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EFFECTS OF STOCKING EARTHEN PONDS WITH PIKEPERCH (*SANDER LUCIOPERCA* (L.)) FINGERLINGS REARED IN RECIRCULATING AQUACULTURE SYSTEMS – EFFECTS OF FISH SIZE AND THE PRESENCE OF PREDATORS

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Abstract

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The aim of the study was to determine the effectiveness of stocking earthen ponds with pikeperch fingerlings reared in recirculating aquaculture systems (RAS). Three “natural” ponds with predators (pike – Pd ponds) and three ponds in which the pikeperch were reared in a monoculture (M ponds) were used. All of the ponds were also stocked with ide, tench and crucian carp (food base for the predatory fish). Each of the ponds was stocked with three pikeperch size groups: fish with body weights (b.w.) of 3.3 g, aged 102 days post hatch (DPH) (group S); b.w. 6.4 g, aged 128 DPH (group M); b.w. 25.4 g, aged 143 DPH (group L). Prior to stocking, each pikeperch size group was tagged with a different colored Visible Implant Elastomer (VIE). The fish were harvested from the ponds in early October. Neither pond type (M or Pd) nor the size/age of the pikeperch was noted to have impacted the final fish b.w. or condition, but both the pond type and the size of the fish impacted increases in stock biomass (MANOVA, $P < 0.05$). Significant differences were noted with regard to survival (Pd ponds – mean 29.7%; M ponds – 81.6%) and fish body deformations of the gill cover and jaw (group S – 14.1%, group M – 14.0%, group L – 31.2%). The results indicate it is more advantageous to stock with material that is smaller in size (b.w. 2–4 g). Tagging juvenile pikeperch with VIE was noted to be highly effective (retention 99.7–100%).

Key words: pikeperch, *Sander lucioperca*, pond stocking, RAS, fish size

Introduction

Fish production in recirculating aquaculture systems (RAS) is developing in two directions. The first is the production of consumer sized fish, while the second is the production of stocking material for use in other systems (e.g., pens, ponds), or for release into natural water bodies (Badiola et al., 2012). Fish production in RAS is one sign of development in the aquaculture sector, while another is the diversification of culture in these types of systems by the introduction of new species. This group includes, among others, the Percidae including pikeperch (*Sander lucioperca* (L.)). Intensive methods for producing this species in RAS, which has been under development in recent years, is particularly promising (Philipsen, 2008; Zakęś, 2009; FAO, 2012). Pikeperch stocking material reared in RAS are used increasingly to stock lakes and rivers (Zakęś, Z., unpubl. data). Traditional methods for producing stocking material of this species are ineffective and production

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is not predictable (Steffens et al., 1996; Zakęś, 2009). The necessity of restocking open waters with this species stems from, among other factors, substantial fluctuations in the abundance of subsequent year classes (Colby and Lehtonen, 1994). Great interest from both recreational and commercial fisheries means that this species is included in restocking programs in many countries (e.g., Ruuhijärvi et al., 1996; Abdolmalaki and Psuty, 2007; Mickiewicz and Wołos, 2012).

Studies that aim to determine the effects of restocking open waters with material produced in RAS are of great scientific and practical significance. The initial stage of determining the usefulness of this type of material could be stocking into earthen ponds. Studies of pike (*Esox lucis* L.) produced in RAS confirmed the suitability of this type of material for stocking earthen ponds. These studies also indicated that the effects of such restocking depend on the age/size of the material released into ponds (Szczepkowski et al., 2012). Another very important element of conducting studies on restocking effectiveness is developing reliable fish tagging methods that permit collecting empirical data. Several tagging methods tested on juvenile pikeperch were not found to be suitable for wider application; these have included hot branding, dye marking (for example with alcian blue), and internal and external tags (Saura, 1996; Hansson et al., 1997; Hopko et al., 2010). Interesting results were obtained when tagging pikeperch with coded-wire tags (CWT) and visible implant elastomer tags (VIE), (Zakęś et al., 2013), and the latter was found to be very effective for short-term tagging (retention of up to six months) of pikeperch released into earthen ponds (Zakęś, Z., unpubl. data).

The aims of the study were as follows: (I) to determine the effectiveness of stocking earthen ponds with three size groups of pikeperch reared in RAS (mean body weight 3.3, 6.4, 25.4 g); (II) to analyze the impact of the presence of predatory fish (pike) on the effects of stocking; (III) to determine the effects of tagging juvenile pikeperch with VIE.

Materials and Methods

Fish and tagging

The study material was obtained from reared pikeperch spawners held in RAS at the Inland Fisheries Institute in Olsztyn (northern Poland; IFI Olsztyn). Out-of-season spawning was conducted according to procedures developed previously (Zakęś and Szczepkowski, 2004; Zakęś, 2007; Zakęś and Demska-Zakęś, 2009). For the first three weeks of rearing in RAS, the pikeperch larvae were fed formulated feed and brine shrimp cysts (*Artemia* sp.). After this, the fish were fed only formulated feed according to procedures developed previously (Szkudlarek and Zakęś, 2007).

During the pikeperch rearing period in RAS, fish samples were chosen at random for tagging. The fish were tagged with visible implant elastomer (VIE), (Northwest Marine Technology, Shaw Island, WA, USA (NMT)). The VIE tags, a viscous polymer with a curing agent, were implanted with a Manual Elastomer Injector with a 0.3 ml syringe according to procedures described in an earlier paper (Zakęś et al., 2013). During tagging, the fish were anesthetized with etomidate in an aqueous solution (Propiscin, IFI Olsztyn) at a dose of 1.0 ml.l^{-1}. Three age/size groups of fish were tagged:

– group S (3 000 individuals), mean body weight (b.w.) 3.3 g, age 102 days post hatch (DPH);

– group M (2 250 individuals), b.w. 6.4 g, age 128 DPH;

– group L (1 500 individuals), b.w. 25.4 g, age 143 DPH (Table 1).

Each fish size group was tagged with a different colored VIE tag: group S – blue; group M – pink; group L – green. After tagging, each fish size group was held for three days in RAS following standard rearing procedures (Zakęś et al., 2013). Then the fish were starved for 24 h, following which they were placed in polyethylene bags (20 l water + 20 l oxygen) and transported to earthen ponds (Berka, 1986). Approximately 500 individuals were in each bag, and transport time was 20 min. The water temperature during transport was adjusted to that in the ponds on the day they were stocked. In May, this was 15.5°C, and in June and July it was 20.5 and 21.5°C, respectively (Table 1).

Earthen ponds and stocking

Stocking material was released into six earthen ponds, each with a surface area of 0.5 ha and a depth of 1.8 m at the outlet box. The ponds were inundated in early April and stocked with six spawning pairs of tench (*Tinca tinca* (L.)), eight pairs of crucian carp (*Carassiuss carassiuss* (L.)), and ide (*Leucis cusidus* (L.)) spawn obtained from three spawning pairs. The ponds were divided into two groups. Three were stocked with three pikeperch size groups (M ponds), while a further three were stocked with pikeperch and pike (Pd ponds). No vascular vegetation occurred in the M ponds, while the shore zone of the Pd ponds was overgrown with common reed (*Phragmites communis*) (the width of the reed strip was up to 2 m). Each of the six ponds was stocked with a total of 1125 pikeperch individuals (2250 fish.ha^{-1}). Pikeperch stocking was conducted in three periods from May 22 to July 2 (Table 1). Pike was stocked into the Pd ponds in mid April at a density of 30 individuals.pond^{-1}. This material had body weights of 0.2 g and had been reared from larvae in a RAS (Szczepkowski, 2009). The fish were harvested in a harvesting pen located behind the outlet box when the ponds were drained in October (Table 1).

Table 1

Characteristics of juvenile pikeperch reared in RAS used in the experimental stocking of earthen ponds

	Fish size group		
	group S	group M	group L
Pond stocking date	May 22	June 17	July 2
Fish age (days post hatch)	102	128	143
Body weight (g)	3.3 ± 0.06	6.4 ± 0.09	25.4 ± 0.13
Stocking density (individual.ha^{-1})	1000	750	500
Visible Implant Elastomer tag color	blue	pink	green
Harvest date		October 8	

Measurements, calculations and statistical analysis

Individual pikeperch body weight (b.w. ± 0.1 g) and standard body length (SL ± 0.1 cm) were measured on the day the fish were tagged (100 fish from each size group) and after they had been harvested (150 fish, 50 fish from each size group (after they were identified by their VIE tags)). The VIE tags were read before the fish were stocked into the ponds (fish transport) and after harvesting them from the ponds. During these manipulations, the fish were anesthetized with etomidate in an aqueous solution (Propiscin, IFI Olsztyn) at a dose of 0.5 ml.l^{-1}. A dedicated UV flashlight (NMT) was used to identify the VIE tags. The tags were identified after the fish had been removed from the water. A tag was considered visible if any or all fragments of it were visible.

The data collected was used to calculate the following for each pikeperch size group:

– biomass growth, B (kg.ha^{-1}) = (weight of harvested fish (kg) – weight of stocked fish (kg)) × pond surface area^{-1} (ha);

– condition coefficient, K = 100 × (body weight (g) × body length SL^{-3} (cm));

– fish survival, S (%) = 100 × (final number of fish (individuals) × initial number of fish^{-1} (individuals));

– percentage of fish with body deformations, D (%) = = 100 × (number of fish with deformations (individuals) × number of all fish^{-1} (individuals));

– tag retention; R (%) = 100 × (number of fish with tags on the final day of the experiment (individuals) × total number of fish on the final day of the experiment^{-1} (individuals)).

The data was analyzed statistically with the STATISTICA (StatSoft®, Kraków, Poland). Homogeneity of variance was tested with Levene's test. Prior to statistical analysis, percentage data were transformed using *arcsin*. Two-way analysis of variance (three fish size groups and two pond types) was applied. Differences were considered statistically significant at $P \leq 0.05$.

Results and Discussion

Short-term VIE tag retention was high at values ranging from 99.7 to 100%. The length of time between tagging and harvest from the ponds was as follows: S group – 140 days; M group – 114 days; L group – 99 days. All of the VIE tags, irrespective of color, were easy to identify using the UV flashlight. In earlier laboratory studies performed on three groups of pikeperch of similar sizes, VIE tag retention after 28 days was also very high at 100%. It was also noted that VIE tagging is minimally invasive and has no impact on either the growth or survival of juvenile pikeperch (Zakęś et al., 2013). Similarly high VIE tag retention was obtained with eel (*Anguilla anguilla* (L.)) after 183 days (Simon, 2007) and with Eurasian perch (*Perca fluviatilis* L.) after 125 days following tag implantation (Goldsmith et al., 2003). Generally, the VIE tagging system is considered to be safe, and it does not contribute to increased mortality among tagged fish (Astorga et al., 2005; Simon, 2007; Simon and Dörner, 2011). Astorga et al. (2005) report that VIE tagging of gilthead seabream (*Sparus auratus* L.) is effective for six to 18 months. Since VIE tag retention decreases three months after the fish are tagged, this method can be recommended for short-term fish tagging (Goldsmith et al., 2003; Soula et al., 2012). The current study indicated that VIE tagging is suitable for material stocked into earthen ponds for periods of several months.

Neither pikeperch age (size group) nor pond type (with or without predators) was noted to have had a significant impact on final body weight. In M ponds it ranged from 40.5 g (group S) to 47.7 g (group L), but in Pd ponds the mean final body weight of subsequent groups decreased and ranged from 45.2 g (group S) to 29.5 g (group L). The differences among the groups were not, however, statistically significant ($P > 0.05$; Table 1). The growth of pikeperch stocking material obtained from RAS in earthen ponds can be considered to be satisfactory, and it was higher than that of this species in traditional pond rearing (Zakęś and Szczerbowski, 1995; Steffens et al., 1996; Zakęś, 2009). Different results were

Table 2

Growth performance, condition factor, body deformities, and survival in three size groups of juvenile pikeperch reared in RAS (groups S, M, and L) and stocked into different ponds (M ponds and Pd ponds) (mean values ± SD) (see Table 1 and Material and methods section)

	Body weight (g)		Condition factor K		Stock biomass increase (kg.ha^{-1})		Body deformities (%)		Survival (%)	
	M ponds	Pd ponds	M ponds	Pd ponds	M ponds	Pd ponds	M ponds	Pd ponds	M ponds	Pd ponds
Group S (n = 3)	40.52 ±5.75	45.22 ±15.57	1.09 ±0.05	1.06 ±0.03	32.43 ±7.44	6.86 ±5.57	11.22 ±8.39	15.90 ±3.59	76.23 ±7.74	22.94 ±17.37
Group M (n = 3)	40.11 ±12.19	33.59 ±16.45	1.10 ±0.05	1.04 ±0.06	27.94 ±23.40	4.11 ±7.43	14.44 ±1.92	13.55 ±3.02	84.17 ±17.56	32.33 ±12.11
Group L (n = 3)	47.70 ±7.55	29.52 ±6.80	1.04 ±0.03	1.03 ±0.03	9.40 ±8.35	-5.10 ±4.47	34.44 ±8.35	27.94 ±9.87	84.33 ±14.96	33.73 ±18.40
MANOVA										
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Size (S)	0.4325	0.6586	2.7147	0.1065	3.9367	0.0484	16.9999	0.0003	0.8601	0.4476
Pond (Ps)	1.5035	0.2437	3.2327	0.0974	15.7069	0.0019	0.1964	0.6655	63.2400	0.0000
S × Ps	1.4758	0.2673	0.5229	0.6057	0.4080	0.6739	1.1177	0.3588	0.0142	0.9854

obtained in an experiment that studied the effectiveness of stocking earthen ponds with three size groups of juvenile pike reared in a RAS (initial b.w. – 1.5, 7.0, 18.5 g). In this instance, after the fall harvest, the mean pike body weight of the smallest group was nearly fourfold higher than that noted in the two other groups (85 *vs* 23 g), (Szczepkowski et al., 2012). This could indicate that older pikeperch stocking material (i.e., that which is held longer in RAS) adapts more readily to new environmental conditions than does pike.

The survival of subsequent pikeperch size groups stocked into M ponds was equal and was within the range of 76.2% (group S) to 84.3% (group L) ($P > 0.05$; Table 2).

The size of pike produced in RAS, at the moment of ponds stocking, was also not noted to impact survival significantly; however, the survival of these fish during fall harvesting was significantly lower (range 26–45%) than that of pikeperch (Szczepkowski et al., 2012, current study). Pikeperch survival in the Pd ponds was decidedly lower in all size groups (range 22.9–33.7%; Table 2). Notably, this is similar to the values obtained for pike (Szczepkowski et al., 2012). Statistical analysis (MANOVA) indicated the absence of a significant impact of initial pikeperch size on survival ($P = 0.4476$), while pond type (M *vs* Pd ponds (additional presence of predators)) had a highly significant impact on the value of this index ($P = 0.0000$; Table 2). It should be noted here that from each of the Pd ponds 10 to 12 pike individuals with individual b.w. 220–420 g were harvested. Comparing pikeperch survival in the M and Pd ponds permitted drawing conclusions about the cannibalism of this species to a certain degree. High values of pikeperch survival in M ponds indicated that this phenomenon increased slightly. This could have stemmed from the stocking densities applied, which, in the end (group S + group M + group L) was 23 individuals.100 m^{-2} pond. This also indicates the necessity of providing the appropriate food base for the pikeperch, which prefer, like pike, fish-prey (cyprinids), (Hart and Hamrin, 1988). The phenomenon of cannibalism was also not confirmed when earthen ponds were stocked with pike produced in RAS (Szczepkowski et al., 2012). The effect of density in this species is very apparent. For example, Sutela et al. (2004) conclude that cannibalism among pike in lakes is not observed at a density of up to 4.7 individuals of pike.100 m^{-2}, but it did occur at abundances of 6.8 to 13.3 individuals of pike.100 m^{-2}. It cannot be ruled out that in our study cannibalism among pikeperch occurred only during the initial period following pond stocking, especially since the stocking density applied of 23 individuals pikeperch.100 m^{-2} was higher than that recommended by Wright and Giles (1987) of 5 individuals.100 m^{-2}, which did not lead to losses from cannibalism. It should be noted that similar densities to those in our study were applied when stocking earthen ponds with pike material reared in RAS and that losses to cannibalism were also small (Szczepkowski et al., 2012). The limited losses from cannibalism in material reared in RAS could also stem from the fact that this material was of similar size, which does not support the occurrence of this phenomenon. It should be noted, however, that three pikeperch sizes were released into each pond (Table 1), and it is not known what the relationships were between the fish sizes that had already been stocked (e.g., group M *vs* group S, or group L *vs* groups M and S) or what the interactions were among subsequent size groups of fish introduced to the ponds.

The large differences in pikeperch survival in M and Pd ponds are interesting (mean of approximately 81 *vs* 30%). High pikeperch survival in M ponds indicates that fish-prey abundance was adequate (a similar complement of fish-prey was applied in the Pd ponds). Additionally, during the fall harvest, a large number of potential fish-prey was harvested from both M and Pd ponds. This could indicate that in the Pd ponds pike preferred pikeperch as its prey. This is obviously just a hypothesis that requires empirical verification, but the results obtained indicate there is significant antagonism between these two predatory fish species. This fact is confirmed by studies conducted in natural conditions in the littoral zone, which is the habitat of juvenile stages of these fish (Łapińska et al., 2001)

Fish size was noted to have a significant impact on the biomass increase on the different pikeperch groups ($P = 0.0484$; Table 2). Fish biomass increase was inversely proportional to fish size, and in M ponds it ranged from 32.43 $kg.ha^{-1}$ (group S) to 9.40 $kg.ha^{-1}$ (group L). The final pikeperch body weight in the different groups was comparable; thus, the value of this indicator depended on the initial fish body weight of the different fish groups and, of course, on survival (Tables 1 and 2). The impact of pond type (M *vs* Pd) had a much more significant impact on this indicator ($P = 0.0019$; Table 2). Low biomass growth must be linked with lower survival stemming from predation and/or cannibalism. Szczepkowski et al. (2012) studied the effectiveness of stocking ponds with three size groups of pike reared in RAS, and only in the group of the smallest fish (initial b.w. 1.5 g) was positive fish biomass growth noted (37 $kg.ha^{-1}$), while values for groups of larger fish (initial b.w.7.0 and 18.5 g) were negative. In this instance, this effect was explained as stemming from differences in growth rates among pike of different size groups. The hypothesis was also put forward that it could have been linked with the difficulties of larger pike, which had been reared in RAS on formulated feed, adapting to consuming live feed, i.e., fish-prey (Szczepkowski, 2009; Szczepkowski et al., 2012). Our observations conducted in both RAS and under natural conditions indicate that among predatory fish species reared in RAS pikeperch best retain the predatory instinct, followed by pike, and then wels catfish (*Silurus glanis* L.), (Zakęś, Z., Szczepkowski, M, unpubl. data). The results of the present study also indicate that pikeperch is a highly plastic species that is adept at adapting to new environmental and feeding conditions.

Body deformations (jaw and gill cover) were noted in all pikeperch size groups, but among fish that had been reared for longer periods in RAS (group L) the percentage share of such individuals was significantly higher ($P = 0.0003$; Table 2). The cause of these anomalies should be sought during the beginning period of rearing fish in RAS. Although, it should be mentioned that body deformations are also noted in pikeperch in the wild, for example in lake populations (Matylla, 1993). The etiology of these anomalies is not fully understood, and some of the factors that are linked with the occurrence of body deformations include genetic disruptions, improperly balanced diets, and disadvantageous environmental conditions. It has been demonstrated that environmental and genetic factors in the early stages of ontogenetic development significantly impact the meristic characters of fish (Favaloro and Mazzola, 2000). However, when producing fish in RAS the most important factor is that of nutrition. Metabolic disturbances (e.g., abnormalities of calcium-phosphate homeostasis) resulting from deficits of calcium, phosphorus, vitamin D, and polyunsaturated fatty acids (PUFA) are dangerous especially during the ossification of cartilage (e.g., Cahu, et al. 2003; Kamler et al., 2008). Body deformation has also been noted in pike reared in RAS, and the share of fish with developmental anomalies was positively correlated with the length of time the fish were held in RAS (Szczepkowski et al., 2012).

Conclusions

The results of the study support the conclusion that pikeperch material produced in RAS can be used to stock earthen ponds. While these results are of a contributive character, they do confirm to a degree that pikeperch reared in RAS are also suitable for restocking open water bodies (Zakęś, Z., Szczepkowski, M., unpubl. data). While this study demonstrated that stocking can be done with various types, or ages/sizes, of pikeperch reared in RAS, because of the costs of rearing fish in RAS, it is recommended to stock with material that is reared for shorter period in these types of systems (b.w. 2–4 g). Using this size fish is also advantageous in terms of its higher biological quality (fewer individuals with body deformations). The suitability of the VIE tagging method (minimally invasive and high tag retention) for short-term pikeperch tagging, e.g., during rearing in earthen ponds, was also confirmed. The predatory pressure of other fish species on the final results of pikeperch rearing in earthen ponds was demonstrated. Future studies should focus on improving the biological quality of larger/older forms of pikeperch stocking material produced in RAS. It would also be advantageous to determine the impact of stocking density on juvenile pikeperch in ponds on losses from cannibalism and predation by other fish species (e.g., pike). Conducting work to determine the effectiveness of stocking open waters with pikeperch produced in various rearing systems, including RAS, is also necessary.

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