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**Original article**

**Reproductive behavior of male rainbow trout (*Oncorhynchus mykiss*) during reproductive period**

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ABSTRACT

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In this study it was described and characterized the reproductive behavior of male rainbow trout during reproductive period. Results indicate that reproductive period is in the months of August until February. Semen production varied at population level, 60% of population presented higher production in September  $33.59 \pm 13.69$  mL, 30% in October  $28.76 \pm 10.22$  mL and 10% in November  $75.53$  mL. Statistical analysis detected significant differences between the months ( $P < 0.05$ ) and at individual level ( $P < 0.05$ ). Spermatozoids concentration  $\text{mL}^{-1}$  varied at individual and population level. Spermatic concentration in 70% of population decreased as reproductive period progressed. August and September were the months with highest concentrations of spermatozoids  $\text{mL}^{-1}$   $7.71 \pm 2.85$ ,  $7.76 \pm 1.85 \times 10^9$  respectively. Statistical analysis detected significant differences between months ( $P < 0.05$ ). Seminal pH was alkaline with light variations at individual and population level. Statistical analysis did not detect significant differences between months nor individual months ( $P < 0.05$ ). This confirms that there is a variation in seminal quality of the same organism. Which is interesting for selection of best breeders, decrease maintenance costs and also reduce number of males.

## 1. Introduction

Rainbow trout (*Oncorhynchus mykiss*; Walbaum, 1972) is one of the most cultivated species worldwide and with a high economic value (Aral et al., 2007). In farms and cultivation centers the reproduction of this specie is based in artificial reproduction, where gametes quality is fundamental to ensure fertilization success. Nevertheless, there are factors that affect gametes quality like culture conditions, gamete collection methods and variations between organisms (Rurangwa et al., 2004; Bobe and Labbé, 2010; Hajirezaee et al., 2010). In males, semen quality control is a problem for aquaculture industry, in well established commercial species production as also in introduction of other species with high commercial interest (Cabrita et al., 2014). Semen quality allows to stablish the fertility potential and according to Bobe and Labbé (2010); Hajirezaee et al. (2010) it is defined as a measurement of spermatozoid capacity to successfully fertilize an egg and any quantifiable physical parameter that is directly correlated with spermatozoids capacity of fertilization, can be used as an indicator for spermatic quality (Rurangwa et al., 2004; Cosson, 2008; Bobe and Labbé, 2010). Volume reflects the performance and spermatic concentration, being the last one with motility the ones that influence in fertilization rates, which can increase significantly by rising pH between 8.0 and 9.0 (Billard and Cosson, 1989; Lahnsteiner et al., 1998; Alavi and Cosson, 2005; Cosson, 2008; Alavi et al., 2008a). Therefore the aim of this investigation is to describe the reproductive behavior concerning semen production, spermatic concentration and seminal pH in male rainbow trout during reproductive period, generating information that details variations in seminal quality at individual level, and also it can be useful for selection and reduction of breeders, decrease maintenance costs and design protocols for a good management that altogether help to optimize fertilization rates.

## 2. Materials and methods

### 2.1. Semen collection

To characterize the reproductive period and evaluate behavior, sampling was made every month during a year (From June 2014 to June 2015) in 10 organisms of three years old, with an average weight of  $2.379 \pm 0.285$  kg and total length of  $54.63 \pm 1.86$  cm, pertaining to Aquaculture Center "El Zarco", Mexico-Toluca, Ocoyoacac, Estado de Mexico. Fish were marked and identified with microchip Avid-System<sup>®</sup> and maintained in a rustic pond of 13.5 m long, 6 m wide and 75 cm depth; with an average water flow of  $121.27 \text{ L min}^{-1}$  and natural photoperiod. Average water temperature was of  $11.31 \pm 1.25$  °C (range of 8.37-13.80 °C). Fish were fed with balanced food for trout WINFISH-ZEIGLER<sup>®</sup> equivalent to 2% of corporal weight  $\text{day}^{-1}$ .

For semen obtainment, organisms were maintained in fast for two days, with the end to prevent semen contamination; to reduce stress during manipulation they were anesthetized with clove essence at a concentration of  $0.05 \text{ mL L}^{-1}$  of water (Keene et al., 1998; Rodríguez et al., 2007). Semen samples were obtained by light abdominal pressure in an operculum-caudal direction, semen was collected in graduated tubes of 50 mL capacity. It was ensured that samples were not contaminated with urine, feces, blood and water (Rodríguez et al., 2007; Ubilla and Valdebenito, 2012; Nynca et al., 2012).

### 2.2. Semen evaluation

Volume was expressed as mL, spermatic concentration was quantified from a solution made from 50  $\mu\text{L}$  of semen, 950  $\mu\text{L}$  of NaCl at 0.7% and 500  $\mu\text{L}$  of formalin at 8%, from which an aliquot was took and charged into a Neubauer chamber, counting was made with program Image-Pro 5.1<sup>®</sup> under a microscope OLYMPUS OPTICAL BX41TF<sup>®</sup> and expressed as number of cell  $\times 10^9 \text{ cell mL}^{-1}$  (Rodríguez, 1992). Semen pH was determined immediately with a potentiometer Hanna HI 9125<sup>®</sup> with an electrode Hanna HI 1330<sup>®</sup>.

Results were processed with descriptive analysis expressed as mean  $\pm$  standard deviation. To evaluate variation of seminal characteristics between organisms it was used a one way variance analysis (ANOVA) followed by a Tukey test to compare means, in all cases with a significance level of ( $P < 0.05$ ).

### 3. Results and discussion

Reproductive period for this population is determined from August to February, in August 80% of population produced semen at the end in February 70% stopped producing semen and in March 100% of population stopped producing. In table 1 it is shown volume, spermatic concentration (spermatozooids mL<sup>-1</sup>) and pH results with mean values and standard deviation, obtained during reproductive period, in figure 1 it is observed that semen production increase as breeding season progresses, it reaches to a maximum value from which a continuous decrease is presented until end of period.

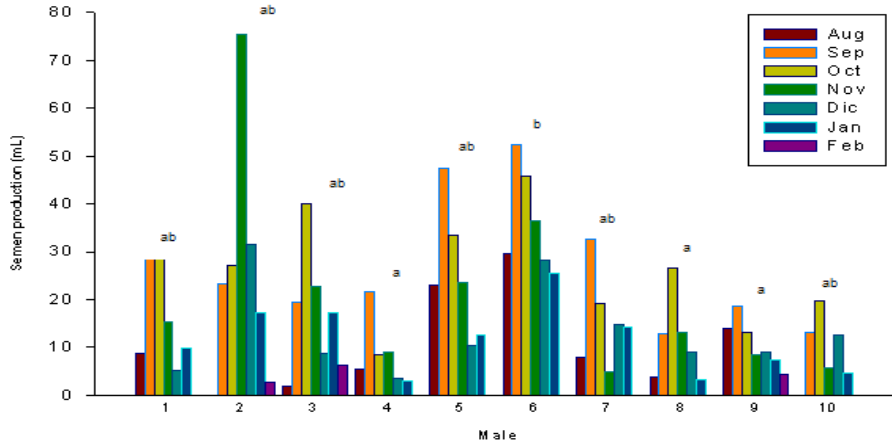
On the other hand, 60% of population presented higher production of semen in the month of September, obtaining in average 33.59 ± 13.69 mL, the 30% in October 28.76 ± 10.22 mL and 10% in November 75.53 mL and an average production during reproductive period of 18.01 ± 14.23 mL, minimum production was of 1.79 mL in august corresponding to organism three and maximum production was of 75.53 mL in November corresponding to organism two (Fig. 1). Male 6 presented higher production during reproductive period 36.30 ± 10.78 mL (Fig. 1). Statistical analysis determined significant differences between months ( $P < 0.05$ ) (Tab. 1) and at individual level between male six and males four, eight and nine ( $P < 0.05$ ) (Fig.1).

**Table 1**  
Semen characteristics of rainbow trout (*O. mykiss*).

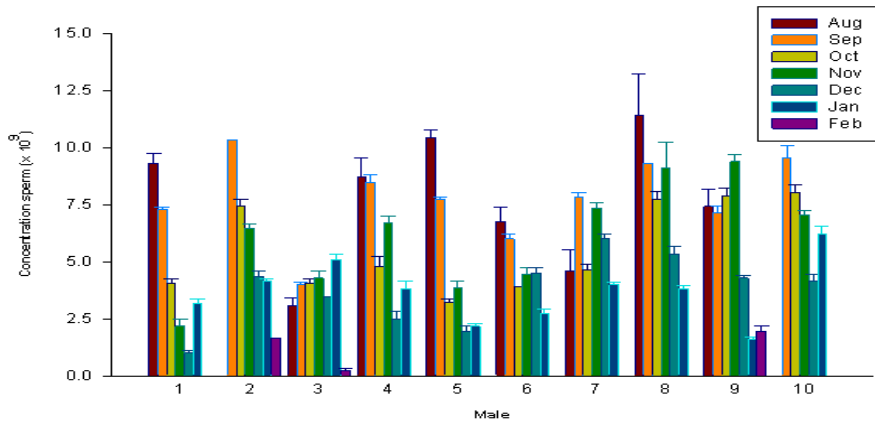
Reproductive period (months)	Weight (kg)	Semen production (mL)	mL kg <sup>-1</sup>	Spermatic concentration (x 10 <sup>9</sup> mL <sup>-1</sup> )	Concentration (x 10 <sup>9</sup> ) kg <sup>-1</sup>	Total concentration (x 10 <sup>9</sup> )	pH
Aug	2.828 ± 0.379	11.82 ± 9.85	3.99 ± 3.00	7.71 ± 2.85 <sup>DJF</sup>	2.73 ± 1.01 <sup>DJF</sup>	95.08 ± 83.61	8.13 ± 0.14
Sep	2.833 ± 0.317	27.06 ± 13.56 <sup>F</sup>	9.46 ± 4.44 <sup>F</sup>	7.76 ± 1.85 <sup>DJF</sup>	2.77 ± 0.72 <sup>DJF</sup>	202.73 ± 92.64 <sup>DJF</sup>	8.22 ± 0.12
Oct	2.882 ± 0.351	26.19 ± 11.58 <sup>F</sup>	9.15 ± 4.10 <sup>F</sup>	5.58 ± 1.94 <sup>F</sup>	1.99 ± 0.68 <sup>F</sup>	136.29 ± 53.56 <sup>F</sup>	8.00 ± 0.21
Nov	2.952 ± 0.231	21.50 ± 21.34	6.97 ± 6.19	6.07 ± 2.34 <sup>F</sup>	2.07 ± 0.85 <sup>F</sup>	120.69 ± 134.93	9.07 ± 2.98
Dec	2.976 ± 0.270	13.27 ± 9.34	4.38 ± 2.78	3.75 ± 1.55 <sup>AS</sup>	1.26 ± 0.52 <sup>AS</sup>	55.42 ± 46.91 <sup>S</sup>	7.74 ± 0.31
Jan	2.868 ± 0.250	11.07 ± 7.74	3.96 ± 2.32	3.67 ± 1.36 <sup>AS</sup>	1.29 ± 0.53 <sup>AS</sup>	38.20 ± 30.09 <sup>S</sup>	8.02 ± 0.31
Feb	2.925 ± 0.380	4.43 ± 1.72 <sup>SO</sup>	1.69 ± 0.86 <sup>SO</sup>	1.28 ± 0.92 <sup>ASON</sup>	0.45 ± 0.34 <sup>ASON</sup>	4.82 ± 3.58 <sup>SO</sup>	8.03 ± 0.65
<b>Mean ± Estandar Deviation</b>	2.895 ± 0.307	18.01 ± 14.23		5.47 ± 2.65		103 ± 95.87	8.19 ± 1.25

Superscripts indicate initial letter of month where there are significant differences ( $P < 0.05$ ).

Like the volume, spermatozooids concentration mL<sup>-1</sup> variated at individual and population level. 50% of population presented higher concentration in august obtaining in average 9.32 ± 1.78 x 10<sup>9</sup>, 30% in September with 9.23 ± 1.36 x 10<sup>9</sup>, 10% in November 9.38 x 10<sup>9</sup> and remaining 10% in January 5.10 x 10<sup>9</sup> spermatozooids mL<sup>-1</sup> (Fig. 2). Spermatic concentration in 70% of population decreased as breeding period progressed (Fig. 2), august and September were the months with higher spermatozooids mL<sup>-1</sup> concentration with 7.71 ± 2.85, 7.76 ± 1.85 x 10<sup>9</sup> respectively and September and October were the months with higher total spermatozooids concentration 202.73 ± 92.64, 136.29 ± 53.56 x 10<sup>9</sup> respectively, general average during reproductive period was of 5.47 ± 2.65 x 10<sup>9</sup> mL<sup>-1</sup> (Tab. 1), minimum concentration of spermatozooids mL<sup>-1</sup> with 0.23 x 10<sup>9</sup> in February was of organism three and maximum spermatozooids mL<sup>-1</sup> concentration was of 11.43 x 10<sup>9</sup> in august of organism eight which presented highest concentration during reproductive period .78 ± 2.79 x 10<sup>9</sup> spermatozooids mL<sup>-1</sup> (Fig. 2). Statistical analysis detected significant differences between months ( $P < 0.05$ ) (Tab. 1) and it did not detected significant differences at individual level ( $P > 0.05$ ) (Fig. 2).



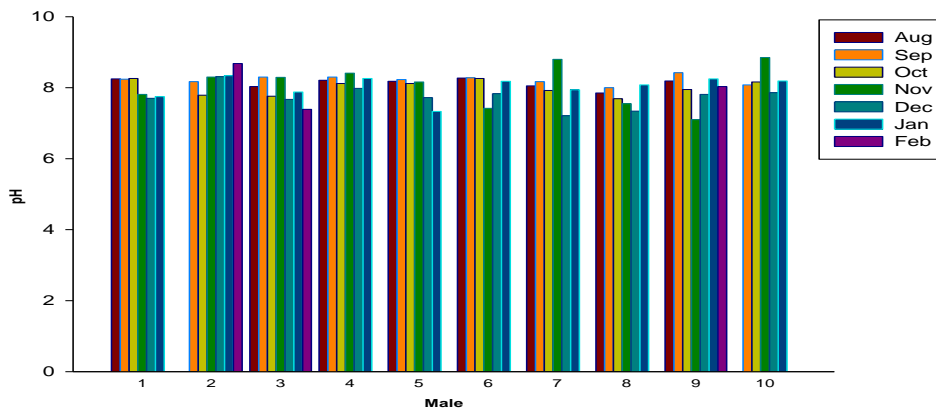
**Fig. 1.** Behavior of semen production during breeding period. Bars with different superscripts indicate significant differences ( $P < 0.05$ ).



**Fig. 2.** Behavior of sperm concentration during breeding period. Significant differences were not detected ( $P > 0.05$ ).

**pH**

During reproductive season, seminal pH was alkaline with light variations at individual and population level with a general average of  $8.19 \pm 1.25$  (Tab. 1), minimum pH was of 7.1 in month of November presented in organism nine and maximum pH was of 8.85 also in November in organism ten. Statistical analysis did not detect significant differences between months at any level ( $P > 0.05$ ) (Fig. 3).



**Fig. 3.** Behavior of seminal pH during breeding period. Significant differences were not detected ( $P > 0.05$ ).

According to consulted literature, this is the first study in Mexico that show reproductive behavior of male rainbow trout at individual level, referring to semen production, spermatic concentration and seminal pH, although knowing individual behavior of organisms allow to select those who have better characteristics as also estimate number of necessary males in aquaculture farms for fertilization in order to optimize production costs (Ramírez et al., 2011; Sahin et al., 2014).

In addition to this, investigation results show that semen production present an increase as reproductive season progresses, which reach a maximum value from which a continuous decrease is presented, until period is finalized (Fig. 1). This behavior is similar to the one obtained by Munkittrick and Moccica (1987); Aral et al. (2005); Sahin et al. (2014) for rainbow trout; but different to the ones reported in other teleost fish as: *Barbus barbuis* (Alavi et al., 2008a), *Carassius auratus* (Zadmajid et al., 2013), and *Pseudoplattystoma metaense* (Ramírez et al., 2011), species where it is initiated with a maximum production from which a decrease is presented until finalizing reproductive period. In *Piaractus mesopotamicus* (Kuradomi et al., 2016) it is reported a continuous rise until period is finalized. Spermatic concentration has been traditionally used for semen quality evaluation, it is consider as an important parameter that have impact on the fertilization success (Cabrita et al., 2014; Nynca et al., 2016) and represents a characteristic feature of specie (Agarwal et al., 2004).

According to results in this investigation, there is a variation in spermatic concentration at individual and population level. 70% of population showed a continuous decrease during reproductive period (start to end), similar behavior to the results reported in rainbow trout by Büyükhatoğlu and Holtz (1984); Munkittrick and Moccica (1987), *Schizothorax richardsonii* (Agarwal and Raghuvansh, 2009), *Barbus barbuis* (Alavi et al., 2008a), *Sparus aurata* and *Dicentrarchus labrax* (Kara and Labeled, 1994) and *Tinca tinca* (Zuromska, 1981). Remaining 30% presented an increase in concentration reaching to a maximum value from which a continuous decrease is presented, until end of period (Fig. 2) which match with obtained results by Aral et al. (2005); Sahin et al. (2014) in rainbow trout and reported in *Piaractus mesopotamicus* (Kuradimi et al., 2016).

Fluctuations in volume and spermatic concentration are related with reproductive period, where at the second and third month it increases and later it decreases, as it occurs in other teleost fish. On the other hand, determined discrepancies regarding to volume and spermatic concentration, according to Ciereszko and Drabrowski (1993); Bobe and Labbé (2010); Hajirezaee et al. (2010); Hidahl et al. (2013); Aragón et al. (2014), are attributed to culture conditions as alimentation, geographic conditions, gametes collection methods, environmental stimulus like temperature and photoperiod and biological characters in breeders as age and genetic origin. According to Kissil et al. (2001) and Campos et al. (2004) from all variables, photoperiod is consider as one of the most important because is related to development; gonadal maturation in organisms and also it can be used to modified reproductive cycle, improve synchrony of sexual maturation and induce to spawning (Campos et al., 2004).

Moreover it is documented that spermatozooids mature and acquire activation capacity in the passage from testicles to spermatic conduct which fluid (seminal plasma) have a basic pH (Billard et al., 1995; Mochida et al., 1999), where they remain immobile due to osmotic pressure,  $K^+$  concentration, sucrose concentration, and seminal plasma pH lower than 7.0; also, seminal pH is important because is related to spermatic motility (Darszon et al., 1999; Cosson et al., 2000). Alavi and Cosson (2005); Alavi et al. (2008b) reported that in salmonids optimum pH for motility and fertilizing capacity must be close to 9.0. Nevertheless, there are studies that show higher motility at a minute with a pH  $\leq 8.0$  (Secer et al., 2004; Bozkurt, 2006; Aral et al., 2007). Reported pH in this study was alkaline with an average value of  $8.19 \pm 1.25$  (Tab. 1), minimum pH of 7.10 and maximum of 8.85 (Fig. 3). However, by not determining spermatic motility it is difficult to prove relation and effect of pH in motility, so it is recommended to make studies of the effect that pH has on spermatic motility.

This confirms that there is a variation in semen production even in the same organism, which is matter of interest for breeders selection and therefore reduce maintenance costs, number of males and design protocols for good management that altogether help to optimize fertilization rates.

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