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### ► To cite this version:

Jinfeng Zhao, Martin Prchal, Antti Kause, Marc Vandeputte, David Gela, et al.. The role of energy reserves in common carp performance inferred from phenotypic and genetic parameters. *Aquaculture*, 2021, 541, pp.736799. 10.1016/j.aquaculture.2021.736799 . hal-03360569

**HAL Id: hal-03360569**

**<https://hal.inrae.fr/hal-03360569v1>**

Submitted on 8 Nov 2024

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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.

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## Highlights

► Hepatopancreas (HP) glycogen traits were positively genetically correlated with survival during the third growing season, unlike HP fat and protein. ► Condition factor and hepatosomatic index provide relatively adequate way of evaluating the energy reserves status. ► Energy status of carp very likely does not correspond to Koi herpesvirus disease resistance.

**Keywords** : Fat, Genetic variance, Glycogen, Heritability, Lipids, Overwintering

## 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). 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 (Fullin 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 tests 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<sup>2</sup> 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<sup>-1</sup>. 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 re-stocked 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 herpes virus (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 5,000 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 1,000 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 ( $10^5 * BW \text{ (g)} / SL^3 \text{ (mm)}$ ). 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<sup>-1</sup> 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 1,000 survived siblings coming from the same pond. Unfortunately, the 3,000 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 1,000 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 1,500 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<sup>3</sup> 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 (Figure 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) 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 (1,500 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:

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

where  $\mathbf{y}$  is the vector of observations for the studied traits,  $\mathbf{b}$  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);  $\mathbf{a}$  is the vector of random additive genetic effect of an animal  $\sim N(0, \mathbf{A}\sigma_a^2)$ ; and  $\mathbf{e}$  is the vector of the random residual effect  $\sim N(0, \mathbf{I}\sigma_e^2)$ .  $\mathbf{X}$  and  $\mathbf{Z}$  are incidence matrices relating observations to the fixed and random effects, respectively.  $\mathbf{A}$  is the additive genetic relationship matrix,  $\mathbf{I}$  is an identity matrix;  $\sigma_a^2$  is the additive genetic variance and  $\sigma_e^2$  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  $p < .05$  of a  $\chi^2$  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 ( $0.50 \pm 0.18$ – $0.87 \pm 0.07$ ) 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 ( $0.36 \pm 0.20$  and  $0.41 \pm 0.25$ , 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 ( $0.05 \pm 0.03$  and  $0.09 \pm 0.04$ , 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. ( $0.05 \pm 0.03$  vs.  $0.41 \pm 0.08$ ) and Rel. HP Glycogen ( $0.14 \pm 0.05$  vs.  $0.50 \pm 0.09$ ) 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. ( $0.42 \pm 0.16$ ) or VSI\_NO ( $0.53 \pm 0.19$ ) were evidenced, while a negative genetic correlation ( $-0.52 \pm 0.19$ ) 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 ( $r_g = 0.59 \pm 0.17$ ,  $0.60 \pm 0.28$  and  $0.55 \pm 0.18$ , 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. ( $0.75 \pm 0.26$ ), Rel. HP Glycogen ( $0.97 \pm 0.04$ ) and Rel. HP Energy ( $0.82 \pm 0.08$ ). 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, 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 ( $r_g = -0.31 \pm 0.13$  vs.  $0.53 \pm 0.19$  in autumn) or between BW and Abs. HP Glycogen ( $r_g = 0.23 \pm 0.07$  vs.  $0.83 \pm 0.07$  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 ( $r_g = 0.55 \pm 0.15$  vs.  $-0.52 \pm 0.19$  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 ( $r_g = 0.24 \pm 0.16$  vs.  $0.50 \pm 0.16$  in autumn) and oppositely, correlation with Rel. HP Energy became significant ( $r_g = 0.59 \pm 0.12$  vs.  $0.23 \pm 0.22$  in autumn). HP Fat conc. became significantly and negatively correlated with relative HP glycogen traits, positively correlated with HP Protein conc. ( $r_g = 0.66 \pm 0.13$  vs.  $-0.21 \pm 0.26$  in autumn) and no correlation was observed with Abs. HP Energy ( $r_g = 0.26 \pm 0.17$  vs.  $0.52 \pm 0.16$  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 (Figure 2, phenotypic correlations; Figure 3, genetic correlations). However, Figure 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 ( $r_g = 0.40 \pm 0.14$ ,  $0.58 \pm 0.12$  and  $0.67 \pm 0.11$ , respectively). HP Protein conc. was negatively correlated with FC\_SpringC2 ( $r_g = -0.43 \pm 0.15$ ) and FC\_C3 ( $r_g = -0.72 \pm 0.16$ ), but 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 first 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  $-0.75 \pm 0.21$ , respectively). Rel. HP Energy was significantly genetically correlated with FC\_SpringC2 and FC\_C3 ( $r_g = 0.35 \pm 0.15$  and  $0.31 \pm 0.15$ , 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; traïra, *Hoplias malabaricus*, Rios et al., 2006; juvenile channel catfish, *Ictalurus punctatus*, Luo et al., 2009; Laval brook charr, *Salvelinus fontinalis*, Crespel et al., 2013).

#### 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  $\times$  pond and genotype  $\times$  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. (Yause 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 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 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 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 ( $0.61 \pm 0.18$ ). 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 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 (Table 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 (Table 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 *Gasterosteus 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 (Table 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 ( $-0.32 \pm 0.14$ ) 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 winter. 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.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

### 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.

### Acknowledgments

The study was financially supported by Ministry of Agriculture through project of NAAR (NAZV) (QK 1710310), by Ministry of Education, Youth and Sports of the Czech Republic through projects CENAKVA (LM2018039) and Biodiversity (CZ.02.1.01/0.0/0.0/16\_025/000/270) and by EU Commission through project of the 7<sup>th</sup> Framework Programme (FP7-2013.1.2-10), grant agreement no. 613611-FISHBOOST.

### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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**Table 1.** Basic phenotypic parameters and heritability estimates for traits before and after the first winter.

Number of observations ( $N$ ), traits mean (Mean  $\pm$  S.D.),  $CV$  (coefficient of variation),  $V_P$  (phenotypic variance),  $V_A$  (genetic variance),  $h^2$  (heritability estimates  $\pm$  S.E.)

Trait	Units	Before the first winter						After the first winter					$P$ val ue	
		$N$	Mean $\pm$ S.D.	$C$ $V$	$V_P$	$V_A$	$h^2 \pm$ S.E.	$N$	Mean $\pm$ S.D.	$C$ $V$	$V_P$	$V_A$		$h^2 \pm$ S.E.
Weight	g	95	13.45 $\pm$	42	31.9	10.3	0.33 $\pm$	93	14.86 $\pm$	35	27.2	9.28	0.34 $\pm$	<
		4	5.69	.3	2	8	0.07	9	5.24	.3	8	0.07	0.01	
FC		95	3.48 $\pm$	9.	0.11	0.04	0.35 $\pm$	93	3.14 $\pm$	7.	0.05	0.03	0.58 $\pm$	<
		4	0.34	6			0.08	9	0.23	4			0.10	0.01
VSI_NO	%	94	9.42 $\pm$	27	6.59	0.29	0.41 $\pm$	93	6.86 $\pm$	17	1.38	0.34	0.25 $\pm$	<
		1	2.56	.2			0.05	5	1.16				0.07	0.01
HSI	%	94	5.88 $\pm$	22	1.78	0.25	0.14 $\pm$	93	4.97 $\pm$	19	0.95	0.41	0.43 $\pm$	<
		6	1.34	.8			0.05	7	0.97	.6			0.09	0.01
Dry matter	%	92	24.46 $\pm$	2.	5.67	1.49	0.26 $\pm$	93	20.06 $\pm$	7.	2.14	0.81	0.38 $\pm$	<
		0	2.19	8			0.07	2	1.45	2			0.08	0.01
M. Fat conc.	mg/g	92	56.29 $\pm$	32	325.	93.3	0.29 $\pm$	93	23.26 $\pm$	40	89.7	42.6	0.47 $\pm$	<
		0	18.21	.4	65	5	0.07	2	9.39	.4	7	2	0.09	0.01
HP Fat conc.	mg/g	94	52.37 $\pm$	37	393.	74.2	0.19 $\pm$	93	20.46 $\pm$	40	67.2	19.8	0.30 $\pm$	<
		1	19.81	.8	11	7	0.06	9	8.23	.2	5	7	0.07	0.01
Abs. HP Fat	mg	93	41.21 $\pm$	62	665.	158.	0.24 $\pm$	93	14.95 $\pm$	57	74.7	21.3	0.29 $\pm$	<
		4	25.78	.5	92	10	0.06	7	8.67	.9	4	4	0.07	0.01



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. Bold numbers indicate significant correlations, numbers in italics moreover represent low genotype by environment ( $G \times E$ ) interaction. See Table 1 for trait abbreviations.

Trait	$r_g \pm S.E.$
<b>Weight</b>	<b>0.69 ± 0.12</b>
<b>FC</b>	<i>0.87 ± 0.07</i>
<b>VSI_NO</b>	<b>0.61 ± 0.18</b>
<b>HSI</b>	0.36 ± 0.20
<b>M. Fat conc.</b>	<b>0.59 ± 0.14</b>
<b>HP Fat conc.</b>	<b>0.50 ± 0.18</b>
<b>Abs. HP Fat</b>	<b>0.52 ± 0.16</b>
<b>HP Glycogen conc.</b>	<b>0.70 ± 0.27</b>
<b>Abs. HP Glycogen</b>	<b>0.58 ± 0.15</b>
<b>Rel. HP Glycogen</b>	<b>0.51 ± 0.19</b>
<b>HP Protein conc.</b>	0.41 ± 0.25
<b>Abs. HP Protein</b>	<b>0.59 ± 0.18</b>
<b>HP Energy conc.</b>	<b>0.54 ± 0.20</b>
<b>Abs. HP Energy</b>	<b>0.60 ± 0.15</b>
<b>Rel. HP Energy</b>	<b>0.51 ± 0.21</b>

**Table 3.** Genetic (above diagonal;  $\pm$  S.E.) and phenotypic (below diagonal) correlations of traits before the first winter. See Table 1 for trait abbreviations.

	Weight	FC	VSI_NO	HSI	M. Fat conc.	HP Fat conc.	Abs. HP Glycogen conc.	Rel. HP Glycogen	Abs. HP Protein conc.	Rel. HP Energy conc.	Abs. HP Energy	Rel. HP Energy
<b>Weight</b>	<b>0.33</b> $\pm$ 0.07	-0.11 $\pm$ 0.18	<b>0.53</b> $\pm$ 0.19	-0.39 $\pm$ 0.19	<b>0.42</b> $\pm$ 0.16	0.39 $\pm$ 0.18	<b>0.83</b> $\pm$ 0.07	-0.33 $\pm$ 0.20	<b>0.72</b> $\pm$ 0.05	0.17 $\pm$ 0.21	<b>0.89</b> $\pm$ 0.05	-0.20 $\pm$ 0.21
<b>FC</b>	-0.09	<b>0.35</b> $\pm$ 0.08	0.18 $\pm$ 0.22	<b>0.59</b> $\pm$ 0.17	0.25 $\pm$ 0.18	-0.16 $\pm$ 0.20	<b>0.60</b> $\pm$ 0.19	<b>0.28</b> $\pm$ 0.18	-0.40 $\pm$ 0.22	0.01 $\pm$ 0.21	-0.13 $\pm$ 0.19	0.08 $\pm$ 0.20
<b>VSI_NO</b>	-0.01	0.05	<b>0.14</b> $\pm$ 0.05	0.06 $\pm$ 0.26	<b>0.56</b> $\pm$ 0.18	<b>0.71</b> $\pm$ 0.17	<b>0.73</b> $\pm$ 0.18	0.17 $\pm$ 0.26	-0.31 $\pm$ 0.26	<b>0.63</b> $\pm$ 0.17	<b>0.90</b> $\pm$ 0.17	<b>0.74</b> $\pm$ 0.18
<b>HSI</b>	-0.26	0.17	-0.24	<b>0.14</b> $\pm$ 0.05	-0.09 $\pm$ 0.22	-0.16 $\pm$ 0.23	<b>0.75</b> $\pm$ 0.22	<b>0.97</b> $\pm$ 0.04	-0.25 $\pm$ 0.26	0.05 $\pm$ 0.26	-0.04 $\pm$ 0.23	0.04 $\pm$ 0.08
<b>M. Fat conc.</b>	0.19	0.12	0.25	-0.08	<b>0.29</b> $\pm$ 0.07	<b>0.55</b> $\pm$ 0.15	<b>0.50</b> $\pm$ 0.16	0.01 $\pm$ 0.22	<b>0.44</b> $\pm$ 0.21	<b>0.52</b> $\pm$ 0.17	<b>0.63</b> $\pm$ 0.14	0.23 $\pm$ 0.22



							<b>14</b>								
							<b>0.</b>								
							<b>74</b>	0.29	-0.24		0.31				
<b>HP Fat</b>							<b>0.18 ±</b>			-0.21 ±		<b>0.97 ±</b>	<b>0.52 ±</b>	0.34 ±	
<b>conc.</b>	0.18	0.003	0.14	0.01	0.35		<b>0.06</b>	-0.37 ±	0.29 ±			<b>0.03</b>	<b>0.16</b>	0.22	
							<b>0.</b>	0.20	0.23		0.21				
							<b>10</b>								
							<b>0.</b>								
							<b>24</b>	<b>0.85</b>	-0.08		<b>0.84</b>				
<b>Abs. HP</b>							<b>0.</b>	-0.04 ±	0.30 ±		<b>-0.56 ±</b>	<b>0.76 ±</b>	<b>0.96 ±</b>	<b>0.39 ±</b>	
<b>Fat</b>	0.63	0.02	0.004	0.20	0.32	0.73	<b>0.</b>	<b>0.08</b>	0.23		<b>0.20</b>	<b>0.11</b>	<b>0.02</b>	<b>0.19</b>	
							<b>06</b>				<b>0.08</b>				
							<b>HP</b>								
							<b>Glycogen</b>	-0.17	0.14	0.05	0.17	-0.07	-0.22	0.	
<b>conc.</b>							<b>0.</b>	<b>0.05 ±</b>	<b>0.03 ±</b>						
							<b>17</b>	0.29	<b>0.10</b>						
							<b>0.</b>								
							<b>Abs. HP</b>								
<b>Glycogen</b>	0.62	0.09	-0.08	0.35	0.10	0.05	<b>0.</b>	0.46			<b>-0.77 ±</b>	0.22 ±	<b>0.95 ±</b>	0.33 ±	
							<b>57</b>				<b>0.14</b>	0.22	<b>0.03</b>	0.21	
							<b>0.</b>								
							<b>Rel. HP</b>								
<b>Glycogen</b>	-0.26	0.19	-0.13	0.77	-0.10	-0.13	<b>0.</b>	0.75	0.53		-0.39 ±	±	-0.16 ±	0.11 ±	<b>0.79 ±</b>
							<b>03</b>				0.23	±	0.26	0.23	<b>0.11</b>
							<b>0.</b>								
							<b>HP Protein</b>								
<b>conc.</b>	-0.08	-0.12	-0.24	-0.21	-0.03	-0.22	<b>0.</b>	-0.34	-0.37	-0.36	<b>0.09 ±</b>	0.47	-0.09 ±	<b>-0.62 ±</b>	-0.29 ±
							<b>16</b>				<b>0.04</b>	±	0.29	<b>0.19</b>	0.27
							<b>0.</b>								
							<b>Abs. HP</b>								
<b>Protein</b>	0.74	-0.05	-0.24	0.23	0.15	0.15	<b>0.</b>	-0.22	0.62	0.02	0.34	±	0.20 ±	<b>0.92 ±</b>	0.17 ±
							<b>63</b>						0.24	<b>0.04</b>	0.25
							<b>0.</b>								
							<b>HP Energy</b>								
<b>conc.</b>	0.08	0.02	0.07	0.004	0.29	0.87	<b>0.</b>	0.12	0.16	0.08	0.21	0.18	<b>0.15 ±</b>	<b>0.49 ±</b>	<b>0.52 ±</b>
							<b>63</b>						<b>0.05</b>	<b>0.18</b>	<b>0.18</b>
							<b>0.</b>								
							<b>Abs. HP</b>								
<b>Energy</b>	0.75	0.03	-0.08	0.31	0.27	0.50	<b>0.</b>	0.01	0.81	0.21	-0.12	0.81	0.45	<b>0.24 ±</b>	0.35 ±
							<b>92</b>							<b>0.06</b>	0.21
							<b>0.</b>								
							<b>Rel. HP</b>								
<b>Energy</b>	-0.21	0.15	-0.18	0.83	0.08	0.47	<b>0.</b>	0.21	0.42	0.69	-0.09	0.30	0.54	0.52	<b>0.13 ±</b>
							<b>53</b>								<b>0.05</b>

**Table 4.**

Genetic

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							<b>73</b>										
<b>HP Fat</b>							<b>0.30 ±</b>	<b>-0.38 ±</b>	-0.31	<b>-0.43</b>		0.31					
<b>conc.</b>	0.08	0.12	0.12	-0.14	0.49		<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	0.26 ±	-0.09 ±		7 / 0
							<b>0.07</b>	<b>0.16</b>		<b>0.13</b>		<b>0.08</b>	0.17	0.19			
							<b>0.</b>		0.17	<b>0.15</b>		0.18					
							<b>09</b>										
							<b>0.</b>										
							<b>29</b>										
<b>Abs. HP</b>							<b>-0.02 ±</b>		0.24	-0.05		<b>0.85</b>					
<b>Fat</b>	0.63	0.11	0.01	0.16	0.32	0.72	<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>0.88 ±</b>	<b>0.85 ±</b>	0.30 ±	5 / 1
							<b>0.</b>	0.18		<b>0.16</b>		<b>0.07</b>	<b>0.05</b>	0.17			
							<b>0.</b>		0.17	0.18		<b>0.06</b>					
							<b>07</b>										
<b>HP</b>							-		<b>0.93</b>	<b>0.99</b>		0.1					
<b>Glycogen</b>	-0.10	0.10	0.12	0.47	0.12	-0.22	0.	<b>0.41 ±</b>	<b>0.08 ±</b>	<b>±</b>	<b>±</b>	<b>±</b>	0.21 ±	<b>0.39 ±</b>	<b>0.89 ±</b>		5 / 3
<b>conc.</b>							07		<b>0.03</b>	<b>0.01</b>		0.19	0.18	<b>0.16</b>	<b>0.05</b>		
							0.		<b>0.40</b>	<b>0.91</b>		<b>0.37</b>					
<b>Abs. HP</b>							0.		<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>±</b>	<b>0.34 ±</b>	<b>0.68 ±</b>	<b>0.87 ±</b>	8 / 3
<b>Glycogen</b>	0.34	0.10	0.003	0.57	0.06	-0.18	25	0.81		<b>0.14</b>		<b>0.16</b>	<b>0.17</b>	<b>0.10</b>	<b>0.06</b>		
							-		<b>0.50</b>			0.04					
<b>Rel. HP</b>							0.		<b>0.84 ±</b>	<b>±</b>	<b>±</b>	<b>±</b>	0.15 ±	<b>0.38 ±</b>	<b>0.91 ±</b>		5 / 2
<b>Glycogen</b>	-0.11	0.12	0.09	0.72	0.15	-0.22	0.		<b>0.07</b>			0.18	<b>0.15</b>	<b>0.04</b>			
							0.		<b>0.09</b>			0.18					
<b>HP</b>							0.					<b>0.50</b>					
<b>Protein</b>	0.18	-0.04	-0.16	-0.40	-0.06	0.25	0.	-0.47	-0.38	-0.50		<b>0.22 ±</b>	0.29 ±	0.19 ±	<b>-0.61 ±</b>		8 / 0
<b>conc.</b>							16		<b>0.06</b>			<b>0.15</b>	0.19	0.19	<b>0.15</b>		
							0.					<b>0.30</b>					
<b>Abs. HP</b>							0.					<b>0.53 ±</b>	<b>0.92 ±</b>	0.26 ±			
<b>Protein</b>	0.84	0.03	-0.16	0.1	-0.04	0.13	67	-0.10	0.34	-0.02	0.45	<b>±</b>	<b>0.15</b>	<b>0.03</b>	0.18		3 / 0
							0.					<b>0.07</b>					
<b>HP</b>							0.					<b>0.24 ±</b>	<b>0.68 ±</b>	<b>0.48 ±</b>			
<b>Energy</b>	0.10	0.12	0.07	0.02	0.37	0.65	52	0.34	0.27	0.27	0.45	0.30	<b>0.06</b>	<b>0.12</b>	<b>0.15</b>		3 / 0
<b>conc.</b>							0.										
<b>Abs. HP</b>							0.							<b>0.34 ±</b>	<b>0.63 ±</b>		
<b>Energy</b>	0.79	0.09	-0.08	0.37	0.11	0.24	79	0.24	0.66	0.32	0.15	0.89	0.44	<b>0.08</b>	<b>0.11</b>		6 / 0
<b>Rel. HP</b>							0.										
<b>Energy</b>	-0.04	0.18	0.04	0.81	0.36	0.26	43	0.57	0.61	0.73	-0.05	0.30	0.59	0.55	<b>0.33 ±</b>		6 / 1
															<b>0.08</b>		

**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.

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
	-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 ± 0.35
	-0.07 ± 0.16	0.13 ± 0.15	-0.12 ± 0.16	0.02 ± 0.17	0.28 ± 0.15	0.24 ± 0.15	<b>0.33 ± 0.14</b>	<b>-0.43 ± 0.15</b>	0.01 ± 0.17	-0.09 ± 0.17	0.10 ± 0.17
ngC2	<b>0.61 ± 0.11</b>	<b>0.40 ± 0.14</b>	<b>0.58 ± 0.12</b>	<b>0.67 ± 0.11</b>	-0.24 ± 0.15	-0.01 ± 0.16	-0.28 ± 0.15	<b>0.54 ± 0.14</b>	<b>0.55 ± 0.13</b>	<b>0.51 ± 0.14</b>	<b>0.48 ± 0.14</b>
	<b>0.67 ± 0.11</b>	-0.11 ± 0.16	0.27 ± 0.16	<b>0.46 ± 0.14</b>	-0.16 ± 0.16	0.08 ± 0.16	-0.22 ± 0.15	<b>0.40 ± 0.16</b>	<b>0.54 ± 0.13</b>	0.31 ± 0.16	<b>0.44 ± 0.14</b>
	-0.42 ± 0.25	-0.08 ± 0.25	<b>-0.59 ± 0.20</b>	<b>-0.51 ± 0.23</b>	<b>0.64 ± 0.20</b>	<b>0.49 ± 0.22</b>	<b>0.69 ± 0.19</b>	<b>-0.75 ± 0.21</b>	-0.37 ± 0.25	-0.33 ± 0.26	-0.13 ± 0.26
	-0.13 ± 0.15	0.05 ± 0.15	-0.12 ± 0.16	-0.02 ± 0.16	<b>0.29 ± 0.14</b>	<b>0.55 ± 0.13</b>	<b>0.52 ± 0.14</b>	<b>-0.72 ± 0.10</b>	-0.07 ± 0.16	-0.08 ± 0.17	0.05 ± 0.17
	<b>0.32 ± 0.15</b>	0.17 ± 0.16	0.25 ± 0.16	<b>0.37 ± 0.15</b>	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 ± 0.17
	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 ± 0.18

**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.

**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.

	Survival C2	Survival C3
<b>FC</b>	-0.19 ± 0.30	0.27 ± 0.23
<b>VSI_NO</b>	0.45 ± 0.35	0.08 ± 0.28
<b>HSI</b>	0.22 ± 0.32	<b>0.72 ± 0.20</b>

**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

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

**Figure 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.

**Figure 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.

Figure 1

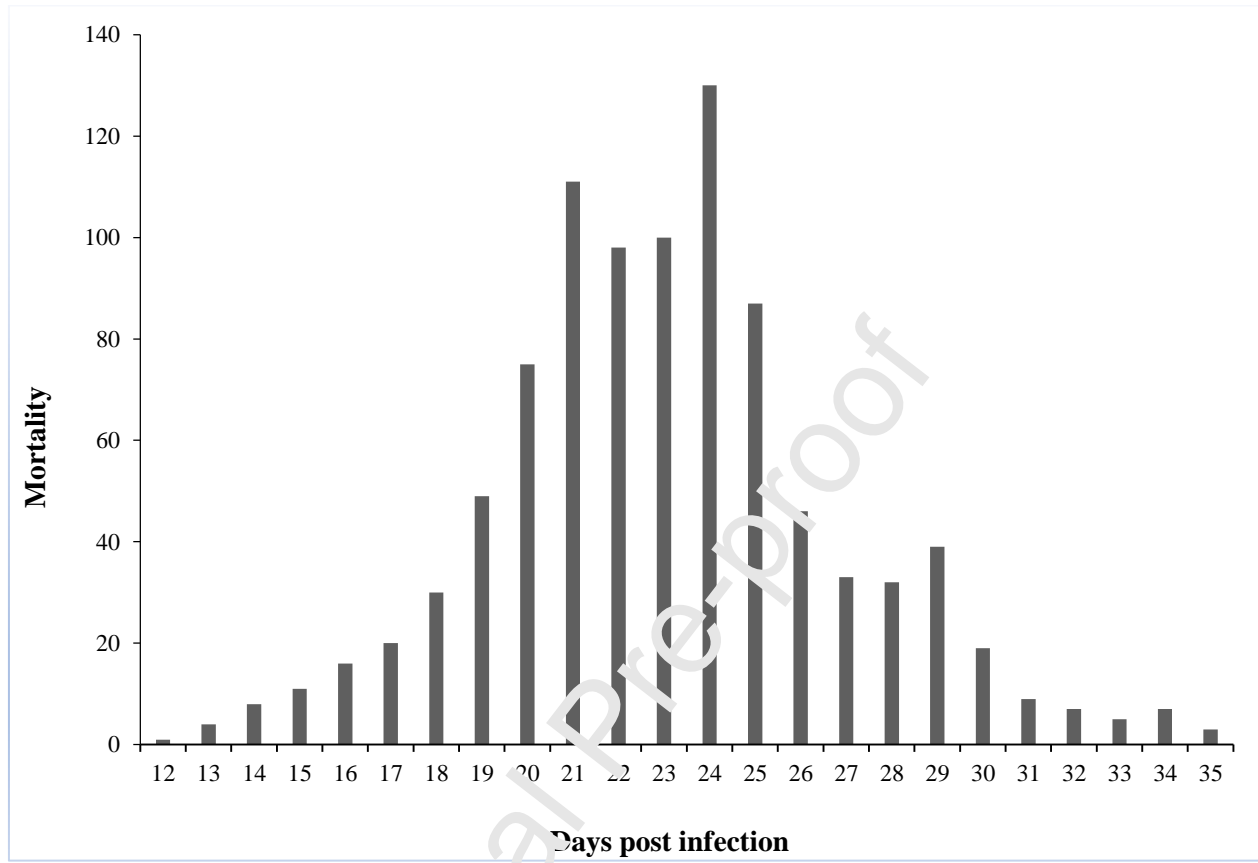


Figure 2

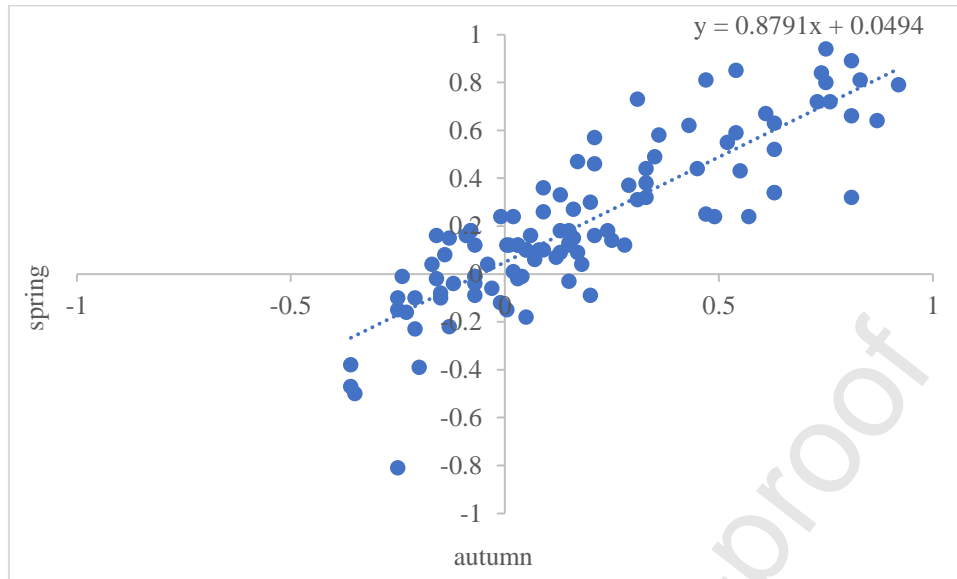
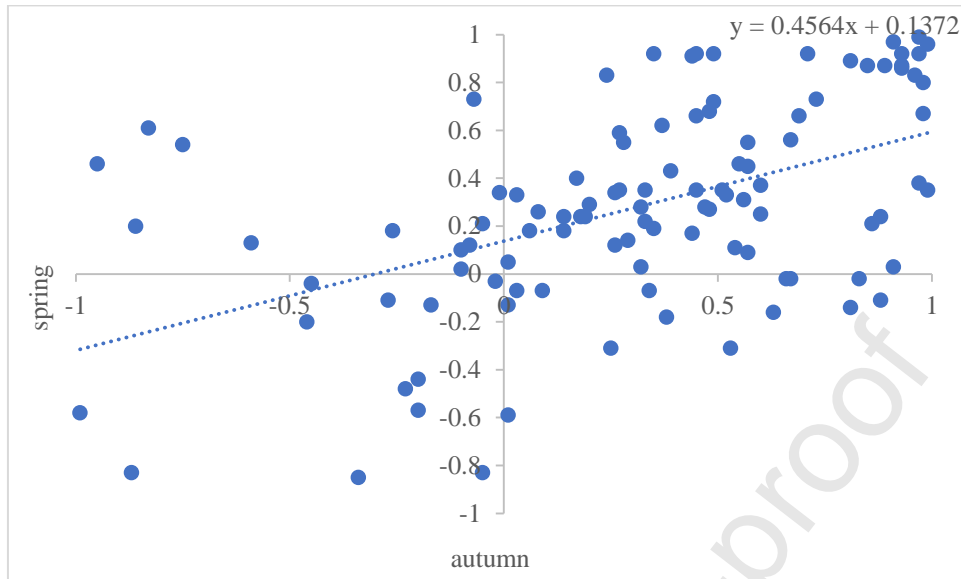


Figure 3





### Highlights:

- Hepatopancreas (HP) glycogen traits were positively genetically correlated with survival during the third growing season, unlike HP fat and protein.
- Condition factor and hepatosomatic index provide relatively adequate way of evaluating the energy reserves status.
- Energy status of carp very likely does not correspond to Koi herpesvirus disease resistance.

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