Fenntartható, innovatív haltermelési és környezetkezelési technológiák fejlesztése és gyakorlati bevezetésének támogatása

MAHOP-2.1.1-2016-2017-00007. számú projekt

ELŐSZÓ

A NAIK Halászati Kutatóintézetben a *Fenntartható, innovatív haltermelési és környezetkezelési technológiák fejlesztése és gyakorlati bevezetésének támogatása”* című projekt keretében olyan feladatokat hajtottunk végre, amelyek a Nemzeti Akvakultúra Stratégiai Terv egyes kiemelt céljainak elérését szolgálják. Kutatás-fejlesztési tevékenységünk eredményei előrelépést jelentenek a haltermelés input igényeinek optimalizálásában, a halolaj- és hallisztfüggőséget csökkentő haltakarmányozási technológiák adaptálhatóságában, a haltermelés környezeti hatásainak megértésében, valamint a sügérfélék termeléstechnológiájának egyszerűsítésében.

Ljubobratović Uroš, Péter Géza, Demény Ferenc, Kugyela Nándor, Horváth Ákos, Pataki Bernadett, Horváth Zoltán, Jakabné Sándor Zsuzsanna, Rónyai András

Arachidonsav tápban való dúsításának hatása először fejt süllőanyák szaporítási mutatóira

Tanulmány háttere és célja

- A kutatás során szaporításra először elkülönített, korábban is teljesen kontrollált környezetben tartott, két-éves (1 ± 0.2 kg) süllőanyákat etettünk két eltérő táppal (kontroll vs. arachidonsavval dúsított)
- A két takarmányozási kezelésbe tartozó anyákat hét hónapig külön neveltük, és mindkét kezelésből három különböző időpontban (a kísérlet 5., 6. illetve 7. hónapjában) oltottunk gonadoliberin hormonnal egy-egy kisebb csoportot. Az oltás időpontjában mértük a petesejt méretét.
- A kísérlet célja annak vizsgálata volt, hogy egyfelől a takarmányozási beállítás, másfelől a petesejt oltás időpontjában való mérete milyen hatással van a szaporítási mutatókra

Tanulmány főbb eredményei

- Életükben először oltott („virgin") süllőanyáknál csak azon csoportok esetében indukál ovulációt az oltás, ahol a petesejt mérete meghaladja 900 μm-t.
- Ugyanakkor az oltás időpontja akár egy hónapnál nagyobb időszakra is elnyújtható, hiszen akkor is sikeresen olthatóak az anyák, amikor a petesejtek mérete 1000 μm körül volt.
- Ez nagy flexibilitást biztosít a mesterséges szaporítást végző gazdaságok számára az ivás időpontjának időzítése tekintetében.
- Az arachidonsavval dúsított tápokkal etetett sülőanyák ikráiban is feldúsul az arachidonsav szintje.
- Ugyanakkor az arachidonsav dúsítás csökkentette az embriómegmaradást a hormonnal indukált ovuláció során.

Az összegzés az alábbi cikkből készült:

Ljubobratović Uroš, Péter Géza, Demény Ferenc, Kugyela Nándor, Horváth Ákos, Pataki Bernadett, Horváth Zoltán, Jakabné Sándor Zsuzsanna, Rónyai András: Reproductive performance in virgin pikeperch (Sander lucioperca L.) females fed different dietary levels of arachidonic acid with respect to the duration of spawning induction AQUACULTURE REPORTS 18 Paper: 100430 (2020)

Reproductive performance in indoor reared virgin pikeperch (*Sander lucioperca* L.) females fed different dietary levels of arachidonic acid with respect to the duration of photo-thermal spawning induction

Uroš Ljubobratović[a#], Géza Péter[a], Ferenc Demény[a], Nándor Kugyela[a], Ákos Horváth[b], Bernadett Pataki[b], Zoltán Horváth[c], Zsuzsanna Jakabne Sándor[a], Andras Rónyai[a†]

[a] *Research Institute for Fisheries and Aquaculture (NAIK-HAKI), Anna-liget 35, 5540 Szarvas, Hungary*
[b] *Szent István University, H-2100 Gödöllő, Péter Károly utca 1, Hungary*
[c] *H&H Carpió Halászati Kft., H-7814 Ócsárd, Kossuth u. 7., Hungary*
† Deceased April 15, 2019
 # Corresponding author, e-mail: u.ljubobratovic@gmail.com
Uroš Ljubobratović, Research Institute for Fisheries and Aquaculture NARIC HAKI, Anna Liget 8, 5540 Szarvas, Hungary. Tel: +36 70 440 31 33; Fax: 36 66 312 142; E-mail: u.ljubobratovic@gmail.com

Abstract

Pikeperch (*Sander lucioperca*) is a species of significant importance for the further development of intensive aquaculture, what makes the total control over species' reproductive system a high priority task. The present study aimed to assess the effect of arachidonic acid (AA) dietary enrichment on the reproductive performance in virgin females kept in fully controlled conditions with respect to different time of hormonal application. Two groups of breeders, ARA and CTRL, were stocked in two tanks of the recirculation system and fed with diets differing in dietary AA levels over the course of seven months. Five, six, and seven months after the start of trial, batches of five pairs of breeders from each group were hormonally treated. Ovulations occurred after six and seven months (oocyte diameter range 912-1030 μm), and samples of dry eggs were taken for the analysis of fatty acid profiles. Among the parameters of reproductive success, only the embryo survival was significantly affected by the diet while there were no effects of spawning induction in both feeding groups. The CTRL group females performed better in the second propagation batch with significantly higher embryo survival (42.6 ± 19.1% in CTRL and 14.4 ± 17.7% in ARA, $p = 0.045$). Likewise, overall survival across both reproduction occasions was significantly lower ($p = 0.036$) in group fed AA enriched diet (35.7 ± 17.1 % and 18.6 ± 13.6 % in CTRL and ARA group, respectively). A significant accumulation of AA was found in ARA eggs in both reproduction batches. Virgin pikeperch breeders appear to keep at least one month plasticity towards the duration of spawning induction in controlled conditions and optimal oocyte diameter range for artificial reproduction is between 900-1000 μm. Levelling dietary AA with eicosapentaenoic acid during the entire photothermal protocol negatively altered the egg quality.

1 Introduction

Total control over the biological cycle has been a subject of high importance in the aquaculture of many species (Devauchelle and Coves, 1988; Pankhurst and Porter, 2003; Wang et al., 2010). The main aim of this technology is independence from outdoor conditions and seasonality, which, in cases of species of annual fecundity and synchronous oocyte development, limits the time of fingerling supply. Since a few decades ago, pikeperch (*Sander lucioperca*) has been described as one of the species of high interest for total control over the reproductive cycle. This species is a promising candidate for fish farming diversification (Fontaine et al., 2009). The latest reports have shown pikeperch farming going from research and pilot-scale to the real industry (Dalsgaard et al., 2013; Steenfeldt et al., 2015). At present, intensive rearing of this species is exclusively achieved in recirculation aquaculture systems (RAS). This farming system is expensive compared to other fish farming possibilities and thus, asks for utmost usage of space and time through maximal yields for the given species (Losordo and Westerman, 1994; Badiola et al., 2012). Therefore, the off-season supply of stocking material is crucial for the feasibility of this production, which makes total control of the reproductive cycle a key subject in pikeperch aquaculture technology.

Pikeperch is a temperate fish; therefore, maturation of its reproductive organs is largely dependent on the autumn and winter conditions characteristic for the temperate climate zone (Teletchea and Fontaine, 2014). In autumn the gradual decrease in temperature, photoperiod, and light intensity stimulates the hypothalamus-pituitary-gonad axis (Lubzens et al., 2010). Via its respective hormones, gonadoliberins, gonadotropins, and finally estradiol in females, this physiological pathway leads to liver secretion and further transport of the egg yolk precursor proteins, vitellogenins. Integration of vitellogenins into the oocyte and its further processing into yolk proteins, together

with the incorporation of lipids and vitamins, defines this developmental stage in secondary oocytes as vitellogenesis or secondary growth (Hara et al., 2016). The most recognizable feature of vitellogenesis is the increase of oocyte size. Therefore, in order to monitor oocyte development, many studies followed the diameter of oocytes through time in order to estimate the progress of secondary growth (Blythe et al., 1994; Kjesbu, 1994; Asturiano et al., 2000). Indeed, the same parameter was used in studies on pikeperch (Żarski et al., 2012; Hermelink et al., 2013, 2016). This is important with the respect that upon completion of vitellogenesis, the oocyte becomes ready for its final developmental stages, maturation, and ovulation. As is the case for many aquaculture species, these last steps of the reproductive cycle are artificially controlled by means of external hormones, a procedure defined as artificial reproduction (Żarski et al., 2015). With respect that maturation follows vitellogenesis, the question becomes, at what size are the oocytes ready for artificial reproduction? In the case of pikeperch, this question is of special interest with regards to the gathered knowledge of breeders cultured in outdoor conditions. In several studies (Rónyai, 2007, Müller-Belecke and Zienert, 2008; Ljubobratović et al., 2019a), these fish were shown to be tolerant to this procedure, yielding gametes of high quality up to three months prior to the natural spawning season. To the best of our knowledge, while the progress of oogenesis was followed in pikeperch in fully controlled conditions (Hermelink et al., 2013, 2016), the aptness for artificial reproduction was not examined.

In addition to the photo-thermal conditions, broodstock nutrition is of major importance for the success of the controlled reproductive cycle (reviewed by Izquierdo et al., 2001). This aspect is poorly documented in pikeperch (Wang et al., 2009; Ben Khemis et al., 2014). Nevertheless, in cases of other teleost species, many nutrient variables were examined, such as proteins (Watanabe et al., 1984), vitamins (Lee and Dabrowski, 2004), and pigments (Torrissen and Christiansen, 1995; Scabini et al., 2010), and their effect on the reproductive performance was shown to different extents. Perhaps the strongest modification of the reproductive system was found in adjusting the fatty acid (FA) content (Bruce et al., 1999; Furuita et al., 2006; Zakeri et al., 2011). In addition to being an energy source, many fatty acids, especially highly unsaturated (HUFA) ones, are either directly or through their derivates responsible for modifying physiological pathways (Murdoch et al., 1993; Nunez et al., 1995; Mercure and Van Der Kraak, 1995). Regarding their derivates, important roles are played by the prostaglandins (PGs), eicosanoid products of 18 and 20 chain FAs (Stacey and Goetz, 1982; Mustafa and Srivastava, 1989). In this sense, the main HUFAs involved are 20:4n-6 arachidonic acid (AA) and 20:5n-3 eicosapentaenoic acid (EPA). These HUFAs affect steroidogenesis in different ways through conversion to their respective PGs, prostaglandin E2 (PGE2) and prostaglandin E3 (PGE3). Due to different metabolic products, these fatty acids compete for cyclooxygenase, an enzyme responsible for their further conversion (Sorbera et al., 2001; Smith, 2005). Thus, the level and the ratio between these fatty acids are among the essential modifiers of reproductive physiology. By adjusting the dietary features of AA and EPA, studies on different teleost species were able to improve the reproductive performance of captive broodstock (Bruce et al., 1999; Bell and Sargent, 2003; Furuita et al., 2003;). Among these, the one closest to the species of interest of this study was performed by Henrotte et al. (2010) on Eurasian perch (*Perca fluviatilis*). These authors found that adjusting AA and EPA to even levels in feed significantly improved the reproductive success of this percid species compared to the commonly found high EPA/AA ratio in commercial diets.

As reviewed, the oocyte diameter increases over the time and could be an important indicator of the readiness of breeders for artificial reproduction. From another side, the dietary AA level and its relationship to EPA could strongly modify the egg quality. Therefore, the main aim of this study was to assess the reproductive success in virgin pikeperch females reared under fully controlled conditions fed with different dietary AA levels and injected at differing moments of photo-thermal spawning induction.

2 Materials and Methods

2.1 Broodstock management

Pikeperch individuals used for the trial originated from wild Körös breeders (Ljubobratović et al., 2018). Following artificial reproduction, five days post-hatch (DPH) larvae were stocked into the ponds for six-week nursing. Subsequently, the juveniles were transferred to the RAS and habituated to formulated diets. The juveniles were reared in RAS at a temperature range of 20-25 °C and exclusively fed with formulated diets. In September 2016, at the age of seventeen months and mean size of 380 g, 77 individuals were transferred to the broodstock chamber, a RAS composed of three 3 m^3 water volume tanks, a bead filter, trickling tower, and oxygen diffusers in each tank. In late March 2017, at the age of 2 years and mean weight of 835 g (range 480 and 1460 g), 72 individuals were tagged with passive integrated transponders. Following tagging, the fish were separated into two tanks, each stocked with 36 fish (19 females and 17 males), representing two treatments:

CTRL – fish fed plain Coppens REPRO commercial broodstock diet;

ARA – fish fed fish fed Coppens REPRO commercial broodstock diet enriched with AA.

Groups were formed in such a way to keep the mean size and size distribution within the tank equal, thus mean weights at stocking were 835 ± 245 g and 829 ± 203 g, while the coefficient of variation was 29.4% and 24.5% in CTRL and ARA group, respectively.

2.2 Photo-thermal conditioning

Since the transfer into the broodstock chamber, fish were kept at a constant temperature of 22°C, while the photoperiod period was gradually reduced from 16:8 LD to 12:12 LD by a 2-minute decrease per day. The temperature reduction started two weeks after the start of the feeding treatment, and over the course of 6 weeks, the temperature was reduced to 10°C. From then on, the temperature in the broodstock chamber was kept constant at 9-10°C for five months. This temperature was chosen as was described as effective for spawning induction in fully controlled conditions (Hermelink et al., 2011) and as limiting for successful spawning in outdoor populations (Müller-Belecke and Zienert, 2008).

Light reduction from 12:12 to 8:16 LD was gradually performed over 11 weeks with a daily decrease of 3-4 minutes. The photoperiod was kept constant for three weeks and then increased gradually to 12:12 LD over 12 weeks with a daily increase in the light period of 3 minutes. The photo-thermal induction from the start of the feeding treatment is detailed in Figure 1.

2.3. Oocyte sampling and artificial reproduction methodology

Starting from late July 2017 (about 4 months since the start of the feeding trial), oocytes from all the females were sampled monthly by biopsy with a catheter. Following the biopsy, the oocytes were immediately placed in separate 6 ml Petri dishes and washed with Serra solution (a mixture of 96% alcohol, 35% formalin and glacial acetic acid in a ration 6:3:1, respectively). About 30 minutes after the sampling, oocytes were photographed using a Nikon ShuttlePix P-400R microscope (Nikon Corporation, Tokyo, Japan) with 20x magnification. Images were further processed using Nikon ShuttlePix Editor Ver3.4.0, and the mean oocyte diameter of each fish was assessed on the sample of 20 oocytes per fish.

Starting from second sampling, five females with the highest diameter of oocytes in both groups and five randomly chosen males from each group were transferred from the broodstock chamber to the spawning room, a RAS of identical features to the broodstock chamber. At the first injection occasion, not any of the injected females ovulated and this groups was excluded from the further analysis. Therefore, two reproduction batches were formed within each feeding treatment group: ARA1, ARA2, and CTRL1, CTRL2.

At the day of transport, the water temperature in spawning room was equal to the broodstock chamber and then gradually heated 1°C per day. Three days later, when the water temperature in spawning room reached 12°C, all the fish were hormonally treated with a single injection. Females received the salmon gonadotropin-releasing hormone analogue sGnRHa [D-Arg6,Trp7,Leu8,Pro9-NEt]-GnRH (Ova-RH; Syndel Laboratories Ltd., Canada) at a dosage of 50 μg kg^{-1}. Males were treated with human chorionic gonadotropin hCG (Choragon, Ferring International Center S.A., Switzerland) at a dosage of 500 IU kg^{-1}. The artificial propagation procedure was carried out, as explained by Ljubobratović et al. (2019a).

Eggs of each females were fertilized with the milt of two males from the same group in a ratio 1 mL of milt per 100g of eggs. Upon fertilisation and adhesiveness elimination with milk and kaolin (Ljubobratović et al., 2019b), eggs from each female were stocked in separate 3 L Zug jars. The eggs were then left to settle and the initial level of eggs in the jar was marked. During incubation, eggs were carefully cleaned from dead eggshells. Thus, 72 h post-fertilisation, eggs were once again left to settle, and the level of eggs was marked again. At the same time, three samples of 100-200 eggs were taken for microscopic evaluation (20× magnification) to assess the proportion of live embryos in the jar. The commercial survival in jars was calculated based on the following equation:

$$\text{Commercial survival} = \frac{\text{volume of eggs 72 hours after fertilisation}}{\text{volume of eggs at the time of stocking into Zug jar}} * \text{mean percentage of live eggs}$$

The Zug jar incubation set was supplied with aerated and well-oxygenated water in a flow-through system. Water temperature and the oxygen saturation of the outflow water were monitored every twelve hours. Mean water temperature during incubation was 14.8 ± 0.2°C, while the mean oxygen saturation was 119.3 ± 6.6%.

Fish handling was performed according to the regulations of the Animal Ethical Panel of the Institute, which was established according to Hungarian State law (10/1999.I.27.).

2.4 Feeding and fatty acid analysis of diets and dry eggs

Since habituated to dry diets until stocking into the broodstock chamber, fish were fed with sturgeon pre-grow and on-grow diets SteCo (Coppens International, The Netherlands) with pellet size adequate for the fish size (1 to 6 mm). Following the transfer to the broodstock chamber, fish were fed with the sturgeon broodstock diet, Coppens REPRO. According to the manufacturer's information, the proximate composition of the feed was as follows: 48% protein, 15% fat, 8.5% ash, 0.9% crude fibre, and 1.2% total phosphorus. Floating 8 mm pellets were supplied by hand at a daily rate of 0.8% of total biomass. In February 2017, freshly produced Coppens REPRO feed was obtained for the trial. An aliquot sample was taken for the analysis of the feed FA composition. In order to obtain an EPA/ARA ratio around 1, feed for the ARA group was enriched with 60 mg kg^{-1}ARA oil (ARASCO™, DSM Nutritional Products, Inc., Basel, Switzerland, AA content 38-42%). Feed enrichment was carried out on a laboratory scale when the oil blends were added to the extruded feed crumbles by vacuum infusion techniques. For vacuum inclusion of oils, the method of configuration of rotary evaporator was used (Rotavapor 215; BÜCHI, Basel, Switzerland). The feeding treatment started in late March a day after the separation of fish into two groups. The feeding rate corresponded to the temperature. In that sense, from 22 to 18°C, fish were given 0.6% of total biomass per day; from 18-11°C, fish were given 0.3% of total biomass per day three days per week; at 10°C and lower, fish were given 0.3% of total biomass per day, two days per week. Prior to the injections, fish were not fed for seven days.

Feed enrichment was done on a monthly basis during the trial. Fatty acid composition analysis was carried out on samples from both ARA and CONTROL feed on three occasions: at the beginning of the trial, two months after the start of the trial, and four months after the start of the trial.

2.5 Fatty acid analysis of feed and eggs

Five grams of freshly stripped dry eggs were taken from all females from each group for each spawning, put into separate vials (one vial for each female), and stored at -80 ºC until analysis.

Lipids were extracted from the samples with a 2:1 mixture of chloroform and methanol. The extracts were purified according to the method by Folch et al. (1957). Aliquots of total lipid samples were trans-esterified using a methanolic solution of HCl (Stoffel et al., 1959). Fatty acid methyl esters (FAME) were separated on fused silica capillary columns (DB-225) in an AGILENT (HP) gas chromatograph system (type "6890N") equipped with a Flame Ionisation Detector (FID) and a mass spectrometer (MS) detector (MSD, type "5973N"). FAME were identified using authentic primary (SUPELCO, Bellefonte, NJ) or secondary standards (e.g., linseed oil, cod liver oil) and by means of the relationship between the logarithms of relative retention times and the carbon number (Cn) of fatty acids. Fatty acid concentrations are expressed as a weight percentage of the FA sample, as assessed by the relative response factor (RRF) and molar concentration of FAME (Ackman and Sipos, 1964a; 1964b).

2.6 Statistical analysis

Data are presented as mean ± standard deviation (SD). Prior to analysis, all the percentage variables were arcsine transformed. Normality assumption for all the variables was checked using the Shapiro–Wilk's test. In cases of two-group comparisons, the differences between the groups were evaluated using the Student's T-test in case of a normal distribution; otherwise, the Mann-Whitney U test was used. In the case of four group comparisons, the homogeneity of variances was additionally checked with the Levene's test. The variables which fulfilled the requirements for one-way ANOVA were further evaluated together with Tukey's post-hoc test, while otherwise non-parametric Dunn test was used to evaluate the differences between the groups. The significance level was set to $p \leq 0.05$. Analyses were performed using JASP 0.12.2 (JASP Team, 2020).

3. Results

3.1 Oocyte growth

The measured diameter of oocytes in all females after 4, 5, 6 and 7 months of trial are presented in Figure 2. While at each following sampling point oocytes diameter was significantly higher than previously, no significant differences were noticed between the dietary groups in any of the sampling points. In each of the sampling points, all of the oocytes were in stage I (germinal vesicle centrally positioned, no visible coalescence of oil droplets).

3.2 Reproductive performance

At the second sampling point five months since the start of the trial oocyte diameter of injected females was 848 ± 36 μm and 867 ± 25 μm in ARA and CTRL group, respectively. None of the females ovulated upon hormonal induction. At the latter two sampling points, six and seven months since the start of the trial, all and all but one female ovulated upon the hormonal injection in CTRL and ARA groups, respectively. Mean oocyte diameter range in injected females was 920-975 μm and 974-1030 μm and in CTRL1 and CTRL2, respectively, while in ARA1 and ARA2 groups the range was 912-930 μm and 924-1018 μm, respectively.

In both reproduction occasions, the embryo survival rate was higher in CTRL groups; however, only in the second reproduction batch difference was significant ($p = 0.045$; Table 2.; Figure 3.). On the basis of the both reproduction batches, embryo survival rate was significantly higher ($p = 0.036$) in CTRL group (35.7 ± 17.1 %) compared to ARA group (18.6 ± 13.6 %).

In terms of induction duration, besides the oocyte diameter, no significant differences were seen with this regard in any of the parameters.

3.3 Fatty acid composition of diets and dry eggs

Considering the diet FA profiles, the two FAs whose shares were significantly altered were 18:2n-6, linoleic acid (LA) and 20:4 n-6 AA, the first being significantly lower and the second significantly higher in the experimental diet in comparison to the control (Table 1.). The FA profiles of dry eggs revealed significant differences in terms of both induction duration and AA supplementation. In the case of the CTRL group, the duration of spawning induction affected total highly unsaturated fatty acids (HUFA) which were significantly lower in CTRL2. Among specific HUFAs, their share differed in 20:3n-6 dihomo-gamma-linolenic acid only, being lower in the reproduction batch 2. In the ARA group, two disagreements were found among MUFAs. Namely, 16:1n-9 hypogeic acid and 16:1n-7 palmitoleic acid concentations reduced with time. Considering the differences between the diets, in the reproduction batch 1, a single disagreement in 16:1n-7 was found among MUFAs, while three n-6 PUFAs differed between the groups. Out of these FAs, only AA was lower in the CTRL1 group. In case of MUFAs in the reproduction batch 2, only one discrepancy was found in 14:1 n-5, and unlike in the first reproduction, this MUFA was higher in the CTRL2 group. In the case of PUFAs differences between the feed treatments in the reproduction batch 2, a single difference was noticed, AA being lower in the CTRL2. In terms of relationships between the FAs, similarly to the feed, EPA/AA was significantly lower in eggs obtained in the ARA group in both reproduction batches. The FA profiles of dry eggs batches are presented in Table 3.

4. Discussion

Defining optimal oocyte developmental stage for artificial reproduction is of crucial importance for the feasibility of the protocol. In case of seasonal pikeperch reproduction, stadiums of final oocyte maturation (FOM) are well elaborated and practically useful tool for this instance (Żarski et al., 2012). However, to answer the question what is the optimal oocyte feature to hormonally induce ovulation in breeders under fully controlled conditions, the FOM classification is of limited usage. Namely, a recent study on the subject by Żarski et al. (2019) found the only commercially applicable procedure for out-of-season pikeperch reproduction could be performed in females with oocytes in stage I, thus prior to germinal vesicle migration and start of the coalescence of oil droplet. Indeed, in the present study, only the FOM stage I was observed in virgin females prior to hormonal treatment. From another side, pikeperch individuals intensively reared in outdoor conditions are rather plastic to the time of application of hormonal induction. Thus, a recent study showed that FOM and ovulation can be induced a few months prior to the natural spawning season with high egg and larval quality (Ljubobratović et al. 2019a). Upon completion of vitellogenesis, oocytes are able to enter their final developmental phase, maturation. Based on the

present study oocyte stage of full vitellogenesis is reached at diameters above 900 μm as overgrowing this critical size oocytes were able to enter the FOM. A month-long exposure to the photothermal induction led to significant increase in oocytes'diameter, however no significant differences were visible in any of the reproductive parameters. Likewise, no progress in oocyte maturation was visible as in both occasions oocytes in all analyzed females were in FOM stage I. Therefore, the oocyte diameters of 900-1000 μm can be suggested as appropriate for artificial reproduction. These values of oocyte diameter agree with earlier reported values in wild breeders at various FOM stages during the spawning season (Żarski et al., 2012). With respect that viable gametes were obtained at two reproduction occasions with one month duration difference in spawning induction, it appears that RAS-reared virgin females maintain a certain level of plasticity towards the time of hormonal injection as was previously described for outdoor reared pikeperch.

The quality of an egg is defined as its potential to develop into a normal embryo (Bobe and Labbé, 2010). The embryo survival obtained in CTRL group is in range with previous studies on cultured pikeperch after the first wintering in semi-controlled (Zakęś et al., 2013) and outdoor conditions (Ljubobratović et al., 2017). Thus, it appears that eggs of such reproductively inexperience fish are generally characterized with a high variability and overall low survival. With such sensitivity, this material can be described as highly informative as the reproduction performance in older classes is in general more advanced and stable (Zakęś et al., 2013; Żarski et al. 2019). Another parameter of high interest with respect to the state of readiness of breeders for the hormonal injection is the latency time, the duration from injection until ovulation. In the case of breeders kept under fully controlled conditions, a unique study from Żarski et al. (2019) found this parameter as the most prevalent one to forecast the female's reproductive performance. Namely, this study showed that breeders which ovulated faster upon injection yielded eggs of diminished quality. In that sense, the border for the low quality of eggs was set at 5 days at 12 °C. In the present study, the pattern was similar as the only reproduction batch with the mean latency time below this border was ARA2 which yielded the eggs with the lowest embryo survival. With respect that embryo survival in CTRL2 was significantly higher, this relationship agrees with the earlier mentioned study. As earlier stated, arachidonic acid is a precursor of PGE2, and both of them are described as promotors of both FOM, specifically germinal vesicle breakdown, and ovulation (Sorbera et al., 2001, Henrotte et al., 2011). Therefore, this physiological pathway might explain the adverse effect of increased dietarry AA on egg quality.

Both the duration of the spawning induction and the AA dietary level had effects on the FA composition. The main FA of interest, AA did not change in content with time, however was significantly higher in ARA group in both reproduction batches. This accumulation of AA is in agreement with previous studies on tongue sole *Cynoglossus semilaevis* (Xu et al., 2017), Senegalese sole *Solea senegalensis* (Norambuena et al., 2013). and Sweet smelt *Plecoglossus altivelis* (Jeong et al., 2002). Thus, this feature of the affinity of gonads to accumulate AA seems conservative to many teleost fish and is to be considered for future studies. When it comes to total HUFAs, they were stable with time in the ARA group, which was not the case in CTRL. These FAs reduced significantly with time in the CTRL group, while the group fed the AA enriched feed was rather stable. Considering that this group of fatty acids is perhaps the most studied and acknowledged with regard to the egg and larvae quality (Bell et al., 1997; Furuita et al., 2000; Mazorra et al., 2003), the FA profiles in eggs obtained from the ARA group might be considered as more favourable. From the other side, there is obviously some physiological process which was disturbed with AA supplementation, perhaps connected to the hormonally induced FOM process, which should be of interest for future studies. Therefore, it might be speculated that a possible favourable effect of AA on egg and larvae quality was diminished by its long and/or untimely administration. Correspondingly, the study on tongue sole (Xu et al., 2017) showed that AA supplementation was more beneficial for immature than mature females and vice versa for males. Therefore, future studies need to address the AA supplementation in pikeperch with concern for the phase of gonadal development.

This study, for the first time in pikeperch, addresses the effects of spawning induction and nutrition on the success of artificial reproduction in virgin pikeperch females kept under fully controlled RAS conditions. The results allow us to conclude that females having oocytes with a diameter over 900 μm may enter the FOM process and ovulation triggered with the use of hormonal treatment. Likewise, we can conclude that induction of FOM and ovulation may be extended over the period of one month with a mean oocytes diameter around 1000 μm. Contrary to the hypothesis, levelling dietary AA to EPA negatively altered the reproductive performance, reducing embryo survival. Therefore, further studies targeting this FA should be more systematic in means of extent and time of application with respect to the gonad development.

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Figure 1. Photo-thermal spawning induction schedule and mean size of the oocyte during the induction.

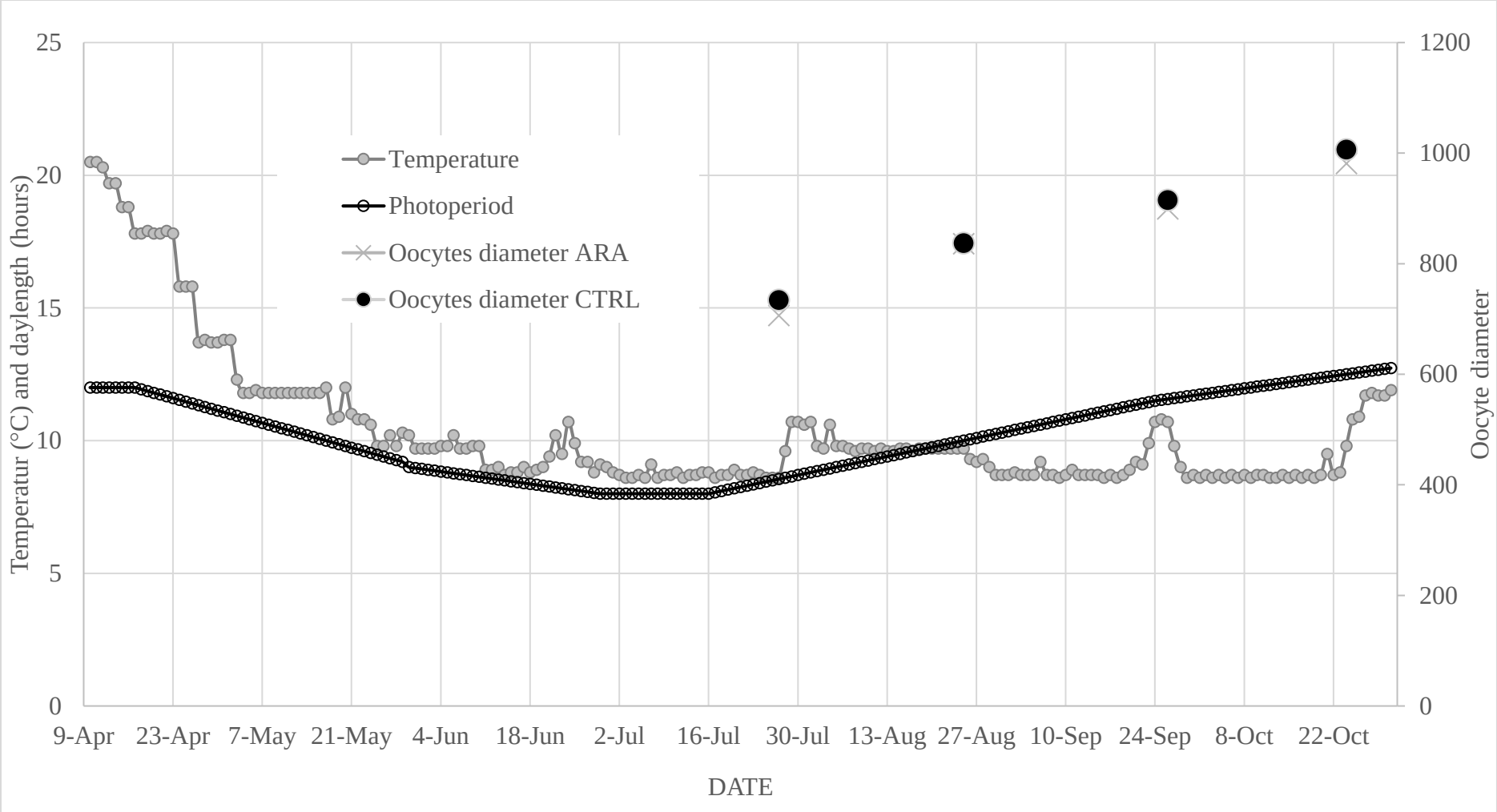


Figure 2. Box plot diagrams of oocyte diameters in dietaty groups enriched with arachidonic acid (ARA) and control (CTRL) after four (July), five (August), six (September) and seven (October) months since the start of the trial.

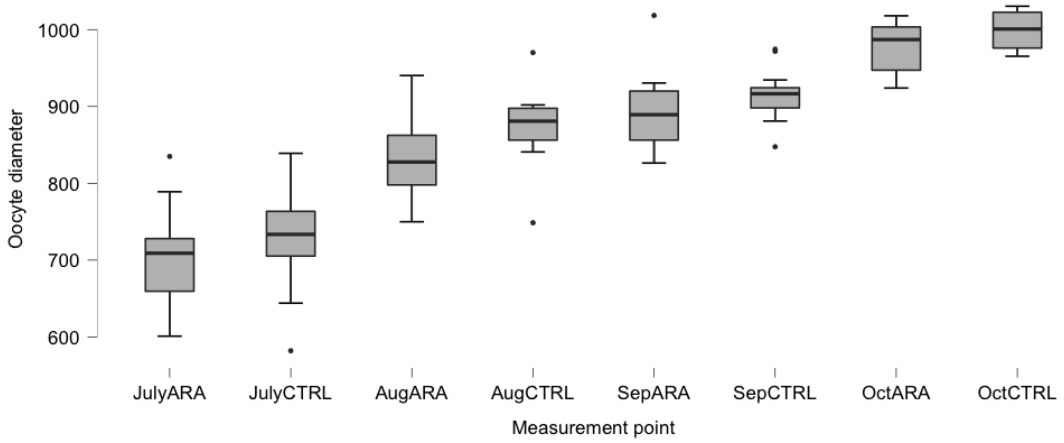
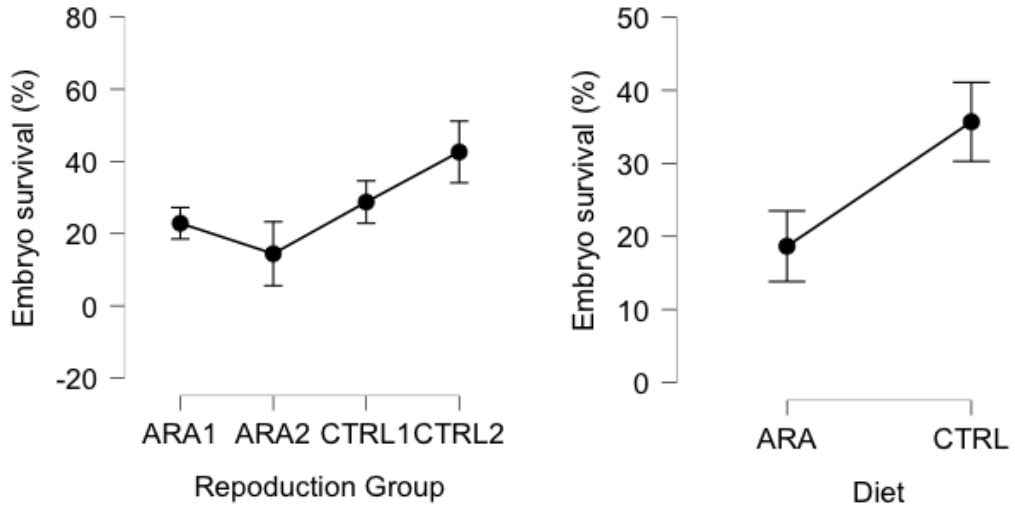


Figure 3. Embryo survival in each reproduction batch (left) and for both reproduction batches (right) with respect to different dietary arachidonic acid level.



Plots are presented as mean ± standard error;

Table 1. Fatty acid profile of control feed (Coppens REPRO) and treatment feed (ARA) enriched with 40% concentrated arachidonic acid oil (ARASCO™) at a concentration of 60 mg kg^{-1}

Fatty acid	CTRL	ARA
14:0	2.4 ± 0.2	2.4 ± 0.1
16:0	12.1 ± 0.2	12.5 ± 0.3
18:0	2.7 ± 0.1	3.1 ± 0.3
22:0	1.2 ± 1.0	1.0 ± 0.9
Total SFA	18.5 ± 0.4	19.3 ± 0.3
16:1n-7	3.3 ± 0.2	3.1 ± 0.2
18:1n-9	33.9 ± 1.2	32.6 ± 0.8
18:1n-7	3.3 ± 0.9	3.2 ± 0.9
20:1n-9	2.8 ± 0.2	2.5 ± 0.1
Total MUFA	46.8 ± 2.5	45.3 ± 2.6
18:2n-6*	15.6 ± 0.2	15.2 ± 0.2
20:4 n-6*	0.4 ± 0.0	3.0 ± 1.1
Total n-6*	17.1 ± 0.5	19.5 ± 1.3
18:3n-3	4.5 ± 0.0	4.0 ± 0.1
20:5n-3	3.1 ± 0.2	2.9 ± 0.2
22:6n-3	4.2 ± 0.3	3.8 ± 0.5
Total n-3	14.1 ± 0.7	12.7 ± 0.2
Total PUFA	31.6 ± 0.5	32.8 ± 0.8
Total n-3/Total n-6	0.7 ± 0.2	0.6 ± 0.1
EPA + DHA	7.3 ± 0.5	6.8 ± 0.7
DHA/EPA	1.3 ± 0.0	1.3 ± 0.1
EPA/AA	8.4 ± 0.5	1.1 ± 0.3
HUFA	10.1 ± 0.9	12.1 ± 1.8

Data are presented as the mean ± SD;
*Parameters with significant difference ($p \leq 0.05$) are marked with superscript;
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acids

Table 2. Reproductive performance in pikeperch females in the spawning batches 1 and 2 of CTRL and ARA groups injected six and seven months after the initiation of trial.

Parameter	CTRL1	ARA1	CTRL2	ARA2
Weight (kg)	1.0 ± 0.4	1.1 ± 0.2	0.9 ± 0.2	1.1 ± 0.1
Oocyte diameter (μm)	934 ± 24[a]	923 ± 7[a]	1006 ± 26[b]	981 ± 42[b]
Ovulated females (%)	100	80	100	80
Latency time (days)	10.0 ± 3.2	11.5 ± 3.3	6.2 ± 4.5	4.8 ± 1.5
PGSI (%)	7.0 ± 2.5	5.1 ± 1.8	7.6 ± 2.9	5.7 ± 3.3
Embryo survival (%)	28.7 ± 13.1[a,b]	22.9 ± 8.7[a,b]	42.6 ± 19.1[b]	14.4 ± 17.7[a]
Hatching (%)	96.6 ± 1.1	95.3 ± 3.9	90.0 ± 6.5	95.3 ± 3.8
Length of larvae (mm)	4.6 ± 0.2	4.5 ± 0.4	4.6 ± 0.1	4.4 ± 0.2

Data are presented as the mean ± SD; A different letter in superscript represents a significant difference ($p \leq 0.05$) between the treatments.
PGSI, pseudo-gonadosomatic index

Table 3. Fatty acid profiles in ovulated eggs stripped from spawning batches 1 and 2 of CTRL and ARA groups injected six and seven months after the initiation of the trial.

Fatty acid	CTRL1	ARA1	CTRL2	ARA2
16:0	3.4 ± 0.2	3.4 ± 0.4	3.5 ± 0.5	3.6 ± 0.5
Total SFA	5.8 ± 0.6	5.5 ± 0.4	5.9 ± 0.9	5.9 ± 0.5
14:1 n-5	1.4 ± 0.2^{a,b}	1.3 ± 0.1^{a,b}	1.5 ± 0.1^{b}	1.3 ± 0.1^{a}
16:1 n-9	0.9 ± 0.1^{a,b}	1.0 ± 0.1^{b}	0.9 ± 0.0^{a,b}	0.9 ± 0.1^{a}
16:1n-7	3.0 ± 0.6^{a}	3.9 ± 0.7^{b}	3.3 ± 0.2^{a}	3.0 ± 0.5^{a}
18:1n-9	13.7 ± 0.6	13.9 ± 0.7	14.5 ± 0.5	13.9 ± 0.4
18:1n-7	1.4 ± 0.3	1.6 ± 0.3	1.3 ± 0.1	1.3 ± 0.2
Total MUFA	22.7 ± 1.4	24.1 ± 1.2	24.2 ± 1.3	22.6 ± 0.8
18:2n-6	14.5 ± 0.6	14.2 ± 0.8	13.9 ± 0.4	14.6 ± 1.1
18:3n-6	16.7 ± 1.8^{b}	14.5 ± 0.8^{a}	15.4 ± 1.3^{a,b}	15.4 ± 1.0^{a,b}
20:3n-6	1.3 ± 0.1^{b}	1.1 ± 0.1^{a}	1.1 ± 0.1^{a}	1.2 ± 0.1^{a,b}
20:4n-6	0.5 ± 0.1^{a}	1.1 ± 0.1^{b}	0.4 ± 0.1^{a}	1.3 ± 0.1^{b}
Total n-6	33.7 ± 2.0	31.6 ± 1.2	31.4 ± 1.9	33.2 ± 2.0
20:5n-3	1.9 ± 0.2	1.8 ± 0.3	1.6 ± 0.3	2.0 ± 0.2
22:6n-3	10.0 ± 1.2	10.6 ± 0.7	8.6 ± 1.1	10.4 ± 0.9
Total n-3	13.6 ± 1.4	14.1 ± 1.1	11.9 ± 1.6	14.2 ± 1.2
Total n-3/Total n-6	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Total PUFA	47.5 ± 2.0	51.6 ± 7.8	43.9 ± 2.6	47.7 ± 2.0
EPA + DHA	11.9 ± 1.3	12.4 ± 0.9	10.2 ± 1.5	12.4 ± 1.1
HUFA	16.0 ± 1.4^{b}	17.0 ± 1.2^{b}	14.3 ± 1.1^{a}	17.5 ± 1.2^{b}
EPA/AA	3.9 ± 0.7^{b}	1.6 ± 0.2^{a}	4.0 ± 0.2^{b}	1.6 ± 0.2^{a}
DHA/EPA	5.3 ± 0.4	6.0 ± 0.5	5.4 ± 0.5	5.2 ± 0.2

Data are presented as the mean ± SD; A different letter in superscript represents a significant difference ($p \leq 0.05$) between the treatments.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acids

Ljubobratović Uroš, Kwiatkowski Maciej, Tóth Flórian, Demény Ferenc

A vízmelegítési fázis megkezdése előtti hormonkezelés hatása az ívási idő szinkronizálására, a petesejt méretére és az ikraminőségre süllő esetében

Tanulmány háttere és célja

- A kutatás általános célja volt, hogy a süllő szezon előtti szaporítási protokollja egyszerűbb legyen, kisebb hormonkezelési költséggel és rövidebb ívási idővel
- Általános gyakorlat, hogy a tavakról behozott anyaállományt előbb felmelegedő hőmérsékleti viszonyok mellett tartják, és csak akkor oltják hormonnal, amikor a kívánt hőmérsékletet (10-14 °C-fok) eléri. Ez az eljárás volt a kísérletben kontrollként alkalmazva.
- Ezzel szemben egy olyan alternatív eljárásnak vizsgáltuk az ovuláció szinkronizálására gyakorolt hatását, amelyben a lazac GnRHa hormonnal történő oltás időpontját előbbre hoztuk az anyaállomány behozatala napjára és ezt követően emeltük a vízhőmérsékletet

Tanulmány főbb eredményei

- A vízmelegítési folyamat megkezdése előtt beadott GnRHa oltással jobb ívás-szinkronizációs teljesítményt lehet elérni anélkül, hogy az ikraminőség romlana
- Ezzel az eljárással a süllő ívási ideje lecsökkenthető 10 napra az anyaállomány behozatalának napjától számítva
- Vizsgálták különböző hormondózisok hatását a szaporítás sikerére, de nem találtunk szignifikáns különbségeket az alacsonyabb és magasabb dózisok hatása között
- A fentiek alapján, optimalizált protokoll gyanánt azt javasoljuk, hogy a süllő anyák oltása azonnal a keletetőházba szállítás után megtörténjen testsúly kg-onkénti 5 µg lazac GnRHa hormon alkalmazásával, és ezt követően legyen a rendszer naponként 1 °C-fokkal melegítve, amíg az el nem éri a 10 °C-fokot.
- Fontos eredménye a tanulmánynak az is, hogy a fiatal anyák esetében a petesejt mérete az oltás időpontjában erősen (pozitív módon) korrelál az embriómegmaradással
- A 900 µm-nél kisebb méretű petesejttel rendelkező anyák esetében a szezon előtti szaporítás már nem vezet embriómegmaradáshoz

Az összegzés az alábbi cikkből készült:

Ljubobratović Uroš, Kwiatkowski Maciej, Tóth Flórian, Demény Ferenc: Effects of hormonal treatment before water warming on synchronisation of spawning time, oocyte size, and egg quality in pikeperch (Sander lucioperca) ANIMAL REPRODUCTION SCIENCE 226 Paper: 106712 (2021)

Injection before warming synchronizes the spawn while oocyte size determines the egg quality in pre-seasonal pikeperch (*Sander lucioperca*) reproduction

Uroš Ljubobratović[#], Maciej Kwiatkowski, Ferenc Demény

Research Institute for Fisheries and Aquaculture (NAIK-HAKI), Anna-liget 35, 5540 Szarvas, Hungary
Corresponding author, e-mail: u.ljubobratovic@gmail.com

Uroš Ljubobratović, Research Institute for Fisheries and Aquaculture NARIC HAKI, Anna Liget 8, 5540 Szarvas, Hungary. Tel: +36 70 440 31 33; Fax: 36 66 312 142; E-mail: u.ljubobratovic@gmail.com

ABSTRACT

Out-of-season reproduction presents important protocol for the feasibility of intensive fish farming. Pikeperch (*Sander lucioperca*) is among most promising candidates for fully controlled aquaculture production in Europe and is often subject of studies on the control of reproductive cycle. Pre-seasonal pikeperch propagation takes places two-three months prior to natural spawning season. The present study aimed to assess effect of thermal schedule and salmon gonadotropin releasing hormone analog (sGnRHa) dosage on final oocyte growth, egg quality, protocol duration and spawning synchronization. Two trials were conducted. The preliminary trial analyzed the effect of thermal schedules, either injection prior or after the warming (WARMING vs. STABLE, respectively), while following trial assessed the sGnRHa dosage (5, 25 and 50 μg kg^{-1}) for the warming schedule. Both trials analyzed the oocyte diameter from the time of injection until late stage of maturation. Reproductive performance was high and stable in the first trial (embryo survival 79.7 ± 12.6 % and 79.5 ± 2.7 % in WARMING and STABLE regime, respectively). Spawning was better synchronized in WARMING schedule reducing all measures of variability in latency time 3 to 6 times depending on measure. In second trial, reproductive performance parameters were variable among groups without any significant differences among treatments. Oocyte diameter at the time of injection was strongly correlated with embryo survival (r = 0.647, P = 0.005) what was further confirmed through analysis of covariance (F = 7.169, P = 0.019). With respect to proven effect of oocyte diameter, robust measures of variability were decisive for optimal sGnRHa dosage in terms of spawn synchronization and thus 5 μg kg^{-1} was found favorable. Selection of breeders according to oocyte diameter, injecting straight upon transport to hatchery with 5 μg kg^{-1} following with 1 °C day^{-1} thermal increment until 10 °C is advised for effective pre-seasonal pikeperch propagation.

1. Introduction

Modern animal farming vitally relies on out-of-season reproduction for year-round supply of market (Fatet et al., 2011; Abecia et al., 2012; Zhu et al., 2017). Recently, it is similar for fish with respect to intensification trend in aquaculture (Edwards et al., 2015; Little et al., 2018; Wilfart et al., 2018). Out-of-season reproduction in fish commonly implies control over the reproductive cycle manipulating environmental cues responsible for triggering the gonadal maturation (reviewed by Mylonas et al., 2010). During the last few decades, a species of increasing interest for farming is pikeperch (*Sander lucioperca*). This is the iteroparous fish with seasonal capital spawning strategy (Feiner, & Höök, 2015). Hence, to obtain viable gametes out-of-season, rearing of a single batch of breeders usually takes place in fully controlled conditions where the management over the reproductive cycle is carried away year-round for a single spawning occasion per year (Fontaine et al., 2015). Therefore, for a constant supply of a material, the number of broodstocks per farm is determined according to needed frequency of stocking material. Presently, pikeperch farming is performed in recirculation aquaculture system (RAS) exclusively (Dalsgaard et al., 2013; Steenfeldt et al., 2015; Policar et al., 2019). Due to high level of environmental control, this system requires continuous stocking and harvesting regime in order to optimize the production from both technological and economical aspects (Watten et al., 1992; Summerfelt et al., 1993). It implies numerous year-round stocking of new juveniles' batches. Pikeperch is a temperate fish and as such requires cold phase for development of its gonads (Wang et al., 2010) what requires additional economical consideration with respect to costs of such year-round thermal manipulation (Hermelink et al., 2011). However, exploring the reproductive biology in wild pikeperch, particularly targeting out-of-season reproduction, early studies realized a rather high potential of this species for prolongation of the period of year when wild fish can be effectively propagated (Zakęś and Szczepkowski, 2004; Rónyai, 2007). Thus, up to three months prior to natural spawning season, breeders were transported from non-controlled conditions into controlled environment and with two to four weeks of photo-thermal manipulation followed with hormonal injection, these fish would ovulate/spermiate yielding high quality

gametes. Further on, the same pattern was demonstrated in domesticated breeders farmed in outdoor units (Ljubobratović et al., 2019). Although not seasonal, this reproduction takes place in a time frame naturally controlled (winter) and therefore instead of out-of-season, such operation is usually termed pre-seasonal or advanced. This specific feature of pikeperch's reproductive biology is from that reason exploitable as it enables three-month-long period when a single batch of breeders kept outdoor can be propagated leading to meaningful cost reduction of broodstock management.

Artificial reproduction in percid fish usually involves hormonal induction of final stages in gonadal development, thus in case of females, final oocyte maturation (FOM) and ovulation (Żarski et al., 2015). With respect to the subject of interest - advanced or pre-seasonal reproduction, the oocyte developmental period preceding FOM, vitellogenesis, is of significant importance. Vitellogenesis in pikeperch is characterized with a relatively sharp increase in oocyte diameter over the course of several months (Hermelink et al., 2017). Thus, the first question which arises is: Taking place two-three months prior to natural season, would the oocyte diameter at the time an upon the injection matter for the pre-seasonal reproduction? From another side, maturation in pikeperch in natural conditions usually takes place during early spring water warming (Lappalainen et al., 2003, Żarski et al., 2012) and growth of the oocyte proceeds even during the FOM until the germinal vesicle breakdown (Tyler and Sumpter, 1993). However, in so far trials on pre-seasonal reproduction, thermal manipulation, namely water warming, took place prior to injection while oocytes still did not start the FOM (Zakęś and Szczepkowski, 2004; Ljubobratović et al., 2019) Therefore, the next question which rises is if warming prior to the injection is necessary and if earlier injection would affect the final oocyte growth and outcome of reproduction? So far attempts for advanced pikeperch spawning were done in thermal regime from 12-18 °C (Zakęś and Szczepkowski, 2004; Rónyai, 2007) what involves rather high temperature gradient from winter ambient (usually about 4 °C) at the time of transport to hatchery. With respect that the warming was done in a manner 1-2 °C daily followed with a period of stable spawning temperature prior to injection, it took three to five weeks period in controlled environment prior to ovulation would finally occur. Thus, the query raising is if this time frame could be reduced by manipulation on lower thermal regime excluding thermal conditioning between the transport and hormonal injection? Finally, the nature and dosage of hormonal preparation are of significant importance. Recently, two hormonal preparations are commonly used in pikeperch hatcheries, human chorionic gonadotropin (hCG) and salmon gonadotropin releasing hormone analogue (sGnRHa). Recent study on out-of-season pikeperch found both hormonal types and their dosages to have insignificant effect on egg quality (Żarski et al., 2019). However, our recent research on this aspect lead us to conclusion that sGnRHa stabilizes the success in less experienced breeders in pre-seasonal reproduction (Ljubobratović et al., 2019). Likewise, compared to hCG, the gonadoliberins were explained as less disturbing for the fish endocrine and immune systems as well as stress response (Falahatkar and Poursaeid, 2014; Żarski et al., 2020). While the hormonal dose was examined in case of gonadotropins (Zakęś and Szczepkowski, 2004), the gonadoliberins were not evaluated yet for pre-seasonal reproduction, so its dose one of the subjects of the present study.

Although the sGnRHa might be beneficial for the fish welfare, due to better spawn synchronization hCG becomes more attractive solution for hatcheries. In so far studies, hCG was found to be more efficient with this regard substantially reducing the deviation among pikeperch females in time duration from injection to the ovulation – latency time (Křišťan et al., 2013; Żarski et al., 2019). Likewise, in our previous study on this subject, time duration from the first to the last ovulated female was only 24 hours in hCG treatment, while in case of sGnRHa this time frame was 96 hours (Ljubobratović et al., 2019). With respect to the increased workload which follows the procedure of pikeperch artificial reproduction (Żarski et al., 2015; Ljubobratović et al., 2018) it leads to the conclusion that using sGnRHa leads to higher labor demand, constraining aspect of European aquaculture (Bostock et al., 2009; Nielsen et al., 2016; Gyalog et al., 2017). Therefore, reduction of timeframe from the first to the last ovulation within the batch is of significant importance for hatcheries' sustainability. Hence, the protocol of interest should secure high reproductive success, yet respecting the economical demands, namely reducing the period of increased labor demand.

The present study aimed to improve the effectiveness of pre-seasonal pikeperch reproduction examining different thermal regimes and hormonal dosages with special attention on oocyte diameter at the time of injection and its further growth. Thus, the preliminary trial aimed to evaluate the effect of thermal regime in means of hormonal injection prior to or after the warming on the oocyte growth dynamics, spawning synchronization and egg quality. The second trial aimed to define optimal sGnRHa dosage for the new thermal method with special emphasis on oocyte diameter at the time of injection.

2. Materials and methods

Fish handling was performed according to the regulations of the Animal Ethical Panel of the Institute, which was established according to Hungarian State law (10/1999.I.27.). Fish were anaesthetized prior to hormonal injection, papilla suture and gamete collection in a 0.3 mL L^{-1} solution of 2-phenoxyethanol. Prior to injection, hormones were diluted in the 0.65% NaCl solution in such a manner that each fish received 1 mL hormonal solution per kg of bodyweight.

2.1. Broodstock management

Breeders used for the study are a part of the gen-bank of Research Institute for Fisheries and Aquaculture (NAIK-HAKI) which are kept in intensive outdoor pond conditions fed with dry broodstock diets. Breeders used for the first trial in 2018 were class 2013, kept in outdoor conditions since and twice reproduced in 2016 and 2017. Breeders used for the second trial in 2019 were class 2016, kept in outdoor conditions since summer of 2017 not reproduced before. All the procedures were carried out in the NAIK-HAKI facilities. Natural photothermal conditions from September until the artificial reproduction are presented in Figure 1.

2.2. Trial 1 design – preliminary testing of new thermal protocol

On January 11, 2018, twelve pairs of breeders were harvested from outdoor pond-unit and transported into the spawning recirculation aquaculture system (RAS) composed of three 4 m^3 tanks. Outdoor and indoor temperature were equalized on 5 °C. Upon transport, randomly chosen six females (group WARMING) were hormonally treated with sGnRHa [D-Arg6,Trp7,Leu8,Pro9-NEt]-GnRH (Ova-RH; Syndel Laboratories Ltd., Canada) in a dose 5 μg kg^{-1}. This low dosage was chosen with assumption that together with temperature increment it will be trigger strong enough to induce FOM and ovulation. Four days later, on temperature of 9 °C, males were treated with the same agent in a dose 25 μg kg^{-1}**.** Finally, five days upon first batch, when temperature reached 10 °C the rest six females were hormonally treated with the same agent in a dose 50 μg kg^{-1} (group STABLE). This dose was chosen as previously recommended in case of constant thermal regime following injection (Żarski, et al., 2019).

2.3. Trial 2 design – evaluation of hormonal dosage for the new thermal protocol

Due to disagreement in hormonal dosages in Trial 1 treatments, Trial 2 was designed in such manner to assess the effect of different hormonal dosage on the reproductive success and spawn synchronization in the WARMING thermal schedule. On January 24, 2019, and pond temperature of 3 °C, 18 females and 12 males were transported into the spawning RAS. Temperature in RAS at the time of transport was 4.4 °C. All the fish were hormonally treated with sGnRHa at the time of stocking into RAS. All males received 25 μg kg^{-1}. Three different hormonal dosages were randomly given to all females, 5, 25 and 50 μg kg^{-1}, thus forming three groups – 5, 25 and 50 – each consisted of six females.

2.4. Thermal regime

In both trials, a common thermal regime was kept. Upon transport to the spawning RAS, temperature was kept stable for a day and further on increased for 1 °C per day. Once the temperature reached 10 °C, temperature was kept stable further on. In Trial 1 all the fish ovulated at 10 °C. However, this was found not practical with respect that the time from stage VI – germinal vesicle breakdown (Żarski et al., 2012) until ovulation was rather excessive, up to 36 hours. Therefore, for the Trial 2., once the female reached stage VI, it was anaesthetized in the water of 11 °C and upon suture stocked into the 12 °C tank until ovulation. In this case, all the fish ovulated within 24 hours upon suture.

2.5. Artificial reproduction procedure

The protocol of artificial reproduction was performed as described by (Ljubobratović et al., 2018). Evaluation of FOM stage following the catheterizations of oocytes using catheter was done at the time of injection and all the fish were in stage I (Żarski et al., 2012). In trial 1, second biopsy was done 120h post-injection and

further on each 24 hours until stage VI. In trial 2, second biopsy was done 96h post-injection and further on each 24-48 hours. Upon oocyte clarification in Serra solution (a mixture of 96% alcohol, 35% formalin and glacial acetic acid in a ration 6:3:1, respectively) FOM stage was evaluated. About 30 minutes after the sampling, oocytes were photographed using a Nikon ShuttlePix P-400R microscope (Nikon Corporation, Tokyo, Japan) with 20x magnification. Images were further processed using Nikon ShuttlePix Editor Ver3.4.0, and the mean oocyte diameter of each fish was assessed on the sample of 20 oocytes per fish. In Trial 1 oocyte diameters were evaluated at the time of transport to the spawning room (injection day for group WARMING), upon warming five days later (injection day for group STABLE) and on the day mean FOM stage of the group was V. In trial 2, oocyte size was evaluated after each biopsy until the first ovulation was noticed.

At the time oocytes reached the stage VI, fish genital papilla was sutured as explained by Żarski et al. (2017) and further on the ovulation was checked each 6 hours. Upon ovulation, females were stripped and eggs were fertilized with milt of two males in ration 1mL of milt per 100g of eggs. Egg adhesiveness removal was done using milk and kaolin solution (Ljubobratović et al., 2019) and finally around 40 minutes post-fertilization eggs were stocked in Zug jars. Eggs of each female were stocked in separate incubator. The eggs were then left to settle and the initial level of eggs in the jar was marked. During incubation, eggs were carefully cleaned from dead eggshells. Thus, 72 h post-fertilization, eggs were once again left to settle, and the level of eggs was marked again. At the same time, three samples of 100-200 eggs were taken for microscopic evaluation (20× magnification) to assess the proportion of live embryos in the jar. The commercial survival in jars was calculated based on the following equation:

$$\text{Commercial survival} = \frac{\text{volume of eggs 72 hours after fertilisation}}{\text{volume of eggs at the time of stocking into Zug jar}} * \text{mean percentage of live eggs}$$

When hatching was noted in the jar, a sample of 100 live eggs from each jar was stocked into separate Petri dishes (size 60 × 10 mm) placed in the 0.5 L plastic bowl filled with plain hatchery water. Bowls were placed in a room with a constant air temperature at 15°C. After placement in the hatching bowls, newly hatched larvae were harvested out of the bowls every 12 hours and counted in order to assess the hatching rate.

The Zug jar incubation set was supplied with aerated and well-oxygenated water in a flow-through system. Water temperature and the oxygen saturation of the outflow water were monitored every twelve hours. Mean water temperature during incubation was 14.4 ± 0.6 °C and 13.5 ± 0.6 °C, while the mean oxygen saturation was 114.7 ± 9.8 % and 110.9 ± 8.5 %, in Trial 1 and 2, respectively.

2.6. Statistical analysis

Data analysis and presentations were carried out in Microsoft Excel (Microsoft Corporation, 2018) and JASP 0.12.2 (JASP Team, 2020). All the percentage variables were arcsin transformed prior to analysis. The normality assumption was checked using the Shapiro–Wilk's test while the homogeneity of variances was validated with Levene's test.

In trial 1, comparison between the treatments was performed using Student's T-test or Mann-Whitney U test with respect to the distribution of the assessed variable. Due to technical problems arose in Trial 1, the parameters embryo survival, hatching rate and hatch index were evaluated in four replications, instead of six as was for the rest of the variables.

In Trial 2, linear relationship among the variables was evaluated using the Pearson's correlation. With respect that oocyte size was strongly correlated with embryo survival, further analysis was carried out using analysis of covariance (ANCOVA) for all the parameters using the oocyte diameter at the time of injection as the covariate. The level of significance was set on $P \leq 0.05$.

3. Results

3.1. Oocyte growth

In Trial 1, there were no differences in oocyte diameter upon transport between the groups and their mean size ranged from 905 to 938 µm among twelve females. Upon reaching 10 °C, females injected at the time of transport (group WARMING) had significantly larger oocytes ($P = 0.026$) compared to the non-injected females

in group STABLE (1000 ± 31 μm and 946 ± 27 μm, respectively). In stage V, no differences were seen between the groups being in both groups over 1 mm of mean diameter. The oocyte diameter and distribution during the trial are shown in boxplot diagrams (Supplementary figure 1)

In Trial 2, no differences in oocyte size were seen between the groups in any of the sampling points, although the trend was stable and oocyte diameter was highest in group 25 and lowest in group 50 in each of the sampling points (Figure 2). Upon transport, mean oocyte diameter ranged from 846 to 948 μm among 18 injected females. In agreement with Trial 1, mean oocyte diameter was over 1mm in mean stage V of FOM without differences between the treatments.

3.2. FOM dynamics, latency time and spawn synchronisation

All injected females in both trials were in FOM stage I at the time of injection and all but one ovulated. The single female which did not ovulate belonged to group 5 in Trial 2 and remained in FOM stage IV until the end of evaluation period of three weeks upon injection. In Trial 1, on days 6 and 7 post-injection significantly higher FOM index was in group STABLE (P = 0.006 and P = 0.017, respectively), while at all other sampling occasions there were no differences between the groups (Figure 3). In trial 2, there were no significant differences in FOM index among groups in any of the sampling points (Figure 4).

There were no statistically significant differences in latency time among the groups in both trials. Mean latency time in both trials ranged from 203 hours in group STABLE to 228 hours in group WARMING. While non-robust measures of variability, standard deviation and range were lowest in groups STABLE and 50, robust median absolute deviation and interquartile range showed lowest latency time variability in groups STABLE and 5. Latency time variability for both trials are presented in Table 1 and Supplementary Figure 2.

3.3. Reproductive performance

In Trial 1 there were no significant differences noticed between the groups. In general, embryo survival was rather high and stable ranging from 66.3 to 93.5 % in WARMING and 77.6 to 83.4% in group STABLE (Table 2).

Reproductive success in Trial 2 was in general rather variable and overall embryo survival among 18 females ranged from 0 to 86.6%, while ranges per hormonal dosage were 0-64.9 %, 18.1-86.6% and 0-76.2% in group 5, 25 and 50, respectively. There were no differences among the treatments in any of the analyzed parameters. The linear correlation analysis among the all assessed parameters showed strong relationship between the oocyte diameter at the time of injection and embryo survival (Table 3). Therefore, for the ANCOVA embryo survival was set as covariate and it showed significant on latency time, embryo survival and hatching index (Table 4).

4. Discussion

Based on the results of the present study, it appears that nor the thermal regime or the hormonal dosage affects the egg quality in pre-seasonal pikeperch artificial reproduction. These results agree with similar studies on hCG and sGnRHa dosages (Zakęś and Szczepkowski, 2004; Żarski et al., 2019) and on thermal regime (Rónyai, 2007; Żarski et al., 2013). However, in agreement with earlier studies including breeders with different reproductive experience (Zakęś et al., 2013; Żarski et al., 2019), young breeders showed high variability in terms of egg quality. Yet, unlike the previously mentioned studies, the present trials approached the egg quality from one additional aspect – oocyte diameter at the time of injection. With this regard, young breeders showed higher variability in mean oocyte diameters being three times greater compared to older group. The correlation analysis showed strong dependence between embryo survival and oocyte diameter at the time of injection in Trial 2, while covariance analysis found this parameter to explain significant share of variance for embryo survival (F = 7.169 and P = 0.019). Thus, to the best of our knowledge, this is the first report which witnessed that oocyte size at the time of injection is strong predictor of spawning success in pre-seasonal reproduction. Therefore, it may be concluded that selection for out-of-season spawning from among reproductively inexperienced females according to the diameter of their oocytes can improve the success of the operation.

The reproductive performance of females used in the first trial of the present study is among the highest reported thus far in intensively cultured pikeperch (reviewed by Żarski et al., 2015). This group of breeders was

already subjected to the artificial reproduction for the third consecutive year and based on so far concluded similar trials it appears to be the main reason for such high and stable eggs quality among females (Zakęś et al., 2013; Żarski et al., 2019). Likewise, younger and reproductively inexperienced females were once more shown to be characterized with variable spawning outcome (Ljubobratović et al., 2017; 2019; 2020). Therefore, in agreement with previous studies, the present study suggests usage of reproductively experienced females. However, it additionally points to increase of oocyte size variation in groups of younger breeders what consequently increased the variability of embryo survival (Supplementary Figure 3). Vitellogenesis precedes FOM and is characterized by most dynamic period of oocyte growth (Tyler & Sumpter, 1996; Hara et al., 2016). Fish in the present study ovulated in mid-January and early-February, what is for conditions of Hungary around two months earlier than natural spawning season. Hence, it is likely that in females with small oocytes vitellogenesis was not complete at the time of injection. Observing the relationship plot between the oocyte diameter and embryo survival in the second trial (Figure 5), a certain border can be noticed at 900 μm. Below this diameter there is no embryo survival. Similar border of readiness for artificial reproduction was recently found for indoor reared breeders searching for the effect that spawning induction duration might have on oocyte growth and spawning performance (Ljubobratović et al., 2020). However, in case of reproductively experienced fish, mean oocyte size was stable above 900 μm in all females, indicating that more experienced individuals are reaching the state of complete vitellogenesis faster and in yet more synchronized manner. In agreement with results of the present study on inexperienced fish, visible oocyte growth variation in young females was reported monitoring oocytes monthly in controlled environment by Ljubobratović et al. (2020). The fact that there might be an intra-group difference in oocyte growth dynamics either within one class or between the classes, indicates that there might be differences in moment when certain females reach state of readiness for artificial reproduction. Thus, either than communally, this study suggests individual approach towards evaluation of maturation state of a female breeder, especially with respect to the recruitment. This issue might be commercially utilized via reproduction of one broodstock maintained in a common system over the time-frame of 2-3 months, yielding two reproduction batches per stock. Therefore, future studies might approach the issue of different reproductive experience and intra-group variation in oocyte growth dynamics in order to optimize the spawning success and yet prolong the period in which a single broodstock can be reproduced.

According to the obtained results, it can be concluded that injection prior to warming where FOM took place in thermal range 5-10 °C and ovulation occurred on 10 and 12 °C is suitable thermal schedule for pikeperch pre-seasonal reproduction agreeing with recent study of Malinovskyi et al. (2018) which showed that ovulations in natural spawning are occurring in thermal range at 8-12 °C. Moreover, the present study showed that hormonal manipulation on lower temperatures does not affect final oocyte growth nor egg quality. Manipulating on lower temperatures straight upon the transport of breeders to hatchery, the duration of the whole procedure was reduced. While usually it took three to five weeks to obtain the eggs (Zakęś and Szczepkowski, 2004; Rónyai, 2007; Ljubobratović et al., 2019) using the warming thermal schedule eggs were obtained already 10 days post-transport. As earlier the eggs are obtained, the time-frame until the seasonal reproduction prolongs, leaving more time for hatcheries to deal with juvenile production procedures prior to the next reproduction batch.

Alongside with egg quality, main emphasis of the present study was given to the spawn synchronization. In case of Trial 1, situation is rather clear, as both robust and non-robust measures of variability in latency time are strongly favoring the WARMING regime with both groups yielding eggs of high and similar quality. Therefore, the second trial went on using this thermal schedule. However, testing different sGnRHa dosages did not lead to straightforward results with respect to latency time variation. While the deviation and range are favoring group injected with 50 μg, robust measure of variability - median absolute deviation and interquartile range - are pointing group 5 to be the most effective in latency time synchronization. There are two reasons why dosage of 5 μg should be rather suggested over 50 μg. First of all, there is an economical background with respect to substantially reduced costs of the procedure. On the other hand, with respect that significant share of variability is explained via oocyte diameter, it appears reasonable to exclude outlier, what robust measures of variability are including (Leys et al., 2013; Sunitha et al., 2014; Vinutha et al., 2018). Accordingly, out of two females with oocytes below set threshold of 900 μm in group 5, one did not ovulate, while the second is outlier in terms of latency time (Figure 6). Therefore, in order to increase spawn synchronization, it is suggested to use WARMING thermal regime following the sGnRHa injection at a dose 5 μg.

The present study found both thermal schedule and hormonal dosage to be unconvincing factors determining egg quality, agreeing with earlier studies on pikeperch. However, WARMING thermal schedule appears to be more effective for spawn synchronization and procedure's time reduction, yet not reducing spawning performance. For the first time, the present study evaluated the oocyte diameter and discovers its value at the time of injection is a reliable predictor of spawning outcome in young females in preseason. Thus, the recommendations for the efficient pikeperch pre-seasonal artificial reproduction are: selection of breeders according to the oocyte diameter (> 900 μm) and sGnRHa injection in a dose of 5 μg kg^{-1} straight upon transport to the hatchery followed

by 1 °C day^{-1} thermal increment to 10 °C. Further studies should approach the issue of oocyte growth variability with respect to reproductive experience and its effect on egg quality and readiness for artificial reproduction in fully controlled conditions.

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Table 1. Descriptive statistics and measures of variability on latency times in hours at different thermal schedules (STABLE and WARMING) and in warming thermal schedule in females injected with different sGnRHa dose (5, 25 and 50 μg kg^{-1})

	Latency time (h)				
Measure of variability	STABLE	WARMING	5	25	50
Mean	203	228	213	226	214
Standard deviation	33	9	27	28	17
Range	76	27	62	72	40
Interquartile range	55	8	17	32	25
Median absolute deviation	28	5	0	16	12

WARMING – sGnRHa injection in a dose of 5 μg kg^{-1} straight upon transport to the hatchery followed by 1 °C day^{-1} thermal increment to 10 °C.
STABLE – sGnRHa injection in a dose of 50 μg kg^{-1} upon thermal increment to 10 °C.

Table 2. Pre-seasonal spawning performance in females in Trial 1 using different thermal schedules.

Parameter	WARMING	STABLE	*p* - value
Female weight (kg)	2.4 ± 0.6	2.1 ± 0.6	0.500
Ovulation (%)	100	100	-
Latency time (h)	228 ± 9	203 ± 33	0.130
Latency time (day degrees)	77 ± 4	83 ± 17	0.867
Relative fecundity (%)	14.5 ± 1.7	14.1 ± 2.7	0.805
Embryo survival (%)	79.7 ± 12.6	79.5 ± 2.7	0.655
Hatching rate (%)	95.2 ± 3.4	92.5 ± 2.4	0.186
Hatching index (%)	75.9 ± 12.0	73.6 ± 4.0	0.481

WARMING – sGnRHa injection in a dose of 5 μg kg^{-1} straight upon transport to the hatchery followed by 1 °C day^{-1} thermal increment to 10 °C.
STABLE – sGnRHa injection in a dose of 50 μg kg^{-1} upon thermal increment to 10 °C.

Table 3. Analysis of covariance in Trial 2 following injection of females with different sGnRHa dosage (5, 25 and 50 μg kg^{-1}) with respect to oocyte diameter at the time of injection

Parameter	5	25	50	*P* - factor	*P* - covariate
Female weight (kg)	1.9 ± 0.4	1.9 ± 0.6	1.8 ± 0.4	0.720	0.447
Oocyte diameter (μm)	914 ± 22	930 ± 24	906 ± 43	0.424*	
Ovulation (%)	83	100	100	-	-
Latency time (h)	213 ± 27	226 ± 28	214 ± 17	0.250	0.043
Relative fecundity (%)	8.3 ± 4.4	11.4 ± 1.3	11.7 ± 1.9	0.099	0.740
Embryo survival (%)	34.1 ± 31.8	61.7 ± 29.4	25.0 ± 29.1	0.298	0.019
Hatching (%)	97.7 ± 0.6	89.7 ± 9.8	94.2 ± 3.6	0.196**	-
Hatching index (%)	27.8 ± 30.9	55.3 ± 27.7	23.5 ± 27.6	0.369	0.011

Factor – sGnRHa dosage
Covariate - oocyte diameter at the time of injection
*ANOVA
**Kruska-Wallis

Fig. 1. Photo-thermal conditions from 1st of September until the pre-seasonal reproduction for Trial 1 (2017-2018) and Trial 2 (2018-2019)

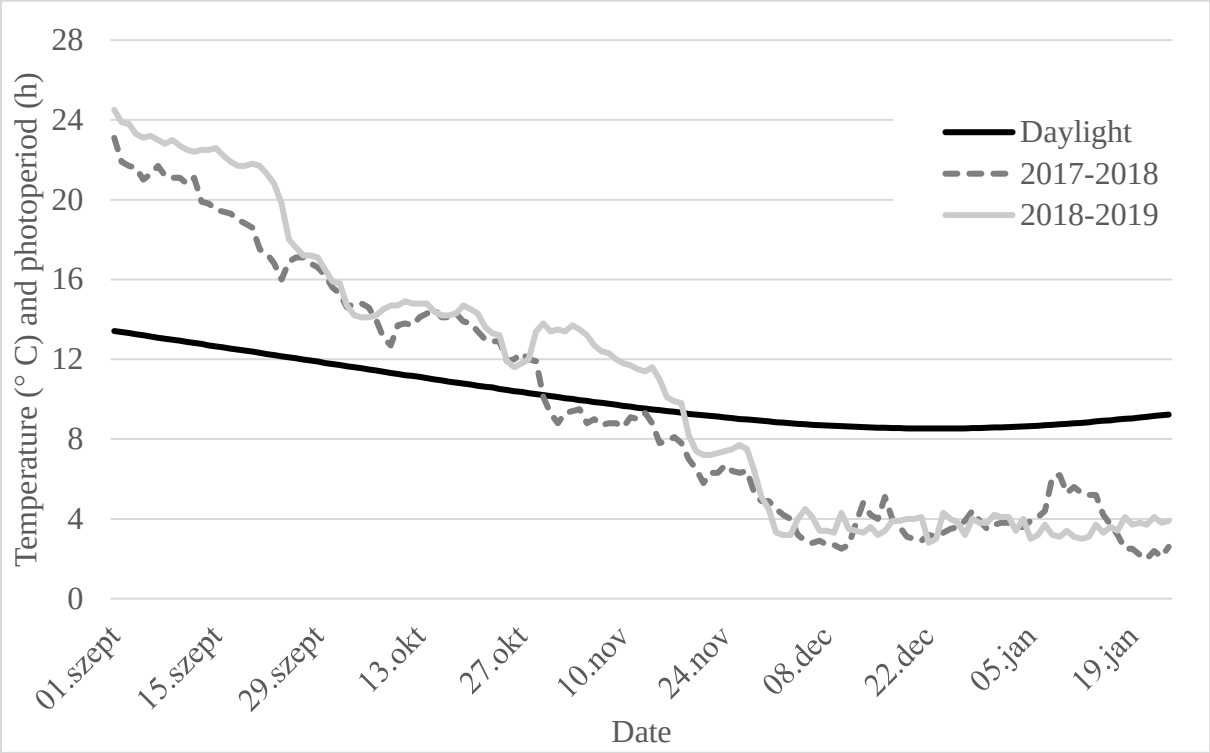


Fig. 2. Scatter plot and linear relationship on mean oocyte diameters at different times following injection in females injected with different sGnRHa dose (5, 25 and 50 µg kg^{-1}).

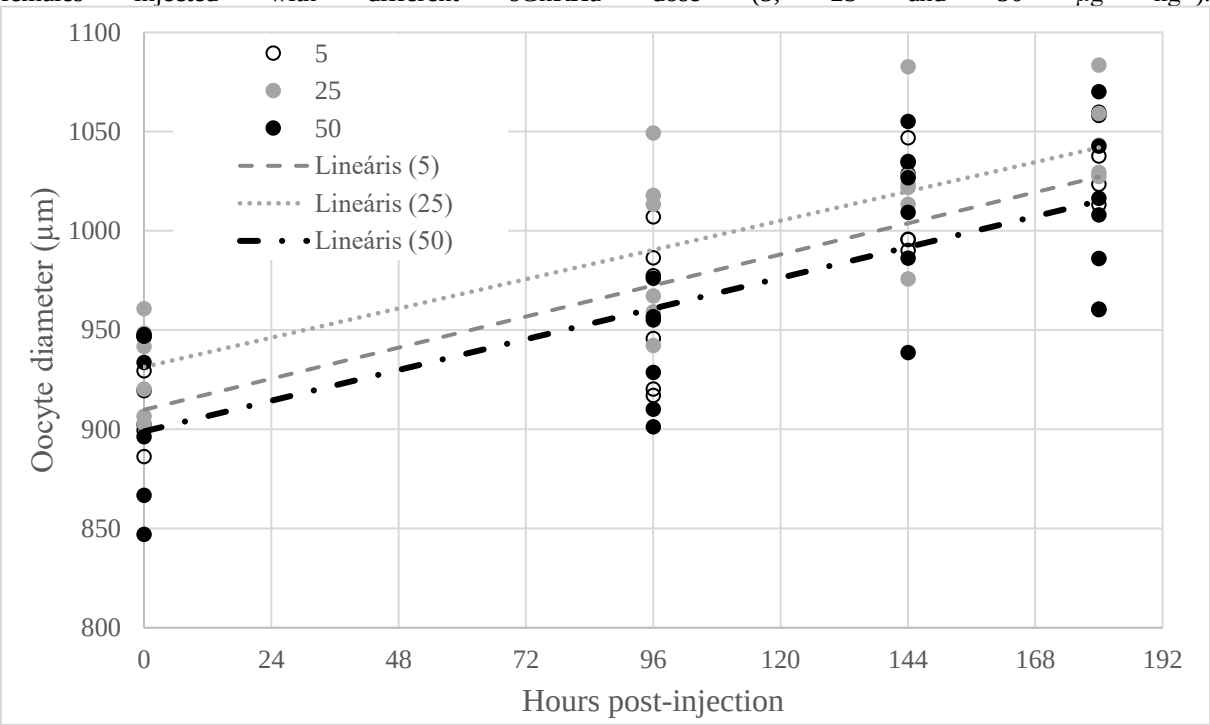
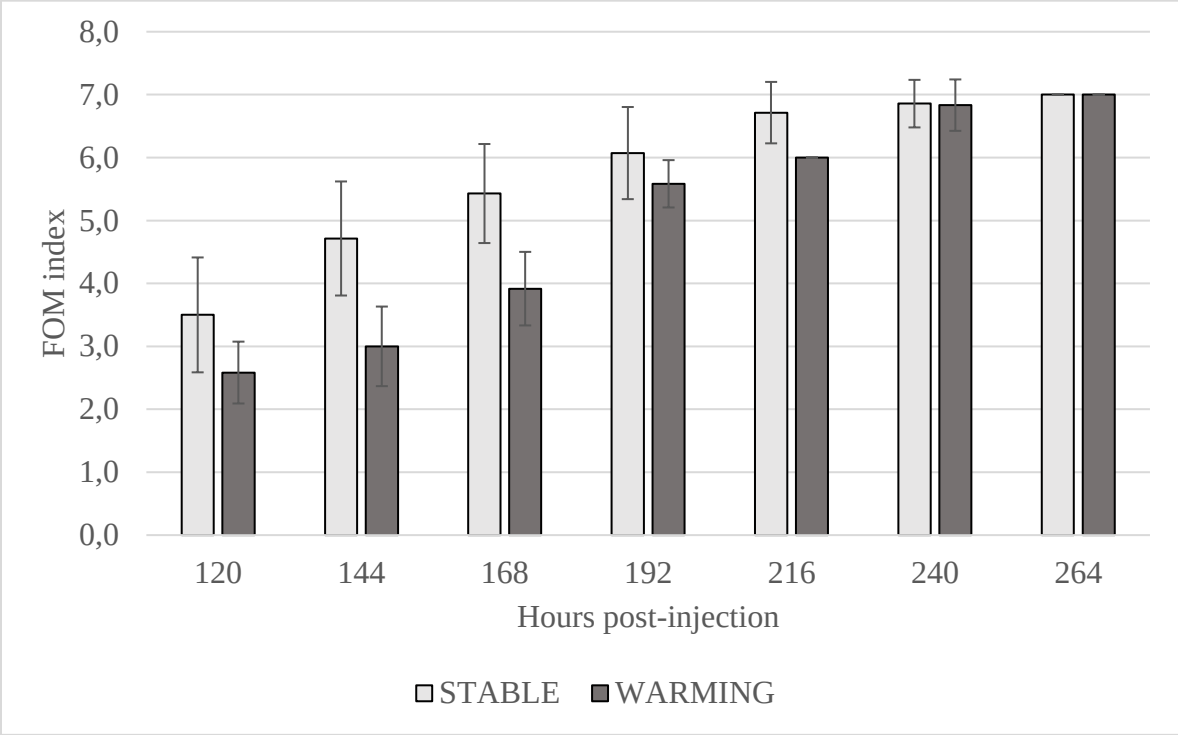


Fig. 3. Mean final oocyte maturation stage (FOM index) following injection in pikeperch females at different thermal regimes (STABLE vs WARMING)



WARMING – sGnRHa injection in a dose of 5 µg kg^{-1} straight upon transport to the hatchery followed by 1 °C day^{-1} thermal increment to 10 °C.
STABLE – sGnRHa injection in a dose of 50 µg kg^{-1} upon thermal increment to 10 °C.

Fig.4. Mean final oocyte maturation stage (FOM index) following injection in pikeperch in females injected with different sGnRHa dose (5, 25 and 50 µg kg^{-1}) at different times following injection.

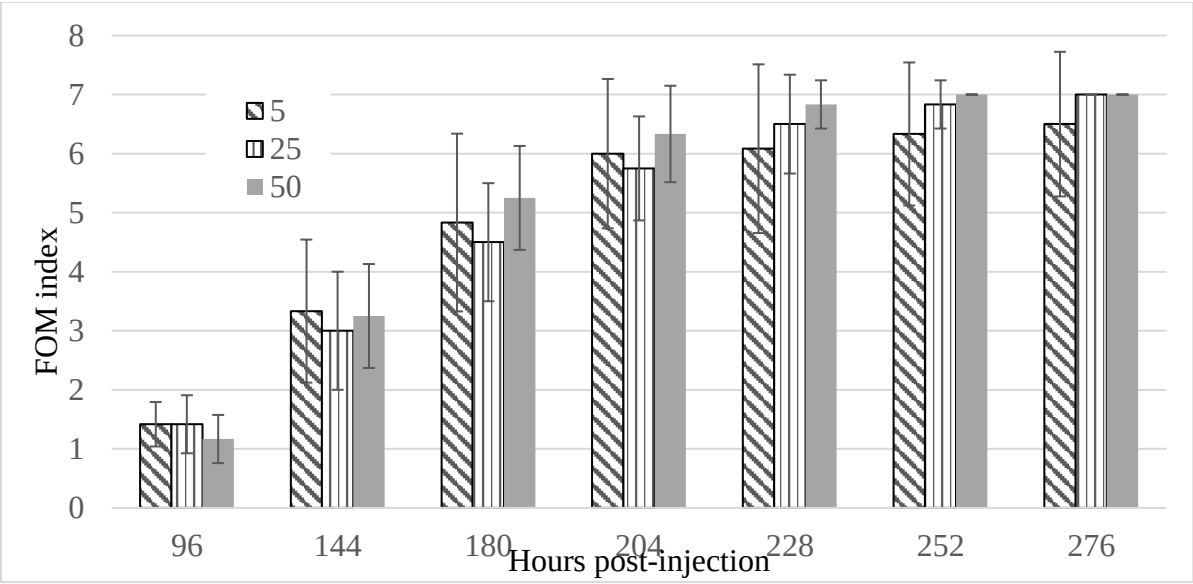
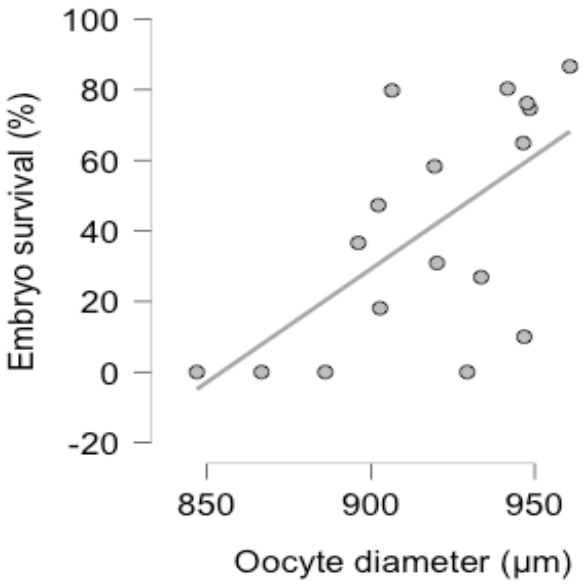


Fig. 5. Scatter plot diagram and linear regression line on oocyte diameter and embryo survival dependence in pre-seasonal pikeperch artificial reproduction in reproductively inexperienced females (Trial 2).

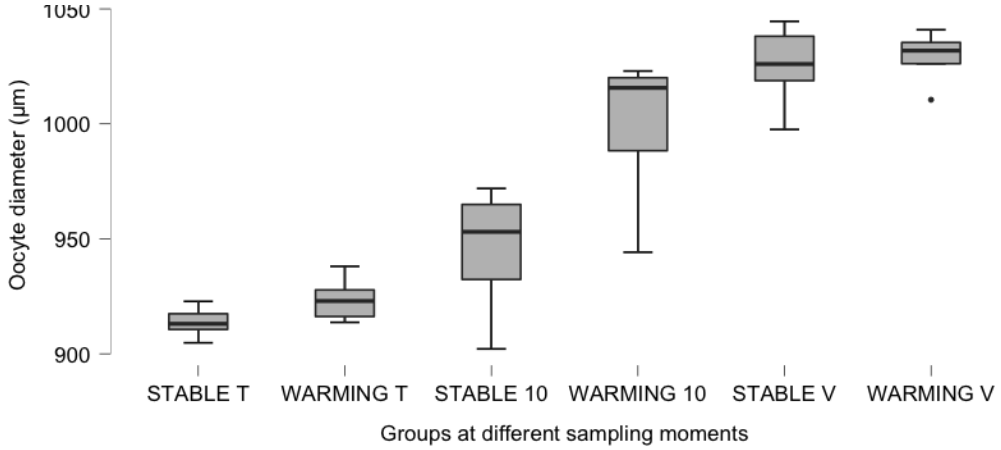


Supplementary Table 1. Pearson correlation explaining dependence of evaluated pre-seasonal reproductive performance in females Trial 2 following injection of 18 females with different sGnRHa dosage (5, 25 and 50 μg kg^{-1}).

Variable		Hormone dose	Female weight	Oocyte diameter	Latency time	Relative fecundity	Embryo survival	Hatching rate	Hatching index
Hormone dose	Pearson's *r*	—							
	p -value	—							
Female weight	Pearson's *r*	-0.204	—						
	p -value	0.418	—						
Oocyte diameter	Pearson's *r*	-0.122	0.199	—					
	p -value	0.629	0.428	—					
Latency time	Pearson's *r*	-0.013	-0.320	-0.410	—				
	p -value	0.962	0.211	0.102	—				
Relative fecundity	Pearson's *r*	0.474	-0.344	0.068	0.383	—			
	p -value	0.055	0.177	0.795	0.130	—			
Embryo survival	Pearson's *r*	-0.156	0.041	0.647**	-0.151	0.066	—		
	p -value	0.551	0.876	0.005	0.563	0.802	—		
Hatching rate	Pearson's *r*	-0.139	0.499	0.153	-0.432	-0.061	-0.150	—	
	p -value	0.650	0.083	0.618	0.140	0.843	0.626	—	
Hatching index	Pearson's *r*	-0.155	0.107	0.680**	-0.186	0.081	0.992***	0.009	—
	p -value	0.552	0.683	0.003	0.476	0.756	< .001	0.977	—

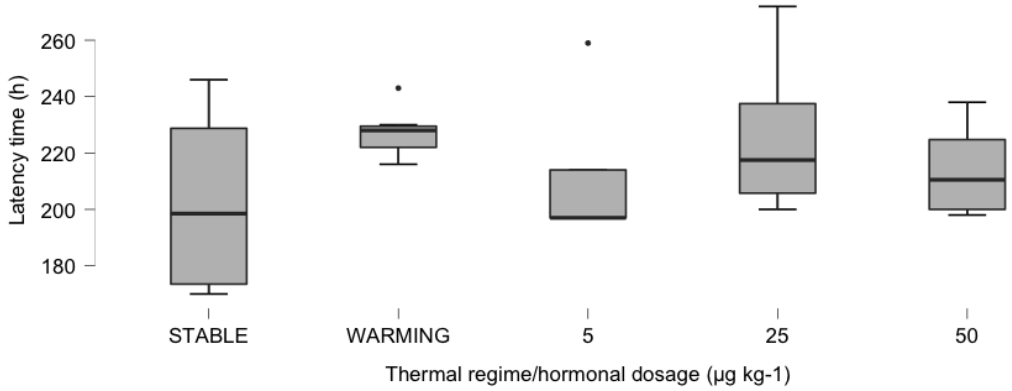
* p < .05, ** p < .01, *** p < .001

Supplementary Fig. 1. Boxplot diagrams on mean oocyte diameter following injection in pikeperch females at different thermal regimes (STABLE vs WARMING) and at different moments of spawning induction.



T – following transport to indoor facility (injection time for group STABLE)
10 – upon reaching 10 °C (time of injection in group WARMING)
V – at the time final oocyte maturation reached stage V.
WARMING – sGnRHa injection in a dose of 5 µg kg^{-1} straight upon transport to the hatchery followed by 1 °C day^{-1} thermal increment to 10 °C.
STABLE – sGnRHa injection in a dose of 50 µg kg^{-1} upon thermal increment to 10 °C.

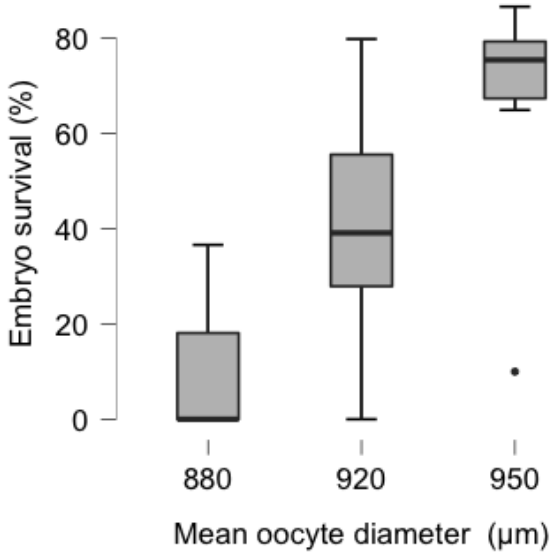
Supplementary Fig. 2. Boxplot diagrams on latency times at different thermal schedules (STABLE and WARMING) and in warming thermal schedule in females injected with different sGnRHa dose (5, 25 and 50 µg kg^{-1})



WARMING – sGnRHa injection in a dose of 5 µg kg^{-1} straight upon transport to the hatchery followed by 1 °C day^{-1} thermal increment to 10 °C.

STABLE – sGnRHa injection in a dose of 50 µg kg^{-1} upon thermal increment to 10 °C.

Supplementary Fig. 3. Boxplot diagram on embryo survival in pre-seasonal pikeperch artificial reproduction in reproductively inexperienced females (Trial 2) classified in three groups (n = 6) according to the oocyte diameter.



Fazekas Georgina, Vass Vivien, Demény Ferenc, Tóth Flórián, Ljubobratović Uroš

Különböző felület-tisztító eszközök hatása az úszóhólyag felfúvódási sikerére a süllő lárvákban

A tanulmány háttere és célja

- A kutatás általános célja volt a vízfelszín tisztítására a legmegfelelőbb módszer azonosítása a süllőlárva nevelés hatékonyságának javítása érdekében. Ugyanis az úszóhólyag felfúvódási sikerét befolyásolja a vízfelszíni olajréteg, azonban a felszíni hozzáférés megkönnyíti a baktériumok és törmelékek bejutását az úszóhólyagba.
- Számos technikai megoldás létezik a vízfelület tisztítására, beleértve az olajszennyeződés emulgeálására szolgáló permetezőt és a felületen lévő olajgömbök csapdázására szolgáló vízfelszíntisztító skimmer-t. Ezeket a módszereket teszteltük az úszóhólyag kezdeti felfúvódási-sikertelenségének arányának szempontjából.
- Kontroll kezelésként semmilyen felületi tisztítóval, csak egy túlfolyó hálóval ellátott kádakat tartalmazó lárvanevelő rendszert alkalmaztunk. Ezzel szemben három kísérleti kezelést állítottunk fel tisztítási módszerek alapján.
    - Két permetezős rendszert: egy keskeny (a kád átmérőjének harmadát fedi le) és egy széles (a kád teljes átmérőjét lefedő) méretű eszközt tartalmazó rendszert, továbbá
    - egy légfúvó felületi tisztító skimmer-t használó kezelést.

Tanulmány főbb eredményei

- Szignifikánsan magasabb úszóhólyag felfúvódási sikert eredményezett a széles permetező méretet (30.6 ± 13.0 %) alkalmazó csoport a másik hárommal szemben (kontrol: 14.7 ± 7.5 %, skimmer: 4.8 ± 1.7 %, keskeny: 12.2 ± 5.1 %).
- Ez alapján két következtetés vonható le:
    - i) a felületi permetezés a legalkalmasabb a süllő lárvák nevelése szempontjából,
    - ii) az azonos hatásmódú, de eltérő kialakítású tisztítóeszközök hatékonyság szempontjából jelentősen eltérhetnek.
- Ugyanakkor nem zárható ki jobb hatékonyságú eltérő felületi tisztító skimmer kialakítása

Az összegzés az alábbi cikk alapján készült:

Georgina Fazekas, Vivien Vass, Ferenc Demény, Flórián Tóth, Uroš Ljubobratović The Effect of Different Surface-Cleaning Devices on the Success of Swim Bladder Inflation in Zander Larvae NORTH AMERICAN JOURNAL OF AQUACULTURE 83 Paper: 10172 (2021)

Short communication: The effect of different surface cleaning devices on the success of swim bladder inflation in pikeperch (*Sander lucioperca* L.) larvae

Georgina Fazekas[1,2*], Vivien Vass[1], Ferenc Demény[1], Flórián Tóth[1], Uroš Ljubobratović[1]

[1]National Agricultural Research and Innovation Centre, Research Institute for Fisheries and Aquaculture NAIK HAKI, H-5540 Szarvas, Anna-liget 35, Hungary;
[2]Animal Husbandry Science Doctoral School, Szent István University, H-2100 Gödöllő, Páter K. 1, Hungary
*Corresponding author: G. Fazekas, Research Institute for Fisheries and Aquaculture NAIK HAKI, Anna-liget 35, 5540 Szarvas, Hungary. Telephone: +36 30 1843602; Fax: 36 66 312 142; E-mail: fazekas.georgina@haki.naik.hu

Abstract
Failure of initial swimbladder inflation (SBI) is one of the main obstacles in pikeperch (*Sander lucioperca* L.) larviculture. Oil layer on the water surface prevents larvae to penetrate it and gulp the air. Numerous technical solutions exist for cleaning the water surface including sprayer to emulsify the oil contamination and skimmer for trapping the oil globules on the surface. In order to investigate the most appropriate method to improve the SBI success three different devices were evaluated in triplicated tanks. Next to the control tanks non-equipped with any surface cleaning device other than overflow mesh, two sprayer designs, narrow, covering a third of the tank's diameter, vs. wide, covering an entire tank's diameter, and air blowing surface skimmer, were set for a 16 days trial. Freshly hatched larvae (7 000 per tank) were divided into four treatment groups in twelve 250 L larval nursing tanks belonging to the common recirculation system. Significantly higher SBI was found in tanks equipped with wide covering sprayer (30.6 ± 13.0 %) compared to control, skimmer and narrow covering sprayer groups (14.7 ± 7.5 %, 4.8 ± 1.7 %, 12.2 ± 5.1 %, respectively). Thus, the results of the present study indicate that sprayer design covering large share of tank's diameter is an appropriate solution to enhance SBI in pikeperch larviculture.

Introduction
Non-inflation of the swim bladder (SB) is a common problem in intensive larval rearing of many fish species (Chatain and Ounais-Guschemann 1990; Chatain 1994; Summerfelt 1996). This incident has a serious effect on development and survival of fish exhibited in reduced growth and survival rate, increased occurrence of spinal deformities due to aberrant energy consuming swimming behaviour (Wooley and Quin 2010; Bagowski et al. 2011; Summerfelt 2013). Pikeperch (*Sander lucioperca* L.) is a physoclistous fish, however its larval stage is physostomous and possess a transient pneumatic duct, an organ enabling initial gulping of air at the water surface to fill the SB (Doroshev et al. 1981; Rieger 1995; Bagowski et al. 2011). However, once this organ atrophies the transient physostomous larvae lose the ability to fill the SB (Demska-Zakęś et al. 2003). Swim bladder inflation (SBI) is a highly temperature-dependent irreversible process and influenced by several biotic and abiotic factors (Blecha et al. 2019). Some of these documented in different species are light intensity, photoperiod and tank colour (Martin-Robichaud and Peterson 1998; Trotter et al. 2003; Kurata et al. 2017; Suchocki and Sepulveda-Villet 2019; Palińska-Żarska et al. 2019). Finally, failure of SBI results from inability of larvae to reach the water surface covered by a layer of oil or if the surface access facilitates enter of bacteria or organic debris to the swim bladder (reviewed by Summerfelt 2013). To the best of our knowledge, other than light intensity (Tielmann et al. 2017) other factors were not evaluated as direct modifiers of the SBI in pikeperch intensive larval rearing.
In larviculture, high viscosity of the water surface stems from the oil residues from excess feed or dead larvae (Szkudlarek and Zakęś 2007) and acts as a barrier to the larvae for reaching the water surface. Therefore, removal of the surface-film is considered to be crucial technique to promote SBI (Trotter et al. 2005). Strategies commonly used in hatcheries include either the surface water spraying for emulsifying the oil film or the oil skimming to capture and retain the oil on the part of surface (Summerfelt 2013). The optimal technique is typically species specific. Thus, while in the case of sea bream (*Sparus aurata)* blower and trap are found to be more appropriate (Chatain and Ounais-Guschemann 1990), in case of pikeperch's North American relative walleye (*Sander viterus*) using surface spray was more advantageous for successful SBI (Clayton and Summerfelt 2010). Each of mentioned strategies can be applied via different apparatus designs. In the case of sprayers, there is a paucity of irrigation sprayers on the market with different design and mode of action. Recommendations for walleye are flat nozzles directed vertical to the surface (Summerfelt 1996; Summerfelt and Johnson 2015). Although the number of sprays per tank surface area was mentioned, the spraying diameter and its ration in the diameter of the tank was not defined.
The aim of the present study was to find the most appropriate method for cleaning the surface in order to improve the efficiency of pikeperch larval rearing. Two different strategies, skimming vs spraying, and two sprayer designs, wide vs. narrow, were tested from the aspect of the rate of SBI.

Materials and methods
The trial was carried out in the experimental recirculation aquaculture system (RAS) of Research Institute for Fisheries and Aquaculture NAIK HAKI, Szarvas, Hungary. Fertilized pikeperch eggs were obtained from artificially propagated wild breeders and after hatching larvae were transferred to the larval nursing RAS composed of twelve 250 L tanks with black walls and white conical bottom. Each tank was stocked with 7000 volumetrically counted newly hatched larvae (mean initial length 4.5 ± 0.1 mm) for a sixteen days rearing. Larval nursing protocol was performed according to earlier studies performed in the facility (Ljubobratovic et al. 2019a; 2019b). Larvae

were fed with newly hatched *Artemia* nauplii each 3-4 hours in amount 100-300 nauplii per larvae per day according to the size of larvae. Photoperiod was set on 14:10 LD with a light intensity at water surface about 10 lux during the light period. Water flow was up-welling with water exchange rate being at 30% per hour at the beginning gradually increased to 75% per hour until the end of the trial.
Larvae were distributed in four treatment groups. Next to the three control tanks non-equipped with any surface cleaning device other than overflow mesh (CONTROL), three different surface cleaning devices were tested on three replicated tanks (Figure 1):

1. Surface skimmer (SKIMMER) – floating PVC frame (20 × 20 cm) with air inlet at one side as described by Moretti (1999.) (Figure 1a);
2. Narrow sprayer (NARROW) – flat fan nozzle sprayer covering a third of the tank's diameter (Figure 1b);
3. Wide sprayer (WIDE) – flat fan nozzle sprayer covering an entire tank's diameter (Figure 1c);

At the end of the experiment, day 16 post-hatch, individual total length was evaluated on a random sample of 50 larvae per tank. Additionally, a random sample of 200 larvae per tank were stocked in a solution of 0.2 mL L^{-1} phenoxyethanol and 10 g L^{-1} of kitchen salt, and fish were counted and graded in terms of whether the swim bladder was inflated or non-inflated, thus float or sank (Steenfeldt, 2015). Finally, all survived fish from each tank were counted.
Water quality parameters were monitored throughout the experiment. The water samples were taken at the outflow of the tanks twice a week and the pH (8.46 ± 0.1), ammonium-nitrogen (0.17 ± 0.1 mg L^{-1}), nitrite-nitrogen (0.03 ± 0.0 mg L^{-1}), nitrate-nitrogen (1.01 ± 1.6 mg L^{-1}) were determined. On the basis of daily measurements in each tank, temperature was maintained at 16.1 ± 0.3 °C while the dissolved oxygen remained at 105.9 ± 1.0 % saturation. Data are presented as mean ± standard deviation. Statistical analysis was based on one-way ANOVA, with respect that all variables fulfilled the conditions of normal distribution and homogenous variances. Differences among treatments were assessed with Duncan's post-hoc test. The level of significance was at $p \leq 0.05$.

Results and Discussion

A single significant difference among the assessed parameters was observed in SBI success (Table 1., Figure 1.). Significantly higher SBI success was seen in WIDE (30.6 ± 13.0 %) compared to CONTROL, SKIMMER and NARROW groups (14.7 ± 7.5 %, 4.8 ± 1.7 %, 12.2 ± 5.1 %, respectively). This outcome leads to two conclusions. One is that surface sprayer is more appropriate surface cleaning device for pikeperch larvae compared the surface skimmer. The second is, however, pointing that even the cleaning device with similar mode of action but different final design might differ significantly in terms of efficacy. Thus, it may be assumed sprayer with wide covering tank diameter is the most appropriate technique for surface cleaning in pikeperch larvae, but the possibility of different surface skimmer design of better efficacy cannot be excluded. However, the results of the present study agree with the similar study of Boggs and Summerfelt (2003) who found significantly higher SBI in walleye larvae reared in tanks enables with sprayer instead of surface skimmer. Likewise, Clayton and Summerfelt (2010) and Barrows et al. (1993) found the sprayer essential for high SBI in this close relative of pikeperch. On the contrary, results in sea bream using sprinkler and hidro-jets had a secondary effect inducing turbulence in the upper layer thus being inaccessible by the most of the larvae (Chatain and Ounais-Guschemann 1990). Generally, the use of surface skimmer significantly contributes to diminishing the problem of noninflation of the swim bladder in sea bass and sea bream culture (Koumoundouros et al. 2000). Finally, rather high SBI in pikeperch using sprayer was earlier reported in a larviculture study evaluating different stocking densities (Szkudlarek and Zakęś 2007). Thus, according to the results of our study and other accessible literature data reviewed, the sprayer seems to be the best fitting solution for pikeperch larviculture. In line with the second outcome of the present study, spray coverage had an important role on SBI in study of Moore et al. (1994). Finally, to the best of our knowledge these are the first data on different surface cleaning methods for pikeperch larviculture. It may be concluded that sprayer design covering large share of tank's diameter is the most appropriate solution for pikeperch larvae.

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Figure 1. Surface cleaning devices applied in the study: a. Surface skimmer (SKIMMER); b. Flat fan nozzle sprayer covering a third of the tank's diameter (NARROW); c. flat fan nozzle sprayer covering an entire tank's diameter (WIDE).

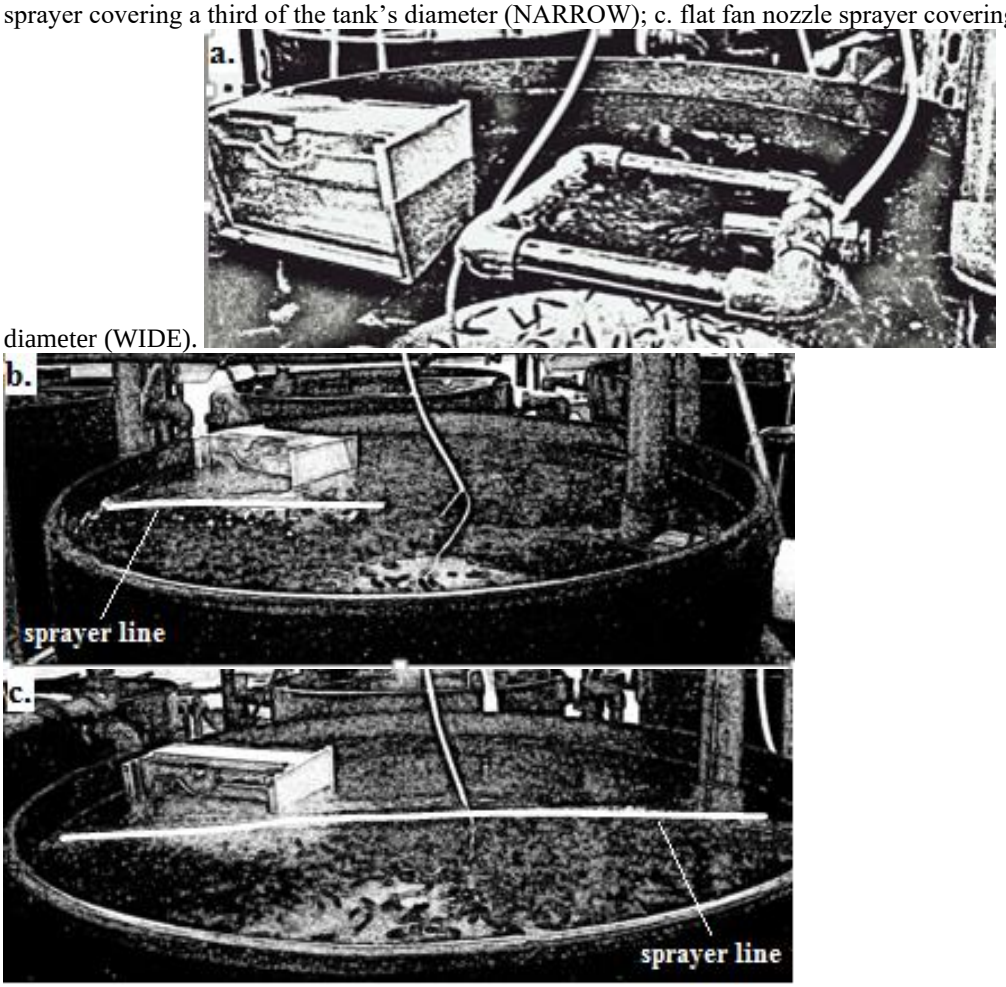
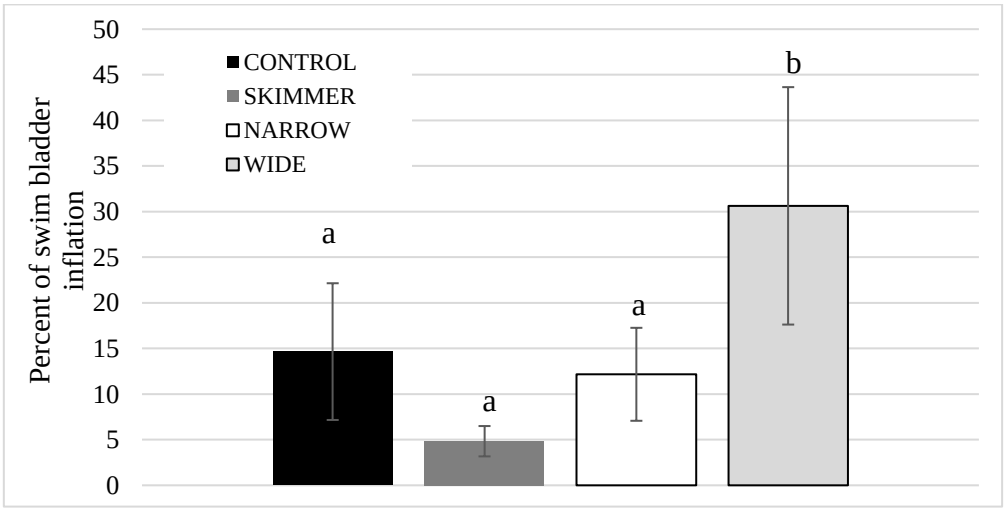


Figure 2. The effect of different surface cleaning devices on the success of swim bladder inflation in 16 days post-hatch larvae.



Significantly different treatments are marked with different letter above the column.

Table 1. Culture performance in 16 days post-hatch larvae (initial length 4.5 ± 0.1)

Parameter	CONTROL	SKIMMER	NARROW	WIDE
Final length	8.4 ± 0.0	8.4 ± 0.2	8.4 ± 0.1	8.4 ± 0.1
Survival (%)	22.9 ± 7.1	21.8 ± 3.2	20.8 ± 4.3	28.3 ± 10.7
SBI (%)	14.7 ± 7.5[a]	4.8 ± 1.7[a]	12.2 ± 5.1[a]	30.6 ± 13.0[b]

Data are presented in form mean ± SD.
Significantly different treatments are marked with different letter in the superscript.
SBI – swim bladder inflation rate – percent of fish with inflated swim bladder.

Varga Mónika, Berzi-Nagy László, Csukás Béla, Gyalog Gergő

Környezeti változók hosszú-távú hatásának dinamikus szimulációs modellezése a halastavi ökoszisztémára és pontyhozamokra

Tanulmány háttere és célja

- Az extenzív halastavi termelés nagy mértékig beágyazott a tavi ökoszisztémába.
- A környezetnek való kitettség, illetve a táplálékhálózatban végbemenő biológiai, kémiai és fizikai folyamatok komplexitása miatt a halastavi hozamok előrejelzése nehéz feladat, de az informatika fejlődésével megoldható
- Célunk volt a Programozható Struktúrák módszerét használva egy szimulációs modell alapjait lefektetni, amely később alapja lehet a precíziós működésnek is

Tanulmány főbb eredményei

- A kreált modell a tavi tápláléklánc folyamatait és a halak növekedését szimulálja napi (24 órás) időlépésben
  - a technológiai beavatkozások (takarmányozás, trágyázás, kihelyezés),
  - valamint a napi szintű időjárási hatások függvényében.
- A következő folyamatok matematikai képletét foglalja magában:
  - tápláléklánc egyes elemei közötti interakciók
  - fizikai tényezők hatása a tápláléklánc egyes elemeinek metabolikus folyamataira (pl vízhőmérséklet, vagy oldott oxigén szint hatása, fitoplankton esetében a fény intenzitás hatása a fotoszintézisre)
  - külső meteorológiai adottságok, valamint a tó fizikai jellegű változói közötti kapcsolat (pl. oxigén be- és kioldódás, hőcsere, párolgás folyamata)
  - külső technológiai beavatkozások
- A tógazdasági folyamatmodellt a szeged-fehértói halastavak tónaplóiban szereplő adatok alapján a 2006 és 2016 közötti időszakra validáltuk.
- A folyamatmodell segítségével a klímaváltozásnak a halastavi hozamokra és a termelési költségekre gyakorolt hatásait is elemezni lehet.
- Vizsgáltuk a párolgás, az oldott oxigén szint, valamint a pontyhozamok szimulált alakulását 2050-ig a NORESM klímamodell RCP 4.5 és RCP8.5 szcenárióját használva bementi adatokként.
- Több technológiai kombináció (10 féle népesítési sűrűség, valamint takarmányozási intenzitás, háromféle mortalitás, három induló egyedsúly) hatását is szimuláltuk a cikkben

Az összegzés az alábbi cikkből készült:

Varga Monika, Berzi-Nagy Laszlo, Csukas Bela, Gyalog Gergo Long-term dynamic simulation of environmental impacts on ecosystem-based pond aquaculture ENVIRONMENTAL MODELLING & SOFTWARE Paper: 104755 (2020)

Long term dynamic simulation of environmental impacts on ecosystem-based pond aquaculture

Monika Varga[✉,1], Laszlo Berzi-Nagy[2], Bela Csukas[1], Gergo Gyalog[2]

✉ M. Varga
E-mail: varga.monika@ke.hu

[1]Research Group on Process Network Engineering, Institute of Methodology, Kaposvar University, 40 Guba S, 7400, Kaposvar, Hungary
[2]Research Institute for Fisheries and Aquaculture, National Agricultural Research and Innovation Centre, 35 Anna-liget, 5540, Szarvas, Hungary

Abstract
The effect of changing environmental conditions on the operation of an ecosystem-based pond aquaculture was analyzed by long-term dynamic simulations. The applied non-conventional framework of Programmable Process Structures supported the appropriate implementation of the trans-disciplinary functionalities in a unified transparent structure. The simplified, but dynamic analysis of the interacting physical, chemical, biological (including ecological) and pond management processes illustrates the necessity and possibility of long term dynamic simulation based analysis of the systems containing complex, non-linear interactions. The process model of medium complexity made possible to study the effects of climate change on fishpond ecosystem and farm management using 50 years long dynamic simulations. We concluded that regardless to the limited amount of data, the approximately validated, but causally established, well-structured and balance based dynamic model may support the design and operation of artificially managed aquatic systems.

1. Introduction

1.1 Pond aquaculture in the world

Aquaculture plays an important role in achieving food and nutrition security. According to FAO statistics, 46% of the 173 million t total fish production came from aquaculture in 2017, and this number is expected to grow to 54% by 2030 (FAO, 2018; FAO 2019). The bulk of fish production comes from pond farms. However, taking into account the scarcity of land and water resources, complex model-based understanding, planning and management of these systems has an increasing importance (Avnimelech et al, 2008), especially if adaptation to climate change is considered (FAO, 2011).
Term of land-based aquaculture basically covers the three typical production systems of flow-through, pond and recirculating systems (Heller, 2017). As semi-intensive ponds come with relatively low environmental risks, they come to the fore (Serpa, 2013). The present work focuses on the dynamic model-based analysis of semi-intensively managed levee-type fishponds in temperate climate.
Carps, which are mainly farmed in ponds, accounted for 39% of global aquaculture production in 2017 (FAO, 2019). Major carp species are herbivorous Grass carp; planktivorous Silver carp and Bighead carp and omnivorous Common carp, contributing to 10%, 9%, 6% and 8% of world fish production, respectively. Typically, these species are produced in polyculture in order to utilize the different niches of the pond food web efficiently. In European pond culture, Common carp is the main target species, other supplementary species such as Grass carp, Bighead carp and Silver carp account for less than 20 % of the output. In Hungary, which is one of the major carp producers in Europe, the ongoing recovery of pond aquaculture sector is highly based on Common carp (Specziar and Eros, 2016) with a share around 78-80% (AKI, 2018). The fish farming method modeled below is a bi-culture of Common carp (*Cyprinus carpio*) and Bighead carp (*Hypophthalmichthys nobilis*), where these species are farmed under semi-intensive conditions meaning that both external feed and natural food contribute to biomass growth. Farmers apply manuring to enhance plankton production, and cereal feed is added to nutritionally supplement the natural food sources. Therefore, fish culture is embedded into the artificially manipulated pond ecosystem (phyto- and zooplankton, benthos, detritus), which has to be fully understood so that input management is optimized. Effective production assumes the proper management of the controllable factors in terms of feeding, manuring, aeration, stocking density and pond level control. Full understanding of food web interactions and environmental impacts requires dynamic modeling and simulation-based analysis of pond farming processes. Model-based analysis has to consider all of the underlying biological, chemical and physical processes (e.g. oxygen production and utilization of phytoplankton under various solar radiation, the prey-predator relations amongst the food web elements, etc.), many of which are governed by environmental and climatic conditions.

1.2 Aquaculture production models

Regarding the determining elementary processes of fishpond models (like growth, mortality, photosynthesis, nitrogen and phosphorus dynamics, etc.), we can rely on significant precedents from literature. Especially in the period of 1970-80, huge efforts were made to describe mathematically the behavior of the ecological communities. Development of knowledge for the particular sub-fields and sub-processes is unbroken since then.

Kaposvar University, 40 Guba S, 7400, Kaposvar, Hungary

Considering growth models, several approaches can be found in the literature for the main species.

- The Thermal Growth Coefficient (TGC) models are based on the assumption that if food is not limited, then the key factor of growth is the water temperature (Jobling, 2003). The simplified TGC formula was widely applied previously, because of its easy usability and flexible adaptation for various specific cases (e.g. Huiwen et al., 2016; Jauralde et al., 2013);
- The biomass-based models do not calculate the weight of the individual fish, but express the growth of the fish biomass as function of available food/feed. These models can handle situations where more than one nutrient source contribute to biomass growth (e.g. Svirezhev et al, 1984 for ponds, containing various fish and biomass components);
- Individual-based models usually calculate the daily growth of the fish as function of the actual individaul body mass. The von Bertalanffy formula is frequently used as a functional basis to model the growth of individuals. Limiting factors of growth such as nutrient availability, temperature and environmental conditions, etc. are typically involved into these models as multiplicative relationships. Functional forms for these limiting factors are usually transformed into standardized formulas taking a maximum value of 1 (Kapetsky and Nath, 1997; Lu, 2003; Poot-Lopez et al., 2014; etc.).

There are also generally accepted relationships for the description of environmental conditions, including but not limited to

- the Lehmann function for the consideration of the temperature for the growth of various organisms (Jorgensen, 1980);
- the relationship between growth and the presence of dissolved oxygen in the water (Svirezhev et al, 1984); or
- the Beer-Lambert formula based consideration of light effect on phytoplankton, etc.

It is to be noted that considerable part of the mentioned relationships were not effectively be utilized at the time of their development, in the lack of effective computational tools. However, they could provide useful background for the development of efficient models with the currently available methods and tools.

1.3 Complex modeling frameworks

Regarding the modeling of large ecosystems, systemic thinking (i.e. systems ecology) is still a rapidly developing area (Borrett et al, 2014), because understanding of complex interactions of ecosystems is an important question in view of the increasing environmental pressures on the overexploited resources at global level. Ecopath with Ecosym (EwE) is a continuously developing tool for the mass balance based analysis of aquatic ecosystems, with a dynamic simulation capability at ecosystem level (Christensen and Pauly, 1992; Christensen and Walters, 2004; Christensen et al., 2005).

Considering the artificially managed pond ecosystems, many attempts appeared relatively early (Wolfe et al, 1986; Hagiwara, 1994) to build complex simulation models. Perhaps Svirezhev et al (1984) developed first a fishpond model, where, besides the farmed species, also plankton, benthos, detritus and oxygen were represented as state variables. Conventional approaches focused on the changes of state elements of the process structures, and the models were described by a set of differential equations. Because of the efforts of pond farmers to intensify production and increase yields, earth pond ecosystems are managed by various interventions that highly affect the natural interactions. Proper consideration of these effects has a keynote importance in modeling and simulation.

The Comprehensive Aquatic Systems Model (CASM) is a generalized and flexible mass-balance based aquatic food web modeling platform that addresses the solution of theoretical and practical problems for a variety of freshwater and coastal aquatic ecosystems (Bartell et al, 1999; CASM, 2013). Many works build on CASM approach (e.g. Schmolke et al, 2019).

Model based decision support systems are also in the forefront of development for various fish farming systems (Halmar Halide et al, 2009; Azadivar et al, 2009; Cobo et al, 2019 for sea cages; etc.).

1.4 Challenges

From modeling point of view, the specific challenge is usually twofold: first, questions arise concerning the applied methodology, second, the availability and reliability of data have to be thoroughly considered.

Regarding methodological side, widely applied and well-developed differential equation solvers sometimes does not fully support the flexible combination of various processes of different spatial and temporal scales that is typical for ecosystems. Spatial multiscale characteristic comes from the variety of physical, chemical, biological processes needed to describe the system, while temporal multiscale character can be mentioned when environmental effects (including climate change) are considered on a longer time horizon.

Challenges related to data availability are also connected to this first issue. As accurate data is not always available right at the start of model building, flexible methodologies that allow stepwise development of various model parts, has an increasing importance. In turn, in line with the experiences, model-based understanding and stepwise discovery of the underlying system could guide the reasonable data collection.

2. Objective

Complex interactions of coexisting biological, environmental, managerial and economic aspects of pond aquaculture evoked the need for long-term dynamic simulation based analysis. Based on data from a Hungarian semi-extensive pond farm from past 10 years, present work aims to implement and validate an easily extensible and reusable dynamic simulation model for pond aquaculture operations.
In the knowledge of the validated model, the dynamic simulation based analysis aims to provide long-term predictions for various climatic scenarios with the consideration of

- the interactions amongst the elements of the food web;
- the dynamic balances of the involved chemical components (O_2, CO_2, nitrogen, phosphorus);
- the managerial interventions of the farmer (stocking density, feeding, fertilization, aeration, pond level control).

Final goal of the work is to evaluate the impact of climate change on carp farming, as well as to establish a comprehensive background for the complex decision support system, under development within ClimeFish project (https://climefish.eu/) for the shallow ponds in Central Europe.

3. Data, expressions and method

3.1 Data from example fishpond

Model development and validation were based on the datasets for the period of 2010-2016 of a production fishpond SzegedFish Ltd., located at the Southern Great Plain in Hungary. The studied fishpond is a 139 ha pond stocked with Common carp and Bighead carp. Considering the food web, we involved those functionally important species (phytoplankton, zooplankton, benthos and detritus) that usually are present in the semi-extensive Hungarian shallow ponds.
Considering the local weather characteristics, theoretically the production season is 214 days long, lasting from April 1 to October 31 in each year.
Water management is in line with the typical levee-type fishpond water level control. Considering the characteristics of the pond bed, leakage and water infiltration were neglected. Accordingly, water level is regulated by evaporation, precipitation and intentional water inlet and drainage. The starting level of water on April 1 is 1 m. The maximally allowed (overflow) level is 1.1 m. The required minimal water level is 0.95 m between April 1 and August 15, and 0.6 m between August 16 and October 31. When the required minimal level is reached (due to evaporative losses), supplementary water use is initiated, and ponds are filled to a level of 1.05 m (between April 1 and August 15) or to 0.9 m (between August 16 and October 31). After harvesting, the fishpond is drained at the end of the production season.
Stratification of the various components was not considered for these shallow lakes.
Recorded data were available from the pond registry for the:

- stocking and harvesting of the main species (Common carp) in the fishpond, with occasional individual weight sampling,
- monthly feeding,
- monthly manuring,
- occasional water supply from three sources (own reservoir, nearby river, excess water),
- occasional water level data (between 2006 and 2016),
- occasional water temperature, pH, ammonium, nitrite, nitrate and phosphate measurements, as well as
- occasional sampling with a plankton net.

3.2 Physical, chemical and biological relationships

The developed dynamic process model, considering the actual or hypothetical meteorological parameters, comprises:

- the hydrological processes for the levee-type fishponds (evaporation, air-water heat exchange, air-water oxygen transport);
- the prey/predator fluxes between the elements of the pond food web, considering their competitive consumption;
- the anabolic/catabolic growth model for the main species (carp and bighead), including their specified feed consumption, growth, respiration, excretion, and mortality;

- the life-related processes (formation, respiration, degradation) of other food web elements;
- the oxygen production of phytoplankton;
- the managerial interventions (stocking, feeding, manuring, water management), as well as
- the related physical and chemical processes (e.g. decomposition of feed and manure, etc.).

In the following parts, we introduce the implemented sub-models of the above summarized interactions.

3.2.1 Growth model of Common carp

3.2.1.1 Core of the anabolic/catabolic model

Main target species of the example fishpond is Common carp, complemented with planktivorous Bighead carp (which is a favorable combination in line with practical observations). Considering the need for the calculation of the individual fish weight for the Common carp, we applied a Bertalanffy kinetic based model. Accordingly, the growth rate of fish increases at a declining rate with weight (W), as a resultant of the anabolic and catabolic processes of different rates (von Bertalanffy, 1938). Accordingly, fish growth rate can be described by two terms with specific exponents ***m*** for anabolism and ***n*** for catabolism:

$$\frac{dW}{dt} = H \cdot W^m - k \cdot W^n \qquad 1.$$

where:

H is the coefficient of anabolism, g^{1-m} day^{-1}
W is the weight of the given species, g
k is the coefficient of catabolism, g^{1-n} day^{-1}

The first term of the Equation can be expanded to consider the daily food intake, the feeding of catabolism and the digestibility of the food (Ursin, 1967), as follows:

$$H \cdot W^m = b \cdot (1 - a) \cdot R \qquad 2.$$

where:

a is the fraction of the assimilated food that is used for feeding catabolism (0–1);
b is the efficiency of food assimilation (0–1); and
R is the so-called "daily ration", g day^{-1}, which is the sum of daily intake of endogenous or natural food (R_n) and supplemental forage feed (R_s).

Based upon previous works (e.g., Winberg, 1960; Ursin, 1967), daily ration (R) can be calculated as a function of fish size (W^m), food availability (f) and environmental conditions (E):

$$R = E \cdot f \cdot h \cdot W^m \qquad 3.$$

where:

f is the relative food availability, i.e. feeding level $0 < f < 1$, dimensionless (see in Section 3.2.1.2),
h is the fish specific coefficient of food consumption, g^{1-m} day^{-1}, and
E considers the environmental conditions (see in Section 3.2.1.3, Eq. 10).

3.2.1.2 Consideration of relative food availability and the choice from the alternative sources

The relative food availability expresses two, quite different effects, namely

- the limited amount of the available feed, and
- the possible choice between the various, simultaneously available alternative natural and artificial feeds (according to the actual food web).

In our model, we assumed the simplified food web according to Fig.1

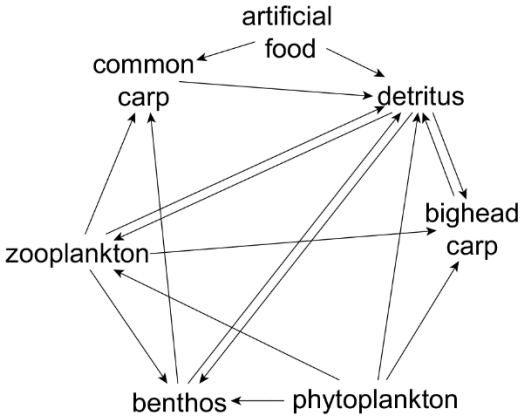


Figure 1. The food web of the studied model

Some authors (e.g. Karimanzira et al, 2016, for RAS) calculate f by various, apparently fish- and case specific heuristic expressions, e.g. based on (Ivlev, 1961). In some other sources (e.g. Kapetsky and Nath, 1997, for ponds) f refers to the level of satiation, combining natural and artificial food

$$1 \geq f = f_n + f_a \qquad 4.$$

where f_n and f_a consider the natural and artificial food levels, respectively. Accordingly, f_a may be calculated in the knowledge of the previously known f_n.

In other fish specific sources (Jamu, 1998; Lu, 2003) f is decomposed to the product of feed preference factor η_j for the various foods j and relative feeding level λ as follows:

$$f = \lambda \cdot \sum_j \eta_j \qquad 5.$$

while

$$\eta_j = \frac{F_j}{K_j + F_j} \qquad 6.$$

where F_j and K_j are the concentration and the satiation coefficient of the feed j, respectively. In another source (Bolte et al, 1995) feeding level λ is defined as

$$\lambda = \max\left(\frac{Critical\ standing\ stock}{Total\ actual\ fish\ biomass}, 1\right) \qquad 7.$$

where the *Critical standing stock* is an ill-defined, heuristic data, depending on pond management and environmental conditions.

It is to be noted that Eq. 6 formally similar to the ration in the Michaelis-Menten satiation kinetics, applied in biomass based food web models. Sometimes the satiation ration is described by the term of

$$\frac{F_j^2}{K_j^2 + F_j^2} \qquad 8.$$

Regarding the interpretation of the fish's preference between the alternative (natural and artificial) feeds, there might be various solutions, as follows:

- Demand driven clear preference: the less preferred food is consumed only in the case of missing more preferred (e.g. Kapetsky and Nath, 1997);
- Supply driven preference: controlled by the ratio of the available alternative feeds;
- Demand weighted supply driven preference: controlled by the weighted ratio of the available alternative feeds (a possible more sophisticated solution with more data demand);
- Application of probabilistic switching functions (e.g. Svirezhev et al, 1984), also with more data demand.

In our model implementation, we utilized a simplified, availability driven heuristics. Instead of taking into consideration the preference of various food web elements, we utilized a formula for the consideration of the availability of the given food source, assuming that the given species does not select, but consume in the proportion of availability. For example, if the main diet of Common carp is based on artificial feed (A), benthos (B) and zooplankton (Z) in line with Fig. 1, then we determine availability rates (A_A, A_B and A_Z), as follows:

$$A_A = \frac{A}{A + B + Z}$$
$$A_B = \frac{B}{A + B + Z} \qquad 9.$$
$$A_Z = \frac{Z}{A + B + Z}$$

3.2.1.3 Consideration of environmental factors

The environmental conditions (E, used in Eq. 3) can be taken into account as follows

$$E = \tau \cdot \delta \cdot v \cdot \beta \quad (10.)$$

where:

τ is the temperature factor, $0 < \tau < 1$, dimensionless,
δ is the DO factor, $0 < \delta < 1$, dimensionless,
v is the unionized ammonia factor, $0 < v < 1$, dimensionless,
β is the BOD factor, $0 < \beta < 1$, dimensionless.

Taking one by one the formulation of these environmental parameters Brett (1979) assumed that the food consumption of fish species increases when the water temperature (T) elevates constantly from the lower limit (T_{min}) to the optimum (T_{opt}). On the other hand, the food consumption decreases when the temperature increases from the optimum to the maximum temperature (T_{max}). The temperature influence on food consumption and anabolism was proposed by Bolte et al, (1995), like the temperature effect is written in term of function (τ)

$$\begin{aligned} \tau &= \exp\{-4.6[(T_{opt} - \mathrm{T})/(T_{opt} - T_{min})]^4\}, && \text{if } \mathrm{T} < T_{\mathrm{opt}} \\ \tau &= \exp\{-4.6[(\mathrm{T} - T_{opt})/(T_{max} - T_{opt})]^4\}, && \text{if } \mathrm{T} \geq T_{\mathrm{opt}} \end{aligned} \quad (11.)$$

Also catabolism (actually the k coefficient of catabolism in Eq. 1) increases exponentially with temperature within the tolerance limits for a given species (Ursin 1967; Cuenco et al., 1985). In the growth model, the exponential function suggested by Ursin to include the lower temperature tolerance limit for the given species was modified by Nath et al. (1997) to assume being equivalent to the above T_{min} as follows:

$$k = k_{min}\, exp\,[s\,(T - T_{min})] \quad (12.)$$

where:

k_{min} is the coefficient of fasting catabolism at T_{min}, $g^{1-n}day^{-1}$; and
s is a constant to describe temperature effects on catabolism, C^{-1}.

The dissolved oxygen (DO) concentration is the dominant factor affecting the food consumption of fish. Bolte et al. (1995) described the effects of DO concentration on food consumption and anabolism in terms of functions with

$$\begin{aligned} \delta &= 1.0 && \text{if } DO > DO_{\mathrm{crit}} \\ \delta &= (\mathrm{DO} - \mathrm{DO}_{min})/(\mathrm{DO}_{crit} - \mathrm{DO}_{min}) && \text{if } DO_{min} \leq DO \leq DO_{crit} \\ \delta &= 0.0 && \text{if } DO < DO_{\mathrm{min}} \end{aligned} \quad (13.)$$

Considering the effect of the ammonia, Cuenco et al., 1985 described how the unionized ammonia (UA) affects food consumption. Accordingly, the food consumption does not affected if the unionized ammonia is less than a critical limit (UA_{crit}) but the food consumption decreases if the unionized ammonia increases. Bolte et al. (1995) described the effects of unionized ammonia on food consumption in term of function v as shown in the equation

$$\begin{aligned} \nu &= 1.0 && \text{if } UA < UA_{crit} \\ \nu &= (\mathrm{UA}_{max} - \mathrm{UA})/(\mathrm{UA}_{max} - \mathrm{UA}_{crit}) && \text{if } UA_{crit} \leq UA \leq UA_{max} \\ \nu &= 0.0 && \text{if } UA > UA_{max} \end{aligned} \quad (14.)$$

while unionized ammonia concentration (UA) can be calculated by the expression

$$UA = \frac{1}{10^{(pKa - pH)} + 1} \cdot TAN \quad (15.)$$

where

TAN is the total ammonia nitrogen concentration, and
pKa is estimated according to (Emerson et al, 1975) as a function of the temperature (T)

$$pKa = 902 + \frac{2730}{T + 273} \quad (16.)$$

The BOD concentration also affects the food consumption of fish. In fact, BOD relates to DO. If BOD is below the critical limit (BOD_{crit}), fish gets benefit in food consumption but the food consumption decreases when BOD increases. If the value of BOD concentration is greater than the critical value (BOD_{max}), fish cannot survive. The function β to represent these BOD effects and is shown in equation

$$\beta = 1.0 \qquad \text{if } BOD < BOD_{crit}$$

$$\beta = (\mathrm{BOD}_{max} - \mathrm{BOD})/(\mathrm{BOD}_{max} - \mathrm{BOD}_{crit}) \quad \text{if } BOD_{crit} \leq BOD \leq BOD_{max}$$
$$\beta = 0.0 \quad \text{if } BOD > BOD_{max} \qquad (17.)$$

All of the utilized parameters for Common carp (together with the source of origin) are listed in Appendix.

3.2.2 Calculation of biomass for the other elements of food web

In the biomass based calculation for other food web elements (Bighead carp, zooplankton, phytoplankton, detritus and benthos), we considered feeding/consumption related growth, metabolism, mortality, as well as the degradation of biomass. In the description of these processes, we relied on the early work of (Shirezhev, et al, 1984), basically.

3.2.2.1 Increase of biomass

In case of other elements of the food web (Bighead carp, phytoplankton, zooplankton and benthos), the consumption related growth is determined by the internal relationships, as the arrows in Fig. 1 refer to the predator/prey relations between the involved components. For example, consumption related growth of zooplankton depends on the concentration of phytoplankton and detritus, as follows:

$$DFZ_0 = UFZ_{max} * \tau * FO * \left(\frac{F^n}{KFZ^n + F^n}\right) * \left(\frac{F}{F + D}\right) * Z * Area * DT$$

and

$$DDZ_0 = UDZ_{max} * \tau * FO * \left(\frac{D^n}{KDZ^n + D^n}\right) * \left(\frac{D}{F + D}\right) * Z * Area * DT \qquad (18.)$$

where:

DFZ_0 and DDZ_0: growth of zooplankton by eating phytoplankton and detritus, respectively, kg,
UFZ_{max} and UDZ_{max}: the maximum uptake rate of phytoplankton and detritus by zooplankton, respectively, 1/day,
τ: the Lehman-function, describing the temperature effects, according to Eq. 11,
F, D, Z: the actual concentration of phytoplankton, detritus and zooplankton, respectively, kg/ha, used for the calculation of availability of each diet elements,
KFZ and KDZ the half saturation parameter of phytoplankton and zooplankton, respectively, kg/ha,
n: the power of Michaelis-Menten like satiation kinetics, dimensionless,
Area: area of the fishpond, ha
DT: prescribed time step of the model, actually 1 day,
FO: the function describing the relationship between growth and the dissolved oxygen:

$$FO = \frac{1}{(1 + \exp(-Lambda * (O_2 - M)))} \qquad (19.)$$

where:

Lambda is the steepness of the oxygen curve for zooplankton, $m^3\ kg^{-1}$;
O_2 is the actual concentration of oxygen, kg m^{-3};
M is the oxygen half-maintenance parameter regarding zooplankton, kg m^{-3}.

3.2.2.2 Decrease and decomposition of biomass

Decrease in biomass (both for the main species and for other food web elements) consists of the

- catabolized portion of consumption (DE, kg), and
- the fecal loss and mortality (DD, kg).

Through the example of zooplankton, catabolized portion of consumption can be described, as follows:

$$DE = Mboz * (DFZ_0 + DDZ_0) \qquad (20.)$$

where:

Mboz: respiration coefficient for zooplankton, dimensionless,
DFZ_0 and DDZ_0: growth of zooplankton by eating phytoplankton and detritus, respectively, kg, calculated by Eq. 18.

Fecal loss and mortality can be calculated by the expression

$$DD = Mbz * (DFZ_0 + DDZ_0) + ODM * Mz * Z * Area * DT \quad 21.$$

where:

Mbz: metabolism parameter for zooplankton, dimensionless,
DFZ_0 and DDZ_0: growth of zooplankton by eating phytoplankton and detritus, respectively, calculated by Eq. 18.
Mz: mortality coefficient of zooplankton, day^{-1},
Z: the actual concentration of zooplankton, kg ha^{-1},
Area: area of the fishpond, ha
DT: prescribed time step of the model, actually 1 day,
ODM: is the factor, describing the dependence of mortality on dissolved oxygen:

$$ODM = 1 + \frac{K_a}{O_2} \quad 22.$$

where:

K_a is the parameter of mortality increase at dissolved oxygen deficit, kg m^{-3};
O_2 is the actual concentration of oxygen, kg m^{-3}.

Decomposition of the considered elements goes into the pool of detritus (see Fig. 1). Next, the calculated contribution of each food web element to the detritus pool (Eq. 23) has to be corrected by the consumption related decrease of detritus by the given food web element, if any (e.g. in case of zooplankton and benthos, because detritus is involved in their diet), accordingly

$$DD_{detr} = DD - DDZ0 \quad 23.$$

where

DD is the fecal loss and mortality, kg, in line with Eq. 21,
DDZ_0: the consumed amount of detritus by zooplankton, kg, according to Eq. 18

Final changes (during a time step) in detritus biomass can be calculated as the sum of growth by consuming phytoplankton and detritus, decreased with the catabolized consumption, as well as with the fecal loss and mortality:

$$DZ = DFZ0 + DDZ0 - DD - DE \quad 24.$$

The final decomposition of dead organic material produces phosphorus and nitrogen (similarly to feed and fertilizer decomposition in Eqs. 37-42, later on).

3.2.3 Photosynthesis of phytoplankton

Natural oxygen supply of fishponds highly depends on production of phytoplankton, so in the description of phytoplankton related processes, we have to consider light dependent oxygen production. In line with the literature (Svirezhev et al, 1984, with reference to Steele, 1962), the light limitation function can be considered with the following equation:

$$FF = \frac{L}{L_{opt}} * exp\left(1 - \frac{L}{L_{opt}}\right) \quad 25.$$

where:

L is the illumination, calculated by the Beer-Lambert formula in Eq. 26,
L_{opt} is the optimal solar radiation.

while

$$L = L_o * exp(-K * PhotDepth) \quad 26.$$

where:

L_0 is the actual total solar radiation, coming from the meteorological database, W m^{-2},
K is the light extinction coefficient, depending on the actual concentration of phytoplankton and detritus in the pond, m^{-1},
PhotDepth refers to the mean photosynthesis depth, m.

while

$$K = Kw + Kf * F + Kd * D * Kpd \quad 27.$$

where:

Kw is the light extinction coefficient in water, m^{-1},
Kf is the self-shading parameter of phytoplankton, ha kg^{-1} m^{-1},
F refers to the actual concentration of phytoplankton in the pond, kg ha^{-1},
D refers to the actual concentration of detritus in the pond, kg ha^{-1},
Kd is the shading parameter of suspended detritus, ha kg^{-1} m^{-1},
Kpd is the fraction of detritus, suspended in the water, dimensionless.

The growth of phytoplankton biomass is calculated as follows:

$$DF0 = UF_{max} * FT * FF * \min\left(\frac{P^2}{K_{PF}^2 + P^2}, \frac{1}{5} * \frac{N^2}{K_{NF}^2 + N^2}\right) * F * Area * DT \quad 28.$$

where:

UF_{max} is the maximum growth rate of phytoplankton, day^{-1}
FT is the temperature factor, dimensionless
FF is the consideration of light limitation, dimensionless
P and N is the actual phosphorus and nitrogen concentration in the pond, kg m^{-3},
K_{PF} and K_{NF} is the half saturation constant for the uptake of phosphorus and nitrogen, kg m^{-3},
F is the mass of phytoplankton, kg ha^{-1},
Area corresponds to the pond surface, ha
DT is the time step, actually 1 day

Decrease of nitrogen and phosphorus was determined in line with the phytoplankton growth, applying the typical Redfield ratio (Perdue, 2009) from the literature. Atomic ratio of C:N:P used to be considered typically 106:16:1. In our case, we utilized the ratio of 106:5:1, suggested by (Svirezhev et al, 1984) keeping the consistency with the other applied parameters. Accordingly, the decrease of nitrogen and phosphorus pool was accounted as follows:

$$DN = -\frac{5}{106} * DF0 \quad 29.$$

$$DP = -\frac{1}{106} * DF0 \quad 30.$$

Phytoplankton related oxygen balance is calculated by

$$DO_2 = Phot * DF0 - Respf * F * Area * DT \quad 31.$$

where:

Phot is assimilation coefficient, dimensionless
DF0 is the growth of phytoplankton, kg,
Respf is the parameter for dissolved oxygen consumption in phytoplankton respiration, 1/day
F is the actual concentration of phytoplankton, kg/ha
Area corresponds to the pond surface, ha
DT is the time step, actually 1 day.

3.3 Environmental (meteorological, hydrological) data

High-precision models for calculation of evaporation are available in the literature (e.g. Saloranta and Andersen, 2007), however, they are designed for the precise modeling of deep-water lakes, with a large input data requirement. Considering the limited availability of necessary data both for the investigated fishponds and in the applied future climate scenario models, we used an approximate modeling approach. The calculation of evaporation (EP) was implemented by the generally accepted empirical expression of Antal and Tóth (1976), developed and validated for the shallow lakes (e.g. Lake Balaton) in Hungary.
The applied formula estimates evaporation with less input data requirement, utilizing the water temperature dependent equilibrium, the actual vapor pressure and the wind speed. In our water balance model, we applied the modified version of the original formula, corrected with a "seasonal" coefficient by VITUKI (1986) as follows:

$$EP = a * (P0_{vap}(T_w) - P_{vap}) * (0.59 + 0.013 * \mathrm{W}) \quad 32.$$

where:

EP is evaporation, mm day^{-1}
$P0_{vap}$ (T_w) is the equilibrium vapour pressure (mbar) in the function of water temperature T_w;
P_{vap} is the actual vapour pressure calculated from the relative air humidity, mbar;

'a' is a seasonal coefficient (in March: 0.7, in April: 0.8, in October: 1.3, in November: 1.4, otherwise: 1) and
W is the daily average of wind speed, m s^{-1}.

For the model development, we used historical data for two geographic areas of the Southern and Northern Great Plain of Hungary. Based on the validated model, NORESM climate scenarios were used to determine predictions for the same two geographic locations. However, using this climate model (that provides only daily air temperature and precipitation data), we had to make some simplifying assumptions.
One of these simplifying assumptions was to approximate the daily average water temperature with daily average air temperature. It is to be noted that, in principle, the surface water temperature should be used for calculation of equilibrium vapor pressure. This surface temperature is the convex combination of air and water temperatures. However, the analysis of data from the period of 2009-2014 confirmed that use of average water temperature is applicable for fishpond production period April-October.
For the calculation of the actual vapor pressure, the relative humidity is also required. As the relative humidity data are not available in the climate scenarios, we utilized 60%, as an average value for the production period, estimated also from the above mentioned 6-year long data series.
Regarding wind speed, our simplifying assumption was to use the average daily value, coming again from the 2009-2014 factual data.
According to the bottom bed characteristics of the studied ponds, seepage was not considered in the hydrological sub-model.

3.4 Consideration of the managerial interventions of feeding and fertilizing

Data for feeding and fertilizing came from the monthly aggregated logbook of the investigated fishpond. In case of feeding, we divided the gross monthly amount into daily portions. Regarding fertilization, in line with the practice, we considered that the organic manure is dispensed in the first three days of every month in the season.
A given part (Alpha) of the forage decomposes directly that decreases the available artificial feed pool (NDA), as well as increases the amount of detritus (DD) at the same time, as follows:

$$DD = Alpha * A * Area * DT \quad (33.)$$

$$NDA = -1 * DD \quad (34.)$$

where
Alpha is the rate of decomposition, day^{-1},
A is the actual concentration of feed in the pond, kg ha^{-1},
Area is the pond surface, ha,
DT is the time step of the model, actually 1 day.

Similarly we took into consideration the decomposition of the supplied organic fertilizer, with a heuristic factor (Beta, day^{-1}):

$$DD = Beta * Fert * Area * DT \quad (35.)$$

The decomposed amount, in addition to the phosphorus (DP) and nitrogen (DN) loss, decreases the fertilizer pool in the pond (and increases the mass of detritus), as follows:

$$NDFert = -1 * DD - DP - DN \quad (36.)$$

Final phosphorus (DP) and nitrogen (DN) formation, associated with organic manure decomposition, can be described as follows:

$$DP = UDP * E1 * E2 * Fert * Area * DT \quad (37.)$$
$$DN = UDN * E1 * E2 * Fert * Area * DT \quad (38.)$$

where:
Fert is the actual concentration of fertilizer in the pond, kg ha^{-1},
Area is the pond surface, ha,
DT is the time step of the model, actually 1 day.
UDP is the phosphorus destruction parameter, day^{-1},

UDN is the nitrogen destruction parameter, day^{-1},
E1 is the dependence of organic matter decomposition on temperature in line with Eq. 39, dimensionless
E2 is the dependence of organic matter decomposition on dissolved oxygen in line with Eq. 40, dimensionless

$$E1 = 2^{\left(\frac{T-20}{10}\right)} \quad 39.$$

$$E2 = \frac{\exp\left(COP * (M_O - O_2)\right)}{1 + \exp\left(COP * (M_O - O_2)\right)} \quad 40.$$

where:

T is temperature, °C,
COP is the parameter of the steepness of oxygen function, dimensionless
M_O is the limit value between aerobic/anaerobic conditions, kg m^{-3},
O_2 is the actual concentration of dissolved oxygen, kg m^{-3}.

Mineralized (sedimental) amount of phosphorus is calculated by:

$$DecrP = SedP * E3 * P * Area * DT \quad 41.$$

where

SedP is the sedimentation parameter of phosphorus, day^{-1}
E3 is the dependence of mineralization on dissolved oxygen, dimensionless, see Eq. 42.
P is the actual concentration of phosphorus, kg m^{-3}

Dependence of the mineralization on the presence of dissolved oxygen is characterized by the following equation:

$$E3 = \begin{cases} 0, & O_2 < Mo \\ \dfrac{O_2 - Mo}{O_2 - COD}, & O_2 \geq Mo \end{cases} \quad 42.$$

where

O_2 is the actual dissolved oxygen concentration, kg m^{-3}
Mo is the threshold between aerobic/anaerobic conditions, kg m^{-3}
COD is the threshold between the oxic/anoxic conditions, kg m^{-3}

3.5 Process modeling by Programmable Process Structures

Programmable Process Structures (PPS) is a non-conventional process modeling and simulation method. It has developed from the former Direct Computer Mapping (DCM, Csukas, 1998; Csukas et al, 2011) in the past years. DCM originally was applied for the solution of various chemical engineering problems (e.g. Csukas et al, 1999; Temesvari et al, 2004; Csukas et al, 2013). The basic characteristic of the method is that the natural building blocks of the elementary states and transitions are mapped onto a unified set of building elements, determining the executable computer code, directly.
The field of application has broadened toward the model based understanding of biosystems, as well as model based analysis and planning of agricultural and environmental process systems in the past years. In these fields we recognized the increasing demand for a flexible and easily extensible modeling framework that is able to generate and visualize the process model, automatically. Accordingly, the original DCM has developed toward Programmable Process Structures, comprising the automatic structure and model generation from the unified description of underlying process systems (Varga and Csukás, 2017a). It was applied for the modeling and simulation of a wide range of process systems in the past years (Varga et al, 2016; Varga and Csukas, 2017b; Varga et al, 2017). The main components of the framework are:

- General kernel of the model generator and simulator (written in platform independent GNU-Prolog);
- GraphML based representation of process structure and with locally editable description of case specific functionalities;
- Connected database of case specific actual and hypothetical meteorological and hydrological data.

The scheme of model building procedure can be seen in Fig. 2. Starting from a conceptual model of the investigated problem (see in Fig. 3), an empty process structure (without the functionalities) is generated from:

- two (one state and one transition) meta-prototypes, that are the general building blocks of the method,

- the standardized description of the investigated problem's process network, and
- the associated set of initial data and parameters.

After the generation of the structure, the next step is the development of functionalities that actually means the formulation of the relationships, in form of local programs, actually formulated in GNU Prolog syntax. GNU Prolog, as a declarative logical language, works on the basis of unification that is useful for knowledge representation. Additional advantages come from with the locally interpretable, reusable, small prototype program codes. It is especially useful for the description of large, multi-scale systems, consisting of many stereotypical parts. It is also important to highlight, that majority of the variables is local, that supports re-usability of program code in similar cases, as well as the easy use of variable names (i.e. consistency of variable names must be ensured only within a given local program, not for the whole code).

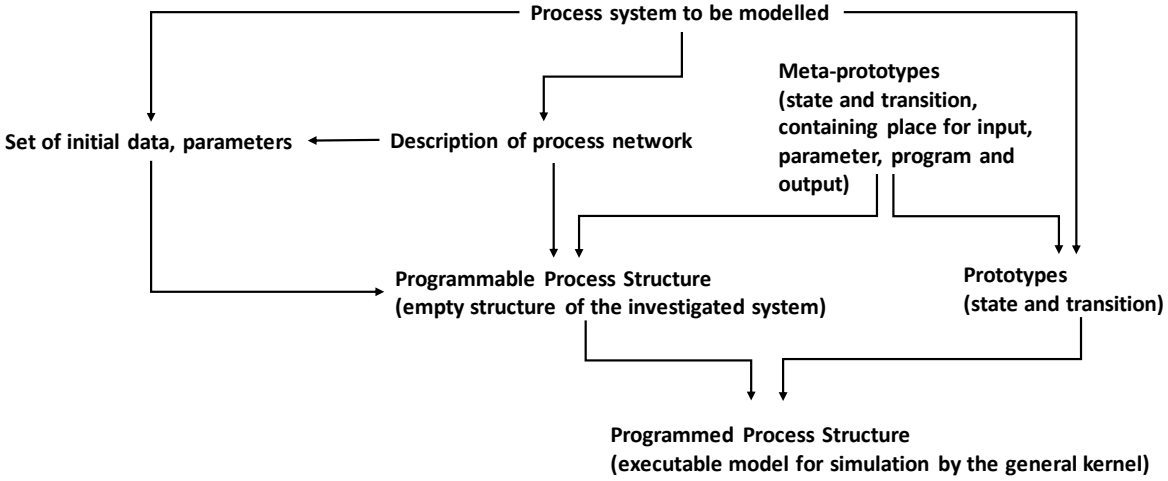


Figure 2. Scheme of model building procedure

Detailed explanation about the characteristics of the building elements (i.e. the architecture of states, transitions and the types of connections between them), the generalized ontology describing the process network, as well as the execution scheme of the simulation model can be found in our former work, in detail (Varga and Csukas, 2017a and 2017b; Varga et al, 2017).

In the next Section, illustration of the generated structure, as well as the implementation of the above (in Section 3.2) described physical, chemical, biological and technological/managerial relationships, embedded in the structure to form an executable model, will be introduced.

4. Results of model development by implementing Programmable Process Structures

4.1 Generation of the parameterized fishpond model, involving a food web

The conceptual model of the fishpond ecosystem, emphasizing the connected environmental and managerial effects is shown in Fig. 3.

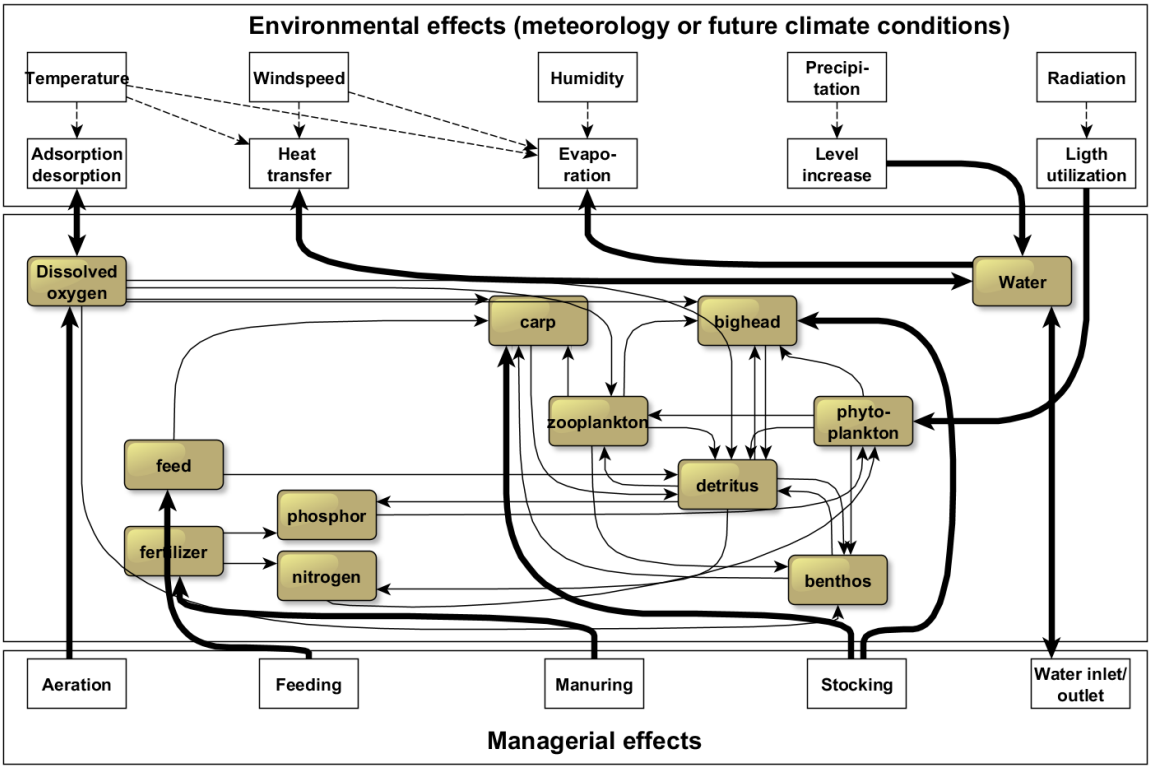


Figure 3. Conceptual model of the fishpond with the connected environmental and managerial effects

In line with the principles of PPS, model building is based on this conceptual model, and starts from the definition of state and transition elements of the underlying processes. The list of state and transition elements are summarized in Table 1. The list of state elements refers to those entities, components, signals that have to be considered in the model. The transition elements represent the underlying physical, chemical and biological processes to be taken into consideration in the calculations.

Table 1. Prototyped state and transition elements of the food web involved fishpond model

State elements	Name of corresponding prototype element in the model	Transition elements	Name of corresponding prototype element in the model
Main fish species: Common carp and Bighead carp in the studied case;	prot_species_main	Life related processes of Common carp: weight-dependent nutrient intake, weight gain, excretion, etc.	prot_t_carp
Other elements of food web: phyto- and zooplankton, benthos, detritus;	prot_species	Life related processes of Bighead carp: weight-dependent nutrient intake, weight gain, excretion, etc.	prot_t_bighead
Forage and fertilizer	prot_component	Phytoplankton related processes (CO_2, N, P metabolization, O_2 and biomass formation, decomposition)	prot_t_phytop
Secondarily formed nutrients: metabolizable nitrogen, metabolizable phosphorus, etc.	prot_component	Zooplankton related processes (metabolization, biomass formation, decomposition)	prot_t_zoop
Chemical components and characteristics of the pond: O_2, CO_2, HCO_3, N, P, water temperature	prot_water	Benthos (biomass formation and decomposition)	prot_t_benthos
Air components: O_2, CO_2	prot_component	Detritus (formation and decomposition)	prot_t_detritus
Meteorological characteristics: air and water temperature, precipitation, relative humidity, wind speed, radiation	prot_datasupply	Heat transfer, evaporation, O_2 and CO_2 transport between air and water body	prot_water_balance
Storages (for forage and fertilizer)		Additional water supply to the pond (considered in line with the recorded data of SzegedFish Ltd.)	prot_water_supply
Heat balance of the pond	prot_wheat	Foraging (various strategies, considered in line with the recorded data of SzegedFish Ltd.)	prot_foraging
		Process of manuring (considered in line with the recorded data of SzegedFish Ltd.)	prot_fertilizing
		Decomposition of forage	prot_forage_decomp
		Decomposition of fertilizer	prot_fertilizer_decomp

General description about model building procedure can be read in former publications (Varga and Csukas, 2017a and 2017b; Varga et al, 2017). However, some important details are highlighted here, in context of model building for fishpond.
The process network was described according to the following syntax:

states(CoordList,StateList).
dcode(StateID,DNameList).
dcodesign(StateID,DNameList).
transitions(CoordList,TransitionList).
trans(TransitionID,InpExtList,InpSignList,OutIntList,OutSignList).

where
CoordList = Coord* (a list of coordinates) and Coord = atomic
StateList = StateID* (a list, consisting of the underlying state elements) and State = symbol
DNameList = DName* (a list, consisting the names of underlying d() triplets of the state elements) and DName = symbol
TransitionList = Transition* (a list, consisting of the underlying transition elements) and Transition = symbol

InpExtList, InpSignList, OutIntList, OutSignList are lists of extensive measures and signals in the input, as well as the intensive measures and signals in the output

The trans() entities (predicates) describe the connections between the involved states() and transitions(). In the state elements, the lower level data structures are the d() triplets, where elements in the triplets refer to an identifier, a quantity and a dimension.
This structure of the process network is conform with state and transition meta-prototypes, which are the two general templates of the method, containing slots with empty lists for the above interpreted inputs, outputs and parameters, as well as a place for local program code or its identifier. The general kernel program generates the empty Programmable Process Structure from these two general meta-prototypes and from the standardized description of the case specific process network.
Also the initial data and parameters can be imported from a database into the PPS during the generation procedure. The most important initial values of the state elements are summarized in Table 2. The parameters of the state and transition elements must be consistent with the symbolic parameters of the local programs, accordingly they can be finalized in the knowledge of the program prototypes (see later). The actually applied parameters are presented in the Appendix.

Table 2. Initial values of the main state elements in the model

Initial values	Value	Dimension	Source of data
Phytoplankton	30.5	kg/ha	estimated value for shallow lakes, based on Bíró, 2002
Zooplankton	72.7	kg/ha	estimated average value, based on some April 1 measurements in SzegedFish Ltd.
Benthos	0.71	kg/ha	estimated value for shallow lakes, based on Bíró, 2002
Detritus	131.9	kg/ha	estimated value for shallow lakes, based on Bíró, 2002
Phosphorus	2.5	mg/l	based on the preparatory fertilization at SzegedFish Ltd.
Nitrogen	15	mg/l	based on the preparatory fertilization at SzegedFish Ltd.
Oxygen	9.76	mg/l	based on Vince et al, 2011

The generated Programmable Process Structure can be seen in Figure 4. Technically, it is in graphml format that supports visualization and flexible further edition of prototypes (including also the calculation determining local program codes).

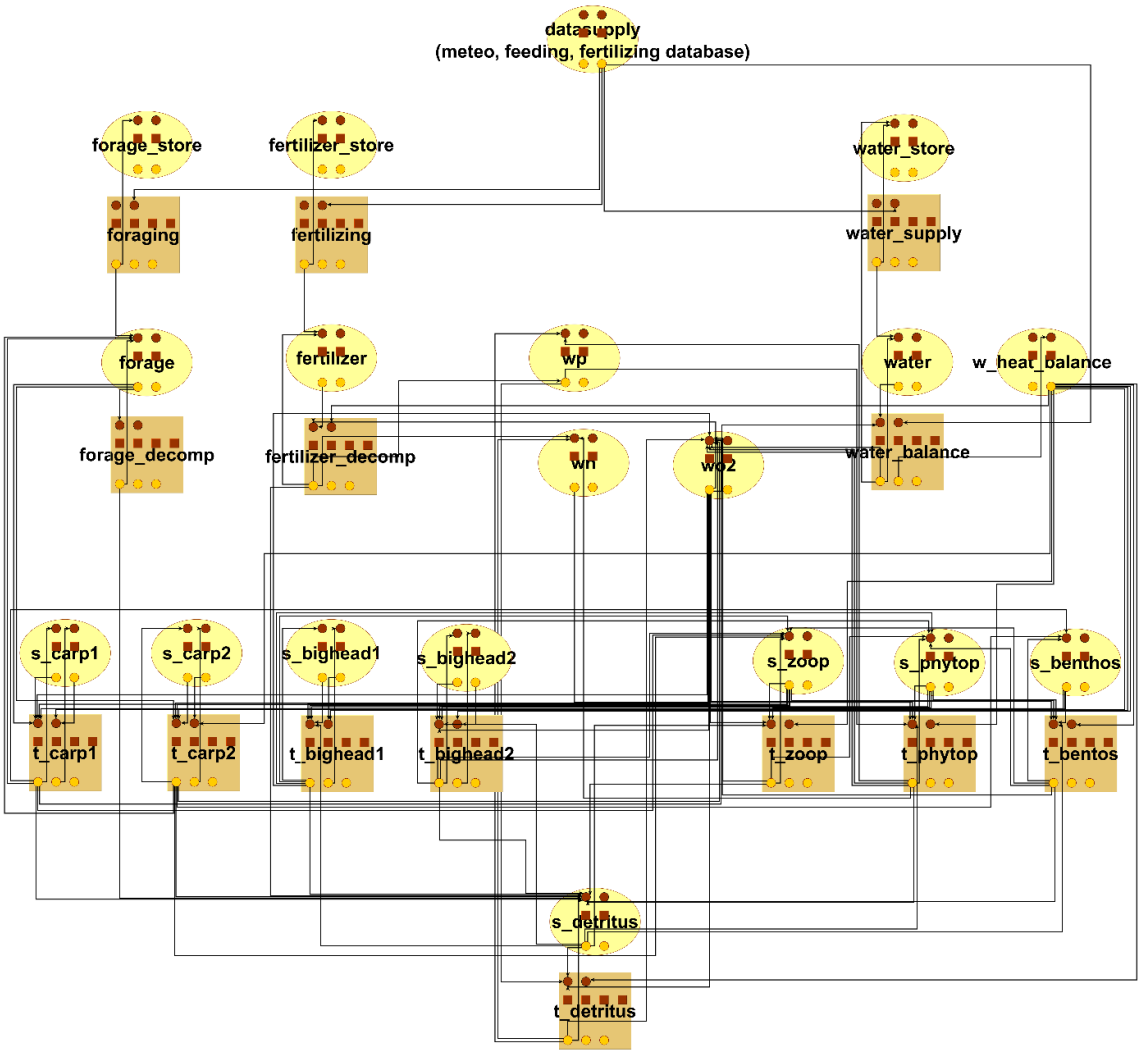


Figure 4. Generated structure of food web involved fishpond model

4.2 Programming of the locally interpretable functional prototypes of the model

As a next step, the parameterized and initialized structure of Figure 4. has to be extended with the calculation determining prototype elements, implementing the introduced models in form of local program codes. The list of the developed prototype elements are summarized in Table 1.
Technically, it means making copies from the meta-prototypes, and the executable program code of calculation can be formulated by the variables of input, parameter and output slots of these prototype elements. Each actual elements within the network have to be associated with one prototype program (as listed in Table 1), as well as in accordance with their own initial data and parameters. (It is to be noted, that usually many general state and transition elements in the network utilize the same prototype program.)
During the execution of the simulation, all of the actual state and transition elements are calculated by the respective prototype program, by unifying (binding) the variables with its own actual data and parameters.
The programming of prototypes will be illustrated through the example of life related processes of Common carp.

Table 3. Example local program for the life related processes of Common carp

Program code of prot_t_carp	**Explanation (designations are in line with the Appendix)**
{program(' prot_t_carp	Opening of the locally executable code.
carp_selection(S_carp,InpConcs),	There are two age group of carps considered in the model (1 and 2 year old carps). “carp_selection()” predicate selects the

	actually calculated group, in line with the below explained definition in this Table.
permutation(InpConcs,[d(S_carp,[C],kg_ha), d(forage,[A],kg_ha), d(s_bentos,[B],kg_ha), d(s_zoop,[Z],kg_ha), d(wo2,[O2],kg_m3)]), permutation(InpSigns,[d(wheat,[T],c),d(S_carp,[PIa],kg_pc)]),	Standard predicate of permutation() is used for appropriate reading of the actually used measures/signals, coming from other elements of the network or from the database, being in any order in the list. These input measures and signals (with variable names) are used in the calculation formulas.
g(dt,DT), g(area,Area)	Global variables of the model.
Carp is C*Area, PNa is Carp/PIa	Calculation of actual mass and the number of carps in the given time step.
ft(T,Topt,Tmin,Tmax,Tau),	The predicate ft() calculates the temperature factor, in line with below explained definition in this Table (see last 5 rows), utilizing Eq. 11.
fo(O2,DOcrit,DOmin,Delta),	The predicate fo() calculates the dissolved oxygen factor, in line with below explained definition in this Table (see last 5 rows), utilizing Eq. 13.
FFA is A/(A+B+Z), FFB is B/(A+B+Z), FFZ is Z/(A+B+Z),	Calculation of availability rations of artificial food, benthos and zooplankton, as a diet of carp, utilizing Eq. 9.
RCalcA is Tau*Delta*Nue*Beta*FFA*H*(PIa*1000)**Mexp*PNa*DT/1000, RCalcB is Tau*Delta*Nue*Beta*FFB*H*(PIa*1000)**Mexp*PNa*DT/1000, RCalcZ is Tau*Delta*Nue*Beta*FFZ*H*(PIa*1000)**Mexp*PNa*DT/1000,	Calculation of the consumed daily amount of artificial food, benthos and zooplankton by carp, in line with Eq. 3.
AvailableA is A*Area, AvailableB is B*Area, AvailableZ is Z*Area,	Calculation of the available amount for each element of the Carp's diet.
DAC0 is min(RCalcA,AvailableA), DBC0 is min(RCalcB,AvailableB), DZC0 is min(RCalcZ,AvailableZ),	The calculated available amount for each element of the diet, utilizing the min() standard predicate.
NDAC0 is (-1)*DAC0, NDBC0 is (-1)*DBC0, NDZC0 is (-1)*DZC0,	Calculated decrease of the diet elements (artificial food, benthos and zooplankton).
PlusDWA is BFCRA*(1-AA)*DAC0, PlusDWB is BFCRB*(1-AA)*DBC0, PlusDWZ is BFCRZ*(1-AA)*DZC0, PlusDW is PlusDWA+PlusDWB+PlusDWZ,	Calculation of the anabolized amount of consumed artificial food, benthos and zooplankton by Carp.
cat_temp(SS,T,Tmin,Coeff),	Calculation of the catabolism coefficient by cat_temp predicate, see below explained definition in this Table.
MinusDW is Kmin*Coeff*(PIa*1000)**Nexp*PNa*DT/1000, DW is PlusDW - MinusDW,	Calculation of the catabolized amount on the basis of the actual carp biomass, in line with the decreasing part of Eq. 1.
chkzero(DW,PNa,DPIa),	Calculation of the mass change in the individual weight of carp. chkzero() predicate of division is embedded in the general kernel program, because it is utilized in many case.
NewPIa is PIa + DPIa,	Calculation of the new individual weight of carp.
DCmort is Mc*C*Area*DT, DC is DW - DCmort,	Calculation of the mortality, as well as of the final accounted weight increase of the given species.
DO2 is (-1)*Respc*C*Area*DT,	Calculation of the utilized dissolved oxygen, in line with the second member of Eq. 31.
DCex is ((1-BFCRA)*DAC0+ (1-BFCRB)*DBC0+(1-BFCRZ)*DZC0) + (BFCRA*AA*DAC0+BFCRB*AA*DBC0+BFCRZ*AA* DZC0) + MinusDW + DO2*(12/44),	Calculation of the excreted mass.

DD is DCmort + DCex,	Calculation of the detritus mass increase (from the excretum and the mortal carps).
Report = [d(plusdw,[PlusDW],kg), d(minusdw,[MinusDW],kg), d(dcmort,[DCmort],kg), d(dc,[DC],kg), d(fo,[Delta],nd), d(ft,[Tau],nd)], OutComps = [d(S_carp,[DC],kg), d(forage,[NDAC0],kg), d(s_bentos,[NDBC0],kg), d(s_zoop,[NDZC0],kg), d(s_detritus,[DD],kg), d(wo2,[DO2],kg)], OutSigns = [d(S_carp,[NewPIa],kg_pc)].	Determination of information to be reported and to be sent to the output measure/signal slots. These measures and signals will be used in the calculation in the next time step.
carp_selection(s_carp,InpConcs) :- member(d(s_carp,_,_),InpConcs),!. carp_selection(s_carp1,InpConcs) :- member(d(s_carp1,_,_),InpConcs),!. carp_selection(s_carp2,InpConcs) :- member(d(s_carp2,_,_),InpConcs),!.	Definition of carp_selection() predicate. This predicate is select between the initial groups (1st year or 2nd year).
ft(T,Topt,Tmin,Tmax,FT) :- T =< Topt, FT is exp(-4.6*((Topt-T)/(Topt-Tmin))**4),!. ft(T,Topt,Tmin,Tmax,FT) :- T > Topt, FT is exp(-4.6*((T-Topt)/(Tmax-Topt))**4),!.	Definition of ft() predicate, defined according to Eq. 11.
fo(DO,DOcrit,DOmin,Delta) :- DO > DOcrit, Delta is 1,!. fo(DO,DOcrit,DOmin,Delta) :- DO =< DOcrit, DO >= DOmin, Delta is (DO-DOmin)/(DOcrit-DOmin),!. fo(DO,DOcrit,DOmin,Delta) :- DO < DOmin, Delta is 0,!.	Definition of fo() predicate, defined according to Eq. 13.
cat_temp(SS,T,Tmin,Coeff) :- T-Tmin > 0, Coeff is exp(SS*(T-Tmin)),!. cat_temp(SS,T,Tmin,Coeff) :- T-Tmin =< 0, Coeff is 1,!.	Definition of cat_temp() predicate, defined according to Eq. 12.
')}	Closing of local code.

In parallel with the programming of prototypes, the actual initial data and parameters of these general prototypes have to be assigned to the actual state and transition elements of process network. The explanation of the utilized global variables, inputs, parameters and outputs are summarized in Appendix.

4.3 Validation of the model by the measured data

Stocking and harvesting data for the main commercialized species (Common carp), occasional individual weight measurements, as well as occasional water level measurements were applied to validate the model.
To evaluate the model predictions, the normalized root mean square error (NRMSE, %) values were calculated by the following expression:

$$\mathrm{NRMSE} = \frac{\sqrt{\frac{\sum_{i=1}^{n}(c_i - m_i)^2}{n}}}{\bar{m}} * 100 \qquad 43.$$

where:
c_i is the i^{th} calculated value,

m_i is the i^{th} measured value,
n is the number of observations,
$\bar{m}$ is the mean of measured values.

The NRMSE values of 6.3% for pond level and 15% for carp biomass prediction show an acceptable prediction ability of the model at the present state of developments (see Figs. 5 and 6). It is to be noted, that in the continuing work there are additional ongoing measurements regarding phytoplankton and zooplankton to improve the model. The comparison of measured and calculated data can be seen in Fig. 5.

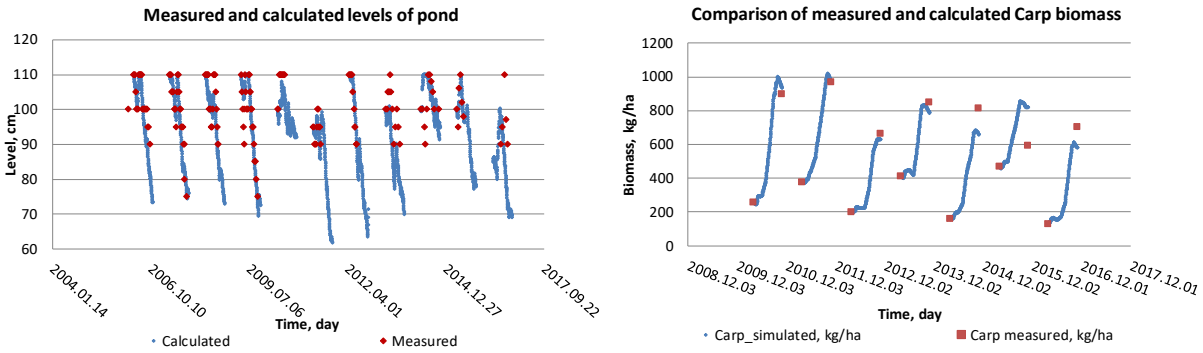


Figure 5. Comparison of measured and calculated data for pond level and carp biomass

The 110 cm upper limit of pond level comes from the applied practice of the fishpond management (that has been added to the model as a rule). For the years of 2014 and 2016, water supply was not recorded (accordingly it was not taken into consideration in the model), causing a significant difference between measured and calculated data.

Regarding carp biomass, there is a remarkable difference for the year 2015 between the measured and calculated data. Measured data in the pond register book showed 468 kg/ha stocking at the beginning of the season, while harvesting data is 593 kg/ha at the end of October. It would not be worth producing such a low net yield; accordingly we assume that an event outside the model boundaries (e.g. high mortalities due to bird predation or disease) caused the extremely low biomass gain.

Figure 6. shows another representation of comparing level and biomass data to illustrate the agreement of measured and calculated values.

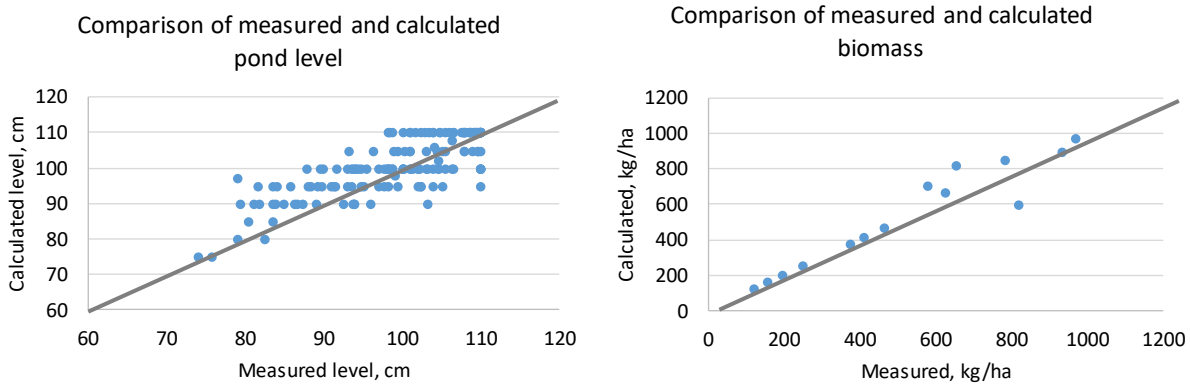


Figure 6. Comparison of measured and simulated data

Figure 7. shows the comparison of occasionally sampled individual weight of carp. Here, comparison results show weaker agreements, however, it is to be noted, that calculated results shows average individual weight, while random sampling is not always reflects to the average weight.

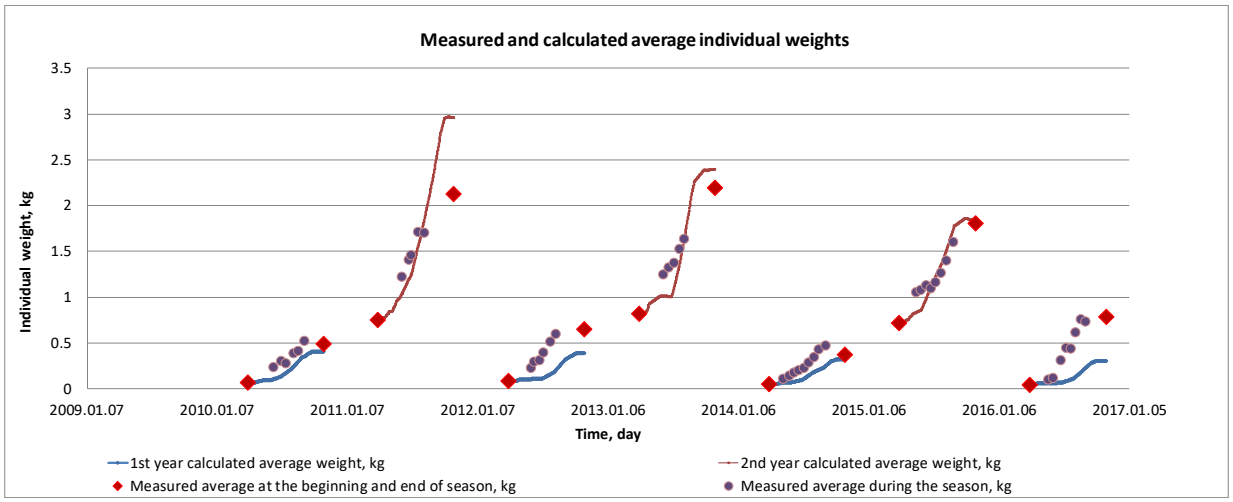


Figure 7. Measured and calculated data of individual weight of Common carp

The available few measurements did not allow the parameter identification for the other food web elements and dissolved components in the fishpond, accordingly we applied those parameters that were suggested in the original literature source. In the continuing work, a series of experiment is planned for the validation of the model in terms of phytoplankton, zooplankton and dissolved components, more accurately.

5. Results and discussion of process simulations

5.1 Overview of possible modes of model execution

One of the final goals of present development was to embed the model into a web based decision support framework, which is under development by ClimeFish project team (https://climefish.eu/). Accordingly, to allow a faster application, the general kernel of simulator was prepared for the following modes of execution:

- simulation of single or multiple past production seasons (from April 1 to October 31) with factual meteorological and management data;
- simulation with single and multiple production seasons (from April 1 to October 31), with factual meteorology but with various hypothetical sets of management scenarios;
- detailed run for climate scenarios (with daily output reporting);
- simplified run for climate scenarios (with end-of-season reporting for each simulated year).

These modes of simulation were developed as extensions of the general kernel program. Accordingly, single or multiple years, started with various sets of parameters, are initiated and executed, automatically, controlled by a configuration script. The simulated outputs are displayed in csv format for flexible further processing by an MS Excel / VBA tool. In the next Sections, some illustrative results from the various simulations will be discussed.

5.2 Simulation results for the 2010-2016 period of the example fishpond

Having applied the simulation mode of "multiple runs for the past production seasons", we can have a deeper insight to the causal relations amongst the various elements in the pond.

Fig. 8 shows the dynamic changes of fertilizer and feed concentration in the fishpond. Having started from the factual data of fertilizer and forage supply, the curves reflect on the actual concentration along time, considering the temperature and dissolved oxygen dependent decomposition of fertilizer (see Eqs. 34-41) and forage, complemented by the actual consumption of Common carp.

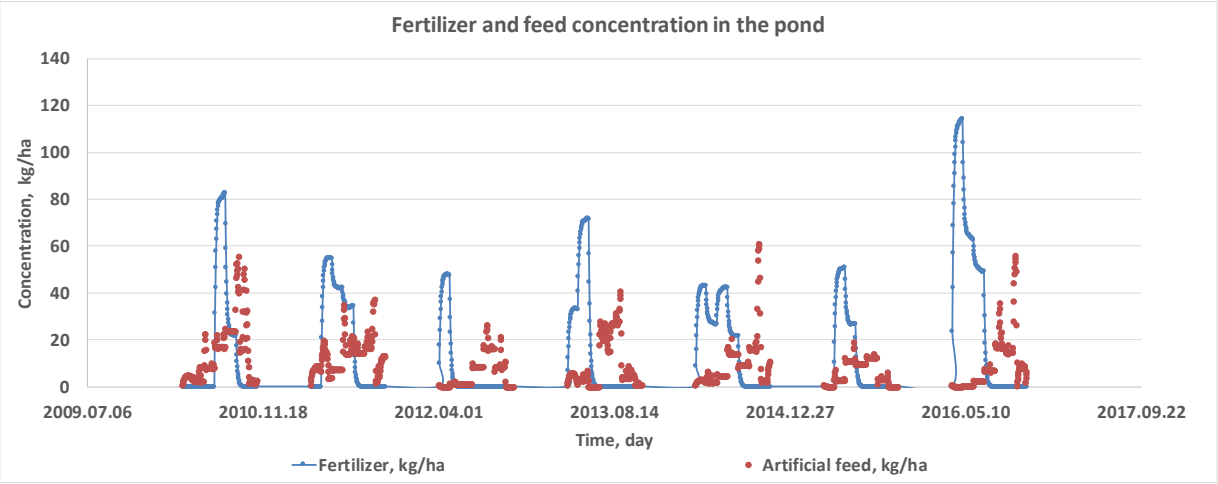


Figure 8. Fertilizer and feed concentration in the fishpond during the production seasons

Fig. 9 shows the biomass of the Common carp and Bighead carp. In the initial data supply and during the calculation both species were taken into consideration with two age groups (1 and 2 year old stockers) in the fishpond. Plots shows the aggregated results for both species.

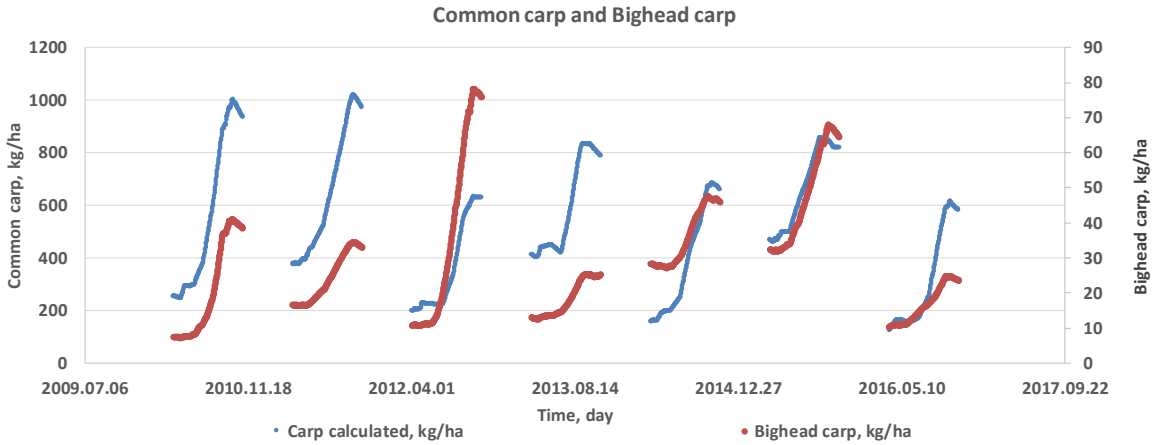


Figure 9. Dynamics in biomass change of Common carp and Bighead carp during production seasons

Dynamic behavior of the food web elements can be seen in Fig. 10. It is to be noted that calculations were based on the parameters from the literature, and further validation with detailed experimental background will continue in the ongoing work. However, it is worth analyzing the trends of the dynamic changes. As the amount of Common carp and Bighead carp starts to increase in each season (Fig. 9), the biomass of zooplankton (Fig. 10) decreases accordingly (being part of the diet of both main species). Hectic changes of phytoplankton biomass (described by Eqs. 26-29) are the joint impact of the increasing mass of their "consumers" (Bighead carp, Zooplankton and Benthos), of the radiation, of the shading effects caused by the increased amount of detritus, as well as of the changing availability of nitrogen and phosphorus in the fishpond. The catabolized and decomposed biomass of living organisms also increase the detritus mass, continuously.

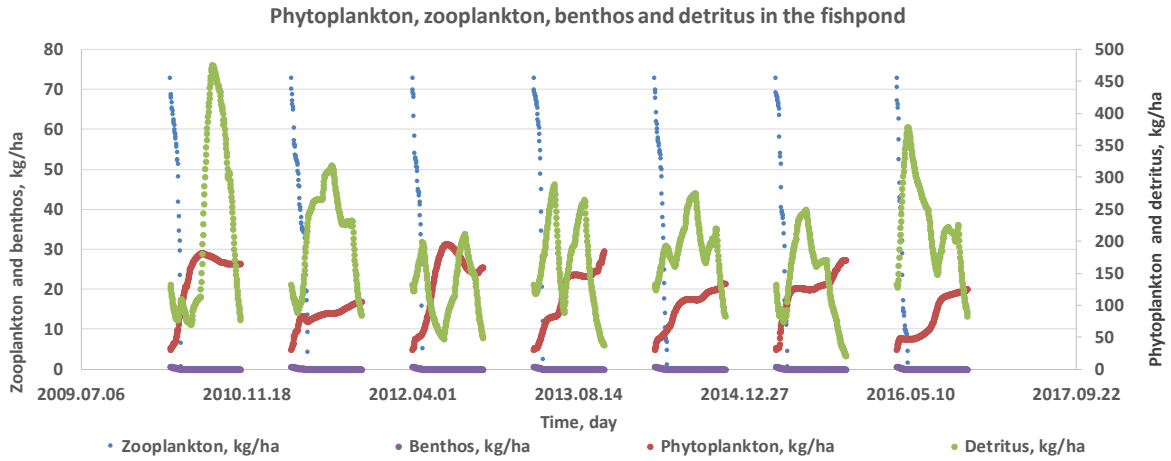


Figure 10. Dynamic changes of the food web elements in the fishpond

In Fig. 11, we can follow the dynamic changes of dissolved oxygen concentration in the analyzed production seasons. The diagram shows the summarized effects of the consumption by various species, the oxygen produced by the phytoplankton biomass, as well as the temperature-dependent air/water oxygen transfer. Aerators were not applied in the investigated fishpond.

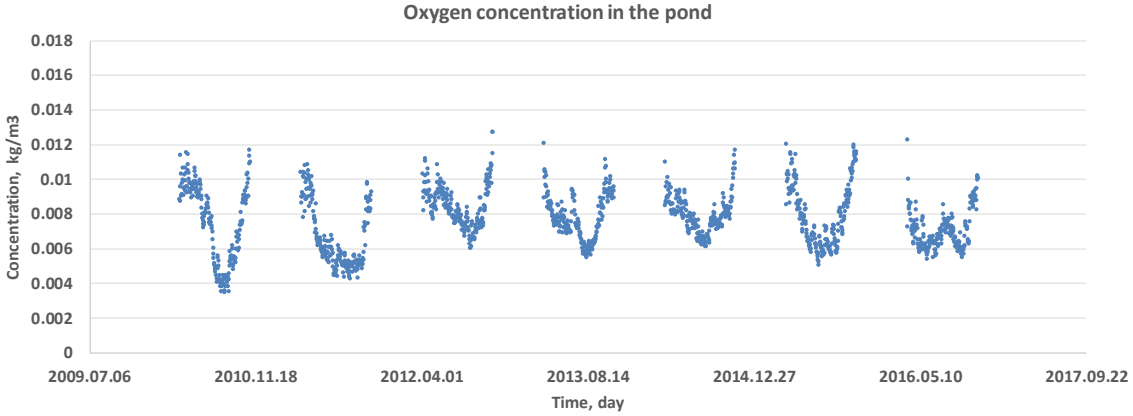


Figure 11. Dissolved oxygen concentration in the fishpond

Dynamic behavior of the nitrogen and phosphorus concentration (Fig. 12) is the cumulated effect of the nutrient utilization by phytoplankton biomass (described by Eqs. 30-31), the monthly supply of organic fertilizer, as well as the continuous degradation of all food web elements within the production period.

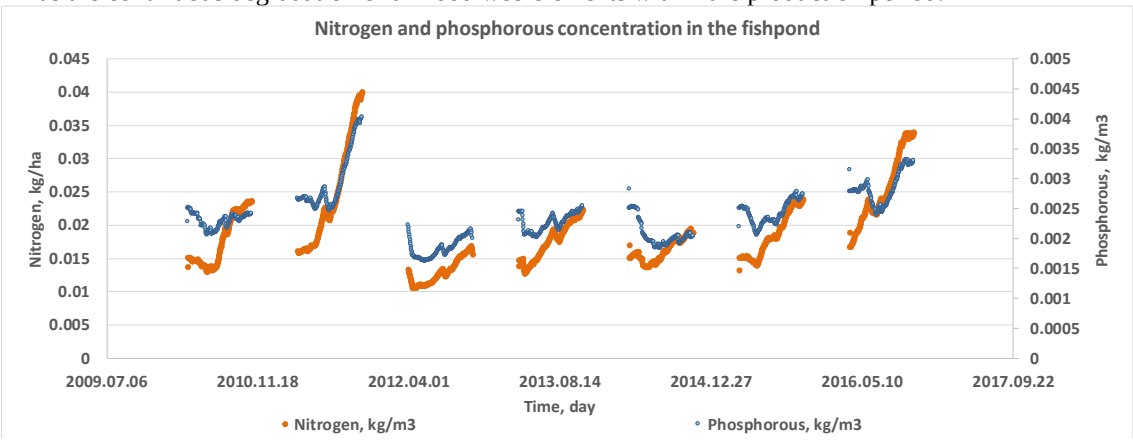


Figure 12. Nitrogen and phosphorus concentration along the production seasons

5.3 Analysis of various pond management scenarios

The previous analysis focused on simulation results for the studied fishpond in SzegedFish Ltd. The respective fish farming data are summarized in Table 4 for the period of 2010-2016. In the following part, we apply the simulation model for studying a hypothetic fishpond, parameterized for the typical Hungarian conditions.

Table 4. Raw data about the studied fishpond

Year	Stocking of Common carp, kg/ha	Gross yield of Common carp (factual), kg/ha	Artificial feed, t/ha/season	Fertilizer, t/ha/season	Av. individual weight gain in the season, kg/piece
2010	254	896	2.45	0.67	3.50
2011	376	966	2.20	1.00	2.60
2012	198	664	1.30	0.30	3.40
2013	411	846	1.60	0.66	2.10
2014	157	814	1.70	0.85	5.20
2015	468	593	1.40	0.49	1.91
2016	124	704	2.30	1.43	5.70

The feeding intensity and stocking density applied in the year 2011 were very close to respective industry-level averages (AKI 2018). For this reason, we selected the technological settings of 2011 as a baseline management scenario, and based on this we compiled a design set of the initial data and parameters for the model (see Table 5), to develop a hypothetical but typical fishpond for the further investigations.

Table 5: Modified initial data and parameters

Assumptions / Initial conditions	Common carp	Bighead carp
Stocking density	376 kg/ha (in line with the data of 2011 for SzegedFish Ltd.)	15 kg/ha (in line with the data of 2011 for SzegedFish Ltd.)
Initial individual weight	475 g (in line with the data of 2011 for SzegedFish Ltd.)	688 g (in line with the data of 2011 for SzegedFish Ltd.)
Mortality	74.5 % survival* (equal to 0.14% daily mortality in parameter MC) *In line with industry-level average mortality (AKI, 2018)	89% survival* (equal to 0.0555 % daily mortality in parameter MC) *In line with industry-level average mortality (AKI, 2018)
Feeding strategy	2163 kg/ha/season In line with the literature recommendation (Vincze et al, 2011), the following distribution for the applied artificial feed (=100%) was applied: April 5%, May 13%, June 19%, July 25%, August 28%, September 10%, October 0%	
Fertilization strategy	Considering 1t/ha/season (in line with the data of 2011 for SzegedFish Ltd.), we applied the general practice (fertilizer is evenly applied on the first three days of each month between May and September in the production season).	

Starting from these initial conditions, model calculations were performed for 80 different pond management scenarios. Pond management scenarios are defined as combination of feeding strategy and stocking density. 10 different stocking densities (see rows in Table 6) and 8 different feeding options (see columns in Table 6) were considered. Red colored numbers in Table 6 refers to the baseline management scenario. Historical meteorological data for the period of 2006-2016 were used in the simulations for two different locations (Szeged and Debrecen, Hungary).

Table 6. Simulated biomass with various stocking densities and feeding rates

Biomass of Common carp, kg/ha	Feeding rate (% and kg/ha)									
Stocking rate (% and kg/ha)		%	0.46	0.69	0.92	1.00	1.16	1.39	1.62	1.85
	%	kg/ha	1000	1500	2000	2163	2500	3000	3500	4000
	0.29	110	447.79	515.41	571.46	579.56	471.15	336.37	268.26	210.02
	0.40	150	492.33	601.31	664.00	684.55	726.01	481.67	356.05	274.72
	0.51	190	525.09	658.19	758.83	779.05	813.51	842.44	565.00	369.94
	0.61	230	553.74	699.91	826.42	860.11	915.06	966.52	912.27	600.03
	0.72	270	579.50	734.14	875.66	917.73	994.03	1069.00	1094.31	996.12
	0.82	310	605.30	764.52	914.97	961.07	1050.78	1159.65	1224.68	1217.13
	0.93	350	630.99	793.62	948.42	997.00	1094.33	1223.87	1322.72	1374.41
	1.00	376	647.67	812.09	969.59	1018.97	1118.86	1256.61	1371.33	1448.77
	1.06	400	663.49	829.22	988.55	1038.92	1140.72	1283.31	1409.18	1504.95
	1.20	450	696.22	864.72	1027.25	1078.77	1184.00	1333.02	1472.37	1594.72

The values in Table 6 refer to the carp biomass at the end of the season. Simulation results here show the average data for the period of 2006-2016

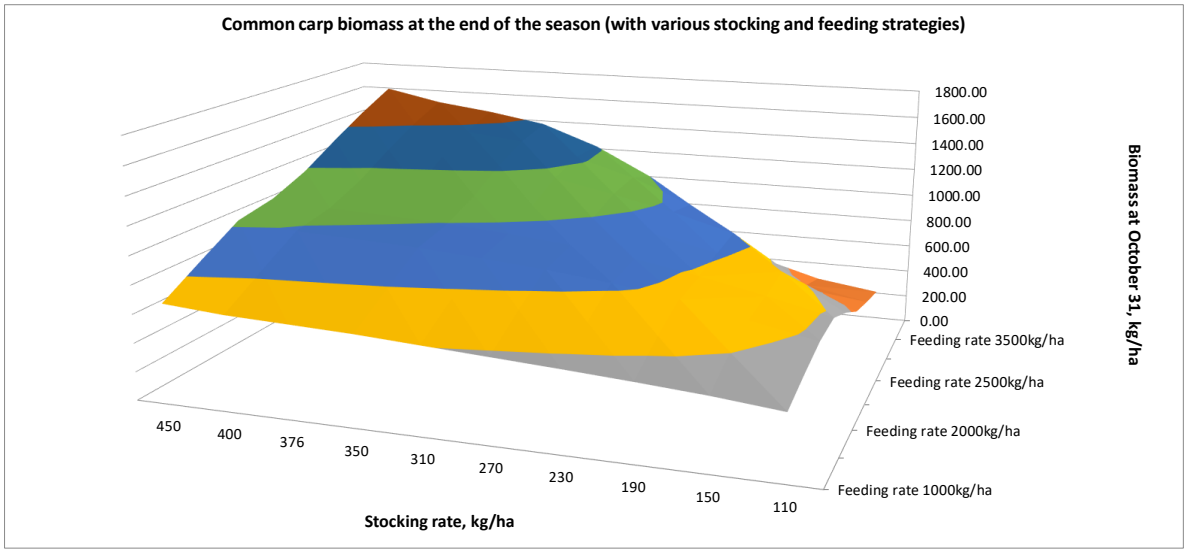


Figure 13. Simulations for biomass yield under various managerial scenarios

Fig. 13 illustrates the tendencies of data in Table 6. It is worth paying attention to the fact that fixing stocking densities at lower values, increasing feeding intensity could lead to lower biomass yield when the degradation of uneaten feed brings about oxygen deficient environment. This situation occurs when the relatively low carp biomass is not able to uptake the large amount of feed present in the pond.

5.4 Simulations for NorESM RCP 4.5 and 8.5 climate scenarios

Finally, predictions for future climate scenarios were performed with the model. In this case, regional climate model of NorESM was applied for the period of 2017-2065, while for the retrospective period of 2006-2016 the factual meteorological data were used. Representative Concentration Pathway (RCP) 4.5 and 8.5 scenarios were utilized, downscaled to a resolution of 0.25x0.25 degrees for two locations of Szeged and Debrecen, Hungary (downloaded from OpenNEX repository, http://opennex.planetos.com/gddp). Forecasted data for precipitation (mm/day) and average air temperature (°C) were utilized as input data for the simulations. It is to be noted that according to previous investigations, air temperature can be utilized, because it is in high correlation with water temperature for shallow (<1.1 m depth) fishponds during the farming season.

The simulations were executed for the period of 2006-2065 for the pond management scenarios presented in Table 6. The simulation mode of "simplified run for various climate scenarios" (see in Section 5.1) was used. Accordingly, 10x8 simulations were made, each of them from 2006 to 2065. The computational time for this amount of calculations was around 6 hours, using an average notebook (CPU 2.4 GHz, 8GB RAM). To fasten simulation, output recording can be restricted for the last day of season (October 31) in each simulation.

Some examples, illustrating the capabilities of the detailed simulation (recorded with daily time step) can be seen in Figs 14-17. Figure 14 shows the dynamic changes of dissolved oxygen concentration in the fishpond along the production seasons for the Szeged location, for the RCP 4.5 climate scenario. Figure 15 shows the dynamics of phytoplankton biomass for the same simulation period and location. In this case, two data series correspond to RCP4.5 and 8.5 climate scenarios. It can be seen that climate change induces increased occurrence of extreme values of oxygen levels and phytoplankton biomass over the next decades.

Figure 16 shows a prediction for the average individual weight of the main target species, the Common carp. A very slowly increasing trend can be observed in the individual weight that can be resulted by the increased anabolic activity. In Figure 17 we show a zoomed period about the same simulation, where the effect of various stocking/feeding densities can be studied. It is worth paying attention to the calculation results under zero feeding conditions (with purple line in Fig. 17). Here, after a slow increase in average weight and the total depletion of natural food of Common carp, average weight start to decrease quickly.

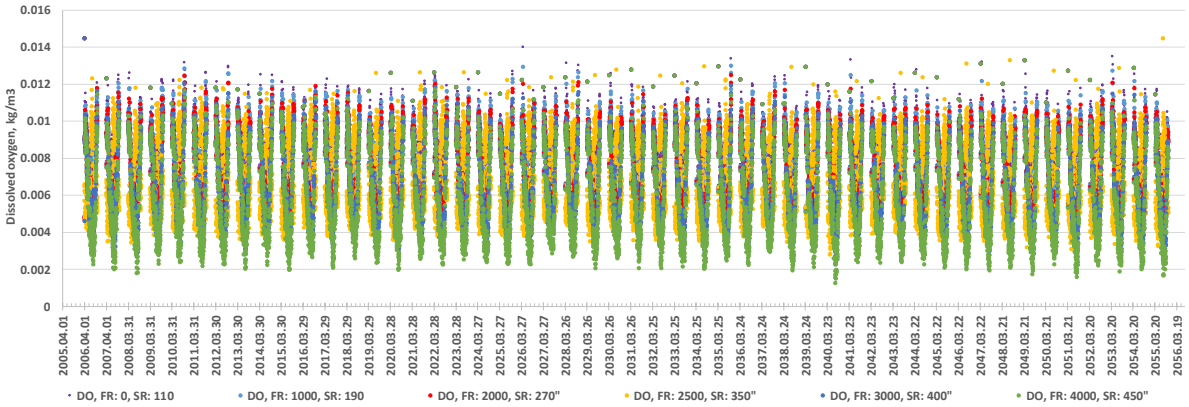


Figure 14. Simulations of dissolved oxygen concentration during the production seasons. Location: Szeged, Hungary; Climate scenario: NorEM RCP4.5, 6 management scenarios with the combination of Feeding Rate and Stocking Rate, in line with the main diagonal of Table 6, extended with zero feeding: (0, 110); (1000, 190); (2000, 270); (2500, 350); (3000, 400); (4000, 450).

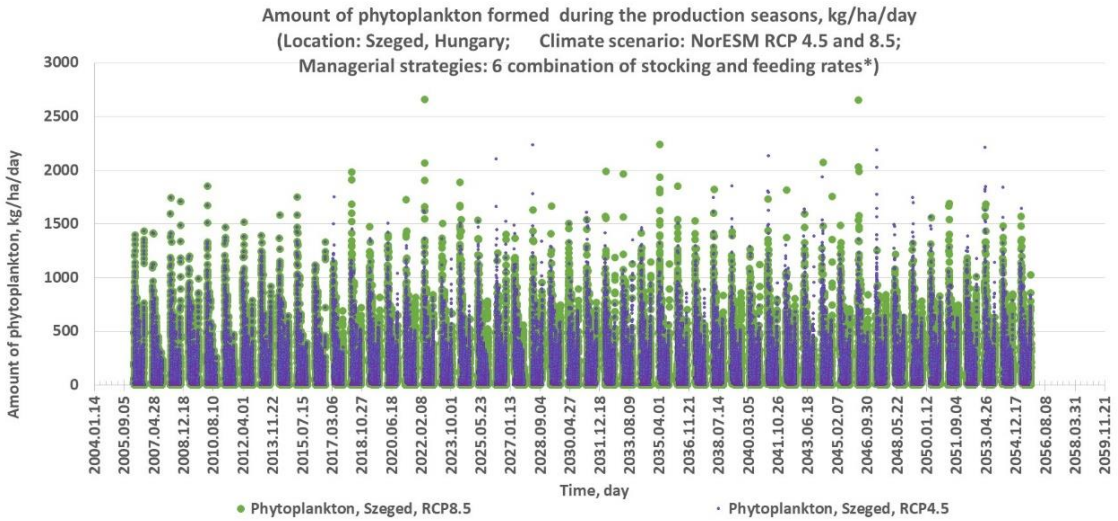


Figure 15. Simulations of phytoplankton biomass
*6 management scenarios with the combination of Feeding Rate and Stocking Rate, in line with the main diagonal of Table 6, extended with zero feeding: (0, 110); (1000, 190); (2000, 270); (2500, 350); (3000, 400); (4000, 450).

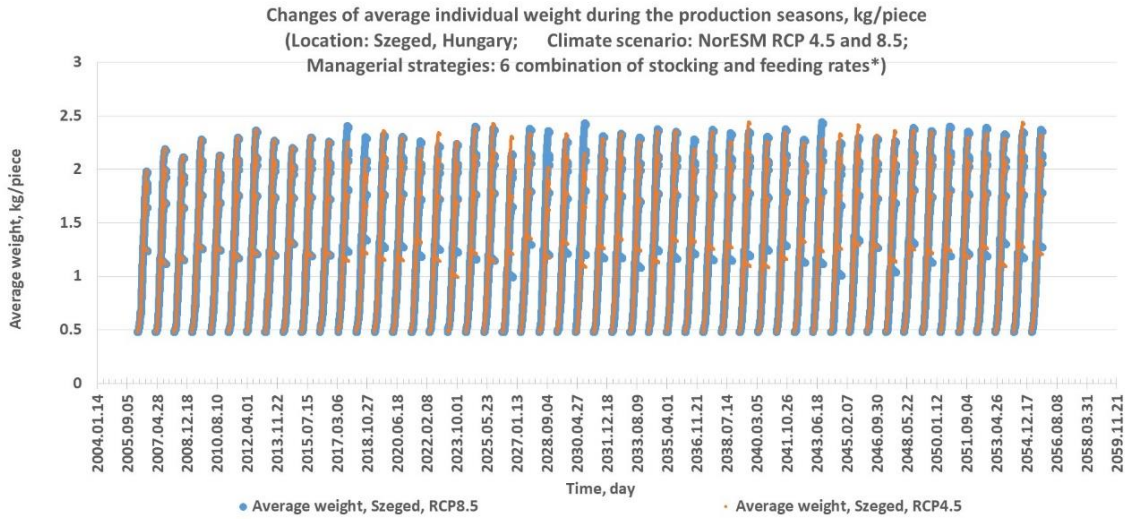


Figure 16. Simulations of average weight of Common carp
*6 management scenarios with the combination of Feeding Rate and Stocking Rate, in line with the main diagonal of Table 6, extended with zero feeding: (0, 110); (1000, 190); (2000, 270); (2500, 350); (3000, 400); (4000, 450).

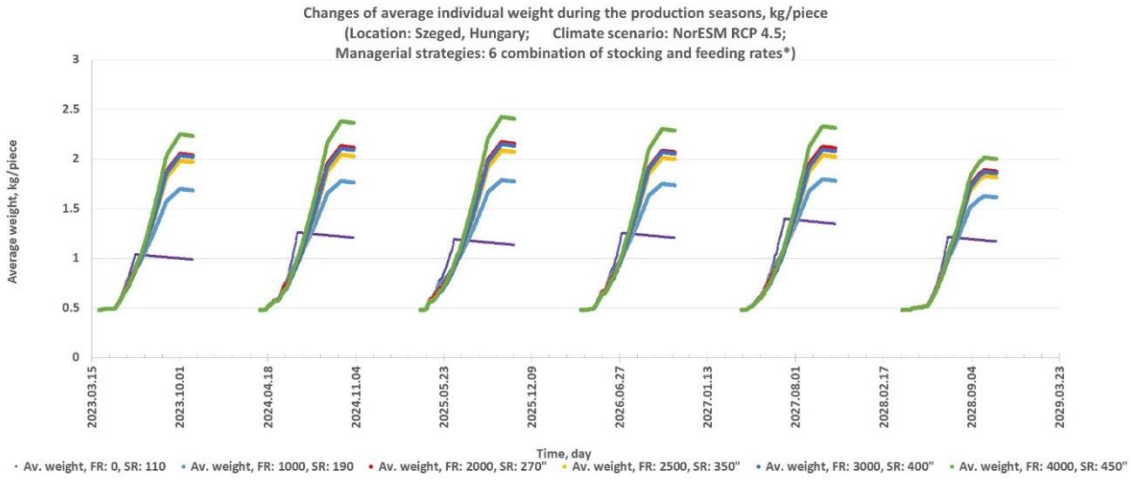


Figure 17. Zooming into a period of 2023-2028 for the simulations of average weight of Common carp
*6 management scenarios with the combination of Feeding Rate and Stocking Rate, in line with the main diagonal of Table 6, extended with zero feeding: (0, 110); (1000, 190); (2000, 270); (2500, 350); (3000, 400); (4000, 450).

In the followings, some complex causal relationships will be illustrated.

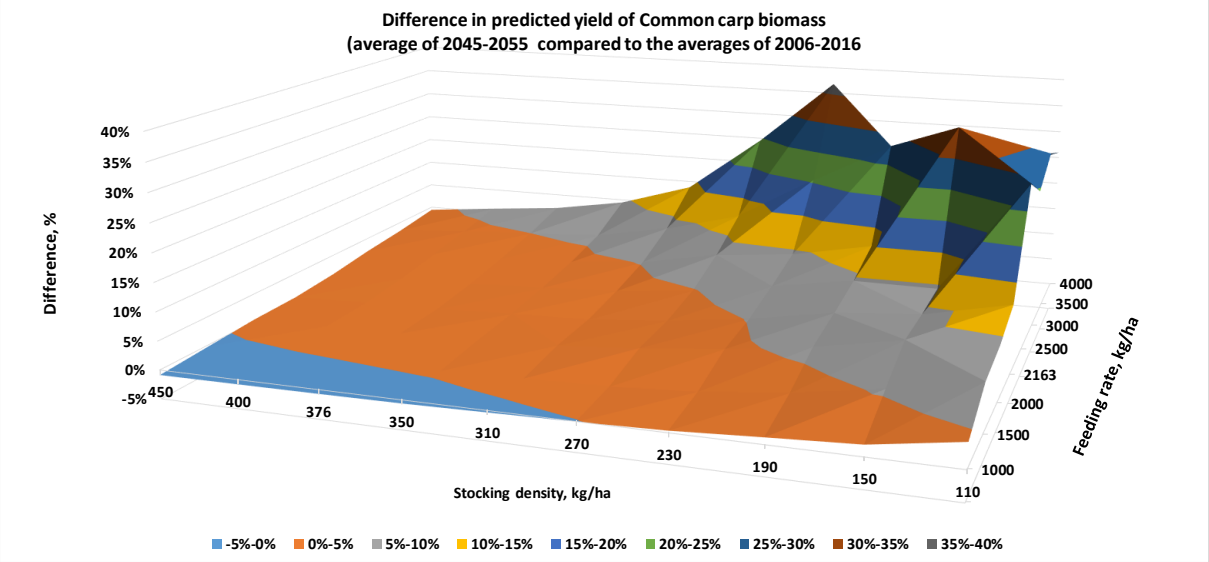


Figure 18. Simulated climate predictions for shorter term (2015-2025) period

Fig. 18 illustrates the predicted difference in average Common carp yields the periods of 2006-2016 and 2045-2055. The calculations were made for Szeged location, utilizing the RCP4.5 climate scenarios. It is worth mentioning that significant differences appear for those management strategies that are characterized by lower (100-250 kg/ha) stocking density and intensive feeding rate (> 3000 kg/ha). However, these input combinations are outside the range of economically optimal pond management strategies, as feed utilization efficiency is very low (Figure 13). The reason for the large increase in predicted yields over the decades is that anabolic activity and appetite of carp will increase with higher temperatures in the period of 2045-55, and for this reason feed utilization will be more efficient. This implies that there would be less waste of forage (see Figure 19), and this will decrease the frequency of oxygen deficient periods.

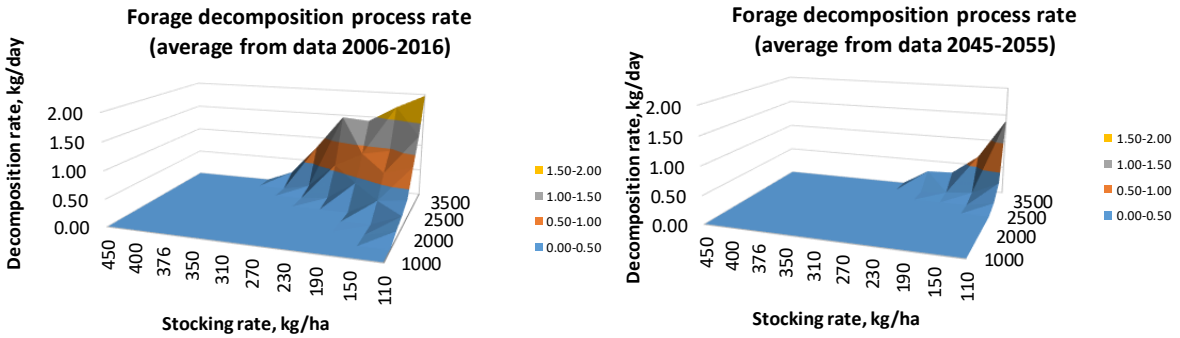


Figure 19. Comparison of forage decomposition rates (that refers to forage loss) in the historical (2006-2016) and in the longer term (2045-2055) simulation period

6. Conclusions and outlook

Management of levee-type fishponds under changing environmental conditions is a difficult challenge because of the complexity of the highly interacting physical, chemical, biological and fish farming technological processes. Accordingly, the proper evaluations and decisions about the management strategies must be based on a possibly most detailed biophysical model.

In the present work, we have developed an ecosystem involved fishpond model. Based on the data from a Hungarian bi-culture semi-extensive pond farm, a reusable dynamic simulation model of levee-type fishponds was developed, considering also the human management activities. At the present state of development, calculated NRMSE values showed acceptable match between measured and calculated data, regarding Common carp biomass and water level.

Using the validated model, the dynamic simulation provided long-term predictions for various climatic scenarios with the consideration of

- the interactions between the food web elements;
- the dynamics of the underlying chemical components (O_2, CO_2, nitrogen, phosphorus); and
- the applied pond managerial actions (stocking density, feeding, fertilizing, aeration, and pond level control).

The applied non-conventional framework of Programmable Process Structures supported the appropriate implementation of the trans-disciplinary functionalities in a unified transparent structure. The process model of medium complexity made possible to study also the effects of climate change on the ecosystem involved fish farming by 50 years long detailed dynamic simulations. We concluded that regardless to the limited amount of data, the approximately validated, but causally established, well-structured and balance based dynamic model may support the design and operation of complex agro-environmental system. The applied modeling method makes possible to revise, to modify and to extend the temporarily applied structure and functionalities for different other cases, conveniently

In the continuing work, a series of experiments is planned for the better validation of the model in terms of phytoplankton, zooplankton and dissolved component concentrations. Another goal of the continuing work is to evaluate the applied managerial scenarios in terms of economic viability, on the basis of the validated model. The model will be embedded in a web based decision support framework that is under development in ClimeFish project (https://climefish.eu/). The aim of the DSS framework is to provide detailed biophysical model based predictions under different climatic scenarios for shallow ponds in Central Europe.

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A fél-intenzív tógazdasági pontytermelés során alkalmazott keveréktápok hatása a vízminőségre

Tanulmány háttere és célja

- Az akvakultúra fenntarthatósága érdekében a halolaj alapú tápokat a növényi alapúak váltják fel, ugyanakkor még kevés információval rendelkezünk, hogy ezen tápok alkalmazása hogyan hat a vízminőségre.
- A fél-intenzív tógazdasági pontytermelés meghatározó szerepet játszik Közép- és Kelet-Európában, így a munkánk során is erre fókuszáltunk és a technológiára jellemző teljes hároméves cikluson keresztül vizsgáltuk a vízminőség alakulását.
- A kutatás célja volt megállapítani, hogy a halolaj alapú, illetve növényi alapú tápok, valamint a hagyományosnak nevezhető gabona használata hogyan hat a vízminőségre.

Tanulmány főbb eredményei

- Míg a nettó hozamban mutatkoztak különbségek az egyes tápok között, a vízminőségben nem tapasztaltunk szignifikáns különbséget az egyes kezelések (tápok) között.
- A vizsgálat évei között szignifikáns különbség volt a teljes nitrogén, teljes foszfor és a szerves anyag mennyiségében is. A mindenevő pontyfélék tápanyagforgalomban betöltött szerepe és a relatív (biomasszára vetített) hatásuk az életkorral jelentősen változik, ami a magyarázattal szolgálhat az évek közötti különbségekre.
- Mind a halolaj, mind pedig a növényi alapú táppal nagyobb hozamokat értünk el, mint a hagyományos gabona alkalmazásával. A két formált táp között azonban nem tapasztaltunk különbséget, ami alapján a növényi alapú tápok releváns alternatívát jelenthetnek a fél-intenzív tógazdaság pontytermelés fenntarthatóságának növelésében.

Az összegzés az alábbi cikk alapján készült:

László Berzi-Nagy, Attila Mozsár, Flórián Tóth, Dénes Gál, Zoltán Nagy, Sándor Alex Nagy, Éva Kerepeczki, László Antal, Zsuzsanna J. Sándor Effects of Different Fish Diets on the Water Quality in Semi-Intensive Common Carp (Cyprinus carpio) farming WATER 13 Paper: 1215 (2021)

Effects of different fish diets on the water quality in semi-intensive common carp (*Cyprinus carpio*) farming

László Berzi-Nagy [1,*], Attila Mozsár [1], Flórián Tóth [1], Dénes Gál [1], Zoltán Nagy [1], Sándor Alex Nagy [2], Éva Kerepeczki [1], László Antal [2,†], Zsuzsanna Jakabné Sándor [1,†]

[1] Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety Research Centre for Aquaculture and Fisheries, Szarvas, Hungary; mozsar.attila@uni-mate.hu (A.M.); jakabne.sandor.zsuzsanna@uni-mate.hu (Zs.J.S.); toth.florian@uni-mate.hu (F.T.); gal.denes.73@gmail.com (D.G.); nagy.zoltan84@uni-mate.hu (Z.N.); eva.kerepeczki@gmail.com (É.K.)
[2] Department of Hydrobiology, University of Debrecen, Debrecen, Hungary; nagy.sandor.alex@science.unideb.hu (S.A.N.); antal.laszlo@science.unideb.hu (L.A.)
* Correspondence: lachus160@gmail.com
† These authors contributed equally.

Abstract
The semi-intensive common carp (Cyprinus carpio) farm technology uses several feed types affecting the growth performance; however, we know less about their long-term effects on water quality. Herein, we evaluated the effects of three commonly used feeds (moderate levels of fish meal and fish oil feed - FF, plant meal and plant oil feed - PF and cereal feed - CF) on the nutrient (total nitrogen - TN, total phosphorus - TP and organic matter - OM) content of the pond water. The experiment was carried out through three consecutive years from juveniles to market sized fish. The type of feed affected the net yields, but generally it did not affect the water quality. The year of sampling however was a significant factor affecting TN, TP and OM, which concentrations decreased during the three years. Our findings highlight that the age of stocked fish on water quality has a more pronounced effect than the nutrient profile of the supplementary feed. Additionally, the plant-based feed could provide comparable net yields as the fish meal-based feed without additional nutrient loading in the water column, reinforcing the sustainability of alternative feeds in semi-intensive carp farming.

1. Introduction

As the human population grows and the demand for aquaculture products increases, the intensification of aquaculture practices [1] and increase in the number of farms are inevitable, both aggravate the pressure on the natural environment in the form of enhanced exploitation of resources and potential organic and inorganic pollutants. The nutrient content and composition of the diet are the main factors affecting nutrient loading by fish [2], thus the dietary practices drive the extent of environmental effect. The combined goals of intensive yields and low pollution potential can be achieved through the use of high-quality feeds, which have good digestive properties and thus are better utilized, resulting in fewer waste nutrients per unit yield [3-7]. This effort created different feed types, the most frequently used ingredients are different terrestrial plants or industrial by-products, based on the cheapest local sources. However, the use of animal or plant-based proteins and lipids and their effects need to be carefully designed on a case-by-case basis for every species [8]. Besides their usefulness for growth, the environmental risk of novel feed types in comparison with traditional cereal feeds or fish meal-based diets is also important to estimate before wide-scale use of the given feed type is encouraged.
Common carp (*Cyprinus carpio*) has a dominant role in Central and Eastern European aquaculture [9]. The most associated production technology with this species is semi-intensive or extensive. The semi-intensive technology uses mainly cereals for supplementary feeding, and fertilization (i.e., manuring) to enhance the natural productivity of the pond [10,11]. In this technology the production period lasts three consecutive years, the ponds are filled in spring (March-April) and are drained and harvested in autumn (September-October). The ponds are left dry in the winter allowing the aeration of the pond sediment, removing anaerobic bacteria and harmful substances, such as ammonia. At the end of the 20th century, an intensification trend and switch from cereal feeds to the artificial fish meal-based feeds [12] and plant-based feeds [13] were observed in carp farming experiments.
Here we aimed to assess the effect of different supplementary feed types on production indices (i.e., survival, specific growth rate, feed conversion ratio, individual weight gain and net yield) and water quality (i.e., total nitrogen, total phosphorus, and organic material). We designed an outdoor pond experiment to monitor the whole (3 years long) production cycle of semi-intensive common carp using three different diets: fish meal/fish oil containing feed (FF), plant-based feed (PF) and cereal feed (CF). Based on the higher quality of artificial feed [14], we expected better production indices in the ponds fed with FF and PF and lower concentrations in the water quality parameters than the ponds fed with CF. Additionally, the substitution of fish meal and fish oil with plant-based nutrients in PF feed can result in lower digestibility [15], and increase the level of antinutritional factors [16-18]. Consequently, we presumed that decreased growth performance and excessive nutrient and organic matter loadings will appear in the PF ponds compared to the FF ponds.

2. Materials and Methods

The 3 year-long study with three diet types using common carp monoculture was completed between 2013 and 2015 at the Research Centre for Aquaculture and Fisheries, Szarvas, Hungary. Two identical earthen ponds (average area: 1772 m^2, depth: 1.3 m) were assigned to each diet type. The water demand of ponds was supplied by an oxbow lake of River Körös. To follow the semi-intensive fish farming protocols, the individuals were introduced to the system in spring and harvested in autumn. In winter, the fish were held in wintering ponds where they stopped feeding and entered a dormant phase. The same fish stock was used during the whole experiment; thus, they were stocked as fingerlings in 2013 and harvested as market sized carps in 2015. In average 20,886; 5,285 and 1,080 individuals/ha, with an average body weight of 0.68 g; 61.46 g and 748 g were introduced into the lakes in the years of 2013, 2014 and 2015, respectively. As the semi-intensive technology requires, cow manure was used to boost the natural production of the ponds. As the earlier age classes of common carp increasingly rely on the natural food sources [19], the earlier years received manure in higher quantities. Each pond individually received 450 kg manure in 2013 and 2014, while 300 kg in 2015.
Two artificial feeds were formulated by a local aquafeed manufacturer company for rearing common carp in semi-intensive monoculture. These diets involved standard ingredients commonly used for fish feeds, were manufactured with extruding technology and they had good sinking properties, as it is common for fishpond diets in Hungary. The different feeds were specifically tailored to be used in the experiment, they were made to be isonutritious to each other and were not available in the commercial feed trade. The FF feed contained moderate levels of fish meal and fish oil, while these were replaced with a mostly soy-bean meal and linseed oil in PF feed (Table 1). These feeds were formulated to contain equal levels of crude protein and lipid (Table 2). The third diet (CF) consisted of grained winter wheat only, which is a traditional feed type used in semi-intensive carp farming in Hungary. The daily feed amount varied between 1-3.5% of MBW (metabolic body weight = $BW^{0,8}$) and was based on periodic stock samplings, which were conducted weekly in 2013, every three weeks in 2014 and every four weeks in 2015. In total 1,970 kg of FF feed, 2,186 kg of PF feed and 1,769 kg of CF feed were distributed during the three years of the experiment.

Table 1. Ingredients (in percentage) of the artificial feeds (FF: feed with fish meal, PF: plant-based feed) used in the experiment, cereal feed (CF) consisted of winter wheat only.

%	**FF**			**PF**		
	2013	**2014**	**2015**	**2013**	**2014**	**2015**
Fishmeal 60	16.00	16.00	14.00	0	0	0
Winter wheat	8.88	10.08	20.50	5.60	8.90	16.50
Maize	30.00	30.73	27.50	29.00	27.00	27.50
Full-fat soy	6.00	4.03	6.50	7.80	9.00	9.50
Extracted soy	25.47	25.36	17.50	40.75	38.30	29.50
Blood meal	5.00	5.00	5.00	8.00	8.00	8.00
Yeast, f.g.	5.00	5.00	5.00	5.00	5.00	5.00
Vit-Min mix	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil	1.65	1.80	2.00	0	0	0
Linseed oil	0	0	0	1.85	1.80	2.00

Table 2. Proximate composition (percentages given for wet weight) of all three feed types (FF: feed with fish meal, PF: plant-based feed, CF: cereal feed).

%	FF			PF			CF		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
dry material	90.95	91.84	91.86	90.65	91.71	92.5	89.32	96.36	89.8
crude protein	33.97	32.7	30.18	34.31	31.72	29.57	11.48	10.05	7.12
crude fat	6.21	6.27	7.38	5.86	5.92	7.43	1.18	1.2	0.24
crude ash	6.92	6.13	5.96	5.67	4.23	4.21	1.68	8.24	2.11
nitrogen	5.44	5.23	4.83	5.49	5.08	4.73	1.84	1.61	1.58
phosphorus	1.02	1.01	0.01	0.79	0.73	0.01	0.40	0.40	0.00
organic matter	0.84	0.86	0.86	0.85	0.87	0.88	0.88	0.88	0.88

The production parameters of fish were calculated based on the following equations:

1. Survival (Survival %) = 100 × (number of fish at harvest) × (number of fish at stocking) $^{-1}$
2. Specific Growth Rate (SGR % day^{-1}) = 100 × ln (average body weight at harvest * (average body weight at stocking) $^{-1}$) × (days) $^{-1}$
3. Feed Conversion Ratio (FCR) = (feed distributed) × (biomass weight gain)$^{-1}$

4. Weight gain (WG g fish^{-1}) = average body weight at harvest (g) – average body weight at stocking (g)
5. Net yield (NY kg/ha) = (weight of biomass at harvest (kg) – weight of biomass at stocking (kg)) × pond area^{-1} (ha)

Integrated water samples from the whole water column were collected weekly in 2013, every two weeks in 2014 and monthly in 2015. The total nitrogen (TN) and the total phosphorus (TP) measurements used peroxodisulfate for digestion and the liberated forms were measured using a spectrophotometer [20], while the organic material (OM) was estimated from the concentration of the volatile suspended solids (VSS), measured by the weight loss-on-ignition after membrane filtering [21]. These measurements were conducted according to the guidelines of the Hungarian Standards Institution [22-24], for total nitrogen, total phosphorus and volatile suspended solids, respectively.
The effects of feed types, the different years and the effects of feed types within the specific years on water quality indices were analyzed using Kruskal-Wallis H tests. Subsequent pairwise comparisons were made on the levels of significant factors. The statistical analyses were performed in the IBM SPSS Statistics software package [25], significance levels were determined at $p < 0.05$.

3. Results

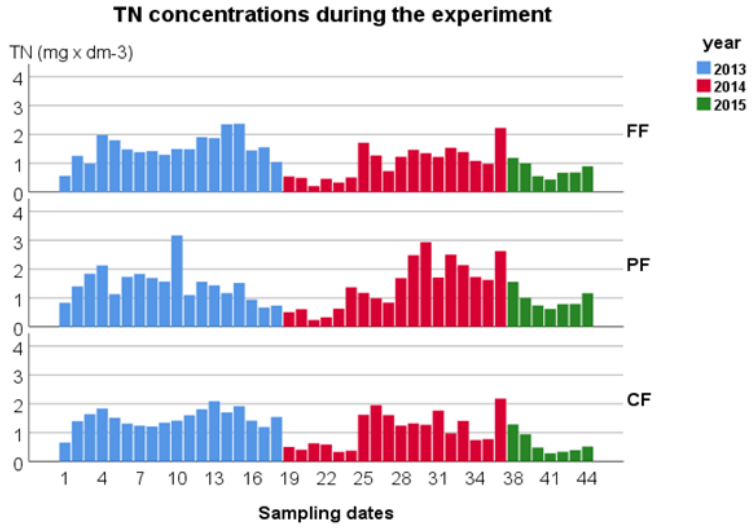
Our results of production indices support that the artificial feed types (FF, PF) performed generally similar to each other but both were better compared to the cereal feed (CF). The difference between the artificial feed types and the control feed was the most conspicuous in the yearly weight gain (WG) and net yield (NY) indices (Table 3). In the second year of the experiment, we observed the lowest survival rates for all groups, especially for the PF group. As the mortality was assessed only at the end of the season, the surviving individuals received relatively more feed in this group and showed somewhat higher individual weight gain. Although the exact reasons for the lower survival are unknown.

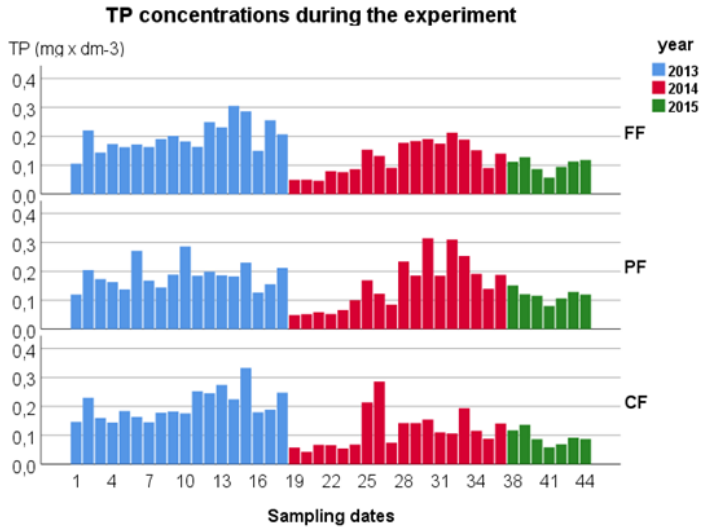
Based on the test results of homogeneity of variance (Levene's test, $p < 0.01$), Kruskal-Wallis test was chosen to analyze differences between groups. Considering water quality parameters, PF ponds had slightly higher average TN (1.38 mg l^{-1}), TP (0.16 mg l^{-1}) and organic material expressed in VSS (19.72 mg l^{-1}) concentrations compared to the ponds with the other feed types (1.18 mg l^{-1}, 0.15 mg l^{-1}, 17.28 mg l^{-1} for FF ponds and 1.21 mg l^{-1}, 0.15 mg l^{-1}, 17.20 mg l^{-1} for CF ponds respectively), however, this difference was not consistent within the specific years. The highest TN values were registered in FF ponds in 2013 (FF: 1.54 mg l^{-1}; PF: 1.48 mg l^{-1}; CF: 1.49 mg l^{-1}), and in PF ponds in 2014 (FF: 1.04 mg l^{-1}; PF: 1.45 mg l^{-1}; CF: 1.09 mg l^{-1}) and 2015 (FF: 0.77 mg l^{-1}; PF: 0.95 mg l^{-1}; CF: 0.61 mg l^{-1}). The highest TP concentrations were in CF ponds in 2013 (FF: 0.198 mg l^{-1}; PF: 0.185 mg l^{-1}; CF: 0.203 mg l^{-1}), and in PF ponds in 2014 (FF: 0.126 mg l^{-1}; PF: 0.153 mg l^{-1}; CF: 0.118 mg l^{-1}) and 2015 (FF: 0.101 mg l^{-1}; PF: 0.118 mg l^{-1}; CF: 0.092 mg l^{-1}). The highest organic material (i.e., VSS) values were in PF ponds in 2013 (FF: 22.45 mg l^{-1}; PF: 26.11 mg l^{-1}; CF: 22.56 mg l^{-1}) and in 2014 (FF: 13.24 mg l^{-1}; PF: 14.39 mg l^{-1}; CF: 11.48 mg l^{-1}) and in CF ponds in 2015 (FF: 13.1 mg l^{-1}; PF: 17.24 mg l^{-1}; CF: 17.37 mg l^{-1}). Ultimately, the differences in water quality parameters were not statistically significant compared between the three feed types. Testing the feed types separately for the given three years however, showed a continuous separating trend between the water quality of the given feeds, which were reflected by decreasing p-values. Only at the third year (2015) TN was significantly ($\chi2(2) = 8{,}688$, $p = 0.013$) different between feed types. Pairwise comparisons showed that this was the result of PF ponds having higher TN than CF fed ponds in 2015. Additionally, the measured water quality variables indicated significant temporal (yearly) variance and decreasing concentrations during the consecutive years of the experiment (Figure 1). Analyzing the year as a grouping variable, and the Kruskal-Wallis test showed it to be highly significant for all water quality variables (TN: $\chi2(2) = 34{,}516$, $p < 0.001$, TP: $\chi2(2) = 73{,}769$, $p < 0.001$, VSS: $\chi2(2) = 19{,}744$, $p < 0.001$) (Figure 2).

Table 3. Yearly production parameters (mean ± SD) by treatments (FF: feed with fish meal, PF: plant-based feed, CF: cereal feed).

		2013	2014	2015
FF	Survival (%)	78.6 ± 3	72.7 ± 11.2	89.5 ± 1.5
	SGR (% day^{-1})	3.37 ± 0.05	1.06 ± 0.08	0.66 ± 0.03
	FCR	1.63 ± 0.11	2.51 ± 0.23	2.5 ± 0.1
	WG (g fish^{-1})	76.6 ± 4.9	641.5 ± 81.8	1686.1 ± 137.5
	NY (kg ha^{-1})	1219 ± 39.1	2034.8 ± 129.4	1669.5 ± 88.9
PF	Survival (%)	75 ± 2	49.2 ± 6.5	92.3 ± 0.2
	SGR (% day^{-1})	3.33 ± 0.12	1.23 ± 0.1	0.6 ± 0.01
	FCR	1.91 ± 0.08	3.54 ± 0.01	2.59 ± 0.01
	WG (g fish^{-1})	60.9 ± 10.1	863 ± 199.4	1735.2 ± 48.6
	NY (kg ha^{-1})	916.2 ± 202.2	1614.5 ± 107.5	1778.5 ± 63
CF	Survival (%)	68.3 ± 2.4	57.8 ± 9	91.9 ± 0.9

SGR (% day^{-1})	3.23 ± 0.08	1.12 ± 0.12	0.57 ± 0.01
FCR	2.12 ± 0.22	3.31 ± 0.22	3.04 ± 0.09
WG (g fish^{-1})	52.7 ± 5.6	609.3 ± 177.1	1282.9 ± 65.3
NY (kg ha^{-1})	732.1 ± 24	1418.5 ± 122.6	1321.4 ± 66.5





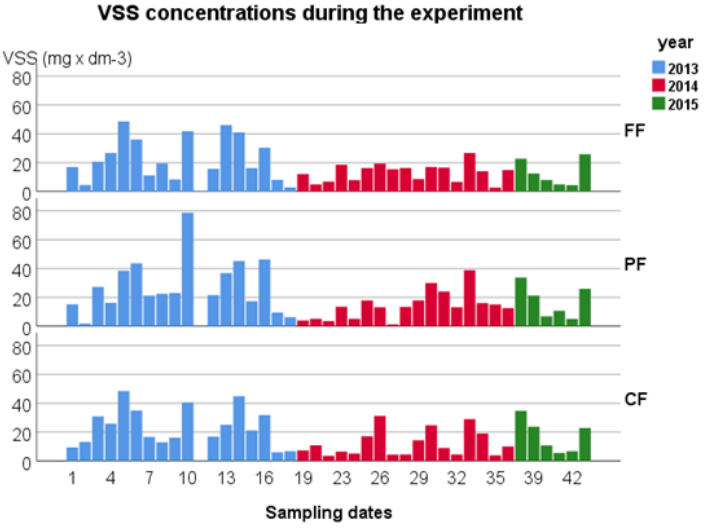


Figure 1. Annual profile of the three water quality variables in case of all three feed types. Figures were generated by the IBM SPSS software package.

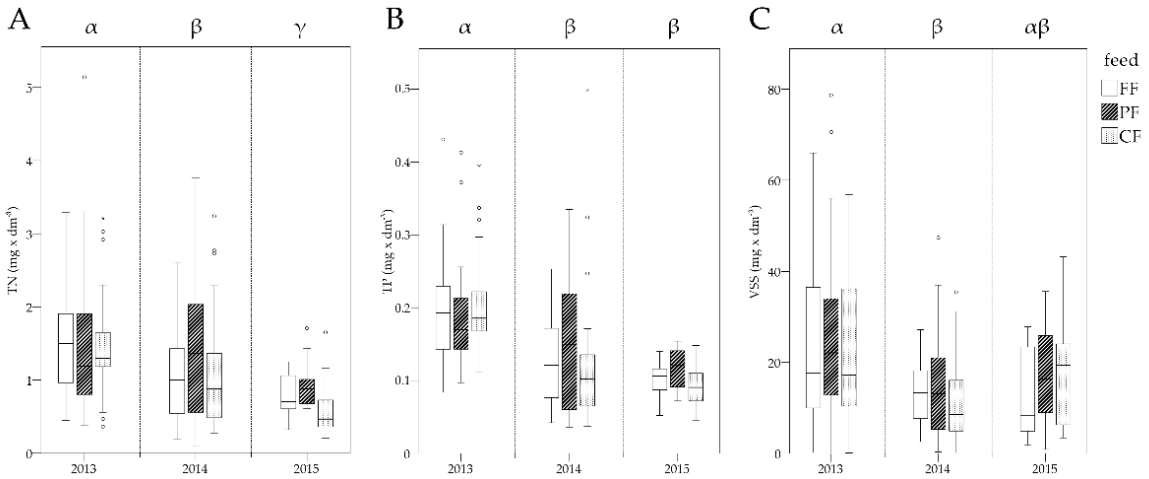


Figure 2. Water quality changes between years and treatments, indicated by total nitrogen (A), total phosphorus (B) and organic material (measured as volatile suspended solids – VSS) (C) concentrations. The different Greek letters (α, β, γ) represent statistically significant ($p < 0.05$) differences between the years for the given water quality variable. Tests and figures were generated by the IBM SPSS software package.

Concluding the results provided by the Kruskal-Wallis tests, the year as a factor significantly affected all water quality parameters, but the feed types resulted in only one difference in TN which was significant in the last year only. This shows the temporal changes on water quality to be dominant over the applied feed types in our experiment. These temporal changes were likely the results of specific effects coming from the characteristic for the weather and the stocked fish in any given year.

4. Discussion

Feeding in aquaculture contributes directly and indirectly to the water quality and ultimately fish welfare as well. As the concentrations of nutrients increase in water, the risk of their potentially harmful solutes in combination with the environmental conditions affect survival and need to be optimized [26]. Intensification in pond farming demands qualitative changes in feeds and necessitate fish feeds with higher protein content compared to the traditional cereal feeds. The most frequent sources of protein in fish feeds are still fish meal and soybean meal. Although the nutritional profile is similar in these artificial diets, their utilization and digestibility efficiency differ and thus different contributions on water quality can be expected. The present study analyses the full production cycle (i.e., from juvenile to market sized) of common carp and in this regard, it provides multi-year data to elucidate changes in water quality under semi-intensive pond farming. In our experiment the year of production was a significant predictor of water quality, however, the type of feed was generally not. The annual changes highlight that the role of fish in environmental nutrient dynamics undergoes substantial changes during ontogeny.

The higher net yields and weight gain in artificial (FF, PF) feeds compared to the cereal (CF) diets indicate the effectiveness of intensification. The higher protein content of FF and PF diets (>30% crude protein) was closer to the nutritional requirements of the carp [27,28]. This compliance to the protein requirements resulted in better FCR and growth rate and consequently higher net yield for artificial feed types. In ponds fed with CF, the protein requirements of the fish could only be temporarily satisfied when zooplankton organisms were in abundance, however when planktonic biomass seasonally decreased, the lack of protein slowed the growth of fish. Having continuous access to protein sources and balanced amino acid profiles contributed to the unhindered growth of fish in FF an PF ponds, especially in the second and third years. This result may suggest, that utilization of high-protein feed types might be most beneficial in the older (1- or 2-year-old) age groups of carp under semi-intensive farming.

Although the formulated diets (FF, PF) were designed to be consumed more efficiently than cereal feeds, these also contained higher N and P concentrations, and these opposing effects seemingly extinguished each other, as there was only one statistically significant difference in the water quality of ponds fed with different feed types during the three-year period of the experiment, which occurred only in TN and only in the third year. The lack of differences disproves our initial notion that the high-quality feed types provide better water quality, but it reinforces that high-protein feed types are adoptable substitutes for the traditional cereal-based diets to increase yield without adverse environmental effects in the ponds under semi-intensive technology. Furthermore, as stagnant ponds can be characterized with relatively quick nutrient mineralization and turnover rate, enhancing natural processes

removing natural pollutants, like phosphorus from water [29], such environmental factors may also contribute to the lack of significant differences in water quality between the different feed types in our experiment. It should be noted as well, that the different diets may not always alter water quality parameters substantially [30], but in certain cases differences in organic matter and chlorophyll-*a* [31] or conductivity [32] were reported in similar experiments.

We also aimed to describe differences in the water quality between FF and PF diets. As the PF diet did not affect the measured nutrient concentrations in the water compared to FF, it supported the idea that PF feeds can potentially fully substitute FF feeds without having negative effects on water quality in the semi-intensive common carp production technology. Alternative protein sources substituting for fish meal and fish oil can support a sustainable future for the expanding aquaculture sector [33].

Lastly, the difference in nutrient concentrations of water column between years could rise from the considerable allometric changes in the role of fish in nutrient dynamics [34,35]. The mass-specific excretion rate is higher in juvenile fish in comparison with adults [35,36], accordingly, the amount of excreted nitrogen and phosphorus per unit biomass was higher in the first year. Additionally, the zooplankton consumption is more emphasized in young carps [37], resulting in less grazing pressure on phytoplankton. The increased phytoplankton density accelerated the internal nutrient cycling and kept the nutrients in the water column [38]. Carps are omnivorous fish species, which reportedly have the potential to increase nutrients in the water column even at young stages of development. In mesocosm experiments with *Carassius auratus* fingerlings Huang et al. [39] reported higher concentrations of TN, TP and TSS. In the following years, the mass-specific excretion rate decreased, and the carp individuals switched from zooplanktivory to benthivory [40]. The former phenomenon decreased the direct contribution of fish to the internal nutrient loading by excretion, while the latter increased zooplankton density and thus also the grazing pressure on phytoplankton. The benthivory, furthermore, increased the turbidity due to the stirring effect exerted along with bioturbation [41] and could adversely affect phytoplankton growth. Although the bioturbation can contribute considerably to the internal nutrient (especially phosphorus) loading, providing a nutrient flux from bottom to water column [42,43], it did not result in an increment in nutrient concentration in this study. On one hand, the factors discussed above presumably suppressed this nutrient flux. On the other hand, the well-oxygenized environment in the ponds did not favor the release and ratio of orthophosphate [44]. Substantial differences in the role of fish in nutrient dynamics occur between juvenile and adult life stages [36], in accordance with this, the first year provided more significant differences in water quality compared to the following years. The role of fish in nutrient dynamics was expressed via numerous pathways, while several of these influence the internal nutrient dynamics in the same direction, others affect inversely [34, 45].

Our findings, that the date of sampling is a more powerful predictor compared to the added feed type is supporting the findings of other authors [14]. However, the amount of added feed and the level of intensification can potentially affect the relationship between the feed types and the water quality [46-50]. In order to explain the phenomenon in more detail, further research on carrying capacity and retention dynamics of the fishponds could be highly valuable. Additionally, the nutritional pathways can be described more accurately if further insight into nutrient concentrations of sediment, or planktonic and benthic biomass calculations are provided. Regardless of the exact underlying mechanism, and the lower survival rates observed in the second year of the experiment, our results support that the highly nutritious feed types can safely increase the yield of semi-intensive pond aquaculture without producing adverse effects on water quality variables. Considering only the net feed costs in our experiment (FF: 0.7 €/kg, PF: 0.5 €/kg and CF: 0.1 €/KG) and assuming the wholesale net selling price of live carp to be 2.5 €/kg [51], we can calculate the profit in our case to be 11,000 € with FF, 9,730 € with PF and 8,437 € with CF through the three years of production. In this case of intensification, the higher quality feed types could have paid off by the higher carp yield they generated compared to the control feed. We need to emphasize, that this equation is only realistic if the market demand for additional fish products is realized for the farmer and the relevant operational costs (e.g., transporting, storing and handling) do not increase with the intensification. Based on our study, the change to alternative, higher quality feed types, does not necessarily increase the expenditure to keep stable water quality in the semi-intensive pond farming. Although the traditional cereal based diets are the most used feeds in the Hungarian inland pond farming, the farmers need to regularly consider their options for intensification in the changing European market. The interchangeability of high-protein feed ingredients was also an important outcome in our experiment, which indicates that fishmeal and fish oil dependence can be reduced without detrimental changes in yield or water quality. The substitution of limited feed ingredients, like fish meal, is an essential endeavor for the sustainability of the ever-expanding pond aquaculture [52]. Furthermore, as the National Fisheries Strategic Plan of Hungary emphasizes the role of fishponds to provide stock for natural waters [53], the trend of a sustainable and intensified inland aquaculture in Hungary could provide additional benefits for national fisheries as well.

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Jakabné Sándor Zsuzsanna, Révész Norbert, Havasi Máté, Kumar Shivendra

Szárított kukoricatörköly (DDGS) hasznosítása a ponty intenzív takarmányozásban

A tanulmány háttere és célja

- A ponty takarmányozása céljából a bioetanol gyártás melléktermeke, a kukorica DDGS vizsgálatára került sor.
- A vizsgálatok kiterjedtek emészthetőségi és komplex takarmányozási kísérletekre, melyek segítségével teszteltük a DDGS alkalmazhatóságát ponty ivadékok esetében.
- Meghatározásra kerültek a DDGS egyes tápanyagjainak emészthetőségi együtthatói különböző vízhőmérsékleteken.

Tanulmány főbb eredményei

- A látszólagos emészthetőségi együtthatók alapján a ponty a kukoricához hasonló mértékben képes emészteni a DDGS-t.
- A foszfor emészthetősége kiemelten magasabb más növényi alapanyagokhoz képest, köszönhetően az alacsony fitát tartalomnak.
- A DDGS tartalmú takarmány emészthetősége romlik a 30°C-os vízhőmérsékleten
- A 40% tartalmú DDGS kísérleti takarmány a legtöbb növekedési, takarmányhasznosítási paraméter esetében előnyösebb, mint a kontroll vagy az alacsonyabb DDGS tartalmú takarmány.
- A vér biokémiai paraméterei és a máj, valamint bélhisztológiai vizsgálatok során káros egészségügyi elváltozást nem figyeltünk meg a halakon.
- Ennek eredményeként megállapítottuk, hogy a DDGS magas arányban is alkalmazható összetett pontytakarmányokban a hagyományos növényi összetevők helyett és alkalmas fiatal ponty korosztályok intenzív nevelésére is.

Az összegzés az alábbi cikkekből készült:

Révész, N.; Kumar, Sh.; Bogevik, A. S.; Fazekas, Gy.; Jeney, Zs.; Hegyi, Á.; J. Sándor, Zs. Effect of temperature on digestibility, growth performance and nutrient utilization of corn distiller's dried grains with soluble (DDGS) in Common carp juveniles. Aquaculture Research, 51 (2): 825-835. (2020)

Norbert Révész, Máté Havasi, Kinga Katalin Lefler, Árpád Hegyi, László Ardó, Zsuzsanna Sándor Jakabné. Protein replacement with dried distiller's grain with solubles (DDGS) in practical diet of common carp (Cyprinus carpio). AACL Bioflux 12 (4), 1174-1188. (2019)

Jakabné Sándor Zsuzsanna, Révész Norbert, Havasi Máté, Andre S. Bogevik, Shivendra Kumar Szárított kukoricatörköly (DDGS) hasznosítása a ponty intenzív takarmányozásban. Állattenyésztés és takarmányozás 69(4) 387-40 (2020)

Effect of temperature on digestibility, growth performance and nutrient utilization of corn distiller's dried grains with soluble (DDGS) in Common carp juveniles

Norbert Révész[1], Shivendra Kumar[1,2*], André S. Bogevik[3], Gyöngyvér Fazekas[1], Zsigmond Jeney[1], Árpád Hegyi[4], Zsuzsanna Jakabné Sándor[1]

[1] National Agricultural Research and Innovation Centre, Research Institute for Fisheries (NARIC - HAKI), H-5540 Szarvas, Anna-liget 8, Hungary
[2] Department of Aquaculture, College of Fisheries, Dr. Rajendra Prasad Central Agricultural University, Dholi-843121, Muzaffarpur, Bihar, India
[3] Norwegian Institute of Food, Fisheries and Aquaculture Research (NOFIMA AS), Fyllingsdalen, Norway
[4] Szent István University, Gödöllő, Hungary

*__Corresponding author__: E-mail: shivdholi@rediffmail.com

Abstract
Three hundred sixty common carp juveniles (average weight, 40±7 g) were distributed in thermo-regulated recirculation water system equipped with twelve 1m3 fiberglass tanks (30 fish per tank), which were allotted to four experimental group in triplicates. Half of the experimental groups were maintained at 20 0C, whereas the other half were exposed to 30 ^{0}C. Juveniles reared under different temperature regimes were fed either of the two diets, with or without DDGS (DDGS diet or reference diet), to evaluate the interaction effect between water temperature and nutrient digestibility of corn DDGS in common carp. Diet and water temperature interaction was effective in modulating the response of dry matter digestibility of DDGS ingredient and found higher in juveniles reared at 20 0C compared to 30 0C. Growth, feed efficiency and protein efficiency were higher at 20 0C compared to 30 0C. Whole body composition of common carp juveniles was found unaffected due to diet and water temperature interaction. Overall, it is concluded that digestibility and growth performance of common carp is better at 20 0C compared to 30 0C and DDGS have high potential for inclusion in diets of common carp.

1. Introduction

Major problem facing by aquaculture industry is the need to obtain a balance between rapid fish growth and optimum use of supplied feed. Since the feed cost accounts approximately 60% of the total farm production costs (Tan and Dominy, 1997), the economic viability of the culture operation depends on the feed. Because of its balanced amino acid, fatty acid composition and palatability, the aqua-feed industries reliant mostly on fish meal protein. However, limited supply and high price of fish meal together stimulated severalstudies to substitute fish meal partially or completely with alternative protein sources (Kaushik, Cravedi, Lalles, Sumpter, Fauconneau & Laroche, 1995; Kaushik, Cove`s, Dutto &Blanc 2004; Fournier, Huelvan & Desbruyeres, 2004). The economic and sustainable protien alternative to fish meal is plant protein, which compete with human food also. That's why there is need to identify protein rich resources other than plant protein for aqua-feed. Therefore, by-products of plant ingredients may be a suitable alternative for replacement of fishmeal as a protein source. Corn distiller's dried grains with soluble (DDGS), major by-product of ethanol production, have attracted considerable attention due to its protein content (26-33%), fat content (9-14%) and phosphorus content (7-10%), as well as increased global production. Fermentation of corn by enzymes and yeast produce ethanol and carbon dioxide and the leftover dry by-product is corn DDGS, and the importance of corn DDGS as feed ingredient is increasing with the increase in biofuel industry (Brown, Schaeffer, Rosentrater, Barnes & Muthukumarappan, 2012; Liu, 2012). DDGS lost almost all starch which were fermented by yeast to produce ethanol, however it contains high quantity of non-starch polysaccharides but devoid of other anti-nutritional factors that are usually present in other plant ingredients, like 24 trypsin inhibitor, glucosinolates, erucic acid and has low level of phytate (Brown et al., 2012; Overland, Krogdahl, Shurson, Skrede & Denstadli, 2013). The main constrains of using DDGS in fish feed are inconsistency of nutrient concentration among different DDGS sources (Liu, 2012) and presence of xanthophylls in corn DDGS that can lead to yellow pigmentation of fillets (Lim, Li & Klesius, 2011). DDGs has been evaluated as feed ingredient for omnivorous fish, due to its protein content close to requirement of the species (Brown et al., 2012). Abo-State, Tahoun & Hammouda (2009) observed that DDGS can be included up to 55%, completely replacing soybean meal, in the diet of Nile tilapia (*Orochromis niloticus*) without affecting growth performance. Similarly, Tidwell, Webster & Yancey (1990) and Webster, Tidwell & Yancey (1991) reported that DDGS can be included up to 40% or 70% in the diet of channel catfish, diets are supplemented with lysine, without compromising growth

performance and feed utilization. However, depression in growth of Nile tilapia was observed after replacement offish meal by corn DDGS (Ali, Mahmoud, Tonsy& Hassouna, 2011). Coyle, Mengel, Tidwell& Webster (2004) also reported depressed growth performance of hybrid tilapia fed diet with 30% DDGS.

Proper utilization of nutrients and energy from feed ingredients is reported to depend largely on the extent of their digestibility (De Silva & Perera 1984; Singh 1992). The nutritional potential of corn DDGS has been evaluated through digestibility studies in different species. Cheng & hardy (2004) reported high indigestible carbohydrate content in DDGS because of its high ADC of protein (88-90%) and lipid (79-89%) and low ADC of dry matter (47-59%) and energy (50-67%) in rainbow trout. Thompson, Rawles, Metts, Smith, Wimsatt, Gannam, Twibell, Johnson, Brady & Webster (2008) observed lower ADC of protein (65%), lipid (69%) and dry matter (10%) in hybrid striped bass. Consequently, ADC of DDGS seems to be dependent on the digestive ability of non-protein energy fraction, which is higher in lower trophic fish species (Castro, Perez-Jimenez, Coutinho, Pousao-Ferreira, Brandao, Oliva-Teles & Peres, 2013). Among the factors known to affect nutrient digestibility, temperature is an important factor that affects evacuation rate, enzyme kinetics, food assimilation and nutrient digestibility (Watanable, Takeuchi, Satoh & Kiron, 1996; Azevedo, Cho, Leeson & Bureau, 1998). ADC of protein and energy in dietary ingredients were found species specific as wellas temperature dependent (Takeuchi, 1991; Takeuchi, Satoh & Kiron, 2002).

Although lots of work has been performed to assess nutritional potential of DDGS in different fish species, data on corn DDGS digestibility in Common carp is very scare. As evaluation of corn DDGS is the basic requirement for aquafeed formulation, the objective of the present study was to evaluate the nutrient digestibility of corn DDGS to develop sustainable low cost feed for Common carp. The present study also aimed to investigate the interaction between water temperature and diets supplemented on the apparent nutrient digestibility of DDGS in Common carp juveniles.

2. Materials and Methods

2.1. Experimental diets

A reference diet was formulated to contain 45% protein and 5% lipids. Yttrium oxide (0.02%) was used as inert digestibility marker in the diet. Fish meal and sunflower meal were used as protein sources and wheat as carbohydrate source in the reference diet. Experimental diet (DDGS diet) was formulated by mixing 70% of the reference diet and 30% of corn DDGS (test ingredient). Pannonia Gold, Hungary supplied Corn DDGS. The feeds were produced at Nofimas feed technology centre (Bergen, Norway) with a Wenger TX 52 extruder (Wenger, USA) and 2 mm size die. Composition, proximate analysis, amino acid profile and fatty acid profile of the both reference diet and DDGS diet are presented in Table 1.

2.2. Digestibility trial

The digestibility trials were performed at the NARIC-HAKI, Hungary, with common carp (*Cyprinus carpio*) juveniles. The trials were conducted according to the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes. The experimental system consisted of a thermo-regulated recirculation water system equipped with twelve 1m3 fiberglass tanks. During the trial, water-flow was established at average 4.5 L/min per tank, dissolved oxygen was kept above 80% of saturation, ammonia-N was below 0.1 mg/l, and pHvaries between 8.6-8.8.Three hundred sixty common carp juveniles (avg. wt. 40±7 g) were distributed in four treatment groups with three replicates each, following a completely randomized design in 12 tanks and allowed to acclimate to the experimental conditions for 15 days. During this period, water temperature in all tanks was 200C and juveniles were daily fed a commercial diet (crude protein – 40%). Thereafter, water temperature of half of the experimental groups (6 tanks) was maintained at 200C, whereas the other half was exposed to 300C. The experiment was continued for four weeks. During this period, juveniles were fed with either of the two diet (reference diet / DDGS diet), by automatic feeder and daily feed were given as per thermal-unit growth coefficient (Cho, 1992). Fish were adapted to the diets during 7 days and then faeces were daily collected by stripping approximately 6 h postprandial (Rawles, Gaylord & Gatlin III, 2006). Fish were gently netted from the tanks and anaesthetized by Norcaicum based anesthesia (Matuk, 1987). Fish were manually stripped onto treatment labeled glass jars. Care was taken to ensure that urine, mucus, or water was not introduced to each sample. Faecal samples were immediately placed on ice for transportation to the laboratory, frozen (-700C) and stored until analysis. Fish handling was performed according to the regulation of Animal Ethical Panel of the Institute, which was established according to the Hungarian Statelaw (10/1999.I.27.) Apparent digestibility coefficients (ADCs) of dry matter, protein and phosphorus of the diets were determined by the following formula:

ADC diet = [1 – ({Ydiet / Yfaeces} × {Dfaeces / Ddiet})] x 100 Where, Ydiet is the dietary yttrium level, Yfaeces is the faeces yttrium level, Ddiet is the dietarynutrient level and Dfaeces is the faeces nutrient level.

The apparent digestibility coefficients of the test ingredient (DDGS) were calculated according to Bureau et al, (1999) as follows:

ADCDDGS ingredient = ADCDDGS diet + [(ADC DDGS diet – ADCreference diet) x (0.7 x Dref / 0.3 DDDGS ingredient]
Where, Dref is the % nutrient (or kJ g−1) of reference diet (dry matter basis) and DDDGS is the % nutrient (or kJ g−1) of DDGS ingredient (dry matter basis).

2.3. Sampling
All fish were weighed (g) individually at every other week interval during the experimental period, not being fed for 16 hours prior, to assess growth parameters. Growth performance of fish such as percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) was calculated based on the following standard formulae: Weight gain (%) = [(Final weight –Initial weight) / Initial weight] x 100; Specific growth rate (SGR) = [(ln(Final weight – ln(Initial weight))/Days in trial)x100]; Feed conversion ratio (FCR)= Feed intake/Biomass growth; Total dry feed given(g)/ wet weight gain (g); Protein efficiency ratio (PER) = Net weight gain (wet weight) /Protein given. Three fish from each tank at the end of experiment were randomly collected for analyses of whole body proximate composition.

2.4. Chemical analysis
Proximate composition of the feed, faeces and fish was analysed by standard methods of AOAC (1995). Dry matter was determined gravimetrically after drying at 105 0C for 4 h. Total nitrogen was determined by Kjeldahl-method using a digestion block (KJELDATHERM, Gerhardt, Germany) and distillation method (VAPODEST 30, Gerhardt, Germany), and crude protein calculated as N X 6.25. Fat was determined by Soxhlet method using a semi-automatic system (SOXTHERM 2000, Gerhardt, Germany) and diethyl ether (boiling point, 40 – 60 0C) as a solvent and ash content was estimated after combustion at 550 0C for 4 h. Crude fibre content was determined in fat extracted feed sample by digestion with sulphuric acid (0.51 mole l-1) and potassium hydroxide 0.89 mole l-1) in a GERHARDT Crude Fibre apparatus (Gerhardt, Germany). Total carbohydrate was calculated by differences as, 1000 – (crude protein + fat + ash + fibre). The gross energy of experimental diets was calculated as described by Halver (1976). The amino acid contents in diets were analysed by the accredited laboratory of the Hungarian Food Chain Safety Office (www.nebih.gov.hu) following the ISO 13903:2005 Official Method. Yttrium and phosphorus content was analysed by ICP-OES. The digestion of samples was made with mixtures of acids, including nitric acid (R.G. 65%) and hydrogen peroxide (R.G. 30%). The extraction was realized by using microwave digestion technique under high pressure. The type of microwave apparatus was Milestone Ethos Plus. The concentrations of elements were measured by Thermo Scientific ICP-OES equipment.

2.5. Statistical analysis
The main effect was analysed by using two-way analysis of variance (ANOVA) with temperature (200C and 300C) and dietary treatment (Reference diet and DDGS diet) as two fixed factors. Where significant interactions were found between main effects, a one-way ANOVA was used to compare simple effects. When results were significant, comparison between means were made using the Duncan's multiple range test (DMRT). The mean valuesfor apparent digestibility coefficient of DDGS ingredients were compared by Student t-test. Means were considered significant at $P<0.05$. Statistical evaluation of the data was carried out using the software SPSS version 22.0.

3. Results
The apparent digestibility coefficient (ADC %) of dry matter and nutrients of the DDGS diet in Common carp juvenile at different temperature are presented in Table 2. The ADC of drymatter and protein in Common carp juveiles were significantly ($P<0.05$) affected by temperature whereas ADC of phosphorus was unaffected ($P<0.05$). Juveniles at 20 0C had a significantly higher ($P<0.05$) dry matter and protein ADC compared to juveniles at 30 0C (Figure 1). Irrespective of temperature, ADC of dry matter and protein were higher ($P<0.05$) in juveniles fed the referene diet than juveniles fed the DDGS diet, however ADC of phosphorus was higher ($P<0.05$) for the DDGS group (Figure 2). Significant interaction was found between temperature and diet on ADC of protein. The dry matter, protein andphosphorus ADC of DDGS ingredient in Common carp juveniles exposed to diffrent temperetaure are presented in Table 3. The dry matter ADC of DDGS ingredient was significantly higher ($P<0.05$) in juveniles at 20 0C compared to 30 0C, whereas ADC ofprotein and phosphorus in DDGS ingredient was unaffected ($P>0.05$) due to different temperature. Growth performance and nutrient utilization of common carp juveniles fed the different diets and at different temperature are summerized in Table 4. Common carp juveniles at 20 0C gain a sigificantly ($P<0.05$) higher weight (%), SGR and PER, and lower FCR compared to juvenile at 30 0C (Figure 3). Irrespective of temperature, juveniles fed
either reference or DDGS diet had no significant effect ($P>0.05$) on weight gain (%), SGR and FCR, whereas significantly ($P<0.05$) higher PER was registered in juveniles fed with DDGS diet.

The effect of corn DDGS and different temperature on the body composition of Common carp juvenile is presented in Table 5. No significant differences were found in whole body compostion of Common carp juveniles either exposed to different temperature (20 0C or 30 0C) or fed with different diets (reference or DDGS diet).

4. Discussion

Quality of grains, fermentation efficiency, temperature, time of drying process and quantity of soluble added to the grains highly affected the nutritional value and nutrient content of corn DDGS (Lim et al., 2011; Liu, 2012). The result of the present study indicate that the dietary protein in both reference and test diets were well digested, however the ADC of dry matter and protein were significantly higher in the reference diet compared to the DDGS based test diet. The digestibility and nutritional value of the protein relates directly to its essential amino acid composition. Proteins are made of amino acids, which are released for absorption into the blood following protein digestion. A protein that does not contain the proper amount of essential amino acid would be considered an imbalanced protein and would have lower digestibility and nutritional value. Sum of essential amino acid (ΣEAA) of reference diet was higher than test diet, which was positively correlated with protein digestibility. Jalal, Ambak, Saad, Hassan & Abol (2000) reported that digestion coefficient of protein in fishmeal was higher than blood and meat meal due to their balanced amino acid profile. However, ADC of phosphorus in DDGS diet was found higher compared to fish meal based reference diet. Higher digestibility of phosphorus in corn DDGS diet may be due to hydrolization of corn during the fermentation process in the ethanol plants, which makes more phosphorus available for absorption.

Temperature has also been reported to influence the digestion and assimilation of nutrients in fish (El-sayed, El-Ghobashy & Al-Amoudi, 1996; Watanable et al., 1996). The data obtained in the present study indicate that ADC of diets were significantly higher at 200C than at 300 C. Previous research outcome on the effect of water temperature on digestibility are controversial; some authors found no effect of temperature on digestibility (Cho, Slinger& Bayley, 1982; Iwata, Kikuchi, Honda, Kiyono & Kurokura, 1994), while others found increase of ADC at higher temperature (Choubert, Fauconneau & Luquet, 1982; Oliva-Teles& Rodrigues, 1991). These inconsistencies may be elucidated by the temperature range examined in the different studies. According to Brauge (1994), the ADC of protein in rainbow trout do not affected by the water temperature within the normal temperature range for the species, but showed differences outside this range. In fact, 20 - 250 C is the optimum range of water temperature for growth and nutrient utilization in common carp (Watanable et al., 1996), the reduction in the digestibility of dry matter and protein by the increase of water temperature (200 C to 300 C) in the present study reveals that there is a direct effect of temperature on digestibility. By contrast, the water temperature did not affect the digestibility of phosphorus. Kim, Breque & Kaushik (1998); Yamamoto, Shima, Furuita, Sugita & Suzuki (2007) also reported that the phosphorus absorption was not influenced by the water temperature.

The current study is basically designed for digestibility study, not for growth performance. However, because of an important response variable for ingredient evaluation study, growth performance parameters were also studied. Results of the present study reflected that inclusion of 30% DDGS in the diet did not affect growth performance and feed efficiency of common carp. Previous studies (Tidwell et al., 1990; Webster, Tidwell, Goodgame, Yancey & Johnsen, 1993; Coyle et al., 2004; Cheng and Hardy 2004) also reported that use of moderate (15 - 30%) inclusion levels of DDGS in channel catfish, tilapia and rainbow trout have been promising. Yet the protein digestibility coefficient of reference diet was higher than DDGS diet, the protein efficiency of DDGS diet is found higher than reference diet. This may be due to lower protein and higher fat level in DDGS diet compare to reference diet. Present study revealed that common carp fed with DDGS along with good protein source does not lead to change the growth performance, as studied in case of de-oiled rice bran (Kumar, Sahu, Gupta, Deo, Shamna & Ranjan, 2017). Furthermore, fish growth is also governed by water temperature. Jobling (1994) and McCarthy & Houlihan (1997) reported that feed intake and metabolism, including protein turnover and therefore the growth efficiency of fish may be directly affected by temperature. Growth of fish follows asymmetric patterns, gradually increasing to a maximum at the optimal temperature, as temperature increases across the thermal tolerance of the species (McCarthy, Moksness, Pavlov, Houlihan, 1999), however increases in temperature above theoptimum, there is sharp decrease in growth efficiency. Similarly, present study also showed decreased growth performance and, feed and protein efficiency of common carp at higher temperature (30 0C) than their optimum range at 20 - 250 C (Watanable et al., 1996). Jobling (1997) explained that increase in temperature over the optmimum temperature range limits the growth potential of fish due to cumulative effect of increased metabolic demand and decrerased oxygen availability.

The proximate composition of whole body fish did not vary significantly either due to inclusion of corn DDGS in the diet or increase in water temperature from 20 0C or 30 0C. Thisis corroborated with the findings of Obirikorang, Amisah, Agbo, Adjei-Boateng, Adjei & Skov (2016) and Kumar et. al. (2017), who found no significant difference in the proximate body composition of nile tilapia fed with different agro-industrial by-products and *Labeo rohita* fed with deoiled rice bran, respectively.

In conclusion, results from the present study demonstrated that ADCs of dry matter and protein of corn DDGS were high, reflecting suitable ingredient for use in common carp diet at least at levels up to 30%. In addition, the dietary inclusion of DDGS contributes with a highly digestible phosphorous source that could reduce the need for supplemental inorganic phosphorus (i.e dicalcium phosphate or monocalcium phosphate). This will not only reduce diet costs but also reduce the quantities of phosphorus that are excreted from the animal. Higher nutrient digestibility and growth performance of common carp at 20 0C compared to30 0C, confirms optimum range of temperature for growth of common carp. Overall, corn DDGS seems to have high potential for inclusion in diets for common carp and further study need to be carried out for its maximum inclusion level.

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digestibility in rainbow trout *Oncorhynchus mykiss* and common carp *Cyprinus carpio*

Table 1: Formulation (%), proximate composition (%, dry matter), amino acid profile and fatty acid profile of the experimental diets used in the experiment.

Ingredients	**Reference diet**	**DDGS diet**
Fish meal[1]	41.38	28.97
Corn DDGS[2]	-	30
Sunflower meal[3]	40	28
Wheat	15	10.5
Vitamin premix[4]	2.86	2
Mineral premix[5]	0.74	0.52
Yttrium oxide[6]	0.02	0.014
Proximate Composition		
Crude Protein (CP)	44.32	39.87
Crude Fat	3.46	5.37
Crude fibre	6.45	6.40
Ash	6.13	8.24
Phosphorus	1.68	1.69
Gross energy (KJ g^{-1})	17.71	18.13
Amino Acid Profile		
Essential Amino Acid		
Lysine	2.61	2.04
Arginine	2.29	1.93
Histidine	2.41	2.04
Isoleucine	1.92	1.53
Leucine	3.26	3.06
Valine	2.79	2.61
Methionine	1.27	1.03
Cysteine	0.55	0.58
Phenylalanine	2.32	1.94
Threonine	1.79	1.49
ΣEAA	**21.21**	**18.25**
Non-Essential Amino Acid		
Tyrosine	1.96	1.56
Aspartic acid	3.79	3.00
Glutamic acid	5.41	4.99
Serine	1.97	1.51
Glycine	2.71	2.09
Alanine	1.76	1.59
Proline	2.06	1.69
Fatty acid profile		
16:0	14.10	14.07
18:2ω6	12.37	32.52
18:3ω3	1.11	1.31
20:4ω6	0.38	0.20
20:5ω3	5.83	3.10
22:6ω3	10.33	5.34
Total SFA	22.05	19.60
Total MUFA	41.47	34.53
Total n-6	13.35	33.01
Total n-3	18.44	10.35
n-3/n-6	1.38	0.31
Total PUFA	31.79	43.36

1Nordsildmel (Bergen, Norway)
2Pannonia Ethanol (Hungary)
3 Pannonia Gold, Hungary
4Wheat from Norgesmoellene, Bergen, Norway
5 Vitamin premix (Vilomix, Hoenefoss, Norway):Added per kg feed: Vitamin D3, 3000 IU; niacin, 200 mg; vitamin C, 200 mg; vitamin E, 160 mg; calcium pantothenate, 60 mg; riboflavin, 30 mg; pyridoxine-HCl, 25 mg; menadione bisulfite, 20 mg; thiamine, 20 mg; folic acid, 10 mg; biotin, 1 mg; vitamin B12, 0,05 mg.
6 Mineral premix (mixed at Nofima Feed Technology Centre): Added per kg feed: Magnesium, 300 mg; potassium, 240 mg; zinc, 48 mg; iron, 30 mg; manganese, 6 mg; copper, 3 mg.
7 Alfa Aesar, VWR[6]

Table 2: Apparent digestibility co-efficient (ADC %) of dry matter and nutrients of the DDGS diet in Common carp.

Temperature	**20 ^{0}C**		**30 ^{0}C**		**Two-way ANOVA**		
Diet	Reference	DDGS	Reference	DDGS	Temperature	Diet	Interaction
Dry matter	$76.23^{a} \pm 0.08$	$68.43^{b} \pm 0.84$	$75.75^{a} \pm 0.08$	$66.76^{c} \pm 0.25$	P<0.05	P<0.05	NS
Crude protein	$94.42^{a} \pm 0.21$	$92.56^{b} \pm 0.24$	$89.54^{c} \pm 0.14$	$88.73^{d} \pm 0.08$	P<0.05	P<0.05	P<0.05
Phosphorus	$51.97^{b} \pm 2.61$	$56.97^{a} \pm 0.32$	$51.58^{b} \pm 2.55$	$56.62^{a} \pm 2.96$	NS	P<0.05	NS

Mean values in the same row with different superscript (a, b, c) differ significantly (P < 0.05).

Table 3: Apparent digestibility coefficients of the DDGS ingredient

Temperature	**20 ^{0}C**	**30 ^{0}C**
Dry Matter	$46.96^{a} \pm 2.83$	$45.49^{b} \pm 0.84$
Crude Protein	86.10 ± 0.74	86.06 ± 0.36
Phosphorus	82.89 ± 1.87	80.86 ± 1.71

Mean values in the same row with different superscript (a, b) differ significantly (P < 0.05).

Table 4: Effect of different treatments on growth performance and nutrient utilization of Common carp juveniles.

Temperature	**20 ^{0}C**		**30 ^{0}C**		**Two-way ANOVA**		
Diet	Reference	DDGS	Reference	DDGS	Temperature	Diet	Interaction
Wt. gain (%)	$40.75^{ab} \pm 4.20$	$46.91^{a} \pm 1.92$	$38.98^{b} \pm 2.16$	$36.08^{b} \pm 4.52$	P<0.05	NS	NS
SGR	$1.10^{ab} \pm 0.10$	$1.24^{a} \pm 0.04$	$1.06^{b} \pm 0.05$	$0.99^{b} \pm 0.11$	P<0.05	NS	P<0.05
FCR	$2.10^{ab} \pm 0.19$	$1.86^{b} \pm 0.10$	$2.33^{a} \pm 0.27$	$2.35^{a} \pm 0.27$	P<0.05	NS	NS
PER	$1.08^{b} \pm 0.10$	$1.35^{a} \pm 0.07$	$0.98^{b} \pm 0.12$	$1.08^{b} \pm 0.13$	P<0.05	P<0.05	NS

Mean values in the same row with different superscript (a, b) differ significantly (P < 0.05).

Table 5: Proximate composition of whole body

Temperature	**20 ^{0}C**		**30 ^{0}C**		**Two-way ANOVA**		
Diet	Reference	DDGS	Reference	DDGS	Temperature	Diet	Interaction
Crude Protein (%)	63.52 ± 1.28	63.14 ± 2.66	61.50 ± 2.64	64.08 ± 1.30	NS	NS	NS
Crude fat (%)	20.06 ± 1.37	19.01 ± 1.71	18.88 ± 2.56	18.13 ± 0.68	NS	NS	NS
Ash (%)	12.58 ± 0.59	12.21 ± 0.31	12.60 ± 1.53	13.49 ± 0.99	NS	NS	NS
Moisture	76.33 ± 0.20	76.14 ± 0.54	76.14 ± 1.21	76.06 ± 0.68	NS	NS	NS

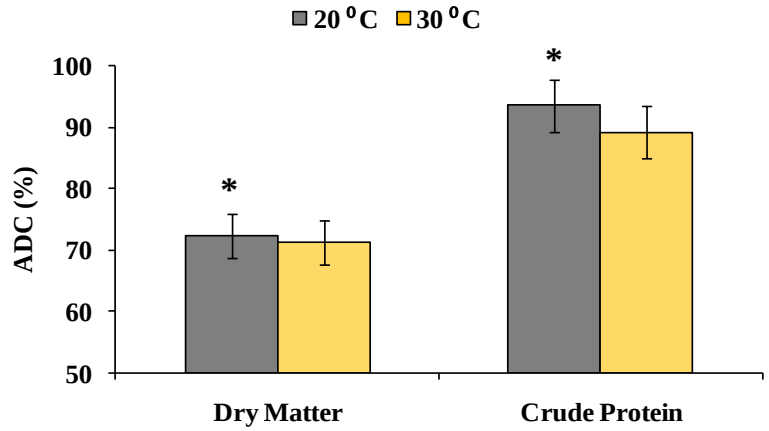

Figure 1: Effect of temperature on apparent digestibility coefficient (%) of dietary dry matter and protein in Common carp juveniles. Significant differences indicated with (*) were determined using Duncan's multiple range test (DMRT) (P<0.05).

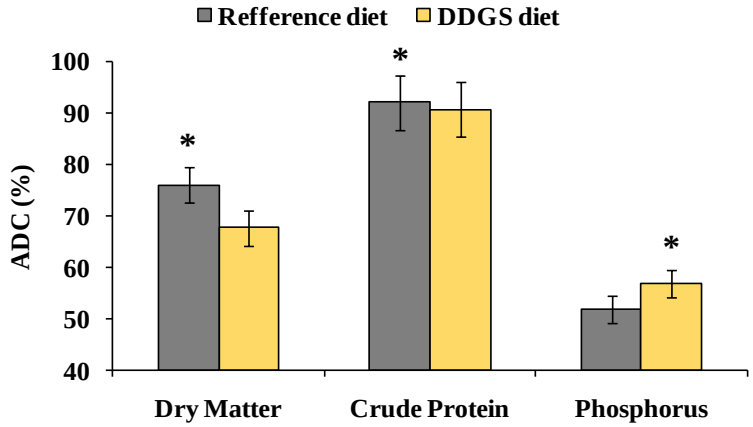

Figure 2:Effect of diet (Refernce diet / DDGS diet) on apparent digestibility coefficient (%) of dry matter, protein and phosphorus in Common carp juveniles. Significant differences indicated with (*) were determined using Duncan's multiple range test (DMRT) (P<0.05).

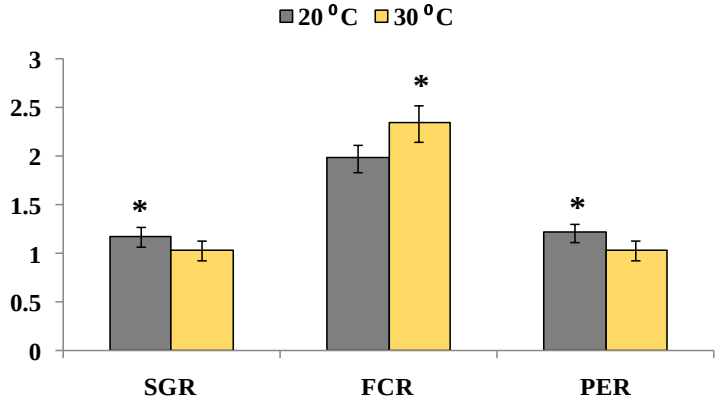

Figure 3: Effect of temperature on growth performance and nutrient utilization of Common carp juveniles. Significant differences indicated with (*) were determined using Duncan's multiple range test (DMRT) (P<0.05).

J. Sándor Zsuzsanna, Révész Norbert, Lefler Kinga, Čolović Radmilo, Banjac Vojislav, Kumar Shivendra

Szárított kukoricatörköly (DDGS) hasznosításának lehetősége a harcsa takarmányozásban

A tanulmány háttere és célja

- A vizsgálatok célja az Európai harcsa számára tervezett keveréktápokban a szója és kukorica helyettesítése volt kukorica DDGS-el.
- Meghatározásra kerültek a DDGS egyes tápanyagjainak emészthetőségi együtthatói harcsa ivadékoknál.
- A DDGS maximális bekeverhetőségi arányának meghatározása céljából emészthetőségi és komplex takarmányozási kísérleteket folytattunk.

Tanulmány főbb eredményei

- Számszerűsítésre kerültek a látszólagos emészthetőségi együtthatók, melynek alapján megállapítottuk, hogy a harcsa a pontyhoz képest gyengébben emészti a DDGS-t.
- A foszfor emészthetősége a harcsa esetében is kiváló más növényi alapanyagokhoz képest.
- A harcsa növekedési lassulás nélkül, kedvezően hasznosította a 10,20, illetve 30 % -os DDGS tartalmú takarmányt.
- A vér biokémiai paraméterei és a máj, valamint bélhisztológiai vizsgálatok során káros egészségügyi elváltozás nem volt megfigyelhető a halakon. Ugyanakkor a növekvő DDGS tartalom hatására csökkent a máj lipidtartalma.
- Megállapításra került, hogy a DDGS 30% -ban alkalmas harcsatakarmányozásra 20%-os halliszt tartalom mellett.

Az összegzés az alábbi cikkből készült:

Sándor, Z. J., Révész, N., Lefler, K. K., Čolović, R., Banjac, V., Kumar, S. Potential of corn distiller's dried grains with solubles (DDGS) in the diet of European catfish (Silurus glanis). Aquaculture Reports, Paper 100653 (2021).

Potential of corn distiller's dried grains with solubles (DDGS) in the diet of European catfish *(Silurus glanis)*

Zsuzsanna Jakabné Sándor[1,*]; Norbert Révész[1], Katalin Kinga Lefler[2], Radmilo Čolović[3], Vojislav Banjac[3], Shivendra Kumar[4]

[1] Research Institute for Fisheries and Aquaculture (HAKI), NAIK, Anna-liget 35., Szarvas, Hungary
[2] Szent István University, Department of Aquaculture, Páter K. str. 1., Gödöllő, Hungary
[3] University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, NoviSad, Serbia
[4] Department of Aquaculture, College of Fisheries, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India

*Corresponding author: Research Institute for Fisheries and Aquaculture NAIK HAKI, Anna Liget Str. 35, 5540 Szarvas, Hungary
Email address: jakabne.sandor.zsuzsanna@haki.naik.hu

Abstract
European catfish (*Silurus glanis*) is an important carnivorous freshwater species in Central and Eastern European aquaculture due to its fast growth, robustness, stress tolerant capability and high market value. Fish meal is the major protein source used for the preparation of commercial feed and alternative sources should be included to reduce this level. A digestibility trial was performed with a by-product of bioethanol production to assess the apparent digestibility of corn DDGS. Afterward four different diets were tested in the course of a nutritional experiment with 0, 10, 20 and 30 % DDGS content in order to assess the effects on the growth, nutrient utilization and metabolism of fish. The feeds were iso-nitrogenous and iso-caloric containing 43 % crude protein and 9 % crude fat. Results of the present study showed that the apparent digestibility of DDGS is auspicious for European catfish. No significant differences were found between the experimental groups regarding growth performance and plasma biochemical parameters. The liver histopathological observations showed that in 20 and 30 % DDGS groups had less vacuolized hepatocytes than the other groups. In conclusion, our study indicates that the European catfish, as a freshwater carnivorous species have the skill to utilize the diets with DDGS up to 30 %.

Introduction

Corn distiller's dried grains with soluble (DDGS) is leftover dry by-product obtained after fermentation of corn by enzymes and yeast which produces bioethanol as major product. Starch available in corn converted to ethanol by fermentation however, other components, like fibre, protein and fat get concentrated in the remaining material i.e DDGS. Therefore, available of relatively high level of energy, protein, amino acid, non-phytate phosphorus and yeast make DDGS a suitable ingredient for fish feed. Nevertheless, its composition varies between different processing plants (**Liu, 2008)** and among the grains used for processing (**Randall and Drew, 2010**). Number of variables in the raw materials and processing factors that have been listed by **Olentine (1986)** contribute to variation in nutrient composition of distiller's by-products viz. soil conditions, applied fertilizers, weather, production and harvesting methods and different processing factors like grind procedures, cooking, conversion, dilution of converted grains, fermentation, etc. Between 2006 and 2015 remarkable peer-reviewed publications have been published, and the composition data are reviewed and summarised by **Zeng et al. (2017).** DDGS became a very attractive ingredient for partial replacement for some of the more expensive traditional energy (corn), protein (soybean meal) and phosphorus (mono- or dicalcium phosphate) ingredients used in animal feeds. Inclusion of DDGS in animal feeds that reported excellent animal performance, health and food product quality.

The growth of the fuel ethanol and biodiesel industries increased the quantities of the corn products and opened new possibilities for feed industry, including aquafeed production. The global biofuels industry produces about 52 million tons of by-products for use in animal feed, and about 85 percent of these by-products are produced by the ethanol industry (**Popp et al., 2016**). The United States ethanol industry is the largest producer of corn co-products, with annual production of about 38 million tons. This amount of corn co-product production is comparable to the amount of soybean meal produced in the U.S. annually, and is being used in large quantities in animal feeds all around the world. (**USGC-DDGS Handbook, 2018**). At present, less than 2 % of total DDGS produced is used in

aquaculture feeds (**Shourson, 2012**). However, expansion of aquaculture followed by rise in global aquafeed consumption potentiates corn DDGS in fish feed.

Major challenges for the successful use of corn DDGS in aquaculture feeds is having limited knowledge of amino acid composition and its digestibility. Despite of relatively high crude protein content (30-32 %), lysine, methionine, threonine and tryptophan concentrations are relatively low in corn DDGS (**(USGC-DDGS Handbook, 2018**). Furthermore, amino acid content and its digestibility are variable due to different period of heating during the DDGS production processes. Consequently, fish diets with high protein levels require synthetic amino acids supplement when significant amounts of DDGS are included. Digestibility of protein, lipid, dry matter and energy have been determined for several fish species like common carp (**Revesz et al., 2020),** rainbow trout (**Overland, 2013**), channel catfish (*Ictalurus punctatus*) (**Lim et al. 2009; Li et al. 2013**), hybrid striped bass (*Moronechrysops x Moronesaxatilis*) (**Thompson, 2008**) and Nile tilapia (*Oreochromis niloticus*) (**Haidar et al.,2016**). However, very few studies have been conducted to determine the amino acid digestibility of corn DDGS in fish such as European seabass (*Dicentrarchus labrax*) and meagre (*Argyrosomus regius*) (**Magalhães et al., 2015),** pompano (*Trachinotus carolinus*) (**Lech and Reigh**, 2012) and rainbow trout (*Ornicorous mykiss*) (**Cheng and Hardy, 2004a**). Apparent digestibility of amino acids in DDGS were found relatively high (90 % for all essential amino acids except threonine) in rainbow trout (*Ornicorous mykiss*) (**Cheng and Hardy, 2004a**).

There are several recommendations for inclusion level of DDGS in the diet of fish. In most of the cases, replacement of fish meal in diet can only be possible with supplementation of synthetic amino acids. For Nile tilapia up to 30 % DDGS could be included without synthetic lysine and methionine supplementation and 8% fish meal in the diet **(Li et. al., 2011)**. In rainbow trout, only 15 % replacement is advised without synthetic lysine and methionine in order to replace 50% fish meal or 22.5 % in case of 75% replacement of fish meal (**Cheng and Hardy**, **2004**). Recently, Ø**verland et al. (2013**) demonstrated that 50 % DDGS inclusion could be advised in the diet with equal amounts of lysine and methionine supplementation. Channel catfish can tolerate higher DDGS in the diet (**Li et al, 2010, 2011b**) with 40 % inclusion in place of soybean with minimum 10% fish meal level in the diet. Moreover, 70 % of DDGS could be utilized without inclusion of any fish meal, but lysine supplementation must be required. The plant ingredients such as soybean or corn other plants, could be replaced much easily in the diets of carnivorous species with DDGS. A maximum dietary DDGS inclusion level of 33 % was established for hybrid striped bass, *Moronechrysops* × *M. saxatilis* (**Trushenski and Gause, 2013**), less (10–20 %), for rainbow trout (**Welker et al., 2014**), for olive flounder, *Paralichthy solivaceus* (**Rahman et al., 2015**), and for turbot (**Diógenes et al., 2018**).

European catfish (*Silurus glanis*) is an important carnivorous freshwater species in Central and Eastern European aquaculture due to their fast growth, robustness, stress tolerant capability and high market value. Recently the protein content of its commercial feeds was based mostly on high quantity of fish meal (ca. 60 %). However, increasing demand, reduced availability and increased price of marine ingredients made it necessary to replace with other alternative sources of protein. Fish meal was successfully replaced with soybean and rendered animal protein by **Havasi et al. (2015) and Kumar et al. (2017),** but replacing the soybean is getting more attention in European feed production. Utilization of agriculture and food industry by products became a current emergent topic. The objective of the present study was to determine the apparent digestibility of nutrients in DDGS for European catfish and to evaluate the effect of partial replacement of fish meal and soybean meal by DDGS in the feeds.

2. Materials and Methods

Two experiments were conducted to evaluate the suitability of corn DDGS as a protein source for European catfish. The first experiment consisted of an *in vivo* digestibility assessment to determine apparent digestibility coefficients for protein, lipid, phosphorus and amino acids available in DDGS. The second experiment was dedicated to eight weeks long nutritional trial with growth performance, nutrient utilization, feed conversion and diet digestibility. The experiments were conducted according to the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes. All animal experiments have been approved by the Ethical Committee of NAIK HAKI, which was established according to Hungarian State law (10/1999. (I. 27) and operated according to different Hungarian State laws concerning animal experiments, transportation of animal, welfare etc. (40/2013. (II.14).

2.1. *In vivo* ingredient digestibility determination

Experimental diets

A reference diet was formulated (Table 1) to contain 43% protein and 9 % fat according to the requirement of European catfish and was supplemented with yttrium oxide as indigestible marker. The test diet was prepared by

mixing 70 % of the reference diet and 30% of the test ingredient- corn DDGS (**Cho et al., 1982; Bureau et al., 1999).** The DDGS was supplied by Pannonia Bio, Hungary. Both diets were produced by extrusion in the pilot plant for animal feed production of the Feed-to-Food Research Centre at the Institute of Food Technology, University of Novi Sad, Serbia. Dry ingredients were ground by a hammer mill (ABC Inženjering, Pančevo, Serbia) equipped with the 0.8 mm sieve and then mixed in Muyang SLHSJ0.2A double-shaft pedal mixer (Muyang, Yangzhou, China). Fish oil was added directly into mixer during mixing of dry ingredients through nozzles that were positioned in the upper part of the mixer, above the mixing pedals. The material was preconditioned by direct addition of steam and water in aforementioned double-shaft mixer until final temperature of the material reached 95 °C. Targeted final moisture content of the material after preconditioning was 25 %. Preconditioned material was extruded using co-rotating twin-screw extruder (Bühler BTSK-30, 7 sections, length/diameter ratio = 28:1, Bühler, Uzwil, Switzerland) with 4 mm die opening (die open area of 12.56 mm^2). The pellets were collected when temperature at the die was in range of 105–110 °C. The pellets were then dried in fluidized bed vibro dryer (FB 500x200, Amandus Kahl, Hamburg, Germany) at 80 °C until product reached final moisture content of approximately 10%. The final pellets were 4.5 mm in diameter and semi-floating.

The digestibility trial was set up in the recirculation system of the institute with European catfish juveniles having average weight of 154.29± 2.73 g. The experimental unit consisted of six 1 m^3 glass fibre tanks. During the trial water flow was established at average 4.5 L/min per tank, dissolved oxygen level was kept above 80 % of saturation, ammonia-N was below 0.1 ml/L and pH varied between 8.6-8.8. Water temperature was set to 24 ± 1°C. A total of 120 catfish juveniles were stocked in tanks with 20 fish/tank stocking density and acclimatized for two weeks. Digestibility trial was carried out in triplicate. The fish were fed *ad libitum* till saturation 3 times per day during the trial.

Faeces collection

After two weeks of feeding, all the fish stock were satisfied in order to collect faeces from intestine (**Austreng, 1978).** Before harvesting fish were anesthetized with Norcaicum/Tonogen based anaesthesia (**Matuk and Gulyás, 1987**). Faecal samples from a given tank were pooled and stored at -20 °C till analysis.

Analytical methods

The chemical compositions of feed and faeces were analysed by standard methods of the **AOAC (1998)** (Table 1). The experimental diet's total carbohydrate (TC) and gross energy (GE) values were calculated as TC = 100 – (crude protein + crude fat + crude fibre + ash), with GE = values of carbohydrates, proteins and lipids of 17.2, 23.6 and 39.5 KJ g^{-1}, respectively (**Halver and Hardy, 2002**). The fatty acid compositions of samples were analysed by capillary gas chromatography (AGILENT 6890N) according to the method by **Folch (1957).** Amino acid contents of the diets and faces samples were analysed by the accredited laboratory of the Hungarian Food Chain Safety Office (www.nebih.gov.hu) following the ISO 13903:2005. Yttrium and phosphorus content were analysed by ICP method. The digestion of samples was made with mixtures of acids, including nitric acid (R.G. 65%) and hydrogen peroxide (R.G. 30%). The extraction was realized by using microwave digestion technique under high pressure. The type of microwave apparatus was Milestone Ethos Plus. The concentrations of elements were measured by Thermo Scientific ICP-OES equipment.

Digestibility equations

Apparent digestibility coefficients (ADCs) of dry matter, protein and phosphorus of the diets were determined by the following formula (Cho et al., 1982; Bureau et al., 1999):

$ADC_{diet} = [1 - (\{Y_{diet}/ Y_{faeces}\} \times \{D_{faeces} / D_{diet}\})] \times 100$

Where, $Y_{diet}$is the dietary yttrium level, Y_{faeces} is the faeces yttrium level, D_{diet} is the dietary nutrient level and D_{faeces} is the faeces nutrient level.

The apparent digestibility coefficients of the test ingredient (DDGS) were calculated according to Bureau et al., (1999) as follows:

$ADC_{DDGS\ ingredient} = ADC_{DDGS\ diet} + [(ADC_{DDGS\ diet} - ADC_{reference\ diet}) \times (0.7 \times D_{ref}/0.3\ D_{DDGS\ ingredient}]$

Where, $D_{ref}$is the % nutrient (or kJ g^{-1}) of reference diet (dry matter basis) and D_{DDGS} is the % nutrient (or kJ g^{-1}) of DDGS ingredient (dry matter basis).

2.2 Nutritional trial

Experimental diets

A control diet meeting the nutritional requirement of European catfish juveniles was formulated to include fish meal and poultry meal in 45 %, soybean defatted product and wheat another 45 % (Table 3.). The experimental diets were formulated with inclusion of DDGS in different ratio (10 %, 20 %, 30 %) by keeping the fish meal and

fish oil level unchanged. Yttrium oxide was incorporated to the diets as inert digestibility marker. The feeds were also produced by twin-screw extruder in the pilot plant for animal feed production of the Feed-to-Food Research Centre at the Institute of Food Technology, University of Novi Sad, Serbia. The diets were produced using same equipment and production parameters as the two diets used in digestibility trial. The feeds were in form of semi-floating pellets with the diameter of 4.5 mm. Amino acid composition and fatty acid profile of the diets are summarized in Table 4.

Experimental design and sampling

20 individuals of catfish juveniles with 272.7±37.8 g initial weight was stocked in 1000 litre glass fibre tanks in a recirculation system in triplicates after 3 weeks of acclimatization. The water temperature was set to 24 ºC, with minimum 8 % oxygen saturation. The fish were fed manually, 3 times per day up to 2.5 % of body weight. The fish were measured every two weeks to follow the growth performance and to adjust the daily feed amount. After 8 weeks of feeding at the end of the experiment, fish were dissected to measure biometrical indices (condition factor, hepatosomatic index, viscerosomatic index) and to take different type of samples. Whole fish and fillet samples were taken for proximate composition, liver samples for histology, gene expression and fatty acid profile analysis, and blood plasma for blood chemistry measurements. One ml of blood was taken from the caudal vein of two fish, using heparinised needles and syringes. Blood samples were put into heparinised microcentrifuge tubes and centrifuged at 1,400 g for 20 minutes at 4°C. After centrifugation, blood plasma was collected and stored at -20°C for further analysis. Finally, faeces samples were collected from the posterior intestine after sacrificing the fish in order to determine the apparent digestibility coefficients of the diets.

Chemical analysis

Proximate analysis, amino acid and fatty acid profile of diets, fish and faeces were determined by standard methods of the AOAC (1998), Folch (1957) and ISO 13903:2005 (Table 2 and 3.). Blood plasma chemistry was done with a Samsung PT10V, semi-automatic clinical chemistry analyser. Plasma enzymes and metabolites, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), lipase, amylase, total cholesterol (TC) and triglyceride (TG) were measured according to IFCC (International Federation of Clinical Chemistry).

Histology

For histological analysis liver samples (n = 6 per treatment) were immediately immersed in Bouin's solution for 16 hours and then transferred to 70% ethanol (**Culling, 1974**). Subsequently, the samples were embedded in paraffin and thin sections (5 μm) were obtained and stained with Mayer's hematoxylin and eosin. The morphological structures of these tissues were observed using an imaging microscope (ECLIPSE 80i, Nikon, Japan).

Calculations and statistical analysis

Growth performance of fish such as percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein production value (PPV) was calculated based on the following standard formulae: Weight gain (%) = [(Final total biomass weight –Initial total biomass) / Initial total biomass] x 100; Specific growth rate (SGR) = (Ln final average weight − Ln initial average weight) × 100 / days; Feed conversion ratio (FCR)= Total dry feed offered (g)/ Weight gain (g); Protein efficiency ratio (PER) = Weight gain / Protein offered. Protein productivity value (%) (PPV) = 100 × (total final biomass (g) × final whole-body crude protein (%) – total initial biomass (g) × initial whole-body crude protein (%)) / (feed protein (%) × total feed offered (g)); Condition factor (CF) = body weight (g) x 100 / body length^3; viscerosomatic index (VSI) = total viscera (g) / body weight (g); hepatosomatic index (HSI) = hepatopancreas weight (g) / body weight (g) x 100; Visceral fat index (VFI) = visceral fat (g) / body weight (g) x 100.

To compare and evaluate the results, we used SPSS 22.0 for Windows. All data were tested with one-way analysis of variance (ANOVA) with Tukey's Post Hoc test. The statistical IDs marked with different letters translate into a deviation on a significance level of $p < 0.05$.

3. Results

3.1. In vivo digestibility

The determined apparent digestibility coefficients (ADC) for dry matter, crude protein, crude fat, phosphorous and for essential amino acids are illustrated in Table 2. Digestibility coefficients of most of the nutrients, except phosphorus, were lower in test diet compared to the reference diet, however significant differences ($p<0.05$) were revealed only for crude fat and dry matter. In respect of essential amino acids, the ADC of cystine, lysine and arginine in test diet were found significantly lower ($p<0.05$) compared to reference diet. The ADC of the DDGS,

as ingredient, had relatively high value for crude protein and crude fat, 73.4 % and 77.4 %, respectively. Meanwhile high phosphorus digestibility was demonstrated with value of almost 88%. Regarding the ADC (%) of amino acid such as lysine, cystine, arginine and histidine, presented reduced values (Table 2.)

3.2 Nutritional trial

After the 8 weeks of feeding trial, statistical differences in terms of growth performance and nutrient protein utilization (Table 5) were not observed for European catfish juveniles. FCR values varied between 1.29 - 1.36 g/g, SGR 1.43 - 1.50 g/day, PER and PPV 1.78- 1.94, and 27.7 - 30.2 % respectively. No mortality was observed during the trial. The examined fish did not showed differences in biometric indices, irrespective of the dietary composition (Table 5). The plasma biochemical parameters such as Glucose, Phosphatase, Ca, Total protein, Globulin, Alanine aminotransferase, Alkaline phosphatase, Cholesterol, Triglyceride and Amylase (Table 6) were not differing significantly. The triglyceride level of the serum presented appreciable differences between DDGS 0 (393 mg/dl) and DDGS 30 (270.8 mg/dl) groups with high standard deviations. Cholesterol level varied between 129-136 mg/dl and glucose 60-64 mg/dl, having the lower value in DDGS 30 group.

Proximate composition was determined in the whole body and filet on dry weight basis. In the filet, crude protein level was found between 79.0-80.5 %, the crude fat between 11.5-12.1 % and crude ash had approximately 5.3 %. Significant differences were not detected between treatments (Table 7.). Crude fat content of the whole fish body differs significantly at $p = 0.070$, the highest level in the DDGS 30 treatment was observed. The crude protein content was similar in all of the treatments.

Structure, shape and consistency of hepatocytes, shape and localization of cell nucleus of liver were studied. The liver histopathological observations (Figure 1) showed that 20 % and 30 % DDGS fed groups had less vacuolized hepatocytes than the other groups. There were no differences were observed between fishes of different experimental groups on gut morphology in respect of length of epithelial cells and number and size of goblet cells. (Figure 2.).

Fatty acid composition of the liver samples presented differences in some of the fatty acids (Table 8.) 16:0 palmitic acid differs significantly in DDGS 20 and 30 to control representing almost 21 % of the fatty acids. Mono-unsaturated fatty acids, as 16:1n-7 and 18:1n-9 decreased significantly with increase of DDGS level in the diet. Consequently, total saturated and total unsaturated fatty acids follow similar trend with individual fatty acids, namely total SFA increased with DDGS inclusion, while total MUFA decreased. Enrichment with total PUFA of the liver is also observed, but this difference is not significantly demonstrated at $p<0.05$. Total lipid content in liver tissue varied between 8.46 and 17.31 mg FA/g, less amount in DDGS 30 treatment was determined.

The apparent digestibility coefficients of the diets determined for different nutrients are presented in Table 9. ADC for dry matter and crude protein were not significantly ($p<0.05$) differing with DDGS inclusion, but for crude fat was highly digestible in all of the diets (ranging between 96-98 %) except DDGS 20 treatment where an outliner values were found (88 %). Phosphorus digestibility was determined for these diets and significant differences found between the treatments. The lowest digestible diet was the control DDGS 0 diet with ADC for P around 29 %, compared to DDGS 10 with 54 %, DDGS 20 with 44 % and DDGS 30 with 47 % phosphorus ADC values.

4. DISCUSSION

Digestibility of DDGS

Dry matter ADC provides an estimate for overall digestibility of the test ingredient, and a low value usually indicates that a high quantity of indigestible material is present in the feedstuff. In the present study, ADC of dry matter determined in DDGS (49.4 %) is comparable with data obtained for channel catfish (50.8 %) (**Li et al., 2013).** The low ADC is due to high level of nitrogen free extract in DDGS, which is mainly composed of non-soluble protein (NSP) and not digestible by carnivorous fish. Recently for protein ADC 86.9 % was reported for channel catfish as long as for European seabream and meagre values were ranging between 92-96 % (**Magalhães et al. 2015**). The ADC for protein in corn DDGS is clearly lower in European catfish (73.4 %) compared to common carp (86.1 %) (**Révész et al., 2020)** and rainbow trout (80.8 %) (**Øverland, 2013a**). The difference in ADC of protein may be due to different feeding habit and metabolism differences of the species.

The digestibility and nutritional value of the protein correlates with amino acid profile of the protein. The digestibility of essential amino acid in the present study is above 80 % for most of the essential amino acid, except the cystine, lysine, arginine and histamine. ADCs in channel catfish determined **by Li et al (2013**) are slightly higher compared to our data. Meanwhile the less digestible AA in European catfish is the lysine (72.4 %). Similarly, ADC for the lysine was the lowest (50.7 %) in corn for stripped catfish, but 94.2 % in the soybean meal (**Da et al., 2013).**

Digestibility of crude fat in corn DDGS is higher (93.8 %) in channel catfish compared to European catfish determined in current study (77.4 %) and in sunshine bass (68.7 %) (**Thompson et al.,2008**). The differences in ADCs of DDGS among different studies may have resulted due to differences in processing conditions, nutrient compositions of diet, faecal collection methods, digestive physiology of different fish species, or experimental conditions.

It is well known that the bioavailability of phosphorus is higher for fish from DDGS (**USGC-DDGS Handbook, 2018)** compared to other feed ingredients of animal and plant origin. Similar result was observed in the present trial for European catfish also. The ADC for phosphorus is dependent on the ingredient like 25-29 % in soy protein concentrate and 34 % in herring meal (Kim et al., 1998), 80-82 % in corn DDGS (**Révész et al., 2020**). However, in the present study, for European catfish, ADC of phosphorus in corn DDGS was registered 88 %. **Prabhu et al. (2019**) obtained 78.1 % ADC of phosphorus in wheat dried distillers for common carp, which represented the highest value within other feed stuffs investigated (whole peas, sunflower meal, hydrolysed feather meal). Higher digestibility of corn DDGS is due to hydrolyzation of corn during the fermentation process which reduces the phytate level. Replacement of fish meal with plant sources in aquafeeds highlighted the role of phytate present in plants seeds, because it has negative impact on growth, nutrient, energy utilization and mineral uptake. From 50 to 80 % of total P in plants is stored in phytate (**Ravindran et al., 1995**), and this form is not bioavailable for fish due to the lack of intestinal digestive enzymes, phytase. Moreover, phytate can reduce digestibility of other nutrients trough connection as cation to the amino acids, lipids, protein (**Kumar et al., 2012**). As a conclusion we can assume that DDGS is good source for available phosphorus and satisfy the requirement for several fish species ranging between 0.3-1.0 % (**NRC, 2011**). Other benefit of using DDGS in aquaculture should be the low phosphorus excretion into the environment due to its relatively high digestible phosphorus content.

Nutritional aspects of DDGS

DDGS is considered as an acceptable ingredient in diets for some of the fish species investigated (**USGC-DDGS Handbook, 2018**). Very few studies have been conducted on the replacement of fish meal with plant ingredients in the diet of European catfish (**Bekcan et al., 2006)**. In the present study different experimental feeds were formulated replacing some ingredients (poultry meal, wheat, soy oil) with increasing amount of DDGS, as long as level of fish meal and fish oil was kept constantly low (20 % and 1.5 %). The diets were isonitrogenous and were enriched with synthetic lysine and methionine in order to balance the potential essential amino acid deficit occurring with DDGS utilization **(Kim et al., 1998)**. The variability of EAA compositions of DDGS may be due to several factors that include differences in non-protein-nitrogen substances, temperature and duration of drying, and the contribution of yeast AA to total AA in DDGS (**Liu, 2011**). The findings of the present result showed that inclusion of 0, 10, 20 and 30 % DDGS did not affect the growth parameters, nutrient utilization and biometric indices of European catfish. Similar study was conducted by **Webster et al. (1993),** when cage reared juvenile catfish were fed diets containing 0, 10, 20, or 30 % DDGS which partially replaced corn and soybean meal in the diets. There were no differences in individual fish weights, survival, feed conversion, carcass composition, carcass waste (head, skin, viscera), and organoleptic properties of the filets among dietary treatments. Results from **Robinson and Li (2008)** study also suggest that adding up to 30 % DDGS to channel catfish diets supports satisfactory growth performance when the diet is supplemented with synthetic lysine. Similarly, **Zhou et al. (2010)** replaced soybean meal and maize meals with DDGS in juvenile hybrid catfish (channel catfish × blue catfish *I. Furcatus*) diets and observed that feeds containing 30 % DDGS provided good growth, feed conversion, and protein retention. Results from these studies indicate that up to 30% DDGS can be added to channel catfish diets without adversely affecting survival, growth or feed conversion and flavour qualities of the filets. Furthermore, when fish meal was replaced in the diet of striped catfish (**Allam et al., 2019**), with High Protein Dried Distilled Grain (HP-DDGS) in different ratio negative tendency was reported in growth and nutrient utilization due to the amino acid imbalance.

Whole body carcass composition and filet composition of European catfish was not affected by DDGS replacement in the present trial. There were no statistical differences in the plasma biochemical parameters, however, DDGS 20 and DDGS 30 groups had less vacuolated hepatocytes in liver. For other species, it has been studied that high dietary DDGS inclusion levels reduced whole body lipids and energy content (**Webster et al., 1993; Robinson and Li, 2008, Diógenes et al.,2018)** that was attributed to a reduction of digestible energy intake. **Diógenes et al., 2018** observed that fish meal replacement by DDGS reduced both plasma triglycerides and cholesterol levels in turbot, and the authors attributed this reduction with lower feed intake of fish fed with DDGS diets. However, when soybean meal was replaced with DDGS, the same parameters increased with inclusion level of DDGS (**Peres et al., 2014**). In present study, both fish meal and soybean meal level were unchanged in the diet, however, lower values for the particular parameters in DDGS 30 group was observed. The reason for decrease in

plasma triglycerides and cholesterol level may be due to lower dietary fat in the DDGS treatment groups. It is well known that dietary replacement of fish oil by vegetable oils affects plasma cholesterol and triglycerides levels of gilthead seabream (**Caballero et al., 2006; Castro et al., 2016**). Moreover, it was hypothesized that the presence of yeast cells in DDGS (**Ingledew, 1999)** may affect plasma lipid profile. **Kumar et al. (2013) and Øverland et al. (2013b)** found a decrease in plasma cholesterol, while, **Mohebbi et al., (2013) reported** increase in plasma cholesterol and decreased triglycerides level when yeast or yeast derived products were utilized. **Allam et al., (2019)** found a decrease in total protein, albumin, globulin and glucose level in the serum of striped catfish fed on HP-DDG with increasing the inclusion level in the diet, but all of the parameters are higher compared to our data obtained in the experiment.Similarly no impact have been reported for gilthead seabream (*Sparus aurata*) juveniles by **Diogenos et al. (2019)** in total protein, globulin and albumin level with dietary inclusion of DDGS as long as triglyceride level and cholesterol were changing significantly compared to the control.

Changes in the structure of hepatocytes are commonly caused by toxicity, but it can induce by nutritional influences. The level of vacuolation may indicate energy stores in form of glycogen or lipid (**Wolf and Wheeler, 2018**). **Bilen and Bilen (2013)** reported that fish in captivity due to less physical activity store much more carbohydrate in the liver than their needs. This symptom has been reported by several authors as it is one of the most common non-infectious nutritional diseases (**Shefat and Karim, 2018**). Besides increasing of DDGS incorporation in feed, present result showed decreasing tendency of liver vacuolation and hypertrophy. However, the experimental diets have been set to isonitrogenous and isoenergetic, the DDGS 30 group had the lowest crude fat content. In this case this small difference and better fatty acid composition due to DDGS resulted less vacuolation, better nutrient utilization and healthier liver tissue. Some ingredients can destroy the health and the integrity of the intestinal epithelia (**Atalah et al., 2007**), but no such observations were found in the present study.

Differences in saturated and mono unsaturated fatty acids level in the liver between different treatments suggest that DDGS inclusion positively affected the fatty acid metabolism of European catfish. Decrease of monoenoic fatty acids in the liver is occurred to the decrease in poultry meal level in the diet, which refers to the usefulness of high amount of rendered animal protein in the diet. High level of MUFA in the DDGS 0 group indicate liver fattening (**Tornstensen et al., 2011**), which is confirmed by higher total lipid (mg FA/g) determined. Similar effect was observed in common carp, when the highest fat deposition in DDGS 0 group have been found. (**Révész et al., 2019**). Nonetheless, based on histological study of the liver and on the ALT and ALP activities in the plasma, damage of the liver tissue was not observed in any of the fish groups. The increase of saturated fatty acids in liver with inclusion level of DDGS in diet could not be explained by the minor differences in saturated fatty acid, crude fat or total lipid content of the diets. Such tendency was not observed in the case of common carp (**Révész et. al, 2019**).

The apparent digestibility coefficients (ADC) of the diet for dry matter and crude protein were similar, this fact pointed out that 30 % inclusion of DDGS in the diet containing 20% fishmeal is preferable and utilizable by European catfish. Moreover, the available phosphorus increases with inclusion of DDGS in the diet, which is a benefit for phosphorus intake and waste discharge.

In conclusion, results from the present study revealed that ADCs of crude fat and phosphorus of corn DDGS were high and reflected in the DDGS as a suitable ingredient for the use in European catfish diet up to 30 %.

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Table 1: Formulation (%), proximate composition (%, wet weight), amino acid profile of the reference and experimental diets used in the digestibility experiment.

Ingredients	**Reference diet**	**DDGS diet**
Wheat flour T850[1]	28.5	20.0
Corn DDGS[2]	-	30.0
Fish meal[3]	40.0	28.0
Soybean flour (defatted, 50% Pr)[4]	20.0	14.0
Fish oil[5]	5.0	3.5
Blood meal[6]	2.5	1.8
Mono-Ca-Phosphate[7]	1.8	1.3
Vitamin premix[7]	1.0	0.70
$CaCO_3$[7]	0.6	0.42
NaCl[7]	0.5	0.35
Yttrium-oxide[8]	0.1	0.07
Proximate Composition		
Crude Protein	43.25 ± 0.29	39.70 ± 0.60
Crude Fat	5.99 ± 0.20	7.68 ± 0.68
Crude fibre	1.37 ± 0.02	3.95 ± 0.05
Crude Ash	10.07 ± 0.12	8.68 ± 0.05
Phosphorus	1.37 ± 0.05	1.24 ± 0.02
Gross energy (KJ g^{-1})	17.76	17.82
Amino Acid Profile		
Essential Amino Acid (EAA)		
Arginine (ARG)	2.30	1.61
Cysteine (CYS)	0.38	0.31
Histidine (HIS)	1.38	1.23
Isoleucine (ILE)	1.90	1.71
Leucine (LEU)	3.04	3.21
Lysine (LYS)	2.65	2.31
Methionine (MET)	1.17	1.25
Phenylalanine (PHE)	1.84	1.64
Threonine (THR)	1.75	1.81
Valine (VAL)	2.15	2.01
ΣEAA	**18.56**	**17.19**
Non-Essential Amino Acid		
Alanine (ALA)	2.16	2.19
Aspartic acid (ASP)	3.76	3.45
Glutamic acid (GLU)	2.25	1.99
Glycine (GLY)	2.25	1.99
Proline (PRO)	2.11	2.05
Tyrosine (TYR)	1.50	1.43
Serine (SER)	2.42	2.22

[1] Union SP Commerce mill, Temerin, Serbia
[2] Pannonia Gold, Dunaföldvár, Hungary
[3] 999 Fish meal LT, TripleNine Fish Protein A/S, Esbjerg, Denmark
[4] SOPRO-TB200, Sojaprotein, Bečej, Serbia
[5] Sardina DOO, Postira, Brač, Croatia
[6] ATEV Fehérjefeldolgozó Zrt., Hungary
[7] Supplied by DTD Ribarstvo, Bački Jarak, Serbia.
[8] Alfa Aesar, Thermo Fisher (Kandel) GmbH, Karlsruhe, Germany

Table 2. Apparent Digestibility Coefficients (ADC) of the feeds and the test ingredient (DDGS)

ADC %	Reference feed	DDGS feed	*p-value*	DDGS ingr.
Dry matter	67.44 ± 0.61	61.97 ± 1.21	0.002	49.42 ± 3.98
Crude protein	82.89 ± 0.41	80.82 ± 0.65	0.010	73.39 ± 2.98
Crude fat	97.35 ± 0.05	90.06 ± 0.32	0.000	77.38 ± 0.86
Phosphorus	60.14 ± 1.59	65.41 ± 1.09	0.009	87.98 ± 5.95
Essential amino acids (EAA)				
Arginine	93.01 ± 0.13	89.41 ± 0.34	0.000	72.64 ± 1.90
Cystine	62.34 ± 0.71	46.97 ± 1.69	0.000	24.49 ± 4.15
Histamine	66.95 ± 0.62	64.74 ± 1.12	0.041	58.00 ± 4.55
Isoleucine	86.94 ± 0.25	86.19 ± 0.44	0.061	83.43 ± 2.05
Leucine	85.28 ± 0.28	86.11 ± 0.44	0.051	87.85 ± 1.37
Lysine	85.56 ± 0.27	82.96 ± 0.54	0.002	66.30 ± 4.02
Metionine	86.06 ± 0.26	86.15 ± 0.44	0.765	86.39 ± 1.56
Proline	85.56 ± 0.27	86.12 ± 0.44	0.137	87.19 ± 1.29
Threonine	82.81 ± 0.32	82.97 ± 0.54	0.682	83.48 ± 2.27
Valine	83.51 ± 0.31	83.36 ± 0.53	0.684	82.82 ± 2.37

Table 3. Formulation and chemical composition of the diets with different inclusion level of DDGS

Ingredients	**DDGS 0**	**DDGS 10**	**DDGS 20**	**DDGS 30**
Poultry meal[1]	25.0	20.5	16.0	12.0
Soybean flour 50% Pr[2]	21.02	22.0	23.0	23.5
Wheat[3]	25.0	18.94	12.8	6.72
Fish meal 60%[4]	20.0	20.00	20.0	20.0
DDGS[5]	**0.0**	**10.0**	**20.0**	**30.0**
Yeast[6]	5.0	5.00	5.0	5.0
Fish oil[7]	1.5	1.50	1.5	1.5
Soybean oil[3]	1.8	1.20	0.6	0.0
Lysin 78%[3]	0.06	0.15	0.22	0.28
Methionine DL 99%[3]	0.04	0.06	0.08	0.10
Premix[3]	0.50	0.50	0.50	0.50
NaCl[3]	0.08	0.15	0.30	0.40
Y_2O_3[8]	0.1	0.1	0.1	0.1
Proximate Composition % wet weight basis				
Crude protein	39.39 ± 0.32	38.40 ± 0.19	37.85 ± 0.16	37.68 ± 0.32
Crude fat	7.58 ± 0.01	6.92 ± 0.01	6.66 ± 0.00	6.09 ± 0.00
Crude fibre	3.32 ± 0.16	3.69 ± 0.20	4.23 ± 0.05	4.46 ± 0.03
Crude ash	8.66 ± 0.08	8.14 ± 0.06	7.86 ± 0.02	7.92 ± 0.03
Gross energy (KJ g^{-1})	19.43	19.26	19.18	18.94
Phosphorus	1.23	1.13	1.08	1.06

1 BRO-MK Processed animal protein, Brovis DOO, Visoko, Bosnia & Herzegovina
2 SOPRO-TB200, Sojaprotein, Bečej, Serbia
3 Supplied by DTD Ribarstvo, Bački Jarak, Serbia.
4 Sardina DOO, Postira, Brač, Croatia
5 Pannonia Gold, Dunaföldvár, Hungary
6 Biofood, Tambou, Russia
7 Sardina DOO, Postira, Brač, Croatia
8 Alfa Aesar, Thermo Fisher (Kandel) GmbH, Karlsruhe, Germany

Table 4. Amino acid and fatty acid composition of the diets (as is)

Essential Amino Acid	**DDGS 0**	**DDGS 10**	**DDGS 20**	**DDGS 30**
Arginine (ARG)	2.68	2.51	2.40	2.41
Cysteine (CYS)	0.56	0.52	0.57	0.52
Histidine (HIS)	1.40	1.48	1.42	1.42
Isoleucine (ILE)	1.84	1.84	1.82	1.77
Leucine (LEU)	2.96	3.03	3.29	3.35
Lysine (LYS)	2.50	2.39	2.48	2.54
Methionine (MET)	0.62	0.57	0.59	0.49
Phenylalanine (PHE)	1.77	1.73	1.94	1.82
Threonine (THR)	1.35	1.37	1.46	1.46
Valine (VAL)	2.26	2.66	2.40	2.57
ΣEAA				
Non-Essential Amino Acid				
Alanine (ALA)	2.28	2.14	2.30	2.30
Aspartic acid (ASP)	4.19	4.21	4.50	4.00
Glutamic acid (GLU)	7.49	6.54	6.67	6,94
Glycine (GLY)	3.11	2.65	2.67	2.32
Proline (PRO)	2.32	2.06	2.69	2.39
Serine (SER)	1.93	2.07	2.06	2.12
Tyrosine (TYR)	1.35	1.37	1.46	1.46
Fatty acids w%				
14:0	1.63 ± 0.14	1.59 ± 0.04	1.75 ± 0.02	1.87 ± 0.02
16:0	16.49 ± 0.64	16.14 ± 0.06	15.80 ± 0.06	15.47 ± 0.21
16:1n-9	0.49 ± 0.27	0.28 ± 0.00	0.26 ± 0.02	0.37 ± 0.14
16:1n-7	3.01 ± 0.07	2.75 ± 0.06	2.57 ± 0.04	2.63 ± 0.05
18:0	4.32 ± 0.08	4.29 ± 0.02	3.97 ± 0.04	3.57 ± 0.06
18:1n-9	23.49 ± 0.71	24.19 ± 0.28	23.24 ± 0.11	22.65 ± 0.38
18:1n-7	2.32 ± 0.58	1.90 ± 0.06	1.81 ± 0.01	2.01 ± 0.36
18:2n-6	28.38 ± 0.49	30.08 ± 0.19	30.31 ± 0.16	29.88 ± 0.33
18:3n-6	0.26 ± 0.20	0.15 ± 0.01	0.12 ± 0.01	0.25 ± 0.17
18:3n-3	3.17 ± 0.11	3.10 ± 0.00	2.81 ± 0.02	2.36 ± 0.08
20:0	0.26 ± 0.02	0.27 ± 0.00	0.29 ± 0.00	0.31 ± 0.02
20:4n-6	0.43 ± 0.01	0.40 ± 0.03	0.39 ± 0.01	0.39 ± 0.06
20:5n-3	1.87 ± 0.05	1.97 ± 0.00	2.24 ± 0.01	2.34 ± 0.03
22:6n-3	4.77 ± 0.29	4.82 ± 0.02	5.52 ± 0.16	5.69 ± 0.23
Total SFA	23.42 ± 0.99	22.97 ± 0.09	25.57 ± 0.06	22.02 ± 0.46
Total MUFA	33.87 ± 0.19	33.87 ± 0.27	33.19 ± 0.09	33.33 ± 0.12
Total n-6	29.66 ± 0.43	31.09 ± 0.25	31.35 ± 0.08	31.19 ± 0.09
Total n-3	10.43 ± 0.17	10.48 ± 0.03	11.22 ± 0.18	11.14 ± 0.18
Total PUFA	40.08 ± 0.26	41.57 ± 0.29	42.57 ± 0.10	42.33 ± 0.27
total lipid mg FA/ g	71.78 ± 6.02	65.54 ± 0.11	66.44 ± 3.46	59.22 ± 4.29

SFA- saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids

Table 5. Growth performance, nutrient utilization parameters (n=20) and biometric indices (n=6)

	DDGS 0	DDGS 10	DDGS 20	DDGS 30	*p-value*
Initial body weight (g)	271.6 ± 35.6	273.6 ± 33.8	273.2 ± 40.2	272.3 ± 41.8	0.964
Final Body weight (g)	629.9 ± 24.04	630.8 ± 110.8	609.3 ± 151.2	629.0 ± 130.5	0.717
Yield (g)	358.3 ± 21.2	357.1 ± 17.2	336.1 ± 23.5	356.7 ± 29.0	0.611
FCR (g/g)	1.29 ± 0.06	1.30 ± 0.04	1.36 ± 0.07	1.29 ± 0.08	0.608
SGR (g/day)	1.50 ± 0.05	1.49 ± 0.04	1.43 ± 0.07	1.49 ± 0.06	0.498
PER (g/g)	1.78 ± 0.12	1.94 ± 0.04	1.93 ± 0.07	1.89 ± 0.08	0.163
PPV (%)	27.68 ± 1.70	29.18 ± 0.50	30.21 ± 1.01	28.87 ± 1.13	0.144
CF (%)	0.60 ± 0.05	0.66 ± 0.08	0.61 ± 0.02	0.60 ± 0.02	0.181
VSI (%)	7.45 ± 0.76	7.66 ± 0.70	7.55 ± 0.25	7.30 ± 0.64	0.777
HSI (%)	1.87 ± 0.34	2.08 ± 0.25	1.84 ± 0.24	1.84 ± 0.15	0.330
VFI (%)	0.78 ± 0.41	0.83 ± 0.40	0.56 ± 0.20	0.53 ± 0.29	0.333
GI (%)	2.89 ± 0.25	2.80 ± 0.25	3.01 ± 0.34	2.79 ± 0.20	0.585
Filleting yield (%)	43.53 ± 2.32	45.07 ± 3.42	43.47 ± 3.40	43.96 ± 3.90	0.824

FCR - feed conversion rate, SGR - specific growth rate, PER - protein efficiency ratio, PPV - protein production value; CF - condition factor; VSI- viscerosomatic index; HSI - hepatosomatic index; VFI - visceral fat index; GI – gut index. Values are means of six replicates.

Table 7. Proximate composition of the whole body and filet (% dry weight basis) (n=6)

Filet	**DDGS 0**	**DDGS 10**	**DDGS 20**	**DDGS 30**	*p-value*
Crude protein	78.96 ± 2.07	80.46 ± 1.55	80.08 ± 1.06	79.0 ± 0.96	0.366
Crude fat	11.58 ± 2.02	11.54 ± 1.39	11.78 ± 2.59	12.07 ± 1.18	0.873
Crude ash	5.28 ± 0.33	5.36 ± 0.38	5.47 ± 0.27	5.49 ± 0.16	0.573
Whole body					
Crude protein	61.32 ± 1.04	61.23 ± 0.88	60.24 ± 1.09	59.39 ± 2.23	0.313
Crude fat	23.87 ± 1.6	23.76 ± 2.29	26.95 ± 1.21	27.45 ± 2.00	0.070
Crude ash	10.27 ± 1.03	9.70 ± 0.13	8.44 ± 1.12	8.56 ± 0.64	0.069

Values are means of six replicates.

Table 6. Biochemical parameters of blood plasma (n=6) at the end of trial

	GLU	PHOS	Ca	TP	GLOB	ALT	ALP	CHOL	TRIG	AMY
	mg/dl	**mg/dl**	**mg/dl**	**g/dl**	**g/dl**	**U/L**	**U/L**	**mg/dl**	**mg/dl**	**U/L**
DDGS 0	64.33 ± 0.45	7.06 ± 0.45	9.92 ± 0.31	2.71 ± 0.40	1.93 ± 0.19	14.00 ± 2.82	88.00 ± 7.21	136.6 ± 18.64	393.2 ± 45.02	18.20 ± 2.16
DDGS 10	62.00 ± 0.32	7.45 ± 0.32	9.90 ± 0.37	2.75 ± 0.20	1.86 ± 0.27	12.50 ± 3.14	88.16 ± 7.73	130.8 ± 11.26	310.2 ± 61.74	17.16 ± 3.18
DDGS 20	62.83 ± 0.76	7.34 ± 0.76	10.22 ± 0.39	2.93 ± 0.21	2.00 ± 0.14	14.50 ± 1.87	90.83 ± 11.85	140.6 ± 21.32	364.5 ± 116.7	19.00 ± 3.08
DDGS 30	60.33 ± 0.28	7.25 ± 0.28	9.81 ± 0.33	2.70 ± 0.17	1.76 ± 0.12	14.66 ± 2.87	90.33 ± 4.76	129.3 ± 10.28	270.8 ± 58.40	18.66 ± 2.06
p- value	0.292	0.406	0.242	0.535	0.181	0.531	0.512	0.483	0.245	0.880

GLU – Glucose, PHOS – Phosphate, Ca – Calcium, TP – Total Protein, GLOB – Globulin, ALT – Alanine aminotransferase, ALP – Alkaline Phophatase, CHOL – Cholesterol, TRIG – Triglyceride, AMY – Amylase

Values are means of six replicates.

Table 8. Fatty acid composition of the liver (w%) (n=6) (as is)

Fatty acids w%	DDGS 0	DDGS 10	DDGS 20	DDGS 30	*p-value*
14:0	0.83 ± 0.09	0.92 ± 0.22	0.75 ± 0.09	1.02 ± 0.34	0.170
16:0	15.47 ± 1.94^{a}	17.19 ± 1.12^{ab}	18.42 ± 0.98^{b}	21.10 ± 0.80^{c}	0.000
16:1n-9	1.04 ± 0.18	0.97 ± 0.10	1.03 ± 0.15	1.02 ± 0.13	0.843
16:1n-7	5.81 ± 2.45^{a}	4.85 ± 1.62^{ab}	3.39 ± 0.51^{ab}	2.69 ± 1.14^{b}	0.013
18:0	7.05 ± 1.14^{a}	7.72 ± 0.40^{a}	9.28 ± 0.69^{b}	10.19 ± 0.55^{b}	0.000
18:1n-9	27.93 ± 7.94^{a}	24.62 ± 4.70^{ac}	18.87 ± 2.05^{bc}	15.90 ± 3.27^{b}	0.002
18:1n-7	4.49 ± 0.93	4.53 ± 0.94	4.04 ± 0.37	3.56 ± 0.92	0.176
18:2n-6	5.40 ± 0.65	4.89 ± 0.70	5.07 ± 0.28	5.11 ± 0.27	0.404
18:3n-6	0.31 ± 0.17	0.38 ± 0.40	0.42 ± 0.47	0.24 ± 0.03	0.578
18:3n-3	0.25 ± 0.09	0.21 ± 0.60	0.28 ± 0.15	0.15 ± 0.09	0.191
20:0	0.09 ± 0.00	0.09 ± 0.01	0.29 ± 0.00	0.12 ± 0.04	0.135
20:4n-6	6.10 ± 1.91	6.83 ± 1.34	8.35 ± 0.55	8.72 ± 2.52	0.052
20:5n-3	0.66 ± 0.20	0.73 ± 0.13	0.79 ± 0.08	0.67 ± 0.18	0.468
22:6n-3	11.69 ± 4.40	13.02 ± 2.65	14.76 ± 0.75	14.86 ± 0.65	0.143
Total SFA	23.74 ± 3.15^{a}	26.20 ± 1.40^{ad}	28.92 ± 1.31^{bd}	32.92 ± 1.21^{c}	0.000
Total MUFA	43.20 ± 11.42^{a}	38.57 ± 7.05^{ac}	30.51 ± 3.24^{bc}	26.00 ± 5.06^{b}	0.003
Total n-6	17.89 ± 3.79	18.55 ± 3.27	21.70 ± 1.53	22.51 ± 4.40	0.075
Total n-3	13.13 ± 4.83	14.57 ± 2.89	16.48 ± 0.92	16.43 ± 0.75	0.164
Total PUFA	31.03 ± 8.60	33.12 ± 6.07	38.17 ± 2.30	38.94 ± 0.27	0.084
Total lipid mg FA/ g	17.31 ± 4.59^{a}	14.42 ± 2.55^{ac}	10.30 ± 2.25^{bc}	8.46 ± 4.81^{b}	0.000

SFA- saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids Values are means of six replicates; values within the same row with different letters are significantly different ($P < 0.05$).

Table 9. Apparent digestibility coefficients of the diets with different inclusion level of DDGS

ADC $_{feed}$ (%)	**DDGS 0**	**DDGS 10**	**DDGS 20**	**DDGS 30**	**p-value**
Dry matter	56.62 ± 1.76	54.65 ± 0.23	55.86 ± 0.23	55.85 ± 0.23	0.140
Crude protein	77.09 ± 1.33	78.60 ± 1.16	74.49 ± 2.32	76.95 ± 0.53	0.054
Crude fat	98.01 ± 0.16^{a}	96.80 ± 0.11^{a}	88.03 ± 1.50^{b}	97.12 ± 0.07^{a}	0.000
Phosphorous	29.59 ± 2.86^{a}	54.66 ± 0.23^{b}	44.69 ± 0.30^{c}	47.21 ± 0.29^{c}	0.000

Values are means of three replicates; values within the same row with different letters are significantly different ($P < 0.05$).

Figure 1. Histological sections of liver

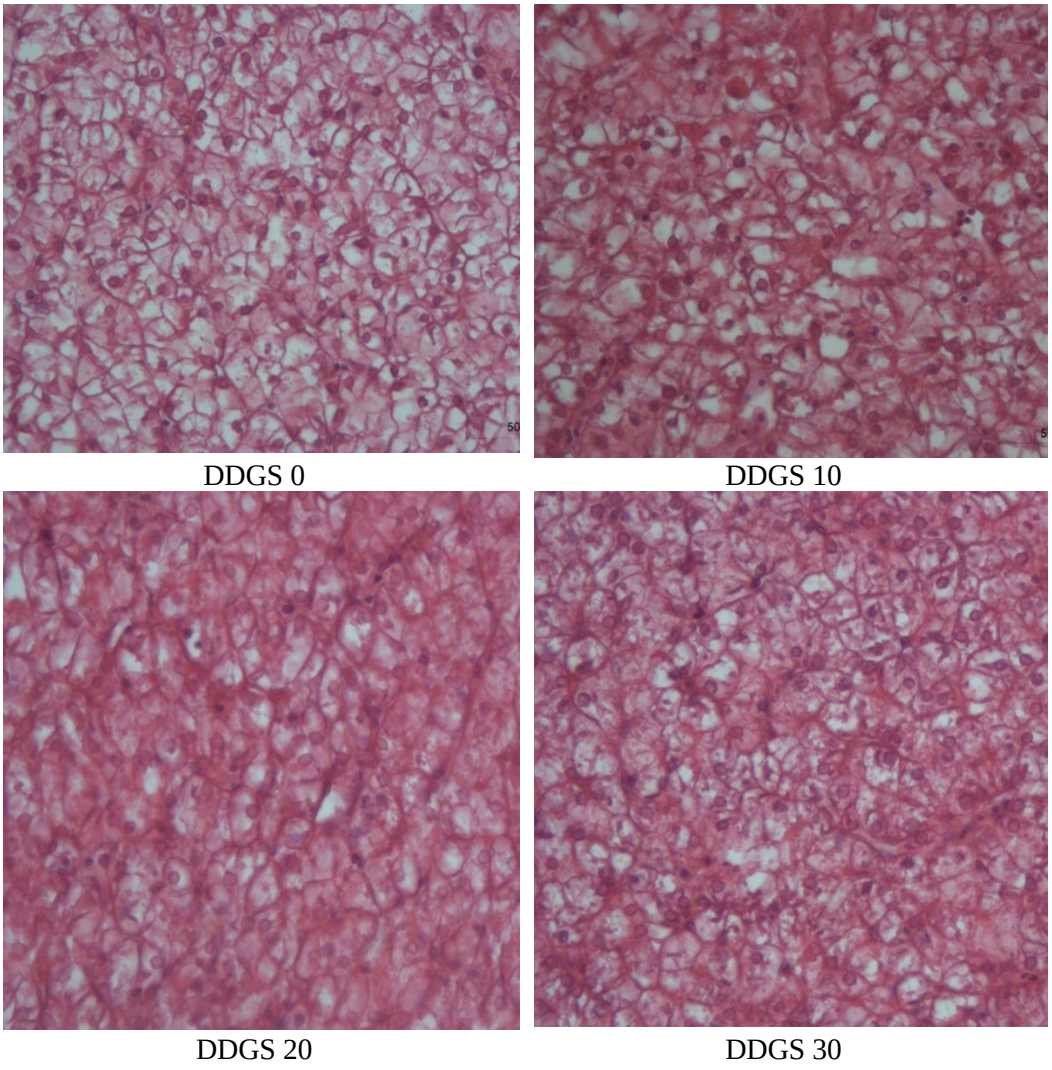
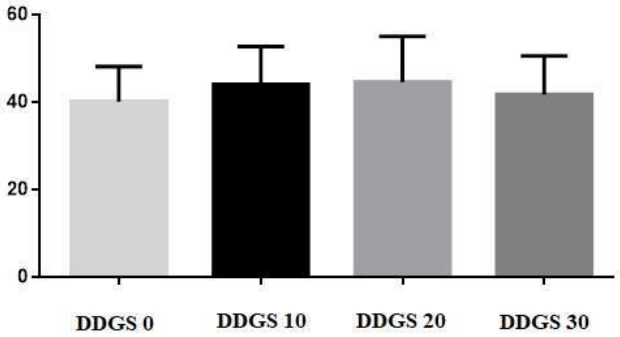


Figure 2. Length of epithelial cells (μm)



Tóth Flórián, Zsuga Katalin, Kerepeczki Éva, Berzi-Nagy László, J. Sándor Zsuzsanna, Körmöczi László

Halastavi zooplankton közösségi összetételében rejlő különbségek eltérő takarmányösszetevők használata mellett

A tanulmány háttere és célja

- A ponty tenyésztése az extenzív és félig intenzív tavi akvakultúrában a természetes táplálékon és a gabonafélék takarmányozásán alapul. Ebben az esetben a haltenyésztő legfontosabb feladata agrotechnikai eszközök felhasználásával a folyamatosan növekvő zooplankton populáció létrehozása és fenntartása.
- Az akvakultúra napjainkban tapasztalható intenzitása kihat a vízi ökoszisztémák és a zooplankton közösségek szerkezetére és dinamikájára.
- Három takarmány: i) egy halliszt- és halolaj alapú, kereskedelemben kapható táp, ii) egy növényi liszt és növényi olaj tartalmú kísérleti táp és iii) gabona, mint kiegészítő takarmány etetésének zooplankton közösségekre gyakorolt hatását vizsgáltuk kanonikus korrespondencia analízissel.

Tanulmány főbb eredményei

- A halastavakban az el nem fogyasztott plankton denzitás és biomassza alapján tipikusan a ponty monokultúrára jellemző mennyiségű (0,06-70 g/m3) és minőségű zooplankton állomány alakult ki, ilyen a Bosmina-Cyclopidae dominancia.
- Halgazdálkodási szempontból a zooplankton közösségek mennyisége és minősége megfelelő volt, a kezelések közötti lényeges különbség a struktúrában nem adódott.
- A környezeti háttérfaktorokat, és a takarmány összetevőket vizsgálva, a különböző takarmányok alig befolyásolták a közösségi összetételt.
- A közösség struktúráját inkább a szezonális jelleg, mintsem a takarmány összetétel határozta meg.
- A kísérleti tápnak nem volt olyan negatív hatása, ami alapján el kellene vetni a használatát.

Az összegzés az alábbi cikkből készült:

Tóth, F., Zsuga, K., Kerepeczki, É., Berzi-Nagy, L.,Sándor, Z. J., Körmöczi, L. The effect of feed composition on the structure of zooplankton communities in fishponds Water, 12(5), 1338 (2020).

The effect of feed composition on the structure of zooplankton communities in fishponds

Flórián Tóth [1,2,*], Katalin Zsuga [3], Éva Kerepeczki [1], László Berzi-Nagy [1], Zsuzsanna Jakabné Sándor [4], and László Körmöczi [2]

[1] Department of Hydrobiology, National Agricultural Research and Innovation Centre – Research Institute for Fisheries and Aquaculture, 5540 Szarvas, Hungary; toth.florian@haki.naik.hu
[2] Department of Ecology, Faculty of Science and Informatics, University of Szeged, 6720 Szeged, Hungary; kormoczi@bio.u-szeged.hu
[3] Agrint Kft, 2100 Gödöllő, Hungary; zsuga.katalin@gmail.com
[4] Department of Fish Biology, National Agricultural Research and Innovation Centre – Research Institute for Fisheries and Aquaculture, 5540 Szarvas, Hungary; jakabne.sandor.zsuzsanna@haki.naik.hu
* Correspondence: toth.florian@haki.naik.hu; Tel.: +36-66-515-300

Abstract: With the intensification of aquaculture the structure and dynamics of aquatic ecosystems are highly affected. At the same time, for a pond fish farmer one of the most important tasks is to establish and maintain a stable and favorable zooplankton populations. In this paper, we assess the effect of different supplementary feed types on zooplankton communities in freshwater fishponds. In an outdoor, experimental fishpond system 2+ years old carp were stocked and fed with a fish meal-based diet, plant meal containing experimental feed and cereals. To compare the diversity of the zooplankton communities we used Shannon diversity index, to assess the effects of environmental factors and the feed ingredients we applied canonical correspondence analysis in R. We described the dynamics of zooplankton communities. The biodiversity of Rotifera and Crustaceans communities differed from each other at different times. Examining the effects of treatments temporal clustering can be observed. The different treatments did not represent special conditions for the zooplankton communities, which would cause a change in their composition in positive or negative directions. Based on these, the experimental feed at the applied level had no negative effect, which makes them suitable for pond feeding in aspect of biodiversity of the ponds.

1. Introduction

The human population is constantly growing, leading to a steady increase in the developments of the agriculture and aquaculture. Nowadays, the aquaculture is the fastest growing livestock sector in the world [1]. In parallel, our ecosystems worldwide are rapidly losing their taxonomic, functional, genetic and phylogenetic diversity due to habitat changes, human exploitation of natural resources, and the spread of pathogens, exotic and domestic animals and plants [2]. In addition to the economic approach to technological development in fisheries, ecological approach should not be overlooked. The global fish production was over 171 million tonnes, 47% of which (around 80 million tonnes) was accounted for by aquaculture in 2016. In this year, in the world aquaculture carp production was 4.5 million tonnes [1]. In recent decades, carp production has intensified in Europe and other parts of the world [3,4]. In Hungary, carp production is dominant in the production of table fish. In 2018, it reached 11,400 tonnes, accounting for 79.5 percent of pond farmed fish [5]. As aquaculture production increases with feed intake and waste, the amount of organic matter, nutrients and suspended solids also increase [6]. There is a growing concern about the potential negative environmental impacts of aquaculture [7], in particular the discharge of nutrients from the effluent of such systems. The impact of feed ingredients on water quality has been widely studied in different

systems inasmuch as those have a definitive effect on growth performance and the receiving water body [8-11]. Originally extensive systems are forced to intensify due to market demands. The sustainable way to produce, is a feed where the highest yields can be achieved with the least nutrient output and ecosystem changes. A traditional practice to achieve that, is supplementary feeding with manuring, which increases both production and natural productivity in the semi-intensive fishpond systems [12,13,3,4]. Replacing cereals with complex feeds makes production more profitable and has a positive impact on fish flesh quality [14-16], however conventional fish feeds are often made from fish meal and oil, and the use of such ingredients is unsustainable. The use of fish meal-based feeds has started to decline in recent years. Compared to the 1990-2000s, the use of fish meal in carp feeds has been reduced by 1-2% for recent years [17-19]. As a result, the aquaculture feed industry has turned to alternative sources [20]. In recent decades, studies have begun to focus on the possibility of using plant-based ingredients to meet the nutritional needs of farmed fish. However, the inclusion of alternative plant ingredients has its own challenges in terms of product quality and environmental impacts [21].

One of the most important finfish species in global freshwater aquaculture is the common carp (*Cyprinus carpio* Linnaeus, 1758). It is the oldest domesticated fish and the most widespread cyprinid species in the aquaculture [22,23]. The most common production technology in Europe is carp monoculture grown in earthen ponds [24]. Farming of carp in extensive and semi-intensive pond aquaculture is based on natural production and feeding with cereals. In this case, the most important task of the fish farmer is to establish and maintain a continuously growing zooplankton population using agro-technological tools [25]. Carp adapts well to semi-intensive pond farming conditions and this technology has less environmental impact than other intensive systems [26]. These fishponds are generally shallow, artificially created water bodies designed for fish production purposes. In addition, they make a major contribution to freshwater biodiversity. It is important to monitor the fishponds physically and chemically to understand their impact on aquatic ecosystems [27]. These fishponds are heavily influenced by human activities and weather conditions [28], but because they are highly controllable in respect of nutrition, we can test the relationship between abiotic parameters and the elements of aquatic ecosystem under experimental conditions [29].

Today's intensification in aquaculture is affecting the structure and dynamics of aquatic ecosystems and those of zooplankton communities. This group of organisms also contributes to the growth of economically important fish species. They are main supporters of the energy transfer between phytoplankton and fish [30]. Zooplankton is considered to be a significant source of amino acids, protein, fatty acids, lipids, enzymes and minerals [32,32]. While cereal feed itself is protein-poor and have high carbohydrate content, zooplankton is low in calories [33]. However, the right amount of zooplankton also acts against the uneven growing [34] and fish health. The composition and abundance of zooplankton communities can be considered both as indicators of water quality and productivity [35], and useful for managing successful and predictable fish yields [36]. Such bioindicators are often used because they show rapid changes due to environmental factors [37]. Zooplankton communities in fishponds have been studied previously, but these were not related to the effects of different feed ingredients. Pechar et al. [38] noted that over the past 50 years, the dominance of fishpond zooplankton species has shifted towards smaller species. Ruttkay [39,40] described the differences between zooplankton communities in mono- and polyculture of carp. Donászy [41] measured the zooplankton biomass formed in the fishponds with different treatments (fish-cum-duck technology, wheat feeding, enhanced fertilization) with a large sample number. Körmendi [42] described the Rotifera fauna of fishponds in Southern Transdanubia region of Hungary. He showed 63 taxa, of which Asplachna and Brachionus species were dominant.

The main goal of this paper is to assess the effect of different supplementary feed types on zooplankton communities. In an outdoor, experimental fishpond system stocked with 2+ years old carps, three different feeds were utilized: (1) fish meal and fish oil based feed (FF) as a conventional

and commercial feed, (2) plant meal and plant oil containing feed (PF) as an experimental, sustainable diet and (3) cereals, the traditional supplementary feed as control (CT). We hypothesized that the impact of experimental feed on community composition does not differ from that of conventional and traditional feeds, and thus experimental feed do not pose a greater environmental burden, and therefore can be recommended as a sustainable alternative to conventional fish meal-based feeds.

2. Materials and Methods

2.1. Experimental design

The experiment was conducted in fishponds of uniform size on the site of the National Agricultural Research and Innovation Centre, Research Institute for Fisheries and Aquaculture (NAIK HAKI), Szarvas, Hungary (Figure 1) in 2015. The feed used for the experiment was compiled according to the semi-intensive breeding conditions of the fish. The proportion of ingredients in the experimental and conventional feeds is shown in Table 1. The two feed types had practically the same crude protein and crude fat concentrations. The third type of feed was cereal (CT), which is traditionally used in Hungary. The experiment was carried out in nine earthen ponds, three replicates per feed. The average area of ponds was 1,754 ± 74 m2 and the average depth was 1.3 meters. The ponds were filled from the nearby oxbow lake of River Körös. Each pond contained 200 individuals of 2+ years old carp (average weight: 745 ± 80 g).

Table 1. The ingredients of the experimental and conventional, commercial feeds.

Fish meal-based feed (FF) (conventional and commercial feed)		**Plant meal-based feed (PF)** (experimental feed)	
Ingredient	%	*Ingredient*	%
Fishmeal 60	14.0	Fishmeal 60	0.0
Winter wheat	20.5	Winter wheat	16.5
Maize	27.5	Maize	27.5
Full-fat Soya	6.5	Full-fat Soya	9.5
Extruded soya	17.5	Extruded soya	29.5
Blood meal	5.0	Blood meal	8.0
Fish oil	2.0	Linseed oil	2.0
Other	7.0	Other	7.0

2.2. Sample collection

The zooplankton community was sampled 3 times in 2015 (June, August, and September). Each time 50 litres of surface water were taken and filtered using 50 μm mesh plankton net, then concentrated to 100 ml. Samples were preserved with added formaldehyde (4% final concentration) and stored at 4 °C until identified by light microscope following standard keys [43-50]. Density was measured with a 5 ml counter chamber. The specific dry mass values needed for biomass estimation were based on literature data [51].

Simultaneously with as the zooplankton sampling, the whole water column was sampled and water chemistry parameter were analysed. Total nitrogen (TN) [52], ammonium nitrogen (TAN) [53], total phosphorus (TP) [54], total suspended solids (TSS) [55], chlorophyll-a (Cl_a) [56] and conductivity (CON) [57] were measured according to the standards of the Hungarian Standards Institution in the National Agricultural Research and Innovation Centre, Research Institute of Irrigation and Water Management (NAIK ÖVKI) Laboratory for Environmental Analytics

Figure 1. The experimental design at the site of the National Agricultural Research and Innovation Centre, Research Institute for Fisheries and Aquaculture (NAIK HAKI); CT-Control; FF- Fish meal-based feed; PF- Plant meal-based feed (PF)

2.3. Diversity evaluation

To compare the diversity of the zooplankton communities of the experimental ponds we used Shannon diversity index in R software environment [58] with vegan package [59].

2.4. Statistics

For statistical evaluation, the effects of environmental factors on the structure of zooplankton communities were analysed by canonical correspondence analysis (CCA) in R with vegan package. Estimated biomass was used for species variables, while environmental variables included concentrations of water chemistry parameters (total ammonium nitrogen, total, nitrogen, total phosphorus, total suspended solids, electrical conductivity, chemical oxygen demand, chlorophyll-a) and total feed components (fish meal and soy component, and feed wheat).

3. Results

3.1. Abundance

The highest density occurred in pond FF1 during the study period (09.22). The bulk of the zooplankton population was the common crustacean, *Bosmina longirostris*, accounting for 31.7% of the total population. Looking at the averages of total number of individuals per treatment, the highest density was measured for the treatment with fish oil-based feed in June. The lowest density was also in the FF1 pond in August, while the lowest average density was for the wheat feed as control at the same time. The density ratio of zooplankton groups varied throughout the year. The proportion of Rotifera in CT1 and PF3 ponds was about 50% in August and 30-40% in June in PF1, PF2, FF2 ponds.

Their share in the zooplankton community was low for ponds FF1, CT2, CT3, FF3 and below 20% for the studied period. In terms of treatments, the Rotifera proportion was between 10-30% with the peak in August, except in the case of feed containing fish meal, where this was seen in June. Cladocera assemblages were highly volatile, but in September they dominated the zooplankton community in all ponds. With each treatment, their proportion was constantly increasing. The density ratio of Copepoda organisms greatly varied between ponds and also seasons (20-90%), but in general for all treatments their dominance in June was reduced by September.

3.2. Biomass

The highest biomass was recorded in pond FF1 (09.22) during the study period, most was given by *Bosmina longirostris*. Examining per treatment, the average biomass of ponds with fish meal feed was the highest in June. On average, this treatment produced almost 1.7 times higher biomass than the other two feed types in the season. At the time of sampling in early August, the ponds had low zooplankton masses, with the smallest value occurring in PF1. There were also low values similar to each other in CT1 and CT2 ponds. The difference between the highest and lowest average biomass was greater than 2.9 fold. The lowest average biomass was found in case of the plant-based feed in August. The proportion of Rotifera was insignificantly low in the total biomass of the studied groups, and it was hardly measurable in comparison to crustaceans. In the zooplankton community, biomass of Cladocera and Copepoda were dominant, but their proportions changed from pond to pond. Ponds CT1 - FF1, PF1 - CT2 - FF2 and CT3 - FF3 - PF3 were the most similar in their tendency, while the annual biomass change was different in PF2.

3.3. Rotifera assemblage

Twenty-three Rotifera taxa were found in the studied fishponds (Table S1), with the smallest number occurring in FF1. The species pool of the other ponds were similar. The dominant elements changed according to the phenological rhythm. The *Asplanchna intermedia* and *Asplanchna pridonta* reached their peak in June, but later only sporadically appeared. The *A. intermedia* did not occur in the ponds treated with fish meal feed from August on, while *A. pridonta* was absent in the control ponds during the whole experiment. As of June, *Brachionus angularis*, *Brachionus calyciflorus*, *Brachionus falcatus* were dominant elements throughout the study period. From this genus *Brachionus diversicornis* was not found in ponds FF in August and September, whereas *Brachionus urceolaris* behaved in a contrary way. Beside them, the populations of the warm stenothermic *Filinia opoliensis*, *Keratella tropica* and *Polyarthra euryptera*, also known as the summer plankton [44,45], were significant. In September, in addition to *Keratella cochlearis*, the presence of *Keratella irregularis*, which is very similar to the former species, could be detected in the Rotifera assemblage in some cases outnumbering *K. cochlearis*. In June 2015 the less common *Brachionus variabilis*, which was not previously registered in Hungary and which was present in large numbers in CT1, FF1 ponds, was found in the Rotifera community, but with little abundance in CT2, CT3, FF3 units. Many species have appeared only sporadically, *Lecane luna*, *Lepadella rhomboids*, *Testudinella patina*, *Pompholyx sulcate*, *Trichocerca pusilla* were present only in one or two ponds, their quantity was low. Similar applies to *Hexarthra mira*, which only occurred in control ponds.

3.4. Cladocera assemblage

Altogether 14 Cladocera taxa were found in the studied fishponds (Table S2) and 3 to 9 taxa per pond formed this assemblage. Among the species, *Bosmina longirostris* was a decisive element of the Cladocera community throughout the year, and in June the proportion of *Daphnia cucullata* was significant, which decreased later. *Moina micrura* dominated in August and September. In a

preliminary survey, we recorded the presence of *Daphnia ambigua* in one of the ponds for which occurrence has not been published from Hungary previously (Zsuga, unpublished). This species was also present in the 2015 study period, and was a stable member of zooplankton community during the May and June sampling, except for pond FF2. Preliminary collections also revealed the occurrence of *Daphnia parvula*, which was previously known in the American continent. It has appeared in several places in Europe in recent decades [60]. In the 2015 collections, a small number of individuals were present in CT1 and FF2 ponds. In the present study, we recorded the presence of a Cladocera species – *Ceriodaphnia rigaudi* (syn: *Ceriodaphnia cornuta f. rigaudi* (Sars, 1896)) – which geographical distribution, according to Bledzki and Rybak [60] was found only in Spain on the European continent. According to our investigation, a significant number of reproductive individuals and stable populations of the species could be found in the ponds during this period. In the population, the summer was characterized by the presence of juveniles and females with subitan eggs. Males were present from the end of August, and in September the proportion of females with resting eggs were significant. The species is small, females are 0.4 mm long in average, with a typical rostriform rostrum on the head. In the ephippium there is one resting egg. The end of the postabdomen is inclined obliquely, with 4-6 spines growing towards the end of the body, the postabdominal claw is smooth.

3.5. Copepoda assemblage

From June onwards, in all ponds, *Acanthocyclops robustus* was a typical, monodominant organism, with a large proportion of the total zooplankton biomass given by individuals of this species at different life stages. Next to it, the presence of *Cyclops vicinus* was detected in two FF ponds in low abundance and only in September therefore, despite its large size, it did not have a significant biomass. This species is especially characteristic in the winter plankton communities.

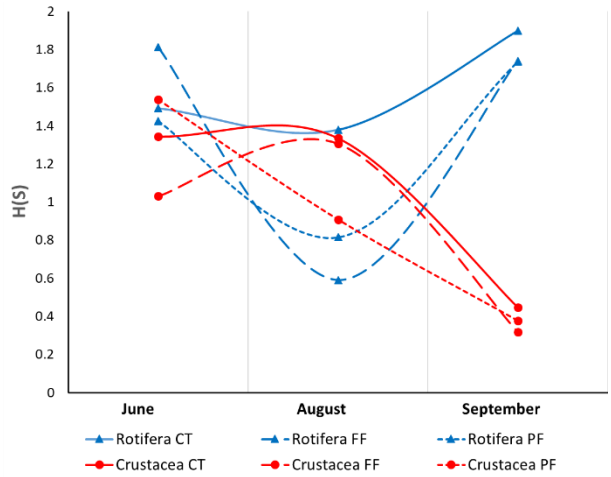


Figure 2. Changes in the Shannon diversity (H(S)) indices of the assemblages of Rotifera and Crustaceans communities

3.6. Diversity evaluation

In terms of the Shannon diversity indices of the assemblages, the biodiversity of Rotifera and Crustaceans (Cladocera and Copepoda) differs from each other at different sampling dates (Figure 2.). At the fishmeal-based treatment, the Rotifera community clearly showed a higher degree of diversity than the other two treatments in June. However, in case of Crustacean communities' diversity, a clear order of plant-based feed > control > fish meal-based feed could be identified. In the other samples, the control has the highest diversity in the case of the Rotifera communities, followed by plant-based

feed and then fishmeal-based feed. In the case of Cladocera and Copepoda communities the control showed the highest diversity. In August, this was approached by FF treatment, while PF was much lower. The difference between the latter two disappeared by September and a reversed order outlined.

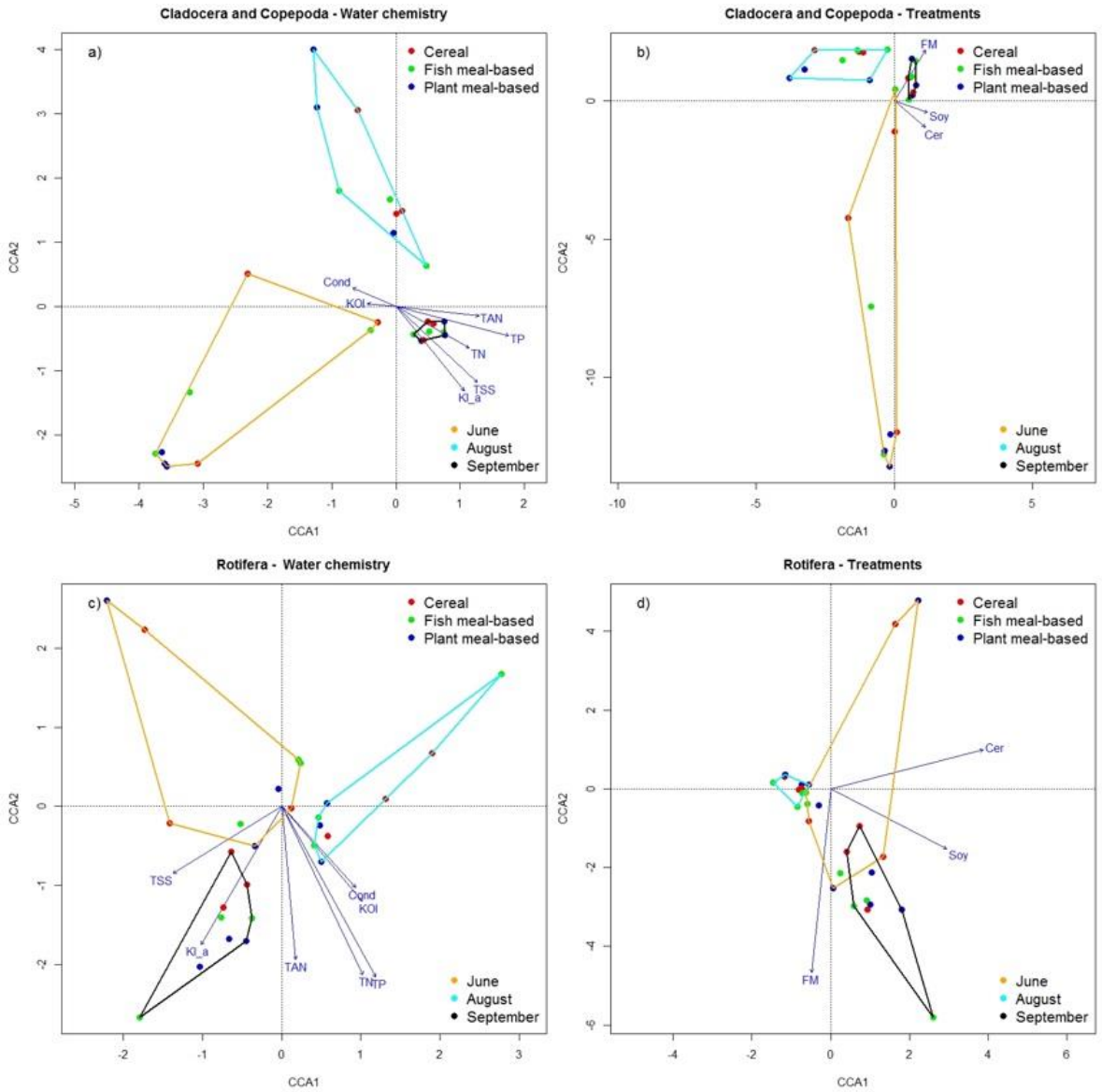


Figure 3. Canonical Correspondence Analysis (CCA) results of the effect of water chemistry and feed ingredient parameters on the distribution of Rotifera and Crustacean (Cladocera and Copepoda) communities; Abbreviations of water chemistry parameters (a, c): TN-Total nitrogen; TAN-ammonium nitrogen; TP-total phosphorus; TSS-total suspended solids; Cl_a-Chlorophyll-a; CON- conductivity. Abbreviations of feed ingredients: Cer-Cereals; FM-Fish meal

3.7. Zooplankton – environment relationships

The effect of seven water chemistry and three feed ingredient parameters was assessed on the distribution of Rotifera and Crustacea (Cladocera and Copepoda) using CCA. The results are shown

along the first and second axes, where species are represented by dots, and environmental variables by arrows. By placing the communities of Crustaceans (Cladocera and Copepoda) of each ponds to the ordination space according to their water chemistry parameters, the individual points are grouped according to the sampling times, but the composition of the feed ingredients had no effect on group formation (Figure 3a). The total ammonium nitrogen, total nitrogen, total phosphorus, total suspended solids and the chlorophyll-a had opposite effect than the electrical conductivity and chemical oxygen demand. The former group positively influenced the community composition in September, while the latter two parameter negatively. Accordingly, water chemistry has little effect on the formation or separation of the community in the other two months. Similarly, a temporal clustering can be observed when examining the effects of treatments (Figure 3b). In this case, the plant content, which was typical to experimental feed and control wheat feeding, negatively correlated with the group of August, while the conventional fish meal feed did the same with the community composition of September. Thus, the plant-based diet might be responsible for the separation of the groups of June and August.

There is also a temporal clustering in the ordination space by examining the effect of water chemistry parameters on sets of points representing Rotifera communities (Figure 3c). The composition of communities of September showed a positive, the communities of August showed a negative correlation with chlorophyll concentration and total suspended solids, while the other parameters showed a negative correlation with the communities of June. Examining the feed components, there is a temporal clustering of Rotifera communities, but there is some overlap (Figure 3d). The samples of August form a discrete set, soya being the one to distinguish it from the September samples, and wheat from June samples. In case of this planktonic group, feed had a smaller effect, but soybean was the most important among the ingredients.

4. Discussion

In an attempt on the replacement of fish meal and fish oil containing aquafeed by a plant-based diet, we investigated the dynamics of zooplankton communities, which represent the natural food source of fishponds. In the ponds, based on "uneaten" zooplankton density and biomass, the communities were formed in quantity (0.06-70 g/m3) [61] and quality (dominance of Bosmina-Cyclopidae [39]) typical to common carp monoculture. Bosmina is too small in size for carp, Cyclopidae's movement is too intensive. The density of larger species of Cladocera (Ceriodaphnia, Daphnia) which ensure primary food source for carp was initially higher, later became sporadic. Due to the faster growth of *Moina micrura*, it can be subdominant throughout the year [40]. After appearance of the alien *Ceriodaphnia rigaudi*, its significant stocks survived in all fishponds. From a fish production point of view, the quantity and quality of zooplankton communities were adequate in the studied ponds, and there was no significant difference in structure between treatments. The number of 23 Rotifera, 14 Cladocera and 2 Copepoda species were similar to the fishpond zooplankton study of Körmendi and Hancz [62] (25, 12 and 1 species, respectively), but 12 common Rotifera species and only 4 common Cladocera species were found in both studies. This distribution of the species number in a nearby natural ecosystem, the River Hármas-Körös was 70, 11 and 2 species, respectively [63]. This river creates the base of the oxbow lakes that are the source of the experimental fishponds.

Several alien species were present in the community composition. Among the Rotifers, Brachionus variabilis was determined to be episodic (adhering to the surface of Daphnia, Ceriodaphnia) or has a free-living lifestyle [43, 45]. At the time of its appearance, *Daphnia cucullata* and *Daphnia longispina* were found in large numbers in the Cladocera assemblages but not adhering to them rather free-living. In the later period it was not recorded and by that time the abundance of Daphnia species was not considerable. We did not find *Brachionus variabilis* in ponds treated with plant-based feeds, while in two of these ponds Daphnia species were present. Based on this, the

connection between the two species cannot be excluded. Among the Cladocera, *Daphnia ambigua* and *Daphnia parvula* are widespread on the American continent [60] but they had only recently appeared in Europe. It can be concluded from the results that establishment, survival and reproduction of Daphnia species was successful in this area. According to a new classification, both organisms are considered invasive species [64,65]. The most surprising occurrence of this zooplankton taxon was *Ceriodaphnia rigaudi*, which, according to the literature, is typical to the warmest, tropical, subtropical zone [66-70]. It has not yet been detected from the filling oxbow lake, but its monitoring is recommended in any case as it may be related to climate change.

Based on the diversity indices, the temporal states showed that Rotifera and Crustacean diversities are different, as was expected. The diversity of each group is quite variable over time, but the treatments have no significant effect, except in August, when the Rotifera diversity in the treated sites was substantially lower than in the controls. In the case of Crustacea, diversity was reduced a lesser extent in PF treatment compared to the other two feeds. Examining the environmental background factors and the feed components, the different treatments had little effect on the community composition, corresponding to our hypothesis. The structure of the community was determined rather by season than by the treatment.

All in all, the different treatments did not represent special conditions for the zooplankton communities, which would cause a change in their composition in both positive and negative directions. Similarly to fish meal-based commercial feed, the typical zooplankton communities for carp monoculture were formed, which corresponds to the fish's natural diet, has been developed using experimental feed. Based on these results, the plant-based experimental feed had no negative effect on planktonic assemblages, which makes it suitable as a sustainable fish feed in pond aquaculture.

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Tóth Flórián, Zsuga Katalin, Kerepeczki Éva, Berzi-Nagy László, Körmöczi László, Lövei Gábor

Akvakultúrából származó elfolyóvíz hatása a Szarvas-Békéksszentandrási (Kákafoki) Holt-Körös szezonális kerekesféreg közösségeire

A tanulmány háttere és célja

- Az intenzív akvakultúra rendszerekből elfolyó víz tápanyagban és lebegőanyagban jellemzően gazdag, ami a természetes befogadó víztestben az eredeti életközösség összetételére módosító hatással lehet akár a táplálékhálózat alapját képező plankton közösségekben.
- Az ilyen élőhelyek közösségszerveződésének megértése elméleti ökológiai, ill. gyakorlati szempontból egyaránt fontos.
- Akvakultúrából származó elfolyóvíz hatását vizsgáltuk a Szarvas-Békésszentandrási (Kákafoki) Holt-Körösben a bioindikátornak tekinthető kerekesféreg (Rotatoria) közösségének szezonális biodiverzitására Rényi-féle diverzitásrendezés segítségével.

Tanulmány főbb eredményei

- A fajszám és egyedszám tekintetében általában nyár - tavasz - ősz sorrend mutatkozott.
- A nagymennyiségű szerves anyagot tartalmazó elfolyóvíz az év egyes szakaszaiban ellentétes hatást gyakorolt a kerekesféreg-közösség diverzitására.
- Tavasszal a befolyási ponttól távolodva a kerekesféreg- közösség diverzitása növekedett, majd egy nyári kiegyenlítődés után őszre megfordult a diverzitások alapján felállított sorrend.
- Összességében elmondható, hogy az elfolyóvíz hozzájárul a közösségszerveződéshez

Az összegzés az alábbi cikkből készült:

Tóth, F., Zsuga, K., Kerepeczki, É., Berzi-Nagy, L., Körmöczi, L., Lövei, G. Seasonal differences in taxonomic diversity of Rotifera communities in a Hungarian lowland oxbow lake exposed to aquaculture effluent Water, 12(5), 1300 (2020).

Seasonal differences in taxonomic diversity of Rotifera communities in a Hungarian lowland oxbow lake exposed to aquaculture effluent

Flórián Tóth [1,2,3,*], Katalin Zsuga [4], Éva Kerepeczki [1], László Berzi-Nagy [1], László Körmöczi [2] and Gábor L. Lövei [3]

[1] Department of Hydrobiology, National Agricultural Research and Innovation Centre – Research Institute for Fisheries and Aquaculture, 5540 Szarvas, Hungary; toth.florian@haki.naik.hu
[2] Department of Ecology, Faculty of Science and Informatics, University of Szeged, 6720 Szeged, Hungary; kormoczi@bio.u-szeged.hu
[3] Aarhus University, Flakkebjerg Research Center, 4200 Slagelse, Denmark; gabor.lovei@agro.au.dk
[4] Agrint Kft, 2100 Gödöllő, Hungary; zsuga.katalin@gmail.com
* Correspondence: toth.florian@haki.naik.hu; Tel.: +36-66-515-300

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Abstract: With the intensification trend of aquaculture technologies, the amount of feed input and waste material is increasing which creates potentially negative impacts on freshwater habitats receiving effluent from such systems. Evaluation of changes in biodiversity of zooplankton communities is an indispensable part of an environmental impact assessment. Rotifers are suitable bioindicators of water quality due to their fast reaction to environmental changes. We examined seasonal changes in the diversity of rotifer communities along a 3.5 km section of the biggest oxbow lake in the Tisza River basin, Hungary, that received inflow from an intensive tank-based aquaculture farm. We detected a species-rich Rotifera community with 26 species. Using the Rényi one-parameter diversity index families, we detected that biodiversity increased away from the point of inflow in spring, but after a summer transition period, the situation became partially reversed during autumn. At the beginning of the study period, the nutrient-rich effluent strengthened the dominance of common species, the difference in dominance decreased in summer but did not disappear. In autumn, the extra nutrient helps the community not to decline at the point of effluent.

1. Introduction

With the growth of the human population, protein production by aquaculture has been greatly increased, it has become the fastest growing animal husbandry sector in the world. [1]. As aquaculture production is intensifying, the amount of fish feed input and waste, including organic matter, nutrients, and suspended solids is also increasing [2]. These create potentially negative impacts on neighboring habitats, especially the freshwater ones receiving outflow from such systems [3].

Wetlands, in general, have suffered a great reduction in Europe during the 20th century [4], and many of the remaining ones, often even areas covered by nature conservation, are under influence from aquaculture [5]. The past river regulation at our study site, the Hungarian lowland, was the biggest such enterprise in Europe during the 19th century and has profoundly modified the lowland's environment and water regime [6]. The regulation aimed to decrease the meandering of the Tisza River and to enhance flood management. After completion, the Tisza became 457 km shorter, but the originally ignored negative ecological impacts became soon evident [7]. An unexpected side benefit was that many of the isolated meanders became oxbow lakes, and developed into valuable wetland habitats in Hungary. The Kákafok Oxbow Lake near to settlements of Szarvas and Békésszentandrás was formed after the regulation of River Körös, (a tributary of the Tisza River) is the largest one of the Tisza watershed, and also represents significant natural value. It has a significant role in the formation of the characteristic landscape of the Körös River basin, and provides different anthropogenic utilization opportunities [8], which influence the composition of the aquatic communities.

Assessment of changes in diversity is an indispensable part of the analysis of anthropogenic effects. A complete inventory of all elements of biodiversity is rarely feasible, thus the selection of a suitable indicator group is an important decision. In water quality assessment, several planktonic

organisms have been suggested as indicator groups. Several authors have used rotifers to indicate eutrophication [9-14] and saprobity [15]. Due to their microscopic size, they are cosmopolitan and ubiquist organisms [16,17], although at a much lower level than previously thought [18] and include several endemisms with narrow biogeographical distribution [19,20] because of this, the communities of rotifers are common objects of faunistic and ecological investigations. Rotifers are microscopic invertebrates that play an important role as the basis of the food web of aquatic ecosystems, transferring energy from a lower to a higher trophic level [21]. They constitute a small, short-lived and the fastest replicating group among the main zooplankton groups [22] and as such, they were suggested as bioindicators of water quality due to their fast reaction to environmental changes [23,24]. Such bioindicators are widely used in environmental assessments because they show fast changes when conditions in their environment change, be those physical, chemical or biological ones [25].

Survey of the zooplankton communities of the Tisza River began in the middle of the 20th century [26]. The research of the Körös River received some attention [27,28] but the rotifers of oxbow lakes are less investigated. A recent study [29] dealt with protected oxbow lakes but left out the biggest one the Szarvas-Békésszentandrás (Kákafok) Oxbow Lake because it is not under nature conservation [29]. A common feature of these studies is that they consider the individual water bodies to be homogeneous and only one or two samples per year were taken, providing information about the overall composition of the rotifer community. On the other hand, seasonal variations are also important, and these assessments did not explore the advantage of rotifers to respond quickly to environmental changes.

The main goal of this article is to describe the seasonal changes in rotifer communities of a section (3.5 km) of the Kákafok Oxbow Lake and to assess the cumulative effect of an aquaculture effluent and other anthropogenic utilisations on the seasonal structure of the communities in a spatially explicit manner. For a synthetic analysis of diversity, we chose the Rényi one-parameter diversity index families [30]. We hypothesized that the effluent affects the community composition but the effect is diluted as the distance from the inlet point increases. We expected, that under oligotrophic conditions, a generally species-rich community develops with low dominance, while there are fewer species with higher abundance under nutrient-rich conditions. The latter conditions tend to favour smaller species with faster reproduction rates and simpler life histories [31,32]. Consequently, our second hypothesis was that lower taxonomic diversity would be recorded near the inflow, while diversity would increase further away from the point of inflow.

2. Materials and Methods

2.1. Study site

The Kákafok Oxbow Lake at Szarvas and Békésszentandrás (N 46° 51' 14.9", E 20° 30' 44.6") is the biggest flood protected oxbow lake of the River Tisza watershed. It is 29 km long, spreads over 207 ha, with an average depth of 2.2 m, holding 4.5 million m^3 of water [8]. The lake is semipaleopotamonic where it is possible to supplement or replace the water, pumping over from the parent river [33]. The oxbow serves for storing inland excess and irrigation water as well as for various activities (fishing, angling, water sports and recreation)[8].

Along a 3.5 km section, we selected five sampling points (K1-K5) at different positions in relation to the combined inflow from a neighboring fish farm producing African catfish (*Clarias gariepinus*) and the experimental fishponds of the National Agricultural Research and Innovation Centre – Research Institute for Fisheries and Aquaculture (HAKI). This inflow, located at site K1, was nutrient-rich, partly of geothermal origin, and before reaching the oxbow lake, was treated by a constructed wetland system. Further sampling points were selected based on the characteristics of the oxbow lake. The K2 point was at 500m, K3 at 2.5km, K4 at 3.0km and K5 at 3.5 km from the influx point. The yearly amount of the effluent of the catfish farm was about 330,500 m^3 [34].

2.2. Chemical water characteristics

In the year of the survey, 1500 ml of effluent water from the African catfish farm was collected monthly and taken to the laboratory at the NAIK Research Institute of Irrigation and Water

Management according to national standards where total Nitrogen [35] and total Phosphorus [36] were measured by spectrophotometry, the total suspended solid [37] by weight measurement.

2.3. Zooplankton Sampling

We sampled Rotifera community nine times (3 times/season) in 2016, at each sampling point, we took 50 l of water from <50 cm below surface, and filtered it using 50 μm mesh plankton net. The filtered samples (100 ml) were put into a 120 ml plastic bottle, preserved by adding formaldehyde (4% final concentration) and taken into the laboratory, where until identification, they were stored at 4 °C. Zooplankton density was assessed using a 5-ml counting chamber. For rotifer identification, a Zeiss light microscope was used following standard keys [38,39].

2.4. Diversity evaluation

To compare rotifer diversity of sample sites, we used diversity ordering with the Rényi generalised entropy function (HR; Eq.1) [30]:

$$HR(\alpha) = \frac{1}{1-\alpha}\log\sum_{i=1}^{S} p_i^{\alpha} \quad (1)$$

where p_i is the relative abundance of the i-th species, S is the total number of species in the sample, and α is the scale parameter without exact biological meaning.

Rényi-function has values of the scale parameter α with special meaning (Table 1.) [40,41]. Thus, for the low values of the scale parameter (close to 0) the index is sensitive to rare species, whereas it becomes gradually more sensitive to the abundant species at higher values of the scale parameter α [41].

Table 1. Special values of scale parameters (α) of the Rényi diversity, * Scale parameter cannot take the exact value of 1, but its limit as α tends to 1

Scale parameter (α)	Rényi diversity (HR)	
0	logarithm of number of species	logS
$\lim_{\alpha\to 1}$*	Shannon diversity	$-\sum_{i=1}^{S} p_i \log(p_i)$
2	logarithm of inverse Simpson diversity	$\log\frac{1}{\sum_{i=1}^{S} p_i^2}$
$\lim_{\alpha\to+\infty}$	logarithm of Berger-Parker index	$\log\frac{1}{p_{max}}$

3. Results

3.1. Nutrient input

During the study period, an estimated 4200 kg year^{-1} of nitrogen, 475 kg year^{-1} of phosphorous and 5220 kg year^{-1} of suspended solids were released into the oxbow lake from intensive aquaculture (Table 2.).

Table 2. The amount of nutrient introduced into the Kákafok oxbow lake

	Total nitrogen (kg)	Total phosphorus (kg)	Total suspended solids (kg)
Spring	1600	168	1135
Summer	830	120	1275
Autumn	1770	188	2810
Total	4200	475	5220

3.2. Assemblage composition

During the sampling period, 26 rotifer species were observed, and further 2 taxa were identified to genus level (Table S1). The highest species richness (14 species) and density (163,272 ind m^{-3}) were registered at the inlet point (K1) in the summer and the lowest values (2 species and 864 ind m^{-3}) were found at the most distant point (K5) in autumn (Table 3.). The most frequent species were *Brachionus calyciflorus* and *Brachionus leydigi*; these were absent from only 2 sites. There were 8 singleton species. The most species-rich genus, Brachionus was represented by 10 species.

In spring, the most species-rich site was K5 (11 species), but this site had the lowest density (7,560 ind m^{-3}). The highest abundance (140,472 ind m^{-3}) was found at K2. *Brachionus nilsoni* and *Brachionus leydigi* were found in the largest amount at the point of inflow and at a distance of 0.5 km.

In summer and autumn, the highest number of species and densities were observed d at the inflow (K1 site). In summer, the abundance varied between 71,928 and 163,488 ind $m^{-3}$and was dominated by *Brachionus angularis* and *B. calyciflorus*. The number of species varied between 7 (K5) and 14 (K1).

In autumn, the highest number of species (12) and individuals (12,096 ind m^{-3}) were detected at K1, and the lowest ones (2 species and 864 ind m^{-3}) at K5.

3.3. Diversity and diversity ordering

3.3.1. Species distributions

The spring assemblages from K1 to K5 had almost the same number of species (8-11), but the evenness increased with increasing distance from the inflow point (Figure 1.). In summer, the species richness was the highest at the inflow. Considering the most frequent and the rarest species, K1 had the highest number of individuals but in the case of the moderately common species, K2, K4, K5 sites showed higher density. In autumn, the assemblage at the inlet point (K1) was the most species-rich with a correspondingly high number of individuals. At K3-K5 the number of species and individuals were lower (Figure 1.). The species distributions at different points in the various seasons followed varying models. In most cases, the Zipf-model fitted the rank-abundance curves best, and the Mandelbrot-model and niche preemption were also frequent what emphasizes moderate species richness, especially in summer (Table 3.).

3.3.2. Rényi diversity profiles

According to the Rényi diversity ordering, in spring the most diverse site was unequivocally the K5 (Figure 2.). The other four assemblages could not be unequivocally ordered. At small values of the scale parameter (emphasis is on rare species), the order was K2>K3>K1>K4. Over most of the range, diversity increased with the distance from the inflow (K4>K3). There was little difference between the first two sites. In summer, the five assemblages did not show any interpretable differences: none of the sites could be unequivocally ordered, and mutual relationships with emphasis on rare species were chaotically different from values under higher influence of moderately common or dominant species. In autumn, three assemblages could be unequivocally ordered (K4>K3>K5). The mutual relationships of the communities became definitive at $\alpha>2$. Thereafter, site K2 had the highest diversity, and K5 the

lowest one (Figure 2.). In fact, four communities could be organized. The two nearby points of the influence could not be distinguished, but they had greater diversity at each scale parameter than the other points.

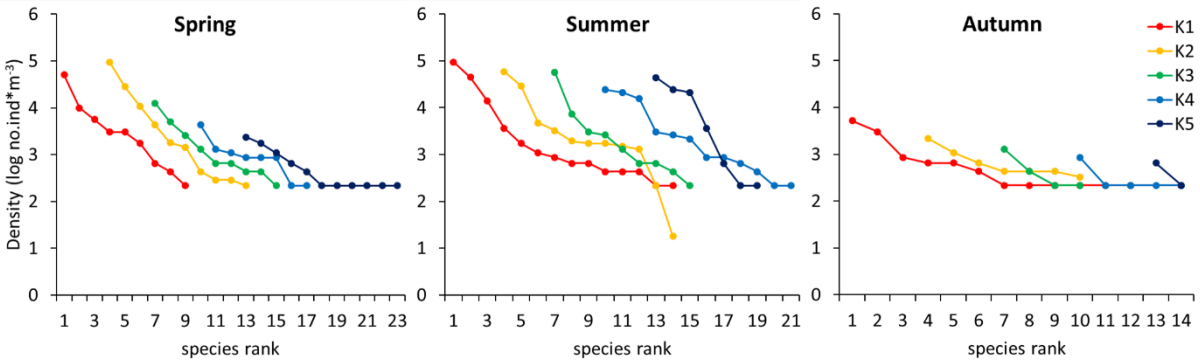

Figure 1. Rank-abundance graphs of the Rotifera assemblages in the different distances (K1-K5) of the inlet point per season. (To decrease overlap, the starting positions of the individual curves are displaced by three ranks.)

Table 3. Models that best fit the rank-abundance curves on Figure 1.

	K1	K2	K3	K4	K5
Spring	Zipf	Lognormal	Mandelbrot	Zipf	Mandelbrot
Summer	Preemption	Mandelbrot	Preemption	Zipf	Preemption
Autumn	Mandelbrot	Zipf	Zipf	Zipf	Null

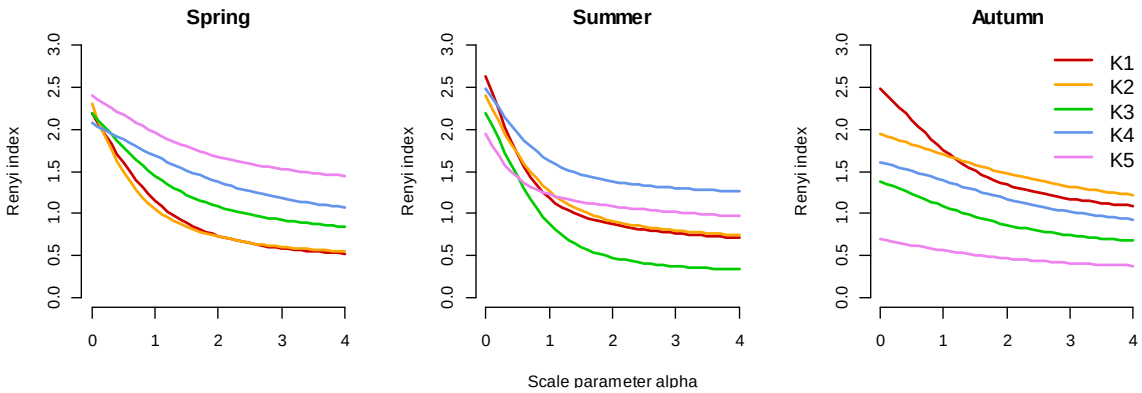

Figure 2. The Rényi diversity profiles of the Rotifera assemblages in the different distances (K1-K5) from the inlet point per season in the Kákafok oxbow lake

Regarding the seasonal biodiversity of the sampling points, a clear order can only be identified for the K5 site. In this case, the spring>summer>autumn order can be seen (Figure 3). At the point of inflow (K1) with decreasing influence of rare species, the order of summer>autumn>spring changed to autumn>summer>spring. Autumn diversity was clearly higher than in spring. For K2, the order of

summer>spring>autumn at α=0 changed to autumn>summer>spring at α=1 and at α=2. Biodiversity in summer was clearly higher than in spring. At the third point (K3), the highest diversity was found in spring, but the order of summer>autumn at α=0 scale parameter reversed at α=1 scale parameter. At the fourth point (K4), autumn biodiversity was clearly the lowest. The other two profiles crossed each other twice. At α=0 scale parameter, diversity was greater in summer than in spring. This was reversed at α=1 and turned back at α=2.

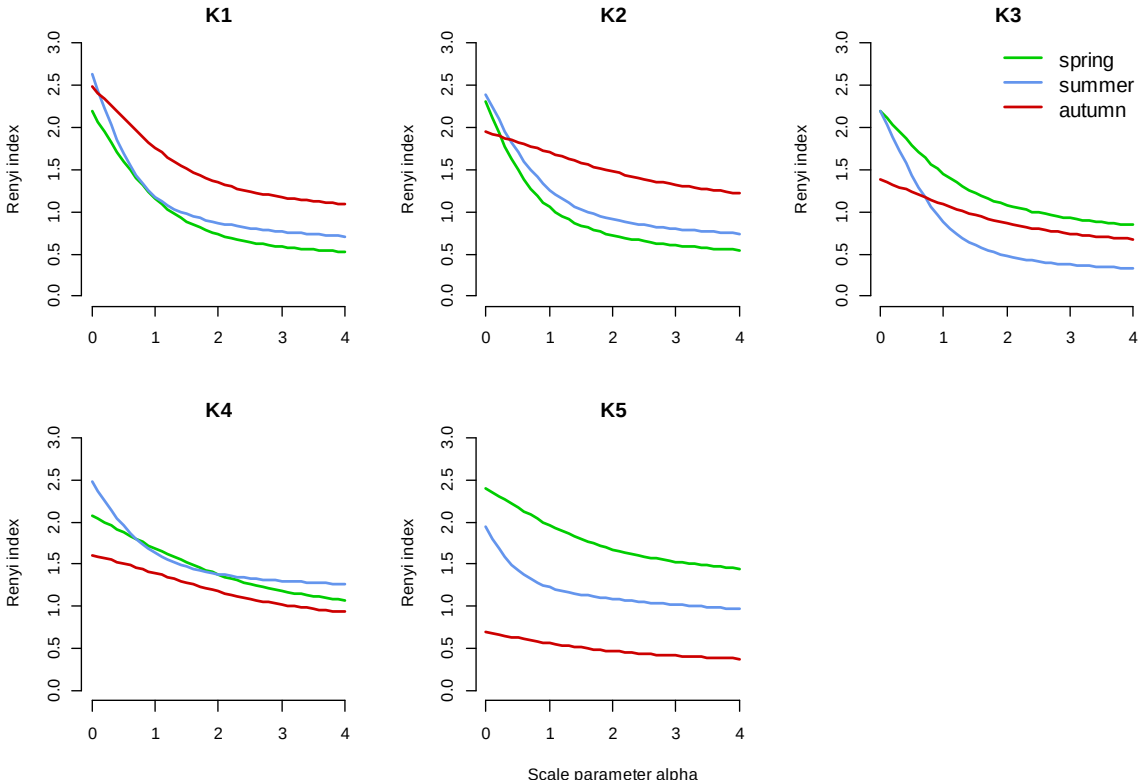

Figure 3. The Rényi diversity profiles of the rotifera assemblages in the different season per distances (K1-K5) from the inlet point in the Kákafok oxbow lake

4. Discussion

The survey indicated a species-rich Rotifera fauna with 26 species (2 identified only to genus level). An earlier survey [29] found 13 and 8 species in the adjacent, protected Aranyos and Borza oxbow lakes, respectively, and 14 species in the similar oxbow lake of Dán-zug. In the Hármas-Körös parent river, 65 species were described 25 years ago [44], but 10 species present in the Kákafok oxbow lake according our results were missing from that specification.

Species found in the Kákafok oxbow lake were categorised as characteristic of oligo- and oligo/β mesosaprobic waters [15] but Brachionus species prefer contaminated, β and β/α mesosaprobic water bodies [15]. These species typically occurred in assemblages with higher species and individual number in summer mainly at K1-K2 points, indicating the impact of the effluent water. We found more Brachionus spp. compared to the two previous studies; which mostly belonged to the β mesosaprobic indicator group [15]. The most frequent (*Brachionus calyciflorus* and *B. leydigi*) and the most common (*B. angularis*) species also belonged to this genus.

Our hypotheses were partially justified. The diversity profile analyses showed more sophisticated and articulated results than the usual one-dimensional diversity indices. In spring, as we expected, the biodiversity increased away from the point of inflow, but after a summer transition period, the situation became partially reversed during autumn. There was no clear seasonal difference between the point at the inflow and the next closest one, indicating that the effect of the

effluent did not decrease at a distance of 500 m. In most cases, that is in spring and autumn, and in summer for rare species, point K3 showed medium diversity, conforming to our hypothesis. By summer, this site became the one of the lowest diversity as a result of more abundant species (=higher values of the scale parameter). The third most distant point (K4) gave surprising results. While by the end of the study period, it could be clearly ranked, in spring it became the second most diverse community by reducing the weight of rare species. In this case, this point was the least diverse in terms of equal weight of rarer species. Unexpectedly, even in summer, diversity was at K4 point significantly higher than nearer and farer from the inlet point. It could not be clearly distinguished only from the point of inflow. The most distant point was the most diverse in spring and the least diverse in autumn. In summer it was not clearly separated from the other points. Comparing the different sampling points, the decreasing abundance was most often observed in Brachionus species, which are indicators of the more saprobic waters among the observed species [15]., At the point of aquaculture inlet (K1) and nearby (K2), Brachionus spp. showed greater dominance in the same species set, leading to a decrease of biodiversity in spring. The species pool was expanded to the maximum in summer, however due to the predominance of more saprobic species, it did not result in a clearly identifiable increase in diversity. In autumn, the number of species and individuals decreased at the more distant points, while at the point of inflow, a relatively rich and abundant community emerged.

Summarizing, we detected a seasonally different effect of the aquaculture effluent in the composition of the rotifera community in the investigated section of the Kákafok Oxbow Lake. This effect was strongest during spring and autumn. In summer, the differences in taxonomic diversity disappeared. At the beginning of the study period, the nutrient-rich effluent strengthened the dominance of common species, the difference in dominance decreased in summer but did not disappear. In autumn, however, the extra nutrient enabled the subsistence of a diverse community at the point of effluent inlet. However, due to the presence of closely related and different saprobiotic indicator species, functional and phylogenetic analysis may provide additional information which is the subject of a subsequent investigation.

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Tóth Flórián, Révész Norbert, Demény Ferenc, Uhljar Attila, Berzi-Nagy László, Molnár Zsuzsanna, Bíró Janka, Gál Dénes, Kerepeczki Éva, J. Sándor Zsuzsanna

Kukoricatörköly, mint ponty takarmány alapanyag hatása a vízminőségre

A tanulmány háttere és célja

- A piaci igények a pontytakarmányozás fejlesztését kényszerítik. A fenntartható intenzitáshoz olyan takarmánytípusok szükségesek, ahol a legmagasabb hozamok érhetők el az ökoszisztéma legkisebb tápanyagterhelésével. Ennek eredményeként az akvakultúra takarmányipara alternatív forrásokhoz fordult. Az ilyen új takarmányösszetevők bevezetését azonban a környezeti hatás vizsgálata kell, hogy megelőzze.
- A vizsgálat során egy kereskedelemben kapható, valamint egy kísérleti, kukoricatörköly (DDGS) alapú ponty takarmány tavi nitrogén- és foszfor-formákra valamint klorofill-a-ra gyakorolt hatása került összehasonlításra.

Tanulmány főbb eredményei

- A nitrogén-formák közül egyedül az összes nitrogén volt szignifikánsan nagyobb koncentrációban a DDGS alapú takarmánnyal kezelt tóban, de a határértéket nem közelítette meg.
- A foszfor-formák esetében nem volt statisztikailag kimutatható különbség.
- A klorofill-a esetében nem volt szignifikáns különbség.
- Összességében elmondható, hogy a DDGS a vízminőség szempontjából lehetséges alternatívát jelenthet, mint haltakarmány alapanyag.

Az összegzés az alábbi cikkből készült:

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Effects of diet containing dried distiller's grain with solubles (DDGS) on water quality of the carp rearing ponds

[1]Flórián Tóth, [1,2]Norbert Révész, [1]Ferenc Demény, [3]Attila Uhljar, [1]László Berzi-Nagy, [1]Zsuzsanna Molnár, [1]Janka Bíró, [1]Dénes Gál, [1]Éva Kerepeczki, [1]Zsuzsanna Jakabné Sándor

[1]Hungarian University of Agriculture and Life Sciences – Institute of Aquaculture and Environmental Safety - Research Centre for Aquaculture and Fisheries, Hungary, H-5540 Szarvas, Anna-liget str 35. Corresponding author: F. Tóth, toth.florian@uni-mate.hu
[2]DSM Nutritional Products, Hungary, H-2367 Újhartyán, Japán fasor 4.
[3] Hungarian University of Agriculture and Life Sciences, Hungary, H-5540 Szarvas, Szabadság út 1 -3.

Abstract
With the intensification of aquaculture, the water quality of the receiving water bodies are highly modified. Thus, it is essential to investigate that when testing an alternative feed ingredient. In this paper, we assess how corn dried distiller's grain with solubles (DDGS) affects the water chemistry parameters and the growth performance of carps (*Cyprinus carpio*). The trial was carried out in an outdoor experimental fishpond system with six earthen ponds, in three concurrent repetitions with one control feed. Each pond was stocked with two-years old and one-year old carp individuals. Total nitrogen, ammonium nitrogen, total inorganic nitrogen, total phosphorus, orthophosphate phosphorus, chlorophyll-a concentrations and production parameters were compared. It was shown that in the ponds receiving DDGS feed, growth rate and final body weight of the fish were higher while daily specific growth rate was significantly higher compared to the control group. No significant difference was found between the treatments for the water chemistry parameters, except for the total nitrogen. The higher total nitrogen concentration observed with DDGS use did not exceed the limit prescribed by the current environmental regulation. In conclusion, DDGS is a promising feed ingredient for carp nutrition, used as supplementary or complete diet in pond culture.

Introduction
The rapid growth of the human population challenges agriculture as well as aquaculture which are trying to meet this with continuous improvements. At present, aquaculture is one of the fastest growing livestock sectors (FAO, 2018). In parallel, human exploitation of natural resources continues, habitats are changing, and pathogens of domesticated and exotic animals and plants are spreading. As a result, ecosystems are losing their genetic and phylogenetic, taxonomic, and functional diversity at a rapid rate and on a large scale (Naeem, Duffy & Zavaleta, 2012).
As aquaculture production increases, it results that the amount of feed intake is also increasing, and side by side its environmental load shows also elevating trend in discharged organic matter, nutrients, and suspended solids amounts (Edwards, 2015), implying a potentially negative environmental impact of aquaculture (Naylor et al. 2000) through the effluent water. Organic matter, phosphorus and nitrogen from feed residuals and metabolites are the most widespread concern in water pollution, such as causing eutrophication and oxygen depletion (Gál et al., 2016). As feed ingredients have a significant effect not only on growth performance but also on the state of receiving water bodies, studies on water quality are of utmost importance (Wahab et al., 2002; Ćirić et al., 2015; Davidson et al., 2016; Nagy et al., 2017).
Common carp (*Cyprinus carpio*) contributes to around 7.7 % of the total aquaculture production globally (FAO 2020). The most widely used common carp production technology in Europe is the polyculture in earthen ponds (Szűcs et al., 2007). Extensive and semi-intensive carp farming is based on natural food resources and supplementary grain feeding. However, carp adapts well to intensive pond fish production conditions, and this technology has a lower environmental impact compared to other intensive systems (Roy et al. 2019; Kestemont, 1995). Carp production dominates in table

fish production in Hungary. In 2018, the production volume reached 18,300 tons, corresponding to 81.6 percent of all pond production. The average yield in 2018 per hectare was 595 kg (Kiss, 2019). Traditionally extensive systems are forced to be intensified due to market expectations. Replacing traditional grain feeding with compound feeds makes production more profitable and have beneficial effects on fish meat quality (Dickson et al., 2016; Marković et al., 2016; Stoycheska et al., 2017). However, the inclusion of a new, alternative plant raw materials in production has its own challenges, both in term of product performance and impact on water quality (Hardy, 2010). The availability and cost of different feed ingredients should also be considered when formulating a diet. From a sustainable and energy-efficient perspective, local by-products from the food and fermentation industries can be particularly important as they are typically cheaper and have a less environmental impact. For these considerations, the Dried Distiller's Grain with Solubles (DDGS), a by-product of bioethanol production, can contribute to the sustainable development of aquaculture. With its medium energy content, medium protein, digestible fibre and accessible phosphorus quantity make it possible to produce high nutritional feed (Lim et al., 2011). An additional advantage over other plant ingredients is the lack of antinutrient factors (Makkar 2012).
Révész et al., (2019, 2020) have demonstrated that DDGS also in high inclusion level is suitable for carp production without any negative effects to the growth and health. Moreover, benefits in fat metabolism and high digestibility of phosphorus have been observed. However, to be able to use DDGS as a sustainable ingredient in the fish feed of the future, examination of its environmental impact is needed as well. The aim of this study is to compare the effect of an experimental DDGS-containing diet and a commercially available conventional carp feed on the main water quality parameters. The effects on nitrogen and phosphorus forms together with chlorophyll-a concentrations were examined. Our hypothesis was that the experimental DDGS-containing diet has not more adverse effect on water quality than a commercially available feed.

Material and Method

Description of the study sites. The trial was accomplished at the experimental site of National Agricultural Research and Innovation Centre, Research Institute for Fisheries and Aquaculture (NAIK HAKI, Szarvas, Hungary). The experiment was carried out in six earthen ponds, in three concurrent repetitions (Figure 1). The ponds had an average surface of 1808 ± 53 m^2 and an average depth of 1.5 m. The ponds were filled from the oxbow lake of River Körös, located next to the institute. Firstly, the water was pumped into a water reservoir, then connected with canals the water flew under control to the experimental ponds by gravity. The six ponds were located parallel, their effluent water was collected in a common dedicated drainage channel. Paddle-wheel aeration devices were placed to the surface of the ponds to keep the dissolved oxygen concentration at a favourable level.
Two different age groups of common carp (1+ and 2+ years old) were stocked into the ponds on the 3rd of May in 2018. Each pond was stocked with 70 two-years old and 1050 one-year old carp individuals, with average weights of 360 g and 50 g, respectively. In order to ensure the proper quantity of natural plankton production, we added cow manure (2 t/ha) to the ponds before the launch of the experiment and other 2 t/ha in the middle of the season. Before the feeding trial, the nutritional design based on the traditional semi-intensive technology where cereals (wheat) and natural resources (zooplankton and zoobenthos) are the food sources of the carp. The semi-intensive feeding with compound feeds started on the 23rd of July. In the half of the ponds, fish were fed with 40 % DDGS-based experimental compound feed, that had 35.04 % crude protein content and 7.8 % crude fat content. In the other three ponds, the fish were fed with a commercial feed as control with similar nutritional characteristics (34.47 % crude protein, 6.60 % crude fat produced by Haltáp Kft., Szarvas, Hungary). The experimental feed has been formulated to replace the plant ingredients of the commercial feed with DDGS still the animal origin ingredient level remained constant. The feeding trial lasted for 174 days during the rearing season. Feeding was done by hand, twice per day with 2-3 % of the fish biomass (depending on water temperature). In total, the fish consumed in control 2370 kg and in experimental DDGS treatment 2484 kg feed, respectively. Every third week the growth and health status of the stock was checked by test harvesting and modified the feeding rate according to the estimated biomass of the ponds. At the end of the trial, the whole stock was

measured to evaluate the growth performance. During the trial, only four fish died, which proves that there were no external harmful effects.
The studies were conducted according to the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes. All animal experiments have been approved by the Ethical Committee of NAIK HAKI, which was established according to Hungarian State law (10/1999. (I. 27.)) and operated according to different Hungarian State laws concerning animal experiments, transportation of animal, welfare etc. (40/2013. (II.14)).

Figure 1. The experimental pond system of Hungarian University of Agriculture and Life Sciences – Institute of Aquaculture and Environmental Safety - Research Centre for Aquaculture and Fisheries. Orange squares indicate the ponds fed with DDGS, while blue squares indicate control ones. (Source: Hungarian University of Agriculture and Life Sciences - Institute of Environmental Sciences - Research Center of Irrigation and Water Management)

Sampling and water chemistry analysis methods. During the trial, water samples were collected every second week from the outlet of the ponds by a column sampler. The water samples were sent to the accredited analytical laboratory of the National Agricultural Research and Innovation Centre, Research Institute of Irrigation and Water Management (NAIK ÖVKI) right after sampling without preservation. Total nitrogen (ISO, 1997), ammonium nitrogen (ISO, 2005), total inorganic nitrogen (ISO, 1996, 2005), total phosphorus (ISO, 2004), orthophosphate phosphorus (ISO, 2004) and chlorophyll-a (ISO, 1992) were analysed according to the given standards of the Hungarian Standards Institution. The vast majority of the water samples were immediately processed in the laboratory. The ones that were not, were cooled down to the temperature of +4 ˚C, and the storage time was 6 hours at maximum. The dissolved reactive phosphorous and nitrogen forms were analysed by spectrophotometer from filtered water samples. The absorbances were measured by a SPEKOL-11 type spectrophotometer in 1 cm cuvettes.
Calculations and statistical analysis. Growth performance of fish such as specific growth rate (SGR, Eq. 1), feed conversion ratio (FCR, Eq. 2) and gross yield (GY, Eq. 3) were calculated based on the following standard formulae:

$$SGR = 100\left(\ln w_f - ln\, w_i\right) * \ t^{-1} \quad (1)$$

where wi and wf are the initial and final average weight (g), and t is the time (day).

$$FCR = TF * \left(w_f - w_i\right)^{-1} \quad (2)$$

where TF is the total feed offered (g), wi and wf are the initial and final average weight (g).

$$GY = (w_f - w_i) * A \quad (3)$$

where wi and wf are the initial and final average weight (t) and A is the area (ha).
For statistical analyses we used Microsoft Excel and „dplyr" (Shimko and Andersen, 2014) and „ggpubr" (Kassambara, 2018) packages of „R" (R Development Core Team, 2013) open source statistic software. Shapiro-Wilk test was used to test the normality of the distribution of the measured water chemistry parameters and the F test was used for the analysis of variance. In the case of normal distributions and corresponding variances, two-sample t-test was used to examine the means for each treatment to be compared, otherwise, Mann-Whitney non-parametric test was used.

Results.

Production parameters. By the end of the feeding experiment, the performance of fish receiving DDGS-containing feed was comparable with fish fed with conventional commercial feed. The specific growth rate for control juvenile fish (1+) were significantly lower (1.46 g/day) than in DDGS group (1.56 g/day). Similar favourable growth was observed for 2-year old stage fish when 0.91 g/day SGR was found compared to 0.86 g/day in the control group. Moreover, in the case of feed conversation ratio (FCR) and gross yield differences were also statistically significant (FCR 1.56 g/g vs 1.78 g/g, gross yield 3.32 t/ha vs 2.85 t/ha in DDGS and control treatments, respectively).

Water chemistry - Total ammonium nitrogen. Ammonium nitrogen concentrations measured in water samples ranged between 0.186-0.283 mg/L for DDGS-fed ponds, with an outlier of 0.549 mg/L (Figure 2. a). This concentration was similar for the control ponds ranging between 0.162-0.258 mg/L. For this treatment, there was also an outlier value (0.536 mg/L) at the same time as the experimental diet fed group. Due to the outliers, we used Shapiro-Wilk test and none of the data sets had a normal distribution, which would be a prerequisite for the t-test. Thus, this value was omitted for statistical analysis, which resulted in normal distribution. Thereafter, the non-parametric Mann-Whitney test showed no significant differences between the effects of the two treatments on water ammonium-nitrogen concentration.

The average concentration of ammonium nitrogen in DDGS fed fish ponds was 0.279 ± 0.123 mg/L. The average concentration of ammonium nitrogen in control ponds was 0.263 ± 0.126 mg/L. The effect of DDGS feed was not statistically different from the commercially available feed in terms of this water chemistry parameter. The ammonium nitrogen concentration did not reach the toxic value of 1 mg/L in either treatment.

Water chemistry - Total inorganic nitrogen. Concentrations ranged of 0.328-0.690 mg/L in "DDGS" ponds and 0.311-0.517 mg/L in control ponds, outlier values were 0.919 and 0.917 mg/L, respectively (Figure 2. b). For control ponds there were outlier values at the same sampling time as in case of ammonium-nitrogen, thus the data sets did not have a normal distribution. Therefore, in the case of total inorganic nitrogen, we analysed the data similarly to ammonium nitrogen sets.
Mann-Whitney test showed no difference between the two feeds in terms of total inorganic nitrogen in the water. The average concentration of total inorganic nitrogen in experimental feed ponds was 0.541 ± 0.205 mg/L. The average concentration of total inorganic nitrogen in control ponds was 0.483 ± 0.207 mg/L. For this water chemistry parameter, the effect of DDGS feed was not different from that of the commercially available feed.

Water chemistry - Total nitrogen. The range of total nitrogen concentration in DDGS ponds was between 1.507-2.197 mg/L, while in control ponds ranged between 1.003-1.790 mg/L (Figure 2. c). The average total nitrogen concentration in DDGS ponds was 1.841 ± 0.264 mg/L, in the control ponds, was 1.384 ± 0.245mg/L. Based on the distributions and variances, it was possible to use t-test for the full data set, which showed that DDGS feed caused a significantly higher total nitrogen level.

Water chemistry - Orthophosphate phosphorus. The average concentration of orthophosphate phosphorus was 0.060 ± 0.026 mg/L in DDGS ponds and 0.053 ± 0.021 mg/L in control ponds (Figure 2. d). As the data set had a normal distribution and the variances were the same, we used two-sample t-test. In the case of the orthophosphate phosphorus concentration of the water, there

was no difference between the two feed types. The effect of DDGS feed was not different from commercially available feed for this water chemistry parameter.

Water chemistry - Total phosphorus. Similar to the orthophosphate phosphorus, the t-test showed no significant difference between the two feed types in terms of total phosphorus concentration in water. The average concentration of total phosphorus was 0.204 ± 0.033 mg/L in DDGS ponds and 0.190 ± 0.030 mg/L in control ponds, respectively (Figure 2. e).

Water chemistry - Chlorophyll-a. The average value was 66.002 ± 41.780 µg/L in DDGS ponds and 50.396 ± 21.664 µg/L in control ponds (Figure 2. f). Mann-Whitney test showed no significant difference between the two treatments in chlorophyll-a concentrations. There was no difference in the effect of the two feeds on the content of chlorophyll-a.

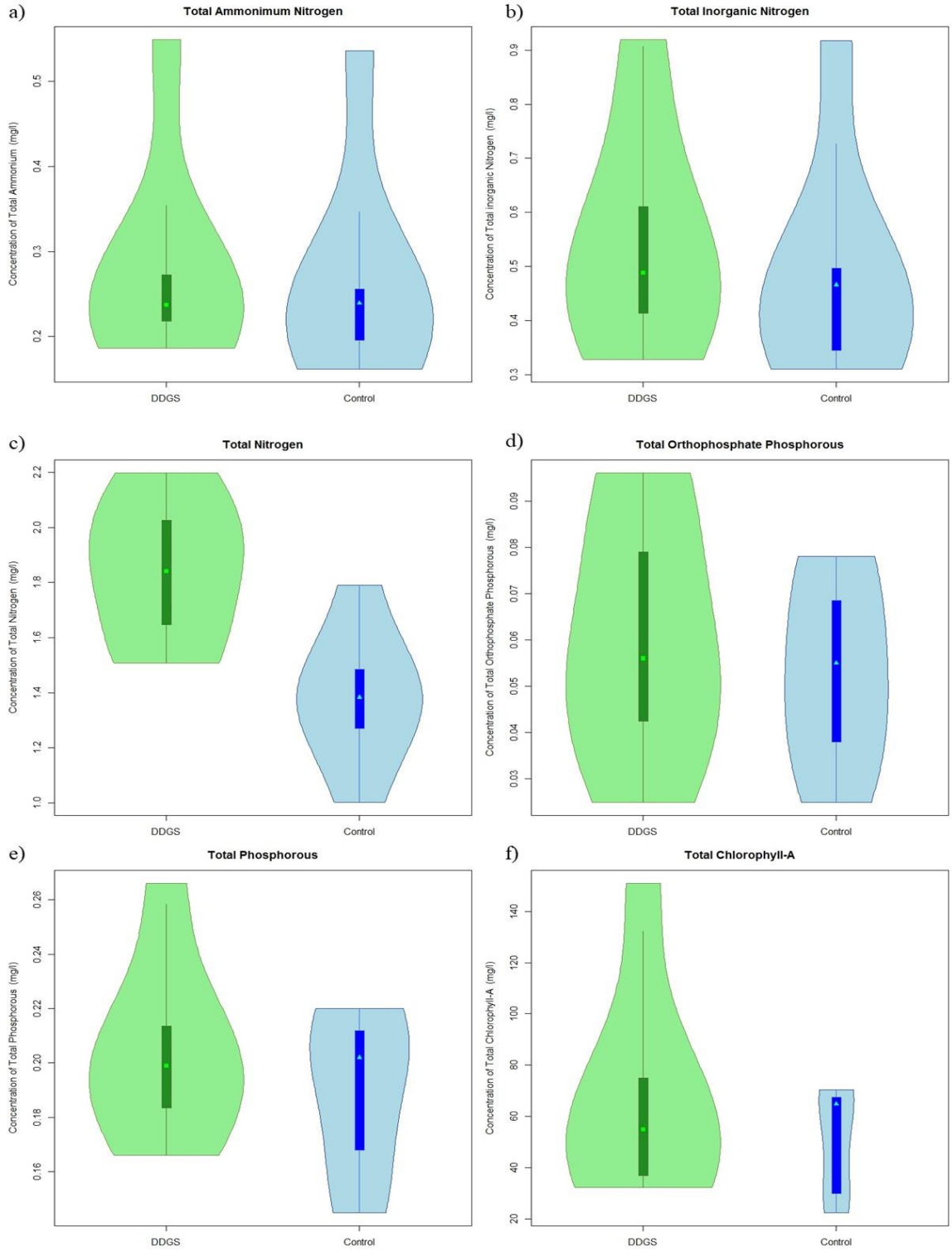

Figure 2. Violin plots of water chemistry parameters (a) Total Ammonium Nitrogen, b) Total Inorganic Nitrogen, c) Total Nitrogen, d) Total Orthophosphate Phosphorous, e) Total Phosphorous, f) Total Chlorophyll-A)

Discussion. The replacement of soybean and corn meal by corn-DDGS seemed to be successful in the diet of common carp. According to the fish growth and production parameters during the rearing season, it was shown that in the ponds that received DDGS feed the growth rate and final body weight of the fish were also higher. The one-year old group nearly tenfold their body weight in both groups during the 155 days of the feeding experiment, but the daily specific growth rate was significantly higher in the experimental group. DDGS-based feed had a positive effect on carp growth and feed utilization under semi-intense pond conditions. Révész at el. (2019) also found better growth parameters for carp fingerlings fed with 40% DDGS diet.

The effect of corn-DDGS based and a commercially available fish feed on the water quality was examined in terms of nitrogen and phosphorous forms as well as chlorophyll-a concentrations. Confirming our hypothesis, the results demonstrated that DDGS-based feed had not higher impact on the water quality than the conventional control fish feed. With regard to nitrogen forms, we detected significantly higher concentrations of total nitrogen in the DDGS-treated ponds but it should be noted that the concentration did not exceed the limit prescribed by the current environmental regulation in Hungary. The permissible limits of 0.4 mg/L for orthophosphate phosphorous and 1 mg/L for ammonium nitrogen have been set for carp culture waters (EU directive 2006/44/E). As no statistical difference was found for the inorganic nitrogen forms between the treatments, it may have caused by the amount of the organic nitrogen forms. The lower essential amino acid level in DDGS compared to those available in soybean can explain the difference which decreased the protein efficiency and increased the non-utilized nitrogen ratio in fish excreta. According to the absence of significant differences in case of the other measured chemical or biological parameters, we can conclude that the DDGS-based experimental carp feed does not pose a more threatening environmental load than conventional fish feed.

The DDGS User Handbook of U.S. Grains Council (2018) presents an overview of the state of DDGS in environmental sustainability and highlighted the moderate position within the feed ingredients. However, such values in aquaculture are not discussed either, while eliminated phosphorus and nitrogen in other farmed animals are included. Henriksson (2017) summarized the environmental impact of feed ingredients in aquatic feeds in Indonesia, and classified them based on global warming, acidification, eutrophication, land use, and freshwater consumption through life cycle assessment. The environmental effects of DDGS were generally moderate among the listed feed ingredients. A study on the effect of a plant-based aquafeed (containing soybean meal and linseed oil) found under semi-intensive fish pond conditions similarly to our results, no significant differences in water chemistry parameters compared to commercial feed or supplementary grain feeding (Berzi-Nagy et al. 2017). For the zooplankton communities as bioindicator organisms at the same experimental setup, no overall difference was detected between plant-based and fish-meal/fish oi based diets (Tóth et al. 2020).

Plant protein meals may have lower digestibility and therefore can cause elevated nutrient levels in the water column, mainly due to antinutritional factors, like fibres and non-starch polysaccharides (Francis et al., 2001; Kokou and Fountoulaki, 2018), however, these indigestible compounds are characterized rather for soybean, pea or rapeseed meals since corn DDGS contains a very low concentration of antinutrient factors (Makkar, 2012, Council, 2018). The 86 % for apparent protein digestibility of DDGS feedstuff reported by Révész et al (2020) is one of the highest value observed for common carp (Roy et al, 2019). The phosphorus digestibility is also high in DDGS compared to other plant ingredients. This is particularly important due to the biological limitation of the stomachless species to digest phosphorous (Hua and Bureau, 2010).

Roy et al (2019) have examined the role of several feed ingredients in carp pond farming as a source for nitrogen and phosphorous saturation of the pond ecosystem. They found that eutrophication caused by an increased level of nitrogen and phosphorus could be mitigated with appropriate pond management and highly digestible feeds including brewery yeast and corn DDGS. Waste nutrients can be absorbed and eliminated by abundant planktonic and benthic microbial communities in earthen ponds and partly recycled into fish biomass. But for this purpose, the key approach is to optimize the size of fish stock in order to allow the zooplankton population to propagate properly (Sommer et al., 2012).

Conclusions. In conclusion, DDGS, a by-product of bioethanol production, is a potential feed ingredient for carp nutrition as a supplementary diet in pond culture. Based on the results obtained for the production performance of carps and water quality parameters of the ponds utilization in semi-intensive technology is promising. Thus, in the future, it may be able to take on a prominent role as ingredients for fish feed instead of soybean or corn meals.

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