Digital Journal of
El Hombre y su Ambiente Department
ISSN: 2007-5782

Vol. 1 (13): 1-9. January to June 2017

Rainbow trout (*Oncorhynchus mykiss*) semen quality from Michoacan batch at the end of reproductive period.

Castro-Castellón A*, González-Villaverde P, Cortés-García A, Martínez-Regalado D, Jiménez-Valencia J.**

Laboratorio de Reproducción Genética y Sanidad Acuícola. Universidad Autónoma Metropolitana Unidad Xochimilco. Calzada del Hueso No. 1100, Col. Villa Quietud, C.P. 04960, Coyoacán, Ciudad de México. Licenciatura en Biología. Módulo Historias de Vida.

Email responsible: **acortes@correo.xoc.uam.mx; *19andrescc@gmail.com

ABSTRACT

Aquaculture center "El Zarco" is dedicated to production of rainbow trout (*Oncorhynchus mykiss*), which reproductive period is from September to February, thereby gametes quality affects percentage of fecundation. The aim of this investigation is to evaluate semen quality, using samples of spawning fish from Michoacan batch at the end of reproductive period. Seven males and a female with an average age of three years were selected; and were sedated with economic anesthetics, once anesthetized, biometric measurements of each organism were obtained and the extraction of semen through light abdominal pressure. Quantitative and qualitative tests were made to determine semen quality and fecundation rate was determined. Average obtained semen volume was of 18.80 ± 7.77 mL. Average spermatozoids number was of 7.7 x 10^7±1.9 x 10^7 with a maximum of 14x10^7 mL^{-1} and a minimum of 38 x10^6 mL^{-1}. Average motility was of 46.25 ± 9.08 s. with a vigorous motility of 22.33 ± 4.34 s. To determine the percentage of fecundation, 421 eggs were used and 98% of viability was obtained. According to obtained results, semen characteristics of males of Michoacan batch at the end of reproductive period are viable for reproduction, being relevant information for aquaculture center for improvement of processes in artificial reproduction.

Key words: Sperm number, motility, fecundation, viability.

RESUMEN

El centro acuícola el Zarco se dedica a la producción de truchas arco iris (*Oncorhynchus mykiss*), cuya época reproductiva es de septiembre a febrero, por lo tanto, la calidad de gametos influye en el porcentaje de fecundación. El objetivo de esta investigación es evaluar la calidad del semen utilizando muestras de reproductores del lote Michoacano a finales de su periodo reproductivo. Se seleccionaron siete machos y una hembra con edad promedio de tres años; fueron sedados con anestésicos económicos, una vez anestesiados se obtuvieron medidas biométricas de cada organismo, así como la extracción del semen mediante una ligera presión abdominal. Se realizaron pruebas cuantitativas y cualitativas para determinar la calidad del semen y también se determinó la tasa de fecundación. Los resultados, en cuanto al volumen de semen promedio, fue de 18.80 ± 7.77 mL. El número de espermatozoides promedio fue de 7.7 x 10^7±1.9 x 10^7 con un máximo de 14x10^7 mL^{-1} y un mínimo de 38 x10^6 mL^{-1}. La motilidad promedio fue de 46.25 ± 9.08 s con una motilidad vigorosa de 22.33 ± 4.34 s. Para determinar el porcentaje de fecundación se utilizaron 421 huevos obteniendo el 98% de viabilidad. De acuerdo con los resultados obtenidos las características del semen del lote Michoacano a finales del periodo reproductivo son viables para la reproducción, siendo información relevante para el centro acuícola para mejorar los procesos de la reproducción artificial.

Palabras clave: Número de espermas, motilidad, fecundación, viabilidad.

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017

Digital Journal of
El Hombre y su Ambiente Department
ISSN: 2007-5782

Vol. 1 (13): 1-9. January to June 2017

INTRODUCTION

From the biological point of view, aquaculture is an activity to increase the productivity of aquatic resources through deliberated manipulation of their processes (Aguilera et al. 1988).

In aquaculture one of the important activities is the production of rainbow trout (*Oncorhynchus mykiss*) which is an important specie that is cultured in many countries, due to its facility of accepting artificial food and adapting to temperature changes. Introduction of rainbow trout to Mexico dates back to the late nineteenth century, bringing social, cultural and economic benefits (FAO 2009, Arredondo and Ponce 2011).

Rainbow trout reproduction is sexual and external, and also cyclical. That is why one of the main goals of aquaculture is to take advantage of resources that can be extracted from this specie throughout all the season outside their reproductive peak. Although, sexual maturation time and fecundation is a slow process, therefore, artificial systems to induce both factors have been made. A viable and widely tested alternative to produce eggs all year, is the application of the artificial photoperiod technique to modify the reproductive cycle, improve synchrony of sexual maturation, induce to spawning snd also resolve overpopulation problems (Kissil et al. 2001, Campos-Mendoza et al. 2004, Biswas et al. 2005). On the other hand, the hormonal induction in artificial control of fish reproduction is another alternative to induce spawning. As in adults, there is generally a lag time between the end of gametogenesis and release of gametes, the resumption of maturation and advance the release of gametes, can be caused through hormone or products with gonadotropic activity injection (Camacho et al. 2000, Bernabé 1996).

Many species like the rainbow trout that are maintained in culture systems, present important reproductive dysfunctions (Vargas 2003), among which an induced spawning in addition to follicular atresia stand out. That is why, commercial exploitation of this salmonid needs a permanent supply of fertilized eggs and/or fingerlings that ensure the trout production. For this it is necessary to maintain a roster of spawning fish with high fertility that ensure the production of fertile eggs and fingerlings, required for the productive system (Bastardo et al. 2004).

Ciereszko and Dabrowski (1995), indicates that semen quality (motility and concentration) is influenced by the content of ascorbic acid in the diet. Scott and Baynes (1980), mentions that trout semen has a high concentration, which ranges from 9 to 26 x 10^9 spermatozoids/mL. In general, salmonids semen is a milky white secretion, in some cases a little viscose, and has a motility of less than 30s seconds after spawning in fresh water (Christen et al. 1987; and Torres et al. 2014).

Because of all the above, in this investigation the semen quality or rainbow trout spawning fish from aquaculture center “El Zarco” is determined, to increase the production, considering the qualitative and quantitative characteristics of semen at the end of reproductive period of the specie.

MATERIAL AND METHODS

Spawning fish selection

Five males were randomly selected from Michoacan batch and also a female of approximately three years old, pertaining to aquaculture center “El Zarco”.

Sedation of spawning fish

To sedate the males, plastic containers were used in which carbonated water at 10% was verted, the organisms were placed in the water during the necessary time for loss of normal balance. While for the female, plastic containers with water were used and it was added 0.05 mL of clove oil per liter of

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017

water, the organism was placed during the necessary time for loss of normal balance.

Biometry

Once the organisms were sedated, with the aim of a modified measuring board of 90 x 18 cm, and a 90° bracket, the total length (TL), pattern length (PL), cephalic length (CL), width and height were obtained. Also, it was registered the weight of each organisms by using a digital balance ADAM d=1g.

Gametes extraction

It was made manually with ventral massages in an operculum-caudal direction, for both the males and female, the semen was collected in graduated tubes of 50 mL, while ovules were placed in dry plastic trays.

Semen evaluation

Qualitative and quantitative characteristics were considered. The qualitative were color (white/yellow) and consistency (milky/creamy/aqueous) (Navarro et al. 2004, Bustamante 2015).

The quantitative characteristic was measurement of volume from the graduated tubes, pH using a potentiometer HANNA HI900, the spermatozoids concentration per milliliter was measured with the aim of a Neubauer chamber and motility was observed with field microscope SWIFT FM-31.

Fecundation

For fecundation, 40 g of ovules and 800 µL of semen were mixed and activated with 40 mL of physiological serum and left to stand for ten minutes, this was made by triplicate. After elapsed time, it was rinsed and hydrated for ten minutes. During incubation time, the fecundated eggs were counted.

Spermatozoids number

For this, 50 µL of semen with 950 µL of physiological serum and 500 µL of formol at 10% were fixed. The counting was made with the aim of a Neubauer chamber by counting five quadrants of the chamber by using an optic microscope SWIFT FM-31 at 40X magnification, it was used a dilution of 100 µL of semen and 900 µL of diluent and if the sample was too dense, it was used a dilution of 10 µL of semen and 990 µL of diluent.

The next formula was used to know the number of spermatozoids per milliliter:

$$\text{No. spermatozoids} = \Sigma(5)(5x10^4)(30)(D)$$

Where:
$\sum(5)$ = The sum of counted quadrants
(5×10^4) = volume of Neubauer chamber
(30)= stock dilution
(D)= dilution

Motility

In a microscope slide it was placed a drop of semen using a Pasteur pipette and activated with physiological serum at 0.9%. It was observed under a field microscope SWIFT FM.31 at 40x magnification. Vigorous activity times and until the sperm stopped moving were registered. This procedure was made by triplicate.

Water physical parameters

During incubation time, it was measured the flow of water, registering the volume per minute and temperature with a mercury thermometer BRANNAN -4 to 50°C. These parameters were measured by triplicate every four hours.

Egg eyed stage

At the 18th day of incubation it was determined the number of eyed stage eggs, determining the percentage of these.

RESULTS

Biometric parameters

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017

Digital Journal of
El Hombre y su Ambiente Department
ISSN: 2007-5782

Vol. 1 (13): 1-9. January to June 2017

Relation Length-Weight

In Table 1 it is shown the obtained results of the biometric parameters of the organisms, where most of organisms are between a similar range of Length-Weight, having an average weight of 2.03 kg and an average length of 52.68 cm. While the organisms M1 with 3.21 kg and 61.8 cm of length, surpass the range that is estimated for most of the organisms.

Spermatic motility

In Fig. 1 it is shown the spermatic motility time of each organism. The male M2 was the one that presented higher vigorous motility tine with an average of 28 ± 6.9 s. The sample that presented higher total motility was male M4 with an average of 56.3 ± 19.55 s. and the lowest total motility time was of M7 with an average of 27 ± 2.6 s. On average, the vigorous activity of sperm samples was of 22.33 ± 4.34 s. while total motility until movement decreases was of 46.25 ± 9.08 s.

Table 1. Biometric parameters of studied organisms.

Batch/ organisms	Total length (cm)	Pattern length (cm)	Cephalic length (cm)	Height (cm)	Width (cm)	Weight (kg)
M1	61.80	57.30	14.70	15.10	6.30	3.21
M2	51.10	45.10	11.00	11.40	6.70	1.59
M4	53.40	48.50	13.20	14.20	7.40	2.23
M5	48.30	42.50	11.10	13.00	6.50	1.51
M7	48.80	44.60	11.60	11.20	6.00	1.61
Average	58.68	47.60	12.32	12.98	6.58	2.03
Deviation	±5.48	±5.83	±1.59	±1.70	±0.52	±0.71

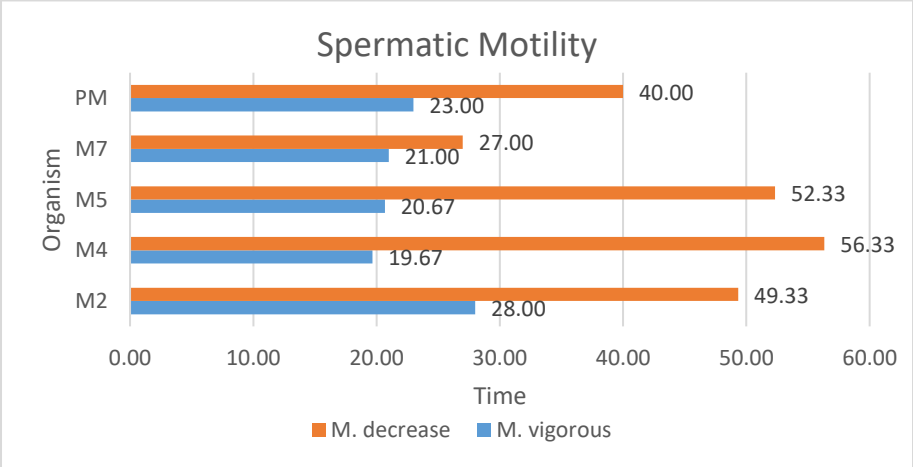

Fig. 1. Average spermatic motility of semen samples from studied organisms.

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017

Spermatic pH

Obtained pH range was of 6.94 to 7.54. Pool sample (PM) was of 7.23.

Volume and spermatozoids number

In Table 2 it is represented the volume and number of spermatozoids, it is observed that the male with more volume was M1 with 30 mL of semen and the male with the lowest obtained semen volume was M2 with 10 mL. Average semen volume was of 18.80 ± 7.77 mL. While the highest number of spermatozoids per volume was obtained by M7 with 25 x 10^8 in 17.5 mL and the lowest spermatozoids per volume was 7.1 x 10^8 in 14 mL obtained from M5. Pool obtained 15 x 10^{10} in 25 mL.

Relation weight per volume

As it is shown in Fig. 2, the organisms that presented higher semen volume, are the ones that have more weight, because the organism M1 that presented more semen volume with 30 mL, it is also the one that has more weight from all organisms with 3.21 kg. Organism M2 is the one that produces fewer semen with 10 mL and a weight of 1.59 kg. The organism with less weight was M5 but it produced 14 mL of semen. In average, the semen volume was of 18.8 ± 7.76 mL, and a weight of 2.03 kg. In total, it was obtained a correlation of R^2= 0.8708 of the organisms and its relation to volume.

Water flow

Water flow during incubation time was in average of 9.22 L min^{-1}, except at 11 and 16 h. where water flow increased in average to 20.36 L min^{-1}. According to variance analysis it was obtained a significant difference (P<0.05) between the hours.

Viability

As it is shown in Fig. 3, the average of fertilized eggs was of 419 ± 1.82, being the 99.52% of the total of eggs and the average of eyed stage eggs was of 66.50%.

Table 2. Volume and spermatozoid number of Michoacan batch.

Organism	A	B	X	DESVEST	mL kg^{-1}	Volume (mL)	Spz vol^{-1}
M1	84x10^6	45x10^6	65x10^6	±28x10^6	9.35	30.0	19x10^8
M2	99x10^6	75x10^6	87x10^6	±17x10^6	6.29	10.0	8.7x10^8
M4	36x10^6	39x10^6	38x10^6	±2.1x10^6	10.09	22.5	8.4x10^8
M5	66x10^6	36x10^6	51x10^6	±21x10^6	9.27	14.0	7.1x10^8
M7	12x10^7	16x10^7	14x10^7	±28x10^6	10.87	17.5	25.0x10^8
PM	65x10^8	57x10^8	61x10^8	±57x10^7		25.0	15.0x10^{10}

Note: A= Number of spermatozoids in chamber A; B= Number of spermatozoids in chamber B; X= average of spermatozoids number; DESVEST = standard deviation of spermatozoids number; mL kg^{-1} = relation weight per volume; Spz= spermatozoids/mL.

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017

Digital Journal of
El Hombre y su Ambiente Department
ISSN: 2007-5782

Vol. 1 (13): 1-9. January to June 2017

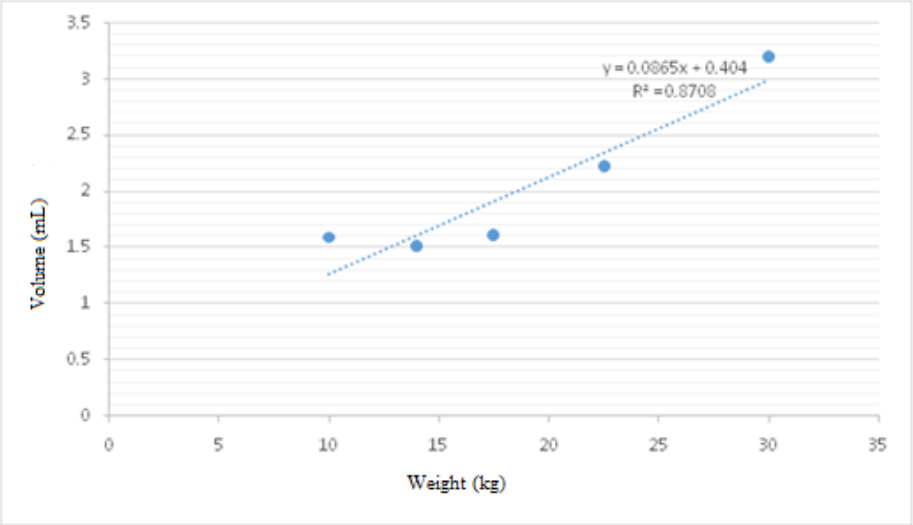

Fig. 2. Correlation of the organism weight against the obtained semen volume of studied organisms.

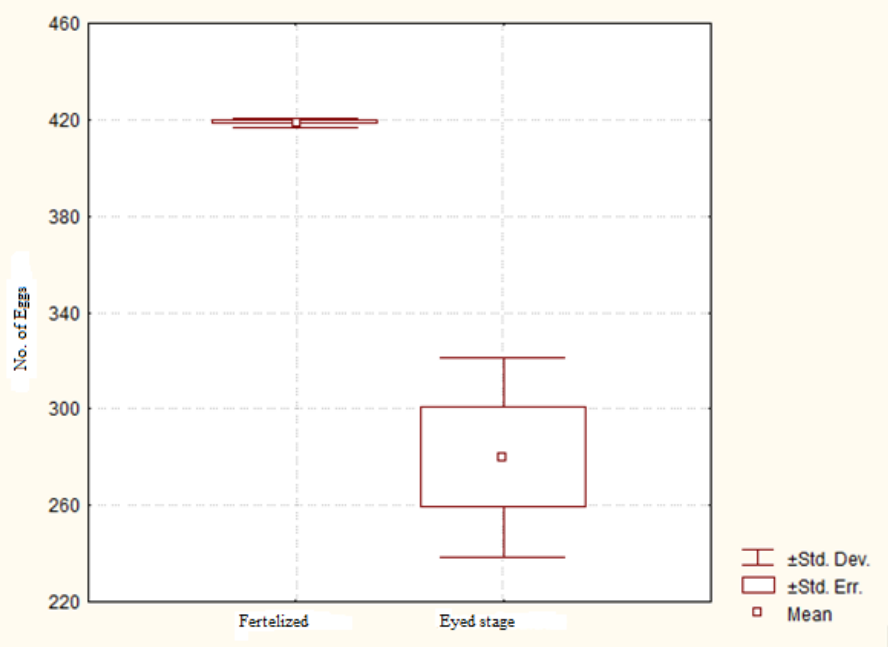

Fig. 3. Variance analysis of fertilized and eyed stage eggs of Michoacan batch.

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017

DISCUSSION

Regarding to Weight-Length relation Olaya-Nieto (2008), in his investigation with *Salminus affinis*, a specie of the same family as rainbow trout, mentions that the relation weight-length is related to the type of growth, taking into account that the size of a fish increase in a dimension, while its weight does it in three dimensions, this accords with the obtained data in this investigation, because the 71.42% of the organisms are concentrated in a range between the weight and total length, therefore having a correlation weight-length, and the rest of the organisms show a significant difference in observed measures, so it can be said that proportions of length and weight are tightly related.

Regarding to spermatic motility, Valdebenito (2007) obtained a higher motility in rainbow trout with an average of 80.8 s., while obtained total motility in this investigation has an average of 46.25 s. On the other hand, Billard and Cosson (1989) had a lower motility in rainbow trout semen, diluted in a solution of NaCl 125 mM and Tris 20 mM at a pH of 9.0, sperm is only motile 20-30 s, but when they added 1mM Ca^{++} the motility was above 30 s; and Vásconez et al. (2011) with five spawning fish obtained a similar motility average to Billard and Cosson (1989) with 33.68 s, lower values than the ones obtained in this investigation. Wanger et al. (2002), obtained a spermatic motility similar to this investigation, with a motility range of 37-56s with organisms also sedated with clove oil. It should be noted that Piñeros y Cara (1991) mention that motility evaluation by using microscope slides affects negatively the movement of spermatozoids, resulting in a movement of low change of position and not in a progressive one.

For the seminal pH, Bastardo et al. (2004), in their investigation of rainbow trout during the reproductive peak (September to January) points out that three-year-old males obtained a pH of 8.2, a higher pH than the one obtained in this investigation, made during the final of reproductive period, with a value of 7.23. Nevertheless, Ciereszko et al. (2010), mentions that in three-year-old rainbow trout males, during reproductive period, obtained a pH of 7, which is a lower value than the one obtained in this investigation.

Valdebenito (2007), mentions that to determine the number of spermatozoids in its investigation it was used a saline solution 0.98% of NaCl to activate the spermatozoids, and obtained $12.7 \pm 1.6 \times 10^9$ spermatozoids per mL. On the other hand, Bastardo (2004), points out that three-year-old males that he observed during the reproductive period, had a concentration of 1.2×10^6 per mL. These investigations obtained lower values when comparing to this investigation where it was obtained 15×10^{10} spermatozoids per mL. According to Bastardo (1992), this difference in the number of spermatozoids between reproductive peak and the end of reproductive period can be due to consumed food and water quality used for culture.

Regarding to relation between weight and volume, in the investigation made by Piñeros and Cara (1991), there is a relation, because the organisms with more weight are the ones that produced a higher semen volume, as in this investigation, where the organisms that

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017

produced more volume with 30 mL, was the one that had a greater weight with 3.21 kg.

Water flow according to SEPESCA (2000), for incubation of rainbow trout in California incubators, must be of 14 L min^{-1}, a relatively high value compared to the water flow in this experiment which was of 9.22 L min^{-1}. But if this one is compared to the peaks of water flow during the investigation, is a lower value because it reached a flow of 20.36 L min^{-1} during the day. On the other hand, FAO (2009), mentions that there must be a water flow of 7-8 L min^{-1}, an average closer to the one obtained. While Bustamante (2013) mentions that to incubate around 40,000 to 50,000 fertilized eggs, there must be a water flow of 16-18 L min^{-1}, an average closer to the water peaks obtained in this investigation.

The fecundation rate compared to the one obtained by Valdebenito (2007) which average of fertilized eggs was of 81.66 ± 5.7% with control sample, while Bastardo (1994), obtained a percentage of 24%, being also a lower value to the one obtained in this investigation (99.52 ± 1.8%), even though it was made during the end of the reproductive period. That is why the obtained information is relevant for the aquaculture center "El Zarco" because this means they can continue the fertilization process in the final stage of the reproductive period and ensure a higher production.

CONCLUSSIONS

According to the obtained results, the spawning males of Michoacan batch present a good quality and quantity of spermatozoids, because it was obtained a high fecundation rate and a 66% of eyed stage egg, noting that it was made at the end of the reproductive period.

ACKNOWLEDMENTS

To the aquaculture center "El Zarco" for allowing the realization of this investigation. To Universidad Autonoma Metropolitana Xoxhimilco, especially to the Biology Department for allowing us to use their laboratories. An also to our classmates of group BE03B of trimester 16-I for their support.

BIBLIOGRAPHY

Aguilera H. P. y C. P. Noriega, 1988. ¿Qué es la acuacultura? Secretaría de Pesca. México.60 p.

Arredondo, J., y Ponce, J. 2011. Bases biológicas para el cultivo de organismos acuáticos de México. Ed. AGT Editor, México. 131 y 134-142 p.

Barnabe. G., 1996. Bases biológicas y ecológicas de la acuicultura. Editorial Acribia. Zaragoza, España. 364-368 p

Bastardo H. 1992. Semen de la trucha arco iris (*Oncorhynchus mykiss*): Concentración y volumen durante un periodo reproductivo en Mérida, Venezuela. Veterinaria Tropical 17: 56-66.

Bastardo H. 1994. Semen de la trucha arco iris (*Oncorhynchus mykiss*): Concentración y volumen durante un periodo reproductivo en Mérida, Venezuela. Veterinaria Tropical 17: 56-66.

Bastardo H, Guedez C y León M. 2004. Características del semen de trucha arco iris de diferentes edades, bajo condiciones de cultivo en Mérida, Venezuela. Zootecnia Tropical 22(3): 277-288.

Billard R y Cosson MP. 1989. Measurement of sperm motility in trout and carp. In: De Pauw, N, Jaspers E, Ackefors H.(Ed.). Aquaculture: a biotechnology in progress 1: 499-503.

Biswas, A.K., Morita, T., Yoshizaki, G., Maita, M. and Takeuchi, T. 2005. Control of reproduction in Nile tilapia *Oreochromis niloticus* (L.) by photoperiod manipulation. *Aquaculture* 243: 229-239 (a).

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017

Bustamante JD. 2013. Evaluación del semen de la trucha arco iris *(Oncorhynchus mykiss)* en organismos de primera y segunda reproducción. Tesis de Licenciatura. Universidad Autónoma Metropolitana. Ciudad de México. 41 p.

Bustamente JD. 2015. Caracterización del periodo reproductivo en machos de trucha arco iris (*Oncorhynchus mykiss*) bajo condiciones de cultivo. Tesis de Maestría Ciencias Agropecuarias. Universidad Autónoma Metropolitana Xochimilco. Ciudad de México. 57 p.

Christen R, Gatti JL, Billard R. 1987. The transient movement of trout sperm is related to changes in the concentration of ATP following the activation of the flagellar movement. Eur. J. Biochem. 166: 1667-671.

Ciereszko A, y Dabrowski K. 1995. Sperm quality and ascorbic acid concentration in rainbow trout semen are affected by dietary vitamin C: an across-season study. Biology of Reproduction, 52: 982-988.

Ciereszko A, Dietrich GJ, Dietrich MA, Nynca J, Kuzminski H, Dobosz S y Grudniewska J. 2010. Effects of pH on sperm motility in several Salmonifers species: *Oncorhynchus mykiss*, *Salvelinus fontinalis*, *Salmo trutta*, *Salmo salar* and *Thymallus thymallus*. Applied Ichthyology 26: 665-667.

Kissil, G.W., I. Lupatsch, A. Elizur, and A. Zohar. 2001. Long photoperiod delayed spawning and resulting increased somatic growth in gilthead seabream (*Sparus aurata*). Aquaculture 200: 363-379.

Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO). 2009. La FAO en México más de 60 años de cooperación 1945- 2009. Roma, Italia. pp. 219-257.

Olaya-Nieto C, Tordecilla-Petro MS, Segura-Guevara S. 2008. Length-weight relationship of rubio (*Salminus affinis steindachner*, 1880) in the Sinu river basin, Colombia. 13(2): 1349-1359.

Piñeros R y Cala P. 1991. Motilidad, morfología, concentración y número de espermatozoides en reproductores de trucha arco iris (*Oncorhynchus mykiss*). Revista Académica Colombiana Ciencia 18: 75-81.

Secretaría de Pesca (SEPESCA). 2000. Guía para el cultivo de trucha. Secretaría de Medio Ambiente, Recursos Naturales y Pesca (SEMARNAP). Ciudad de México. 30 p.

Scott AP y Baynes SM. 1980. A review of the biology, handling and storage of salmonid spermatozoa, J. Fish Biol., 17: 707-739.

Torres JM, Maíz RA, y Castellano JJ. 2014. Aspectos de la reproducción anual de semen de trucha arcoiris (*Oncorhynchus mykiss*) en los andes tropicales venezolanos. Mundo Pecuario 1: 09-14.

Valdebenito I. 2007. Efecto de la Cafeína en la Motilidad y Fertilidad Espermática de Trucha Arcoiris (*Oncorhynchus mykiss*). Información tecnológica 18(2): 61-65.

Vargas R. 2003. Evaluación de trucha arco iris (*Oncorhynchus mykiss*) producida en Costa Rica. Agronomía Mesoamericana 14: 123-127.

Vásconez MJ, Ortiz J, Giacometti (2011). Criopreservación de semen de trucha arco iris *(Oncorhynchus mykiss)* en el programa de mejoramiento genético de truchas en Ecuador.

Wagner, E., R. Arndt and B. Hilton, 2002. Physiological stress responses egg survival and sperm motility for rainbow trout broodstock anesthetized with clove oil tricainemethansulfonate or carbon dioxide. *Aquaculture*, 211: 353-366.

Receive: November 11th 2016 Accepted: April 05th 2017 Published: June 30th 2017