J. Asiat. Soc. Bangladesh, Sci. 40(1): 121-131, June 2014

EFFECTS OF TERTIARY AMYL ALCOHOL, 2-PHENOXYETHANOL, QUINALDINE AND BENZOCAINE ON THE MORTALITY AND RNA: DNA RATIO IN CATLA FINGERLINGS

MAHMUD HASAN[1], ARIFA RAHMAN, S. M. MAHBUBUR RASHID[2] AND SALIM AHMED[2]

Department of Fisheries, University of Dhaka, Dhaka-1000, Bangladesh

[2]*Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh*

Abstract

The efficacy of tertiary amyl alcohol (TAA), 2-Phenoxyethanol (2 PE), quinaldine and benzocaine was evaluated to determine their optimal dosages for rapid induction and recovery in the first experiment. The second experiment focused on the determination of effects of 2 PE, quinaldine and benzocaine with oxygen for 1, 3 and 6 h on the mortality, water quality and RNA:DNA ratio at 400 g L^{-1} in catla *Catla catla* fingerlings in a truck transport simulation. TAA at 1.5 mL L^{-1}, 2 PE 200 µL L^{-1}, quinaldine (4%) 200 µL L^{-1} and benzocaine 30 mg L^{-1} were found to be effective in inducing complete immobilization and recovery within 4 minutes. 2 PE and benzocaine treatments did not have any immediate mortality, while control and quinaldine treatment, respectively, resulted in 20 and 7% immediate mortality at 6 h. Similarly, 2 PE and benzocaine treatments did not result in any delayed mortality, however, control and quinaldine treatments, respectively, had nearly 7 and 2% delayed mortality observed 24 h after transport. Results of this study suggest that catla fingerlings can be transported at 400 g L^{-1} for 6 hours by using low dose 2 PE, benzocaine and quinaldine with no or little mortality.

Key words: Tertiary Amyl Alcohol, 2 Phenoxyethanol, Quinaldine, Benzocaine, DNA:RNA ratio, Catla fingerlings

Introduction

Catla is a high value cultured carp in Bangladesh. Indian major carps including catla contribute nearly 21% of the country's fish yield (DoF 2008). Currently nearly 80% of the total fingerling is being transported by truck using plastic drum (200 L) covering distances of some 50-500 km from the seed market or farm gate. Typically, the drums are filled with 130 L subsurface clean water and loaded with 25 kg catla fingerlings. The total quantity of seed weighs near 400 kg per trip in 16 drums by a standard truck (5 mt). During transport of the fingerlings in each drum aeration is given by hand splash by a day labourer. The drums are refilled with river or pond water to cover lost-water during transport due to jolting mobility. Mortality resulting from handling and transport amounts to be 5-25% (Hasan 2009). Sometimes the death tolls may be the entire lot. Hasan and

[1] Corresponding author: Email: mhasan@du.ac.bd

Bart (2006) have estimated that the annual loss (6200 mt) of carp fingerling resulting from immediate and delayed mortality of traditional transport method in Bangladesh which is equivalent to 13.62 million US$. Traditional transport without compressed oxygen or mechanical aeration and sedatives results in physical injury, scale loss, fin bifurcation and finally deaths of the fingerling. In addition, because of no oxygenation or aeration, the loading density is maintained low, which increases transport cost and low income for the seed traders. Therefore, an improvement of transport technique with the aid of oxygen and sedatives could have increased the loading density and reduce transport cost which would have sustained the development of aquaculture by providing healthy, strong and less stressed fingerlings and steady supply of fish seed to the end users in desired quantity on time.

Use of sedatives during transportation of fish fingerlings has not been fully explored. Several sedatives, including MS 222 (tricaine methanesulfonate), benzocaine, 2 phenoxyethanol, quinaldine, clove oil and metomidate have been evaluated for aquaculture practices (Marking and Meyer 1985, Gilderhus and Marking 1987 and Coyle *et al*. 2004). When used at appropriate doses, sedatives can effectively reduce metabolic rate, physiological stress, oxygen consumption, and carbon dioxide and ammonia production during transport (Wedemeyer 1997 and Ross and Ross 1999), thereby reducing mid-transport and post-transport mortality (Hoskonen and Pirhonen 2004 and Hasan and Bart 2007a).

Use of sedatives generally depends on their efficacy, availability, cost-effectiveness and safety. Quinaldine, one of the cheapest sedatives, was found to be more effective in alkaline water, efficient at low dose and less toxic with a shorter recovery period (Bell 1964, Sills and Allen 1973 and Lambert 1982). Several reports have been published on the efficacy of quinaldine. Quinaldine (25 mg L^{-1}) induces light sedation in tilapia, *Sarotherodon melanotheron*, (3-13 cm) fingerlings (Sado 1985); quick lethargy and recovery in sea bream, *Sparus sarba* fingerlings (3-3.5 g) compared to other sedatives (e.g., quinate, MS-222, benzocaine-98%, and 2-phenoxyethanol; Hseu *et al*. 1998) which was also supported by Munday and Wilson (1997). Durve (1975) achieved 97% survival of mullet *Mugil cephalus*, *Liza tade* fry/fingerlings by using quinaldine during 24 h transport while Lambert (1982) recommended a lower dose (1-4 mg L^{-1}) to minimize handling stress in yearling Atlantic mackerel, *Scomber scrombrus*. However, the clearance rate of quinaldine in fish is 24 h of withdrawal (Hunn and Allen 1974).

Benzocaine, the least expensive sedative, is chemically and functionally similar to MS-222 and quinaldine sulfate (Gilderhus and Marking 1987, Mattson and Riple 1989 and Gilderhus *et al*. 1991). but found more effective at lower concentrations than MS-222 when tested in chinook salmon, *Oncorhynchus tshawytscha;* in Atlantic salmon, *Salmo salar* during spawning (Gilderhus 1990); and in juvenile tambaqui, *Colossoma macropomum* (Gomes *et al*. 2001). Benzocaine was found to anesthetize and allow rapid

recovery in juvenile striped bass, *Morone saxatilis* at higher temperature (Gilderhus *et al*. 1991). In addition, sedation of benzocaine was observed in tilapia, *Tilapia marie* fingerlings (Ross and Geddes 1979); in rainbow trout, *Oncorhynchus mykiss* and in largemouth bass, *Micropterus salmoides* (Allen 1988). Basavarju *et al*. (1998) have demonstrated that benzocaine (40 mg L^{-1}) was effective in inducing sedation on catla fingerlings (<20 g), while 150 mg L^{-1} to be optimum for common carp, *Cyprinus carpio* fry and advanced fingerlings (<10 and 20-60 g).

2-Phenoxyethanol is an opaque, oily and aromatic liquid. It is relatively inexpensive and remains active in the diluted state for 3 days. It's efficacy varies with the size of the fish and with the temperature of the water. It is a moderately water-soluble clear or straw-colored liquid widely used for sedation and anesthesia (Hseu *et al*. 1998).

While use of mechanical aerator in a moving truck bed requires 220 V electric current if 12 V batteries are not used, use of compressed oxygen does not require any energy but provides the most valuable oxygen required for the survival of the fingerlings. However, uses of 2 PE, quinaldine and benzocaine with oxygen in the live transport of catla fingerlings have never been reported.

RNA:DNA ratios have been used as short term growth and condition factor in bluegill, *Lepomis macrochirus* (Bullow *et al*. 1978 and 1981), in Atlantic cod, *Gadus morhua* (Buckley 1979) and in channel catfish, *Ictalurus punctatus* (Peterson and Brown-Peterson 1992). Although DNA adducts and DNA strand breakage have been used in few previous studies as the indicators of environmental stressors (Thomas 1990), however, RNA:DNA ratio has never been used as an indicator of physical stress in catla fingerlings elsewhere in the world.

The objectives of this study were to determine the efficacy of tertiary amyl alcohol (TAA), 2 PE, quinaldine and benzocaine and their effects on the immediate and delayed mortality, water quality variables and RNA:DNA ratio in catla fingerlings.

Materials and Methods

Catla fingerlings (10.46 ± 2.51 cm; 12.82 ± 1.37 g; mean ± SE) used in this experiment were obtained from "Maa Fatema Fish Hatchery", Chachra, Jessore, Bangladesh. Before loading for transport simulation, fingerlings were held in a flow-through-tank system and feeding was stopped 12 h before loading into the plastic drum used as the experimental system.

Efficacy test: To determine the optimal dosages of sedatives with least or no mortality, efficacy test was undertaken before doing simulated transport experiment. The safe and optimal sedative dosage required for inducing sedation with least mortality of catla fingerlings were determined by a series of preliminary tests. Immobilization and recovery period including cost and availability of the anesthetics with least or no mortality were

considered to determine the optimal dosages. Several aluminum vessels of 10 liters capacity with 1 L well water were loaded with 15 catla fingerlings. Several dosages of 2 PE (100, 150, 200, and 250 μL L^{-1}), benzocaine (25, 30, 35, 40 and 45 mg L^{-1}); TAA (1.0, 1.5, 2.0, 2.5 and 3.0 mL L^{-1}) and quinaldine (4%) (150, 175, 200, and 225 μL L^{-1}) were applied to determine the optimal dosages. DO concentration and temperature of the water in the vessels were monitored at 1, 3 and 6 hour of exposure. Time required for immobilization was recorded. Behavioral responses of fingerlings were also observed and recorded during 1, 3, and 6 hour duration. Recovery periods of fingerlings were observed too. Finally, considering cost and availability, 2 PE, quinaldine and benzocaine were used in the transport simulation experiment.

Transport experiment: The experiment was simulated by using a truck. A 4 × 3 factorial design was used with three replicates. The experimental variables were four transport methods and three transport durations. The control did not receive any sedative. The indicator variables were immediate and delayed mortality, and RNA: DNA ratio. Water quality variables such as dissolved oxygen concentration and temperature were also measured. Fingerlings were placed into 12 200 L plastic drums. Each drum was filled with 100 L subsurface clean water and mixed with sedatives and given oxygen injection by two air stones. Before loading, for simulation transport purpose, each drum was stocked with 40 kg fingerlings at the rate of 400 g L^{-1} loading density.

TAA, 2 PE and benzocaine were obtained from Sigma-Aldrich, Germany while quinaldine 4% as TRANCE was collected from Argent Laboratories, Redmond, WA, USA. Solutions of all four sedatives were prepared by adding to the respective transport drum until the desired concentration was achieved.

Oxygen cylinder containing 9.8 m^3 oxygen at 2200 PSI with 99.9% purity was used as the source of pure oxygen. Oxygen was released at 8 PSI into transport drum water.

Dead fingerlings were removed and counted to determine the immediate mortality rate at 1, 3 and 6 h after transport. Samples were also drawn for RNA: DNA at the time of dead fingerling counting. Water quality variables were also measured at the time of fingerling sampling for RNA: DNA quantification. After collection, samples were held in frozen condition at -20^0 C. For determination of delayed mortality after simulation experiment, dead fingerlings were removed from stocking hapa and counted 24 hours after the experiment began.

Dissolved oxygen concentration and temperature of transport water were measured using HACH portable DO (HACH sension^{TM} 6, USA) after 1, 3 and 6 h of transport simulation.

To determine RNA:DNA ratio, total nucleic acid (DNA and RNA) was isolated by CTAB (Cetyltrymethylammoniumbromide, a non-ionic detergent) method, first developed by Murry and Thompson (1980). The original protocol was modified by not using RNase at the last step of DNA isolation so that RNA remains intact with genomic

DNA. Moreover, the overall laboratory setup and experimental conditions were ensured as DNase and RNase free. Since a single experiment was undertaken for quantification of both DNA and RNA from a single weighed sample, there was almost zero chance of any biasness in quantification as well as the ratio of DNA and RNA.

Quantity of DNA and RNA was measured by Flurometry method by using a Qubit® 2.0 Flurometer (Invitrogen, USA). Qubit® 2.0 flurometer is sensitive than UV mediated technologies, accurate and reproducible mentioned in many articles such as Bakos *et al*. (2009) and Cavalieri *et al*. (2008).

All percent data were transformed into square root before statistical analysis. Treatments were compared by anova followed by Tukey's HSD post hoc for multiple comparisons. Data were analyzed by using spss software version 10.0 with the level of significance at $p<0.05$.

Results and Discussion

Efficacy: Of five doses, TAA 1.5 mL L^{-1} was found to induce complete immobilization (Table 1). Four doses of 2 PE were applied in which optimum result was obtained from 200 µL L^{-1}. Similarly, when benzocaine was applied in five doses, 30 mg L^{-1} was found to be the best. Quinaldine 200 µL L^{-1} was found optimum among the tested four doses.

When efficacies of sedatives were tested, TAA and 2 PE showed the best performance considering the recovery period for catla fingerlings (Table 1). Benzocaine was the cheapest sedative followed by 2 PE and quinaldine but TAA was very expensive.

Low dose TAA (1.5 mL L^{-1}), 2 PE (200 µL L^{-1}), benzocaine (30 mg L^{-1}) and quinaldine (200 µL L^{-1}) were found effective in catla fingerlings over six hour exposure to impart sedation, above which significant loss of equilibrium and mortality has been observed. Light sedation has been found to be sufficient and desirable over deeper sedation to facilitate handling and transport of fingerlings (Wedemeyer 1997 and Golovanova *et al*. 2006). Hasan and Bart (2007a) have demonstrated that low dose quinaldine (rohu: 250 µL L^{-1}; silver carp: 100 µL L^{-1}) and benzocaine (rohu: 30 mg L^{-1}; silver carp: 15 mg L^{-1}) are effective in improving survival (100%) of rohu and silver carp fingerlings during transportation. Durve (1975) also confirmed that of all different stages of behavioral responses of fish to different levels of anesthesia, only light sedation is suitable for live fish transport.

Table 1. Optimal dose, time of induction, recovery period and cost (Bangladeshi Taka = BDT; 1 US$ = 82 BDT) of sedatives per kg catla fingerlings transport.

Sedatives	Dose	Time required to be immobilized (min)	Recovery time (min)	Cost BDT kg^{-1}
TAA	1.5 mLL^{-1}	2.0-3.0	2.0 to 3.0	97.50
2-Phenoxyethanol	200 µLL^{-1}	1.5-2.0	2.0 to 3.0	7.60
Benzocaine	30 mg L^{-1}	3.0-4.0	3.5 to 4.0	1.95
Quinaldine (4%)	200 µLL^{-1}	3.0-3.5	3.0 to 4.0	6.00

Effects of Transport Methods on the Immediate and Delayed Mortality Rate, Dissolved Oxygen Concentration, Temperature and RNA:DNA ratio: In the control group which received only oxygen injection, only 6 h sample resulted in nearly 20% immediate mortality while other two durations did not have any mortality. In the 2 PE and benzocaine treatment groups, transport durations had no mortality. While no immediate mortality was found up to 3 hour, nearly 8% mortality was observed at 6 h duration in the quinaldine treatment group (Table 2).

Similar to immediate mortality, benzocaine and 2 PE treatment groups did not have any delayed mortality. In the control group, only 6 h treatment resulted in nearly 7% delayed mortality but the other two treatment durations did not have any mortality. As with the control group, the quinaldine treatment group had no delayed mortality upto 3 h but resulted in nearly 2% mortality after 6 h (Table 2).

Table 2. Immediate and delayed mortality rates (%) of catla *Catla catla* fingerlings sampled from four transport methods. Control did not receive any sedative.

Duration (h)	Immediate mortality rates (%) observed in four transport methods sampled at 1, 3 and 6 h in catla fingerlings			
	Control (O_2 only)	2 PE	Quinaldine	Benzocaine
1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6	19.95 ± 1.15	0.00 ± 0.00	7.20 ± 0.63	0.00 ± 0.00
Cumulative	19.95 ± 1.15[a]	0.00 ± 0.00	7.20 ± 0.63[b]	0.00 ± 0.00
	Delayed mortality			
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
6	7.41±0.35	0.00±0.00	1.99±0.18	0.00±0.00
Cumulative	7.41±0.35	0.00±0.00	1.99±0.18	0.00±0.00

Within row means (± SEM) with different superscript letters indicate significant differences ($p<0.05$).

High level of DO concentrations (above 12 mg L^{-1}) was found in the quinaldine and in the 2 PE treatment groups throughout the entire experimental duration. In the control group DO detected at 1 h was above 6 mg L^{-1} but less than 2 in the other two duration treatments. Benzocaine had nearly 8-times higher DO measured at 1 h while very low level (<2 mg L^{-1}) was observed at 3 and 6 h transport durations (Table 3).

The cumulative immediate mortality was nearly 20 % and the delayed mortality was low (7%) in the control group in this study, whereas much higher levels of immediate (38 and 83-92%) and delayed mortality (>90%) have been observed in carps, largemouth bass, *Micropterus salmoides*, freshwater drum, *Aplodinotus grunniens* and striped bass (Lewis *et al*. 1996). Hasan and Bart (2007b) have found low level of immediate mortality (4-12%) and high level of delayed mortality (27-49%) in a transport simulation experiment

of rohu fingerlings given hand splash for aeration. The low levels of mortality as observed in this study may possibly be explained by the quality of the water remaining within the tolerance limit and less immediate effects of stress at a life threatening level during transport. The observed no immediate and delayed mortality in the benzocaine and 2 PE treatment groups could be due to the effectiveness of sedatives (Hasan and Bart 2007a).

Table 3. Dissolved oxygen concentration and temperature of the transport water sampled at 1, 3 and 6 h after transport from four transport methods.

Duration (h)	Dissolved oxygen concentration (mg L^{-1}) measured from four transport methods at 1, 3 and 6 h in catla fingerlings			
	Control (O_2 only)	2 PE	Quinaldine	Benzocaine
Pre-loading	27.60±0.00[a]	27.60±0.00 [a]	27.60±0.00 [a]	27.60±0.00 [a]
1	6.13±0.30[b]	16.89±0.28[b]	15.02±0.19[b]	12.11±0.07[b]
3	1.38±0.08[c]	16.40±0.22[b]	14.28±0.19[b]	1. 55±0.22[b]
6	1.21±0.17[c]	12.69±0.35[c]	12.36±0.25[c]	1.53±0.12[c]
Temperature				
1	27.67±0.12	27.67±0.03	27.70±0.06	27.67±0.03
3	28.73±0.03	28.10±0.06	28.10±0.06	28.18±0.03
6	29.03±0.03	28.70±0.06	29.10±0.10	28.71±0.10

Within column means (± SEM) with different letters of DO denote significant differences ($p<0.05$).

No deaths of the fingerlings up to 3 h duration that has been observed in the control group could be associated with good environmental condition indicated by DO concentration. However, the observed 20% immediate mortality and 7% delayed mortality after 6 h of transport could have resulted from reduced DO concentration and subsequent hyper activity of the fingerlings in confined space. Physical injury due to social competition of the fingerlings for space may also be responsible for this mortality. Hasan and Bart (2007a) have demonstrated that the highest mortality (about 29%) of rohu fingerlings has resulted when no sedative was used but given mechanical aeration only. Low levels of DO concentration (<2 mg L^{-1}) detected at 3 and 6 h of transport durations can be explained by the increased oxygen consumption due to hyperactivity of the fingerlings for longer duration without sedative.

In the quinaldine treatment group, the observed no mortality up to 1 and 3 h transport duration could be associated with the effects of sedative. But low level of immediate mortality (7%) as observed at 6 h denotes loss of effectiveness. Little or no mortality was observed in rohu and silver carp fingerlings when they were treated with quinaldine and benzocaine and given mechanical aeration (Hasan and Bart 2007a). Loss of effectiveness of quinaldine at 6 h could be related to the observed 2% delayed mortality. Sedative effectiveness of 2 PE and benzocaine might have helped in maintaining better environmental condition which in turn assisted in less consumption of oxygen by

reducing metabolic activity and hyperactivity, and thus eliminating undue injuries (Mcfarland 1959).

In all four transport methods water temperature was found to be almost similar (Table 3).

Ratios of RNA:DNA in the fingerlings sampled before loading (resting fish) was significantly lower as compared to the control and treatment groups which were given sedatives. Values of RNA:DNA ratio in the 2 PE group was significantly higher than in the other treatment groups, while level of RNA:DNA ratio sampled from quinaldine and benzocaine treatment groups was similar to that of the control group. In durations, significantly lower levels of RNA:DNA ratio was measured at 6 h and non transported resting fingerlings than did 1 and 3 h treatment groups. However, the levels of RNA:DNA ratio detected at 1 h and 3 h were similar (Table 4).

Table 4. RNA:DNA ratio detected in the fingerlings sampled from four transport methods at 1, 3 and 6 h durations.

Duration (h)	RNA:DNA ratio observed in four transport methods sampled at 1,3 and 6 h after simulation				Overall
	Control (O_2 only)	2 PE	Quinaldine	Benzocaine	
Resting fish	0.44±0.04^{b}	0.44±0.04^{b}	0.44±0.04	0.44±0.04	0.44±0.04^{b}
1	0.56±0.19^{b}	1.80±0.37^{a}	0.67±0.09	0.84±0.14	0.97±0.20^{a}
3	0.92±0.07^{a}	1.99±0.30^{a}	0.81±0.07	0.38±0.09	1.02±0.23^{a}
6	0.94±0.06^{a}	0.46±0.10^{b}	0.76±0.16	0.56±0.13	0.68±0.08^{ab}
Overall	0.80±0.09$_{b}$	1.42±0.33$_{a}$	0.75±0.06$_{b}$	0.59±0.10$_{b}$	0.86±0.10

Within row for overall and within column means (± SEM) with different letters indicate significant differences ($p<0.05$).

In the control, RNA:DNA values observed in the fingerlings sampled at 3 and 6 h was significantly higher than that of 1 h transport duration and resting fingerlings. In 2 PE treatment group, levels of RNA:DNA ratio measured in the fingerlings sampled at 1 and 3 h after simulation transport were significantly higher than that of 6 h and of resting fish. However, the level of RNA:DNA ratio sampled at 6 h was similar to that of resting fish. In quinaldine and benzocaine treatment groups, levels of RNA:DNA ratio was not different between three treatment durations and non transported resting fish (Table 4).

It is possible that the lower RNA:DNA ratio in the resting fingerlings as compared to the control and the treatment groups was probably be due to the increase expression of stress related genes. The stress may even start from initial hauling and weighing. The observed higher RNA:DNA ratios at 1 and 3 h duration treatment in the 2 PE group than that of 6 h and of resting fingerlings indicate maximum effectiveness of sedation which resulted in an increase expression of genes associated with normal body function and metabolism. Organisms in good condition has been demonstrated to have higher RNA:DNA ratios in

the than those in poor condition (Robinson and Ware 1988 and Clemmesen 1994). However, similar RNA:DNA ratio observed at 6 h to that of resting fish might be due to the partial loss of sedation over duration. The observed no differences in RNA: DNA ratio between duration treatments in quinaldine and benzocaine treatment groups and non transported resting fingerlings denotes similar stress level.

Catla fingerlings at 400 g L^{-1} can be transported with low dose 2 PE (200 μLL^{-1}) and benzocaine (30 mg L^{-1}) with compressed oxygen for a period of 6 hours without any mortality. Benzocaine lost its effectiveness 3 h after inception making it more appropriate for shorter haul. 2 PE and quinaldine maintain high DO concentration throughout the entire study period by reducing the oxygen consumption. 2 PE also helps in maintaining good physiological condition indicated by higher RNA: DNA ratio.

Acknowledgement

We thank Ministry of Education, Govt. of the People's Republic of Bangladesh for their financial support in conducting this study. The authors are also thankful to Dr. Ruhul Kuddus for his critical suggestions.

References

Allen, J.L. 1988. Residues of benzocaine in rainbow trout, largemouth bass, and fish meal. *The Prog. Fish-Cult.*, **50**: 59-60.

Bakos, J., N. Hlavacova, M. N. Rajman, K. M. Ondicova, C. K. Koros, E. C. Kitraki, E. H.W. Steinbusch and D. Jezova. 2009. Enriched environment influences hormonal status and hippocampal brain derived neurotrophic factor in a sex dependent manner. *Neuroscience*. **164**(2): 788-797.

Basavarju, Y., G.C. Mair, G. Mukthayakka, B.S.R Devi and D.J. Penmann. 1998. The use of benzocaine as an anesthetics and sedative for carps. *Proc. Nat. Acad. Sci.*, *India. Section B, Biol. Sci*., **68**(3/4): 231-240.

Bell, G.R. 1964. A guide to properties, characteristics and uses of some general anesthetics for fish. *Bull. Fish. Res. Board Can.*, **148**: 11.

Buckley, L.J. 1979. Relationships between RNA-DNA ratio, pray density, and growth rate in Atlantic cod *Gadus morhua* larvae. *J. Fish. Res. Board Can.* **22**: 405-409.

Bullow, F.J., C.B. Jr. Coburn and C.S. Cobb. 1978. Comparisons of two bluegill populations by means of the RNA-DNA ratios and liver-somatic index. *Trans. Am. Fish. Soc.,* **107**: 799-803.

Bullow, F.J., M.E. Zeman, J.R. Winningham and W.F. Hudson. 1981. Seasonal variations in RNA-DNA ratios and in indicators of feeding, reproduction. Energy storage, and condition in a population of bluegill, *Lepomis macrochirus* Rafinesque. *J. Fish Biol.,* **8**: 237-244.

Cavalieri, V., Di Bernardo, M. L.Anello and G. Spinelli. 2008. cis-Regulatory sequences driving the expression of the Hbox12 homeobox-containing gene in the presumptive aboral ectoderm territory of the *Paracentrotus lividus* sea urchin embryo. *Dev. Biol.*, **321**(2): 455-469.

Clemmesen, C**.** 1994. The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. *Mar. Biol.,* **118**: 377-382.

Coyle, S.D., R.M. Durborow and J.H. Tidwell. 2004. Anesthetics in aquaculture. Southern Regional Aquaculture Center. Publication No. 3900. 6 pp.

DoF (Department of Fisheries). 2008. *Fisheries Statistical Yearbook of Bangladesh*. Fisheries Resources Survey System. Ministry of Fisheries and Livestock, Govt. of the People's Republic of Bangladesh. 44 pp.

Durve ,V.S. 1975. Anesthetics in the transport of mullet seed. *Aquaculture.* **5:** 53-63.

Gilderhus, P.A. 1990. Benzocaine as a fish anesthetic: Efficacy and safety for spawning-phase salmon. *The Prog. Fish-Cult.* **52:** 189-191.

Gilderhus, P.A. and L.L. Marking. 1987. Comparative efficacy of 16 anesthetic chemicals on rainbow trout. *North Am. J. Fish. Man*. **7:** 288-297.

Gilderhus, P.A., C.A. Lemm, C.A. and L.C. Woods III. 1991. Benzocaine as an anesthetic for striped bass. *The Prog. Fish-Cult.* **53**: 105-107.

Golovanova, T.S., S.I. Nikonoov and M.A. Moise. 2006. Evaluation of potential anesthetics for the Caspian inconnu (*Stenodus leucichthys* G1772) juveniles. Meeting Abstract, World Aquaculture Society, Aqua. Florence, Italy. pp.1139

Gomes, L.C., A.R Chippari-Gomes, N.P. Lopes, R. Roubach and C.A.R.M. Araujo-Lima. 2001. Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. *J. World Aquacult. Soc.* **32**(4): 426-431.

Hasan, M. and A.N. Bart. 2007b. Effects of capture, loading density and transport stress on the mortality, physiological responses, bacterial density and growth of rohu *Labeo rohita* fingerling. *Fish Physiol. Biochem.* **33:** 241-248.

Hasan, M. 2009. Carp seed transport in Bangladesh. *Asia-Pacific J. Rural Dev.* **19**(1): 155-168.

Hasan, M. and A.N. Bart. 2006. Carp seed traders in Bangladesh: sources of livelihoods and vulnerability resulting from fish seed mortality. *Asia-Pacific J. Rural Dev.* **16**(2): 101-124.

Hasan, M. and A.N. Bart. 2007a. Improved survival of rohu, *Labeo rohita* and silver carp, *Hypophthalmichthys molitrix* fingerlings using low dose quinaldine and benzocaine during transport. *Aquacult. Res.* **38**: 50-58.

Hoskonen, P. and J. Pirhonen. 2004. Temperature effects on anaesthesia with clove oil in six temperate-zone fishes. *J. Fish Biol.* **64**: 1136-1142.

Hseu, J.R., S.L. Yeh, Y.T. Chu and Y.Y. Ting. 1998. Comparison of efficacy of five anesthetics in goldlined Sea Bream, *Sparus sarba*. *Acta Zool. Taiwanica*, **9**(1): 35-40.

Hunn, J.B. and J.L. Allen. 1974. Urinary excretion of quinaldine by channel catfish. *The Prog. Fish-Cult.* **36**(3): 157-159.

Lambert, T.C. 1982. Techniques for the capture and handling of Atlantic mackerel with special reference to the use of quinaldine. *The Prog. Fish-Cult.* **44** (3): 145-147.

Lewis, D.J., G.D. Wood and R. Gregory. 1996. *Trading the silver seed*. University press limited. Dhaka-1000, Bangladesh. 199 pp.

Marking, L.L. and F.P. Meyer. 1985. Are better anesthetics needed in fisheries? *Fisheries,* **10**(6): 2-5.

Mattson, N.S. and T.H. Riple. 1989. Metomidate, a better anesthetic for Cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol, and phenoxyethanol. *Aquaculture.* **83**: 89-94.

McFarland, W.N. 1959. A study of the effects of anesthetics on the behavior and physiology of fishes. *Pub. Ins. Mar. Sci.* **6**: 23-55.

Munday, P.L. and S.K. Wilson. 1997. Comparative efficacy of clove oil and other chemicals in anesthetization of *Pomacentrus amboinensis*, a coral reef fish. *J. Fish Biol.* **51**(5): 931-938.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight DNA. *Nucleic Acids Res.* **8**: 4321-4325.

Peterson, M.S. and N. Brown-Peterson. 1992. Growth under stressed conditions in juvenile channel catfish *Ictalurus punctatus* Rafinesque as measured by nucleic acids. *Comp. Biochem. Physiol.* **103**A: 323-327.

Robinson, S.M. and D. Ware. 1988. Ontogenetic development of growth rates in larval Pacific herring. Clupea harengus pallasi, measured with RNA:DNA ratios in the Strait of Georgia. British Columbia. *Can .J. Fish. Aquat. Sci.* **45**: 1422. 1429.

Ross, L.G. and B. Ross. 1999. *Anesthetic and sedative techniques for aquatic animals*. 2nd ed. Blackwell, London, UK. 159 pp.

Ross, L.G. and J.A. Geddes. 1979. Sedation of warm-water fish species in aquaculture research. *Aquaculture.* **16**: 183-186.

Sado, K.E. 1985. Influence of the anesthetic quinaldine on some tilapias. *Aquaculture.* **56**: 55-62.

Sills, J.B. and J.L. Allen. 1973. The influence of pH on the efficacy and residues of quinaldine. *Trans. Am. Fish. Soc.,* **100**: 544-545.

Thomas, P. 1990. Molecular and biochemical responses of fish to stressors and their potential use in environmental monitoring. *Am.Fish.Soc. Symp.,* **8**: 9-28.

Wedemeyer, G.A. 1997. Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In: *Fish stress and health in Aquaculture*. Iwama G.K., A.D. Pickering, J.P. Sumpter and C.B. Schreck (ed.). *Society for experimental biology seminar series*, **62**: 35-71.

(Received revised manuscript on 23 January 2014)