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Investigation on the Response of Ornamental Fish *Etroplus maculatus* to Anaesthetization for Live Fish Transport

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ABSTRACT: In the worldwide fish trade, fish are stressed during capture, handling, packaging and shipping. This state of disturbance of homeostasis arouses the stress response. Accordingly, variations occur in various blood constituents. In the present study, Handling and packing stress was evaluated for *Etroplus maculatus*. Anaesthetic efficiency of 2-Phenoxyethanol, Clove oil and Lemon grass oil were analysed.

It was observed that all stress indicators evaluated indicated an alteration in all experiments during the 48-hour study period. The plasma cortisol value increased during the first six hours of transportation but it decreased after unpacking after 48 hours of transportation. The plasma glucose also followed the trend of cortisol. In the case of Hemoglobin and Erythrocytes the values remained high even after 48 hours after unpacking. However, stress was identified to be adaptive in the fish as the cortisol and glucose returned to basal values after 48 hours of the experiment.

Keywords: Live Food fish, Ornamental fish, Anaesthetization, Natural oil, Fish transport.

INTRODUCTION

Currently there is great demand for live food fish and ornamental fish. Treatment and handling of fish for fish transport is an important aspect of fish trade. The process of capture and quarantine, loading density, transit time, metabolic waste built-up upsets the homeostasis in fish creating stress (Lim *et al.,* 2003). Stress induces weakness, disease, reduces reproductivity and even causes mortality.

Anaesthetics are medications that cause a reversible loss of consciousness. Anesthetics are commonly used in the aquaculture industry to reduce stress, prevent mortality of fish during transportation and handling and during the invasive or surgical procedures (Martins *et al*., 2019; Adel *et al*., 2016). Use of anaesthetics during fish transportation minimises injury and reduces mortality as the metabolic activity decreases in the anaesthetised state (Wurts, 1995; Durve *et al*., 1966).

An ideal anesthetic should induce anesthesia rapidly with minimum hyperactivity or stress and when animal is removed from the anaesthetic, recovery should be rapid. While, first generation anesthetics like chloroform, have been ineffective, new chemicals have taken its place like MS-222, benzocaine, quinaldine, Phenoxyethanol, metomidate, clove oil (Coyle *et al*., 2004).

Studies on anaesthetic activity are crucial for identifying the effectiveness of the anaesthetic.

In the present study the effect of 2-Phenoxyethanol, Clove oil and Lemongrass in reducing stress in transport of Ornamental fish *Etroplus maculatus* which is also used as a food fish was investigated. Here a study on the efficiency of anaesthetics on *Etroplus maculatus* through analysis of temporal variation of the blood parameters were conducted.

MATERIALS AND METHODS

The fish were acclimatized in large tanks after capture for conducting the transportation experiment. They were fed with commercial pelleted food. After Prophylactic treatment they were starved for 24 hours before all the packing experiments. The length of the Plastic bags used were 40cm and width 20cm, the size of the polythene bag was so selected on the basis that, one-fourth of the bag could be filled with water and fish and three fourths with oxygen. The blood was collected from the caudal region by puncturing of the caudal vein.

A. Estimation of Cortisol

12 polythene bags were filled with one litre water. Into each bag 12 fishes of 10±1g weight were introduced. The bags were grouped into 4 groups as A, B, C, D with each group with three bags. Into three groups (A, B, C) the three anaesthetics 2- Phenoxyethanol, Clove oil, Lemon grass with the respective effective concentration of 60mg/l, 12mg/l and 8mg/l was introduced aerated, sealed and packed in cartons. The three bags of the fourth group (D) were prepared without anaesthetic and twelve fish were introduced aerated, sealed and packed. All the bags, that is the

three experimental groups with anaesthetic (A, B, C) and control (D) were maintained at 26 ±1°C.

After six hours of starting the experiment one bag from each of the four groups was opened. Blood was taken from the fish from the caudal region and cortisol content was estimated by *Chemiluminescence immunoassay* method. After 24 hours blood was collected from the second set of four groups and the procedure was repeated. After 48 hours of experimentation the fish in the last set of the four groups were released to unanaesthetised water. Again after 48 hours after the experiment, blood was taken from fish and cortisol contents were estimated. The experiment was done in triplicate.

B. Estimation of Glucose, haemoglobin and RBC

24 polythene bags were taken and they were filled with one litre water. These bags were grouped into 4 groups A, B, C and D with 6 bags each. To one group of 6 bags 60mg/l, 2- Phenoxy ethanol was added, to the second group of 6 bags 12mg/l clove oil was added, to the third group 8mg/l lemongrass oil was added, the fourth group was filled with unanaesthetised water.

12 fishes of 10±1g were introduced to each bag. The bags were aerated, sealed and kept in 26 ±1°C temperature. One bag each from the four groups were opened after 6 hours of starting experiment. Analyses of secondary stress was done by collecting Blood from the fishes by caudal puncture.

After 12 hours of starting the experiment the blood was collected again and analysed. The same procedure was repeated at 24 hours, 36 hours and 48 hours. The fish in the last set of four bags was released to unanaesthetised water after 48 hours of packing experiment and blood was collected and analysed after 48 hours after the experiment. The experiments were conducted in triplicate. Estimation of Glucose was done by colorimetric method. Haemoglobin was estimated by Cyanomethemoglobin method and Total RBC was estimated with Neubaur chamber.

RESULT AND DISCUSSION

Table 1 represents the variation in plasma Cortisol of *Etroplus maculatus* in the experiment. It was found that Six hours after packing the cortisol content was increased from 7.17 to 38.8, 29.20, 16.61, 17.7 in Control, Clove oil, 2-Phenoxy ethanol and Lemon grass oil treated fish respectively. It was found that it decreased at 24 hours but 48 hours after transportation the values were found to be stabilised. On conducting the Two way Analysis of variance for the cortisol, the interaction between period and between treatments was found to be significant at one percent level (Table 5).

Therefore, a comparison of treatment was conducted separately for each period with the help of Duncan multiple range test (DMRT). The results indicated that the value of cortisol was highest in control in all the periods.

Table 2 provides data on the Variation of plasma Glucose during packing and transportation of *Etroplus maculatus*. The basal value was 32 it was found that it increased to 206.3, 142, 139.6 and 116.6 after 48 hours of experiment for Control, Clove oil, 2-Phenoxy ethanol and Lemon grass oil respectively. However, the values decreased 48 hours after the unpacking.

Table 6 indicates that the Results of Two-way ANOVA for glucose between period and between treatments was significant at one percent level. Results of Duncan test indicate that in all periods the glucose was highest for the control. At 6th and 12th hour there was no difference between the different anaesthetic treatments. However, after the 12th hour glucose value for the four treatments varied considerably.

Table 1: Variations in plasma Cortisol during packing and transportation of *Etroplus maculatus*

Treatment	Cortisol (µg/dl)			
	0 Hours after packing	6 Hours after packing	24 Hours after packing	48 Hours after unpacking
Control	7.17	38.87^{a}	21.87^{a}	11.27^{a}
Clove oil		29.20^{b}	15.57^{c}	10.13^{b}
2-Phenoxy ethanol		16.61^{d}	17.85^{b}	10.13^{b}
Lemon grass oil		17.7^{c}	18.20^{b}	10.77^{a}

Means having same letter as superscript are homogeneous

Table 2: Variations in plasma Glucose during packing and transportation of *Etroplus maculatus.*

Treatment	Glucose (mg)						
	0 Hours after packing	6 Hours after packing	12 Hours after packing	24 Hours after packing	36 Hours after packing	48 Hours after packing	48 Hours after unpacking
Control	32	74.67^{a}	87.00^{a}	97.00^{a}	155.67^{a}	206.33^{a}	155.67^{a}
Clove oil		67.67^{b}	69.67^{b}	89.67^{b}	99.67^{b}	142.00^{b}	138.00^{b}
2-Phenoxy ethanol		67.33^{b}	71.33^{b}	76.00^{d}	83.33^{c}	139.6^{b}	135.67^{b}
Lemon grass oil		66.33^{b}	73.00^{b}	84.00^{c}	84.00^{c}	116.6^{c}	107.00^{c}

Means having same letter as superscript are homogeneous

Table 3 indicates the variation in haemoglobin during packing experiment. The basal value was 6.2, it increased to 10.4, 8.6, 10.3 and 10.2 for Control, Clove oil, 2-Phenoxy ethanol and Lemon grass oil treatments at 48 hours after the packing.

On conducting the Two way Analysis of variance for the Haemoglobin it showed that the result for between period and between treatments was at one percent level according to Table 7. It was found that only the clove oil treatments showed considerable difference from the control values at the 48th hour and 48 hours post treatment values. It was found that the values of erythrocytes (Table 4) also varied during the experiment. The basal value was 1.6, but it increased to 2.6 at 48 hours for control and it reached a value of 2.2 for 2-Phenoxy ethanol.

Results of Two-way ANOVA for Erythrocytes between period and between treatments show that it was significant at one percent level according to Table 8.

Table 3: Variations in Haemoglobin during packing and transportation of *Etroplus maculatus.*

Treatment	Haemoglobin (g%)						
	0 Hours	6 Hours after packing	12 Hours after packing	24 Hours after packing	36 Hours after packing	48 Hours after packing	48 Hours after unpacking
Control	6.2	8.60^{a}	8.77^{a}	8.93^{a}	9.37^{c}	10.47^{a}	10.47^{a}
Clove oil		8.12^{b}	7.80^{b}	8.10^{b}	8.23^{d}	8.6^{b}	9.33^{b}
2-Phenoxy ethanol		7.37^{c}	7.37^{c}	7.37^{c}	10.27^{a}	10.3^{a}	10.33^{a}
Lemon grass oil		8.23^{b}	6.20^{d}	6.20^{d}	10.27^{a}	10.2^{a}	10.57^{a}

Means having same letter as superscript are homogeneous

Table 4: Variations in Erythrocytes during packing and transportation of *Etroplus maculatus.*

Treatment	Erythrocytes (mill/cumm)						
	0 Hours after packing	6 Hours after packing	12 Hours after packing	24 Hours after packing	36 Hours after packing	48 Hours after packing	48 Hours after unpacking
Control	1.6	1.43^{a}	1.43^{a}	2.27^{a}	2.63^{a}	2.6^{a}	2.73^{a}
Clove oil		1.57^{a}	1.33^{a}	1.30^{c}	1.53^{c}	1.67^{c}	1.67^{c}
2-Phenoxy ethanol		1.33^{b}	1.39^{a}	1.77^{b}	1.73^{b}	2.20^{b}	2.17^{b}
Lemon grass oil		1.37^{b}	1.37^{a}	1.27^{c}	1.73^{b}	1.73^{c}	1.57^{c}

Means having same letter as superscript are homogeneous

Table 5: Comparison of Cortisol between period and between treatments.

Source	df	Sum of squares	Mean square	F-value	P-value
Between period	2	1355.44	677.72	1879.297**	< 0.001
Between treatment	3	465.42	155.14	430.199**	< 0.001
Period x treatment interaction	6	593.59	98.93	274.334**	< 0.001
Error	24	8.66			
Total	35	2423.11			

** significant at 0.01 level

Table 6: Comparison of Glucose between period and between treatments.

Source	df	Sum of squares	Mean square	F-value	P-value
Between period	5	65804.44	13160.89	797.63**	< 0.001
Between treatment	3	17351.61	5783.87	350.538**	< 0.001
Period x treatment interaction	15	11572.56	771.50	46.758**	< 0.001
Error	48	792.00			
Total	71	95520.61			

** significant at 0.01 level

Table 7: Comparison of Haemoglobin between period and between treatments.

Source	df	Sum of squares	Mean square	F-value	P-value
Between period	5	74.73	14.95	532.255**	< 0.001
Between treatment	3	10.28	3.43	121.999**	< 0.001
Period x treatment interaction	15	25.39	1.69	60.284**	< 0.001
Error	48	1.35			
Total	71	111.75			

** significant at 0.01 level

Table 8: Comparison of Erythrocytes between period and between treatments.

Source	df	Sum of squares	Mean square	F-value	P-value
Between period	5	5.86	1.17	131.828**	< 0.001
Between treatment	3	5.38	1.79	201.766**	< 0.001
Period × treatment interaction	15	3.32	0.22	24.878**	< 0.001
Error	48	0.43			
Total	71	14.98			

** significant at 0.01 level

The control experiment setups had the highest value. The values for clove oil and lemon grass oil treatments was found to vary considerably from control values at 48th hours. Erythrocytes value was considerably low for clove oil and lemon grass oil treatments in the 48th hour and post treatment study.

DISCUSSION

In the ornamental fish industry, fish collection, handling, and shipping are inevitable. Fish handling and transportation-related trauma and stress have an impact on overall fish quality and survival (Crosby *et al.*, 2011; Sandodden *et al.*, 2001). Stress boosts the fish's metabolic rate, blood flow, and ion exchange. The elevation of adrenaline is the first sign of the stress reaction, followed by a rise in blood cortisol. Perry *et al*., (1999) proposed that the sympathetic pre-ganglionic neuronal cholinergic route, as well as non-cholinergic pathways with neuronal and humoral origins, are the mechanisms that cause catecholamine release.

It was found that the basal value of cortisol at capture was 7.17ng ml/l. This is according to Grutter *et al.* (2000) who observed that at capture 3 ng ml/1 was the basal cortisol levels in *H. melapterus*. Vijayan *et al.* (1994) reported that in sea ravens during stress, plasma cortisol levels increased considerably after one hour and persisted at elevated levels for four hours, whereas, plasma glucose levels increased after half an hour and persisted at elevated levels for up to twenty-four hours.

In the current investigation, it was discovered that cortisol levels increased steadily beginning six hours into the trial. Yet, compared to the control, the anaesthetic treatments showed a lower value. On intense handling similar increase in plasma cortisol was observed in Rainbow trout (Barton *et al*., 1982). Pirhonen *et al*. (2003) also observed that plasma cortisol increased after 48 hours after treatment with MS 222 and clove oil.

Depending on the stressors that the fish are exposed to, different species and anaesthetics take different amounts of time for recovery from stress. According to the cortisol results of the post-transport investigation, the fish in the current study appeared to have recovered following the trial.

Despite the fact that hypercortisolemia is a sign of stress, it cannot be used as the only diagnostic of stress (Barton, 2000) since various fish species generate cortisol in varying amounts.

During stressful situations, glucose levels also rise; however, sometimes there is no change, and occasionally even a fall is seen. Blood glucose levels vary by species, food type, life stage, sickness and length of time since the previous meal. That is yet another crucial sign of stress. Mc Donald *et al*. (1997) state that adrenaline increases 1000 times, cortisol by 200 times and glucose by three fold with recovery by 48 hours but Acerete *et al*. (2004) observed only a 3-fold increase in cortisol while glucose increased by 1.5 fold during the first week after transport.

Following a rise in cortisol, glucose levels rose in the current research as well. Hyperglycaemic cortisol is known to prepare the body for the fight, flight, or freeze response. The glucose level rose from 32 to 206.3 in the control group, but it rose less in all of the treatments.

Stress has a negative impact on blood components as it causes changes in haematology (Mohammadizarejabad *et al*., 2010; Hattingh *et al*., 1974; Pramod *et al*., 2010). According to the current study also handling and packaging have a range of effects on haematology. Erythrocytes and haemoglobin were seen to rise as the duration of the transportation experiment lengthened in the current investigation.

Caruso *et al.* (2005) reported that variations in haematological and biochemical values are useful indicators in assessing the condition of chronic stress. However, Hur *et al.* (2007) reported that recovery was slow taking more than 24 hours as for normal physical metabolism to resume after stress it would take longer time than a 24-hour period. Therefore, repeating fish

transport within a 24-h period is dangerous and would cause increase in mortality

Similar findings were identified in the current investigation, which showed that recovery was a very slow process. Fish that were collected were stressed during handling, packing and with accumulation of metabolic products in the transport medium.

Carneiro *et al.* (2002) reported that stress response is transient but according to Barton (2002) the stress response is an adaptive mechanism to deal with stresses and preserve homeostasis

The current study also shows that after the experiment, the values of stress indicators returned to normal levels, confirming that the stress response is a healthy adaptive mechanism to maintain equilibrium for the benefit of the organism. It also shows that the three anaesthetics are significantly effective at reducing stress.

However, Iversen *et al.* (2003) reinforced the importance of organic anaesthetics as they are safe for environment and therefore clove oil and lemon grass oil are ideal with their added advantage of low cost, safety, availability. Natural plant products like clove oil and Aloe vera extract reduced primary and tertiary stress symptoms in fish (Iversen *et al*., 2003; Ross *et al.*, 2007).

This work emphasis the importance of green anaesthesia as clove oil and lemon grass oil could effectively be used as alternatives to MS222, benzocaine and quinaldine which are expensive and hazardous.

2-phenoxyethanol and clove oil are commonly used by the local traders. Usually 2-phenoxyethanol is directly added to the transporting water. However, the observations in the present study reveals that mixing of 2-phenoxyethanol with equal quantity of ethanol before adding to water increases the solubility and is most effective. Therefore, the three anaesthetic agents were effective with respect to *Etroplus maculatus,* an ornamental and food fish with immense trade potential.

CONCLUSION AND FUTURE SCOPE

Biochemical changes are ideal indicators of stress that can correlate to status and future condition of fish. In the present study it was observed that 2-phenoxyethanol and clove oil were effective in producing anaesthesia in *Etroplus maculatus.* The present work discovers the anaesthetic property of lemongrass oil as an effective anaesthetic with the property of induction and recovery. However, such studies on different fish species are essential. Further studies on gender, reproductive state and life history stages are to be done. Therefore, according to the present investigation the three anaesthetic agents were effective and exhibited a good margin of safety with respect to *Etroplus maculatus,* an ornamental and food fish with immense trade potential.

Conflict of Interest: None.

REFERENCES

Acerete, L., Balasch, J. C., Espinosa, E., Josa, A. and Tort, L. (2004). Physiological responses in Eurasian perch (*Perca fluviatilis*, L.) subjected to stress by transport and handling. *Aquaculture, 237*(1), 167-178.

Barton, B. A. (2000). Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. *North American J. of Aquaculture*, *62*(1), 12-18.

Barton, B. A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and comparative biology, 42*(3), 517-525.

Barton, B. A. and Peter, R. E. (1982). Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anaesthesia and cold shock. *J. of Fish Biology*, *20*(1): 39-51.

Caruso, G., Genovese, L., Maricchiolo, G. and Modica, A. (2005). Haematological, biochemical and immunological parameters as stress indicators in *Dicentrarchus labrax* and *Sparus aurata* farmed in off-shore cages. *Aquaculture International, 13*(1-2), 67-73.

Coyle, S. D., Durborow, R. M. and Tidwell, J. H. (2004). *Anesthetics in aquaculture* (Vol. 3900). Texas: Southern Regional Aquaculture Center.

Crosby, T. C., Hill, J. E., Martinez, C. V., Watson, C. A., Pouder, D. B. and Yanong, R. P. (2011). On-farm transport of Ornamental fish. *Fisheries and aquatic sciences department, Florida Cooperative Extension Service,* Institute of Food and Agricultural Sciences, University of Florida. *Fact Sheet FA-119.*

Durve, V. S. and Dharmaraja, S. K. (1966). Effects of anaesthetics on the behaviour of mullet fingerlings and the scope of using these in different fishery procedures. *J. of Marine Biological Association of India, 8*(1 and 2), 28-56.

Grutter, A. S. and Pankhurst, N. W. (2000). The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus*. *J. of Fish Biology, 57*(2), 391-401.

Hattingh, J. and Van Pletzen, A. J. J. (1974). The influence of capture and transportation on some blood parameters of fresh water fish. *Comparative Biochemistry and Physiology Part A: Physiology, 49*(3), 607-609.

Iversen, M., Finstad, B., McKinley, R. S. and Eliassen, R. A. (2003). The efficacy of metomidate, clove oil, Aqui-S™ and Benzoak® as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts and their potential stress-reducing capacity. *Aquaculture, 221*(1), 549-566

Lim, L. C., Dhert, P. and Sorgeloos, P. (2003). Recent developments and improvements in ornamental fish packaging systems for air transport. *Aquaculture Research*, *34*(11), 923-935.

McDonald, G. and Milligan, L. (1997). Ionic, osmotic and acid-base regulation in stress. *Fish stress and health in aquaculture*, *62*, 119-145.

Mohammadizarejabad, A., Bastami, K. D., Sudagar, M. and Motlagh, S. P. (2010). Haematology of great sturgeon (Huso huso Linnaeus, 1758) juvenile exposed to clove powder as an anaesthetic. *Comparative Clinical Pathology*, *19*(5), 465-468.

Perry, S. F. and Bernier, N. J. (1999). The acute humoral adrenergic stress response in fish: facts and fiction. *Aquaculture*, *177*(1), 285-295.

Pirhonen, J. and Schreck, C. B. (2003). Effects of anaesthesia with MS-222, clove oil and CO2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*) *Aquaculture, 220*(1), 507-514.

Pramod, P. K., Ramachandran, A., Sajeevan, T. P., Thampy, S. and Pai, S. S. (2010). Comparative efficacy of MS-222 and benzocaine as anaesthetics under simulated transport conditions of a tropical ornamental fish

Puntius filamentosus (Valenciennes). *Aquaculture research, 41*(2), 309-314.

Ross, L. G., Sanchez Blanco, J., Martínez-Palacios, C., Racotta, I. S. and Toledo Cuevas, M. (2007). Anaesthesia, sedation and transportation of juvenile *Menidia estor* (Jordan) using benzocaine and hypothermia. *Aquaculture Research, 38*(9), 909-917.

Sandodden, R., Finstad, B. and Iversen, M. (2001). Transport stress in Atlantic salmon (*Salmo salar* L.): anaesthesia and recovery. *Aquaculture Research, 32*(2), 87-90.

Vijayan, M. M. and Moon, T. W. (1994). The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. *Canadian J. of Zoology, 72*(3), 379-386.

Wurts, W. A. (1995). Using salt to reduce handling stress in channel catfish. *World Aquaculture, 26*(3), 80-81.

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