AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 11, No 4, pp 323-326, 2019
DOI: 10.15547/ast.2019.04.054

Production Systems

Induction and recovery from anaesthesia in fry of European catfish (*Silurus glanis* L.) exposed to clove oil

V. Krasteva*, A. Zaikov

Institute of Fisheries and Aquaculture, 4003 Plovdiv, Bulgaria

(Manuscript received 20 June 2019; accepted for publication 11 August 2019)

Abstract. *The purpose of the study is to establish the efficacy of different concentrations of clove oil and the time needed for induction and recovery from anesthesia of European catfish (Silurus glanis L.) fry. The experiment was carried out in laboratory conditions with 50 specimens with body weight 1.11±0.25g and body length 5.45±0.60cm. For the aim of this paper the following five concentrations of clove oil are used: 0.01 ml.l^{-1}, 0.02 ml.l^{-1}, 0.03 ml.l^{-1}, 0.04 ml.l^{-1} and 0.05 ml.l^{-1}. At the lowest concentration (0.01ml.l^{-1}) the effect is sedative and the fry do not reach the phase of complete immobilization. The application of 0.02 ml.l^{-1} concentration of clove oil is not beneficial for practical usage, with only 30% of the fish reaching phase 4 of anesthesia. At the highest concentrations (0.04 ml.l^{-1} and 0.05 ml.l^{-1}) all of the fry were anesthetized for a short time, but the process of recovery was not successful for all of the fry. All of the fry reach phase of anesthesia and recovery without loses at 0.03 ml.l-1. This concentration is advisable for anesthesia of European catfish fry during different manipulations.*

Keywords: European catfish *(Silurus glanis* L.*)*, fry, anaesthesia, clove oil

Introduction

Anesthesia has been used in fish farming for over 60 years (Velisek et al., 2006; Brown, 2011) as a method of reducing stress and protecting fish from injury, gaining even wider application in aquaculture (Hamachkova et al., 2006; Hajek et al., 2006; Zaikov et al., 2008; Mikodina et al., 2011). Currently, an increasing number of anesthetizing agents are being applied in fish aquaculture. Some of them have synthetic origin, such as MS-222, benzocaine and 2-phenoxyethanol (Marking and Mayer, 1985; Gilderhus and Marking, 1987; Coyle et al. 2004; Hamachkova et al., 2004, 2006; Mikodina et al., 2011; Githukia et al., 2016; Hoseini et al., 2018; Priborsky and Velisek, 2018). Others are natural products, such as essential oils of plant origin (Hoseini et al., 2018). They are considered an alternative to synthetic preparations (Readman et al., 2013), mainly due to lower prices, easy access, efficiency and safety of the environment.

The most commonly used anesthetic of natural origin in aquaculture is the clove oil, which Anderson et al. (1997) determine as an alternative to MS-222. It covers completely the qualities of a good anesthetic described by Coyle et al. (2004) and Brown (2011). Some of them include: the application of low concentrations, quick induction and recovery of anesthesia, soluble in water and alcohol, etc. Clove oil is a natural product with application in medicine, cosmetics and food industry (Taylor and Roberts, 1999; Mikodina et al., 2011). According to Bullerman et al. (1977) and Hamachkova et al. (2006) it also has antibiotic, antiseptic, antimycotic and antibacterial effect, blocks the action of common aflatoxins in compound feed, does not accumulate in fish tissues and does not cause allergies.

Clove oil is dark brown liquid, which is distilled from the buds, leaves and stems of the clove tree *Eugenia caryophyllata*. The interest in clove oil in fish farming as an anesthetic is high. For this reason, there are many studies on its effects with different species of fish: rainbow trout (*Oncorhynchus mykiss*) (Anderson et al., 1997; Hamackova et al., 2006; Yildiz et al., 2013), carp (*Cyprinus carpio)* (Velisek et al., 2005; Hajek et al., 2006; Kamble et al., 2014), pike (*Esox lucius*) (Peake, 1998; Hamackova et al., 2006; Zaikov et al., 2008;), sander (*Sander lucioperca*) (Rozynski et al., 2016); channel catfish (*Ictalurus punctatus*) (Waterstrat, 1999; Small, 2003; Small and Chatakondi, 2005; Zaikov et al., 2009), African catfish (*Clarias gariepinus)* (Ogretmen and Gokçek, 2013; Adeshina et al., 2016; Diyaware et al., 2017), etc.

The research on the anesthetic effect of clove oil on European catfish are relatively few (Velisek et al., 2006; Gokcek et al., 2016) with no studies on its effect on the fry stage of development. Velisek et al. (2006) determined a concentration of 0.028 mg.l^{-1} as the most efficient and not having negative side effects. Zaikov and Iliev (2007) conducted an experiment related to the anesthesia of juveniles of European catfish. The aim of this study is to establish the efficacy of different concentrations of clove oil and the time needed for induction and recovery from anesthesia of European catfish fry.

Material and methods

The research was carried out at the Institute of Fisheries and Aquaculture, Plovdiv in 2017. For the aim of the study 50 specimens were used with an average body weight and length showed in Table 1. The fish were cultured in a flow-through production system at water temperature 23.0±1.5ºC.

*e-mail: vasilka_mitrova@abv.bg

Table 1. Body length and body weight of the fry

	Total body length (TL), cm	Body weight (BW), g
X	5.45	1.11
SD	0.60	0.25
Cv, %	11.86	22.87
Lim.	4.51-6.74	0.74-1.52

The experiment was conducted with the following five concentrations of clove oil: 0.01 ml.l^{-1}, 0.02 ml.l^{-1}, 0.03 ml.l^{-1}, 0.04 ml.l-1 and 0.05 ml.l^{-1}. Prior to the preparation of the solution, the clove oil was dissolved in ethanol (95%) in a 9:1 ratio and added to the experimental tanks while stirring. The fry were treated in small tanks with volume of 10L without aeration. In each tank, with different concentration of clove oil, were set 10 specimens. The count of the specimens and the time in which they enter the following phase of anesthesia or recovery was registered. The anesthetic effect of clove oil was tested at exposure of 15min, with water temperature of 23.5-24.0ºC and dissolved oxygen 7.5-7.7 mg.l^{-1}. For the recovery from the anesthesia the fish were transferred into a tank with micro compressors. The time for the induction of anesthesia and the recovery was measured with a stopwatch. The behavior of the fish was observed and analyzed according to the phases of anesthesia described by Hamackova et al. (2006) shown in Table 2. The main focus of the research was the time needed for the fry of European catfish to reach phases 2, 3, 4 and 5 of anaesthesia.

Table 2. Phases of induction and recovery from anesthesia

Phase	Anaesthetizing	Phase	Recovery
	Behaviour of the fish		Behaviour of the fish
1	Acceleration of the opercular movements, increased respiratory activity.	1	Uncoordinated locomotion.
2	Decreased respiratory activity accompanied by uncoordinated locomotion.	2	Normal position of the body. Normal locomotion activity.
3	Loss of equilibrium, decreased opercular movements, the fish still react to strong external stimuli.		
4	Complete immobilization, the fish lie on the bottom and do not react to handling.		
5	Complete cessation of opercular movements, the fish die if they remain in the solution.		

Results and discussion

The fish exposed to the lowest concentration of 0.01 ml.l^{-1} did not reach phase 4 of complete immobilization (Figure 1). At the 8th min 6 specimens entered phase 2 of uncoordinated locomotion and decreased respiratory activity. At the 15th min 9 specimens reached phase 3 of loss of equilibrium, lying on the bottom, moving their fins without swimming and reacting to external stimuli. The stage of recovery was relatively short. At the 4th min 7 fish reached phase 1 of recovery, characterized by weak and uncoordinated movements. At the 5th min 8 specimens were swimming actively and at the 10th min all of the fry regained normal locomotion activity.

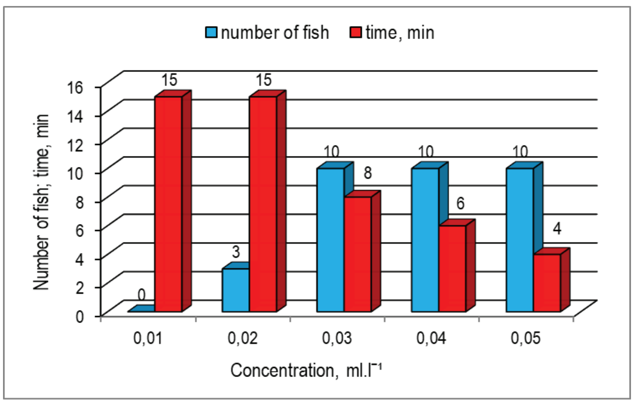

Figure 1. Time and number of fry entering phase 4 at different concentrations of clove oil at maximum exposure of 15min

At a concentration of 0.02 ml.l^{-1}, at the 12th min 6 specimens entered phase 2 of decreased respiratory activity. At the 15th min 7 specimens lost equilibrium, laid on the bottom and turned around periodically. Only 3 specimens reached phase 4 of complete anesthesia and they were not reacting to external stimuli (Figure1). As opposed to the results mentioned by Zaikov and Iliev (2007) in the present study, the concentration of 0.02 ml.l^{-1} had an anesthetic effect for some of the treated fish.

The stage of recovery took the same period of time (10min) as in the lowest concentration of 0.01 ml.l^{-1}. At the 2nd min 8 specimens made uncoordinated movements. At the 4th min 6 of the fry had decreased locomotion activity and 4 specimens regained normal body position and activity.

The behavior of the fry exposed to concentration of 0.03 ml.l^{-1} of clove oil was completely opposite to the behavior of the fish exposed to the lower concentrations of the oil. They responded to the anesthetic immediately after they were placed in the tank with rapid, accelerating movements. The first 2 fish were anesthetized after 3min. At the 7th min 6 specimens reached phase 4 of complete immobilization, while 4 specimens remained in phase 3 of loss of equilibrium and still reacted to strong external stimuli. At the 8th min all of the catfish fry were completely anesthetized. At 0.03 ml.l^{-1} concentration of clove oil the time needed for regaining normal position of the body of all fish was 18min, which is longer compared to the lower concentrations where the fish recovered for 10min.

The behavior of the fish placed at concentration of 0.04

$ml.l^{-1}$ was similar to 0.03 $ml.l^{-1}$ – the fish reacted to the solution with vigorous movements. 3 fish were anesthetized for 2 min, 5 specimens lost equilibrium and 2 fish remained in phase 2 of decreased respiratory activity. At the 5th min 6 specimens reached phase 4 of complete immobilization, while 4 lost equilibrium, laid on the bottom and reacted to external stimuli. At the 6th min all of the fry reached phase 4 of complete immobilization. The time needed for reaching phase 4 of complete anesthesia was shorter than at 0.03 $ml.l^{-1}$ concentration of clove oil. The stage of recovery of all of the fry took 20min, with 1 fish remaining in phase 5 of complete cessation of opercular movements.

At concentration of 0.05 $ml.l^{-1}$ of clove oil the response of the fry was vigorous movements. In comparison with the previous two concentrations (0.03 $ml.l^{-1}$and 0.04 $ml.l^{-1}$), at the highest concentration of clove oil the reaction of the fish to the solution was the most vigorous. At the 3rd min 5 specimens were anesthetized, the rest of the fish remained in phase 3 lying on the bottom and still reacting to external stimuli. At the 4th min all of the fry reached phase 4 of complete anesthesia.

The stage of recovery was the longest for all experimental concentrations of the clove oil. At the 10th min 7 specimens entered phase 2 of decreased locomotion activity and 3 fish remained in phase 1. After 25min 6 specimens regained normal position of the body and normal locomotion activity. For 4 fish the process of anesthesia was lethal.

The results from the experiment showed that the lowest concentration of 0.01 $ml.l^{-1}$ had sedative and not anesthetizing effect on the fry, as they do not reach phase 4 of complete immobilization. The sedative effect was manifested in phase 3 of the process of anesthesia in which the fish were immobilized but reacted to external stimuli. The efficacy of the concentration of 0.02 $ml.l^{-1}$ of clove oil is approximately the same with that of 0.01 $ml.l^{-1}$ with only 3 specimens anesthetized and the rest remaining in sedative state.

The anesthesia of all specimens, as well as the subsequent recovery without losses, was achieved at concentration of 0.03 $ml.l^{-1}$ of clove oil. At the concentrations 0.04 $ml.l^{-1}$ and 0.05 $ml.l^{-1}$ all the fry entered phase 4 of anesthesia in relatively short period of time, respectively for 6min and 4min. The stage of recovery was the longest and it is related to the mortality rate at the highest concentrations. At the higher experimental concentrations (0.03 $ml.l^{-1}$, 0.04 $ml.l^{-1}$ and 0.05 $ml.l^{-1}$) the individuals immediately responded after they were placed in the tank. The highest concentrations induced the highest movement rate.

Based on the results, it was established that the time needed for anesthesia significantly decreased with an increase in the concentration of clove oil. The lowest concentrations at which fish do not reach phase 4 of complete anesthesia make an exception. On the other hand, the time needed for recovery increased with an increase in the anesthetic concentration. These correlations are confirmed by Gokcek et al. (2016) for the three types of anesthetics: clove oil, 2-phenoxyethanol and benzocaine and also by other authors about different species of fish (Zaikov and Iliev, 2007; Zaikov et al., 2008; Mikodina et al., 2011).

Zaikov and Iliev (2007) researched the efficacy of clove oil as an anesthetic for juveniles of *Silurus glanis* from two weight groups (WG I: 11.92±2.46g and WG II: 128.2±1.65g) which are treated individually. The individuals from WG I, exposed to concentration 0.02 $ml.l^{-1}$ of clove oil, lost equilibrium within 4 min, but opposite to the results from the present study, they did not reach phase 4 of complete immobilization. The effect of treating the fish from WG II was only decreased locomotion activity.

At the concentration of 0.04 $ml.l^{-1}$ of clove oil the fish weighing 11.92g reached phase 4 of anesthesia within 3.95-5.15 min while the specimens weighing 128.2g were anesthetized within 4.25-7.17 min. (Zaikov and Iliev, 2007). The recovery took 3.75-8.0 min for WG I and 5.0-9.5 min, respectively. During the present research 20min were necessary for the recovery of all specimens. Therefore, at this concentration the three weight groups of European catfish reached phase 4 of complete anesthesia, but the period of time was different. The recovery of the fry was the longest (20min), while the juveniles of European catfish from the two weight groups regained their normal locomotion activity within 8 and 13min, respectively (Zaikov and Iliev, 2007).

Based on the comparison of the present results with these by Zaikov and Iliev (2007), it can be concluded that the weight of the fish directly affects the time needed for the process of anesthesia and recovery. At the same concentrations smaller fish reach phase 4 of complete anesthesia faster and the period of recovery is longer. The larger the individuals, the faster they are anesthetized and the slower they recover. Similar correlation between age, weight of the specimens and the concentration of clove oil for anaesthetizing various fish species has been established by other authors (Keene et al., 1998; Hoskonen and Pirhonen, 2004; Bosworth et al., 2007).

In regard to the concentration, it can be assumed that with increasing the concentration of the clove oil, the time necessary for anesthesia decreases and the period of recovery increases (Hajek et al., 2006; Kamble et al., 2014).

Conclusion

The results from the present study showed that the lowest experimental concentration of 0.01 $ml.l^{-1}$ of clove oil did not have an anesthetizing effect on the fry of European catfish. The lowest concentration induced sedative state in which the specimens lost equilibrium and did not react to external stimuli. The effect of concentration 0.02 $ml.l^{-1}$ for exposure of 15min was 70% sedative and 30% anesthetizing. At the application of concentration of 0.04 $ml.l^{-1}$ and 0.05 $ml.l^{-1}$ all of the fry were anesthetized in relatively short period of time, but some specimens did not recover successfully. Thus, these concentrations are not advisable for anesthesia of fry of European catfish. The most applicable concentration of clove oil is 0.03 $ml.l^{-1}$ at which all of the fry reached phase 4 of complete anesthesia and recovered without loses.

References

Adeshina I, Adewale A and Yusuf O, 2016. *Eugenia cayrophyllata* oil as anesthetic in cultured African catfish (*Clariasgariepinus*, Burchell 1822) juveniles. Nigerian Journal of Fisheries and Aquaculture, 4, 8-17.

Anderson W, McKinley R and Colavecchia M, 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management, 17, 301-307.

Bosworth B, Small B, Gregory D, Kim J, Black S and Jerret A, 2017. Effect of rested-harvest using the anesthetic aqui-s on channel catfish, *Ictalurus punctatus*, physiology and fillet quality. Aquaculture, 262, 302-318.

Brown L, 2011. Anaesthesia for fish. Vietfish, 8, 1-3.

Bullerman B, Lieu Y and Seir A, 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils; cinnamic aldehyde and eugenol. Journal of Food Science, 4, 1107-1116.

Coyle S, Durbalov M and Tidwell J, 2004. Anesthetic in aquaculture. Southern Regional Aquaculture Center publication, 3900, 1-6.

Diyaware M, Suleiman S, Akinwande A and Aliyu M, 2017. Anesthetic effects of clove (*Eugenia aromaticum*) seed extracton *Clarias gariepinus* (Burchell, 1822) fingerlings under semi-arid conditions. Journal of Agricultural Sciences, 62, 411- 421.

Githukia C, Kembenya E and Opiyo M, 2016. Anaesthetic effects of sodium bicarbonate at different concentrations on African catfish (*Clarias gariepinus*) juveniles. Journal of Aquaculture Engineering and Fisheries Research, 2, 151-158.

Gokcek K, Ogretmen F and Kanyilmaz M, 2016. Efficacy of clove oil, 2 phenoxyethanol and benzocaine on European catfish (*Silurus glanis* Linnaeus, 1758). Turkish Journal of Fisheries and Aquatic Sciences, 16, 129-133.

Guilderhus P and Marking L, 1987. Comparative efficacy of 17 anaesthetic chemicals on rainbow trout. North American Journal of Fisheries Management, 7, 288-292.

Hajek G, Klyszejko B and Dziaman R, 2006. The anaesthetic effect of clove oil on common carp (*Cyprinus carpio*). Acta Ichtyologica et Piscatoria, 36, 93-97.

Hamachkova J, Kouril J, Kozak P and Stupka Z, 2006. Clove oil as an anaesthetic for different freshwater fish species. Bulgarian Journal of Agricultural Science, 12, 185-194.

Hamachkova J, Lepicova A, Kozak P, Stupka Z, Kouril J and Lepic P, 2004. The efficacy of various anaesthetics in tench (*Tinca tinca*) related to water temperature. Veterinary Medicine Czech Republic, 49, 467- 472.

Hoseini S, Mirghaed A and Yousefi M, 2018. Application of herbal anaesthetics in aquaculture. Reviews in Aquaculture, 0, 1-15.

Hoskonen P and Pirhonen J, 2004. Temperature effects on anaesthesia with clove oil in six temperate-zone fishes. Journal of Fish Biology, 64, 1136-1137.

Kamble A, Saini V and Ojha M, 2014. The efficacy of clove oil as an anesthetic in common carp (*Cyprinus carpio*) and its potential metabolism reducing capacity. International Journal of Fauna Biological Studies, 1, 01-06.

Keene J, Noakes D, Moccia R and Soto C, 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus-mykiss* (Walbaum). Aquacuatic Research, 29, 89-101.

Mikodina E, Kouril J, Sedova A and Hamackova J, 2011. Manual for using of the anesthetics "clove oil" in aquaculture. The Russian Institute of Fisheries and Oceanography, Publishing, 1-58 (Ru).

Ogretmen F and Gokçek K, 2013. Comparative efficacy of three anesthetic agents on juvenile African catfish, *Clarias gariepinus* (Burchell, 1822). Turkish Journal of Fisheries and Aquatic Sciences, 13, 51-56.

Peake S, 1998. Sodium bicarbonate and clove oil as potential anaesthetics for non-salmonid fishes. North American Journal of Fisheries Management, 18, 919-924.

Priborsky A and Velisek J, 2018. Review of three commonly used fish anesthetics. Reviews in Fisheries Science and Aquaculture, 26, 417- 442.

Readman GD, Owen SF, Murrell JC and Knowles TG, 2013. Do fish perceive anaesthetics as aversive? PLOS ONE, 8, 737-739.

Rozynski M, Ziomek E, Zakes K and Zakes Z, 2016. Propiscin-A safe anaesthetic for pikeperch (*Sander Lucioperca* L.). Acta Veterinaria Hungarica, 64, 415-424.

Small B and Chatakondi N, 2005. Routine measures of stress are reduced in mature Channel catfish during and after AQUI-S anesthesia and recovery. North American Journal of Aquaculture, 67, 72-78.

Small B, 2003. Anesthetic efficacy of methomidate and comparison of plasma cortisol responses to tricaine methane sulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus.* Aquaculture, 218, 177-185.

Taylor PW and Roberts SD, 1999. Clove oil: An alternative anesthetic for aquaculture. North American Journal of Aquaculture, 61, 150-155.

Velisek J, Svobodova Z, Piackova V, Groch L and Nepejchalova L, 2005. Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.). Veterinary Medicine Czech Republic, 50, 269-275.

Velisek J, Wlasow T, Gomulka P, Svobodova Z, Novotny L and Ziomek E, 2006. Effects of clove oil anaesthesia on European catfish (*Silurusglanis*). Acta Veterinaria Brno, 75, 99-106.

Waterstrat P, 1999. Induction and recovery from anesthesia in channel catfish (*Ictalurus punctatus*) fingerlings exposed to clove oil. Journal of the World Aquaculture Society, 30, 250-255.

Yildiz M, Kayim M and Akin S, 2013. The anesthetic effects of clove oil and 2-phenoxyethanol on rainbow trout (*Oncorhynchus mykiss*) at different concentrations and temperatures. Iranian Journal of Fisheries Sciences, 12, 947-961.

Zaikov A and Iliev I, 2007. The efficacy of clove oil as an anesthetic for wels (*Silurus glanis*, L). Suleyman Demirel Universitesi Egirdir Su Urunleri Fakultesi Degrisi, 3, 30-36.

Zaikov A, Iliev I and Hubenova T, 2008. Induction and recovery from anesthesia in pike (*Esoxlucius* L.) exposed to clove oil. Bulgarian Journal of Agricultural Science, 14, 165-170.

Zaikov A, Iliev I and Hubenova T, 2009. The efficacy of clove oil as an anaesthetic for channel catfish (*Ictalurus punctatus* Raf.). In: Proceeding of the IVth International Conference on Fish Farming, pp. 365-370.