Short communication

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Hatching rate of striped murrel *Channa striatus* (Bloch 1797) eggs treated with formalin, hydrogen peroxide and copper sulphate pentahydrate

JAMES MILTON[1], MOHAMMED ABDULKATHER HANIFFA[2], PAULASIR JEYA SHEELA[3], KUMARESAN KAVITHA[4] and JAFAR SATHIK[5]

Huazhong Agricultural University, Wuhan, Hubei, 430 070, PR China

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Striped murrel, *Channa striatus* is a native freshwater fish of tropical Africa and Asia. Bacterial and fungal diseases are major threat to murrel eggs. In particular, Aquatic fungus (Saprolegniales) ubiquitous in natural water, often cause serious diseases in fishes and their eggs. Bacterial infections are common to hatcheries with temperature higher than 28ºC whereas fungal infections are prevalent below 26ºC. At this juncture, management techniques viz., temperature manipulation, jar-hatching of eggs with adhesive matrix removed, sanitation and aeration are helpful in minimizing fish mortality but not cent percent cure is assumed. Mechanically removing dead and infected eggs will be time consuming and chemical treatments can be proved effective. The beneficial effects of various disinfectants were evaluated by aquaculturists of different countries using different fish species viz., hydrogen peroxide by Rach *et al.* (1998) in warm and cold water fishes, hydrogen peroxide by Mitchell *et al.* (2009) in channel catfish, formalin by Akpoilih and Adebayo (2010) in African catfish and copper Sulfate by Straus *et al.* (2011a, b, c) in channel catfish. As there is a continued need for therapeutants to control fungal infestations, aquatic fungicides viz. formalin, hydrogen peroxide (H_2O_2) and copper sulphate ($CuSO_4$. $5H_2O$) were investigated in the present study.

Ten pairs of gravid *C. striatus* (700 – 800 g mean weight) were selected and acclimatized without food in four fibre tanks (1m × 1m × 1 m) with a density of 3 fish per tank at CARE Aquafarm. Brooders were injected with natural hormone, human chorionic gonadotrophin (HCG) at 4,000 IU/kg and allowed to spawn. Fertilized egg masses were collected and sorted out as 150 healthy eggs per tank filled with 500 L of water at 28°- 30°C, for treatments with formalin, hydrogen peroxide and copper sulphate each with triplicate for 15 min exposure time (Table 1). Hatching and survival rates were calculated as follows:

% hatching rate = Number of hatched eggs / Total number of eggs × 100.

% survival = Number of larvae alive / total number of hatched larvae × 100.

Following egg disinfection, 0.2 ml of eggs was subjected to fungal and bacterial counting on plates containing nutrient agar and czapadox agar respectively. Percentage of egg hatching, larval survival to first feeding, larval size at hatching and number of microbial colonies were compared using ANOVA and Tukey's multiple comparison test. All percentages were appropriately transformed using arc sin-square root. The statistical software package STATISTICA was used.

H_2O_2 and Cu75 treatments resulted in the lowest egg hatching rates ($P<0.05$) compared with the other treatments; whereas F60 showed the highest hatching rate (82.1%) and survival rate (90.5%) ($P>0.05$). Fry started to hatch after 24h, and hatching was over by 48 h. Most of the F60 treated eggs hatched (> 82%) by day 3, but the other treatments resulted in <60% hatching. Fry survival in the control was only 25% whereas 90.5±3.5 % survival was noticed in 60 mg/L Formalin treatment and the difference in survival was significant between treatments ($P=0.005$).

Mitchell *et al.* (2009) reported that treatments of eggs with 2000 mg/l H_2O_2 and above caused complete egg

Table 1. Dose of the four chemicals used to disinfect eggs of *Channa striatus*

Treatment	Abbreviation	Chemical	Dose (mg L^{-1})
1	F20	Formalin	20
2	F40	Formalin	40
3	F60	Formalin	60
4	H20	Hydrogen peroxide	20
5	H40	Hydrogen peroxide	40
6	H60	Hydrogen peroxide	60
7	Cu50	Copper sulphate	50
8	Cu75	Copper sulphate	75
9	Cu100	Copper sulphate	100
10	CONT	Control	NA

Present address: [1](milton.sxc@gmail.com), College of Fishery, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education. [2]UGC Emeritus Scientist and Director (haniffacare@gmail.com), [3](sheelacare.11@gmail.com) [4](kavicare2014@gmail.com), [5](jafiazi@gmail.com), Centre for Aquaculture Research and Extension (CARE), St. Xavier's College, Palayamkottai.

mortality, while treatment with 1,000 mg/L H_2O_2 resulted in 11% survival. However, Rach *et al.* (1998) reported survival of 78%, 68% and 0% at 1,000, 3,000 and 6,000 µl/l H_2O_2, respectively.

The high concentration of $CuSO_4$ (Cu100) was toxic to eggs of *C. striatus*. In addition to the low cost of $CuSO_4.5H_2O$, it does not have the human health implications or storage precautions associated with hydrogen peroxide or formalin. Several reasons can be suggested for the difference in hatching rates viz. susceptibility of an individual spawn to saprolegniasis, the number of unfertilized eggs in an individual spawn and other pathogens infecting an individual spawn (e.g., bacteria, ciliates).

As tabulated (Table 2), no bacterial colonies were detected among treatments Cu75 and Cu100. For the different treatments regarding bacterial load, there was a significant difference (P<0.05) between the control and the treated groups. In previous effectiveness studies under similar conditions, control showed hatching rate of 2% (Straus *et al*. 2009a), 8% (Straus *et al.* 2009b) and 4% (Straus *et al.* 2011). Leonard *et al.* (2000) reported that recirculating systems released approximately10^4 cfu m/l into the rearing medium and the UV disinfection unit kept the number of bacteria stable. Ozonation has been frequently used for pre-treating water supply to fish farms where water-borne pathogens are significant problems (Summerfelt 2003).

Eggs treated with formalin showed the highest hatching rate and larval survival rate for each treatment. The F100 treatment has been used successfully in the culture of other freshwater fishes such as *Clarias gariepinus* (Akpoilih 2010).

The high dosage treatment of formalin produced a better hatching rate than other antifungal agents, although, the effective concentration that can be used depend on the time of application, type of fish and kind of parasite targeted (Herwig 1979). There were significant differences in the hatching and survival rates of early larvae between the control and all 4 formalin concentrations (P<0.05), but the application of 100 mg/l of $CuSO_4$. $5H_2O$ resulted in total egg mortality (0% hatching rate). Because of the highest survival and hatching rates, formalin (F60) is recommended for farm use.

Table 2. Microbial load (mean±SD) for *C. striatus* eggs exposed to different disinfection treatments and a control

Treatment	No. of colonies ($\log_{10}$ cfu/ml)
F20	2.65±0.07**
F40	1.83±0.10**
F60	0.59±0.08**
H20	3.24±0.07**
H40	2.46±0.02**
H60	1.08±0.01**
Cu50	0.56±0.10**
Cu75	0
Cu100	0
CONT	14.53±27**

** Significant level P<0.05.

SUMMARY

The effect of formalin, hydrogen peroxide and copper sulphate pentahydrate on the hatching rate of eggs of the striped murrel, *Channa striatus* and subsequent survival of the larvae were investigated using range finding and definitive tests. Four treatments were used to define the threshold limit of tolerance of eggs to formalin, hydrogen peroxide and copper sulphate pentahydrate. There were significant differences in the hatching rate and survival of early larvae between the control and all four formalin.

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REFERENCES

Akpoilih B U and Adebayo O T. 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* **14**(4): 31 – 34.

Herwig. 1979. *Handbook of Drugs and Chemicals Used in the Treatment of Fish Diseases. A manual of Fish Pharmacology and Materia Medica*. pp 272. Charles C. Thomas, Springfield, Ill.

Leonard N, Blancheton J P and Guiraud J P. 2000. Populations of heterotrophic bacteria in an experimental recirculating aquaculture system. *Aquaculture Engineering* **22** (1–2): 109–20.

Mitchell A J, Radomski A A, Straus D L and Carter R. 2009. The effect of hydrogen peroxide on the hatch rate and *Saprolegnia* spp. infestation of channel catfish eggs. *North American Journal of Aquaculture* **71**: 276–80.

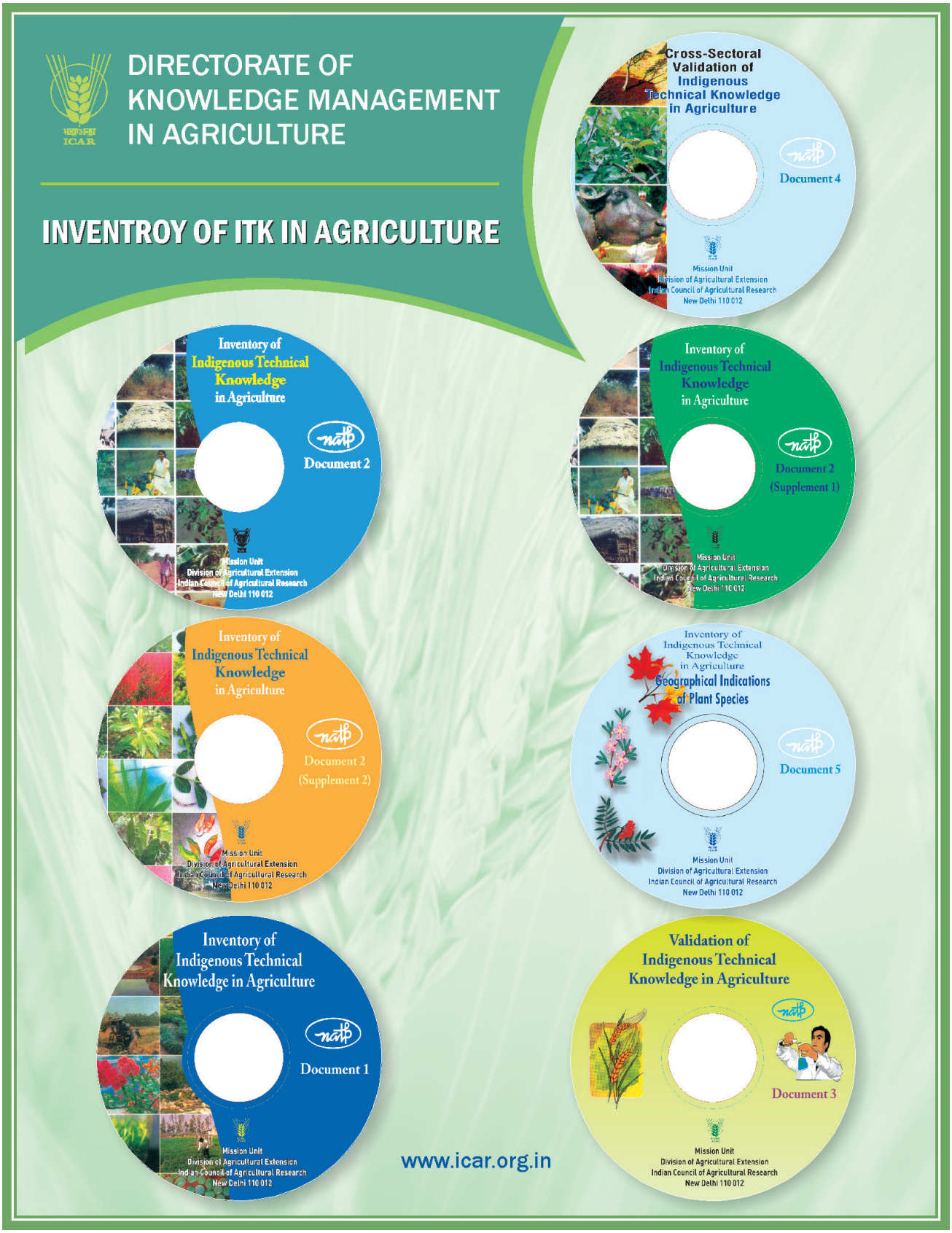
Rach J J, Gaikowski M P, Howe G E and Schreier T M. 1998. Evaluation of the toxicity and efficacy of hydrogen peroxide treatments on eggs of warm and cool water fishes. *Aquaculture* **165**: 11–25.

Straus D L, Mitchell A J, Carter R R and Steeby J A. 2011. Dose-confirmation of copper sulfate for treating fungus on channel catfish eggs at a commercial hatchery. *Journal of Applied Aquaculture* **23** (3): 199–206.

Straus D L, Mitchell A J, Carter R R and Steeby J A. 2009. Optimizing copper sulfate treatments for fungus control on channel catfish eggs. *Journal of Aquatic Animal Health* **21**: 91–97.

Straus D L, Mitchell A J, Carter R R, Radomski A A and Steeby J A. 2009. Laboratory dose confirmation of copper sulfate for treating fungus on channel catfish eggs. *North American Journal of Aquaculture* **71**: 333–38.

Summerfelt S T. 2003. Ozonation and UV irradiation an introduction and examples of current applications. *Aquaculture Engineering* **28**: 21–36.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