THE EFFECT OF DIFFERENT PRESPAWN HOLDING TEMPERATURES ON THE DEGREE OF STERLET (*ACIPENSER RUTHENUS*) BROODSTOCK FEMALES OOCYTE RIPENESS

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ABSTRACT

In this study, there are results of temperature treatment of female sterlet spawners with accordance to sum of effective temperatures of water expressed in degree-days (°D) presented. Research took place in 2012 and 2013 on fish located under conditions of Rybníkářství Pohořelice a.s. The aim was to assess the degrees of oocyte ripeness in the prespawn period in two variants with heated and not heated water. To identify the degrees of oocyte ripeness, the PI (polarization index) was used. Fish in variants with heated water collected more degree-days in both years 2012 and 2013 and the average PI was lower in comparison with the variants with not heated water. It is possible to influence the oocyte ripeness and to synchronize the spawning by the water heating at prespawn period.

Key words: nucleus moving (%), polarization index (PI), prespawn holding, synchronous spawning

Acknowledgments: The authors thank to colleagues and students from the Section of Fishery and Hydrobiology MENDELU in Brno for the help by realization of this experiment and we also thank to Rybníkářství Pohořelice,a.s. for offering sterlet research material and cooperation. This work was supported by internal grand agency TP6/2013.

INTRODUCTION

It is necessary to understand the gametogenic stage and oocyte ripeness at sterlet female´s prespawn period. The synchronous spawning is one of the most important phases at broodstock management. It avoids use of overriped or immature fish. The prespawn gametogenic stage describes (Conte F.S. *et al*., 1988) by the presence of cytoplasm filled with platelets and oil droplets and containing melanin pigment granules in the cortex area. Envelope consists of two-layered zona radiata and thick gelatinous coat. As follicle ripens, the egg becomes polarized and the enlarged nucleus (germinal vesicle) migrates to the animal pole. Degree of this migration is expressed by the polarization index (PI). There are more options to provide the measurements of values for PI calculations described by (Van Eenennaam J.P. *et al.*, 1996 and Rodina M. 2006). The index of oocyte polarization (PI) values recorded during biopsy gonad examination is the basic criterion routinely used at sturgeon hatcheries for proper female prespawn holding regime selection. For example, females with PI < 0.09 may ovulate eggs at spawning temperatures after hormonal administration without prespawn holding. Duration of prior-to-spawn holding of other female groups is determined on the basis of the sum of effective temperatures of water (expressed in degree-days) (Dettlaff T.A., Ginsburg A.S., Schmalhausen O.I. 1993).

MATERIAL AND METHODS

Female broodstock research took place in 2012 and 2013. Monitored fish were kept in concrete troughs in natural water temperature. The fish were transferred only at prespawn period into troughs with a temperature controlled environment. Fish were divided into variants with heated and not heated water. These individuals were tagged individually by means of chip marks. The temperature was recorded on a digital wireless thermometer Minikin (EMS Brno) with an accuracy of 0.2 ° C. Three biopsies were performed in 2012: 28th February at 1,8 °C water temperature, 4th April at 9,5 °C water temperature and 16th April in groups with heated water at 16,4 °C and not heated water 10 °C. Two biopsies were performed in 2013: 28th February at 3,2 °C water temperature and 19th April in groups with heated water at 14,5 °C and not heated water 12,6 °C. The first biopsy was prepared at the laboratory of Physiology of Research Institute of Fish Culture and Hydrobiology Vodňany. Following biopsies were made at the Section of Fishery and Hydrobiology MENDELU in Brno. Biopsy was performed after the methodic by (Gela D. 2008). For needs of oocyte ripeness degree, the Polarization index (PI) was calculated. To provide accurate determination of the nuclear position via the digital image of the oocyte, ImageJ software was used for analysis. Pictures were taken using a binocular microscope coupled with a digital camera which was connected to computer. The PI was calculated using equation (PI = l/L*100) of the distance between animal pole and vegetal pole (L), as well as the distance between animal pole and the outer edge of the nucleus germinal vesicle of the oocyte (l). Immediately before the spawning, the PI value for calculations was set as PI 3. For exact expression of nucleus moving in percent the equation [% PI = (first PI - second PI) / first PI * 100] was used. Also the temperatures of water in degree-days (°D) were calculated as the sum of the mean daily effective temperatures.

RESULT AND DISCUSSION

2012

Fish between first biopsy with 69 individuals and second biopsy with 21 individuals collected 207 °D, between second and third biopsy with 48 individuals 316°D at the variant with not heated water and 472 °D at the variant with heated water. The data of polarization indices and nucleus moving (%) are shown in Tab.1.

Tab. 1: Summarized values of the first polarization index (1.PI), second polarization index (2.PI), third polarization index (3.PI) and the nucleus moving (%) between indices.

	First biopsy		Second biopsy	
	1.PI	% 1.PI → 2.PI	2.PI	% 2.PI → spawning
min	8,9	0	3,3	10
max	33,4	41,4	7,9	62,3
mean	17,6	22,7	5,8	45,8
SD	7	12,1	1,2	13,1

	Third biopsy - both variants		Third biopsy - not heated water		Third biopsy - heated water	
	3.PI	% 1.PI → 3.PI	3.PI	% 1.PI → 3.PI	3.PI	% 1.PI → 3.PI
min	4,9	0	6,4	12,2	5	0
max	25,5	57,2	22,5	52,6	25,5	57,2
mean	11,3	35,1	11,5	31,8	11,1	37,03
SD	4,8	13,8	4,8	10,5	4,8	15,1

Difference between polarization indices of fish in not heated water was 5,7 at 316°D with 55,4 °D per one polarization index. At the variant with heated water the polarization index was 6,8 at 473 °D with 69,6 °D per one polarization index. This relation is clearly visible at the linear trend line in Fig 1. Value difference between the variants is PI 1,1 with 157°D per one polarization index. The nucleus moving in percent is shown in Fig. 2.

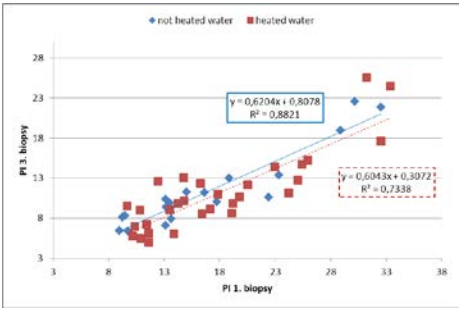

Fig. 1: Relation between polarization indices of the first (PI 1. biopsy) and third biopsy (PI 3. biopsy) at variant with and with not heated water

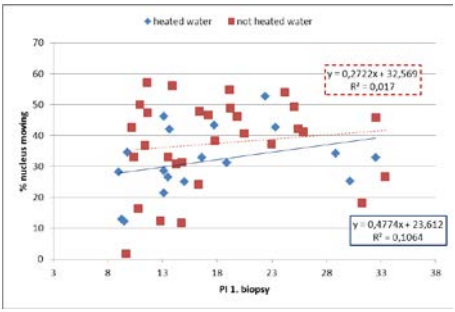

Fig. 2: Nucleus moving (% nucleus moving) between first and third biopsy at variants with and with not heated water in correlation with the polarization index of the first biopsy (PI 1. biopsy)

2013

Fish between first biopsy with 73 individuals and spawning with 25 individuals collected 181 °D. Fish between first and second biopsy with 48 individuals collected 224 °D at the variant with not heated water and 442 °D at the variant with heated water. Immediately before the spawning, the PI value was for calculation set as 3. The Polarization index data and moving of nucleus(%) are shown in Tab. 2.

Tab. 2: Summarized values of the first polarization index (1.PI), second polarization index (2.PI) and the nucleus moving (%) between both indices (% 1.PI → 2.PI)

First and second biopsy - both variants			
	1.PI	% 1.PI → 2.PI	2.PI
min	5,6	0	3,6
max	21,6	64,7	16,5
mean	10,3	19,5	8,1
SD	3,5	14,6	2,2
First and second biopsy - not heated water			
	1.PI	% 1.PI → 2.PI	2.PI
min	5,6	0	3,6
max	20	51	16,5
mean	9,6	17,2	7,9
SD	3,4	14,3	2,5
First and second biopsy - heated water			
	1.PI	% 1.PI → 2.PI	2.PI
min	8,1	3,8	5,3
max	21,6	64,7	12,4
mean	11,3	22,7	8,3
SD	3,4	14,6	1,6

At the variant with not heated water the difference between the polarization indices was PI 1,7 at 224 °D with 131 °D per one polarization index. At the variant with heated water the polarization index was PI 2,9 with 443 °D with 69,6 °D per one polarization index. Value difference between the variants is PI 1,2 with 219 °D required in addition to variant with not heated water. Summarized data from 2013 are shown at Fig. 3 and 4.

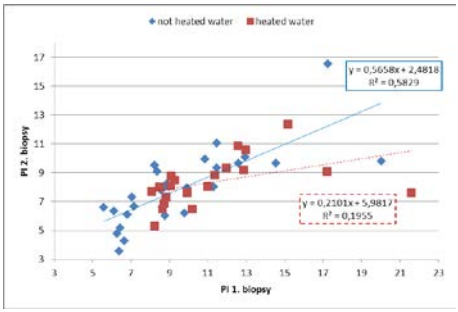

Fig. 3: Relation between polarization indices of the first (PI 1. biopsy) and second biopsy (PI 2. biopsy) at variant with and with not heated water

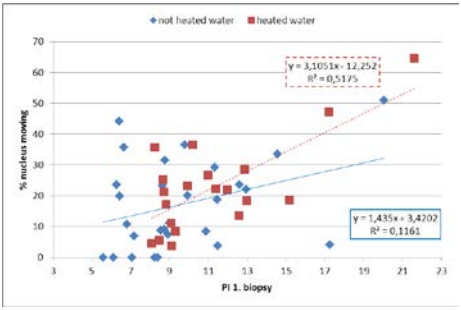

Fig.4: Nucleus moving (% nucleus moving) between first and second biopsy at variants with and with not heated water in correlation with the polarization index of the first biopsy (PI 1. biopsy)

There is higher variability in the movement of nucleus to index of real ripeness at the fish with higher PI than 8. On the contrary, it is possible to let broodstock females to collect temperature in the range of PI 8 to PI 13 according to our results, so their movement in polarisation index reaches the values which are favourable for spawning. Slow increase of temperature above the optimum in prespawning period causes faster movement of nucleus to animal pole and acceleration of ripeness. This result confirms (Dettlaff T.A., Ginsburg A.S., Schmalhausen O.I. 1993) and recommends to held less mature fish at lower spawning temperatures and to lower the increase of gradient of temperature prior the hormonal stimulation. Violation of this requirement causes desynchronization in oocyte maturation resulting in poor hatchery quality of eggs. (Dettlaff T.A., Ginsburg A.S., Schmalhausen O.I. 1993) reports the requirement of 25 °D in oocyte ripeness 10 PI per one polarisation index with fluent rising till the 18 PI value with requirement of 80 °D per one polarization index. An average requirement is 52°D per one polarization index. It is not recommend to apply temperatures over 16 – 18 °C in prespawning period. Temperature applied in our experiment in variant with heated water did not exceed 16 °C. An average requirement of degree-days per one polarization index was higher in 2012 than 2013. This might be caused by increasing of degree-days requirement due to increasing of polarisaton index, because there were more fish with higher polarization index than 18 PI in 2012. Not every movement is constant. Broodstock female with index movement from PI 6 to PI 4 will have her index tied to movement PI 2 the same way as broodstock female with index moved from PI 10 to PI 8. Nucleus will cross different

distance comparing to initial state of first biopsy. For comparison the percentage formulation of movement calculated as difference between first and second biopsy divided by first biopsy multiplied by one hundred [% PI = (first PI - second PI) / first PI * 100] can be used. This calculation is a real movement of nucleus per observation period. Also, it indicates the ability of nucleus to move slowly or quickly towards animal pole due to physiological need of ripening. That is the reason why the comparison of particular polarization indices of first and second or first and third biopsy more apt for nucleus movement estimation.

CONCLUSIONS

The biopsy is for Rybníkářsví Pohořelice a.s. and for other fishing operations the indispensable method for broodstock females of sturgeons determination. But it is very time-consuming procedure as well. We can influence the degree of oocyte ripeness of sterlet broodstock females by tempering the environment. This knowledge can be used at synchronizing of ripening and following spawning. Also it can be helpful for more effective prediction of ripeness according to first biopsy and by elimination of need of following biopsies. It is more suitable to infer the movement of nucleus from the trend when comparing polarization indices of particular biopsys than percentage formulation of movement when predicting the movement of nucleus towards the ripeness for spawning.

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