Volume 7 Issue 2 2021

ISSN 2454-3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 7, No. 2, 637-646 (2021)

International Journal of Zoological Investigations
ISSN: 2454-3055
Contents available at Journals Home Page: www.ijzi.net

Herbal Feed Additives for Gonadal Maturity and Milt Quality in Males of Snow Trout (*Schizothorax richardsonii*)

Sharma Sheetal[1*], Bisht H.C.S.[1], Pandey N.N.[2] and Vishwakarma Bipin K.[1]

[1]Zoology Department, D.S.B Campus, Kumaun University, Nainital 263002, Uttarakhand, India
[2]ICAR-Directorate of Coldwater Fisheries Research, Bhimtal-263136, Nainital, Uttarakhand, India

**Corresponding Author*

Received: 10th September, 2021; **Accepted:** 27th September, 2021; **Published online:** 30th September, 2021

https://doi.org/10.33745/ijzi.2021.v07i02.048

Abstract: The present study was conducted to investigate the influence of dietary supplementation of root powder of Ashwagandha (*Withania somnifera*), flower powder of *Butea monosperma*, seed powder of *Mucuna pruriens* and dried powder of Jaiphal (*Myristica fragrans*) on gonadal maturity and milt quality in male brood stock of commercially important indigenous coldwater fish, snow trout, *Schizothorax richardsonii* in captive condition. Experimental fish of 3 years age were reared in FRP tanks (n=6) of 1200 L capacity with stocking density of n=60 in each tank and fed with control (CD) and experimental (TD) diets in duplicate at the feeding rate of 3-5% of their body weight twice a day. Gonadosomatic index (GSI) and Hepatosomatic index (HSI) were recorded for annual breeding cycle coupled with breeding indices and sperm density in breeding season along with regular monitoring of water quality parameters. In males, the GSI values ranges from 0.49 to 7.92 and 0.85 to 11.83 in control and treated groups, respectively. Two peaks of GSI values reflects the two breeding season of the species during the month of March and September. Similarly, HSI values vary from 0.41% to 0.52% and 0.44% to 0.58% in control and treated groups, respectively. In captive condition, 92% specimen were observed mature for spawning in treated group, while only 32% males were ready to oozing the milt in control stock. The fertilization rate (September 2019) was observed as 94.2% in treated group, while it was 86% in control group.The mean value of sperm count per ml. of milt (September 2019) was $3.64\pm0.66\times10^8$/ml in control group and $4.44\pm0.64\times10^8$/ml in treated group. The study revealed that 1.0 % supplementation of blend of herbs in the diet is beneficial for gonadal maturity in males and quality milt production of *Schizothorax richardsonii* in captivity under coldwater condition.

Keywords: Herbal diet, Gonadal maturity, Gonadosomatic index, Hepatosomatic index, Milt, Spawning, Snow trout

Citation: Sharma Sheetal, Bisht H.C.S., Pandey N.N. and Vishwakarma Bipin K.: Herbal feed additives for gonadal maturity and milt quality in males of snow trout (*Schizothorax richardsonii*). Intern. J. Zool. Invest. 7 (2): 637-646, 2021.
https://doi.org/10.33745/ijzi.2021.v07i02.048

Introduction

Schizothorax richardsonii (Gray, 1832), which is a member of the family Cyprinidae and subfamily Schizothoracinae, is an commercially important, indigenous food fish that thrives in Himalayan and sub-Himalayan streams and rivers. This species is widely distributed in Asia's mountain ranges, primarily in the Himalayan and central Asian highlands (above an altitude of 670 masl) (Mirza and Saeed, 1988). *Schizothorax richardsonii* locally known as "Asela" in the central Himalayas and is a

key catch fishery in the Uttarakhand region, India. It was realized that the population of this species in its natural habitat had declined significantly (Das and Josh 1993; Sharma 1989; Sehgal, 1999), mainly due to over fishing, destructive fishing and increased human pressure and the species is categorized under vulnerable category (Vishwanath, 2010). Since, this is a most preferable food fish in hill region; this species has aquaculture prospects and potential to be a candidate species for culture in the coldwater bodies of Indian Himalaya. Its dwindling wild population also requires propagation in the natural coldwater bodies of Indian Himalaya (Sehgal, 1998; Petr, 2002; Agarwal *et al.*, 2009). Presently, snow trout culture and breeding is not in practice due to its slow growth and constraints in gonadal maturation under captivity. As a result, breeding and seed production of this species is a challenge in the hills, which necessitate the scientific approach for captive maturation and spawning. Therefore, considerable emphasis has been given for the development of reproductive techniques viz. induced breeding, artificial fertilization, egg rearing, hatching and milt cryo-preservation for snow trout species (Vass *et al.,* 1979; Joshi and Sunder, 1995; Agarwal 1996; Sehgal, 1998; Agarwal *et al*., 2004, 2007). For the efficient application of these techniques information about proper gonadal maturity and quality of the milt is prerequisite. It is well accepted in the literature that broodstock nutrition has significant impact on reproductive performance (Izquierdo *et al*., 2001; Babe and Labbe 2010). Intensive feeding care is required for development of brooder in captive condition. Dietary compounds affect the endocrine system and consequently reproductive parameters, such as fecundity (Tyler and Sumpter 1996), quality of gametes, and larvae (Izquierdo *et al.,* 2001). In recent years, plant-based additives in aquaculture has been one of the methods used to promote weight gain, feed efficiency in cultured fish (Dada, 2015) and also helps to improve fertility (Salman and Adesokan, 2007). Therefore, present study was focused to evaluate the efficacy of herbal feed additives for gonadal maturity and improved milt quality in snow trout *Schizothorax richardsonii* under captive condition.

Materials and Methods

Experimental Site:

The field study was carried out at ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand (Latitude 29^0 21'N, Longitude 79^0 34'E, 1370 masl) during May 2019 to April 2020. The original research reported herein was conducted under ethical guidelines approved by the ethical committee of the institution.

Collection and Acclimation of Experimental Fish:

Experimental male fish of the age of three years having average weight (82.6±3.18 g) were procured from fish ponds of ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, India, acclimatized for two days, stocked in each tank and fed with experimental diets at the rate of 3-5% of their body weight with feeding frequency of twice a day. Water quality parameters were monitored daily (temperature, pH, dissolved oxygen, free carbon dioxide) and fortnightly (total alkalinity, Nitrate, Phosphate, Ammonia). Water quality parameters were analysed by using standard methods (APHA, 1998).

Formulation of Experimental Diets:

The main focus of the study was to evaluate the efficacy of herbal feed additives in gonadal maturity and captive breeding of snow trout (*Schizothorax richardsonii*). For the purpose, two diets were formulated-- (i) control diet or basal diet (Fish meal, 10%; Rice bran,45%, mustard oil cake 25% and Soyabean oil cake, 25%) having 30% protein level, and (ii) test diet having basal diet and 1% supplementation of the blend of tested herbs such as root powder of Ashwagandha (*Withania somnifera*), flower powder of *Butea monosperma,* seed powder of *Mucuna pruriens* and dried powder of Jaiphal (*Myristica fragrans*).

Experimental Set-up:

Experimental fish were reared in FRP tanks (n=6) of 1200 L capacity. Each tank was stocked with 3 years old fish (n=60) and fed with control (CD) or test diet (TD) in duplicate at the feeding rate of 3-5% of their body weight twice a day.

Estimation of reproductive parameters:

Three fish samples were randomly taken from each treatment tank, and testis and liver were removed and weighed for the determination of Gonadosomatic Index (GSI) and Hepatosomatic Index of male brood stock.

Gonado somatic index:

Gonadosomatic index was calculated by the following formula (Afonso-Dias *et al*., 2005) on monthly basis.

$$GSI = \frac{\text{Weight of gonad (g)}}{\text{Total body weight (g)}} \times 100$$

Hepatosomatic index (HSI):

The hepatosomatic index (HSI) was calculated on monthly basis to observe the energy status for growth and gonadal development as liver is an important store of energy reserve in many fishes (Wotton *et al.*, 1978; Campbell and Love, 1978). Male specimens were dissected and liver was taken out and weighed. HSI was calculated by using following formula:

$$HSI = \frac{\text{Weight of liver (g)}}{\text{Total body weight (g)}} \times 100$$

Fertilization rate:

Fertilization rate was calculated using the following formulae:

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs in a batch}} \times 100$$

Evaluation of Milt quality (sperm count, milt volume and motility):

At the end of the feeding trial, 3 male fish were randomly selected from each dietary treatment and the testes were removed to determine milt quality indices (milt volume, motility duration, percentage motility and spermatozoa concentration). Milt quality is difficult to assess in a quantitative and conclusive manner (Rurangwa *et al*., 2004). The milt quality in fishes is primarily examined by determining the sperm density and percentage of motile sperm. Milt volume and fertilization success are the other factors for consideration for assessing milt quality.

To determine the milt volume, small incision was made into the lobes of the testes and the milt squeezed out into a Petri dish. This was then measured with plastic syringe in ml. The motility duration was determined by placing 1 µl of milt from each male on a Neubauer hemocytometer. A drop of distilled water was then added and it was covered with slip. The sperm activity was viewed under Olympus microscopic at 100 x magnification to see when all the sperm stop (Mims, 1991). Percentage motility was estimated using light microscope at 400x magnification immediately after addition of 20 µl distilled water as an activating solution. During spermatozoa activation, immotile sperm cells (ISC) were counted. When the activation stopped, whole sperm cells (WSC) were counted (Canyurt and Akhan, 2008). The motile sperm cells (MC) was calculated as:

$$MC = WSC - ISC$$

$$\%MC = MC / WSC \times 100$$

Where, MC = Motile sperm cells, WSC= Whole sperm cells, ISC= Immotile sperm cells

Concentration of sperm was determined by counting the number of spermatozoa in sample dilute with distilled water (100 x) in a Burker haemocytometer, under 400x magnification (Rainis *et al.*, 2003).

Sperm count was made by using a haemocytometer. Sperm in undiluted semen are difficult to count due to high concentration. Thus semen was diluted with Neutral Buffered Formalin (NBF) in two steps (Step I - 50 µl of semen in 2 ml of NBF; Step II - 50 µl of first dilution in 10 ml of

NBF). Spermatozoa were counted in 10 squares at random after loading in counting chamber under a phase-contrast microscope. The mean value for sperm number is calculated. The number of spermatozoa per ml of milt was determined for each sample by using the following formula-

Sperm density (ml) = X × 40 × 200 × 1000

Where, X=mean value of sperm number, 40=First step dilution, 200=Second step dilution and 1000=Conversion factor from $1mm^3$ to ml or cm^3.

Spermatozoa density was finally determined by taking the mean of sperm count in five aliquots of diluted semen samples.

Statistical analysis:

One way analysis of variance (ANOVA) with 5% level was accepted for statistical significance.

Results and Discussion

Males of the *Schizothorax richardsonii* get partial gonadal maturity in captive condition and the volume of the milt remain in lower side rather than the wild male fish. In present study, 92% specimen of the herb supplemented diet reared males were observed with oozing the milt during breeding season, while it was only 32% in control group. Gonadosomatic Index (GSI) is considered as reliable estimate for gonadal maturity and spawning of any species. The GSI increased with the maturation of fish and reaches to its maximum at the peak period of maturity during the breeding season. GSI and volume of the gonad was suggested as indicators of gonadal state by Saksena (1987) for Indian fresh water goby, *Glossogobius giuris*. The GSI has also taken to assess the gonadal maturity in *Mystus gulio* (Sarkar *et al.*, 2002), in *Labeo dyocheilus* (Pandey and Ali, 2011), in *Labeo rohita* (Alam and Pathak, 2010) and in *Neolissocheilus hexagonolepis* (Mahapatra and Kumar, 2011).

In the present study, changes in values of GSI were observed in males for one complete year (May 19 to April 2020) and depicted in Figure 1. In treated group, gonadosomatic index (GSI) increased significantly ($P<0.05$). The GSI values of male snow trout ranged from 0.49 to 7.92 and 0.85 to 11.83 in control and treated groups, respectively. GSI value attained two peaks in complete maturation cycle, during the month of March and September and reached its lowest level during the month of May and December.

Hepatosomatic index (HSI) has often been used as indicator of energy status in relation to gonadal development and growth of fish (Wotton *et al.,* 1978; Campbell and Love, 1978; Shankar and Kulkarni, 2007). The correlative changes between liver weight and gonadal activity have been shown to be associated with energy requirement of the ovary for the development of oocytes (Htun-Han, 1978). Patil and Kulkarni (1996) had also shown that GSI and HSI have relationship and this relationship is directly related to gonadal activity. In present study, HSI values vary from 0.41% to 0.52% and 0.44% to 0.58% in control and treated group, respectively, lowest during the month of March and September and highest during the month of May to June and December to February, with similar trend in both, treated and control groups (Fig. 2). A gradual decrease in HSI was observed from May to September and January to March in captive reared males of *Schizothorax richardsonii.* HSI differed significantly ($p<0.01$) between control and treated fish and also varied significantly ($p<0.01$) in relation to months in both the groups. The difference in HSI of both groups showed significant ($p<0.05$) correlation between dietary herbal supplementation and reproductive status. It was observed that when the HSI values were at its minimal, the GSI values were highest and this condition suggests the point that the liver has a weight loss during reproduction which may indicate the mobilization of hepatic reserve for gonads maturation (Zin *et al*., 2011) and therefore, the same period might be the pre-spawning period of this fish.

During the present experimentation, milt of the captive reared males were used to fertilize the

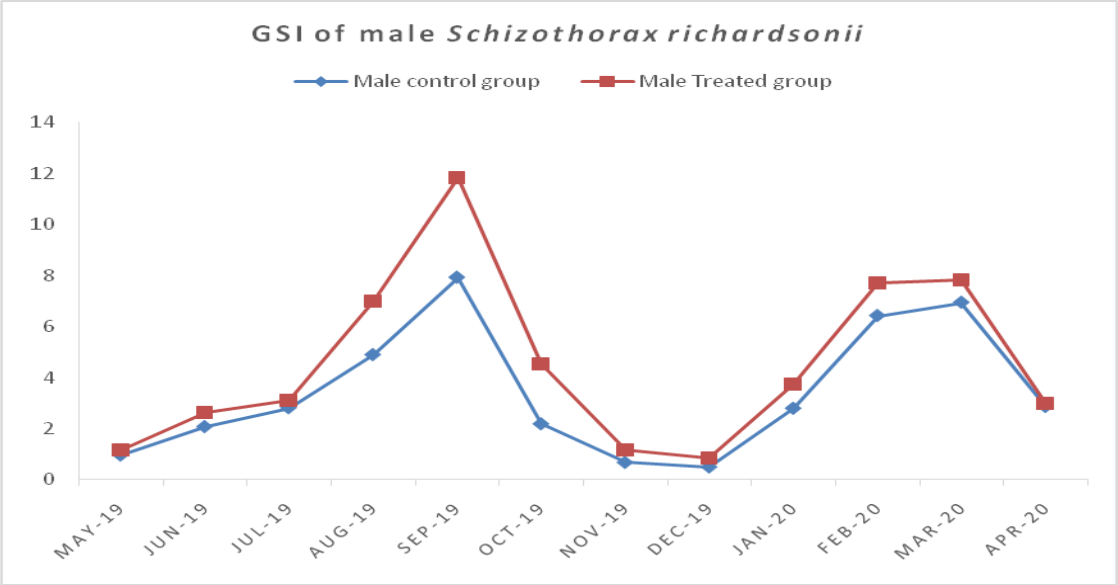

Fig. 1: Monthly fluctuation in the Gonadosomatic index of *Schizothorax richardsonii* males.

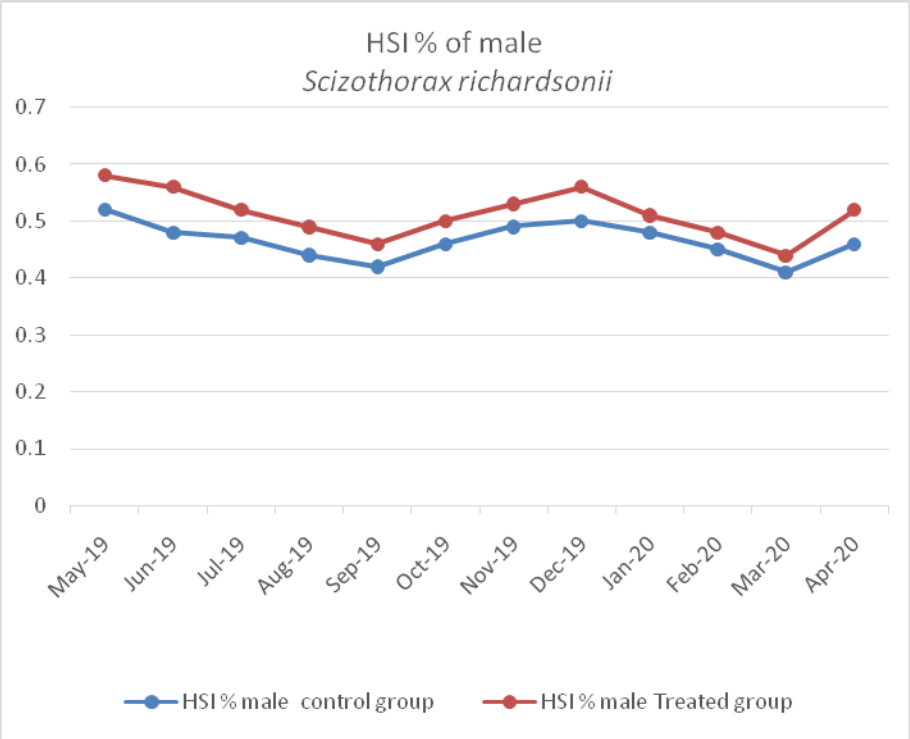

Fig. 2: Monthly fluctuation in the hepatosomatic index of *Schizothorax richardsonii* males.

eggs, which reflects the fertilization rate as 94.2% in treated group, while it was 86% in control group. There is increase in fertilization rate, increasing milt volume and increase in sperm motility in treated group as a result of inclusion of blend of herbs in the diet of fish (Table 1).

Composition of the fish milt and their physical characteristics have been found varying with the species and are important in milt quality perspective (Honeyfield and Krise, 2000; Kruger *et al.*, 2006). The milt quality even also varies considerably among the individuals of the same

Table1: Comparative milt quality parameters in control and treated group.

	Control group		Treated group	
Date of Stripping/Date of breeding operation	23th Sept. 2019	25th March 2020	23th Sept. 2019	25th March 2020
Avg. weight of male fish (g)	89.28±8.02	75.93±6.52	92.16±5.24	87.29±6.30
Milt volume (ml)	0.64±0.04	0.60±0.05	0.80±0.06	0.76±0.06
Per cent of motility (%)	62.0±4.54	60.0±5.16	88.0±6.43	86.5±7.24
Sperm density (x 10^8/ml)	3.64±0.66	3.44±0.68	4.44±0.64	4.20±0.74
Fertilization rate (%)	86.0±2.8	80.0±2.6	94.2±3.4	90.0±3.0

Data expressed as Mean ± SE, n=3

species (Piironen, 1985). There are various external factors such as the feeding regime, the quality of feed and the rearing temperature/spawning season of the males, which influence directly or indirectly the quality of milt (Bromage and Roberts,1995; Rakitin *et al*., 1999; Rurangwa *et al*., 2004; Aral *et al*., 2007). The concentration of sperm or sperm density is generally used for the assessment of milt quality. This parameter directly impacts the fertilization rate and is a characteristic feature of a species (Agarwal *et al*., 2004; Agarwal, 2005). In the present study, the mean value of sperm count/ml of milt was 3.64±0.66×10^8/ml in control group and 4.44±0.64×10^8/ml in treated group (Table 1). There is 22.0% better sperm density due to the inclusion of blend of herbs in the diet of snow trout. However, these values are comparatively lower than the previously observed sperm count of closely related other snow trout species viz. *Schizothorax curvifrons*, 6.89±1.09×10^8/ml and *Schizothoraichthys progastus*, 8.67 ±0.50×10^8/ml (Agarwal, 2005). The sperm density of this species is also in lower side in comparison to the other coldwater fish species and fresh water carps and other marine fish species. Agarwal (2005) recorded sperm density as 1.70 ± 0.29 × 10^9/ml in *Tor putitora* and it is 4.90–7.45 ×10^9/ml in *Tor khudree* (Basavaraja *et al*., 1998). In Indian major carps, sperm density was recorded from 2 × 10^7 to 3.5 × 10^7/ml and highest sperm density was recorded up to 50–65×10^9/ml in Bluefin Tuna (Doi *et al*., 1982). Agarwal and Raghuvansi (2009) also reported the direct impact of age and advancement of breeding season on the sperm density of *Schizothorax richardsonii.*

The leaves, roots and seeds of medicinal plants contain phytochemicals and antioxidants which have the tendency to increase sperm count, motility, and enhance sperm morphology (Javeed *et al.,* 201; Singh *et al*., 2013). Dada *et al*. (2019) reported impact of dietary inclusion of *Prunus amygdalusdulcis* on sperm quality in *Clarias gariepinus*, such as spermatozoa concentration, percentage motility, milt volume and motility duration. Several studies (Daramola *et al*., 2015; Suresh *et al*., 2009) reported that *Mucuna pruriens* seed extracts enhance reproductive performance in animals. Seed of this herb contains oleic acid, linoleic acid and palmitic acid (Adebowale *et al*. 2005), allegedly having aphrodisiac activities (Suresh *et al.,* 2009). Ahmad *et al.* (2008) documented increased fertility potential of *Mucuna* seed meal in men. Also, the ethanolic extract of *Mucuna pruriens* seeds has been reported to significantly increase testosterone, LH, FSH and prolactin hormone levels, sperm count and motility in infertile obese mutant rat models (Kumar *et al.,* 2011). Dada *et al*. (2011) observed impact of *Mucuna pruriens* seed powder on improved reproductive performance and sperm motility in *Clarias gariepinus*. However, Dhas *et al.* (2015) observed an increase in GSI, fertilization and hatching of *Etroplus suratansis* brood stock

treated with herbal maturation diet prepared from *Mucuna pruriens: Withania somnifera: Moringa oleifera* (150:300:150 mg kg^{-1}). The findings of the present study are consistent with this study. Babu (1999) prepared an herbal maturation diet containing *Withania somnifera, Mucuna pruriens, Ferula asafoetida* and *Piper longum* which was fed to *Penaeus monodon* through bioencapsulated Artemia. The herbal-enriched Artemia feeding increased the fecundity (42%), reduced the intermoult period and increased gonad weight by 38%. Malpani *et al.* (2011) reported the use of water extract of *Butea monosperma* gum as an antifungal agent as well as a strong exhibitor to prevent and protect the histological damage observed in gonads.Tajuddin *et al*. (2003, 2005) reported use of *Myristica fragrans* to increase libido and potency in mice. The current study also demonstrates that a dietary supplement comprising a blend of herbal diet has a beneficial and significant impact on the gonadal maturity and spawning of snow trout in captivity.

The water temperature during the captive rearing of brooders varied from 8.4-24.2 C. Dissolved oxygen value ranged from 6.6-8.8 mg/l, pH value from 7.2-7.4, free CO_2 from 0.0-1.9 mg/l, total alkalinity from 82-157 mg/l. Ammonia and nitrate-N level were very much alike and ranged from 0.01-0.04 mg/l and 0.11 to 0.16 mg/l, respectively. Phosphate level was also in the range of 0.11 to 0.18 mg/l. Pandey *et al*. (2010) observed similar results of water quality parameters during breeding of *Schizothorax richardsonii.*

Conclusion

The study revealed a positive and significant impact of dietary inclusion of herbs on reproductive performance of male *Schizothorax richardsonii* in captivity under coldwater condition. Better gonadal maturity and quality milt can be achieved with 1.0 % supplementation of blend of *Withania somnifera*, *Butea monosperma, Mucuna pruriens* and *Myristica fragrans* in the diet of fish.

Acknowledgements

The authors express their sincere gratitude to ICAR-Directorate of Coldwater Fisheries Research Bhimtal (Uttarakhand) for providing all facilities to carry out the work successfully and smoothly. Authors are also indebted to Head, Department of Zoology, DSB Campus, Kumaun University, Nainital (Uttarakhand) for providing facilities to carry out the study.

References

Adebowale YA, Adeyemi IA and Oshodi AA. (2005) Functional and physico-chemical properties of flours of six Mucuna species. Afr J Biotechnol. 4(12): 1461-1468.

Afonso-Dias I, Reis C and Andrade P. (2005) Reproductive aspects of *Microchirus azevia* (Risso, 1810) (Pisces: Soleidae) from the south coast of Portugal. Sci Mar. 69: 275-283.

Agarwal NK. (1996) Fish Reproduction. A.P.H. Publishing Corporation, New Delhi, pp.160.

Agarwal NK. (2001) Reproductive strategies of snowtrout with reference to the environment of river Bhagirathi and Bhilangana of Tehri Garhwal. Final Report of ICAR scheme, pp. 90.

Agarwal NK. (2005) Developing cryopreservation protocols for the spermatozoa of snowtrout and other endangered coldwater species. Final report, ICAR Scheme, pp.105.

Agarwal NK and Raghuvanshi S. (2009) Spermatocrit and sperm density in snow trout (*Schizothorax richardsonii*): Correlation and variation during the breeding season. Aquacult. 291: 61-64.

Agarwal NK, Raghuvanshi S, Saini V and Rawat US. (2004) Milt quality and short term storage of snow trout (*Schizothorax richardsonii*) sperm. J Inland Fish Soc India 36 (2): 13-18.

Agarwal NK, Raghuvanshi S, Saini V. (2009) Cryopreservation of snow trout (*Schizothorax richardsonii*) milt as a means of propagation and ex-situ conservation of species. In: Fish Genetic Resources, (eds.) Lakra W.S. and Singh A.K. and Mahanta P.C., Narendra Publishing House, p. 273-284.

Agarwal NK, Thapliyal BL and Raghuvanshi S. (2007) Induced breeding and artificial fertilization of snow trout, *Schizothorax richardsonii* through the

application of ovaprim. J Inland Fish Soc India 39 (1): 12-19.

Ahmad MK, Mahdi AA, Shukla KK, Islam N, Jaiswar SP and Ahmad S. (2008) Effect of *Mucuna pruriens* on semen profile and biochemical parameters in seminal plasma of infertile men. Fertil Steril. 90 (3): 627-635.

Alam M and Pathak JK. (2010) Assessment of fecundity and gonadosomatic index of commercially important fish, *Labeo rohita* from Ramganga River. Int J Pharma Biosci. 1(3): 1-6.

APHA. (1998) Standard methods for the examination of water and waste water. 20th Edition, American Public Health Association, American Water Works Association and Water Environmental Federation, Washington DC.

Aral F, Şahinoz E and Dogu Z (2007) A study on the milt quality of *Oncorhynchus mykiss* (Walbaum, 1972) and *Carasobarbus luteus* (Heckel, 1843) in Atatürk Dam Lake, South eastern Turkey. Turk J Fish Aquat Sci. 7: 41-44.

Babe J and Labbe C. (2010) Egg and sperm quality in fish. Gen Comp Endocrinol. 165(3): 535-548.

Babu MM. (1999) Developing bioencapsulated ayurvedic product for maturation and quality larval production in *Penaeus monodon*. Ph.D. Thesis, MS University, Tirunelveli.

Basavaraja N, Ahmed I and Hegde SN. (1998) Short term and long term preservation of fish gametes with special reference to Mahseer (*Tor khudree*) spermatozoa. Fish Chimes 18 (6): 17-19.

Bromage NR and Roberts RJ. (1995) Preservation of gametes. In: Broodstock Management and egg and larval quality, (eds.) Bromage N.R., Bromage R. and Roberts R.J., Blackwell Science Publications, pp. 53-75.

Campbell S and Love RM. (1978) Energy reserves of male and female haddock (*Melanogrammus aeglefinus*) from the Moray Firth. J Cons Int Expl Mer. 38: 120-211.

Canyurt MA and Akhan S. (2008) Effect of ascorbic acid supplementation on sperm quality of rainbow trout (*Onchorynchus mykiss*). Turkish J Aquat Sci. 8: 171-175.

Dada A and Ogunduyile FD. (2011) Effects of Velvet Bean (*Mucuna pruriens*) on sperm quality of African Catfish, *Clarias gariepinus* (Burchell, 1822) Broodstock. J Fish Aquat Sci. 6(6): 655-661.

Dada A, Elemuo CC and Oke IO. (2019) Dietary effects of almond (*Prunus amygdalusdulcis*) seed powder on the reproductive indices in male African catfish (*Clarias gariepinus*) Broodstock. Asian J Fish Aquat Res. 5(2): 1-11

Dada A. (2015) Use of fluted pumpkin (*Telfairia occidentalis*) leaf powder as feed additive in African catfish (*Clarias gariepinus*) fingerlings. Int J Biol Chem. Sci. 9(1): 301-307.

Daramola JO, Abiona JA, Olusiji FS, Olubukola AI, Sowande OS, Olaniyi MO and Olanite JA. (2015) Effect of Mucuna (*Mucuna pruriens*) on spermiograms of west African dwarf bucks. Trop Subtrop Agroecosyst. 18:145-150.

Das P and Joshi KD. (1993) Why sustainable fish harvesting? Intensive Agricult. 31:18-22.

Doi M, Hoshino T, Taki Y and Ogasawara Y. (1982) Activity of the sperm of the bluefin tuna *Thunnus thynnus* under fresh and preserved conditions. Bull Jpn Soc Sci Fish 48: 495-498.

Honeyfield DS and Krise WF. (2000) Measurement of milt quality and factors affecting viability of fish spermatozoa. In: Cryopreservation in Aquatic Species, (eds.) Tiersch T.R. and Mazik, P.M., World Aquaculture Society, Baton Rouge, Louisiana, pp. 49-58.

Htun H. (1978) The reproductive biology of the dab, *Limanda limanda* L. in north sea: Gonadosomatic index, hepatosomatic index and condition factor. J Fish Biol. 13: 369.

Izquierdo MS, Fernandez-Palacios H and Tacon AGJ. (2001) Effect of broodstock nutrition reproductive performance of fish. Aquacult. 197:25-42.

Javeed AN, Rajeshwara N, Achur R and Nema K. (2011) Phytochemical screening and aphrodisiac property of *Asparagus racemosus*. Int J Pharm Sci Drug Res. 3(2):112-115.

Joshi CB and Sunder S. (1995) Breeding and culture of snow-trout, *Schizothorax richardsonii* (Gray) in Kumaon Himalayas. Uttar Pradesh J Zool Soc India 15 (2): 136-140.

Kruger JCD, Smit GL, Van Vuren JHJ and Ferreira JT. (2006) Some chemical and physical characteristics of the semen of *Cyprinus carpio* L. and *Oreochromis mossambicus* (Peters). J Fish Biol. 24 (3): 263-272.

Kumar S, Ali MY, Sailaja P, Mahesh S, Surekha M.V, Giridharan NV and Harishankar N. (2011) Male reproductive enhancing activity of *Mucuna pruriens* Linn. seed extract in WNIN/GR-OB obese rats – an infertile obese mutany rat model with prediabetes. Int J Curr Res. 33:323-327.

Mahapatra BK and Kumar V. (2011) Reproductive biology and artificial propagation of chocolate

mahseer *Neolissocheilus hexagonolepis* (McClelland) in Meghalaya, India. Indian J Fish. 58(2):35-40.

Malpani MO, Rajput PR, Mane VD and Dhabe PS. (2011) Phytochemical Screening, antifungal activity and curative impact on *Channa punctatus* fish by *B. monosperma* (LAM) flower, leaves and gum. Der Pharmacia Lett. 3(5): 271-280.

Mims SD. (1991) Evaluation of activator solutions, motility duration and short-term storage of Paddle fish spermatozoa. J World Aquacult Soc. 22: 224-229.

Mirza MMR and Saeed T. (1998) A note on the systematics of the genus *Schizothorax* Heckle, (Pices: Cyprinidae). Pakistan J Zool. 20(3): 312-314.

Pandey NN and Ali S. (2011) Rainbow trout farming in India R&D perspectives. Bulletin no. 23 ICAR-DCFR, Bhimtal, Nainital (Uttarakhand).

Pandey NN, Kumar P, Srivastava SK, Ali S, Kunjwal S and Simran (2010) Domestication and breeding of asela, snow trout (*Schizothorax richardsonii*). In: Coldwater Fisheries Management, (eds.) Mahanata P.C. and Sarma D., DCFR Publications, pp. 49-50.

Patil M and Kulkarni RS. (1996) Annual biochemical changes of ovary and liver in relation to vitellogenesis in the female fish, *Puntius sarana* (Ham.). J Parasit Appl Anim Biol. 5(2):61-65.

Petr T. (2002) Cold water fish and fisheries in countries of the high mountain arc of Asia (Hindu Kush-Pamir- Karakoram- Himalayas). A review. In: Cold water fisheries in the trans-Himalayan countries, (eds.) Petr T. and Swar S.B., FAO Fisheries Technical paper No. 431 Rome, pp. 1-38.

Piironen J. (1985) Variation in the properties of milt from the Finnish landlocked salmon (*Salmo salar* m. sebago Girard) during the spawning season. Aquacult. 48: 337-350.

Rainis S, Mylonas CC, Kyriakou Y and Divanach P. (2003) Enhancement of spermiation in European sea bass (*Dicentrarchus labrax*) at the end of the reproductive season using GnRHa implants. Aquacult. 19:873-890.

Rakitin A, Fegruson MM and Trippel EA. (1999) Spermatocrit and spermatozoa density in Atlantic cod (*Gadus morhua*): correlation and variation during the spawning season. Aquacult. 170: 349-358.

Rurangwa E, Kime DE, Ollevier F and Nash JP. (2004) The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquacult. 234:1-28.

Salman JM and Adesokan AA. (2007) Sperm quality of male rats treated with aqueous extracts of *Enantia chlorantha* stem bark. Afr J Biotechnol. 7(7): 865-867.

Sarker PK, Pal HK, Rahman MM and Rahman MM. (2002) Observation on the fecundity and gonado-somatic index of *Mystus gulio* in brackish waters on Bangladesh. Online J Biol Sci. 2(4): 235-237.

Sehgal KL. (1988) The Ecology and Fisheries of Mountain Streams of N.W. Himalayas. D. Sc. Thesis, Meerut University, India.

Sehgal KL. (1999) Coldwater fish and fisheries in the Indian Himalayas: rivers and streams. In: Fish and fisheries at higher altitudes-Asia. FAO Fisheries technical paper no. 385. (ed.) Peter T., FAO, Rome. pp. 41-63.

Shankar DS and Kulkarni RS. (2007) Tissue cholesterol and serum cortisol level during different reproductive phases of the female freshwater fish *Notopterus notopterus* (Pallas). J Environ Biol. 28: 137-139.

Sharma BP. (1989) Status of *Schizothorax* spp. in India-Chinese Sub-continent. FAO. Fisheries Report. No. 405 (Suppl.): FAO, Rome. 405: 90-94.

Singh R, Ashraf A, Gaurav G, Alok S and Jeyabalan G. (2013) Some medicinal plants with aphrodisiac potential: A current status. J Acute Dis. 179-188.

Suresh S, Prithiviraj E and Prakash S. (2009) Effect of *Mucuna pruriens* on oxidative stress mediated damage in aged rat sperm. Int J Androl. 33: 22-32.

Tajuddin AS, Latif A and Qasmi IA. (2003) Aphrodisiac activity of 50% ethanolic extracts of *Myristica fragrans* Houtt. (nutmeg) and *Syzygium aromaticum* (L.) Merr. & Perry. (clove) in male mice: a comparative study. BMC Complement Altern Med. 3: 6.

Tajuddin AS, Latif A, Qasmi IA and Amin KMY. (2005) An experimental study of relationships function improving effect of *Myristica fragrans* Houtt. (nutmeg). BMC Complement Altern Med. 5(1): 1 – 7.

Tyler CR and Sumpter JP. (1996) Oocyte growth and development in teleosts. Rev Fish Biol Fish. 6: 287-318.

Vass KK, Raina HS and Sunder S. (1979) On the breeding behaviour of *S. niger* (Heckel) in Dal Lake. J Bombay Nat Hist Soc. 76:171-184.

Vishwanath W. (2010) *Schizothorax richardsonii.* The IUCN Red list of Threatened Species: eT166525A6228314.

Wootton RJ, Evans GW and Mills LA. (1978) Annual cycle in female three spined stickleback (*Gasterosteus*

aculeatus) from an upland and lowland population. J Fish Biol. 12: 331-343.

Zin T, Than AA and Naing TT. (2011) Fecundity (F), gonadosomatic Index (GSI), hepatosomatic index (HSI), condition factor (K) and length weight relationship (LWR) in (*Channa orientalis*) Bloch and Schneide, 1801. Uni Res J. 4: 47-62.