Asian Fisheries Science **26** (2013): 156-166 ©Asian Fisheries Society ISSN 0116-6514 E-ISSN: 2073-3720 https://doi.org/10.33997/j.afs.2013.26.3.003



Efficacy of Copper Sulphate as a **Prophylactic Agent for Fungal Infection on Egg, and its Effect on Hatching and Early Growth of** *Clarias gariepinus* (Burchell 1822)

ATAGUBA G.A., OKOMODA V.T^{*}. and UNDE E.S.

Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria P.M.B. 2373, Makurdi, Benue State, Nigeria

Abstract

This study was undertaken to determine the efficacy of prophylactic treatment of North African catfish, *Clarias gariepinus* (Burchell 1822) eggs with copper sulphate (CuSO₄) and its effect on hatch rate, survival to first feeding and growth of fry. Copper sulphate concentrations were: 2 mg L⁻¹, 4 mg L⁻¹, 6 mg L⁻¹, 8 mg L⁻¹ and 10 mg L⁻¹. The study employed two treatment methods termed "dip treatment" and "continuous treatment". A homogeneous mass of eggs (2.4 g) from the same fertilized batch treated with either the dip or continuous method were used to determine the hatching success of the eggs. With a mean fertilization rate of 63.2% in all treatments, significant differences in the hatchability between treated eggs and the control was observed. However an increase in concentration had *Saprolegnia* growth compared to those incubated in the continuous treatment. Survival rates were significantly higher in eggs treated with concentrations above 6 mg L⁻¹ compared to the control and lower concentrations. The use of the continuous treatment for rearing fry beyond first feeding is recommended at 2 mg L⁻¹ CuSO₄; this concentration exhibited the best growth performance and survival.

Introduction

The North African catfish *Clarias gariepinus* (Burchell 1822) is highly appreciated as a good aquaculture species because of its resistance to disease, ability to tolerate a wide range of environmental parameters and relative fast growth rate (Goos and Richter 1996). In many parts of the world, *C. gariepinus* is firmly entrenched as one of the most important aquaculture species. With increased emphasis on fish culture in Nigeria and the advent of modern techniques, *C. gariepinus* has become even more valuable to man (AIFP 2005). It is among the most widespread freshwater fishes in Africa (Nguyen and Janssen 2002). Fingerling production and availability of quality fish feeds have been bottlenecks for development of fish farming in Nigeria for over 40 years (NSPFS 2006). Over the past several years, private sector fingerling production has increased from 3 million fingerlings per year in 2001 to more than 30 million per annum in 2006 with several large producers delivering more than 300,000 fingerlings monthly (NSPFS 2006). However, the Federal Ministry of Agriculture plans to increase fingerling production to 1.25 billion fingerlings annually beginning

^{*}Corresponding author. E-mail address: okomodavictor@yahoo.com

from 2013 (Nigerian Tribune 2013). Farming of the species in Nigeria is limited by problems of high mortality in fingerlings and the resulting fry scarcity. Despite the high fecundity, the hatch rates of eggs in many hatcheries in Africa are erratic, ranging from 8-70% depending on the degree of sophistication of management in the hatcheries (Macharia et al. 2005). One probable cause of erratic hatching is the parasitization of eggs. In the United States, it has been shown that unfertilized channel catfish, *Ictalurus punctatus* (Rafinesque 1818) eggs contribute to egg mortality because these eggs are easily colonized by fungus (USDA 2003). The common practice in hatcheries is to routinely control fungal infection by using anti-fungal agents (Barnes and Gaikowski 2004).

In Nigeria's aquaculture industry, fungicides used include malachite green, formalin and sodium chloride (Adeyemo et al. 2011). They are used together or separately as anti-parasite treatments against ectoparasites such as Gyrodactylus, Dactylogyrus, Ichthyobodo, Trichodina, Chilodonella and Ichthyophthirius (Adeyemo et al. 2011). Malachite green is effective for the control of ichthyophthiriasis and other protozoan parasites, but is prohibited for the treatment of eggs of herbivorous fish and tench; in some countries around the world, this prohibition generally covers food fish (Citek et al. 1998) because eggs of these fish species are very sensitive to malachite green and treatments leave the residual leucomalachite in flesh. Hence efforts are being made to find alternative chemicals for treating fish diseases. Considerable research has been undertaken on the suitability of copper sulphate (CuSO₄) in aquaculture operations. The effectiveness of CuSO₄ in controlling ichthyophthiriasis and saprolegniasis in channel catfish and other temperate fish species has been demonstrated (Straus and Tucker 1993, Schlenk et al. 1998, Straus et al. 2008a; Straus et al. 2009a). However, little is known about its use in the control of fungus in C. gariepinus hatcheries. Today, CuSO₄ is approved as an algicide and molluscicide by the U.S. Environmental Protection Agency (EPA) but not approved by the FDA for therapeutic use in aquaculture (Straus et al. 2009b). Regulatory action on CuSO₄ has been deferred pending the outcome of ongoing research at the Harry K. Dupree-Stuttgart National Aquaculture Research Center (SNARC) (Straus et al. 2009b). In light of the need to find a suitable substitute for conventionally prophylactic use in aquaculture, the present study was designed to determine the effectiveness of CuSO₄ to control fungus infection in C. gariepinus.

Materials and Methods

The broodstock (400-500 g) used for the present study were obtained from the research farm at the University of Agriculture in Makurdi (Benue State, Nigeria) and transferred to the north core hatchery of the University of Agriculture Makurdi where the experiment was conducted. Copper sulphate (Sigma-Aldrich Corporation, Saint Louis, Missouri, USA) solutions were prepared in five concentrations. A stock solution of CuSO₄ was aliquoted into 10 L of water to give 2, 4, 6, 8 and 10 mg⁻ L⁻¹. Alkalinity of the water source was determined and found to be 37 mg⁻ L⁻¹, which was above the minimum of 20 mg⁻ L⁻¹ recommended by Masser and Jensen (1991) for the administration of CuSO₄ as therapeutic agent on fish. Two treatment methods were applied: "dip treatment" which involved spreading the eggs on an incubating raft and dipping in the appropriate water solution with each concentration for 20 sec and "continuous treatment" where the eggs were left to incubate in the $CuSO_4$ solution until hatching. The study had three replications. Fertilization of eggs was determined using the methods of Ella (1987) after which percentage fertilization was calculated.

The hatch rate was determined using a simple proportion and assuming a normal distribution of fry in water. The bowl containing the fry was stirred to create an even spread and triplicate sample of 200 mL water was collected in a conical flask. The number of fry in 200 mL was counted three times. From this, total number of fry in a litre of water was calculated.

Percent survival was determined by counting the number of live fry at first feeding and after 2 weeks of feeding, this was expressed as percent of the initially hatched fry.

Fungal infestation was not induced but it occurred naturally. Fungi infestation was verified by visual examination of the eggs for a fungal mass, after which samples were examined under a microscope for the typical mycelia and hyphal development (presumptive identification). Fungus samples from the control treatment were morphologically identified to genus via reproductive structures. After the absorption of the endogenous food, the second experiment was set up to determine the growth rate of *C. gariepinus* fry exposed to various concentrations of CuSO₄ for 12 days after hatching using the same concentrations. The routine weighing and cleaning of the bowls were done every 3 days; water in each bowl was renewed with the appropriate concentration of CuSO₄ accordingly. Three hundred fry were stocked for each concentration of CuSO₄ used. Fish were bulk weighed on a balance and mean weights were obtained after counting.

Growth parameters of fry were determined using the relation shown below:

- Final mean weight (mg)
- Weight gain: $W_1 W_0$ (mg)
- Growth rate: $\frac{W_1 W_0}{t}$ and
- Specific growth rate (SGR) : $\frac{LnW_1 LnW_0}{t} \times 100$

Water quality parameters (dissolved oxygen, temperature, hardness, free CO_2 , ammonia, sulphide and pH) were analysed using the methods of APHA (1998).

Descriptive statistics for all the treatment means was done with Minitab 14 software (Minitab Inc., State College, Pennsylvania, USA). Students t-test was used to determine differences in hatch rate and survival to first feeding between each treatment methods (dip and continuous treatments) using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, California, USA). Hatch rate, survival, and growth parameters were subjected to one-way ANOVA to determine significant differences between the means in the various concentrations for the dip and continuous treatment, as well as the growth experiment.

Results

Eggs were infested with fungi between 9-24 h of incubation in the control and in all the dip treatments. There were no visible fungi growth on dead eggs incubated in continuous treatments while those in dip treatments had observable growth (Fig. 1). Fungi were identified as Saprolegnia spp. Species-level determination could not be made because of lack of oogonial development. The study revealed that hatch rate was statistically similar between dip and continuous treatments across all concentrations (Table 1), but significantly higher than the control. Survival to first feeding between dip and continuous treatments was higher (P<0.05) in the continuous treatment for 4 mg L^{-1} CuSO₄ and higher when compared to the dip treatment. Dip treatments had the highest survival in the 8 mg L^{-1} (74. 7%) and 10 mg L^{-1} (82.64 %) CuSO₄ concentrations while the lowest survival was observed in the 0 mg^{-L-1} (33.3 %) treatment. Continuous treatments with concentrations above 4 mgL^{-1} CuSO₄ had similar survival, but differed from the values observed for the 0 mgL⁻¹ and 2 mg^{-L⁻¹} (P<0.05) CuSO₄ treatments (Table 2). Ranges of water quality parameters in the dip treatment during incubation and rearing to first feeding include: free CO₂: 3.5 mg L^{-1} to 6.5 mg L^{-1} , pH: 6.7 to 7.4, NH₃: 0.3 mg L⁻¹ to 0.7 mg L⁻¹, hardness: 53.0 mg L⁻¹ to 59.5 mg L⁻¹, SO₃: 0.04 mg L⁻¹ to 0.12 mg L^{-1} , dissolved oxygen: 5.5 mg L^{-1} to 6.2 mg L^{-1} and temperature: 25.7 °C to 26. 5 °C. In the continuous treatment during egg incubation and rearing to first feeding, water quality parameter ranges were: free CO₂: 4. 3 mg L⁻¹ to 5.2 mg L⁻¹, pH: 6.4 to 7.2, NH₃: 0.2 mg L⁻¹ to 0.4 mg L⁻¹, hardness: 46.5 mg L⁻¹ to 53.5 mg L⁻¹, SO₃: 0.005 mg L⁻¹ to 0.15 mg L⁻¹, dissolved oxygen: 5.4 mg L⁻¹ to 6.0 mg⁻L⁻¹ and temperature: 25.5 °C to 25.95 °C. Growth and survival of *C. gariepinus* fry raised for 12 days in the continuous treatment at different concentrations are presented in Table 3 and reveals that mean final weight (MFW) ranged from $23.0 \pm 0.1 \text{ mg} (10 \text{ mg} \text{L}^{-1})$ to $38.4 \pm 0.6 \text{ mg} (2 \text{ mg} \text{L}^{-1})$ $mg'L^{-1}$) with significant differences between the concentrations (P<0.05). The general trend in growth reveals that fry raised in the control $(0 \text{ mg}\text{L}^{-1})$ were significantly larger than those exposed to 8 mg L^{-1} and 10 mg L^{-1} and were statistically similar with those exposed to 4 mg L^{-1} and 6 mg L^{-1} . However, fry raised in 2 mg l⁻¹ had the best growth. Percent survival decreased as the level of concentration of CuSO₄ increased and the highest value of 60.1% was observed at the lowest concentration of $CuSO_4$ (2 mgL⁻¹) while the lowest survival recorded was 19.8% for the control. Water quality was also monitored and ranges were: free CO₂: 3.7 mg L⁻¹ to 4.2 mg L⁻¹, pH: 6.6 to 7.3, NH₃: 0.4 mg·L⁻¹ to 0.8 mg·L⁻¹, hardness: 46.0 mg·L⁻¹ to 51.0 mg·L⁻¹, SO₃: 0.015 mg-.L⁻¹ to 0.11 mg L⁻¹, dissolved oxygen: 5.5 mg L⁻¹ to 6.7 mg L⁻¹ and temperature: 25.8 $^{\circ}$ C to 26.5 $^{\circ}$ C.

Treatment	% Hatch			% Survival to 1 st feeding		
	Dip	Continuous	P-Value	Dip	Continuous	P-Value
$2 \text{ mg} \text{L}^{-1}$	59.7 ± 1.4	67.5 ± 12.3	0.7125	44.3 ± 9.7	54.8 ± 7.9	0.4862
$4 \text{ mg} \cdot \text{L}^{-1}$	59.3 ± 5.4	53.1 ± 1.5	0.3864	50.0 ± 1.9	97.8 ± 2.2	0.0038**
$6 \text{ mg} \text{L}^{-1}$	53.4 ± 1.0	53.9 ± 1.8	0.5101	60.8 ± 13.3	92.9 ± 23.1	0.0361*
$8 \text{ mg} \cdot \text{L}^{-1}$	53.1 ± 0.5	61.3 ± 2.1	0.0604	74. 7 \pm 1.5	99.4 ± 0.2	0.027*
10 mg^{-1}	54.9 ± 3.6	$52.5\pm1.~7$	0.1961	82.6 ± 6.3	96. 9 ± 2.1	0.05*

Table 1. Hatch rate and survival to first feeding (comparison between dip and continuous treatments).

*Student T-test significant at P<0.05

** Student T-test significant at P<0.01

Table 2. Hatch rate and survival to first feeding (comparison among concentrations for dip and continuous treatments).

Treatmont	Dip tre	eatment	Continuous treatment		
Treatment	% Hatch	% Survival	% Hatch	% Survival	
0 mg l ⁻¹	42.1 ± 1.5^{b}	$33.3 \pm 1.8^{\circ}$	38.1 ± 1.5^{b}	33.3 ± 1.8^{b}	
2 mg l^{-1}	$59.7\pm1.4^{\rm a}$	44.3 ± 9.7^{bc}	67.5 ± 12.3^{a}	54.8 ± 7.9^{b}	
4 mg l ⁻¹	$59.3\pm5.4^{\rm a}$	50.0 ± 1.9^{bc}	$53.1\pm1.5^{\rm a}$	$97.8\pm2.2^{\rm a}$	
6 mg l ⁻¹	$53.4\pm1.0^{\rm a}$	60.8 ± 13.3^{ab}	$53.9\pm1.8^{\rm a}$	72.9 ± 23.1^{a}	
8 mg l ⁻¹	$53.1\pm0.5^{\rm a}$	74. 7 ± 1.5^{a}	$61.3\pm2.1^{\rm a}$	$99.4\pm0.2^{\rm a}$	
10 mg l ⁻¹	$54.9\pm3.6^{\rm a}$	$82.6\pm6.3^{\rm a}$	52.5 ± 1.7^{a}	96. 9 ± 2.1^{a}	

Means in the same column with different superscripts differ significantly (P<0.05).

Table 3. Growth and survival of *C. gariepinus* fry in the continuous treatment with different concentrations of CuSO₄ for 12 days.

Treatment	MIW (mg)	MFW (mg)	MWG (mg)	SGR	% Survival
0 mg l^{-1}	9.1 ± 0.1	33.5 ± 0.1^{b}	$24.3\pm0.3^{\text{b}}$	$10.8\pm0.2^{\mathrm{b}}$	$19.8\pm0.2^{\rm d}$
2 mg l ⁻¹	9.2 ± 0.2	$38.4\pm0.6^{\rm a}$	$29.3\pm0.5^{\rm a}$	12.0 ± 0.0^{a}	60.1 ± 0.1^{a}
4 mg l ⁻¹	9.3 ± 0.1	31.7 ± 0.7^{b}	22.4 ± 0.7^{b}	10.2 ± 0.0^{b}	$58.8\pm0.8^{\rm a}$
6 mg l ⁻¹	9.1 ± 0.1	32.1 ± 0.1^{b}	23.0 ± 0.2^{b}	10.5 ± 0.1^{b}	$53.6\pm0.6^{\text{b}}$
8 mg l ⁻¹	9.3 ± 0.1	24.4 ± 0.1^{c}	$15.3\pm0.0^{\rm c}$	8.2 ± 0.1^{c}	40.1 ± 0.1^{c}
10 mg l ⁻¹	9.1 ± 0.1	23.0 ± 0.1^{d}	13.7 ± 0.0^{d}	$7.5\pm0.1^{\text{d}}$	40.2 ± 0.2^{c}

(MIW= Mean Initial Weight, MFW= Mean Final Weight, MWG= Mean Weight Gain, SGR= Specific Growth Rate). Means in the same column of treatments followed by different superscripts differ significantly (P<0.05).



Continuous treatment, 2 mg^{-1}



Dip treatment, 2 mg⁻L⁻¹



Continuous treatment, 4 mg⁻L⁻¹



Dip treatment, 4 mg⁻L⁻¹



Continuous treatment, 10 mg⁻L⁻¹



Dip treatment, 10 mg⁻L⁻¹

Fig. 1. Unfertilized *Clarias gariepinus* eggs at 72 hours treated continuously or by dip with CuSO₄.

Fungus samples from this study were identified morphologically as *Saprolegnia* spp. Common genera implicated in saprolegniasis are mainly *Saprolegnia* spp. and *Achlya* spp (Tucker and Robinson 1990; Noga 1996; Hawke and Khoo 2004). *Aphanomyces* spp. was however reported by Straus et al. (2009b), hence suggesting that species of fungus causing saprolegniasis on catfish eggs are more diverse and probably are defined by locality and other environmental factors. Despite Fig.1 elucidating the effectiveness of the continuous use method of CuSO₄ in the control of *Saprolegnia* spp. growth on eggs during incubation, it was observed that hatch rate was statistically similar between dip and continuous treatment methods and across all concentrations for both methods, but significantly differed from the control. However, survival to first feeding was statistically higher in the 4 mg L⁻¹ continuous treatment and higher concentrations compared to the dip treatment (Table 1).

Differences in fungal susceptibility and other non fungal pathogens (i.e., bacteria, ciliates, etc.) probably caused by the application of the different treatments may have led to differences in survival recorded in both treatments across the concentration (Straus et al. 2009a). The mean egg survival in the US catfish industry has been estimated to be approximately 60% (Wolters 2001) when along with other best management practices, formalin and iodine are used as needed to control fungal infections. The present study confirms this with hatch rates between 53.1-67.5% in all treatments administered with copper sulphate. A 10 mg L⁻¹ CuSO₄ concentration was recommended for sufficient fungi control on eggs of channel catfish I. punctatus by Straus et al. (2009b); a survival rate of 67.4% was reported for hatched fry in the CuSO₄-treated eggs while severe fungal growth in the control treatments resulted in 8% survival. Mitchell et al. (2010) demonstrated the effectiveness of CuSO₄ in the control of fungi as lesser film of fungal growth (2.9 cm) was reported on eggs treated in 10 mg L^{-1} CuSO4 compared to 7.2 cm coverage of fungi observed in the control. Akpoilih and Adebayo (2010) also reported a similar trend with formalin in preventing fungal growth on eggs. Copper sulphate is usually administered daily during the egg incubation period of channel catfish (USDA 2003; Steeby and Avery 2005) until the eggs become "eyed" (eye pigment is first seen in the embryos). However with appreciable mortality rates recorded in the present study, copper sulphate can be used continuously to ensure optimum production of cultured C. gariepinus (Teugels 1986). Straus et al. (2009b) reported that numerous unfertilized eggs contributed greatly to fungal development and consequently mortalities as they provide a suitable substrate for fungus to grow. The present study observed statistically similar hatching rates and consequently the same dead egg proportion resulting from a statistically similar fertilization rate, the difference in the results obtained for survival must have been due to the effectiveness of the prophylactic application of the copper sulphate at different levels. Though hatch rate among both methods and treatment concentration did not differ, the 20 second dip treatment did not prevent the growth of fungi. According to Straus et. al. (2009a), Saprolegnia spp. are present in most water sources. The usual egg/fry management practices of refreshing the water quality through water dilution with fresh water during the incubation and rearing to first feeding under static method of fish breeding would have led to reinoculation of the experimental units with new fungi spores from the fresh water. However, this

was not the case with the continuous treatment as the $CuSO_4$ concentrations were added at every water change.

Reports of prophylactic administration for other commonly used chemicals are highly varied according to species administered and stage of development. Mbaru et al. (2011) suggested the use of formalin for 15 min bath dip at 500 mg⁻L⁻¹ for yolk sac fry, and for 15 min at 600 mg⁻L⁻¹ for free-swimming fry to reduce fungal and parasitic outbreaks. Martin (1968) recommended the use of 1 mg L⁻¹ malachite green dip for 5 min to control Saprolegnia parasitica (Coker 1923) in fish and fish eggs. For the treatment of carp eggs infected with Saprolegnia spp., a 1 h treatment in a malachite green bath at 4-5 mg L^{-1} proved effective (Citek et al. 1998). Fish egg treatments with concentrations of 5-10 mg L^{-1} were recommended in the Czech Republic to be applied for 5–30 min, once or twice daily, or twice weekly (Citek et al. 1998). However the present study has demonstrated that continuous exposure of African catfish eggs to up to 10 mg L^{-1} CuSO₄ for 24 h and hatched fry for 3 days significantly increased the hatch rate, the effectiveness of fungal control and caused appreciable survival of larvae to first feeding. Meyer and Jorgenson (1983) reported that a malachite green bath treatment of rainbow trout eggs delayed the time of sack-fry hatching by 5 to 8 days compared with controls. Furthermore, they observed that treated eggs exhibited an increased frequency of abnormalities (malformation of the head and jaws, spine deformation or missing fins). The present study observed neither delay in incubation and hatching period (though the African catfish have a considerably shorter hatching period of 24 h compared to the rainbow trout) nor deformity in hatched larvae; hence this compound may be considered better than malachite green. Straus (2008b) reported that treatments of channel catfish eggs with copper sulphate was stopped at the eyed stage because embryos within the same rearing unit may be hatching and the doses of the chemical is toxic to the fry. Hence, differences in the sensitivity of larvae of the different species to Cu toxicity may have led to higher survival of the African catfish even in the continuous exposure method.

The mean water quality parameters obtained in the present study indicated fouling in the dip treatment with an elevated level of ammonia differing significantly among the concentrations compared to the continuous treatment. The level of NH_3 observed in this study was at a range of 0-2 mg⁻L⁻¹ which is not detrimental to tropical fish (Stone and Thomforde 2004). Results obtained for water quality in the continuous treatment were similar to the findings of Akpoilih and Adebayo (2010) who reported a decrease in DO as the concentration of $CuSO_4$ increases. This decrease may be due to complete eradication of photosynthetic activity as a result of the copper sulphate therapeutic effect on non-target microscopic flora in the water.

Growth performance of fry showed that $2 \text{ mg}\text{L}^{-1}$ CuSO₄ is the best concentration for raising fry for 2 weeks after first feeding. Beyond this level mortality increased while growth decreased.

Conclusion

The present study provides base line information on the prophylactic use of $CuSO_4$ in hatchery production of *C. gariepinus*. The study has not only indicated the suitability of $CuSO_4$ for prophylactic use in aquaculture but suggested continuous exposure as a better treatment procedure for increased hatch rate, growth and survival of eggs and fry. However, efforts should be intensified to further study the best use of $CuSO_4$ on a long-term basis and considerable attention should be focused on possible long-term residual effects.

References

- Adeyemo, O.K., S.A. Alarape and B.O. Emikpe. 2011. Reprotoxic effect of malachite green on African catfish *Clarias gariepinus* (Burchell 1822). Journal of Fisheries and Aquatic Science. 6:563-570.
- AIFP. 2005. Aquaculture and Inland Fisheries Project (AIFP) Farming Nigeria's water. A compilation of Newsletters of the Aquaculture and Inland Fisheries project of the National Special Programme for Food Security in Nigeria. FAO Office, Abuja. Technical Note No. 16: June 2005 pp 1-2
- Akpoilih, B.U. and O.T. Adebayo. 2010. Effect of formalin on the hatching rate of eggs and survival of larvae of the African catish (*Clarias gariepinus*). Journal of Applied Science and Environmental Management JASEM . 14:31-34.
- American Public Health Association (APHA). 1998. Standard methods for the examination of water and waste water. 20th Edn., Washington DC. 1161 pp.
- Barnes, M.E. and M.P. Gaikowski. 2004. Use of hydrogen peroxide during incubation of landlocked fall chinnok salmon eggs in vertical-flow incubator. North American Journal of Aquaculture. 66:29-34.
- Citek J., Svobodova Z., Tesarcik J. 1998. Diseases of Freshwater and Aquarium Fish (in Czech). Informatorium, Prague. 218 pp
- Ella M.O. 1987. Simple calculations in fish farming. Wusen Press Ltd. Calabar, Nigeria. 64 pp.
- Goos, H.J.T. and C.J.J. Richter, 1996. Internal and external factors controlling reproduction in the African catfish, *Clarias gariepinus*. Aquatic Living Resources 9:46-58.
- Hawke, J. P. and L.H. Khoo. 2004. Infectious diseases. In: Biology and culture of channel catfish (eds. C.S. Tucker and J.A. Hargreaves), pp. 387-443. Elsevier, Amsterdam.
- Macharia, S.K., C.C. Ngugi and J. Rasowo. 2005. Comparative study of hatching rates of African catfish (*Clarias gariepinus, Burchell 1822*) eggs on different substrates. Naga World Fish Center Quarterly 28 (3&4):23-26.
- Masser, M.P. and J.W. Jensen 1991. Calculating treatments for ponds and tanks. Southern Regional Aquaculture Center, Pub. No. 410, Stoneville, Mississippi. USA. 7 pp
- Martin R. 1968. The effect of malachite green as a fungicide. The Ohio Journal of Science 68:118-121
- Mbaru, E.K., T.K. Mkare and J.O. Rasowo. 2011. Tolerance of yolk sac and free swimming fry of African catfish (*Clarias gariepinus*, Burchell 1822) to chemotherapeutic doses of formalin. African Journal of Agricultural Research 6:323-330.

- Meyer F.P. and F.A. Jorgenson. 1983. Tetratological and other effects of malachite green on development of rainbow trout and rabbits. Transactions of the American Fisheries Society 112:818-824.
- Mitchell, A.J., D.L. Straus, B. Farmer and R. Carter. 2010. Comparison of percent hatch and fungal infestation in channel catfish eggs after copper sulfate, diquat bromide, formalin, and hydrogen peroxide treatment. North American Journal of Aquaculture 72:201-206.
- Nigerian Tribune. 2013. 'Nigeria's local fish output low'. Monday March 11, 2013. <u>http://tribune.com.ng/news2013/index.php/en/component/k2/item/7053-nigeria-s-local-fish-output-low</u> Accessed August 18, 2013
- Nguyen, L.T.H. and C.R. Janssen. 2002. Embryo-larval toxicity tests with the African catfish (*Clarias gariepinus*): Comparative sensitivity of endpoints. Archives of Environmental Contamination Toxicology. 42: 256-262.
- Noga, E.J. 1996. Fish disease diagnosis and treatment. Mosby, St. Louis, Missouri. 367 pp.
- NSPFS. 2006. Natural special programme for food security (NSPFS). Manual on catfish hatchery and production: a guide for small to medium scale hatchery and farm producers in Nigeria. Kamathorn Potangkam and Jim miller (FAO TechnicalAdviser) Aquaculture and Inland Fisheries project. 78 pp.
- Schlenk, D., J. L. Gollon, and B. R. Griffin. 1998. Efficacy of copper sulfate for the treatment of ichthyophthiriasis in channel catfish. Journal of Aquatic Animal Health 10:390-396.
- Steeby, J. and J. Avery. 2005. Channel catfish broodfish and hatchery management. Southern Regional Aquaculture Center, Publication 1803, Stoneville, Mississippi. 8pp.
- Stone, N.M. and Thomforde H.K.. 2004. Understanding your fish pond water analysis report. FSA9090-PD-7-04N. University of Arkansas Cooperative Extension Service, Little Rock, Arkansas. 4pp
- Straus, D.L., A.J. Mitchell, R.R. Carter and J.A. Steeby. 2009a. Optimizing copper sulfate treatments for fungus control on channel catfish eggs. Journal of Aquatic Animal Health. 21:91-97.
- Straus D.L., A.J. Mitchell, A.A. Radomski, R.R. Carter and J.A. Steeby. 2009b. Laboratory dose confirmation of copper sulfate for treating fungus on channel catfish eggs. North American Journal of Aquaculture 71:333-338,
- Straus, D.L. 2008a. Comparison of copper sulfate concentrations to control ichthyophthiriasis in fingerling channel catfish. Journal of Applied Aquaculture 20:272-284.
- Straus, D. L. 2008b. Copper sulfate toxicity to channel catfish fry: yolk sac fry versus swim-up fry. North American Journal of Aquaculture 70:323–327.
- Straus, D. L., and C. S. Tucker. 1993. Acute toxicity of copper sulfate and chelated copper to channel catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society 24:390-395.
- Tucker, C. S. and E.H. Robinson, 1990. Channel catfish farming handbook. Van Nostrand Reinhold, New York. 468pp.

USDA. 2003. Part I: Reference of fingerling catfish health and production practices in the United States, 2003 USDA:APHIS:VS, CEAH, National Animal Health Monitoring System, Fort Collins, CO #N406.1103. Available on the world wide web at http://www.extension.org/mediawiki/files/7/72/UDSA_Catfish_2004_Part_I_Reference_of_fingerling_Catfis h_Hea.pdf Accessed June 28, 2013

Wolters, W. R. 2001. Improving catfish spawning success. National Warmwater Aquaculture Center News 4(1):2.

Received: 10/07/2013; Accepted: 19/08/2013 (MS13-44)