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Effect of Egg and Sperm Quality in Successful Fish Breeding

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Abstracts: *Fish egg quality is the ability of the egg to be fertilized and subsequently develop into a normal embryo. While sperm quality can be defined as its ability to successfully fertilize an egg and subsequently allow the development of a normal embryo. Presently there is a concern about the quality of eggs and milt collected for breeding, therefore the evaluation of egg and milt quality is essential so as to increase the efficiency of artificial fertilization. Studies have shown that qualitative parameters of the milt (sperm motility, sperm lobe length, milt volume and count) and egg can be influenced by several factors such as feeding regime, environmental factors, quality of the feed, variations between individual, age, weight, length of the fish, season of the year, stress, pollutant, fungi, and bacteria, uptake of nutritive and genetic materials, physiochemical properties of water (pH, salinity and temperature and dissolve oxygen). In this paper the role of the egg and sperm in fish farming was reviewed and the factors that affect the success of fish breeding. Understanding of these factors that affect both egg and sperm quality could reduce poor breeding process and increase fingerlings production for a sustainable aquaculture.*
Key words: *Fish breeding, sperm quality, egg quality, artificial fertilization.*

I. Introduction

Fish is the major source of protein for human, providing significant portion of nutrient to a large proportion of people particularly in the developing world (Ochokwu et al., 2014).

Increasing demands on wild fish stocks by commercial fishing has caused widespread over-fishing and depletion of wild stocks. Some estimates predict that, at the current rate, 90% of our fisheries will be completely fished out by 2050, Paul, (2013). Fish require good quality fish feed in high proportion in order to obtain quality eggs and sperm, so as to achieve maximum protein intake needed for proper growth (Hassan, 2001). Ayinla (2003) reported that feeds accounts for at least 60% of the total cost of fish production in Africa, and Kassahun et al. (2012) reported that fish feeds accounts about 40-60% of the total cost of fish production in Africa which to a large extent determines the viability and profitability of fish farming enterprise. Fish nutrition has advanced in recent years with the development of new balanced commercial diets that promote optimal growth, egg, sperm viability and fish health (Craig and Helfrich, 2002). According to Abowei et al. (2011) the development of new species specific diet formulations supports the aquaculture industry as it expands to satisfy the increasing demand for affordable, safe and high quality fish and sea food products. Capture fisheries and aquaculture supplied the world with about 106 million tons of fish in 2004 and aquaculture accounted for 43% (FAO, 2007).

Aquaculture is a fast growing sector in Nigeria contributing not less than 5% of the total fish supply but at a growth rate of about 2% per year (Moses, 2006). Fish also supply 50% of total animal protein consumed in the developing countries and less than 50% in developed countries (Omitoyin, 2007). In fish production under intensive method, attempts are made to obtain quality eggs and sperm, to produce highest possible number of good quality seeds. Several factors that affect fish seeds quality includes different strains, genetics, nutrition, contents of feed, P^H, temperature and activities of modern aquaculture which have introduced several substances such as organic matter, chemicals such as fertilizers and insecticides into the water used as cultured medium (Canyurt and Akhan, 2008). Common practices in hatcheries such as transportation, handling, cleaning, use of chemicals, overstocking; water quality problems are also factors that may negatively influence reproduction (Adeparusi et al., 2010). These common factors affect fertilization success in both artificial and natural reproduction. As a result of these factors, low quality fish seeds are produced (Adeparusi et al., 2010).

The need for high quality fish seed has necessitated research into various ways of improving egg quality and enhancing fertility of sperm to meet the world protein demand. However the continuing expansion of aquaculture requires shifting from synthetic drugs to natural plant. According to Adedeji et al. (2006) wild fruits that have potentials in enhancing egg quality and sperm fertility that are not recognized and valued, are now been investigated, evaluated and included into fish feeds with little or no side effects on the fish. Hence the use of wild fruits as medium to improve egg quality and enhance fertility is now receiving some attention. Dada and Ajilore (2009) used extract of Garcinia kola seed to enhance fertility in Clarias gariepinus, and Adeparusi et al (2010), used Kigelia Africana fruit meal to enhance fertility in male C. gariepinus and Ochokwu et al., (2014) used Azanza garckeana pulp to enhance fertility in C. gariepinus Broodstock.

Nash (2011) defines Aquaculture as the rational rearing of fish and other aquatic organism in control water body. The area in which the Fisheries potential of Nigeria could be exploited is depended upon factors such as availability of good quality and inexpensive feed ingredients for the formulation of high nutritive compounded feed supplement that will improve the quality of egg and sperm fertility and feed supplement bring greater yield in the control condition than when the fish are left to depend upon natural aquatic feed (Ochokwu et al., 2014). The aim of this review is to point out the effect of egg and sperm quality in fish breeding, the objective is to identify some of the possible factors that lead to poor egg quality and possible reasons while low sperm are obtained and while majority of the fish farmers in Nigeria have low fertility and poor hatching after incubation. Feeds, poor egg quality and inability of the sperm to fertilise the egg contribute to poor aquaculture development in Nigeria (Personal Communication, 2013**).**

Importance of Quality Egg and Sperm

Fish populations, both farmed and wild, are dependent upon the production of good quality eggs and sperm. Poor egg quality and sperm is one of the major constraints in the expansion of aquaculture of both marine and many freshwater fish species. In the fish farming industry, good quality eggs have been defined as those exhibiting low mortalities at fertilization, hatching and first feeding (Bromage et al., 1992), Bobe and Labbe (2010) defined fish egg quality as the ability of the egg to be fertilized and subsequently develop into a normal embryo while (Coban et al., 2011) define egg quality as the potential of an egg to hatch into a viable larva. Egg quality is significant for the production of high quality fish larvae and for economical utilization of hatcheries. In fish culture, egg quality control is necessary in species that have recently been introduced in fish culture (Lahnsteiner and Patarnello, 2004). Sperm quality was also defined by Bobe and Labbe (2010) as its ability to successfully fertilise an egg and subsequently allow the development of a normal embryo,

Egg and sperm quality is significant for the production of high quality fish larva and for economical utilization of hatcheries (Coban et al., 2011). Egg and sperm generally plays an important role in the quality of the fish farming, good quality eggs and sperm increases fish production, all year round availability of fish, thereby increasing market demand for fish and fish protein, it also increases the revenue of the country and individuals directly involves in it.

Fish feed nutrients for good quality egg and sperm

Good nutrition in animal production systems is essential to economically produce a healthy, high quality egg and sperm (National Research Council 1993). Prepared or artificial diets may either be complete or supplemental. Complete diets supply all the ingredients (protein, carbohydrates, fats, vitamins, and minerals) necessary for the optimal growth and health of the fish (Michelle, (2009). Feed nutrients for good quality egg and sperm includes protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (< 8.5%), phosphorus (< 1.5%), water (< 10%), and trace amounts of vitamins, and minerals (Robinson et al., 1998). When fish are reared in high density indoor systems or confined in cages and cannot forage freely on natural feeds, they must be provided a complete diet. In contrast, supplemental (incomplete, partial) diets are intended only to help support the natural food (insects, algae, small fish) normally available to fish in ponds or outdoor raceways. Supplemental diets do not contain a full complement of vitamins or minerals, but are used to help fortify the naturally available diet with extra protein, carbohydrate and lipid. Fish, especially when reared in high densities, require a high-quality, nutritionally balanced diet to grow rapidly and remain healthy so that finally there will be a good quality egg and sperm for breeding.

Techniques/Methods of Fish Breeding

Fish breeding is synonymous to fish propagation which simply means multiplication. Breeding entails all the various techniques or methods which can be used or at least maintain a fish stock (i.e. methods of fish seed production). This concept in fish production is important when an increase demand for fish and for culture is considered (Rhett 2013). Also, in the tropics, many fish species breed only once a year. Therefore, a continuous demand for fish seed makes fish propagation a necessity. There are various techniques which differ from different parts of the countries depending on local conditions and available local facilities. For instance, in developing countries where facilities are not available, fish seeds are obtained from the wild while in advanced countries where there are facilities, propagation is completely artificial. Basically there are three types of fish breeding/propagation, Natural propagation/breeding techniques, naturally induced breeding/pond or enclosure propagation, artificial propagation through hypophysation.

Process of artificial propagation through hypophysation techniques includes the use pituitary gland to induce spawning in fish; this can be carried out at any time of the year and under any environmental conditions. The technique ensures fish seed availability at all times of the year. For instance, using this technique a single common carp (Cyprinus carpio) has been induced to spawn five times within a year at intervals of 60,62,41 and 186 days between successive spawning, even though carp breeds naturally only once a year (Brooks et al., 1997) . Artificial propagation was first described in 1765, but was neglected until 1842 when it was described again. A number of experiments were carried out and by 1937 artificial propagation at commercial level was attained in 1964, it has spread to many parts of Europe, America, Japan, China, Israel but to date there are increased trials in Nigeria with varying degrees of success (Omoniyi et al., 2013). It was first reported in Panyan fish farm and Agodi fish farm where carp propagation was successful. Other privately owned fish farms have tried hypophysation using catfishes e.g. Clarias gariepinus, Heterobranchus bidorsalis. For the purpose of easy description and discussion, artificial propagation can be divided into six stages as: Selection of brooders, Maturation of the brooders Stripping i.e. obtaining eggs and sperms (milt) from the brooders, Fertilization, Incubation of fertilized eggs to ensure that they hatch, Rearing of the larvae/fry up to fingerling stage. On the whole, more fry can be obtained from an individual fish through artificial propagation involving stripping after hypophysation when compared to what is obtained through other propagation techniques.

Advantages of Artificial Propagation

- Fish seed is guaranteed all the year round, Fish seed is obtained outside the natural environment of fish, It increases the survival rate of the fry, It improves quality by crossing two different species (i.e. hybridization) can be obtained.

Disadvantages of Artificial Propagation

- The donor fish has to be sacrificed in most cases and hence a loss of fish, The whole process is laborious and highly technical, Very expensive in that it requires proper housing, constructions of tanks, installation of jars in a close circulatory system. It should be noted that artificial (i.e. naturally induced or through hypophysation) production of fish seed are carried out in enclosures known as Hatcheries which may be an indoor or outdoor facilities and they require inputs such as brood stock, adequate water supply and suitable feed.

Cultivable Fish Species in Nigeria

The following are some of the fish species that have been identified to be cultivable in Nigeria **-** Clarias gariepinus, Heterobranchus bidorsalis, Heterobranchus longifilis, Heterotis niloticus, Oreochromis niloticus, Sarotherodon galilaeus Gymnarchus niloticus, Lizajalcipinnis, Liza grandisquamis, Elopes lazeta, Tilapia melanopleura, Chrysichthys nigrodigitatus, Macrobrachium macrobranchium Macrobrachium vollenhoveni, Clarias anguillaris (**NU**DLN, 2013)

Factors That Affect Fish Quality Egg and Sperm

The quality of both egg and sperm is highly variable and depends on various external factors such as feeding regime, the quality of the feed (Cerovsky et al., 2009), (Jacyno et al., 2009), while (Bezdicek et al., 2010), (Strapak et al., 2010), (Hanus et al., 2011), (Stolc et al., 2009), reported that variations between fish, age of the fish are also a factor that determine the quality of egg and sperm, environmental conditions (Wolf and Smital, 2009), and season of the year (Hajirazaee et al., 2010). Sperm quality is determined by sperm motility and fertilization potentials. (Karouni et al., 2011) reported the three main elements that determine the health of sperm cells, sperm quality, and sperm quantity and sperm motility. It is the number of sperm cells present in 1mililiter of semen, in healthy semen an analysis will show tens of millions of cells in just 1 milliliter. Also fish feed nutrients and feeding regime are one of the factors that determines the egg and sperm quality (Tahoun et al., 2008), also hormones play a role in larval development and thus may affect egg quality. (Brooks et al., 1997) stated that environmental and genetic factors can also affect egg and sperm quality. They are pollutant, fungi, and bacteria, uptake of nutritive and genetic materials, physiochemical properties of water pH, salinity and temperature.

Hormones and Egg Quality

Hormones play a role in larval development and thus may affect egg quality. Hormones could be supplied to the egg before fertilization, in which case they must enter by maternal transfer, or they could be synthesized at any time after fertilization (Brooks et al., 1997). Studies looking at the ontogeny of the endocrine system (Leatherland and Barrett, 1993; Tanaka et al., 1995) have so far indicated that fish larvae are physiologically immature, with little or no capacity to produce certain enzymes, growth factors and hormones, until at or around the end of yolk re-absorption. Thus, fish larvae appear to be dependent on exogenous sources (mother or live food) for the supply of these regulatory factors, rather than the synthesis in the egg /larvae (Lam, 1994). Indeed, hormones have been shown to pass into fish oocytes from the maternal circulation (Greenblatt et al., 1989). This store of maternal hormones may fulfill the regulatory needs of fish larvae for growth, development, osmo regulation, stress responses and other physiological functions prior to the functional development of their own endocrine glands (Lam, 1994). Knowledge on the hormonal content of eggs prior to fertilization, however, is limited to a very few hormones, including the thyroid hormones, thyroxine (Tagawa and Hirano, 1991) and triiodothyronine (Tagawa and Hirano, 1987); Brooks et al., 1995) and several sex steroids (Feist et al., 1990). Thyroid hormones of maternal origin are deposited in egg yolk, and they may have significant effects on fish embryo development (Lam, 1994).

Egg Size in Successful Fish Breeding

In aquaculture, historically there has been the perception that, in terms of quality, bigger eggs are better. Egg size may vary both within a species and between populations of the same species (within the limits set by their genes: Beacham and Murray, 1985. Ecological explanations for differences in egg size of fish in different Populations include temporal and spatial changes in food particle size and in food quality availability to larvae, and predation. Age at maturity may also affect egg size in fish (Sargent et al., 1987), with a larger body size often resulting in the production of larger eggs (L'Abee Lund and Hindar, 1990; DeMartini, 1991). Egg size in fish is also affected/ modulated by the nutritional status of the female during ovarian recrudescence (Tyler et al., 1994) and in asynchronous spawners, the size of eggs ovulated in the later batches is often smaller, a phenomenon associated with the diminishing resources of the female (Hsiao et al., 1994). In Atlantic cod, their spawning strategy indicates that egg size takes priority over fecundity (Kjesbu et al., 1996). It has been suggested that, in the wild, larger hatchlings that result from larger eggs, have a survival advantage during the first few days of their lives, as they have larger yolk reserves (Blaxter and Hempel, 1963), may have higher growth rates (Moodie et al., 1989), are able to avoid predators more effectively (Wootton, 1994), and eat a wider variety of food items (Webb, 1986). Other authors, however, indicate that this is not necessarily so, arguing that larger offspring would be more noticeable as prey (Kjesbu et al., 1996). Furthermore, as larger eggs often take longer to hatch than smaller eggs, they are at risk from predation or adverse abiotic conditions for longer periods of time; the smaller eggs hatch earlier and the mobile larvae may then avoid adverse conditions (Miller et al., 1988). In cultured rainbow trout, egg size does not appear to be an important indicator of egg quality (Bromage et al., 1992). Bromage et al. (1992) showed that larger eggs did produce larger fry, but this size advantage was soon masked by other environmental determinants of growth.

Age of Fish and Egg Quality

Studies on mammals have shown that reproductive age can affect egg quality (Navot et al., 1994). For example, in pigs, the incidence of embryo mortality in pubertal gilts (experiencing the first oestrous cycle) is greater than that of gilts mated after one or more oestrous cycles (Archibong et al., 1992). Cytogenetic evaluation of ova from pubertal and third-oestrus gilts revealed that gilts at first oestrus ovulated a greater proportion of immature ova than gilts at third oestrus. In addition, the estimated frequency of meiotic non disjunction was greater for gilts at first oestrus than at third oestrus (Koenig and Stormshak, 1993). In older humans, the reverse is seen; there is a 50% decrease in human female fecundity from age 25 to 35, which appears to be linked to deterioration in oocyte quality (Navot et al., 1994).

Studies of this nature in fish have not been reported, but recent work in the rainbow trout indicated that over the first two spawning seasons, females produce better quality eggs in the second season (Brooks et al., 1997). Similarly, Bromage and Cumaranatunga (1988) found that the survival to eyeing of eggs from female rainbow trout ovulating for the second time (as 3-year-olds) was significantly higher compared with eggs from females spawning for the first time (as 2-year-olds; 75% versus 58% survival, respectively). A general improvement in egg quality, quantified as survival to hatching, in successive spawning seasons has also been observed in European sea bass (Navas et al., 1995).

Environmental Influences on Egg and Sperm Quality

Comparisons of wild and captive fisheries show, consistently, that egg quality and sperm is higher in wild fish compared with that in captive stocks. As an example, studies on wild and captive Atlantic salmon (Salmo salar, Salmonidae) have shown that eggs from wild salmon have up to 25% higher fertilization and hatching success, which is associated with greater size and survival of embryos, compared with their captive counterparts (Srivastava and Brown (1991), Variation in sperm and egg quality may be due to sex ratio, stocking density, age, size, nutrition and feeding regime; (Tahoun et al., 2008). The superior quality of wild eggs over farmed eggs is believed to be largely a function of environmental influences. Spherical and very ellipsoidal oil globules are indicators of low embryonic survival and therefore of low egg quality while eggs with slightly ellipsoidal oil globules indicate high embryonic survival (Lahnsteiner et al., 2008). Environmental factors that may affect egg quality and sperm quality in fish include the diet of the Brood fish and the physiochemical conditions of the water in which the eggs are incubated (temperature, salinity and pH of the water, etc.). In aquaculture, the photoperiod to which the brood fish have been exposed and the quality of the husbandry - factors such as the level of stress to which the broodstock are exposed, the fertilization procedures adopted, over ripening of eggs in the body cavity and bacterial colonization of fertilized eggs - can all affect egg quality. In both wild and captive fisheries, exposure of maturing females and male, or exposure of the eggs and sperm or developing embryos to environmental pollutants may affect egg, sperm and fry survival (Miller, 1993). More subtle features of the environment may also affect spawning. For example, in the ayu

(Plecoglossus altivelis, Plecoglossidae), sensitivity to hormone signals is affected by the physical features of the spawning environment (Soyano et al., 1993).

Diet

Difference in egg and sperm quality as a consequence of diet, especially lipid content, is one of the most researched aspects concerning egg quality and sperm viability. Dietary components as diverse as polar and non-polar lipids (Watanabe et al., 1991), fatty acids (Harel et al., 1994), protein (Washburn et al., 1990) and ascorbic acid (Dabrowski and Blom, 1994) have all been shown to affect egg and embryo survival. Egg quality was found to be improved in the European sea bass by altering the lipid composition of broodstock diet (Carrillo et al., 1995). Eggs considered to be of better quality had a higher content of total n-3 fatty acids, which enhanced levels of both decosahexaenoic acid and eicosapentaenoic acid. In gilthead sea bream, broodstock fed on a diet deficient in n-3 highly unsaturated fatty acids for a period of 10 days shortly before spawning, produced eggs with a reduced viability. Further, it was shown that the levels of n -3 highly unsaturated fatty acids supplied in the diet were directly correlated with levels in both the polar and neutral fractions of egg lipid (Harel et al., 1994). In contrast to these studies, an analysis of the lipid and fatty acid composition of Atlantic halibut eggs showed that batches of eggs with widely differing viabilities had very similar lipid compositions (Bruce et al., 1993). Dietary proteins and carbohydrate also appear to influence egg quality (Washburn et al., 1990; Harel et al., 1994, 1995), although these components have received less study than the lipids. In fish, although carbohydrates are relatively poorly utilized, and the main sources of energy are protein and lipid (Walton and Cowey, 1982), in rainbow trout, broodstock fed on a diet low in carbohydrate had a reduced relative fecundity (they produced fewer eggs per kg body weight), and the eggs had a reduced survival to the eyeing stage and a reduced hatchability (Washburn et al., 1990). Proteins act as a source of amino acids and as a reservoir of materials used during the many biosynthetic activities that are essential for the early stages of embryogenesis (Metcoff, 1986). Successful embryonic development in fish has been shown to be dependent on the balance of amino acids present in the egg (Fyhn, 1989). Most studies on diet and egg quality in fish have focused on the bulk dietary components; that is, the proteins, fats and carbohydrates.

Photoperiod and Physiochemical Properties of the Water

It has been suggested that environmental factors such as photoperiod, temperature, salinity and pH of the water influence egg and sperm quality (Brown et al., 1995). Photoperiod manipulation is commonly used in aquaculture as a method for advancing or delaying spawning, to obtain a year-round supply of eggs of salmonids and other fish species. Delaying spawning of pink salmon (Oncorhynchus gorbuscha, Salmonidae) by light manipulation led to increased egg mortality by the eyed stage from 5% (in the controls) to between 60% and 80% (Dabrowski and Blom, 1994). In contrast, Pohl-Branscheid and Holtz (1990) found only minor differences in egg quality - estimated as percentage survival at the eyed stage in female rainbow trout exposed to an artificially compressed light regime that induced four spawning in 2 years; under a natural photoperiod, rainbow trout spawn once a year. Gillet (1994) found that delaying ovulation in the Arctic charr (Salvelinus alpinus, Salmonidae), by changing the photoperiod regime, improved egg quality. However, this was probably because the delay in ovulation meant that the eggs were ovulated when the water was 28^{o}C colder than normal, which reduced over-ripening of the eggs.

Pollutants

Fish oocytes and eggs are particularly sensitive to environmental pollutants. As an example, fish have a 100-fold greater sensitivity compared with mammals to induction of embryonic development in fish also specific mutations during oogenesis (Walker and Streisinger, 1983). There is considerable experimental evidence that the quality of fish eggs can be adversely affected by pollution. Malformations and impaired development and viability may result from exposure to a variety of environmental contaminants, including insecticides (van Leeuwen et al., 1986), organ chlorinated biphenyls (Smith and Cole, 1973) and polychlorinated biphenyls (Mauck et al., 1978; Matsui et al., 1992).

Exposure to these pollutants, and their accumulation in the oocyte/egg, may occur when the oocytes are developing in the ovary (Miller, 1993), or subsequently, when the eggs are released into the aquatic environment. Gonads with high concentrations of chlorinated hydrocarbons yield eggs with lower hatching success than less contaminated eggs (Westin et al., 1985). Some persistant environmental chemicals, for example chlorinated hydrocarbons, polychlorinated biphenols, and some known endocrine disrupters (e.g. DDT) are lipophilic and may be transported with lipid reserves into the developing oocyte (Ungerer and Thomas, 1995). Rates of mortality and deformities become particularly pronounced when larvae from organ chlorine-exposed females start to use their lipid reserves (Burdick et al., 1964).

Husbandry of Captive Fish

There is little doubt that poor husbandry results in poor reproductive success of cultured fish. It is surprising; therefore, that more attention has not been paid to the effects of husbandry practices on egg quality. Key husbandry factors that are likely to have major effects on egg quality are: to what extent the brood fish have been stressed, the fertilization practices adopted, egg over-ripening, and bacterial colonization of eggs (Bromage et al., 1992). In captivity, confinement and over stocking of fish may affect egg quality. Both chronic confinements experienced during the final stages of reproductive development, and periods of acute stress, have been shown to disrupt the endocrinology underpinning normal growth and development of the ovary in trout, and may result in significantly lower progeny survival rates (Campbell et al., 1994). Stress can lead to irregular spawning intervals, low fertilization rates and increased occurrence of abnormal embryos in Atlantic cod (Kjesbu, 1989; Wilson et al., 1995). How stressing brood fish leads to deleterious effects on egg quality has not been established. Some fish, when reared in captivity, do not usually release their eggs; the eggs will then age or over-ripen within the body cavity. The time interval between ovulation and fertilization will also affect the ripeness of the eggs. In rainbow trout, there is approximately a one-week window for successful fertilization, in Atlantic halibut the window is 4 to 6 hours, but in tilapia, over-ripening of eggs occurs very quickly and the window for successful fertilization is only an hour or so post-ovulation (Bromage et al., 1994). It is important, therefore, that brood fish are checked regularly to ensure that they are stripped and the eggs are fertilized shortly after ovulation. For both cultured fish species that release (oviposit) their eggs, and for wild fish, the time to fertilization will depend upon the presence of a mature male. Over-ripening of eggs is perhaps the most common reason for poor egg quality in captive brood fish. After fertilization, dying or dead eggs become colonized with bacteria/ fungus, and if these eggs are not removed quickly from the incubation trays, viable eggs may also be colonized. By incubating eggs in water of high quality, and by `picking' colonized eggs at regular intervals, egg survival (and, therefore, overall egg quality) can be enhanced considerably (Bromage et al., 1994).

Genetic selection may alter sperm quality and fertility and may become an actual concern. Zohar (1996) reported that selection of interesting commercial strains of salmon resulted in a reduction by 20 times the sperm volume produced by males and Agnèse et al. (2005) reported a decrease of fertility at the fourth generation of domestication in Heterobranchus longifilis.

Sperm Concentration

The concentration of spermatozoa in the semen is easily accessed by different techniques such as microscopy counting, spectrophotometer; flow cytometry and determination of spermatocrit values (Alavi, et al., 2008). However the different methods proposed present some inconveniences. Counting (which is the basic method) allows assessing sperm number with an acceptable variability due to dilution and counting errors and evaluated to 6% for 300 spermatozoa per observation when counting of 1/500 diluted sperm was repeated 3 times (Suquet et al., 1992). It is the cheapest way to assess concentration of sperm but the main concern of this method is that it is time consuming and may not be compatible with the further use of sperm such as conditioning for preservation or aliquoting for multiple crosses in genetic studies.

The requirement of a fast assessment of spermatozoa concentration can be fulfilled by different techniques such as photometry, cytometry including Coulter counter and centrifugation. Photometric evaluation was reported for different species using different wavelengths of visible lights for which the apparent absorption of the light is mostly due to the turbidity of the medium (Cieresko and Dabrowski, 1993). The scanning of sea bass sperm absorption along the light spectrum revealed a constant increase from 500 to 300nm then a stronger increase occurred with a peak at 260 nm and at last the absorption rises up to its maximum at 210 nm. The best correlations between sperm counts and sperm optical densities were observed for 260 nm (Fauvel et al., 1999). Although spermatocrit has been used for sperm concentration assessment (Alavi et al., 2008). As a conclusion for this parameter, sperm concentration can be assessed precisely and quickly by means of sophisticated and costly tools which can provide other sperm characteristics about viability (see below) but new cheap applications such as small mobile single wavelength (even in UV) photometers or image analysis can successfully be used with an acceptable time lag for further use of sperm.

Sperm Morphology

Spermatozoa are isolated cells subject to variable environments from the testis to the fertilizing medium. Moreover they can be damaged by xenobiotics, altered by genetic mutation or ageing and at last they can be partially degraded by conservation protocols.

Under these different causes, modifications of sperm structure such as flagellum length or sperm head size have been observed in mammals. The recent techniques of image analysis were adapted in the 90's to describe mammal sperm morphology through the development of ASMA (Automated Sperm Morphology Analysis). This method was first applied to fish sperm by Van Look and Kime (2003) studying the effect of increasing dose of mercury on sperm quality. Later on, ASMA was used to assess the effects of HCG on spermiation in European eel (Asturiano et al., 2006), the effects of dilution media for cryopreservation (Penaranda et al., 2008) and the possible correlation between sperm morphology and swimming performance. (Tuset et al., 2008).

However attention must be paid to the possible distortions of fish sperm morphology due to the necessary fixation protocols

Sperm Motility

Sperm motility evaluation technologies have been regularly and exhaustively reviewed in the past five years (Bobe and Labbe 2009). The simple observation of sperm under a microscope at a magnification between x10 and x25 using a glass slide with or without cover slip, has been so far extensively used in order to qualify spermatozoa properties before their use in fertilization trials. This type of observation provides a coarse evaluation of sperm quality by motility criteria since it allows only assessing classes in terms of percent of motile sperm and motility duration defined by the time period leading to cessation of any progressive movement.

Although inaccurate and subjective, such a method highlighted the difficulties to objectively analyze the motility of spermatozoa and allowed defining the bases for further individual analysis. A reliable motility assessment actually requires), a two step dilution with a predilution in a non activating medium and ii) a high final dilution rate(>1/1000) in the activating medium adapted to either final sperm concentration and motility efficiency in order to avoid heterogenous triggering of motility (Billard & Cosson, 1992). The use of misfit activation conditions in the past may have driven to erroneous data. However, if this type of assessment provides the general features of fish sperm motility by a batch observation, it does not allow quantifying movements objectively and prevents establishing correlations between sperm movements and fertility and also correlations between factors determining sperm quality and sperm movement.

Description of Motility Process

The motility of fish sperm has been objectively described by different ways according to the use of phase contrast or dark field microscopy improved considerably the possibilities to observe both the head and the flagellum of the spermatozoa. The application of different types of high speed video recording or the application of stroboscopic light sources provided high quality static pictures of a same flagellum in different successive positions. This also allowed describing the frequency of flagella beating, the shape, amplitude and number of flagella waves and their variations and propagation responsible for the progression of the spermatozoa. Such techniques describing very accurately the initiation and the future variations of flagella beats showed that waves initiate at the junction between head and flagellum and progress towards the tip of the flagellum while, on the contrary, the decrease of motility efficiency observed for fish spermatozoa during their motility period is mostly due to a dampening from the distal part of the flagellum, which seems to be a general feature for fish species studied so far (Cosson et al., 2008a, b and Cosson, 2010).

II. Conclusion

In a successful fish breeding, the quality of egg and viability of sperm is very essential. Environmental factors that may affect egg quality and sperm quality in fish include the diet of the Brood fish and the physiochemical conditions of the water in which the eggs are incubated (temperature, salinity and pH of the water, etc.). In aquaculture, the photoperiod to which the brood fish have been exposed and the quality of the husbandry **-** factors such as the level of stress to which the broodstock are exposed, the fertilization procedures adopted, over ripening of eggs in the body cavity and bacterial colonization of fertilized eggs **-** can all affect egg quality. In both wild and captive fisheries, exposure of maturing females and male, or exposure of the eggs and sperm or developing embryos to environmental pollutants may affect egg, sperm and fry production. When all these factors are monitored and averted there will be improved and sustainability in aquaculture system in Africa.

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