BIO Web of Conferences **113**, 05038 (2024) https://doi.org/10.1051/bioconf/202411305038
INTERAGROMASH 2024

Assessment of the sedative effect of various anesthetics in Coregonus Peled (Gmelin, 1789) in aquaculture

Yulia Tolmacheva[1*], *Elena* Borisova[2], *Elena* Dzyuba[3], *Lyubov* Sukhanova[3], *Ivan* Nebesnykh[1], *Inna* Demyanovich[1], and *Konstantin* Demyanovich[2]

[1] Irkutsk GAU, Molodezhny settlement, Irkutsk district, Irkutsk region, Russia
[2] Shelekhovsky Veterinary Diagnostic Center, Shelekhov, Irkutsk region, Russia
[3] FGBUN Limnological Institute SB RAS, Irkutsk, Irkutsk region, Russia

Abstract: The sedative effect of four anesthetic agents in whitefish was studied. It is shown that the effect of anesthetics causes a number of consistent behavioral and physiological reactions in fish, reflecting the change in the state of their body. For most technological fish-breeding processes, it is necessary for fish to stay in a state of muscle relaxation while maintaining respiratory rhythm. These requirements are met by the sedation stage, which is observed with all types of anesthesia in peled. It has been established that the use of some of the tested drugs is incorrect in anesthesia in general in whitefish and in pelage in particular. Thus, the use of sodium thiopental is unacceptable, due to the uncontrolled suppression of the respiratory function of fish under the action of this agent. The use of lidocaine is characterized by prolonged induction, which does not correspond to the temporary norms of fish anesthesia. Among the tested drugs, propofol has sufficient anesthetic efficacy, which is confirmed by the temporary indicators of induction/ recovery, the depth and manageability of anesthesia, and low indicators of respiratory depression. Eugenol can serve as an alternative to propofol, which is cost-effective when working with mass material that requires large financial costs. In general, the choice of anesthetic and the adjustment of its doses depend on the specific task facing the fish breeder-ichthyopathologist.

1 Introduction

Lifetime monitoring of the condition of fish producers of repair and brood stock, including preventive and therapeutic and diagnostic manipulations, is one of the modern tasks of artificial reproduction and obtaining sustainable aquaculture. At the same time, it is worth considering the damaging effect of manipulation stress on biochemical and physiological processes, tissue structures of fish (Soldatov, 2021). As a strategy to prevent stress, fish anesthesia is used, which allows you to prevent or significantly reduce damage. At the same time, the type of narcotic agent used and the physiological aspects of its action are of no small importance. Currently, researchers have summarized data on the functional aspects

[*] Corresponding author: tjul78@mail.ru

of the effect on the fish body of more than 50 types of chemicals and physical factors used for anesthesia in fish (Zahl et al., 2012; Readman et al., 2017; Martins et al., 2019; Soldatov, 2021; Anaesthesia ..., 2021). It is shown that many of them cause a functional response at the level of individual physiological systems and change the nature of the course of metabolic processes in the body of animals.

A significant difference in morpho-physiological features between different ecological and taxonomic groups of fish leads to the fact that approaches that work for some fish species may be ineffective for others. In this regard, in each specific case, it is necessary to adjust the combination of drugs, anesthesia regimen, registration of basic physiological parameters and other components for the successful implementation of preventive, diagnostic and therapeutic manipulations.

In this regard, the purpose of this work is to screen some anesthetic substances and evaluate their sedative effectiveness, using the example of Coregonus peled (Gmelin, 1789).

2 Materials and methods

The object of the study: 43 copies served as the object of the study. peladas incubated in artificial conditions of the Freshwater Aquarium Complex of the Limnological Institute of the Siberian Branch of the Russian Academy of Sciences in the spring of 2020 [1-25].

The studied individuals were kept in 1.5 m^2 tanks in a water recirculation system with mechanical and biological filtration. Water renewal by 50% of the system volume was carried out twice a week. The water temperature in the pool was maintained within 12-16 °C. The fish were fed ADVANCE (0.8-1.2 mm) for individuals up to one year old and SUPREME - 22 (3.0 mm) - after one year.

Methodology of the anesthesiological experiment. A series of experiments were carried out with four drugs for anesthesia, using their different working doses (Table 1).

Table 1. Characteristics of anesthetic substances

Drug	Pharmacological effect	Doses used in the experiment
Eugenol	Local antiseptic, analgesic and sedative. Muscle relaxant. As an anesthetic, it is used only in aquaculture.	400 mg/l 500 mg/l 600 mg/l
Lidocainum	Local analgesic and anesthetic agent. It inhibits ion flows involved in the formation of an irritant, due to which an analgesic effect is achieved. Reversibly blocks the conduction of impulses in the peripheral nervous system. In case of overdose, it is cardio- and neurotoxic. Causes convulsions, respiratory arrest, cardiovascular collapse.	80 mg/l 120 mg/l 160 mg/l
Propofol	Hypnotic, has no analgesic activity. It quickly penetrates into the brain, due to lipophilicity, reduces cerebral blood flow and oxygen consumption by the brain, inhibits reflexes, depending on the dose, causes sedation, amnesia, sleep, apnea. Causes vasodilation and myocardial depression, a decrease in heart rate.	10 mg/l 20 mg/l 40 mg/l

Thiopentalum Natrium	Hypnotic, anticonvulsant. Lipophilene, quickly penetrates the blood-brain barrier. It inhibits the activity of the cerebral cortex. Reduces the blood flow of the brain, causes clinically significant depression of the respiratory and vasomotor centers. Causes myocardial depression, vasodilation, compensatory tachycardia. Even with deep anesthesia, reactions to external stimuli may persist.	4 mg/l 6 mg/l 10 mg/l

For each experiment, the fish were distributed randomly into groups (n = 15). Anesthetics were used in the form of baths, each individual was placed once in glass tanks with a dissolved preparation. The volume of the experimental tanks was 10 liters, the dose of the drugs was calculated relative to the volume of water used. After the experiment, the fish were placed in a permanent habitat tank and their condition was monitored for 72 hours.

The following criteria were taken into account when evaluating the anesthetic effectiveness:

1) Narcotic activity – the working concentration of the substance;

2) Depth of anesthesia – reaching a certain stage of anesthesia;

3) Respiratory activity – respiratory rate, the degree of decrease in respiratory norm, the onset of apnea.

4) Controllability of anesthesia – induction and recovery after sedation, adjustability of the depth of anesthesia with a change in the concentration of the anesthetic;

The induction time was recorded from the beginning of the experiment to the achievement of stage 2, the recovery time – from the moment of immersion in clean water to the normalization of respiratory function and motor activity. The respiration rate of fish was recorded by counting the movements of the gill cap per minute (Alvarenga and Volpato, 1995). The respiratory norm in peled is 120-140 respiratory movements per minute (at rest).

When assessing the depth of anesthesia, the following parameters were taken into account: muscle tone, changes in the nature of breathing and motor reactions. The stages of anesthesia were determined according to the previously modified scale for whitefish (Tolmacheva, Demyanovich, 2023):

Stage I. Reassurance. The overall activity of fish and the frequency of respiratory movements are slightly reduced. There is no reaction to most external stimuli, except tactile ones (loss of fear).

Stage II. Sedation. Loss of balance and muscle tone, decreased frequency of respiratory movements. Fish continue to respond to strong tactile and vibrational stimuli.

Stage III. Deep anesthesia. The rate of respiratory movements is reduced. There is no reaction to external stimuli and reflex reactivity.

Stage IV. Collapse. There is no reaction to external stimuli and reflex reactivity. The respiratory rate decreases up to apnea. Resuscitation measures are required (enhanced aeration in clean water).

Digital and graphical data processing was carried out using the Microsoft Ecxel 2010 program.

Table 2 – Time indicators of anesthesiological support of peled for different drugs

Dose of the drug (mg/l)	Time (sec)			
	Anesthesia, stages			Recovery
	I	II	III	
Eugenol				
400	102 91-110	437 412-445	Absent	394 381-408
500	70 62-81	320 309-351		340 280-381
600	50 42-56	280 271-304		301 240-338
Lidocainum				
100	470 458-495	Absent	Absent	Absent
120	390 377-402	721 749-781		274 258-288
160	350 337-361	708 696-715		311 300-321
Thiopentalum Natrium				
4	280 271-288	360 349-368	Absent	40 33-55
6	158 147-166	281 270-298		70 57-83
10	113 104-118	207 195-218		130 118-141
Propofol				
5	10 7-18	40 31-50	110 99-118	220 175-241
10	~5	30 25-33	75 64-81	283 195-320
20	Absent	~5	28 22-33	389 372-496
40		~2	21 17-24	400 389-411

Thiopentalum Natrium is a hypnotic with a pronounced dose-dependent sedative effect. When immersed in an anesthetic solution of 4-10 mg / l in fish, there is a weak inhibition of motor activity (stage I), which progresses to the sedation stage (stage II), which has distinctive features of the development of an anesthetic effect. The pharmacological feature of this drug is the suppression of the respiratory center, which is expressed in fish in a sharp decrease in the respiratory rate (up to 58-70%) (Fig. 1). Upon cessation of respiratory activity, the tested individuals continue to respond to tactile stimuli, but there is no transition to deeper stages of anesthesia. Thiopentalum Natrium belongs to the hypnotic agents of ultrashort action and causes a state of drug-induced sleep (stage III) by inhibiting the functions of the cerebral cortex. It is likely that the full provision of deep anesthesia in fish cannot be realized, due to the primitive structure of the brain in this group of animals. An increase in the working dose (> 10 mg / l) or the duration of stay in the anesthetic solution (> 3 min) leads to an increase in the toxic effect, asphyxia and death of fish. After extraction from the anesthetic solution, recovery occurs almost immediately and averages 1-2 minutes. Mortality of fish during anesthesia with timely resuscitation measures, as well as after 72 hours was not observed.

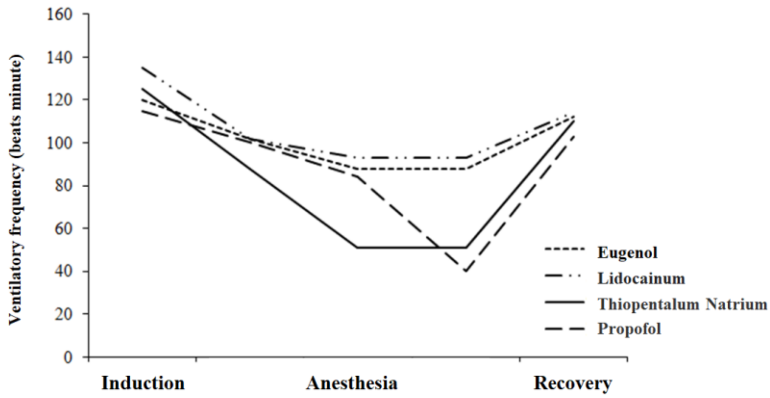

Fig. 1. Dynamics of respiratory activity of fish at different stages of anesthesia with the use of various anesthetics

Propofol belongs to the group of ultra-short-acting hypnotics. It has a pronounced dose-dependent sedative and narcotic effect when used in anesthesiological practice in whitefish in general and in peled in particular (Tolmacheva, Demyanovich, 2023). When immersed in an anesthetic solution with a dosage of 5-10 mg / l, there is a short-term stage of "sedation" (stage I), which quickly passes into deeper stages of anesthesia. When using higher concentrations of 20-40 mg / l, the onset of stage II occurs immediately after immersion in the solution (5-10 seconds), after which the fish move to stage III. When a state of deep anesthesia is reached, fish have a decrease in muscle tone and a complete lack of reaction to external stimuli. Unlike Thiopentalum Natrium, propofol is characterized by a smooth inhibition of respiratory activity; at the sedation stage, the respiratory rate decreases to 25-33%, during the transition to deep anesthesia to 55-71%. In this case, the tendency of a gradual decrease in the respiratory rate is due to a slowdown in physiological reactions in a state of narcotic sleep, and not depression of the respiratory center. With an increase in the period of stay in the propofol solution for more than 5-7 minutes, stage IV occurred, requiring urgent resuscitation measures. It is obvious that increasing the dose (> 40 mg / l) and the exposure time (> 3-5 min) is impractical, due to the possible increase in the toxic effect, respiratory depression and death. Recovery time after anesthesia averaged about 5-7 minutes. Fish mortality during anesthesia and after 72 hours was not observed.

3 Results and discussion

Modern anesthesia strives to minimize the exposure time of the narcotic agent and increase its effectiveness. According to the literature data, with effective anesthesiological support, the development of sedation should occur in no more than 180 seconds, and the restoration of respiratory and motor functions should not exceed 300 seconds. (Keene et al., 1998; Ross, Ross, 2008).

A comparative analysis of the data obtained showed that propofol corresponds to the "optimal" time parameters, the indicators of induction and recovery of which practically do not go beyond the recommended limits (Fig. 2). Moreover, this drug, referring to anesthetics of central action, is characterized by pronounced dose dependence, which allows you to control the depth of anesthesia by changing the concentration of the solution and exposure time. The respiratory norm, with anesthetic accompaniment with propofol, tends to gradually decrease, which makes it possible to control the risks of apnea. Numerous studies of this drug on other species confirm the optimality of choosing propofol as the leading anesthetic in the anesthesia of adult fish (Gholipour et al., 2013; Gholipour Kanani H., Ahadizadeh S., Gomułka et al., 2015; Balko et al., 2017; Martins et al., 2019; Obirikorang et al., 2020, Owen, Kelsh, 2021; Davis et al., 2022)

The duration of induction and recovery when using sodium thiopental also corresponds to the time norms of the "optimal anesthetic", since it refers to anesthetics of central action. However, the use of this drug for fish is undesirable for a number of reasons, the main of which is uncontrolled progressive depression of respiratory function, leading to asphyxia. Studies on the use of this drug in fish are isolated (Strebkova, 1970), and currently barbiturate preparations are not used in ichthyopathological practice.

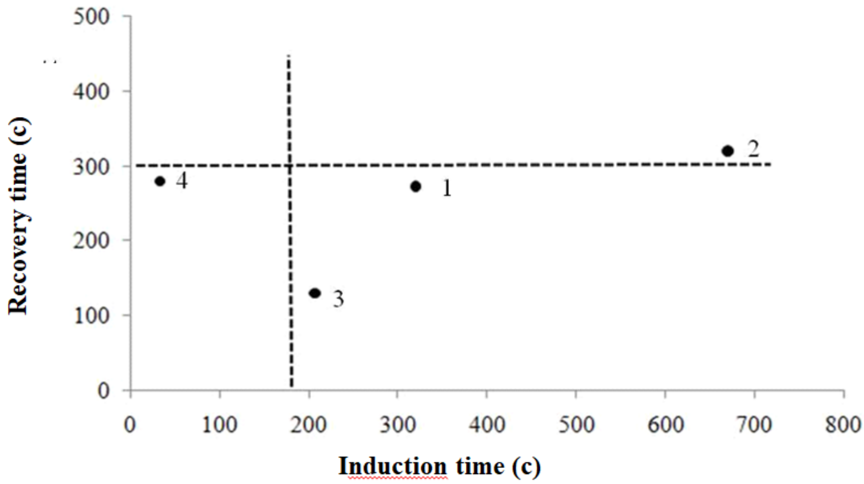

Fig. 2. Temporary anesthetic parameters of various drugs during peled anesthesia: 1) **Eugenol** 600 mg/l; 2) Lidocaine 160 mg/l; 3) **Thiopentalum Natrium** 10 mg/l; **Propofol** 10 mg/l. Note: the dotted line indicates the boundaries of the recommended temporary anesthetic parameters

The effect of eugenol on peled was characterized by a longer induction. The depth of anesthesia was limited by the effect of muscle relaxation without progression into deeper stages of anesthesia. Unlike other anesthetics, when fish were immersed in a solution of eugenol, a short-term stage of excitation was observed, which is probably due to the irritating effect of essential oils on the epithelium of the gill petals. Previously, it was found that clove oil covers the gill epithelium, can block the diffusion of gases (Sladky et al., 2001) and cause damage to the gills (Waristha et al., 2011). However, the use of eugenol has become widespread and is justified by its availability and economic efficiency, especially when working with a large volume of material at fish hatcheries (Inoue et al., 2005; Mikodina et al., 2010; Balamurugan et al.; 2016, Ferreira et al., 2021; Didorenko et al., 2022). When choosing anesthetics, eugenol can be attributed to an alternative option in the implementation of anesthesia in peled.

The use of lidocaine as an anesthetic in the present experiment has shown its inconsistency, although some works mention its use in fish farming practice (Zavyalova et al., 2012; Didorenko et al., 2022). However, the analysis of our own and literature data allows us to conclude about its low anesthetic and economic efficiency due to the high consumption of the drug, the duration of induction and recovery period, and a weak sedative effect.

The effect of anesthetics causes a number of consistent behavioral and physiological reactions in fish, reflecting the change in the state of their body. Knowledge of the regularities of the mechanism of development of these reactions allows you to predict the behavior of the object and plan certain preventive and therapeutic diagnostic measures using certain sedatives.

When the fish were immersed in a solution of anesthetics, all individuals passed into the **"calming" stage (stage I),** which is characterized by the absence of a fright reaction and slowing down of movements (Soldatov, 2005; Iwama et al., 2011; Jorge et al., 2021). A decrease in the motor activity of fish contributes to a decrease in oxygen consumption, which makes it possible to use the first plane of anesthesia for transporting fish at a high landing density (Zavyalova et al., 2012). For long-term maintenance of light anesthesia without progression into deeper stages, we propose to use low doses of anesthetics, mainly muscle relaxants like lidocaine, novocaine and tricaine), which are characterized by prolonged induction.

The stage of **"sedation" (stage II)** was noted with all types of anesthesia in peladi and is characterized by a loss of balance, muscle tone and a decrease in respiratory rate, the development of this stage of anesthesia is usually associated with blocking neuromuscular transmission (Oswald, 1978), which ensures effective muscle relaxation of the object. Respiratory activity decreased by an average of 30% in all tested anesthetics, with the exception of sodium thiopental. With timely extraction of fish from the anesthetic solution, there is no transition to deeper stages of anesthesia or respiratory depression. In general, the sedation stage provides partial immobilization of fish and is convenient for short technological processes that are not accompanied by pain syndrome. For example, clinical examination, bonitirovka, vaccination, chipping, taking scrapings from the outer covers. The optimal time indicators of induction and recovery, as well as controlled respiratory rhythmics for peled are characterized by anesthetic support with the use of eugenol (400-600 mg /l) or low dosages of propofol (5-7 mg/l).

The stage of deep anesthesia is characterized by loss of motor activity, lack of response to pain stimuli and decreased but stable respiratory rhythmics (Soldatov, 2005; Iwama et al., 2011; Jorge et al., 2021). In our experiments and fish farming practice, this stage is achieved only with the use of propofol (10-15 mg/ l), which provides deep anesthesia of fish while maintaining respiratory activity. The stage of deep anesthesia provides complete immobilization of fish and is necessary for complex technological processes such as surgery, ultrasound and X-ray diagnostics, endoscopy, biopsy of internal organs.

The stage of deep anesthesia is followed by a progressive decrease in the frequency of respiratory movements, which can lead to the development of asphyxia and the death of individuals. In order to avoid a fatal outcome, it is necessary to monitor the stages of anesthesia and timely transplantation of fish into a tank with clean water and enhanced aeration.

4 Conclusions

The analysis of the results obtained and the literature data showed that propofol is a drug with sufficient sedative effect for both pelage and other fish species, which is confirmed by its temporary induction/recovery characteristics, the depth and controllability of anesthesia, and low rates of respiratory depression. At the same time, its optimal dose should be adjusted depending on the type, age and state of health of the individual to be anesthetized. In general, it is impossible to deny the advantages of other drugs, since the choice of anesthetic depends on specific tasks and must be economically justified. When organizing simple technological processes that do not require deep anesthesia (fish feeding, transportation, preventive examination), more affordable or cost-effective drugs, for example, eugenol, should be used. With prolonged manipulations of a complex combined nature, accompanied by pain syndrome, propofol is used, if necessary, in combination with a local anesthetic.

Taking into account scientific practice makes it possible to reduce the side functional effects caused by manipulation measures, as well as the use of certain narcotic agents. This makes it possible to obtain more reliable results, especially when conducting experimental work.

Acknowledgements

The work was carried out on the basis of the NIL "Aquaculture and protection of aquatic biological Resources" of the Irkutsk State Agrarian University and the UNU "Experimental Freshwater Aquarium Complex of Baikal Hydrobionts (PAK LIN SB RAS)" and the Shelekhov Veterinary Diagnostic Center with financial support under the theme of the State Task of the Ministry of Agriculture of the Russian Federation No. 1220413001259, the State Task of the LIN SB RAS No. 121032300224-8, RFBR and MOKNSM in the framework of scientific project No. 20-54-44017.

References

1. Zavyalova, E. A., Droshnev, A. E., Gulyukin, M. I., Kalinina, N. R. (2012). Russian Veterinary Journal, **(4),** 22-24.
2. Mikodina E.V., Sedova M.A., Pyanova S.V., Kourzhil Ya. // Fish farming. 2010: № **3-4.** – p.46-47
3. Strebkova T.P. 1972. The effect of anesthetic substances (sodium thiopental) on the physiological parameters of mirror carp fingerlings // Vopr. ichthyology. Vol. 12. No. 2. p. 397
4. Tolmacheva Yu.P., Demyanovich K.A. The use of propofol for anesthesia of whitefish in aquaculture // Materials of the National scientific and practical conference. "Readings dedicated to the 100th anniversary of the birth of Nikolai Sergeyevich Sviridov" Molodezhny, 2023. from 109-114.
5. Anaesthesia of laboratory, aquaculture and ornamental fish: Proceedings of the first LASA-FVS Symposium / Schroeder P. [et al.] // Lab Anim. – 2021. - V. **55**. - № 4
6. Balko JA, Wilson SK, Lewbart GA, Gaines BR, Posner LP. propofol AS AN immersion anesthetic and in a minimum anesthetic concentration (MAC) reduction model in goldfesh (Carassius auratus). J Zoo Wildl Med. 2017 Mar;**48(1):**48-54. doi: 10.1638/2016-0079.1.
7. Davis AK, Garner JP, Chu DK, Felt SA. Propofol Immersion As a Euthanasia Method for Adult Zebrafish (Danio Rerio). Comp Med. 2022 Jun 1;**72(3):**204-209. doi: 10.30802/AALAS-CM-22-000050.
8. Ferreira JM, Félix L, Jorge S, Monteiro SM, Olsson IAS, Valentim AM. Anesthesia Overdose Versus Rapid Cooling for Euthanasia of Adult Zebrafish. Zebrafish. 2022 Aug;**19(4):**148-159. doi: 10.1089/zeb.2022.0001.
9. Gholipourkanani H, Ahadizadeh S. Use of propofol as an anesthetic and its efficacy on some hematological values of ornamental fish Carassius auratus. Springerplus. 2013 Dec;**2(1):**76. doi: 10.1186/2193-1801-2-76.
10. Gomułka P, Fornal E, Berecka B, Szmagara A, Ziomek E. Pharmacokinetics of propofol in rainbow trout following bath exposure. Pol J Vet Sci. 2015;**18(1):**147-52. doi: 10.1515/pjvs-2015-0019.

11. Jorge S, Ferreira JM, Olsson IAS, Valentim AM. Adult Zebrafish Anesthesia: A Study of Efficacy and Behavioral Recovery of Different Anesthetics. Zebrafish. 2021 Oct;**18(5):**330-337. doi: 10.1089/zeb.2021.0023.
12. Owen JP, Kelsh RN. A suitable anaesthetic protocol for metamorphic zebrafish. PLoS One. 2021 Mar 5;**16(3**):e0246504. doi: 10.1371/journal.pone.0246504.
13. Readman GD, Owen SF, Knowles TG, Murrell JC. Species specific anaesthetics for fish anaesthesia and euthanasia. Sci Rep. 2017 Aug 2;**7(1):**7102. doi: 10.1038/s41598-017-06917-2.
14. Ferreira, A.L., Favero, G.C., Boaventura, T.P. et al. Essential oil of Ocimum gratissimum (Linnaeus, 1753): efficacy for anesthesia and transport of Oreochromis niloticus. Fish Physiol Biochem **47**, 135–152 (2021). https://doi.org/10.1007/s10695-020-00900-x
15. Martins T, Diniz E, Fe´lix LM, Antunes L (2018) Evaluation of anaesthetic protocols for laboratory adult zebra fish (Danio rerio). PLoS ONE **13(5):** e0197846. https://doi.org/10.1371/journal. pone.0197846
16. Soldatov A.A. Functional effects of the use of anesthetics on teleostean fishes (review) // Inland Water Biology. 2021. T. **14**. № 1. C. 67-77. DOI: 10.31857/S0320965220060169
17. Zahl IH, Samuelsen O, Kiessling A. Anaesthesia of farmed fish: implications for welfare. Fish Physiol Biochem. 2012 Feb;**38(1):**201-18. doi: 10.1007/s10695-011-9565-1.
18. Ross LG, Ross B (2008) Anaesthetic and sedative techniques for aquatic animals, 3rd edn. Blackwell Science, United Kingdom, Oxford
19. Keene JI, Noakes DIG, Moccia RD, Soto GC (1998) The efficacy of clove oil as an anesthetic for rainbow trout, Oncorhynchus mykiss (Walbaum). Aquac Res **29**:89–101. https://doi. org/10.1046/j.1365-2109.1998.00927.x
20. Alvarenga CMD, Volpato GL (1995) Agonistic profile and metabolism in alevins of the Nile tilapia. Physiol Behav **57**:75– 80. https://doi.org/10.1016/0031-9384(94)00206-K
21. Iwama G.K., Mcgeer J.C., Pawluk M.P. 2011. The effects of five fish anaesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout // Can. J. Zool. V. **67**. Iss. 8. P. 2065.
22. Adewale AY, Adeshina I, Yusuf OY (2017) Anaesthetic effect of Ocimum gratissimum extract on Oreochromis niloticus juveniles. Eur Exp Biol 7(2–7):10.21767/2248-9215.100007
23. Didorenko S.I., Etingova A.A., Fialkov V.A., Kupchinsky A.B., Kondratov I.G., Denikina N.N., Dzyuba E.V. Anaesthesia procedure for two species of Baikal golomyankas (Cottoidei: Comephoridae) // Limnology and Freshwater Biology. 2022. **– № 1.** – C. 1210-1216. DOI: 10.31951/2658-3518-2022-A-1-1210
24. Sladky K.K., Swanson C.R., Stoskopf M.K. et al. 2001. Comparative efficacy of tricaine methanesulfonate and clove / Limnology and Freshwater Biology 2022 (1): 1210-1216 1216 oil for use as anesthetics in red pacu (Piaractus brachypomus). American Journal of Veterinary Research **62**: 337-342. DOI: 10.2460/ajvr.2001.62.337
25. Waristha A., Kingkaew W., Kumthorn T. 2011. Research Journal of Chemistry and Environment **15(2):** 139-146.