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Research Article

FECUNDITY AND OOCYTE DIAMETER OF *PHOXINELLUS PSEUDALEPIDOTUS* (TELEOSTEI: CYPRINIDAE), AN ENDEMIC AND ENDANGERED SPECIES FROM MOSTARSKO BLATO (NERETVA RIVER BASIN, BOSNIA AND HERZEGOVINA)

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ABSTRACT

Mostarsko Blato is a karst field in western Herzegovina and is a habitat for the endangered fish species *Phoxinellus pseudalepidotus* (Bogutskaya and Zupančič, 2003). Present data suggest that species is restricted only to wetland of the Mostarsko Blato but it is considered to be distributed more widely in the River Neretva basin. According to IUCN Red List, *P. pseudalepidotus* is listed as Vulnerable D2 ver. 3.1. The purpose of this study is to analyze fecundity and oocyte diameter of *P. pseudalepidotus* in the area of Mostarsko Blato. The research was conducted at the area of Mostarsko Blato from January to December 2009. The samples were collected using gill nets, and "krtol". Males and females were separated based on morphological characteristics of the gonads. Gonads were preserved in formaldehyde solution 4%. The absolute fecundity ranged from 965-4740 oocytes, with the mean value and standard deviation of 2524.7±820.63. The diameter of the oocytes inside the gonads varies. The diameter of oocytes of *P. pseudalepidotus* was between 10.02 µm and 1367.10 µm. The average diameter of the oocyte of *P. pseudalepidotus* reaches its maximum in January with a value of 446.59±351.74 µm, while the minimum average size was measured in November with a value of 188.05±138.94 µm. The average diameter of oocytes of *P. pseudalepidotus* during the spawning period was 315.01±284.16 µm. The type of *P. pseudalepidotus* spawning was categorized as 'partial spawning'.

Keywords: *Phoxinellus pseudalepidotus*, Fecundity, Oocyte diameter, Partial spawner, Mostarsko Blato.

INTRODUCTION

Mostarsko Blato is a karst field in western Herzegovina and is a habitat for an endangered fish species, *P. pseudalepidotus* (Bogutskaya & Zupančič, 2003). Mostar minnow, *P. pseudalepidotus*, is a freshwater fish endemic to the Neretva River basin. Present data suggest that species is restricted only to wetland of the Mostarsko Blato but it is considered to be distributed more widely in the Neretva River basin (Bogutskaya & Zupančič, 2003). *P. pseudalepidotus* inhabits streams or shallow canals with little current and clean water (Crivelli, 2006; Bogutskaya & Zupančič, 2003). During unfavorable periods, it lives in subterranean waters (Markotić, 2013). According to IUCN Red List, *P. pseudalepidotus* is listed as Vulnerable D2 ver. 3.1 (Crivelli, 2006; IUCN). It is endangered because of the extremely limited range of distribution, river regulation, and influence of non-indigenous species (Mihinjač et al., 2014).

Knowledge on aspects of fish reproduction is basic requirement to plan conservation, strategies for management of fishery resource, life story, domestication, and aquaculture (Amin et al., 2008; Solomon et al., 2011; Muchlisin et al., 2011; Ramos et al., 2016; Adite et al., 2017; Bobori et al., 2018; Jayadi et al., 2019). Information on the reproduction aspect of *P. pseudalepidotus* is scarce. It has extended its period of spawning which lasts from

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January to May when water temperature is between 9.6 and 23.9 ºC. During spawning season, females lay around 2500 eggs in more than one portion. The period of spawning coincides with flooding of the Mostarsko Blato (Markotić, 2013).

The information on fecundity and variation in oocyte diameter of *P. pseudalepidotus* is very important because it relates to the survival of these fish. Knowledge of fecundity may also be used to assess the spawning stock (Amin et al., 2008) while information on the spawning season becomes the basis of knowledge regarding life history (Ramos et al., 2016) and species conservation (Muchlisin et al., 2010). Hence, the objective of present study is to evaluate the fecundity and variation in oocyte diameter of *P. pseudalepidotus* in the area of Mostarsko Blato.

MATERIAL AND METHODS

Description of the Study Area

Mostarsko Blato (43°19'55"N 17°41'53"E) is an enclosed karst field in the Neretva River basin, SW Bosnia and Herzegovina. The area of Mostarsko Blato characterizes sub-Mediterranean climate, i.e. temperately warm humid climate with hot summers (Cfa), according to Köppen's climatic classification. In general, winters are mild and rainy; while summers are hot and relatively dry, (there is no specific minimum like in Csa climatic zones, such as the neighboring littoral and insular part of Southern Croatia). Pluviometric regime is maritime, with most of precipitation concentrated in fall and winter (primary maximum), and in April and May (secondary maximum). The influence of the Adriatic Sea reaches from three sides; southeast, east and south respectively. The strongest inflow of air arrives through the Neretva River valley, and over low Varda ridge, which separates lower alluvial plain of Mostarsko Blato from higher Mostarsko Blato Field. This inflow relieves the influence of colder air coming from the mountains in the north. During fall, winter and spring, the most part of Polje is flooded, but during summer, almost all water springs dry out (Mostarsko Blato Hydropower Plant, Feasibility Study, EP HZHB, Salzburg, 2000).

Limestone hills of Orlovac, Mikuljača, Virača, Trtla, and Varda surround the area of Mostarsko Blato, through which the Lištica River flows. On average, the field is flooded for 5 to 6 months during the year. In addition to permanent water sources in Mostarsko Blato, significant amounts of periodic waters of rivers Ugrovača, Orovnik, Mokašnica, and a number of small torrents are active during major rainfall seasons. In order to reduce the floods, the tunnel Varda was built in 1947. Additionally, water from Mostarsko Blato is managed also by the following sinkholes: Krenica, Košina, Renkovača, Kruševo, and the Velika Jama (Great Hole). These sinkholes are important for *P. pseudalepidotus* life cycle (Bogut et al., 2007).

Collection of Fish Samples

Sampling was carried out monthly from January to December 2009 by gill nets (7 m in length, 0.7 m in height, and with a mesh of 7 mm in size) and “krtol”, a traditional fishing tool in the area of Mostarsko Blato (Neretva River Basin, Bosnia and Herzegovina). Samples were studied at the Biology Laboratory of the Faculty of Science and Education of the University of Mostar, Bosnia and Herzegovina. The sex of each specimen was identified based on morphological characteristics of the gonads. The samples were measured for total length, body weight and gonad weight. Sampling was performed surgically via the abdomen using a scalpel and scissors to remove the gonad. The gonads were preserved in 4% formalin solution for analysis of fecundity and oocyte diameter. The study area with sampling sites is shown in Figure 1.

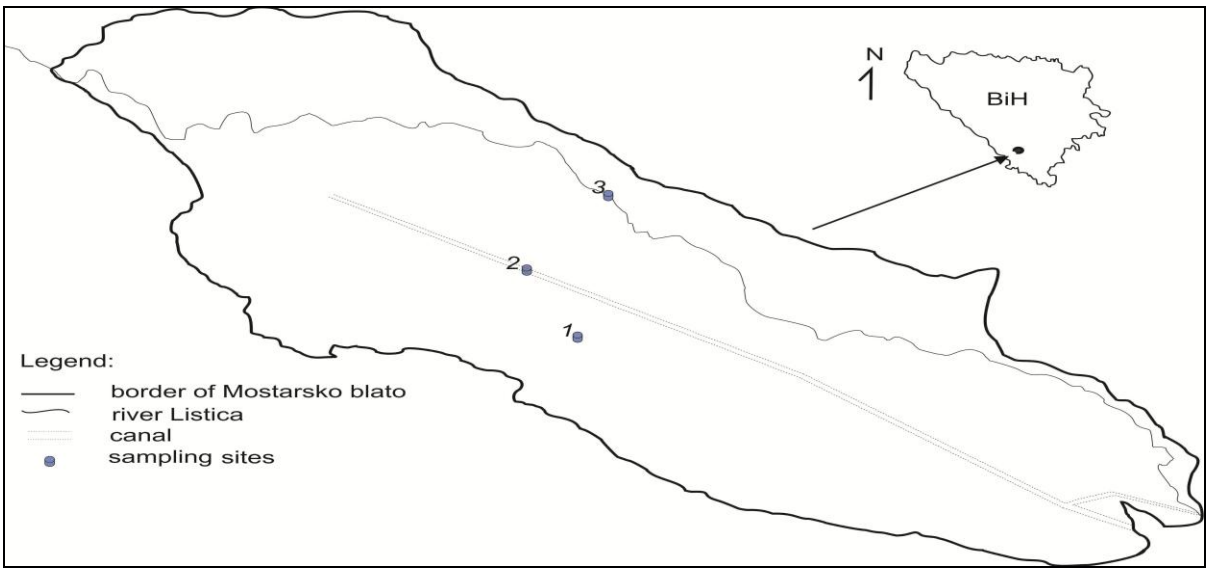

Figure 1. Map of sampling sites: Station 1 (Pisak), Station 2 (Međurić) and Station 3 (Pološki gaz).

Determination of Absolute Fecundity (F)

For the fecundity study, 100 female individuals of different age categories were taken and the content of their ovaries was analyzed. Only individual parts of the ovary were counted and weighed, and fecundity (F) was estimated according to the formula:

F = wet mass of ovary (g) / wet mass of the counted part of the ovary (g) x number of mature oocytes in the counted part of the ovary.

The relationship between individual fecundity and total body length was expressed according to the regression equation: $F = a L^b$, where: F = fecundity; L = total length of the fish (cm); a and b = constants.

By analyzing the logarithmically transformed data, the relationships between fecundity and length, mass and gonadal mass of females were obtained.

Measurement of Oocyte Diameter

In order to obtain a graphical representation of the diameter of oocytes in the ovaries of the species *P. pseudalepidotus*, 200 oocytes were measured each month during 2009.

RESULTS AND DISCUSSION

Absolute fecundity ranged from 965 to 4740 oocytes with a mean value and standard deviation of 2524.7±820.63. The relationship between fecundity and total length (Lt), total body mass (Wt), and gonadal mass (Wg) is graphically shown. The equations, or parameters, of these relationships are as follows: $F = 1134.6Lt^{0.1126}$; $R^2 = 0.0464$; $p = 0.046$ (Figure 2), $F = 1533.7Wt^{0.1192}$; $R^2 = 0.1179$; $p = 0.001$ (Figure 3), and $F = 1637.2Wg^{0.4372}$; $R^2 = 0.2319$; $p = 0.000$ (Figure 4). The regression of the above relationships is statistically significant at a significance level of $p < 0.05$ (F-regression test).

The relationship between the fecundity of *P. pseudalepidotus* and total length (Lt) indicates an increase in fecundity with an increase in total length (but with a low potency of 0.1126), that is, shorter females produce a smaller number of mature oocytes compared to females in higher length classes (Figure 2). Analyzing the relationship between fecundity and total body mass (Wt), i.e. gonad mass (Wg), it was also determined that individuals with higher body and gonad mass have a higher number of mature oocytes (Figures 3 and 4).

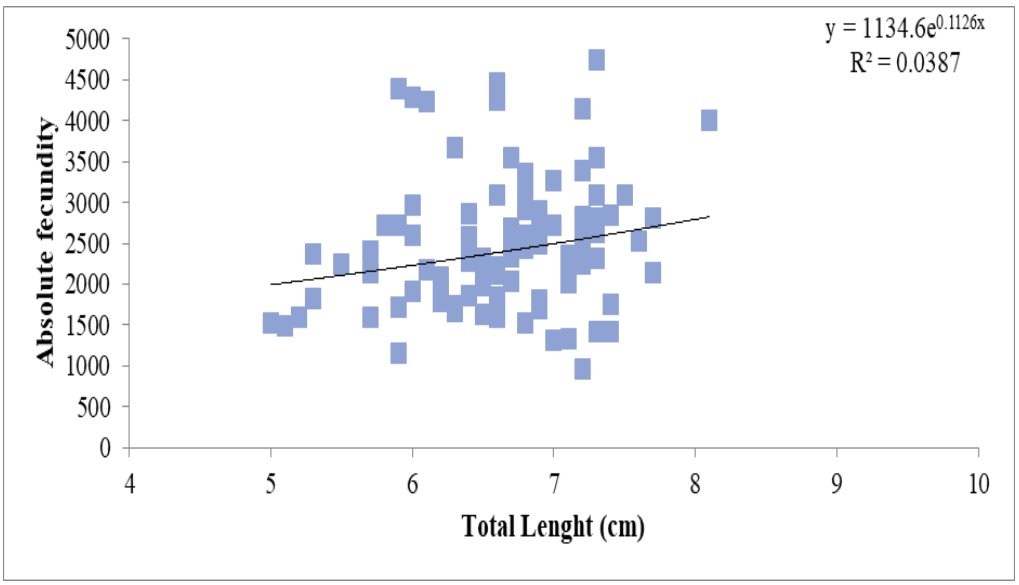

Figure 2. Relationship between fecundity and total body length of *Phoxinellus pseudalepidotus* in the Mostarsko Blato area during 2009.

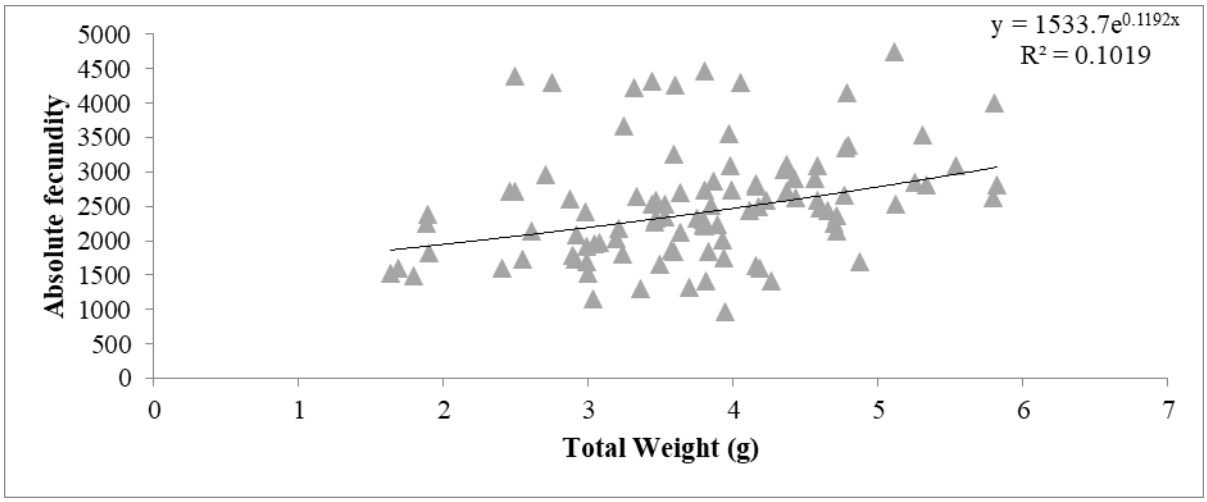

Figure 3. The relationship between fecundity and total body mass of *Phoxinellus pseudalepidotus* in the Mostarsko Blato area during 2009.

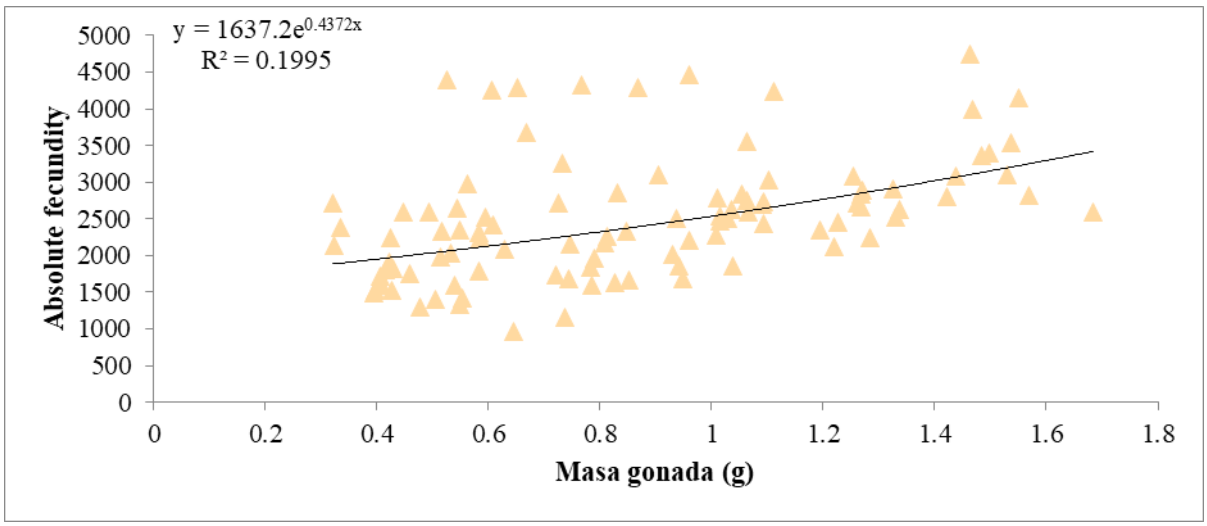

Figure 4. The relationship between fecundity and gonadal mass of *Phoxinellus pseudalepidotus* in the Mostarsko Blato area during 2009

In order to obtain a graphical representation of the diameter of oocytes in the ovaries of *P. pseudalepidotus*, two hundred oocytes were measured each month during 2009. During January, in ovaries of *P. pseudalepidotus* oocytes with diameters ranging from 24.32 µm to 1367.10 µm are observed and the most common are oocytes from 50 to 100 µm (Figure 5). In February, oocytes with diameters ranging from 38.78 µm to 639.15 µm are present in the ovaries. The most common oocytes are those with a diameter of 50 to 100 µm, followed by those with a diameter of 100 to 150 µm, followed by those with a diameter of 250 to 300 µm, then those with a diameter of 400 to 450 µm, and those with a diameter of 450 to 500 µm. In March, the ovaries of *P. pseudalepidotus* contain oocytes ranging from 33.68 µm to 498.14 µm, and in April from 86.88 µm to 1237.20 µm. In May, oocytes with a diameter ranging from 75.81 µm to 921.50 µm are present, and the most common are oocytes with diameter ranging from 200 to 250 µm and oocytes with diameter ranging from 150 to 200 µm. During June, oocytes with a diameter ranging from 124.50 µm to 523.53 µm are present in the ovaries of *P. pseudalepidotus* and in July from 73.43 µm to 820.18 µm. In August, oocytes with

a diameter ranging from 93.12 µm to 841.26 µm are present in the ovaries of *P. pseudalepidotus*. The most common are oocytes ranging from 100 to 150 µm and oocytes ranging from 150 to 200 µm. In September, oocytes with a diameter ranging from 104.82 µm to 893.09 µm are present in the ovaries of *P. pseudalepidotus* and in October those with a diameter ranging from 85.38 µm to 985.81 µm. During November, oocytes with a diameter ranging from 10.02 µm to 697.23 µm are present in the ovaries, with oocytes with a diameter ranging from 50 to 100 µm prevailing. In December, oocytes with a diameter ranging from 42.68 µm to 1188.89 µm are present in the ovaries of *P. pseudalepidotus*, with oocytes with a diameter ranging from 50 to 100 µm being the most abundant (Figure 5). In the ovaries of *P. pseudalepidotus*, oocytes that are in the process of vitellogenesis as well as previtelline oocytes that are just beginning the development cycle are constantly present (Figure 5).

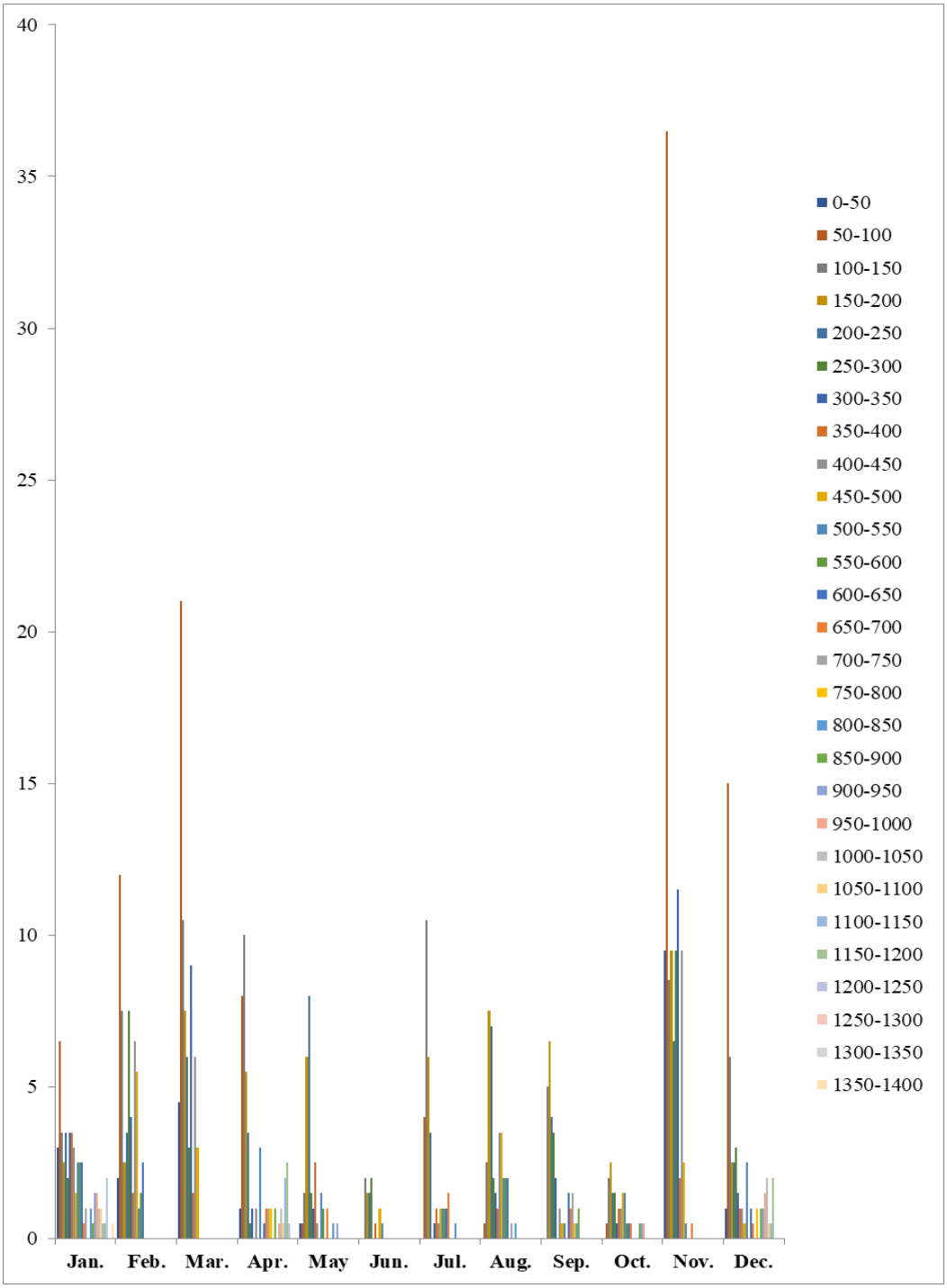

Figure 5. Diameter of oocytes in the ovaries of *Phoxinellus pseudalepidotus* in the Mostarsko Blato area during 2009 (legend = size of oocytes in µm).

The average monthly size of *P. pseudalepidotus* oocytes ranged from 188.05±138.94 µm in November to 446.59±351.74 µm in January. In 2009, the largest average diameter of oocytes of *P. pseudalepidotus* was measured in January (446.59±351.74 µm), April (418.31±378.06 µm), October (380.76±238.52 µm) and December (361.94±359.41 µm). The average oocyte diameter in February was 267.54±173.07 µm, in March, it was 193.73±133.51 µm, in May it was 326.02±186.95 µm, and in June, it was 258.18±126.53 µm. In July, the average oocyte diameter was 244.61±192.21 µm, in August it was 331.99±172.37 µm, and in September it was 327.98±229.13 µm (Figure 6).

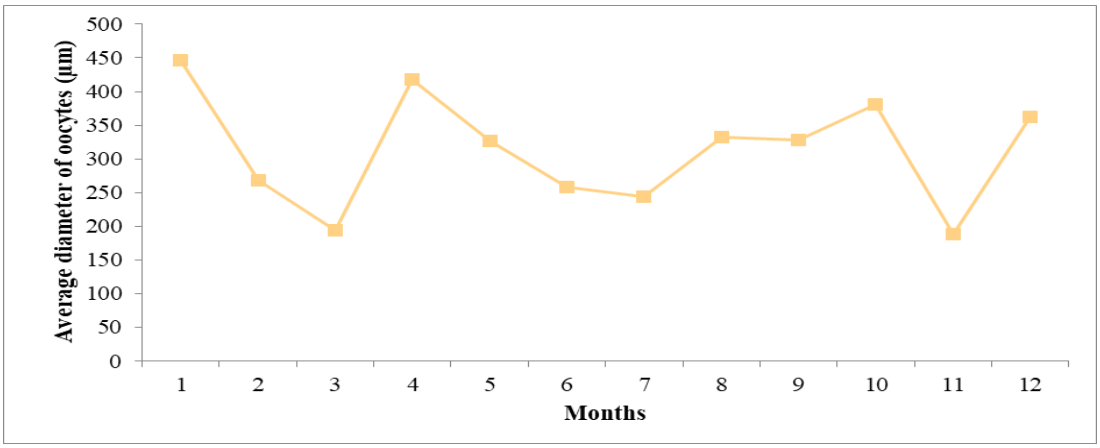

Figure 6. Average diameter of oocytes in the ovaries of *Phoxinellus pseudalepidotus* in the Mostarsko Blato area during 2009.

In order to compare the results obtained, data from different sources were taken. Knowledge about the biology and ecology of the two most closely related species, *P. alepidotus* and *P. dalmaticus*, is very scarce and insufficient. Therefore, the data from this paper were also compared with other taxa from related endemic genera. Namely, despite the taxonomic difference, all of these are species that are related to *P. pseudalepidotus*, that occupy very similar, if not identical, ecological niches, and that live in almost identical ecological conditions and in very similar habitats. The fecundity of *P. pseudalepidotus* ranged from 965 to 4740 oocytes, with a mean value and standard deviation of 2524.7±820.63.

Comparing the obtained fecundity values with the recorded values for other species from related genera (*Telestes croaticus*: 2000 oocytes, Trgovčević, 1905; *Delminichthys jadovensis*: 1200-1500 oocytes, Kottelat & Freyhof, 2007; *Delminichthys krbavensis*: 1000-2000 oocytes, Mrakovčić et al., 2006; *Delminichthys ghetaldii*: 1000-2000 oocytes, Mrakovčić et al., 2006; *Telestes ukliva*: 504-1180, Zanella, 2003; *Telestes souffia*: 503-3216, Vuković, 1985), large oscillations of fecundity values are observed in *P. pseudalepidotus* and species belonging to related genera. Significant differences in fecundity between species often reflect different reproductive strategies (Pitcher & Hart, 1982; Helfman et al., 1997). Within certain species, fecundity may vary because of different adaptations to environmental conditions (Witthames et al., 1995). Bagenal (1967) also states that, in some cyprinid species, it takes a longer time for oocytes to mature and those oocytes in the ovaries are of different sizes. Even within a genus, fecundity is known to vary annually (Horwood et al., 1986) and to be correlated with length (Wootton, 1998; Erdoğan et al., 2002), mass, age and gonadal mass (Erdoğan et al., 2002). It also varies between populations of the same species and does not remain the same from year to year (ŞaŞi, 2004). Although many factors complicate the interpretation of fecundity: fertility, spawning frequency, parental care, oocyte size, population density and environmental factors (Bagenal, 1978), the higher fecundity in *P. pseudalepidotus* determined in this study, compared to the recorded fecundity of species belonging to related genera, can be attributed to biological differences between these species, differences in body length and mass of the studied species, and environmental factors. In addition, the oscillations of fecundity values in the species *P. pseudalepidotus* and species belonging to related genera can be explained as a result of different interpretations of fecundity. Some authors define fish fecundity as the number of mature oocytes in the body of the female just before spawning (Bagenal, 1967), while other authors write about fecundity as the number of released mature eggs (oocytes) during spawning. The relationship between fecundity of *P. pseudalepidotus* and total body length (Lt) indicates an increase in fecundity with an increase in total length but with low potency. Also, by analyzing the relationship between fecundity and total body mass (Wt), that is, gonad mass (Wg), it was determined that individuals with higher body and gonad mass have a higher number of mature oocytes. Oocyte size is directly correlated with gonadal development stages. The ovaries of *P. pseudalepidotus* have multiple oocyte size classes, i.e. oocytes in multiple stages of gonadal

development. At least two developmental stages of oocytes are present in the ovary. Oocyte size varied depending on the developmental stages of the gonads. In this study, the diameter of oocytes in the studied individuals of *P. pseudalepidotus* varied from 10.02 to 1367.10 µm. The average diameter of oocytes of *P. pseudalepidotus* reaches its maximum in January with a value of 446.59±351.74 µm, while the minimum average size was measured in November with a value of 188.05±138.94 µm.

The average diameter of oocytes of *P. pseudalepidotus* during the spawning period was 315.01±284.16 µm. The diameter of oocytes during the spawning period of the species *Telestes dabar*, distributed in the Opačica and Vrijeka rivers in Bosnia and Herzegovina, varied from 1.3 to 1.7 mm (Bogutskaya et al., 2012). The largest diameter of oocytes of the species *Telestes ukliva* of the Cetina River was 1.517 mm, while the average diameter was 1.09 mm (Zanella, 2003). The average diameter of oocytes of *Chondrostoma knerii*, distributed in the Hutovo Blato area, was 1.78±0.72 mm, with a range of variation from 1.51 to 2.16 mm (Glamuzina et al., 2007). Many researchers have noted that oocyte size increased with fish length, mass, and age (Nikolsky, 1963; Jellyman, 1980; Epler et al., 2001).

CONCLUSION

The absolute fecundity of *P. pseudalepidotus* varies between 965-4740 oocytes, with mean value and standard deviation of 2524.7±820.63. The diameter of the oocytes inside the gonads varies. The diameter of oocytes in the ovaries of *P. pseudalepidotus* ranged from 10.02 µm to 1367.10 µm. The average diameter of oocytes of *P. pseudalepidotus* reached its maximum in January with a value of 446.59±351.74 µm, while the minimum average size was measured in November with a value of 188.05±138.94 µm. The average diameter of oocytes of *P. pseudalepidotus* during the spawning period was 315.01±284.16 µm. The type of *P. pseudalepidotus* spawning was categorized as 'partial spawning'.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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