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COMPARISON OF EFFICIENCIES OF USING TRAINER FISH AND SHAPE OR TASTE MODIFIED FEED FOR ENCHANCING DIRECT WEANING OF PIKEPERCH (*Sander lucioperca* L.) YEARLINGS ON DRY FEED

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ABSTRACT

Two practical methods aimed at the enhancement of efficiencies of weaning pond-raised pikeperch (*Sander lucioperca* L.) yearlings on dry feed were compared in two consecutive experiments. The first method was applying pellet-feeding fish as trainers/teachers for naive fish. As trainers, either pikeperch or perch (*Perca fluviatilis* L.) were used. The treatments and control (without trainer fish) were run in triplicates (9 aquaria, 40 fish/aquarium). Pikeperch yearlings were not able to learn to eat dry feed by social learning. In contrast, the presence of trainers decreased the success of weaning of naive fingerlings non-significantly (2.2% with pikeperch trainers; 7.8% with perch trainers; 8.3% with only naive fish).

The second method was to modify the taste and shape of the pellet. Bullet-form feed pellets were ground and reshaped as "artificial blood worms" (ABW). ABW were flavored either by Chironomus mixture or by a commercial aroma. Non-flavored ABW served as a control. Commercial aroma flavored ABW significantly increased the percentage of weaned fish (16.7%) in comparison to the bloodworm flavored (12.2%) and non-flavored (4.4%) ones.

INTRODUCTION

Over the last 15-20 years there has been a growing interest for the farming of European percid fishes (Ljunggren et al., 2003; Kestemont and Mélard, 2000). In case of pikeperch (*Sander lucioperca* L.), farmers notice high demands on markets, due to its good meat quality. The catches in commercial fisheries are getting lower and lower, due to overfishing (Dil, 2008). In Eastern Europe, the pond production of pikeperch is very unstable and it is unlikely to increase with traditional culture methods (Hilge and Steffens, 1996). Therefore, the only chance to increase production is by using intensive systems. The main bottleneck of pikeperch production is supplying the grow-out phase with fingerlings, weaned to artificial diet (Schram, 2008; Policar et al., 2012; Ljunggren at al., 2003). Numerous information is at hand on how to wean pikeperch fry (Ostaszewska et al., 2005; Zienert and Heidrich, 2005; Kestemont et al., 2007; Szczepkowski et al., 2011; Szkudlarek & Zakęś, 2007; FAO, 2012) and fingerlings (Zakes and Demska, 1998; Ljunggren et al., 2003; Zienert & Heidrich, 2005; Bódis et al., 2007; Policar et al., 2012; FAO, 2012) onto an artificial diet, but little is known about weaning pikeperch into

bigger sizes, as young-of-the-year fish, otherwise called autumn fingerling (10-30 g). This size of fish is also suitable for intensive production. By this, the Eastern European ponds could also serve the year-round production of weaned fingerling without the difficulties of out of season spawning (Zakes and Demska, 2009; Zakes, 2007) and larvae weaning.

There are only a few studies discussing the weaning ability of autumn pikeperch. Zienert and Heidrich (2005) trained 200 pikeperch (28 g). 100 fish were trained directly and 100 were trained by mixing bloodworm (*Chironomus* sp.) with feed. This method, using *Chironomus* sp. mixed with pellets, is stated as the best weaning procedure for fingerlings of pikeperch (Zienert & Heidrich, 2005; Bódis et al., 2007). There were no replicates in the experiment. The group with the mixing of feed and bloodworm reached 80% while the one with direct weaning resulted in only 10% of the weaning rate. This result suggests that the weaning of 28 g fish can be done. In our previous studies, 10% of weaning rate was found by direct weaning. Interestingly, Policar et al. (2012) mention that, according to their unpublished experience, working with older and bigger pikeperch is not suggested (11-13 cm; 6.5–16 g) due to their observed low survival rates (25-30%) during the weaning process. They do not mention which method was used for weaning. Altun et al. (2008) worked with pikeperch fingerlings, caught from wild stock (6.21 g, 7.8 cm). They offered six types of transitional-foods before feeding dry feed, however, the best results were achieved with immediate dry feed offering. Their results do not agree with that of Zienert and Heidrich (2005), or Policar et al. (2012).

Production of young-of-the-year pikeperch (10-30 g) mostly on plankton and insect larvae in pond is relatively simple. By the economics of the weaning process, it is logical to find a way for an effective, direct and easy-to-use weaning technology for young-of-the-year pikeperch (10-30 g). It is simply because we cannot wean as many fish from one-year-old fish in the same fish tank as from the fingerlings (0.4-1 g). With this technology, trained pikeperch could be produced during autumn, without the need for out-of-season propagation. In order to develop this technology, two approaches were considered. The first approach was to apply "teacher" fish at the weaning process.

Many studies show that fish have the ability of social learning. This ability is mainly studied for restocking programs where survival in the wild depends on how the stocks - grown in hatcheries - can learn from their native, wild-grown mates the predation on prey fish (Brown and Laland, 2001; Reiriz et al, 1998; Suboski and Templeton, 1989; Sundstrom and Johnsson, 2001; Brown and Laland 2011). In the first experiment the opposite of the above was studied, i.e. whether naive/pond reared pikeperch yearlings learn to accept dry feed from feed consuming trainer fish in artificial conditions.

Our second approach was to modify the pellets to imitate natural food. Many studies show that some properties of the food particles play an important role in the weaning process of fish. These are the following: colour (Ginetz and Larkin, 1973; Willis and Flickinger, 1981; Masterson and Garling, 1986), shape (Stradmeyer, 1989), texture (Kubitza and Lovshin, 1999; Ottera et al., 2003) and taste (Kubitza et al., 1997; Kolkovski et al., 2000; Kasumyan and Doving 2003). Colour, shape and taste are the easiest to modify, without decreasing the shelf life of the feed. Moisturized feed is less stable over time (Kubitza and Lovshin, 1999). In the second experiment, the effect of two of the above properties, the taste and the shape of the pellet, was studied aiming to increase the weaning rate with the modified taste and shape feeds to a percentage acceptable for farming purposes (>40-50%).

MATERIALS AND METHODS

Origin of fish and conditions of keeping

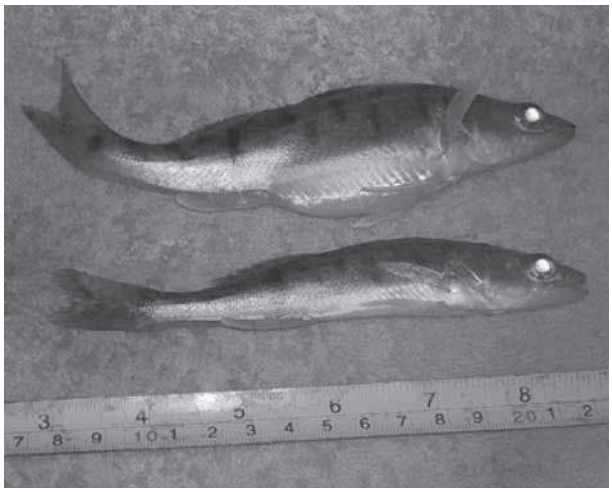
The fish (340 specimens) arrived at the fish laboratory of the Georgikon Faculty at the University of Pannonia on 10 October, right after the fish farm harvest. Each fish was measured for total length (14.82 $\pm$ 1 cm), weight (23.7 $\pm$ 1.05 g) and given a bath treatment (20 g NaCl/ liter of water, 10 minutes) for removing any possible parasites. Fish were then randomly distributed between 9 aquaria, with 30 fish in each. The rest remained as reserve in a separate tank. On the first day, the aquaria were covered with black plastic which was removed after 2 days. The light conditions were adjusted according to Luchiari et al. (2009) and Kozlowski et al. (2010) using dark and dim conditions. For that red light bulbs above the aquaria were used. The light period was 16 hours and the dark period was 8 hours. The salinity of the water was maintained between 2-3 ppt, because it helps prevent the outbreak of parasites (Németh et al., 2013) and promotes faster recovering from acute stress (Barton and Zitzow, 1995). The first nine days served as an acclimatization period to allow fish to adapt to the new environment. During these days, the feeding was low; fish only got 30 grams of frozen water fleas (*Daphnia magna*) in each tank daily. This was done

in order to teach them that the feed will come from the surface of the water.

Both experiments were conducted in the same recirculation system (total volume 4500 l) with 9 aquaria (300 l each). For disinfecting the water 2 UV lamps (18 W each) connected in series were used. The water flow was set to 10 $l.min^{-1}$ per tank. The fish were kept with a grid close to the inlet of the tanks to maintain the 20 $kg.m^{-3}$ stocking density, which proved to be the best according to our previous experience in fish tanks.

In both experiments, each fish tank was siphoned daily to remove the remaining uneaten feed or excrement particles. Total length and body weight of each fish was measured at the start and end of the experiments with 0.5 cm and 0.1 g accuracy, respectively. Oxygen levels and water temperature were recorded daily.

The success of weaning was easily determined by visual observation. See Picture 1.



Picture 1. Weaned fish vs. un-weaned fish

Experiment I. - The effect of trainers

During the 9-day acclimatization, 21 individuals died due to post harvest injuries. The dead fish were replaced from the reserve stock. At the beginning of the experiment, the length and weight of individual fish were measured again. Three treatments with three replicates were set.

In treatment (PST) ten - formerly weaned on dry feed – pikeperch were added as trainers to the naive ones. In treatment (PPT) ten perch (*Perca fluviatilis* L.) – formerly weaned on dry feed – were added to the naive ones, while in the control (CON) ten new naive pikeperch from the reserve supplemented the number (Table 1).

The experiment lasted for 29 days. In the first 3 days the amount of water fleas was decreased and the amount of feed increased (1/4, 2/4, 3/4, daphnia/feed ratio). The fish were fed with clockwork belt feeders over 12 hours from 8 am until 8 pm. The feeding ratio was 4% of the body weight daily, resulting in that fish were over fed for the interest of the weaning period. The feed used was from Skretting, sized 1.7 mm (49% protein, 20% fat).

Experiment II. - The effect of modified feed

The second experiment was aimed at enhancing the weaning of pikeperch directly with dry feed, which was modified in shape and taste. Before applying dry feed, the non-weaned fish from the previous experiment were conditioned by feeding bloodworm (*Chironomus* sp.) 3 times (50 grams/aquaria each time) a day for 12 days. At the end of this conditioning period, fish were individually measured and randomly distributed into 9 aquaria (Table 2) again. The experiment lasted for 21 days.

Table 1. Average weight at the arrival and start of Experiment I
(CON) – control – no trainers added; (PST) – groups with pikeperch trainer; (PPT) – treatment where perch was the trainer

Treatment	Type of fish	Number/ aquaria	Weight at arrival (g) (Mean + SD)	Weight at the start of the experiment (g) (Mean + SD)
CON (no trainers added)	**Wild pikeperch**	40	24.1 + 5.3	22.1 + 5.5
PST (with Sander trainers)	**Wild pikeperch**	30	24.0 + 5.8	22.1 + 5.3
	Teacher pikeperch	10	-	23.7 + 4.8
PPT (with Perca trainers)	**Wild pikeperch**	30	23.3 + 4.6	21.6 + 4.2
	Teacher perch	10	-	12.6 + 3.8

Table 2. The initial weight of fish at the start of Experiment II

Treatment	Number/ replicates	Initial weight (g)
Control (C)	30	20.4 + 4.9
Bloodworm treatment (B)	30	19.8 + 4.3
Aroma (F)	30	20.6 + 4.4

Three treatments were compared in 3 replicates:

- (C) Long thin pellets with commercial dry feed taste - commercial feed with modified shape,
- (B) Long thin pellets with bloodworm taste - commercial feed with modified shape and bloodworm taste,
- (F) Long thin pellets with commercial aroma made to catch predatory fish (Dynamite Baits – Killer fish scent - DYNAMITE BAITS, The Development Centre, Fosseway, Cotgrave, Nottingham, NG12 3HG) – commercial feed with modified shape and artificial aroma.

The compositions of the feeds are given in Table 3. In each feed, commercial feed (49% protein, 20% fat) was grounded as meal and mixed with egg protein powder. The mixture was moisturized by water in order to be able to form bloodworm-shape particles. The form (1.5 mm diameter and 1.0-1.5 cm in length) was given by using a pasta machine. Freshly pressed semi-moisture feed was gently dried for 6 hours at 50 °C and sprayed with the taste modifier so that the taste molecules remained on the surface of the feed. Ten minutes after spraying – letting the spray absorb into the external layer – the feeds were kept in plastic bags in small portions at -15 °C until use.

Table 3. Composition of the feeds – Exp. II

Feed composition	Commercial feed (C)	Bloodworm flavor (B)	Aroma flavor (F)
Feed preparation:			
Feed (g)	2000	2000	2000
Egg powder (g)	100	100	100
Water (l)	0.6	0.2	0.45
Flavor (mixed with water) (l)	-	0.4	0.15
Flavor sprayed on the feed:			
Water (l)	0.2	0.1	0.1
Flavor (mixed with water) (l)	-	0.1	0.1

Before filling up the belt feeders, the feed was warmed in small bags to prevent vapor. The feeding was done by clockwork belt feeders over 12 hours. The feeding started at 8 am. Daily feeding ratio was 4% of body weight.

Statistical data analysis

To detect difference between treatments, the non-parametric Kruskal-Wallis test was used. The minimum levels of significance were set at $P<0.05$.

Windows Office Excel 1997 was used for organizing data. Basic statistical results were calculated with Excel software. Statistical tests were done using IBM SPSS 20.0.

RESULTS

Experiment I – The effect of trainers

During the experiment, temperature (22.6 ± 1.1 °C) and oxygen (7.73 ± 0.7 mg/l) saturation were at acceptable levels and were maintained constantly. There was no mortality in any of the experimental groups.

Figure 1 shows the average initial and final weight of the fish resulted by the different treatments. Please note that data of the trainer fish were treated separately. In each naive group the weight loss was more or less the same, the small differences were caused by the weight of the newly weaned pikeperch. In each treatment each trainer fish fed normally during the experiment. The pikeperch eating dry feed grew 17.9 g on average over the 29 days (1.94% SGR=(Ln(41.6)-Ln(23.7))/29*100). Perch trainers had a weight gain of 10.8 g and the SGR was 2.13% (=(Ln(23.4)-Ln(12.6))/29*100).

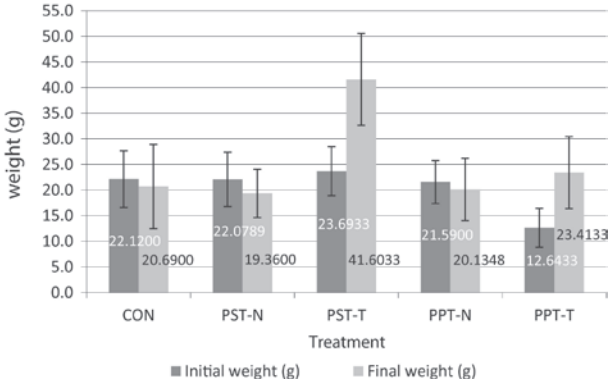


Fig 1. The average initial and final weight of the fish in different treatments in Exp. I

((CON) – control – no trainers added; (PST-N) naive pikeperch of the PST treatment where pikeperch was the trainer; (PST-T) – the trainers of PST groups, (PST-N) – naive pikeperch of the PPT treatment where perch was the trainer; (PE-T) – teachers of the PPT groups))

There were 10 weaned fish in the control treatment, 2 in the pikeperch treatment and 7 fish in the perch treatment. Figure 2 shows the resulted weaning rates. In the PST treatment, 2.2% of the fish got weaned in all 3 replicates. This was the worst result. The best weaning rate was in the control group (8.3%). The PPT treatment was slightly lower, 7.8%. None of the differences were significant at p<0.05.

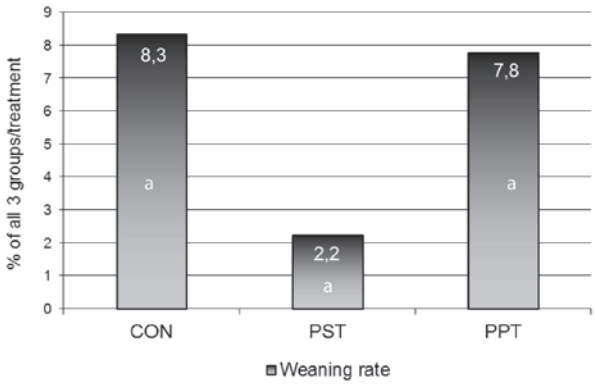


Fig 2. Weaning rates between the treatments in Exp. I

((CON) – control; (PST) – treatment with pikeperch trainers; (PPT) – treatment with perch trainers; same letters indicate that there is no significant difference between means (Pb<0.05))

Experiment II - The effect of bloodworm like feeds

During the second experiment, the temperature was higher at 0.6 °C (23.2 ± 0.5 °C) and thus the oxygen saturation (7.35 ± 0.54 mg/l) was lower. Nonetheless, each parameter was at acceptable levels and was constantly maintained. In this experiment, mortality, which was caused by starvation, was present in each treatment. During the first 50 days (including the first 9 days of acclimatization, 29 days of the first experiment and 12 days of conditioning) the non-weaned fish lost 5.4 grams (22% of their initial body weight), even with the 12 days of bloodworm feeding between the two experiments. At the end of the experiment, the fish were very weak. In the C treatment there was 4.4%, in the B there was 5.6% and in the F treatment there was 7.5% mortality.

Figure 3 demonstrates the average initial and final weight of the fish between treatments. In the C treatment there was the highest weight loss (2.1 g). In the F treatment, on average there was a slight growth (0.1 g). In the B treatment there is also a slight decrease in weight (0.5 g).

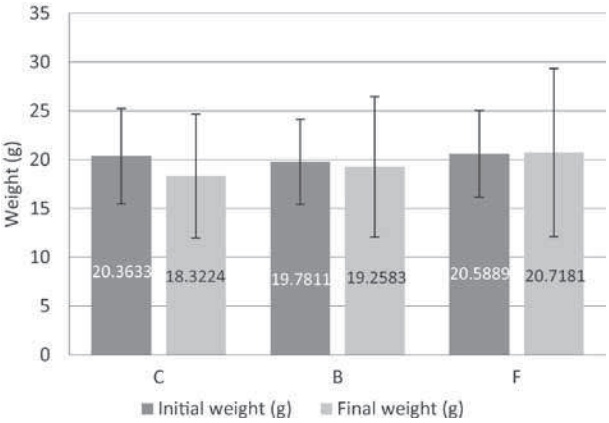


Fig 3. The average initial and final weight of the fish in different treatments in Exp. II

((C) – commercial feed; (B) – bloodworm; (F) – commercial flavor aroma))

In the C groups there were 4, in the B groups there were 11 and in the F groups there were 15 weaned fish. The weaning rates are shown in Figure 4. The highest rate was in the F treatment with 16.7%, the lowest was in the C treatment (4.4%) which showed significant differences (<0.008). The B treatment resulted in 12.2% between other treatments without statistically proved difference.

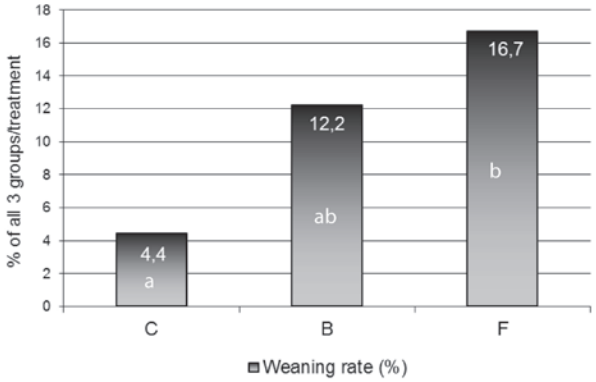


Fig 4. Weaning rates between the treatments in Exp. II

((C) – commercial feed; (B) – bloodworm; (F) – commercial flavor; different letters indicate a significant difference between means (Pb<0.05))

DISCUSSION

Experiment I – The effect of trainers

Our results in this experiment showed that pikeperch is not able to learn to eat dry feed by social learning. In some cases, with addition of trained pikeperch, negative effects on the weaning success were found. The possible explanation is that

the trained fish continuously grew and increased in size, but the non-feeders did not, and even got weaker. Probably the social stress from the trainers towards the non-feeders was stronger in these groups.

In the groups where the perch trainers were present, the weaning rate didn't differ much from the control groups. It was still slightly lower, meaning that social learning of this ability cannot be done from other fish species in the case of pikeperch either (20-30 g).

Experiment II - The effect of modified feed

In this experiment our results showed that by enhancing the atractiveness of feed in taste or shape, the weaning success of pikeperch can be enhanced. Interestingly, the bloodworm favored feed did not have the best effect. However, this was expected as between the two experiments the fsh were fed with bloodworms.

In the groups where the feed enhanced with the commercial aroma was used, the best effect was achieved, which was a surprise. Unfortunately, we are not familiar with the content of the commercial feed atractant as it was not provided by the supplier.

Experiment I-II

If the results of the two experiments are added together, the best weaning rate (25%) of the same stock was achieved in Experiment I - in the control groups with normal commercial feed (8.3% weaning rate) and plus in Experiment II - in A treatment with longthin pellets with commercial aroma taste (16.7% weaning rate). Based on these results, direct weaning had a very low success rate, compared to Ljunggren et al. (2003) who succeeded in weaning 70% of the fish by direct weaning, though the experiment was done with juveniles. Compared to the results of Zienert and Heidrich (2005) whose experiment was done with the same autumn pikeperch our results are also much lower. They got a weaning rate of 80% of the stock; however, weaning was done by mixing bloodworm with the feed during their trials, which is known to be one of the best weaning methods for pikeperch. They also tried direct weaning and received 10% of the weaning rate, which is close to our results in the control treatment of the first experiment. This 10% could be increased with aromatic substances, based on the outcome of the second experiment in the treatments B and F. Even then the weaning rate was still low and economically questionable.

If the trainer fish had been smaller or the trained fish been removed after two weeks in the first experiment where pikeperch was the trainer, it would be possible to have better weaning rates, by reducing the social stress caused by the stronger dominant weaned pikeperch during the weaning.

As a main conclusion, the success of direct weaning of pikeperch could not be enhanced drastically with the two methods used. Negative non-signifcant effect of co-rearing with eaters and a positive efect of feed with an enhanced taste and shape was found. With these results, there can be an aromatic substance which enhances the weaning rate of pikeperch, hopefully without changing the consistency of the feed.

Further investigations will continue to find a feed atractant that increases the weaning rate to higher levels.

Sažetak

USPOREDBA UČINKOVITOSTI KORIŠTENJA RIBA-TRENERA I PROMJENE OKUSA I OBLIKA PELETA U NAVIKAVANJU JEDNOGODIŠNJEG SMUĐA (*Sander lucioperca* L.) NA SUHU HRANU

U dva uzastopna pokusa uspoređene su dvije praktične metode usmjerene na unapređenje učinkovitosti navikavanja jednogodišnjeg smuđa (*Sander lucioperca* L.), uzgojenog u ribnjaku, na suhu hranu. Prva je metoda primjena riba-trenera, hranjenih peletama, za neiskusne ribe. Kao ribe-treneri korišteni su smuđ i grgeč (*Perca fluviatilis* L.). Tretmani i kontrola (bez riba-trenera) izvođeni su u tri primjerka (u 9 akvarija, 40 riba po akvariju). Jednogodišnji smuđ nije prihvatio suhu hranu primjenom "socijalnog poučavanja". Štoviše, prisutnost riba-trenera smanjila je uspješnost navikavanja neiskusne mlađi, ali tek neznatno (2,2% sa smuđem kao trenerom; 7,8% s grgečem kao trenerom; 8,3 % samo s neiskusnim ribama).

U drugoj metodi je izmijenjen okus i oblik peleta za hranidbu. Pelete u obliku zrnaca su smrvljene i preoblikovane u "umjetne krvne crve" (ABW). Krvni crvi su začinjeni mirisom trzalaca ili komercijalnom aromom. Nezačinjeni krvni crvi korišteni su kao kontrolna skupina. Krvni crvi začinjeni komercijalnom aromom značajno su povećali postotak naviknute ribe (16,7%) u odnosu na one začinjene mirisom trzalaca (12,2%) i one nezačinjene (4,4%).

Ključne riječi: smuđ, "socijalno poučavanje", navikavanje, suha hrana

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