African Journal of Biotechnology Vol. 9 (54), pp. 9148-9154, 29 December, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Review

Fish milt quality and major factors influencing the milt quality parameters: A review

Saeed Hajirezaee*, Bagher Mojazi Amiri and Alireza Mirvaghefi

Faculty of Natural Resources, Department of Fisheries and Environmental Sciences, University of Tehran, P.O. Box 31585-4314, Karaj, Iran.

Accepted 8 December, 2010

In commercial fish production, the evaluation of milt quality is essential in order to increase the efficiency of artificial fertilization. Numerous studies have demonstrated that qualitative parameters of milt (i.e. seminal fluid composition, spermatozoa motility and sperm production) could be influence by several factors including biological characteristics of brooders (age, weight and length), rearing conditions of brooders (temperature, photoperiod, nourishment, undesirable components and animal welfare and health), artificial induction of spawning, spawning season (repeated milt collection and spermiation time) and post stripping factors (chemical properties of diluents and short-term and long-term storage of milt). In the present paper, we review the roles of these factors on quality of milt fish. On the whole understanding of the factors that affect milt quality could be useful for adjustment and efficient management of these factors in order to obtain good milt for fertilization.

Key words: Fish production, milt quality, artificial fertilization.

MILT QUALITY

The use of high quality gametes from fish brood stock is of great importance for ensuring the production of viable larvae for aquaculture (Kjorsvik et al., 1990; Bromage and Roberts, 1995). Milt quality is a measure of the ability of sperm to successfully fertilise an egg which such ability mostly depends on qualitative parameters of milt i.e. composition of seminal fluid, milt volume, sperm density and sperm motility (Rurangwa et al., 2004). Fish seminal fluid has a unique composition regarding the presence of the organic and inorganic components which support the viability of spermatozoa (Hajirezaee et al., 2010a). Sperm motility and sperm density determine the fertilization capability of spermatozoa and often are used to estimate milt quality (Suquet et al., 1982; Billard, et al., 1993; Linhart et al., 1994a; Krol et al., 2006) because of chemical properties of seminal fluid, fish spermatozoa are immotile in seminal fluid (Billard, 1986). During natural spawning, fish spermatozoa are rendered motile after discharge into the aqueous environment (in oviparous species) or the female genital tract (in viviparous and ovoviviparous species) (Stoss, 1983; Billard, 1986; Billard and Cosson, 1990). After activation of motility, spermatozoa move towards micropyles in the surface of eggs and then fertilization is done. When a dense milt (i.e. containing more counts of sperm cells) sample is used for fertilization, it is obvious that the chance of collision of a sperm with an egg is higher than a milt sample containing a lower density of spermatozoa.

*Corresponding author. E-mail: S.Hajirezaee@gmail.com.

WHAT FACTORS AFFECT QUALITATIVE PARAMETERS OF MILT?

In fish farms and hatcheries, the various factors affect milt parameters that are dependent on complex interactions between genetic, physiological and environmental factors. These factors may affect either at different levels of the aquaculture production process or during collection and storage of sperm *in vitro* prior to fertilization and at activation after spawning. Factors which affect milt quality in farmed fish are briefly reviewed in the following sections. Understanding of the factors that affect sperm quality could be useful for adjustment and efficient management of them. These factors have been split into effects of biological characteristics of brooders (age,

weight and length), rearing conditions of brooders (temperature, photoperiod, nourishment, undesirable components and animal welfare and health), artificial induction of spawning, spawning season (repeated milt collection and spermiation time) and post stripping factors (chemical properties of diluents and short-term and long-term storage of milt).

Biological characteristics of brooders (age, weight and length)

The age of brood stock has a significant influence on the sperm quality and may affect the success of storing sperm (Vuthiphandchai and Zohar, 1999). Büyükhatipoglu and Holtz (1984) observed that second-season spawners of rainbow trout produce better quality of milt than first-season spawners in terms of milt volume and sperm concentration. In captive reared striped Bass (*Morone saxatilis*), 3-year-old fish had higher sperm quality than the 1-or 12-year-old fish, based on higher sperm production and increased sperm longevity during short-term storage. However, the fertilizing capacity of virgin and repeat spawners was comparable in Atlantic cod (*Gadus morhua*) (Trippel and Neilson, 1992). The positive correlations were found between volume of milt and body size (weight and length) in Atlantic salmon (*Salmo salar*) and rainbow trout (Gjerde, 1984).

Rearing conditions

Rearing photoperiod and temperature

In aquaculture, various light regimes are used in order to accelerate or slow the gonadal development so that fish spawn at a convenient time of the year for the aquaculturist (Nash, 1999). However, the data about the role of photoperiod and temperature on quality of fish milt especially commercial species are rare. Tate and Helfrich (1998) observed the photoperiod-depended changes of milt parameters in the sunshine bass (*Morone chrysops* × *M. saxatilis*) exposed to different light periods (6 to 9 to 12 months). In this regard, duration of milt production was proportional to the cycle length, lasting 38, 42 and 91 days in the 6, 9 and 12-month cycles. In gold fish (*Carassius auratus*) (Iigo and Aida, 1995) and wolffish (*Anarhichas minor*) (Pavlov et al., 1997) photoperiod manipulation had no effect on sperm production.

A few studies have shown that temperature can affect the milt parameters. In a study of Hajirezaee et al. (2010c), it was observed that the sperm motility (percentage and duration) decrease coincident with the decline of water temperature during spawning season, although this decrease was significant only at 2°C (that is end of season). This result was contrary to results on cultured Siberian sturgeon (*Acipenser baeri*) where highest spermatozoa motilities were obtained at 10°C and the lowest at 17.5°C respectively (Williot et al., 2000). It is likely that optimal temperature range for best sperm motility is tantamount to the water temperature range which spawning occurs in wild.

Nourishment of brooders

Several studies have showed that the quality of sexual materials varies depending on quality of food composition. Labbe et al. (1995) observed that dietary lipids alter the composition but not the fluidity of the sperm plasma membrane of rainbow trout and increase their fertilization capacity. Enrichment of commercial pelleted diet with fish oil significantly increased milt volume, sperm concentration, survival rate of embryos and larvae in Sea bass (*Dicentrarchus labrax*) compared to those fed on a non- enriched diet. In another study, enrichment of diets with polyunsaturated fatty acid (PUFAs) enhanced reproductive efficiency of male sea bass (Astuarino et al., 2001). It was revealed that dietary ascorbic acid (Vitamin C) can protects sperm cells from adverse effects of antioxidants on cell membrane lipids by reducing the risk of lipid peroxidation. As well as, dietary ascorbic acid had important role on male fish fertility of rainbow trout (Ciereszko et al., 1996a; Dabrowski and Ciereszko, 1996). Antinutritional factors can adversely affect milt quality. *In vivo* treatment of male lamprey (*Petromyzon marinus*) with gossypol (a naturally occurring compound in cotton seeds) reduced sperm motility (Rinchard et al., 2000) and *in vitro* assays confirmed it's toxicity to perch spermatozoa (Ciereszko and Dabrowski, 2000).

Undesirable components in Water, food and genital materials

Existence of some materials (such as some steroids and heavy metals) in food and water can affect milt quality. Spermiation was suppressed after 7 months of exposure in 17β-estradiol-fed Juvenile black porgy (*Acanthopagrus schlegeli*) (Chang et al., 1995). Genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one) adversely decreased the sperm motility and concentration in a dose-dependent manner in rainbow trout (Bennetau-Pelissero et al., 2001). Dietary mercury at levels that are found in North American lakes impaired gonadal development in male juvenile wall eye (*Stizostedion vitreum*) (Friedmann et al., 1996). In wild roach (*Rutilus rutilus*), it was revealed that sperm quality changes negatively in relation to sewage effluents (Jobling et al., 2002). In some fish species, contamination of milt by urine during stripping and probably ejaculation is unavoidable due to the close proximity of sperm duct and ureter, or the presence of a single urogenital pore through which both milt and urine are released. Urine stimulates the spontaneous activation of spermatozoa in

seminal fluid before applying activation solution (or diluents) or water (Linhart and Kvasnicka, 1992; Linhart and Billard, 1994b; Billard et al., 1995b; Perchec et al., 1995, 1998; Linhart et al., 1995; Billard, 1998; Dreanno et al., 1998; Linhart et al., 1999). This may make spermatozoa immotile before fertilization can occur if contact with eggs be delayed. In Atlantic salmon, urine contamination increased the variability of the seminal plasma composition and reduced the osmolality as well as the concentration of potassium (Rana, 1995). Contamination of Carp (*Cyprinous carpio*) milt with urine decreased the energetic stores of spermatozoa and motility (Perchec et al., 1995, 1998). Generally, it is suggested that milt quality be assayed before it's usage since the prevention of milt from urine contamination is unavoidable in many times.

Animal welfare and health

Under culture condition, adult fish are exposed to various types of stressors such as confinement, crowding, handling, biopsy, transportation and hormonally induced spawning. Stress has adverse effects on reproduction process ranging from depression of endocrine function to reduction of gamete and larval quality (reviewed in Pankhurst and Van Der Kraak, 1997). In rainbow trout, confinement stress had suppressive effects only on testosterone (T) levels not GtH (Gonadotropin hormone), indicating possible act of stress at the level of GtH signaltransduction (Pankhurst and Van Der Kraak, 2000). Similar results were found in male sockeye salmon (*Oncorhynchus nerka*) where the levels of T and 11-Ketotestosterone decreased in response to confinement stress (Kubokawa et al., 1999). Few studies showed that stress can also affect milt quality. Decreases in seminal fluid osmolality and sperm motility of white bass after transportation to freshwater were attributed to confinement stress (Allyn et al., 2001), although these could be due to the hypotonicity of freshwater environment, possibly causing the hydration and dilution of milt (Morisawa et al. 1979; Hajirezaee et al., 2010c).

In rainbow trout, repeated acute stress during reproductive development prior to spawning significantly decreased sperm density (Campbell et al., 1992). In Persian sturgeon (*Acipenser persicus*), although milt quality (sperm motility and density) decreased during spermiation period in response to repetition of a management stressor (i.e. milt collection), but this decrease might also be in relation to low levels of sex steroids (Hajirezaee and Rafiee, 2010b; Hajirezaee et al., 2011). The males of striped bass produced milt with non-motile sperm under confinement conditions in freshwater (Berlinsky et al., 1997). Since aquaculture involves many procedures that are usually stressful and unavoidable, thus, determination of role of different management stressors on milt quality could be useful for design the good methods in order to minimize the consequences of stress on milt quality.

Appling the anaesthetics are a usual method for reduction of stress-dependent effects. However, it was recognized that these anaesthetics may affect the milt quality when handling the fish. In rainbow trout, the duration of motility decreased as anaesthetic concentration increased (Wagner et al., 2002).

Other factors that may affect the milt quality are diseases of brooders. Infectious pancreatic necrosis (IPN) virus has been reported to attach to sperm cells of rainbow trout (Rodriguez et al., 1993) which could affect sperm quality, although no confirmatory or experimental data is available yet.

Artificial induction of spawning

Hormonal induction of spawning is a good strategy in order to shorthen and synchronizes of gamete maturation in hatcheries. As well as, it is revealed that hormone-therapy can affect milt quality. In European catfish (*Silurus glanis*), both injected carp pituitary extract (CPE) and both implanted GnRHa (Gonadotropin releasing hormone analogue) increased the sperm density, although CPE had more effect (Linhart and Billard, 1994b). Similar results were found when common carp males treated with oral and intraperitoneal administration of salmon gonadotropin hormone-releasing hormone analogue (sGnRHa) and Pimozide (Pim) (Roelants et al., 2000). In yellow tail flounder (*Pleuronectes ferrugineus*), sperm production, milt volume, sperm motility and seminal plasma pH were increased by GnRHa treatment (Clearwater and Crim, 1998). Increased milt volume and prolonged spermiation were also observed in seabass (*Dicentrarchus labrax*) administered GnRHa (Sorbera et al., 1996). GnRHa-microspheres increased significantly sperm production in Atlantic salmon, S. salar and stripped bass *(Morone Saxatilis*) (Mylonas et al., 1995).

Seasonal variations of milt quality

Several studies have focused on seasonal aspects of milt quality (Büyükhatipoglu and Holtz, 1984; Kruger et al., 1984; Piironen, 1985; Munkittrick and Moccia, 1987; Hajirezaee et al., 2010c) and suggested the characteristics which can influence milt quality. The values of milt volume, sperm density and spermatocrit declined in Atlantic salmon (Aas et al., 1991) and caspian brown trout (*Salmo trutta caspius*) (Hajirezaee et al., 2010c) over the course of spawning season, although these parameters increased in landlocked salmon (*Salmo salar* M. sebago girard) (Piironen, 1985) and rainbow trout (Sanchez-Rodriguez et al., 1978) during spawning season. In addition to these parameters, the concentration of some chemical components of seminal fluid (including: Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Cl^{-} and total protein) decreased also in caspian brown trout (Hajirezaee et al., 2010c). In most fish species a

decline in milt quality throughout the spawning season has been reported (Legendre and Billard, 1980; Piironen, 1985; Munkittrick and Moccia, 1987; Aas et al., 1991) due to ageing of spermatozoa (Rana 1995; Suquet et al., 1998; babiak et al., 2006). As a consequence of ageing, several sperm features may be modified, including cell morphology (Billard, 1984; Suquet et al., 1998), composition of seminal fluid (Aas et al., 1991), spermatozoa concentration (Büyükhatipoglu and Holtz, 1984; Zuromska, 1981), percentage and duration of sperm motility (Billard et al., 1977; Benau and Terner, 1980; Montalembert et al., 1980; Büyükhatipoglu and Holtz, 1984; Methven and Crim, 1991; Slominska and Gluchowska, 1994; Suquet et al., 1998).

In another study, it was revealed that milt quality of Caspian brown trout can make changes based on spermiation time (Hajirezaee et al., 2010d). In this regard, the percentage of motile spermatozoa, duration of motility, sperm density, milt osmolality and also the concentrations of Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+} and total protein content in seminal fluid were significantly higher in mid-mature males compared to pre-mature and late-mature individuals.

Post stripping factors

Properties of activator solution or diluent

For fertilization, it is essential that fish spermatozoa become active in water or a diluent (so called activator solution). Numerous studies have showed that the chemical properties of activators can affect the milt quality. These conditions depolarize the cell membrane, they may affect the capacity of sperm tails for flagellar motility and may stimulate this motility (Morisawa and Suzuki, 1980; Morisawa et al., 1983). In salmonids, it is well recognized that sperm motility is inhibited after dilution with solutions containing high potassium concentrations. (Scheuring, 1925; Kusa, 1950; Morisawa et al., 1983; Billard et al., 1987; Tanimoto et al., 1994) which this condition could be reversed with adding of Na^+, Ca^{2+} and Mg^{2+} to diluent (Scheuring, 1925; Baynes et al. 1981; Billard et al. 1987). These results suggest that spermatozoa should be active in a diluent with low K^+ concentration at the time of fertilization in salmonids. In cyprinids, it is revealed that sperm motility is regulated more by osmolality than K^+ ion and K^+ regulates merely the induced motility by hypo-osmotic medium (Krasznai et al., 1995, 2000). In fact, carp spermatozoa are less K^+ sensitive than those of trout. Hypoosmotic shock results in hyperpolarization of the cell membrane (Krasznai et al., 1995), which re-activates the Ca^{2+} channels. For this event, existence of external Ca^{2+} in diluent is crucial (Krasznai et al., 2000).

In conclusion, the high osmolality of diluent and absence of Ca^{2+} can suppress the carp spermatozoa at the time of fertilization. Similar to salmonids, the sperm motility is inhibited by K^+ concentrations of diluent in sturgeons (Acipenseridae) and paddlefish (Polyodontidae) (Gallis et al., 1991; Cosson and Linhart, 1996; Toth et al., 1997), although inhibitory levels of K^+ ion varies depending on species. For example, Paddlefish spermatozoa are sensitive to very low K^+ concentrations (Cosson and Linhart, 1996). Inhibitory effects of Na^+ on sturgeon motility may be in high concentrations. For example, the concentrations more than 10 and 25 mM in lake sturgeon (*Acipenser fulvescens*) (Toth et al. 1997) and Persian sturgeon (*Acipenser persicus*) (Alavi et al., 2004) respectively. In contrast to Cyprinidae and Salmonidae, the literature on the effect of ions on sperm motility in marine fishes is fairly rare and species-specific. In tilapia (Linhart et al. 1999) and herring (Vines et al. 2002), existence of extracellular Ca^{2+} is necessary for initiation of motility.

In addition to ions, other factors such as temperature and pH of diluent can affect milt quality. In the sperm of salmonid and cyprinid fish, temperature affects the sperm beat frequency (Cosson et al., 1985). In trout, higher temperature increased the beat frequency and decreased the duration of forward movement (Billard and Cosson, 1992) while the lower temperature that trout experience during natural spawning (4 to 10°C) increases the duration of sperm movement (Van Look, 2001). In African catfish, low temperature (4°C) also prolonged motility and viability of spermatozoa compared to the culture temperature (25°C) (Mansour et al., 2002). In salmonids, alkaline conditions, (i.e. pH similar to or greater than seminal fluid) apparently enhance the percentage of motile cells and the fertilizing ability of spermatozoa (Billard et al., 1974; Billard, 1981).

On the whole, knowledge about the effects of the above factors (ions, pH and Temperature) on milt quality may be useful for adjustment of suitable diluents so that good sperm motility be obtained at the time of fertilization.

Effects of Short-term and long-term storage of milt

The storage of sexual materials aimed at increasing post spawning gamete longevity, may improve hatchery management, minimize problems resulting from inbreeding and provide synchronous brooder maturation (Bromage and Roberts, 1995). Nevertheless, the milt quality can change after a storage period. In short-term storage, a low storage temperature (4°C) is suitable, although anaerobic conditions and associated microbial contaminations may reduce sperm motility and viability. In Channel catfish, after 10 days successful storage at 4°C, the sperm quality decreased due to the increasing load of bacterial infection and subsequently the production of extracellular enzymes and consumption of oxygen (Jenkins and Tiersch, 1997). In non-sterile solution, the motility was completely lost after 3 days. This reveals that antibiotics (e.g. gentamicin and ampicillin) in a suitable dosage can lengthen storage time of refrigerated spermatozoa by preventing the sperm cells from bacterial infection

At now, Fish sperm is routinely held frozen in liquid nitrogen for long periods (McAndrew et al., 1993). Mostly, three factors including freezing and thawing rates and chemical composition of cryoprotectants and extenders determine the quality of cryopreserved sperm. In this regard, numerous data is available, thus, it is suggested that readers referred to recent reviews for further details (Leung and Jamieson, 1991; McAndrew et al., 1993; Tiersch and Mazik, 2000).

ACKNOWLEDGMENT

The authors express their sincere appreciation to the people who gave their time, advice, and support to this study.

REFERENCES

Aas GH, Refstie T, Gjerde B (1991). Evaluation of milt quality of Atlantic salmon. Aquaculture, 95: 125-132.

Alavi SMH, Cosson J, Karami M, Abdoulhay H, Mojazi Amiri B (2004). Chemical Composition and osmolality of seminal plasma of *Acipenser persicus*; their Physiological relationship with sperm motility. Aquacult. Res. 35: 1238 43.

Allyn ML, Sheehan RJ, Kohler CC (2001). The effects of capture and transportation stress on white bass semen osmolarity and their alleviation via sodium chloride. Trans. Am. Fish. Soc. 130: 706-711.

Astuarino JF, Sorbera LA, Carrillo M, Zanuy S, Ramos J, Navarro JC, Bromage N (2001). Reproductive performance in male European seabass (*Dicentrarchus labrax, L.*) fed two PUFA-enriched experimental diets: a comparison with males fed a wet diet. Aquaculture, 194: 173-190.

Babiak I (2006). Quantitative characteristics of Atlantic halibut, *Hippoglossus hippoglossus* L., semen throughout the reproductive season. Theriogenology, 65: 1587-1604.

Baynes SM, Scott AP, Dawson AP (1981). Rainbow trout, Salmo gairdneri, spermatozo a: effects of cations and pH on motility. J. Fish Biol. 19: 259-267.

Benau D, Terner C (1980). Initiation, prolongation, and reactivation of the motility of salmonid spermatozoa. Gamete Res. 3: 247-257.

Bennetau-Pelissero C, Breton B, Bennetau B, Corraze G, LeMenn F, Davail-Cuisset B, Helou C, Kaushik SJ (2001) Effect of genistein-enriched diets on the endocrine process of gametogenesis and on reproduction efficiency of the rainbowtrout *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 121: 173-187.

Berlinsky DL, William K, Hodson RG, Sullivan CV (1997). Hormone induced spawning of summer flounder *Paralichthys dentatus*. J. World Aquac. Soc. 28: 79-86.

Billard R (1981). Short-term preservation of sperm under oxygen atmosphere in Rainbow trout, Salmo gairdneri. Aquaculture. 23: 287-293.

Billard R (1984). La conservation des game`tes et l'inse´mination artificielle chez le bar et la daurade. In: Barnabe G, Billard R, Eds. L'Aquaculture du Bar et des Sparide´s. Paris: INRA; pp. 95-116.

Billard R (1986) Spermatogenesis and spermatology of some teleost fish species. Reprod. Nutr. Dev. 2: 877-920.

Billard R (1998). Initiation of carp spermatozoa motility and early ATP reduction after milt contamination by urine. Aquaculture, pp. 317-328.

Billard R, Cosson G, Perchec G, Linhart O (1995b) Biology of sperm and artificial reproduction in carp. Aquaculture. 129: 95-112.

Billard R, Cosson J, Crime L (1993). Motility of fresh and aged halibut sperm. Aquat. Living Resour. 6: 67-75.

Billard R, Cosson MP (1990). The energetics of fish sperm motility. In: Gagnon C, ed. Controls of sperm motility, biological and clinical aspects. Boca Raton, Florida: CRC Press; 153-173.

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

Billard R, Cosson MP, Christen R (1987). Some recent data on the biology on trout spermatozoa. Proceedings of the third international symposium on reproductive physiology of fish, StJohn's, New foundland; pp. 187-190.

Billard R, Dupont J, Barnabe´ G (1977). Diminution de la motilite´ et de la dure´e de conservation du sperme de *Dicentrarchus labrax* L. (Poisson, teleoste´en) pendant la pe´riode de spermiation. Aquaculture, 11: 363-367.

Billard R, Petit J, Jalabert B, Szollosi D (1974). Artificial insemination in trout using a sperm diluent. In: Blaxter OHS, ed. Symposiumon the early lifehistory of fish. Berlin: Springer; pp. 715-723.

Bromage NR, Roberts RJ (1995a). Preservation of gametes. broodstock Management and Egg and Larval Quality, Oxford: Blackwell; 53-75.

Bromage NR, Roberts RJ (1995b). Broodstock Management and Egg and Larval Quality. Oxford: Blackwell Science Ltd, p. 424.

Büyükhatipoglu S, Holtz W (1984). Sperm output in rainbow trout *(Salmo gairdneri)* effect of age, timing and frequency of stripping and presence of females. Aquaculture, 37: 63-71.

Campbell PM, Pottinger TG, Sumpter JP (1992). Stress reduces the quality of gametes produced by rainbow trout. Biol. Reprod. 47: 1140-1150.

Chang CF, Lau EL, Lin BY (1995). Stimulation of spermatogenesis or of sex reversal according to the dose of exogenous estradiol-17β injuvenile males of protandrous black porgy, *Acanthopagrus schlegeli*. Gen. Comp. Endocrinol. 100: 355-367.

Ciereszko A, Dabrowski K (2000) *In vitro* effect of gossypolacetate on yellow perch (*Perca flavescens*) spermatozoa. Aquat. Toxicol. 49: 181-187.

Ciereszko A, Liu L, Dabrowski K (1996a). Effects of season and dietary ascorbic acid on some biochemical Characteristics of rainbow trout (Oncorhynchus mykiss) semen. Fish Physiol. Biochem. 15: 1-10.

Clearwater SJ, Crim LW (1998) Gonadotropin releasing hormone-analogue treatment increases sperm motility, seminal plasma pH and sperm production in yellow tail flounder *Pleuronectes ferrugineus*. Fish Physiol. Biochem. 19: 349-357.

Cosson J, Linhart O (1996). Paddlefish, *Polyodon spathula*, spermatozoa: Effects of Potassium and pH on motility. Folia Zool. 45: 361-370.

Cosson MP, Billard R, Gatti JL, Christen R (1985) Rapid and qualitative assessment of trout sperm motility using stroboscopy. Aquaculture, 46: 71-75.

Dabrowski K, Ciereszko A (1996). Ascorbic acid protects against male infertility in teleost fish. Experientia 52: 97-100.

Dreanno C, Suquet M, Desbruyeres E, Cosson J, Delliou H, Billard R (1998). Effect of urine on semen quality in turbot (Psetta maxima). Aquaculture, 169: 247-262.

Friedmann AS, Watzin MC, Brinck-Johnsen T, Leiter JC (1996). Low levels of dietary methyl mercury Inhibit growth and gonadal development in juvenile wall eye (Stizostedion vitreum). Aquat. Toxicol. 35: 265-278.

Gallis JL, Fedrigo E, Jatteau P, Bonpunt E, Billard R (1991). Siberian sturgeon spermatozoa: effects of dilution, pH, osmotic pressure, sodium and potassium ion sonmotility.In: Williot P,ed. *Acipenser Bordeaux*. Cemagref; pp. 143-151.

Gjerde B (1984) Variation in semen production of farmed Atlantic salmon and rainbow trout. Aquaculture, 40: 109-114.

Hajirezaee S, Mojazi Amiri B, Mirvaghefi AR (2010b). Relationships between the Chemical Properties of Seminal Fluid and the Sperm Motility Characteristics of Caspian Brown Trout, *Salmo Trutta Caspius* (A Critically Endangered Salmonid Fish). Res. J. Fish. Hydrobiol. 5: 27-31.

Hajirezaee S, Mojazi Amiri B, Mirvaghefi AR (2010c). Changes in sperm production, sperm motility, and composition of seminal fluid in Caspian brown trout, *Salmo trutta caspius*, over the course of a spawning season. J. Appl. Aquacul. 22: 157-170.

Hajirezaee S, Mojazi Amiri, Mirvaghefi AR, Sheikh Ahmadi A (2010d) Evaluation of semen quality of endangered Caspian brown trout (*Salmo trutta caspius*) in different times of spermiation during a spawning season. Czech J. Anim. Sci. 55(10): 445-455.

Hajirezaee S, Rafiee GR (2010a). Stress responses of Persian sturgeon, *Acipenser persicus* to repetition of a management stressor

(handstripping of milt). J. Appl. Biol. Sci. 4: 9-12.
Hajirezaee S, Rafiee GR, Hushangi R (2011). Comparative analysis of milt quality and steroid levels in blood and seminal fluid of Persian sturgeon males, *Acipenser persicus* during final maturation induced by hormonal treatment. Biologia. 66(1): 160-169.
Iigo M, Aida K (1995). Effects of season, temperature and photoperiod on plasma melatonin rhythms in the Gold fish (Carassius auratus). J. Pineal Res. 18: 62-68.
Jenkins JA, Tiersch TR (1997). A preliminary bacteriological study of refrigerated channel catfish sperm. J. World. Aquac. Soc. 28: 282-288.
Jobling S, Coey S, Whitmore JG, Kime DE, VanLook KJW, McAllister BG, Beresford N, Henshaw AC, Brighty G, Tyler CR, Sumpter JP (2002). Wild intersex roach (Rutilus rutilus) have reduced fertility. Biol. Reprod. 67: 515-524.
Kjorsvik E, Mangor-Jensen A, Holmefjord I (1990). Egg quality in fishes. In: Blaxter JHS, Southward AJ, eds. Adv. Mar. Biol. 26: 71-113.
Krasznai Z, Marian T, Balkay L, Gasper RJ, Tron L (1995). Potassium channels regulate hypoosmotic shock induced motility of Common Carp, Cyprinus carpio, sperm. Aquaculture, 129: 123-128.
Krasznai Z, Marian T, Izumi H, Damjanovich S, Balkay L, Tron L, et al., (2000). Membrane hyperpolarization removes inactivation of Ca channels leading to Ca influx and initiation of sperm motility in the common carp. Biophysics, 97: 2052-2067.
Krol J, Glogowski J, Demska-Zakes K, Hliwa P (2006). Quality of semen and histological analysis of testes in Eurasian perch *Perca luviatilis L*. during a spawning period. Czech J. Anim. Sci. 51: 220-226.
Kruger JC, Smit DeW, Van GL, Vuren JHJ, Ferreira JT (1984). Some chemical and physical characteristics of the semen of *Cyprinus carpio* L. and *Oreochromis mossambicus*. J. Fish Biol. 24: 263-272.
Kubokawa K, Watanabe T, Yoshioka M, Iwata M (1999). Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breedingseason. Aquaculture, 172: 335-349.
Kusa M (1950). Physiological analysis of fertilization in the egg of the salmon, Onchorynchus keta. I. Why are the eggs not fertilized in isotonic Ringer solution. Annot. Zool. Jpn. 24: 22-28.
Labbe C, Maisse G, Muller K, Zachowski A, Kaushik S, Loir M (1995). Thermal acclimation and dietary lipids alter the composition, but not fluidity, of trout sperm plasma membrane. Lipids, 30: 23-33.
Legendre M, Billard R (1980). Cryopreservation of rainbow trout sperm by deep-freezing. Reprod. Nutr. Dév. 20: 1859-1868.
Leung LKP, Jamieson BGM (1991). Live preservation of fish gametes. In: Jamieson BGM. Ed. Fish Evolution and Systematics: Evidence from Spermatozoa. Cambridge: Univ. Press, Cambridg; pp. 245-295.
Linhart O, Billard R (1994b). Spermiation and sperm quality of European catfish (Silurus glanis) after implantation of GnRH analogs and injection of carp pituitary extract. J. Appl. Ichthyol. 10: 182-188.
Linhart O, Billard R, Proteau JP (1994a). Cryopreservation of European catfish (*Silurus glanis L*.) spermatozoa. Aquaculture, 115: 340-359.
Linhart O, Kvasnicka P (1992). Artificial insemination of tench (Tinca tinca L.). Aquac. Fish. Manage. 23: 125-130.
Linhart O, Mims SD, Shelton WL (1995). Motility of spermatozoa from shovelnose sturgeon and paddlefish. J. Fish Biol. 47: 902-909.
Linhart O, Walford J, Sivaloganathan B, Lam TJ (1999). Effects of osmolality and ions on the motility of Stripped and testicular sperm of freshwater- and seawater-acclimated tilapia, Oreochromis mossambicus. J. Fish Biol. 55: 1344-1358.
Mansour N, Lahnsteiner F, Patzner RA (2002). The spermatozoon of the African catfish: fine structure, motility,viability and its behaviour in seminal vesicle secretion. J. Fish. Biol. 60: 545-560.
McAndrew BJ, Rana KJ, Penman DJ (1993). Conservation and preservation of genetic variation in Aquatic organisms. In: Muir JR, Roberts RJ, eds. Recent Advances in Aquaculture, pp. 297-336.
Methven DA, Crim LW (1991). Seasonal changes in spermatocrit, plasma sex steroids, and motility of sperm from Atlantic halibut (*Hippoglossus hippoglossus*). In: Scott AP, Sumpter JP, Kime DE, Rolfe MS, eds. Proceedings of the Fourth International Symposium on Reproductive Physiology of Fish. Sheffield: Fish Symp. p. 170.
Montalembert Gde, Marcel J, Billard R (1980). La spermiation chez le brochet. l'Evolution de la quantite´ de sperme re´colte´ au cours de la saison de reproduction. *Bulletin Franc¸ais de Pisciculture*, 276: 90-103.
Morisawa M, Hirano T, Suzuki K (1979). Changes in blood and seminal plasma composition of the mature salmon (*Oncorhynchus keta*) during adaptation to freshwater. Comparative Biochemistry and Physiology. 64: 325-329.
Morisawa M, Suzuki K (1980). Osmolality and potassium ions: their roles in initiation of sperm motility in teleosts. Science, 210: 1145-1147.
Morisawa M, Suzuki K, Shimizu H, Morisawa S, Yasuda K (1983). Effect of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes. J. Exp. Zool. 107: 95-103.
Munkittrick K, Moccia D (1987). Seasonal changes in the quality of rainbow trout (*Salmo gairdneri*) semen: effect of delay in stripping on spermatocrit, motility, volume and seminal plasma constituents. Aquaculture, 64: 147-156.
Mylonas CC, Tabata Y, Langer R, Zohar Y (1995). Preparation and evaluation of polyanhydride microspheres containing gonadotropin-releasing hormone (GnRH),for inducing ovulation and spermiation in fish. J. Control. Release, 35: 23-34.
Nash JP (1999). Seasonal reproduction in fish. In: Knobil E, Neill JD eds. Encyclopaedia of Reproduction. NewYork: Academic Press; pp. 329-340.
Pankhurst NW, Van Der Kraak G (1997). Effects of stress on reproduction and growth of fish. In: Iwama GK, Pickering AD, Sumpter JP, Schreck CB, eds. fish stress and Health in aquaculture. Society for experimental Biology Seminar Series. Cambridge: Cambridge Univ. Press; pp. 73-93.
Pankhurst NW, Van Der Kraak G (2000). Evidence that acute stress inhibits ovarian steroidogenesis in rainbow trout in vivo, through the action of cortisol. Gen. Comp. Endocrinol. 117: 225-237.
Pavlov DA, Knudsen P, Emelyanova NG, Moksness E (1997). Spermatozo aultrastructure and sperm production in wolffish (Anarhichas lupus), a species with internal fertilisation. Aquat. Living Resour. 10: 187-194.
Perchec G, Cosson J, Andre F, Billard R (1995). Degradation of the quality of carp sperm by urine contamination during stripping. Aquaculture, 129:135-136.
Perchec G, Paxion C, Cosson J, Jeulin C, Fierville F, Billard R (1998). Initiation of carp spermatozoa motility and early ATP reduction after milt contamination by urine. Aquaculture, 160: 317-328.
Piironen J (1985). Variation in the properties of milt from the Finnish landlocked salmon (*Salmo salar m. sebago Girard*) during a spawning season, Aquaculture. 48: 337-350.
Rana K (1995) Preservation of gametes. In: Bromage NR, Roberts RJ. Eds. Broodstock Management and Egg and Larval Quality. Oxford:Blackwell; pp. 53-75.
Rinchard J, Ciereszko A, Dabrowski K, Ottobre J (2000). Effects of gossypol on sperm viability and plasma Sex steroid hormones in male sea lamprey, *Petromyzon marinus*. Toxicol. Lett. 111: 189-198.
Rodriguez SJS, Prierto SIP, Minondo MPV (1993). Flow cytometric analyses of infectious pancreatic necrosis virus attachment to fish sperm. Dis. Aquat. Org. 15: 153-156.
Roelants I, Mikolajczyk T, Epler P, Ollevier F, Chyb J, Breton B (2000). Induction of spermiation in common carp after enhanced intestinal uptake of sGnRH-A and Pimozide. J. Fish. Biol. 56: 1398-1407.
Rurangwa E, Kime DE, Ollevier F, Nash JP (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture, 234: 1-28.
Sanchez-Rodriguez M, Escaffre AM, Marlot S, Reinaud P (1978). The spermiation period in the rainbowtrout_*Salmo gairdneri*.. Plasma gonadotropin and androgen levels, sperm production and biochemicalchanges in the seminal fluid. Ann. Biol. Anim. Biochim. Biophys. 18: 943-948.
Scheuring L (1925). Biologische und physiologische untersuchungen am Forellensperma. Arch. Hydrobiol. 4: 187-318.
Slominska I, Gluchowska E (1994) Quality parameters of common carp eggs and milt. In: Muir J, Sevila F, eds. Measures For Success European Aquaculture Society Special Publication. pp. 168-169.
Sorbera LA, Mylonas CC, Zanuy S, Carrillo Zohar M (1996). Sustained administration of GnRHa increases milt volume without altering sperm counts in the sea bass. J. Exp. Zool. 276: 361-368.
Stoss J (1983). Fish gamete preservation and spermatozoan

physiology. In: Hoar WS, Randall DJ, Donaldson EM, eds. Fish physiology. NewYork: Academic Press; pp. 305-350.
Suquet M, Dreaano C, Dorange G, Normant Y, Quemener L, Gaignon JL, Billard R (1998). The ageing phenomenon of turbot spermatozoa: effects on morphology, motility and concentration, intracellular ATP content, fertilization, and storage capacities. J. Fish Biol. 52: 31-41.
Suquet M, Omnes MH, Fauvel C (1982). Rapid assessment of sperm quality in Turbut. *Scophtalmus maximus*. Aqualulture, 101: 177-185.
Tanimoto S, Kudo Y, Nakazawa T, Morisawa M (1994). Implication that potassium Flux and increase in intracellular calcium are necessary for the initiation of sperm motility in salmonid fishes. Mol. Reprod. Dev. 39: 409-414.
Tate AE, Helfrich LA (1998). Off-season spawning of sunshine bass (Morone chrysops × M. saxatilis) exposed to 6-or 9-month phase-shifted photothermal cycles. Aquaculture, 167: 67-83.
Tiersch TR, Mazik PM (2000). Cryopreservation in aquatic species. Advances in World Aquaculture.World Aquaculture Society. p. 439.
Toth GP, Ciereszko A, Christ SA, Dabrowski K (1997). Objective analysis of spermmotility in the Lake sturgeon, Acipenser fulvescens: activation and inhibition conditions. Aquaculture, 154: 337-348.
Trippel EA, Neilson JD (1992). Fertility and sperm quality of virgin and repeat-spawning Atlantic cod (Gadus morhua) and associated hatching success. Can. J. Fish. Aquat. Sci. 49: 2118-2127.
Van Look KJ (2001). The development of sperm motility and morphology techniques for the assessment of the effects of heavy metals on fish reproduction. PhD thesis. University of Sheffield.
Vines CA, Yoshida K, Griffin FJ, Pillai MC, Morisawa M, Yanagimachi R, et al., (2002). Motility initiation in herring sperm is regulated by reverse sodium calcium exchange. Proc. Natl. Acad. Sci. USA, 99: 2026-2031.
Vuthiphandchai V, Zohar Y (1999). Age-related sperm quality of captive striped bass *Morone saxatilis*. J. World Aquac. Soc. 30: 65-72.
Wagner E, Arndta R, Hilton B (2002). Physiological stress responses, egg survival and sperm motility for rainbow trout broodstock anesthetized with cloveoil, tricaine methanesulfonate or carbon dioxide. Aquaculture, 211: 353-366.
Williot P, Kopeika EF, Goncharo BF (2000). Influence of testis state, temperature and delay insemen collection on spermatozoa motility in the cultured Siberian sturgeon (*Acipenser baeri* Brandt). Aquaculture, 189: 53-61.
Zuromska H (1981). Effect of different thermal regimes on reproductive cycles of tench, *Tinca tinca* (L.). Part VI. Estimation of milt quality. Polish Arch. Hydrobiol. 28: 229-241.