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Effect of density and mixed culture of largemouth bass (*Micropterus salmoides*) and pikeperch (*Sander lucioperca*) on growth, survival and feed conversion rate in intensive culture

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Abstract: In this study, two experiments were performed with the aim to optimize intensive aquaculture of largemouth bass (*Micropterus salmoides*). In the first 140-day experiment the effect of the initial fish density was assessed at three levels: low density (LD) 23 kg/m^3, medium density (MD) 35 kg/m^3 and high density (HD) 46 kg/m^3. All three densities provided the same final Fulton's condition coefficient (FC = 1.24–1.28), specific growth rate (SGR = 0.22–0.24%/day) and survival rate (97–100%). No cannibalism was observed at all tested densities. Feed conversion ratio (FCR = 1.39 ± 0.21 g/g) was the lowest for LD and the highest (1.61 ± 0.08 g/g) for MD. The highest fish biomass (25.7 ± 2.7 kg/m^3) was obtained at HD and this density was considered as the most effective density of all tested ones during the intensive culture of largemouth bass. The second 60-day experiment tested the effect of largemouth bass and pikeperch (*Sander lucioperca*) monoculture and biculture of both species on production efficiency. Higher size heterogeneity was obtained in both (mono- and bicultural) groups of pikeperch (308.91–314.56‰/day) compared to the groups of largemouth bass (279.26–284.05 ‰/day). The higher FC (1.09) was found in both types of culture in largemouth bass compared to both methods of culture in pikeperch (0.74–0.78). The lowest SGR was evident in both types of largemouth bass cultures (1.20–1.28%/day). In contrast, the highest SGR was achieved in the bicultural pikeperch (1.88%/day). Similar results like for SGR were also assessed for FCR, where the highest value of FCR was in both cultures of largemouth bass (1.44–1.48 g/g) compared to the lowest FCR in the bicultural of pikeperch (0.73 g/g). Largemouth bass in both tested types of culture had higher survival rates (99.95–99.99%) compared to pikeperch (98.61–98.63%).

Keywords: biculture; growth performance; feed conversion ratio; high-quality fish production; monoculture; survival rate

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Current European inland aquaculture production is largely dominated by two species (rainbow trout – *Oncorhynchus mykiss* and common carp – *Cyprinus carpio*) with slow development and increasing rate compared to the Asia-Pacific region. European inland aquaculture needs further technology innovations providing higher production of high-value fish species (Policar and Adamek 2013) such as pikeperch – *Sander lucioperca* (Yanes-Roca et al. 2020), Eurasian perch – *Perca fluviatilis* (Policar et al. 2019), burbot – *Lota lota* (Kucharczyk et al. 2018) and also largemouth bass – *Micropterus salmoides* (Fischer et al. 2021) if this species aquaculture is to be well established in Europe. Effective and profitable intensive aquaculture of the above-mentioned species using RAS technology can eliminate needs for land and water resources and is able to support economically and environmentally sustainable all-year-around marketable fish production located near to markets and customers (Nebesky et al. 2016; Policar et al. 2019).

The largemouth bass, a popular carnivorous freshwater centrarchid, is a native species to North America (Park et al. 2015). Outside of its native area, it has been introduced into South America, Europe, Africa and Asia (Petit et al. 2001). In China it has become a major freshwater product in aquaculture (Bai et al. 2008). It is an economically very promising and valuable freshwater species for the consumption (Bai and Li 2018). The introduced largemouth bass is common in freshwater fish ponds of southern Europe (Lorenzoni et al. 2002), where it has also a positive economic effect in aquaculture production (Rodrigues 2017). Aquaculture in such conditions opens the possibility of introducing new warm-water fish species, the largemouth bass certainly belongs to (Han et al. 2017). Optimum water temperature for its growth is 20–30 °C (Bai and Li 2018). The general determinants of its successful culture are optimal temperature, good water quality, appropriate compound feed, light regime and stocking density (Engle et al. 2020).

The pikeperch is a very popular fish species among anglers, which is native to central and eastern Europe, but also it is widespread in western Europe (Argillier et al. 2012). New farms for its intensive aquaculture have been built mainly in western and central Europe (Policar et al. 2019). The declining production of pikeperch by fishing from the lakes and its high popularity among consumers and sport fishermen have caused a lack of supply of this species to the market, particularly in western Europe (Fontaine and Teletchea 2019). This phenomenon, of course, caused the increasing sales price in the whole Europe (Policar et al. 2013).

The intensive freshwater aquaculture using the recirculating aquaculture systems (RAS) is a new approach to getting more efficient, stable, profitable and high-quality marketable fish production which is currently mainly supported by the traditional pond and water through culture methods (Martins et al. 2010). The main advantage of the RAS is the continuous control of water quality and the cultured stock providing the production cycle around the year (Martins et al. 2010; Dalsgaard et al. 2013). This way of culture makes it easy to monitor fish behaviour, to improve or positively influence their condition, nutrition, health, welfare and fitness (Martins et al. 2011). These activities then result in the creation of optimal environmental conditions for the rapid growth and high survival rate of farmed fish without seasonal fluctuations (Martins et al. 2010; Clarke and Bostock 2017).

Polyculture intensive fish farming is often used in pond aquaculture. The polyculture in aquaculture consists in exploiting different dietary demands of farmed fish species, such as planctonophagous, bentophagous, herbivorous and predatory species (Azim and Little 2006). The use of polyculture in the RAS with similar requirements for the environment and with different behaviour, swimming and feeding activity offers a great potential to more effectively use the capacity of this kind of aquaculture (Thomas et al. 2020). The applications of bicultural intensive aquaculture in the RAS have already been studied and tested for brook trout (*Salvelinus fontinalis*) with black sea trout (*Salmo labrax*) (Bascinar et al. 2010), Abant trout (*Salmo abanticus*) with brook trout (Onder and Khan 2016), rainbow trout (*Oncorhynchus mykiss*) with brown trout (*Salmo trutta*) (Karatas et al. 2017) and rainbow trout with Siberian sturgeon (*Acipenser baerii*) (Ak et al. 2019).

The aim of this study was to optimize the intensive largemouth bass culture in the RAS and to monitor the impact of the initial density on the efficiency of its production, as well as to compare the impact of the biculture of largemouth bass and pikeperch on their production efficiency with the monoculture of both species.

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MATERIAL AND METHODS

Effect of initial fish density on production

For 140-day intensive culture, the effect of different initial density of largemouth bass (low: 23 kg/m^3; medium: 35 kg/m^3; high: 46 kg/m^3) on the growth rate, biomass production, survival and cannibalism rates was observed during the first part of this study. In total, 3 303 experimental fishes, produced by the combination of pond and RAS aquaculture according to Policar et al. (2013), were stocked to three different density groups [low with total length (TL) = 221.3 ± 19.7 mm and body weight (BW) = 148.6 ± 43.6 g, medium with TL = 225.2 ± 17.4 mm and BW = 157.1 ± 38.7 g, and high with TL = 217.8 ± 18.2 mm and BW = 139.9 ± 37.2 g]. Each density was tested in three replications, i.e. in nine tanks connected to the large-scale experimental RAS of University of South Bohemia, Faculty of Fisheries and Protection of Waters at Vodňany (USB, FFPW), Czech Republic. This system has the total water volume of 30 m^3, with 15 m^3 of ten tanks used for fish culture and 15 m^3 are used for mechanical and biological filtration and water treatment and distribution. In total, nine tanks of 1.5 m^3 in capacity were used for this study, when three tanks were used for each tested density as triplicates, and one tank was empty.

All laboratory experimental procedures complied with valid legislative regulations in the Czech Republic (Act No. 166/1996 and No. 246/1992); the permit was issued to No. 2293/2015-MZE-17214 and No. 55187/2016-MZE-17214 in NAZV QK1710310 project. All samplings were carried out with the relevant permission from the Departmental Expert Committee for Authorization of Experimental Projects of the Ministry of Education, Youth and Sports of the Czech Republic (Permit No. MSMT 4394/2017-2).

These water quality parameters were maintained: water temperature (22.9 ± 1.8 °C), oxygen level (125.7 ± 14.7% in the morning and 109.3 ± 21% in the evening) and pH (6.9 ± 0.2), concentrations of total ammonia (0.36 ± 0.1 mg/l) and nitrite (0.3 ± 0.12 mg/l) with a light regime of 12 h light (from 7:00 am to 7:00 pm) and 12 h of darkness (from 7:00 pm to 7:00 am). The light intensity of 120 lux on the water surface was used during the first 8 h of light regime (from 7:00 am to 3:00 pm) and for another 4 h (from 3:00 pm to 7:00 pm) the reduced light intensity of 70–80 lux was applied in order to offer the largemouth bass optimal light conditions and support the fish sufficiently by feed and maintain all routines in RAS. Water quality parameters such as water temperature and oxygen level were measured using a portable YSI ProODO oximeter (YSI Inc., Yellow Springs, OH, USA) twice daily at 7:00 am and 7:00 pm. The pH values were measured with a portable pH meter from WTW 3310 (WTW, měřicí a analytická technika, s.r.o., Prague, Czech Republic) once a day at 8:00 am. The light intensity was checked daily using a UNITEST 93514 digital luxmeter (Beha-Amprobe GmbH, Glottertal, Germany). The concentration of total ammonia was determined once a day at 8:30, using a simple titration and colorimetric reference kit using Nessler's reagent and Seignett salt. Nitrite concentration was analysed with a handheld titration and colorimetric kit using sulphanilic acid ($C_6H_7NO_3S$) and NED solution [*N*-(1-naphthyl)ethylenediamine dihydrochloride]. Using these kits, the approximate concentration of ammoniacal nitrogen (NH_4^+–N) in mg/l and nitrite nitrogen (NO_2^-–N) in mg/l was determined. Subsequently, the concentration of both parameters was recalculated using coefficients for the concentration of total ammonia and nitrite. Both above-mentioned sets were obtained at the Faculty of Fisheries and Protection of Waters at Vodňany, Czech Republic, in the Laboratory of Aquatic Toxicology and Ichthyopathology. Every day, 5% of the total water volume of the system was replaced by tap water. The water flow of 3 m^3 per hour was used as water renewal twice per hour in each used tank.

Experimental fish of all groups in all tanks were fed 50% by hand and 50% by belt feeder from 8:00 am to 7:00 pm with actual calculated daily feeding rate (DFR) at a 28-day interval. At the beginning of the experiment, DFR was set at 1.5% of fish biomass and DFR was reduced to 0.75% of fish biomass from the middle of the experiment (from 72^{nd} day of this experiment). The fish were fed using artificial pelleted floating feed Europa F-15 with a pellet size of 2 mm (at the beginning of the experiment) to 3 mm (at the end of the experiment) from Skretting, Stavanger, Norway. The nutritional composition of the used feed is presented in Table 1. Throughout the experiment, the pelleted feed used in all experimental groups was enriched with vitamin C, A and E using Vitamin C PG 100% plv (Pharmagal CZ s.r.o., Nové Město na Moravě, Czech Republic) in order to support

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Table 1. Nutritional composition of feeds for intensive rearing of largemouth bass (*Micropterus salmoides*) under different initial densities (Skretting), monoculture and biculture with pikeperch (*Sander lucioperca*) in the RAS (BioMar)

Main parameters	Skretting Europa F-15	BioMar INICIO Plus
Size of particles (mm)	2.0 and 3.0	1.5 and 2.0
Proteins (%)	55.0	52.0–54.0
Fat (%)	16.0	21.0–24.0
Carbohydrates (NFE %)	16.5	7.2–13.0
Fibre (%)	0.6	0.4–1.0
Ash (%)	10.0	7.6–11
Straight energy (MJ/kg)	19.4	20.0–20.7

NFE = nitrogen-free extract

the good condition of the fish, keep their high survival rate and efficiency of this culture.

At the beginning and at the end of this experiment, the total length and standard length (SL) of a representative sample of 100 stocked experimental fishes from each tank were measured by a conventional gauge in millimetres and body weight by laboratory Mettler AE 2000 digital scales (Mettler Toledo s.r.o., Prague, Czech Republic) to the nearest 0.01 g. Before each biometric measurement, the fish were anesthetized by immersing then into a bath containing clove oil in a concentration of 0.03 ml per litre of water (Kristan et al. 2014).

During this experiment, fish mortality was monitored daily in each tank and actual consumption of the applied feed was registered. For these purposes, the amount of food that was not consumed remained on the water surface for 45 min after feeding. Such pelleted feed was collected, and all uneaten pellets were counted. Previously, 500 dry pellets of the feed used were individually weighed using a KERN-ABT 220-SDM analytical scale (Kern & Sohn GmbH, Balingen, Germany) to the nearest 0.1 mg. Finally, the average weight of one pellet was determined. From the number of collected remaining pellets and the average weight of one pellet, the amount of uneaten feed per day was found and this amount was deducted from the DFR. This DFR adjusted for uneaten feed was then used to calculate the feed conversion ratio (FCR). At the end of each 28-day rearing period, the number of all surviving fish was counted and their biomass was weighed using the CAS PB 100/200 kg scale (CAS Corporation, East Rutherford, NJ, USA) to the nearest 0.01 kg to update the DFR. Thanks to the identified biometric data of fish at the beginning and at the end of the culture, the amount of eaten feed, the number of surviving fish and their final biomass, the following production parameters were calculated for each tank of each group:

$$\mathrm{FC} = (\mathrm{BW}/\mathrm{TL}^3) \times 100 \quad (1)$$

$$\mathrm{SGR} = [(\ln \mathrm{BW_F} - \ln \mathrm{BW_I})/d] \times 100 \quad (2)$$

$$\mathrm{FCR} = F/(\mathrm{BW_F} - \mathrm{BW_I}) \quad (3)$$

$$\mathrm{SR} = (N_\mathrm{F}/N_\mathrm{I}) \times 100 \quad (4)$$

$$\mathrm{CR} = [(N_\mathrm{E} - N_\mathrm{F})/N_\mathrm{I}] \times 100 \quad (5)$$

where:
FC – Fulton's condition coefficient;
BW – the average body weight (g);
TL – the average total length of farmed fish at the beginning and at the end of the experiment (cm);
SGR – specific growth rate (%/day);
FCR – feed conversion ratio (g/g);
SR – survival rate (%);
CR – cannibalism rate (%);
$\ln \mathrm{BW_F}$ – natural logarithm for the final body weight of fish at the end of the experiment;
$\ln \mathrm{BW_I}$ – natural logarithm for the initial body weight of fish;
d – duration of the experiment (days);
F – the total eaten feed (g);
$\mathrm{BW_F}$ – the final body weight of fish at the end of the experiment (g);
$\mathrm{BW_I}$ – the initial body weight of fish at the beginning of the experiment (g);
N_F – the final number of surviving fish (individuals);
N_I – the initial number of stocked fish (individuals);
N_E – the number of expected surviving fish according to daily records of dead fish (individuals).

Comparison of the effect of monoculture and biculture on fish production

The experiment tested the effect of mixed culture of largemouth bass and pikeperch compared to monoculture of both species during 60-day intensive aquaculture. For the purpose of this experiment, experimental "old model" RAS of the Faculty of Fisheries and Protection of Waters at Vodňany,

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Czech Republic was used. This RAS included nine quadrilateral tanks with the volume of 0.6 m^3 and it was also equipped with a mechanical drum filter (IN-EKO TEAM s.r.o., Tišnov, Czech Republic), fluid biological filter with the volume 2 m^3, two retention plastic tanks with the total volume of 1.5 m^3, UV lamp, oxygen column, and pump submerged in one retention tank with the total water exchange once per 20 minutes. In the nine tanks, three experimental groups were tested: monoculture of largemouth bass (monocultural largemouth bass), monoculture of pikeperch (monocultural pikeperch) and biculture of both species (bicultural largemouth bass and pikeperch) with three replications.

The same age fingerlings of largemouth bass (TL = 103.8 ± 3.5 mm; BW = 12.5 ± 2.5 g) and pikeperch (TL = 121.7 ± 5.5 mm; BW = 12.2 ± 1.9 g) that were produced by a combination of pond and RAS culture according to Policar et al. (2013) were used for this experiment. In total, 700 fishes were stocked in each tank (in monoculture only one species and in biculture both species at a 50 : 50 ratio, i.e. 350 individuals per each species) with the initial density of 1.17 fish and 14.5–14.8 g per litre. At the beginning and at the end of this experiment, TL and SL of 100 individuals of representative stocked experimental fish from each tank and species (in total 300 individuals per each species and group) were measured in the same way as during the first experiment. Before the experiment, all experimental fish of both species were size sorted and only the fish with minimum size differences (9.0–16.0 g) in individual body weight were selected for this study. At the beginning of each biometrical measurement, the fish were anesthetized by immersing into the same bath containing clove oil as in the previous experiment. All stocked fish in each tank of all tested groups were weighed using the same above-mentioned scale. The fish were divided into eight different size groups with an interval of 1.0 g as follows: ≤ 9.0; 9.1–10.0; 10.1–11.0; 11.1–12.0; 12.1–13.0; 13.1–14.0; 14.1–15.0; 15.1–16.0. Detailed frequency of fish in each size group was found at the beginning of this experiment. At the end of the experiment, all the fish were weighed again, and the frequency of the fish in all obtained size group with the same 1 g interval of the final size of fish (11.1–75.0 g) was calculated again. From this data, the final size heterogeneity of both species in different types of culture was compared thanks to the calculated coefficient of variation (CV) and the specific variation rate (SHR, ‰/day):

$$CV = \sigma/\mu \quad (6)$$

$$SHR = [(\ln CV_2 - \ln CV_1)/(t_2 - t_1)] \times 100 \quad (7)$$

where:

σ – the standard deviation;

μ – the arithmetic mean;

CV_2 – the final coefficient of variation of body weight;

CV_1 – the initial coefficient of variation of body weight;

t_2 – the final number of days of the experiment;

t_1 – the initial number of days of the experiment.

Also, the frequency of the final individual body weight classes was compared with the frequency of the initial individual body weight classes after the experiment. At the end of the experiment the following production parameters of all tested groups (FC, SGR, FCR, survival rate, cannibalism rate and specific heterogeneity variation rate) were calculated in the same way as in the first experiment of this study.

The water quality parameters were maintained (related to the first experiment of this study), such as water temperature (22.58 ± 0.75 °C), oxygen level (104.6 ± 7.9% in the morning and 96.1 ± 7.7% in the evening), pH (6.5 ± 0.3), total ammonia (0.5 ± 0.2 mg/l), nitrite (0.4 ± 0.15 mg/l) with a light regime of 12 h of light and 12 h of darkness (from 7:00 am to 7:00 pm) with a light intensity of 80 lux on the water surface. All water quality parameters were measured in the same way as during the first experiment.

All experimental groups of monocultural and bicultural largemouth bass and pikeperch were fed DFR of 2% of fish biomass using an artificial sinking pelleted feed from BioMar INICIO Plus (BioMar SAS, Nersac, France) with a pellet size of 1.5–2 mm (Table 1). These pellet sizes were initially used at a 1 : 1 ratio, and only the 2 mm pellets were used from the middle of the experiment. During this experiment, the used feed was enriched with vitamin C, A and E in the same way as during the first experiment of this study. The DFR was applied 50% by hand and 50% by a belt feeder to the fish during the light part of the day.

Statistical analysis

All collected data are presented as mean ± standard deviation in this study. Data from both experiments

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were analysed using one-way analysis of variance (ANOVA). Tukey's post-hoc test, with significance at $P < 0.05$, was chosen to determine differences in biometric data and production parameters under different densities of the largemouth bass (in the first experiment) and the effect of monoculture and biculture of largemouth bass and pikeperch on their production parameters (in the second experiment). Selected statistical tests were evaluated in the statistical software Statistica 12 (StatSoft Inc., Prague, Czech Republic).

RESULTS

Effect of initial fish density on production

At the end of 140-day experiment, the increased biomass was achieved in all tested fish groups (Table 2): the gain of biomass was observed at high density of fish (25.7 ± 2.7 kg/m^3), at medium density (18.0 ± 1.9 kg/m^3) and at low density (15.8 ± 1.3 kg/m^3). The final Fulton's condition coefficient (FC) was without significant differences compared to all densities at the beginning (1.33–1.35) and at the end (1.24–1.28) of the experiment (Table 3).

The lowest feed conversion ratio (FCR) was found for low density (1.39 ± 0.21 g/g) and the highest (1.61 ± 0.08 g/g) for medium density. FCR for high density (1.50 ± 0.17 g/g) was not different from the other two tested densities. The following results were obtained without statistical differences between the tested groups: comparably low SGR (0.22–0.26%/day), high survival rate (97.27–99.87%) and zero cannibalism rate (Table 2). The highest density was considered as the most effective density at the end of this experiment providing the highest fish production with good feed conversion rate and high survival rate of largemouth bass under good conditions.

Effect of monoculture and biculture with pikeperch on fish production

The differences between measured parameters at the beginning and at the end of the experiment indicate a noticeable gain in the measured parameters (TL, SL and BW) especially in monocultural and bicultural pikeperch. At the end of the experiment, the bicultural pikeperch had the higher parameters (TL = 172.53 mm, SL = 147.55 mm and BW = 40.92 g) than the monocultural pikeperch (TL = 160.94 mm, SL = 102.91 mm and BW = 31.29 g) and both mono- and bicultural largemouth bass (TL = 131.95–132.43 mm, SL = 109.6–110.8 mm

Table 2. Comparison of initial and final biomass, SGR, FCR, survival rate and cannibalism rate between different stocking densities of largemouth bass (*Micropterus salmoides*) (mean ± SD) during 140-day experiment

Stocking density	Biomass (kg/m^3)		Gain of biomass (kg/m^3)	SGR (%/day)	FCR (g/g)	SR (%)	CR (%)
	initial	final					
Low	23.0 ± 0.1	34.1 ± 4.5	15.8 ± 1.3^c	0.26 ± 0.07^a	1.39 ± 0.21^b	99.87 ± 0.44^a	0^a
Medium	35.0 ± 0.2	49.1 ± 2.2	18.0 ± 1.9^b	0.22 ± 0.05^a	1.61 ± 0.08^a	98.68 ± 1.02^a	0^a
High	46.0 ± 0.3	66.3 ± 1.7	25.7 ± 2.7^a	0.24 ± 0.03^a	1.50 ± 0.17^{ab}	97.27 ± 0.71^a	0^a

CR = cannibalism rate; FCR = feed conversion ratio; SGR = total length; SR = survival rate

$^{a–c}$Different letters in the same column indicate statistical differences ($P < 0.05$)

Table 3. Comparison of initial and final TL, SL, EW and FC (mean ± SD) between different stocking densities of largemouth bass (*Micropterus salmoides*; n = 300) during 140-day experiment

Stocking density	Initial				Final			
	TL (mm)	SL (mm)	BW (g)	FC	TL (mm)	SL (mm)	BW (g)	FC
Low	221.3 ± 19.7^a	174.5 ± 16.7^a	148.6 ± 43.6^a	1.34 ± 0.16^a	252.0 ± 18.7^a	209.9 ± 17.3^a	205.7 ± 50.1^a	1.26 ± 0.05^a
Medium	225.2 ± 17.4^a	177.6 ± 14.3^a	157.1 ± 38.7^a	1.35 ± 0.09^a	254.0 ± 18.5^a	209.2 ± 16.1^a	211.2 ± 49.3^a	1.28 ± 0.07^a
High	217.8 ± 18.2^b	171.0 ± 14.2^b	139.9 ± 37.2^b	1.33 ± 0.01^a	253.1 ± 17.0^a	208.2 ± 14.6^a	207.9 ± 43.4^a	1.24 ± 0.26^a

BW = body weight; FC = Fulton's condition coefficient; SL = standard length; TL = total length

^{a,b}Different letters in the same column indicate statistical differences ($P < 0.05$)

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and BW = 12.37–13.09 g). The bicultural pikeperch had 1.07, 1.06 and 1.31 times significantly higher TL and SL and BW than monocultural pikeperch and 1.30–1.31, 1.33–1.35 and 1.61 times higher TL, SL and BW than bicultural and monocultural largemouth bass (Table 4).

Comparison of frequencies in the representation of individual weight classes between monocultural and bicultural largemouth bass is shown in Figure 1, 2 and pikeperch in Figure 3, 4. Both figures present an increasing fish size and its heterogeneity for each group although the initial body weights of stocked fish were well balanced. The size heterogeneity of all experimental fish in both species was observed in the range of 11.1–78.1 g at the end of the experiment. The highest range of final body weight was found in the bicultural pikeperch (with the range of body weight 12.7–78.1 g), where the most frequently represented pikeperch (43.8%) was in the size group of 29.1–40.0 g. The second highest range of final body weight was found in the monocultural pikeperch (with the range of body weight 11.4–63.0 g), where most fish (41.2%) were included in the size group pf 23.1–30.1 g. On the contrary, the lower and very same range of final body weight (11.1–36.0 g and 12.1–39.0 g) was seen in both groups of largemouth bass. Most fish in monocultural (51.3%) and bicultural (61.9%) largemouth bass were in the body weight range of 16.1–25.0 g and 18.1–27.0 g, respectively. Size heterogeneity presented by CV_2 (24.32) and SHR (314.56 ‰/day) was the highest in monocultural pikeperch at the end of the experi-

Table 4. Biometric parameters of monocultural and bicultural largemouth bass (*Micropterus salmoides*; n = 300) and pikeperch (*Sander lucioperca*; n = 300), including their production parameters (mean ± SD) after 60 days of their intensive culture

Main parameters	Monocultural largemouth bass	Bicultural largemouth bass	Monocultural pikeperch	Bicultural pikeperch
Initial				
TL (mm)	101.02 ± 6.94^b	103.83 ± 6.43^b	121.71 ± 5.53^a	122.94 ± 5.93^a
SL (mm)	84.44 ± 9.70^b	86.79 ± 5.45^b	102.91 ± 4.88^a	103.95 ± 5.16^a
BW_{total} (g)	13.09 ± 1.91^a	12.37 ± 1.73^b	12.19 ± 1.90^b	13.09 ± 1.91^a
BW_{min} (g)	9.00	9.00	9.00	8.50
BW_{max} (g)	16.00	16.00	16.80	17.60
CV_1	16.28	13.99	15.62	14.60
FC	1.10 ± 0.07^a	1.10 ± 0.07^a	0.67 ± 0.07^b	0.70 ± 0.06^c
Final				
TL (mm)	131.95 ± 7.49^c	132.43 ± 7.39^c	160.94 ± 11.51^b	172.53 ± 11.52^a
SL (mm)	109.60 ± 6.32^c	110.80 ± 6.34^c	138.25 ± 10.76^b	147.55 ± 10.89^a
BW_{total} (g)	25.36 ± 4.55^c	25.39 ± 4.37^c	31.29 ± 7.62^b	40.92 ± 9.40^a
BW_{min} (g)	12.10	11.10	11.40	12.70
BW_{max} (g)	39.00	36.00	63.00	78.10
CV_2	17.94	17.06	24.32	22.96
FC	1.09 ± 0.04^a	1.09 ± 0.04^a	0.74 ± 0.05^b	0.78 ± 0.05^c
SGR (%/day)	1.28 ± 0.36^c	1.20 ± 0.27^c	1.54 ± 0.48^b	1.88 ± 0.43^a
FCR (g/g)	1.48 ± 0.29^a	1.44 ± 0.21^a	1.03 ± 0.02^{ab}	0.73 ± 0.01^b
SR (%)	99.99 ± 0.03^a	99.95 ± 0.05^a	98.61 ± 1.30^b	98.63 ± 1.23^b
CR (%)	0	0	0	0
SHR (‰/day)	284.05	279.26	314.56	308.91

BW_{max} = maximum body weight; BW_{min} = minimum body weight; BW_{total} = total body weight; CR = cannibalism rate; CV_1 = initial coefficient of variation of weight; CV_2 = final coefficient of variation of weight; FC = Fulton's coefficient; FCR = feed conversion ratio; SGR = specific growth rate; SHR = specific heterogeneity variation rate; SL = standard length; SR = survival rate; TL = total length

[a–c]Different letters in the same row indicate statistical differences (P < 0.05)

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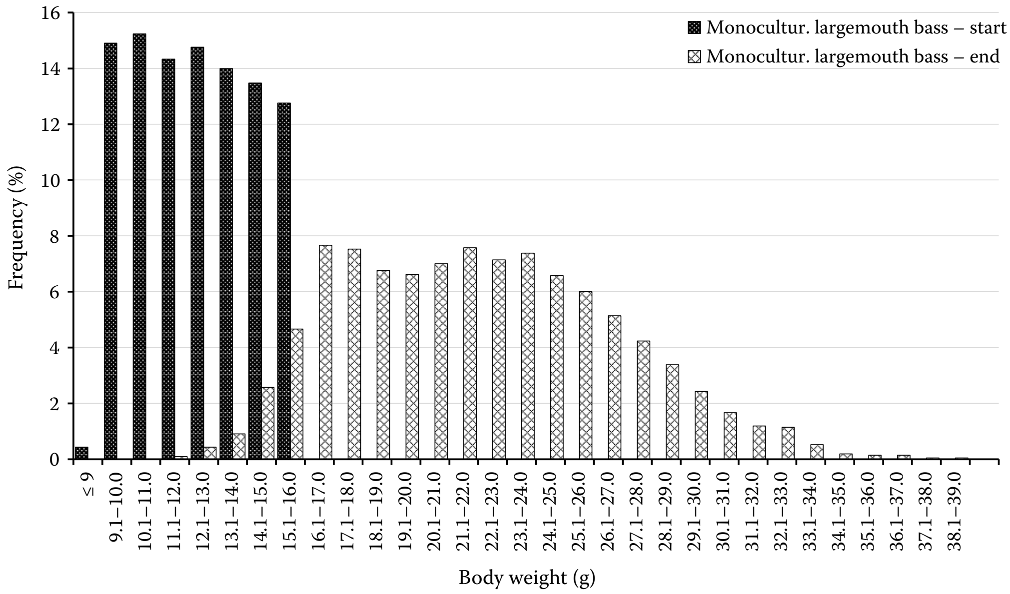

Figure 1. The range of initial and final body weight in monocultural largemouth bass (*Micropterus salmoides*; *n* = 2 100) divided at an interval of 1 gram at the beginning and at the end of 60-day intensive culture

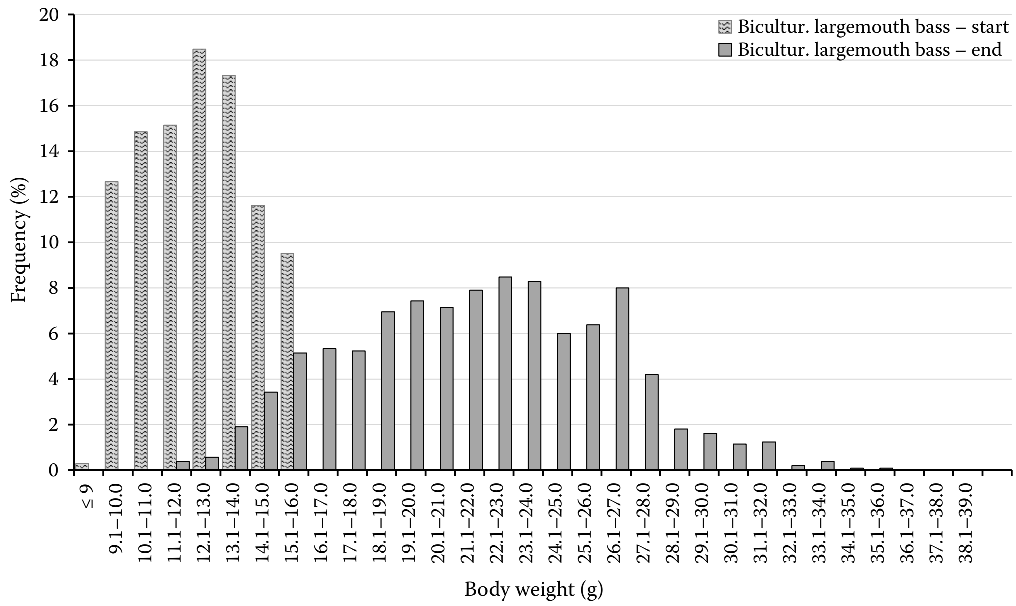

Figure 2. The range of initial and final body weight in bicultural largemouth bass (*Micropterus salmoides*; *n* = 1 050) divided at an interval of 1 gram at the beginning and at the end of 60-day intensive culture

ment. The second highest size heterogeneity was observed in bicultural pikeperch by CV_2 (22.96) and SHR (308.91‰/day). The lowest and very same size heterogeneity was evident in both groups of largemouth bass (CV_2 = 17.06–17.94 and SHR = 279.26–284.05‰/day).

Higher Fulton's coefficient at the end of this experiment was determined in both groups of largemouth bass (1.09 ± 0.04) compared to monocultural (0.74 ± 0.05) and bicultural (0.78 ± 0.05) pikeperch (Table 4). These results are not related to the poor condition of pikeperch, but rather to different body shape and condition in both tested fish species.

The highest SGR was achieved in the bicultural pikeperch (1.88 ± 0.43%/day) and the lowest SGR was found in bicultural and monocultural largemouth bass (1.20 ± 0.27%/day and 1.28 ± 0.36%/day). Monoculture pikeperch had medium SGR

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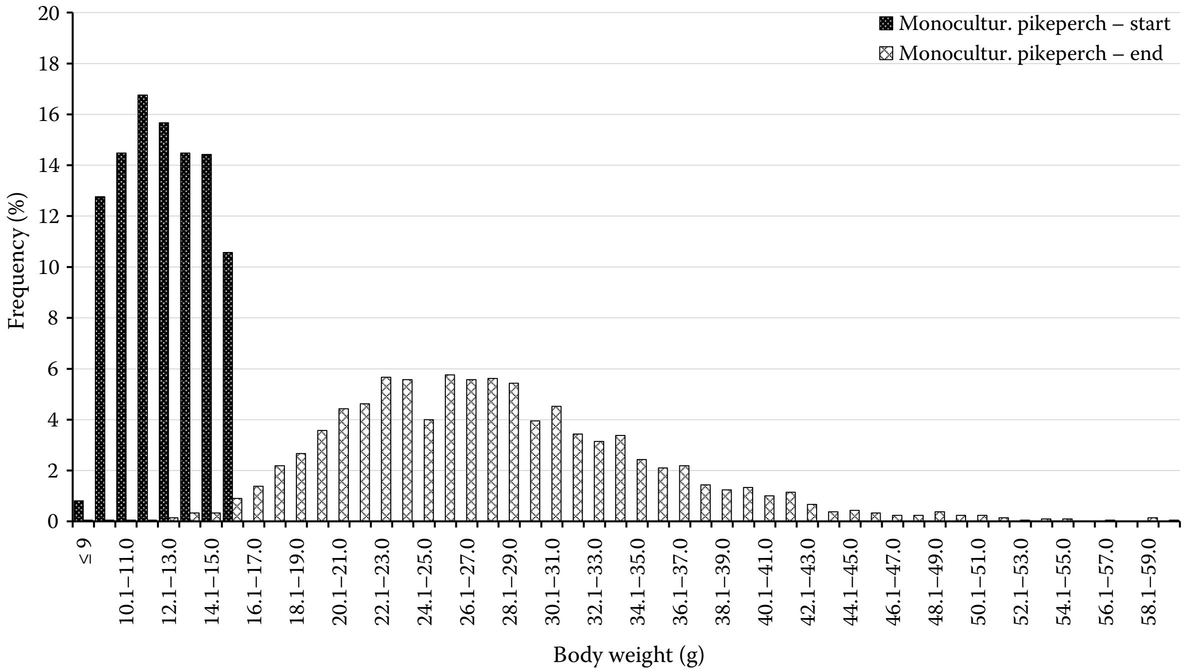

Figure 3. The range of initial and final body weight in monocultural pikeperch (*Sander lucioperca*; *n* = 2 100) divided at an interval of 1 gram at the beginning and at the end of 60-day intensive culture

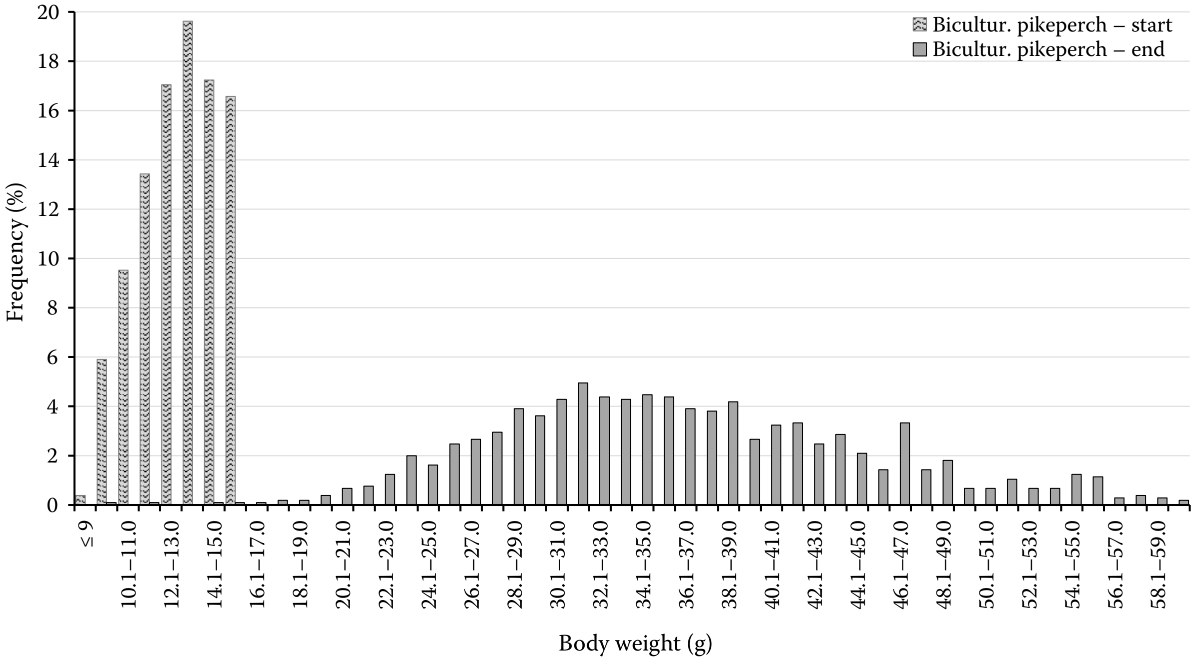

Figure 4. The range of initial and final body weight in mono- and bicultural pikeperch (*Sander lucioperca*; *n* = 1 050) divided at an interval of 1 gram at the beginning and at the end of 60-day intensive culture

(1.54 ± 0.48%/day) which was different from the above-mentioned groups (Table 4).

Similar tendencies like for SGR were also obtained for FCR (Table 4). Within the evaluated FCR, the significantly higher feed conversion was observed for pikeperch (0.73 ± 0.01 g/g) cultured in biculture with largemouth bass which had the highest FCR (1.44 ± 0.21 g/g), similarly like largemouth bass cultured in monoculture (1.48 ± 0.29 g/g). In addition to these results, it can be stated that pikeperch cultured in monoculture (1.03 ± 0.02 g/g) showed no difference in feed

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conversion from both largemouth bass groups and bicultural pikeperch.

At the end of this experiment, a high survival rate from 98.61% to 99.99% was observed for both tested fish species in both tested culture types. The course of cumulative survival during the experiment is shown in Figure 5. The statistical comparison of survival rates emphasized a lower survival rate in both cultured pikeperch (98.61–98.63%) compared to a better survival rate in largemouth bass (99.95–99.99%). At the end of the experiment, zero cannibalism rate was determined for all tested groups, which characterized the very well size sorted fish of both species at the beginning of the experiment. No cannibalism rate provided the high survival rate of all tested groups. The cannibalism had no negative effect on the success of the tested experimental culture with gradual size heterogeneity in all experimental groups (Table 4).

In addition, different largemouth bass behaviour compared to the behaviour of pikeperch in all types of culture (mono- and biculture) was an interesting fact from this experiment. After only a few days of culture, it was clear that both species typically use different space in the tanks. The largemouth bass used the space at the surface in the tank more often, and the pikeperch tended to stick to the bottom or in the lower parts of the tanks. The pikeperch within the bicultural groups were less timid, received better feed and made better use of the middle space in the tanks. This changed behaviour compared to the monoculture stocks of pikeperch, where the fish used only the bottom and lower parts of the tanks, was caused by the fact that largemouth bass swam in the upper parts of the tank at the water level and thus covered the pikeperch. They were then calmer and probably suffered less stress, received better feed, which they also used better. It is even highly probable that the pikeperch used part of the administered feed at the expense of coproduced largemouth bass, which, however, was not possible during bicultural rearing in one tank.

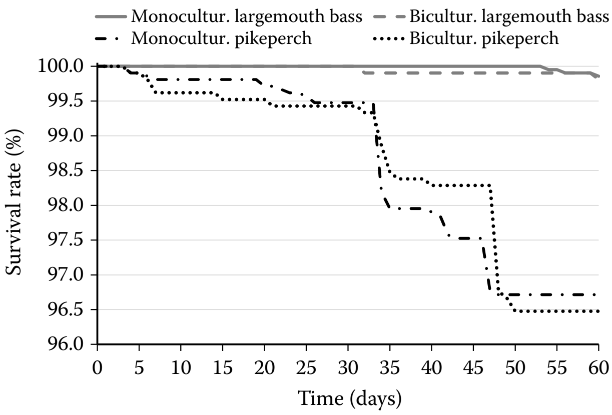

Figure 5. The course of cumulative survival rate of mono- and bicultural largemouth bass (*Micropterus salmoides*) and pikeperch (*Sander lucioperca*) during 60-day intensive culture under RAS

DISCUSSION

The stocking density of cultured fish affected physiological and morphological processes such as growth and survival rates, size heterogeneity, food intake, behaviour, welfare, fin condition (Petit et al. 2001; Park et al. 2015; Ronald et al. 2014; Policar et al. 2019) and also cost effectiveness and profitability in commercial fish production (Nebesky et al. 2016). Therefore, the optimal, as high as possible fish density is a very important fact of successful intensive aquaculture in many species (Ronald et al. 2014). Higher density of intensively cultured juvenile Eurasian perch (0.5–15 g) from 400 fish/m^3 to 10 000 fish/m^3 increased growth rate and decreased growth heterogeneity. This positive correlation between fish density and growth rate was applicable until perch juveniles reached 10–16 g. In bigger perch juveniles up to 16 g, increasing density from 20 kg/m^3 to 60 kg/m^3 decreased growth rate (Melard 1996a). In this study, no effect of different density on growth rate was found because probably the highest acceptable density was not applied in largemouth bass. In the present study the initial density from 23 kg/m^3 to 46 kg/m^3 for 140–157 g largemouth bass and the final density of 34–66 kg/m^3 for fish weighing 205–211 g were tested. Melard (1996a) published the optimal fish density of 35 kg/m^3 in 5-gram fish for maximal Eurasian perch production and of 80 kg/m^3 in 150-gram fish. Results of this study also showed that the biggest fish production (25.7 kg/m^3) was reached at the highest fish density (from 46–66 kg/m^3). However, no negative effect of this highest density on fish production was found. These results suggest that largemouth bass can probably accept higher density compared to the already tested level. This statement is also in line with survival and cannibalism rates reached in this study which were not affected by different tested largemouth bass densities. This study can

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state that largemouth bass is a very tolerant and easily cultured fish species, providing excellent survival rate without any culture and technological problems. This species has no problem with higher density which reached up to 66 kg/m^3. However, FCR (feed conversion rate) was negatively affected by medium density (35–49 kg/m^3) compared to the lowest density (23–34 kg/m^3) but no clear effect was found at the highest density (46–66 kg/m^3) on FCR, which was not different from the other two tested densities. It can be concluded that the highest density provided the highest fish production, low FCR, high survival rate and no cannibalism (Policar et al. 2019). These results can be explained by the fact that largemouth bass can be cultured under higher density than it was tested and these higher densities should be tested in future research. Finally, it is necessary to know the maximal density of largemouth bass which starts to reduce fish growth and survival rates and feed conversion and welfare for the future optimization of largemouth bass successful and profitable aquaculture.

The aim of bi- or polyculture farming is to increase overall production through different species that have different dietary or spatial requirements for the environment (Bascinar et al. 2010; Onder and Khan 2016; Ak et al. 2019). This positive relationship can have an effect on the higher survival rate of cultured fish. These facts were also found in this study, especially in bicultural pikeperch, which demonstrated high fish production, growth and survival rate in culture with largemouth bass. However, bi- or polycultural farming is not yet very common in intensive aquaculture using RAS (Thomas et al. 2020).

Largemouth bass behaviour and its preference to occupy upper parts of tanks probably provided the significantly highest growth rate in bicultural pikeperch which occupied middle and bottom parts of thanks as well monoculture pikeperch which had lower SGR at the same time. This benefit in bicultural pikeperch was found in this study when sinking BioMar pellets were used for the feeding of both species. Thanks to the above-mentioned different behaviour of both species cultured in mono- and biculture different kind of pellets (sinking or floating) can be preferable separately for both species. Sinking pellets can be preferable for mono- and bicultural pikeperch that is mainly concentrated in the middle or bottom space of tanks. However, largemouth bass and its preference to surface and middle parts of tanks probably require using floating pellets. Application of sinking pellets in the present study could also be one reason for the significantly lower growth rate of largemouth bass which presented very poor efficiency of its intensive aquaculture production in both populations (monocultural and bicultural). The second reason for the lower SGR in largemouth bass could be its high degree of inbreeding, because largemouth bass is not a native species to the Czech Republic and this species was introduced there at the end of the 19th century in limited numbers of fish (Kouril and Klimes 1999). However, this statement must be confirmed during future genetic research according to the study published by Wang et al. (2019). Finally, it should be mentioned that SGR differed between the two experiments due to different size of experimental fish and also rearing conditions such as feed type (sinking and floating), feed composition produced by different companies BioMar and Skretting, different RAS and tank types (Melard et al. 1996b; Molnar et al. 2004; Ronyai and Csengeri 2008; Policar et al. 2019).

The results show that the fish survival rate during the experiment was significantly lower for the pikeperch than for the largemouth bass. There may be several reasons for these differences in the survival of both tested species, which have slightly different physiological processes, their tolerance and adaptation to conditions of intensive farming (Bell 2013).

CONCLUSIONS

It can be concluded that largemouth bass with the initial body weight of about 140 grams can be successfully intensively cultured under RAS conditions without substantial technological problems. The highest initial fish density (46 kg/m^3) provided the highest fish production (25.7 kg/m^3) without any negative effect on the growth, survival and cannibalism rates, food conversion and condition of farmed fish. This density was considered as the most effective density for all tested groups in this study.

This study also showed that it is possible to intensively culture largemouth bass in intensive biculture with pikeperch. This culture method mainly supports pikeperch growth and its heterogeneity because largemouth bass helps to increase pikeperch activity, growth rate, production and feed conversion.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Ak K, Kurtoglu IZ, Serezli R, Kayis S, Yandi I. Introduce the Siberian sturgeon (Acipenser baerii) to Turkish aquaculture industry: Duoculture possibility with rainbow trout. Ege J Fish Aquat Sci. 2019 Sep 15;36(3):211-7.

Argillier C, Barral M, Irz P. Growth and diet of the pikeperch Sander lucioperca (L.) in two French reservoirs. Arch Pol Fish. 2012 Jan;20(3):191-200.

Azim ME, Little DC. Intensifying aquaculture production through new approaches to manipulating natural food. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour. 2006 Nov;1(62):1-23.

Bai J, Li S. Development of largemouth bass (Micropterus salmoides) culture. In: Gui JF, Tang Q, Li Z, Liu J, De Silva SS, editors. Aquaculture in China: Success stories and modern trends. Oxford: John Wiley & Sons; 2018. p. 421-9.

Bai J, Lutz-Carrillo DJ, Quan Y, Liang S. Taxonomic status and genetic diversity of cultured largemouth bass Micropterus salmoides in China. Aquaculture. 2008 Jun 10; 278(1-4):27-30.

Bascinar N, Atasaral-Sahin S, Kocabas M. Effect of duoculture on growth performance of brook trout (Salvelinus fontinalis Mitchill, 1814) and Black Sea trout (Salmo trutta labrax Pallas, 1811) in tank reared condition. Kafkas Univ Vet Fak Derg. 2010 Jun;16(3):249-54.

Bell G. Evolutionary rescue and the limits of adaptation. Philos Trans R Soc B. 2013 Jan 19;368(1610): 6 p.

Clarke R, Bostock J. Regional review on status and trends in aquaculture development in Europe – 2015. In: FAO Fisheries and Agriculture circular No. 1135/1. Rome: Food and Agriculture Organization of the United Nations; 2017. 41 p.

Dalsgaard J, Lund I, Thorarinsdottir R, Drengstig A, Arvonen K, Pedersen PB. Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquac Eng. 2013 Mar 1;53:2-13.

Engle CR, Kumar G, van Senten J. Cost drivers and profitability of U.S. pond, raceway, and RAS aquaculture. J World Aquac Soc. 2020 Aug;51(4):847-73.

Fischer H, Romano N, Jones J, Howe J, Renukdas N, Sinha AK. Comparing water quality/bacterial composition and productivity of largemouth bass Micropterus salmoides juveniles in a recirculating aquaculture system versus aquaponics as well as plant growth/mineral composition with or without media. Aquaculture. 2021 May 30;538.

Fontaine P, Teletchea F. Domestication of the Eurasian perch (Perca fluviatilis). In: Teletchea F, editor. Animal domestication [Internet]. London: IntechOpen; 2019 May 14 [cited 2021 June 16]. 23 p. Available from: https://www.intechopen.com/chapters/66267.

Han AX, Berlin C, Ellerby DJ. Field swimming behavior in largemouth bass deviates from predictions based on economy and propulsive efficiency. J Exp Biol. 2017 Sep 15;220(18):3204-8.

Karatas B, Arabaci M, Onalan S. Comparison of rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo trutta ssp) dual culture in different ratios and growth performances. Pak J Zool. 2017 Nov 1;49(6):2257-61.

Kouril J, Klimes J. Rozmnozovani a odchov nasadoveho materialu okounka pstruhoveho [Reproduction and culture of stocking fish in largemouth bass]. Ed. metodik (technol. rada). [Vodnany]: FROV JU; 1999 Sep. 9 p.

Kristan J, Stara A, Polgesek M, Drasovean A, Kolarova J, Priborsky J, Blecha M, Svacina P, Policar T, Velisek J. Efficacy of different anaesthetics for pikeperch (Sander lucioperca L.) in relation to water temperature. Neuroendocrinol Lett. 2014 Jan 1;35(2):81-5.

Kucharczyk D, Nowosad J, Kujawa R, Dietrich G, Biegaj M, Sikora M, Luczynski MJ. Comparison of spontaneous and hormone-induced reproduction of burbot (Lota lota L.) under hatchery conditions. Aquaculture. 2018 Feb 2;485:25-9.

Lorenzoni M, Dorr AJM, Erra R, Giovinazzo G, Mearelli M, Selvi S. Growth and reproduction of largemouth bass (Micropterus salmoides Lacepede, 1802) in Lake Trasimeno (Umbria, Italy). Fish Res. 2002 Apr 1;56(1):89-95.

Martins CIM, Eding EH, Verdegem MCJ, Heinsbroek LTN, Schneider O, Blancheton JP, Roque d'Orbcastel E, Verreth JAJ. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquac Eng. 2010 Nov 1;43(3):83-93.

Martins CIM, Eding EH, Verreth JAJ. Stressing fish in recirculating aquaculture systems (RAS): Does stress induced in one group of fish affect the feeding motivation of other fish sharing the same RAS? Aquac Res. 2011 Nov 18; 42(9):1378-84.

Melard C, Baras E, Mary L, Kestemont P. Relationships between stocking density, growth, cannibalism and survival rate in intensively cultured larvae and juveniles of perch (Perca fluviatilis). Ann Zool Fennici. 1996a Nov;33:643-51.

https://doi.org/10.17221/59/2021-CJAS

Melard C, Kestemont P, Grignard JC. Intensive culture of juvenile and adult Eurasian perch (P. fluviatilis): Effect of major biotic and abiotic factors on growth. J Appl Ichthyol. 1996b Dec;12(3-4):175-80.

Molnar T, Hancz C, Bodis M, Muller T, Bercsenyi M, Horn P. The effect of initial stocking density on growth and survival of pike-perch fingerlings reared under intensive conditions. Aquac Int. 2004 Mar;12(2):181-9.

Nebesky V, Policar T, Blecha M, Kristan J, Svacina P. Trends in import and export of fishery products in the Czech Republic during 2010–2015. Aquac Int. 2016;24(6):1657-68.

Onder MY, Khan U. Effects of mono-and duoculture on the survival and growth rate of juvenile abant trout (Salmo trutta abanticus) and brook trout (Salvelinus fontinalis). Pakistan J Zool. 2016 Jun 1;48(3):895-8.

Park J, Renukdas N, Luna T, Roy LA. The effects of biomass density on size variability and growth performance of juvenile largemouth bass, Micropterus salmoides, in a semi-closed recirculating system. J World Aquac Soc. 2015 Jun 1;46(3):283-91.

Petit G, Beauchaud M, Buisson B. Density effects on food intake and growth of largemouth bass (Micropterus salmoides). Aquac Res. 2001 Jun;32(6):495-7.

Policar T, Adamek Z. Diversification in inland aquaculture. Preface. Aquac Int. 2013 Aug 3;21(Spec issue 4):737-8.

Policar T, Stejskal V, Kristan J, Podhorec P, Svinger V, Blaha M. The effect of fish size and stocking density on the weaning success of pond-cultured pikeperch Sander lucioperca L. juveniles. Aquac Int. 2013 Aug 1;21(4):869-82.

Policar T, Schaefer FJ, Panana E, Meyer S, Teerlinck S, Toner D, Zarski D. Recent progress in European percid fish culture production technology–Tackling bottlenecks. Aquac Int. 2019 Jul 16;27(4):1151-74.

Rodrigues AM. Recent research on largemouth bass production and fillet nutritional characterization of freshwater fish species in Portugal. J Aquac Res Dev. 2017 Mar;8(2 Suppl): 1 p.

Ronald N, Gladys B, Gasper E. The effects of stocking density on the growth and survival of nile tilapia (Oreochromis niloticus) fry at son fish farm, Uganda. J Aquac Res Dev. 2014;5(2): 7 p.

Ronyai A, Csengeri I. Effect of feeding regime and temperature on ongrowing results of pikeperch (Sander lucioperca L.). Aquac Res. 2008 Jun;39(8):820-7.

Thomas M, Lecocq T, Abregal C, Nahon S, Aubin J, Jaeger C, Wilfart A, Schaeffer L, Ledore Y, Puillet L, Pasquet A. The effects of polyculture on behaviour and production of pikeperch in recirculation systems. Aquac Rep. 2020 Jul 1;17: 6 p.

Wang D, Yao H, Li YH, Xu YJ, Ma XF, Wang HP. Global diversity and genetic landscape of natural populations and hatchery stocks of largemouth bass micropterus salmoides across American and Asian regions. Sci Rep. 2019 Nov 13;9(1): 10 p.

Yanes-Roca C, Holzer A, Mraz J, Vesely L, Malinovskyi O, Policar T. Improvements on live feed enrichments for pikeperch (Sander lucioperca) larval culture. Animals. 2020 Mar;10(3): 13 p.

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