Hazards Affecting Fish Fecundity (Reproductivity) and Adjusting Biosecurity Measures of Aquaculture

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Abstract

Fecundity refers to the total ripen (well matured) eggs formed in fish ovary and are capable to fertilization. Male sperm density resembles the number of well matured normal sperms formed in fish testis. Fecundity and sperm density at sexual maturity, during prespawning and spawning are essential biological items that give a real picture of fish reproductive performance. Their data helps to predict the reproductive capability of fish production. In this chapter, Fecundity and Sperm Density were proved to be highly affected by pathogenic or nonpathogenic factors. These pathogenic factors were bacteria or fungus or parasites affecting fish. Nonpathogenic factors such as climatic conditions or pollution were also proved to highly affect fish reproductive performances. The authors studied the harmful effects exerted on fish of both sexes, especially the gonads and liver (vitellogenin precursor). Some treatment trials were held proving fecundity and sperm density remedy. Whereas, some factors were proved to exert untreatable drastic effects on fish gonads or liver. Fish species had proved great variety in their influence.

Keywords: Fecundity, Ovary, Testis, Bacteria, Fungus, Pollution

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Introduction

Over the last few years, fish farming has multiplied dramatically in many regions of the world. Fish cultivation has consequently grown to be a significant global industry. FAO (2024) registered few countries in the global aquaculture industry including Egypt accounted for over 89.8% of the world's total aquaculture production. This emphasizes the concentration of aquaculture activity among few nations specially Egypt and their significant role in the global supply of farmed seafood. Correctly organized and managed aquaculture provide sustainable source of protein. Gender is recognized as an important factor influencing intestinal microbiota in several fish species (Wang et al., 2020).

Absolute Fecundity was defined by Gerking (1978) as the number of total well matured ova present in the female just prior to spawning which is produced by the action of yolk material (vitellogenin) taken by the perifollicular cells to form well matured ova filled with vitellogenin. Fecundity provide data significant to predict the reproductive capability of fish broodstock which is helpful to estimate the number of offsprings produced in a spawning season, by Jamali et al. (2016). Recently Hasan et al. (2020) specified that spawning and sexual maturity are reproductive activities known through fecundity estimation which include relative fecundity to gonadal weight, total fish length and body weight.

Schulz et al. (2010) had defined Spermatogenesis as a developmental process in which few spermatogonial stem cells produce a large number of highly differentiated spermatozoa. Factors affecting fish reproduction including Sperm Quality (sperm concentration and percent of dead to living), Morpho - anatomical parameters (hepatosomatic and gonadosomatic indexes), Sex hormonal levels as testosterone, estrogen. In addition to climatic conditions as water temperature, Ph and salinity. Water Quality: chemical constituents as unionized ammonia, nitrite by and sulphate. Besides, water pollution with heavy metals, petroleum, insecticides, pesticides, Chlorinated hydrocarbons or organic phosphorus compounds which might act as Endocrine Disruption Chemicals, also infectious Agents including bacteria, fungi or parasites.

- Fish Fecundity is Affected by: Pathogenic and Non-pathogenic Items

1. Pathogenic Items Include

i. Bacterial Infection

Aeromonas hydrophila isolated from apparently healthy *Oreochromis niloticus* ovaries, testes, livers, fry and unfertilized eggs. Infected males showed severe drop of sperm density and living spermatozoa. Females Relative and Absolute fecundity registered highly significant drop (Elias & Husein, 2004). In addition, *Aeromonas hydrophila* caused a decrease of gonado and hepato somatic indices together with Total protein level. Elias & Husein (2004) were the first to throw light on *Aeromonas hydrophila* vertical transmission to unfertilized eggs and affecting fry. *Vibriosis* being one of the prevalent enzootic fish diseases, it rapidly spread among fish in heavily stocked condition. Elias & Husein (2009)

examined *O. niloticus* infected with *Vibrio anguillarum*. Infected ovaries of prespawning and spawning fish appeared either hemorrhagic ruptured or empty. *Vibrio anguillarum* caused mortality between 70 – 80 %. The research recorded pronounced significant decrease in gonadosomatic index as well as relative and absolute fecundity. Owing to the destructive effects of *V. anguillarum* on the liver cells hepatosomatic index, and Total protein decreased. *Pseudomonas flouresence* and *Citrobacter spp.* isolated from *O. niloticus* gonads at pH 9 with decrease in relative and absolute fecundity of females in addition to impairment of male reproductive performance (Abd - Elhalim & Elias, 2017). They proved that pH 5 was far more harmful where *Aeromonas hydrophila* was isolated from ovaries and testes revealing drastic drop in fecundity of infected *O. niloticus* both sexes. The first trial discussing effect of ECPs of *Yersinia ruckeri* injected in healthy female *Clarias gariepinus* through intraperitoneal (I/P) injection registered by Elias & Abdellatief (2009). Mortality rate of was 75%, occurring between 48-72-hour post-injection. The results demonstrated a highly significant increase in ovarian weight (WG), coupled with congestion and severe hemorrhages in the ovaries, which negatively impacted fecundity relative to gonad weight (F.O.W).The administration of ECPs from *Y. ruckeri* resulted in Absolute fecundity (the total egg number (T. Egg No.)) significant reduction with a majority of the ova appearing either completely or partially devoid of vitellogenin. Histopathological analysis of the ovaries revealed scattered hemorrhagic patches among the ova, with many ova exhibiting signs of emptiness rather than containing vitellogenin. Furthermore, extensive hemorrhages were noted across the entire contour of the ova, accompanied by ruptures in the ovarian theca and the subsequent oozing of vitellogenin. These observations provide a coherent explanation for the considerable decline in absolute fecundity (T. Egg No.) observed in the studied fish. The researchers added that treatment trial with the immunostimulator Levamisole succeeded in decreasing mortality and repair fish fecundity as all ova were well matured filled with vitellogenin. and Total Protein level. Being an immune-modulator Levamisole increased IgM level thus activating the non – specific defense mechanism to protect fish against microbes. Levamisole decreased stress in infected fish indicated by decreasing glucose level (stress indicator). Meng et al. (2022) found ovaries and testicles swamps eels (*Monopterus albus*) were examined using 16S rRNA gene sequences to investigate the exact differences of gonadal microorganisms which are related to the eels' sex. Detected microorganisms included *Erysipelotrichia, Clostridia, Escherichia, Flavobacteriia, Bacillales, Romboutsia, Lactobacillus, Pseudomonas*, and *Actinomycetes* with sex percentages variation. Moreover (Meng et al., 2022) showed that lysozyme significantly increased in the testis, indicating that more bacterial microorganisms inhabited in testis. Lysozymes are an antimicrobial peptides distributed widely in teleost and acting as antibacterial defense (Kumaresan et al., 2015; Yu et al., 2021).

ii. Fungus Infection

Fungi often provides an important ecological role by breaking down decaying organic matter. They could, however, become an issue if fish are under stress from illness, unfavorable environmental circumstances, malnourishment, injuries, or even during spawning. These conditions weaken the fish, making it easier for the fungus to attack Roberts (2004). The fungus harm fish at every stage of life, which results in reduced fish and fry output in farms (Kwanprasert et al., 2007). The first experiment to assess the fecundity and reproductive performance of male *O. niloticus* fish infected with the *Icthyophonus* fungus was conducted in Egypt (Elias & El Khatib, 2003). Infection prevalence was high (53 %). *Icthyophonus hoferi* showed white nodules (resting spores) in *O. niloticus* males' testes with decrease in gonadosomatic index, significant drop in sperm density as well as living percent. In Females *Icthyophonus hoferi* showed macro and microscopic white nodules in tissue of ovary, El Khatib & Elias (2003). Absolute fecundity, gonadosomatic indices and total protein showed highly significant drop among *O. niloticus* more than *Cyprinus carpio* infected with *Icthyophonus*. According to Neish & Hughes (1980), *Fusarium* species, a well-known plant pathogen in terrestrial habitat, are imperfect fungi that can cause significant illnesses in freshwater fish.

Skin lesions in Nile catfish and Tilapia species induced by *Fusarium* species were described by Abd El Ghany (1998). Furthermore, it has been demonstrated by Zinedine et al. (2007) that *F*usarium caused reproductive problems and hyper estrogenic syndromes in both sexes. In addition to examining the impact of extracts from toxicogenic fungus isolates on the fecundity of Nile tilapia (*O. niloticus*). According to Elias & Abd El Ghany (2008) *Fusarium moniliform* infection caused *Clarias gariepinus* to develop severe skin hemorrhages, mouth redness, erosion of fins, tail cleavage, and muscle ulcers. Clinical signs appeared as 100 % and 70 % among both females and males respectively. Males suffered of vacuolar degeneration of spermatocytes together with thickening of seminiferous tubules lumen containing very few sperms and high percent of dead sperms because *Fusarium* and its toxins decreased the pituitary response to gonadotropin – releasing hormone. Moreover, *Fusarium* and its toxins reduced ovarian development and sperm number due to its estrogen like activity. Total protein, liver enzymes levels and relative and absolute fecundity registered highly significant decrease in both sexes. Treatment with garlic (*Allium sativum)* powder at concentration of 30 g / Kg. diet, enhanced growth and relative fecundity but failed to achieve complete remedy of absolute fecundity.

Abd El Ghany & Elias (2009) had arranged to identify one of the *Fusarium* mycotoxins T-2 toxin, in fish meals. Several fungal pathogenic toxins as well as T-2 mycotoxin proved to induce drastic effects on fish fecundity. Among *O. niloticus* it resulted in highly significant drop in relative and absolute fecundity beside total protein level. In females T-2 toxin proved its immunosuppressive effect through highly decreasing total globulins. T-2 toxin exerted much stress which was expressed in elevation of glucose level among both sexes. Moreover, treatment with Ginger proved to be effective only among males as it highly improved reproductive performance, the number of sperms as well as its live percent.

Aflatoxins (AFB1) are compounds with minute molecular weight produced by *Aspergillus flavus* and *Aspergillus parasiticus* moulds, found specially in hot and humid climates. Aflatoxins (AFB1) caused no changes in body weight, neither mortality nor abnormal behaviour. However, Huang et al. (2014) proved that Aflatoxin B_1 affected gonadosomatic index (GSI), and inhibited gibel carp reproduction through decreasing fish fecundity and ova size.

iii. Parasitic Infestation

Elias & El Khatib (2003) studied the effect of Eimeria spp. (coccidia) on male *O. niloticus*. White nodules were found between testicular tissues containing fragile walled oocysts which resulted in severe significant decrease in sperms number as well as living percent. Treatment trial of coccidia registered complete eradication of parasite with non – significantly improve of fish reproductive performance.

Also, El Khatib & Elias (2003) revealed significantly drop of absolute and relative fecundity of female apparently healthy *O. niloticus* and *Cyprinus carpio* infected with *Myxobolus spp*. Parasite prevalence was proved to be higher in *O. niloticus* proving its more sensitivity. Myxosporean parasite appeared as white large, rounded spores localized in the ovarian tissue causing drop of gonado and hepato somatic indices. The sever significant drop of total protein indicated vitellogenin decrease.

Yousef & Elias (2017) showed that absolute fecundity (Total ripen eggs) decreased severely resulting in Encysted metacercaria infestation. In addition, serum total protein, globulin and liver enzymes (AST and ALT) also severely decreased in the infested *Clarias gariepinus*. Oppositely, glucose level significantly increased as stress indicatior.

2. Non-pathogenic items Include

Pollution

Fish are continually exposed to adverse water quality conditions, including chemical contaminants which act as integrators of stressors. Metals in water may change the physiological and biochemical parameters of fish blood and tissues. Thus, water quality parameters significantly affect the growth, survival and reproduction of fish at various developmental stages, making it crucial to determine optimal water physical and chemical parameters for aquaculture.

Endocrine Disruptor Chemicals

Silent killers, threatening aquaculture on a large scale. They are foreign substances or mixture of substances that affectinge, consequently harming individuals' life or their offspring. These chemicals inhibit oestrogens, including pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and plasticisers. Synthetic oestrogens may also contaminate the aquatic environment. They are lipophilic and bioaccumulate in aquatic organisms. Wildlife suffers of physiological effects that are indicative of exposure to oestrogenic chemical contaminants, including feminisation, Similarly, male fish living in rivers in the UK might induce a physiological response (vitellogenin synthesis) that depends on oestrogen. Endocrine disrupting chemicals were divided by (Mills et al., 2001) into two classes. The first are anti-androgenic as Octylphenol causing increase estradiol without vitellogenin production. Oppositely the second (as DDT) having an estrogenic effect that decreased both testosterone (T) and estrogen with vitellogenesis induction. This class interacting with estrogen receptor was termed by Iwanowicz & Blazer (2014) as estrogenic endocrine disrupting chemicals (EEDCs) from which are pesticides.

Vitellogenin, a precursor of egg-yolk in fish and other oviparous animals, may be used as a biomarker for 'oestrogen' exposure. Tyler et al. (1996) revealed that during vitellogenesis process the yolk protein (VTG) is synthesized in the liver and transferred to the growing oocytes. Thus Vitellogenin being an estrogen dependency, is used as biomarker for estrogen exposure by endocrine disruptor chemicals. Males also contain Vitellogenin gene. Eoestrogen is female specific and present highly during spawning while it is normally detected in plasma of male fish due to its role in sperms maturation. It was evident by Schulz et al. (2010) the fact that both pituitary gonadotropins LH and FSH stimulate gonadal sex steroid hormone production, which is activated by Leydig cells,

i. Pollution with Heavy Metals

Heavy metals are naturally present in rocks but due to manmade developing processes, nowadays are found in soils resulting from industrial, civilized development and transport activities. These human activities raise heavy metals' concentrations resulting in serious environmental issues ending with chronic toxicity. Accordingly, Ishak et al. (2020) proved that heavy metal pollution in aquatic ecosystem had become critical issue. Elias (2007a) had proved the drastic effects of heavy metals pollution on fish reproduction. Apparently healthy females *O. niloticus* polluted with Lead, Cadmium, Copper and Zinc showed rudimentary ovaries although being apparently healthy and during spawning season. According to ovarian weight functioning and rudimentary. Pollution with heavy metals resulted in significantly impairment of fecundity among both functioning and rudimentary groups compared to unpolluted. In addition, total protein and liver enzymes levels highly affected due to liver dysfunction.

Whereas Elias (2007b) added that the overdose of copper sulphate caused significant drop in total egg number and sperm density of *Clarias gariepinus* both sexes within breeding season. The ovarian and testicular weight severely decreased with dropped relative fecundity. Hormonal steroidal levels (Testosterone and 17β methyl Estradiol) registered highly significant drop. Total protein increased due to increase of metal – binding proteins (transferrin and metallothionein) which acted as heavy metals carriers. Chromium toxicity was proved by Elias & Mohamed (2009) on *O. niloticus*. Testes showed seminiferous tubules atrophy and severe drop of sperm density. Female absolute fecundity registered highly significant decrease. Chromium caused vacuolar degeneration with hemosiderosis of liver represented in highly significant drop of Total protein. Treatment with Selenium as antioxidant showed progress in female fecundity while on the contrary, with male it was ineffective. Sperm density remained decreased due to seminiferous tubules necrosis. Sangita et al. (2012) studied Cadmium toxicity *Colisa fasciatus* caught from lake. Cadmium is one of the nonessential heavy metals, however, being the highest toxic water contaminant and could cause toxicity at each level in fishes. It caused drop in Total protein and body weight during testicular and ovarian cycles.

ii. Pollution with Pesticides

Haassan & Nemr (2020) defined "pesticides" as compounds destroying or regulating pests including herbicides (weeds) or insecticides (insects) or fungicides (fungi). Pesticides in low doses might not kill fish but impair their survival and reproduction.

Butachlor Herbicide

Aquatic systems might be subjected to several pollutants released from effluents discharged from sewage treatment plants or different industries, or agricultural drainage. Butachlor is herbicide used for grasses or weeds control especially in rice crops. It causes inhibition of mitosis and cell division by blocking protein synthesis.

Females *O. niloticus* polluted with Butachlor Soliman (2010) induced harmful effects on absolute and relative fecundity and oocytic atrasia in ovaries. Testosterone "T" and estradiol "E_2" hormones decreased significantly. Among males, one or two oogonia per field were seen in testes. The anti- androgenic action of Butachlor caused significant drop of sperm density ending with male infertility or sex reversal. Females' liver examination revealed clear tumor (hepatoadenocarcinoma) in an aggregation form scattered. They suggested that the highly significant drop of male reproductive performance most probably was irreversible.

Thiobencarb Herbicide

Thiobencarb is an Edocrine disrupting chemical (EDC) used to control grasses and weeds especially in rice crops. Eid (2015) organized research to shed light on the mismatch between Thiobencarb pollution and *O. niloticus* reproductive activity. Thiobencarb caused about 25-30% mortality rate in acute period while with chronic it increased gradually reaching 90 - 95% after 21 days. Ovaries showed fragmentation of oocytes' cytoplasm, shrinkage atresia with much thickening of ovarian wall.

Majority of spermatocytes revealed necrosis together with cystic formation and rare formation of spermatids. In addition, islands of proliferated spermatocytes are seen in focal aggregation with fibrous connective tissue formation (carcinogenic stage). Deformity of sperms appeared. Also, one or two oogonia were seen. Relative fecundity, Total Ripen Egg No for female, sperm density, live and dead % in male and Total protein revealed significant decrease in acute and chronic experiment comparing with the control.

Sex hormones (estradiol E2 and testosterone T) and matured oocytes decreased with increase in atretic follicles. Vitellogenin fractionation proved disappearance of the two bands among female polluted *O. niloticus*, while males showed several oogonial cells in the testes approved by presence of female specific band of vitellogenin.

Atrazine Herbicide

Al Saedy (2018) had registered mortality rate 25 % in *Clarias gariepinus* polluted with Atrazine. Atrazine exerted severe decrease in ovarian weight (Wg) and gonadosomatic index (I_G) showing many oocytes in the previtellogenic stage and atretic vitellogenic oocytes. Atrazine resulted in a significant drop in relative and absolute fecundity. Liver showed hypertrophy of hepatocytes and vacuolar degeneration expressed as highly significant drop in liver enzymes after Atrazine exposure for 9 and 15 days.

iii. Pollution with Phenol

Phenol originates from industrial wastewater, most often found in higher concentrations in fish Das et al. (2013). Soliman (2020) found that phenol residue in water from Nile River (Helwan branch of) was 0.02 ppm and in collected *O. niloticus* females' and males' organs (liver, ovary and testis) was 1.4 ppm. World Health Organization (WHO) prescribed 0.001 mg/L the most permissible concentration of phenol in water. Phenol presence presented by Soliman et al. (2020) was due to the industrial pollution resulted from sugar factories wastes in this area. Tilapia of both sexes were exposed to phenol (1/10 LC50) for 45 days chronic or long term exposure while fish samples were examined on intervals throughout the experiment. Mortality rate due to phenol experiment was about 25% and 40% in females and males respectively at the end of 45 days (total experimental period). In addition to the unsymmetrical and deformity in testes and ovaries. In females, Egg yolk oozing out of the ova. Thus, gonado-somatic index, absolute fecundity (Total Ripen Egg No.) and Estradiol (E2) hormone in females from Tebin or experiment showed significant decrease comparing with control.

Males showed more significant impairment than females which indicated steroidogenesis. Testicular tubules showed obvious degenerative changes including pyknosis and sloughing of spermatocytes with complete loss of spermatogenic contents. Sperm density, sperm live %, Relative fecundity, and globulin severely decreased. On the contrary, Estradiol (E2) hormone significantly increased. In contrast, glucose (stress indicator) showed highly significant increase in both sexes. Hepato-somatic index decrease was reflected in liver enzymes, Total protein and globulin elevations among both sexes. Vitellogenin fractionation by Electrophoretic analysis of female *O. niloticus* controlled group two distinct protein bands appeared, the second of which was specified as female specific. Among females, naturally and experimentally it disappeared indicating loss of vitellogenin. In experimentally males second band (female specific) appeared after 45 days.

iv. Climatic Changes

Climatic changes decrease fish health, fecundity and hatchability. It also causes a drop of dissolved oxygen leading to respiratory disturbances.

a. Ph (hydrogen ion concentration)

Physico-chemical parameters have induced a strong influence on growth, survival and reproductive performance among different stages. Favorable water quality parameters is essential for fish farming. Water Ph is considered one of the main factors affecting water quality. Scott et al. (2005) registered that Ph fluctuations are toxic to fish because of exerting ionic disequilibrium as well as impairing the circulatory system. Low water Ph decreases dissolved inorganic phosphorus and carbon dioxide used by phytoplankton during photosynthesis.

Elias & Abd- Elhalim (2009), conducted an experiment on tilapia influenced by Ph 5 and 9. Concerning females' fecundity, Ph 5 proved to be far more harmful as it exerted highly significant drop in the relative and absolute fecundity than those influenced by Ph 9. Moreover, most ova appeared net-like free from vitellogenin. However, although males' reproductive performance was highly significantly dropped by both experienced Ph values still it was more pronounced with the Ph 9. Testes appeared thread like producing watery milt of about 80% dead sperms.

Low Ph 5 and deviation to 9.5 resulted in highly significant drop of absolute and relative fecundity of female *O. niloticus* as well as drop of male reproductive performance. With Ph 9.5 testes contained watery milt and about 80 % dead sperms, Abd - Elhalim & Elias (2017). Marinuthu (2020) examined effect of several Ph levels on African catfish (*Clarias gariepinus)* reproductivity. Ph levels of (6.7–

7.6) recorded the maximum hatching rate compared to those of lower and higher Ph. At low pH levels (3.1 to 3.4) and at high Ph (10) hatching was completely disabled. They clarified the important role of Water Ph in adjusting fish homeostasis, acid-base and ionic regulation as well as ammonia excretion.

b. Temperature

Global warming is a phenomenon existing from the evolution of toxic gases such as carbon dioxide, sulphate and nitrogen resting inside the earth thus increasing temperature degrees. Temperature was defined by Pushkar et al. (2010) as one of the important abiotic environmental factors controlling all fish vital processes. Global warming scenarios will affect survival, growth, physiological behavior and other body functions. Water temperature (25 - 27ºC) is the most suitable temperature for rearing Nile tilapia. At 32ºC growth reduced and mortality increased. Low temperatures delayed maturation. Temperatures far beyond the optimal stopped gametogenesis and induced atresia.

Lopes et al. (2020) studied the effect of high temperature and low pH on reproductive performance of marine species, *Gobiusculus flavescens*. Reproduction was highly significantly impaired by elevated high temperatures. High temperature had 3% to 10% hatching success only and eggs number decreased up to 30% which were up to 20% smaller compared to fish spawned in favorable temperature. The climate models predicted that global temperature is likely to increase 1.4 - 6.4 ºC in this century, (Keller et al., 2015). Temperature, as one of the most effective environmental factors, exerts harmful effects on the different fish species. Moreover, they proved that climate changes might affect steroid estrogen concentration.

Fish, being cold blooded creatures achieve the same water temperature whereas this heat stress affects fish hypothalamus pituitary function causing complete fish impairment. Eid (2019) collected *O. niloticus* at temperatures 27±2 ºC and 36 ºC (survey group). Experimental both sexes were divided into four groups: control group at room temperature and water temperatures: 30ºC, 33 ºC and 36 ºC respectively for two weeks. At 33ºC and 36ºC fish showed nervous manifestation, abnormal swimming as well as suffocation. At 36ºC majority of testis appeared thread like.

Females Relative and absolute fecundity, and males Sperm Density and live to dead percent significant decrease at the three experimental water temperature degrees compared to the control group. Relative fecundity males increased during experiment. Both sexes Total Protein and Globulin of survey and experimented groups decreased significantly. Glucose increased at 33 ºC and 36 ºC. Estrogen hormone decreased at survey group and all experimented males' groups and only at 36 ºC group with females. Testosterone hormone increased significantly at 30 ºC, 33ºC, 36ºC compared to control among both sexes.

c. Photoperiod

Al Emran et al. (2024) throw light on Photoperiod which regulates fish feed intake, metabolism and hormonal secretion needed for hemostasis. Among some species Long-day photoperiod improves growth. Moreover, Short-day photoperiod exert either positive and negative effects on growth and reproduction in farmed fish species. In rainbow trout increasing spring photoperiod altered fish maturation and in turn vitellogenesis/spermatogenesis. Decreasing autumn photoperiod dragged final maturation, ovulation and spermiation.

Probioticss

B. subtilus gram –ve bacteria being ubiquitous in nature either obligate or facultative aerobes, it is one of the best prokaryotes in terms of molecular and cell biology. Elias & Husein (2009) found *Bacillus subtilus* showed highly improvement in gonadosomatic index, absolute and relative fecundity among *O. niloticus* fish during prespawning phase. From the immunological point of view, *B.subtilus* increased total globulins and decreased mortality among prespawning fish fed *B.subtilus* and challenged with *V. anguillarum*. *B.subtilus* caused increased body weight as it germinate in the intestine and produce wide range of digestive enzymes.

Biosecurity Measures in Fish Farms

It is necessary to apply Standard Operating Procedures including safety aquaculture practices at hatcheries:

- Good practices for feed, drug and chemical storage.
- Schedule for each aquarium history (hatching, growth rate, diseases, date and type of medication and harvesting).
- Obtaining national strategies on aquatic animal health, disease surveillance and reporting as well as contingency planning.
- HACCP; good management practices [good aquaculture practices (GAP) and good hygienic practices (GHP).
- Well training of workers, using proper personnel protective equipment and enriching their awareness on risks/hazards.
- Complete separation of utensils for each aquarium. Dead or diseased fish are quickly removed and burnt in incinerator.
- Quarantine for any imported fish or fries.
- Sustainable management is only applicable with accurate and efficient stock assessment data is available.

Recommendations

- Heavy metals might accumulate in fish organs (gonads or liver) causing their damage despite being within permissible limits in water.
- Frequent misuse or overuse of chemicals, pesticides or herbicides or faulty behaviors in fish farms impair fish health and reproduction.
- Immunostimulants or probiotics are advisably added to fish meals, especially broodstocks as prophylaxis against different pathogens.

Conclusion

Importance of periodical examination for fish, especially broodstocks for early diagnosis of any pathogens affecting fish reproduction. Since water is the environment of fish, thus the schedule examination for water physical and chemical parameters is important. Beside securing the farm's water source to avoid water pollution with any chemicals or pesticides. Importing fish must be of well-known source to assure their

reproductive high performance. Some pathogens' effects might be irreversible. The suitable quarantine for imported fish is a necessity. In addition, implementation of sanitary culture systems as well as biosafety and biosecurity measures are the only way for obtaining healthy highly reproductive broodstocks. Well training for all workers. Necessity for timetabling the farm and aquaria cleaning and maintenance. Usage of probiotics, immunomodulators is preferable. Further research is required for cryopreservation or sperm bank establishment. Finally, studying reproductive performance for all fish species helps estimate the number of offsprings per spawning season.

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