Leibniz-Institute of Freshwater Ecology and Inland Fisheries

Length rather than year-round spawning, affects reproductive performance of RAS-reared F-generation pikeperch, *Sander lucioperca* (Linnaeus, 1758) – Insights from practice

Fabian J. Schaefer https://orcid.org/0000-0003-3699-4791, Julia. L. Overton, Werner Kloas and Sven Wuertz

DOI
doi:10.1111/jai.13628

Original publication date
3 February 2018 (Version of record online)

Document version
Accepted version

Published in
Journal of Applied Ichthyology

Citation
Schaefer FJ, Overton JL, Kloas W, Wuertz S. Length rather than year-round spawning, affects reproductive performance of RAS-reared F-generation pikeperch, Sander lucioperca (Linnaeus, 1758) – Insights from practice. Journal of Applied Ichthyology. 2018;34(3):617-21.

Length and not year-round spawning, affects reproductive performance of RAS-reared F-generation pikeperch, *Sander lucioperca* (Linnaeus, 1758) – Insights from practice

Running title: Reproduction of F-generation pikeperch

Fabian J. Schaefer[1,2*], Julia L. Overton[3], Werner Kloas[1,2,4], Sven Wuertz[1,2]

[1] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Ecophysiology and Aquaculture, Müggelseedamm 310, 12587 Berlin, Germany

[2] Humboldt-Universität zu Berlin, Faculty of Life Sciences, Thaer Institute of Agricultural and Horticultural Sciences, Invalidenstraße 42, 10115 Berlin, Germany

[3] AquaPri Denmark A/S, Lergårdvej 2, Egtved, Denmark

[4] Humboldt-Universität zu Berlin, Faculty of Life Sciences, Department of Biology, Invalidenstraße 110, 10115 Berlin, Germany

* Corresponding author: Fabian J. Schaefer Email: schaefer@igb-berlin.de

Summary

The continuous production of large numbers of high quality gametes is essential for aquaculture, particularly in candidate species, such as pikeperch, *Sander lucioperca* (L.). The common practice of year-round reproduction is under suspicion of inflicting adverse effects on the quality of the gametes through the disturbance of endogenous rhythms. We hypothesized that such perturbation does not affect RAS-reared F-generation broodstock. Reproductive performance (number of eggs) and gamete quality (fertilization and hatching rate) were assessed over the course of three years covering six independent, photothermal shifted spawning seasons in a commercial pikeperch hatchery (n = 31 egg batches of F-generation fish in total). No substantial differences in fertilization or hatching rates could be detected between the individual spawning seasons. Fecundity varied, but there are indications for a size effect on female fecundity with intermediate sized females producing higher number of eggs (~65 – 70 cm). Low egg quality could be detected in batches of very large fish. In conclusion, size-specific broodstock composition, but not year-round reproduction of F-generation pikeperch spawners affects the reproductive performance.

Keywords: egg quality; fertilization; oocyte; out-of-season; sperm quality

1. Introduction

The continuous production of large numbers of high quality gametes is essential for aquaculture and often constitutes the first major bottleneck, particularly in candidate species such as pikeperch, *Sander lucioperca*. Protocols for the reproduction of pikeperch in recirculating aquaculture systems (RAS) have been established, which are applied on a commercial level (Fontaine et al., 2015; Żarski et al., 2015). Through the photothermal control of sexual maturation and parallel rearing of several broodstocks, reproduction of pikeperch can be achieved in RAS at any given time of the year (Hermelink et al., 2011, 2013; Żarski et al., 2015), with or without the use of hormone treatments (Müller-Belecke et al., 2008; Zakęś & Demska-Zakęś, 2005). Maturation is induced once per year in every broodstock, but the timing is shifted to achieve year-round supply of larvae. In contrast, these freshwater percids reproduce only during spring under natural conditions (Lappalainen et al., 2003). Still, similar to other emerging cultured fish, the reproductive performance (number of fertilized eggs, hatching success) is highly variable in pikeperch spawners and supply of stocking material is presently often insufficient and inconsistent (Overton et al., 2015; Schaerlinger & Żarski, 2015).

Various factors affecting the reproductive performance of fish in aquaculture have been identified, including fish size and condition, hormone treatment, broodstock nutrition or handling stress (Brooks et al., 1997; Izquierdo et al., 2001; Bobe & Labbé, 2010; Migaud et al., 2013; Valdebenito et al., 2015). It is very likely that reproduction of pikeperch is similarly compromised by these factors, especially since this species has yet to overcome candidate status. For example, there are no species-specific broodstock diets available and selective breeding programs are just being launched. While differences in gamete quantity and quality have been mainly attributed to broodstock rearing and maternal characteristics, it remains unknown to what extent these parameters might change in response to a disturbance of endogenous rhythms. Similar to other species, the neuroendocrine regulation of reproduction in pikeperch is triggered by environmental factors, most importantly temperature and – to a certain extent – photoperiod (Hermelink et al., 2011, 2013). The regulation of environmental cues, as commonly practiced to achieve year-round spawning, may result in such a disturbance affecting the homeostasis (endocrine system, metabolism) and subsequently modulate the reproductive performance (Brooks et al., 1997; Schaerlinger & Żarski, 2015).

Interestingly, knowledge of the consequences of this aquaculture practice on the reproductive performance is limited. Only a few studies comprehensively addressed the effects of year-round on egg quality in temperate fish species and this has yet not been evaluated in pikeperch. To date, studies on the effects of photothermal manipulation on gamete quality in pikeperch mainly addressed wild-caught broodstocks, which are adapted to the natural cycle (e.g., Khemis et al., 2014; Müller-Belecke et al., 2008; Zakęś & Demska-Zakęś, 2005). There are indications that shifting the reproductive season through such manipulation has adverse effects on the reproduction of wild fish, for example in Eurasian perch, *Perca fluviatilis* (Żarski et al., 2011). We hypothesized that year-round reproduction does not affect reproductive performance of RAS-hatched F-generation spawners, which are not adapted to a natural photothermal cycle, but rather to continuous high temperatures suppressing gonad maturation during grow-out (Hermelink et al., 2011, 2013). To gain insights from practice on how environmental manipulation affects broodstocks, this hypothesis was tested during routine reproduction at a commercial pikeperch farm.

2. Materials and Methods

2.1 Animal rearing and reproductive protocol

Four separated broodstocks (mixed sex) were kept on the site (AquaPri, Egtved, Denmark). Maturation was induced by wintering below 14 °C for 12 weeks and subsequent warming to ~16 °C to trigger ovulation and spermiation. Assessment of maturation stage and detection of ovulation in female spawners was monitored using the biopsy technique: several eggs were obtained form anaesthetized females (Kalmagin 20%; Centrovet, Santiago de Chile, Chile) using a catheter and were analyzed for the maturation stage (cf. Żarski et al., 2015). Reproduction was induced at different time points (spring, summer, fall, winter) once per year in each group in accordance with EU and National legislation for animal welfare in fish production with approximately three months time in-between the broodstocks (no hormone treatments applied). The applied photothermal protocol, broodstock feed (mix of commercial broodstock diets) and the handling personal did not differ in-between spawning periods. Spawners originate from grow-out facilities and were introduced to the broodstock prior to the previous reproduction period. They were allowed to acclimatize for one entire photothermal cycle prior to first stripping.

2.2 Sampling and egg incubation

Before stripping, the fish were anesthetized. The standard length (SL) of females was measured to the nearest cm and the number of eggs per females was calculated using egg counts of a 2 mL subsample. A total of 31 egg batches of F-generation pikeperch (same genetic origin) were collected covering a total of six independent spawning periods (Summer 2013, S1, n = 6; Autumn 2013, S2, n = 5; Winter 2014, S3, n = 5; Summer 2014, S4, n = 7; Winter 2015, S5, n = 5; Spring 2015, S6, n = 3). Eggs were fertilized (dry) with freshly stripped sperm of one to three males (sperm activation visible). After fertilization, eggs were transferred to Zug-jars and incubated (15 – 16 °C). Fifty 50 eggs were monitored in triplicates per batch 2 h after fertilization and during hatching (from day 4 post-fertilization) for the determination of fertilization and hatching rates (%) using a Stereozoom IT-TR microscope (Gundlach, Harlev, Denmark). Female reproductive performance was assessed as total number of eggs and respective rates of fertilization and hatching. Fertilization rate was used as approximation to assess male reproductive performance.

2.3 Data analysis

Data are presented as mean ± standard deviation (SD). Groups of non-parametric data (Kolmogorov-Smirnov normality test) were compared with Kruskal-Wallis tests and Dunn's post-hoc test. For the comparison of two paired non-parametric groups Wilcoxon matched-pairs test was carried out. Correlations were tested with Spearman's (non-parametric data) correlation. Data analysis was performed with PRISM software (version 4.03; GraphPad, Irvine, CA, USA). Due to hatchery related working operations, not all performance parameters could be obtained in individual cases.

3. Results

Average number of eggs was 491.400 ± 161.609 per female ranging from 160.000 to 840.000 eggs. Average fertilization rate was 90.2 ± 14.8% with a minimum of 40.0 and a maximum of 100.0%. Hatching rates were 77.4 ± 5.7% on average ranging from 40.0 to 99.0% and were significantly lower than fertilization rates (Wilcoxon matched-pairs test; $p < .0001$). Group comparison of the six different spawning periods observed showed no substantial differences in fertilization and hatching rates (Fig. 1). It needs to be noted that in spawning period S6 only two batches could be assessed until fertilization. Differences in the number of eggs per female were similarly marginal with only one season (S1) producing on average lower egg counts compared to S2 and S3. The size composition of the broodstocks partially explained for these observations. Against our expectations, large females (> 70 cm) showed increased variability in reproductive performance and fecundity was not higher compared to intermediate sized fish (Fig. 2). While there were no significant differences in the size composition of the broodstocks (Kruskal-Wallis test: p = .69), the spawning periods S1 and S6 were strongly influenced by this pattern with large females (> 70 cm) producing comparably low number of eggs with variable quality. Consequently, correlation coefficients were negative for both, average fertilization (Spearman's r = -0.45; p = .007) and hatching rates (Spearman's r = -0.27; p = .077) versus female SL.

4. Discussion

Reproductive performance in terms of gamete quality was relatively high confirming good hatchery practice. Previous studies on embryo viability, assessed as survival at 72 h post-fertilization or survival until the eyed stage, typically reported survival rates of ~50 to 80% on average in wild-caught pikeperch breeders (Müller-Belecke et al., 2008; Zakęś & Demska-Zakęś, 2005; Żarski et al., 2012). These results seem comparable, since mortalities in pikeperch embryos mainly occur during early embryogenesis and not within 48 h prior to hatching (Schaefer et al., 2016). Similar to egg quality, high sperm quality was confirmed by fertilization rates. Fertilization success is commonly regarded as ultimate measure of sperm quality in fish (Rurangwa et al., 2004). Here, low fertilization success (< 50%) could only be observed in individual egg batches and it remains unknown whether these observations were caused by low sperm or egg quality. Despite the overall high observed gamete quality, there was quite a high variability especially in hatching rates and the number of eggs produced per female. In line with our hypothesis, this variability can not be attributed to adverse effects of year-round reproduction. Consequently, there were no substantial adverse effects on the reproductive performance in-between the six spawning periods. Therefore, there seems to be no perturbation of endogenous rhythms affecting homeostasis in F-generation spawners, which were not adapted to a natural photothermal cycle.

Rather than effects of year-round reproduction, the maternal size composition in the respective broodstock seems to affect variability in the female reproductive performance representing an optimum-driven relationship. Especially an intermediate fish SL (~65 to 70 cm) was associated with high and stable number and quality of eggs. In contrast, very large females above a size of 70 cm showed exhaustion effects, which are reflected in variable egg quality and in stagnating numbers of eggs per female. In fish below ~65 cm on the other hand, chances for low individual egg counts were higher. These findings contrast sharply with the common convincement that large spawners produce more eggs of higher quality, which is supported by numerous studies (Feiner & Höök, 2015) and is recognized for fisheries management (Birkeland & Dayton, 2005). In freshwater percids a similar positive relation of absolute fecundity and/or egg quality with female age and/or size was observed (Żivkov & Petrova, 1993; Wiegand et al., 2004; Johnston et al., 2012). In pikeperch, however, the relative fecundity does not always show such a clear linear relationship. Similar to out observations, a decrease of fecundity above a female size of ~65 cm has been reported in some populations (Lappalainen et al., 2003). This might represent a species-specific pattern in pikeperch, which might affect population dynamics and fisheries management. It needs to be noted that age-specific effects are masked in RAS, since spawner age is unknown and cannot easily be identified in isothermally reared fish. Here, F-generation spawners of very large lengths showed decreased reproductive performance, which may indicate that fast growth and reproduction are competitive traits.

Conclusively, it is recommended to focus hatchery efforts during year-round reproduction on intermediate sized F-generation females (65-70 cm). The results suggest that the inherent circadian rhythm is of negligible importance. These insights from hatchery practice present promising research perspectives in regard to selective breeding and the optimization of broodstock management.

Acknowledgements

This study was funded by the German Research Foundation (DFG KL 745/6-1). We would like to thank our cooperation partner AquaPri and the IGB working group Sustainable Aquaculture and Applied Physiology.

References

Birkeland, C., & Dayton, P.K. (2005). The importance in fishery management of leaving the big ones. *Trends in Ecology & Evolution*, 20, 356-358. *doi:*

Bobe, J., & Labbé, C. (2010). Egg and sperm quality in fish. *General and Comparative Endocrinology*, 165, 535-548. *doi:*

Brooks, S., Tyler, C. R., & Sumpter, J.P. (1997). Quality in fish: what makes a good egg? *Reviews in Fish Biology and Fisheries*, 7, 387-416. *doi:*

Fontaine, P., Wang, N., & Hermelink, B. (2015). Broodstock Management and Control of the Reproductive Cycle. In P. Kestemont, K. Dabrowski, & R.C. Summerfelt (Ed.), *Biology and Culture of Percid Fishes*. (pp. 103-122). Dordrecht, Netherlands: Springer Verlag. *doi:*

Hermelink, B., Wuertz, S., Trubiroha, A., Rennert, B., Kloas, W., & Schulz, C. (2011). Influence of temperature on puberty and maturation of pikeperch, *Sander lucioperca*. *General and Comparative Endocrinology*, 172, 282-292. *doi:*

Hermelink, B., Wuertz, S., Rennert, B., Kloas, W., & Schulz, C. (2013). Temperature control of pikeperch (*Sander lucioperca*) maturation in recirculating aquaculture systems-induction of puberty and course of gametogenesis. *Aquaculture,* 400, 36-45. *doi:*

Izquierdo, M. S., Fernández-Palacios, H., & Tacon, A. G. J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture,* 197, 25-42. *doi:*

Johnston, T.A., Lysack, W., & Leggett, W.C. (2012). Abundance, growth, and life history characteristics of sympatric walleye (*Sander vitreus*) and sauger (*Sander canadensis*) in Lake Winnipeg, Manitoba. *Journal of Great Lakes Research,* 38, 35-46. *doi:*

Khemis, I. B., Hamza, N., Messaoud, N. B., Rached, S. B., & M'Hetli, M. (2014). Comparative study of pikeperch *Sander lucioperca* (Percidae; Linnaeus, 1758) eggs and larvae from wild females or from captive females fed chopped marine fish. *Fish Physiology and Biochemistry,* 40, 375-384. *doi:*

Lappalainen, J., Dörner, H., & Wysujack, K. (2003). Reproduction biology of pikeperch (*Sander lucioperca* (L.)) - a review. *Ecology of Freshwater Fish,* 12, 95-106. *doi:*

Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., Herraez, M. P., & Carrillo M. (2013). Gamete quality and broodstock management in temperate fish. *Reviews in Aquaculture,* 5, 194-223. *doi:*

Müller-Belecke, A., & Zienert, S. (2008). Out-of-season spawning of pike perch (*Sander lucicoperca* L.) without the need for hormonal treatments. *Aquaculture Research,* 39, 1279-1285. *doi:*

Overton, J. L., Toner, D., Poliçar, T., & Kucharczyk, D. (2015). Commercial Production: Factors for Success and Limitations in European Percid Fish Culture. In P. Kestemont, K. Dabrowski, & R.C. Summerfelt (Ed.), *Biology and Culture of Percid Fishes.* (pp. 881-890). Dordrecht, Netherlands: Springer Verlag. *doi:*

Rurangwa, E., Kime, D. E., Ollevier, F., & Nash, J. P. (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, 234, 1-28.

Schaefer, F. J., Overton, J. L., & Wuertz, S. (2016). Pikeperch *Sander lucioperca* egg quality cannot be predicted by total antioxidant capacity and mtDNA fragmentation. *Animal Reproduction Science,* 167, 117-124. *doi:*

Schaerlinger, B., & Żarski, D. (2015). Evaluation and Improvements of Egg and Larval Quality in Percid Fishes. In P. Kestemont, K. Dabrowski, & R.C. Summerfelt (Ed.), *Biology and Culture of Percid Fishes.* (pp. 193-226). Dordrecht, Netherlands: Springer Verlag. *doi:*

Valdebenito, I. I., Gallegos, P. C., & Effer, B.R. (2015). Gamete quality in fish: evaluation parameters and determining factors. *Zygote* 23, 177-197. *doi:*

Wiegand, M. D., Johnston, T. A., Martin, J., & Leggett, W.C. (2004). Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). *Canadian Journal of Fisheries and Aquatic Sciences,* 61, 110-121. *doi:*

Zakęś, Z., & Demska-Zakęś, K. (2005). Controlled reproduction of pikeperch *Sander lucioperca* (L.): a review. *Archives of Polish Fisheries,* 17, 153-170. *doi:*

Żarski, D., Palińska, K., Targońska, K., Bokor, Z., Kotrik, L., Krejszeff, S., Kupren, K., Horváth, Á., Urbányi, B., & Kucharczyk, D. (2011). Oocyte quality indicators in Eurasian perch, *Perca fluviatilis* L., during reproduction under controlled conditions. *Aquaculture,* 313, 84-91. *doi:*

Żarski, D., Kucharczyk, D., Targońska, K., Palińska, K., Kupren, K., Fontaine, P., & Kestemont, P. (2012). A new classification of pre-ovulatory oocyte maturation stages in pikeperch, *Sander lucioperca* (L.), and its application during artificial reproduction. *Aquaculture Research,* 43, 713-721. *doi:*

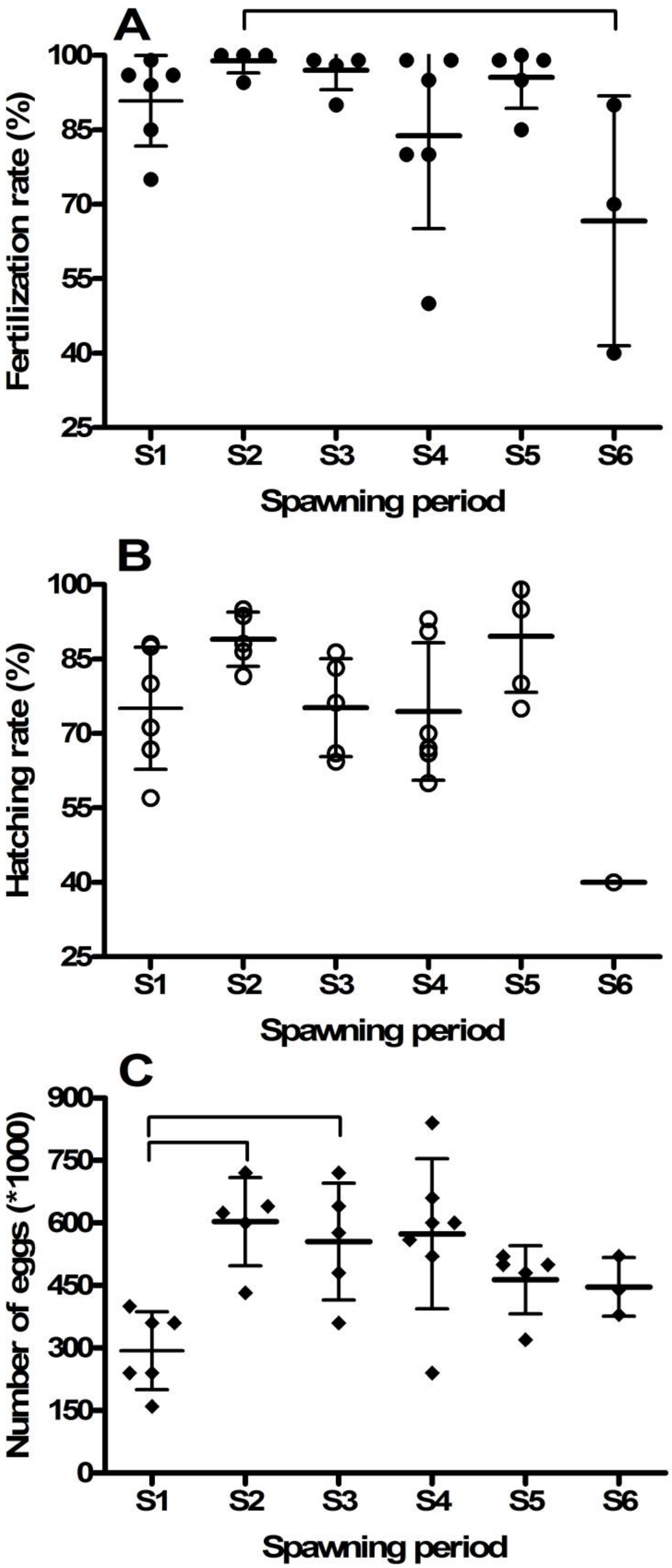
Żarski, D., Horváth, Á., Held, J. A., & Kucharczyk, D. (2015). Artificial Reproduction of Percid Fishes. In P. Kestemont, K. Dabrowski, & R.C. Summerfelt (Ed.), *Biology and Culture of Percid Fishes.* (pp. 123-162). Dordrecht, Netherlands: Springer Verlag. *Doi:*

Živkov, M., & Petrova, G. (1993). On the pattern of correlation between the fecundity, length, weight and age of pikeperch *Stizostedion lucioperca*. *Journal of Fish Biology*, 43, 173-182. *doi:*

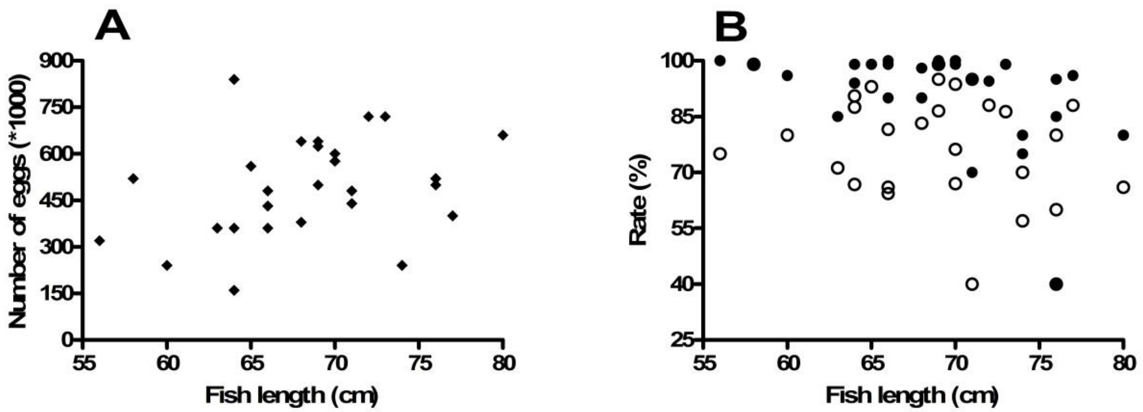
Figure legends

Fig. 1. Fertilization rates (A; n = 30), hatching rates (B; n = 29) and number of eggs (C; n = 30) of pikeperch egg batches during respective spawning periods. The horizontal lines indicate the mean values and whiskers indicate the standard deviation. Significant differences in-between individual spawning periods are indicated by brackets (Dunn's multiple comparison: $p < .05$).

Fig. 2. Number of eggs per female (A; n = 30) and fertilization (open circles; n = 30) and hatching rates (filled circles; n = 29) (B) versus standard length of female pikeperch for all six observed spawning periods.


A
Fertilization rate (%)
100
85
70
55
40
25
S1
S2
S3
S4
S5
S6
Spawning period
B
Hatching rate (%)
100
85
70
55
40
25
S1
S2
S3
S4
S5
S6
Spawning period
C
Number of eggs (*1000)
900
750
600
450
300
150
0
S1
S2
S3
S4
S5
S6
Spawning period

266


A
Number of eggs (*1000)
900
750
600
450
300
150
0
55
60
65
70
75
80
Fish length (cm)
B
Rate (%)
100
85
70
55
40
25
55
60
65
70
75
80
Fish length (cm)