

Anaesthesia procedure for two species of Baikal golomyankas (Cottoidei: Comephoridae)

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ABSTRACT. This study aimed to develop the anaesthesia procedure for *Comephorus baicalensis* (Pallas, 1776) and *Comephorus dybowskii* Korotneff, 1904 to eliminate stress effects during various manipulations in aquarium exposition. Clove oil preparation and lidocaine solution were tested in various concentrations as anaesthetics. The experimental studies revealed optimal lidocaine concentration of 600 mg/L for *C. baicalensis* and 450 mg/L for *C. dybowskii*. The concentration of clove oil sufficient to achieve the rest phase was 150 mg/L for both species. Clove oil preparation took a faster effect on golomyankas, and the fish recovered from anaesthesia in a shorter period than after anaesthesia with lidocaine. However, the presence of the excitation phase and excessive mucus secretion with the use of clove oil preparation indicated that fish were under stress. At this stage of research, the use of lidocaine for anaesthesia of golomyankas during regular manipulations is preferable.

Keywords: Cottoidei, Comephoridae, clove oil, eugenol, lidocaine, anaesthesia

1. Introduction

Scientific interdisciplinary research based on the experimental aquarium complexes can significantly optimize many aspects of modern biotechnology for the cultivation of aquatic organisms. The aquarium exposition of Baikal Museum of ISC SB RAS includes various fish species, the manipulations with which during the catch, transportation and subsequent adaptation to artificial conditions are the main stress factors (Pastukhov, 2010). Endemic members of the family Comephoridae (suborder Cottoidei) are the most numerous non-commercial species in Lake Baikal (Taliev, 1955; Koryakov, 1972; Starikov, 1977; Sideleva, 1995). The keeping of these species in artificial conditions is necessary not only for demonstration purposes but also for many scientific studies. In this regard, the development of the optimal keeping conditions for Baikal golomyankas (oilfish), big *Comephorus baicalensis* (Pallas, 1776) and small *Comephorus dybowskii* Korotneff, 1904, is a relevant area of research.

A fundamentally new method of maintaining the activity of Baikal golomyankas at normal atmospheric pressure increased their life expectancy in conditions

of aquarium exposition from three-four days to one month and a half (Didorenko et al., 2020). In the course of these experiments, the refusal of golomyankas to eat become one of the main problems. The problem of animals refusing to self-feed in artificial and/or experimental conditions is solved by using forced feeding (Henao Duque and Núñez Rangel, 2016; Siers et al., 2018). The scientific literature describes methods of forced feeding using various anaesthetics (Aas et al., 2017; Brezas and Hardy, 2020), which actions primarily involve sedation, immobilization, or paralytic effects. Moreover, anaesthesia of fish is becoming an ethical requirement in various field and experimental studies to reduce stress (Sloman et al., 2019).

Fish are anaesthetised with a wide range of drugs, including MS -222 (tricaine methane sulfonate), benzocaine hydrochloride, metomidate, clove oil (AQUI-S 20E, eugenol or 4-allyl-2-methoxyphenol), and lidocaine hydrochloride. In many countries, MS -222 remains the only legally approved anaesthetic for use in edible fish and is, therefore, commonly used for fish transport (Topic Popovic et al., 2012) and invasive surgical procedures (Priborsky and Velisek, 2018). The listed agents have also been described for marine and freshwater members of the suborder Cottoidei, which

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include the Baikal golomyankas studied in this paper: MS -222 for euthanasia (Sapozhnikova et al., 2021), benzocaine for anaesthesia during measurements, labelling procedures (Goto, 1986) and euthanasia (Lau et al., 2019), lidocaine for euthanasia (Kim et al., 2007), and clove oil for anaesthesia (Ovidio et al., 2009; Rocaspana and Aparicio, 2017). Unfortunately, these studies do not describe phases/stages of anaesthesia and the features of fish recovery but indicate that fish were released into the natural environment (Goto, 1986; Rocaspana and Aparicio, 2017) where they were observed for several months.

Some of these agents have many significant disadvantages. MS-222 is expensive and has been associated with numerous side effects, including hypoxia, increased catecholamine and cortisol levels (King et al., 2005; Carter et al., 2011) as well as suppressive effects on peripheral and central neurons (AVMA Guidelines..., 2020; Putland et al., 2020). Benzocaine or metomidate may also be cost-prohibitive, as some fish species require higher concentrations of these agents for anaesthesia (AVMA Guidelines..., 2020). Therefore, clove oil and lidocaine may be considered an alternative anaesthetics for Baikal golomyankas because they are effective in a wide range of water temperatures, available in many pharmacies and cost little (Javahery et al., 2012; Çağıltay et al., 2017; Putland et al., 2020).

Lidocaine is effective for rapid anaesthesia of fish (Abbas et al., 2006) and shows promise as an anaesthetic in surgical procedures (Collymore et al., 2014). The main advantage of clove oil is the absence of an excitation phase, the short transition of fish into resting phases and the restoration of natural behavioural responses (Fernandes et al., 2017; Soldatov, 2021). Clove oil has also been proven to be an effective alternative for sedation of larval, juvenile and adult fish (tambaqui, *Colossoma macropomum* (Roubach

et al., 2005), guppy, *Poecilia vivpara* (Bolasina et al., 2017), and freshwater angelfish, *Pterophyllum scalara* (Oliveira et al., 2019)) compared to other available anaesthetics. However, there is a considerable variation in response to clove oil and lidocaine between species, with some species requiring higher doses or secondary measures to ensure a lack of stress. Therefore, the choice of anaesthetic should be individualised for each fish species (Martins et al., 2019). Accordingly, this study aims to develop an anaesthetic procedure using clove oil and lidocaine for two species of Baikal golomyankas to eliminate stress effects during different manipulations in museum exposition: effectiveness for sedation and immobilization effects and the optimal safe doses of agents.

2. Materials and methods

Fish were caught during the ice period from 2019 to 2020 in Listvennichny Bay at depths of 400 to 300 m. The gear, fishing method, conditions for transporting and keeping fish in aquariums were previously described in detail (Didorenko et al., 2020). The experiments were carried out with adult individuals that after 96 hours of adaptation did not show any signs of loss of balance and motor activity. The total zoological length of small golomyanka ranged from 105 to 125 mm, and that of big golomyanka – from 185 to 200 mm. All individuals of small golomyanka (three specimens) were at the bottom, occasionally swimming from place to place. The females of big golomyanka (11 specimens) were at the bottom in the ‘head-down’ position and made characteristic undulatory movements with their fins (Fig. 1).

The staying of golomyankas at the bottom in this position was previously described based on the visual observations (Abramov et al., 1979), and we took it as ‘normal/natural’ behaviour during the experiments.



Fig.1. *Comephorus baicalensis* (Pallas, 1776) in an aquarium.

Working solutions for anaesthesia were prepared several minutes before the experiments. The following anaesthetics were used in the study:

1. The 2% lidocaine solution (lidocaine “Bufus”, Renewal, Russia). The working solution was prepared in a 6 L plastic container with the addition of water from the aquarium, in which fish were kept (inhabited aquarium). The volume of the working solution for both golomyanka species was 4.5 L. The experimental concentrations of lidocaine are shown in Table 1.

2. The pure essential clove oil (“Naturalnyie Masla”, Russia) comprised 82% eugenol (active ingredient) that was dissolved in 95% ethanol to the final concentration of 67 mg/ml, taking into account the recommendations in (Devi and Kamilya, 2019). To obtain a working solution, 10 ml of the resulting concentrated solution of clove oil in ethanol were added to 4.5 L of water from an inhabited aquarium. The solution was prepared in a 6 L plastic container. The volume of the solution for both golomyanka species was 4.5 L with a final clove oil concentration of 150 mg/L.

Anaesthesia. The individual was transferred from the inhabited aquarium to the container with anaesthetic using a 1.5 L plastic rectangular container with rounded corners. Simultaneously, contact of the fish with warm (10–13°C) air in laboratory was excluded. The fish was transferred to the anaesthetic solution with the minimum amount of water. The container with anaesthetic was placed floating in the container with running Baikal water to maintain the temperature of 3.5 to 4.5°C and covered with a lid to prevent fish from jumping out. Continuous aeration of the solution was carried out using a compressor. The behavioural responses, in particular, decrease in the intensity of movements or complete immobilization (rest phase), served as the criterion for achieving a satisfactory degree of anaesthesia. Additionally, the absence of the reaction to the touch of the manipulator, a needle with a plastic coating, was recorded. The duration of the anaesthesia phases was recorded in minutes from the end of the previous phase to the start of the next one. The anaesthetic was washed off in individual plastic containers with water from the inhabited aquarium. The containers covered with a lid were placed floating to the container with running water and temperature of 3.5 to 4.5°C. Water aeration was continued. After the partial restoration of motor activity, the fish was returned to inhabited aquariums.

Recovery of golomyankas after anaesthesia. Behavioural reactions of fish were observed in washing containers and inhabited aquariums: the ability to maintain balance, movements of opercula and fins as well as swimming. After anaesthesia, fish were observed in inhabited aquariums for up to one month and a half.

3. Results and discussion

Anaesthesia with lidocaine. In the solutions with concentrations of 150 and 300 mg/L, fish of both species did not reduce their motor activity within one

Table 1. Experimental concentrations of lidocaine

Final concentration of lidocaine, mg/L	Amount of 2% lidocaine in mL per 4.5 L of working solution
150	33.75
300	67.5
450	101.25
600	135.0

hour. With an increase in the lidocaine concentration in the working solution to 450 mg/L, big golomyanka did not show complete immobilization, and the rest phase occurred in small golomyanka within 30 to 40 minutes. Therefore, the necessary sedation effect was achieved for small golomyanka at the lidocaine concentration of 450 mg/L. In big golomyanka, the rest phase occurred within 45 to 60 minutes only in the solution with the concentration of 600 mg/L.

In our experiments, the optimal concentrations of lidocaine were 450 mg/L within 30 to 40 minutes for small golomyanka and 600 mg/L within 45 to 60 minutes for big golomyanka. This is more than two and three times, respectively, higher than the concentration of 200 mg/L recommended for the rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (Zavyalova et al., 2012). The sedation effect of both golomyanka species developed slower than in rainbow trout (Zavyalova et al., 2012). The excitation phase was absent in both golomyanka species. There were no visible changes in the skin of the fish during anaesthesia.

Previously, when lidocaine was tested on the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), the toxicity values differed and depended mainly on the concentration and time of exposure (Abbas et al., 2006). In the experiments on anaesthesia of rainbow trout, lidocaine was characterized by a long time of fish staying in the anaesthetic solution without harm to health (Zavyalova et al., 2012). Additionally, lidocaine had been previously been found to leave relatively low tissue residues and was not expected to pose any deferred hazards to fish (AVMA Guidelines..., 2020).

Anaesthesia with clove oil. The calculation of the concentration of clove oil optimal for golomyankas was based on that recommended for rainbow trout amounting to 500 mg/L (Zavyalova et al., 2012). At the initial stage of the experiment, a low concentration of 150 mg/L was chosen for both golomyanka species. At this concentration, the fish were completely immobilized within 3 to 15 minutes, and the required anaesthetic effect was achieved. Therefore, we did not carry out further experiments on the selection of other concentrations.

When the fish were placed into the anaesthetic solution, their motor activity increased for a short time, and their opercula moved rapidly; the fish opened their mouths wide and made ‘yawning’ and ‘coughing’ movements. The excitation phase passed into the rest phase within 3 to 15 minutes. The immobilized fish showed no anxiety during manipulations. However, the skin of all fish excessively secreted mucus.

In our experiments, the concentration of clove oil sufficient to achieve the rest phase was 150 mg/L for both golomyanka species. This was more than three times lower than the concentration recommended for rainbow trout amounting to 500 mg/L (Zavyalova et al., 2012). We recorded the excitation phase for both golomyanka species, as was previously indicated for other fish species (Zavyalova et al., 2012).

Recovery of fish after anaesthesia. All individuals survived successfully after the experiments and were viable for at least one and a half month. Evaluation of the recovery of fish after anaesthesia should be of special attention (Valentim et al., 2016). We recorded the recovery time for the ability of the fish to maintain balance and move after they were placed in the container for washing off from anaesthetic. The recovery stages were as follows (Table 2).

After anaesthesia with lidocaine, partial recovery of motor activity (stages I and II) occurred within 5 to 30 minutes in big golomyanka, and within 15 to 60 minutes in small golomyanka. Visual observation after placing the fish in inhabited aquariums revealed that the complete recovery of the ability to maintain balance and normalization of motor activity (stage III) occurred in both species within 3 to 8 hours after anaesthesia.

After anaesthesia with clove oil preparation, the first two recovery stages occurred in both species within 5 to 15 minutes, and stage III – within 1 to 3 hours.

Assumed effects of anaesthesia. In this study, clove oil and lidocaine yielded similar primary behavioural changes as those earlier observed with MS-222 (Cho and Heath, 2000; Putland et al., 2020). Compared to lidocaine, clove oil preparation took effect faster on golomyankas, and they recovered from anaesthesia for a shorter time. Although it has been suggested that the listed anaesthetic mechanism involves dilatation of neuronal cell membranes, the exact action of their effects in different fish has not been reported yet (Burka et al., 1997). However, it is known that anaesthesia may involve varying degrees of loss of consciousness and anaesthetic depth, according to the Guedel's classification: from analgesia and disorientation (often local anaesthesia without loss of consciousness) to sedation and immobilization with the paralytic effect, which may also involve wide-awake anaesthesia with loss of consciousness. Nevertheless, depending on the species and anaesthetics, some stage components are unknown (Harms, 1999; Stetter, 2001). On the contrary, some signs attributed to anaesthetic effects are reactions to stress.

After anaesthesia with clove oil preparation, the presence of the excitation phase and excessive secretion of skin mucus indicated that the fish were under stress. The increased secretion of skin mucus is one of the most common reactions of fish to stress (Vatsos et al., 2010; Fernández-Alacid et al., 2019). Previous studies in other species showed that low concentrations of clove oil caused a slow anaesthetic effect, contributing to the increase in glucose and cortisol levels in plasma (Hoseini and Nodeh, 2013; Mirghaed et al., 2018), i.e. stress. At the same time, fish exposed to high

Table 2. Recovery stages of Baikal golomyankas after anaesthesia.

Stages	Recovery of motor activity
I	The sedation effect decreased in the washing container. The fish stayed motionless, gradually starting to move with their opercula and fins. There were no reactions to the touch of the manipulator.
II	The fish started to swim but still could not maintain balance. There were weak reactions to the touches.
III	Complete recovery of golomyankas took place in inhabited aquariums. The body of the fish was in a natural position; they kept their balance, swam and avoided being touched.

concentrations can make intensive movements of the opercula (Neiffer and Stamper, 2009).

In our experiments, these symptoms were manifested in both golomyanka species: when the fish were placed to the solution of clove oil preparation, there was a short-term increase in their motor activity; the fish rapidly moved their opercula, opened their mouths wide and made 'yawning' and 'coughing' movements. Clove oil covers gill epithelium, can block the diffusion of gases (Sladky et al., 2001) and cause damage to the gills (Waristha et al., 2011). The concentration of clove oil of 150 mg/L was sufficient to achieve the rest phase in golomyankas. Nevertheless, the excessive mucus secretion indicated stress in the fish, and 'cough' evidenced that it is high for them. Baikal golomyankas are comparatively inactive fish. The area of the respiratory surface of gills in golomyankas per body weight is from five to six times smaller than in more active benthopelagic Baikal members of the genus *Cottocomephorus* (Jakubowski, 1996). In this respect, the gills of golomyankas may be more sensitive to the effects of clove oil than, for example, the gills of rainbow trout as active swimmers, and clove oil concentration may correlate directly with reduced ventilation and loss of equilibrium but may not provide sufficient sedation.

In our experiments, anaesthesia with lidocaine did not cause excessive mucus secretion, inflation of opercula and 'coughing' in fish. Lidocaine is less aggressive than clove oil (Readman et al., 2013), and no histological changes indicating its high toxicity were identified in fish (Collymore et al., 2016). Cold-water fish species respond to lower concentrations of anaesthetics than warm-water ones (Coyle et al., 2004). Moreover, the skin thickness and the presence of scales influence the anaesthetic effect (Ferreira et al., 1984). Thus, thin skin and the absence of scales in Baikal golomyankas should have been the factors contributing to the shorter period of the onset of the rest phase and lower lidocaine concentration to achieve it. Lidocaine, thus, most likely resulted in an analgesic effect in the early stages, and sedation or immobilisation of the fish required a higher concentration and longer exposure

times. Moreover, the higher concentration could also be due to the physiological peculiarities of Baikal golomyanka. Lidocaine is highly soluble in lipids such as subcutaneous fat, and this explains its slow entry into the bloodstream (Rosenberg et al., 1986). A unique feature of big golomyanka is its high content of lipids (Kozlova and Khotimchenko, 2000), which explains the slow effect of lidocaine and the longer period of fish recovery after anaesthesia. To achieve the rest phase, lidocaine concentration for big golomyanka was higher than for small golomyanka. This can be also associated with the characteristics of these species because the lipid content in small golomyanka is much lower than in big golomyanka (Kozlova and Khotimchenko, 2000).

Conclusions

There is limited information about the effects of various analgesics on fish species in the field and experimental setting. The development of an anaesthesia procedure for Baikal golomyankas and the choice of optimal anaesthetics were caused by the need to use them for eliminating stress effects during various manipulations, in particular, forced feeding. The forced feeding procedure implies a certain frequency; thereby the safety of regular use of anaesthetics becomes the main problem. Although the use of anaesthesia for fish in experimental studies is now becoming an ethical requirement and should be a routine procedure, there is no data on the effectiveness of anaesthetics as well as their optimal and maximum safe doses for many fish species. In our experiments, clove oil preparation took a faster effect on golomyanka, and the fish recovered from anaesthesia in a shorter period than after anaesthesia with lidocaine. At the same time, the presence of the excitation phase and excessive mucus secretion with the use of clove oil indicated that the fish were under stress. At this stage, the use of lidocaine for anaesthesia of golomyankas during regular manipulations is preferable.

For further work, we propose that by combining clove oil and lidocaine with different properties, a more complete anaesthesia with different desired effects can be achieved than it can be possible with a single agent alone. Furthermore, synergistic effects between these different compounds could also allow a reduction in the dosage of each agent compared to individual administration, resulting in smoother induction, faster recovery and a lower incidence of adverse postponed effects. The results obtained will be used in future experimental studies on the physiology of *Comephorus* members. However, a better understanding of the effects of anaesthesia requires the incorporation of more modern methods, particularly changes in catecholamine and cortisol levels, suppressive effects on afferent neural activity, and transcriptome analysis.

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Conflict of interests

The authors declare that they have no competing interests.

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