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EVALUATION OF SEXUAL MATURITY IN MALE COMMON CARP (*Cyprinus carpio*, Linnaeus, 1758) IN MEXICO UNDER GROWING CONDITIONS

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Abstract: The tent in Mexico was aimed at the low-income social sector; Its use in bodies of water where it is produced generates food and economic resources for the communities. At the Tezontepec de Aldama Aquaculture Center, Hidalgo, in charge of its propagation, a study was carried out to determine if the males maintained the production and quality of semen during the year, determining: volume, milliliters/weight of the organism, number of spermatozoa per milliliter and density. The statistical analysis of the results determined that there are significant differences (P<0.5) between the variables throughout the year, confirming the maturity period in the warm months, but maintaining the seminal production throughout the year.
Keywords: Seminal volume, Volume per kilogram of weight, number of spermatozoa per milliliter, density.

INTRODUCTION

The common carp: *Cyprinus carpio,* is the species with the longest history, the first treatise on its cultivation was written in the 5th century BC. C by Fan Li, and since then, it has been distributed throughout the world due to its biological characteristics such as: high fecundity and survival rate, resistance in all phases of development, rapid adaptation to unfavorable situations and variety of climates, rapid growth and ability to populate large expanses of water, reasons that determined that developing countries imported it to provide food in rural communities (Cházari, 1884; Baumann et al., 1983; Bardach et al., 1993; Balon, 1985).

In 2018, the world production of freshwater fish placed it in fourth place, with: 4,189.5 X 10^6 tons, which represented 7.7% of total production (FAO, 2020). On the other hand, it constitutes a species of scientific interest, since its origin, morphological and physiological diversity determine that it is one of the most studied internationally.

Mexico, in 1876, imported it from the United States of North America, where it arrived from Germany and was destined for extensive production in support of the rural community, to provide them with protein and economic resources, since due to its flavor it would not be appreciated by other sectors; however, currently in the States of Mexico, Hidalgo, Michoacán and Tlaxcala, carp-based dishes are prepared with regional demand (Rodríguez et al., 2016).

The Tezontepec de Aldama Aquaculture Center, (CATA), Hidalgo state, since 1965, was a center for the reproduction and distribution of baby carp throughout the country and due to the importance and positioning of the species, interest arose to delve into its physiology, due to its adaptability in different regions of the world where it is cultivated. Due to this, the present study describes the sexual maturity in males throughout the year, through the study of the volume and seminal quality.

METHODOLOGY

The present investigation was carried out in CATA, a total of 193 organisms were sampled in different months of the year from rustic ponds supplied with spring water and with an average temperature of 22 ± 1 °C, fed with concentrated food, suspended when they were sampled for avoid contamination with feces.

The selection of organisms was carried out when they emitted semen under gentle pressure, stage V, Nikolsky's Empirical Maturation Scale (1963).

The manipulation of each organism was performed under deep anesthesia, with xylocaine solution (Rodríguez-Gutiérrez and Esquivel-Herrera, 1992), weight, standard length (LP), height (H) were taken, and semen extraction was performed.

The semen was collected in a graduated centrifuge tube, kept refrigerated until the sperm evaluations were carried out, which consisted of: determining the volume (quantified directly from the centrifuge tube), and considering the weight of the organism, mL/kg was calculated; the number of spermatozoa/mL (using diluent solution) and counting in the Neubauer Chamber; total number of spermatozoa, which was calculated from the number of spermatozoa per milliliter multiplied by total volume; Density was determined from the ratio of the weight of 100 µL of semen, and motility evaluated by direct observation of the percentage of sperm motility under an optical microscope, mass motility, taking two times: the first with the greatest vigor, T1 and T2, until it ceases (Rodríguez y Esquivel 1992).

The statistical analysis was carried out with the JMP V.10 software, by means of a multivariate ANOVA using the months of the annual cycle as a contrast factor; with Tukey's post hoc test ($p<0.05$) to delimit subsets, results are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

The diversity of teleosts due to the conditions where they develop determines that some species have reproductive cycles defined by the climatic seasons. The indicator of sexual maturity in males is the emission of semen, which, in a mature organism, depending on the reproductive season, can be collected in variable quantities (Billard and Cosson, 1992; Bustamante et al., 2018).

Taking the above into account, the results of the selected variables are summarized in Table 1, which presents mean values and standard deviation (SD). With respect to size, the heaviest organisms were sampled in December, April and September, in which it is observed that, if the size corresponded to maturity, the largest seminal volumes must have been collected in these months; however, this was not the case, except for April.

The analysis of semen volume per month with Tukey's post hoc test, (Table 1), significant differences were determined ($p<0.05$), the lowest volumes were obtained in the autumn and winter months, which according to the reports of other authors there is no semen production under their conditions (Bekh et al., 2008); however, in CATA, semen was obtained throughout the year; on the other hand, the SD of semen volume was higher in April and December, which denotes the variability of sexual maturity in the study population (Bustamante et al., 2018).

This was corroborated by the measurements of the seminal volume (mL/kg), in this regard the analysis of variance showed significant differences ($p<0.05$) between months and the Tukey post hoc test indicated that January, February, September, October November and December are significantly different from April, May, June, and July. The foregoing shows that the months that reached the highest values were April, May, June and July, which correspond to the reproductive season reported by authors such as Rodríguez and Marañón, (1993); Cruz and Murillo, (1992); Bekh et al., (2008) determined by the maturity of the females (fig. 1).

On the other hand, various authors have reported that the seminal volume presents high variability during the reproductive period, in some species, at the beginning and end of the season it decreases; while in others the same amount is produced on average, which may be affecting the heterogeneity of the results obtained (Rodríguez et al., 1991; Bekh et al., 2008; Bustamante et al., 2018).

In this regard, it is worth mentioning that

Variable	January	February	April	May	June	July	Sep.	October	November	December.
Weight (g)	220.6 ^{ab}	223.6 ^{ab}	502.7^{d}	237.3^{b}	136.3^{a}	154.5 ^{a}	438.9^{cd}	328.3^{bc}	205.7 ^{a}	673.0^{e}
	±46.5	±74.7	±148.6	±131.3	±28.1	±49.0	±177.6	±138.0	±58.2	±106.6
Volume (mL)	0.30^{ab}	0.38^{ab}	6.02^{d}	2.27^{ab}	1.25^{a}	1.07^{a}	1.18^{cd}	0.50^{bc}	0.59^{a}	3.50^{e}
	±0.20	±0.22	±5.43	±0.92	±1.00	±0.87	±0.87	±0.37	±0.61	±3.27
mL/K	1.53^{a}	2.01^{a}	12.35^{c}	11.60^{c}	8.46^{b}	7.00^{a}	2.62 ^{a}	1.72^{a}	3.06^{a}	5.04 ^{a}
	±1.31	±1.69	±9.21	±5.67	±7.93	±5.05	±1.63	±1.42	±3.19	±4.46
Density	1.12^{cd}	1.21^{d}	0.87^{a}	0.93^{abc}	0.89^{ab}	1.09^{bcd}	0.90 ^{abc}	1.01^{abc}	1.02^{abcd}	0.91^{abc}
	±0.11	±0.28	±0.11	±0.07	±0.12	±0.15	±0.11	±0.16	±0.17	±0.10
Number of spermatozoa: X 10*^{6}	32338.8^{a}	33148.39^{a}	24950.00^{a}	37985.16^{b}	36632.52^{a}	40053.74^{b}		28164.28^{a}	30881.00^{a}	
	±9142.52	±13804.6	±8060.00	±7559.07	±12467.95	±12614.94		±7099.80	±7526.36	
Total number of spermatozoa: X 10*^{9}	8659.70	12358.36	141355.00	84017.33	41912.53	41216.06		15818.57	17921.21	
	±2854.90	±8627.99	±129010.67	±33593.10	±31600.71	±32691.46		±13906.35	±22976.63	

Table 1. Summary of variables with descriptive statistics (mean, SD±) of common carp (*Cyprinus carpio*) during an annual cycle. (Different letters as superscripts in the means indicate significant differences, Tukey's post hoc test).

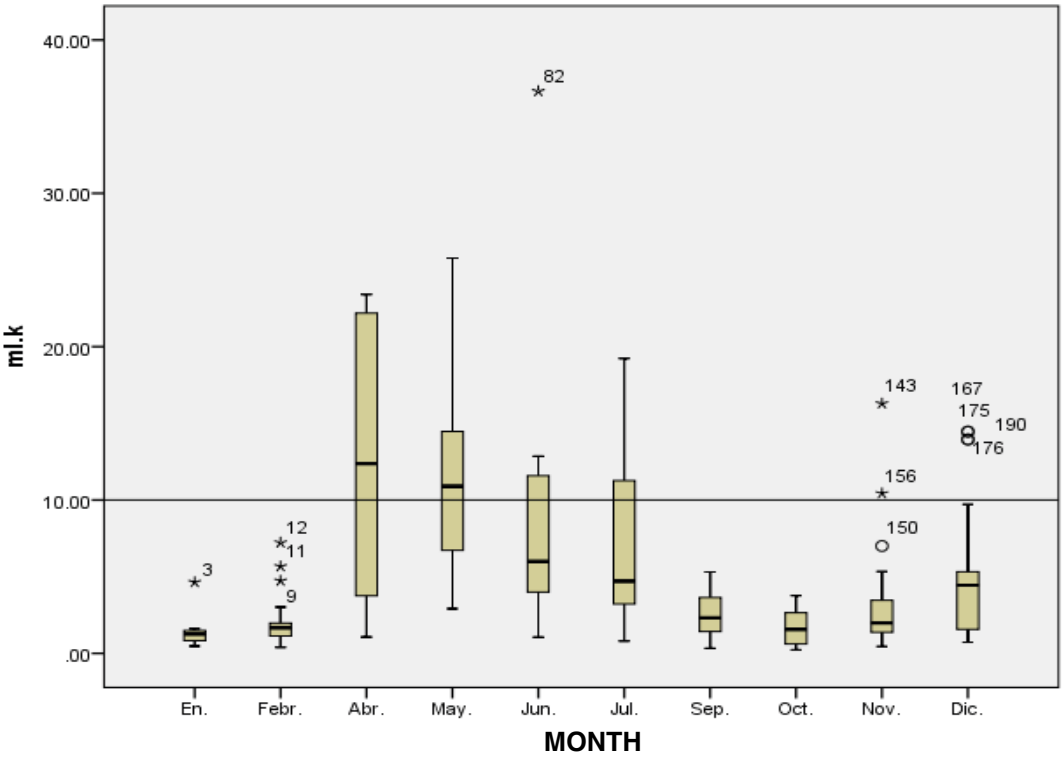

Figure 1. Box plot of milliliters of semen/kg of *C. carpio* in different months of the year.

during the annual cycle it was not possible to sample the same organisms, which makes interpretation and analysis difficult, since semen production depends on seasonality, duration of the reproductive period, the moment of sampling with respect to the cycle. reproductive that is different for each species and determined by the production of eggs, which is not always synchronous with that of sperm (Cruz and Murillo, 1992).

When comparing semen volume other authors such as Rodríguez and Marañón, (1993) in organisms of 200 g, in the different seasons of the year, obtained 1.5 mL in spring; 0.8 mL in summer; 0.4 mL in autumn and 0.5 mL in winter and although in this study it was by months, when grouping them by season of the year, on average, 4.1 mL was obtained for spring, 1.1 mL in summer, 0.72 mL in autumn and 1.39 mL in winter, being in all cases, averages higher than those indicated previously.

Although the growing conditions are similar throughout the year, the results reflect the influence of the photoperiod on the seminal production, proposing a period from April to July as a period of higher production and a lower one from September to February, a situation different from countries with marked climatic conditions. such as the Czech Republic, in which the reproductive season of this species is limited to the month of May (Horvárth et al., 1986a; Bekh et al., 2008), while in Mexico and particularly under the climatic conditions of cultivation of CATA where the water temperature is maintained at 22 ± 1°C; semen can be obtained throughout the year, although in less quantity in the cold months, probably due to inactive spermatogenesis, as Barry et al. (1990); Billard and Cosson (1992).

Regarding the number of spermatozoa per milliliter, an average of 34.5 X 10^6 was determined (Table 1, Fig. 2) and the analysis of variance reported significant differences ($p<0.05$) between months and Tukey's post hoc test established that April, October, November, January, February and June are different from May and July, however, the average value is within the range reported by other authors for the species, as has been indicated by Rodríguez and Marañón, (1993); Cruz and Murillo, (1992); Bekh et al., (2008).

The variability of the number of spermatozoa has been reported by several authors and even in the same individual, and has a physiological explanation in gametogenesis, caused by spermiation that determines sperm hydration, degree of fluidity, and, therefore, density. and variability in the number of spermatozoa per milliliter, in addition to the lack of a seminal vesicle that accumulates them (Clemens and Grant, 1965; Billard et al., 1995).

The variability with respect to the number of spermatozoa per milliliter, this also depends on the variety, age, size of the organisms, type of diet, physicochemical conditions in which they are kept, as well as whether they are individuals of first reproduction and the phase of the cycle. reproduction in which they were sampled, the method and diluent used for the determination (Biegniewska et al., 2010).

The annual average number of spermatozoa per milliliter determined in the present investigation was 34.5 X 10^9, being different from that determined by Billard et al., (1983), who established 20.9 and 23.1 X 10^9 spermatozoa per milliliter, quantified by optical density; Saad and Billard (1987a) report 25 X 10^9; Barry et al., (1990) determined 1.52 X 10^9 and 2.02 X 10^9 by counting with a hemocytometer; while Rodríguez and Marañón (1993) quantified 37.3 X 10^9 with Cámara de Neubauer and Biegniewska et al., (2010) 18.7 ± 3.1 X 10^9.

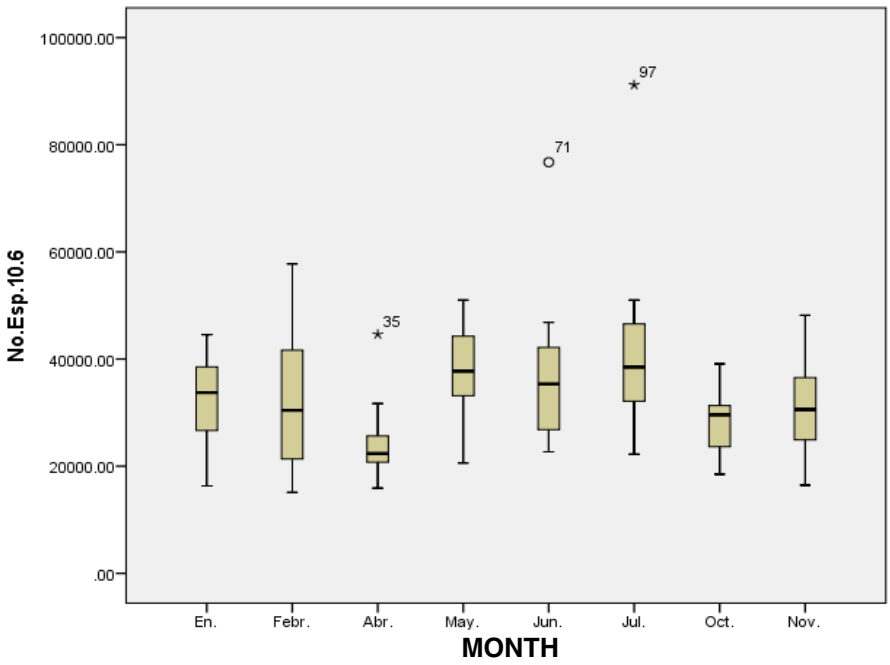

Figure 2. Box plot of the number of spermatozoa/mL in *C. carpio* in the different months of the year.

Total sperm production is a function of volume and number per milliliter; Table 1 shows the average values with the standard deviation obtained during the year.

This is relevant in aquaculture farms when they use artificial fertilization in any of the methods: wet, dry or super dry, where authors such as Rothbard (1981) indicate that 2 to 3 milliliters of semen per liter of ovules are necessary; while Horváth and Tamás (1986) recommend 10 milliliters per kilogram of egg. Under experimental conditions, Saad and Billard (1987b) found that 17,000 spermatozoa are necessary to fertilize an egg.

The amounts of semen recommended by these authors are excessive, when related to the number of spermatozoa per milliliter, since one liter has 650,000 ovules and one kilo 500,000, due to this, a male is capable of fertilizing several females; since a milliliter of semen has the order of 34.5 X 10^9 spermatozoa, therefore, the number of breeding males in intensive aquaculture can be reduced, after selection, which reduces production costs.

The average results of semen density in the different months of the annual cycle are shown in table 1. The lowest average value was presented in April with 0.87; and the highest of 1.21 was determined in February. In the rest of the months considered cold, the value exceeded unity, except in December and in the months considered warm it was less than unity; which suggests that hydration is higher in the warm months, so it is assumed that the adequate density for reproduction is less than one. There are few reports on density, Stancey et al., (1994) pointed out: one for semen density in *Cyprinus carpio*.

Density is directly related to the number of spermatozoa per milliliter and inversely related to volume, due to the semen hydration process prior to ejaculation, since in the case of teleosts and particularly carp there are changes in sperm density. semen related to maturity, presenting fluid semen in the spawning season (Clements and Grant, 1965; Billard, 1970).

The reproduction period from April to September, determined by the maturity of the females, has been reported for carp by Huet (1983); Woynarovich and Horvárth (1980) who point out that it is possible to have more than one spawning per year as long as the water temperature is higher than 22º C; and that the day is longer than the night, conditions that are fulfilled in the CATA, being then the temperature and the photoperiod the triggering stimuli of spermatogenesis, since according to Barry et al., (1990); Billard and Cosson (1992), temperature plays a determining role in the complex endocrine process that triggers spermatogenesis.

In CATA it was observed that males reach sexual maturity at small sizes, since organisms less than one year old and 100 g mature were found; while, in Poland, Billard and Cosson (1992) report that they mature when they weigh 187 g and are thirteen months old; in Israel they reach it in six months and in the south of France in the second year (Billard, 1995).

Another characteristic of the semen evaluation is sperm motility, since numerous studies suggest that the duration in fish is related to the fertilization capacity, since it is limiting in fertilization, because the sperm locate and fertilize depends on it. to the ovule, which keeps the micropyle open for one minute (Kudo, 1982; Biegniewska et al., 2010; Berois et al., 2011).

It is generally accepted that sperm motility in freshwater species is short-lived, only one to two minutes, on the other hand, teleost sperm are immobile within the testicles and remain immobile when diluted in solutions. isotonic, but when exposed to hyposmotic media, they are immediately activated as is the case in carp (Billard and Cosson, 1992; Bastani et al., 2010; Bustamante et al., 2018).

Regarding motility, the duration of T1 was 45.3 s and T2, 107.8 s, it is suggested that the decay of the flagellar movement that starts T2 is associated with the decrease in intracellular ATP (Morizawa et al. 1983; Billard and Cosson 1992).

The results obtained by Alavi and Cosson (2006) and Bastami, et al., (2010) these authors indicate that carp sperm motility is activated in media with osmotic pressure lower than 150 - 200 mOsmolkg, and that sperm exposure to extreme osmotic conditions it produces changes in morphology and therefore in movement capacity, a common situation when it is contaminated with urine in artificial fertilization.

The way to store semen must be carefully selected in order to keep the ATP concentration and the energy of adenylate as long as possible close to physiological values (Inaba, 2003; Cosson et al., 2008).

The amount of extender used to quantify motility depends on the species, Billard and Cosson (1992), Bustamante et al., (2018), recommend 1:1000 for the simultaneous activation of all spermatozoa; while Ciereszko and Dabrowski, (1993) use 10 µl of semen per 100 µl of activating solution, Bastami, et al., (2010), propose that for uniform activation, the semen is first prediluted in isotonic solution, hence it takes 10 µl in 2000 µL, to form a stock solution and add the solutions to be evaluated.

The variation in the rate and duration of motility between different males can also be attributed to the effect of contamination of the semen during extraction, either with water, fecal matter or urine, and the lack of environmental conditions for storage that cause activation (Rodríguez et al., 2009; Bastami, et al., 2010). Although there are also references that indicate that motility varies in force and duration, between males and even between the same male depending on maturity, feeding conditions,

age, environmental factors, period of reproduction or dilution and of course the ionic composition and the osmolarity of the solution used (Ciereszko and Dabrowski, 1993).

Taking the above into account, the motility estimate is influenced by multiple factors, that is, it depends on whether the sperm cells are many or few, which determines that the motility percentage may be overestimated or underestimated; it also explains the diversity of motility times reported for the same species.

CONCLUSIONS

From the analysis of the results, it can be affirmed that the volume of seminal fluid is a good indicator of sexual maturity, especially when relating it to mL/kg of body weight.

Given the climatic and water conditions of the Tezontepec Aquaculture Center, it is possible to extract semen all year round, although in a greater proportion in the warm months, considered the "natural" spawning season, determined by the maturity of the females, than in the summer. case of cold months.

Males produce semen not based on their size, but rather on maturity, influenced by the season of the year, so it is possible to find small and large organisms that have good amounts of semen, or that lack it.

The number of spermatozoa per milliliter presents variability due to the state of maturity of the organism, caused by sperm hydration and climatic conditions.

The average number of spermatozoa per milliliter determined for common carp was $34.5 \text{ X } 10^6$.

The average semen density in the natural spawning season is less than 1.

Regarding motility, it is established that in water the T1 of greatest activity lasts 45s and T2 ends at 107s.

REFERENCES

Alavi, S., y Cosson, J. (2006). ***Sperm motility in fishes (II) effects of ions and osmolality: A review.*** Cell Biology International. 30 1–14.

Balon, E. K. (1995). ***Origin and domestication of the wild carp, Cyprinus carpio: from Roman gourmets to the swimming flowers***. Aquaculture. 129. 3-48.

Bastami, K. D., Imanpour, M. R., y Hoseinifar, S.H. (2010). ***Sperm of feral carp Cyprinus carpio: optimization of activation solution***. Journal International. 18: 771-776.

Bardach, J., Ryther, J., y McLarney, W. (1986)**.** ***Acuacultura. Crianza y Cultivo de Organismos de Agua Dulce y Marinos.*** AGT Editor. México. 884 pp.

Barry, T., Katsumi, A., Takuji, O., y Isao, H. (1990). ***The shift from C-19 to C-21 steroid synthesis in spawning male common carp, Cyprinus carpio, is regulated by the inhibition of androgen production by progestogens produced by spermatozoa***. Biology of Reproduction. 43: 105 -112.

Baumann, P., Jaeger, J. y Elliot, K. (1983). ***Freshwater Companion Fisherman's***. Van Nostrand Reinhold Company Limited. 203 pp.

Bekh, V., Hrytsynyak, I. Bogeruk, A., Lukanova, I., Bialowas, H. Irnazarow, I., Rakus, K. Jurecka, P. Pilarczyk, A. Kurkubet, G., Domanchuk, V., Jeney, Z., Gorda, S., Bakos, J., Kocour, M., Gela, D., Slechtova, V., Kopecka, J., Slechta, V., Rodina, M., Flajshans, M., Tarazevich, E., Semenov, A., Kniga, M. Sazanov, V., Dudarenko, A. y Vashkevich, L. (2008*).* ***Catalogue of carp breeds (Cyprinus carpio L.) of the countries of central and Eastern Europe.*** Russian. Ministry of Agriculture of the Russian Federation.

Berois, N., Arezo, M., y Papa, N. (2011*).* ***Interacciones de gametos en peces teleósteos: la envoltura del huevo. Estudios básicos y perspectivas como biomonitor ambiental.*** Biol. Res. 44: 119-124.

Biegniewska, A., Zi, M., Rurangwa, E., Ollevier, F., Swierczynski, J., y Skorkowski, E. F. (2010). ***Some differences between carp (Cyprinus carpio) and African catfish (Clarias gariepinus) spermatozoa motility***. J. Appl. Ichthyol. 26: 674–677.

Billard, R. (1970). ***Ultrastructure compareé de spermatozoides de quelques poissons Téléosteéns. In: Comparative Spermatology***. Ed. B. Baccetti. Academic Press, New York. Págs. 71 – 77.

Billard, R. (1995). ***Les carpes. Biologíe et élevage***. INRA, Paris. 387 pp.

Billard, R., y Cosson, J. (1992). ***Some problems related to the assessment of sperm motility in freshwater fish***. J. Exp. Zool. 261:122 – 131.

Billard, R., Cosson, J., Crim, L., y Suquet, M. (1995). ***Sperm physiology and quality. In: Broodstock management and eggs and larval quality***. Edit. Bromage, N., Roberts, R. Blackwell Science. Great Britain. pp. 25 – 52.

Billard, R., Choisis, J. P., y Reinaud, P. (1983*).* ***Stimulation of spermiation in carp in response to LHRH and D-ALA 6-LHRH 6thylamide***. Aquaculture, 35, 173-176.

Bustamante-González J. D., A. Cortés-García y M. Rodríguez-Gutiérrez. (2018). ***Crecimiento y calidad espermática en trucha arcoíris Oncorhynchus mykiss (Teleostei: Salmonidae) durante la temporada reproductiva***. Hidrobiológica 28 (1): 163-170.

Ciereszko, A., y Dabrowski, K. (1993). ***Estimation of sperm concentration of rainbow trout, white fish and yellow perch using a spectrophotometric technique***. Aquaculture. 109: 367-373.

Clemens, H., y Grant, B., (1965). ***The seminal thinning response of carp (Cyprinus carpio) and rainbow trout (Salmo gairdneri) after injections of pituitary extracts***. COPEIA. No. 2.

Cosson, J. (2008)**.** ***Methods to Analyse the Movements of Fish Spermatozoa and their Flagella.*** In: Fish spermatology. Eds. Alavi, S.M.H., Cosson, J., Coward, K., Rafiee, G. Alpha Science, Oxford, pp. 64-102.

Cruz, G., y Murillo, L. (1992). ***Estrategias para el manejo de reproductores de ciprínidos.*** *In*: Temas actuales sobre reproducción de teleósteos. Edit. Martha Rodríguez. Secretaria de Pesca. Universidad Autónoma Metropolitana. México. 37 -56 pp.

Cházari, E. (1884). ***Piscicultura de Agua Dulce***. Secretaría de Pesca. Edición Facsimilar. (1984). México. 828 pp.

FAO. (2020). ***El estado mundial de la pesca y la acuicultura. La sostenibilidad en acción.*** Roma 243 pp. https://doi.org/10.4060/ca9229es

Horváth, L, Tamás, G., y Coche, A. (1986). ***La carpa común. Parte 1. Producción masiva de huevos y prealevines.*** Organización de las Naciones Unidas para la Agricultura y la Alimentación. Roma. 87 pp.

Huet, M. (1983). ***Tratado de piscicultura***. Ediciones Mundi Prensa, Madrid. 728 pp.

Inaba, K. (2003). ***Molecular architecture of the sperm flagella: molecules for motility and signaling.*** Zoolog. Sci.20: 1043-1056.

Kudo, S. (1992). ***Ultraestructure and ultracytochemistry of fertilization envelope formation in the carp eggs.*** Develop. Growth and Devr. 24 (4): 327 – 339.

Morizawa, M., Suzuki, K., Shimizu, H., Morisawa, S., y Yasuda, K. (1983). ***Effects of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes***. J. Exp. Biol. 107: 95 – 103.

Nikolsky, G. (1971). ***The ecology of fishes***. New York, Academic Press. 342 pp.

Rothbard, S., (1981). ***Induced reproduction in cultivated cyprinids-the common carp and the group of Chinese carps. 1. The technique of induction spawning and hatching.*** Bamidgeh. 22: 42 -47.

Rodríguez, M. (1992). ***Técnicas de Evaluación Cuantitativa de la Madurez Gonádica en Peces***. México: AGT Editor. 79 p.

Rodríguez M., Contreras, D., Rodríguez, K., Cortés, A., y Hernández, H. (2009). ***Evaluación de la reproducción inducida en Ciprínidos. In: Contribuciones Metodológicas al Conocimiento de los Recursos Naturales***. Edit. Luis Ayala; Raúl Gío; Nuri Trigo. Universidad Nacional Autónoma de México; Universidad Autónoma Metropolitana – Xochimilco. México. 115- 126 pp.
Rodríguez, M., Cortés, A., y Guardiola, K. (2016), ***Carpa común (Cyprinus carpio). Bases de la reproducción inducida.*** CBC. UAM. 49. México. 125 pp.

Rodríguez, M., Garza, G., y Marañón, S. (1991)**.** ***Evaluación anual del líquido seminal de Cyprinus carpio rubrofuscus en organismos de primera reproducción***. Universidad Ciencia y Tecnología 1(4): 53 – 59.

Rodríguez-Gutiérrez, M.; Esquivel-Herrera, A. (1995). ***Evaluation of the repeated use of xylocaine as anesthetic for handling of beeding carp (Cyprinius carpio).*** Aquaculture, v. 129, n.1-4, p.431-436. <http://dx.doi.org/10.1016/0044- 8486(94)00280-2>

Rodríguez, M., y Marañón, S. (1993). ***Relación del factor de condición múltiple con la reproducción de machos en la carpa Cyprinus carpio.*** Anales del Instituto de Ciencias del Mar y Limnología. Vol. 20 (1):105 -113.

Saad, A., y Billard, R. (1987a). ***Spermatozoa production and volume of semen collected after hormonal stimulation in the carp, Cyprinus carpio.*** Aquaculture. 65: 67 – 77.

Saad, A., y Billard, R. (1987b). ***The composition and use a sperm diluent in the carp, Cyprinus carpio.*** Aquaculture. 66: 329 -345.

Stancey, N., Zheng, W., y Cardwell, J. (1994). ***Milt production in common carp (Cyprinus carpio): stimulation by a goldfish steroid pheromone***. Aquaculture. 127: 265 – 276.

Woynarovich, E., y Horváth, L. (1980). ***The artificial propagation of warm – water finfishes – a Manual for Extension.*** FAO Fisheries Technical Paper No. 201. Rome. 183 pp.