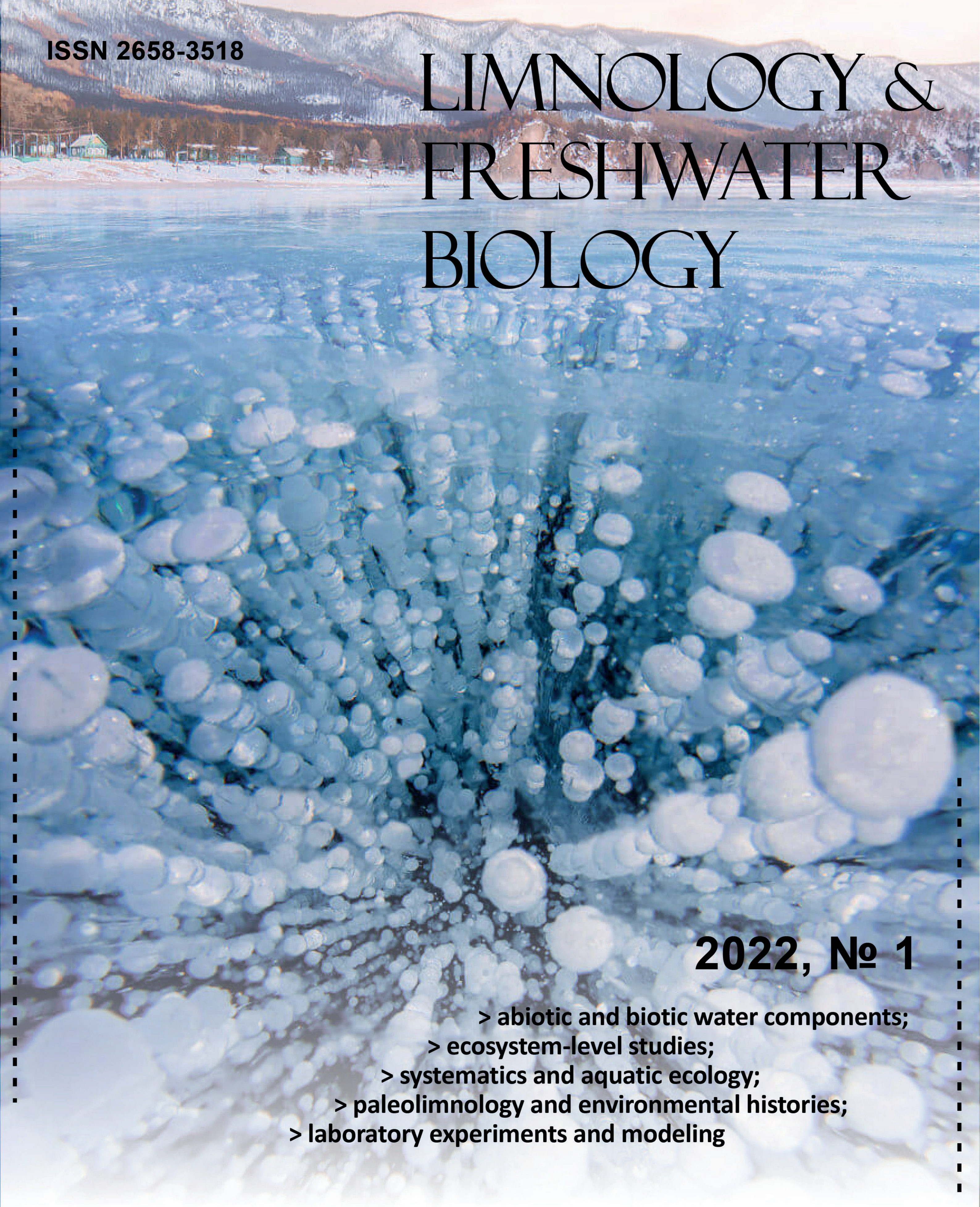

ISSN 2658-3518

LIMNOLOGY & FRESHWATER BIOLOGY

2022, № 1

> abiotic and biotic water components;
> ecosystem-level studies;
> systematics and aquatic ecology;
> paleolimnology and environmental histories;
> laboratory experiments and modeling

Limnology and Freshwater Biology 2022 (1): 1205-1208 DOI:10.31951/2658-3518-2022-A-1-1205

Short communication

ISSN 2658-3518

LIMNOLOGY FRESHWATER BIOLOGY

www.limnolfwbiol.com

The first finding of aegagropilious, or algal balls, in the oldest freshwater Lake Baikal

Volkova E.A.[1,2]*

[1] Limnological Institute Siberian Branch of the Russian Academy of Sciences, Ulan-Batorskaya Str. 3 Irkutsk, 664033, Russia
[2] Institute of Biological Sciences, University of Rostock, Albert Einstein Str. 3 Rostock, 18059, Germany

ABSTRACT. Free-floating algal aggregations, or aegagropilious, are a rare phenomenon known from some freshwater or marine environments worldwide. In September 2014, unusual green algal balls were washed ashore in Ludar Bay on the west coast of the northern basin of Lake Baikal. The paper describes these algal aggregations and characterizes their composition. The Baikal algal balls mainly consisted of green filamentous algae of the family Cladophoraceae, such as *Cladophora glomerata*, *Rhizoclonium* sp., and *Chaetomorpha* sp. Other prominent components of the balls were numerous filaments of *Spirogyra* and *Oedogonium.* These taxa are known for their rapid growth in response to nutrients increase. As the result of the hyperproduction of filamentous benthic algae in the coastal zone of Lake Baikal, new living forms such as aegagropilious-like aggregations have been occurring. The algal balls signify the adaptive capacities of the Lake Baikal algal communities and might retain their functioning in the natural ecosystem's self-purification processes.

Keywords: algal balls, aegagropilious, photogranules, FAB, green filamentous algae*, Spirogyra*, Lake Baikal

1. Introduction

Free floating, detached spherical masses of algae were described from many parts of the world in freshwater and marine habitats (Ballantine et al., 1994; Thiel and Gutow, 2005; Wakana et al., 2005; Boedeker and Immers, 2009; Boedeker et al., 2010; Babich and Zaika, 2011; Cooke et al., 2015; Mathieson et al., 2015; Tsutsui et al., 2015). Algae that form free-floating balls are called by the term aegagropilious (Linnaeus 1753), which refers to their resemblance to bezoar, the masses found in the gastrointestinal tracts of goats, *Capra aegagrus* (Erxleben) (Cooke et al., 2015). The most famous algal balls are formed by an extremely rare freshwater alga, *Aegagropila linnaei* (Kützing), also known as lake balls, or Marimo, popular in the souvenir and aquarium trades and depicted on postage stamps from Japan and Iceland (Wakana et al., 2005; Boedeker and Immers, 2009; Boedeker et al., 2010; Togashi et al., 2014).

In addition to *A. linnaei*, several aegagropilious algae are known to form aggregations like loose-lying balls and spherical forms. Among them, there are at least 18 green, 11 brown, and 25 red taxa (Bach and Josselyn, 1978; Kurogi, 1980; Hoek van den et al., 1984; Littler et al., 1989; Ballantine et al., 1994; Cooke et al., 2015; Mathieson et al., 2015; Tsutsui et al., 2015).

Ball formation occurs by the mechanical action of free-floating thalli rolled against a substratum, either by rocking at the lake bottom or by more vigorous wave action in shallow marine environments (Hoek van den et al., 1984; Togashi et al., 2014; Cooke et al., 2015; Mathieson et al., 2015).

Under the conditions of the active hydrodynamic regime in the Lake Baikal littoral zone, the well-developed benthic algal flora is represented by attached mats forming so-called algal belts, the design of which varies in depth depending on the taxa prevailing in their composition. Traditionally, in the open littoral zone of Lake Baikal, there are five algal belts dominated by certain species (Izhboldina, 2007). Although the same species can form both mat-like and ball-like aggregations (Togashi et al., 2014; Cooke et al., 2015; Tsutsui et al., 2015, etc.), the latter are not typical for the Lake Baikal algal communities.

The present short communication aims to characterize the first findings of the green algal aegagropilious-like aggregations discovered in Lake Baikal.

*Corresponding author.
E-mail address: ekaterina.volkova@uni-rostock.de (Volkova E.A.)

Received: March 25, 2022; ***Accepted:*** March 30, 2022;
Available online: April 21, 2022

2. Materials and methods

Algal balls were found on the shore of Ludar Bay (55°27'01.0"N 109°11'10.1"E) on the west coast of the northern basin of Lake Baikal in September 2014. The following instruments measured the surface water temperature, conductivity, and pH: HI 98501 Checktemp; Hanna Instruments, Woonsocket, Rhode Island, USA. About 10 algal balls were collected from an area of 60 x 20 m.

Twenty algal balls were collected and dried at room temperature; five algal balls were kept in a glass jar with filtrated lake water in natural daylight for the microscopic analysis. In the laboratory, samples were analyzed under an Olympus CX 21 light microscope using a ToupView 3.7 digital camera at magnifications ranging from ×40 to ×400. Species were identified using keys (Izhboldina, 2007; Rundina, 1998; Timoshkin, 2001; Popovskaya et al., 2002). The taxonomy is given according to (Guiry and Guiry, 2022).

3. Results and discussion

The coast of Lake Baikal where green algal balls were observed neither was inhabited nor was exposed to any regular infrastructure or use. Boulders and pebbles were a prevailing substrate at the study site. The surface near-shore water temperature was 9.2 ^{o}C; conductivity – 122.4 µs cm^{-1}, and pH 8.3. On the day of sampling at the study site, the wave height was about 1-1.5 m. The algal balls were not numerous; their distance from each other was about 2 m, most of them were 1.5-2 m above the shoreline. The aggregations had a near-spherical shape with the diameter varying from 3 to 12 cm; they were free-floating, and most of them were just washed ashore (Fig. 1). The fresh balls, after draining, weighed 28.7 ± 12.3 g (n = 4) and 7.02 ± 2.8 g (n = 4) when air dried. The size and structure of the balls was rather similar to those reported in the literature (e.g., Togashi et al., 2014; Cooke et al., 2015; Mathieson et al., 2015). All freshly washed algal balls had a bright green color, were not hollow and consisted of tightly intertwined filamentous thalli of *Cladophora glomerata* (Linnaeus) Kützing, *Chaetomorpha* sp. Kützing, *Rhizoclonium* sp. Kützing, *Spirogyra* sp. ster. 1 Link, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Oedogonium* sp. ster. Link ex Hirn, and *Cladophora* cf. *floccose* C. Meyer. All these taxa, except for *Spirogyra* and *Oedogonium*, are historically common for the first vegetative belts in Lake Baikal (Izhboldina, 2007). The vegetative cells of *Spirogyra* sp. ster. 1 were 34.0-47.5 µm wide, 84.0-284.0 µm long, with 3-5 chloroplasts, and plane transverse walls. The vegetative cells of *Spirogyra* sp. ster. 2 were 38.0-41.0 µm wide, 60.0-80.0 µm long, with 1 chloroplast, and plane transverse walls. The vegetative cells of *Spirogyra* sp. ster. 3 were 85.0-92.0 µm wide, 200.0-520.0 µm long, with one chloroplast, and plane transverse walls. The vegetative cells of *Oedogonium* sp. ster. were 30.0-35.5 µm wide and 50.0-67.0 µm long. Due to a lack of fertile specimens, the species identification of conjugates was not possible.

Filaments of *Ulothrix zonata* (Weber et Mohr) Kützing, *Stigeoclonium tenue* (C. Agardh) Kützing were less abundant but rather regular in the algal balls. Another constant components were fragments of *Nitella flexilis* (Linnaeus) C.Agardh. To a lesser extent, there were *Chara contraria* A.Braun ex Kützing and *Chara* cf. *fragifera* Durieu de Maisonneuve.

Many colonies of *Didymosphenia* M. Schmidt were present in the algal balls. Cells of *Cymbella* C. Agardh, *Encyonema* Krammer, *Cocconeis* Ehrenberg, *Fragilaria*

Fig.1. Photographs of algal balls in situ washed ashore and collected in Ludar Bay on the west coast in the northern basin of Lake Baikal, September 2014.

Lyngb. were abundant on the thalli of *Cladophora* spp. and *U. zonata*. Among other diatoms that were present in a noticeable amount, there were *Navicula tripunctata* (O.F. Mueller) Bory, *N. cryptocephala* Kützing, and *N. radiosa* Kützing.

The upper layer of the bigger-sized algal balls included needle leaf fragments, fine particles of detritus and sand. The scares qualitative analysis of the fauna in the algal balls revealed various macro- and meiofauna taxa belonging to Oligochaeta, Amphipoda, Gastropoda, Polychaeta, Crustacea, Nematoda, and Harpacticoida.

Some algal balls were found dried on the shore; they lacked pigmentation, and their upper layers were rather fragile. Supposedly, these algal balls were washed ashore and already partly dried some time before our observations. However, after exposure of these balls in a jar with filtrated Lake Baikal water for a few months at natural light source, individual filaments and pulls of filaments of *Oedogonium* sp. ster., *Spirogyra* spp., and *Rhizoclonium*-like began to protrude from the balls by several centimeters. This observation indicated that the algal balls retained enough water for the species to last for some time even after being washed and kept ashore. Apparently, after reoccurring in the water or being exposed to water splashes, at least the filamentous algae contained in the algal balls have the potential to persist.

Most of the known reports of algal balls indicate the predominance of taxa belonging to the family Cladophoraceae (Wakana et al., 2005; Boedeker and Immers, 2009; Boedeker et al., 2010; Cooke, et al., 2015; Tsutsui et al., 2015). As a rule, such balls are represented by a single species, although often include fragments of other algae and higher aquatic plants (Ballantine et al., 1994; Babich and Zaika, 2011; Togashi et al., 2014). Baikal algal balls were mainly represented by taxa of the Cladophoraceae family such as *C. glomerata*, *Rhizoclonium* sp. and *Chaetomorpha* sp. Mass development of these species, which is also often referred to as filamentous algal bloom (FAB) (Vadeboncoeur et al., 2021), is associated with eutrophication of aquatic ecosystems, leading to irreversible successional processes not only in coastal communities but also in the entire aquatic ecosystem (e.g., Higgins, 2008; Ozersky et al., 2013).

In addition to *Cladophora* spp., Baikal algal balls always had numerous filaments of *Spirogyra* and *Oedogonium*, which, as indicated above, continued to vegetate even after several months of keeping the balls under laboratory conditions. These taxa are known for their rapid growth in response to increasing nutrients input into the environment (Rundina, 1998; Gubelit and Berezina, 2010). The atypical mass proliferation of *Spirogyra* during the past decade along a substantial part of the Lake Baikal shoreline (Kravtsova et al., 2014; Timoshkin et al., 2015; 2016; Volkova et al., 2018), as well as a local mass development of *C. glomerata* in some areas of the lake (Kobanova et al., 2016), are evidence of algal hyperproduction in response to the high anthropogenic load on the shallow Lake Baikal zone (Kobanova et al., 2016; Kulikova et al., 2021).

4. Conclusions

The algal hyperproduction in the coastal zone of Lake Baikal might have led to occurrence of new living forms such as metaphyton or free-floating algal mats (Volkova et al., 2018), photogranules consisting of filamentous cyanobacteria and filamentous green algae (Volkova et al., 2020), and green algal balls described here. The emergence of such aggregations may indicate the adaptivity of coastal communities and might retain their functioning in the natural self-purification processes of Lake Baikal shallow zone.

Acknowledgments

I thank Dr. O.A. Timoshkin for the opportunity to join a ship field trip on Lake Baikal for collecting material. Many thanks to Dr. M.M. Penzina and my friend O.V. Medvezhonkova for their help with sampling. This study was carried out as a part of the project no. 0279-2021-0007 “Comprehensive studies of the coastal zone of Lake Baikal: long-term dynamics of communities under the influence of various environmental factors and biodiversity; causes and consequences of negative environmental processes”. The article was prepared with the financial support from the Russian Foundation for Basic Research and from the government of the Irkutsk region as part of the scientific project No. 20-44- 380014 r_a.

Conflict of interests

The author declares no conflict of interests.

References

Babich E.I., Zaika V.E. 2011. Fauna of the algal balls of the South-Eastern Sivash Bay. Hydrobiological Journal 48(1): 107-109. DOI: 10.1615/HydrobJ.v48.i1.130

Bach S., Josselyn M.N. 1978. Mass blooms of the alga *Cladophora* in Bermuda. Marine Pollution Bulletin 9(2): 34-37. DOI: 10.1016/0025-326X(78)90529-5

Ballantine D.L., Aponte N.E., Holmquist J.G. 1994. Multi-species algal balls and potentially imprisoned fauna: an unusual benthic assemblage. Aquatic Botany 48: 167-174.

Boedeker C., Eggert A., Immers A. et al. 2010. Global decline of and threats to *Aegagropila linnaei*, with special reference to the lake ball habit. BioScience 60: 187-198. DOI: 10.1525/bio.2010.60.3.5

Boedeker C., Immers A. 2009. No more lake balls (*Aegagropila linnaei* Kützing, Cladophoraphyceae, Chlorophyta) in the Netherlands? Aquatic Ecology 43: 891-902. DOI: 10.1007/s10452-009-9231-1

Cooke J., Lanfear R., Downing A. et al. 2015. The unusual occurrence of green algal balls of *Chaetomorpha linum* on a Beach in Sydney, Australia. Botanica Marina 58(5): 401-407. DOI: 10.1515/bot-2015-0061

Gubelit Y.I., Berezina N.A. 2010. The causes and consequences of algal blooms: the *Cladophora glomerata* bloom and the Neva Estuary (eastern Baltic Sea). Marine Pollution Bulletin 61(4-6): 183-188. DOI: 10.1016/j.marpolbul.2010.02.013

Guiry M.D., Guiry G.M. 2022. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. URL: https://www.algaebase.org

Higgins S.N., Malkin S.Y., Howell E.T. et al. 2008. An ecological review of *Cladophora glomerata* (Chlorophyta) in the Laurentian Great Lakes. Journal of Phycology 44: 839-854. DOI: 10.1111/j.1529-8817.2008.00538.x

Hoek C van den., Ducker S.C., Womersley H.B.S. 1984. *Wittrockiella salina* Chapman (Cladophorales, Chlorophyceae), a mat and ball forming alga. Phycologia 23: 39-46.

Izhboldina L.A. 2007. Atlas i opredelitel' vodorosley bentosa i perifitona ozera Baykal (meyo- i makrofity) s kratkimi ocherkami po ikh ekologii [Atlas and key to benthic and periphyton of Lake Baikal (meyo- and macrophytes) with short essays on their ecology]. Novosibirsk: Science Center. (in Russian)

Kobanova G.I., Takhteev V.V., Rusanovskaya O.O. et al. 2016. Lake Baikal ecosystem faces the threat of eutrophication. International Journal of Ecology 2016(401). DOI: 10.1155/2016/6058082

Kravtsova L.S., Izhboldina L.A., Khanaev I.V. et al. 2014. Nearshore benthic blooms of filamentous green algae in Lake Baikal. Journal of Great Lakes Research 40(2): 441-448. DOI: 10.1016/j.jglr.2014.02.019.

Kulikova N.N., Chebykin E.P, Volkova E.A. et al. 2021. Elementniy sostav vodorosley roda *Spirogyra* kak indicator zagryazneniya pribrezhnoy zony Baikala khozyaistvenno-bytovymi stokami [Element composition of algae of the genus *Spirogyra* as the indicator of pollution of the Baikal near-shore zone with domestic sewage]. Geografia i Prirodnyie Resursy [Geography and Natural Resources] 42(2): 79-91. DOI: 10.15372/GIPR20210209 (in Russian)

Kurogi M.E. 1980. Lake ball “Marimo” in Lake Akan. Japanese Journal of Phycology 28: 168-169.

Littler D.S., Littler M.M., Bucher K.E. et al. 1989. Marine plants of the Caribbean. Washington DC: Smithsonian Institution Press.

Mathieson A.C., Dawes C.J., Lull W.W. 2015. Mystery beach balls foul Long Island, NY, beaches. Rhodora 117(969): 92-97. DOI: 0.3119/14-11

Ozersky T., Barton D.R., Hecky R.E. et al. 2013. Dreissenid mussels enhance nutrient efflux, periphyton quantity and production in the shallow littoral zone of a large lake. Biological Invasions 15: 2799-2810. DOI: 10.1007/s10530-013-0494-z

Popovskaya G.I., Genkal S.I., Likhoshvai E.V. 2002. Diatomovye vodorosli planktona ozera Bajkal [Diatoms of the plankton of Lake Baikal]. Novosibirsk: Nauka. (in Russian)

Rundina L.A. 1998. Zignemovye vodorosli Rossii [The Zygnematales of Russia: (Chlophyta: Zygnematophyceae, Zygnematales)]. Saint-Petersburg: Nauka. (in Russian)

Thiel M., Gutow L. 2005. The ecology of rafting in the marine environment. I. The floating substrata. Oceanography and Marine Biology: an Annual Review 42: 181-264. DOI: 10013/epic.22063.d001

Timoshkin O.A. 2001. Lake Baikal: diversity of fauna, problems of its immiscibility and origin, ecology and “exotic” communities. In: Timoshkin O.A. (Ed.), Index of animal species inhabiting Lake Baikal and its catchment area. Novosibirsk: Nauka, pp. 74-113. (in Russian)

Timoshkin O.A., Bondarenko N.A., Volkova E.A. et al. 2015. Mass development of green filamentous algae of the genera *Spirogyra* and *Stigeoclonium* (Chlorophyta) in the littoral zone of the Southern part of Lake Baikal. Hydrobiological Journal 51: 13-23. DOI: 10.1615/HydrobJ.v51.i1.20

Timoshkin O.A., Samsonov D.P., Yamamuro M. et al. 2016. Rapid ecological change in the coastal zone of Lake Baikal (East Siberia): is the site of the world’s greatest freshwater biodiversity in danger? Journal of Great Lakes Research 42: 487-497. DOI: 10.1016/j.jglr.2016.02.011

Togashi T., Sasaki H., Yoshimura J.A. 2014. Geometrical approach explains lake ball (Marimo) formations in the green Alga, *Aegagropila linnaei*. Scientific Reports 4(1). DOI: 10.1038/srep03761

Tsutsui I., Miyoshi T., Sukchai H. et al. 2015. Ecological and morphological profile of floating spherical *Cladophora socialis* aggregations in Central Thailand. PLoS ONE 10(4). DOI: 10.1371/ journal. pone.0124997

Vadeboncoeur Y., Moore M.V., Stewart S.D. et al. 2021. Green bottoms: benthic filamentous algal blooms are an emerging threat to clear lakes worldwide. BioScience 71. DOI: 10.1093/biosci/biab049

Volkova E.A., Bondarenko N.A., Timoshkin O.A. 2018. Morphotaxonomy, distribution and abundance of *Spirogyra* (Zygnematophyceae, Charophyta) in Lake Baikal, East Siberia. Phycologia 57(3): 298-308. DOI: 10.2216/17-69.1

Volkova E.A., Sorokovikova E.G., Belykh O.I. et al. 2020. Photogranules formed by filamentous cyanobacteria and algae of the genus *Spirogyra* Link in the coastal zone of Lake Baikal. Izvestiya Baikal’skogo Gosudarstvennogo Universiteta [Bulletin of Baikal State University] 30(1): 14-22. DOI: 10.17150/2500-2759.2020.30(1).14- 22 (in Russian)

Wakana I., Kawachi M., Hamada N. et al. 2005. Current situation and living environment of an endangered algal species Marimo (*Aegagropila linnaei*) in Takkobu marsh, Kushiro-Shitsugen wetland. Journal of Plant Research 118: 64.

Short communication

ISSN 2658-3518

LIMNOLOGY FRESHWATER BIOLOGY

www.limnolfwbiol.com

Diatoms: life in glass houses

Pickett-Heaps J.D., Pickett-Heaps J.*

Cytographics, http://www.cytographics.com/

ABSTRACT. Diatoms are an extraordinarily successful group of organisms and their many thousands of species have diversified and specialized so as to occupy every environmental niche. Part of their success derives from their unique protective cell walls, beautifully elaborate structures made of pure silica. However, living inside walls made of this rigid, refractory material creates many problems. Just how diatoms circumvent these problems provides the major theme in this spectacular exploration of the biology of living cells. "Diatoms: Life in Glass Houses" is a 60 minute video covering all aspects of living diatoms, with emphasis on how they get around problems of living within cell walls made of pure silica.

Keywords: diatoms, silica, frustule

"Diatoms: Life in Glass Houses" is a 60-minute video describing various aspects of the structure and life of diatoms, including:

1. Fossil diatoms, the siliceous frustules of which form diatomite deposits.
2. Advantages and problems of a cell living inside silicon glass walls.
3. Morphology and symmetry of the diatom frustules.
4. Structure and movement of the chloroplasts.
5. Description of cytoplasmic vacuoles and their movement.
6. Microtubule cytoskeleton and the Microtubule Center.
7. Motility of centric and pennate diatoms.
8. Phototaxis (the ability to respond to light).
9. Secretion of holdfasts and adhesives.
10. Labiate processes ("Rimoportulae").
11. Colony formation.
12. Planktonic diatoms: effects of size, shape and colony formation.
13. Chitin secretion; strutted processes ("fultoportulae").
14. Mitosis and cleavage.
15. Species specific valve morphogenesis.
16. Morphogenesis of spines. Turgor pressure.
17. Sexual reproduction in centric diatoms.
18. Sexual reproduction in pennate diatoms.
19. Resting spores.

This film was created in 2003 but the main ideas and questions are still relevant today, and the film is very useful for beginning biologists who want to learn about these wonderful organisms. More information about this video is available in (Pickett-Heaps, 2003). The film in DVD and avi formats is available at:

- http://limnolfwbiol.com/files/share/Pickett-Heaps&Pickett-Heaps2003.zip (film in DVD)
- http://limnolfwbiol.com/files/share/Pickett-Heaps_Glass_House.avi (avi format)

under Creative Commons Attribution 4.0 International Public License.

Conflict of interests

The authors declare that they have no competing interests.

References

Pickett-Heaps J. 2003. Teacher's guide. Diatoms: life in glass houses. Melb. Cytographics. URL: www.cytographics.com/resource/catalog/tapes/in-dia.pdf

*Corresponding author.
E-mail address: jph@cytographics.com (J. Pickett-Heaps)

Received: April 14, 2022; ***Accepted:*** April 14, 2022;
Available online: April 21, 2022

Limnology and Freshwater Biology 2022 (1): 1210-1216 DOI:10.31951/2658-3518-2022-A-1-1210

Original Article

ISSN 2658-3518

LIMNOLOGY FRESHWATER BIOLOGY
www.limnolfwbiol.com

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

Didorenko S.I.[1], Etingova A.A.[1], Fialkov V.A.[1], Kupchinsky A.B.[1], Kondratov I.G.[2], Denikina N.N.[3,*], Dzyuba E.V.[3]

[1] *Baikal Museum, Irkutsk Scientific Center, Siberian Branch of the Russian Academy of Science, Academicheskaya str., 1, s. Listvyanka, Irkutsk region, 664000, Russia*
[2] *Research Center for Family Health and Human Reproduction, Karl Marx st., 3/2, Irkutsk, 664003, Russia*
[3] *Limnological Institute of the Siberian Branch of the Russian Academy of Sciences, Ulan-Batorskaya Str., 3, Irkutsk, 664033, Russia*

ABSTRACT. This study aimed to develop the anaesthesia procedure for *Comephorus baicalensis* (Pallas, 1776) and *Comephorus dybowski* 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. dybowski*. 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 dybowski* 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-methanoxyphenol), 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

*Corresponding author.
E-mail address: denikina@lin.irk.ru (N.N. Denikina)

Received: January 28, 2022; ***Accepted:*** April 11, 2022;
Available online: April 21, 2022

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; Çağiltay 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 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.

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

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).

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.

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 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.

Acknowledgements

The study was carried out within the framework of the State Projects of BM ISC SB RAS (No. 0285-2021-0001) and LIN SB RAS (No. 0279-2021-0005) and supported by the RFBR grant, project No. 190700322.

Conflict of interests

The authors declare that they have no competing interests.

References

Aas T.S., Sixten H.J., Hillestad M. et al. 2017. Measurement of gastrointestinal passage rate in Atlantic salmon (*Salmo salar*) fed dry or soaked feed. Aquaculture Reports 8: 49-57. DOI: 10.1016/j.aqrep.2017.10.001

Abbas H.H.H., Abdel-Gawad A.S., Akkr A.A. 2006. Toxicity and efficacy of lidocaine as an anesthetic for Nile tilapia; *Oreochromis niloticus*. Pakistan Journal of Biological Sciences 9: 2236-2242. DOI: 10.3923/pjbs.2006.2236.2242

Abramov V.A., Brovko V.P., Maksimov V.N. et al. 1979. The organization of work at the underwater vehicles and some results of observations during individual diving. In: Monin A.S. (Ed.), Geologo-geofizicheskiye i podvodnyye issledovaniya ozera Baikal [Geological-geophysical and underwater studies of Lake Baikal]. Moscow: Inst. Okeanologii AN SSSR, pp. 48-64. (in Russian)

AVMA (American Veterinary Medical Association) guidelines for the euthanasia of animals. 2020. Available online: https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (accessed on April 11, 2022).

Bolasina S.N., de Azevedo A., Petry A.C. 2017. Comparative efficacy of benzocaine, tricaine methanesulfonate and eugenol as anesthetic agents in the guppy *Poecilia vivipara*. Aquaculture Reports 6: 56-60. DOI: 10.1016/j.aqrep.2017.04.002

Brezas A., Hardy R.W. 2020. Improved performance of a rainbow trout selected strain is associated with protein digestion rates and synchronization of amino acid absorption. Scientific Reports 10: 4678. DOI: 10.1038/s41598-020-61360-0

Burka J.F., Hammel K.L., Horsberg T.E. et al. 1997. Drugs in salmonid aquaculture – a review. Journal of Veterinary Pharmacology and Therapeutics 20: 333-349. DOI: 10.1046/j.1365-2885.1997.00094.x

Çağiltay F., Atanasoff A., Sağlam M. et al. 2017. Comparison of different anesthetic protocols for morphometric measurements of carp (*Cyprinus carpio*). Advanced Research in Life Sciences 1(1): 81-84. DOI: 10.1515/arls-2017-0014

Carter K.M., Woodley C.M., Brown R.S. 2011. A review of tricaine methanesulfonate for anesthesia of fish. Reviews in Fish Biology and Fisheries 21: 51-59. DOI: 10.1007/s11160-010-9188-0

Cho G.K., Heath D.D. 2000. Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum). Aquaculture Research 31: 537-546. DOI: 10.1046/j.1365-2109.2000.00478.x

Collymore C., Tolwani A., Lieggi C. et al. 2014. Efficacy and safety of 5 anesthetics in adult zebrafish (*Danio rerio*). Journal of the American Association for Laboratory Animal Science 53(2): 198-203.

Collymore C., Banks E.K., Turner P.V. 2016. Lidocaine hydrochloride compared with MS222 for the euthanasia of zebrafish (*Danio rerio*). Journal of the American Association for Laboratory Animal Science 55(6): 816-820.

Coyle S.D., Durborow R.M., Tidwell J.H. 2004. Anesthetics in aquaculture. Southern Regional Aquaculture Center 2004: 3900.

Devi A.A., Kamilya D. 2019. Efficacy and effects of clove oil and MS-222 on the immune-biochemical responses of juvenile rohu *Labeo rohita*. Aquaculture Research 50: 957-963. DOI: 10.1111/are.13980

Didorenko S.I., Etingova A.A., Fialkov V.A. et al. 2020. Development of a method for keeping deep-water oilfish of the Lake Baikal (*Comephorus baikalensis* (Pal.), C. dybowskii Korotn.) at normal atmospheric pressure. In: Bychkov I.V., Kazakov A.L. (Eds.), Aktual'nyye problemy nauki Pribaykal'ya [Current Problems of Science in the Baikal Region]. Irkutsk, pp. 157-162. (in Russian)

Fernandes I.M., Bastos Y.F., Barreto D.S. et al. 2017. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. Brazilian Journal of Biology 77(3): 444-450. DOI: 10.1590/1519-6984.15015

Fernández-Alacid L., Sanahuja I., Ordóñez-Grande B. et al. 2019. Skin mucus metabolites and cortisol in meagre fed acute stress- attenuating diets: correlations between plasma and mucus. Aquaculture 499: 185-194. DOI: 10.1016/j.aquaculture.2018.09.039

Ferreira J.T., Schoonbee H.J., Smit G.L. 1984. The uptake of the anesthetic benzocaine hydrochloride by the gills and the skin of three freshwater fish species. Journal of Fish Biology 25: 35-41. DOI: 10.1111/J.1095-8649.1984.TB04848.X

Goto A. 1986. Movement and population size of the river sculpin *Cottus hangiongensis* in the Daitobetsu River of Southern Hokkaido. Japanese Journal of Ichthyology 32(4): 421-430.

Harms C.A. 1999. Anesthesia in fish. In: Fowler M.E., Miller R.E. (Eds.), Zoo and wild animal medicine: current therapy. Philadelphia, pp. 158-163.

Henao Duque A.M., Núñez Rangel V. 2016. Maintenance of red-tail coral snake (*Micrurus mipartitus*) in captivity and evaluation of individual venom variability. Acta Biológica Colombiana 21(3): 593-600. DOI: 10.15446/abc.v21n3.51651

Hoseini S.M., Nodeh A. 2013. Changes in blood biochemistry of common carp *Cyprinus carpio* (Linnaeus), following exposure to different concentrations of clove solution. Comparative Clinical Pathology 22: 9-13. DOI: 10.1007/s00580-011-1362-9

Jakubowski M. 1996. Gill structure and morphometry of its respiratory components in the Baikalian golomyankas (Comephoridae; Cottoidei; Pisces). Journal of Morphology 228(1): 19-26. DOI: 10.1002/(SICI)1097-4687(199604)228:1<19::AID-JMOR2>3.0.CO;2-K

Javahery S., Nekoubin H., Moradlu A.H. 2012. Effect of anaesthesia with clove oil in fish (review). Fish Physiology and Biochemistry 38: 1545-1552. DOI: 10.1007/s10695-012-9682-5

King W.V., Hooper B., Hillsgrove S. et al. 2005. The use of clove oilmetomidate, tricaine and phenoxyethanol for inducing anaesthetics on their effect on the cortisol stress response in black sea bass (*Centropristis striata*). Aquaculture Research 36: 1442-1449. DOI: 10.1111/j.1365-2109.2005.01365.x

Kim E.-M., An H.-S., Park I.-S. 2007. Cytogenetic study of *Pleuronectes obscurus*, *Konosirus punctatus* and *Pseudoblennius percoides*. Journal of Fisheries Science and Technology 10(1): 24-29. DOI: 10.5657/fas.2007.10.1.024

Koryakov E.A. 1972. Pelagicheskie bychkovye Baikala [Pelagic Cottoidei of Lake Baikal]. Moscow: Nauka. (in Russian)

Kozlova T.A., Khotimchenko S.V. 2000. Lipids and fatty acids of two pelagic cottoid fishes (*Comephorus* spp.) endemic to Lake Baikal. Comparative Biochemistry and Physiology, Part B 126: 477-485. DOI: 10.1016/s0305-0491(00)00207-8

Lau G.Y., Arndt S., Murphy M.P. et al. 2019. Species- and tissue-specific differences in ROS metabolism during exposure to hypoxia and hyperoxia plus recovery in marine sculpins. Journal of Experimental Biology 222. DOI: 10.1242/jeb.206896

Martins T., Valentim A., Pereira N. et al. 2019. Anaesthetics and analgesics used in adult fish for research: a review. Laboratory Animals 53(4): 325-341. DOI: 10.1177/0023677218815199

Mirghaed A.T., Yasari M., Mirzargar S.S. et al. 2018. Rainbow trout (*Oncorhynchus mykiss*) anesthesia with myrcene: efficacy and physiological responses in comparison with eugenol. Fish Physiology and Biochemistry 44: 919-926. DOI: 10.1007/s10695-018-0481-5

Neiffer D.L., Stamper M.A. 2009. Fish sedation, analgesia, anesthesia, and euthanasia: considerations, methods, and types of drugs. ILAR Journal 50(4): 343-360. DOI: 10.1093/ilar.50.4.343

Oliveira C.P.B., Lemos C.H.P., Felix e Silva A. et al. 2019. Use of eugenol for the anaesthesia and transportation of freshwater angelfish (*Pterophyllum scalare*). Aquaculture 513: 734409. DOI: 10.1016/j.aquaculture.2019.734409

Ovidio M., Detaille A., Bontinck C. et al. 2009. Movement behaviour of the small benthic Rhine sculpin *Cottus rhenanus* (Freyhof, Kottelat & Nolte, 2005) as revealed by radio-telemetry and pit-tagging. Hydrobiologia 636: 119-128. DOI: 10.1007/s10750-009-9941-3

Pastukhov V.V. 2010. Methods and conditions of keeping hydrobionts of Lake Baikal. In: International Scientific Conference "Actual Problems in the Activity of Academic Natural-Scientific Museums", pp. 131-134. (in Russian)

Priborsky J., Velisek J. 2018. A review of three commonly used fish anesthetics. Reviews in Fisheries Science & Aquaculture 26: 417-442. DOI: 10.1080/23308249.2018.1442812

Putland R., Rogers L., Giuffrida B. et al. 2020. Anesthetic effects of AQUI-S 20E® (eugenol) on the afferent neural activity of the oyster toadfish (*Opsanus tau*). Fish Physiology and Biochemistry. 46(6): 2213-2226. DOI: 10.1007/s10695-020-00874-w

Readman G.D., Owen S.F., Murrell J.C. et al. 2013. Do fish perceive anaesthetics as aversive? PLoS One 8(9): e73773. DOI: 10.1371/journal.pone.0073773

Rocaspana R., Aparicio E. 2017. Population trends and current status of the endangered Pyrenean sculpin *Cottus hispaniolensis* in the Spanish part of the Garonne drainage. Knowledge and Management of Aquatic Ecosystems 418. DOI: 10.1051/kmae/2017017

Rosenberg P.H., Kyttä J., Alila A. 1986. Absorption of bupivacaine, etidocaine, lignocaine and ropivacaine into n-heptane, rat sciatic nerve, and human extradural and subcutaneous fat. British Journal of Anaesthesia 58: 310-314. DOI: 10.1093/bja/58.3.310

Roubach R., Gomes L.C., Leão Fonseca F.A. et al. 2005. Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). Aquaculture Research 36(11): 1056-1061. DOI: 10.1111/j.1365-2109.2005.01319.x

Sapozhnikova Y.P., Koroleva A.G., Yakhnenko V.M. et al. 2021. Sex associated effects of noise pollution in stone sculpin (*Paracottus knerii*) as a model object in the context of human-induced rapid environmental change. Biology (Basel) 19(10): 1063. DOI: 10.3390/biology10101063

Sideleva V.G. 1995. Pelagic Cottoidei – cottoid fishes. In: Atlas i opredelitel' pelagobiontov Baikala (s kratkimi ocherkami po ikh ekologii) [Atlas and keys to Baikal pelagobionts (with brief essays on their ecology)]. Novosibirsk: Nauka, pp. 523-538. (in Russian)

Siers S.R., Yackel Adams A.A., Reed R.N. 2018. Behavioral differences following ingestion of large meals and consequences for management of a harmful invasive snake: a field experiment. Ecology and Evolution 8(20): 10075-10093. DOI: 10.1002/ece3.4480

Sladky K.K., Swanson C.R., Stoskopf M.K. et al. 2001. Comparative efficacy of tricaine methanesulfonate and clove 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

Sloman K.A., Bouyoucos I.A., Brooks E.J. et al. 2019. Ethical considerations in fish research. Journal of Fish Biology 94(4): 556-577. DOI: 10.1111/jfb.13946

Soldatov A.A. 2021. Functional effects of the use of anesthetics on bony fish (a brief overview). Inland Water Biology 1: 55-66. DOI: 10.31857/S0320965220060169

Starikov G.V. 1977. Golomyanki Baikala [Comephoridae of Lake Baikal]. Novosibirsk: Nauka. (in Russian)

Stetter M.D. 2001. Fish and amphibian anesthesia. Veterinary Clinics of North America. Exotic Animal Practice 4(1): 69-82. DOI: 10.1016/s1094-9194(17)30052-x

Taliev D.N. 1955. Bychki-podkamenshchiki Baikala [Cottoidei of Lake Baikal]. Moscow: AN SSSR Publ. (in Russian)

Topic Popovic N., Strunjak-Perovic I., Coz-Rakovac R. et al. 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. Journal of Applied Ichthyology 28: 553-564. DOI: 10.1111/j.1439-0426.2012.01950.x

Valentim A.M., Félix L.M., Carvalho L. et al. 2016. A new anaesthetic protocol for adult zebrafish (*Danio rerio*): propofol combined with lidocaine. PLoS One 11(1): e0147747. DOI: 10.1371/journal.pone.0147747

Vatsos I.N., Kotzamanis Y., Henry M. et al. 2010. Monitoring stress in fish by applying image analysis to their skin mucous cells. European Journal of Histochemistry 54: e22. DOI: 10.4081/ejh.2010.e22

Waristha A., Kingkaew W., Kumthorn T. 2011. Acute toxicity of clove oil and effects on histopathological changes in gill of Siamese fighting fish *Betta splendens*. Research Journal of Chemistry and Environment 15(2): 139-146.

Zavyalova E.A., Droshnev A.E., Gulukin M.I. et al. 2012. Anesthesia of the Rainbow Trout. Rossiyskiy Veterinarnyy Zhurnal. Sel'skokhozyaystvennyye Zhivotnyye [Russian Veterinary Journal] 2012(4): 22-24. (in Russian)

Zavyalova E.A., Droshnev A.E., Gulukin M.I. et al. 2012. Anesthesia of the Rainbow Trout. Russian Veterinary Journal 4: 22-24.