

# Effects of Clove Seed as Anaesthetic Agents in Two Species of Grey Mullet *Liza falcipinnis* and *Liza grandisquamis*

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**Abstract** The effects of Clove Seed extracts as anaesthetic agents in two species of grey mullets *Liza Falcipinnis* and *Liza grandisquamis* were assessed. A total of 120 of these species comprising 60 *L. Falcipinnis* with 30 each of fingerlings (mean length 9.86cm  $\pm$  1.11 SD; mean weight 21.20g  $\pm$  2.10 SD) and juveniles (mean length 17.24cm  $\pm$  1.88 SD; mean weight 66.24g  $\pm$  4.24SD) and 60 *L. grandisquamis* also consists of 30 each of fingerlings (mean length 10.24cm  $\pm$  1.92 SD; mean weight 23.41  $\pm$  2.21 SD) and juveniles (mean length 18.21cm  $\pm$ SD; mean weight 71.21g  $\pm$  4.12 SD) were sampled from the recruitment ponds at African Regional Aquaculture Center, (ARAC), Buguma, Rivers State and were exposed to clove seed extracts at different concentrations of 0.0mg/L (control), 5.0, 10.0, 15.0, 20.0 and 25.0mg/L. The results obtained indicated a size related response, to Clove seed extracts. The induction time in both species were found to decreased significantly ( $P < 0.05$ ) as the concentrations of the clove extracts increased with the shortest induction time 55.61(s) observed in fingerlings of *L. grandisquamis*, while the longest 210.61 (s) was recorded in juveniles of *L. falcipinnis*. However, the recovery time increased significantly with increasing concentration of the clove seed, with the highest recovery time of 350.11 (s) observed in *L. falcipinnis*, at 25.0mg/L concentration and the lowest 246.91(s) in *L. grandisquamis* at 5.0mg/L concentration of the clove extracts, with the optimum dosage of 10.0mg/l and 15.0mg/l for fingerlings and juvenile sizes respectively. Interestingly, the survival of the exposed fish during the trial was 100% in both sizes of the two species, as no mortality was recorded in all concentrations of the plant extracts. The findings of this trial, indicated that clove seed extracts was effective, producing minimum stress and zero mortalities and therefore can be recommended as an effective anaesthetic for use in aquaculture.

**Keywords:** anaesthetics, clove, seed mullets aquaculture

## 1. Introduction

Brackish water aquaculture is becoming more important in recent years, as a result of increasing awareness in its potential in supplying food fish to the people living in the coastal areas of the country [1]. Farmed fish in culture medium are subjected to various management procedures such as, confinement, transportation, handling and other farm operations from the hatchery to the final commercial stage [2]. These on farm practices, as crucial as they are produce some level of disturbances, which can elicit a stress response leading to decreased fish performance, increased its susceptibility to disease and in extreme cases results in mortality [3,4].

Anaesthetics are widely used in routine aquaculture activities to reduce incidence of stress by sedating and immobilizing fish before performing any task in aquaculture [5]. The desirable attributes of anaesthetics used for fin fish include, short induction and recovery time, non-toxicity to fish and humans, no lasting physiological effects, rapid clearance from the body, high solubility in

fresh and salt water, availability and cost effectiveness (Marking and Meyer 1985). Conventional anaesthetics such as tricaine methane sulphonate (MS-222), benzocaine, quinaldine and 2-phenoxyethanol are expensive, toxic and not readily available in some countries including Nigeria. To solve this problem, biologists and aquaculturists alike have been searching for alternative anaesthetics, that are less toxic, readily available, efficacious and safe for humans [7].

Cloves (*Syzygium aromaticum*) are the aromatic dried flower buds of a tree in the family *Myrtaceae* [7]. They are widely used in dentistry, where the essential oil of clove is used for dental emergencies [8]. In addition, the cloves are anti-mutagenic, anti-inflammatory and anti-parasitic in nature [9,10]. According to the USFDA [11], the constituting ingredients of clove are considered safe as a substance that can be used in food industry. And in many parts of the country, clove seed is being used as a spice in preparation of food and local drinks.

The grey mullets, *Liza falcipinnis* and *Liza grandisquamis* are two important species of the family *Mugilidae*, that are widely distributed in the Niger delta region of Nigeria [12]. Their culture is becoming more

popular among fish farmers in the coastal areas of the region, where they are being transported regularly from the wild to the stocking site [13]. Because of their importance as good aquaculture species, especially in brackish water, *L.falciipinnis* and *L. grandisquamis* are ideal species to examine the suitability of clove seed as anaesthetics. The aim of this study therefore, is to determine the efficacy and optimum concentration of aqueous extracts of clove seed in different sizes of *L.falciipinnis* and *L. grandisquamis* which hitherto has not been reported.

## 2. Materials and Methods

A total of 120 grey mullets comprising 60 *L. falciipinnis* with 30 each of fingerlings (mean length 9.86cm  $\pm$ 1.11 SD; mean weight 21.20g  $\pm$  2.10 SD) and juveniles (mean length 17.24cm  $\pm$ 1.89 SD; mean weight 66.24g  $\pm$ 4.24 SD) and 60 *L. grandisquamis*, also consists of 30 each of fingerlings (mean length 10.24cm  $\pm$ 1.92 SD; mean weight 23.41  $\pm$ 2.2 SD) and juveniles (mean length 18.21cm  $\pm$ 3.14 SD; mean weight 71.21g  $\pm$  4.12 SD) were sampled from the recruitment ponds at African Regional Aquaculture Center, (ARAC), Brackish Water Research Station, Buguma, Rivers State, Nigeria. They were immediately transferred into 200L tanks (for each species) in the hatchery, where they were acclimated to laboratory conditions for a period of 14 days, during this period they were fed at 3% body weight with ARAC feed (35.0 % C.P), with the water in acclimation tanks being renewed every two days.

Dried buds of clove plant (*Syzygium aromaticum*) were purchased from Choba market, in Obio-Akpor Local government area of Rivers State, Nigeria. Plant authentication was done using the keys of Agbaje [14]. The buds were later taken to the laboratory where they were ground into powder using a kitchen blender (Model, H112, Kenwood, Japan). The milled clove buds were sieved into fine powdery form using 0.1 $\mu$  nylon mesh. Graded series of ground and filtered seeds of *S.aromaticum* were weighed and applied directly at (5.0, 10.0, 15.0, 20.0 and 25.0mg/L) into the water in the 20L experimental tanks filled to 5L mark, the mixture was stirred vigorously to allow homogenous mixing. Fish were randomly caught by hand-net in the holding tanks and transferred immediately into the experimental tanks containing clove seed. The fish were observed for opercula movement, balance and there after loss of response to stimulus. The fish were removed when they exhibited loss of equilibrium, no spinal reflexes and imperceptible opercula movements [15].

Induction and recovery times were obtained using a stop watch. Induction of anaesthetics was assumed as complete, when fish lost equilibrium and reflex activity [16]. Once the fish was immobilized it was removed from

the experimental tanks and transferred to a clean brackish (without clove seed powder) in a similar tank. Recovery was considered complete in all groups, when fish were able to regain upright position and swim normally. Recovery and induction time were recorded for each fish and left for 48 hours for potential mortality.

During the study, the following water quality parameters were monitored in the experimental tanks: temperature, pH dissolved oxygen, nitrite, ammonia and sulphide. The water temperature were taken by using mercury in glass thermometer, p<sup>H</sup> was determined by the use of a pH meter (Model H1 9812, Hannah Products, Portugal). Salinity was measured by hand held refractometer (Model HRN -2N, Atago Products, Japan). While the dissolved oxygen levels in the experimental tanks were determined by the Winkler method [17,18,19] and nitrite, ammonia and sulphide were measured using Lamotte salt water test Kit (Model AQ -4). Chestown, Maryland (U.S.A).

Data from the experiments were subjected to one way analysis of variance (ANOVA) test at 0.05 % probability and differences among means when existed were determined by Tuckeys multiple comparison test [18].

## 3. Results

The mean values of water quality variables obtained in exposure tanks of *L. facipinnis* and *L. grandisquamis* to clove seed powder are shown in Table 1, all the parameters analyzed were within the same range with no significant difference ( P > 0.05), comparable to the control. The induction and recovery time in both juvenile and adult sizes of the two species of *L. facipinnis* and *L. grandisquamis* (Table 2 and Table 3) exposed to clove seed extracts, indicated a size related responses, with the induction and recovery time in the juvenile consistently higher than the fingerlings. The induction time in both species of fish was found to decrease significantly (P < 0.05) as the concentrations of the clove seed extracts increased. The shortest induction time (55.81  $\pm$  8.92) was observed in fingerlings of *L. grandisquamis* (Table 3) while the longest (210.61  $\pm$  8.14) was recorded in juvenile of *L. falciipinnis* (Table 2). However, the recovery time for two sizes in both species increased significantly with increasing concentration of clove seed, with the highest recovery time (350.11  $\pm$  12.90) observed in *L. falciipinnis* exposed to clove seed at 25.mg/L concentration and the lowest (246.91  $\pm$  12.14) in *L. grandisquamis* at 5.0m/lL concentration of the clove extract.

Interestingly, the survival of the exposed fish during the trial was 100 % in both sizes of the two species (Table 2 and Table 3) no mortality was recorded in all concentrations of the plant extract.

**Table 1. Water Quality Parameters in Experimental Tanks of Grey mullets Exposed to Clove Powder Extracts (Mean $\pm$ SD)**

Parameters	Concentration of Clove extract mL <sup>-1</sup> )					
	0.00	5.00	10.00	15.00	20.00	25.0
Temperature (°C)	28.80 $\pm$ 0.34 <sup>a</sup>	29.10 $\pm$ 0.36 <sup>a</sup>	29.10 $\pm$ 1.64 <sup>a</sup>	28.73 $\pm$ 0.41 <sup>a</sup>	29.00 $\pm$ 0.32 <sup>a</sup>	29.00 $\pm$ 1.11 <sup>a</sup>
pH	6.91 $\pm$ 0.17 <sup>a</sup>	6.80 $\pm$ 0.16 <sup>a</sup>	6.80 $\pm$ 0.16 <sup>a</sup>	6.68 $\pm$ 0.15 <sup>a</sup>	6.93 $\pm$ 0.36 <sup>a</sup>	6.98 $\pm$ 0.41 <sup>a</sup>
Dissolve Oxygen (mgL <sup>-1</sup> )	6.89 $\pm$ 0.14 <sup>a</sup>	6.93 $\pm$ 0.35 <sup>a</sup>	6.93 $\pm$ 0.35 <sup>a</sup>	6.89 $\pm$ 0.25 <sup>a</sup>	6.79 $\pm$ 0.66 <sup>a</sup>	6.78 $\pm$ 1.25 <sup>a</sup>
Nitrite (mgL <sup>-1</sup> )	0.047 $\pm$ 0.02 <sup>a</sup>	0.0053 $\pm$ 0.02 <sup>a</sup>	0.0053 $\pm$ 0.02 <sup>a</sup>	0.0060 $\pm$ 0.03 <sup>a</sup>	0.0047 $\pm$ 0.03 <sup>a</sup>	0.0061 $\pm$ 0.02 <sup>a</sup>
Ammonia (mgL <sup>-1</sup> )	0.31 $\pm$ 0.03 <sup>a</sup>	0.32 $\pm$ 0.02 <sup>a</sup>	0.32 $\pm$ 0.02	0.32 $\pm$ 0.04	0.036 $\pm$ 0.03 <sup>a</sup>	0.0036 $\pm$ 0.03
Sulphide (mgL <sup>-1</sup> )	0.04 $\pm$ 0.01 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.01 <sup>a</sup>	0.14 $\pm$ 0.03 <sup>a</sup>	0.04 $\pm$ 0.03 <sup>a</sup>

Mean within the row with different superscripts are significant (P<0.05)

**Table 2. Induction, Recovery and Survival of *L. falcipinus* exposed to Clove seed extraction (Mean±SD)**

Life State	Parameters	(Concentration of Clove extract mL <sup>-1</sup> )					
		0.0	5.0	10.0	15.0	20.0	25.0
Fingerlings	Induction time (s)	0.0	130.44±6.41	104.69±8.39	90.40±4.21	71.31±6.11	60.14±1.11
	Recovery time (s)	0.0	189.62±7.31	215.71±6.43	245.66±7.10	259.74±11.11	286.12±12.60
	Survival (%)	100.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.00±0.01
Juveniles	Induction time (s)	0.0	210.61±8.14	186.41±11.62	141.24±18.18	137.8±7.11	119.00±12.7
	Recovery time (s)	0.0	251.82±11.66	279.81±12.91	30.47±14.14	336.81±12.14	350.1±12.9
	Survival (%)	100.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00

Mean within the row with different superscripts are significant (P<0.05)

**Table 3. Induction, Recovery and Survival of *L. falcipinus* exposed to Clove seed extraction (Mean±SD)**

Life State	Parameters	(Concentration of Clove extract mL <sup>-1</sup> )					
		0.0	5.0	10.0	15.0	20.0	25.0
Fingerlings	Induction time (s)	0.0	126.81±10.12 <sup>c</sup>	98.61±7.18 <sup>b</sup>	89.64±9.12 <sup>ab</sup>	72.61±7.11 <sup>ab</sup>	55.61±8.92 <sup>a</sup>
	Recovery time (s)	0.0	172.66±9.21 <sup>a</sup>	201.74±8.12 <sup>ab</sup>	231.69±9.11 <sup>b</sup>	244.71±8.61 <sup>b</sup>	269.14±11.72 <sup>b</sup>
	Survival (%)	100.0	100.0±0.00 <sup>b</sup>	100.0±0.00 <sup>b</sup>	100.0±0.00 <sup>b</sup>	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>
Juveniles	Induction time (s)	0.0	200.78±11.12	178.6±9.34	135.64±8.14	125.6±11.11	100.28±10.6
	Recovery time (s)	0.0	246.91±12.14	271.4±10.11	300.14±9.22	324.12±11.21	340.16±11.21
	Survival (%)	100.0	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Mean within the row with different superscripts are significant (P<0.05)

## 4. Discussion

The qualities required of an anaesthetic agent in sedating fish varies, depending on the nature, mode of application and species of fish. Most importantly, a quick induction and recovery time which allows for maximum manipulation of fish in culture medium is desirable by many aquaculturists, despite this, anaesthetics in aquaculture, should be cheap, safe, easy to handle, readily available and accessible for the fish farmers in different parts of the country.

The present study revealed that clove seed extracts acted as an anaesthetic agent in sedating both sizes of *L. falcipinnis* and *L. grandisquamis*. It acted by widespread depression of the central nervous system produced by an action on nerve axons, transmitter release or membrane excitability. The induction time recorded in this work, reduced as the dosage of clove seed extracts increased, while the recovery of anaesthetized fish increased notably with increasing concentrations of the extracts. This trend followed the pattern of typical fish anaesthetics [19]. In terms of induction time and recovery time to anaesthetic, the aqueous extracts of clove seed met Marking and Meyer (1985) criterion with the optimum dosage of 10.0mg/L and 15.0mg/L for fingerlings and juveniles sizes respectively. Clove seed appears to meet many of the criteria used in evaluation of an ideal anaesthetic and compared favourably with conventional anaesthetics like MS-222, metomidate and 2-phenoxethanol. Its main advantages lie in its availability all the year round, low cost, easy to use and high safety margins to humans [20].

Results of the numerous studies on clove oil, revealed that effective concentration of anaesthetic vary with fish body size [21,22,23] with the smaller fish being more responsive than the larger ones [24]. In this study, the induction and the recovery time were found to be lower in fingerlings, compared to the juvenile size for both species of *L. falcipinnis* and *L. grandisquamis*, this result is in line with the report of Weber *et al.* [25] in Senegalese sole (*Solea senegalensis*) anaesthetized with MS-222 and clove oil. The shorter induction time may be due to the small body size and reduced surface area of the gill of fingerlings, when compared to the juvenile sizes [26].

The 100% survival of exposed fish, during the trial, agrees with the findings of Agokei and Adebisi [27] in Nile tilapia *Oreochromis niloticus* exposed to tobacco leaf extracts, but contradicts that of Mgbenka and Ejiofor [28] in *Clarias gariepinus* and *Heterobranchus longifilis* fingerlings exposed to extracts of *Erythrophleum suaveolens*, this difference in observation may be due to narrow safety margin of *E. Suaveolens* for small fish [29].

## 5. Conclusion

The use of clove seed as an anaesthetic agent in grey mullets *L. falcipinnis* and *L. grandisquamis*, can be recommended for use in transportation of grey mullets fingerlings and juveniles from the wild to the stocking site with little or no stress which will enhance its performance in culture medium and leads to optimum yield of these species in aquaculture.

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