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Broader insight into mutual genetic and phenotypic relationships of production-related quantitative traits in common carp

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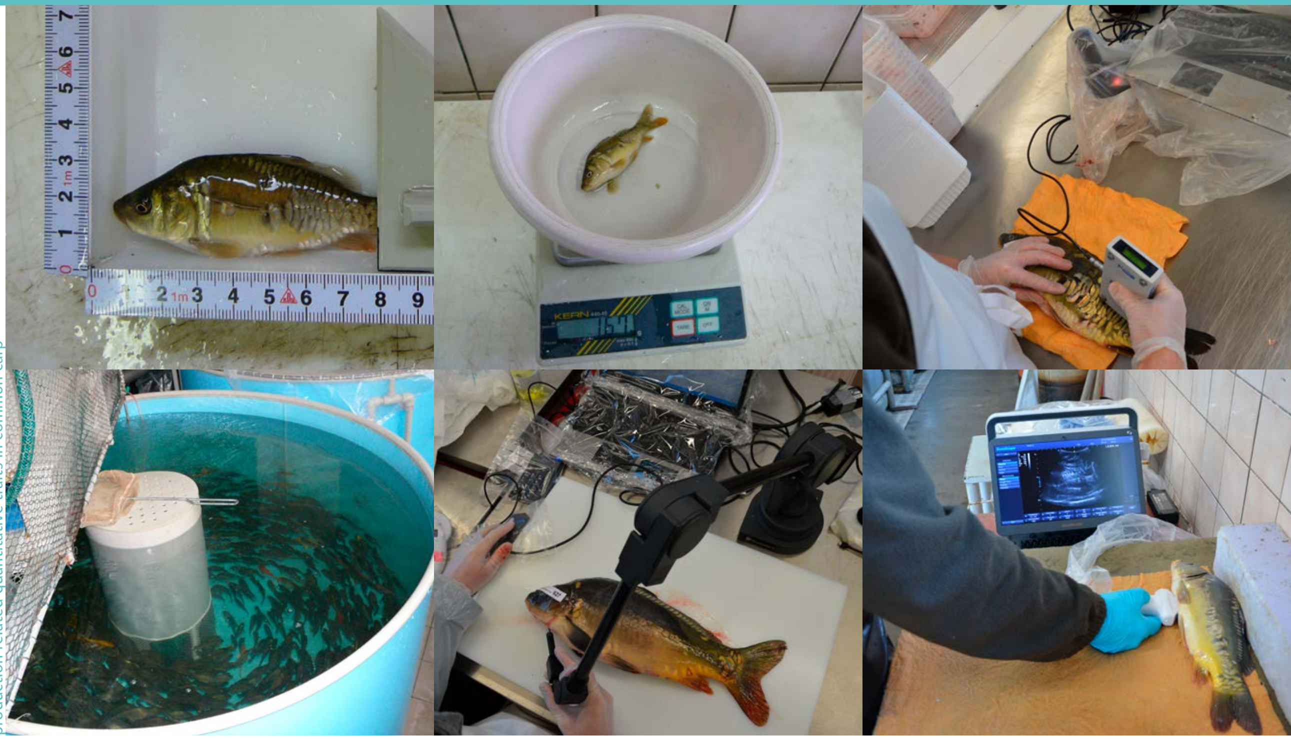

Doctoral thesis by
Jinfeng Zhao

Czech Republic, Vodňany, 2021

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1. Breeding of common carp

Common carp (*Cyprinus carpio*) is one of the most cultured and economically important species in aquaculture. Common carp also belongs to intensively studied fish species in many research areas including genetic improvement of quantitative traits. Studies on genetic improvement of quantitative traits in common carp have history longer than 50 years and from the beginning they have concerned three main directions – selection (Moav and Wohlfarth, 1976), genome manipulations (Cherfas et al., 1996; Gomelsky, 2003; Kocour et al., 2003, 2005b) and crossbreeding (e.g., Wohlfarth, 1993; Hulata, 1995; Kocour et al., 2005a). At present, the crossbreeding is still the main method widely used to improve performance of market stocks of common carp. Unlike most other economically important fish species selective breeding is not commonly applied in common carp. Crossbreeding had received much attention in this species, maybe because selection experiments in Israel (Moav and Wohlfarth, 1976), very well-developed country, did not show satisfactory results. The authors did not achieve any improvement in growth performance of a domesticated European carp breed over five generations of mass selection. On the other hand, heterosis for growth and survival was found to be quite common phenomenon (e.g., Wohlfarth, 1993; Linhart et al., 2002; Nielsen et al., 2010). In crossbreeding non-additive genetic variation plays a role, but it is not a suitable method for long-term genetic improvement. On the contrary, selective breeding exploits additive genetic variation, thus the genetic gain is cumulative and permanent over multiple generations (Gjedrem and Rye, 2018). Application of selective breeding is widely recognized as a key factor for the efficient development of sustainable and economically profitable aquaculture productions. For example, there are rainbow trout (*Oncorhynchus mykiss*) (Donaldson and Olson, 1955; Gjedrem, 1985; Morkramer et al., 1985), chinook salmon (*Oncorhynchus tshawytscha*) (Donaldson and Menasveta, 1961), Atlantic salmon (*Salmo salar*) (Gjedrem et al., 1991; Friars et al., 1995), gilthead seabream (*Sparus aurata*) (Knibb et al., 1997; Brown, 2003) and other main fish species in which selective breeding is used. So, in recent years selective breeding in common carp has attracted again widespread attention. The technological development has enabled economically accessible individual fish tagging and parentage assignment of fish reared in communal stocks from the larva stage and heritability estimates from recent studies in common carp (Vandeputte et al., 2004, 2008; Wang et al., 2006; Kocour et al., 2007; Nielsen et al., 2010; Yousefian et al., 2011; Dong et al., 2015) suppose that selective breeding should be an effective way for improvement of growth related traits even in this species. Gjedrem and Robinson (2014) reviewed the average genetic gain has been greater than 12% per generation for growth rate and disease resistance in some aquaculture breeding programs and illustrated selective breeding is very effective for aquatic species. Selective breeding programs of common carp have been developing in Asia, especially China, Indonesia and Vietnam (Nguyen, 2016). In Europe, there are currently no systematic selective breeding programs for common carp used at commercial level.

In Asia, conventional selective breeding approaches have resulted in significant improvement in fish productivity, with genetic gains ranging from 8 to 20% per generation for carp species (Nguyen, 2016). The selection program resulted in a remarkable improvement in growth performance in the common carp population (Ninh et al., 2013; Dong et al., 2015). In study of Ninh et al. (2011), the authors practiced two rearing scheme: i) separate families until the fish were large enough to be physically tagged, and ii) early communal rearing from very soon after hatching. The separate early rearing (SER) is that individual families of both the selected and control lines were reared separately from larvae to juveniles. The communal early rearing (CER) is that about three days after hatching, fry from all families were communally reared in a pond at a specific stocking density. Finally, they concluded that CER conditions are more similar to typical farming practice than SER and the fish under the CER scheme grew faster and achieve greater genetic gain than under SER. Moreover, the CER also reduces common environmental effects and hence warrants its implementation when resources make it possible (Ninh et al., 2011, 2013). This should be remembered when doing selective breeding in fish species cultured in ponds or similar environments. The superiority of CER relative to SER benefits exactly from accessibility of molecular parentage assignment as a key tool in practical selective breeding programes. Recently, new genomic methods could accelerate the progress of genetic improvement of carp through genomic selection using genome-wide genetic markers, marker-assisted selection using significant quantitative trait loci (QTLs) or candidate genes (Lv et al., 2016; Peng et al., 2016; Zheng et al., 2016; Lu et al., 2017; Wang et al., 2018).

In European aquaculture, as mentioned above selective breeding in common carp plays a minor role in carp breeding (Vandeputte, 2003; Janssen et al., 2015; 2017) and most researches are still at experimental phase. Vandeputte et al. (2004) concluded that selective breeding for increased weight gain may be successful in juvenile carp, even using indirect selection for length. However, the facts that heritability was estimated at 8 weeks of age and not at harvest weight, as well as a possible amplification of additive variance by competition, are limitations to the applicability of the present results. Subsequently, Kocour et al. (2007) showed theoretical feasibility of growth and processing traits for genetic improvement in common carp at market size. Vandeputte et al. (2008) showed that although selection for growth seems to be feasible in common carp, it would be a long-term process before results are visible. Nielsen et al. (2010) estimated genetic parameters for weight, length and survival traits for common carp measured at different periods in commercial farm conditions, indicating that selective breeding for these traits could be successful in common carp. Ødegård et al. (2010) found there is good prospect for joint improvement of Koi herpesvirus (KHV) resistance and pond survival in common carp through genetic selection. Palaiokostas et al. (2018a) observed no significant quantitative trait locus (QTL) for growth by restriction site associated DNA sequencing in common carp, but genomic prediction of breeding values outperformed traditional pedigree-based prediction. At the same time, Palaiokostas et al. (2018b) found a genome-wide significant QTL affecting resistance to KHV on linkage group 44 in common carp, which indicates the potential for genomic selection to improve economically important traits in common carp breeding programs. Subsequently, the prediction accuracy of identifying KHV disease resistant carp using genomic selection was between 8 and 18% higher than pedigree best linear unbiased predictor (Palaiokostas et al., 2019). The genetic basis of KHV was investigated and then QTL regions affecting KHV survival were identified (Tadmor-Levi et al., 2017, 2019). Overall, present research results may help to improve disease resistance to KHV in common carp. Nonetheless, there is still much space for studying on the potential of selective breeding for the genetic improvement of common carp under pond management conditions.

It is important to create the synthetic strains with high genetic variability, which may be a good material to start selective breeding programs in common carp (Vandeputte, 2003; Nielsen et al., 2010). In studies within this dissertation, Amur mirror carp (AM) was used for establishing experimental stocks that displayed sufficient within-breed genetic variation. The partial factorial mating designs were used to produce experimental families and the communal early rearing scheme was used to rear experimental stock. A diagram that shows the overall set-up of the experiments from Chapter 2 to Chapter 5 is shown as Figure 1. AM (Vodňany line) is a new Czech breed (Flajšhans et al., 2015) that is 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. This breed is used in crossbreeding programs and crossbreds with AM have high performance with superior growth and survival when compared with other groups (Kocour et al., 2012). Pure breed showed to be also promising as basic population for selective breeding program because main performance traits are highly heritable (Palaiokostas et al., 2018a,b; Prchal et al., 2018). Moreover, the breed, and even its crossbreds, displays very good non-specific resistance against stress situations and diseases including Koi herpes virus disease (KHVD) (Piačková et al., 2013).

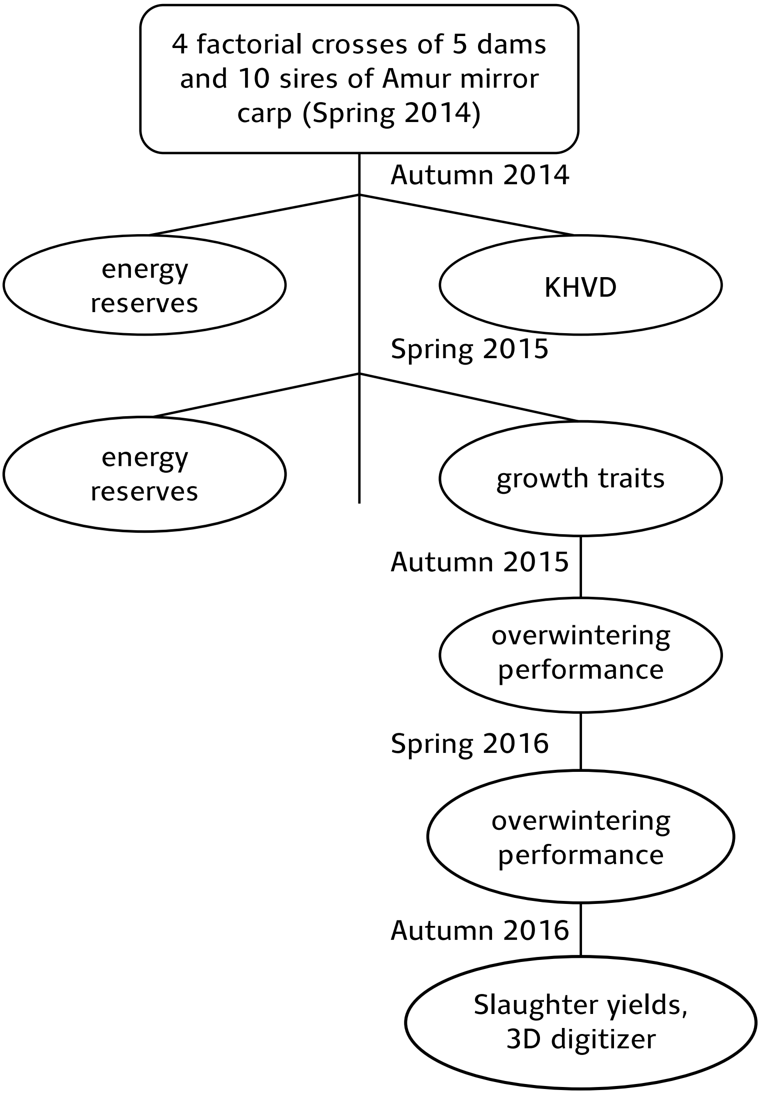

Figure 1. *A diagram showing the data record of the experimental traits from Chapter 2 to Chapter 5.*

2. Use of molecular markers in common carp

Genetic methods considered to be important for maintaining common carp strains began with allozymes, which reported protein variability in common carp breeds in Czech Republic (Šlechtová et al., 2002). This measure was used to create synthetic strains with high genetic variability, which has provided good material to start selective breeding programs. However, allozyme markers were found to be useless in some aspect of breeding owing to their low variability. So, the attention gradually shifted to microsatellite markers (Kohlmann et al., 2003). Genetic parameters estimates using microsatellite markers for parentage assignment have been paid of higher attention in common carp (e.g., Vandeputte et al., 2004, 2008; Kocour et al., 2007; Ninh et al., 2011; 2013; Prchal et al., 2018). This approach enables estimates of genetic parameters under CER, as also mentioned above, which does not bias the estimates by common environmental effects and ensures the higher reliability of the genetic parameters evaluated. Genetic characterization of carp strains/populations by microsatellite variability is also a reliable tool for understanding the inter- and intra-relationships among the investigated populations and could be of great benefit in other breeding strategies as crossbreeding (Hulak et al., 2010). Knowledge of inter-population relationships may be also beneficial when founding a basic (starting) population for selective breeding program. Single nucleotide polymorphism (SNP) is another available molecular marker to estimate relatedness (Ritland, 1996; Lynch and Ritland, 1999). SNP is a variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population. The genome sequence helps to discover large numbers of genetic variations such as SNPs which is one of the fundamental reasons why individuals of same species perform differently from one another. There are a few instances of using SNPs (Palaiokostas et al., 2018a,b; 2019) for parentage assignment in genetic improvement programs of common carp. In comparison to microsatellite markers, the number of SNPs is much larger and estimated at many millions (Halushka et al., 1999), and SNPs can be used successfully to replace microsatellites for the purposes of parentage assignment and pedigree verification due to their lower error rate (Trọng et al., 2013). However, large data sets of SNPs (from genome-wide association study or sequencing) may be used mainly for estimating genomic heritability, searching of Quantitative Trait Loci (QTLs), etc.

3. Genetic variation

The estimation of genetic variation is important for making decisions regarding design and implementation of selective breeding programs. The main estimated parameters are mean, standard deviation, heritability and genetic and phenotypic correlations between the traits of interest (Gjedrem, 1983). Understanding the interactions between the economically important traits is of particular importance if we are to control the product quality in terms of body composition and flesh quality. Heritability, which is defined as the ratio of the heritable (or additive) genetic variance to the total phenotypic variance, is therefore central genetic parameter in fish breeding programs. Heritability is classified as either broad sense or narrow sense heritability. Broad sense heritability is the ratio of the total genetic variance to the phenotypic variance, while narrow sense heritability is the ratio of the additive genetic variance to the phenotypic variance. Broad sense heritability contains dominance and epistatic effects that do not respond to selection and is not often used (Gjedrem, 2005). Heritability, particularly narrow sense heritability, has been considered as the primary genetic parameter in quantitative genetics. The magnitude of estimated heritability was established, following the classification of Cardellino and Rovira (1987) reported by Navarro et al. (2009a) as low

(0.05–0.15), medium (0.20–0.40), high (0.45–0.60), and very high (>0.65). Reliable estimates of heritability can provide very valuable information for determining reasonable breeding plans and for predicting selection response and breeding values of candidate traits.

Correlation, which measures the relative magnitude of covariance between traits, has phenotypic and genetic levels. Phenotypic and genetic correlations are important parameters in quantitative genetics analysis. Genetic correlation, which demonstrates the heritable part in the phenotypic correlation, is also an important parameter in quantitative genetics analysis. The estimates of correlation can indicate changes in the traits that may not be used in selection when selection is practiced on candidate traits. Correlations were classed as low (0–0.40), medium (0.45–0.55) and high (0.60–1), regardless of the sign (Navarro et al., 2009a). Due to linkage and pleiotropy of gene, there are various degree or direction of correlations between each trait. Using of correlation can provide convenience for selective breeding. For example, when some target traits are difficult to measure or have low heritability, and it is difficult to achieve the expected responses by direct selection, it may be easier to obtain better selection breeding response by indirect selection for other higher additive related traits. Therefore, determining the correlation between different traits is one of the main contents of quantitative genetics research.

As farming of common carp is growing as an aquaculture industry, in order to design a practical and efficient selective breeding program for European common carp, the need to estimate the genetic (co)variances of traits is of increasing importance. Quantitative genetic studies are required to design effective breeding programmes, specific to a given population, which are aimed at improving the economic value and quality of the fish. Previous genetic studies have revealed moderate levels of genetic variation for production traits (growth, slaughter yields, disease resistance and main biometrical indices) of common carp that show high potential for application of systematic selective breeding to genetic improvement (e.g., Vandeputte et al., 2004; Wang et al., 2006; Kocour et al., 2007; Nielsen et al., 2010; Prchal et al., 2018; Palaiokostas et al., 2018a,b). In contrast to extensive evaluation in the heritability, correlation estimate was seldom conducted for the common carp. For some traits, such as feed efficiency or carcass quality, are known for the difficulties in selection. Consequently, genetic improvement of such traits can rely on correlated responses through selection for other correlated trait. In selective breeding, selection for a single trait can lead to unwanted genetic changes in other traits. Some genetic and phenotypic correlations among production traits have been reported for common carp (e.g., Vandeputte et al., 2004; Kocour et al., 2007; Nielsen et al., 2010; Dong et al., 2015). However, broader insight into mutual genetic and phenotypic relationships of production-related quantitative traits needs to be known in common carp.

4. Important traits within aquaculture

A breeding programme is a long-term process aimed at producing incremental improvements of economically important traits by changing their genetic abilities. These traits are determined by the requirements and wishes from customers or the society which might change over time. Growth rate is the main breeding target trait that is selected in all aquaculture breeding programs. However, as growth rate increases and production intensifies, other traits become increasingly important such as disease resistance, non-specific mortality, feed efficiency, flesh yield and quality. Fish quality is usually a question of size, meatiness, fat percentage, fat distribution, flesh color, fillet yield, structure, firmness, taste, shape of body, dressing percentage and so on. Moreover, some of the traits are rather difficult to be measured or judged, such as meatiness, taste, structure and firmness (Gjerde et al., 2007). The most preferable criteria for inclusion of traits in the breeding programme are their current value and future potential. The traits for selection must hold following prerequisites: economic and ethical importance, genetic variance, possibility of measuring at a reasonable cost (Gjedrem, 2005). According to Gjerde et al. (2007), efficient and sustainable genetic improvement work involves: balanced selection for traits that primarily reduce production cost (growth rate, feed conversion efficiency, survival); traits related to health and functionality of fish (disease resistance and reproduction); and traits related to product quality (e.g., pigmentation, fat deposition and content).

All traits discussed here are considered to be quantitative traits with many genes involved for each trait. Implemented breeding programs evolve with new traits being added over time to increase the productivity (growth, disease resistance, processing yield), but also to meet the needs of consumers (fish morphology, product quality, etc.). The traits of interest can be recorded directly on candidate breeders, as in the case of growth, morphology and some reproduction-related traits, or on their sibs when the trait measurement requires sacrifice of a fish, e.g., assessment of disease resistance or processing yield or energy reserves.

4.1. Growth-related traits

Body weight and length are important traits in all species and are usually the main breeding goal in selection programs. Estimates of heritability for growth-related traits are necessary in fish breeding programs to assess the utility of selective breeding for growth-related traits. A number of heritability estimates of growth-related traits in common carp have been widely carried out (e.g., Wang et al., 2006; Kocour et al., 2007; Vandeputte et al., 2004, 2008; Nielsen et al., 2010; Yousefian et al., 2011). However, combining reviews of Vandeputte (2003) and Wang (2009) and recent study reports on common carp show that heritability estimates vary widely among experiments and methods used. For example, the heritability ranges from 0 to 0.70 for body weight and from 0.01 to 0.80 for standard length. It is worth knowing that most estimates show moderate to high heritability, suggesting that additive genetic variation exists in the growth-related traits and genetic improvement for such traits is feasible and it should be possible to obtain a positive response to selection in common carp. Contrary to extensive heritability estimates, there are few correlation estimates for common carp. Since Vandeputte et al. (2004) first reported the genetic correlation of 0.98 between weight and length in common carp. From then on, phenotypic and genetic correlations for the growth-related traits have been frequently estimated in this species (e.g., Li et al., 2006; Wang et al., 2006; Kocour et al., 2007; Vandeputte et al., 2008; Nielsen et al., 2010). Wang (2009) stated that generally the genetic correlations between body weight and length are 0.52–0.98 and phenotypic correlation are 0.54–0.97. In some cases, the correlations between body weight and length is very close to unity, and the heritabilities of body weight and length are very similar. So, selection on length (which might be easier to apply in the field) should give approximately the same results on body weight as direct selection on body weight in the common carp (Vandeputte et al., 2004; Nielsen et al., 2010). Moreover, the correlations among body weight, height, and width are moderate to high (Li et al., 2006; Wang et al., 2006). These results indicate that positive responses to selection for body weight can occur if the length, height, and width would be used as indirect breeding indices in practice. In addition, experimental studies with common carp report that selection for increased body weight may cause changes in production-related traits. For example, selection for increased body weight, should slowly change body shape towards more rotund fish and may improve processing and quality traits (Kocour et al., 2007), but will increase survival until harvesting (Nielsen et al., 2010) and did not have detrimental effect on fitness related traits, such as survival during grow-out (Dong et al., 2015). Many studies have been conducted to estimate the correlations between growth-related traits and other production-related traits in other fishes. For instance, selection for better growth is expected to produce favourable changes in fillet weight but unfavourable changes in fillet fat percentage in Atlantic salmon (*Salmo salar*) (Powell et al., 2008) and will improve fillet weight in gilthead seabream (*Sparus auratus* L.) (Navarro et al., 2009b). Such observations are similar to those reported in common carp. On the other hand, unlike results reported in common carp selection for better growth may affect bony tissue development in large all-female rainbow trout (*Oncorhynchus mykiss*) (Haffray et al., 2012), but should not affect fillet yield and fat content in male Nile tilapia (*Oreochromis niloticus*) (Garcia et al., 2017).

4.2. Energy reserves

Many animals in temperate environments seasonally accumulate energy reserves. The energy reserves–lipids, proteins or glycogen are then expended in energetically demanding activities during periods with lack of food or when fish reduce feed intake. For temperate-zone fishes the most critical period is overwintering, which may last up to six months (e.g., Oliver et al., 1979; Sogard and Olla, 2000; Finstad et al., 2004; Heermann et al., 2009; Crespel et al., 2013). Especially, energy mobilization during the first winter of life is an important physiological trait since it could crucially affect survival (Sogard, 1997; Crespel et al., 2013). It is important to note that investigations of genetic bases of energy reserves accumulation and mobilization during the overwintering are often conducted by sampling different individuals at each season and not the same individuals repeatedly because of sacrificing of fish. It is also impossible to directly select such traits, so maybe some related traits can be selected to change these traits. In general, the dynamics of energy reserves utilization can be expressed in part by monitoring indirect indices (e.g., condition factor, relative weight), also known as morphometric indices, or direct indices (e.g., hepato-somatic, visceral-somatic, gonado-somatic index), however, these changes of bioindicators occur as a result of the mobilization of endogenous lipid, protein, and glycogen. One of the most frequently used morphometric indices is the Fulton's condition factor, which however has species-specific links with energy reserves in fish (Mozsár et al., 2015). More specifically, the condition factor was positively correlated with whole-body crude lipid, crude protein and gross energy during winter periods in juvenile lake herring *Coregonus artedi* (Pangle and Sutton, 2005); the mobilization of muscle protein and fat was reflected in a decrease of the condition factor during food deprivation in juvenile roach *Rutilus rutilus* (Van Dijk et al., 2005). In addition, the relative weight index, which measures the variation between individual fish weight and a length-specific standard weight, may be used for estimating body composition – gross energy and for predicting reserve energy (visceral fat) of overwintering in juvenile striped bass *Morone saxatilis* and hybrid striped bass (Brown and Murphy, 1991); the changes in the levels of liver lipid, protein, and glycogen reflected those observed in the hepato-somatic index (HSI) during starvation of the golden perch *Macquaria ambigua* (Collins and Anderson, 1995); the change of visceral index (VSI) directly indicated stored lipids in the viscera utilized over the winter for basal metabolic demands, gonad maturation, and potentially liver maintenance in largemouth bass *Micropterus salmoides* Lacepede (Adams and Mclean, 1985). Additionally, significant genetic correlations were observed between morphometric indices like the condition factor, body mass and HSI with energy reserve indices (i.e., visceral fat, relative liver glycogen and total liver energy content) during the first winter of life in Laval brook charr *Salvelinus fontinalis* (Crespel et al., 2013). These reported results indicated strong relationships between performance of fish and energy reserves during the winter period or food deprivation. Therefore, the measurement of both bioindicators and biochemical parameters probably determines the sequence of utilization and degree of maintenance of endogenous energy reserves during overwintering or food deprivation. Thus, estimation of genetic parameters of such traits can help to understand energy mobilization. To date, the relationship between energy condition and performance traits has rarely been studied in common carp. The first study on the role of energy reserves in common carp performance inferred from phenotypic and genetic parameters of energy reserve-related trait in yearlings and from mutual correlations of relevant traits up to market size was carried out within work on topic of this dissertation (Chapter 2).

4.3. Muscle fat

Body composition and carcass quality traits, including meat color, fat content, intermuscular bones and texture of flesh, directly influence quality of final product and consumer preference for common carp. Fat deposition within fish body influences both production efficiency and product quality. In addition, fat deposits may be an asset for overwintering survival in carp (Steffens, 1996). It is generally considered that the fat content in fish fillets exceeding 16–18% is too high. High fat content, which is thought to have a detrimental effect on meat texture and quality traits such as coloration, is also thought to affect the processing characteristics of the carcass, making handling more difficult and errors more likely to occur (Rye and Gjerde, 1996). At present, quantitative genetic analyses of fat content have been reported extensively in fish species. The heritability estimates were moderate to high for the fat content of muscle (e.g., Saillant et al., 2009; Kause et al., 2011; Garcia et al., 2017; Janhunen et al., 2017), indicating that the trait should respond to selection. The importance of product quality means that most breeding programs for fish species should incorporate quality traits along with body weight as selection criteria (Gjedrem, 1997; 2000). Body weight showed positive phenotypic and genetic correlations with muscle fat content in Coho salmon (*Oncorhynchus kisutch*) (Iwamoto et al., 1990; Neira et al., 2004), in sea bass (*Dicentrarchus labrax*) (Saillant et al., 2009), in Atlantic salmon (*Salmo salar*) (Rye and Gjerde, 1996; Quinton et al., 2005; Powell et al., 2008), in gilthead sea bream (*Sparus aurata* L.) (García-Celdrán et al., 2015), in common carp (Kocour et al., 2007) as well as in European whitefish (*Coregonus lavaretus*) (Kause et al., 2011; Janhunen et al., 2017). In contrast, negative or no genetic correlation between body weight and fat content of fillet was observed in Nile tilapia (*Oreochromis niloticus*) (Garcia et al., 2017). Knowing correlations would allow us to properly predict the correlated response on fat content when selection is practiced for increased growth rate. Observations of high positive correlations between body weight and muscle fat content were unfavorable as selection for increased body weight would lead to increased muscle fat percentage. In such cases, when selection for increased body weight is applied, a restricted selection index (Cameron, 1997) to control the expected increase in fat percentage should be used in breeding programs to maintain the quality of fish product. So, in selection programs, all traits that can negatively affect each other, should be optimally included in the selection index with the relevant correlations and economic weights. If that is done, it should be possible to get response to selection for body weight without significant affecting muscle fat percentage.

In common carp high heritability (0.58) of muscle fat content was observed when using Fish Fatmeter (Kocour et al., 2007), a non-invasive method that is done on live breeding candidates. This observation was in accordance with results found in other fish (Quillet et al., 2005; Saillant et al., 2009; Janhunen et al., 2017). This high heritability suggests that direct selection for muscle fat content using a Distell Fish Fatmeter is feasible. Yet, a little is known about the genetic variation of muscle fat content and its impact on other performance traits of carp. The study on the phenotypic and genetic variation of muscle fat content obtained using Fish Fatmeter and its relation to important performance traits (growth, slaughter yields, winter survival) during the second overwintering and the successive growing period until the market size was performed when working on topic of this dissertation (Chapter 3).

4.4. Disease resistance

In fish farming, diseases are quite common, and their occurrences reduce profitability and sustainability. Due to the fact that improvement in economic efficiency of aquaculture largely depends on the disease prevention and control, a large number of breeding programs that include disease resistance within the breeding objective have already emerged in recent years. It is of great importance to increase the natural disease resistance of farmed fish. The development of disease resistant fish will be the most effective method in preventing and curing diseases. Fjalestad et al. (1993) explained three possible ways of measuring disease resistance: 1) Rate of survival as a complex trait, a desirable breeding goal in dealing with resistance; 2) Challenge test when disease resistance is recorded based on the exposing the fish to specific disease agents; 3) Immunological or physiological parameters as indirect measurements of disease resistance as all factors that enable fish to resist the pathogens in a specific environment are entities of a disease resistance complex. Selection schemes could be designed either by direct selection or indirectly by selecting for correlated traits to disease resistance. Heritability estimates for survival to the diseases by direct selection indicate a significant additive genetic variance. This was confirmed by later estimates for resistance to columnaris disease in rainbow trout (Evenhuis et al., 2015), to *Piscirickettsia salmonis* in rainbow trout (Bassini et al., 2019), to *Piscirickettsia salmonis* in Coho salmon (Barría et al., 2019), to Viral Nervous Necrosis in European sea bass (Doan et al., 2017), to *Streptococcus iniae* and *S. agalactiae* in Nile tilapia (Shoemaker et al., 2017) and to koi herpesvirus in common carp (Ødegård et al., 2010; Palaiokostas et al., 2018b). The levels of genetic variation determined for resistance to disease demonstrate the feasibility of improving these traits through artificial selection. An increasingly important strategy for disease control and sustainable aquaculture development is applying selective breeding programs producing stocks with improved resistance to certain pathogens, exploiting significant heritability of disease resistance due to naturally occurring genetic variation for resistance in farmed aquaculture populations (Yáñez et al., 2014; Gjedrem, 2015; Houston, 2017).

It is worth mentioning again that, even with respect to disease resistance, genetic correlations are necessary to be known for each fish species before setting up a systematic selective breeding program. Numerous studies have reported various genetic correlations between resistance to diseases and growth rate. Some examples from recent years follow. Yáñez et al. (2016) and Barría et al. (2019) estimated the same negative genetic correlation (-0.50±0.13) between *Piscirickettsia salmonis* resistance and harvest weight for Coho salmon by different resistance measurement pattern. Doan et al. (2017) demonstrated moderate negative genetic correlation (-0.35±0.14) between Viral Nervous Necrosis resistance and body weight in European sea bass. Some non-significant genetic correlations between resistance to disease and growth rate were found in rainbow trout (Silverstein et al., 2009; Flores-Mara et al., 2017; Bassini et al., 2019). The absence of significant genetic correlations between these two commercially important traits suggests that artificial selection for resistance to disease will not influence growth rate and vice versa. Many studies on disease resistance for aquatic species are continuing, however, the successful development of selective breeding programs still faces a number of challenges. According to Gjedrem and Robinson (2014), when taking into account all major diseases, it is possible to obtain an average genetic gain of 12.5% per generation for disease resistance by applying challenge tests and thus to generate strains of fish and shellfish with high resistance. Thereby in the future it should be possible to reduce the use of antibiotics as well as vaccines in aquaculture and to produce healthier and more economically beneficial aquatic food.

Koi herpesvirus disease (KHVD), also known as Cyprinid herpesvirus-3 (CyHV-3) disease is a major threat to carp farming over the world. Increasing genetic resistance to KHVD via selective breeding may be important for the long-term sustainability of carp aquaculture. At present, high heritability estimates for KHVD resistance have been reported in common carp (Ødegård et al., 2010; Palaiokostas et al., 2018b), showing the feasibility of genetic improvement through selection. However, no genetic correlation between KHVD resistance and pond survival was found (Ødegård et al., 2010), the genetic correlations between KHVD and other performance traits are rarely reported in common carp. Accordingly, study on the genetic correlations between koi herpesvirus disease (KHVD) resistance and production traits in common carp was performed within work on topic of this dissertation (Chapter 4).

4.5. Processing yields

Processing yields such as fillet yield and headless carcass yield are the most frequent fish products and thus of high interest, especially for fish species sold in processed form (Kankainen et al., 2016). Processing traits influencing their profitability are more interesting for fish producers (Saillant et al., 2009). However, the direct measurement of these processing traits on live breeding candidates involves animal slaughter. Consequently, it may lose breeding potential within the stock. At present, processing yields are mostly genetically improved through indirect selection with correlated traits (e.g., Kause et al., 2007; Kocour et al., 2007; Melo et al., 2013), sib selection, indirect selection on some morphological predictors of processing yields (e.g., Haffray et al., 2013; Prchal et al., 2018; Vandeputte et al., 2017, 2019). In order to indirectly select animals for slaughter yields by measuring other characteristics, it is necessary to verify the relationships of these important traits. Previous studies have reported heritabilities estimated for biometrical traits and processing traits and their genetic correlations in some fish species (Neira et al., 2004; Rutten et al., 2005; Kause et al., 2007; Kocour et al., 2007; Powell et al., 2008; Navarro et al., 2009b; Nguyen et al., 2010; Gjerde et al., 2012; Haffray et al., 2012). Simple marker assisted selection could not be successfully applied for genetic improvement of slaughter yields due to polygenic architecture of this trait in fish species (Tsai et al., 2015; Gonzalez-Pena et al., 2016; Yoshida et al., 2019). In contrast, the use of genomic selection could be seen as future possibility for the genetic improvement of processing traits with better selection accuracy, for example, fillet yield in Nile tilapia breeding (Yoshida et al., 2019) and carcass and quality-related traits in Banana shrimp (Nguyen et al., 2020). However, genomic selection is still too costly and thus useful only for fish species with well-developed breeding programs e.g. Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*) (Robledo et al., 2018). For common carp (*Cyprinus carpio* or *Cyprinus rubrofuscus*) there is no available commercial SNP assay for such traits.

Moreover, morphological predictors of slaughter yields based on non-lethal selection criteria from body measurements (Bosworth et al., 1998; Cibert et al., 1999; Bosworth et al., 2001; Rutten et al., 2004; Van Sang et al., 2009), internal ultrasound and external 2D landmarks (Haffray et al., 2013; Vandeputte et al., 2017; Prchal et al., 2018) could be an effective option to improve processing yields, as they can be used on live candidates without need for sib or genomic information. In a previous study on common carp, genetic potential of slaughter yield predictors based on 2D image analysis and ultrasound measurements, and their use in carp breeding programs were investigated (Prchal et al., 2018). A high accuracy of predictors and a favourable genetic relationship to the real yields were observed. However, digitization of 2D landmarks requires post processing of images and cannot measure variation in body width. So, this method is incomplete and time consuming, which is a major technical limitation for practical breeding programs. Alternatively, 3D collection of body landmarks could speed up digitization of potentially relevant morphological predictors and take into account the variability of carp body width. A variety of 3D imagery systems have been used in pigs (Tillett et al., 2004), chickens (Mortensen et al., 2016) and cattle (Cappai et al., 2019; Le Cozler et al., 2019), as well as in rainbow trout (Vandeputte et al., 2019). However, their potential for real time slaughter yield prediction directly on live fish has been rarely studied. Thus, 3D digitation usability for prediction of slaughter yields in common carp and selective program purposes was studied within this dissertation (Chapter 5).

Even if 3D slaughter yield predictors seem to work well, their usage is still quite demanding for equipment and skillfulness of personals. Thus, further simplification was looked for. It was found that ultrasound tomography might be simply and efficiently used for indirect selection of slaughter yields (Bosworth et al., 2001; Haffray et al., 2013; Vandeputte et al., 2017; Prchal et al., 2018). Hence, within topic of this dissertation it was investigated more in deep if and how this tool could be applied in common carp culture under pond conditions of Central Europe (Chapter 6).

5. The aims of the thesis

The overall aim of this thesis was to get broader insight into mutual genetic and phenotypic relationships of production-related quantitative traits in common carp. The main focus was given to estimation of genetic parameters of traits that were not studied intensively in this fish species but that are of high importance with respect to production. So, traits related to energy reserves, fitness such as survival and resistance to the koi herpes virus and slaughter yields were studied in order to provide information for designing a practical and efficient selective breeding program for common carp.

The specific objectives were to:

1. Investigate the way of managing energy reserves that might relate to performance of the fish by studying phenotypic and genetic parameters of relevant traits and their genetic correlations.
2. 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.
3. Estimate genetic correlations of KHVD resistance with other important production traits in Amur mirror carp from yearlings to market size.
4. Determine the best morphological predictors and a simple predictor of slaughter yields using combination of 3D landmarks and ultrasound imagery or ultrasound imagery only in common carp, to predict the genetic progress achievable with their application in a hypothetical selection scheme.

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CHAPTER 2

THE ROLE OF ENERGY RESERVES IN COMMON CARP PERFORMANCE INFERRED FROM PHENOTYPIC AND GENETIC PARAMETERS

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The role of energy reserves in common carp performance inferred from phenotypic and genetic parameters

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ABSTRACT

In temperate zones, energy reserves of fish are closely related to survival during the first winter of their life. In this study, the genetic and phenotypic background of the accumulation, mobilization and utilization of energy reserves was investigated in Amur mirror carp. To achieve this, the role of traits related to energy reserves on fish performance during the first winter and further periods of rearing was investigated. The experimental stock was established by four full-factorial matings of 5 dams and 10 sires to generate up to 200 full-sibling families. The offspring were sampled before and after the first winter rearing period. Seasonal variation in direct and indirect measures of energy status was examined using Fulton's condition factor (FC), hepato-somatic index (HSI), visceral index (VSI_NO), glycogen, fat and protein in hepatopancreas (HP) and muscle fat content. Other performance traits were also recorded (weight, resistance to koi herpesvirus disease). All traits related to energy reserves, except HP protein, were significantly lower after the first winter. Overall, HP glycogen and fat from muscle, HP and viscera were mobilized during winter. However, genetic correlations between same traits recorded in autumn and spring were lower than 0.8 for most of the traits, implying that not all families responded to overwintering in a similar manner. Heritability also differed before and after the first winter. Before the first winter, all traits had low to medium heritability (0.05–0.35), but after the winter the same traits were moderately or highly heritable (0.22–0.58). Interestingly, HP glycogen traits, unlike HP fat and HP protein, and HSI recorded in yearlings were positively genetically correlated with survival during the third growing season (r_g = 0.49–0.72). This study provides the first evidence of a genetically based strategy for energy mobilization related to overwintering of common carp. Measuring of FC and HSI could be used to monitor the energy status of common carp and to provide a supplementary tool for management of carp stocks.

1. Introduction

Common carp (*Cyprinus carpio*) is one of the most widely cultivated freshwater fish species all over the world (FAO, 2018). It is also one of the most intensively studied fish species. In general, the first winter period is thought to be a critical period for common carp in temperate zones, in which catastrophic mortality may occur (Grote et al., 2018). Survival of fish in temperate zones during the first winter of their life is closely related to energy reserves, particularly glycogen, lipid and protein stores (e.g., Cunjak and Power, 1986; Henderson et al., 1988).

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Energy reserves may influence resistance to stressors (Schreck, 2010) or diseases (Choi et al., 1989; Encomio et al., 2005). As such, overwintering is an important period for the aquaculture industry of common carp in the Central Europe. Yearlings are more susceptible due to their greater vulnerability to winter stressors. Overwintering carp in temperate regions restrict their activity, reduce metabolism and feeding, resulting eventually in a decrease of weight (Bauer and Schlott, 2004; Hurst, 2007). However, the mechanisms by which overwintering carp use energy reserves, what mutual interactions exist among them, and what is their importance in terms of survival, are not well known.

Temperate-zone fishes rely on their ability to store and mobilize endogenous energy reserves during growing season in order to compensate low energy intake during overwintering that may last from four to six months (e.g., Oliver et al., 1979; Sogard and Olla, 2000; Finstad et al., 2004; Heermann et al., 2009; Crespel et al., 2013). Especially, energy mobilization during the first winter of fish life is an important physiological trait since it could crucially affect survival (Sogard, 1997; Crespel et al., 2013). The general pattern of energy reserve mobilization in many fishes when feeding activity ceases is first characterized by the acute depletion of glycogen (inducing a reduction of liver mass), followed by the use of lipids until reaching a critical threshold, and ultimately mobilization of protein (Jobling, 1980; Collins and Anderson, 1995; Hung et al., 1997; Rios et al., 2006). The mobilization of energy reserves is influenced by a variety of environmental factors, including primarily water temperature and dissolved oxygen levels (Pastoureaud, 1991; Bauer and Schlott, 2004). Mild water temperatures (above 8 °C) promote energy depletion and increase the metabolic rate suggesting that higher mortality may occur in warmer winter than in colder winter microenvironments (Pullin and Bale, 1989; Zani, 2008; Zani et al., 2012). These results indicate that warmer winters caused by global climate change have the potential to negatively affect fitness of some animals.

Fish stock with a known family structure is an effective way to study mobilization of energy reserves. Firstly, families can be sampled repeatedly, and even destructively, before and after the winter, to assess the changes of energy status at a family level. This is very difficult to perform at an individual level. Secondly, the family structure allows to estimate the degree of genetic variation in the traits and the genetic correlations with other performance traits. For example, significant genetic correlations were observed between morphometric indices like the condition factor, body mass and HSI with energy reserve indices (i.e., visceral fat, relative liver glycogen and total liver energy content) during the first winter of life in Laval brook charr, *Salvelinus fontinalis* (Crespel et al., 2013). Therefore, estimation of genetic parameters can help to understand the degree to which the energy mobilization is genetically determined. To date, the relationship between energy reserves and performance traits has rarely been studied in common carp.

The aim of the present study was to investigate the way of managing energy reserves in common carp that might relate to performance of the fish by studying phenotypic and genetic parameters of relevant traits and their genetic correlations. We focused on i) investigating the status of energy reserves before and after the first winter, ii) estimating genetic and phenotypic parameters of traits related to the first overwintering, and iii) examining how the energy reserves were genetically correlated to common carp performance up to market size including resistance to a disease.

2. Materials and methods

2.1. Ethics statement

The entire experiment was performed according to the law on the protection of animals against cruelty (Act No. 246/1992 Coll. of the Czech Republic) upon its approval by the expert committee of the Institutional Animal Care and Use Committee (IACUC). People conducting the trait measurements on live animals and challenge test were qualified to conduct and manage experiments on the live animals according to the above-mentioned act.

2.2. Establishment and rearing of experimental stocks

The reproduction of common carp was performed in 2014 at the fish hatchery of the Genetic Fishery Centre of University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters (USB, FFPW) in Vodňany, Czech Republic, while carp were cultured at USB FFPW and Klatovy Fish Farm, JSC. The Amur mirror carp breed, newly recognized in the Czech Republic (Flajšhans et al., 2015), was chosen as broodstock fish. In May 2014, gametes from 20 dams and 40 sires were used for artificial spawning according to the protocol by Vandeputte et al. (2004) and crossed by four full-factorial series of 5 dams and 10 sires to generate an expected 200 full-sibling families. From each of the parents used in the cross, a fin clip of 0.5 cm^2 from the caudal fin was collected and stored in 98% ethanol at room temperature for later parentage assignment of the offspring. After fertilization, the eggs from each of the four series 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. The experimental stock was then created by pooling equal quantities (estimated volumetrically) of larvae from all four post-hatching incubators and stocked in two earthen nursery ponds at a density of 150,000 larvae. ha^{-1}. Thereafter, the progenies were reared communally under semi-intensive pond management conditions with the fish being fed on natural food. From age of 8 weeks supplementary feeding was served 2–3 times until water temperature decreased below 8 °C (October 2014). Both stocks were kept in ponds during the first growing period and the first overwintering. Fish out of both ponds were harvested and restocked before the first winter to perform i) evaluating of energy reserves status on one stock and ii) random sampling of part of the second stock for a koi herpesvirus (KHV) disease challenge test (Palaiokostas et al., 2018; Zhao et al., 2020).

2.3. Phenotypic recording before the first winter

Before the first winter (November 2014) stock from a pond with the higher mean weight of fish (13.5 g) and with average survival rate of 17.2% was selected for evaluating the status of the energy reserves. Before that 5000 fish out of 34,400 harvested fish were randomly selected and individually PIT-tagged, fin-clipped for later parentage recognition, weighed and measured for standard length and stocked back to a pond for overwintering period and phenotypic recording after the first winter. Another random sample of 1000 individuals was anesthetized to death using 2-phenoxyethanol (dose of 0.8 mL per 1 L of water) and also individually fin-clipped for later pedigree reconstruction. Every individual was recorded for body weight (BW) (to the nearest 0.1 g) and for standard length (SL) (to the nearest mm). Fulton's condition factor (FC) was calculated according to the equation (10^5 * BW (g)/SL^3 (mm)). Subsequently, the hepatopancreas (HP) was excised and weighed to determine the hepato-somatic index (HSI: hepatopancreas weight/body weight * 100) and stored at −80 °C until further analysis. Furthermore, the intestine was also excised and weighed to determine the visceral index (VSI_NO = intestine (without HP) weight/body weight * 100). In addition, the left half of the body muscle was excised and homogenized using a TissueLyser II (Qiagen) and part of the homogenate was dried at 105 °C for determining its dry matter. Rest of the homogenate was stored at −80 °C until fat analysis. Dry matter percentage was determined according to the equation (weight of dried material/weight of wet material * 100).

Glycogen, crude protein and total fat were analyzed from the HP. With regards to the muscle homogenate only the total fat was analyzed as it was previously shown that muscle crude protein and glycogen in carp do not play a significant role during food deprivation (Blasco et al., 1992; Binner et al., 2008). HP glycogen concentration (conc.) was measured as described by Roe and Dailey (1966). The procedure involved digestion of tissue in boiling sodium hydroxide, deproteinization of the alkaline digest with trichloroacetic acid, precipitation with ethanol, washing with ethanol, and color production with anthrone reagent. Total fat concentration in muscle and HP was analyzed using the sulfo-phospho-vanillin method according to Zoellner and Kirsch (1962) with modifications according to Saborowski and Buchholz (1996). Protein was extracted from liver and muscle samples as described by Munro and Fleck (1966) and concentrations were determined by the Bradford method (Bradford, 1976) with a commercial protein assay (Bio-Rad). Absolute weight of fat, glycogen and protein in the entire hepatopancreas were calculated by their respective concentration multiplied by HP weight. HP glycogen content per gram of fish weight (Rel. HP Glycogen) was calculated as the ratio between absolute HP glycogen and weight of fish. Energy content in HP was estimated by using conversion factors of 39, 23.5, and 17 kJ/g of lipids, proteins, and glycogen, respectively (Jobling, 2017). HP energy content was expressed on both a relative and an absolute basis. HP energy concentration (HP Energy conc.) is energy stored per gram of HP tissue. Absolute HP energy is energy stored in the whole HP tissue and was calculated as HP Energy conc. * HP weight. Specific energy content per gram of fish weight (Rel. HP Energy) was calculated as the ratio between absolute HP energy and weight of fish.

2.4. Phenotypic recording after the first winter

As collecting tissues for the evaluation of energy reserves before winter needed sacrificing the individuals, traits after winter were thought to be recorded on 1000 survived siblings coming from the same pond. Unfortunately, the 5000 PIT-tagged siblings disappeared during overwintering (the reason was not found out but predation or environmental conditions in the pond are the most probable). Therefore, 1000 siblings coming from the second pond were taken and processed the same way and recorded for the same traits as the fish before winter.

2.5. Koi herpesvirus disease (KHVD) challenge test

In autumn 2014, a 1500 fish subset of the same stock as the one sampled after the first winter was created at random. All individuals were PIT-tagged and fin-clipped for subsequent pedigree construction. These fish were then acclimatized together with koi (*Cyprinus rubrofuscus*) ($n = 215$) for five days to 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. Hereafter, the fish were transferred to the Veterinary Research Institute (VRI) in Brno (Czech Republic) to perform the KHVD challenge test. The experimental procedure was the same as described in Palaiokostas et al. (2018). Cohabitation challenge protocol was performed in a 1.4 m^3 tank with recirculation and biological filtration. 20 fish out of the koi received an intraperitoneal injection with KHV culture established according to the standardized protocol of Piačková et al. (2013) and were cohabited with Amur mirror carp and the rest of koi. Mortality of individual fish was recorded twice a day until 35 days post infection (dpi) when mortality was negligible. First mortalities appeared at 12 dpi, reached a maximum between 21 and 24 dpi (Fig. 1) and then they continuously decreased. The percentage of overall mortality in the KHVD challenge test for the Amur mirror carp was 66%. For statistical analysis resistance was recorded as 0 for dead fish and 1 for alive fish. Presence of KHV on a sample of dead fish ($n = 100$) was confirmed by nested PCR as described by Pokorova et al. (2010).

2.6. Phenotypic recordings in older fish

Three thousand individuals from the remaining part of the stock that was used for the KHVD challenge test and the determination of the energy reserves status after the first winter were PIT-tagged and fin-clipped in spring 2015. Fish were measured for BW and SL and stocked in a pond of 1 ha for the second growing season. All survivors were stocked in November 2015 for the second winter in a 0.2 ha pond and in March 2016 they were stocked in a 3 ha pond for the third and last growing season. The following traits, survival, BW, SL and muscle fat before and after the second winter and at market size (after the third growing season) were recorded. Survival after the second winter (Survival_C2)

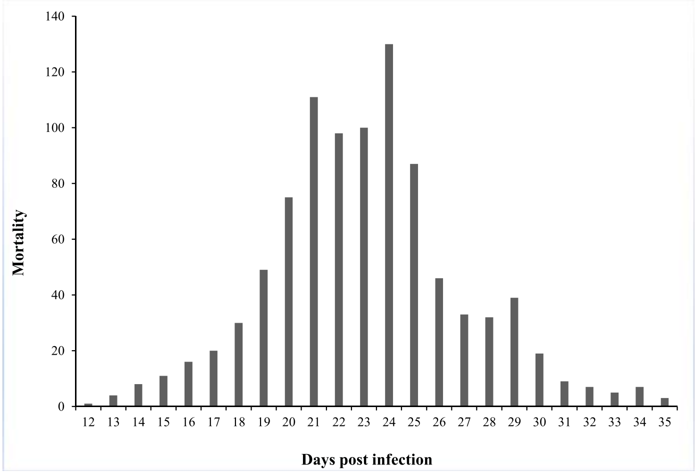

Fig. 1. Daily mortality of fish (number of died individuals) during the KHVD challenge test.

and during the third growing season (Survival_C3) were coded with 1 given for survived fish and 0 for fish not found during the trait recordings. FC was also calculated as described above and muscle fat percent was measured on live fish using the Distell Fish Fat Meter (Model FFM-992).

2.7. *Parentage assignment*

Parentage assignment of the first year fish (energy reserves related traits), the second and the third year fish (production traits) was based on the analysis of 12 microsatellite loci labelled as CCE46 (Wang et al., 2007), HLJE265, HLJ2241, HLJ2346, HLJ2382, HLJ24657, HLJ2544, HLJ334, HLJ526, HLJ534 (Zheng et al., 2011), J58 (Yue and Orban, 2002), KOI 57–58 (David et al., 2001) and performed using the AccurAssign software, applying a maximum-likelihood method (Boichard et al., 2014). Parentage assignment of challenged fish to KHVD (1500 fish) was done by SNP genotype data (12,311 SNPs grouped in 50 linkage groups) using hsphase v2.0.2 (Ferdosi et al., 2014) package of R v3.6.1 (R Core Team, 2019).

2.8. *Data analysis*

The fact that data before winter and after winter came from two separated stocks (coming from different ponds) affected or did not allow certain parameter estimations and decreased the power of some results and conclusions. For instance, winter survival during the first winter could not be correlated with traits of energy reserves, real trends in change of mean values in many traits before and after winter, especially those concerning the energy reserves, could not be observed. That is why we focused more on the genetic background of energy reserves and correlations of energy reserves after the first winter with i) survival in further periods of rearing cycle, ii) survival during the KHVD challenge test done on subset of siblings coming from the same stock and iii) other important performance traits in further years of rearing until market size.

Descriptive statistics were used to present traits recorded before and after the first winter. All trait values were checked for outliers that may indicate errors during measurements and recordings. Basic data statistical analysis was completed and differences between the trait means were tested by Welch two sample *t*-test using R v3.6.1 (R Core Team, 2019). A significance level of $\alpha = 0.05$ was used in all statistical tests.

Heritability estimates as well as phenotypic and genetic correlations (r_p and r_g, respectively) were evaluated with a restricted maximum likelihood (REML) approach in DMU statistical software (Madsen and Jensen, 2013). An animal model was applied as follows:

$\boldsymbol{y} = X\boldsymbol{b} + Z\boldsymbol{a} + \boldsymbol{e}$,

where $\boldsymbol{y}$ is the vector of observations for the studied traits, $\boldsymbol{b}$ is the vector of fixed effects for traits recorded before and after the first winter and KHVD resistance (intercept) and traits recorded after the second winter and at market size (sex); $\boldsymbol{a}$ is the vector of random additive genetic effect of an animal $\sim N(0, \mathbf{A}\sigma_a^2)$; and $\boldsymbol{e}$ is the vector of the random residual effect $\sim N(0, \mathbf{I}\sigma_e^2)$. X and Z are incidence matrices relating observations to the fixed and random effects, respectively. **A** is the additive genetic relationship matrix; **I** is an identity matrix; σ_a^2 is the additive genetic variance and σ_e^2 is the residual variance.

The heritability was calculated as $h^2 = \sigma_a^2 / \sigma_a^2 + \sigma_e^2$.

The estimated heritability values were considered significant when the difference of additive genetic effect 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 correlations were estimated using multivariate models. Correlations among traits before and after the first winter and among traits after the first winter and further periods were measured on siblings (different individuals). Thus, residual covariances between these traits were set to zero. Correlations among traits calculated within given recordings had identical set of individuals and so residual covariances were included in the estimates. Estimated genetic correlation was considered significant when $|r_g| - |1.96 * \text{S.E.}|$ was higher than zero (two-tailed hypothesis) (Coolidge, 2013).

3. Results

3.1. *Mean values of traits before and after the first winter*

For a total of 16 traits including those concerning energy reserves status, the mean values differed between autumn and spring (Table 1). The stock of fish sampled in spring displayed higher mean weight. Nevertheless, most spring recorded traits were significantly lower after the first winter. The values of most energy reserves (muscle fat concentration, HP glycogen and fat traits and HP energy contents) were lower with almost halved values ($p < 0.05$). Only, HP protein was found to be higher after the winter than before the winter.

3.2. *Genetic correlation between spring and autumn measurements*

Almost all traits before the first winter were significantly genetically correlated (0.50 ± 0.18–0.87 ± 0.07) with the recordings after the first winter. Still, most genetic correlations were lower than 0.80 (Table 2) meaning interactions between spring and autumn measurements. On the other hand, HSI and HP Protein conc. before and after the first winter were not significantly genetically correlated (0.36 ± 0.20 and 0.41 ± 0.25, respectively).

3.3. *Heritability estimates*

The estimated heritability for all traits were higher after the first winter recordings (Table 1). Before winter, low heritability estimates for HP Glycogen and Protein conc. were estimated (0.05 ± 0.03 and 0.09 ± 0.04, respectively). Moreover, the other traits were low to medium heritable (0.13–0.35). However, after winter, all traits were moderately or highly heritable (0.22–0.58). Interestingly, the heritability for HP Glycogen conc. (0.05 ± 0.03 vs. 0.41 ± 0.08) and Rel. HP Glycogen (0.14 ± 0.05 vs. 0.50 ± 0.09) before winter were remarkably lower than after winter, although the mean value of traits was much lower after winter.

3.4. *Genetic and phenotypic correlations of traits before the first winter*

Genetic and phenotypic correlations among traits before the first winter are presented in Table 3. Generally, phenotypic correlations were much lower than genetic correlations. Most phenotypic correlations were lower than 0.7 in absolute value. Those above 0.7 were mostly expected as they were related to some absolute and relative values of HP energy reserves, or to BW and absolute values of HP energy reserves. It was also shown that HSI is a good indicator of Rel. HP Energy ($r_p = 0.83$). On the other hand, low phenotypic correlations were observed between M. Fat conc. and HP Fat, HP Glycogen even HP Protein traits (−0.03–0.35).

Many significant genetic correlations values were observed (Table 3). Positive genetic correlations between BW and M. Fat conc. (0.42 ± 0.16) or VSI_NO (0.53 ± 0.19) were evidenced, while a negative genetic correlation (−0.52 ± 0.19) was observed between BW and HP Protein conc. No significant genetic correlation was observed between FC and BW or M. Fat conc., but correlations between FC and HSI, HP Glycogen conc. and Rel. HP Glycogen (r_g = 0.59 ± 0.17, 0.60 ± 0.28 and 0.55 ± 0.18, respectively) were positive and significant. Correlations between VSI_NO and other traits were positive and significant, except with FC, HSI, HP Glycogen conc., Rel. HP Glycogen and HP Protein conc. HSI was positively genetically correlated only with HP Glycogen conc. (0.75 ± 0.26), Rel. HP Glycogen (0.97 ± 0.04) and Rel. HP Energy (0.82 ± 0.08). In terms of genetic correlations among traits of energy reserves, the highest values were observed between muscle and HP fat traits. Nonsignificant correlations were observed between the relative fat and glycogen traits. Finally,

Table 1

Basic phenotypic parameters and heritability estimates for traits before and after the first winter. Number of observations (*N*), traits mean (Mean ± S.D.), *CV* (coefficient of variation), V_P (phenotypic variance), V_A (genetic variance), h^2 (heritability estimates ± S.E.)

Trait	Units	Before the first winter						After the first winter						*P* value
		N	Mean ± S.D.	*CV*	V_P	V_A	h^2 ± S.E.	*N*	Mean ± S.D.	*CV*	V_P	V_A	h^2 ± S.E.	
Weight	g	954	13.45 ± 5.69	42.3	31.92	10.38	0.33 ± 0.07	939	14.86 ± 5.24	35.3	27.28	9.28	0.34 ± 0.07	<0.001
FC		954	3.48 ± 0.34	9.6	0.11	0.04	0.35 ± 0.08	939	3.14 ± 0.23	7.4	0.05	0.03	0.58 ± 0.10	<0.001
VSI_NO	%	941	9.42 ± 2.56	27.2	6.59	0.89	0.14 ± 0.05	935	6.86 ± 1.16	17	1.38	0.34	0.25 ± 0.07	<0.001
HSI	%	946	5.88 ± 1.34	22.8	1.78	0.25	0.14 ± 0.05	937	4.97 ± 0.97	19.6	0.95	0.41	0.43 ± 0.09	<0.001
Dry matter	%	920	24.46 ± 2.39	9.8	5.67	1.49	0.26 ± 0.07	932	20.06 ± 1.45	7.2	2.14	0.81	0.38 ± 0.08	<0.001
M. Fat conc.	mg/g	920	56.29 ± 18.21	32.4	325.65	93.35	0.29 ± 0.07	932	23.26 ± 9.39	40.4	89.77	42.62	0.47 ± 0.09	<0.001
HP Fat conc.	mg/g	941	52.37 ± 19.81	37.8	393.11	74.27	0.19 ± 0.06	939	20.46 ± 8.23	40.2	67.25	19.87	0.30 ± 0.07	<0.001
Abs. HP Fat	mg	934	41.21 ± 25.78	62.5	665.92	158.10	0.24 ± 0.06	937	14.95 ± 8.67	57.9	74.74	21.34	0.29 ± 0.07	<0.001
HP Glycogen conc.	mg/g	919	100.16 ± 22.02	22	485.02	25.83	0.05 ± 0.03	932	49.48 ± 22.67	45.8	522.82	212.83	0.41 ± 0.08	<0.001
Abs. HP Glycogen	mg	911	76.34 ± 33.48	43.9	1137.11	284.24	0.25 ± 0.07	930	37.09 ± 23.53	63.4	558.75	222.16	0.40 ± 0.08	<0.001
Rel. HP Glycogen	mg/g BW	911	5.93 ± 1.99	33.6	3.94	0.54	0.14 ± 0.05	931	2.55 ± 1.43	56	2.08	1.04	0.50 ± 0.09	<0.001
HP Protein conc.	mg/g	937	66.86 ± 13.06	19.5	170.30	15.54	0.09 ± 0.04	931	71.89 ± 13.95	19.4	194.65	42.14	0.22 ± 0.06	<0.001
Abs. HP Protein	mg	929	50.26 ± 18.74	37.3	352.45	53.14	0.15 ± 0.05	899	52.34 ± 23.04	44	532.26	160.01	0.30 ± 0.07	0.03
HP Energy conc.	kJ/g HP	905	5.31 ± 0.78	14.8	0.62	0.09	0.15 ± 0.05	899	3.34 ± 0.49	14.5	0.24	0.06	0.24 ± 0.06	<0.001
Abs. HP Energy	kJ	898	4.04 ± 1.63	40.3	2.69	0.65	0.24 ± 0.06	897	2.44 ± 1.02	42.8	1.03	0.35	0.34 ± 0.08	<0.001
Rel. HP Energy	kJ/g BW	898	0.31 ± 0.08	27.8	74.92	10.09	0.13 ± 0.05	897	0.17 ± 0.04	24.2	16.39	5.47	0.33 ± 0.08	<0.001

Legend: FC (Fulton's condition factor) = 10^5 * weight (g)/standard length^3 (mm), VSI_NO (visceral index without HP) = intestine (without hepatopancreas) weight/weight of fish, HSI (hepato-somatic index) = HP weight/weight of fish, M. Fat conc. = fat in muscle (mg per gram of muscle tissue), HP Fat conc. = concentration of fat in hepatopancreas (mg per gram of HP tissue), Abs. HP Fat = Absolute fat content in whole HP (HP Fat conc. * HP weight), HP Glycogen conc. = concentration of glycogen in hepatopancreas (mg per gram of HP tissue), Abs. HP Glycogen = Absolute glycogen content in whole HP (HP Glycogen conc. * HP weight), Rel. HP Glycogen = Content of HP Glycogen per g of fish (Absolute HP glycogen/fish weight in g), HP Protein conc. = concentration of protein in hepatopancreas (mg per gram of HP tissue), Abs. HP Protein = Absolute protein content in whole HP (HP Protein conc. * HP weight), HP Energy conc. = Sum of fat, glycogen and protein energy in hepatopancreas per gram of HP tissue, Abs. HP Energy = Total energy in HP (HP Energy conc. * HP weight), Rel. HP Energy = Energy level of HP per g of fish (Absolute HP Energy/fish weight in g).

Table 2

Genetic correlations of traits between two sibling stocks of Amur mirror carp before and after the first winter. Underline numbers indicate significant correlations, numbers in italics moreover represent low genotype by environment (G × E) interaction. See Table 1 for trait abbreviations.

Trait	*Rg* ± S.E.
Weight	$\underline{0.69 \pm 0.12}$
FC	$\underline{\mathit{0.87 \pm 0.07}}$
VSI_NO	$\underline{0.61 \pm 0.18}$
HSI	0.36 ± 0.20
M. Fat conc.	$\underline{0.59 \pm 0.14}$
HP Fat conc.	$\underline{0.50 \pm 0.18}$
Abs. HP Fat	$\underline{0.52 \pm 0.16}$
HP Glycogen conc.	$\underline{0.70 \pm 0.27}$
Abs. HP Glycogen	$\underline{0.58 \pm 0.15}$
Rel. HP Glycogen	$\underline{0.51 \pm 0.19}$
HP Protein conc.	0.41 ± 0.25
Abs. HP Protein	$\underline{0.59 \pm 0.18}$
HP Energy conc.	$\underline{0.54 \pm 0.20}$
Abs. HP Energy	$\underline{0.60 \pm 0.15}$
Rel. HP Energy	$\underline{0.51 \pm 0.21}$

the relationships among relative HP protein traits and HP glycogen or fat-related traits were mostly significant and negative (Table 3).

3.5. Genetic and phenotypic correlations of traits after the first winter

Genetic and phenotypic correlations among traits after the first winter are listed in Table 4. For phenotypic correlations, most features were similar for both periods – before and after the first winter. The differences were connected to HP glycogen traits and Rel. HP Energy. For instance, in spring, unlike autumn, correlation between Abs. HP Glycogen and HP Glycogen conc. was higher (r_p = 0.81 vs. 0.46 in autumn), as well as correlation between Abs. HP Glycogen and Rel. HP Glycogen (r_p = 0.84 vs. 0.53 in autumn) or correlation between Rel. HP Energy and HP Glycogen conc. (r_p = 0.57 vs. 0.21 in autumn). On the other hand, correlation between Abs. HP Glycogen and Abs. HP Fat was lower in spring (r_p = 0.25 vs. 0.57 in autumn).

In terms of BW the biggest changes were seen in the corresponding correlations with VSI_NO, Abs. HP Glycogen and HP Protein conc. Following the first winter, nonsignificant correlation between BW and VSI_NO (r_g = −0.31 ± 0.18 vs. 0.53 ± 0.19 in autumn) or between BW and Abs. HP Glycogen (r_g = 0.23 ± 0.17 vs. 0.83 ± 0.07 in autumn) were observed. On the contrary, significant and negative genetic correlation between BW and HP Protein conc. before the first winter changed to significant positive correlation after the first winter (r_g = 0.55 ± 0.15 vs. −0.52 ± 0.19 in autumn). Regarding FC, no significant correlation was observed with Rel. HP Glycogen and conversely, the correlation between FC and Rel. HP Energy became significant. Unlike before the first winter, no correlations of VSI_NO with other traits were significant. For M. Fat conc., among others, correlation with Abs. HP Glycogen became insignificant (r_g = 0.24 ± 0.16 vs. 0.50 ± 0.16 in autumn) and oppositely, correlation with Rel. HP Energy became significant (r_g = 0.59 ± 0.12 vs. 0.23 ± 0.22 in autumn). HP Fat conc. became significantly and negatively correlated with relative HP glycogen traits, positively correlated with HP Protein conc. (r_g = 0.66 ± 0.13 vs. −0.21 ± 0.26 in autumn) and no correlation was observed with Abs. HP Energy (r_g = 0.26 ± 0.17 vs. 0.52 ± 0.16 in autumn).

3.6. Relationship of correlations in autumn and spring

The scatterplot extracted from Table 3 and Table 4 for the relationships of all phenotypic and genetic correlations of autumn and spring showed a positive and linear relationship (Fig. 2, phenotypic correlations; Fig. 3, genetic correlations). However, Fig. 3 showed a weaker correlation which means that globally, the phenotypic correlations between autumn and spring were more consistent than the genetic correlations.

3.7. Genetic relationship among first winter energy reserves traits and performance traits in further rearing periods

Genetic correlations among energy reserves parameters in yearlings after the first winter and traits in older siblings are shown in Table 5. % M Fat_SpringC2 showed significant genetic correlations with M. Fat conc., HP Fat conc. and Abs. HP Fat (r_g = 0.40 ± 0.14, 0.58 ± 0.12 and 0.67 ± 0.11, respectively). HP Protein conc. was negatively correlated with FC_SpringC2 (r_g = −0.43 ± 0.15) and FC_C3 (r_g = −0.72 ± 0.10), but

Table 3
Genetic (above diagonal; ±S.E.) and phenotypic (below diagonal) correlations of traits before the first winter. See Table 1 for trait abbreviations. Underline numbers indicate statistically significant correlations.

	Weight	FC	VSI_NO	HSI	M. Fat conc.	HP Fat conc.	Abs. HP Fat	HP Glycogen conc.	Abs. HP Glycogen	Rel. HP Glycogen	HP Protein conc.	Abs. HP Protein	HP Energy conc.	Abs. HP Energy	Rel. HP Energy
Weight		−0.11 ± 0.18	0.53 ± 0.19	−0.39 ± 0.19	0.42 ± 0.16	0.39 ± 0.18	0.85 ± 0.07	−0.26 ± 0.29	0.83 ± 0.07	−0.33 ± 0.20	−0.52 ± 0.19	0.92 ± 0.05	0.27 ± 0.21	0.89 ± 0.05	−0.20 ± 0.21
FC	−0.09		0.18 ± 0.22	0.59 ± 0.17	0.25 ± 0.18	−0.16 ± 0.20	0.02 ± 0.19	0.60 ± 0.28	0.26 ± 0.19	0.55 ± 0.18	−0.40 ± 0.22	−0.01 ± 0.21	−0.13 ± 0.21	0.08 ± 0.19	0.39 ± 0.20
VSI_NO	−0.01	0.05		0.06 ± 0.26	0.56 ± 0.18	0.71 ± 0.17	0.78 ± 0.17	0.34 ± 0.32	0.73 ± 0.18	0.17 ± 0.26	−0.31 ± 0.26	0.63 ± 0.24	0.90 ± 0.17	0.74 ± 0.18	0.54 ± 0.24
HSI	−0.26	0.17	−0.24		−0.09 ± 0.22	−0.16 ± 0.23	−0.08 ± 0.23	0.75 ± 0.26	0.17 ± 0.22	0.97 ± 0.04	−0.25 ± 0.26	−0.05 ± 0.26	−0.04 ± 0.26	0.04 ± 0.23	0.82 ± 0.08
M. Fat conc.	0.19	0.12	0.25	−0.08		0.55 ± 0.15	0.60 ± 0.14	0.20 ± 0.29	0.50 ± 0.16	0.01 ± 0.22	−0.47 ± 0.21	0.44 ± 0.18	0.52 ± 0.17	0.63 ± 0.14	0.23 ± 0.22
HP Fat conc.	0.18	0.003	0.14	0.01	0.35		0.74 ± 0.10	−0.37 ± 0.29	0.29 ± 0.20	−0.24 ± 0.23	−0.21 ± 0.26	0.31 ± 0.21	0.97 ± 0.03	0.52 ± 0.16	0.34 ± 0.22
Abs. HP Fat	0.63	0.02	0.004	0.20	0.32	0.73		−0.04 ± 0.30	0.85 ± 0.08	−0.08 ± 0.23	−0.56 ± 0.20	0.84 ± 0.08	0.76 ± 0.11	0.96 ± 0.02	0.39 ± 0.19
HP Glycogen conc.	−0.17	0.14	0.05	0.17	−0.07	−0.22	−0.17		0.26 ± 0.29	0.90 ± 0.10	−0.58 ± 0.27	−0.00 ± 0.33	−0.28 ± 0.34	0.17 ± 0.30	0.56 ± 0.29
Abs. HP Glycogen	0.62	0.09	−0.08	0.35	0.10	0.05	0.57	0.46		0.22 ± 0.22	−0.77 ± 0.14	0.94 ± 0.05	0.22 ± 0.22	0.95 ± 0.03	0.33 ± 0.21
Rel. HP Glycogen	−0.26	0.19	−0.13	0.77	−0.10	−0.13	0.03	0.75	0.53		−0.39 ± 0.23	0.03 ± 0.25	−0.16 ± 0.26	0.11 ± 0.23	0.79 ± 0.11
HP Protein conc.	−0.08	−0.12	−0.24	−0.21	−0.03	−0.02	−0.16	−0.34	−0.37	−0.36		−0.47 ± 0.27	−0.09 ± 0.29	−0.62 ± 0.19	−0.29 ± 0.27
Abs. HP Protein	0.74	−0.05	−0.24	0.23	0.15	0.15	0.63	−0.22	0.62	0.02	0.34		0.20 ± 0.24	0.92 ± 0.04	0.17 ± 0.25
HP Energy conc.	0.08	0.02	0.07	0.004	0.29	0.87	0.63	0.12	0.16	0.08	0.21	0.18		0.49 ± 0.18	0.52 ± 0.18
Abs. HP Energy	0.75	0.03	−0.08	0.31	0.27	0.50	0.92	0.01	0.81	0.21	−0.12	0.81	0.45		0.35 ± 0.21
Rel. HP Energy	−0.21	0.15	−0.18	0.83	0.08	0.47	0.53	0.21	0.42	0.69	−0.09	0.30	0.54	0.52	

Table 4
Genetic (above diagonal; ±S.E.) and phenotypic (below diagonal) correlations of traits after the first winter. See Table 1 for trait abbreviations. Underline numbers indicate statistically significant correlations.

	Weight	FC	VSI_NO	HSI	M. Fat conc.	HP Fat conc.	Abs. HP Fat	HP Glycogen conc.	Abs. HP Glycogen	Rel. HP Glycogen	HP Protein conc.	Abs. HP Protein	HP Energy conc.	Abs. HP Energy	Rel. HP Energy	A/S deviations
Weight		0.02 ± 0.17	−0.31 ± 0.18	−0.16 ± 0.17	0.15 ± 0.17	0.35 ± 0.17	0.82 ± 0.07	−0.14 ± 0.18	0.23 ± 0.17	−0.15 ± 0.17	0.55 ± 0.15	0.96 ± 0.02	0.51 ± 0.16	0.84 ± 0.05	0.11 ± 0.18	6/1
FC	−0.02		0.30 ± 0.17	0.36 ± 0.15	0.26 ± 0.15	0.01 ± 0.17	0.15 ± 0.17	0.12 ± 0.16	0.14 ± 0.16	0.19 ± 0.16	−0.04 ± 0.18	0.22 ± 0.17	0.08 ± 0.18	0.22 ± 0.16	0.35 ± 0.16	2/0
VSI_NO	−0.12	0.16		0.30 ± 0.18	0.07 ± 0.19	0.00 ± 0.20	−0.08 ± 0.20	0.26 ± 0.18	0.04 ± 0.19	0.26 ± 0.18	−0.26 ± 0.20	−0.18 ± 0.19	0.09 ± 0.21	−0.10 ± 0.19	0.27 ± 0.19	10/0
HSI	−0.11	0.15	0.01		0.35 ± 0.15	−0.50 ± 0.15	−0.08 ± 0.18	0.89 ± 0.05	0.80 ± 0.07	0.95 ± 0.03	−0.86 ± 0.08	0.05 ± 0.18	−0.01 ± 0.19	0.35 ± 0.16	0.89 ± 0.04	5/1
M. Fat conc.	−0.11	0.18	0.15	0.18		0.57 ± 0.13	0.63 ± 0.12	0.21 ± 0.17	0.24 ± 0.16	0.23 ± 0.16	0.11 ± 0.19	0.35 ± 0.16	0.70 ± 0.11	0.48 ± 0.14	0.59 ± 0.12	7/0
HP Fat conc.	0.08	0.12	0.12	−0.14	0.49		0.73 ± 0.09	−0.38 ± 0.16	−0.31 ± 0.17	−0.43 ± 0.15	0.66 ± 0.13	0.31 ± 0.18	0.81 ± 0.08	0.26 ± 0.17	−0.09 ± 0.19	7/0
Abs. HP Fat	0.63	0.11	0.01	0.16	0.32	0.72		−0.02 ± 0.18	0.24 ± 0.17	−0.05 ± 0.18	0.52 ± 0.16	0.85 ± 0.06	0.88 ± 0.07	0.85 ± 0.05	0.30 ± 0.17	5/1
HP Glycogen conc.	−0.10	0.10	0.12	0.47	0.12	−0.22	−0.07		0.93 ± 0.03	0.99 ± 0.01	−0.84 ± 0.08	0.01 ± 0.19	0.21 ± 0.18	0.39 ± 0.16	0.89 ± 0.05	5/3
Abs. HP Glycogen	0.34	0.10	0.003	0.57	0.06	−0.18	0.25	0.81		0.91 ± 0.03	−0.58 ± 0.14	0.37 ± 0.16	0.34 ± 0.17	0.68 ± 0.10	0.87 ± 0.06	8/3
Rel. HP Glycogen	−0.11	0.12	0.09	0.72	0.15	−0.22	−0.01	0.94	0.84		−0.86 ± 0.07	0.04 ± 0.18	0.15 ± 0.18	0.38 ± 0.15	0.91 ± 0.04	5/2
HP Protein conc.	0.18	−0.04	−0.16	−0.40	−0.06	0.23	0.16	−0.47	−0.38	−0.50		0.50 ± 0.15	0.29 ± 0.19	0.19 ± 0.19	−0.61 ± 0.15	8/0
Abs. HP Protein	0.84	0.03	−0.16	0.16	−0.04	0.13	0.67	−0.10	0.34	−0.02	0.45		0.53 ± 0.15	0.92 ± 0.03	0.26 ± 0.18	3/0
HP Energy conc.	0.10	0.12	0.07	0.02	0.37	0.65	0.52	0.34	0.27	0.27	0.45	0.30		0.68 ± 0.12	0.48 ± 0.15	3/0
Abs. HP Energy	0.79	0.09	−0.08	0.37	0.11	0.24	0.79	0.24	0.66	0.32	0.15	0.89	0.44		0.63 ± 0.11	6/0
Rel. HP Energy	−0.04	0.18	0.04	0.81	0.36	0.26	0.43	0.57	0.61	0.73	−0.05	0.30	0.59	0.55		6/1

positively with % M Fat_SpringC2 ($r_g = 0.54 \pm 0.14$) and BW_SpringC2 ($r_g = 0.40 \pm 0.16$). Glycogen related traits were genetically related to higher survival during the third growing season (r_g = 0.49–0.69). In contrary, fat and protein traits in hepatopancreas were correlated to lower survival during the last growing season ($r_g = -0.59 \pm 0.20$ and -0.75 ± 0.21, respectively). Rel. HP Energy was significantly genetically correlated with FC_SpringC2 and FC_C3 ($r_g = 0.35 \pm 0.15$ and 0.31 ± 0.15, respectively).

3.8. Genetic relationship between non-energy reserves related traits and survival traits

Genetic correlations of other traits in yearlings after the first winter and survival of their older siblings are presented in Table 6. High positive genetic correlations were observed between HSI in one-year old carp after the first winter and survival during last (third) growing season ($r_g = 0.72 \pm 0.20$). FC and VSI_NO were not correlated with survival after the second winter and last growing season.

3.9. Genetic relationship between energy reserves and KHVD resistance

No significant genetic correlations were observed between KHVD resistance and the energy reserves related traits before and after the first winter season (Table 7).

4. Discussion

In this study, we investigated the accumulation, mobilization and usage of energy reserves in common carp during the first winter, the most critical period for survival of carp in the temperate zone. Furthermore, we examined the role of the energy reserves-related traits and estimated their genetic parameters. To the best of our knowledge this study is the first reporting heritability values and genetic correlations for traits related to energy reserves in common carp.

4.1. Mobilization of energy reserves during overwintering

Even though the stock sampled after the first winter had a moderately higher weight (14.9 g) than the stock before the first winter (13.5 g), most traits related to energy reserves were significantly lower after the first winter. Phenotypic as well as genetic correlations between body weight and absolute values of energy reserves traits in both periods were positive and quite high. Thus, there was a real reduction of mean values of traits related to fat and HP glycogen during winter even if autumn and spring data came from fish sampled in different ponds. During winter, mainly muscle and HP fat as well as visceral fat and HP glycogen were mobilized and suspected to have played the most important role. Similar patterns of energy mobilization have been observed in other fish species during starvation or limited energy intake (golden perch, ***Macquaria ambigua***, Collins and Anderson, 1995; white sturgeon, ***Acipenser transmontanus***, Hung et al., 1997; traira, ***Hoplias malabaricus***, Rios et al., 2006; juvenile channel catfish, ***Ictalurus punctatus***, Luo et al., 2009; Laval brook charr, ***Salvelinus fontinalis***, Crespel et al., 2013).

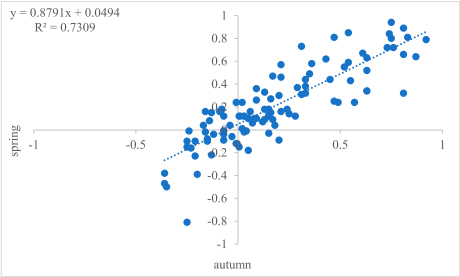

Fig. 2. Linear relationship between phenotypic correlations before and after the first winter. X-axis represents values of coefficients of correlations between pairs of traits in Table 4 (below diagonal); the Y-axis represents values of coefficients of correlations between pairs of traits in Table 3 (below diagonal); each point in the figure is connection between values of coefficient of variation before and after the first winter for the given pair of traits.

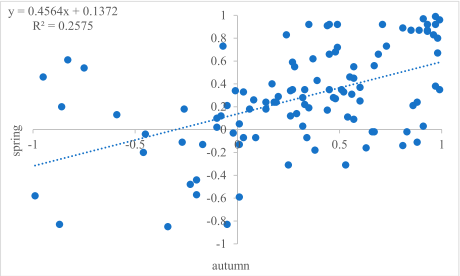

Fig. 3. Linear relationship between autumn and spring genetic correlations. X-axis represents values of coefficients of correlations between pairs of traits in Table 4 (above diagonal); the Y-axis represents values of coefficients of correlations between pairs of traits in Table 3 (above diagonal); each point in the figure is connection between values of coefficient of variation before and after the first winter for the given pair of traits.

4.2. Genetics of energy use during overwintering

The presence of genotype-by-environment interaction is considered unimportant when genetic correlation among the same trait recorded in different environments is higher than 0.8 (Robertson, 1959). Genetic correlations between the same traits measured in autumn and spring were lower than 0.8 for most of the traits in our study, implying that not all families responded to overwintering in a similar manner. This could have two main reasons: genotype × pond and genotype × season interactions. Interestingly, HSI and HP Protein conc. before and after the first winter were not correlated at all. Moreover, the heritability of those traits was very low before the first winter but became significant and moderate after the first winter. Protein concentration exhibits low heritability in rainbow trout (Tobin et al., 2006) and in European whitefish *Coregonus lavaretus* L. (Kause et al., 2009). Crespel et al. (2013) observed very low heritabilities of 0.05 for HSI in Rupert strain and of 0.01 for liver protein concentration in Laval strain of brook charr. Similarly, there was a very low heritability of 0.061 for HSI in Atlantic cod *Gadus morhua* L. (Kristjánsson and Arnason, 2016). Based on that it can be hypothesized that the storing of glycogen in HP is maximized before winter, with low genetic variance between individuals, while the ability to use it, mobilize and/or restore it during winter is genetically variable. On the other hand, the ability to store and use the fat (in muscle as well as HP) is genetically variable at all periods of the year.

4.3. Importance of fat for carp performance

Muscle, HP and visceral fat play an important role as energy reserves during starvation and/or winter period of several fish species (Adams and McLean, 1985; Black and Love, 1986; Collins and Anderson, 1995; Van Dijk et al., 2005; Rios et al., 2006). However, recent results show that excessive fat reserves might be sometimes disadvantageous. Based on our study, no positive response to survival when selecting for higher muscle fat would be expected. Moreover, if selection for higher HP fat would be performed, survival could be negatively affected, especially during the last growing season. This confirms recent observations by

Table 5
Genetic correlations among energy reserves parameters in yearlings after the first winter and performance traits in older siblings of the same stock. See Table 1 for trait abbreviations. Underline numbers indicate statistically significant correlations.

Selected traits of older carp siblings	Traits after the first winter											
	Weight	M. Fat conc.	HP Fat conc.	Abs. HP Fat	HP Glycogen conc.	Abs. HP Glycogen	Rel. HP Glycogen	HP Protein conc.	Abs. HP Protein	HP Energy conc.	Abs. HP Energy	Rel. HP Energy
Survival_C2	−0.06 ± 0.32	0.25 ± 0.33	−0.20 ± 0.32	−0.09 ± 0.33	0.50 ± 0.31	0.30 ± 0.32	0.43 ± 0.31	−0.37 ± 0.34	−0.04 ± 0.33	0.07 ± 0.35	0.07 ± 0.33	0.23 ± 0.34
FC_SpringC2	−0.07 ± 0.16	0.13 ± 0.15	−0.12 ± 0.16	0.02 ± 0.17	0.28 ± 0.15	0.24 ± 0.15	0.33 ± 0.14	−0.43 ± 0.15	0.01 ± 0.17	−0.09 ± 0.17	0.10 ± 0.16	0.35 ± 0.15
% M Fat_SpringC2	0.61 ± 0.11	0.40 ± 0.14	0.58 ± 0.12	0.67 ± 0.11	−0.24 ± 0.15	−0.01 ± 0.16	−0.28 ± 0.15	0.54 ± 0.14	0.55 ± 0.13	0.51 ± 0.14	0.48 ± 0.13	−0.03 ± 0.17
BW_SpringC2	0.67 ± 0.11	−0.11 ± 0.16	0.27 ± 0.16	0.46 ± 0.14	−0.16 ± 0.16	0.08 ± 0.16	−0.22 ± 0.15	0.40 ± 0.16	0.54 ± 0.13	0.31 ± 0.16	0.44 ± 0.14	−0.13 ± 0.17
Survival_C3	−0.42 ± 0.25	−0.08 ± 0.25	−0.59 ± 0.20	−0.51 ± 0.23	0.64 ± 0.20	0.49 ± 0.22	0.69 ± 0.19	−0.75 ± 0.21	−0.37 ± 0.25	−0.33 ± 0.26	−0.13 ± 0.26	0.47 ± 0.24
FC_C3	−0.13 ± 0.15	0.05 ± 0.15	−0.12 ± 0.16	−0.02 ± 0.16	0.29 ± 0.14	0.55 ± 0.13	0.32 ± 0.14	−0.72 ± 0.10	−0.07 ± 0.16	−0.08 ± 0.17	0.05 ± 0.16	0.31 ± 0.15
% M Fat_C3	0.32 ± 0.15	0.17 ± 0.16	0.25 ± 0.16	0.37 ± 0.15	0.002 ± 0.16	0.10 ± 0.16	−0.05 ± 0.16	0.17 ± 0.18	0.28 ± 0.16	0.28 ± 0.17	0.31 ± 0.15	0.02 ± 0.17
BW_C3	0.15 ± 0.16	0.04 ± 0.16	0.07 ± 0.17	0.13 ± 0.17	0.10 ± 0.16	0.13 ± 0.16	0.05 ± 0.16	−0.13 ± 0.18	0.10 ± 0.17	0.07 ± 0.18	0.13 ± 0.17	0.06 ± 0.17

Legend: Survival_C2 = survival during the second winter, FC_SpringC2 = Fulton's condition factor after the second winter, % M Fat_SpringC2 = muscle fat percent after the second winter, BW_SpringC2 = body weight after the second winter, Survival_C3 = survival during the last growing season, FC_C3 = Fulton's condition factor at market size, % M Fat_C3 = muscle fat percent at market size, BW_C3 = body weight at market size.

Prchal et al. (2018) where higher muscle fat content was genetically correlated to lower survival before the second winter ($r_g = -0.59 \pm 0.26$) and during the third growing season ($r_g = -0.53 \pm 0.19$). This is in contrary to the expected positive effect on survival when selecting fish for higher fillet lipid content in European whitefish *Coregonus lavaretus* (Kause et al., 2011). In addition, during mild winters when fish do not hibernate properly, they are stimulated to higher activity. In our case the winter was very mild, and from October 2014 to March 2015 the average air temperature was about 2.2 °C higher when compared to long-term average temperature. For such winters the excessive fat content might be disadvantageous. Excessive lipid deposition negatively affects not only feed conversion ratio but also protein-retention efficiency (Kause et al., 2016).

In this study, before the first winter, fat in muscle, HP and viscera (VSI_NO) was moderately correlated with body weight. Likewise, Abs. HP Fat, Glycogen, Protein and Energy were highly related to body weight. So, muscle, HP and visceral fat as well as absolute energy storage of fish would indirectly increase by selection on higher weight. This is in line with a positive allometric relationship between body size and energy reserves in the form of lipids observed at the onset of winter in several fish species (Henderson et al., 1988; Cargnelli and Gross, 1997; Sutton and Ney, 2001; Biro et al., 2004; Huss et al., 2008; Heermann et al., 2009). After the first winter, VSI_NO, the parameter including both intestine and visceral fat, was not genetically correlated with any other trait even if the genetic correlation between VSI_NO before and after the first winter was quite high (0.61 ± 0.18). So, the most logical explanation is that most of visceral fat was spent during winter and thus the intestine ratio itself, although heritable, is not correlated to other traits. Stored lipids in the viscera were utilized over the winter for basal metabolic demands also in largemouth bass, *Micropterus salmoides* Lacepede (Adams and McLean, 1985) and in Rupert brook charr (Crespel et al., 2013). On the contrary, body weight is typically positively correlated with fat deposition during the growing period (Neira et al., 2004; Kause et al., 2007b; Prchal et al., 2018). During winter this relationship is reduced as fat is utilized for

Table 6
Genetic correlations of energy status-related indices in yearlings after the first winter and survival of their older siblings coming from the same stock. See Table 1 for trait abbreviations. Underline numbers indicate statistically significant correlations.

	Survival C2	Survival C3
FC	−0.19 ± 0.30	0.27 ± 0.23
VSI_NO	0.45 ± 0.35	0.08 ± 0.28
HSI	0.22 ± 0.32	0.72 ± 0.20

Table 7
Genetic correlations of energy reserves related traits in yearlings before and after the first winter and KHVD resistance of their siblings. See Table 1 for trait abbreviations.

	KHVD resistance	
	Before the first winter	After the first winter
FC	−0.25 ± 0.17	−0.09 ± 0.16
VSI_NO	−0.07 ± 0.21	−0.28 ± 0.17
HSI	−0.11 ± 0.21	0.04 ± 0.17
M. Fat conc.	−0.13 ± 0.18	−0.02 ± 0.17
HP Fat conc.	0.25 ± 0.19	0.02 ± 0.18
Abs. HP Fat	0.17 ± 0.18	0.12 ± 0.18
HP Glycogen conc.	−0.36 ± 0.26	0.18 ± 0.17
Abs. HP Glycogen	−0.08 ± 0.19	0.22 ± 0.16
Rel. HP Glycogen	−0.21 ± 0.21	0.15 ± 0.16
HP Protein conc.	0.05 ± 0.24	0.10 ± 0.19
Abs. HP Protein	0.05 ± 0.21	0.17 ± 0.18
HP Energy conc.	0.21 ± 0.21	0.28 ± 0.18
Abs. HP Energy	0.12 ± 0.19	0.23 ± 0.17
Rel. HP Energy	0.02 ± 0.22	0.18 ± 0.17

maintenance. Fat reserves accumulated during the previous summer and autumn are used for both reproduction and winter survival in the sand smelt, *Atherina boyeri* Risso (Henderson et al., 1988).

From the above it can be concluded that fat in muscle, viscera or hepatopancreas were used as energy source during the first winter of common carp, and higher fat content in fish body might be under certain conditions surprisingly disadvantageous for survival during the winter or subsequent rearing periods.

4.4. Importance of HP glycogen for carp performance

HP glycogen is the chief energy reserve during the early phases of food deprivation in several fish species (Black and Love, 1986; Sheridan and Mommsen, 1991; Collins and Anderson, 1995; Rios et al., 2006). HP Glycogen conc. and Rel. HP Glycogen were highly genetically correlated with HSI in both periods (Tables 3 and 4). Moreover, HSI after the first winter was positively genetically correlated to survival during the third growing season (Table 5). Similarly, all HP glycogen values after the first winter were highly positively genetically correlated to survival during the third growing season. It suggests that selecting the fish that keep higher HSI during winter or fish having higher HP glycogen levels could positively influence the overall survival. That higher winter survival is primarily related to liver glycogen has been reported for instance in small crucian carp *Carassius carassius* (Vornanen et al., 2011) and side-blotched lizards *Uta stansburiana* (Zani et al., 2012). It might be hypothesized that fish with higher HP glycogen values after the first winter either i) consumed more food during winter as suggested by Hochachka (1961) and Valtonen (1974), or ii) were able to effectively restore the glycogen from other energy reserves as reported by Sheridan and Mommsen (1991) and Collins and Anderson (1995) or iii) spent more other energy reserves than glycogen.

In short, our results indicate that selecting fish that would keep higher HP glycogen traits during winter might improve the overall survival of the common carp stocks, at least under conditions of mild winters.

4.5. Importance of HP protein for carp performance

HP protein is also an endogenous energy reserve during food deprivation (Sheridan and Mommsen, 1991; Collins and Anderson, 1995; Rios et al., 2006). However, the protein content of fish typically does not change until the majority of the fat reserves have been utilized during long-term starvation, at which point protein is catabolized to maintain basal metabolic demands (Jobling, 1980; Collins and Anderson, 1995; Pangle and Sutton, 2005; Bar, 2014). Here significant negative genetic correlation between HP Protein conc. and weight before the first winter (Table 3) changed to significantly positive correlation after the first winter (Table 4). This change may be due to changes in the role of HP protein, from the conservation of tissue glycogen during fasting to the maintenance of cell function and integrity, as reported by Collins and Anderson (1995). In addition, HP Protein conc. after the first winter was genetically negatively correlated with FC in siblings after the second winter and at market size and with survival during the last growing season (Table 5). This observation was opposite to HP glycogen traits. Moreover, HP Protein conc. was highly negatively genetically correlated with HP Glycogen conc. before and after the first winter and highly positively genetically correlated with HP Fat conc. after the first winter. Genetic correlations among HP Fat, HP Protein and HP Glycogen shows that these three energy reserves somehow “compete” about the space in HP during winter and in spring. There might be a genetic predisposition for either a type of energy reserve to be stored and used or different behavioral approaches during winter with respect to movement and feeding activity. In both cases, fish that would keep higher HP Glycogen conc., but not high HP Protein and HP Fat conc., tend to have better survival. It seems also that selecting fish with higher FC in two-year old or market-sized fish should have a similar effect. Similar results were observed by Prchal et al. (2018) when higher FC was genetically correlated with better winter performance and higher muscle fat was related to lower survival. Prchal et al. (2018) also suggested that selection of carps for faster growth should be done after the second growing season. This practice should have a similar effect on growth as selection done at market-sized fish, but it is technically much easier. However, the results in this study show that when selecting Amur mirror carp for faster growth after the second growing season, unlike selecting at market size, fish with higher HP protein conc. strategy would be preferred. Unfortunately, it could have an indirect negative impact on survival. So, this problem needs to be considered when establishing the selective breeding schemes.

It can be concluded that HP Protein conc. after the first winter seems to vary in the same direction as HP Fat conc. and opposite to HP Glycogen conc., and in this respect strategy for keeping excessive HP Protein conc. is disadvantageous for carp survival, at least under conditions where ability of keeping higher level of HP Glycogen conc. is better.

4.6. Energy status-related indices and their relation to carp performance

FC is a morphometric based condition index that has already been used as an efficient predictors of energy reserves in different fish species (Mozsár et al., 2015). In the present study, FC was genetically positively correlated with HP Glycogen conc. and Rel. HP Glycogen before the first winter, with Rel. HP energy after the first winter and with HSI in both periods (Tables 3 and 4). Moreover, FC in older siblings (after the second winter and during the third growing season) was genetically positively correlated with some HP glycogen traits and negatively correlated with HP Protein conc. (Table 5). Thus, FC might be a good indicator and indirect selection trait for higher total energy state of the fish and for fish with ability of higher HP glycogen maintenance. Similar results have been presented in males of three-spined stickleback *Gusterosteus aculeatus* L. by Chellappa et al. (1995), in cod *Gadus morhua* by Lambert and Dutil (1997) and in Atlantic salmon *Salmo salar* juveniles by Persson et al. (2018).

HSI was strongly genetically and phenotypically moderately positively correlated with HP glycogen traits and Rel. HP Energy in both periods and significantly negatively phenotypically and genetically correlated with HP Fat and HP Protein conc. after the first winter (Tables 3 and 4). Moreover, it was also positively genetically correlated with survival of siblings during the last growing season. So, higher HSI, similar to FC, may be used as indirect selective trait for increasing the rate of fish with higher potential for HP glycogen maintenance and for better survival. HSI is a commonly used indicator of energy reserves in other fishes (e.g., Htun-Han, 1978; Chellappa et al., 1995; Lambert and Dutil, 1997; Binder et al., 2015), but knowledge about the composition and mutual rate of individual nutrients is missing. In an endangered catfish, *Steindachneridion parahybae*, HSI was phenotypically positively correlated with hepatic glycogen, and negatively correlated with hepatic protein (Tolussi et al., 2018). Similar strong relationship between HSI and hepatic glycogen was observed by Crespel et al. (2013). Particularly, Blasco et al. (1992) found the early phase of fasting in carp had a reduction in the HSI mainly due to glycogen mobilization. Lambert and Dutil (1997) observed, similarly as we did, that the higher HSI was indirectly related to better survival in Atlantic cod (*Gadus morhua*). The problem with HSI is that its determination needs sacrificing of fish. Thus, it may be used only in an indirect selection scheme using sib selection, similarly as e.g. slaughter yields of fish (Kause et al., 2007a). Alternatively, a strong indirect predictor for HSI values should be found.

We previously mentioned that VSI_NO includes not only viscera, but also visceral fat. Interestingly, phenotypic correlations among VSI_NO and absolute values of energy reserves were very low or negative. On the other hand, before the first winter high positive genetic correlations between VSI_NO and absolute values of all energy reserves (HP Fat, HP Glycogen and HP Protein) were observed. Thus, if fish were selected for higher VSI_NO, there should be a higher proportion of individuals in the next generations that tend to cumulate higher absolute values of energy reserves. However, only concentrations of fat in muscle and HP would increase. It verifies that VSI_NO is closely related to overall fat storage ability.

4.7. Relation of energy reserves traits with KHVD resistance

Energy reserves may influence resistance to diseases in aquatic organisms. For instance, Choi et al. (1989) and Encomio et al. (2005) found that oysters (*Crassostrea virginica*) with higher level of energy reserves may be more resistant to *Perkinsus marinus* disease. In the present study, genetic correlations between KHVD resistance and energy reserves related traits before and after the first winter showed that selection for such traits would not affect the susceptibility of fish to KHVD and opposite selection for increased KHVD resistance would not affect the energy reserve traits in fish. It seems that resistance of fish to KHVD is not related to any source of energy, type of energy reserve, or other performance traits of fingerling carp. A low but significant negative genetic correlation (−0.32 ± 0.14) was observed between FC after the second winter and KHVD resistance (Zhao et al., 2020). In this study higher FC seems to be rather advantageous for fish vitality. So, the importance of FC seems to be a bit contradictory for overall survival and KHVD survival. Zhao et al. (2020) showed that FC in their study could in fact measure the proportion of the Amur wild carp ancestor genome in the Amur mirror (AM) carp individuals, knowing that Amur wild carp has both a low FC and a high resistance to KHVD. So, resistance of the breed to KHVD should be checked in case of significant increase of FC in future generations.

5. Conclusions

The results of the present study extend our understanding of the overwintering strategy in common carp. Survival of carp during winter and/or spring is closely related to the way glycogen stores are changing in the hepatopancreas. Results of this study verified the previous and quite surprising observation that higher fat (in muscle or in HP) might be linked under certain circumstances to lower survival. In addition, it seems that keeping higher HP glycogen traits is linked to higher chance for survival of fish at any age stage of common carp, at least under conditions of mild winters. Condition factor and the hepatosomatic index provide simple and relatively adequate way of evaluating the energy reserves status of Amur mirror carp. Energy status of carp very likely does not correspond to KHVD resistance. This study identifies i) interesting biological mechanisms of energy reserves management with respect to performance of fish and ii) possible selective breeding goals to improve the overwintering performance of carp.

Author contributions

AK, MV and MK proposed the experimental design of the study. JZ, MP, DG and MK shared on establishing and rearing the experimental stocks, PIT tagging and fin clipping. VP participated on the KHVD challenge experiment and monitored health status of experimental stocks. JZ, MP, MV, HKK, VP, PŠ, CS and MK shared on phenotyping of fish. JMA, CP and RDH carried out parentage assignment of experimental stocks. JZ and MP performed statistical analyses of data. All authors contributed to drafting the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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CHAPTER 3

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

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My share on this work was about 5%.

RESEARCH ARTICLE

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

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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.

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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].

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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, $SGR_{1-2} = (\ln w_t – \ln w_0)/t^{-1} * 100$, 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, $FatCh_{1-2} = Fat_2 – Fat_1$, where Fat_2 is the percent fat content after wintering and Fat_1 is the percent fat content before wintering, iii) relative fat change, % $FatCh_{1-2} = (Fat2 – Fat_1) / Fat_1 * 100$, and iv) survival, during overwintering, $Surv_{1-2}$, 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_{2-3}, $FatCh_{2-3}$, % $FatCh_{2-3}$, and $Surv_{2-3}$ 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].

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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.

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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.

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SGR$_{1-2}$ showed high genetic correlations with BW$_3$ and FC$_3$ ($r_g = 0.62 \pm 0.10$ 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$_3$ ($r_g = -0.50 \pm 0.14$) and with % Fill ($r_g = -0.38 \pm 0.16$). Oppositely, % FatCh$_{1-2}$ was significantly correlated with % FatCh$_{2-3}$ ($r_g = -0.37 \pm 0.16$). 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 ($\mathrm{Surv}_{1\text{-}2}$) and fish weight ($\mathrm{BW}_1$). 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 $\mathrm{Surv}_{1\text{-}2}$ (-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 $\mathrm{FatCh}_{1\text{-}2}$ as well as % Fat_1 and % $\mathrm{FatCh}_{1\text{-}2}$ 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, $\mathrm{SGR}_{1\text{-}2}$, $\mathrm{FatCh}_{1\text{-}2}$, % $\mathrm{FatCh}_{1\text{-}2}$ and $\mathrm{Surv}_{1\text{-}2}$. 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 $\mathrm{SGR}_{1\text{-}2}$. 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 ($\mathrm{FatCh}_{1\text{-}2}$) as well as relative values (% $\mathrm{FatCh}_{1\text{-}2}$). 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: $\mathrm{SGR}_{1\text{-}2} = 0.50$) 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)

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CHAPTER 4

GENETIC RELATIONSHIP BETWEEN KOI HERPESVIRUS DISEASE RESISTANCE AND PRODUCTION TRAITS INFERRED FROM SIBLING PERFORMANCE IN AMUR MIRROR CARP

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Genetic relationship between koi herpesvirus disease resistance and production traits inferred from sibling performance in Amur mirror carp

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Genetic correlation

ABSTRACT

Koi herpesvirus disease (KHVD) is currently the most serious threat to global carp farming. Prevention is a sensible strategy for tackling this disease and improved genetic resistance of carp strains is a desirable breeding goal. To study the potential for multitrait selection, the objective of the current study was to estimate the genetic correlations between KHVD resistance and production traits in Amur mirror carp. A total of 1500 fingerlings from four factorial crosses of five dams and ten sires were challenged with Koi herpesvirus (KHV). Juvenile growth-related traits were collected on the same individuals before the challenge test. Production traits were measured on siblings of the challenged population at different life stages (yearling to market size). The estimated heritability for resistance to KHVD was 0.43 ± 0.08 on the observed scale and 0.72 ± 0.13 on the underlying liability scale. Most genetic correlations between KHVD resistance and important production traits were insignificant, showing that selection for improved production traits would not increase susceptibility to KHV and vice versa. However, resistance to KHVD was negatively correlated with Fulton's condition factor (FC) after the second overwintering and relative head length (RHL), relative body height (RBH) and relative body width (RBW) from the second growing season to the market size, with a more prolonged body shape of Amur mirror carp (genes from Amur wild scaly carp, *Cyprinus rubrofuscus*) being associated with higher KHVD resistance. Intermediate favorable genetic correlations between KHVD resistance and log-log residuals of headless carcass yield (0.37 ± 0.14) and fillet yield (0.44 ± 0.13) at market size suggested that selection for improved yields of edible body parts might indirectly lead to a slight improvement in KHVD resistance and vice versa.

1. Introduction

Common carp (*Cyprinus carpio*) is one of the most important cultured fish species globally. However, carp farming faces serious challenges due to disease outbreaks (Haenen et al., 2004; Ødegård et al., 2010; Adamek et al., 2018, 2019; Su and Su, 2018). Currently, the most serious one is the koi herpesvirus disease (KHVD) that is caused by *Cyprinid herpesvirus-3* (CyHV-3), also called Koi herpesvirus

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(KHV). KHVD outbreaks have been reported both in European and Asian countries (Haenen et al., 2004; Rakus et al., 2013). The severity of KHVD is pinpointed by its listing as a notifiable disease by the European Union (Taylor et al., 2010) and the World Organization for Animal Health (OIE, 2018). The disease occurs most commonly at water temperatures between 17 °C and 26 °C (Haenen et al., 2004). Morbidity is often 100%, but mortality can vary due to different factors and is more likely to occur at higher temperatures (23 °C and 28 °C) (Hedrick et al., 2000; Yuasa et al., 2008). Therefore, looking to prevent KHVD via selective breeding to produce strains of carp with increased genetic resistance is important for the long-term sustainability of carp aquaculture.

Aquaculture breeding programs are increasingly focusing on genetic improvement of disease resistance (Gjedrem and Robinson, 2014; Yáñez et al., 2014; Gjedrem and Rye, 2018; Robledo et al., 2018). Previous studies have laid the foundation for breeding of KHV-resistant carp strains and have shown that breeding programs could produce carp strains with high level of resistance (Shapira et al., 2005; Piačková et al., 2013; Tadmor-Levi et al., 2017; Palaiokostas et al., 2018). Estimation of genetic correlations between resistance to KHVD and other production traits that may be included in breeding goals, such as growth, body composition and fish welfare traits is of paramount importance. Most evaluations of genetic correlation of disease resistance with other traits in fish have been focused on growth-related traits, particularly in salmonids (e.g. Yáñez et al., 2016; Flores-Mara et al., 2017; Barría et al., 2019; Gjerde et al., 2019). Prior results demonstrated different genetic relationships between resistance to disease and growth. Therefore, genetic correlations are necessary to be known for each fish species before systematic selective breeding program.

The main aim of this study was to estimate genetic correlations of KHVD resistance with other important production traits in Amur mirror carp from yearlings to market size. Such knowledge will assist in developing a sustainable breeding program for genetic improvement of disease resistance alongside multiple production traits.

2. Materials and methods

2.1. Ethics statement

The entire experiment was performed 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 the expert committee of the Institutional Animal Care and Use Committee (IACUC). All people conducting the trait measurements and challenge test were qualified to conduct and manage such kind of experiments on the live animals.

2.2. Establishment and rearing of experimental stocks

The details of the stocks have been described previously (Palaiokostas et al., 2018; Prchal et al., 2018a,b). In brief, an experimental population of Amur mirror carp was established at the hatchery of Faculty of Fisheries and Protection of Waters of University of South Bohemia in České Budějovice, Vodňany, Czech Republic in May 2014 from artificial spawning according to Vandeputte et al. (2004) using four factorial crosses of five dams and ten sires (20 dams and 40 sires were used in total) allowing up to 200 full-sib families. Randomly sampled progenies from each cross were pooled using approximately equal total volume and stocked into nursery earthen ponds at stocking density of 150,000 larvae/ha. Fish were then reared under semi-intensive pond conditions through the first growing season until October 2014. Following pond harvest a subset of the stock was used for KHVD challenge test (see below). Prior to the test, fish were phenotyped for growth-related traits. In addition, siblings of the challenged stock were grown up to the market size. Stocking density was 6000 ind./ha (mean weight of 336.1 g, coefficient of variation of 19.2%) during the second growing season and 500 ind./ha (mean weight of 1910 g, coefficient of variation of 14.6%) during the last (third) growing season (see boxplots for weight of experimental stocks in S1 figure). The aforementioned 3000 fish were individually tagged using Passive Integrated Transponders (PITs) and then phenotyped for the main production traits after each growing season and each overwintering. At the end of the third growing season, the fish were slaughtered and phenotyped for processing traits.

2.3. KHVD challenge test

A total of 1500 fish were taken at random and PIT-tagged and a sample of fin tissue was taken in autumn 2014 for subsequent DNA analysis (parentage allocation and genomic analysis). These fish were then acclimatized together with Koi carp ($n = 215$) 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) only for experimental fish to eliminate ectoparasites. Soon after, the fish were transferred to the Veterinary Research Institute (VRI) in Brno in order to perform the KHVD challenge test.

The experimental procedure was the same as described in Palaiokostas et al. (2018). Cohabitation challenge protocol was performed in a tank of 1.4 m^3 with recirculation and biological filtration. 20 fish out of the Koi carp received an intraperitoneal injection with KHV culture established according to standardized protocol by Piačková et al. (2013) and were cohabited with Amur mirror carp and the rest of Koi carps. Mortality of individual fish was recorded twice a day for a period of 35 days post infection (dpi) when mortalities were negligible. Resistance was recorded as 0 for dead fish and 1 for surviving fish. Presence of KHV on samples of dead fish ($n = 5$) was confirmed in seven time points from 10 to 35 dpi by nested PCR as described by Pokorova et al. (2010).

2.4. Parentage assignment of experimental stocks

Parentage assignment of challenged fish was done on 1500 fish by SNP genotype data (12,311 SNPs grouped in 50 linkage groups) using R software version 3.6.1 (R Core Team, 2019) with hsphase package version 2.0.2 (Ferdosi et al., 2014). Parentage assignment of the second and the third year fish (production traits) was based on the analysis of 12 microsatellite loci and performed using the AccurAssign software, applying a maximum-likelihood method (Boichard et al., 2012). For more details see Palaiokostas et al. (2018) and Prchal et al. (2018b).

2.5. Production traits recording on siblings

Juvenile growth-related traits (body weight – BW and Fulton's condition factor – FC) were measured in the same tagged individuals ($n = 1500$) from the challenge test. Each individual was weighed (to the nearest 0.01 g) and measured for standard length (SL) (to the nearest 0.1 mm). FC was calculated according to formula: $FC = 10^5 * (BW / SL)^3$.

After the first overwintering (March 2015), a random sample of 3000 siblings coming from the same stock as the disease challenged animals (being reared in the same pond, i.e. identical environmental conditions) were phenotyped. Recorded traits included standard length (SL), body length (BL), head length (HL), body height (BH), body width (BWI), and body weight (BW). In addition, biometric indices were calculated as follows: relative body height: RBH = BH/SL, relative head length: RHL = HL/SL and relative body width: RBW = BWI/SL. The survival of those individually tagged fish was recorded before and after the second overwintering period as well as at the end of third growing season (market size). At each of these time points, the following individual traits were recorded: body weight (g), muscle fat by Distell fish fat meter (%), absolute and relative muscle fat change, weight change expressed as specific growth rate (SGR), RBH, RHL, RBW, FC. At the final sampling, the natural logarithm of the weight of each slaughter body part was calculated and regressed on the logarithm of body weight to obtain growth-independent allometry residuals that fix the bias of ratio traits. The log-log residuals of headless carcass yield and fillet yield were recorded as Logr_hl-Carss and Logr_Fill, respectively. Specific details about traits and their calculation are shown in Prchal et al. (2018a, b).

2.6. Estimation of genetic parameters

The heritability of KHVD resistance and genetic correlations between KHVD resistance (1214 fish assigned to a parental pair, forming 195 full-sib families) and production traits (1879 fish assigned to a parental pair, forming 199 full-sib families) were estimated using DMU statistical software (Madsen and Jensen, 2013). The following linear animal model was used:

$$\boldsymbol{y} = X\boldsymbol{\beta} + Z\boldsymbol{a} + \boldsymbol{e},$$

where $\boldsymbol{y}$ is the vector of the observations of KHVD resistance and production traits, X and Z are the corresponding design matrices for the fixed effects and the additive genetic effects of the animal, $\boldsymbol{\beta}$ is the vector of the fixed effects for KHVD resistance (with mating design – four crosses) and production traits (with sex), $\boldsymbol{a}$ is the vector of random animal additive genetic effect that is distributed $N(0, \mathbf{A}\sigma_a^2)$, and $\boldsymbol{e}$ is the vector of the random residual effect that is distributed $N(0, \mathbf{I}\sigma_e^2)$. $\mathbf{A}$ is the relationship matrix, $\mathbf{I}$ is an identity matrix, σ_a^2 is the additive genetic variance and σ_e^2 is the residual variance. Heritability for KHVD resistance was firstly estimated on the observed scale as $h^2 = \sigma_a^2/\sigma_a^2 + \sigma_e^2$. Subsequently, this value was transformed to the underlying normally distributed liability scale using the formula by Dempster and Lerner (1950). All traits were recorded from the same families and had a common pedigree (family structure), but KHVD resistance and other performance traits were recorded on different individuals. As a result, residual covariance was set to zero between these traits. Estimated genetic correlation was considered significant at $p < .05$ when $|r_g| - |1.96 \times \text{S.E.}|$ was not zero or less (two-tailed hypothesis) (Coolidge, 2013).

3. Results

3.1. Experimental challenge test

Fish experimentally infected with KHV presented typical clinical and pathological patterns. These symptoms included behavioral changes (lethargic, loss of equilibrium and disorientation), pale discoloration of the skin and gills or reddened skin, focal or total loss of epidermis, overproduction of mucus on the skin and gills, sunken eyes, hemorrhages of the skin and fins, and fin erosion. During the 35 days of challenge, mortalities began to appear at 12 dpi, then reached a maximum between 21 and 24 dpi (Fig. 1). The percentage of total mortality for the Amur mirror carp was 66%.

3.2. Heritability and genetic correlations among KHVD resistance and production traits

The estimated heritability for resistance to KHVD was moderate (0.43 ± 0.08) on the observed scale and high (0.72 ± 0.13) on the underlying liability scale. Genetic correlations between KHVD resistance and important production traits were in general statistically insignificant (Tables 1–4). However, genetic correlation between FC after the second overwintering and KHVD resistance was significantly different from zero and negative ($r_g = -0.32 \pm 0.14$). Likewise, genetic correlations between KHVD resistance and biometrical indices (relative head length, relative body height, relative body width) were negative and significant when the traits were measured during the period before the second winter until market size (Tables 3–4) and at the edge of significance for RBW at market size (Table 4). On the other hand, intermediate positive and significant genetic correlations were found between KHVD resistance and slaughter yields (log-log residuals of headless carcass yield and fillet yield, respectively) ($r_g = 0.37 \pm 0.14$ and 0.44 ± 0.13, respectively). Phenotypic correlations could not be estimated due to the fact that KHVD resistance and other production traits were measured on different individuals.

4. Discussion

The heritability estimate for resistance to KHVD was high on both observed and the underlying scale (0.43 ± 0.08 and 0.72 ± 0.13, respectively). Ødegård et al. (2010) observed even higher heritability (0.79 ± 0.14) for KHVD resistance on the underlying scale in common carp. In their case probably high overall mortality of challenged fish (94% vs. 66% in our study) and significant survival differences among tested full-sib families (91 full-sib families vs. 195 full-sib families in our study) could have increased the heritability value. Using a subset of the current data set, heritability of KHVD resistance as measured by survival on the underlying scale for the pedigree and genomic relationship matrix was 0.61 and 0.50, respectively (Palaiokostas et al., 2018). However, we used a different animal model and statistic program that showed a slightly higher pedigree heritability (0.72). Similar significant heritability estimates of resistance to diseases have been reported, which demonstrated the feasibility of genetic improvement through selection (e.g. Doan et al., 2017; Shoemaker et al., 2017; Barría et al., 2019; Gjerde et al., 2019). More specifically, there is a strong potential to improve resistance to KHVD by a breeding program. Still, it would be very useful to find a reliable way of selecting resistant candidates (to predict KHVD resistance) without challenging the fish to KHV. Furthermore, challenge tests to diseases are problematic with respect to animal welfare and sometimes even to further utilization and rearing of challenged survivor fish as broodstock, which is the cheap but risky solution to select for a disease without need for family information (e.g. KHV is on the list of notifiable diseases, so challenged fish could not be reared in outer pond conditions any more).

In our data, almost all genetic associations between KHVD resistance and production traits were insignificant. Similar to Ødegård et al. (2010), we found no genetic correlation between KHVD resistance and pond survival. So, genetic improvement of KHVD resistance is not genetically related to better general survivability in the absence of disease outbreaks. Similarly, we found insignificant genetic correlations between disease resistance and growth-related traits, as have been found in other fishes (e.g. Silverstein et al., 2009; Yáñez et al., 2014; Flores-Mara et al., 2017; Bassini et al., 2019). This means that selection of fish for growth, the trait typically given the highest weighting in a carp breeding program, should not increase susceptibility of fish to diseases. The absence of significant genetic correlations among commercially important production traits (e.g. growth, % muscle fat, survival) and KHVD in Amur mirror carp suggested that selective breeding program should not affect the resistance of carp to KHVD and vice versa.

Significantly negative genetic correlation was observed between FC after the second overwintering and KHVD resistance, suggesting that selection for lower FC could improve the resistance of fish to that disease. Likewise, RBH, RBW and RHL were significantly negatively related to KHVD resistance from the second growing season to the third growing season. This is in accordance with the fact that FC in Amur mirror carp is closely genetically related to the body shape and to relative body height and relative head length (Prchal et al., 2018b). So, such associations implied that Amur mirror carp with typically more prolonged body shape of Amur wild scaly carp, which was used to establish Amur mirror carp (Flajšhans et al., 2015), is well known as one of the most resistant carps to KHVD (Piačková et al., 2013). So, selection for lower FC, RHL, RBW or RBH would indirectly increase KHVD resistance probably due to the fact that such fish would bear more genes from wild scaly carp that might be responsible for resistance to KHVD

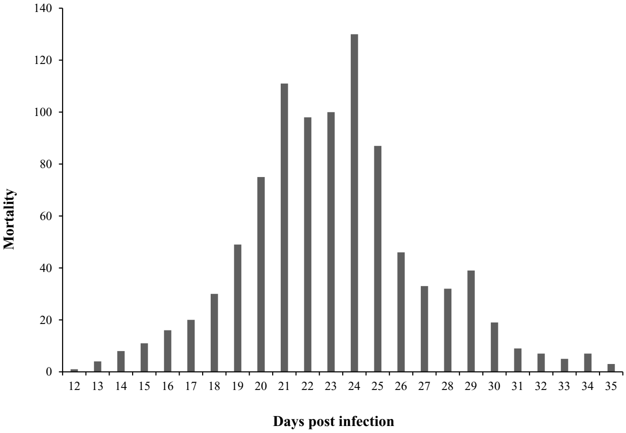

Fig. 1. Daily mortality of fish during the KHV challenge experiment.

Table 1
Genetic correlations (±standard error) between KHVD resistance and body weight (BW) and condition factor (FC) in challenged fish.

	KHVD resistance
BW	−0.02 ± 0.18
FC	−0.16 ± 0.17

Table 2
Genetic correlations (± standard error) between KHVD resistance and production traits of siblings after the first overwintering.

	KHVD resistance
BW	0.06 ± 0.18
FC	−0.07 ± 0.17
RBH	−0.26 ± 0.16
RBW	−0.23 ± 0.19
RHL	−0.15 ± 0.21

BW = body weight, **FC** = Fulton's condition factor, **RBH** = relative body height, **RBW** = relative body width, **RHL** = relative head length.

Table 3
Genetic correlations (±standard error) between KHVD resistance and production traits of siblings related to the second overwintering.

	KHVD resistance
Fatch	−0.17 ± 0.19
% Fatch	−0.17 ± 0.19
SGR	−0.10 ± 0.17
Winter Surv	−0.19 ± 0.32
FC_B	−0.28 ± 0.15
FC_A	**−0.32 ± 0.14**[*]
% Fat_B	0.15 ± 0.16
% Fat_A	0.07 ± 0.16
BW_B	−0.07 ± 0.17
BW_A	−0.05 ± 0.17
RBH_B	**−0.45 ± 0.13**[*]
RBH_A	**−0.35 ± 0.14**[*]
RBW_B	**−0.39 ± 0.15**[*]
RBW_A	**−0.38 ± 0.15**[*]
RHL_B	**−0.39 ± 0.15**[*]
RHL_A	**−0.41 ± 0.14**[*]

B = the trait was recorded before the second overwintering, **A** = the trait was recorded after the second overwintering, **Fatch and % Fatch** = absolute and relative fat change, **SGR** = specific growth rate, **Winter Surv** = winter survival, **FC** = Fulton's condition factor, **% Fat** = muscle fat content, **BW** = body weight, **RBH** = relative body height, **RBW** = relative body width, **RHL** = relative head length.

[*] Confidence limit | r_g | - |1.96 × S.E.| not being zero or less represents significant correlations at $p < .05$.

and these genes are linked to those responsible for prolonged body shape or might show certain pleiotropic effect, similarly like scaliness pattern in relation to fitness traits in common carp (Casas et al., 2013). Moreover, such selection could indirectly further improve slaughter yields at market size as there is a relationship that prolonged body shape is genetically related to higher dress out traits in common carp (Prchal et al., 2018a). That is why selection of fish for log-log residuals of slaughter yields could positively affect KHVD resistance and vice versa.

We should also take into consideration that any selection (e.g. for increased KHVD resistance or dress out yield) that might indirectly lead to significant decrease of RHL might also negatively impact the animals welfare. Gills control vital respiration and osmoregulation processes, as such a significant decrease of RHL might impair their function. Moreover, any selection that might indirectly lead to a significant change of less favorable body shape for market acceptance should also be under close monitoring (Haffray et al., 2012; Fraslin et al., 2018; Prchal et al., 2018a). Therefore, further studies are needed to identify real effect of long-term selective breeding program focused on improved KHVD resistance and/or slaughter yields on body shape and allometry.

5. Conclusion

In conclusion, we identified negative genetic correlations between KHVD resistance and body shape traits, and the favorable genetic

Table 4
Genetic correlations (±standard error) between KHVD resistance and market size production traits of siblings.

	KHVD resistance
Market Surv	0.19 ± 0.24
% Fat	0.16 ± 0.15
FC	−0.20 ± 0.14
BW	−0.19 ± 0.15
Logr_hl-Carss	**0.37 ± 0.14***
Logr_Fill	**0.44 ± 0.13***
RBH	**−0.29 ± 0.14***
RBW	−0.26 ± 0.15
RHL	**−0.35 ± 0.14***

Market Surv = market size survival, **% Fat** = muscle fat content, **FC** = Fulton's condition factor, **BW** = body weight, **Logr_hl-Carss** = log-log residuals of headless carcass yield, **Logr_Fill** = log-log residuals of fillet yield, **RBH** = relative body height, **RBW** = relative body width, **RHL** = relative head length.

* Confidence limit $|r_g|$ - |1.96 × S.E.| not being zero or less represents significant correlations at p < .05.

correlations between KHVD resistance and slaughter yields. In addition, the absence of significant genetic correlations between other production traits and KHVD resistance indicates that selection for improved production traits is not expected to increase the susceptibility of fish to KHVD and vice versa.

Author contributions

MP, DG and MK shared on establishing and on-growing the experimental stock, juvenile phenotyping, PIT tagging, and fin clipping of fish. TV, DP, VP and LP carried out the challenge experiment. JZ, MP, MV, AV, JB, AB, HKK and MK shared on final trait recordings. CP, RH and LG carried out DNA extractions and parentage assignment. MP introduced JZ to the quantitative genetic analysis. JZ and MP estimated the genetic parameters. All authors contributed to drafting the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2020.734986.

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CHAPTER 5

MORPHOLOGICAL PREDICTORS OF SLAUGHTER YIELDS USING 3D DIGITIZER AND THEIR USE IN A COMMON CARP BREEDING PROGRAM

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Morphological predictors of slaughter yields using 3D digitizer and their use in a common carp breeding program

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ABSTRACT

Slaughter yields are traits of high interest especially for fish species sold in processed form like headless carcass or fillets, as well as with regard to increasing consumer preference for easy-to-prepare fish products. However, slaughter yields cannot be measured on live fish and thus their genetic improvement through mass selection is impossible. The usual alternatives are sib selection and/or indirect selection on correlated traits or morphological predictors of slaughter yields. In the present study, we assessed the possibility of using a combination of 3D digitized landmarks and ultrasound measurements in genetic improvement of slaughter yields in common carp. DNA – pedigreed market-size carp ($n = 1553$ fish) were produced from a partial factorial design of 20 dams and 40 sires. Morphological predictors were recorded in real-time using a 3D digitizer and ultrasound tomography, and combined by multiple linear regression to predict slaughter yields. The 3D model-predicted headless carcass and fillet yields explained 59% and 50%, respectively, of the total phenotypic variation in slaughter yields. Genetic parameters of model-predicted yields and of the best individual predictor (3D_P$_2$ – ratio between abdominal fillet thickness – E8 and external 3D ventral height) were similar or slightly lower when compared to previous 2D-based predictors (Prchal et al., 2018a, 2018b, 2018c). This was also the case for the expected genetic gain using indirect selection on the same simple predictor for fillet yield improvement (0.48% fillet units for 3D vs. 0.52% for 2D). 3D model-predicted yields and especially simple predictors thus have a solid potential for genetic improvement of slaughter yields in common carp. While they are not better than 2D predictors, they are much more convenient and faster to collect in the field, as they do not imply post-processing of images. These practical aspects should be taken into account in the future carp breeding program.

1. Introduction

Fish traits defined as ratio of inputs and outputs (such as feed efficiency), or ratio of edible high-valued biomass relative to total fish weight (such as fillet yield) are fundamental efficiency-related traits for aquaculture operations. They are of high economic value (Kankainen et al., 2016), yet tricky to measure and include in breeding programs (Haffray et al., 2013; Vandeputte et al., 2017; De Verdal et al., 2018; Fraslin et al., 2018).

Slaughter yields are traits of high interest especially for fish species sold in processed form like headless carcass or fillets (Kankainen et al., 2016), as well as with regard to increasing consumer preference for easy-to-prepare fish products (FAO, 2018). However, slaughter yields cannot be measured on live fish and consequently genetic improvement through mass selection on live breeding candidates is impossible. Similarly, the potential for marker-assisted selection (MAS) is significantly limited due to the polygenic structure of slaughter yields in fish species (Tsai et al., 2015; Gonzalez-Pena et al., 2016; Yoshida et al.,

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2019). Yet, genomic selection might be seen as future possibility for the genetic improvement of yields, as it allows a better precision on sib-recorded traits (Yoshida et al., 2019). However, genomic selection is still too costly and thus useful only for fish species with well-developed breeding programs e.g. Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*) (Robledo et al., 2017) and for common carp (*Cyprinus carpio* or *Cyprinus rubrofuscus*) there is no available commercial SNP assay for such trait. Presently, edible part yields are commonly genetically improved by sib selection or by indirect selection via traits which are genetically correlated to slaughter yields (Kause et al., 2007; Gjedrem, 2010). Morphological predictors of slaughter yields based on non-destructive recording of external 2D landmarks and internal measures using ultrasound tomography could be an effective option to select for improved yields, as they can be used on the candidates without need for (costly) sib or genomic information (Cibert et al., 1999; Bosworth et al., 2001; Rutten et al., 2004; Van Sang et al., 2009; Haffray et al., 2013; Vandeputte et al., 2017; Prchal et al., 2018a).

Common carp is an important fish species in world aquaculture, though its breeding programs are mainly focused on utilization of heterotic effect by crossbreeding (Vandeputte, 2003; Janssen et al., 2017). This is the case despite the evidence for high genetic potential in using additive effect by genetic selection (Vandeputte et al., 2004; Kocour et al., 2007; Vandeputte et al., 2008; Nielsen et al., 2010; Ninh et al., 2011; Ninh et al., 2013; Hu et al., 2017; Prchal et al., 2018b). In a previous study with common carp, we investigated phenotypic and genetic potential of slaughter yield predictors based on 2D image analysis and ultrasound measurements, and their use in carp breeding programs (Prchal et al., 2018a). We observed a high accuracy of predictors and a favourable genetic relationship to the real yields. However, digitization of 2D landmarks requires post processing of images and cannot measure variation in body width. So, this method is at the same time incomplete and time consuming, which is a major technical limitation for practical breeding programs. Alternatively, 3D collection of body landmarks could speed up digitization of potentially relevant morphological predictors and take into account the variability of carp body width. A variety of 3D imagery systems have been used in pigs (Tillett et al., 2004), chickens (Mortensen et al., 2016) and cattle (Cappai et al., 2019; Le Cozler et al., 2019). Moreover, 3D digitizers like the MicroScribe (Solution Technologies Inc., Oella, MD, USA) are often used for research related to direct 3D morphological digitization of animal skeletons (Drake, 2011; Owen et al., 2014; Hanot et al., 2017). However, their potential for real time digitization of slaughter yield predictors directly on live fish has never been studied.

In the present study, a 3D digitizer was used to collect landmarks on the fish body, instead of 2D digitizing from post-processing images. Thus, we aimed to i) determine the best morphological predictors of slaughter yields using combination of 3D landmarks and ultrasound imagery, ii) estimate genetic parameters of slaughter yield predictors and their association to the real yields, and iii) predict and compare expected genetic gain in response to selection for slaughter yield predictors based on 2D and 3D measurements and their practical implication in the carp breeding program.

2. Material and methods

2.1. 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), 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 16OZ19179/2016–17,214). At market size the fish were humanely euthanized by trained person for subsequent processing and slaughter yield evaluation.

2.2. Production and rearing of experimental stock

The fish are the same as those used in Prchal et al. (2018a). In short, an experimental stock of Amur mirror carp was produced at the Genetic Fishery Centre of University of South Bohemia (USB) in České Budějovice, Faculty of Fisheries and Protection of Waters (FFPW) in Vodňany, Czech Republic. Twenty dams and forty sires were propagated and crossed in a partial factorial design with four series of 5 dams and 10 sires. Each parental fish was DNA sampled (fin tissue) for later parentage assignment of the offspring fish. At the swimming stage, the experimental stock was created by pooling equal volumes of larvae. These larvae were released (150,000 larvae. ha^{-1}) to the prepared nursery ponds at the Klatovy fish farm and reared communally in ponds under a semi-intensive culture system typical for Central Europe. At one-year old, a random sample of 3000 fish from one 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 marked by PIT-Tags and DNA sampled for parentage assignment. The fish were harvested after the second growing period and the second overwintering. In October 2016 the stock (mean weight = 1910 g) was harvested and moved to a storage pond before final traits recording at fish slaughter house of USB FFPW in České Budějovice, Czech Republic. A total of 1622 individuals were humanely killed by a hit on the head and bled by cutting the gills according to the law on the protection of animals against cruelty (Act no. 246/1992).

2.3. Final data collection

Briefly, as previously reported (Prchal et al., 2018a), 1622 fish were phenotyped for total length (TL), standard length (SL), body length (BL), head length (HL), body height (BH) and body width (BWI) with an in-house electronic ruler (to nearest 0.1 mm), and body weight (BW) was recorded with an electronic scale (to nearest 0.1 g). To describe the shape of the body, the head and the lateral line, a total of 15

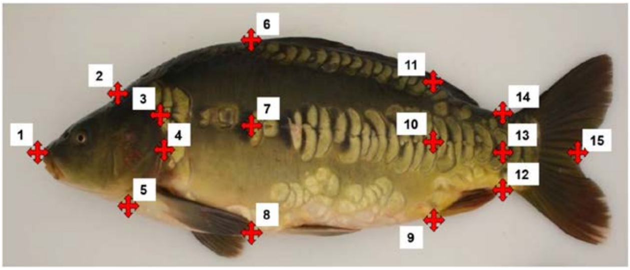

Fig. 1. 3D landmarks place on each carp.
1: head extremity; **2**: end of the head beginning of the fillet on the back; **3**: intersection between opercula and lateral line; **4**: opercula at the maximum length from the landmark 1; **5**: end of the head beginning of the fillet on the ventral part; **6**: beginning of the dorsal fin; **7**: intersection between the lateral line and the vertical of landmark 6; **8**: intersection of the ventral part and the vertical of point 7; **9**: beginning of the anal fin; **10**: intersection between lateral line and vertical of point 9 towards the carp back; **11**: vertical of point 10 on the back; **12**: end of anal fin; **13**: intersection of lateral line and vertical of 12; **14**: vertical of point 13 on the carp back; **15**: end of the caudal fin at the fork.

coordinates (Fig. 1.) of morphological points were digitized in 3 dimension (X,Y,Z) using a 3D digitizer (MicroScribe G2LX) connected to a computer, to which raw data were exported and stored in real time with a home-made software. The 11 landmarks and 4 semi landmarks (point 7, 10, 11 and 13) were chosen to be both easy to collect based on anatomical features (nose, operculum, fin, anus, lateral line) and evenly distributed all along the body to describe the whole body shape.

Four muscular thicknesses from anterior (E4), intermediate (E5, E8) and posterior (E6) were collected using ultrasound imagery (Hospimedi LC1000, 7.5 MHz). For more details see Haffray et al. (2013) and Vandeputte et al. (2017) that preceded this study.

The total muscle fat content (% Fat) was recorded using a Fish Fatmeter FM 692 (Distell Ltd., UK), using calibration option 'CARP – 1'. Biometrical indicators were calculated as Fulton's condition factor: FC = 10^5 * [BW (g) / SL^3 (mm)], 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 fillet, viscera, gonad, left fillet skin, half carcass, left fillet 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 fillet + left skin + left ribs and trimmings + half carcass], fillet weight with skin [FilletW = (left fillet + left fillet skin) * 2]. The slaughter yields expressed in % were calculated as: headless carcass yield % [% hl-Carss = (hl-CarssW / BW) *100], and fillet yield [% Fill = (left fillet + left skin) * 2 / BW * 100]. The natural logarithm was calculated for the weight of each slaughtered part and regressed on the logarithm of body weight to obtain growth-independent allometry residuals in order to provide genetic and phenotypic parameters giving reasonable estimates of predicted gains in slaughter yield (Gunsett, 1984, 1987; Vandeputte et al., 2014). Therefore, for % headless carcass and % fillet yield, the surrogate traits are defined as log-log residuals (Logr) and termed as Logr_hl-Carss and Logr_Fill, respectively. In addition, logarithm of weight of all body portions was regressed on the logarithm of body weight to visualize body allometry (See Supplementary Material in Prchal et al., 2018a).

2.4. 3D morphology and prediction models of slaughter yields

The association of the variation in carp morphology to the variation in processing yields was analyzed using the MorphoJ software (Klingenberg, 2011) as described previously in Prchal et al. (2018a) but using 3D landmarks coordinates instead of 2D. The wireframe visualization was performed on the side (X and Y) and dorsal (X and Z) view of the fish. The R Package 'geometry' was used to calculate areas and volumes from 3D coordinates raw data. A multiple linear regression using the reg.best function of the FactoMineR of R software package was performed using external morphology descriptors, ultrasound measurements and fat meter value as independent variables and the Logr_hl-Carss and Logr_Fill as dependent variables. List of predictors calculated and initially included in the multiple linear regression are shown in Supplementary Table S1.

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_Fill for fillet yield. Models were cross validated using the crossval function of the bootstrap package in R software (Efron and Tibshirani, 1993).

2.5. Parental allocation

The 60 parents and 2035 offspring were genotyped with 12 microsatellites loci at LABOGENA-DNA, the French laboratory for livestock genotyping (ISO 170025 accredited, Jouy-en-Josas, France). Parental allocation was performed using the AccurAssign software, applying a maximum-likelihood method (Boichard et al., 2014).

2.6. Estimation of genetic parameters

The data set was checked for potential outliers and the final genetic model was applied on 1553 individuals assigned to a single parental pair with a complete set of variables. Heritability (h^2), phenotypic and genetic correlations (r_{P}, and r_g, respectively) were estimated in multivariate mixed models using the restricted maximum likelihood method in DMU statistical software (Madsen and Jensen, 2013). The univariate (for heritability) and multivariate analysis (for genetic correlations) were on the following animal model:

$$\mathrm{Y_{ijk}} = \mathrm{\mu_i} + \mathrm{sex_{ij}} + \mathrm{anim_{ik}} + \mathrm{e_{ijk}}$$

where Y_{ijkl} is the measured phenotypic value of each analyzed trait, μ_i is the overall mean for trait i, sex_{ij} is the fixed effect of sex (j = female, male, unidentified sex) for trait i, $anim_{ik}$ is the random genetic effect of an animal k with pedigree based on parentage assignment ($k = 1, 2, \ldots, 1553$) for trait i, and e_{ijk} is the random residual. Heritability estimates were calculated as the ratio of additive genetic variance (V_{A}) divided by the total phenotypic variance (V_{P}), $h^2 = V_{\mathrm{A}} / V_{\mathrm{P}}$. The likelihood ratio test (LRT) was used for comparing the goodness of fit of two models (including vs. excluding the animal genetic effect). The heritability estimates were considered significant when the difference of additive genetic effect in -2Log-likelihood was higher than the threshold value for $p < .05$ of a χ^2 distribution with 1 degree of freedom (Pinheiro and Bates, 2000). Genetic correlations were considered significant if $|r_g|$-$|1.96$ x S.E. $|$was higher than zero (Coolidge, 2013).

The genetic gains (ΔG) per generation were estimated using the breeder's equations from Falconer and MacKay (1996) under a mass (MS), full-sib (FSS) and indirect (IS) selection responses for fillet yields. The theoretical genetic gain under mass selection (although it cannot be applied in practice) was calculated as $\Delta G_M = i\ h^2\ \sigma_P$, 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 response to selection of FSS was estimated as $\Delta G_{FS} = \frac{i \times \sigma_P \times h^2 \times n \times r}{\sqrt{n(1+(n-1)\,t)}}$, where n is the number of sibs sampled per family ($n = 10$), 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 estimated genetic gain for indirect selection criteria was calculated as $\Delta G_I = i$ x h_1 x h_2 x r_g x σ_{P2}, where ΔG_{I} 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. Finally, the real genetic gain was scaled back to the percent body weight units by multiplying ΔG by the real mean fillet yield in the present experimental stock (50%). The selection intensities were set up of 10% and 30%, with 10 sibs per family in FSS as the most practical intensities for potential common carp breeding program.

3. Results

3.1. Representation of families

The 1553 fish used in this study arise from 197 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.

3.2. Slaughter yields percentage

Percentage of headless carcass was 66% ± 2.19 and fillet yield was 50% ± 1.95. Such values were higher than usual values in common carp, most likely due to the specific experimental processing which was different from the commercial one but more valuable for studying the variation in the biological characteristics of the traits.

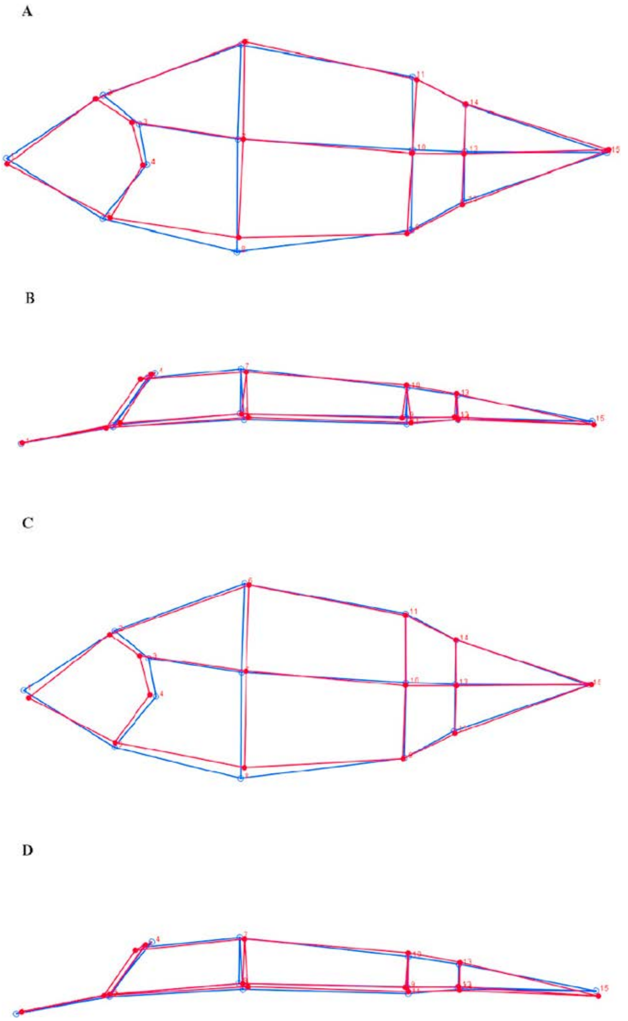

Fig. 2. A graphical visualization of body and ventral part morphology associated to low (blue line) and high (red line) yield for Logr_hl-Carss (A, B) and Logr_Fill (C, D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. 3D morphology and prediction equations of slaughter yields

A graphical visualization of body and ventral part morphology associated to low (blue line) and high (red line) yield for Logr_hl-Carss and Logr_Fill is shown in Fig. 2. The greatest differences were observed on the abdominal part of the fish and on the head. Fish with higher yields present a lower ventral area, mainly under the dorsal fin, and also have a smaller head area.

The most informative morphological predictors (3D_P$_{1–7}$) included into two prediction equations (3DMod_hl-Carss and 3DMod_Fill), and their R^2 and Fisher test values (F) are listed in Table 1. The selected 3D morphological landmarks (1–15) of carp body are shown on Fig. 1.

Logr_hl-Carss was best predicted with a model combining five simple predictors (3D_P$_2$, 3D_P$_3$, 3D_ P$_4$, 3D_P$_5$, 3D_P$_6$). 3D_P$_2$ = the ratio of abdominal fillet thickness (E8) to height between the lateral line and the aligned ventral point 7–8; 3D_P$_3$ = 3D area between 2–3–7–10–11–6 divided by 3D area 3–4–5–8–9–10–7; 3D_P$_4$ = volume between 2–3–7–10–11–6 divided by volume 3–4–5–8–9–10–7,

Table 1
Multiple linear regression models to predict Logr headless carcass and Logr fillet yields in common carp including predictors characteristics, regression statistics R^2, F - Fisher test value and prediction equations.

Headless carcass yield 3D model	Predictor characteristics
3D_P$_2$	Ratio between E8 and 3D height between points 7–8
3D_P$_3$	3D area between 2–3–7–10–11–6 divided by area 3–4–5–8–9–10–7
3D_P$_4$	Volume between 2–3–7–10–11–6 divided by volume 3–4–5–8–9–10–7
3D_P$_5$	Width at point 7 divided by width at point 4
3D_P$_6$	Width at point 10 divided by width at point 4
Regression statistics: R^2 = 0.59, F = 449.7	
3DMod_hl-Carss = −0.18 + 5.88 3D_P$_2$ + 0.21 3D_P$_3$–0.13 3D_P$_4$–0.02 3D_P$_5$ + 0.09 3D_P$_6$	

Fillet yield 3D model	Predictor characteristics
3D_P$_1$	3D head area divided by total area (except the 14–15–12–13)
3D_P$_2$	Ratio between E8 and 3D height between points 7–8
3D_P$_6$	Width at point 10 divided by width at point 4
3D_P$_7$	Volume between 6–11–10–7 divided by volume 7–10–9–8
Regression statistics: R^2 = 0.49, F = 381.9	
3DMod_Fill = −0.10 - 0.46 3D_P$_1$ + 5.88 3D_P$_2$ + 0.11 3D_P$_6$ + 0.02 3D_P$_7$	

3D_P$_5$ = width at point 7 divided by width at point 4 and 3D_P$_6$ = width at point 10 divided by width at point 4. 3DMod_hl-Carss explains 59.2% (R^{2}CV = 58.8%) of total phenotypic variation in Logr_hl-Carss.

Logr_Fill was best predicted by a model combining four predictors. Two of them were the same as for Logr_hl-Carss (3D_P$_2$, 3D_P$_6$) and the different ones were 3D_P$_1$ = 3D head area divided by total area (except the 14–15–12–13) and 3D_P$_7$ = volume between 6–11–10–7 divided by volume 7–10–9–8. 3DMod_Fill explains 49.6% (R^{2}CV = 49.3%) of total phenotypic variation of Logr_Fill.

3.4. *Heritability estimates and genetic correlations*

Heritability estimates of the single predictors (3D_P$_1$ – 3D_P$_7$), Logr slaughter yields (Logr_hl-Carss and Logr_Fill) and model-predicted slaughter yields (3DMod_hl-Carss and 3DMod_Fill) are given in Table 2. All heritabilities were significantly different from zero and achieved moderate to high values in the range of 0.29–0.66. Heritability estimates and genetic correlations of yield-related phenotypes (BW, % Fat, FC, RelBH, RelHL) are detailed in Prchal et al., 2018a.

The genetic correlations between individual predictors, Logr and 3DMod slaughter yields are listed in Table 2. 3D_P$_1$ was highly negatively correlated to 3D_P$_6$ (r_g = −0.70). 3D_P$_3$ and 3D_P$_4$ were highly correlated to each other (0.98) as well as to 3D_P$_7$ (r_g = 0.87, 0.85, respectively). Besides, 3D_P$_3$ and 3D_P$_4$ were also negatively genetically associated to 3D_P$_5$ and 3D_P$_6$. Moreover, 3D_P$_5$ and 3D_P$_6$ were in moderately high genetic relationship (r_g = 0.73). Only 3D_P$_1$, 3D_P$_2$ and 3D_P$_6$ achieved favourable genetic relationship with Logr slaughter yields (r_g = |0.44–0.80|). Likewise, 3D model-predicted slaughter yields showed high genetic associations to the real yields to be predicted (r_g = 0.84–0.88). Besides, residual weights to be predicted (Logr) as well as model-predicted (3DMod) slaughter yields were highly correlated to each other (r_g = 0.84–0.97).

The genetic correlations of yield-related phenotypes to the most informative simple predictors and 3D model-predicted yields are presented in Table 3. The predictors 3D_P$_1$ and 3D_P$_6$ were in absolute values in the same genetic pattern to all yield-related phenotypes. Thus, these predictors were genetically related to lower BW and FC (low correlation), RelBH (medium correlation) and RelHL (strong correlation). Oppositely, selecting for such predictors might lead to a slightly higher muscle fat (r_g = |0.31–0.37|). 3D_P$_2$ was not significantly correlated to BW, FC and RelBH but was also positively genetically associated to % Fat and negatively but at the edge of significance with RelHL. Regarding model-predicted yields, genetic correlations were similar to 3D_P$_1$ and 3D _P$_6$ predictors but generally stronger for 3DMod_Fill especially in relation to BW, FC (insignificant for 3DMod_hl-Carss) and RelHL (r_g = −0.47 vs. -0.67, respectively).

3.5. *Expected genetic gain*

Expected genetic gains (Table 4.) were calculated for fillet yield and compared among mass (MS), full-sib (FSS) and indirect selection (IS) scheme using 3D model (3DMod_Fill) and 3D single predictors (3D_P$_1$ – 3D_P$_7$).Genetic gain calculated for hypothetical mass selection (MS) was 0.70% (10% selection intensity) and 0.46% (30% selection intensity) per generation. Genetic gain for full-sib selection (FSS) with 10 sibs selected per family (10% and 30% selection pressure) was slightly lower (0.61% and 0.40%) than for MS. Estimated genetic gain achieved by indirect selection on the 3D model-predicted fillet yields (3DMod_Fill) was 0.65% and 0.43% for 10% and 30% selection intensity, respectively. Genetic gains of the most effective 3D predictors (3D_P$_1$, 3D_P$_2$ and 3D_P$_6$) ranged from 0.27% to 0.47%. Other predictors showed much lower values (0.01%–0.19%). Relative genetic changes of yield-related traits were calculated for fillet yield improvement using IS scheme (Supplementary Table S2).

4. Discussion

In the present study, we showed i) favourable phenotypic prediction accuracy of real slaughter yields, ii) moderate to high heritability estimates of simple 3D predictors and 3D model-predicted yields; iii) strong genetic correlations of 3D predictors / models with the real slaughter yields suggesting that the indirect selection could be strong enough to be used in genetic improvement of slaughter yields. Moreover, iv) potential genetic gain based on indirect selection of the 3D model (3DMod_Fil) was similar to that achieved by previously developed 2D model (2DMod_Fil) (Prchal et al., 2018a), however, best individual 3D predictor – 3D_P2 (E8/ 3D height in the ventral part) achieved less favourable genetic parameters than the same predictor in 2D – 2D_P2 (E8/ 2D height) (Prchal et al., 2018a). Still, 3D models / predictors have a solid potential for genetic improvement of slaughter yields in common carp as they are much faster evaluated on live breeding candidates, so that breeding program would be simple, efficient and sustainable, compared to previously used methods of external and internal measures (Cibert et al., 1999; Bosworth et al., 2001; Rutten et al., 2004; Van Sang et al., 2009; Haffray et al., 2013; Vandeputte et al., 2017; Prchal et al., 2018a).

The 3D model-predicted yields explained 59% of the phenotypic variation in the real headless carcass yield, and 50% in real fillet yield. Accuracy to predict headless carcass yield using 3D was slightly lower than the accuracy in a previous study (63%) that used 2D and ultrasound measurements (Prchal et al., 2018a). Yet, prediction accuracy of

Table 2
Heritability (± standard error) estimates (diagonal) in bold, phenotypic (below the diagonal) and genetic correlations ± standard error (above the diagonal) in common carp for simple predictors (3D_P$_1$ – 3D_P$_7$), log-log residuals (Logr) of slaughter yields and 3D models (Mod) to predict slaughter yields.

	3D_P$_1$	3D_P$_2$	3D_P$_3$	3D_P$_4$	3D_P$_5$	3D_P$_6$	3D_P$_7$	Logr_hl-Carss	Logr_Fill	3DMod_hl-Carss	3DMod_Fil
3D_P$_1$	**0.50 ± 0.08**	−0.25 ± 0.15	0.27 ± 0.15	0.38 ± 0.14	−0.46 ± 0.12	−0.70 ± 0.08	0.17 ± 0.16	−0.54 ± 0.11	−0.59 ± 0.10	−0.47 ± 0.12	−0.72 ± 0.08
3D_P$_2$	−0.07	**0.44 ± 0.08**	−0.02 ± 0.16	−0.09 ± 0.16	−0.25 ± 0.15	0.18 ± 0.15	0.12 ± 0.16	0.80 ± 0.06	0.73 ± 0.08	0.93 ± 0.02	0.80 ± 0.06
3D_P$_3$	0.15	0.25	**0.39 ± 0.07**	0.98 ± 0.01	−0.39 ± 0.14	−0.41 ± 0.13	0.87 ± 0.05	−0.10 ± 0.16	−0.19 ± 0.16	−0.07 ± 0.16	−0.22 ± 0.15
3D_P$_4$	0.17	0.21	0.95	**0.42 ± 0.08**	−0.44 ± 0.13	−0.51 ± 0.12	0.85 ± 0.05	−0.20 ± 0.16	−0.29 ± 0.15	−0.17 ± 0.16	−0.33 ± 0.15
3D_P$_5$	−0.41	−0.06	−0.17	−0.22	**0.48 ± 0.09**	0.73 ± 0.08	−0.37 ± 0.14	−0.01 ± 0.16	0.17 ± 0.15	−0.01 ± 0.16	0.23 ± 0.15
3D_P$_6$	−0.49	0.15	−0.16	−0.19	0.56	**0.66 ± 0.09**	−0.25 ± 0.15	0.44 ± 0.12	0.58 ± 0.10	0.49 ± 0.12	0.69 ± 0.08
3D_P$_7$	0.09	0.30	0.63	0.70	−0.19	0.02	**0.29 ± 0.06**	0.05 ± 0.16	−0.03 ± 0.16	0.12 ± 0.16	−0.01 ± 0.16
Logr_hl-Carss	−0.23	0.65	0.21	0.14	0.05	−0.23	0.22	**0.46 ± 0.08**	0.97 ± 0.01	0.86 ± 0.04	0.85 ± 0.05
Logr_Fill	−0.38	0.54	0.04	0.01	0.19	−0.38	0.17	0.77	**0.50 ± 0.08**	0.84 ± 0.05	0.88 ± 0.04
3DMod_hl-Carss	−0.18	0.94	0.30	0.20	0.05	−0.18	0.30	0.70	0.60	**0.46 ± 0.08**	0.94 ± 0.02
3DMod_Fil	−0.53	0.83	0.12	0.08	0.26	0.53	0.26	0.66	0.67	0.90	**0.56 ± 0.09**

3D and ultrasound values for fillet yield prediction was equal to 2D and ultrasound recording in common carp (Prchal et al., 2018a) but higher than in rainbow trout (Haffray et al., 2013) and European sea bass (*Dicentrarchus labrax)* (Vandeputte et al., 2017). So, model-predicted yields using 2D or 3D showed almost similar phenotypic prediction accuracy of real slaughter yields in common carp, explaining 49%–63% of phenotypic variation. Nevertheless, in common carp headless carcass yield was only predicted using three simple 2D predictors (Prchal et al., 2018a) but the best models of headless carcass yield used six predictors in rainbow trout (Haffray et al., 2013), nine predictors in European sea bass (Vandeputte et al., 2017) and five predictors in present study. It might have been caused by different digitization procedure between 2D and 3D, as the previous 2D study in carp involved additional landmarks in the caudal part (Prchal et al., 2018a). So, comparison between 2D and 3D predictions might be affected by the number of landmarks digitized (15 in 3D vs. 20 in 2D) as more landmarks may lead to more precise measurements (especially areas) but on the other hand require more time for acquisition. Yet, the time required for post processing of morphological 2D landmarks and the lack of information on body width are the main limitations of 2D prediction. Moreover, Logr_Fill could be predicted only with 4 predictors using 3D instead of 5 2D predictors in Prchal et al. (2018a) and 6 predictors in Vandeputte et al. (2017), suggesting a simplified model of fillet yield using 3D digitization. 3D model-predicted yields have generally a strong prediction accuracy similar to 2D models, but 3D predictions are more practical for using in the field on a large sample of fish, due to the possibility to acquire data in real time. On the other hand, the initial cost of 3D digitizer is higher than collection of 2D images by camera (10,000 € vs 1000 €). However, when taking into account time required for post processing by one skilful person (almost one month), the return on this investment and practical use of such device is fast and clear. Briefly, during own recording it takes about 1.5 min. of skilful person to get 3D coordinates and no further post-processing is required. To have 2D picture it takes about 1 min. (correct positioning of fish to take an informative picture, checking the result) and further 1 min. to process the image later. So, 2D is about 30 s slower than 3D. However, the most important is that 3D coordinates we have immediately and can select the fish directly during one manipulation, meanwhile when using 2D we need to manipulate with all fish again later to select proper candidates. It represents further time and more stress for fish (two manipulations, longer short-term storage of fish in tanks). So, being able to select the fish during one manipulation is a crucial task. However, both 2D and 3D model-predicted yields rely on precise coefficients for linear combination of predictors. So, directly evaluating the potential of simple predictors is therefore potentially much simpler for practical indirect selection.

Heritability estimates of 3D model-predicted yields were high (0.46 for hl-Carss and 0.56 for fillet) and slightly lower than the estimates predicted using 2D digitization (0.48, 0.63, respectively; Prchal et al., 2018a). Yet, the heritability estimates for 3D traits were higher when compared to other fish species (Van Sang et al., 2012; Haffray et al., 2013; Vandeputte et al., 2017). Predicted yields of both 2D and 3D models were strongly genetically correlated to the real yields (0.84–0.88), showing their strong potential for indirect selection to improve edible part yields. It is also necessary to know the genetic correlations between the selected yield traits with other traits such as growth, body composition and fish welfare traits, as these traits may be changed indirectly by selection on yield traits. Some examples can be clearly seen in the previous studies that focused on improvement of yield that would indirectly lead to degradation of the flesh quality in cattle (Feitosa et al., 2017) and in common carp (Prchal et al., 2018c), pulmonary disease in broiler chicken (Hocking, 2010; Muir et al., 2014) or loss of flavour in tomatoes (Tieman et al., 2017).

In this study, we observed that selecting for 3D model-predicted yields would indirectly lead to several undesirable impacts similarly as in case of 2D-based predictors (Prchal et al., 2018a). Thus, such

Table 3
Genetic correlations ± standard error between most informative simple predictors, 3D model-predicted yields and yield-related traits.

	3D_P$_1$	3D_P$_2$	3D_P$_6$	3DMod_hl-Carss	3DMod_Fil
r_g BW	0.36 ± 0.13	−0.10 ± 0.15	−0.32 ± 0.13	−0.21 ± 0.14	−0.30 ± 0.14
r_g FC	0.47 ± 0.11	−0.05 ± 0.14	−0.36 ± 0.12	−0.18 ± 0.13	−0.30 ± 0.13
r_g % Fat	−0.37 ± 0.13	0.37 ± 0.13	0.31 ± 0.13	0.43 ± 0.13	0.49 ± 0.11
r_g RelBH	0.50 ± 0.11	−0.13 ± 0.13	−0.51 ± 0.10	−0.31 ± 0.13	−0.43 ± 0.11
r_g RelHL	0.86 ± 0.04	−0.27 ± 0.13	−0.61 ± 0.08	−0.47 ± 0.11	−0.67 ± 0.08

Table 4
Genetic gains (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 fillet yield improvement.

Trait selected	Type of selection	Genetic gain (10%)	Genetic gain (30%)
Logr_Fill	MS	0.70	0.46
Logr_Fill	FSS	0.61	0.40
3DMod_Fill	IS	0.65	0.43
3D_P$_1$	IS	0.41	0.27
3D_P$_2$	IS	0.48	0.32
3D_P$_3$	IS	0.12	0.08
3D_P$_4$	IS	0.19	0.12
3D_P$_5$	IS	0.09	0.06
3D_P$_6$	IS	0.47	0.31
3D_P$_7$	IS	0.02	0.01
2DMod_Fill	IS	0.66	0.43
2D_P$_1$[a]	IS	0.33	0.22
2D_P$_2$[a]	IS	0.52	0.34

[a] Cited from Prchal et al. (2018a).

selection would more likely increase muscle fat, slightly decrease body weight and cause fast change to an oblong-like body shape with a limited head size. A similar negative effect was also observed in other studies as a genetic consequence of the selection for improved slaughter yields (Kocour et al., 2007; Nguyen et al., 2010; Haffray et al., 2012; Janhunen et al., 2017; Fraslin et al., 2018). However, such changes could lead to negative fitness effects in a long-term breeding program (Fraslin et al., 2018). Therefore, these undesirable genetic relationships must be accounted for when breeding goal would be focused on increased slaughter yields.

Yields predicted by multitrait models are constructed from several simple individual predictors and the collection of all of them requires time and precision of measurements. Our results showed that recording of suitable individual predictors seems to be efficient for a simplified breeding program. Seven individual predictors from which the yield predictors were estimated achieved moderate to high heritability (0.29–0.66). Three of them (3D_P$_1$, P$_2$, P$_6$) could be eventually used in a breeding program due to their high heritabilities (h^2 = 0.44–0.66) and significant genetic correlations with the Logr yields (r_g = |0.44–0.80|). In addition, 3D_P$_1$ and 3D_P$_2$ are the same morphological predictors as the previous 2D predictors 2D_P$_1$ and 2D_P$_2$ differing only in a way of digitization (Prchal et al., 2018a).

The best simple predictor seems to be 3D_P$_2$ (ratio between abdominal fillet thickness – E8 and external ventral height measured between points 7–8 in 3D), similar to 2D predictor P$_2$ in Prchal et al. (2018a) and ratio of E8 to E23 (depth of the peritoneal cavity) in rainbow trout (Haffray et al., 2013). 3D_P$_2$ is a highly heritable predictor, has genetic association to edible part yields showing its strong potential to be used in a breeding program as "*quick-to measure*" indirect selection criterion for improvement of yields in common carp. In addition, selection for that predictor would not lead to such significant decrease on head size and general body shape as in case of selection on 3D model predicted yields. On the other hand, both yield models and simple predictors are positively genetically correlated to muscle fat. Hence, a selection program focused on improvement of slaughter yields should check muscle lipid level and eventual change of feeding strategy would be necessary to keep high flesh quality with respect to beneficial fatty acids (Prchal et al., 2018c). 3D_P$_1$ as well as 2D_P$_1$ are defined as a ratio between head area to total body area with a negative genetic correlation to the real yields. Nevertheless, 3D_P$_1$ has a higher heritability than 2D_P$_1$ (h^2 = 0.50 vs. 0.34 in 2D) and is also more correlated to Logr yields (r_g = −0.54 to −0.59 vs. -0.52 – −0.57 in 2D). 3D_P$_6$ (width at point 10 divided by width at point 4) was a new simple predictor with very high heritability (0.66) and favourable genetic relationship to the slaughter yields (0.44–0.58). However, selection based on these simple predictors could indirectly lead to several unwanted changes similarly like in case of selection on 2D/3D yield models already discussed above. Therefore, the use of predictors in a selection index including shape and fat content would be a suitable breeding scenario to avoid a negative impact of indirect selection for fillet yield on other traits of interest.

The calculations of expected genetic gains showed that 3D model-predicted yields or simple predictors are interesting candidates for a selection program. The highest genetic gain in fillet yield was observed by hypothetical mass selection (MS) on real fillet yield (0.70% for 10% selection intensity). Such selection method is typically used as benchmark value to compare with other selection schemes, though it is not possible in the breeding program. Slightly lower genetic gain was observed using sib selection (FSS) on real fillet yield, a typical approach applied for traits requiring destructive recording (slaughter yields, meat quality) or disease resistance (Gjedrem, 2010). However, costs for FSS are higher and in our case cover also parentage assignment and processing costs of sib groups. Thus, our work confirm that indirect selection may be an interesting alternative to establish a lower cost and sustainable breeding program. Indeed, indirect selection on 3D model-predicted fillet yields showed even better genetic progress than FSS. This result was also in accordance with previous 2D fillet model yields (Prchal el., 2018a) that showed the same expected response to selection. However, main limitations of using model-predicted yields in a breeding program have been already discussed above. Expected genetic gains of simple predictors were generally lower than from 2D or 3D fillet yield models but still significant enough to be included in a selection program. 3D_P$_1$ achieved a better gain than the same predictor in 2D (0.41% vs. 0.33%). Alternatively, new 3D_P$_6$ predictor was better for genetic progress than 3D_P$_1$ but these predictors are genetically related to unfavourable consequences that might be considered as reasonable biological limits in a long-term breeding program. The best genetic progress using a simple predictor would be obtained by 3D_P$_2$ (0.48%), similarly to our previous 2D experiment where the gain was even higher (0.52%) (Prchal et al., 2018a). Moreover, this predictor is more favourably genetically connected to other phenotypes and easy and especially quick to record in the field and thus a very practical simple trait for indirect selection of slaughter yields in common carp.

5. Conclusions

The accuracy of the phenotypic prediction of slaughter yields by 3D models is high and almost similar to 2D prediction models. Likewise, expected genetic progress to be obtained by selection on model-predicted yields and on the best individual predictor (3D_P$_2$) were similar or only slightly lower when compared to the 2D-based models and the best 2D simple predictor (Prchal et al., 2018). In conclusion, model-predicted yields and especially simple 3D predictors have a solid potential for genetic improvement of slaughter yields in common carp. While such predictors are not better than 2D predictors, they are much more convenient and faster to collect in the field, as they do not imply post-processing of images. These practical aspects should be taken into account in the future carp breeding program and we expect to verify the applicability of such predictors in a practical selection response experiment.

Authors' contributions

MP, DG, and MK established and reared the experimental stock. MP and MK PIT tagged and sampled the DNA from finclips. PH and MV provided the methodology and equipment. MP, JB, MV, AV, JZ, DG, AB, VK, and MK shared on final trait recordings. AK introduced MP to the quantitative genetic analysis. JB carried out 3D digitization and the phenotypic prediction of slaughter yields. LG performed the DNA extractions and parentage assignment. MP estimated the genetic parameters. All authors contributed to drafting the manuscript and approved the final version.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

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CHAPTER 6

SIMPLIFIED METHOD FOR GENETIC SLAUGHTER YIELDS IMPROVEMENT IN COMMON CARP UNDER EUROPEAN POND CONDITIONS

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My share on this work was about 20%.

Simplified method for genetic slaughter yields improvement in common carp under European pond conditions

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Abstract

Demand for edible part yields, usually offered as headless carcass or fillets, is continuously increasing in all fish species. Therefore, slaughter yields are interesting traits for genetic improvement. However, phenotyping of slaughter yields cannot be done on live breeding candidates and the suitable alternative seems to be utilization of simple yield predictors in indirect selection recorded by ultrasound tomography. In this study, we evaluated the possibility of using ratio of abdominal fillet thickness (E8) either to the abdominal depth (E23) or as alternative to E23 – 2D_d abdominal depth (length between lateral line and abdominal part vertical to the dorsal fin) as predictors for genetic improvement of slaughter yields in common carp. The predictors were recorded on each fish twice at two years old (E8/E23_2, E8/2D_d_2) and three years (market size) old (E8/E23_3, E8/2D_d_3). An experimental stock was established from a partial factorial design of 27 dams and 29 sires. Phenotyping of simple yield predictors, headless carcass and fillet with skin yields (only market size), and other performance traits was done on 987 (second year) and 773 (market size) individuals, uniquely assigned to their parental pair. The predictors recorded on market size fish showed slightly better heritability (0.41, 0.44), genetic correlations to the slaughter yields (0.78–0.86) and expected genetic gains (1.29%–1.54%) of the slaughter yields. Still, predictors recorded on two-year old fish showed a solid genetic potential for slaughter yields improvement ($h^2 = 0.27–0.40$; $r_g = 0.64–0.72$, expected genetic gain = 0.93%–1.35%). It suggests that the indirect selection for improved slaughter yields could be performed using simple and quickly-to-measure yield predictors, also applicable in two-year old fish that are much easier for handling and manipulation. These newest results could significantly simplify carp breeding program focused on genetic improvement of slaughter yields.

Key words: *yield predictor, ultrasound tomography, indirect selection, selection program*

1. Introduction

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 et al., 2012). Since that time, selective breeding has become one of the most useful breeding method 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 (Janssen et al., 2017). Most of them are primarily focused on growth rate but new production efficiency traits are increasingly studied or even included in selection programs (Gjedrem and Rye, 2016). Among those traits, disease resistance (Yáñez et al., 2014; Gjedrem, 2015; Houston, 2017) and feed conversion efficiency (Daulé et al., 2014; De Verdal et al., 2018) play a majority. However, slaughter yields (processed carcass or fillets) are also traits of high-economic value and their importance for genetic improvement is thus unquestionable (Kankainen et al., 2016).

On the other hand, slaughter yields as traits to be genetically improved by selection has been criticized until recently. Several studies concluded that slaughter yields (defined as percent ratio of headless carcass or fillet weight to body weight) have low heritability, probably due to low phenotypic variation, and cannot be improved independently of body weight (Powell et al., 2008; Nguyen et al., 2010; Gjerde et al., 2012; Van Sang et al., 2012). However, recent simulation study from the field data clearly confirmed that moderate genetic gains on fillet yield in fish are possible (Fraslin et al., 2018). Nevertheless, genetic parameters have to be estimated using linear selection index (combining fillet weight and body weight) or alternatively as residual fillet weight (the residual of the regression of fillet weight on body weight) that can be both used as a reliable surrogate for fillet yield (Vandeputte et al., 2020).

However, phenotyping of slaughter yields is lethal and cannot be recorded on live fish and therefore mass selection on live breeding candidates is impossible. Therefore, slaughter yields are commonly selected through sib selection (Kause et al., 2007), or might be indirectly selected through traits which are highly genetically correlated to slaughter yields (Rutten et al., 2005; Kocour et al., 2007; Saillant et al., 2009; Haffray et al., 2012). For instance, ultrasound tomography might be simply and efficiently used for indirect phenotypic prediction of slaughter yields in breeding programs of fish (Bosworth et al., 2001; Haffray et al., 2013; Vandeputte et al., 2017). In addition, ultrasound tomography is also used to obtain other non-lethally phenotypes in tambaqui (*Colossoma macropomum*) (Perazza et al., 2015, 2017).

Common carp is the third most produced species in aquaculture worldwide (FAO, 2018). However, its breeding programs are still simple, mostly based on crossbreeding, while selective breeding programs are rare in spite of their high genetic gain potential (Kocour et al., 2007; Nielsen et al., 2010; Ninh et al., 2013; Hu et al., 2017; Prchal et al., 2018b). Carp is mainly sold as whole fish, thus alive or freshly killed (bled and gutted, alternatively without scales and skin). Slaughter yields are usually offered as chilled headless carcass or fillets with skin at the markets and their preference is becoming increasingly important. Therefore, slaughter yields are interesting traits for a breeding program. In our previous studies with market sized common carp, we observed high phenotypic and genetic potential of slaughter yield models and predictors based on 2D and 3D digitization of morphological landmarks and ultrasound measurements (Prchal et al., 2018a; Prchal et al., 2020). Multiple linear models composed of simple predictors showed the best estimates of slaughter yields. However, slaughter yield models rely on precise coefficients for linear combination of several simple predictors, and thus they are not so suitable for simple selection program for improved slaughter yields performed on large sample of fish. Nevertheless, simple predictors themselves are also efficient traits for indirect selection of slaughter yields. We observed that ratio of abdominal fillet thickness (E8 – measured by ultrasound tomography) to length between the lateral line and the aligned ventral point recorded by 3D digitizer ($3D\_P_2$) might be a useful carp yield predictor (Prchal et al., 2020). However, a slight limitation might be seen in high initial costs of 3D digitizer and requirement of further working place with a trained person. Alternatively, ultrasound predictor defined as ratio of E8 to the abdominal depth (E23) suggested a strong potential for fillet yield improvement on rainbow trout (Haffray et al., 2013; *Oncorhynchus mykiss*). Moreover, recent selection experiment (Vandeputte et al., 2019) performed also on rainbow trout using combination of mentioned predictor (E8/E23) and information from sibs of the selection candidates, showed clearly positive response to selection for up-selected fish (1.16% units difference in fillet percentage to down-selected fish). However, all studies on slaughter yields were conducted on market sized fish and information about possibilities to select younger and smaller breeding candidates is still missing. Indeed, selection of such fish can be advantageous from the technical point of view due to much easier handling and lower capacity needs for short-term storage of fish. Similarly, a reasonably high genetic correlation (0.74) between the weight of two-year old and market-size common carp was observed (Prchal et al., 2018b).

In the present study, we investigated genetic potential of simple slaughter yield predictor defined as ratio of E8/E23 or alternatively E8/2D_d measured twice in two-years old Amur mirror carp and the same fish at market size. Thus, we aimed to i) estimate genetic variation of simple slaughter yield predictors, basic phenotypes and slaughter yields, ii) estimate genetic correlations of simple yield predictors to the real slaughter yields iii) calculate expected genetic gains based on simple yield predictor recorded on two- and three-year old fish and iv) suggest simplified methodology of carp breeding program.

2. Material and methods

2.1. 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 16OZ19179/2016-17214). At market size the fish were humanely sacrificed by trained person for subsequent processing and slaughter yield evaluation.

2.2. Establishment of base population

In May 2017, the base population of Amur mirror carp was established at the Genetic Fishery Centre (GFC) 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, a new Czech common carp breed (Flajšhans et al., 2015; Prchal et al., 2018b), was chosen as the broodstock fish. Each parental fish was fin-clipped before own spawning for later parentage assignment of the offspring. Artificial spawning of the broodstock was performed using the same methodology as described in Prchal et al., 2018. Within one day, gametes from 27 dams and 29 sires (different parents than used in the previous studies Prchal et al., 2018a, 2020) were individually collected and a partial factorial design with three rounds of gametes coming from 8 dams and 7 sires in each was applied. In the fourth round, sperm from 6 sires were used only while eggs came from 8 dams but 3 out of them were used already in the previous crossings. After fertilization, the eggs (400 g per dam) from each round were incubated in four separate Zuger jars.

2.3. Fish rearing and phenotyping

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 population was created by pooling equal quantities (estimated volumetrically) of larvae from all four post-hatching incubators to the two plastic bags under oxygen atmosphere. Larvae from both bags were released separately (150,000 larvae. ha^{-1}) into the two nursery ponds at the Experimental Fish Culture and Facility at FFPW USB. Thereafter, the progenies were reared communally under semi-intensive pond management conditions with the fish being fed on natural food (zooplankton and zoobenthos). From age of 8 weeks supplementary plant-based pellets composed of wheat, corn and soybean meal (dry matter: 88.8%, crude protein: 17.9%, carbohydrates: 58.9% and total fat: 3.7%) were served 2-3 times until water temperature decreased below 8 °C in doses of 5–10% of the fish stock biomass per feeding day according to the month, relative oxygen level in the water and water temperature.

After first growing season (October 2017), both ponds were harvested and fish stock out of pond with better fish survival (50% survival, mean weight 27.3±3.6 g) were taken for the further steps of the experiment. The fish were transferred to manipulation tanks of GFC at USB FFPW facility in Vodňany. A random sample of 2000 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 parentage assignment. Likewise, each tagged individual was weighed (to the nearest 0.1 g) and measured for standard length (to the nearest mm). Those fish were then released into a 2 ha pond at GFC. During the second and also the third growing season carp were fed with natural food developing in ponds and pelleted feeding replaced later with wheat grain (dry matter: 88.3%, crude protein: 10.4%, carbohydrates: 72% and total fat: 2.4%). The supplemental feed was served three times a week in doses of 1.5–3% of the fish stock biomass per feeding day according to the month, relative oxygen level in the water and water temperature.

After second overwintering (March 2019), all survived fish (n = 1,064) were harvested and again transferred to manipulation tanks at GFC of USB FFPW in Vodňany. Each identified individual with assigned parental pair (n = 987) (fish without P.I.T. or unknown parental pair were excluded from the experiment) was phenotyped for standard length (SL), head length (HL), body height (BH), body width (BWI), 2D abdominal depth (2D_d_2) and body weight (BW). 2D_d_2 was recorded as a distance between the lateral line and the abdominal part of body at the vertical from the highest point of body height (Figure 1.) down towards the abdomen and was studied as a "quickly-to-measure" alternative to ultrasound measurement E23. Biometrical indicators were calculated as follows: Fulton's condition factor: $FC=10^5\times[BW (g)/SL^3 (mm)]$, relative body height: RelBH=BH/SL, relative body width: RelBWI=BWI/SL and relative head length: RelHL=HL/SL. The total muscle fat content (% Fat) was recorded using a Fish Fatmeter FM 692 (Distell Ltd., UK), using calibration option 'CARP – 1'. Abdominal wall thickness (E8_2) and abdominal depth (E23_2) were recorded by ultrasound tomography (SonoScape E2, 10 MHz) using curved array (2–6 MHz). E8_2 was measured on halfway between the lateral line and the abdomen at the vertical from the highest point of body height (Figure 1 and 2A.) down towards the abdomen. E23_3 was recorded as the distance between the abdomen and the end of body cavity as the vertical from the highest point of body height towards the dorsal fin (Figure 1 and 2B.). E8, E23 and 2D abdominal depth were all measured by one person across the experiment to eliminate potential measuring biases. The yield-related predictors were calculated as ratio of E8/E23_2 or E8/2D_d_2.

Then, experimental fish were released back to the same pond for the third growing season and in November 2019 market size stock (mean weight = 1,619 g) was harvested again and transferred to a storage pond before final processing (December 2019) at fish slaughter house of USB FFPW in České Budějovice, Czech Republic.

A total of 773 survived individuals were humanely sacrificed by a hit on the head and bled by cutting the gills according to the law on the protection of animals against cruelty. Standard length (SL), head length (HL), body height (BH), body width (BWI), 2D abdominal depth (2D_d_3) and body weight (BW), % Fat and internal measurements (E8_3 and E23_3) were recorded the same way as after the second overwintering. The yield-related predictor was created again as ratio of E8/E23_3 or E8/2D_d_3. Biometrical indicators were calculated similarly as after the second overwintering. After biometric recordings, each fish was processed as described in Kocour et al. (2005) and the following body portions were weighed (to nearest 1 g): left fillet without skin, left skin, right fillet with skin, head, body skeleton with pectoral fin girdle and remnants. Sex was identified macroscopically as male (M), female (F) or unspecified (U). The weight of slaughter body parts was calculated by combining the previous body portions: headless carcass weight [hl-CarssW = left fillet + left skin + right fillet with skin + body skeleton with pectoral fin girdle and remnants], fillet weight with skin [FilletW = left fillet without skin + left skin + right fillet with skin]. The slaughter yields expressed in % were calculated as: headless carcass yield % [% hl-Carss = (hl-CarssW/BW)×100], and fillet yield [% Fill = FilletW/BW×100]. The natural logarithm was calculated for the weight of each slaughtered part and regressed on the logarithm of body weight to obtain growth-independent allometry residuals in order to provide genetic and phenotypic parameters giving reliable and precise prediction of genetic gains in slaughter yield (Vandeputte et al., 2020). Therefore, for % hl-Carss and % Fill the log-log residuals (Logr) termed as Logr_hl-Carss and Logr_Fill, respectively were defined as surrogate traits to typical % yields.

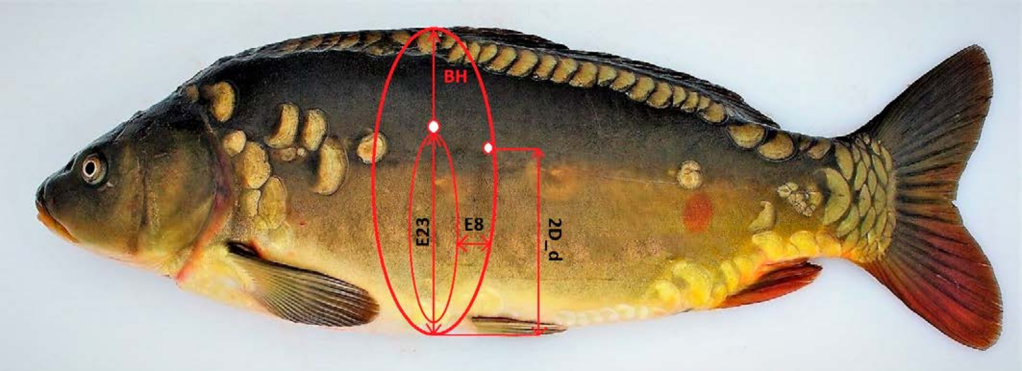

Figure 1. *Illustration scheme for measuring locations of ultrasound values (E8, E23) and 2D depth (2D_d)*

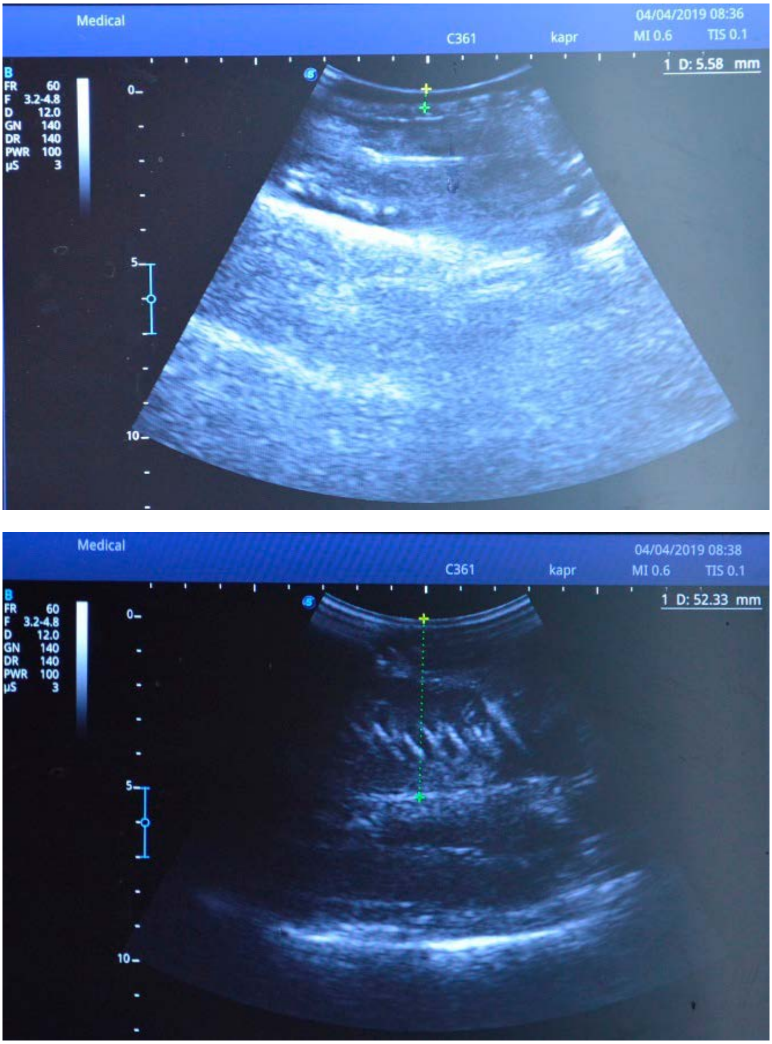

Figure 2. *Ultrasound images. Scans of (A) abdominal fillet thickness (E8) and (B) the abdominal depth (E23)*

2.4. Parental allocation

The 56 parents and 1064 offspring were genotyped using 12 microsatellites loci labelled as CCE46 (Wang et al., 2007), HLJE265, HLJ2241, HLJ2346, HLJ2382, HLJ24657, HLJ2544, HLJ334, HLJ526, HLJ534 (Zheng et al., 2011), J58 (Yue and Orban, 2002), KOI 57–58 (David et al., 2001) at LABOGENA-DNA, the French laboratory for livestock genotyping (ISO 170025 accredited, Jouy-en-Josas, France). Parental allocation was performed using the AccurAssign software, applying a maximum-likelihood method (Boichard et al., 2014) with default thresholds set up combining 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).

2.5. Estimation of genetic parameters

The raw data were carefully checked by initial regression analysis of correlated traits revealing potential outliers and such errors were excluded from the statistical model. Likewise, fish whose body parts sum was over the 100% or lower than 95% of the initial body weight (5% tolerance for loss of body liquids, scales etc. during slaughtering process) were excluded from the final data set. Heritability (h^2), phenotypic and genetic correlations ($r_{p,}$ and r_g, respectively) were estimated in multivariate mixed models using the restricted maximum likelihood method in DMU statistical software (Madsen and Jensen, 2013). The univariate (for heritability) and multivariate analysis (for genetic correlations) were on the following animal model:

$$Y_{ijk}=\mu_i+sex_{ij}+anim_{ik}+e_{ijk}$$

Where Y_{ijkl} is the measured phenotypic value of each analyzed trait, μ_i is the overall mean for trait *i*, sex_{ij} is the fixed effect of sex (*j*=female, male, unidentified sex) for trait *i*, $anim_{ik}$ is the random genetic effect of an animal *k* with pedigree list of studied fish based on parentage assignment (*k*=1, 2, ..., *n*) for trait *i*, and e_{ijk} is the random residual. Heritability estimates were calculated as the ratio of additive genetic variance (V_A) divided by the total phenotypic variance (V_P), $h^2=V_A/V_P$. The likelihood ratio test (LRT) was used for comparing the goodness of fit of two models (with or without the animal genetic effect). The heritability estimates were considered significant when the difference of additive genetic effect 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 correlations were considered significant if $|r_g| - |1.96\times S.E.|$ was higher than zero (Coolidge, 2013).

The genetic gains (ΔG) per generation were estimated using the breeder's equations (Falconer and MacKay, 1996) under a mass (MS), full-sib (FSS) and indirect (IS) selection responses for slaughter yields. The theoretical genetic gain under mass selection (although it cannot be applied in practice) was calculated as $\Delta G_M=i\times h^2\times\sigma_P$, where *i* is a table value of selection intensity for 10% selection pressure (*i*=1.755) and h^2 and σ_P are the heritability and phenotypic standard deviation of the trait under selection, respectively. The response to selection of FSS was estimated as $\Delta G_{FS} = \frac{i \times \sigma_P \times h^2 \times n \times r}{\sqrt{n(1+(n-1)t)}}$, where *n* is the number of sibs sampled per family (*n*=10), *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 expected genetic gain for simple yield predictor was calculated as $\Delta G_I=i\times h_1\times h_2\times r_g\times\sigma_{P2}$, where ΔG_I is the expected 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. Finally, the real genetic gain was scaled back to the percent body weight units by multiplying ΔG by the real mean headless carcass or fillet yield [$\Delta G(yield\%) = \Delta G(Logunits) \times average\_yields\%$] in the present experimental stock (62.4% and 43.3%, respectively). The selection pressure was set up of 10% as the most practical selection pressure for potential carp selective breeding program.

3. Results

3.1. Representation of families

Out of the 1064 offspring genotyped, 987 (92.8%) could be assigned to a single parental pair, 44 (4.1%) had two possible parental pairs and were considered unassigned, 17 (1.6%) could not be assigned to any parent pair and 16 (1.5%) had DNA quality problems and thus no exploitable genotype. Out of 987 uniquely assigned fish, 971 individuals had adequate phenotypes after removal of outliers.

The 971 fish used in the analysis and recorded after the second winter originated from 191 out of the possible 216 full-sib families. The number of progeny per sire varied from 13 to 61, the average was 36. The number of progeny per dam varied from 4 to 65, the average was 33. The 704 fish used in the analysis and recorded at market size originated from 182 out of the possible 216 full-sib families. The number of progeny per sire varied from 5 to 50, the average was 27. The number of progeny per dam varied from 4 to 54, the average was 29.

3.2. Descriptive statistics of traits

Mean ± standard deviation (S.D.), number of observations (*n*) and coefficient of variation (*CV*) of yield-related traits and slaughter yields are listed in Table 1. The mean values of measured performance traits fitted to common range of respective age category of common carp cultured under European pond conditions.

3.3. Heritability estimates and genetic and phenotypic correlations

Heritability estimates of the slaughter yield predictors in two-year and market size fish, main market size performance traits and biometrical indicators, and Logr slaughter yields are given in Table 2. All heritabilities were significantly different from zero and achieved moderate to high values in the range of 0.27–0.60. Heritability estimates of second year performance traits and genetic and phenotypic correlations between the second-year performance traits and Logr slaughter yields are listed in Table 3 The values of heritability were low (RelHL=0.20) to high (RelBH=0.60) and only % Fat and RelHL showed significant genetic correlation to the slaughter yields. Genetic correlations of the same performance traits between second year and third year were high (r_g=0.78–0.93; see Table 4).

The phenotypic and genetic correlations between slaughter yield predictors, performance traits at market size and Logr slaughter yields are shown also in Table 2. All yield predictors (recorded in two-year old – E8/E23_2; E8/2D_d_2 and market size fish – E8/E23_3; E8/2D_d_3) were moderately to highly genetically correlated to the both Logr slaughter yields (0.64–0.86). The slaughter yield predictors were highly correlated to each other (r_g=0.84–0.97) and showed similar genetic associations to % Fat (r_g=0.40–0.50) and RelHL (r_g=-0.49 – -0.50). Slaughter yield predictors recorded in two-year old fish were significantly related to the harvest weight (r_g=0.39). Slaughter yields (Logr_hl-Carss and Logr_Fill) were highly correlated to each other (r_g=0.96) and achieved the similar genetic correlations to the % Fat (r_g=0.48–0.49) and RelHL (r_g=-0.37 – -0.40) as slaughter yield predictors.

Table 1. *Traits means, standard deviations (S.D.), number of observations (n) and coefficients of variation (CV) for simple yield predictors and performance traits recorded in two- and three- year old fish.*

	Two -year old				
	Units	**Mean**	**S.D.**	***n***	***CV***
E8/E23_2		0.09368	0.0114	963	12.12
E8/2D_d_2		0.1006	0.0139	963	13.80
BW	g	639	152	971	23.78
FC		2.68	0.21	971	7.96
%Fat	%	5.11	1.63	971	31.83
RelHL		0.2542	0.0129	971	5.08
RelBH		0.3399	0.0169	971	4.98
RelBWI		0.2019	0.0114	971	5.67
	Three-year-old				
		Mean	**S.D.**	***n***	***CV***
E8/E23_3		0.1098	0.0118	751	10.72
E8/2D_d_3		0.1215	0.0140	751	11.54
BW	g	1619	346	751	21.37
FC		2.88	0.24	751	8.37
%Fat	%	13.46	2.86	751	21.27
RelHL		0.2418	0.0113	750	4.67
RelBH		0.3346	0.0193	751	5.76
RelBWI		0.1850	0.0097	749	5.23
Logr_hl_Carss		-0.00001548	0.03387972	704	
Logr_Fill		0.00006676	0.04807252	704	
% hl-Carss	%	62.3762	2.344401	704	3.76
%Fill	%	43.3481	2.271634	704	5.24

E8/E23 and E8/2D_d = simple yield predictors, BW = body weight, FC = Fulton's condition factor, %Fat = muscle fat, RelHL = relative head length, RelBH = relative body height, RelBWI = relative body width, Logr_hl_Carss = logarithmic of residuals between BW and headless carcass weight , Logr_Fill = logarithmic residuals between BW and fillets weight, % hl-Carss – headless carcass yield, % Fill – filet yield.

Table 2. *Heritability (± standard error) estimates (diagonal) in bold and diagonal, phenotypic (below the diagonal) and genetic correlations (value ± standard errors above the diagonal) in common carp for simple yield predictors recorded in two- (2) and three- (3) year-old fish, market size performance traits and log-log residuals (Logr) of slaughter yields. Underlined values represent genetic correlations significantly different from zero.*

	E8/E23_2	E8/2D_d_2	E8/E23_3	E8/2D_d_3	BW	FC	%Fat	RelHL	RelBH	RelBW	Logr_hl_Carss	Logr_Fill
E8/E23_2	**0.40±0.08**	0.97±0.02	0.89±0.06	0.84±0.08	0.39±0.16	0.01±0.18	0.44±0.15	-0.63±0.13	-0.25±0.17	-0.03±0.19	0.64±0.12	0.72±0.10
E8/2D_d_2	0.84	**0.27±0.07**	0.86±0.08	0.84±0.08	0.39±0.16	0.07±0.18	0.40±0.16	-0.60±0.14	-0.14±0.18	0.04±0.19	0.66±0.12	0.71±0.11
E8/E23_3	0.39	0.35	**0.41±0.09**	**0.98±0.02**	0.27±0.17	0.08±0.17	0.47±0.15	-0.59±0.14	-0.22±0.17	0.03±0.19	0.80±0.09	0.78±0.09
E8/2D_d_3	0.37	0.35	0.73	**0.44±0.09**	0.29±0.17	0.14±0.18	0.50±0.14	-0.49±0.15	-0.12±0.17	0.08±0.19	0.86±0.07	0.78±0.09
BW	0.17	0.22	0.12	0.15	**0.40±0.09**	0.39±0.15	0.17±0.18	-0.35±0.16	0.17±0.17	0.44±0.16	0.11±0.18	0.02±0.19
FC	-0.01	0.01	-0.01	0.03	0.46	**0.51±0.10**	-0.03±0.18	0.37±0.16	0.88±0.04	0.97±0.03	0.04±0.18	0.01±0.17
%Fat	0.19	0.20	0.20	0.19	0.35	0.21	**0.50±0.10**	-0.52±0.14	-0.13±0.17	0.02±0.18	0.48±0.14	0.49±0.14
RelHL	-0.24	-0.23	-0.19	-0.16	-0.32	0.11	-0.33	**0.47±0.10**	0.56±0.13	0.28±0.18	-0.37±0.16	-0.40±0.16
RelBH	-0.14	-0.07	-0.10	-0.15	0.20	0.72	0.13	0.19	**0.60±0.10**	0.92±0.04	-0.14±0.17	-0.16±0.17
RelBWI	-0.06	-0.01	-0.03	-0.08	0.20	0.70	0.07	0.16	0.63	**0.38±0.09**	-0.05±0.18	-0.06±0.18
Logr_hl_Carss	0.23	0.24	0.38	0.45	0.03	0.04	0.28	-0.22	0.09	-0.03	**0.53±0.10**	0.96±0.02
Logr_Fill	0.28	0.27	0.36	0.38	0.03	0.05	0.27	-0.25	0.04	-0.01	0.84	**0.50±0.10**

Table 3. *Heritability estimates (h^2) of selected performance traits in two-year old fish and phenotypic (r_p) and genetic (r_g) correlations between those traits and Logr slaughter yields. Underlined values represent genetic correlations significantly different from zero.*

	h^2	r_p Logr_hl_Carss	r_g Logr_hl_Carss	r_p Logr_Fill	r_g Logr_Fill
BW	0.35±0.07	-0.19	-0.14±0.18	-0.18	-0.24±0.17
FC	0.53±0.10	0.04	-0.09±0.17	0.01	- 0.11±0.17
%Fat	0.49±0.09	0.16	0.41±0.15	0.20	0.54±0.13
RelHL	0.20±0.06	-0.08	-0.39±0.18	-0.13	-0.47±0.17
RelBH	0.60±0.10	0.10	-0.16±017	0.03	-0.24±0.17
RelBWI	0.32±0.07	-0.03	-0.28±0.17	-0.04	-0.31±0.17

Table 4. *Phenotypic and genetic correlations of performance traits between two-year- and three-year-old fish. Underlined values represent genetic correlations significantly different from zero.*

	r_p	r_g
BW	0.81	0.78±0.07
FC	0.61	0.94±0.04
%Fat	0.47	0.77±0.08
RelHL	0.41	0.92±0.03
RelBH	0.66	0.93±0.03
RelBWI	0.37	0.89±0.07

3.4. Expected genetic gains

Expected genetic gains (Table 5.) were calculated for headless carcass (% hl-Carss) and fillet yield (% Fill) and compared among mass (MS), full-sib (FSS) and indirect selection (IS) scheme using simple yield predictors (E8/E23_2, E8/2D_d_2, E8/E23_3, E8/2D_d_3) with the 10% selection pressure. Generally, calculated expected genetic gains were mostly higher for headless carcass than for fillet yield. Genetic gain calculated for hypothetical mass selection (MS) was 1.97% for % hl-Carss and 1.83% for % Fill per generation. Expected genetic gains for full-sib selection (FSS) with 10 sibs selected per family were similar to genetic gains achieved by MS (1.91% for % hl-Carss and 1.83% – for % Fill). Expected genetic gain achieved by indirect selection on the simple yield predictors (E8/E23_2, E8/2D_d_2) recorded on two-year old fish showed still favorable values for both slaughter yields (0.93% – 1.18%) and estimates calculated from measurement of abdominal depth by ultrasound (E23_2) were higher. Higher values of genetic gain for the same yield predictors (E8/E23_3, E8/2D_d_3) were achieved at market size and interestingly this time the estimates were higher for ratio created by 2D_d_3 (1.54% for % hl-Carss, 1.34% for % Fill) than by ultrasound value E23_3 (1.35% for % hl-Carss, 1.29% for % Fill).

Table 5. *Estimated relative genetic gains per generation with 10 % selection pressure using mass (MS), full sib (FSS), and indirect (IS) selection for slaughter yields improvement when selecting according to different traits. 2 and 3 refer to yield predictor recorded in two- or three-year old fish, respectively.*

Trait selected	Real yield trait	Type of selection	Selection int.	Genetic gain (%)
Logr_hl-Carss	% hl-Carss	MS	10%	1.97
Logr_Fil	% Fil	MS	10%	1.83
Logr_hl-Carss	% hl-Carss	FS	10%	1.91
Logr_Fil	% Fil	FS	10%	1.83
E8/E23_2	% hl-Carss	IS	10%	1.09
E8/E23_2	% Fil	IS	10%	1.18
E8/2D_d_2	% hl-Carss	IS	10%	0.93
E8/2D_d_2	% Fil	IS	10%	0.95
E8/E23_3	% hl-Carss	IS	10%	1.35
E8/E23_3	% Fil	IS	10%	1.29
E8/2D_d_3	% hl-Carss	IS	10%	1.54
E8/2D_d_3	% Fil	IS	10%	1.34

4. Discussion

Previously, a high genetic potential of 2D and 3D yield predictors for genetic improvement of slaughter yields in Amur mirror carp was observed (Prchal et al., 2018a, 2020). In this study, we investigated a different stock of Amur mirror carp to verify a power and efficiency of the predictors for the carp breed as the whole and provided new and easier methodology applicable generally for carp breeding program using a simplified yield predictor E8/E23 (ratio of fillet thickness – E8 to abdominal depth – E23 accessed with ultrasound imagery), or its alternative E8/2D_d (ratio of E8 to abdominal depth measured by a standard scale). We showed i) a moderate to high genetic variation of slaughter yield predictors, standard performance traits and Logr slaughter yields, ii) strong genetic correlations of tested yield predictors recorded on market sized fish with the Logr slaughter yields and iii) strong genetic correlations of the slaughter yield predictors measured at market size and in two-year old fish suggesting that the indirect selection for improved processing yields might be performed on younger and thus smaller fish and that iv) expected genetic gains of slaughter yields (carcass and fillets with skin) calculated based on indirect selection using a slaughter yield predictor recorded on two-year old fish were lower but still reasonable to that achieved by the same predictor measured on market sized fish. So, the results supported us to suggest an upgrade of breeding program scheme in carp.

In the present study, we primarily focused on simple yield predictor calculated as ratio of ultrasound measurements E8/E23. This predictor was found as key indirect trait for rainbow trout breeding program based on slaughter yields improvement (Haffray et al., 2013; Vandeputte et al., 2019). In common carp E8/E23 yield predictor was studied for the first time. We previously suggested as suitable slaughter yield predictor the ratio between E8 (measured by ultrasound tomography) and 2D digitized length between lateral line and abdominal part aligned to the dorsal fin (E8/2D) that requires post-processing of images taken by a camera (Prchal et al., 2018a). However, phenotyping of such predictor is technically quite difficult and time-consuming and thus not very suitable for a breeding program performed on a large number of breeding candidates. In the recent study (Prchal et al., 2020), ratio between E8 and 3D digitized length (E8/3D) was found as the best simple predictor of slaughter yields.

This ratio is similar to the one calculated using 2D digitized length mentioned above but it takes into account the natural roundness of the carp body. Moreover, the E8/3D ratio can be calculated directly during measurement of fish and immediate selection of fish can be performed without further manipulation, but this method needs initial high costs for purchasing of 3D digitizer and requires another working place with a skilful person to get 3D coordinates. Therefore, phenotyping of simple slaughter yield predictor using ultrasound tomography only could be significantly accelerated and simplified.

We observed lower phenotypic correlations between studied simple predictors (E8/2D or E8/E23) recorded at market size and Logr slaughter yields in comparison to 3D yield predictor (0.45 and 0.36 vs. 0.65 and 0.54, respectively; Prchal et al., 2020) but they were similar to values observed on rainbow trout using E8/E23 predictor (0.43 and 0.33; Vandeputte et al., 2019). Even lower phenotypic correlations were found between simple predictors measured on two-year old fish and slaughter yields (0.28 and 0.24). On the other hand, worse and more technically complicated prediction of fillet yield were suggested in previous studies on common carp (Cibert et al., 1999; Maas et al., 2020). Still, genetic parameters of new slaughter yield predictors and especially their relation to the Logr slaughter yields are promising for a breeding program focused on slaughter yields improvement in common carp using indirect selection.

A high genetic variation for slaughter yields and all other performance traits (h^2=0.38–0.53) was observed. It shows a strong potential for their genetic improvement via selection. Indeed, all recent studies performed on common carp under European pond aquaculture (Kocour et al., 2007; Nielsen et al., 2010; Prchal et al., 2018b,c) suggest that selection programs focused on improvement of a given trait of interest (e.g. growth, body shape, muscle fat) should be successful. Therefore, establishment of a sustainable and long-term breeding program in common carp is the main breeding goal for carp culture not only in the Central Europe.

Heritability estimates of simple slaughter yield predictors in this study (E8/2D_d and E8/E23) were intermediate to high for those measured on two-year old fish (0.27–0.40) and high for those recorded on market size fish (0.41–0.44). This is comparable to the 3D slaughter yield predictor (0.44 – Prchal et al., 2020) and even higher when compared to heritability of E8/E23 observed on rainbow trout (0.24 – Haffray et al., 2013; 0.21 – Vandeputte et al., 2019). Besides, simple predictors recorded on market size fish were strongly genetically associated (0.78–0.86) to the slaughter yields. Compared to other studies, E8/E23 evaluated on carp achieved stronger genetic relationship to slaughter yields than previous 3D predictor (0.73–0.80; Prchal et al., 2020) and E8/E23 evaluated on rainbow trout (0.41–0.51 – Vandeputte et al., 2019; 0.72 – Haffray et al., 2013). Likewise, alternative predictor E8/2D_d_3 showed similar high genetic correlations to the slaughter yields. 2D abdominal depth simply measured by a scale was tested also for the first time as suitable alternative for ultrasound value E23 which might be difficult to be recorded quickly if not done by a very skilful and trained person and by an ultrasound device with good technical parameters. However, E23 defined as depth of the body cavity was strongly correlated to 2D_d_3 (r_g = 0.95, data not shown). As a result, 2D abdominal depth could be easily measured on live fish during selection process and E8/2D_d_3 seems to be suitable yield predictor recorded on market size fish. However, our initial research question was to verify if the same yield predictors recorded at market size could be successfully used on two-year old fish as well. We observed only slightly lower genetic correlations between simple slaughter yield predictors (0.64–0.66 for E8/2D_d_2 and 0.71–0.72 for E8/E23_2) measured on two-year old fish and Logr slaughter yields but still strong enough to be used for a genetic improvement of carp slaughter yields. This result can change the future strategy of common carp breeding program based on slaughter yields. Thus, phenotyping and subsequent selection of breeding candidates using simple slaughter yield predictors could be performed on two-year old fish that are smaller (around 200–700 grams) in contrast to market size carps (around 1,500–2,000 grams) and manipulation with such fish is much easier, faster and requires less tank capacities for short-term storage. This is in an agreement with previously suggested selection strategy focused on genetic improvement of body weight when high genetic correlation (0.74) between weight of two-year old and market sized fish was observed (Prchal et al., 2018b). Similarly, a high genetic correlation (0.78) between weight of two-year old and market sized fish was observed in this study. So, carp breeding program focused on two main traits (body weight and slaughter yields) under the European pond conditions might be performed using phenotypes of fish after the second wintering. This measure may improve profitability of the breeding program i) when e.g. combined with methodology to shorten generation interval in carp from five to three years, ii) due to lower short-term storage capacity needs and faster fish phenotyping and selection. Still, the best selective breeding strategy for each enterprise should be chosen based on an economic calculation.

Unfortunately, selection for growth and slaughter yields should be performed either independently or using index selection method. Results in the present study show that selection on slaughter yield predictors might itself slightly improve even body weight as positive and significant genetic correlations between slaughter yield predictors and harvest weight was observed (0.39) which is favourable. However, the correlation was low and even simulation showed that 10% selection pressure, e.g. selecting 100 breeding candidates out of 1,000 heads of fish), of two-year old fish based on the highest value of yield predictor would cover at the same time only 8% (only 8 fish within the 100 selected breeding candidates) of the heaviest candidates that would be selected under the same pressure in case of weight-oriented selection program. Moreover, other studies showed zero or even low negative genetic correlations between the best simple slaughter yield predictors and body weight in the same carp breed (Prchal at al., 2020) or in rainbow trout (Haffray et a., 2013; Vandeputte et al., 2019).

Other genetic correlations of traits showed that selection for slaughter yield predictors might lead indirectly to a reduction of head (r_g=-0.49 – -0.63) and increasing of muscle fat (r_g=0.40–0.50). Relative head size was suggested as general indirect selection criterium for improved slaughter yields in common carp (Kocour et al., 2007; Prchal et al., 2018a, 2020), European seabass (*Dicentrarchus labrax*) (Saillant et al., 2009), rainbow trout (Haffray et al., 2012; Vandeputte et al., 2019) and Nile tilapia (*Oreochromis niloticus*) (Rutten et al., 2005). However, reduction in head size could lead to capacity disruption of respiratory organs and decreasing of general fitness (Haffray et al., 2012). Yet, Vandeputte et al. (2019) despite observed negative genetic correlation between slaughter yield predictors and relative head length did not see any correlated response in head size after selection for increased or decreased fillet yield in the first selected generation. However, it is difficult to predict if this stays or changes in the future generations. Moreover, selection for higher slaughter yields should lead to higher fat storage in common carp, rainbow trout (Vandeputte et al., 2019), European seabass (Sailant et al., 2009), striped catfish (*Pangasianodon hypophthalmus)* (Van Sang et al., 2012) and Atlantic salmon (*Salmo salar*) (Powell et al., 2008). Hence, slaughter yields improvement in common carp cultured under Central Europe pond management might lead to a significant fat storage and gradual reduction of beneficial fatty acids in muscle unlike feeding strategy changes (Prchal et al., 2018c). Therefore, in case of selection for improved slaughter yields in common carp using of alternative supplemental feeding with higher rate of rapeseed, linseed or hempseed (Mráz and Pickova, 2011; Mráz et al., 2012) should be considered. In general, when selecting for improved slaughter yields shifts in relative head size and muscle fat shall be monitored.

The expected genetic gains of slaughter yields were calculated using the most realistic and economic 10% selection pressure on both headless carcass and fillet yields using mass (MS), full-sib (FSS) and indirect selection (IS). The highest genetic gains were observed by hypothetical mass selection (MS) (1.97% for headless carcass and 1.83% for fillet yields). Almost similar expected genetic progress (1.83%–1.91%) was observed using sib selection (FSS) that is commonly applied for these traits (Kause et al., 2007; Gjedrem, 2010). However, FSS is costly as it requires i) knowing of sibling's relationships and ii) precise phenotyping of slaughter yield parameters by sib groups. On the other hand, genetic gains estimated using simple slaughter yield predictors were lower than by FSS but still interesting to be used in a breeding program when considering practical, technical and economic aspects. Indirect selection using simple slaughter yield predictors would lead to an interesting selection response ranging from 0.93% to 1.54% per generation in absolute values. Estimated values in this study were higher than previously estimated absolute gains (0.48%–0.52%) using best indirect selection criteria in the same common carp breed (Prchal et al., 2018a, 2020). It can be explained by different stock of Amur mirror carp and number of fish studied, by common variability of environmental conditions (pond effect) and other factors during phenotyping of fish (different methodology of slaughter yields processing) including human errors. It verifies the fact that expected genetic gain is not fixed due to common variation of parameters that enter into genetic gain calculations. In this study mean values of body weight at harvest, slaughter yields, FC, muscle fat (tested by one-way ANOVA) as well as estimated genetic variance (heritability) (tested by one typing error for chi-square test) and genetic correlations were significantly different from those recorded in study by Prchal et al. (2018a, 2020).

It was shown that genetic gains of slaughter yields calculated on yield predictors at market size were logically higher than those measured in two-year old fish, However, the differences were not too high and still interesting. In this study genetic gains of slaughter yields calculated from 10% of fish with the best slaughter yield predictor values measured in two-year old fish were still higher than genetic gains calculated the same way at market size in the study by Prchal et al. (2020). Thus, improvement of slaughter yields in common carp based on selection of fish according to E8/E28_2 (or alternatively E8/2D_d_2) seems to be an interesting alternative if considered that handling and manipulation with two-year old fish is much easier and it might save time and need for tank capacity for short-term storage of fish, rearing capacity and human power. Similarly, selecting the two-year old carps when focusing on genetic improvement of weight under the Central European pond management was suggested as an effective alternative based on results by Prchal et al. (2018b). However, it must be stressed that in any case no economic calculation was performed to specify the best strategy for selective breeding program. To do that specific conditions at each institution should be taken into account including traits of interest (slaughter yields only, weight only, both), product offered (live fish only, processed fish only, both) etc.

5. Conclusions

A simple slaughter yield predictor defined as ratio of ultrasound values (alternatively ultrasound value and 2D abdominal depth) has a strong potential for genetic improvement of slaughter yields in common carp. Moreover, such predictor can be efficiently applied either on market size fish or on two-year old fish. In conclusion, our new finding might lead to a significant simplification of yield predictor phenotyping and its use in a sustainable long-term breeding program. However, the efficiency of simple slaughter yield predictors needs to be verified by real response to selection in genetically improved generations and economic calculation for specific conditions of each institution shall be done to decide about the best selective breeding strategy. Moreover, monitoring of other performance traits (e.g. harvest weight, relative head length, body shape, muscle fat) shall be done to see real effect of selection on such traits.

Competing interests

The authors declare that they have no competing interests.

Author contributions

MK coordinated the study. MP, DG, and MK established and reared the experimental stock. MP and JZ PIT tagged the progeny; MP, JZ and MK phenotyped and fin-clipped the fish during on-growing. MP, JZ, DG, JK, PL and MK shared on final traits phenotyping. All authors contributed to drafting the manuscript and approved the final version.

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CHAPTER 7

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLEDGEMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING THE STUDY
CURRICULUM VITAE

General discussion

Recent studies have reported considerable genetic variation of several production traits in common carp (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; Palaiokostas et al., 2018a,b). Significant heritability of main production traits (weight, slaughter yields and main biometrical indices) as well as their genetic and phenotypic relationships were estimated repeatedly in common carp. Results show high potential for application of systematic selective breeding aiming at enhancing of i) fish performance and thus ii) profitability in commercial aquaculture. However, in selective breeding, selection for a single trait can lead to unwanted genetic changes in other traits. Hence, it is necessary to know in a broader way the genetic relationships among economically important production traits. Faster growth, muscle fat content, slaughter yields, survival and resistance to koi herpesvirus disease (KHVD) are supposed to be the most important production traits for common carp at present time. Accordingly, the role of energy reserves-related traits to fish performance during the first winter and further periods of rearing were investigated from phenotypic but mainly genetic variance and correlations among various traits (Chapter 2). At the same time, KHVD resistance and its relationships to different production traits was studied from their siblings (Chapter 4). Other siblings were used to study overwintering performance (winter survival, muscle fat level) in two-year old common carp and its link to the market size traits (Chapter 3) and to assess the potential of morphological predictors in genetic improvement of carp slaughter yields (Chapter 5 and 6).

1. Growth and its relationships to other traits

Body weight and growth rate have generally been assigned as traits with the highest importance in animal selection programmes. The body weight was found to correlate with several other traits. In studies described in Chapter 2 and 3, body weight was moderately genetically and lowly phenotypically correlated with muscle fat content before the first winter (0.42±0.16, 0.19) and before the second winter period (0.32±0.13, 0.28). At same time, body weight after the first winter was genetically related to muscle fat content after the second winter (0.61±0.11) and at market size (0.32±0.15). However, no significant correlations were found between these two traits in all other periods. So, it seems that selection for body weight in common carp may affect muscle fat content, but the real change will depend on conditions (e.g. term of selection, age of fish). Moreover, body weight was negatively significantly genetically and/or phenotypically correlated to Logr slaughter yields and was not correlated to survival and KHVD resistance. Similarly, growth in most fish species was positively correlated with muscle fat (Neira et al., 2004; Kause et al., 2007b; Hamzah et al., 2016) and was not significantly genetically correlated with disease resistance (e.g., Silverstein et al., 2009; Flores-Mara et al., 2017; Bassini et al., 2019). Unlike the findings in this thesis, body weight was significantly positively genetically correlated with i) survival in common carp in study by Nielsen et al. (2010) and in other fishes (Rye et al., 1990; Luan et al., 2008; Gitterle et al., 2005), ii) slaughter yields in most fish species (Neira et al., 2004; Gjerde et a., 2012; Haffray et a., 2012), and was not correlated to Logr slaughter yields (Haffray et a., 2012; Vandeputte et al., 2017). This points to the fact that such correlations are probably specific for breed and species and modified by environmental, biological and/or genetic phenomena between the traits.

It can be concluded that in case of selection to improved slaughter yields in Amur mirror carp body weight should be integrated in a selection index to avoid a negative impact of such selection on growth. On the other side there is no need to worry that mass selection for faster growth performed in two-year old fish, as suggested in Chapter 3–4 of this thesis, would not negatively affect general survival, muscle fat content at market size and resistance of fish to KHVD.

2. Muscle fat and its relation to other traits

Many fish species, including common carp, must adapt for wide water temperature range during a year in temperate zones. Common carp belongs to warm-water fishes with optimal temperature for live at about 20–25 °C and thus overwintering period in temperate zones is considered a critical period for this species, especially in younger fish. Overwintering carps in temperate regions restrict their activity, reduce metabolism and feeding which eventually result in a decrease of weight (Bauer and Schlott, 2004; Hurst, 2007). Despite reduced metabolism during the overwintering, low feed intake results in utilizing of energy reserves that become essential for winter survival. In addition, various energy reserves strategies exist in different fish species which deal with the overwintering. Muscle, hepatopancreas (HP) and visceral fat and muscle and HP glycogen play an important role as energy reserves during starvation and/or winter period of several fish species including common carp (Adams and McLean, 1985; Black and Love, 1986; Collins and Anderson, 1995; Steffens, 1996; Van Dijk et al., 2005; Rios et al., 2006). However, muscle fat is generally important production trait in fish, such as in Atlantic salmon (*Salmo salar*) (Powell et al., 2008), in European whitefish (*Coregonus lavaretus*) (Kause et al., 2011; Janhunen et al., 2017), in gilthead sea bream (*Sparus aurata* L.) (García-Celdrán et al., 2015) and in Nile tilapia (*Oreochromis niloticus*) (Garcia et al., 2017). Content of muscle fat in common carp is quite variable (0.9–11.56%) (Mráz and Pickova, 2009; Mráz et al., 2012; Zajic et al., 2013; Prchal et al., 2018b) and depends on many factors internal as well as external (Mráz and Pickova, 2011; Mráz et al., 2012; Zajic et al., 2013; Zheng et al., 2016; Prchal et al., 2018c). Thus, under certain conditions it may be needed to select for genetic change of muscle fat content. Fat reserves stored in the white muscles have different purpose than fat bodies stored in viscera (visceral fat). The former serves as an insurance to energy as well as physiological functioning (e.g. muscle fluidity, membrane components, hormone synthesis, etc.) while the latter was presumed to be more of an emergency leverage or insurance of storing energy in excess (if it is needed later on, e.g. feed deprivation). The former is more closely related to product quality itself, whereas the latter to production efficiency because visceral fat is often considered as waste (Tobin et al., 2006). One other thing is that most common freshwater fish species do not have a clear, large proportion of white muscle and red muscle in body; white muscle predominates in freshwater fish species. As muscle fat plays an important role in biological functions of common carp, there is a need to know how this trait affect other production traits. It was investigated in the Chapter 2 and 3.

As said before, before the first and second overwintering muscle fat content were positively moderately genetically and lowly phenotypically correlated with body weight, muscle fat content after the second winter and at market size were positively genetically related to body weight after the first winter and all other correlations of weight with the muscle fat were nonsignificant in other periods. So, muscle fat of common carp would indirectly increase in all subsequent periods by selection on higher body weight in all periods before the second winter. This is in line with a positive allometric relationship between body size and energy reserves in the form of lipids observed at the onset of winter in several fish species (Henderson et al., 1988; Cargnelli and Gross, 1997; Sutton and Ney, 2001; Biro et al., 2004; Huss et al., 2008; Heermann et al., 2009). It also showed that muscle fat content would indirectly increase by selecting for higher body weight in Coho salmon (Neira et al., 2004), in rainbow trout (Kause et al., 2007b), in European whitefish (Kause et al., 2011) and in gilthead sea bream (García-Celdrán et al., 2015). Positive significant genetic correlation between weight and visceral fat was evidenced before the first winter, nonsignificant correlation was found after the first winter. So, selection on higher body weight in all periods before the second winter, muscle fat of common carp would indirectly increase in all subsequent periods, but visceral fat would only increase before the first winter and during winter this relationship is reduced as visceral fat is utilized for maintenance. In addition, we found that final muscle fat content would slightly decrease by selection for higher condition coefficient (FC) after the second winter as an indirect indicator (r_g=-0.27±0.13) (Chapter 3).

In Chapter 2, when selecting for higher muscle fat no positive response to survival would be expected. In Chapter 3, we found that muscle fat content before and after the second overwintering is negatively moderately genetically and very lowly phenotypically correlated to survival during the second overwintering period and the third growing season (-0.59±0.26, -0.53±0.19, -0.01, -0.02). Hence, selection for higher muscle fat might lead surprisingly to lower survival. This is contradicting to previous observations in which the fish with lower energy reserves before overwintering use those reserves more rapidly, resulting in higher mortalities as reported in common carp (Steffens, 1996) and other fish species (Sogard and Olla, 2000; Biro et al., 2004). While a certain level of muscle fat plays an important role in biological functions of fish (Steffens et al., 1996; Ali et al., 2003; Tocher, 2003; Kause et al., 2016), our findings demonstrate that an excess of fat might adversely affect survival. This is also in contrary to the expected positive effect on survival when selecting fish for higher fillet lipid content in European whitefish (Kause et al., 2011). However, it must be said that conditions during winter period may change the suitability of having higher or lower muscle fat. In case of studies described in Chapter 2 and 3 the winters were very mild (2–3 °C above average air temperatures). For such winters, the excessive fat content might be disadvantageous. It was also shown that excessive lipid deposition negatively affects not only feed conversion ratio but also protein-retention efficiency (Kause et al., 2016).

It can be concluded that that high muscle fat might not be the best for good overwintering in common carp and that selective breeding for restricted fat content may increase survival without affecting harvest weight.

3. Survival and resistance to KHVD and their relationships to other traits

Genetic improvement programs for common carp should also be tailored to improving traits related to fitness such as survival and resistance to the koi herpesvirus which has become a problem in common carp production (Haenen et al., 2004). Survival is an important economic trait as it may significantly vary in common carp pond culture of the Central Europe especially during the first and the second year and thus it influences production of fish per a unit of pond area. Survival and resistance of carp to KHVD were investigated in the Chapter 2–4.

As mentioned before, general survival might be negatively affected in further generations in case that fish with higher muscle fat content would be preferred. Generally, it was found that survival is genetically connected to energy reserves status of Amur mirror carp. In Chapter 2 of this thesis, it was described that survival of further generations could be positively affected if selection for lower HP fat and protein after the first winter would be possible. Similarly, selecting the fish that keep higher HSI during winter or fish having higher HP glycogen levels after the first winter could positively influence the overall survival (r_g=0.64±0.20 and 0.72±0.20, respectively). Correlations between higher winter survival and higher liver glycogen were reported also in small crucian carp *Carassius carassius* (Vornanen et al., 2011) and side-blotched lizards *Uta stansburiana* (Zani et al., 2012). So, it seems that strategy for keeping excessive muscle fat, HP fat and protein are disadvantageous for carp survival, at least under conditions where ability of keeping higher level of HP glycogen and HSI is better.

Condition coefficient (FC) was found also as trait that is genetically connected to survival. Selection for higher FC after the second winter should lead to slight increase of survival (r_g=0.45±0.21) during the third growing season (Chapter 3).

In Chapter 2, genetic correlations between KHVD resistance and energy reserves related traits (FC, HSI, muscle and visceral fat and glycogen, fat and protein in hepatopancreas) before and after the first winter showed that selection for status of energy reserves would not affect the susceptibility of fish to KHVD and opposite. In Chapter 4 it was described that FC after the second overwintering had a quite low but significant negative genetic correlation with KHVD resistance (-0.32±0.14), suggesting that selection for lower FC might improve the resistance of fish to that disease. So, it seems that features that share on final FC value include somehow those that influence survival. Unfortunately, for general survival higher FC value is positive, the same is negative with respect to resistance to KHVD. Still, FC is a valuable trait in common carp that should be monitored on the regular basis and under certain conditions even included into selective breeding programs. In addition, relative head length (RHL), relative body height (RBH) or relative body width (RBW) were significantly negatively correlated with KHVD resistance from the second growing season to the third growing season (Chapter 4). These traits are also highly positively genetically correlated with FC (Prchal et al., 2018a). Hence, selection of fish for lower FC, RHL, RBH or RBW could indirectly increase KHVD resistance of fish probably due to the fact that such fish would bear more genes from wild scaly carp which has prolonged body shape and was found to be as one of the most resistant carps to KHVD (Piačková et al., 2013). Selecting the fish for prolonged body shape could indirectly improve slaughter yields at market size as prolonged body shape is genetically correlated to higher dress out traits in common carp (Prchal et al., 2018a). That is why resistance to KHVD could be positively influenced in Amur mirror carps that would be selected for improved slaughter yields (Chapter 4).

Presented information highlights the need to carefully monitor through generations of selection all traits that were found to be significantly correlated to any trait of interest in order to estimate the real impact of selection on such traits.

4. Slaughter yields and their relationships to other traits

Unlike some important performance traits as seen above, genetic improvement of slaughter yields is much tricky and impossible on live fish. Thus, their direct genetic improvement through mass selection is impossible. Slaughter yields are usually genetically improved through indirect selection with correlated traits (e.g., Kause et al., 2007a; Kocour et al., 2007; Melo et al., 2013) or sib selection and indirect selection on some morphological predictors (e.g., Haffray et al., 2013; Prchal et al., 2018a; Vandeputte et al., 2019). Morphological predictors of slaughter yields based on external 2D or 3D landmarks and ultrasound tomography that can be measured on live candidates and that correlate with real slaughter yields thence remain as an attractive alternative. It has shown potential for genetic improvement of slaughter yields through selective breeding using morphological predictors of 2D and ultrasound measurements in common carp (Prchal et al., 2018a). Accordingly, in order to predict the application potential of 3D digitizer or simple predictor, we assessed the possibility of using a combination of 3D digitized landmarks and ultrasound measurements or ultrasound only for genetic improvement of slaughter yields in common carp in Chapter 5 and 6.

The high heritabilities for real slaughter yields (0.46–0.53) were observed in common carp, showing a strong potential for their genetic improvement via selection (Chapter 5 and 6). The heritabilities for real processing yields were high (0.47–0.55) in rainbow trout (Haffray et al., 2013) and low to high (0.21–0.57) in European sea bass *Dicentrarchus labrax* (Vandeputte et al., 2017). In terms of common carp, we achieved that heritability estimates of 3D model-predicted yields were high (0.46 for headless carcass yield and 0.56 for fillet yield) and slightly lower than the estimates predicted using 2D digitization (0.48, 0.63, respectively; Prchal et al., 2018a). Yet, they were higher in comparison with the heritability estimates of predicted slaughter yields in other fish species (Van Sang et al., 2012; Haffray et al., 2013; Vandeputte et al., 2017). Likewise, heritability estimates of simple slaughter yield predictors were found to be also high, showing its strong potential to be used in a breeding program as "quick-to measure" indirect selection criterion for improvement of yields in common carp.

In Chapter 5 and 6, similar to results reported by Prchal et al. (2018a) using 2D models, predicted yields of 3D models were strongly genetically and highly phenotypically correlated to the real yields (0.84±0.05–0.88±0.04, 0.60–0.70) and simple predictors recorded on two-year old fish and market size fish were highly genetically and moderately phenotypically associated to the slaughter yields (0.64±0.12–0.86±0.07, 0.23–0.45), showing their strong potential as indirect selection criterions to improve edible part yields in common carp.

We observed that both prediction yield models and simple predictors are positively moderately genetically and lowly phenotypically correlated to muscle fat content. So, selection for higher slaughter yields should lead indirectly to higher muscle fat content in common carp, also in rainbow trout (Vandeputte et al., 2019), European seabass (*Dicentrarchus labrax*) (Saillant et al., 2009), striped catfish (*Pangasianodon hypophthalmus*) (Van Sang et al., 2012) and Atlantic salmon (Powell et al., 2008). Hence, a selection program focused on improvement of slaughter yields in common carp cultured under Central Europe pond management should check muscle fat level and eventual change of feeding strategy would be necessary to keep high flesh quality with respect to beneficial fatty acids (Prchal et al., 2018c).

It is worth mentioning that selection for both yield models and simple predictors might lead to a reduction of head length. Relative head length was suggested as general indirect selection criterium for improved slaughter yields in common carp (Kocour et al., 2007; Prchal et al., 2018a), European seabass (Saillant et al., 2009), rainbow trout (Haffray et al., 2012; Vandeputte et al., 2019) and Nile tilapia (Rutten et al., 2005). However, reduction of head size could lead to capacity disruption of respiratory organs and decreasing of general fitness (Haffray et al., 2012). Vandeputte et al. (2019) despite observed negative genetic correlation between slaughter yield predictors and relative head length, there was no any correlated response to head size after selection for increased or decreased fillet yield in the first selected generation. However, it is difficult to predict if this stays or changes in the future generations. Therefore, the use of predictors in a selection index including shape and fat content would be a suitable breeding scenario to avoid a negative impact of indirect selection for slaughter yields on other traits of interest.

Morphological predictors, especially simple predictors of slaughter yields used for indirect selection might be strongly recommended and implemented in selection program of common carp. Due to good genetic correlations between simple predictors in age of two and three years, selection of Amur mirror carp could be done at size of fish that requires less storage capacity and time. However, in future research realized selection response for such predictors need to be evaluated in a practical selection experiment.

5. Conclusions and future prospects

Results presented in this thesis provide a basis for establishing systematic selective breeding programs in common carp and show more broader insight into exploiting genetic variation in a long-term breeding under semi-intensive pond conditions. The specific conclusions are as follows:

- Almost all traits in Amur mirror breed (AM) of common carp achieved a sufficient genetic variation and heritability demonstrating the feasibility for their genetic improvement through selective breeding.
- Selection for faster growth in AM, may affect muscle fat content but the real change will depend on conditions and slaughter yields and it would not affect survival, susceptibility of fish to KHVD (Chapter 2–6).
- Fat might not be the best for good overwintering in common carp due to negative genetic correlation between muscle fat content and survival during the second and third growing season. Keeping excessive muscle fat, HP fat and protein might be disadvantageous for carp survival, at least under conditions where ability of keeping higher level of HP glycogen and HSI is better (Chapter 2 and 3).
- Including condition coefficient into selection program of common carp could be advantageous for general fish vitality and performance but may have reverse effect on resistance of fish to KHVD (Chapter 2–4).
- Selection for more prolonged body shape (lower condition factor, RHL or RBH) and higher slaughter yields in AM could positively affect KHVD resistance and vice versa (Chapter 4).
- Slaughter yields predicting models as well as some simple parameters used for their calculations in common carp were highly heritable and strongly genetically related to real yields and may be used in indirect selection program. Due to good genetic correlations between simple predictors in age of two and three years, selection of Amur mirror carp could be done at size of fish that requires less storage capacity and time. However, it should be remembered that such selection may affect body shape, muscle fat content and resistance of fish to KHVD (Chapter 5 and 6).

Future prospects should be focus on designing a practical and efficient selective breeding program for common carp based on obtained estimates of genetic parameters of the traits of interest. Selection index methology should be applied in the breeding theory for computing total breeding values. The breeding program should utilize combined selection (e.g. mass selection, sib selection and indirection selection) for several traits (e.g. growth rate, muscle fat content, slaughter yields, survival and disease resistance), has been practised for salmon and rainbow trout. Realized heritabilities, real genetic gains and total production from the pond area unit should be verified, which shows a real impact of selective breeding on performance traits under the traditional pond management. Introducing selective breeding programs in European common carp would provide potential for increased productivity and production efficiency in the industry. Another important area for future research will be to make sustainable selective breeding program by shortening of carp generation cycle, keeping the high-quality flesh and decreasing of production costs but oppositely increasing of profitability from genetically improved fish stocks. New investigations are needed to develop appropriate and economical technology for measuring some traits and to investigate the magnitude of genetic variation. Especially for some traits about flesh quality of carp, such as taste, meatiness, shape, dressing precent and intermuscular bones, it is difficult to make objective measurements. Thus, knowledge of the magnitude of the genetic variation for these traits and estimates of genetic correlations between these traits was very limited. Moreover, new technologies like genomic selection should be used to breeding programs for this species that might improve selective efficiency.

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English summary

Broader insight into mutual genetic and phenotypic relationships of production-related quantitative traits in common carp

Jinfeng Zhao

Selective breeding has large potential for genetic improvement of production traits in the aquaculture and made considerable progress for aquatic species. However, the successful development of selective breeding programs for common carp is facing a number of challenges. Basic knowledge is lacking about phenotypic and genetic parameters for economically important traits. In selective breeding, selection for a single trait can lead to unwanted genetic changes in other traits. Faster growth, muscle fat content, survival, resistance to KHVD and slaughter yields are supposed to be the most important production traits for common carp at present. Therefore, the present study focuses on genetic and phenotypic relationships of production-related quantitative traits in Amur mirror breed of common carp under the pond management condition.

In all presented studies, communal rearing of families was used and the parentage assignment was based on analysis of the microsatellites or single nucleotide polymorphism markers. The genetic parameters were estimated using multivariate mixed models in DMU statistical software. Most of the analyzed production traits achieved sufficient and significant genetic variation (h^2=0.12–1.0) suggesting a good possibility for genetic improvement using selective breeding.

Growth is usually the first goal in breeding programs of different species. Selection for faster growth, may affect muscle fat content but the real change will depend on conditions and slaughter yields and it would not affect survival, susceptibility of fish to KHVD. In addition, body weight should be integrated in a selection index to avoid a negative impact on growth when selecting for slaughter yields.

Muscle fat content is a production trait playing an important role in biological functions of common carp. Muscle fat of common carp would indirectly increase in all subsequent periods by selection on higher body weight in all periods before the second winter. High muscle fat might not be the best for good overwintering in common carp due to negative genetic correlation between muscle fat content and survival during the second and third growing season and that selective breeding for restricted fat content may increase survival without affecting harvest weight.

Survival is an important economic trait related to performance of common carp. Keeping excessive muscle fat, HP fat and protein might be disadvantageous for carp survival, at least under conditions where ability of keeping higher level of HP glycogen and HSI is better. In addition, selection for higher FC after the second overwintering should lead to slight increase of survival.

KHVD is currently the most serious threat to global carp farming. Improvement of genetic resistance of carp strains through selection can be the most effective method. It suggested that most genetic correlations between KHVD resistance and important production traits (e.g., growth, muscle fat content, winter survival and energy reserves) were insignificant, showing that selective breeding program for important production traits would not affect the KHVD resistance of carp and vice versa. Selection for lower FC, relative head length, relative body height or relative body width, with a more prolonged body shape of Amur mirror carp being associated with higher KHVD resistance and selection for improved slaughter yields could positively affect resistance to KHVD and vice versa. It showed that including FC into selection program of common carp could be advantageous for general fish vitality and performance but may have reverse effect on resistance of fish to KHVD. Hence, resistance of the breed to KHVD should be checked in case of significant increase of FC in future generations.

Slaughter yields are traits of high interest especially for fish species sold in processed form like headless carcass or fillet. Due to impossible direct measurement on live breeding candidates for slaughter yields, sib selection and/or indirect selection on correlated traits or morphological predictors as usual alternatives are used for genetic improvement of such traits. It was found that the model-predicted predictors and simple predictors of slaughter yields recorded using combination of 3D landmarks and ultrasound imagery or ultrasound only were highly heritable and significantly genetically correlated with real yields and may be used in indirect selection program. Due to good genetic correlations between simple predictors in age of two and three years, selection of Amur mirror carp could be done at size of fish that requires less storage capacity and time. However, it should be remembered that selection for slaughter yields may affect body shape, muscle fat content and resistance of fish to KHVD.

The present findings might significantly contribute to establishing a systematic carp breeding program. However, the real impact of selective breeding should be verified by reliable estimates of heritability and achieved genetic gain within the given performance traits in genetically improved generations.

Czech summary

Hlubší náhled do genetických a fenotypových korelací mezi kvantitativními znaky se vztahem k produkci u kapra obecného

Jinfeng Zhao

Selekční šlechtění má velký potenciál pro genetické zlepšování produkčních znaků v akvakultuře a díky této metodě bylo v chovech mnoha vodních živočichů dosaženo značného pokroku. Úspěšný rozvoj programů zaměřených na selekční šlechtění v chovu kapra obecného však čelí řadě výzev. V mnoha případech stále chybí základní informace o fenotypových a genetických parametrech ekonomicky významných znaků. Při selekci na jeden znak můžeme bez hlubších znalostí o genetických korelacích mezi znaky způsobit nežádoucí genetické změny v jiných znacích. Rychlejší růst, obsah tuku ve svalovině, přežití, odolnost vůči koi herpes viróze (KHV) a jateční výtěžnost jsou v současnosti nejdůležitějšími produkčními znaky kapra obecného. Proto se tato práce hlouběji zaměřuje na genetické a fenotypové korelace mezi produkčními kvantitativními znaky u plemene kapra obecného s názvem amurský lysec (AL) chovaného v rybničních podmínkách.

Ve všech prezentovaných studiích bylo využito odchovu všech rodin ve společných podmínkách a vzájemné příbuzenské vztahy mezi experimentálními rybami byly stanoveny s využitím genetických analýz pomocí mikrosatelitních márkerů nebo polymorfismu jednotlivých nukleotidů (SNPs). Genetické parametry byly odhadnuty pomocí vícerozměrných smíšených regresních modelů pomocí statistického software DMU. Většina analyzovaných produkčních znaků vykázala významné a dostatečně vysoké genetické variace (heritabilita, h^2=0,12–1,0), což naznačuje možnost efektivního genetického zlepšení takových znaků pomocí selekčního šlechtění.

Zvýšení rychlosti růstu je obvykle hlavním cílem ve šlechtitelských programech většiny druhů zvířat. U AL bylo zjištěno, že selekcí na rychlejší růst můžeme ovlivnit i) obsah svalového tuku, ale skutečná změna obsahu tuku bude záviset na určitých podmínkách, a dále ii) výtěžnosti (podíly) jedlých částí těla. Naopak selekcí na rychlejší růst pravděpodobně neovlivníme přežívání ryb a jejich odolnost vůči KHV. Doporučuje se, aby dosažená hmotnost byla zahrnuta jako váha do selekčního indexu v případě realizace selekce na zvýšení podílu jateční výtěžnosti.

Obsah svalového tuku je další produkční znak, který hraje důležitou roli v biologických a fyziologických procesech kapra obecného. U AL bylo zjištěno, že svalový tuk kapra obecného by se nepřímo zvýšil ve všech následujících obdobích při selekcí ryb na vyšší tělesnou hmotnost po druhém roce odchovu. Zajímavé bylo zjištění, že vyšší obsah tuku ve svalovině nemusí být vždy tím nejlepším pro dobré přezimování kaprů, neboť byla nalezena negativní genetická korelace mezi obsahem svalového tuku a přežitím během druhého a třetího vegetačního období. Selekce na nižší obsah tuku tak naopak může zvýšit přežití ryb bez ovlivnění rychlosti růstu ryb.

Přežití je dalším důležitým ekonomickým ukazatelem souvisejícím s užitkovostí kapra obecného. Podpora zvyšování množství tuku ve svalovině, a s tím související zvyšování obsahu tuku a bílkovin v hepatopankreatu (HP) může být pro přežití kapra nevýhodné, a to přinejmenším za podmínek, kdy je výhodnější udržovat si v HP vyšší hladinu glykogenu a s tím související vyšší hodnoty hepatosomatického indexu (HSI). Lepší přežívání AL bychom mohli nepatrně ovlivnit selekcí ryb na vyšší Fultonův kondiční koeficient (FC) po druhém zimním období.

KHV je v současné době asi nejzávažnější virové onemocnění v chovu kaprů. Jednou z metod zvýšení odolnosti ryb vůči KHV je rovněž selekce. Bylo zjištěno, že většina genetických korelací mezi odolností ryb vůči KHV a dalšími důležitými produkčními znaky (např. růst, obsah svalového tuku, zimní přežití a energetické zásoby) byla zanedbatelná. To ukazuje, že program selekčního šlechtění na důležité produkční znaky by neměl zhoršit odolnost AL vůči KHV. Naopak při selekci na nižší FC, relativní délku hlavy (RDH), relativní výšku těla (RVT) nebo relativní šířku těla (RŠT), tedy znaky související s protáhlejším tvarem těla u AL, a dále při selekci na vyšší podíly jedlých částí těla, bychom mohli odolnost vůči KHV mírně ovlivnit v pozitivním směru. Z tohoto pohledu se ukázal být zajímavým ukazatel FC. Při selekci na jeho vyšší hodnotu u AL bychom mohli pozitivně ovlivnit celkovou vitalitu a užitkovost růstu ryb, ale opačný účinek bychom mohli pozorovat s ohledem k odolnosti ryb vůči KHV. Proto by se při nárůstu hodnoty tohoto ukazatele v dalších generacích měla zkontrolovat odolnost plemene vůči KHV.

Ukazatele jateční výtěžnosti jsou znaky zajímavé u těch druhů ryb, které se prodávají ve zpracovaném stavu (např. jako opracovaný trup či filety). Problémem je, že tyto znaky nemůžeme zjišťovat přímo na živých rybách, jež chceme využívat pro další šlechtění. Proto se při selekci na tyto znaky využívá nepřímá selekce, a to dle užitkovosti sourozenců nebo dle tzv. morfologických prediktorů. Bylo zjištěno, že nejen predikce výtěžností s využitím kombinovaných morfologických ukazatelů a matematických modelů při využití 2D a 3D dokumentačních a zobrazovacích metod a ultrazvuku ale i poměrně jednoduché ukazatele měřitelné ultrazvukem či měrnou deskou vykazují vysokou dědivost a významně geneticky korelují se skutečnými výtěžnostními podíly a lze je využít v selekčních programech. Vzhledem k dobrým genetickým korelacím mezi jednoduchými prediktory ve věku ryb dvou a tří let je možné provádět selekci na vyšší podíly jedlých částí těla u AL u ryb ve dvou letech, což má výhodu v menší potřebě krátkodobých skladovacích kapacit a snadnější realizaci selekce (čas a potřeba lidských zdrojů). Je však třeba mít na paměti, že při selekci na vyšší podíly jedlých částí těla můžeme ovlivnit tvar těla a obsah svalového tuku.

Výsledky prezentované v rámci této disertační práce mohou významně pomoci při zavádění systematických selekčních programů u kapra obecného. Skutečný dopad selekčního šlechtění na popisované znaky je však nutné ověřit při vlastním programu realizovanou dědivostí a skutečným pozorovaným genetickým ziskem.

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List of publications

Peer-reviewed journals with IF

Zhao, J., Prchal, M., Kause, A., Vandeputte, M., Gela, D., Kroupová, K. Hana, Piačková, V., Šauer, P., Steinbach, C., Allamellou, J-M., Palaiokostas, C., Houston, R.D., Kocour, M., 2021. The role of energy reserves in common carp performance inferred from phenotypic and genetic parameters. Aquaculture 541, 736799. (IF 2020 = 4.242)

Prchal, M., Kocour, M., Vandeputte, M., Kause, A., Vergnet, A., **Zhao, J.**, Gela, D., Kašpar, V., Genestout, L., Bestin, A., Haffray, P., Bugeon, J., 2020. Morphological predictors of slaughter yields using 3D digitizer and their use in a common carp breeding program. Aquaculture 520, 734993. (IF 2019 = 3.225)

Zhao, J., Prchal, M., Palaiokostas, C., Houston, R.D., Kause, A., Vandeputte, M., Vergnet, A., Bugeon, J., Bestin, A., Veselý, T., Pokorová, D., Piačková, V., Pojezdal, L., Genestout, L., Gela, D., Kocour Kroupová, H., Kocour, M., 2020. Genetic relationship between koi herpesvirus disease resistance and production traits inferred from sibling performance in Amur mirror carp. Aquaculture 520, 734986. (IF 2019 = 3.225)

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Huang, J., Su, Y., **Zhao, J**., Liu, X., Wang, Z., 2016. The complete mitogenome sequence of *Pseudohynobius jinfo* (Urodela: Hynobiidae). Mitochondrial DNA A 27, 3141–3142. (IF 2015 = 1.760)

Zhao, J., Xu, D., Zhao, K., Diogo, R., Yang, J., Peng, Z., 2016. The origin and divergence of Gobioninae fishes (Teleostei: Cyprinidae) based on complete mitochondrial genome sequences. Journal of Applied Ichthyology 32, 32–39. (IF 2015 = 0.783)

Zhao, J., Zhao, K., Peng, Z., 2014. Development and characterization of eleven microsatellite markers for an endangered cavefish (*Triplophysa rosa* Chen and Yang, 2005) using 454 sequencing. Journal of Applied Ichthyology 30, 1056–1058. (IF 2014 = 0.867)

Manuscripts

Prchal, M., **Zhao, J.**, Gela, D., Kašpar, J., Lepič, P., Kašpar, V., Kocour, M., 2021. Simplified method for genetic slaughter yields improvement in common carp under European pond conditions. (manuscript)

International conferences

Prchal, M., Bugeon, J., Vandeputte, M., Kause, A., Vergnet, A., **Zhao, J.**, Gela, D., Genestout, L., Bestin, A., Haffray, P., Kocour, M., 2019. Morphological predictors of slaughter yields using 3D digitizer and their use in a common carp breeding program. Aquaculture Europe 2019. 8–10 October 2019, Berlin, Germany.

Zhao, J., Prchal, M., Palaiokostas, Ch., Houston, R., Kause, A., Bugeon, J., Vandeputte, M., Vergnet, A, Bestin, A., Vesely, T., Pokorová, D., Piačková, V., Pojezdal, L., Genestout, L., Kroupová, H., Kocour, M., 2019. Genetic relationship between koi herpesvirus disease resistance and production traits inferred from sibling performance in Amur mirror carp. Aquaculture Europe 2019. 8–10 October 2019, Berlin, Germany.

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Training and supervision plan during study

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Ph.D. courses	**Year**
Pond aquaculture	2017
Fish genetics	2017
Applied hydrobiology	2017
Basic of scientific communication	2018
Ichthyology and fish taxonomy	2018
English language	2018
Biostatistics	2019

Scientific seminars	**Year**
Seminar days of RIFCH and FFPW	2017 2018 2019 2020

International conferences	**Year**
Zhao, J., Prchal, M., Palaiokostas, Ch., Houston, R., Kause, A., Bugeon, J., Vandeputte, M., Vergnet, A, Bestin, A., Vesely, T., Pokorová, D., Piačková, V., Pojezdal, L., Genestout, L., Kroupová, H., Kocour, M., 2019. Genetic relationship between koi herpesvirus disease resistance and production traits inferred from sibling performance in Amur mirror carp. Aquaculture Europe 2019. 8–10 October 2019, Berlin, Germany.	2019
Prchal, M., Bugeon, J., Vandeputte, M., Kause, A., Vergnet, A., **Zhao, J.**, Gela, D., Genestout, L., Bestin, A., Haffray, P., Kocour, M., 2019. Morphological predictors of slaughter yields using 3D digitizer and their use in a common carp breeding program. Aquaculture Europe 2019. 8–10 October 2019, Berlin, Germany.	2019
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. Aqua 2018. 25–29 August 2018, Montpellier, France.	2018
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

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Beatriz Villanueva Ph.D., The National Institute for Agricultural Research and Experimentation, Madrid, Spain (3 months, Practicing calculations of simulated breeding programs and genome-wide association analysis).	2020

Pedagogical activities	**Year**
Leading of project entitled Phenotypic and genetic variance of scaly patterns in Amur mirror carp and their association to fish performance at International Summer school	2019
FAO workshop at USB FPPW: Preparation of presentation and lecturing entitled “Potential of hybridization programs for improvement of fish yield” (15h)	2019
Share of preparation of presentations (.ppt) on given topic for courses: Genetics in Fisheries and Disorders of Endocrine System in Aquatic Animals (55h)	2018–2019
Updating information for bachelor course: Principles of Genetics and Reproduction in Fish according to instructions of the guarantor (10h)	2019
Extraordinary practicing of summer school student Olena Bielikova in field of molecular biology outside the topic of her summer school project (10h)	2019

Curriculum vitae

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EDUCATION

2016–present	Ph.D. student in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Czech Republic
2012–2015	M.Sc., in Hydrobiology, Southwest University, China
2008–2012	B.Sc., in Biology Science, Longdong University, China

Ph.D. COURSES

Pond aquaculture, Fish genetics, Applied hydrobiology, Basic of scientific communication, Ichthyology and fish taxonomy, English language, Biostatistics

TRAINING

08.01–20.01.2018	Workshop on Genomics 2018, Český Krumlov, Czech Republic.
06.11–08.11.2018	Advanced Aquaculture breeding training workshop, Montpellier, France.
26.08–30.08.2019	AQUAEXCEL2020 Training Course: "Introductory Bioinformatic Course to Sequencing Data Processing", Stirling, UK.

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