E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(6): 203-209

www.fisheriesjournal.com
Received: 25-09-2019
Accepted: 27-10-2019

Djiman Lederoun
Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01 BP: 526 Cotonou, Benin

Imaculé S Baglo
Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01 BP: 526 Cotonou, Benin

Eliane de Pedicepuis
Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01 BP: 526 Cotonou, Benin

Armel Gougbedji
Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01 BP: 526 Cotonou, Benin

Mohammed NA Fagbemi
Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01 BP: 526 Cotonou, Benin

Philippe A Lalèyè
Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01 BP: 526 Cotonou, Benin

Corresponding Author:
Djiman Lederoun
Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01 BP: 526 Cotonou, Benin

Efficacy of clove oil and benzocaine as anesthetics for *Protopterus annectens* (Owen, 1839) (Acanthopterygii: Protopteridae)

Djiman Lederoun, Imaculé S Baglo, Eliane de Pedicepuis, Armel Gougbedji, Mohammed NA Fagbemi, and Philippe A Lalèyè

Abstract
One of the most important in aquaculture is the anesthesia of fish to facilitate their handling. That is why many experiments have been carried out with that goal in mind for species. But for those to be introduced in fish farming, a foundation still needs to be established. That's the case of the African lungfish *Protopterus annectens* on which two anesthetics, clove oil and benzocaine, were tested. Pure clove oil was diluted directly in water to obtain concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ml per liter of water For benzocaine, the solution obtained by diluting 100 g of powder in 1 L of alcohol was further diluted in water to obtain concentrations of 0.85, 0.9, 0.95, 1.2, 1.5, 1.8, 2.1, 2.4, 2.5, 2.6 and 2.7 ml L^{-1}. The experiment consisted in placing a fish in a container filled with water containing a specific concentration of the anesthetic of interest. The time taken by the fish to reach each stage of anesthesia was then timed. This procedure was repeated ten times for all treatments. In order to determine the recovery time, the anesthetized specimen was transferred to a container filled with only plain water. The fish used for the experiment are wild specimens acclimated for four weeks with an average weight of 50.3±5.5 g and an average size of 22.1±0.4 cm. The results show that clove oil is the most suitable anesthetic for African lungfish and performs better than benzocaine as it is effective at lower concentrations. Recovery time of the equilibrium position is significantly longer with clove oil than with benzocaine. The optimum concentrations are between 0.6 and 0.9 ml L^{-1} for clove oil and between 1.8 and 2.6 mlL^{-1} for benzocaine.

Keywords: Aquaculture, anesthetic, clove oil, benzocaine, African lungfish

Introduction
West African lungfish, *Protopterus annectens*, is a species highly exploited by populations living near the Mono River [1]. However, the control of the technical route of production of a species passes through the restraint of the specimens in a controlled environment. Anesthetics such as 2-phenoxy ethanol, benzocaine, tricaine methanesulfonate (MS-222) and clove oil have often been used to facilitate handling, sorting, tagging, artificial reproduction procedures and surgery, and to suppress sensory systems during invasive procedures [2-17]. Generally, the dosages are unique to each species [6]. Induction and recovery times during application are very dependent on the dose used but also on the water's temperature [10]. That's why, depending on the species, in recent years, experiments have been conducted to control the anesthesia of several farmed species to avoid mortality after handling. Exact data on the anesthesia of *P. annectens* with the most widely used anesthetics are not yet available in the literature. The fragmentary data available often refer to the doses used on an ad hoc basis to maintain the fish under anesthesia during handling (see [18] for benzocaine) with no precision on the stage of anesthesia [4]. It is well known that when choosing an anaesthetic, a number of considerations are important, such as efficacy, cost, availability and ease of use, as well as toxicity to fish, to humans and to the environment [19, 20]; on the other hand, choice of the anesthetic may also depend on the nature of the experiment and on the fish species concerned [21, 22]. In the fish farms of Benin, 2-phenoxy ethanol used to be the most commonly used product to anesthetize fish. But in recent years, it was abandoned because the narrow margin between the induction dose and the lethal dose, the toxicity potential and the significant effects on the cardiovascular system as well as the physiological response to

stress, make this anesthetic not an ideal compound for use in fish [23]. MS-222 did not get to be popular because it is expensive, and a 21-day withdrawal period following anaesthesia treatment is necessary before the fish can be safely consumed or released into natural habitats [24, 25]. In contrast, benzocaine has low toxicity to humansand recovery times can be prolonged in older or gravid specimens [26]. Also, clove oil has become an attractive anaesthetic for aquatic organisms because of its low price, ready availability, and safety while handling [2]. The aim of the present study was to investigate the right doses of clove oil or benzocaine to anesthetize African lungfish.

Materiel and methods

Study area and living material

The experiments were conducted between September and October 2018 at the Agro-Fish Training Center of Benin (CeFAP-Benin) in Benin. This center is located not far from the Mono River, more precisely in the flood plain of its lower reach where the African lungfish is often exploited. For this study, the fish used are just wild individuals of African lungfish from the flood plain of the lower Mono. These fish, which were collected through traditional fishing protocol using traps, have an average weight of 50.3±5.5 g and an average size of 22.1±0.4 cm.

Collection and acclimation of the fish

For capture, traps were placed between 3 and 4 PM on the first day and picked up the next morning between 7 and 8 AM. The collected specimens were put in a plastic bucket and transported to CeFAP-Benin for storage in the ponds of the said center at a density of 10 individuals per m^2. Hoses were introduced into the ponds to provide hiding places, as it has been found that captive individuals are injured and cannibalism develops at high density. The collected individuals being wild, they were acclimatized for four weeks before the start of the experiment. During this period, they were fed the first two weeks with food leftovers, palm nuts, cooked cassava and yam roots because feeding trials using granulated feed for *Clarias gariepinus*were not conclusive. Three-quarters of the foods used in the first two weeks were replaced with corn meal dough and one-quaretr was replaced with black soldier fly larvae (*Hermetia illucens*) produced at the Laboratory of Hydrobiology and Aquaculture of the Faculty of Agricultural Sciences of the University of Abomey-Calavi (LHA/FSA/UAC).

Protocol and experimentation

Due to its incomplete solubility in water, pure clove oil (100% eugenol) is usually dissolved in ethyl alcohol (92.8%) at a ratio of 1:9 (clove oil ethyl alcohol) when the temperature of the water is below 15°C [2]. This solution is often subsequently diluted in water to obtain different concentrations to be tested. However, since the water temperature was well above 15°C in the study area (26.3±1.2°C), the clove oil obtained at the pharmacy was not diluted in this study. In addition, a pre-test performed after dilution gave much longer anesthesia times. The pure oil was therefore diluted directly in water to obtain concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.9 and 1 ml of clove oil per liter of water.

For benzocaine, the solution obtained by diluting 100 g of powder in one liter of alcohol was diluted in water to obtain concentrations of 0.85, 0.9, 0.95, 1,2, 1.5, 1.8, 2.1, 2.4, 2.5, 2.6 and 2.7 ml of benzocaine per liter of water. These concentrations were selected based on data obtained during pre-tests.

Running the experiment

Chemical anesthesia of a fish is characterized by five different stages [4]; thoseare: (1) partial loss of balance with "normal" swimming activity, (2) loss of balance with "normal" swimming activity, (3) partial loss of swimming activity, (4) total loss of swimming activity with small operculum movements, (5) absence of opercular movements. Stage 4 is the one generally required for handling in aquaculture. Stage 5 is an overdose and leads to death.The experiment consisted in placing a fish in a container filled with 1 liter of water containing a specific concentration of solution of clove oil or benzocaine. The time taken by the fish to reach each stage of anesthesia was timed. This procedure was repeated 10 times for all treatments. In order to determine the recovery time, the anesthetized individual was transferred to a container filled with water only to record the time necessary for the fish to make the first movement and recover the balanceand regular movements. For clove, individuals who showed no vital signs after 1 h and 45 min were considered dead. The choice of this time is justified by the fact that even after 1 h and 30 min athigh doses, anesthetized fish would hardly have recovered equilibrium and would not havepresented regular movements. For each dose and treatment,when the fish were anesthetized, total length was measured to the nearest 0.1 cm using a measuring-tapeand the weight determined to the nearest 0.01 g using an electronic precision balance. After recovery, the individuals were returned to the breeding ponds. Dead fish during the experiment were fixed in a 10% formalin solution and, two weeks later, were placed in a 70% alcohol solution and stored in the LHA collection.

Statistical analyzes

Exponential regressions were used to study, for each anesthetic, the relationship between the average induction timesof the different stages of anesthesia (1 to 4) and the different doses tested on the one hand, and between the average recovery times (first movement and resumption of normal activity) and the different doses tested on the other hand. The one-way ANOVA test was performed to see if there is a significant difference between the times recorded per anesthetic. When a significant difference is found, the Least Signicant Difference (LSD) test was performed to make two-by-two comparison of the different doses and for each anesthetic.

Results

Anesthesia time

For clove oil, the highest average time to stage 4, i.e.24 min,was observed at the lowest dose tested which was 0.1 ml L^{-1}while the lowest time,i.e.2 min and 55 s, was obtainedat the highest dose which was 1 ml L^{-1}(Table 1). The one-way ANOVAtest shows that there is a significant difference between the different doses for stage 4 induction times (p<0.05). The LSD test shows that this difference is between the induction times forlow doses (0.1 to 0.5 ml L^{-1}) and those forhigh doses (0.6 to 0.9 ml L^{-1}) (Table 1).

For benzocaine, the highest average time forthe induction of stage 4, i.e.11 min,was observed with the lowest dose tested which was 0.85 ml L^{-1}, while the lowest one,i.e.1 min and 54 s,was observed with the largest dose tested which was 2.7 ml

L^{-1}(Table 1). The One-way ANOVA test shows that there is a significant difference between the different induction times of stage 4 ($p<0.05$). The two-by-two comparison of the doses using the LSD test shows that the difference is between the low doses (0.85 to 1.5 ml L^{-1}) and high doses (1.8 to 2.7 ml L^{-1}) of stage 4 induction times (Table 1).

Table 1: Induction and recovery times for *Protopterus annectens* anaesthetized with different concentrations (n = 10 per replicate) of two anaesthetic agents. Data are presented as mean±sd. BW: body weight; TL: total length

Anesthetic	TL (cm)	BW (g)	Induction time (s)				Recovery time (s)	
			Stage 1	Stage 2	Stage 3	Stage 4	First movement	Equilibrium
Clove oil (ml L^{-1})								
0.1	21.0±1.0	45.91±9.51	167.6±61.8^{a}	312.6±80.0^{a}	552.1±129.8^{a}	1446.3±376.7^{a}	186.5±114.2^{d}	989.9±396.2^{e}
0.2	22.4±2.2	49.87±14.30	72.8±25.1^{bc}	179.0±20.0^{b}	294.5±26.8^{b}	774.9±192.3^{b}	237.4±63.6^{d}	1340.3±256.0^{e}
0.3	21.9±1.6	52.80±5.87	79.8±31.5^{b}	169.9±50.5^{b}	292.3±109.1^{b}	509.9±104.4^{c}	799.5±163.8^{c}	1563.3±281.0^{e}
0.4	21.7±1.2	52.02±6.16	56.3±13.3^{bcd}	111.9±13.0^{c}	212.1±46.8^{c}	456.8±70.1^{cd}	972.3±213.7^{c}	2393.9±348.7^{d}
0.5	22.1±1.4	48.90±11.00	50.2±31.3^{de}	88.0±27.6^{cd}	169.4±38.1^{cd}	347.7±75.5^{cde}	1049.9±235.0^{c}	3237.8±554.6^{c}
0.6	22.2±2.3	49.36±14.87	42.6±15.1^{cde}	75.2±21.4^{cde}	129.5±17.2^{de}	241.1±61.5^{de}	1225.2±296.0^{c}	3474.5±427.9^{c}
0.7	21.8±1.5	49.67±7.39	52.6±17.6^{bcd}	87.0±28.5^{cd}	133.9±33.1^{cde}	310.0±55.8^{cde}	2153.3±1221.5^{b}	3414.6±1013.7^{c}
0.8	21.9±2.0	49.60±13.70	46.9±9.6^{cde}	93.8±17.1^{cd}	144.7±34.6^{cde}	268.4±49.4^{de}	3227.0±466.7^{a}	4321.8±955.1^{b}
0.9	22.5±1.1	52.90±5.00	28.1±4.1^{de}	52.1±15.5^{de}	103.7±24.6^{de}	202.6±37.5^{e}	3547.4±749.8^{a}	5659.3±1199.0^{a}
1	22.1±1.3	50.34±7.00	19.5±3.5^{e}	37.0±11.3^{e}	69.0±11.3^{e}	175.5±40.3^{e}	Dead	Dead
Benzocaine (ml L^{-1})								
0.85	21.9±1.2	44.45±7.93	57.5±25.3a	176.1±54.5a	365.3±88.7a	694.4±100.9a	43.3±16.1cd	139.7±35.2bcd
0.9	22.7±1.2	55.52±5.57	56.7±11.7^{ab}	150.7±46.0^{ab}	258.7±72.7^{a}	676.1±118.1^{a}	35.4±14.4^{d}	126.0±42.8^{cd}
0.95	21.6±1.2	45.13±5.31	64.1±28.0^{a}	151.0±36.8^{ab}	333.5±141.3^{a}	621.0±201.9^{a}	63.2±12.2^{abc}	159.7±29.3^{bcd}
1.2	21.5±1.6	44.39±8.66	62.5±21.1^{a}	132.5±28.1^{b}	235.6±28.5^{b}	466.1±145.3^{b}	53.6±26.9^{bcd}	186.5±105.1^{ab}
1.5	22.0±1.4	44.34±8.41	36.0±9.5^{cd}	81.6±18.7^{c}	145.9±48.8^{c}	314.3±53.1^{c}	47.8±30.4^{cd}	159.9±77.5^{bcd}
1.8	21.7±1.3	44.66±7.23	43.4±11.1^{bc}	78.3±10.3^{c}	130.6±23.4^{cd}	289.1±70.6^{cd}	76.0±47.7^{ab}	219.2±77.7^{a}
2.1	22.1±1.2	55.47±8.09	29.3±7.8^{d}	59.7±14.3^{cd}	92.1±13.6^{cd}	197.2±64.8^{cd}	38.8±15.9^{bcd}	147.0±52.9^{abc}
2.4	22.1±1.4	55.81±8.26	31.0±8.3^{cd}	60.6±4.7^{cd}	91.7±5.4^{cd}	176.7±21.0^{cd}	74.3±9.5^{a}	167.0±30.0^{abc}
2.5	22.8±1.0	55.45±8.65	27.0±10.9^{de}	57.6±12.4^{cd}	84.5±27.9^{d}	161.9±34.3^{d}	59.0±17.5^{bcd}	170.5±34.6^{abc}
2.6	22.4±1.4	54.73±7.00	23.5±7.8^{de}	44.6±10.0^{d}	80.2±15.2^{d}	139.9±30.1^{d}	51.5±16.3^{bcd}	161.7±58.9^{bc}
2.7	22.1±1.0	55.40±6.91	15.4±4.0^{e}	42.2±9.3^{d}	71.5±12.3^{d}	114.2±30.0^{d}	45.6±13.7^{cd}	102.7±16.3^{d}

In all columns, means with different superscripts are significantly different from each other ($p<0.05$).

In all columns, means with different superscripts are significantly different from each other ($p<0.05$).

The time taken by fish to reach anesthesia stages 1 to 4 are strongly dose-related as indicated by the high R^2 valuesof each exponential regression (Figs 1 & 2). For both anesthetics, the higher the concentration, the faster the fish go asleep (Figs1 & 2). The relationship between dose and anesthesia time results in the negative exponential function (Figs1 & 2).

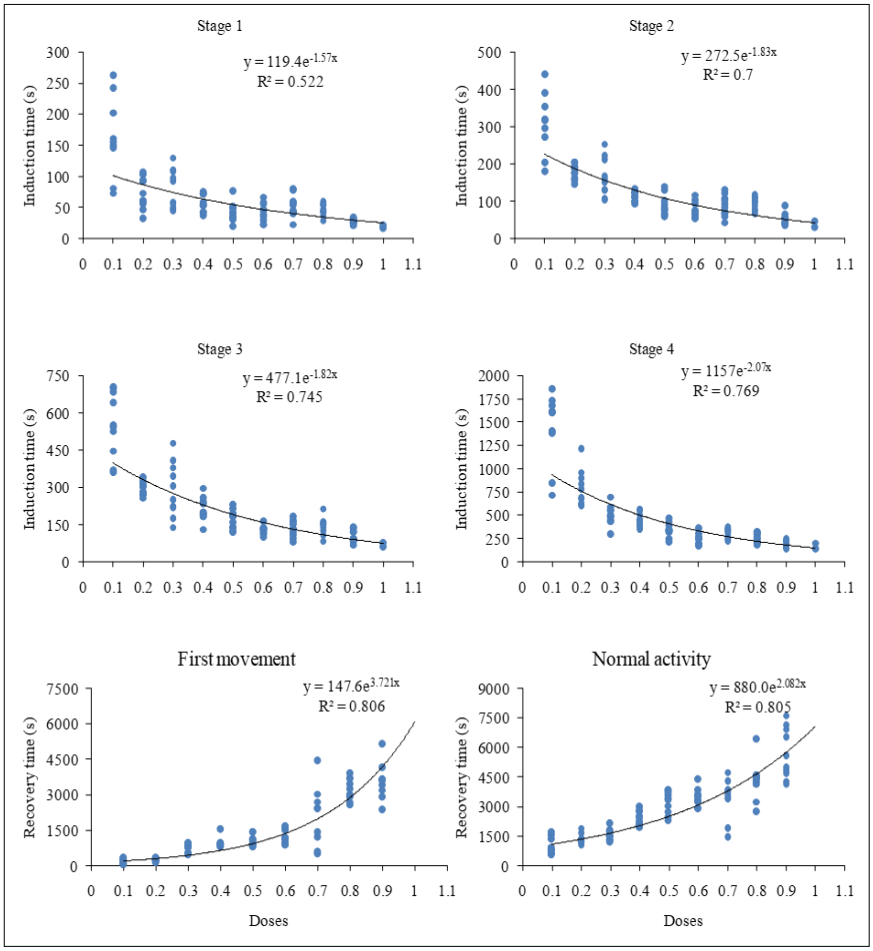

Fig 1: Induction and recovery times (s) relation to clove oil concentrations for *Protopterus annectens* (n=10 for each trial).

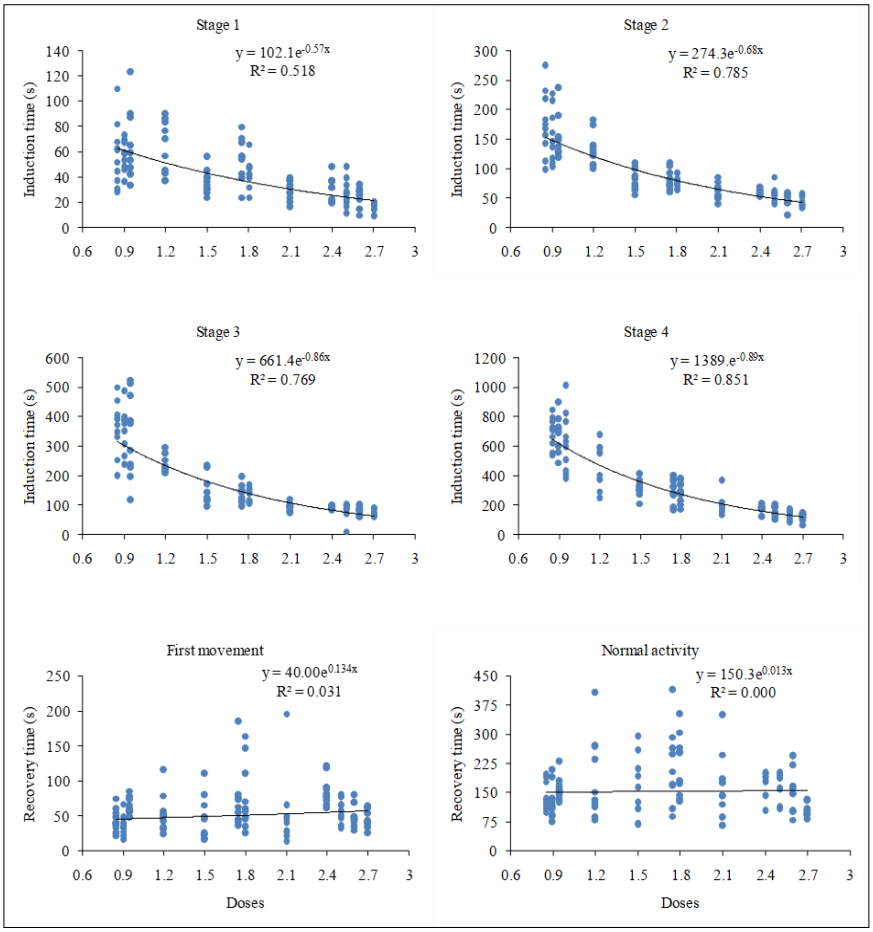

Fig 2: Induction and recovery times (s) relation to benzocaine concentrations for *Protopterus annectens* (n=10 for each trial).

Recovery time

For clove oil, the lowest mean recovery time, i.e.16 min and 30 s, was obtained with the 0.1 ml L^{-1} dose, while the highest time,1 h and 34 min, was observed with the 0.9 ml L^{-1} dose (Table 1). The One-way ANOVA test shows that there is a significant difference between the recovery times of the different doses ($p<0.05$). The LSD test showss that this difference is located between the recovery times of the doses ranging from 0.1 to 0.3 ml L^{-1} and those of the other doses (0.4 to 0.9 ml L^{-1}) (Table 1). For doses ranging from 0.1 to 0.3 ml L^{-1}, there is no significant difference between the recovery times of anesthetized fish. Forranging from 0.4 to 0.8 ml L^{-1},there are no significant differences in recovery times of anesthetized fish. In contrast, mean wake-up time of anesthetized fish with the 0.9ml L^{-1} dose was significantly different from all other doses, i.e. 0.1 to 0.8 ml L^{-1} (Table 1). The recovery time is strongly related to the anestheticconcentration as indicated by the high R^2 values of the linear regression of the first movement time and the recovery time of the normal activity as a function of the concentration (Fig. 1). The higher the concentration of clove oil, the faster the fish wakes up (Fig. 1). This is a positive exponential function.As for benzocaine, the minimum average time of recovery, i.e. 1 min and 43 s, is obtained with the 2.7-ml L^{-1}dosewhile the highest one, i.e.3 min and 39 s, is observed with the 1.8-ml L^{-1}dose (Table 1). The One-way ANOVA test shows that there is a significant difference between the recovery times of the different doses ($p<0.05$). The LSD test reveals that this difference is only between the recovery times of anesthetized fish with the 2.7- ml L^{-1}dose and those of the other doses tested (Table 1). In contrast to clove oil, the time taken by the fish to perform the first movement and the time to resume normal activity are not related to the benzocaine concentration as indicated by the R^2 values for each regression exponential(0.031and 0.000, respectively for first movement and resumption of normal activity) (Fig.2).

Adequate doses

The lowest doses of the two anesthetics (0.1 to 0.5 ml L^{-1} for clove oil and 0.85 to 1.5 ml L^{-1} for benzocaine) do not appear to be satisfactory (Table 1). Indeed, as far as benzocaine is concerned, the fish are difficult to handle, the majority of them move on the measuring-tape or on the scales while stage 4 of the anesthesia has been reached. As for the lowest doses of clove oil, the average induction times of stage 4, greater than 5 min in all cases, are significant and induce relatively long awakening times (Table 1).With regard to high doses (0.6 to 1 ml L^{-1} for clove oil and 1.8 to 2.7 ml L^{-1} for benzocaine), mean times of induction of stage 4, all times beingless than or equal to 5 min, seem conclusive (Table 1).In fact, for benzocaine, the 1,8-, 2.1-, 2.4-, 2.5-, 2.6- and 2.7- ml L^{-1} doses provoke sleep (stage 4) in the fish between 1 and 5 min on average (Table 1), with an average recovery time between 2 and 4 min, to the exception ofthe 2.7- ml L^{-1} dose which induces an average wake-up time of less than 2 min (Table 1).The latter does not allow easy handling of the fish because they move on the measuring-tapeor scaleas in the case of low doses. For benzocaine, only doses ranging from 1.8 to 2.6 ml per liter of water may therefore be recommended as adequate doses to anesthetize *P. annectens* juveniles.

For clove oil, the 0.6-, 0.7-, 0.8-, 0.9- and 1-ml L^{-1}doses make it possible to put the fish to sleep between 3 and 5 min on average (Table 1), but with a very high mean waking time compared to that of benzocaine (57 min and 54 s to 1h and 34 min, Table 1). Only these doses can be recommended to anesthetize African lungfish juveniles. In fact, the specimens used for doses of 1-ml L^{-1} and above show no vital signs after 1h and 45min in the wakening water (Table 1). They were therefore considered dead. Doses greater than or equal to 1 ml of clove oil per liter of water are therefore not suitable for anaesthetizing African lungfish juveniles.

Discussion

Anaesthesia is a biological state with the partial or complete loss of sensation or loss of voluntary neuromotor control induced by chemical or nonchemical means [10, 21]. Studies have shown the anesthetic properties of several products on farmed fish [4, 6, 19, 22, 28-30]. It is clearly established that efficacy is function of both biological factors (species, the stage of life cycle, age, size, weight, lipid content, body content and disease status) [31] and environmental factors (temperature and pH) [26, 32]. Anaesthesia suppresses pain in fish and induces a calming effect followed by loss of equilibrium, mobility and consciousness [21]. But an ideal anesthetic for fish farming requires several characteristics, including: (1) to causing rapid stilling of the fish (3 min or less), (2) allowingrapid recovery (5 min or less), (3) being non-toxic to fish or humans, (4) havinga large margin of safety, (5) allowing a "reasonable" exposure time, (6) not producing cumulative effects following repeated exposures [6, 21, 33].At the termination of experiments, faster induction of and recovery from anaesthesia were obtained from the benzocaine treatment at concentrationsof 2.4 ml L^{-1} to 2.6 ml L^{-1}. As for clove oil, any concentrating leading to a rapid induction leads to a longer waking time. Nevertheless, the concentration that allowed the fastest induction is 0.9 ml L^{-1}. The difference betweenstilling and recovery times of the two anesthetics has already been reported for many fish species (*Salmo salar*, *Siganus rivulatus*, *Clarias gariepinus*, *Barbus grypus*, etc.) [11, 31, 34, 35]. Clove oil seems to be more effective. The anesthetic power of clove oil has already been recognized in a wide variety of species including, medaka (*Oryzias latipes*), goldfish (*Carrassius auratus*), carp (*Cyprinus carpio*), rabbitfish (*Siganus lineatus*), channel catfish (*Ictalurus punctatus*), Atlantic (*Salmo salar*), sockeye (*Oncorhynchus nerka*), salmon (*Salmo salar)* and longfinned eel (*Anguilla reinhardtii*) [6, 19, 28-30, 36-38]. For both clove oil and benzocaine, the mean induction time versus anesthetic dose is a negative exponential function. The higher the anesthetic concentration, the sooner the fish are asleep. A concentration-dependent regimen is well known for these two types of anesthetics and for many others, so that increasing the anesthetic dose results in decreased induction time [35, 39-44]. Recovery times for African lungfish are significantly higher with clove oil than with benzocaine. This result highlights the importance of using clove oil as an anesthetic for operations requiring a significant amount of time to fall asleep. It appeared that, with clove oil, the fish that took the longest time to recover their equilibrium position were those that were exposed to the highest concentrations. This results in a positive exponential function. Several authors [35, 45-48] have already found thatrecovery times were positively correlated with increasingconcentrations of anaesthetics. However, decreasing recovery times with an increase in concentration of clove oil and 2-phenoxyethanol for European sea bassand gilthead seabream has been reported by [41]. The explanation put forward by these authors is that with the highest

concentration, the fish is not in contact with the anaesthetic for long, which allows faster recovery [34, 49].Unlike clove oil, the recovery times of fish exposed to different doses of benzocaine are not dose-dependent, which means that fish exposed to less concentrated anesthetic baths can wake up at the same time as those subjected to more concentratedbaths. Other authors [50] also reported thatincreasing the concentration did not affect the recovery time.In terms of efficacy, clove oil is a more potent anesthetic than benzocaine because it works at significantly lower doses than benzocaine (Table 1). For the adequate dose ranges of the two anesthetics, the extreme (minimum and maximum) doses of benzocaine are multiples of three of the extreme doses of clove oil (Table 1). He therefore deduce that for a satisfactory result obtained with clove oil, a dose of benzocaine equivalent to three times that of clove is needed to obtain similar results.

Protopterus annectens is a very resistant species. As an illustration, to anesthetizeits juveniles in 3 min, a dose greater than or equal to 0.9 ml L^{-1} is required. [34] have shown that the optimal dose of clove oil for the juveniles of African catfish in 3 min is 50 mg L^{-1} (i.e. 0.05 ml L^{-1}), thus 18 times less than that of *P. annectens*. This can be explained not only by the resilience of *P. annectens* but also by its breathing pattern. Indeed, Africanlungfish lives in areas very poor in oxygen. Its ability to breathe oxygen from the air allows it to avoid the absorption of the anesthetic solution as much as possible by keeping its head above the solution, which is a form of resistance to the anesthesia.

Conclusion

The present study compared the efficacy of clove oil and benzocaine to relax African lungfish during handling. The results indicate that both anesthesics tested can be used, but clove oil appears to be more suitable than benzocaine. Especially, the long recovery time makes clove oil a better choice for transporting fish from hatchery to farm.

References

1. Lederoun D, Agbohessou P, Vandewalle P, Lalèyè PA. Données préliminaires sur quelques aspects de la biologie de *Protopterus annectens* (Owen, 1839) dans le bassin du fleuve Mono au Bénin (Afrique de l'Ouest). In: VI^{ème} Colloque des Sciences, Cultures et Technologies de l'Université d'Abomey-Calavi, 25-30 Septembre 2017, Abomey-Calavi, Bénin, 2017, 515.
2. Anderson WG, Mickinley RS, Colavecchia M. The use of clove oil as an anesthesic for rainbow trout and its effects swimming performance. North American Journal of Fisheries Management. 1997; 17:301-307.
3. Keene JL, Noakes DLG, Mocela RD, CGS. The efficacy of clove oil as an anaesthesic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research. 1998; 29:89-101.
4. Peake S. Sodium bicarbonate and clove oil as potential aanesthesics for nonsalmonid fishes. North American Journal of Fisheries Management. 1998; 18:919-924.
5. Taylor PW, Roberts SD. Clove oil: an alternative anaesthetic for aquaculture. North American Journal of Aquaculture. 1999; 61(2):150-155.
6. Chanseau M, Bosc S, Galiay E, Oules G. L'utilisation de l'huile de clou de girofle comme anesthésique pour les smolts de saumon atlantique (*Salmo salar* L.) et comparaison de ses effets avec ceux du 2-phenoxyethanol. Bulletin Français de la Pêche et de la Pisciculture. 2002; 365-366:579-589.
7. Gullian M, Villanueva J. Efficacy of tricaine methanesulphonate and clove oil as anaesthetics for juvenile cobia *Rachycentron canadum*. Aquaculture Research. 2009; 40:852-860.
8. Heo GJ, Shin G. Efficacy of benzocaine as an anaesthetic for Crucian carp (*Carassius carassius*). Veterinary Anaesthesia and Analgesia. 2010; 37:132-135.
9. Gomes LC, Chippari-Gomes AR, Lopes NP, Roubach R, Araujo-Lima CAR. Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. Journal of the World Aquaculture Society. 2011; 32:426-431.
10. Javahery S, Nekoubin H, Moradlu AH. Effect of anaesthesia with clove oil in fish (review). Fish Physiology and Biochemistry. 2012; 38(6):1545-1552.
11. Ghanawi J, Samer M, Imad PS. Anesthetic efficacy of clove oil, benzocaine, 2 phenoxyethanol and tricaine methanesulfonate in juvenile marbled spinefoot (*Siganus rivulatus*). Aquaculture Research. 2013; 44:359-366.
12. Kamble AD, Saini VP, Ojha ML. The efficacy of clove oil as anesthetic in common carp (*Cyprinus carpio*) and its potential metabolism reducing capacity. International Journal of Fauna and Biological Studies. 2014; 1(6):01-06.
13. Nair VMS, Williams ES. Comparative study of 2-phenoxy ethanol and clove oil on its efficiency as anesthetics in anesthetizing *Hypselobarbus kurali*. Banat's Journal of Biotechnology. 2015; 6(12):15-19.
14. Kucuk S, Coban D. Effects of tricaine as an anaesthetics on goldfish, *Carassius auratus* (Linnaeus 1758) at different salinities and concentrations. Turkish Journal of Fisheries and Aquatic Sciences. 2016; 16:611-661.
15. Bolasina SN, Azevedo AD, Petry AC. Comparative efficacy of benzocaine, tricaine methanesulfonate and eugenol as anesthetic agents in the guppy Poecilia vivipara. Aquaculture Reports. 2017; 6:56-60.
16. Fernandes A, Makoś P, Khan JA, Boczkaj G. Pilot scale degradation study of 16 selected volatile organic compounds by hydroxyl and sulfate radical based advanced oxidation processes. Journal of cleaner production. 2019; 208(2):54-64.
17. Okey IB, Keremah RI, Gabriel UU. The efficacy of clove (*Eugenia caryophyllata*) powder as anaesthesia on African catfishes (*Clarias gariepinus* and *Heterobranchus bidorsalis*) fingerlings. Journal of Aquaculture & Marine Biology. 2018; 7(4):182-188.
18. Christensen CB, Christensen-Dalsgaard J, Madsen PT. Hearing of the African lungfish (*Protopterus annectens*) suggests underwater pressure detection and rudimentary aerial hearing in early tetrapods. The Journal of Experimental Biology. 2015; 218:381-387.
19. Soto CG, Burhanuddin S. Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). Aquaculture. 1995; 136:149-152.
20. Akbulut B, Çakmak E, Aksungur N, Çavdar Y. Effect of exposure duration on time to recovery from anaesthesia of clove oil in juvenile of Russian sturgeon. Turkish Journal of Fisheries and Aquatic Sciences. 2011; 11(3):463-467.
21. Summerfelt RC, Smith LS. Anesthesia surgery and related techniques. In: Schreck CB Moyle PB, editors. Methods for Fish Biology. Bethedsa: American Fisheries Society, 1990, 213-272.

22. Munday PL, Wilson SK. Comparative efficacy of clove oil and other chemicals in anesthetisation of *Pomacentrus amboinensis*, a coral reef fish. Journal of Fish Biology. 1997; 51:931-938.
23. Ackerman PA, Morgan JD, Iwama GK. Anesthetics. In Supplement to the Guidelines on: the care and use of fish in research, teaching and testing. Canadian Council on Animal Care (CCAC), Ottawa, Ont. Available from http://www.ccac.ca/Documents/Standards/Guidelines/Add_PDFs/Fish_Anesthetics.pdf.Google Scholar, 2005.
24. Pirhonen J, Schreck CB. Effects of anaesthesia with MS-222, clove oil and CO2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). Aquaculture. 2003; 220(1-4):507-514.
25. Yamamoto Y, Woody CA, Shoji T, Ueda H. Olfactory nerve response of masu salmon (*Oncorhynchus masou* Brevoort) and rainbow trout (*O. mykiss* Walbaum) to clove oil and MS-222. Aquaculture Research. 2008; 39(10):1019-1027.
26. Ross LG, Ross B. Anesthetics and sedative techniques fro aquatic animals. Blackwell Science, Oxford, 1999, 159.
27. Sladky KK, Swanson CR, Stoskopf MK, Loomis MR, Lewbart GA. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). American journal of veterinary research. 2001; 62(3):337-342.
28. Endo T, Ogishima K, Tanaka H, Ohshima S. Studies on anesthetic effect of eugenol in some fresh water fishes. Bulletin of the Japanese Society of Scientific Fisheries. 1972; 38(7):761-767.
29. Hikasa Y, Takase K, Ogasawara T, Ogasawara S. Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopemal sodium in the carp, *Cyprinus carpio*. Japanese Journal of Veterinary Science. 1986; 48:341-351.
30. Waterstrat PR. Induction and recovery from anesthesia in channel catfish *Ictalurus punctatus* fingerlings exposed to clove oil. Journal of the World Aquaculture Society. 1999; 30(2):250-255.
31. Iversen M, Finstad B, Mac Kinley RS, Eliiassen RS. 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. 2003; 221:549-566.
32. Burka JF, Hammell KL, Horsberg TF, Johnson GR, Rainnie DJ, Speare DJ. Drugs in salmonid aquaculture. Journal of Veterinary Pharmacology and Therapeutics. 1997; 20:333-349.
33. Marking LL, Meyer FP. Are better anesthesics needed in fisheries? Fisheries. 1985; 10(6):2-5.
34. Öğretmen F, Gökçek K. Comparative Efficacy of Three Anesthetic Agents on Juvenile African Catfish, *Clarias gariepinus* (Burchell, 1822). Turkish Journal of Fisheries and Aquatic Sciences. 2013; 13:51-56.
35. Öğretmen F, Gölbasi S, Kutluyer F. Efficacy of clove oil, benzocaine, eugenol, 2-phenoxyethanol as anaesthetics on shabbout fish (*Barbus grypus* Heckel, 1843). Iranian Journal of Fisheries Sciences. 2016; 15(1):470-478.
36. Walsh CT, Pease BC. The use of clove oil as an anaesthetic for the longfinned eel, *Anguilla reinhardtii* (Steindachner). Aquaculture Research. 2002; 33(8):627-635.
37. Woody CA, Nelson J, Ramstad K. Clove oil as an anaesthetic for adult sockeye salmon: field trials. Journal of Fish Biology. 2002; 60(2):340-347.
38. Holloway AC, Keene JL, Noakes DG, Moccia RD. Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. Aquatic Research. 2004; 35:1025-1030.
39. Mattson NS, Riple TH. Metomidate, a better anaesthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol and phenoxyethanol. Aquauculture. 1989; 83:89-94.
40. Hseu JR, Yeh SL, Chu YT, Ting YY. Comparison of efficacy of five anaesthetic goldlined sea bream, *Sparus sarba*. Acta Zoologica Taiwanica. 1998; 9:35-41.
41. Mylonas CC, Cardinaletti G, Sigelaki I, Polzonetti-Magni A. Comparative efficacy of clove oil and 2-phenoxyethanol as anaesthetics in aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus auratus*) at different temperatures. Aquaculture. 2005; 246:467-481.
42. Roubach R, Gomes LC, Fonseca F, Luis Val A. Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). Aquaculture Research. 2005; 36:1056-1061.
43. Weber RA, Peleterio JB, Garcia Martin LO, Aldegunde M. The efficacy of 2- phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup, 1858). Aquaculture. 2009; 288:147-150.
44. Heo GJ, Shin G. Efficacy of benzocaine as an anaesthetic for Crucian carp (*Carassius carassius*). Veterinary Anaesthesia and Analgesia. 2010; 37:132-135.
45. Smit GL, Hattingh J. Anaesthetic potency of MS 222 and neutralized MS 222 as studied in three freshwater fish species. Comparative Biochemistry and Physiology. 1979; 62:237-241.
46. Limsuwan C, Grizzle JM, Plumb JA. Etomidate as an anesthetic for fish: its toxicity and efficacy. Transactions of the American Fisheries Society. 1983; 112:544-550.
47. Velisek J, Svobodova Z, Piackova V. Effects of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*). Acta Veterinaria Brno. 2005; 74:139-146.
48. Sudagara M, Mohammadizarejabada A, Mazandarania R, Pooralimotlagha S. The efficacy of clove powder as an anesthetic and its effects on hematological parameters on roach (*Rutilus rutilus*). Journal of Aquaculture Feed Sciences Nutrion. 2009; 1:1-5.
49. Pawar HB, Sanaye SV, Sreepada RA, Harish V, Suryavanshi U, Tanu *et al*. Comparative efficacy of four anaesthetic agents in the Yellow Seahorse, *Hippocampus kuda* (Bleeker, 1852). Aquaculture. 2011; 311:155-161.
50. Malmström BG, Aasa R. The nature of the CuAcenter in cytochrome c oxidase. FEBS letters. 1993; 325(1-2):49-52.