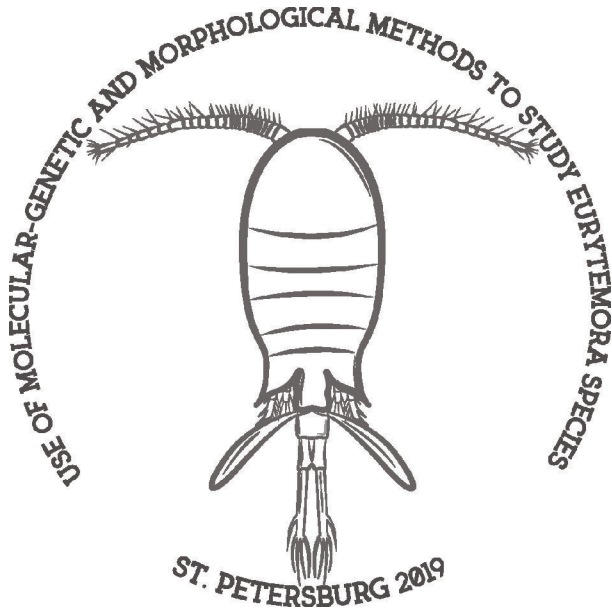


# International Scientific Conference



## Book of Abstracts



Use of molecular-genetic and morphological  
methods to study the taxonomy, phylogeny,  
biogeography, and ecology  
of *Eurytemora* species

International Scientific Conference  
May 13–17, 2019. Saint Petersburg, Russia

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# Programme

Monday, May 13. Arrival and Welcome Party

Tuesday, May 14

10.00 Registration

11.00 Opening ceremony

Speech by the Director of ZIN RAS Sergei Sinev

Session 1: Phylogeny (Chairs: Carol Eunmi Lee, Victor Alekseev)

11.20 Carol Eunmi Lee. “Species” concepts in the *Eurytemora affinis* species complex

12.00 Coffee break

12.20 Vjacheslav Ivanenko, Korzhavina O.A., Nikinin M.A.  
Integrative taxonomy and phylogeny of copepods

13.00 Natalia I. Abramson. Genome — wide association studies:  
new challenge in ecological and evolutionary studies

13.40 Lunch

14.40 Tour of the Zoological Museum

15.30 Natalia Sukhikh, Carol Eunmi Lee, Ekaterina Abramova,  
Vincent Castric, Elena Fefilova, Sami Souissi, Victor Alekseev.  
A comparative analysis of genetic differentiation of *Eurytemora*  
species using CO1, ITS and 18SrRNA genes with an emphasis  
on *E.affinis* species complex

15.50 Imran Parvez, Md. Ashraful Alam. Phylogenetic relationships of  
cyprinid fishes (Cyprinidae) inferred from morphological traits  
and mitochondrial gene cytochrome b in Bangladesh

16.10 Coffee break

Session 2: Biogeography (Chairs: Jiang-Shiou Hwang, Elena Kochanova)

16.30 Victor Alekseev. Cosmopolite species versus cryptic species two  
paradigms in copepod biogeography in molecular-genetic epoch  
and the future of morphological taxonomy

- 17.10 Elena Kochanova, Natalia Sukhikh, Victor Alekseev. Phylogeography of the freshwater copepods: two ways of distribution in the European area
- 17.30 Ekaterina Abramova, Natalia Sukhikh, Elena Fefilova, Aleksandr Novikov, Waldemar Schneider and Paul Overduin. *Eurytemora* species (Copepoda, Calanoida) in the Lena river delta — Laptev Sea region: Composition, distribution and ecology
- 17.50 Poster session  
(Chairs: Ekaterina Abramova, Jonna Engström-Öst)
1. Victor Alekseev, Ksenia Kaskova, Olga Chaban. Use of integumental pore analyze for sibling species separation in the genus *Eurytemora*
  2. Evgeny Barabanshchikov. Distribution, dynamics of abundance and biomass of species of the genus *Eurytemora* in water bodies of Primorye Territory
  3. Anna Demchuk, N. Sukhikh, S. Golubkov. The role of invasive *Eurytemora carolleae* and native *Eurytemora affinis* (Copepoda: Crustacea) in the diet of coastal fish in the eastern Gulf of Finland
  4. Elena G. Krupa. The ecological preferences of *Eurytemora affinis* (Poppe, 1880) in the Kazakhstan's water bodies (Central Asia)
  5. Valentina I. Lazareva. Distribution of the copepod *Eurytemora caspica* Sukhikh et Alekseev, 2013 (Crustacea: Calanoida) in the reservoirs of the Volga river and Don river basins
  6. Nataliya Polyakova, Aleksey Starkov. *Eurytemora* sp. in the rock pools of the White Sea islands
  7. Li-Chun Tseng, Yan-Guo Wang, Guang-shan Lian, Jiang-Shiou Hwang. A multi-year investigation of the Temoridae (Crustacea: Copepoda) assemblage succession with the interplay waters in the northern South China Sea
  8. Anna Semenova. Seasonal and interannual dynamics of *Eurytemora affinis* (Poppe, 1880) as key species in Vistula Lagoon of the Baltic Sea
- 18.30 Discussion of the day

Wednesday, May 15.

Session 3: Taxonomy. (Chairs: Natalia Sukhikh, Łukasz Sługocki)

- 10.00 Natalia Sukhikh, Victor Alekseev. On morphological peculiarities and species distribution in the *Eurytemora affinis* species complex
- 10.40 Dmitry Lajus, Natalia Sukhikh, Victor Alekseev. Random phenotypic variation in *Eurytemora* species and other crustaceans
- 11.20 Coffee break
- 11.40 Elena Fefilova, Natalia Sukhikh, Ekaterina Abramova, Ilya Velegzhaninov. About the systematics of Palaearctic *Eurytemora* (Calanoida, Copepoda) on base of their morphological analysis
- 12.00 Łukasz Sługocki, Robert Czerniawski. Variability of mandible shape in freshwater glacial relict *Eurytemora lacustris*
- 12.20 Vezhnavets Vasil, Anastasiya Litvinova. The genus *Eurytemora* in the waterbodies of Belarus
- 12.40 Denis Zavarzin, Natalia Sukhikh, Victor Alekseev. Some aspects of ecology of *Eurytemora cf. affinis* from Sakhalin, Russia, in comparison with Hokkaido, Japan
- 13.00 Lunch

Session 4: Ecology. (Chairs: Gesche Winkler, Dmitry Lajus)

- 14.00 Gesche Winkler, Manuelle Beaudry-Sylvestre and David Ouellet. Biological traits of co-occurring sibling species of the *Eurytemora affinis* complex in an important fish nursery zone of the St. Lawrence estuary, Canada
- 14.40 Petr Strelkov, Skazina M., Gagarina A., Dzhelali P., Ivanova A., Kireeva M., Genelt-Yanovsky E., Malavenda S., Polyakova N., Masharsky A., Shunatova N. Genetic consequences of landlocking for marine invertebrates and fishes from marine lake
- 15.20 Solvita Strake, Astra Labuce, Inta Dimante-Deimantovica. Calanoid copepod *Eurytemora affinis* (Poppe, 1880) in the Gulf of Riga, Baltic Sea – some aspects of behavior and ecology
- 15.40 Alexander Timpe, Bart De Stasio. Comparison of an electron transport system (ETS) enzyme-mediated assay and total

respiration rate of the invasive copepod *Eurytemora carolleeae* in Green Bay, WI, USA

16.00 Coffee Break

16.20 Astra Labuce, Anda Ikaunieca, Solvita Strake, Anissa Souissi. Survey of presence of non-indigenous *Eurytemora carolleeae* in the Gulf of Riga (Baltic Sea) five years after its first discovery

16.40 Nikolaos V. Schizas, Camille Amaro Berrocal, Govind Nadathur. Biodiversity of deep-sea zooplankton off the southwest coast of Puerto Rico

17.00 Jonna Engström-Öst, *Eurytemora affinis* in the Western Gulf of Finland — responses to environmental change

17.20 Cabrol J., Tremblay R., Gesche Winkler. Niche separation in the cryptic species complex *Eurytemora affinis*: eco-physiological response in an “in-situ” reciprocal transplant experiment

17.40 Discussion of the day

18.30 Festive dinner

Thursday, May 16. Boat trip to Peterhof Museum

Friday, May 17.

10.00 Natalia Abramson, Evgeniy Genelt-Yanovsky, Semyon Bodrov, Olga Bondareva. Molecular-genetic master-class on *Eurytemora* species in «Taxon» Research Resource Center of ZIN RAS

13.30 Lunch and transfer to the main building of ZIN RAS

15.00 Victor Alekseev, Elena Fefilova, Ekaterina Abramova, Natalia Sukhikh. Taxonomic training on *Eurytemora* species

18.30 Discussion of the day and the Closing ceremony

# Abstracts



## “Species” concepts in the *Eurytemora affinis* species complex

CAROL EUNMI LEE

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Species concepts are notoriously difficult to define, given that species are dynamic entities that are continuously evolving. Moreover, rates of molecular evolution, morphological differentiation, and reproductive isolation are often discordant and idiosyncratic. Many crustacean species form species complexes where boundaries are difficult to define. Thus, it is important to include all genetically distinct clades in a comprehensive analysis, which includes data on genetic divergence, and morphological descriptions, and reproductive isolation among the clades.

In this talk I will present existing and new data on patterns of speciation within the *Eurytemora affinis* species complex. The data set includes genomic divergence among clades, morphological differentiation among populations, patterns of reproductive isolation both within and between clades, and behavioral mating preference.

While there is evidence for speciation within the *E. affinis* species complex, drawing “species” boundaries is problematic given the complexity of the patterns. For many well-studied taxa, such as *Daphnia pulex*, *Tigriopus californicus*, and *Hyalella azteca*, investigators have opted to leave the species designation as “complex.” If we do wish to define species boundaries, *all* the data and their complexities need to be taken into account in a thoughtful and comprehensive manner.

## Genome — wide association studies: new challenge in ecological and evolutionary studies

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The quick development of DNA technologies and their successful application in the field of biodiversity studies in the end of XX – beginning of XXI century dramatically changed both the conventional pipeline of zoological and botanical studies and issues in the focus of the studies. The application of this new technique allowed uncovering a large amount of cryptic species, past demographic histories of species and populations, post Pleistocene colonization routes and genesis of contemporary distribution ranges for many taxa alongside with influence of Pleistocene glaciations during the latter opening a whole new scientific discipline — Phylogeography. Application of mitochondrial and nuclear molecular markers drastically altered views on phylogeny and systematics in many taxa. However simultaneously with accumulation of molecular data these newly arisen trends of investigations faced serious challenges.

A large portion of studies was based on single mitochondrial markers not always adequate for revealing phylogenetic relationships within groups of high taxonomic rank, the other complication in using mitochondrial markers in phylogenetic and phylogeographic studies is related to frequent mitochondrial DNA introgression and with often amplification of nuclear copies of mitochondrial genes (NUMT), thus leading to paraphyly in the species trees. The latter may be caused both by past hybridization events and incomplete lineage sorting. The inclusion of nuclear markers in phylogenetic research often lead to more robust phylogenetic trees but at the same time discrepancy between different markers was also common place. It should be underlined that all studies mentioned above using PCR and Sanger sequencing were relying mostly on randomly chosen markers. The choice itself was most often in favor of already used markers in closely related taxa and one that most widely represented in the GenBank. In phylogenetic studies thus the most complicated were cases of rapid

diversifications that almost always resulted in unresolved trees representing polytomy, or so called bush-like phylogenies. The increase in the number of independent markers may to some extent improve the robustness but rarely resolve the tree in a whole. The other point in the reviewed studies with individual markers is that due to random choice of the markers, the mechanisms of adaptive evolution and the genetics of adaptation process in non- model organisms remained elusive.

The face and pipeline of most areas of evolutionary biology and ecology dramatically changed with the input of new generation sequencing data (NGS) and their wide. The greatest change related to genome wide association studies (GWAS). The greatest change is in that it became possible to identify genetic loci responsible for adaptive evolution in non-model organisms. Thus the accent eventually was shifted from descriptive towards causative side of the study and from neutral to selective paradigm in molecular evolution and genetics of adaptation and speciation processes. These studies above all provide a number of bright examples when earlier unresolved rapid radiations received a complete resolution. In this communication the brief review of such studies is provided.

A comparative analysis of genetic differentiation of *Eurytemora* species using CO1, ITS and 18SrRNA genes with an emphasis on *E. affinis* species complex

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VINCENT CASTRIC<sup>4</sup>, ELENA FEFILOVA<sup>5</sup>, SAMI SOUISSI<sup>6</sup>, VICTOR ALEKSEEV<sup>2</sup>

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Genus *Eurytemora* was derived by Giesbrecht from *Temora* in 1881 from the Kiel Fjords. As a type was chosen species *E. affinis* Poppe 1881 with the type Locality in the Elbe River. Just few *Eurytemora* species were known in those times. Today we have 23–26 species within *Eurytemora* genus depending on the source (<http://copepodes.obs-banyuls.fr/> and/or <http://www.marinespecies.org/>). Historically *Eurytemora* is a challenging group of species, there is a number of species, especially problematic: for instance *E. affinis* (Poppe). Today *E. affinis* is a species complex.

At least six different species and subspecies were described within *E. affinis* since the first species description by Poppe in 1880. Some of the descriptions became a valid species; some of them were reduced to synonyms.

In this work, we will discuss the genus from the genetic point of view. 16 species of *Eurytemora* genus were studied with three genes in this work. Mitochondrial cytochrome C oxidase 1 part of gene (CO1) and nuclear genes ITSn and 18SrRNA were used for analysis of *Eurytemora affinis* species complex. Twelve species were searched with CO1 and 18SrRNA genes: *E. americana* Williams; *E. arctica* Wilson & Tash;

*E. bilobata* Akatova; *E. brodsky* Kos; *E. canadensis* Marsh *E. composita* Keiser; *E. foveola* Johnson, *E. gracilicauda* Akatova; *E. herdmani* Thompson & Scott; *E. lacustris* Poppe; *E. pacifica* Sato; *E. velox* Lilljeborg.

All available CO1, ITSn and 18SrRNA sequences were retrieved from the international GenBank base. Totally about 540 sequences were analyzed, most of them are original.

For this study, the Federal Collection of Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) was used. The work was conducted in accordance with the national initiative AAAA-A19-119020690091-0 and supported by grant from Russian Foundation for Basic Research (RFBR 17-04-00027A and 19-04-00217); partly the work was done in frames of the State Tasks of the Animals Ecology Department of the Institute of Biology, Komi SC UrD RAS (AAAA-A17-117112850235-2).

## Phylogenetic relationships of cyprinid fishes (Cyprinidae) inferred from morphological traits and mitochondrial gene cytochrome b in Bangladesh

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For the very first time in Bangladesh, the evolutionary relationships of cyprinidae fishes were inferred from the morphological traits and retrieved nucleotide sequences of mitochondrial gene cytochrome b (CytB). The morphological traits were collected from 30 taxa and used for phylogenetic tree reconstruction by maximum parsimony method using Mesquite (v.2.6) software. The nucleotide sequences of CytB of selected cyprinid fishes were retrieved from NCBI genbank databases. The nucleotide composition, transition/transversion bias, evolutionary divergence between sequences, phylogenetic trees and timetree were analyzed by using the Molecular Evolutionary Genetic Analysis (MEGA ver. 6.01) software. The average nucleotide compositions were 28.86% (A), 28.22% (T/U), 28.46% (C), and 14.46% (G) where GC content (42.92%) was lower than the AT content (58.08). The rate of transition/transversion ratios were  $k_1 = 2.894$  (purines) and  $k_2 = 4.126$  (pyrimidines) with an overall transition/transversion bias  $R = 1.842$ , where  $R = [A * G * k_1 + T * C * k_2] / [(A + G) * (T + C)]$ . The transitional substitutions (64.24) were higher than the transversional substitution (35.76). The highest and lowest estimates of evolutionary divergence between CytB sequences were found between *Rasbora daniconius* and *Securicula gora* (0.359), and *Catla catla* and *Labeo rohita* (0.065) respectively. In both phylogenetic trees only monophyletic lineage was supported for leuciscinae subfamily where other sub-families garrinae, schizothoracinae, rasborinae, cultrinae, cyprininae were polyphyletic. From the time tree, the highest divergence time 10.42 MYA was observed between *B. bendelisis* and *L. goniis* and the lowest divergence time 1.35

MYA was observed between *H. molitrix* & *A. nobilis*. This study revealed the complex evolutionary relationship of cyprinid fishes in Bangladesh, detail phylogenetic and evolutionary analyses of cyprinid fishes by molecular sequencing of mitochondrial genes of all the available cyprinid fishes of Bangladesh are required.

## Cosmopolite species versus cryptic species two paradigms in copepod biogeography in molecular-genetic epoch and the future of morphological taxonomy

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Areal of species is one of the most important characteristics of any organism in the world. It combines many biological, historical and geographical features. Among those the most important are as following evolution and adaptations to food ability and predator pressure, geographical barriers and species adaptations to dispersal, climate fluctuation and continental drift. Copepoda as many other aquatic Maxillopoda are very ancient organisms. Copepods can be possibly traced till Pangea period similar to modern species paleontological lineages in Ostracods better preserved in sediments (Vegnerer 1926; Alekseev & Starobogatov 1996). Observation of copepod remains in oil infiltrate recently obtained from North America seems like confirm morphological stability of taxonomically important signs in Copepods. From another point, the short history of copepod taxonomical studies (less than 200 years) and relatively small public interest to microscopic crustaceans as well as a few numbers of real experts in this group all living in Europe became reasons for low progress in taxonomy and systematics of Copepoda. As a result, in other continents and geographically isolated areas, the same names were used for similar or close related but not scientifically separated species. Morphological similarity and unification many geographically separated organisms within the same taxon were titled cosmopolitism or species cosmopolite paradigm.

As an antagonist of this paradigm the cryptic species theory was erected in the 20th century with elaborating of new instruments for species identification (enzyme analyses, DNA hybridization approach,



barcoding etc.). In accordance with this theory, most of the described species living in isolated localities, in reality, were presented with flocks of species missing stable morphological differences. Interactions between these opposite views, their effects on species identification, richness, manifestation, the role of "new cosmopolitanism" human mediated global species transportation in breaking of zoogeographical barriers is discussed on examples of copepods with emphasis on *Eurytemora*.

This study was supported with RFBR grant 17-04-00027, by Government Program #AAAA-A19-119020690091-0, by Russian Academy of Science topics 65.4 and 65.5 and the Federal Zoological Collection.

## Phylogeography of the freshwater copepods: two ways of distribution in European area

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Current tools enable the tracing of biogeographical and phylogenetic histories of organisms and have significantly changed our concepts of the extent and background of the diversity in different taxa. Previous analyses of molecular phylogeny and phylogeography of freshwater copepods revealed the mosaic structure among conspecific populations: cosmopolitan and widespread species had been discovered to be cryptic complexes. This study aims to investigate phylogeographic patterns of morphological and genetic variation among three widespread copepod species (*Canthocamptus staphylinus* Jurine, *Attheyella crassa* Sars, and *Eucyclops serrrulatus* Fischer) to reveal possible ways of colonisation in Europe. Analysis of morphological traits and genetic data from mitochondrial and nuclear DNA showed the presence of two patterns of distribution among three species: the congruent model of division into East and West populations for *A. crassa* & *E. serrrulatus*, and separation into Fennoscandian and Continental populations for *C. staphylinus*. The latter pattern can be explained by the formation of Yoldia Sea in Fennoscandian area (10300–9500 years BP) and gradual ice retreat at the continental part of Europe and consequently the development of distinct phylogenetic lineages. At the same time, similar subdivisions into Eastern and Western races have been often noticed among other taxa (mammals, invertebrates, and fish) in the area. Those distinctions have been traced back to geographically disjunct refugia in Southern Europe (13000–15000 years BP, which are thought to have been critical for the accumulation and maintenance of divergence. Thus, two different patterns of phylogeographic distribution based on past geological events have been observed among widespread freshwater copepod species.

For this work, the Federal Collection of Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) was used. This work was conducted in accordance with the national initiative AAAA-A19-119020690091-0 and supported by grant from Russian Foundation for Basic Research (RFBR 17-04-00027A and 19-04-00217).

*Eurytemora* species (Copepoda, Calanoida) in the Lena river delta — Laptev Sea region: Composition, distribution and ecology

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More than 20 *Eurytemora* species are known from the world's fauna, most of which inhabit brackish waters and only a few occupying fresh waterbodies (Kos 1977; Borutskii et al. 1991). Five *Eurytemora* species have previously been reported from the Lena River Delta: *Eurytemora canadensis* Marsh, 1920; *E. raboti* Richard, 1897; *E. gracilis* Sars, 1898; *E. lacustris* (Poppe, 1887) and *E. affinis* (Poppe, 1880) (Pirozhnikov and Shulga, 1957; Kerer 1968). Most of these species have broad aerial distributions throughout the Northern Hemisphere. Our own investigations over long-term period (1990–2018) revealed nine *Eurytemora* species in the various waterbodies on the Lena River Delta — Laptev Sea shelf area. Some of these (*E. bilobata* Akatova, 1949; *E. gracilicauda* Akatova, 1949; *E. foveola* (Jonson, 1966); *E. arctica* Wilson and Tash, 1966; *E. composita* Keiser, 1929 and *E. richingsi* (Heron and Damkaer, 1976)) are a new species for the investigated region. The first two have previously been reported from the Kolyma River region, on the northeast coast of Kamchatka & from western Alaska (Akatova, 1949; Heron, 1964). Two another *E. arctica* and *E. foveola*, are new species to Palearctic pelagic fauna, these species had previously only been reported from Alaska, Nearctic region.

Clear differences were noted between the *Eurytemora* composition in the different water bodies, depending on their hydrological and hydrochemical regimes. Only *E. richingsi* was recorded as a marine species from the Laptev Sea shelf. *E. canadensis*, *E. raboti* and *E. composita* mostly inhabit the brackish estuary parts of the channels, lagoons and lakes on the edge of the delta under the salinity of about 0 to 4-6 ‰. They are also occurring from the open part of the Olenek Tumat, Yana and Tiksi bays, where they are confined within the upper freshened water layer. *E. gracilis* and *E. foveola* were found in both fresh and brackish waters. *E. bilobata*, *E. arctica* and *gracilicauda* were recorded only in freshwater environments in the Lena Delta.

Of special interest was the first documented presence of *E. Gracilicauda* in the pelagic fauna of the Lena River Delta. The appearance of *E. gracilicauda* in this area may be the result of passive transport (from Alaska or from other Arctic coastal regions) with seasonal bird migrations (Bennike, 2000; Alerstam et al., 2007). The great concentration of birds in the delta area maximizes the potential for their contact with copepods. Latent stages of crustaceans are extremely resistant to mechanical damage and to oxygen and nutrient deficiencies, and have a good chance of surviving for long periods in hostile conditions (Santer, 1998). Alternatively, it is possible that *E. gracilicauda* reached the delta from more southerly regions during spring floods; even though no *Eurytemora* species have been recorded in the river water this possibility cannot be excluded. Furthermore, recent climatic warming is likely to have supported biological invasions by species originating from warmer regions (Adrian et al., 2009; Rautio et al., 2011). The recent spread of these copepods may also have been facilitated by human activities. The variety of possible mechanisms that could have been involved in the invasion of these island waterbodies by *Eurytemora* and its subsequent persistence highlight its considerable ecological flexibility (Saunders, 1993). *E. arctica* was described from small, shallow, permanent, unnamed bodies of fresh water and nearby smaller, less permanent pools and ponds situated in wet *Carex* marsh and meadows in the Cape Thompson region, Alaska (Wilson and Tash, 1966). We found *E. arctica* in small permanent polygon ponds, in water-filled

## Session 2: Biogeography

ice-cracks, and in small, less permanent waterbodies on the floodplain of Lena River Delta islands. These sound very similar to the fresh waterbodies described by Wilson and Tash (1966) for Alaska.

The situation is, however, different for the distribution of *E. foveola* in the Lena Delta area. This species had previously only been reported from the brackish landlocked lagoons along the coastline in the Cape Thompson region of Alaska (Johnson, 1961). Nothing more was known concerning its habitat or distribution elsewhere in the world (Borutskiy et. al., 1991). In the Lena River Delta *E. foveola* is one of the dominant species in brackish water area around the delta and it is present in vast number in some deep freshwater oxbow lakes, where it breed successfully every year in the middle-end of summer. It would appear that *E. foveola* has occupied these freshwater reservoirs for a long time and their distribution in the Arctic regions (as well as the distribution of *E. arctica* and *E. bilobata*) probably relates directly to the last glaciation. It has been previously noted that glaciation had a marked effect on the biogeography of freshwater crustaceans in circumpolar areas (Samchyshyna et al. 2008). It seems so that Eurytemora species originally (probably at the time of the last interglacial transgression) had a very broad range but their distribution subsequently became more restricted and a number of local brackishwater and freshwater populations of these species developed, as was the case for *Senecella calanoides* Juday (Pirozhnikov, 1958).

The distribution patterns of *E. arctica* and *E. foveola* in the pelagic fauna of water bodies in the Lena Delta and Alaska support the above theory. The fact that *E. arctica* and *E. foveola* have only been recorded from these two locations suggests that there may now be two isolated populations, one in Alaska and the other in Siberia. Many faunal affinities are known between northern Alaska and eastern Siberia and it is therefore not surprising that the small-crustacean faunas of these adjacent areas also show similarities, not only with each other but also with those of other Arctic areas (Reed 1962). The high diversity of *Eurytemora* species in the Lena River Delta can probably be attributed to the paucity of collections in other regions rather than to distributional peculiarities. The number of zooplankton collections from the Lena Delta

over last two decades is high compared to other areas at similar latitudes in Russia, and probably in the entire world.

The authors would like to express their gratitude for the opportunity to carry out material processing at the “Samoylov Island” scientific station (EPPG RAS, Novosibirsk) and for the technical support provided. The study was supported by the RFBR grant 17-04-00027-a.

## Use of integumental pore analyze for sibling species separation in the genus *Eurytemora*

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Integumentary system in copepods is presented by numeral pores, sensiles and outer cover variations in shape, width. It represents several organs and can be used by the animals in different ways including chemical communications between sexes. It was shown in several copepod genera both in Cyclopoida (*Eucyclops*, *Thermocyclops*) and Calanoida (*Epischura*) that combinations of pore/sensile numbers with their position in outer copepod skeleton are unique for species and can be used for delineation of morphologically close forms (Alekseev et al 2003; Alekseev & Naumova 2007). Integumentary system in *Eurytemora* has not been studied yet so our preliminary results obtained in several close related species of so named *affinis*-group and in *E. velox* are the first data on this organ presence as well as became the first attempt to apply the express method of integumental pore staining to this genus. To our mind this method possibly can be successively used in American *affinis*-tribe of sibling species discrimination and description.

This study was supported by RFBR grant 17-04-00027, by Government Program #AAAA-A19-119020690091-0, by Russian Academy of Science topics 65.4 and 65.5 and the Federal Zoological Collection.



## Distribution, dynamics of abundance and biomass of species of the genus *Eurytemora* in water bodies of Primorye Territory

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The collection of zooplankton in the estuary systems of the Primorsky Territory was carried out from 1993 to 2002. Studies were conducted on the entire coast from the rivers of the south part of the Khasansky district to the north part of the Terney district. The work was carried out throughout the year in the lower, middle and upper parts of the internal estuary of rivers, lagoon lakes, and the sea coast in the outer estuarine zone of water bodies. Usually, the collection of zooplankton was carried out in the regime of daily work. Charges were carried out every 4 hours, from 12-00 of the first day to 12-00 of the next day. For collections, the Nansen network was used with an inlet opening area of 0,05 m<sup>2</sup>, made of gas with a sieve No. 63-77. Sampling was carried out totally from the bottom to the surface of water. The environment setting (temperature and salinity) were measured in parallel.

In the estuary systems of the Primorsky Territory, two species of calanoid copepods of the genus *Eurytemora* are noted. These are *Eurytemora pacifica* Sato, 1913 and *E. americana* Williams, 1906. The first species is most numerous in the southern parts of Primorsky Territory. The second species prevails in the waters of the central and northern parts of eastern Primorye. These species in estuarine systems were noted mainly along with other brackish-water species of calanoid copepods *Pseudodiaptomus* (= *Scmackeria*) *inopinus* Burckhardt, 1913 and *Sinocalanus tenellus* (Kikuchi, 1928). In total, about 200 animal taxa – freshwater, brackish-water and marine — were recorded in the zooplankton community of the estuary systems.

Species genus *Eurytemora* in the estuary of the river is concentrated

in its lower part. They are noted throughout the inland estuary, as well as in the coastal zone of the sea, but their numbers there are very low. During the year, two abundance peaks are observed: the first in May-June, the second in September-November, depending on the region of Primorsky Territory and the hydrological conditions of the year. As a rule, the number of crustaceans of the genus *Eurytemora* is very low compared with other types of brackish-water of calanoid copepods. Usually it is several hundred specimens per cubic meter of water, rising during the period of peaks to 2-3 or more thousands individuals / m<sup>3</sup>. At the same time, its biomass amounts to several mg/m<sup>3</sup>, which increases to 30-50 mg/m<sup>3</sup>. The highest biomass of both types of species genus *Eurytemora* with a predominance of *E. americana* was noted in the Kievka River on the eastern coast of the Primorsky Territory. During the spring peak in May, its biomass increased to 5 g/m<sup>3</sup> in the lower part of the estuarine zone. The rest of the time its amount is small. Thus, the highest values of the quantitative parameters of species genus *Eurytemora* are noted in the zones where the maximum overlap of the ranges of species occurs. Such a zone in the Primorsky Territory was the southern part of the central region, where the estuary zone of the Kievka River is located.

As noted when performing daily work (collections were carried out every 4 hours starting from 12.00) in the estuary systems of the Primorye Territory, the highest values of the number and biomass of plankton animals, including the calanoid copepods of the genus *Eurytemora*, were observed at night. During the daytime, they preferred to be in the lower, bottom layer.

In winter, the number of species genus *Eurytemora* sharply decreased, since there was a period of diapause, and they were noted individually. Mostly these species were represented by copepodites at stages III-IV.

## The role of invasive *Eurytemora carolleeae* and native *Eurytemora affinis* (Copepoda: Crustacea) in the diet of coastal fish in the eastern Gulf of Finland

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Invasive American species *Eurytemora carolleeae* Alekseev et Souissi was detected by molecular markers in the Gulf of Finland in 2007. This species is morphologically similar to the native *Eurytemora affinis* (Poppe). As a result, before this finding, this species was detected as *E. affinis* together with native one. *E. affinis* is a dominant pelagic species and constitutes the main food source for animals at high trophic levels of estuaries including the Gulf of Finland (e.g. Devreker et al., 2008, 2010; Dur et al., 2009; Lee, 2000; Sukhikh et al., 2018). The purpose of the study was to assess the role of both mentioned *Eurytemora* species in the feeding of adults and juveniles of some fish species, based on the intestinal tract content analysis.

Samples of coastal fish species and zooplankton were collected in the Neva Estuary (eastern Gulf of Finland) in 2014 & 2015. In the surveyed area, food composition of adult and juvenile three-spined sticklebacks (*Gasterosteus aculeatus* L.) and juvenile perch (*Perca fluviatilis* L.) was investigated. This two species are one of the most abundant fish species in studied area, which diet mostly consist of planktonic organisms (Demchuk et al., 2017; Golubkov et al., 2018).

Group of species of genus *Eurytemora* was most important prey items among planktonic organisms in the diet of sticklebacks and perches. For sticklebacks, *Eurytemora* spp. were 60% from the total stomach contents, for perches it was about 40%. Frequency of occurrence of *Eurytemora* spp. in all non-empty stomachs was about 60% for both fish species.

Cladoceran *Daphnia cucullata* G.O. Sars, *Bosmina* sp. and copepods of genus *Eurytemora* spp. formed a dominant group of zooplankton species, presented in both years in zooplankton community with high abundance. The share of *Eurytemora* spp. in zooplankton was in average about 25%

in 2014 and 45% in 2015. Three different species of genus *Eurytemora* spp. were present. They were *E. carolleae*, *E. affinis* and *Eurytemora lacustris* (Poppe). *E. affinis* was dominant and accounted 60% of all genus *Eurytemora*. *E. lacustris* was not included in this study due to its relatively low presence in zooplankton community (not more than 10%) and in fish diet (about 5% in average).

The investigation of fish feeding showed that there was no selectivity in consuming of *E. affinis* & *E. carolleae*. However, the species *E. affinis* was dominant in the stomach content of perch and stickleback both in juveniles and adult fish. Probably, this was due to the dominance of the native *E. affinis* in zooplankton during the studied period.

Adults of three-spined stickleback preferred to consume *E. affinis* females, while their juveniles, as well as perch juveniles fed, preferably, on males and latest stages of copepodites of this species. This fact probably may be explained by a dimensional selectivity, since the males and latest stages of copepodites of *E. affinis* are smaller than females and are probably the more suitable food for fish juveniles.

In conclusion, further studies of the fish feeding by the *Eurytemora* species are needed to improve the understanding of their trophic relationships and coastal plankton-eating fish in the eastern Gulf of Finland.

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## The ecological preferences of *Eurytemora affinis* (Poppe, 1880) in the Kazakhstan's water bodies (Central Asia)

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Information about *Eurytemora* from the water bodies of Kazakhstan is extremely scarce. According to literary data, 5 species of this genus are known — *E. affinis* (Poppe, 1880), *E. velox* (Lilljeborg, 1853), *E. lacustris* (Poppe, 1887), *E. grimmi* (Sars, 1897), and *E. minor* Behning, 1938 (Krupa et al., 2016). Until the early 90s of the last century, *E. grimmi* and *E. minor* were a constant component of the zooplankton of the Middle and South Caspian (Hossieni et al., 1996). The occurrence of ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 in the Caspian Sea led to a reduction in the *Eurytemora* abundance (Roohi et al., 2008). In recent decades, there is no reliable information about the findings of these species in the Caspian Sea. Two other species, *E. velox* and *E. lacustris*, are also rarely found in shallow brackish water bodies of the West Kazakhstan.

Compared with other *Eurytemora* species, *E. affinis* is a more common component of the zooplankton, but in the literature there is no information on its environmental preferences in this region (Krupa et al., 2016). This paper, whose goal was to analyze the distribution of *E. affinis* in the gradient of environmental factors, fills this gap.

Zooplankton samples were taken in 128 water bodies of Kazakhstan in the summer of 1997–2018. *E. affinis* was found in 17 water bodies. Most of them belong to the Nura River Basin (Central Kazakhstan). These are the reservoirs of the upper and middle currents and the delta lakes of the lower reaches of the river. *E. affinis* was also found in shallow ponds located to the north of the mineralized Lake Teniz. In addition to Central Kazakhstan, *E. affinis* is occasionally found in the Emba River (Western

Kazakhstan), from where it is brought into the Northern Caspian.

In water bodies inhabited by *E. affinis*, the water temperature reached 17.5–23.5°C. Maximum depths were equal to 1.0–4.0 m. Water transparency was 0.1–4.0 m. The mineralization changed from 0.2 to 7.1 g/dm<sup>3</sup>, at the pH value of 8.2–9.2. The content of NH<sub>4</sub><sup>+</sup> was 0.00–9.1, NO<sub>3</sub><sup>-</sup> – 0.001–3.42, NO<sub>2</sub><sup>-</sup> – 0.004–0.4 mg/dm<sup>3</sup>. The Cd and Zn concentrations varied from trace values to 0.0019 mg/dm<sup>3</sup> and to 0.004 mg/dm<sup>3</sup> respectively. The amount of Cu changed from 0.003 to 0.150 mg/dm<sup>3</sup>. The concentrations of Pb reached 0.003–0.035 mg/dm<sup>3</sup>.

In the Nura and Emba Rivers, as well as the channels connecting the delta lakes the *Eurytemora* abundance was at a low level — 4.0–10.1 thousand ind/m<sup>3</sup>. In the reservoirs the number of its populations reached 2.7–19.9 thousand ind/m<sup>3</sup>; in the delta lakes was 3.1–111.6 thousand ind/m<sup>3</sup>. The high abundance of *E. affinis* was recorded in the shallow ponds – 66.4–242.3 thousand ind/m<sup>3</sup>. The number of mature individuals of *E. affinis* varied from 0.02 to 1.6 thousand ind/m<sup>3</sup>. Males dominated most often. Their abundance was 1.2–5.5 times more than of the females.

According to the results of the correlation analysis, the relationship between the abundance of *E. affinis* and environmental factors (mineralization, the content of nutrients and heavy metals) was not statistically significant. Analysis of the Scatterplots Diagrams showed the nonlinearity of the crustacean's distribution in the gradient of external factors. In lakes and reservoirs the highest abundance of mature individuals and the total abundance of populations of *E. affinis* were recorded at the water salinity of 1.0–3.0 g/dm<sup>3</sup>. In the Caspian Sea, this species was found only in the coastal zone with the salinity of 0.2–0.7‰. The heavy metals Cd, Pb, and Zn did not have a significant effect on the *E. affinis* populations. The decrease in the abundance of crustaceans has been revealed at the Cu content more than 0.02 mg/dm<sup>3</sup>.

Thus, in Kazakhstan *E. affinis* is found locally. One of the reasons for the relatively rare findings of this species is obviously the climatic conditions of the region. According to the literature, copepods prefer temperatures from 12 to 14°C (Berenike et al., 2012). In experiments after temperature increased to 24°C, the population remained viable, but copepod size was significantly decreased as well as female's fecundity (Souissi et al., 2016). Within the above mentioned narrow range, *E. affinis*

populations were numerous at the temperature of 23.5–24.0°C, which may be the upper thermal boundary for this species. The absence of copepods in the water bodies of the South Kazakhstan may be due precisely to the high summer temperature (28–32°C).

In addition to the temperature, the fauna of water bodies is determined by salinity (Khlebovich, 2013). *E. affinis* is considered a wide euryhaline species (Karlsson et al., 2018), with an optimal range of 5 to 15‰ (Cailleaud et al., 2007; Ishikawa et al., 1999). Only the most extreme salinities (0 and 35‰) had a negative effect on naupliar survival (Devreker et al., 2007). In the Baltic Sea copepods were constantly encountered at the salinity of 2.4–9.3‰ (Paturej, Gutkowska 2015), but according to (Viitasalo et al., 1994), they avoided salinity above 6.5‰. In seas with oceanic salinity (30–35‰), *E. affinis* were recorded in desalinated coastal zones (Borutsky et al., 1991).

The salinity of the open part of the Caspian Sea (9–13‰) is within the optimum for this species. However, *E. affinis* preferred the coastal zone influenced by the runoff of the Ural and Emba Rivers, with the salinity of 0.2–0.7‰. This may be associated not only with the peculiarity of the chemical composition of the Caspian Sea waters (Alekin, Lyakhin, 1984), but also with more favorable feeding conditions in the zone of the influence of the river flow. According to the experimental data, the amount of food influenced this Copepod's adaptation to salinity, but not to temperature (Hammock et al., 2015).

In the examined lakes and reservoirs of Kazakhstan, *E. affinis* was registered at the salinity of 0.5–7.0 g/dm<sup>3</sup>, which confirms its euryhalinity. These copepods were the most numerous at 1.0–3.0 g/dm<sup>3</sup>. This indicates a shift in the optimal range of mineralization in continental waters towards fresher waters than indicated for the seas and established in experiments.

The described features of the biology of *Eurytemora* can be associated both with the heterogeneity of its populations from different regions and with the existence of a complex of related species called "*Eurytemora affinis*" (Lee, 2000).

Distribution of the copepod *Eurytemora caspica*  
Sukhikh et Alekseev, 2013 (Crustacea: Calanoida)  
in the reservoirs of the Volga and Don rivers basins.

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In August 2015–2018, in the course of the complex research expeditions of the Institute for Biology of Inland Waters, Russian Academy of Sciences, zooplankton was studied in all eight reservoirs of the Volga River and in its unregulated part between the cities of Volgograd and Astrakhan. In August 2016, three reservoirs of the Kama River and in August 2018, the Volga-Don Canal and Tsymlyansk Reservoir in the Don River were studied. During the first year it was found that a recently described species *Eurytemora caspica* Sukhikh et Alekseev, 2013 from the Caspian Sea inhabited the Volga River downstream of the Kama River mouth (southward of 55°12' N). *E. caspica* has inhabited the Volga River since the 1980s; before 2015 the species was identified as *E. affinis* (Poppe, 1880). In 2016, *E. caspica* was first recorded in the Kama River from its mouth to the middle part of the Kama Reservoir (58°52' N) within 1000 km from the Volga River. In 2018, the taxonomic status of crustaceans from the Volga and Kama rivers was confirmed by the researchers who first described them and the results of the molecular genetic analysis [Sukhikh et al., 2018].

In 2018, the species was first recorded in the western part of the Volga-Don Canal: in small Varvarovka (48°29' N, 44°14' E), Breslavka (48°37' N, 44°06' E), and Karpovka (48°38' N, 43°40' E) reservoirs (45–90 km from the Volga River and 6–50 km from the Don River), and in the Tsymlyansk Reservoir (47°43' N, 42°22' E). In the Tsymlyansk Reservoir *E. caspica* was reported as a mass species since the end of the 1970s [Svistunova, Sayapin, 2010], up to the present the species was identified as *E. affinis*. The taxonomic status of crustaceans from these habitats



requires confirmation.

In the second half of the summer *E. caspica* was a common (occurrences 70–90% of samples) inhabitant in the pelagial zone of the Kuibyshev and Volgograd reservoirs in the Volga River and in the unregulated part of the river downstream of the city of Volgograd (50% of samples) but the species was not detected in the Saratov Reservoir in the studied years. In the Kama River in the pelagial zones of the Votkinsk and Kama reservoirs the species was recorded everywhere (>90% of samples) and in 65% of samples in the Nizhnekamsk Reservoir. In the Don River *E. caspica* was detected almost in the entire area of the Tsymlyansk Reservoir except its upper part upstream of the town of Kalach. The analysis of the published data indicates that the species inhabits the Don River at a distance of 480 km from the Sea of Azov to the middle course of the river (about 48°40' N). The species abundance (adults, copepodites, and nauplii) vary in the area of the studied water bodies from 5–10 to 6000 ind./m<sup>3</sup>; high values (>1000 ind./m<sup>3</sup>) are recorded in the Volga-Kama reach of the Kuibyshev Reservoir; middle parts of the Volgograd, Votkinsk and Varvarovka reservoirs.

*E. caspica* is the smallest copepod among Ponto-Caspian copepods inhabiting the Volga River and Don River. The length of mature crustaceans varies between water bodies. The largest females (a body length of 1.1–1.3 mm) and males (1.0–1.1 mm) were recorded in the Kama and Votkinsk reservoirs; the smallest specimens (♀ 1.0–1.1 mm, ♂ 0.8–1.0) were recorded in the Kuibyshev Reservoir and in the unregulated part of the Volga River. Specimens from the northern Caspian Sea also have a small body length (♀ 0.90–0.98 mm, ♂ 0.88–0.96 mm) [Sukhikh, Alekseev, 2013]. Small-sized populations of *E. caspica* similar in the body length to the Caspian populations inhabit the Volga-Don Canal

0.88–1.0 mm, ♂ 0.84–0.96 mm) and the Tsymlyansk Reservoir  
0.88–0.96 mm, ♂ 0.88–0.96 mm).

In August, adult reproducing specimens formed 10–70% of the abundance of *E. caspica* populations in the Volga, Kama, and Don rivers. The maximum individual fecundity (10–30 eggs in the egg sac) was recorded in comparatively large crustaceans from the Kama River. It was the maximum (10–25 eggs) in the Kuibyshev Reservoir; decreased twice (7–10 eggs) in the Volgograd Reservoir; and the species did not

reproduce in the unregulated part of the river downstream of the city of Volgograd where only adult males and juvenile females were, mainly, recorded. The individual fecundity of *E. caspica* (8–23 eggs/♀) in the Volga-Don Canal was similar to that in the Kuibyshev Reservoir. The absolute individual fecundity is considerably lower (6–15 eggs) and is similar to that recorded in the Lower Volga. *E. caspica* in this reservoir is the most abundant in July; it forms about 70% of the zooplankton biomass along with *Heterocope caspia* Sars, 1897 [Svistunova, Sayapin, 2010]. According to the data [Timokhina, 2000], the peak of the species abundance in the Kuibyshev Reservoir occurs in June; during this month its abundance is twice as much as the abundance of the Ponto-Caspian invader *H. caspia* which is the most numerous in the Volga River.

Thus, to date, *Eurytemora caspica* is a widespread and abundances species in the Lower and Middle Volga River from the mouth of the Kama River; in the lower and middle courses of the Don and Kama rivers, and in water bodies of the Volga-Don shipping canal. The species has inhabited the Don and Volga rivers since 1970–1980s and it has recently penetrated to the Kama River; its first finding was recorded in 2016.

## *Eurytemora* sp. in the rock pools of the White Sea islands

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Rock pools of the various water salinity are widely distributed on islands of the Kandalaksha Bay, White Sea. The one of the features of ecosystems of this type is the extreme living conditions, which is due to the small size of watersheds and temporal drastic fluctuations of physicochemical parameters. This is particularly in the case of offset area with no permanent connection to the sea. Water accumulation is facilitated here from atmosphere and surface run-off on one side, and from the sea on another. Depending the weather conditions and number of other causes, the salinity fluctuations may achieve here 50‰ during one season.

In 2014–2018 period we studied about 100 rock pools of over 10 islands. Zooplankton was sampled with hydrobiological net (gauze 70) from 1–10 L of filtrated water.

The mean summer salinity in the studied pools was between 0 and 25‰, though often the band of salinity in a pool was 2 to 40 ‰.

This is because of absence of regular connection of pools with the sea and, consequently, during dry season salinity grows up with evaporation, and it plummets after rains.

There were found 55 zooplankton species, including 26 of rotifers, 25 of crustaceans. It is necessary to note the low frequency of species within sufficient fauna list.

Within crustaceans the widely distributed is *Tigriopus brevicornis* (O.F. Müller, 1776), fixed in the most pools, as well as representatives of genus *Eurytemora*. These last ones are developed in mass in ponds, where the mean salinity was 4-10‰ during the season, or slightly exceeding the upper limit of the diapason. In such pools the maximal abundance of zooplankton is fixed both with the maximal disparities. In some large pools the high number of zooplankton abundance are stem from mass development of eurytemora, especially of its juveniles. Its ratio was 90% of the total number. In 18 pools of two islands of monitoring

the species of *Eurytemora* were constantly in three pools characterized with the stability of salinity.

These large pools, depth of 1 m, are situated in the southern side of the Medianka and Sedlovataya islands, the wash of sea here is lower. *Eurytemora sp.* is here a dominant form, estimating the number and biomass of zooplankton community. With it the eurytemoras are presented in three more pools but due to the salinity fluctuations their presence there during season is not constant.

Similar patterns of *Eurytemora sp.* occurrence are on the other islands, but the monitoring was intermittent there.

Crustaceans of genus *Eurytemora* found particularly in the pools during the whole summer season and their number fluctuated from ones to 500 ind./l. But if the salinity was over 16‰ (which is within the limits of mysomesogalin group) they absent. In pools with physical and chemical features slightly changing within summer, there was shown the vertical halinic stratification. Crustaceans of the genus *Eurytemora* stay only in the surface 20-cm sheet and barely found in deeper saltier water levels.

During water freshening there is a change of community to the freshwater, and with the grow of salinity *Eurytemora sp.* are replaced with *T. brevicornis*. In 2012-2014 there was an extensive study of maximally freshened pools. These pools are well isolated from the sea but in contrast to freshwater pools, they have periodical grows of salinity, mainly due to the wind spraying or foul offsets. Salinity varies here usually within 0 and 3‰, rises periodically up to 5-10‰, and seldom as high as 22‰. With the offset of the marine water, the freshwater fauna suppression occurs, and the quantity goes down to 100 ind./l or less, as well the specimens mean body size reduces, which is especially seen in specimens of the genus *Daphnia*. There is often fixed the full elimination of communities after the marine water offset. It happens usually if the salinity exceeds 5‰ and the rainfalls absence within or over two weeks. In this case was observed a full or particular change of community with predominance of brackish fauna, in particular, of specimens of the genus *Eurytemora*, which is of the constant presence of dormant stages and adaptations of the species for having the adverse conditions, and possibility to distribution between watersheds.

## A multi-year investigation of the Temoridae (Crustacea: Copepoda) assemblage succession with the interplay waters in the northern South China Sea

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The South China Sea is the largest marginal sea in the world. The northern South China Sea has a complex hydrographic system affected by interplay waters of the Kuroshio branch and the South China Sea water. To understand the community structure of the planktonic calanoid copepod family Temoridae with the interplay waters, we collected zooplankton samples in the northern South China Sea from depths of the surface (2-0 m) during November 2001 to January 2007 on 24 research cruises using the standard North Pacific zooplankton net (with a mesh size of 200  $\mu\text{m}$ , 1.8 m length, and a mouth diameter of 0.45 m). Among a total of 256 samples, 5 species belonging to 3 genera of Temoridae were identified: *Eurytemora pacifica* Sato, 1913, *Temora discaudata* Giesbrecht, 1889, *Temora stylifera* (Dana), 1849, *Temora turbinata* (Dana), 1849, and *Temoropia mayumbaensis* Scott T, 1894. The 3 most abundant species were: *T. turbinata* (relative abundance, RA: 56.06%; mean density  $\pm$  standard deviation, MD:  $32.81 \pm 101.26$  inds  $\text{m}^{-3}$ ), *T. stylifera* (RA: 33.48%, MD:  $19.6 \pm 144.38$  inds  $\text{m}^{-3}$ ), and *T. discaudata* (RA: 7.62%, MD:  $4.46 \pm 12.02$  inds  $\text{m}^{-3}$ ). The top 3 frequently occurring species were: *T. turbinata* (occurrence ratio, OR: 52.73%), *T. discaudata* (OR: 35.94%), and *T. stylifera* (OR: 9.77%). A noteworthy discovery in our samples was *E. pacifica* a marine and brackish water species, collected from 3 stations located near to the Kuroshio area in April 2003. This is the only month when this species was recorded during the investiga-

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tion period. The variation composition of dominant Temoridae species shows a clear pattern of seasonal succession. Total abundance was significantly higher in the third quarter (Sept.–Nov.,  $116.98 \pm 314.49$  inds  $m^{-3}$ ) than in the fourth quarter (Dec.-Feb.,  $24.26 \pm 47.72$  inds  $m^{-3}$ ) ( $p = 0.016$ , one-way ANOVA). In general, the present results demonstrate that the assemblages of the Temoridae are very much structured by the water masses of the Kuroshio Current and the South China Sea.

## Seasonal and interannual dynamics *Eurytemora affinis* (Poppe, 1880) as key species in Vistula Lagoon of the Baltic Sea

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The Vistula Lagoon is one of the largest shallow brackish water lagoons (area 838 km<sup>2</sup>; water capacity 2.3 km<sup>3</sup>; average depth 2.7 m) of the Baltic Sea which is strongly affected by anthropogenic pressure. *Eurytemora affinis* (Poppe, 1880) is a key species of Vistula Lagoon according to all previous and current research (Schödler, 1866; Vanhöffen, 1917; Riech, 1927; Róžańska, 1963; Adamkiewicz-Chojnacka, 1983; Krilova, 1985; Naumenko, 2008, 2010, 2016; Dmitrieva, Semenova, 2012). The purpose of this study is to analyze the seasonal and interannual dynamics, as well as the spatial distribution of relative mortality parameters *Eurytemora affinis* of Vistula Lagoon.

Plankton samples were taken in 2008–2018 from March till December in the central part of the lagoon at 9 standard monitoring stations of AtlantNIRO using 5–6-liter Van Dorn water sampler from the depths of 0.5, 1–1.5, and 2–3 m. Zooplankton was filtered using a plankton net with a mesh size of 64 µm. Immediately after sampling, the zooplankton was stained with aniline blue to distinguish dead individuals from living ones (Dubovskaya, 2008; Bickel et al., 2008; Dubovskaya et al., 2003; Gladyshev et al., 2003; Seepersad, Crippen, 1978). The staining was performed aboard the vessel, so as to exclude the additional mortality of zooplankters during the transport of samples. Over 700 zooplankton samples were collected and processed in the course of the study. Proportions (%) of the abundance/biomass of dead individuals in the total abundance/biomass of living and dead zooplankters were used as parameters of zooplankton mortality (Dubovskaya, 1987; Dubovskaya et al., 1999).

From March till December 2008–2018 *E. affinis* formed 19.8% of total

abundance and 47.5% total biomass of zooplankton. The maximal proportion *Eurytemora affinis* in zooplankton community was in spring 34% of total abundance and 72% of total biomass of zooplankton, with a maximum in May (50% of total abundance and 89% of total biomass). In summer, the share of *E. affinis* decreased to 18% of total abundance and 27% of total biomass, with a minimum in June (6.6% of total abundance and 15.3% of total biomass), by autumn the share increased to 24% of total abundance and 50% of total biomass. In 2010, invasion bivalve mollusk *Rangia cuneata* in the benthic community of the Vistula lagoon occurred (Rudinskaya, Gusev, 2012), as a result of which restructuring and significant changes occurred in the plankton community of the lagoon. In the period after the invasion of *Rangia cuneata*, the proportion of *E. affinis* in zooplankton in the summer period decreased by 3–5 times compared with the period before the invasion.

The percentage of dead individuals in the population of *E. affinis* was 8.8% on average during the growing season, the minimum proportion of dead individuals was observed in spring, it increased in summer and autumn. After the invasion of *Rangia cuneata*, the proportion of dead individuals decreased by 3 times. The proportion of dead individuals varies by station - the maximum values of the proportion of dead individuals was at stations located near the strait, at which high salinity, at these stations, the proportion of dead individuals was higher than at other more freshwater stations in 2–2.5 times.

The maximum fecundity of *Eurytemora affinis* was observed in the spring period in March–April - 32–35 eggs per female, in the summer period it decreased. Also, the maximum fertility was noted at more freshwater stations, at stations with high salinity, fertility was also reduced.

*Eurytemora affinis* was unequally distributed in the lagoon area, the maximum abundance and biomass values were observed at the most freshwater stations, the minimum at stations located closer to the strait connecting the lagoon with the Baltic Sea.

The seasonally average abundance and biomass of *Eurytemora affinis* were 54.3 thousand specimens/m<sup>3</sup> and 1.02 g/m<sup>3</sup>. The maximum values of abundance and biomass was observed in the spring period in May, then in June–July they decreased and increased again in the autumn.



The seasonally average abundance and biomass values of *E. affinis* did not decrease after the invasion of the mollusk *Rangia cuneata*, there was a decrease of abundance and biomass in summer period of maximal pressure of *Rangia cuneata*, but in spring abundance and biomass of *E. affinis* were increased in compensating these changes.

Thus, the seasonal and interannual dynamics and spatial distribution of *E. affinis* depends on many factors, both abiotic, the main of which are salinity and biotic — the introduction of new species and changes in the food base, as well as the pressure of fish.

## On morphological peculiarities and species distribution in the *Eurytemora affinis* species complex

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Our understanding of the systematics and species richness in *Eurytemora affinis* complex has evolved at a fast pace over the last decades. Formerly considered as a complex of cryptic species, it includes now three valid species: *Eurytemora affinis* (Poppe), *Eurytemora carolleae* Alekseev et Souissi and *Eurytemora caspica* Sukhikh et Alekseev.

Such a way formerly Holarctic distribution has been divided into 3 different areas occupied by each species. *E. caspica* inhabits the North part of the Caspian Sea along with The Volga River basin. The main area of *E. affinis* is European estuaries. The native area of *E. carolleae* is North American Atlantic coast. Areas of *E. affinis*, *E. carolleae* also overlap in the Baltic Sea, where American invader *E. carolleae* was found in 2007. *E. cf. affinis* from Russian Far East and Japan is different morphologically and genetically so in the nearest time it will be described as a new species.

Differences among these *Eurytemora* species were based on measurements and features of mandibles, caudal rami, genital somite and some structures in P4 and P5 in both sexes. Moreover, some of given characters along with additional fine details indicate the presence of at least four morphological groups within the European populations of *E. affinis*. Those morphological differences in these forms along with partly isolated/separated areas of their distribution to our mind correspond to subspecies level.

Morphological variability was estimated in the number of sites:

1. Seine Estuaries, France; Elbe Estuary, Germany;
2. Baltic Sea: Luga Estuary, the Gulf of Finland and Vistula Lagoon, Russia; Gulf of Riga, Latvia;
3. Gironde, Loire, France;
4. Guadalquivir, Spain.

Morphological variability was not random, but grouped in accordance with geographic distribution. Similar groups was obtained using part of Cytochrome-C oxidase 1 gene analysis. Only excluding is Spain population, which was not analyzed with genetic tools.

For this work, the Federal Collection of Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) was used. It was conducted in accordance with the national initiative AAAA-A19-119020690091-0 and supported by grant from Russian Foundation for Basic Research (RFBR 17-04-00027A and 19-04-00217).

## Random phenotypic variation in *Eurytemora* species and other crustaceans

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Variation of any phenotypic trait is traditionally considered to be caused two factors – parental genotypes (genotypic component) and heterogeneity of environmental conditions during period of the trait formation (phenotypic plasticity). However, since the 1930s (Astaurov 1930), it became clear that there is also a third component of phenotypic variation, standing on equal footing with the former two, and caused by developmental instability, which is the inability of an organism to produce an “ideal” form consistently under the same environment (random or stochastic component). The understanding that phenotypic variation includes three but not two components gradually is becoming more and more common.

Traditionally, the random phenotypic variation is studied on bilaterally symmetrical morphological traits. Such traits are never perfectly symmetrical, and minor deviations from such symmetry are called fluctuating asymmetry (FA). FA is often used as an indicator of stress because it was shown that FA increases under stress of different nature – genetic, dealing with disruption of gene co-adaptation, and environmental, due to departure from optimal conditions. FA may attribute rather high proportion of variation of morphological traits, in crustaceans sometimes up to half of the total variance. Studies of FA on *Eurytemora* species are so far very limited, but show that samples of three species *E. caspica*, *E. carolleae* and *E. affinis* are different in FA, which therefore can be considered as a useful technique for revealing morphological heterogeneity (Lajus et al., 2015). It was found nine studies samples can be subdivided to three groups based on FA level. The lowest FA was observed in the sample of *E. caspica* from the Caspian Sea, intermediate — in *E. affinis* from three Baltic populations and populations from Loire and Gironde estuaries, and highest FA was found in *E. affinis* from northern Seine and Elbe estuaries, and from *E. carolleae* from the Chesapeake Bay (USA).

Interestingly, that FA was higher in native *E. carolleeae* (Chesapeake Bay), compared to invasive *E. carolleeae* (Gulf of Finland), and the latter had the same level of FA as native *E. affinis* from the same area. It may mean that first, FA of *Eurytemora* is defined rather by environmental conditions than genotype, and second, that in the new range, the crustacean found the more favorable conditions than in their native range, in particularly, lower temperature, and more stable temperature and salinity conditions due to absence of tidal events in the Gulf of Finland. This could facilitate the invasion. The other evidence of stressfulness of estuarine conditions with significant tidal events is provided by high FA level of native *E. affinis* population from the Seine estuary.

Random phenotypic variation is usually considered to be non-adaptive, as it is a consequence of inability of developmental control mechanisms to produce the perfect phenotype. In some cases, however, this type of variation may have adaptive significance. Such example is a bet-hedging. Bet-hedging is a risk-spreading strategy borrowed from plant biology (equivalent to not placing all your eggs in one basket). Originally bet-hedging was used to explain the seed bank phenomenon, a reservoir of ungerminated seeds in the soil, which allows plants to mitigate risks of unfavorable conditions. If a drought kills the germinated plants, those with seeds remaining in the seed bank will have a fitness advantage in comparison with plants whose seeds germinate immediately. Therefore, development of a bet-hedging strategy may have an evolutionary significance based on adaptive significance of random phenotypic variance because it increases probability of survivorship in a situation of unpredictable environmental changes. It is of special significance for animals producing resting eggs, which need to find a trade off between adjusting their hatching to environmental cues and leaving some number of eggs unhatched if photoperiod or temperature signals do not coincide with favorable environmental conditions. Bet-hedging strategy is considered to be especially important adaptation allowing to mitigate risks of organisms with resting eggs, which often face unpredictable environmental conditions. This strategy also was considered while studying patterns of hatching of diapausing eggs of *Eurytemora* (Glippa & all 2011). Being a manifestation of random phenotypic variation, magnitude of phenotypic variation associated with the bet-hedging strategy, should

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increase under more stressful conditions due to increasing developmental instability. Such pattern can even increase adaptive significance of this strategy as bet-hedging strategy should play even more important role under stressful environmental conditions.

Therefore, studies of the random phenotypic variation, in particularly such as fluctuating asymmetry of morphological traits and bet-hedging strategy may bring important information for population biology of *Eurytemora* species.

For this study, the Federal Collection of Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) was used. The work was conducted in accordance with the national initiative AAAA-A19-119020690091-0 and supported by grant from Russian Foundation for Basic Research (RFBR 17-04-00027A and 19-04-00217A).

## About the systematics of Palaearctic *Eurytemora* (Calanoida, Copepoda) on base of their morphological analysis

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The first descriptions of the *Eurytemora* species (Temoridae, Calanoida, Copepoda) obtained in the former century do not contain information on their variability (Williams, 1906; Akatova, 1949; Borutsky, 1949). This problem is characteristic of, at least, Russian key books and identification tables for the genus representatives (Borutsky et al., 1991; Stepa-nova, 2010; Kos, 2016) and complicates the diagnostics of *Eurytemora* by morphological parameters. The recent descriptions of these copepods include information on the variability of their principle morphometric parameters, the structure of fine formations (mouth parts) and are often added with the analysis results of molecular-genetic variability for species and populations, ecological and behavior signs (Lee, 1999; Lee, Frost, 2002; Fefilova, 2008; Dodson et al., 2010; Alekseev, Souissi, 2011; Moon et al., 2016; Sukhikh et al., 2016 a, b). This study approach is highly required as *Eurytemora* easily colonize new habitats, quickly distribute and change their habitation limits. These changes complicate the research work on composition of local faunas and produce co-existence of numerous intraspecific morpho- and haplotypes, as well as interspecific hybrids (Lee, Frost, 2002).

The goal of our studies is to-species determination of morphologically similar *Eurytemora* representatives inhabiting fresh-water coastal

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waterbodies of the White Sea, the Korovinskaya Bay of the Pechora Sea and the Lena River delta and the stability and changeability research of diagnostically important signs.

The individuals from Siberia and the Pechora River delta have been first identified by us as *Eurytemora gracilicauda* (Akatova), specimens from the White Sea basin – as *Eurytemora brodskyi* Kos. For determination, we have used key books and morphological descriptions of E.V. Borutsky with co-authors (1991) and M.S. Kos (2016). But by the molecular-genetic study results of maxillopods from three populations, they belong to one species and show a low variability index among populations by the studied parts of mitochondrial DNA. To study the morphologic changeability of this species (*Eurytemora gracilicauda*), we take 13 morphometric characteristics of caudal rami and the fifth pair of thoracal legs (P5) for females, morphometric characteristics of caudal rami for males, as well as qualitative and quantitative signs characterizing these formations which are highly important in the *Eurytemora* systematics (Borutsky et al., 1991; Kos, 2016). Additionally, we survey the structure of distal thoracal, genital, and anal somites of females and males, armatures of segments of geniculate antennules for males.

Finally, we specified the *Eurytemora gracilicauda* morphologic characteristics. Females of three populations had the majority of studied signs being stable with little variations (variation coefficient (CV) is < 10%). For any females, the back angles of the distal thoracal somite were elongated into triangle outgrowths, the genital somite was without protrudences or narrowings, the anal somite and caudal rami were covered with spinules. Females from the Lena River delta demonstrated the longest caudal rami ( $0.373 \pm 0.009$  mm) and females from the White Sea basin – the shortest caudal rami ( $0.251 \pm 0.009$  mm). The Lena delta females also had the most elongated segments and spines of P5. Both spines of distal segment of females P5 of any study sampling groups were covered with very small spinules. By the earlier data (Kos, 2016), for the *Eurytemora gracilicauda* diagnosis was important that only long inner spines is covered by spinules, and in the *Eurytemora brodskyi* diagnosis both apical annexa of female P5 are without spinules naked (Kos, 1993, 2016).



The morphological signs of the studied *Eurytemora* males were variable in contrast with characteristics of females. A half of males (from the White Sea basin and the Pechora River delta) had caudal rami and the anal somite without spinules according to the *Eurytemora gracilicauda* diagnosis in the identification keys of M.S. Kos (2016). The other half of males (from the White Sea basin and the Pechora River delta) had caudal branches and the anal somite covered with seldom spinules along external edges according to the *Eurytemora brodskyi* diagnosis (Kos, 2016). The number of spinules on P5 of males largely varied: on the left P5 – from 2 to 3, on the right P5 – from 1 to 6 (CV = 14.2-43.5 %). Only males from the Lena River delta and one male from the Pechora River delta had a finger-like outgrowth on side of the genital somite. This sign was known as varying also for other *Eurytemora* species (Kos, 2016). Basipodites of only left P5 or both P5 of males from any population had a group of very small spinules on the external edge. The stable characteristics of males also were P5 shape, elongated caudal rami (shorter than female rami), no outgrowths on the end thoracal somite, and no long annexa on the 8–12<sup>th</sup> segmentes of geniculate antennule.

Thus, we introduced some additions to the *Eurytemora gracilicauda* diagnosis: identified the changeability limits of several morphometric, qualitative and quantitative signs of the species. The variability of signs may be related with different environmental conditions. But we do not exclude the possibility of hybridization. The sampling group from the Lena River delta had *Eurytemora gracilicauda* together with the other species of the genus as *Eurytemora arctica* Wilson M.S. & Tash, *E. lacustris* (Poppe), *E. roboti* Richard. In the lake of the Pechora delta, the analyzed species was accompanied by *Eurytemora lacustris*. The hybrids of calanoids are known to be seldom met in natural ecosystems. They are similar to one of parents phenotypically but can have morphologic (normally morphometric) changes (Chen et al., 1997; Parent et al., 2012).

The question on the lifetime of the study European populations of *Eurytemora* was open. This question was earlier discussed in relation to *Eurytemora prope brodskyi* (Sukhikh et al., 2016 a) from the White Sea basin. The species was first registered here in 1993 (Kos, 1993). Until recently, the areal of *Eurytemora gracilicauda* was meant to be

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limited by the Far East and the northern part of the Pacific coast in North America (Borutsky et al., 1991). But from the early 2000-s, this species along with *Eurytemora arctica* and *E. foveola* (Johnson M. W.) was found in plankton samples from the Lena delta (Abramova et al., 2017). *Eurytemora gracilicauda occidentalis* Fefilova was found on the Vaigach Island only in 2004. For the Pechora Sea, *Eurytemora gracilicauda* was first noted by us in 2016, 2017. These findings may evidence both a rapid areal widening of the species from east to west or just be additional information on the genus systematics and composition of native plankton communities in the regions.

The work was done in frames of the national themes of the Animals Ecology Department of the Institute of Biology, Komi SC UrD RAS and of the freshwater laboratory of ZIN RAS (AAAA-A17-117112850235-2 and AAAA-A19-119020690091-0 correspondingly), UrD RAS Complex Program (AAAA-A18-118011390005-9) and financially supported by the RFBR grants: 17-04-00027\_a, 18-44-110017 p\_a. For this work, the Federal Collection of Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) was used.

## Variability of mandible shape in freshwater glacial relict *Eurytemora lacustris*

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A typical habitats of *Eurytemora lacustris* are a deep and clear-water lakes. Extensive agriculture and anthropogenic origin nutrients cause acceleration of eutrophication of waters hence nutrient rich waters become serious threat for *E. lacustris* populations. Therefore in some of north European countries *E. lacustris* is considered as an endangered species. Rapid changes in the trophic state and climate changes may force the organisms for urgent adaptation to new conditions. If the species is not phenotypically fitted, it loses chances of winning competition with other species. Organisms have to be successful in feeding, survival and reproduction in order to thrive. Therefore, a few questions have been asked about trait related to feeding, which have a major impact on the species success in a rapidly changing environment. Mandibles have specific shapes therefore are used to classify feeding mode of the certain species. Hence, the shape, number and position of the teeth are characters that are closely linked to the feeding preference of copepods and have been used to clarify their niches in food web. The aim of this study is to determine morphological variability of mandible shape in freshwater calanoid *E. lacustris*. Regard to the aim of this study we tried to answer i) whether the mandible shape differ from larva to adult, ii) whether the shape of the mandible depend on sex and iii) whether the shape of the mandible depends on environmental conditions. Our studies allows to classify the species as an omnivore and show that shape of *E. lacustris* mandible is characterized by large plasticity.

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## The genus *Eurytemora* in the waterbodies of Belarus

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Only one alien species of the genus *Eurytemora* of Ponto-Caspian origin — *E. velox* Lilljeborg, 1853 — is known in the waterbodies of Belarus. Since the time of glacial transgressions, there have existed a number of the relict species of crustaceans, which are also of marine, but Northern origin, in the fauna of Belarus. One of these species belonging to this genus is *Eurytemora lacustris* Pope 1887. It is classified as a rare and endangered species and included in the Red Books of the Republic of Belarus and neighboring Baltic States. Thus, 2 species of *Eurytemora* genus of different geographical origin (one of which is alien species, the other is aboriginal relict one) are represented in the recent fauna of Belarus. The integration and comparison of data on the biology of these species, as well as the establishment of trends in their populations are of general biological significance as a contribution to the understanding of the formation of freshwater communities during the penetration of brackish-water representatives of faunas completely different in genesis.

*E. velox* has now inhabited the main large rivers of the South of Belarus and their accessory reservoirs belonging to the basins of the Baltic and Black seas. The species has penetrated into the reservoirs of Belarus along the central water European invasive corridor, as evidenced by its high occurrence in the rivers of the Dnieper basin. The highest frequency of occurrence is observed in the Pripyat river, where the species has been found all over its Belarusian part from the town of Mikashevichi in the West to the town of Narovlya in the South-East. *E. velox* is not yet registered in the tributaries of the Pripyat river (with the exception of the Pina river) and in the main riverbed of the Dnieper (with the exception of the Sozh river).

The relict species *E. lacustris* has been found only in two mesotrophic dimictic lakes on the territory of the Belarusian Lake District belonging

to the Baltic Sea basin — Volchin (maximum depth 32.9 m) and Vecheliye (35.9 m); this is the southern boundary of the European part of the area of this species.

On the territory of Belarus, the predominant settlement of *E. velox* is observed in the coastal biotopes of watercourses and shallow waterbodies. In river ecosystems, the density is 4.3 times higher in the thicket near-shore coast compared with the current. In standing waterbodies, the density of *E. velox* is 8.5 times higher in the littoral zone compared to the pelagial. The preference for coastal biotopes allows probably avoiding a competition due to spatial disconnection with the pelagic species of diaptomids and is one of the factors for a successful colonization of new habitats. The species is characterized by age differences in spatial distribution depending on the type of a coastal biotope. The naupliar stages of development keep predominantly open areas of water, and the copepodites are in overgrown zones, since it is likely that more adult individuals use thickets as a refuge from predators.

*E. lacustris* is characterized by uneven distribution in the water column and location in deep water layers. The limiting factors of distribution in the water column are temperature and the content of dissolved oxygen. The main part of individuals keep in water layers with temperatures below 13°C, which indicates stenotherm and cold-loving nature of this species. The vertical daily crustacean distribution varies from year to year and correlates with the oxygen concentration in the hypolimnion. The average annual depth of the population habitat is 14.6 m. The younger stages of development keep at a lower depth. With increasing age, the depth of immersion increases: the average annual depth of the nauplius habitat is 11.5 m, copepodites — 19.9 m.

For *E. lacustris* have been established seasonal and direct daily vertical migrations: in the dark time, individuals rise to higher horizons, and during the day they descend into the deep layers of water. The main movements occur in the low-temperature layers of water. With age, the amplitude of migrations increases, reaching values of more than 10 m in adults.

The density of *E. velox* in waterbodies and streams varies widely: from 20 to 12,000 ind./m<sup>3</sup>. The average density of *E. velox* is 1631 ind./m<sup>3</sup>, the share in zooplankton does not exceed 1%. Population density in the

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waters of the Baltic Sea basin is higher than in the rivers of the Black Sea basin. The density of alien species in the freshwater bodies of Belarus is lower than in the brackish ones of the original range, and is comparable to the indicators for other freshwater habitats in the acquired range.

In recent years in the lake Vecheliye, the average density of *E. lacustris* in the water column has been about 2000 ind./m<sup>3</sup>, while the share in zooplankton has been 7%. In the lake Volchin in the same period, the density has been 2–5 times lower, and the share in plankton has not exceeded 2%. The difference in the values of indicators is due to the characteristics of the temperature and oxygen regimes, transparency and morphometry of lakes.

For the first time for *E. lacustris*, a morphological description has been given and the linear sizes of individuals at all stages of development have been determined. It has been established that lake eurythemora males have a longer body length in comparison with females due to the relatively longer abdomen length, which is not typical for other species of freshwater plankton copepods and can be considered as a distinctive feature of this species. The body length of mature individuals is comparable to that indicated for the waterbodies of Russia and some countries of Western Europe. The values of the individual fecundity of *E. lacustris* in the reservoirs of Belarus vary from 8 to 48 eggs (at the average value – 13), which is lower than the values known for other points of the range. The egg sizes of this species are  $100.53 \pm 1.733 \mu\text{m}$ .

From April to December, there were recorded 4 peaks in the dynamics of the density of *E. velox* population: one weakly expressed in mid-July and three with high numbers (August-October). Based on the dynamics of the density of individual stages, we can state polycyclicality. The breeding season is extended and timed to the summer-autumn season. The breeding process is continuous, and generations overlap.

According to the presence of two maximum numbers of mature individuals and nauplius of the first stage in the year, *E. lacustris* is a dicyclic species. The development time of both generations is about six months. Two generations develop during the year: one in spring, the second in autumn. The duration of generation development is about six months, since the reproduction is stretched, they partially overlap.

The development of the crustaceans of the second generation takes place in winter at low water temperatures, which also confirms the cold-loving nature of the species.

Alien eurybiont species *E. velox* inhabits waterbodies with a large gradient of habitat conditions. An increase in the number of this crustacean on sites with low water quality (third class, moderately polluted water) has been established. Since this species is characterized by eurybiontity and the ability to endure unfavorable conditions at the stage of resting eggs, we should expect its further settlement and colonization of new habitats.

According to long-term data, a gradual decrease in the population number of relict copepod *E. lacustris* has been established. The processes of eutrophication or pollution can lead to the disappearance of this species from the fauna of the lakes of Belarus, which has already been established for neighboring countries. *E. lacustris* cannot naturally inhabit new lakes with suitable habitat conditions due to stenobiontity (dwelling in the deep water layers) and the lack of the resting stages of development. Despite this, according to the materials of recent years of studies, the observed populations remain viable and consistently reproducible.

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## Some aspects of ecology of *Eurytemora cf. affinis* from Sakhalin, Russia, in comparison with Hokkaido, Japan

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*Eurytemora affinis* complex — is widely distributed group of species. Until recently *Eurytemora* from Sakhalin Island and Japan was identified as *Eurytemora affinis* (Poppe). Our preliminary morphological searches and results of genetic analysis showed significant differences of oriental *Eurytemora* from the *Eurytemora affinis* specimens in the type locality in the Elbe River. More likely it is new species, which is morphologically close to *E. carolleae* Alekseev et Souissi and *E. caspica* Sukhikh et Alekseev.

This species inhabits oligohaline and fresh water bodies, having a connection with the seas. It was observed in both pelagial and littoral areas, where it can be an epibenthic grazer. It was not found at a salinity of more than 8‰ (the upper boundary of the horohalimum). Seasonal dynamic has been studied in Tunaicha Lake in 2002–2003 years. This is a meromictic lagoon lake with oligohaline upper layer — 2.4–2.6‰. Dates on *Eurytemora* from Japan lakes are taken from literature sources.

Population peaks are present at different times in different regions: in Lake Ohnuma (South Hokkaido) the maximum abundance was observed in early June (study 1984-1985 (Ban and Minoda 1989)), in Lake Tunaicha (South Sakhalin) — in September (2002-2003). In both lakes, Nauplii appeared in mid-April, when adults were not observed. Adult females carrying eggs occurred in early May. In Lake Tunaicha in February–March *Eurytemora* was absent in the plankton (Zavarzin 2005). No occurrence was found in winter also in Hokkaido waterbodies: Lake Ohnuma and Ishikari river oxbow lakes. Females produced diapause eggs and hibernated as the egg stage here (Ban & Minoda 1989). The production of diapause eggs in Lake Ohnuma was observed in spring in addition to winter (Ban 1992a). According to Ban (1992b),



diapause egg production is related to short day length, low temperature, and high copepod population density. Eggs produced in spring can remain in the sediment for at least a year; the accumulation of the eggs in the sediment may serve as an “egg bank” that can provide an important source of nauplii for recruitment into the planktonic population (Ban 1992a).

Ban and Minoda (1989) with reference to Matoda (1950) note that before the 1950s the occurrence of *E. affinis* in Japan was probably restricted to some brackish waters. They suggested that an artificial introduction of smelt fish *Hypomesus nipponensis* from brackish-water lakes played an important role in *Eurytemora* invasion in fresh waters. If the invasion of the freshwaters of Japan is related to human activity, then in the north of Sakhalin this is apparently the natural process of migration from the estuaries because there was no artificial introductions of fish or other aquatic organisms into freshwater lakes Panitu and Sladkoe, at that this lakes are located not far from the sea.

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Biological traits of co-occurring sibling species  
of the *Eurytemora affinis* complex in an important  
fish nursery zone of the St. Lawrence estuary, Canada

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Estuarine transition zones (ETZs), being the receiving end of lentic systems as well as being influenced by the ocean, are recognized as unique ecosystems. Sharp spatial gradients create complex and diverse spatial patterns at the biogeochemical level and in biological communities. ETZs are important fish nursery zones, due to high biological productivity. Especially copepods play a major role in channelling energy from primary producers to higher trophic levels such as mysids, shrimps and larval fishes. Although we may already provide a general description of ETZ food web structure of the St. Lawrence River, we are still far from understanding the underlying processes involved in supporting high productivity. Increasing evidence shows that genetic diversity influences a range of ecosystem functions and properties, such as productivity and that intraspecific genetic diversity may replace species diversity in taxonomically poor systems such as ETZs. However, only few studies have examined the ecological consequences of genetic diversity in estuaries and the impact of intraspecific genetic diversity of zooplankton on biological traits and productivity patterns remains unknown. Here, we focus on the estuarine copepod sibling species complex *Eurytemora affinis* that is the dominant calanoid copepod of the ETZ. Genetically divergent sibling species may exhibit distinct habitat preferences and/or divergence in life history traits. The St. Lawrence ETZ is the secondary contact zone of two ancestral sibling species of *Eurytemora affinis*, *E. carolleeae* and the *E. affinis* North Atlantic clade (NA-clade). The two sibling species strongly segregated spatially throughout the ETZ and this segregation is partially maintained by

physical features, such as estuarine circulation, advection and dispersal patterns in the ETZ. *E. carolleae* occupies predominantly tidal freshwater and hypersaline habitats, whereas NA-clade is limited to brackish water habitats. The aim of this study was to test if the two sibling species show differences in biological traits that might be important to understand the overall production patterns of this species complex in the ETZ. We hypothesized that the cost of wide ecophysiological tolerance of the *E. carolleae* translates into smaller size and lower egg production compared to *E. affinis*. *Eurytemora* was sampled at two different locations in the St. Lawrence ETZ at Berthier sur mer (freshwater) and St. Jean Port Joli (brackish) from May to August 2018. In the laboratory, the NA-clade and *E. carolleae* were identified and sex ratios, female to ovigerous female ratio, clutch size and female size were determined. In addition, egg production experiments were carried out over 24h. Both species followed the “temperature-size rule” as they experienced a decrease in body size with rising water temperatures throughout the summer. In general, the NA-clade was longer than *E. carolleae*. The NA-clade and showed higher length width ratio, translating into a slenderer body form than that of *E. carolleae*. However, our hypothesis that clutch sizes were smaller in *E. carolleae* was rejected. In contrast, we found smaller body length and bigger mean clutch sizes in this species. *E. carolleae* expressed a reproductive strategy of producing more, but smaller eggs, whereas the NA-clade showed the opposite. Ratios of egg-carrying female to female without an egg sac was above 50% in both sibling species from June to August, with slightly higher percentages of egg-carrying females in the NA-clade than in *E. carolleae*. During the experiments spawning rates were also higher in *E. carolleae* compared to NA-clade and these were temperature dependent. Decreasing spawning rates were found at temperatures > 20 ° C. Preliminary estimates of egg production (EPR) revealed that *E. carolleae* showed higher EPRs than NA-clade. Maximum rates for both species were found in the middle of June at temperatures of 16.6 and 18.7 °C in the brackish and tidal freshwater habitat, respectively. Our study revealed substantial differences in biological traits of both sibling species in the ETZ. Despite higher overall production rate of *E. carolleae*, based on the short term incubation experiments,

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densities in the field were mostly lower than in NA-clade, suggesting that other parameters might also influence observed copepod standing stock. Bottom-up and top-down control such as food limitation or high predation will be discussed in the light of both sibling species of the *Eurytemora affinis* complex in this important nursery zone of the St. Lawrence Estuary.

## Genetic consequences of landlocking for marine invertebrates and fishes from marine lake

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Populations of islands are subject to evolutionary dynamics that are determined in part by the degree of isolation. In the marine realm marine lakes (i.e. natural coastal lakes, saline or brackish, containing marine biodiversity) most of all resemble islands, in the biogeographic sense. Marine lakes communicate with the Sea either at the surface (“landlocked waters”) or under the ground (“anchialine lakes”). Up to date only individual representatives of marine lake communities like golden jellyfishes from tropical anchialine lakes or Atlantic cod from Arctic landlocked waters have been studied genetically. Here we ask about the degree of isolation of the macro community of Arctic anchialine lake Mogilnoe (Kildin Island, the Barents Sea). We analyzed mitochondrial variation in lacustrine and neighbor oceanic populations of eleven species including fishes, bivalves, scyphozoan jellyfish, parasitic worm, Gammarus shrimp and mesoplanktonic crustaceans. We demonstrate that most lacustrine populations differ from oceanic ones showing signatures of genetic isolation and drift. Both physical isolation (water exchange between the lake and the Sea occurs by means of filtration through the microporous rock) and isolation by environment (the lake is stratified and meromictic) could contribute to biological isolation.

We conclude that marine lakes indeed could be an “islands of the Ocean”, in the biogeographic sense. The study was supported by Russian Geographical Society project 13/2018-P.

Calanoid copepod *Eurytemora affinis* (Poppe, 1880)  
in the Gulf of Riga, Baltic Sea — some aspects  
of behavior and ecology

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There are only few “key” species in zooplankton communities in a low biodiversity ecosystem — semi-enclosed brackish Baltic Sea. *Eurytemora affinis* is one of those “key” species — dominant copepod in the Gulf of Riga as a resource for top predators. The entire Gulf of Riga area is favorable for this species development as to salinity, temperature and trophic state conditions. Nevertheless, results revealed differences in population’s behavior and ecology in littoral, sublittoral and open waters through the year. For instance, there is a difference between female egg production in spring and summer populations. Diel vertical migrations occurred in two different patterns: adult copepods and copepodite stages IV-V migrated actively through the thermocline, while nauplii and copepodite stages I-III migrated between the thermocline and surface. It also turned out that C:N ratio for adults and copepodites is different from other Baltic Sea sub-regions. Grazing experiments in spring with adults of *E. affinis* and microbial food web elements (autotrophic (ANF) and heterotrophic (HNF) nanoflagellates, and picocyanobacteria) under in situ conditions enhanced grazing pressure mainly on the heterotrophic nanoflagellates hence promoting the increase of picocyanobacteria.

Comparison of an electron transport system (ETS) enzyme-mediated assay and total respiration rate of the invasive copepod *Eurytemora carolleae* in Green Bay, WI, USA

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The use of aquatic resources for agriculture, trade, and recreation adds stress to water-dwelling organisms. Rapid changes in abiotic conditions, such as warming due to climate change and nutrient loading from agricultural runoff and urban areas, threaten to induce profound alterations to aquatic environments. These changes affect interspecific community interactions and may cause an aquatic resource to lose its functionality that is valuable to humans. Studying organisms such as plankton that form an ecosystem's foundation is an important step towards understanding the entire food web and predicting how it may or may not be able to respond to a changing environment. One important planktonic species in the Laurentian Great Lakes is the invasive calanoid copepod *Eurytemora carolleae* (formerly considered part of the *Eurytemora affinis* species complex). This study analyzes the metabolic activity of *E. carolleae* in Little Sturgeon Bay, WI, USA using two different methods, over a range of temperatures from 9° to 26°C. Total oxygen consumption was measured directly using a micropulse oxygen probe, and the activity of aerobic metabolic enzymes in the electron transport system (ETS) was quantified using the in vitro reduction of iodinitrotetrazolium chloride (INT). We find that the respiration rate of *E. carolleae* increases linearly from 9° to 26°C. We also find that the copepod's metabolic enzymes have an Arrhenius activation energy of 12.3 kJ/mole and experience a thermal maximum between 22° and 26°C. This thermal limit has implications for the future success of this species, as the combination of warmer temperatures and the disappearance of oxygenated colder-water refuges may limit *E. carolleae*'s success in the Green Bay system.

## Survey of presence of non-indigenous *Eurytemora carolleae* in the Gulf of Riga (Baltic Sea) five years after its first discovery

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In the brackish ecosystem of the Gulf of Riga, the calanoid copepod *Eurytemora affinis* is a key species but recently a new non-indigenous species *Eurytemora carolleae* was discovered in the region.

In the present study, we aimed to validate the presence of *E. carolleae* in the southern part of the Gulf of Riga five years after its first discovery. The study area is the closest region to the Riga harbour – the main source of non-indigenous species arrival in the Gulf. Actually, recent studies projected the possible potential of *E. carolleae* invasion due to its physiological plasticity. Male and female specimens of *Eurytemora* were collected in spring, summer and autumn of 2013, and then an analysis based on three morphological indicators was conducted. As a result, despite more effective reproduction rates of *E. carolleae*, this intrusive species does not seem to succeed in establishing during five year period after its first discovery in the Gulf of Riga, and hence do not threaten the native *E. affinis* population in the study area.



## Biodiversity of deep-sea zooplankton off the southwest coast of Puerto Rico

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The vast majority of research in the Caribbean tailors towards the coastal ecosystems because of the presence of coral reefs and the concerns about their rapid degradation rates. There are other eco-systems that contain both high levels of biodiversity and could be susceptible to rapid changes such as the zooplankton. Our knowledge though is limited, especially the deeper zooplankton of the Caribbean. Here, we report our ongoing studies on the biodiversity of deep-sea zooplankton from samples collected off the southwest coast of Puerto Rico during 2018. We collected a wide array of zooplankton using nets of various mesh sizes at approximately 500–600m depth during night. The most abundant taxon was the calanoid copepods, followed by decapod larvae, chaetognaths, ostracods, mysids, isopods, and amphipods, among others. We are developing a photographic catalogue that will be accompanied by DNA barcoding information to assign species names, especially to juveniles stages of zooplankton. We are also using metatranscriptomics to study the diverse zooplankton population metabolic activities based on RNA-seq. Our study highlights the rich zooplankton biodiversity of the deep-sea of Puerto Rico and through traditional and modern methods is developing a learning guide for the students of zooplankton biology.

## *Eurytemora affinis* in the Western Gulf of Finland — responses to environmental change

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*Eurytemora affinis* belongs to one of the most important copepod species in the western Gulf of Finland, apart from *Acartia biflosa*. We have studied *E. affinis* in a number of experiments, focussing mainly on responses to environmental changes, such as algae blooms, warming, ocean acidification and salinity change. Our results show that egg hatching and survival peaked in salinities that are higher than ambient salinity, suggesting other factors than salinity limit the species to spread into other areas. *E. affinis* oxygen consumption was negatively correlated with salinity, suggesting that the copepod originally is adapted to higher salinity. *E. affinis* also seems fairly robust to algae blooms and decreasing pH. In an ocean acidification mesocosm experiment, effects of near-future CO<sub>2</sub> levels were found, on neither offspring, nor antioxidant production. Food quantity, however, did affect nauplii production in the ocean acidification experiment. In a study comparing *Eurytemora* responses to cyanobacteria blooms in the Gulf of Finland vs. Michigan Lake, the Baltic *Eurytemora* was more sensitive, as survival was reduced in cyanobacteria filtrate, and smaller nauplii were produced. These findings further our knowledge of how a common species like *E. affinis* can respond in the face of changing local selection pressures from natural and anthropogenic stressors.

Niche separation in the cryptic species complex  
*Eurytemora affinis*: eco-physiological response in an  
“in-situ” reciprocal transplant experiment

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Spatial segregation exists between two cryptic species of *Eurytemora affinis* throughout the St. Lawrence estuarine transition zone (ETZ) arising questions on divergence of their ecological niches. The *E. carolleae* inhabits tidal freshwater and hypersaline habitats, whereas the *E. affinis* North-Atlantic clade dominates the brackish water stretch of the ETZ. This distribution pattern is intriguing because in laboratory studies *E. carolleae* is known as extremely euryhaline performing well from freshwater to hypersaline conditions. Thus the realized niches might be a result of environmental conditions and differential ecophysiology of both cryptic species. Furthermore, the ETZ is characterized by strong tidal currents, so that advection of these zooplankton species should homogenize distribution throughout the ETZ. Physical features were shown to enhance separation of these two species, however it could not entirely explain segregation patterns. Thus the goal of this study was to evaluate the ecophysiological capacity of the two cryptic species “in situ” and when arriving in a “new” habitat under the assumption of being dispersed by tidal currents. First, we characterised the environmental envelop of the tidal freshwater and the brackish water habitats in terms of environmental conditions such as temperature, salinity and food sources. Second, we performed “in situ” reciprocal transplant experiments in the freshwater and the brackish water habitats to mimic arrival of individuals in a new habitat. To quantify the ecophysiological condition of both cryptic species, we determined their fatty acids composition and concentration, their survival rate and reproduction efficiency under the different “in situ” experimental conditions. The two habitats differed in

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abiotic conditions showing mean temperature of 23°C and 0.1 psu salinity in the fresh-water habitat and the brackish habitat was cooler at 21°C with a higher mean salinity of 3.5 psu. However, food composition between the habitats was similar. Reciprocal transplant experiments revealed significantly different survival rates between cryptic species and habitats.

*E. carolleae* had similar survival rates in both habitat and showed a higher survival rate of 86% d<sup>-1</sup> than the *E. affinis* NA clade (57% d<sup>-1</sup>). Survival rate of *E. affinis* NA clade decreased by 26% in the “new” freshwater habitat comparatively to its native brackish water habitat. Despite the fact that total fatty acid content did not change significantly during the transplantation, the *E. affinis* NA clade showed a strong decrease of its reproduction rate in the “new” freshwater habitat, suggesting a trade-off between survival and reproduction in order to maximize available energy for survival. Overall, *E. carolleae* showed better performances in terms of survival and reproductive efficiency compared to the *E. affinis* NA clade in both habitats, suggesting a wide eco-physiological niche. In contrast, the *E. affinis* NA clade showed evidence of eco-physiological limitations in freshwater under summer conditions that might reduce its intrusion in the freshwater habitat at this time period. However, results of this reciprocal transplant experiment would suggest wide distribution of *E. carolleae* throughout the ETZ. Thus the absence of *E. carolleae* in the brackish water habitat, could not be explained by its eco-physiology and might be related to other control mechanisms, such as competition and predation or mechanisms acting on early life stages, such as nauplii and copepodites, which might show different eco-physiological tolerance than adults.

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