects, u the vector of random animal effects $\sim N(0, A\sigma_g^2)$ with A corresponding to the pedigree-based relationship matrix or the genomic relationship matrix G (VanRaden, 2008), σ_g^2 the additive genetic variance, e the vector of residuals $\sim N(0, I\sigma_e^2)$ and σ_e^2 the residual variance.

Heritability was estimated using the following formula:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}.$$

Bivariate models with the same fixed and random effects as in Equation (1) were used in order to estimate genetic correlations between the phenotypes of weight and length.

Genome-Wide Association Analysis

To test the association between individual SNPs and growth (only length records were utilized), GWAS was performed using R/gaston (Hervé and Dandine-Roulland, 2018). The mixed model applied had the same format as in Equation (1) with the addition of each tested SNP as a fixed effect. The genome-wide significance threshold was calculated using a Bonferroni correction ($0.05/N$), where N represents the number of QC-filtered SNPs.

Genomic Selection

The accuracy of prediction of genomic breeding values (GEBVs) was calculated and benchmarked against the accuracy of prediction for EBVs using traditional pedigree-based best linear unbiased prediction (BLUP) (Henderson, 1975). GEBVs were estimated with GBLUP (Meuwissen et al., 2001) using the BLUPF90 suite (Misztal et al., 2002) updated for genomic analyses (Aguilar et al., 2011). Pedigree-based BLUP was applied to calculate breeding values using the same software. The general form of the fitted models was as in Equation (1).

A fivefold cross-validation was performed in order to test prediction accuracy. The data set was randomly split into sequential non-overlapping training (n = 972 individuals) and

validation sets (n = 242). The fivefold cross-validation procedure was repeated 10 times in order to reduce random sampling effects. The accuracy of the estimated breeding values was approximated as:

$$r = (EBV, y)/h,$$

where y is the vector of recorded phenotypes and h the square root of the heritability. GEBVs were used to approximate accuracy in the case of GBLUP. Additionally, five different scenarios were used in order to test the effect of reduced genotyping densities on the obtained prediction accuracies. In these scenarios, GBLUP was performed as above using subsets of SNPs selected by progressive increase of a minimum threshold for MAF. The tested scenarios involved MAF thresholds of 0.1 (8,237 SNPs), 0.2 (4,950 SNPs), 0.3 (2,744 SNPs), 0.4 (1,182 SNPs), and 0.45 (530 SNPs). Bias in the form of the regression coefficient of the phenotypic trait on (G)EBV was estimated for both PBLUP and all tested scenarios of GBLUP.

RESULTS

Descriptive Statistics

The mean weight of the genotyped carp juveniles after approximately 4 months of growth (**Supplementary Table S1**) was 16.3 g (SD 4.6) and the mean SL was 77 mm (SD 7.1). The Pearson correlation coefficient between length and weight was r = 0.93 (**Figure 1**).

SNP Identification and Genotyping

Animals with fewer than 25% missing SNP genotypes were retained for downstream analysis (corresponding to 60 parental and 1,400 offspring samples). The total number of raw reads passing the QC filters was 6.89 (SD 1.33) M for the parental samples and 3.68 (SD 1.28) M for the offspring. The mean number of RAD loci identified was 57,983 (SD 1,573) and 57,235 (SD 5,224) for parents and offspring, respectively, with mean coverage of 60 (SD 10) X and 29 (SD 8) X, respectively. A total of 22,756 putative SNPs

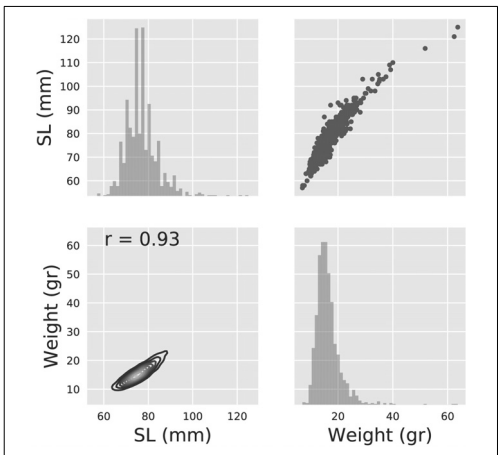

FIGURE 1 | Descriptive statistics of the phenotypic data (weight, length).

TABLE 1 | Number of QC-filtered SNPs per linkage group.

Linkage group	Size (cM)	Number of markers
1	102	350
2	91	350
3	76	325
4	77	322
5	90	319
6	77	315
7	77	311
8	77	292
9	75	290
10	84	287
11	83	283
12	71	277
13	81	272
14	70	263
15	69	260
16	78	259
17	105	257
18	78	256
19	92	249
20	75	249
21	106	246
22	75	245
23	84	241
24	70	241
25	76	240
26	77	239
27	71	238
28	84	237
29	93	237
30	81	236
31	71	229
32	77	229
33	78	227
34	70	227
35	87	226
36	77	225
37	88	222
38	77	221
39	73	215
40	71	213
41	74	212
42	72	212
43	72	208
44	79	203
45	80	199
46	67	193
47	73	175
48	71	169
49	75	163
50	69	157
Total	3,944	12,311

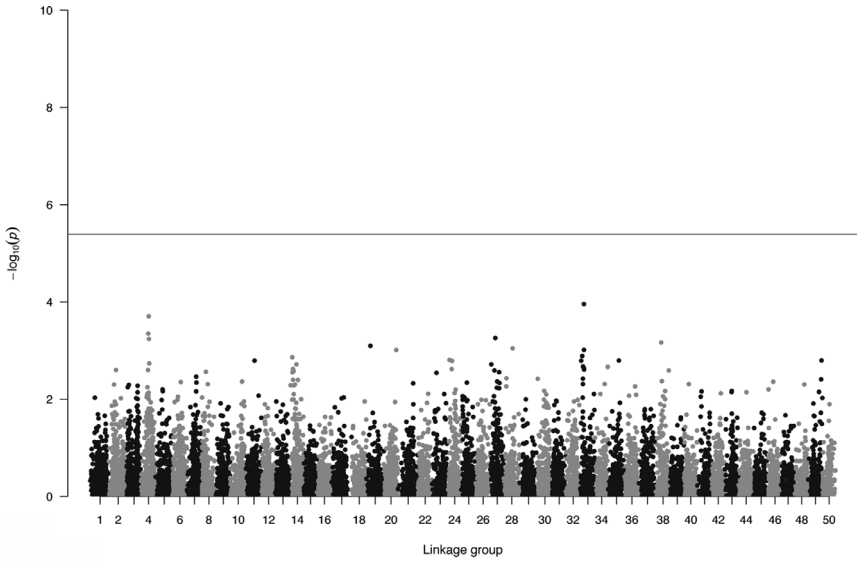

FIGURE 2 | Genome-wide association plot for body length.

were identified, of which 20,039 SNPs passed QC filters and were retained for downstream analysis (**Supplementary Table S2**).

Parentage Assignment

The carp progeny was assigned to unique parental pairs allowing for a maximum genotypic error rate of 4%. In total 1,214 offspring were uniquely assigned, forming 195 full-sib families (40 sires, 20 dams) ranging from 1 to 21 animals per family with a mean size of 6 (SD 4). The individual dam contribution to the population ranged from 9 to 99 animals with a mean of 61 (SD 23), while the sire contribution ranged from 7 to 53 animals with a mean of 30 (SD 12).

Linkage Map Construction

The linkage map constructed using the aforementioned families consisted of 12,311 SNPs (**Table 1**) that were grouped into 50 linkage groups (in accordance with the expected karyotype). The length of the consensus linkage map was 3,944 cM (**Supplementary Table S3**). The number of SNPs per chromosome ranged from 157 to 350 (mean = 246; SD = 45), while linkage group length ranged between 67 and 106 cM (mean = 79; SD = 9).

Heritability Estimation

The estimated heritabilities for weight and length were 0.26 (SE 0.05) and 0.33 (SE 0.05), respectively, and were consistent between the pedigree and genomic models. Genetic correlation between body weight and SL was 0.94 (SE 0.02), and as such only length data were used for downstream analyses.

Genome-Wide Association Study (GWAS) – Genomic Selection (GS)

Genome-wide association study and GS were performed using only the SL data. GWAS did not identify SNPs surpassing the genome-wide significant threshold (**Figure 2**), indicating that juvenile growth is likely to be a polygenic trait. Using the cross-validation approach, breeding value prediction accuracy was estimated to be 0.60 (SE 0.03) for PBLUP, as opposed to 0.71 (SE 0.03) with GBLUP. Accuracies obtained through GBLUP using the various reduced SNP densities ranged from 0.66 to 0.71 (**Figure 3**). GBLUP using only SNPs with a minimum MAF of 0.45 (530 SNPs) had approximately a 10% accuracy improvement compared to PBLUP. SNP densities of a minimum MAF of 0.3 had practically the same prediction accuracies as the full data set (**Figure 3**). Estimated bias of (G)EBVs ranged between 0.78 and 1.02. The scenario using SNPs with minimum MAF of 0.45 was found to produce the most biased GEBVs (**Table 2**).

DISCUSSION

Traditional selective breeding relies on well-documented pedigree, which is relatively straightforward in livestock, but

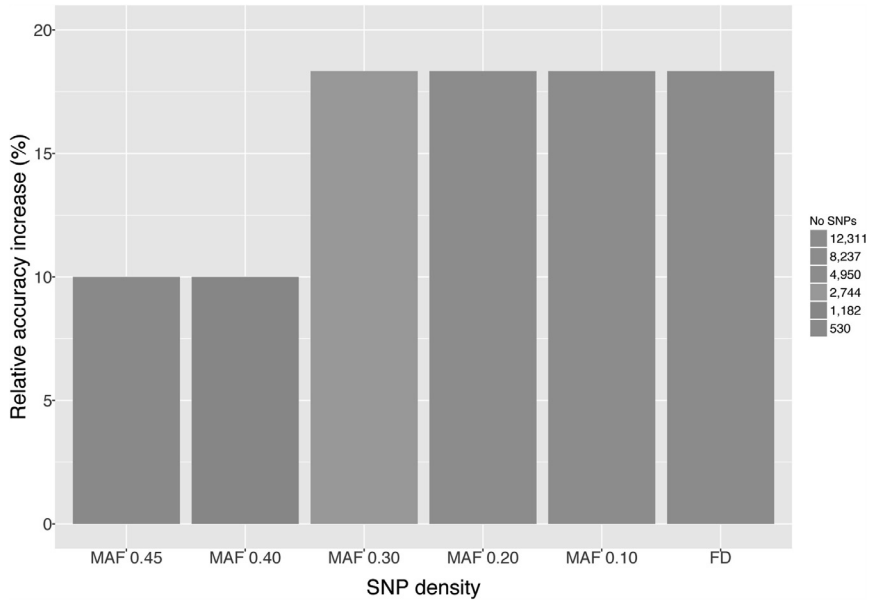

FIGURE 3 | Mean accuracies of GBLUP and PBLUP. GBLUP accuracies were obtained using either all available SNPs (FD; 12,311), SNPs with MAF >0.10 (8,237), SNPs with MAF >0.20 (4,950), SNPs with MAF >0.30 (2,744), SNPs with MAF >0.40 (1,182), and SNPs with MAF >0.45 (530).

TABLE 2 | Mean accuracy of pedigree BLUP (PBLUP) and GBLUP using SNP of varying MAF obtained from 10 repeats of fivefold cross-validation.

Reps	FD[1] (bias)	MAF[2] > 0.1 (bias)	MAF[3] > 0.2 (bias)	MAF[4] > 0.3 (bias)	MAF[5] > 0.4 (bias)	MAF[6] > 0.45 (bias)	PBLUP (bias)
1st	0.71 (1.05)	0.71 (1.04)	0.71 (1.05)	0.71 (1.06)	0.68 (1.03)	0.66 (0.93)	0.59 (1.02)
2nd	0.70 (1.03)	0.71 (1.02)	0.70 (1.02)	0.70 (1.03)	0.66 (0.99)	0.65 (0.90)	0.59 (1.01)
3rd	0.73 (1.05)	0.73 (1.04)	0.73 (1.05)	0.73 (1.05)	0.67 (0.99)	0.68 (0.92)	0.61 (1.03)
4th	0.69 (0.98)	0.69 (0.97)	0.69 (0.99)	0.69 (0.99)	0.64 (0.95)	0.63 (0.84)	0.60 (1.02)
5th	0.69 (1.04)	0.68 (1.00)	0.70 (0.99)	0.69 (1.02)	0.66 (0.98)	0.66 (0.91)	0.58 (1.02)
6th	0.71 (1.03)	0.71 (1.02)	0.71 (1.03)	0.71 (1.03)	0.67 (1.00)	0.66 (0.90)	0.61 (1.05)
7th	0.70 (1.03)	0.70 (1.03)	0.70 (1.03)	0.70 (1.02)	0.65 (0.97)	0.65 (0.87)	0.60 (1.02)
8th	0.71 (1.04)	0.71 (1.04)	0.71 (1.04)	0.71 (1.05)	0.66 (1.00)	0.66 (0.92)	0.60 (1.03)
9th	0.71 (1.01)	0.71 (1.02)	0.71 (1.03)	0.71 (1.04)	0.67 (1.00)	0.67 (0.92)	0.61 (1.04)
10th	0.70 (1.04)	0.70 (1.02)	0.70 (1.03)	0.70 (1.03)	0.65 (0.98)	0.65 (0.88)	0.60 (1.02)
Mean	0.71 (1.03)	0.71 (1.02)	0.71 (0.98)	0.71 (0.87)	0.66 (0.99)	0.66 (0.90)	0.60 (1.03)

[1]12,311 SNPs, [2]8,237 SNPs, [3]4,950 SNPs, [4]2,744 SNPs, [5]1,182 SNPs, [6]530 SNPs.

more challenging in aquaculture species. Genetic markers have the potential of addressing this issue, facilitating the practical implementation of breeding programs via effective parentage assignment and circumventing the requirement of rearing the fish in separate tanks until tagging is possible (Vandeputte and Haffray, 2014). In the current study, the utility of RAD-seq data for enabling selective breeding for a polygenic trait in common carp was investigated. Using the RAD SNP data, approximately 86% of genotyped animals could be uniquely assigned to parental pairs. Following pedigree reconstruction, moderate heritability estimates of 0.26 and 0.33 were obtained for juvenile weight and length, respectively. These estimates are in line with the previous heritability estimates of weight/length obtained from juvenile carp (Vandeputte et al., 2004; Ninh et al., 2013; Hu et al., 2017). However, one limitation of the current study lies in the fact that trait measurements were taken in juveniles, and the correlation between growth in early life with growth to harvest size is unknown for this population. Contradicting

evidence is available regarding this, with studies recording high positive phenotypic correlations between growth-related traits at juvenile and harvest stage (Ninh et al., 2013), moderate positive correlations (Vandeputte et al., 2008; Nielsen et al., 2010), or correlations near to zero (Hu et al., 2017). However, common carp juvenile weight and length in the current study is used as an exemplar polygenic trait, with broader implications for the use of genomic data to improve other economically important traits, in particular for those typically not measurable directly on selection candidates (disease resistance or fillet traits, e.g.).

Genetic markers can be a valuable addition to selective breeding, but the optimal strategy for their application depends on the underlying genetic architecture of the trait of interest. Where a trait is primarily controlled by one or several major QTL, it may be most effective to use marker-assisted selection with low-density markers flanking QTL regions. This is the case for resistance to the Infectious Pancreatic Necrosis virus in Atlantic salmon for example, where almost all genetic variation is explained by a single QTL (Houston et al., 2008; Moen et al., 2009). However, most traits of economic importance have a polygenic architecture and GS is likely to be the most effective use of genetic markers to improve these traits. In the current study, the GWAS results implied that the juvenile growth traits were polygenic in nature. Previous studies using linkage analysis have reported QTL related to growth in common carp. A study on a single full-sibling family of common carp detected 14 QTL distributed in five different LGs, including regions associated with the hypothalamic–pituitary–gonadal and GH/IGF-I axis that regulates development, cell-proliferation, energy metabolism, and growth (Peng et al., 2016). Additionally, a study on eight full-sib families of common carp reported 38 growth-related QTL, although no QTL was detected in all of the families (Lv et al., 2016). Therefore, due to the lack of consistent major effect QTL, it is unlikely that MAS will be an effective approach for selecting the best breeding candidates for this and similar economically important traits, and GS is a promising alternative.

The results from the testing of GS in the carp population used in the current study were encouraging, with prediction accuracy obtained through cross-validation analyses using GBLUP being 0.71. This signifies an approximate 18% improvement of accuracy compared to pedigree BLUP, suggesting major potential benefits for selection accuracy and genetic gain for complex economic traits in carp. Our results are in agreement with other studies in aquaculture species where a major benefit to using the genomic models was demonstrated for disease resistance in, e.g., Atlantic salmon (Tsai et al., 2015, 2016), rainbow trout (Vallejo et al., 2017), and gilthead sea bream (Palaiokostas et al., 2016). GS benefits from increased sample size of the reference population (Vallejo et al., 2017) indicating that further improvements of prediction accuracies could be expected by increasing the number of genotyped animals in the current study. In typical aquaculture breeding designs, including mass spawning species, where the reference and validation sets are closely related use of genetic markers can be highly effective for capturing within-family genetic variation (Lillehammer et al., 2013). However, given that prediction accuracy is likely to be highly reliant on genetic relationships, this implies that genomic prediction in distant relatives to the reference population is likely to be substantially more challenging, as observed in terrestrial livestock (Daetwyler et al., 2013).

It is noteworthy that GBLUP resulted in increased prediction accuracies compared to pedigree prediction using relatively sparse (∼500 SNPs) genotype data, especially considering the large genome size (∼1.8 Gb) of common carp. Similar results were recorded for Atlantic salmon where similar prediction accuracies were obtained from 5K SNPs as for 112K SNPs (Tsai et al., 2015). Both common carp and Atlantic salmon have large genomes, and the effectiveness of genomic prediction at low marker density is again likely to reflect the aforementioned close relationships between the training and validation animals. Nonetheless, this may have economic benefits, since low cost sparse genotyping could be sufficient for improving prediction accuracies in a breeding program. This could be important for driving implementation of GS, since breeding candidates of aquaculture species are of lower economic value compared with livestock, making the application of costly high-density genotyping approaches difficult to justify. Genotype imputation approaches have major potential to drive this genotype density and cost down further, and have already shown significant promise in Atlantic salmon (Tsai et al., 2017). While verification of the results of the current study using harvest size carp (or other economically important complex traits) would be a logical next step, the results of the current study suggest that GS has potential for substantial improvement in prediction accuracy in carp breeding, and RAD-seq is one method of generating the marker data to enable this improvement.

CONCLUSION

The results from the current study demonstrated that the use of SNP markers generated via RAD-seq is an efficient approach for investigating and potentially improving a polygenic trait in a common carp breeding population. These SNPs enabled pedigree assignment, genetic parameter estimation, GWAS, and GS within a single experiment. GS resulted in improved prediction accuracy versus pedigree approaches even when only relatively sparse marker information was utilized.

AUTHOR CONTRIBUTIONS

MK, MP, and RH conceived the study and contributed to designing the experimental structure. MK and MP shared on establishing and on-growing the experimental stock, PIT tagging, phenotype data recording, and fin clipping the fish. CP carried out the DNA extractions, RAD library preparation, and sequence data processing. CP and RH carried out parentage assignment and the quantitative genetic analyses. All authors contributed to drafting the manuscript.

FUNDING

The authors were supported by funding from the European Union's Seventh Framework Programme (FP7 2007-2013) under Grant Agreement No. 613611 (FISHBOOST). MK and MP were also supported by the Ministry of Education Youth, and Sports of the Czech Republic (projects CENAKVA – CZ.1.05/2.1.00/01.0024 and CENAKVA II – LO1205 under the NPU I program) and the Grant Agency of the University of South Bohemia (Project No. 125/2016/Z).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2018.00082/full#supplementary-material

TABLE S1 | Parentage and phenotypic information of juvenile common carp.

TABLE S2 | Detailed information of identified SNP through RADseq.

TABLE S3 | Linkage map of common carp.

REFERENCES

Aguilar, I., Misztal, I., Legarra, A., and Tsuruta, S. (2011). Efficient computation of the genomic relationship matrix and other matrices used in single-step evaluation. *J. Anim. Breed. Genet.* 128, 422–428. doi: 10.1111/j.1439-0388.2010.00912.x

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., et al. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One* 3:e3376. doi: 10.1371/journal.pone.0003376

Balon, E. K. (1995). Origin and domestication of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. *Aquaculture* 129, 3–48. doi: 10.1016/0044-8486(94)00227-F

Barría, A., Christensen, K. A., Yoshida, G. M., Correa, K., Jedlicki, A., Lhorente, J. P., et al. (2017). Genomic predictions and genome-wide association study of resistance against *Piscirickettsia salmonis* in coho salmon (*Oncorhynchus kisutch*) using ddRAD sequencing. *G3*. doi: 10.1534/g3.118.200053 [Epub ahead of print].

Baxter, S. W., Davey, J. W., Johnston, J. S., Shelton, A. M., Heckel, D. G., Jiggins, C. D., et al. (2011). Linkage mapping and comparative genomics using next-generation RAD sequencing of a non-model organism. *PLoS One* 6:e19315. doi: 10.1371/journal.pone.0019315

Campbell, N. R., LaPatra, S. E., Overturf, K., Towner, R., and Narum, S. R. (2014). Association mapping of disease resistance traits in rainbow trout using restriction site associated DNA sequencing. *G3* 4, 2473–2481. doi: 10.1534/g3.114.014621

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., and Postlethwait, J. H. (2011). Stacks: building and genotyping Loci de novo from short-read sequences. *G3* 1, 171–182. doi: 10.1534/g3.111.000240

Daetwyler, H. D., Calus, M. P. L., Pong-Wong, R., de los Campos, G., and Hickey, J. M. (2013). Genomic prediction in animals and plants: simulation of data, validation, reporting, and benchmarking. *Genetics* 193, 347–365. doi: 10.1534/genetics.112.147983

Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., and Blaxter, M. L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat. Rev. Genet.* 12, 499–510. doi: 10.1038/nrg3012

de los Campos, G., Hickey, J. M., Pong-Wong, R., Daetwyler, H. D., and Calus, M. P. L. (2013). Whole-genome regression and prediction methods applied to plant and animal breeding. *Genetics* 193, 327–345. doi: 10.1534/genetics.112.143313

Dong, Z., Nguyen, N. H., and Zhu, W. (2015). Genetic evaluation of a selective breeding program for common carp *Cyprinus carpio* conducted from 2004 to 2014. *BMC Genet.* 16:94. doi: 10.1186/s12863-015-0256-2

Etter, P. D., Bassham, S., Hohenlohe, P. A., Johnson, E. A., and Cresko, W. A. (2011). SNP discovery and genotyping for evolutionary genetics using RAD sequencing. *Methods Mol. Biol.* 772, 157–178. doi: 10.1007/978-1-61779-228-1-9

FAO (2015). *FishStat Database*. Available at: http://faostat.fao.org/site/629/default.aspx

Ferdosi, M. H., Kinghorn, B. P., van der Werf, J. H. J., Lee, S. H., and Gondro, C. (2014). hsphase: an R package for pedigree reconstruction, detection of recombination events, phasing and imputation of half-sib family groups. *BMC Bioinformatics* 15:172. doi: 10.1186/1471-2105-15-172

Gjedrem, T. (2000). Genetic improvement of cold-water fish species. *Aquac. Res.* 31, 25–33. doi: 10.1046/j.1365-2109.2000.00389.x

Goddard, M. E., and Hayes, B. J. (2009). Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nat. Rev. Genet.* 10, 381–391. doi: 10.1038/nrg2575

Henderson, C. R. (1975). Best linear unbiased estimation and prediction under a selection model. *Biometrics* 31, 423–447. doi: 10.2307/2529430

Hervé, P., and Dandine-Roulland, C. (2018). *Gaston: Genetic Data Handling (QC, GRM, LD, PCA) & Linear Mixed Models Version 1.5 from CRAN*. Available at: https://rdrr.io/cran/gaston/

Hickey, J. M., Chiurugwi, T., Mackay, I., Powell, W., and Implementing, Genomic Selection in CGIAR Breeding Programs Workshop Participants. (2017). Genomic prediction unifies animal and plant breeding programs to form platforms for biological discovery. *Nat. Genet.* 49, 1297–1303. doi: 10.1038/ng.3920

Houston, R. D., Davey, J. W., Bishop, S. C., Lowe, N. R., Mota-Velasco, J. C., Hamilton, A., et al. (2012). Characterisation of QTL-linked and genome-wide restriction site-associated DNA (RAD) markers in farmed Atlantic salmon. *BMC Genomics* 13:244. doi: 10.1186/1471-2164-13-244

Houston, R. D., Haley, C. S., Hamilton, A., Guy, D. R., Tinch, A. E., Taggart, J. B., et al., (2008). Major quantitative trait loci affect resistance to infectious pancreatic necrosis in atlantic salmon (*Salmo salar*). *Genetics* 178, 1109–1115. doi: 10.1534/genetics.107.082974

Hu, X., Li, C., Shang, M., Ge, Y., Jia, Z., Wang, S., et al. (2017). Inheritance of growth traits in Songpu mirror carp (*Cyprinus carpio* L.) cultured in Northeast China. *Aquaculture* 477, 1–5. doi: 10.1016/j.aquaculture.2017.04.031

Hulata, G. (1995). A review of genetic improvement of the common carp (*Cyprinus carpio* L.) and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture* 129, 143–155. doi: 10.1016/0044-8486(94)00244-I

Janssen, K., Chavanne, H., Berentsen, P., and Komen, H. (2017). Impact of selective breeding on European aquaculture. *Aquaculture* 472, 8–16. doi: 10.1016/j.aquaculture.2016.03.012

Kocour, M., Mauger, S., Rodina, M., Gela, D., Linhart, O., and Vandeputte, M. (2007). Heritability estimates for processing and quality traits in common carp (*Cyprinus carpio* L.) using a molecular pedigree. *Aquaculture* 270, 43–50. doi: 10.1016/j.aquaculture.2007.03.001

Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359. doi: 10.1038/nmeth.1923

Lillehammer, M., Meuwissen, T. H. E., and Sonesson, A. K. (2013). A low-marker density implementation of genomic selection in aquaculture using within-family genomic breeding values. *Genet. Sel. Evol.* 45:39. doi: 10.1186/1297-9686-45-39

Lv, W., Zheng, X., Kuang, Y., Cao, D., Yan, Y., and Sun, X. (2016). QTL variations for growth-related traits in eight distinct families of common carp (*Cyprinus carpio*). *BMC Genet.* 17:65. doi: 10.1186/s12863-016-0370-9

Meuwissen, T., Hayes, B., and Goddard, M. (2013). Accelerating improvement of livestock with genomic selection. *Annu. Rev. Anim. Biosci.* 1, 221–237. doi: 10.1146/annurev-animal-031412-103705

Meuwissen, T. H. E., Hayes, B. J., and Goddard, M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157, 1819–1829.

Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T., and Lee, D. H. (2002). "BLUPF90 and related programs (BGF90)," in *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production*, Montpellier, 21–22.

Moav, R., and Wohlfarth, G. (1976). Two-way selection for growth rate in the common carp (*Cyprinus carpio* L.). *Genetics* 82, 83–101.

Moen, T., Baranski, M., Sonesson, A. K., and Kjøglum, S. (2009). Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in atlantic salmon (*Salmo salar*): population-level associations between markers and trait. *BMC Genomics* 10:368. doi: 10.1186/1471-2164-10-368

Nielsen, H. M., Ødegård, J., Olesen, I., Gjerde, B., Ardo, L., Jeney, G., et al. (2010). Genetic analysis of common carp (*Cyprinus carpio*) strains. I: genetic parameters and heterosis for growth traits and survival. *Aquaculture* 304, 14–21. doi: 10.1016/j.aquaculture.2010.03.016

Ninh, N. H., Ponzoni, R. W., Nguyen, N. H., Woolliams, J. A., Taggart, J. B., McAndrew, B. J., et al. (2013). A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): responses to selection. *Aquaculture* 40, 152–159. doi: 10.1016/j.aquaculture.2013.06.005

Odegård, J., Moen, T., Santi, N., Korsvoll, S. A., Kjøglum, S., and Meuwissen, T. H. E. (2014). Genomic prediction in an admixed population of Atlantic salmon (*Salmo salar*). *Front. Genet.* 5:402. doi: 10.3389/fgene.2014.00402

Palaiokostas, C., Ferraresso, S., Franch, R., Houston, R. D., and Bargelloni, L. (2016). Genomic prediction of resistance to Pasteurellosis in gilthead sea bream (*Sparus aurata*) using 2b-RAD sequencing. *G3* 58, 3693–3700. doi: 10.1534/g3.116.035220

Palti, Y., Vallejo, R. L., Gao, G., Liu, S., Hernandez, A. G., Rexroad, C. E., et al. (2015). Detection and validation of QTL affecting bacterial cold water disease resistance in rainbow trout using restriction-site associated DNA sequencing. *PLoS One* 10:e0138435. doi: 10.1371/journal.pone.0138435

Peng, W., Xu, J., Zhang, Y., Feng, J., Dong, C., Jiang, L., et al. (2016). An ultra-high density linkage map and QTL mapping for sex and growth-related traits of common carp (*Cyprinus carpio*). *Sci. Rep.* 6:26693. doi: 10.1038/srep26693

Rastas, P., Paulin, L., Hanski, I., Lehtonen, R., and Auvinen, P. (2013). Lep-MAP: fast and accurate linkage map construction for large SNP datasets. *Bioinformatics* 29, 3128–3134. doi: 10.1093/bioinformatics/btt563

Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P., and Houston, R. (2017). Applications of genotyping by sequencing in aquaculture breeding and genetics. *Rev. Aquac.* doi: 10.1111/raq.12193 [Epub ahead of print].

Sargolzaei, M., Chesnais, J. P., and Schenkel, F. S. (2014). A new approach for efficient genotype imputation using information from relatives. *BMC Genomics* 15:478. doi: 10.1186/1471-2164-15-478

Sonesson, A. K., and Meuwissen, T. H. E. (2009). Testing strategies for genomic selection in aquaculture breeding programs. *Genet. Sel. Evol.* 41:37. doi: 10.1186/1297-9686-41-37

Tsai, H.-Y., Hamilton, A., Tinch, A. E., Guy, D. R., Bron, J. E., Taggart, J. B., et al. (2016). Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations. *Genet. Sel. Evol.* 48:47. doi: 10.1186/s12711-016-0226-9

Tsai, H.-Y., Hamilton, A., Tinch, A. E., Guy, D. R., Gharbi, K., Stear, M. J., et al. (2015). Genome wide association and genomic prediction for growth traits in juvenile farmed Atlantic salmon using a high density SNP array. *BMC Genomics* 16:969. doi: 10.1186/s12864-015-2117-9

Tsai, H.-Y., Matika, O., Edwards, S. M., Antolín-Sánchez, R., Hamilton, A., Guy, D. R., et al. (2017). Genotype imputation to improve the cost-efficiency of genomic selection in farmed Atlantic salmon. *G3*, 1377–1383. doi: 10.1534/g3.117.040717

Vallejo, R. L., Leeds, T. D., Fragomeni, B. O., Gao, G., Hernandez, A. G., Misztal, I., et al. (2016). Evaluation of genome-enabled selection for bacterial cold water disease resistance using progeny performance data in rainbow trout: insights on genotyping methods and genomic prediction models. *Front. Genet.* 7:96. doi: 10.3389/fgene.2016.00096

Vallejo, R. L., Leeds, T. D., Gao, G., Parsons, J. E., Martin, K. E., Evenhuis, J. P., et al. (2017). Genomic selection models double the accuracy of predicted breeding values for bacterial cold water disease resistance compared to a traditional pedigree-based model in rainbow trout aquaculture. *Genet. Sel. Evol.* 49:17. doi: 10.1186/s12711-017-0293-6

Vandeputte, M. (2003). Selective breeding of quantitative traits in the common carp (*Cyprinus carpio*): a review. *Aquat. Living Resour.* 16, 399–407. doi: 10.1016/S0990-7440(03)00056-1

Vandeputte, M., and Haffray, P. (2014). Parentage assignment with genomic markers: a major advance for understanding and exploiting genetic variation of quantitative traits in farmed aquatic animals. *Front. Genet.* 5:432. doi: 10.3389/fgene.2014.00432

Vandeputte, M., Kocour, M., Mauger, S., Dupont-Nivet, M., De Guerry, D., Rodina, M., et al. (2004). Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). *Aquaculture* 235, 223–236. doi: 10.1016/j.aquaculture.2003.12.019

Vandeputte, M., Kocour, M., Mauger, S., Rodina, M., Launay, A., Gela, D., et al. (2008). Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio* L.): heritability estimates and response to selection. *Aquaculture* 277, 7–13. doi: 10.1016/j.aquaculture.2008.02.009

VanRaden, P. M. (2008). Efficient methods to compute genomic predictions. *J. Dairy Sci.* 91, 4414–4423. doi: 10.3168/jds.2007-0980

Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y., et al. (2014). Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. *Nat. Genet.* 46, 1212–1219. doi: 10.1038/ng.3098

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CHAPTER 6

MAPPING AND SEQUENCING OF A SIGNIFICANT QUANTITATIVE TRAIT LOCUS AFFECTING RESISTANCE TO KOI HERPESVIRUS IN COMMON CARP

Palaiokostas, Ch., Robledo, D., Vesely, T., Kocour, M., Prchal, M., Pokorova, D., Piackova, V., Pojezdal, L., Houston, R.D., 2018. Mapping and sequencing of a significant quantitative trait locus affecting resistance to Koi herpesvirus in common carp. G3: Genes Genom. Genet. In press.

It was allowed by publisher on 21st April 2018 to include the manuscript in this Ph.D. thesis.

My share on this work was about 10%.

MAPPING AND SEQUENCING OF A SIGNIFICANT QUANTITATIVE TRAIT LOCUS AFFECTING RESISTANCE TO KOI HERPESVIRUS IN COMMON CARP

Christos Palaiokostas[1,2], Diego Robledo[1], Tomas Vesely[4], Martin Kocour[3], Martin Prchal[3], Dagmar Pokorova[4], Veronika Piackova[3], Lubomir Pojezdal[4], Ross D. Houston[1]

[1]The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, Scotland, United Kingdom

[2]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7090, 750 07 Uppsala, 7 Sweden

[3]University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, 389 25 Vodnany, Czech Republic

[4]Veterinary Research Institute, Hudcova 70, Brno 62100, Czech Republic

ABSTRACT

Cyprinids are the most highly produced group of fishes globally, with common carp being one of the most valuable species of the group. Koi herpesvirus (KHV) infections can result in high levels of mortality, causing major economic losses, and is listed as a notifiable disease by the World Organisation for Animal Health. Selective breeding for host resistance has the potential to reduce morbidity and losses due to KHV. Therefore, improving knowledge about host resistance and methods of incorporating genomic data into breeding for resistance may contribute to a decrease in economic losses in carp farming. In the current study, a population of 1,425 carp juveniles, originating from a factorial cross between 40 sires and 20 dams was challenged with KHV. Mortalities and survivors were recorded and sampled for genotyping by sequencing using Restriction Site-Associated DNA sequencing (RAD-seq). Genome-wide association analyses were performed to investigate the genetic architecture of resistance to KHV. A genome-wide significant QTL affecting resistance to KHV was identified on linkage group 44, explaining approximately 7% of the additive genetic variance. Pooled whole genome resequencing of a subset of resistant (n = 60) and susceptible animals (n = 60) was performed to characterize QTL regions, including identification of putative candidate genes and functional annotation of associated polymorphisms. The TRIM25 gene was identified as a promising positional and functional candidate within the QTL region of LG 44, and a putative premature stop mutation in this gene was discovered.

1. INTRODUCTION

Common carp (*Cyprinus carpio* and *Cyprinus rubrofuscus*), is one of the most highly produced aquaculture fish species globally (FAO, 2016), being farmed in a wide variety of environments and production systems (Balon, 1995). However, in common with many aquaculture species, only a minority of farmed carp are derived from family-based selective breeding programs (Vandeputte, 2003; Janssen et al., 2017). The potential for selective breeding to enhance production in carp is highlighted by several studies, but much of the production of commercial stock is still generated via intra / Interspecific crossbreeding (Kocour et al., 2005, 2007; Vandeputte et al., 2008; Nielsen et al., 2010; Prchal et al., 2018).

Koi herpesvirus (KHV), also known as Cyprinid herpesvirus-3 (CyHV-3), is one of the main threats to carp production. The first major outbreaks were recorded in 1998 (Hedrick et al., 2000), and subsequent outbreaks in many carp producing countries were reported worldwide (Haenen et al., 2004). The seriousness of the KHV threat is highlighted by its listing as a notifiable disease by the European Union (Taylor et al. 2010) and the World Organization for Animal Health (OIE 2018). Selective breeding is a valuable tool for contributing to sustainable food production through the prevention and management of infectious outbreaks in a wide range of species (Bishop and Woolliams, 2014). This may be particularly true in aquaculture species, due to moderate to high heritabilities of disease resistance documented in numerous cases (Ødegård et al. 2011; Houston 2017), and successful examples of disease control using marker-assisted breeding, e.g. the case of the IPN virus in Atlantic salmon; Salmo salar (Houston et al., 2008; Moen et al., 2009).

Several studies have investigated the genetic basis of KHV resistance in carp (utilizing data and samples collected from disease challenge trials), showing encouraging results with large variation in survival both between-families (Dixon et al. 2009; Tadmor-Levi et al. 2017) and between strains (Shapira et al., 2005; Piačková et al., 2013). Results from candidate gene association studies have suggested a possible role for polymorphism in MHC loci (Rakus et al., 2009) and Interleukin-11 (Kongchum et al., 2011) in host resistance to KHV. Taken together, these studies indicate that selective breeding has the potential to increase resistance to KHV, with potential downstream benefits for the carp aquaculture industry and fish welfare. However, to date, genome-wide polymorphisms have not been applied to investigate the genetic architecture of resistance to KHV.

Restriction-site associated DNA sequencing (RAD-seq) (Baird et al., 2008) and similar genotyping by sequencing techniques have been widely applied to generate genome-wide SNP markers due to their cost-efficiency in a wide range of aquaculture species (Robledo et al., 2017), including common carp (Palaiokostas et al., 2018a). Various genome wide association studies (GWAS) using this technique have been published in aquaculture species (e.g. Campbell et al., 2014; Palti et al., 2015). GWAS have been used to study disease resistance in various aquaculture species including salmonids (Correa et al., 2015, 2017; Vallejo et al., 2017; Barría et al., 2018; Robledo et al., 2018), catfish (Zhou et al., 2017), European sea bass (Palaiokostas et al., 2018b) and Pacific oyster (Gutierrez et al., 2018) amongst others. With the notable exception of the aforementioned case of IPN resistance in salmon, the GWAS results have pointed to a polygenic or oligogenic architecture for disease resistance in aquaculture species. The main aim of this study was to investigate genetic resistance to KHV in common carp using a RAD-seq approach. Classical genome wide association study (GWAS) and weighted genomic best linear unbiased predictor (WGBLUP) approaches were taken to examine the genetic architecture of resistance. Finally, pooled whole genome sequencing (PWGS) was performed in a subset of samples with divergent resistance and susceptibility to characterize and annotate QTL regions, and to identify potential gene candidates and polymorphisms involved in KHV resistance.

2. MATERIALS AND METHODS

2.1. Sample collection and disease challenge

A population of Amur Mirror Carp was created at the University of South Bohemia in České Budějovice, Czech Republic in May 2014 using artificial insemination (Vandeputte et al., 2004) involving four factorial crosses of five dams x ten sires (20 dams and 40 sires in total). Incubation of eggs was performed in 9 L Zugar jars at 20°C. At the first swimming

stage, randomly sampled progeny from each mating (of approximately equal total volume) were pooled and stocked into several nursery earthen ponds at stocking density of 150,000 larvae / ha and reared under semi-intensive pond conditions throughout the growing season (from May to September). Before the challenge test a random sample of 1,500 fish described above were tagged and fin clipped for DNA extraction. These fish were the same as those described in Palaiokostas et al. (2018a). These animals were acclimatized for five days at water temperature of 22 °C and bathed in FMC solution (formalin, malachite green, methylene blue using a dose of 2 mL per 100 L of water) to eliminate ectoparasites. Subsequently, the fish were transferred to Veterinary Research Institute (VRI) in Brno (Czech Republic) to perform the KHV disease challenge test. A small (n = 215) sample of koi carp were challenged alongside the Amur mirror carp as a positive control, since Koi carp are highly susceptible to KHV.

A cohabitation challenge was performed in a 1,400 L tank equipped with recirculation and biological filtration. Koi carp received an intraperitoneal injection with 0.2 mL culture medium containing 104 TCID 50 / mL KHV at day 0 and were added into the tank with challenged fish. Mortality of individual fish was recorded twice a day for a period of 35 days post infection (dpi). Presence of KHV on a sample of dead fish (n = 100) was confirmed by PCR according to guidelines by the Centre for Environment, Fisheries & Aquaculture Science, UK (Cefas) (Pokorova et al., 2010). The experiment was run until mortalities were negligible, implying that survivors were resistant. The entire experiment was conducted in accordance with the law on the protection of animals against cruelty (Act no. 246/1992 Coll. of the Czech Republic) upon its approval by Institutional Animal Care and Use Committee (IACUC) of the VRI and appropriate state authority. All people conducting the experiment hold a certificate about qualification to conduct experiments on the live animals, and the VRI is accredited for the culture of experimental animals according to the aforementioned law.

2.2. Library preparation and sequencing

The RAD library preparation protocol followed the methodology originally described in Baird et al. (2008) and presently in detail in Palaiokostas et al. (2018a). Briefly, template DNA was digested using the SbfI (recognizing the CCTGCA|GG motif) high fidelity restriction enzyme (New England Biolabs; NEB). DNA shearing was conducted with a Pico bioruptor (Diagenode). Following a final gel elution step into 20 μL EB buffer (MinElute Gel Purification Kit, Qiagen), 66 libraries (24 animals each) were sent to BMR Genomics (Italy), for quality control and high-throughput sequencing. RAD libraries were run in fourteen lanes of an Illumina NextSeq 500, using 75 base paired-end reads (v2 chemistry).

Whole genome sequencing libraries (n = 4) from pooled DNA samples (30 animals each library) of susceptible and resistant animals were constructed using the Illumina TruSeq DNA PCR free kit (350bp insert). Sequencing was performed in Edinburgh Genomics facilities using two lanes of Illumina HiSeq 4000. The reads were deposited at the NCBI Sequence Read Archive (SRA) under the accession PRJNA414021.

2.3. SNP discovery and genotyping

The process of obtaining the SNP genotype data from the RAD-seq reads was described in detail in Palaikostas et al. (2018). Briefly, sequenced reads were aligned to the common carp reference genome assembly version GCA_000951615.2 (Xu et al., 2014) using bowtie2 (Langmead and Salzberg, 2012). The aligned reads were sorted into RAD loci and SNPs were identified using the Stacks software 1.4 (Catchen et al., 2011). The SNPs were detected

using a minimum stack depth of at least ten or five for the parental and offspring samples respectively. SNPs with minor allele frequency (MAF) below 0.01, greater than 20 % missing data, and deviating from expected Hardy-Weinberg equilibrium in the parental samples (P < 1e-06) were discarded. R/hsphase (Ferdosi et al., 2014) software was used for parentage assignment allowing for a maximum genotyping error of 4 %. The pedigree obtained was further validated for possible erroneous assignments using FImpute (Sargolzaei et al., 2014). In total, 1,214 offspring were uniquely assigned, forming 195 full-sib families (40 sires, 20 dams). Since the carp reference genome assembly is currently very fragmented, a medium density linkage map of 12,311 SNPs grouped in 50 linkage groups was created (Palaiokostas et al., 2018a), and used to orientate the results from the GWAS.

2.4. Heritability estimation

The probit link function was used to connect the observed binary phenotype (0 = dead, 1 = alive) with the underlying liability scale. Variance components were estimated using the R/BGLR (Pérez and de Los Campos, 2014)e.g., the number of marker effects software with the following animal model:

$$\mathbf{l} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}, \quad (1)$$

where **l** is the vector of latent variables, **b** is the vector of the fixed effects (intercept, tank), **X** is the incidence matrix relating phenotypes with the fixed effects, **Z** is the incidence matrix relating phenotypes with the random animal effects, **u** is the vector of random animal effects ~ $N(0, \mathbf{A}\sigma_g^2)$ [where **A** corresponds to the pedigree-based relationship matrix and is replaced by G for analyses using the genomic relationship matrix (VanRaden 2008)967 bulls and 50,000 markers distributed randomly across 30 chromosomes. Estimation of genomic inbreeding coefficients required accurate estimates of allele frequencies in the base population. Linear model predictions of breeding values were computed by 3 equivalent methods: 1 and σ_g^2 is the additive genetic variance], **e** the vector of residuals ~N(0, **I**) where is the residual variance.

The parameters of this model were estimated through Markov chain Monte Carlo (MCMC) using Gibbs sampling (11 M iterations; burn-in: 1 M; thin: 1,000). Convergence of the resulting posterior distributions was assessed both visually (inspecting the resulting MCMC plots) and analytically using R/coda v0.19-1 (Plummer et al., 2006)Bayesian inference with Markov Chain Monte Carlo (MCMC. Heritability for the trait of survival during the KHV challenge (on the underlying liability scale) was estimated using the following formula:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

where σ_g^2 is the previous estimated additive genetic variance and the residual variance. Residual variance on the underlying scale is not identifiable in threshold models (Goldstein et al., 2002; Nakagawa and Schielzeth 2013) and was therefore fixed to 1.

2.5. Genome wide association analysis (GWAS)

To test the association between individual SNPs and resistance to VNN, a classical genome wide association study (CGWAS) was performed using R/gaston (Perdry and Dandine-Roulland, 2018). The mixed model applied for overall survival had the same format as in (1) with the addition of including each SNP as a fixed effect. The variance components were estimated using the penalized quasi-likelihood approach (Chen et al., 2016). The genome-wide significance threshold was calculated using a Bonferroni correction (0.05 / N), where N represents the number of tested SNPs.

Weighted genomic best linear unbiased predictor (WGBLUP) was performed (Wang et al., 2012) using windows of ten adjacent SNPs for estimating SNP effects and direct genomic values (DGV) instead of genomic estimated breeding values (GEBV) to reduce bias (Lourenco et al., 2015; Zhang et al., 2016)almost 3.4 million on postweaning gain (PWG. The weighted genomic relationship matrix was initially created following VanRaden (2008) as:

$$\mathbf{G^{*} = ZDZ'q}$$

where **Z** *is* the design matrix relating genotypes of each locus, **D** is a weight matrix for all SNPs, and **q** is a weighting vector derived from observed SNP frequencies. SNP weights were calculated using the nonlinearA method (VanRaden, 2008). Briefly the steps for performing WGBLUP were as follows (Wang et al., 2012):

a) Initialize **D** = **I** and t=1, where **I** the identity matrix and t is the iteration number.
b) Calculate $\mathbf{G}^{*}$.
c) Estimate DGVs.
d) Estimate SNP effects from GEBVs: $\hat{\boldsymbol{\alpha}} = \mathbf{qDZ'}\boldsymbol{G}^{*}\hat{\mathbf{u}}$, where $\hat{\boldsymbol{\alpha}}$ the vector of SNP effects and $\hat{\mathbf{u}}$ the vector of DGV
e) Calculate the weight for each SNP: $d_{ii}^{(t+1)} = 1.125^{\frac{|a_i|}{sd(a)}^2}$, where $\hat{\boldsymbol{\alpha}}_i$ the estimated SNP effect (VanRaden 2008)967 bulls and 50,000 markers distributed randomly across 30 chromosomes. Estimation of genomic inbreeding coefficients required accurate estimates of allele frequencies in the base population. Linear model predictions of breeding values were computed by 3 equivalent methods: 1.
f) Normalize SNP weights so the total genetic variance remains constant.
g) Loop to step d) until convergence (10^{-14}).

Convergence of SNP weights was tested using the convergence criterion BLUPF90 uses for variance components estimation

$$C = \frac{\Sigma_i(\theta_i - \xi_i)^2}{\Sigma_i \theta_i^2}$$

Percentage of additive genetic variance was estimated by non-overlapping windows of 10 adjacent SNPs as follows:

$$\frac{\mathrm{Var}(a_i)}{\sigma_\alpha^2} \times 100\% = \frac{\mathrm{Var}(\Sigma_i^{i=10} z_i\ u_i)}{\sigma_\alpha^2} \times 100\%$$

The weighted GBLUP analyses were performed using THRGIBBSF90 for estimating DGVs (Misztal et al., 2002) combined with of PreGSF90 and PostGSF90 (Aguilar et al., 2011) until convergence (10^{-14}).

2.6. Pooled whole genome sequencing analysis

Pools of genomic DNA (25 ng / ul) from 60 survivors and 60 mortalities from the disease challenge experiment were prepared. These animals originated from 20 full-sib families, and the family structure was balanced between the resistant and susceptible pools. Libraries were prepared using the TruSeq DNA PCR free kit (350 bp insert size) and sequenced in two lanes of an Illumina HiSeq 4000 using paired-end sequencing by Edinburgh Genomics.

Reads were QC-filtered (phred score above 30) and trimmed to 140 bp long using Trimmomatic v0.36 (Bolger et al., 2014). Reads were aligned to the carp reference genome GCA_000951615.2 (Xu et al., 2014) using bowtie2 (Langmead and Salzberg, 2012). SNP identification was performed using Burrows-Wheeler Aligner v0.7.8 (BWA-mem, Li 2013).

Pileup files describing the base-pair information at each genomic position were generated from the alignment files using the mpileup function of Samtools v1.6 (Li et al., 2009) requiring minimum mapping and base quality of 20. A Cochran-Mantel-Haenszel test was performed to test the significance of the allele frequency differences using Popoolation 2 v1.201 (Kofler et al., 2011). Only those genomic positions with at least 6 reads of the alternative allele across all pools and a maximum coverage of 50 reads and a minimum of 8 in all pools were considered SNPs. All QC-filtered SNPs were annotated using SNPeff (Cingolani et al., 2012).

2.7. Data availability

Raw reads were deposited in the National Centre for Biotechnology Information (NCBI) repository under project ID SRP081498.

3. RESULTS

3.1. Disease challenge

Mortalities began at 12 dpi reaching a maximum between 21 and 24 dpi (98 – 130 mortalities per day) decreasing thereafter with no mortalities observed after 35 dpi (Figure 1). The overall mortality in the KHV challenge experiment for the Amur Mirror Carp was 66 %. All observed mortalities displayed typical KHV symptoms (e.g. weakness, lethargy, loss of equilibrium, erratic swimming, sunken eyes, excessive mucous production, increased respiratory rate, discoloration, and hemorrhagic lesions on the skin and gills). The presence of KHV was confirmed in all tested samples (n = 100).

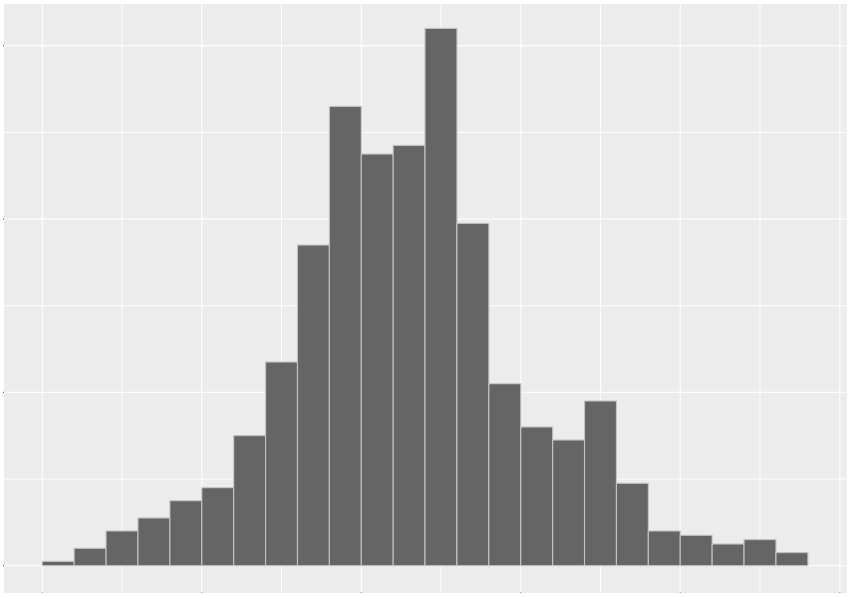

Figure 1. *Daily mortality levels of fish during the KHV challenge experiment.*

3.2. Heritability estimation

There was marked between-family variation in survival rate for both sires (6 – 83 %) and dams (0 – 52 %), suggesting the existence of considerable genetic variation for host resistance. Heritability estimates of overall survival for the pedigree and genomic relationship matrix on the underlying scale were 0.61 (HPD interval 95%: 0.42 – 0.80) and 0.50 (HPD interval 95%: 0.38 – 0.63) respectively.

3.3. Genome wide association approaches - SNP annotation in QTL region

The three SNPs with the highest association (exceeding genome wide significance level) according to classical GWAS were located on linkage group 44 (chromosome 33; P < 1e-07; denoted by stars in Figure 2). This QTL was also identified using the WGBLUP approach (Figure 3) suggesting it accounted for approximately 7% (convergence obtained after 5 iterations) of the additive genetic variance on the underlying scale. In addition the WGBLUP identified QTLs explaining more than 1% of the additive genetic variance in linkage groups 34 (~ 2.5%) and 42 (~ 1.1%). Whole genome sequencing data from the pools of resistance and susceptible animals was used to discover and annotate additional SNPs in the QTL region (Figure 4), and potential candidate genes were identified. Further, SNPs with significant allele frequency differences (P-value < 0.05) between the two groups were identified. A SNP coding for a putative premature stop codon was identified in gene TRIM25 (Glu258*), an E3 ubiquitin ligase with a major role in initiation of intracellular antiviral response to herpesviruses (Gupta et al. 2018).

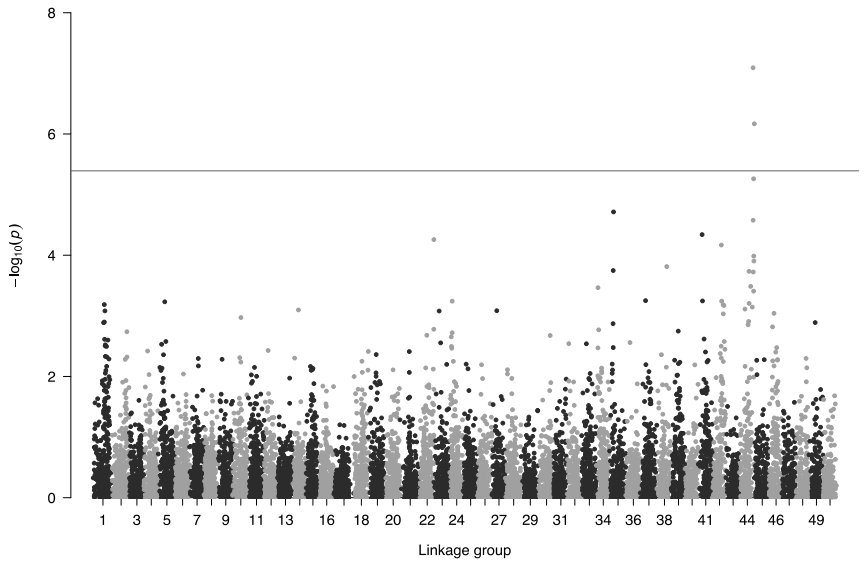

Figure 2. *Classical Genome wide association plot for overall survival during the KHV challenge.*

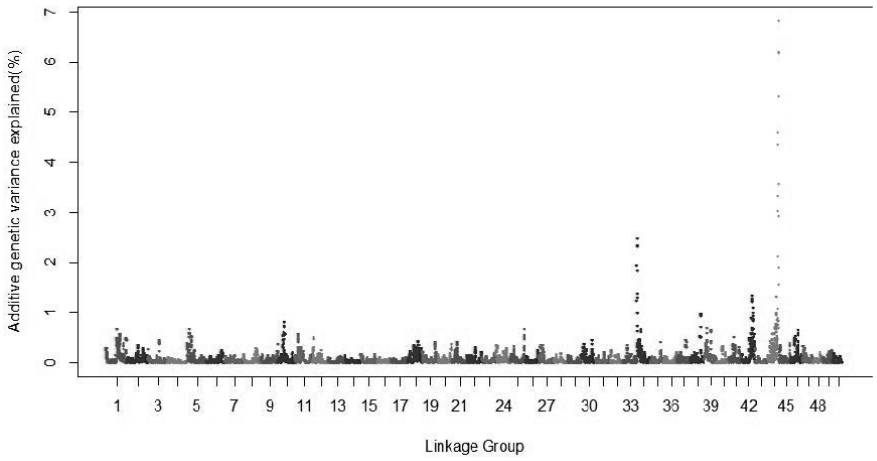

Figure 3. *WGBLUP for resistance to KHV. The additive genetic variance explained was calculated using windows of 10 adjacent SNPs.*

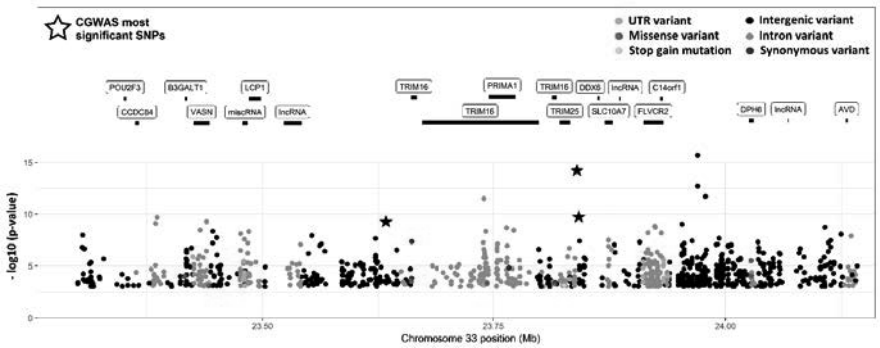

Figure 4. *Annotation of the QTL region on LG 44 including identification of putative genes in the region, functional annotation of SNPs.*

4. DISCUSSION

In the current study, high throughput sequencing was applied to study genetic resistance of common carp to KHV. While genomic data in the form of genetic markers can be a valuable addition to selective breeding for disease resistance, how to apply this data depends on the underlying genetic architecture. In the case of traits controlled by a major QTL, it may be most effective to use marker-assisted selection, while in the case of polygenic traits genomic selection is likely to be preferable. Modern genomic tools also facilitate high resolution study of the genomic regions underpinning genetic resistance, facilitating identification and annotation of promising functional candidate genes which may play a direct role in differential host response to infection.

Following pedigree reconstruction using the RAD SNP data, the heritability of resistance as measured by survival on the underlying scale was estimated to be 0.61 (pedigree) and 0.50

(genomic). This is an unusually high heritability estimate, but is comparable to the estimated of 0.79 that was previously documented for this trait (Ødegård et al., 2010). These independent high estimates of heritability of resistance to KHV highlight that selective breeding has major potential for producing carp with increased resistance. Additionally, in a recent study of introgression of KHV resistance from a wild carp strain to a farmed carp strain, significant additive genetic variation in resistance was detected (Tadmor-Levi et al. 2017). Furthermore, the authors showed that resistant carp do become infected, implying that resistance is due to an effective host response to infection (Tadmor-Levi et al., 2017). Early stage host response to KHV infection is likely to have a major interferon pathway component, with Interferon αβ, and interleukin 12 suggested to play a major role in Koi and Red common carp (Hwang et al., 2017).

The CGWAS resulted in the identification of genome-wide significant QTL on linkage group 44. While this test is the most commonly used association analysis, it fails to utilize all available information since it does not consider linkage disequilibrium between adjacent SNPs, resulting in reduced statistical power as opposed to methods where all SNPs are used simultaneously (Wang et al., 2012). The WGBLUP approach incorporates multiple SNPs and combines the computational efficiency of GBLUP with an increased statistical power for QTL detection (Zhang et al., 2016). However WGBLUP has limitations as well, like the heuristic influence regarding optimal number of iterations and the difficulty to determine appropriate significance levels for the identified QTL (Wang et al., 2012; Lourenco et al., 2015; Zhang et al., 2016). The recent implementation of nonlinearA (VanRaden, 2008) in PostGSF90 (Misztal et al. 2018) may help circumvent the issue of optimal number of iterations due to its better convergence properties. NonlinearA benefits particularly in situations where a non-normal prior distribution more accurately describes the trait under study (VanRaden, 2008). In the current study, both CGWAS and WGBLUP provided significant evidence for the existence of a QTL associated with resistance to KHV on linkage group 44, explaining approximately 7% of the genetic variation in a highly heritable trait. The SNP with highest association in the CGWAS was located ~6.5 Kb upstream of TRIM25, an E3 ubiquitin ligase with a major role in initiation of intracellular antiviral response to herpesviruses. Autoubiquitinisation of TRIM25 is a viral strategy for functional inactivation of the pattern recognition protein RIG1, and subsequent cellular interferon response (Gack et al., 2008). In the PWGS, majority of the SNPs with significant allele frequency differences between the resistant and susceptible pools were annotated as 'intergenic'. However, interestingly, a putative premature stop mutation in position 258 of the carp TRIM25 protein was identified. TRIM25 has 649 - 682 amino acids (isoform dependent), and therefore this stop mutation is highly likely to result in loss of function. The premature stop causing allele is rare in the population, but reads of this allele were more common in the susceptible (n = 11) than the resistance (*n* = 3) pools, albeit the Cochran-Mantel-Haenszel test p-value for this SNP was only nominally significant (0.049). This may fit with a loss of function of TRIM25 in susceptible fish, being unable to trigger an appropriate antiviral response.

It will be interesting to study whether this single genome-wide significant QTL for resistance to KHV has an effect in other carp populations and strains. Follow up functional studies of candidate genes in the QTL region, including assessment of gene expression response to infection and the differential response between alternate QTL types, may be a fruitful avenue to shortlist functional candidate genes. Currently, TRIM25 and its premature stop mutation seem to be the most promising candidates, and additional genotyping of this SNP alongside directed functional studies may help to test if it may be causative for the QTL. While the QTL identified in the current study was highly significant, the proportion of genetic variation explained was relatively moderate, implying multifactorial causal mechanisms underlying host

resistance. Nonetheless, it is plausible that genetic markers within the QTL region may have value for marker-assisted selection, either directly or via a genomic prediction strategy with increased weighting on QTL-region SNPs.

5. CONCLUSIONS

In conclusion, the results from the current study demonstrate that SNP markers generated via RAD-seq are effective at studying the genetic variation in resistance to KHV in a common carp breeding population. The RAD-derived SNPs facilitated the identification of a genome-wide significant QTL on LG 44 affecting resistance to KHV. The sequencing and annotation of the QTL regions provided candidate functional genes and polymorphisms for future study to understand the mechanisms underlying the QTL. This QTL may have value for selective breeding via incorporation into marker-assisted or genomic selection, albeit genetic resistance to KHV in common carp appears to be multifactorial in nature.

Author contributions

TV, MK, MP, VP, RH conceived the study, contributed to designing the experimental structure. MK, MP, VP share on establishing and on-growing the experimental stock, PIT tagging and fin clipping the fish. TV, DP, LP carried out the own KHV challenge experiment. CP carried out DNA extractions, RAD library preparation and sequence data processing. CP and RH carried out parentage assignment and the quantitative genetic analyses. All authors contributed to drafting the manuscript.

Acknowledgements

The authors are supported by funding from the European Union's Seventh Framework Programme (FP7 2007-2013) under grant agreement no. 613611 (FISHBOOST). MK, MP, VP were also supported by the Ministry of Education, Youth, and Sports of the Czech Republic (projects CENAKVA – CZ.1.05/2.1.00/01.0024 and CENAKVA II – LO1205 under the NPU I program and project Biodiverzita (CZ.02.1.01/0.0/0.0/16_025/0007370) and the Grant Agency of the University of South Bohemia (project no. 125/2016/Z).

REFERENCES

Aguilar, I., Misztal, I., Legarra, A., Tsuruta, S., 2011. Efficient computation of the genomic relationship matrix and other matrices used in single-step evaluation. J. Anim. Breed. Genet. 128, 422–428.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson E.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE 3, e3376.

Balon, E.K., 1995. Origin and domestication of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. Aquaculture 129, 3–48.

Barría, A., Christensen, K.A., Yoshida, G.M., Correa, K., Jedlicki, A., Lhorente, J.P., Davidson, W.S., Yáñez, J.M., 2018. Genomic predictions and genome-wide association study of resistance against *Piscirickettsia salmonis* in coho salmon (*Oncorhynchus kisutch*) using ddRAD sequencing. G3-Genes Genom. Genet. 8, 1183-1194.

Bishop, S.C., Woolliams, J.A., 2014. Genomics and disease resistance studies in livestock. Livest. Sci. 166, 190–198.

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

Campbell, N.R., LaPatra, S.E., Overturf, K., Towner, R., Narum, S.R., 2014. Association mapping of disease resistance traits in rainbow trout using restriction site associated DNA sequencing. G3-Genes Genom. Genet. 4, 2473–2481.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J.H., 2011. Stacks: building and genotyping Loci de novo from short-read sequences. G3-Genes Genom. Genet. 1, 171–182.

Chen, H., Wang, C., Conomos, M.P., Stilp, A.M., Li, Z., Sofer, T., Szpiro, A.A., Chen, W., Brehm, J.M., Celedón, J.C., Redline, S., Papanicolaou, G.J., Thornton, T.A., Laurie, C.C., Rice, K., Lin, X., 2016. Control for Population Structure and Relatedness for Binary Traits in Genetic Association Studies via Logistic Mixed Models. Am. J. Hum. Genet. 98, 653–666.

Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X., Ruden, D.M., 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. Fly 6, 80–92.

Correa, K., Lhorente, J.P., López, M.E., Bassini, L., Naswa, S., Deeb, N., Di Genova, A., Maass, A., Davidson, W.S., Yáñez, J.M., 2015. Genome-wide association analysis reveals loci associated with resistance against *Piscirickettsia salmonis* in two Atlantic salmon (*Salmo salar* L.) chromosomes. BMC Genom. 16, 854.

Correa, K., Lhorente, J.P., Bassini, L., López, M.E., Di Genova, A., Maass, A., Davidson, W.S., Yáñez, J.M., 2017. Genome wide association study for resistance to *Caligus rogercresseyi* in Atlantic salmon (*Salmo salar* L.) using a 50K SNP genotyping array. Aquaculture 472, 61–65.

Dixon, P.F., Joiner, C.L., Way, K., Reese, R.A., Jeney, G., Jeney, Z., 2009. Comparison of the resistance of selected families of common carp, *Cyprinus carpio* L., to koi herpesvirus: preliminary study. J. Fish Dis. 32, 1035–1039.

FAO, 2016. FishStat Database. Available at: http://faostat.fao.org/site/629/default.Aspx

Ferdosi, M.H., Kinghorn, B.P., van der Werf, J.H.J., Lee, S.H., Gondro, C., 2014. hsphase: an R package for pedigree reconstruction, detection of recombination events, phasing and imputation of half-sib family groups. BMC Bioinform. 15, 172.

Gack, M.U., Kirchhofer, A., Shin, Y.C., Inn, K.S., Liang, C., Cui, S., Myong, S., Ha, T., Hopfner, K.P., Jung, J.U., 2008. Roles of RIG-I N-terminal tandem CARD and splice variant in TRIM25-mediated antiviral signal transduction. PNAS 105, 16743–16748.

Goldstein, H., Browne, W., Rasbash, J., 2002. Partitioning Variation in Multilevel Models. Understanding Statistics 1, 223–231.

Gupta, S., Ylä-Anttila, P., Callegari, S., Tsai, M.H., Delecluse, H.J., Masucci, M.G., 2018. Herpesvirus deconjugases inhibit the IFN response by promoting TRIM25 autoubiquitination and functional inactivation of the RIG-I signalosome. PLoS Pathogens 14, e1006852.

Gutierrez, A.P., Bean, T.P., Hooper, C., Stenton, C.A., Sanders, M.B., Paley, R.K., Rastas, P., Bryrom, M., Matika, O., Houston, R.D., 2018. A genome-wide association study for host resistance to Ostreid Herpesvirus in Pacific oysters (*Crassostrea gigas*). G3-Genes Genom. Genet. 8, 1273–1280.

Haenen, O.L.M., Way, K., Bergmann, S.M., Ariel, E., 2004. The emergence of koi herpesvirus and its significance to European aquaculture. Bull. Eur. Ass. Fish Pathol 24, 293–307.

Hedrick, R.P., Gilad, O., Yun, S., Spangenberg, J.V., Marty, G.D., Nordhausen, R.W., Kebus, M.J., Bercovier, H., Eldar, A., 2000. A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. J. Aquat. Anim. Health 12, 44–57.

Houston, R.D., Haley, C.S., Hamilton, A., Guy, D.R., Tinch, A.E., Taggart, J.B., McAndrew, B.J., Bishop, S.C., 2008. Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). Genetics 178, 1109–1115.

Houston, R.D., 2017. Future directions in breeding for disease resistance in aquaculture species. R. Bras. Zootec 46, 545–551.

Hwang, J.A., Kim, J.E., Kim, H.S., Lee, J.H., 2017. Immune Response to Koi Herpesvirus (KHV) of Koi and Koi × Red Common Carp (*Cyprinus carpio*). Dev. Reprod. 21, 361–370.

Janssen, K., Chavanne, H., Berentsen, P., Komen, H., 2017. Impact of selective breeding on European aquaculture. Aquaculture 472, 8–16.

Kocour, M., Gela, D., Rodina, M., Linhart, O., 2005. Testing of performance in common carp Cyprinus carpio L. under pond husbandry conditions I: top-crossing with Northern mirror carp. Aquacult. Res. 36, 1207–1215.

Kocour, M., Mauger, S., Rodina, M., Gela, D., Linhart, O., Vandeputte, M., 2007. Heritability estimates for processing and quality traits in common carp (*Cyprinus carpio* L.) using a molecular pedigree. Aquaculture 270, 43–50.

Kongchum, P., Sandel, E., Lutzky, S., Hallerman, E.M., Hulata, G., David, L., Palti, Y., 2011. Association between IL-10a single nucleotide polymorphisms and resistance to cyprinid herpesvirus-3 infection in common carp (*Cyprinus carpio*). Aquaculture 315, 417–421.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359.

Lourenco, D.A.L., Tsuruta, S., Fragomeni, B.O., Masuda, Y., Aguilar, I., Legarra, A., Bertrand, J.K., Amen, T.S., Wang, L., Moser, D.W., Misztal, I., 2015. Genetic evaluation using single-step genomic best linear unbiased predictor in American Angus. J. Anim. Sci. 93, 2653–2662.

Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T., Lee, D.H., 2002. BLUPF90 and related programs (BGF90), In: Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, pp. 21–22.

Misztal, I., Shogo, T., Lourenco, D., Aguilar, I., Legarra, A., Vitezica, Z., 2014. Manual for 509 BLUPF90 family of programs. Univ. Georg. Athens, USA.

Moen, T., Baranski, M., Sonesson, A.K., Kjøglum, S., 2009. Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. BMC Genomics 10, 368.

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R 2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142.

Nielsen, H.M., Ødegård, J., Olesen, I., Gjerde, B., Ardo, L., Jeney, G., Jeney, Z., 2010. Genetic analysis of common carp (*Cyprinus carpio*) strains. I: Genetic parameters and heterosis for growth traits and survival. Aquaculture 304, 14–21. Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011. Methodology for genetic evaluation of disease resistance in aquaculture species: Challenges and future prospects. Aquacult. Res. 42, 103–114.

Ødegård, J., Olesen, I., Dixon, P., Jeney, Z., Nielsen, H.M., Way, K., Joiner, C., Jeney, G., Ardó, L., Rónyai, A., Gjerde, B., 2010. Genetic analysis of common carp (*Cyprinus carpio*) strains. II: Resistance to koi herpesvirus and *Aeromonas hydrophila* and their relationship with pond survival. Aquaculture 304, 7–13.

Ødegård, J., M. Baranski, B. Gjerde, and T. Gjedrem, 2011 Methodology for genetic evaluation of disease resistance in aquaculture species: Challenges and future prospects. Aquacult. Res. 42, 103–114.

OIE, 2018. OIE-Listed diseases, infections and infestations in force in 2018. World Organization for Animal Health.

Palaiokostas, C., Kocour, M., Prchal, M., Houston, R.D., 2018a. Accuracy of genomic evaluations of juvenile growth rate in common carp (*Cyprinus carpio*) using genotyping by sequencing. Front. Genet. 9, 82.

Palaiokostas, C., Cariou, S., Bestin, A., Bruant, J. S., Haffray, P., Morin, T., Cabon, J., Allal, F., Vandeputte, M., Houston, R.D., 2018b. Genome-wide association and genomic prediction of resistance to viral nervous necrosis in European sea bass (*Dicentrarchus labrax*) using RAD sequencing. Genet. Sel. Evol. 50, 30.

Palti, Y., Vallejo, R.L., Gao, G., Liu, S., Hernandez, A.G., Rexroad III, C.E., Wiens, G.D., 2015. Detection and Validation of QTL Affecting Bacterial Cold Water Disease Resistance in Rainbow Trout Using Restriction-Site Associated DNA Sequencing. PLoS ONE 10, e0138435.

Perdry, H., Dandine-Roulland, C., 2018. gaston: Genetic Data Handling (QC, GRM, LD, PCA) & Linear Mixed Models version 1.5 from CRAN (https://cran.r-project.org).

Pérez, P., de Los Campos, G., 2014. Genome-Wide Regression and Prediction with the BGLR Statistical Package. Genetics 209.

Piačková, V., Flajšhans, M., Pokorová, D., Reschová, S., Gela, D., Čížek, A., Veselý, T., 2013. Sensitivity of common carp, *Cyprinus carpio* L., strains and crossbreeds reared in the Czech Republic to infection by cyprinid herpesvirus 3 (CyHV-3; KHV). J. Fish Dis. 36, 75–80.

Plummer, M., Best, N., Cowles, K., Vines, K., 2006. CODA: convergence diagnosis and output analysis for MCMC. R News 6, 7–11.

Pokorova, D., Reschova, S., Hullova, J., Vicenova, M., Vesely, T., Piackova, V., 2010. Detection of Cyprinid Herpesvirus-3 in field samples of common and koi carp by various single-round and nested PCR methods. J. World Aquac. Soc. 41, 773–779.

Prchal, M., Kause, A., Vandeputte, M., Gela, D., Allamelou, J.M., Girish, K., Bestin, A., Bugeon, J., Zhao, J., Kocour, M., 2018. The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. PLoS ONE 13, e0191624.

Rakus, K.Ł., Wiegertjes, G.F., Adamek, M., Siwicki, A.K., Lepa, A., Irnazarow, I., 2009. Resistance of common carp (*Cyprinus carpio* L.) to Cyprinid herpesvirus-3 is influenced by major histocompatibility (MH) class II B gene polymorphism. Fish Shellfish Immunol. 26, 737–743.

Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P., Houston, R., 2017. Applications of genotyping by sequencing in aquaculture breeding and genetics. Rev. Aquacult. 0, 1–13.

Robledo, D., Matika, O., Hamilton, A., Houston, R.D., 2018. Genome-Wide Association 556 and Genomic Selection for Resistance to Amoebic Gill Disease in Atlantic Salmon. G3-Genes Genom. Genet. 8, 1195–1203.

Sargolzaei, M., Chesnais, J.P., Schenkel, F.S., 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15, 478.

Shapira, Y., Magen, Y., Zak, T., Kotler, M., Hulata, G., Levavi-Sivan, B., 2005. Differential resistance to koi herpes virus (KHV)/carp interstitial nephritis and gill necrosis virus (CNGV) among common carp (*Cyprinus carpio* L.) strains and crossbreds. Aquaculture 245, 1–11.

Tadmor-Levi, R., Asoulin, E., Hulata, G., David, L., 2017. Studying the genetics of resistance to CyHV-3 disease using introgression from feral to cultured common carp strains. Front. Genet. 8, 24.

Taylor, N.G.H., Dixon, P.F., Jeffery, K.R., Peeler, E.J., Denham, K.L., Way, K., 2010. Koi herpesvirus: distribution and prospects for control in England and Wales. J. Fish Dis. 33, 221–230.

Vandeputte, M., 2003. Selective breeding of quantitative traits in the common carp (*Cyprinus carpio*): a review. Aquat. Living Resour. 16, 399–407.

Vandeputte, M., Kocour, M., Mauger, S., Dupont-Nivet, M., De Guerry, D., Rodina, M., Gela, D., Vallod, D., Chevassus, B., Linhart, O., 2004. Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). Aquaculture, 235, 223-–236.

Vandeputte, M., Kocour, M., Mauger, S., Rodina, M., Launay, A., Gela, D., Dupont-Nivet, M., Hulak, M., Linhart, O., 2008. Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio* L.): Heritability estimates and response to selection. Aquaculture, 277, 7–13.

VanRaden, P.M., 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91, 4414–4423.

Wang, H., Misztal, I., Aguilar, I., Legarra, A., Muir, W.M., 2012. Genome-wide association mapping including phenotypes from relatives without genotypes. Genet. Res. 94, 73–83.

Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y., Xu, J., Zheng, X., Ren, L., Wang, G., 2014. Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. Nat. Genet. 46, 1212–1219.

Zhou, T., Liu, S., Geng, X., Jin, Y., Jiang, C., Bao, L., Yao, J., Zhang, Y., Zhang, J., Sun, L., 2017. GWAS analysis of QTL for enteric septicemia of catfish and their involved genes suggest evolutionary conservation of a molecular mechanism of disease resistance. Mol. Genet. Genomics 292, 231–242.

CHAPTER 7

1. GENERAL DISCUSSION

Amur mirror carp (AM) were chosen for studying the potential of selective breeding approaches in improving most performance traits in common carp under pond management conditions. Accordingly, overwintering performance (winter survival, lipid deposition, loss of lipid and change in growth), and its link to the market size traits was studied (Chapter 2). The same batch of fish was also used to study i) the potential of morphological predictors in genetic improvement of carp slaughter yields (Chapter 3) and ii) genomic mapping for deeper insight into the potential of marker-assisted (MAS) and genomic (GS) selection in growth and resistance to KHV on juvenile common carp (Chapter 5, 6). In addition, Hungarian synthetic mirror carp (HSM) were used for studying the genetic parameters of the composition of fatty acids and their relation to production traits at market size (Chapter 4).

1.1. GENETIC VARIATION

Genetic variation of all analysed traits across all studies was also estimated using additional random effect common to dams (non-genetic maternal effect) to assess the impact of maternal effect on heritability estimates. However, this effect was negligible for all traits similarly as in the previous experiments where early communal rearing of common carp families was applied (Vandeputte et al., 2008; Ninh et al., 2011). However, sex and mating had significant effect on several traits, and were involved in the genetic model when applicable. It was observed that most performance traits (including survival and resistance to KHV) across experiments showed sufficient heritability in Amur mirror carp (Chapters 2,3,5 and 6) and Hungarian synthetic mirror carp (Chapter 4) with a clearly increasing pattern over the studied age of the fish (Chapter 2). In general, our estimates were in the upper range when compared to the previous studies made on various breeds, strains and lines of common carp under various rearing and climatic conditions (Vandeputte et al., 2004, 2008; Kocour et al., 2007; Nielsen et al., 2010; Ødegård et al., 2010; Ninh et al., 2011; Dong et al., 2015; Nguyen, 2016; Hu et al., 2017; Tadmor-Levi et al., 2017), suggesting interesting potential for the genetic improvement of most performance traits via selective breeding. Moreover, genetic variation of performance traits in common carp is on the same level or even higher in comparison with other fish species (Vandeputte, 2003; Gjedrem and Baranski, 2009; Wang, 2009; Gjedrem et al., 2012; Gjedrem and Robinson, 2014; Nguyen, 2016). However, carp selection programs are still at the beginning unlike other fish species in which selection for growth performance, processing yield, product quality and disease resistance is commonly applied (Chavanne et al., 2016; Janssen et al., 2017). In the past, Vandeputte et al. (2008), Ninh et al. (2013) and Dong et al. (2015) applied mass selection for growth in common carp and reported response to upward selection (7% – 21.4%). Likewise, considerable selection response was also reported for survival to dropsy (Ilyassov, 1987; Kirpichnikov et al., 1993) and furunculosis (Schäperclaus, 1962). Thus, selective breeding in common carp could be successful.

Based on the results, the optimal stage for selection of several performance traits under Central European climatic conditions seems to be after the second overwintering. At this stage, the fish are still small enough for easy handling and short-term storage, and in particular, there is a high genetic correlation between traits at this stage and traits at market size (e.g. body weight, fat content, condition factor). This is important as it shows solid potential for a successful selection program in common carp. However, this issue should be under further research interests in order to verify our conclusion after applied selection through realized heritability and real genetic gain.

1.2. OVERWINTERING PERFORMANCE AND ITS IMPACT ON TRAITS AT HARVEST

Winter is known to be a critical period in the production of common carp (Bauer and Schlott, 2004). Each winter, around 10-50% fish die under commercial production conditions. This causes significant economic losses to producers and reduces animal welfare (Horváth et al., 1992). Common carp use lipid stores to maintain their body functions, and thus lipids are taken as essential reserves for winter survival (Steffens, 1996). Thus, the main research item was to verify if a certain level of muscle fat and its dynamics is also genetically related to the winter survival of carp.

We found that muscle fat content before overwintering is negatively correlated to winter survival (r_g = -0.59), and thus selecting for higher muscle fat before winter would lead to lower survival. This is contradictory to our original hypothesis and also to previous observations in which fish with less energy reserves before overwintering burn those reserves more rapidly and this results in higher mortalities in common carp (Steffens, 1996) as well as other fish species (Sogard and Olla, 2000). Nevertheless, it was previously observed in some studies that larger/fatter individuals of rainbow trout, unlike smaller/leaner ones, consumed more of their lipid reserves than predicted by standard metabolic allometry (Biro et al., 2004). We observed that heavier fish before winter were slightly fatter (r_p = 0.28) and that selection for heavier fish would lead to a slight increase in muscle fat content (r_g = 0.32). However, phenotypic correlations between muscle fat before winter and winter fat change (both in absolute and relative values) were negative. Thus, it also shows that fatter fish burned their lipid reserves more than leaner ones. Accordingly, it is assumed that due to the mild winter fish were more active than normally, they needed more energy, and thus they looked more for food as seen from the slight weight gain after the winter. Similar winter foraging behaviour was observed by Bauer and Schlott (2004) in three-year-old carp. Nevertheless, it should be pointed that our findings might be relevant only for the mild winter conditions that appeared during the study. On the other hand, milder winter periods may be expected more often in Europe in the future due to climate changes (IPCC, 2014).

Likewise, other results support the negative effect of high muscle lipid level on overwintering performance. Selection on higher muscle fat content before winter may lead to spending more muscle fat during winter in absolute value together with lower winter growth (lower SGR). Accordingly, the results showed that selection for lower decreases in weight and muscle fat content may lead to better winter performance similarly as in previous studies (Bernard and Fox, 1997; Pratt and Fox, 2002). As already mentioned, during a mild winter, leaner fish may perform better, so they can maintain their lipid stores and weight more effectively. A similar strategy of leaner fish was also reported in Atlantic salmon (Johansen et al., 2002). Conversely, fatter fish without stimulation to look for food would be disadvantaged during a mild winter and, due to higher metabolic activity, they would lose more lipid stores and body weight, which may affect their winter survival. In any case, our research points to the fact that fish may be able to identify their lipid reserves status, termed as lipostatic regulation (Thompson et al., 1991; Jobling and Johansen, 1999; Johansen et al., 2002; Ali et al., 2003; Eckmann, 2004; Bell, 2012; Brodersen et al., 2014) and that being fatter does not necessarily mean having a greater chance of surviving the winter period.

Interestingly, a similar negative genetic correlation was also found between muscle fat after winter and survival during the last growing season. It may be concluded that while a certain lipid level plays an important role in several biological functions of fish (Steffens, 1996; Ali et al., 2003; Tocher, 2003; Kause et al., 2016), a lipid excess may have a contradictory negative effect for further survival. Likewise, selection leading to increasing of the level of muscle fat could also negatively influence flesh fatty acid composition as observed in Chapter 4.

With regard to survival and other traits, the condition factor (FC) may be an interesting selection trait. Selecting for higher FC in the second spring would lead to an increase in i) growth (SGR) and survival during the third growing period and ii) weight at harvest. A similar positive correlation between FC and harvest weight was observed by Haffray et al. (2012) in rainbow trout and European seabass (Saillant et al., 2009). Furthermore, FC could be also a valuable selection trait for increasing the yield of edible parts as seen from the genetic correlations in Chapter 4. Likewise, SGR during overwintering was positively genetically correlated to harvest weight (r_g = 0.62) and condition factor (r_g = 0.54). Accordingly, Hu et al. (2017) observed that selection for lower relative weight loss during winter should gradually enhance growth performance in the successive growing period. On the other hand, selection for higher SGR would also result in a slight decrease in muscle fat content and slaughter yields. A similar trend with muscle fat and slaughter yields would be visible when selecting for higher absolute fat change during winter (lower decrease or slight increase). A possible explanation is that fish which lost more muscle fat during winter compensated the muscle fat during the growing season. In addition, higher muscle fat content also negatively affected the growth (protein biosynthesis; Kause et al., 2016) but had slight positive impact on slaughter yields. However, if selection for winter SGR were in the breeder's interests, the negative effect with dress-out yields may be overcome by the use of multitrait selection , for instance, predictors of edible part yields similarly as in rainbow trout (Kause et al., 2007; Haffray et al., 2013), European seabass (Vandeputte et al., 2017), and common carp (Chapter 3).

Our observations could contribute towards enhancing overwintering performance in common carp. However, due to the warmer winter in this study a repeat of the study design under different winter conditions could bring additional information that may lead to a deeper insight into the genetics of overwintering including genotype by environment interactions.

1.3. SLAUGHTER YIELD PREDICTORS

Though important performance traits in common carp could be genetically improved as seen above, genetic improvement of slaughter yields is much trickier. It can be done only indirectly using either sib selection based on values of slaughtered fish, or selection on correlated traits recorded *in vivo* (Kause et al., 2007; Gjedrem and Rye, 2016). Morphological predictors that can be measured on live fish and that correlate with real slaughter yields hence remain another attractive alternative (Haffray et al., 2013; Vandeputte et al., 2017). As a result, external (phenotyping, 2D imaging) and internal measurements (ultrasound imagery) were combined by linear regression to predict log-log residuals (Logr) of slaughter yields (headless carcass and fillet yields) in common carp (Chapter 3). We observed that phenotypic prediction was high for headless carcass yield (R^2 = 0.63) and intermediate for fillet yield (R^2 = 0.49). Our predictions were generally higher when compared to similar-constructed studies on rainbow trout (Haffray et al., 2013) and European seabass (Vandeputte et al., 2017). Hence, slaughter yields may be solidly predicted on live breeding candidates in common carp. In addition, the headless carcass is both easier and more accurately predicted, and strongly phenotypically and genetically correlated to fillet yields (both in real and predicted form). Therefore, the headless carcass may be recommended as a trait to be predicted to select for improved fillet yields.

From the genetic point of view, slaughter yields expressed as log-log residuals achieved high heritability estimates (0.46 – 0.50). Interestingly, heritability estimates of predicted yields were higher (0.48 – 0.63) in comparison with the previous heritability estimates of predicted slaughter yields (Van Sang et al., 2012; Haffray et al., 2013; Vandeputte et al., 2017). The

results show that there is a good possibility of using predicted yields as an indirect selection criterion. This is also supported by high genetic correlations between Logr and predicted yields (0.84 – 0.88). Likewise, some individual predictors, of which the prediction models were constructed, were highly heritable and favourably correlated to the Logr slaughter yields. Hence, it suggests their alternative application for slaughter yield improvement. The best predictor seems to be the ratio between the ultrasound measurement of abdominal thickness (E8) and external belly height (2D points), that was highly positively associated to Logr yields (r_g = 0.76 – 0.83). Similarly, in rainbow trout (Haffray et al., 2013), the ratio between the ultrasound measurements E8 and E23 (depth of body cavity) was recognized as an efficient slaughter yield predictor. However, in our case, 2D digitization of morphological landmarks is time-consuming work (approximately one month in this study) that should be done by the same person to eliminate the individual effect during point picking. Therefore, we could also indicate just the simple ultrasound measurements as other potential predictors that were both highly inherited and correlated to Logr edible part yields. The high potential of using yield predictors in genetic improvement is also seen from the expected genetic gains. Both predicted yields and individual predictors achieve almost the same genetic gain in comparison to hypothetical mass selection, and even higher gain in comparison to gains obtained by sib selection. Thus, selection on trait predictors has an interesting perspective for *in vivo* genetic improvement of slaughter yields that will become more and more actual with the increasing rate of common carp sold in processed form. However, our study was done only on market size Amur mirror carp. Therefore, it raises further research question for phenotypic and genetic validation of the predictors before their utilization on other carp breeds, strains, lines, and size categories. Moreover, real response to selection, focused on indirect improvement of slaughter yields, would show true impact of predictors in a carp breeding program.

1.4. SELECTIVE BREEDING AND CHANGE IN FLESH QUALITY

Carp flesh is an important source of omega-3 polyunsaturated fatty acids (n-3 PUFAs, mainly eicosapentaenoic acid – EPA and docosahexaenoic acid – DHA) with a favourable ratio of omega-6/omega3 PUFA ratio (n-6/n-3) and thus, it is part of a healthy human diet (Mráz et al., 2012ab). Unfortunatelly, current carp pond management depends on cereal feeding that worsens the fatty acid (FA) composition of carp flesh. Therefore, high attention has been paid to the improvement of FA content in the flesh of common carp through nutrition (Mráz et al., 2012ab; Steffens, 2016). Nonetheless, data has been missing about the genetic variation of market size common carp FA composition and its relation to performance traits. To fill this gap we focused on this problem in Chapter 4. Despite a potential limitation when a relatively small sample size was used (n = 158), moderate and significant heritability estimates were observed (h_2 = 0.24 – 0.47) for several FA groups presented as relative values (e.g. PUFA, n-3 PUFA, EPA, n-6/n-3) and moderate (muscle fat, condition factor, slaughter yields) to high (body weight) for performance traits (h^2 = 0.23 – 0.62). Results in other fish differ. In Nile tilapia low and insignificant heritability was found in FA groups (Nguyen et al., 2010). Conversely, high heritability was found in the flesh for n-3 PUFAs (relative value) in Atlantic salmon (Leaver et al., 2011), and for EPA and DHA in rainbow trout (Overturf et al., 2013).

Genetic correlations among FA groups and performance traits seemed to be much interesting information. Our results across all studies show that performance traits (e.g. body weight, slaughter yields, condition factor, muscle fat) may be improved by selection. However, such selection would also most likely lead to worse flesh quality as the relative amount of n-3 PUFAs would decrease and the n-6/n-3 PUFA ratio would increase. This is in accordance with

similar unfavourable phenotypic and genetic correlations observed between performance traits (growth-related traits, fillet weight and yield) and EPA, n-3 PUFA and n-3/n-6 ratio in Atlantic salmon and Nile tilapia (Nguyen et al., 2010; Leaver et al., 2011). It indicates that the flesh quality of farmed fish may shift towards an unfavourable direction when selection for improved performance is applied (Janhunen et al., 2017). The reason for this effect in common carp is likely the fact that better performed fish feed more on supplemental feeds (wheat, barley, triticale). It was proven several times that the fatty acid profile of fish feed significantly affects the final composition of FA composition in fish flesh (Mráz and Pickova, 2011; Marković et al., 2016; Trbović et al., 2017). The supplemental feeds lack n-3 PUFAs, and this makes the FA profile in fish of lower quality – lower level of n-3 PUFAs, higher n-6/n-3 ratio (Mráz et al., 2012b). On the other hand, natural food (zooplankton and zoobenthos) is rich in n-3 PUFAs (Horváth et al., 1992). Weight gain of common carp should be created by natural food and supplemental feeding in a ratio of 1:1 in order to sustain unchanged carp flesh quality. It is unclear whether genetically improved carp stocks could also utilize natural food more effectively than unimproved stocks. If not, fish would require more supplemental feeding, leading to a decrease in flesh quality. It seems that selection for better growth will require modification of the feeding strategy. Such a diet should be composed of EPA and DHA precursors (rapeseed, linseed or hempseed) during the last growing season before reaching market size (Mráz et al., 2012ab). Then, the carp stocks could positively respond to selection for better performance (body weight, slaughter yields) and to keep high quality flesh within a sustainable selection program.

Correlations between growth-related traits and carp flesh quality show that a change in breeding strategy without a complex approach to the whole production cycle and product quality may have unfavourable consequences. Good examples may be seen in worsening of the flesh quality in cattle (Feitosa et al., 2017) or loss of flavour in tomatoes (Tieman et al., 2017). Moreover, the cost-effectivity of carp farming depends on the carp biomass yield per area unit of a pond. The biomass yield is essentially linked to the mutual relationship among natural productivity of the pond (natural food), the stocking density of fish and the average weight gain of fish (Horváth et al., 1992; Kocour et al., 2007; Vandeputte et al., 2008). Thus, this phenomenon must be taken into account when setting up the carp production technology when selective breeding approaches are applied. Therefore, it would be valuable to analyse the fatty acid profile of carp the next generation after selection and to verify the sustainability of selective breeding. In addition, future research could focus on genotype by nutrition interactions that could also contribute towards adjusting production technology of genetically improved carp stocks.

1.5. MODERN GENOMIC TOOLS

The studies presented above showed a relatively solid view to selective breeding strategy in common carp under pond conditions. However, modern genomic approaches may give an even deeper insight into the potential of genetic variation in performance traits. Thus, genetic markers can be valuable tools for modern breeding methods but the optimal strategy for their application depends on the underlying genetic architecture of the trait of interest. Thus, if variation of any trait is significantly related to only one or several QTLs or candidate genes, marker-assisted selection (MAS) may be effective. Otherwise, genomic selection (GS) is another possible tool for utilizing genetic information for the improvement of traits (Sonesson, 2007; Yáñez et al., 2015; Robledo et al., 2017). The utilization of restriction-site-associated DNA sequencing (RAD-seq; Baird et al., 2008) for growth improvement (Chapter 5)

and Koi herpesvirus (KHV) resistance (Chapter 6) in common carp was investigated. RAD-seq generated SNP markers (single nucleotide polymorphism) that enabled pedigree assignment, genetic parameter estimation, construction of a medium density genetic map (12,311 SNPs) and for a first time estimation of effectivity of genomic selection.

It was found that juvenile growth (Chapter 5) is of polygenic genetic architecture (no significant QTL – quantitative trait loci). However, genomic prediction of breeding values outperformed pedigree-based prediction, resulting in an 18% improvement in prediction accuracy in genetic improvement in common carp breeding programs. Such a result point to the potential for GS to improve economically important traits in common carp breeding programs. In previous studies, several growth-related QTLs have been detected (Lv et al., 2016; Peng et al., 2016; Wang et al., 2018) but none of them was significant enough to be used in MAS. Similarly, no major QTLs for feed conversion (Lu et al., 2017) and muscle fat content (Zheng et al., 2016) have been found until now.

Regarding the resistance to KHV (Chapter 6), heritability measured by survival on the underlying scale was very high (0.61 – pedigree; 0.50 – genomic) but in accordance to the previous estimates (Ødegård et al., 2010; Tadmor-Levi et al., 2017). In addition, significant QTL affecting resistance to KHV was identified on linkage group (LG) 44. The QTL explains approximately 7% of the additive genetic variance. Conversely, the majority of genetic variation of resistance to the infectious pancreatic necrosis (IPN) virus in Atlantic salmon was explained by a single QTL (Houston et al., 2008; Moen et al., 2009).

Hence, it seems that genetic improvement of investigated traits via simple marker-assisted selection would be most likely less efficient. Thus, genomic selection may be a more promising breeding method for exploiting overall genetic variation of traits under polygenic control (Sonesson, 2007; Yáñez et al., 2015; Robledo et al., 2017). Interestingly, using pooled whole genome resequencing of a subset of resistant (n = 60) and susceptible (n = 60) animals, a putative premature stop mutation in position 258 of the carp TRIM25 protein (Gack et al., 2008) was identified within the same QTL region of LG 44. The premature stop causing allele was more common in susceptible fish. This may fit with a loss of function of TRIM25 in susceptible fish, being unable to trigger an appropriate antiviral response.

1.6. CONCLUSIONS AND FUTURE PROSPECTS

The results presented in this Ph.D. thesis support and extend recent knowledge concerning the potential of selective breeding in common carp and show more complex insight into relationships among various traits during the whole production cycle of common carp under semi-intensive pond conditions. In addition, the thesis proposes some strategies for introducing novel findings into the breeding program of common carp. The specific conclusions are as follows:

- Heritability estimates of most investigated traits were high enough to propose genetic improvement of such traits by selective breeding.
- Muscle fat content plays an important role in the biological functions of common carp during winter and growing period. Surprisingly, selection for i) lower fat content before and after winter, ii) lower decrease in muscle fat content and/or body weight during winter, may both lead to better survival and growth of fish during the third growing season.
- Selection for higher condition factor (after winter – Chapter 2 or at harvest – Chapter 4) may result in better performance during the winter, and mainly during the third growing

season and at market size. However, it should be remembered that this would lead to a slow change in the appearance of fish to a less favourable square-like body shape.
- There is potential for genetic improvement in processing yields through *in vivo* indirect selection (Chapter 3).
- Genetic improvement in growth via selective breeding under Central European pond conditions without changing production technology would very likely negatively affect carp flesh quality with respect to FA composition (Chapter 4).
- Genomic data analysed by restriction-site-associated DNA sequencing (RAD-seq) did not reveal any significant QTL for growth (Chapter 5), but genomic prediction of its breeding value may outperform traditional pedigree-based prediction. On the other hand, 7% of additive genetic variation in KHV resistance (Chapter 6) may be explained by QTL located on LG 44. In this case the TRIM25 gene was found as a promising positional and functional candidate within the QTL region. Nonetheless, construction of a SNP array is likely the only possibility for prediction of KHV resistance in carp individuals.

Although the obtained results are interesting and in most cases unique and useful for common carp breeding, potential weaknesses might be seen in a relatively smaller (one generation) data set in comparison to studies performed previously on larger (multigenerational) data sets e.g. in rainbow trout, tilapia or Atlantic salmon. Therefore, future prospects should be mainly focused on verification of the accuracy of genetic parameters by calculation of realized heritabilities, real genetic gains and total fish biomass production from a pond area unit of carp stocks established through selective breeding. In addition, results of this Ph.D. thesis have raised further possibilities for optimizing the future breeding program of common carp. Hence, the genetics and breeding strategy enhancing overwintering performance should be verified by a new study under different winter conditions. Likewise, the efficiency of slaughter yield predictors should be validated on other carp strains with a different body shape. Furthermore, as predictors combine several sources of information, further information on the resulting responses to selection would be valuable in the future to quantify the expected progress. Another important area of further research should also concern modification of rearing technology in order to maximally utilize the advantages of selective breeding in common carp production, to maintain or improve the product quality with respect to fatty acid composition and to set up processes in broodstock management to minimize inbreeding.

REFERENCES

Ali, M., Nicieza, A., Wootton, R.J., 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish. 4, 147–190.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE 3, e3376.

Bauer, C., Schlott, G., 2004. Overwintering of farmed common carp (*Cyprinus carpio* L.) in the ponds of a central European aquaculture facility — measurement of activity by radio telemetry. Aquaculture 241, 301–317.

Bell, R.J., 2012. Winter feeding as an overwintering survival strategy in young-of-the-year winter flounder. Trans. Am. Fish. Soc. 141, 855–871.

Bernard, G., Fox, M.G., 1997. Effects of body size and population density on overwinter survival of age-0 pumpkinseeds. N. Am. J. Fish. Manage. 17, 581–590.

Biro, P.A., Morton, A.E., Post, J.R., Parkinson, E.A., 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 61, 1513–1519.

Brodersen, J., Chapman, B.B., Nilsson, P.A., Skov, C., Hansson, L.A., Brönmark, C., 2014. Fixed and flexible: Coexistence of obligate and facultative migratory strategies in a freshwater fish. PLoS ONE 9, e90294.

Dong, Z., Nguyen, N.H., Zhu, W., 2015. Genetic evaluation of a selective breeding program for common carp *Cyprinus carpio* conducted from 2004 to 2014. BMC Genet. 16, 94

Eckmann, R., 2004. Overwinter changes in mass and lipid content of *Perca fluviatilis* and *Gymnocephalus cernuus*. J. Fish Biol. 65, 1498–1511.

Feitosa, F.L.B., Olivieri, B.F., Aboujaoude, C., Pereira, A.S.C., de Lemos, M.V.A., Chiaia, H.L.J., Berton, M.P., Peripolli, E., Ferrinho, A.M., Mueller, L.F., Mazalli, M.R., de Albuquerque, L.G., de Oliveira, H.N., Tonhati, H., Espigolan, R., Tonussi, R.L., de Oliveira Silva, R.M., Gordo, D.G.M., Magalhães, A.F.B., Aguilar, I., Baldi, F., 2017. Genetic correlation estimates between beef fatty acid profile with meat and carcass traits in Nellore cattle finished in feedlot. J. Appl. Genet. 58, 123–132.

Gack, M.U., Kirchhofer, A., Shin, Y.C., Inn, K.S., Liang, C., Cui, S., Myong, S., Ha, T., Hopfner, K.P., Jung, J.U., 2008. Roles of RIG-I N-terminal tandem CARD and splice variant in TRIM25-mediated antiviral signal transduction. PNAS 105, 16743–16748.

Gjedrem, T., Baranski, M., 2009. Selective Breeding in Aquaculture: an Introduction. Springer, Dordrecht, The Netherlands, pp. 221.

Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. Aquaculture 350, 117–129.

Gjedrem, T., Robinson, N., 2014. Advances by selective breeding for aquatic species: a review. Agricult. Sci. 5, 1152.

Gjedrem, T., Rye, M., 2016. Selection response in fish and shellfish: a review Reviews in Aquaculture 0, 1–12.

Haffray, P., Bugeon, J., Pincent, C., Chapuis, H., Mazeiraud, E., Rossignol, M.N., Chatain, B., Vandeputte, M., Dupont-Nivet, M., 2012. Negative genetic correlations between production traits and head or bony tissues in large all-female rainbow trout (*Oncorhynchus mykiss*). Aquaculture 368, 145–152.

Haffray, P., Bugeon, J., Rivard, Q., Quittet, B., Puyo, S., Allamelou, J.M., Vandeputte, M., Dupont-Nivet, M., 2013. Genetic parameters of in-vivo prediction of carcass, head and fillet yields by internal ultrasound and 2D external imagery in large rainbow trout (*Oncorhynchus mykiss*). Aquaculture 410–411, 236–244.

Horváth, L., Tamás, G., Seagrave, C., 1992. Carp and pond fish culture including Chinese herbivorous species, pike, tench, zander, wels, catfish and goldfish. Oxford, Fishing News Books Ltd, pp. 170.

Houston, R.D., Haley, C.S., Hamilton, A., Guy, D R., Tinch, A.E., Taggart, J.B., McAndrew B.J., Bishop, S.C., 2008. Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar).* Genetics 178, 1109–1115.

Hu, X., Li, C., Shang, M., Ge, Y., Jia, Z., Wang, S., Zhang, Q., Shi, L., 2017. Inheritance of growth traits in Songpu mirror carp (*Cyprinus carpio* L.) cultured in Northeast China. Aquaculture 477, 1–5.

Chavanne, H., Janssen, K., Hofherr, J., Contini, F., Haffray, P., Komen, H., Nielsen, E.E., Bargelloni, L., 2016. A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry. Aquacult. Int. 24, 1287–1307.

Ilyassov, Y.L., 1987. Genetic principles of fish selection for disease resistance. In: Tiewes K (ed.) Selection, Hybridization and Genetic Engineering in Aquaculture. I, Heenemann Verlagsgegesellshaft, Berlin, Germany, pp. 455–469.

IPCC. 2014. Climate Change: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (Eds]. IPCC, Geneva, Switzerland, pp. 151.

Janhunen, M., Nousiainen, A., Koskinen, H., Vehviläinen, H., Kause, A., 2017. Selection strategies for controlling muscle lipid content recorded with a non-destructive method in European whitefish, *Coregonus lavaretus*. Aquaculture 481, 229–238.

Janssen, K., Chavanne, H., Berentsen, P., Komen, H., 2017. Impact of selective breeding on European aquaculture. Aquaculture 472, 8–16.

Jobling, M., Johansen, S., 1999. The lipostat, hyperphagia and catch-up growth. Aquacult. Res. 30, 473–478.

Johansen, S., Ekli, M., Jobling, M., 2002. Is there lipostatic regulation of feed intake in Atlantic salmon Salmo salar L.? Aquacult. Res. 33, 515–524.

Kause, A., Paananen, T., Ritola, O., Koskinen, H., 2007. Direct and indirect selection of visceral lipid weight, fillet weight, and fillet percentage in a rainbow trout breeding program. J. Anim. Sci. 85, 3218–3227.

Kause, A., Kiessling, A., Martin, S.A., Houlihan, D., Ruohonen, K., 2016. Genetic improvement of feed conversion ratio via indirect selection against lipid deposition in farmed rainbow trout (*Oncorhynchus mykiss* Walbaum). Br. J. Nutr. 116, 1656–1665.

Kirpichnikov, V.S., Ilyasov, Ju. I., Shart, L.A., Vikhman, A.A., Ganchenko, M.V., Ostashevsky, A. L., Simonov, V.M., Tikhonov, G.F., Tjurin, V.V., 1993. Selection of Krasnodar common carp (*Cyprinus carpio* L.) for resistance to dropsy: principal results and prospects. Aquaculture 111, 7–20.

Kocour, M., Mauger, S., Rodina, M., Gela, D., Linhart, O., Vandeputte, M., 2007. Heritability estimates for processing and quality traits in common carp (*Cyprinus carpio* L.) using a molecular pedigree. Aquaculture 270, 43–50.

Leaver, M.J., Taggart, J.B., Villeneuve, L., Bron, J.E., Guy, D.R., Bishop, S.C., Houston, R.D., Matika, O., Tocher, D.R., 2011. Heritability and mechanisms of n–3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. Comp. Biochem. Physiol. Part D Genomics Proteomics 6, 62–69.

Lu, C., Laghari, M.Y., Zheng, X., Cao, D., Zhang, X., Kuang, Y., Li, C., Cheng, L., Mahboob, S., Al-Ghanim, K.A., Wang, S., Wang, G., Sun, J., Zhang, Y., Sun, X., 2017. Mapping quantitative trait loci and identifying candidate genes affecting feed conversion ratio based onto two linkage maps in common carp (*Cyprinus carpio* L). Aquaculture 468, 585–596.

Lv, W., Zheng, X., Kuang, Y., Cao, D., Yan, Y., Sun, X., 2016. QTL variations for growth-related traits in eight distinct families of common carp (*Cyprinus carpio*). BMC Genet. 17, 65.

Marković, Z., Stanković, M., Rašković, B., Dulić, Z., Živić, I., Poleksić, V., 2016. Comparative analysis of using cereal grains and compound feed in semi-intensive common carp pond production. Aquacult. Int. 24, 1699–1723.

Moen, T., Baranski, M., Sonesson, A. K., Kjøglum, S., 2009. Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. BMC Genom. 10, 368.

Mráz, J., Pickova, J., 2011. Factors influencing fatty acid composition of common carp (*Cyprinus carpio*) muscle. Neuroendocrinol. Lett. 32, 3-8.

Mráz, J., Zajíc, T., Pickova, J., 2012a. Culture of common carp (*Cyprinus carpio*) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy. Neuroendocrinol. Lett. 33, 60–67.

Mráz, J., Máchová, J., Kozák, P., Pickova, J., 2012b. Lipid content and composition in common carp - optimization of n-3 fatty acids in different pond production systems. J. Appl. Ichthyol. 28, 238–244.

Nguyen, N.H., Ponzoni, R.W., Yee, H.Y., Abu-Bakar, K.R., Hamzah, A., Khaw, H.L., 2010. Quantitative genetic basis of fatty acid composition in the GIFT strain of Nile tilapia (*Oreochromis niloticus*) selected for high growth. Aquaculture 309, 66–74.

Nguyen, N.H., 2016. Genetic improvement for important farmed aquaculture species with a reference to carp, tilapia and prawns in Asia: achievements, lessons and challenges. Fish Fish. 17, 483–506.

Nielsen, H.M., Ødegård, J., Olesen, I., Gjerde, B., Ardo, L., Jeney, G., Jeney, Z., 2010. Genetic analysis of common carp (*Cyprinus carpio*) strains. I: Genetic parameters and heterosis for growth traits and survival. Aquaculture 304, 14–21.

Ninh, N.H., Ponzoni, R.W., Nguyen, N.H., Woolliams, J.A., Taggart, J.B., McAndrew, B.J., Penman, D.J., 2011. A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): estimation of genetic parameters. Aquaculture 322–323, 39–46.

Ødegård, J., Olesen, I., Dixon, P., Jeney, Z., Nielsen, H.M., Way, K., Joiner, C., Jeney, G., Ardó, L., Rónyai, A., Gjerde, B., 2010. Genetic analysis of common carp (*Cyprinus carpio*) strains. II: Resistance to koi herpesvirus and *Aeromonas hydrophila* and their relationship with pond survival. Aquaculture 304, 7–13.

Overturf, K., Welker, T., Barrows, F., Towner, R., Schneider, R., LaPatra, S., 2013. Variation in rainbow trout, *Oncorhynchus mykiss*, to biosynthesize eicosapentaenoic acid and docosahexaenoic acid when reared on plant oil replacement feeds. J. World Aquacult. Soc. 44, 326–337.

Peng, W., Xu, J., Zhang, Y., Feng, J., Dong, C., Jiang, L., Feng, J., Chen, B., Gong, Y., Chen, L., Xu, P., 2016. An ultra-high density linkage map and QTL mapping for sex and growth-related traits of common carp (*Cyprinus carpio).* Sci. Rep. 6, 26693.

Pratt, T.C., Fox, M.G., 2002. Influence of predation risk on the overwinter mortality and energetic relationships of young-of-year walleyes. Trans. Am. Fish. Soc. 131, 885–898.

Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P., Houston, R., 2017. Applications of genotyping by sequencing in aquaculture breeding and genetics. Rev. Aquacult. 0, 1–13.

Saillant, E., Dupont-Nivet, M., Sabourault, M., Haffray, P., Laureau, S., Vidal, M.-O., Chatain, B., 2009. Genetic variation for carcass quality traits in cultured sea bass (*Dicentrarchus labrax*). Aquat. Liv. Resour. 22, 105–112.

Schäperclaus, W., 1962. Trate de Pisciculture en Etang. Vigot Freres, Paris 208, 208–227.

Sogard, S.M., Olla, B.L., 2000. Endurance of simulated winter conditions by age-0 walleye pollock: effects of body size, water temperature and energy stores. J. Fish Biol. 56, 1–21.

Sonesson, A.K., 2007. Within-family marker-assisted selection for aquaculture species. Genet. Sel. Evol. 39, 301.

Steffens, W., 1996. Protein sparing effect and nutritive significance of lipid supplementation in carp diets. Arch. Tierenahr. 49, 93–98.

Steffens, W., 2016. Aquaculture produces wholesome food: cultured fish as a valuable source of n-3 fatty acids. Aquacult. Int. 24, 787–802.

Tadmor-Levi, R., Asoulin, E., Hulata, G., David, L., 2017. Studying the genetics of resistance to CyHV-3 disease using introgression from feral to cultured common carp strains. Front. Genet. 8, 24.

Tieman, D., Zhu, G., Resende, M.F., Lin, T., Nguyen, C., Bies, D., Rambla, J.L., Beltran, K.S.O., Taylor, M., Zhang, B., Ikeda, H., Liu, Z., Fisher, J., Zemach, I., Monforte, A., Zamir, D., Granell, A., Kirst, M., Huang, S., Klee, H., 2017. A chemical genetic roadmap to improved tomato flavor. Science 355, 391–394.

Thompson, J.M., Bergersen, E.P., Carlson, C.A., Kaeding, L.R., 1991. Role of size, condition, and lipid content in the overwinter survival of age– 0 Colorado Squawfish. Trans. Am. Fish. Soc. 120, 346–353.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184.

Trbović, D., Živić, I., Stanković, M., Živić, M., Dulić, Z., Petronijević, R., Marković, Z., 2017. Dependence of the common carp (*Cyprinus carpio* L.) fatty acid profile on diet composition in a semi-intensive farming system: tissue and time variability. Aquacult. Res 48, 3121–3133.

Van Sang, N., Klemetsdal, G., Ødegård, J., Gjøen, H.M., 2012. Genetic parameters of economically important traits recorded at a given age in striped catfish (*Pangasianodon hypophthalmus*). Aquaculture 344–349, 82–89.

Vandeputte, M., 2003. Selective breeding of quantitative traits in the common carp (*Cyprinus carpio*): a review. Aquat. Living Resour. 16, 399–407.

Vandeputte, M., Kocour, M., Mauger, S., Dupont-Nivet, M., De Guerry, D., Rodina, M., Gela, D., Vallod, D., Chevassus, B., Linhart, O., 2004. Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). Aquaculture 235, 223–236.

Vandeputte, M., Kocour, M., Mauger, S., Rodina, M., Launay, A., Gela, D., Dupont-Nivet, M., Hulak, M., Linhart, O., 2008. Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio* L.): Heritability estimates and response to selection. Aquaculture 277, 7–13.

Vandeputte, M., Puledda, A., Tyran, A.S., Bestin, A., Coulombet, C., Bajek, A., Baldit, G., Vergnet, A., Allal, F., Bugeon, J., Haffray, P., 2017. Investigation of morphological predictors of fillet and carcass yield in European sea bass (*Dicentrarchus labrax*) for application in selective breeding. Aquaculture 470, 40–49.

Wang, C., 2009. Quantitative genetic estimates of growth-related traits in the common carp (*Cyprinus carpio* L.): A review. Front. Biol. China 4, 298–304.

Wang, X., Fu, B., Yu, X., Qu, C., Zhang, Q., Tong, J., 2018. Fine mapping of growth-related quantitative trait loci in Yellow River carp (*Cyprinus carpio haematoperus*). Aquaculture 484, 277–285.

Yáñez, J.M., Newman, S., Houston, R.D., 2015. Genomics in aquaculture to better understand species biology and accelerate genetic progress. Front. Genet. 6, 128.

Zheng, X., Kuang, Y., Lv, W., Cao, D., Sun, Z., Sun, X., 2016. Genome-wide association study for muscle fat content and abdominal fat traits in common carp (*Cyprinus carpio*). PLoS ONE 11, e0169127.

ENGLISH SUMMARY

Estimation of genetic variation of performance traits in common carp to predict potential of selective breeding under pond management conditions

Martin Prchal

Selective breeding is the most common breeding method used in the genetic improvement of European fish stocks. Unfortunately, for genetic improvement of common carp intra / interspecific crossbreeding is widely used. On the other hand, several research issues that could contribute towards establishing a selection program in common carp are still unaddressed. The main aim of this thesis was to study the genetic variation of several performance traits under pond management conditions and to estimate future perspective of sustainable selective breeding program in common carp. In the present study, Amur mirror carp (AM) and Hungarian synthetic mirror carp (HSM) were used. Partial or full factorial mating schemes were used to establish experimental stocks. Furthermore, communal rearing of families was applied and the pedigree was reconstructed by microsatellite or by single nucleotide polymorphic markers (SNPs). The genetic and genomic parameters were estimated using common statistical software by multivariate mixed models.

Most of the studied performance traits showed sufficient genetic variation (h^2 = 0.12 – 1.0), suggesting a good potential for genetic improvement of traits through selective breeding. It was shown that selection of common carp under pond management conditions should be optimally applied after the second overwintering. At this stage, handling and short-term storage of fish is much easier, and in particular, there is high genetic correlation between traits at this stage and at the market size.

Winter survival is often a bottleneck for common carp production. As a result, the genetic background of winter survival and traits that might be correlated to the survival was studied. Main focus was given to the muscle fat and body weight and their dynamics through winter period. Moreover, correlation of traits between winter period and successive growing period was estimated. It was found that selection for i) lower fat content before and after winter, ii) lower decrease in muscle fat content and/or body weight during winter, may both lead to better survival during both winter and the third growing period and growth of fish during the third growing season. Likewise, we found in two studies that the condition factor could be an important selection trait supporting better performance during winter, the third growing period and at market size. However, selection on the condition factor would indirectly lead to a less favourable square-like body shape.

Slaughter yields of edible parts are also of high economic importance, and thus they are interesting traits for a breeding program. However, direct selection for slaughter yields is impossible on live breeding candidates. Therefore, morphological predictors that can be measured *in vivo* are considered as an interesting alternative. As a result, external and internal measures were combined on 1553 fish by linear regression to predict log-log residuals (Logr) of slaughter yields. It was found that the accuracy of the prediction of slaughter yields may be solid. From the genetic point of view, model-predicted (h^2 = 0.48 – 0.63) and even individual predictors (h^2 = 0.34 – 0.72) of slaughter yields were highly heritable and favourably genetically correlated to the Logr yields. In addition, the predicted headless carcass yield may be used as an efficient surrogate (easier and more precise to predict, high genetic correlation to fillet yields) for improvement of fillet yield. Hence, selection on trait predictors has an interesting perspective for genetic improvement of slaughter yields in common carp.

For a sustainable selection program it is also essential to know how genetic improvement of performance traits under certain management conditions affect the flesh quality represented by fatty acid (FA) composition. As a consequence, genetic parameters of fillet fatty acid content and performance traits in market size common carp cultured under semi-intensive pond conditions were estimated. For flesh FA composition analysis 158 individuals were dressed out and selected. Heritability estimates of total muscle fat, some FA groups and most performance traits were moderate to high (0.23 – 0.62). Interestingly, genetic correlations showed that genetic improvement of growth via selective breeding under Central European pond conditions without changing the production technology would very likely negatively affect carp flesh quality with respect to FA composition. The results should be remembered when selecting for faster growth in order to maintain the quality of carp meat.

Deeper insight into genetic variation of performance traits may be further studied using genotyping by sequencing (GBS) techniques. Hence, restriction-site-associated DNA sequencing (RAD-seq) was used to identify and genotype SNPs markers for subsequent parentage assignment, construction of a medium density genetic map (12,311 SNPs), and testing of efficiency of marker-assisted (MAS) and genomic selection (GS) for growth and Koi herpes virus (KHV) resistance. No genome-wide significant QTL was identified for growth. However, genomic prediction of its breeding value may outperform the traditional pedigree-based prediction, resulting in an 18% improvement in prediction accuracy. On the other hand, genome-wide significant QTL affecting resistance to KHV was identified on linkage group (LG) 44 explaining approximately 7% of the additive genetic variation. Importantly, using pooled whole genome resequencing of a subset of resistant and susceptible fish, a promising positional and functional candidate gene (TRIM25 protein) within the same QTL region was identified.

The presented results add further evidence supporting the application of selective breeding in common carp cultured even under traditional pond management conditions. However, it is evident that the rearing technology will need suitable modifications. In addition, real economic impact of selective breeding on carp culture should be verified by calculation of realized heritabilities, real genetic gains and the total fish biomass yield of genetically improved stocks from a pond area unit.

CZECH SUMMARY

Odhad genetické variance užitkových vlastností kapra obecného s cílem předpovědět potenciál selekčního šlechtění v rybničních podmínkách chovu

Martin Prchal

Selekční šlechtění je nejběžnější šlechtitelskou metodou, která se používá pro tzv. „genetické zlepšování" evropských druhů ryb. Bohužel u kapra obecného se pro genetické zlepšování používá především vnitrodruhové / mezidruhové křížení. Nicméně několik otázek, které by mohly přispět k založení selekčního programu kapra obecného, zůstalo stále nezodpovězených. Hlavním cílem této práce bylo prostudovat genetickou varianci užitkových znaků kapra obecného v rybničních podmínkách chovu a na jejich základě odhadnout perspektivy dlouhodobě udržitelného selekčního programu. Ve všech prezentovaných studiích byl použit Amurský lysec (AL) a syntetická linie maďarských lysců (HSM). Experimentální obsádky byly založeny s využitím částečného či úplného faktoriálního schématu páření. Rodiny byly chovány ve společných podmínkách a jejich rodokmen byl vytvořen s využitím mikrosatelitů či jednonukleotidového polymorfismu (SNP). Genetické a genomické parametry byly odhadnuty běžnými statistickými programy pracujícími s multivariačními smíšenými modely.

Většina studovaných užitkových znaků vykázala dostatečnou genetickou varianci (h^2 = 0.12 – 1.0), což ukazuje na dobrý potenciál pro jejich genetické zlepšování s využitím selekčního šlechtění. Navíc se ukázalo, že selekce kapra obecného by měla být v rybničních podmínkách optimálně prováděna po druhém zimování. V této fázi umožňuje velikost ryb snazší manipulaci i krátkodobé držení ryb mimo chovné prostředí a je zde vysoká genetická korelace mezi znaky v tomto období a v tržní velikosti.

Zimní období (přezimování) je často kritickým obdobím v chovu kapra. Z tohoto důvodu bylo studováno genetické pozadí znaků, jež mohou mít vliv přezimování. Nejvyšší pozornost byla věnována obsahu tuku ve svalovině a hmotnosti ryb a jejich dynamice v průběhu zimního období. Rovněž byla sledována závislost znaků mezi zimním a následným (třetím) vegetačním obdobím. Bylo zjištěno, že selekce na i) nižní obsah tuku před a po zimě, ii) nižší pokles obsahu tuku a hmotnosti těla během zimy mohou vést k obecně lepšímu přežití ryb a také k lepšímu růstu během třetí vegetační sezony. Dále jsme zjistili ve dvou studiích, že kondiční faktor (FK, Fultonův kondiční koeficient) by mohl být důležitým selekčním znakem podporujícím vyšší užitkovost během zimy, vegetační sezony i v tržní velikosti. Nicméně selekce na FK by nepřímo vedla k méně žádoucímu čtvercovému tvaru těla.

Jateční výtěžnosti jedlých podílů těla mají také velký ekonomický význam a jsou tak zajímavými znaky pro šlechtění. Bohužel přímá selekce na výtěžnost není možná na živých kandidátech. Nicméně morfologické prediktory, které se mohou měřit *in vivo*, jsou považovány za zajímavou alternativu. Z tohoto důvodu byla zkombinována externí a interní měření z 1 553 ryb a pomocí lineární regrese byly předpovězeny log-log reziduály (Logr) výtěžnostních ukazatelů. Bylo zjištěno, že přesnost předpovědi jateční výtěžnosti může být spolehlivá. Z genetické hlediska jsou kombinované modely pro odhad výtěžnosti (h^2 = 0.48 – 0.63) a dokonce i jednotlivé prediktory h^2 = (0.34 – 0.72) vysoce dědivé a příznivě korelované s Logr jatečními výtěžnostmi. Kromě toho zle předpokládat, že výtěžnost opracovaného trupu těla může být použita (jednodušší a přesnější předpověď, vysoký genetický vztah k filetům) i pro zlepšení výtěžnosti filetů. Lze tedy říci, že selekce s využitím předpovězených hodnot má zajímavou perspektivu pro genetické zlepšování jateční výtěžnosti kapra obecného.

Pro udržitelný selekční program je rovněž nezbytné znát, jak zlepšení užitkových vlastností v určitých chovatelských podmínkách ovlivní kvalitu produktu vyjádřenou složením mastných

kyselin ve svalovině ryb. Z tohoto důvodu byly studovány genetické parametry obsahu mastných kyselin ve filetě a užitkových vlastností tržního kapra obecného chovaného v polointenzivních podmínkách. Pro analýzu mastných kyselin bylo zpracováno a vybráno 158 jedinců. Míra dědivosti obsahu tuku ve svalovině, některých skupin mastných kyselin a většiny užitkových znaků byla střední až vysoka (0.23 – 0.62). Genetické korelace ukázaly, že genetické zlepšování růstu selekčním šlechtěním ve středoevropských podmínkách chovu by bez změny technologie chovu pravděpodobně negativně ovlivnilo kvalitu masa. Výsledky by proto měly být vzaty v úvahu při selekci na vyšší růst, aby kvalita kapřího masa zůstala zachována.

Hlubší pohled do genetické variance užitkových znaků může být dále studován pomocí genotypování s využitím rozsáhlejšího sekvenování genomu (GBS). Proto byla využita metoda založená na náhodném sekvenování úseků po štěpení DNA s využitím restrikčních enzymů (tzv. RAD sekvenování). Byly konstruovány středně-husté genetické mapy (12 311 SNPs) a nalezené SNPs byly využity pro určení rodičovství a testování účinnosti selekce s využitím těchto markerů (tzv. MAS, marker assisted selection) pro rychlost růstu a odolnost vůči Koi herpes viróze (KHV). Pro růst nebyl identifikován žádný významný lokus (tzv. QTL, quantitative trait locus). Nicméně genomická predikce plemenné hodnoty pro rychlost růstu by mohla překonat selekci založenou na rodokmenu a zvýšila by efektivitu selekce až o 18%. Na druhou stranu byl ve vazebné skupině 44 identifikován poměrně významný QTL pro odolnost vůči KHV vysvětlující přibližně 7% aditivní genetické variance tohoto znaku. Navíc s použitím směsného celogenomového sekvenování podskupiny rezistentních a vnímavých ryb ke KHV byl v oblasti QTL identifikován kandidátní gen pro protein TRIM25, jehož sekvence může mít přímou souvislost s odolností ryb vůči této nemoci.

Prezentované výsledky v této práci přinášejí další důkazy podporující využití selekčního šlechtění kapra obecného i v tradičních podmínkách rybničního chovu. Je však zřejmé, že technologie chovu kapra bude vyžadovat vhodné úpravy. Skutečný dopad selekčního šlechtění na ekonomiku chovu by měl být navíc ověřen výpočtem realizovaných dědivostí, skutečným genetickým ziskem a celkovým výnosem rybí biomasy geneticky zlepšených populací z jednotky plochy rybníka.

ACKNOWLEDGEMENTS

Foremost, I would like to thank my supervisor Assoc. Prof. Martin Kocour for his perfect leading, constructive criticism, advices, comments, editing of papers and hard-working access that motivated me to finish this Ph.D. thesis.

I would like to thank also to my lab colleagues, especially to Prof. Martin Flajšhans who believed in me and gave me a chance to finish my study. Likewise, I thank to our technicians Maruška Pečená and Ivana Samková for their help and time plasticity during the experiments. Last but not the least, I thank to MSc. Jinfeng Zhao for her help with collecting morphological landmarks and with editing the references.

I am also indebted to David Gela, Ph.D. and staff at Genetic Fisheries Center of FFPW USB for their help during establishment of experimental stocks, harvesting and data collection and also to staff at Experimental Fish Culture Facility of FFPW USB that helped us during ongrowing of fish, harvesting and dress-out event.

Furthermore, I would like to thank to director of Klatovy fishery Dipl-Ing. Václav Voráček and his staff for providing facility and participation in studies that are presented in this thesis.

My special thanks belongs to Antti Kause, Ph.D., Marc Vandeputte, Ph.D., Jerome Bugeon, Ph.D., and Anna K. Sonesson, Ph.D. and also to other FISHBOOST partners for their huge help during running the FISHBOOST experiments, and especially to Antti, Marc and Anna, who have been teaching me all things concerning estimation of genetic parameters. Likewise, I would like to thank to LABOGENA staff for genotyping the fish and colleagues from University of Edinburgh who participated on genomic oriented part of our experiments.

My greatest gratitude goes to my family, to my parents, wife's parents and especially to my grandparents that give me a strong support during the bad days.

Finally, I would like to thank my wife for staying with me during my never-ending work and I really appreciate that she understood that the research needs the victims and self-sacrifice.

I also appreciate the financial support from the following projects that enabled funding of different parts of the research summarized in this dissertation:

- Ministry of Education, Youth and Sports of the Czech Republic - projects "CENAKVA" (No. CZ.1.05/2.1.00/01.0024), "CENAKVA II" (No. LO1205 under the NPU I program) and "Biodiverzita" (CZ.02.1.01/0.0/0.0/16_025/0007370)
- European Union's Seventh Framework Programme (KBBE.2013.1.2-10) under grant agreement no. 613611 FISHBOOST (http://www.fishboost.eu/)
- Grant Agency of the University of South Bohemia (project no. 059/2015/Z and 125/2016/Z)
- Ministry of Agriculture - project of the Czech NAAR (NAZV) no. QK1710310

LIST OF PUBLICATIONS

Peer-reviewed journals with IF

Prchal, M., Kause, A., Vandeputte, M., Gela, D., Allamelou, J.M., Girish, K., Bestin, A., Bugeon, J., Zhao, J., Kocour, M., 2018. The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. PLoS ONE 13, e0191624. (IF 2017 = 2.766)

Prchal, M., Bugeon, J., Vandeputte, M., Kause, A., Vergnet, A., Zhao, J., Gela, D., Genestout, L., Bestin, A., Haffray, P., Kocour, M., 2018. Potential for genetic improvement of the main slaughter yields in common carp with *in vivo* morphological predictors. Front. Genet. 9, 283. (IF 2017 = 4.151)

Prchal, M., Vandeputte, M., Gela, D., Doležal, M., Buchtová, H., Rodina, M., Flajšhans, M., Kocour, M., 2018. Estimation of genetic parameters of fatty acids composition in flesh of market size common carp (*Cyprinus carpio* L.) and their relation to performance traits revealed that selective breeding can indirectly affect the flesh quality. Czech J. Anim. Sci. 63, 280 – 291. (IF 2017 = 0.955)

Palaiokostas, Ch., Kocour, M., **Prchal, M.**, Houston, R.D., 2018. Accuracy of genomic evaluations of juvenile growth rate in common carp (*Cyprinus carpio*) using genotyping by sequencing. Front. Genet. 9, 82. (IF 2017 = 4.151)

Palaiokostas, Ch., Robledo, D., Vesely, T., Kocour, M., **Prchal, M.,** Pokorova, D., Piackova, V., Pojezdal, L., Houston, R.D., 2018. Mapping and sequencing of a significant quantitative trait locus affecting resistance to Koi herpesvirus in common carp. G3: Genes Genom. Genet. In press. (IF 2017 = 2.742)

International conferences

Prchal, M., Kause, A., Vandeputte, M., Gela, D., Allamelou, J.M., Girish, K., Bestin, A., Bugeon, J., Zhao, J., Kocour, M. 2017. The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. Aquaculture Europe 2017. 17–20 October 2017, Dubrovnik, Croatia.

Palaiokostas, Ch., Vesely, T., Kocour, M., **Prchal, M.,** Pokorova, D., Piackova, V., Pojezdal, L., Houston, R.D., 2017. Investigation the potential of breeding KHV resistant common carp *Cyprinus carpio* using RAD-sequencing. Aquaculture Europe 2017. 17–20 October 2017, Dubrovnik, Croatia.

Kocour, M., **Prchal, M.,** 2017. FISHBOOST: results, plans and implications in common carp. 4th Carp Conference, 21–22 September 2017, Zagreb, Croatia.

Veselý, L., Boukal, D., Buřič, M., Kuklina, I., Fořt, M., Yazicioglu, B., **Prchal, M.,** Kozák, P., Kouba, A., Sentis, A., 2017. Temperature, prey availability and predator diversity jointly influence surplus killing in a freshwater food web. In: 5th meeting of Fresh Blood for FreshWater. 9–13 April 2017, České Budějovice, Czech Republic.

Prchal, M., Kause, A., Sonesson, K, A., Vandeputte, M., Kroupová, K. H., Kumar, G., Gela, D., Piačková, V., Genestout, L., Kocour, M., 2016. Estimation of genetic parameters of growth and fat related traits in one year common carp before and after winter. Aquaculture Europe 2016. 20–23 September 2016, Edinburgh, Scotland.

Prchal, M., Flajšhans, M., Gela, D., Kašpar, V., Linhart, O., Rodina, M., Kocour, M., 2015. Breeding work in common carp in the Czech Republic: recapitulation of last 15 years and future prospects. In: Book of Abstracts – 3rd Carp Conference, 3–4 September 2015. Vodňany, Czech Republic, pp. 86–90.

Veselý, L., Sentis, A., Kuklina, I., Buřič, M., Fořt, M., Yazicioglu, B., **Prchal, M.,** Boukal, D., Kouba, A., 2015. Effect of temperature and nutrient enrichment on prey-predator complex system. In Abstract volume, European Crayfish conference: Research and Management, 9–12. April 2015, Landau, Germany, pp. 17.

TRAINING AND SUPERVISION PLAN DURING STUDY

Name	Dipl.-Ing Martin Prchal
Research department	2013–2018: Laboratory of Molecular, Cellular and Quantitative Genetics of FFPW
Supervisor	Assoc. Prof. Martin Kocour
Period	30th October 2013 until 20th September 2018

Ph.D. courses	**Year**
Biostatistics	2014
Basic of scientific communication	2014
Fish genetics	2014
Ichthyology and fish taxonomy	2015
English language	2016

Scientific seminars	**Year**
Seminar days of RIFCH and FFPW	2014 2015 2016 2017

International conferences	**Year**

Prchal, M., Kause, A., Vandeputte, M., Gela, D., Allamelou, J.M., Girish, K., Bestin, A., Bugeon, J., Zhao, J., Kocour, M. 2017. The genetics of overwintering performance in two-year old common carp and its relation to performance until market size. Aquaculture Europe 2017. 17–20 October 2017, Dubrovnik, Croatia.	2017
Palaiokostas, Ch., Vesely, T., Kocour, M., **Prchal, M.,** Pokorova, D., Piackova, V., Pojezdal, L., Houston, R.D., 2017. Investigation the potential of breeding KHV resistant common carp *Cyprinus carpio* using RAD-sequencing. Aquaculture Europe 2017. 17–20 October 2017, Dubrovnik, Croatia.	2017
Kocour, M., **Prchal, M.,** 2017. FISHBOOST: results, plans and implications in common carp. 4th Carp Conference, 21–22 September 2017, Zagreb, Croatia.	2017
Veselý, L., Boukal, D., Buřič, M., Kuklina, I., Fořt, M., Yazicioglu, B., **Prchal, M.,** Kozák, P., Kouba, A., Sentis, A., 2017. Temperature, prey availability and predator diversity jointly influence surplus killing in a freshwater food web. In: 5th meeting of Fresh Blood for FreshWater. 9–13 April 2017, České Budějovice, Czech Republic.	2017
Prchal, M., Kause, A., Sonesson, K, A., Vandeputte, M., Kroupová, K. H., Kumar, G., Gela, D., Piačková, V., Genestout, L., Kocour, M., 2016. Estimation of genetic parameters of growth and fat related traits in one year common carp before and after winter. Aquaculture Europe 2016. 20–23 September 2016, Edinburgh, Scotland.	2016
Prchal, M., Flajšhans, M., Gela, D., Kašpar, V., Linhart, O., Rodina, M., Kocour, M., 2015. Breeding work in common carp in the Czech Republic: recapitulation of last 15 years and future prospects. In: Book of Abstracts – 3rd Carp Conference, 3–4 September 2015. Vodňany, Czech Republic, pp. 86-90.	2015
Veselý, L., Sentis, A., Kuklina, I., Buřič, M., Fořt, M., Yazicioglu, B., **Prchal, M.,** Boukal, D., Kouba, A., 2015. Effect of temperature and nutrient enrichment on prey-predator complex system. In Abstract volume, European Crayfish conference: Research and Management, 9–12. April 2015, Landau, Germany, pp. 17.	2015
Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Antti Kause, Ph.D., Natural Resources Institute, Jokionen, Finland (Luke) (1 month, estimation of genetic parameters of performance traits in aquaculture species).	2016
Pedagogical activities	**Year**
Leading of project entitled Statistical processing of quantitative data in economically important fish species in the Czech Republic at International Summer school	2017
Announcing the project entitled Selective breeding in common carp: Statistical processing of quantitative data at International Summer school	2016
Lecturing of students of master study, discipline Fishery at USB FFPW in range of 50 teaching hours	2014–2017
Training of students in basics of breeding work in fish in range of 40 teaching hours	2014–2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name:	Martin
Surname:	Prchal
Title:	Dipl-Ing.
Born:	31st December 1988, Pelhřimov, Czech Republic
Nationality:	Czech
Languages:	English (B2 level – FCE certificate), Czech (native speaker)
Contact:	mprchal@frov.jcu.cz

RESEARCH INTEREST

Fish breeding, Quantitative genetics, Genetic and genomics parameters, Common carp
Selective breeding in common carp
Intra / interspecific crossbreeding in common carp

EDUCATION

2013 – present	Ph.D. student in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice, Czech Republic
2011–2013	Dipl.-Ing. in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice, Czech Republic
2008–2011	B.Sc. in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice, Czech Republic

PROFESSIONAL EXPERIENCE

2017 – present	worker in biological sciences, Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice, Czech Republic

COMPLETED COURSES

Biostatistics, Basic of scientific communication, Fish genetics, Ichthyology and fish taxonomy, English language

TRAINING

10.3. – 14.3. 2014	Basic methods in molecular biology
26.5. – 30.5. 2014	Certificate of qualification pursuant for designing experiments and experimental projects within section 15 of Act No 246/1992 Coll. on Protection of Animals against Cruelty, No. CZ 01704
16.4. – 17.4. 2018	PIT Tag methodologies and monitoring systems in fish

RESEARCH STAY

17.11. – 15.12. 2016	Antti Kause, Ph.D., Natural Resources Institute (Luke), Jokioinen, Finland