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Effect of Anaesthetics, Temperature and Aeration in Live Transportation of Tilapia (*Oreochromis mossambicus*) (Peters, 1852)

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Abstract

Fish processing and preservation is mainly concerned with improving the quality and value of seafood and one of the most reliable methods for maintaining the quality of fish is to keep them alive till consumption. This guarantees freshness and more consumer demand unlike the other processed commodities. However the stress associated with the live fish handling and transportation is a major challenge and minimizing this can successfully improve the survival rate and associated biochemical quality changes of the fish. Anaesthetics are known for reducing stress and related problems during live fish transportation. Hence, a study was carried out to understand the efficacy of anaesthetics *viz.,* MS 222, AQUI-S and clove essential oil on the survival of common tilapia (*Oreochromis mossambicus*). Results indicated that these sedatives had differential effect on live tilapia, with distinctly diverse induction and recovery time as well rate of survival. Among the three anaesthetics used, highest dosage was required for MS 222, moderate levels for AQUI-S and lowest for essential oil for the same extent of sedation in live tilapia. However more intense studies are required to understand the dosage levels for long term transportation. Similarly toxicological studies on the use of essential oil are to be intensely explored to standardize their permissible concentrations in food fishes. Further, trials on the individual and combined effect of low temperature and aeration conditions on the survival rate of live tilapia proved, temperature and aeration to play a significant role in influencing the survival of tilapia.

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Introduction

Seafood has emerged as an integral constituent in the human diet due to its richness in nutrients but at the same time its chemical composition paves to be an ideal substrate for microbial activity affecting its shelf stability. In this context proper hygienic handling and preservation is very important for making aquatic products available to the target population in the desired quality (Getu et al., 2015). One of the major difficulties in this regard is the unpredictability in catch resulting in lack of timely preservation possibilities especially in case of capture fisheries. The major problems in fish marketing *viz.,* high perishability and heterogeneity of samples, storage and transportation cost, lack of guarantee in quality and quantity of commodity has resulted in an increased demand for the development of effective supply chain protocols. Different processing techniques are employed for enhanced stability of fish and of these the most guaranteed option is keeping the fish live till marketing. Live fish, indicative of supreme quality, attracts more customers and hence more market value realization in comparison to the processed counterparts. One of the greatest challenges in handling with live fish transportation and storage is the stress associated with the fishes and minimizing it can successfully improve the survival rate and associated biochemical quality changes (Barton, 1997). Careful handling practices together with thorough knowledge on the tolerancy conditions of fishes are mandatory for an effective transportation and storage protocol. Stress can be due to several external or internal conditions. Carmichael et al. (2001) have reported that though fishes are carefully handled and transported, a group of mild stressors might act together resulting in fatal conditions.

During live transportation, anaesthetics are widely employed prior to and during transport to slow the metabolism of the fish, thus reducing the rate of oxygen uptake, release of carbon dioxide, and ammonia production. Reports by Wedemeyer (1996) suggest that anaesthetics assist in reducing the stress response caused by increased activity and handling. It also makes the handling procedures easier and reduces the mortality of fish during handling and transportation. Important factors to be considered for the selection of anaesthetics include its efficacy, cost, availability, ease of use, safety aspects and finally the nature of the experiment and fish species employed (Mylonas et al., 2005). A number of safe and food grade anaesthetics are used for this purpose, which cause temporary loss of sensation. The proper dosage is critical and in general, it vary widely with the type of anaesthetics, species employed as well as size and condition of the fish. Moyle & Cech (1988) have reported worthwhile to note variations *viz.,* osmoregulatory and water-quality sensitivity among marine and freshwater species during the designing of live transportation protocol. Reports by Harmon (2009) also suggested the relevance of maintaining proper water quality during transport as it reduces physiological stress and indicated these parameters might vary within a species depending on life stage, health and previous holding conditions. Hence as sensitivity, resistance and endurance towards the sedative vary in these regards, prior optimization studies need to be done, as even the closely related species may differ very much in this respect. Studies on the application of anaesthetics on response and survival of live fishes during transportation is limited and hence the present study focused on optimization of the dosage levels of three different anaesthetics on live tilapia (*Oreochromis mossambicus*) for their enhanced survival during transportation. Further the individual and combined effect of temperature and aeration conditions on the survival rate of live tilapia was also studied.

Materials and Methods

Uniformly sized live tilapia (*Oreochromis mossambicus*) having an average weight of 250 g were procured from Fisheries Research Station, Puthuvype and were transported in oxygenated plastic bags to the laboratory and further carefully transferred to well aerated concrete tanks. The health status of fish is a major decisive factor influencing its survival during live transporation and hence prior to the study, weak, injured and diseased fishes were removed. Further they were starved for 48 h to ensure their digestive tract clearance to prevent regurgitation and associated water quality degradation. For each trials, a sampling size of five (n = 5) was considered.

Anaesthetics *viz.,* MS 222, AQUI-S and clove essential oils were selected for the experiment. These anaesthetics are considered effective for use on food fish with short induction time and rapid recovery time. Sedation pattern is divided into four stages and to generalize, light sedation is characterized by slow movement without losing equilibrium and with response to external stimuli whereas deep sedation is characterized by gill movement with no body movement, loss of equilibrium and no response to external stimuli (Woods III et al., 2008). Tilapia were tested at different concentrations of anaesthetics *viz.,* MS-222 (20, 30, 40, 100, 150 ppm), AQUI-S (30, 45, 60 ppm), and clove essential oils (5, 10, 20, 30, 40 ppm). All anaesthetization studies were done in plastic tanks of 10 l capacity. The anesthetics were completely dissolved in the pre-contained water and the tanks were aerated with a fine-bubble air diffuser throughout the experiment. Further the experimental fish was quickly transferred from the holding tank into the anaesthetic bath. The time required for anaesthetising, anesthesia stage, period of recovery as well as survival rate of the experimental fishes were noted.

Further to understand the influence of temperature and aeration conditions on the survival of tilapia, control and fishes anaesthetized under optimized clove oil concentration were subjected to ambient (28^{o}C) and low temperature (20^{o}C) conditions as well as with and without aeration conditions. Fishes were randomly selected for the all the experimental trials over the course of 1-2 days.

Results and Discussion

The efficacy of an anesthetic is related to the dosage required as well as the period taken to completely anaesthetize the subject (Small & Chatakondi, 2005). The present study indicated entirely different pattern of response for the fish to different anaesthetics *viz.,* MS-222, AQUI-S and clove essential oil. MS-222 has limited application, as USFDA demands fishes treated with MS-222 to be held for a minimum of 21 days before human consumption. The dosage used for MS-222 depend on size and species of the fish, the reason for sedation, with exposure times ranging from a few minutes for high concentrations to upto 48 h for low concentrations (Topic Popovic et al., 2012). In the present investigation, a series of trials were conducted with different concentrations of this anaesthetic at ambient temperature and studies indicated a higher MS-222 concentration to bring about deep sedation in common tilapia. A concentration of 20-40 ppm resulted in minimized movement in tilapia (Stage 1). Fish anaesthetized at a concentration of 100 ppm resulted in sedation on exposure for 10±3.2 min but the subjected ones were observed to recover in the anaesthetized solution on average exposure for about 35±6 min. Further trials with higher concentrations indicated a dosage of 150 ppm to be optimum (Table 1). A recovery period in solutions containing various concentrations of MS-222 was observed as 4.0±0.7 min (15 ppm) and 6.0±1.6 min (50 ppm). However on further exposure to a solution concentration of 100 ppm, indicated no recovery with symptoms like very slight fin movement and 125 ppm showed complete sedation, hence suggesting possibilities of initial anaesthetization at higher dose of 150 ppm and further transportation at lower doses of 100 ppm. Previous reports suggest an induction time ranging from 40-150 ppm in MS-222 for sedation of tilapia and an average exposure time of 3 min (Coyle et al., 2004; Barreto et al., 2007).

AQUI-S, with isoeugenol (2-methoxy-4-propenylphenol) as the active ingredient together with Polysorbate 80 as an inert surfactant (Woods III et al., 2008), is relatively a new anaesthetic and has approval for use with food fish in Australia, Chile and New Zealand with no withholding period. In the present study, the effect of this anaesthetic on common tilapia under ambient conditions was assessed (Table 1). A concentration of 30 ppm only minimized the movement (Stage 1) and was not sufficient to bring about deep sedation in the species. Previous studies by Small & Chatakondi (2005) on AQUI-S application in channel catfish indicated a loss of equilibrium at 8.0, 3.9, and 3.7 min when anesthetized in 20, 40 and 60 ppm AQUI-S, respectively. Similarly reports on market sized striped bass indicated an induction time of 8.8, 6.4 and 6 min, respectively when AQUI-S concentrations in the range of 25, 35 and 45, respectively were applied at 20^{o}C (Woods III et al., 2008). However for the present study, tilapia required a higher sedation time which must be because of the higher tolerance of the species as well as the lower temperature adopted in the reported studies which signified the anaesthetic effect.

Clove oil, a natural anaesthetic is considered superior to a number of anesthetics and is comparatively less expensive to other anesthetics used in fish. In addition, USFDA has classified it as GRAS compound although presently it is not approved for use in treating fish (Mylonas et al., 2005). Further USFDA in its National Toxicological Programme has established the constituents of clove oil to have carginogenic effect. Hence, adequate toxicological studies are mandatory to understand the permissible concentrations of the same in food fishes. Present study indicated that the species response to this sedative followed an inverse pattern with increasing doses resulting in a decreased sedation time. A dose of 30 ppm and higher was found to be beyond the tolerance limit of the species. Average exposure time for deep sedation of tilapia at ambient temperature with different clove oil concentrations (Table 1) indicated a dose of 5 ppm as optimum with an average sedation period of 6 ± 0.9 min. Further

Table 1. Anaesthetic concentration and sedation time requirement of *Oreochromis* mossambicus

Anaesthetic	Concentration (ppm)	Average time for deep sedation (mins)	Recovery time (mins)
MS 222	150	4.5±0.5	3.0±1.2
AQUI-S	60	4.0±2.9	5.2±0.9
	45	6.7±0.5	2.0±0.4
Clove oil	20	2.0±1.2	8.0±1.2
	10	4.0±1.6	2.0±0.7
	5	6.0±0.9	1.0±0.6

Values are expressed as Mean±SD; n = 5

after exposure to this dosage for sedation, a lower concentration of 2.5 ppm was found optimum for live transportation of this species upto 24 - 52 h. Immediate recovery was observed after 24 h of transportation at 2.5 ppm whereas the average recovery period was 30 ± 8 min after 52 h. Studies suggest sedation of fish in higher dose of anaesthetics in a holding facility for a particular time period before loading and further exposure to a lower concentration of sedative during transport (Wedemeyer, 1997).

Being poikilotherms, the surrounding water is decisive to the physiological reactions in fishes. The changes in their body temperature have a direct effect on the rates of biochemical reactions. Hence cooling the surrounding water slows the fish metabolism which reduces ammonia production and toxicity, oxygen consumption and increases oxygen solubility. Studies suggest lowering the hauling water temperature by 5-10°C assist in reducing the oxygen consumption and ammonia production by 50% in most of warm water fishes (Wedemeyer, 1997). Hence further to study the effect of low temperature on fishes during transportation, the experiment was repeated with optimised concentration of clove oil at a temperature of 20°C for varying stocking densities (1:2 (w/v) and 1:4 (w/v) of fish and water, respectively) under non-aerated and aerated conditions. For comparison purpose, ambient temperature (28°C) was kept as control. The survival rate of tilapia stocked at low density (1:4 (w/v)) (Fig. 1) with different conditions is depicted in Table 2.

Observations on the effect of low temperature (20°C) on the survival of tilapia for a study period of 24 h remarked absolute survival under both anaesthetised and unanaesthetised conditions on coupling with aeration. However lack of aeration resulted in fish mortality at both ambient and low temperature conditions. Hence the results indicated that anaesthetisation had a negligible effect on survival rate at lower stocking densities. However temperature and aeration seemed to play a significant role. Further it was also observed that the survival rate was lower in anaesthetized fish in comparison to control which indicate that after exposure to 5 ppm for anaesthetizing, a lower holding/transportation dose is to be adopted, as previously mentioned. Further experiments under higher stocking density without anaesthetisation substantiated aeration to be the major limiting factor affecting the survival of tilapia (Table 3). Studies on the variation pattern of DO levels in the holding tank containing tilapia under different stocking

Fig. 1. HDPE boxes with live common tilapia at low stocking density (1:4 (w/v) fish: water) and low temperature (20°C)

Table 2. Survival rate of common tilapia stocked at low density under different conditions

Anaesthetised	Aerated	Room Temp	20°C	Survival
No	No	Yes	-	3 h
No	No	-	Yes	7 h
No	Yes	Yes	-	24 h
No	Yes	-	Yes	24 h
Yes	No	Yes	-	2 h
Yes	No	-	Yes	5 h
Yes	Yes	Yes	-	24 h
Yes	Yes	-	Yes	24 h

*24 hours indicate 100% survival

Table 3. Survival rate of common tilapia stocked at high density under different conditions

Aerated	Room Temp	20°C	Survival (24 h)
No	Yes	-	3 h
No	-	Yes	7 h
Yes	Yes	-	24 h
Yes	-	Yes	24 h

density as well as temperature were assessed. The DO values indicated decreasing trend during 24 h of stocking. However, these parameters were within the critical limit which must be on account of continuous aeration (Fig. 2). However the reduction in DO was directly related to stocking density as well as temperature with reduced rate of reduction for low stocking density *viz.,* 1:4 ((w/v) fish:water) as well as low temperature (20°C) in comparison to their counterparts *viz.,* stocking densities (1:3; 1:2 (w/v)) and ambient temperature (28°C), respectively. Previous studies also suggest dissolved oxygen (DO) to be the single most limiting factor in any fish-holding systems and hence maintenance of proper DO is critical during live fish transportation. Harmon (2009) have reported the solubility of oxygen to be inversely related to the water temperature.

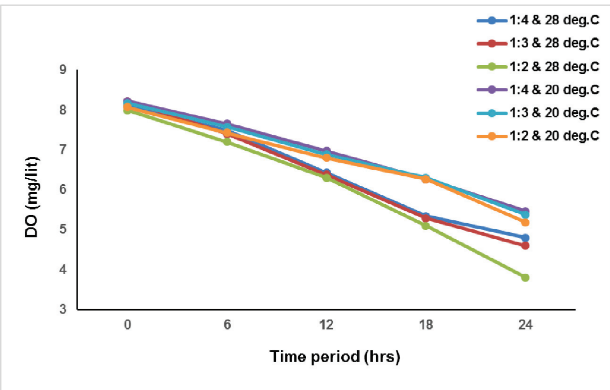

Fig. 2. Variations in Dissolved Oxygen content in holding water tank stocked with common tilapia in different densities and temperature under aeration

The present study indicated that sedatives *viz.,* MS-222, AQUI-S and essential oil had differential effect on live common tilapia, with distinctly diverse induction and recovery time as well as rate of survival. Among the three anaesthetics used, highest dosage was required for MS-222, moderate levels for AQUI-S and lowest for essential oil for the same extent of sedation in live tilapia. However more intense studies are required to understand the dosage levels for long term transportation of this species. Similarly during the study variations in the responses were observed in the samples which must be related to the individual diverseness on account of factors *viz.,* stress conditions, metabolic rates, size etc. Likewise the tolerance levels towards different anaesthetics as well as temperature may vary significantly between fish species. Further adequate toxicological studies are essential to realise the allowable concentrations of clove essential oil for application in food fishes. The direct implication of the observed results indicated that anaesthetisation had negligible effects on survival rate, rather temperature and aeration had a more significant role. Furthermore, fish health also has a critical role in survivability during and after live transport. Hence the procedures prior to, during as well as after transportation play an important role in addressing the success of live-fish transports. Hence further studies need to be carried out in this angle for effective transportation protocols.

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