**Quantitative assessment of mixed settlements of blue mussels Mytilus edulis and M. trossulus in a small subarctic inlet (Tyuva Inlet, the Barents Sea Murman Coast).**

**Highlights**

**Abstract (294)**

The knowledge about Mytilus of the Barents Sea Murman Coast, at the northeastern frontier of Atlantic littoral communities, is mainly based on data from a century ago. Since that time the Arctic environment, the sublittoral sampling methods and the taxonomy of mussels have changed. A single species, M. edulis (ME) was divided into ME and M. trossulus (MT). We provide results of an extensive survey of mussels in a small, anthropogenically undisturbed small Tyuva inlet (Kola Bay) performed in 2004-2018. Parameters analyzed were density, biomass, age structure, maximal size, size-at-age index and proportions of ME and MT among middle aged mussels (4-7 years old) predicted by their shell morphology. Mussels were omnipresent in the littoral and upper sublittoral. Their characteristic habitats were littoral rocks in the mouth of the inlet, tidal shoals in its freshed top, the very river mouth with a large littoral-sublittoral mussel bed, and sublittoral kelp forests. Intriguingly, no mussels were recorded in kelps in historic studies. Against a background of high variability among settlements the next spatial patterns were observed. Densities of young (<4 years old) mussels decreased with the distance from the mouth of the inlet. Mussel size-at-age index increased both with distance from the mouth and with depth. Proportions of ME positively correlated with the depth; this species also dominated the bed. Between 2004 and 2010 settlements experienced unidirectional changes: proportions of young mussels increased (by 40 % on average) while proportions of MT among middle aged mussels decreased (by 22%). By 2018, proportions of MT decreased even more. Indirect evidence suggests that during recent decades, that were unusually warm, mussels were not more abundant on the littoral than a hundred years before. Our study provide the first insight into local scale habitat segregation of widespread “cryptic” ME and MT and interannual dynamics of their mixed settlements.

**Introduction**

In recent decades, much effort has been expended on the studying of populations of boreal blue mussels *Mytilus* in the high Arctic (Northeastern Alaska, Feder et al. 2003; Spitzbergen, Berge et al. 2005, Leopold et al. 2019; the Pechora Sea, Sukhotin et al. 2008; Northwestern Greenland, Blicher et al. 2013, Thyrring et al. 2015 ) as well as in Antarctic (the South Shetland Islands, Cárdenas et al. 2020). These geographical populations, most of which were previously unknown or did not even exist are so interesting because they represent the very cold front of boreal mussels distribution and polarward expansion under warming climate. As opposed to high Arctic populations, the more southern - subarctic ones have received less attention (but see studies of Khaitov, Lentsman 2016 on the White Sea, Bodkin et al. 2018 on the northern Gulf of Alaska and Khalaman et al. 2019 on the Sea of Okhotsk mussels) primarily because they are not so interesting from a biogeographical point of view, have long been known and have usually been studied at one time or another. Populations of the Barents Sea coast of Kola Peninsula (broadly known as Murman) at 68-70 degrees north and 31-40 east (Fig. 1) are a case in point.

Murman Coast is washed by a warm Atlantic Murman Coastal Current which defines relatively high sea surface temperatures (SST) for such high latitudes (the long-term SST of August 8.0, of February 3.2, www.pinro.ru) and limited winter ice cover. The Barents Sea is strongly affected by climate oscillation with SST varies several degrees at annual and decadal time scales (Matishov et al. 2012). Murman is the very NE border of typical North Atlantic littoral communities with canopies of fucoid algae, crusts of barnacles and mussels on the hard bottoms (ESM Fig.?; Zatsepin et al. 1948; Genelt-Yanovsky et al. 2019 and references therein).

Due to the extensive phenomenological studies undertaken in the early 20s century, mainly by the former Murman Biological Station in Alexandrovsk (Yekaterininskaya Havan, Kola Bay), broad data on coastal macrobiota of Murman including mussels are available (Guryanova, 1924; Guryanova et al, 1928; Guryanova et al, 1929; Zatsepin et al. 1948, Matveeva 1948; note that 1948 papers are all based on the prewar data). According to these studies, mussels were quite numerous on sheltered shorelines being ultimately the most abundant, in terms of biomasses, and conspicuous littoral animal species there. At the same time mussels were rare in sublittoral except the very mouths of the rivers where mussel beds have been observed (Derjugin, 1915; Guryanova et al, 1929). In particular, targeted research has shown that they were nearly absent from the sublittoral kelp forests in Kola Bay (Guryanova, 1924). Later on, few studies, all published in Soviet “grey literature”, considered mussels. The main conclusion of these studies was that mussel abundances have drastically decreased between 1960th and 1970th along the entire coast and no recovery occurred until 1980th (Antipova et al. 1984) or even late 1990th (Strelkov et al. 2001). This decrease was related to a period of low sea water temperatures started in 1960th, supposedly unfavorable to boreal mussels (Antipova et al. 1984).

Therefore, the knowledge on ecology of Murman mussels is mainly based on centuries-old data and it is unknown how well this data can reflect the current situation. In the aftermath there have been changes in methods of mussel sampling, in the environment and in the taxonomy of mussels. Earlier, the diving method was not available and only dredges were used for sublittoral sampling which may have influenced the inferences. The first two decades of the current century were extremely warm in the Barents Sea (Marshall et al. 2016; ???). Under the hypothesis that mussel abundance positively depends on temperature, the recovery after supposed population decline in 1960th, and high mussel abundance are expected. In late 1980th the singular boreal mussel species Mytilus edulis was divided into M. edulis (ME) and M. trossulus (MT) basing on genetic data (Koehn et al., 1984; McDonald, Koehn, 1988; Varvio et al., 1988). ME and MT co-occur in many areas of Atlantic and neighboring Arctic, including Murman (VS 2011; Wenne et al. 2020). In the Barents and White Seas ME is considered as native species while MT as a recent invader (VS 2011). Since ME and MT are “cryptic” species – lacking diagnostic morphological characters, little is known about their ecological peculiarities anywhere. Salinity, degree of wave exposure, depth and type of fouling substrates were considered as factors of species segregation in sympatry in other geographical regions (Bates, Innes, 1995; Ridgway, Naewdal, 2004; Riginos, Cunningham, 2005; Dias et al., 2009; Tam, Scrosati, 2014; Katolikova et al., 2016; Michalek et al., 2021). Recently it was shown that in the Barents Sea ME and MT differs by frequencies of shell morphotypes defined as presence or absence of an uninterrupted prismatic strip under the ligament on the inner side of the shell: in low saline localities (<30 ppt arbitrary) differences approach 70% while in high saline localities – 31% (note however that … hybrids). With such small differences as in high saline localities individual mussel assignment into species by morphotypes is unreasonable but taxonomic structure of samples (i.e. proportions of species) could be predicted accurately by morphotype frequencies using provided formulas (Khaitov et al. 2021).

The lack of up-to-date data on Murman mussels and general lack of data on habitat preferences of M. edulis and M. trossulus in sympatry led us to undertake a new phenomenological study of Murman mussels. Tyuva inlet at the entrance to Kola Bay was chosen as a polygon of the study. Apart from transport accessibility, it was chosen for the following reasons. Firstly, by its morphology and oceanographic features Tyuva is a typical Murman small inlet (Derjugin, 1915). Secondly, Tyuva is the closest “pristine” – slightly anthropogenically disturbed - inlet to Yekaterininskaya Havan (6 km across the Kola Bay, Fig. 1) where the former Biological Station was used to be up to 1933 and where many classical studies involving mussels have been centered (Guryanova, 1924; Guryanova et al, 1928; Guryanova et al, 1929). Since that time Yekaterininskaya Havan itself has turned into a militarized area and is inaccessible. Finally, both M. edulis and M. trossulus were recorded in Tyuva by geneticists (VS 11; Khaitov et al. 2021) which makes the inlet a suitable place for a comparative study of sympatric mussels.

Using broad material (78 quantitative samples and 15 genetic samples) accumulated through 2004-2018 we are looking for answers to the following questions. How taxonomic structure and basic demographic parameters of mussel settlements – density, biomass, age structure, size-at-age index varies across habitats, with depth and other environmental gradients? What are the patterns of decadal-scale dynamics of mussel settlements? To what extent do observed mussel abundance and patterns of distribution meet expectations based on classical studies?

На основе обширного материала (78 количественных проб и 15 генетических сборов) собранного в 2004-2018 гг., мы попытались описать как связаны таксономический состав и базовые демографические показатели (плотность поселения, биомасса, возрастная структура, size-at-age index) с ключевыми факторами среды. Дополнительно мы сделали попытку оценить варьирование этих показателей во времени на протяжении 14 лет наблюдений. И, наконец, используя старые публикации о поселениях мидий в регионе, мы сопоставили паттерны наблюдаемые в настоящее время, с теми закономерностями, которые были описаны в прошлом.

**Materials and Methods.**

***Tyuva Inlet*.**

Tyuva Inlet in the NE Kola Bay is 3.6 km long and 0.7 km wide at its widest with a surface area of about ?? km 2(Fig. 1, Fig. 2). The shores of the outer part of the Inlet are steep, littoral zone is narrow, dominant bottoms are boulders and rocks. Littoral fucoid algae are abundant. Towards the top of the Inlet, the shores become more indented. The inner part of the Inlet is shallow, with unconsolidated bottoms and broad intertidal **flats** up to 400 m wide. Fucoids are scarcer. The river (the common bed of Bolshaya Tyuva and Malaya Tyuva rivers that fuse in the very top of the Inlet), with annual runoff of about 0.7 km3, flows across the shoals. The Inlet is covered by ice during cold winters. The tidal amplitudes in the northern Kola Bay are 1.1 – 3.7 m. Summer surface salinity at a distance from the mouths of rivers about 31-32 ppt while in the top of the inlet is about 20 ppt (Derjugin 1915, Gur’yanova et al. 1928, Mytilaev 2014, Shavykin 2018).

***Mussel sampling***

Tyuva was visited in July … 2004, … 2005, …2009, …2010, …2012 and July 2018.

In 2004-2005, distribution of mussels on the littoral was preliminary mapped from the shore and, by SCUBA divers, in the sublittoral of the inner part of the Inlet where a large littoral-sublittoral mussel bed was founded (hereinafter the Bed).

23 quantitative samples (sampling area 0.01-0.03 m2, replicate samples 1-8, area and number of replicates varied to account to mussel density) of mussels were collected by hand across the Inlet, mostly from the littoral (?Fig. 2).

In 2004-2005 (thereafter, the period I), intertidal mussel distribution was preliminary visually mapped by shoreline surveys during low tide. SCUBA divers were used for mapping of mussel settlements in the upper part of the inlet where extensive mussel bed (thereafter, Bed) was found. Several settlement types were visually outlined and 1-8 samples with corer of 0.01-0.03 m2 were took in each of them (totally 23 quantitative samples).

The more detailed surrey was performed in 2009-2010. The search for mussels in the sublittoral was extended to the outer part of the Inlet where mussels were founded in kelp forests at 0-4 m depth. Mussels were sampled at low water (in order to accurately determine the depth of sampling sites basing on the tide table for Ekaterininskaya Hawan) along seven vertical transects perpendicular coastline? placed across the whole zone inhabited by mussels in different parts of the Inlet. Transects and sampling sites were chosen to account for diversity of mussel habitats and for the 2004 collection points.

Transects MoN and MoS were placed in the mouth of the Inlet, MidN in its central part, SN and SS – from the opposite coasts across the seaside border of the Bed and BS and SS – across the central part of the Bed. Mussels were also sampled from the most riverside littoral settlement (locality R+05) (Fig. 2). Sampling localities and corresponding mussel settlements are denoted hereinafter by transect names and sampling depths (e.g. BS+05 – littoral part of the Bed on the south shore, fig. 2), except for a single locality R+05 in the very top of the Inlet.

At each of 43 sampling localities at least 3 quantitative samples of mussels were taken randomly in the same manner as in 2004-2005, and type of bottoms, dominant algae species and total cover abundance of macrophytes were visually registered. During brief visits in 2012 and 2018 several settlements were resampled, amongst them BS+05 which was studied on five visits (?Fig. 2). Additional qualitative sampling for allozyme analysis were also conducted at each localities in 2012 and 2018 (2012 data were used in Khaitov et al 2021).

The more detailed survey was performed in 2009-2010 (observation period II). In this period additional mussel settlement mapping was carried out in the outer part of the inlet where mussels were found in kelp forests at 0-4 m depth. We founded several vertical transects to make quantitative assessment of mussels settlements (Fig. 2). Each transect was oriented perpendicularly to coastline and consisted of several samples placed at different tidal levels. Samples on the most transects were collected at 6 levels in relation to zero-depth: +2, +1.5, +1, +0.5, -0.5, -1, -1.5 m (negative values denote sublitoral position). Transects positions were chosen to cover the diversity of mussel habitats revealed in the observation period I. Sampling localities and corresponding mussel settlements are denoted hereinafter by transect names and sampling level (e.g. BS+05 – littoral part of the Bed on the south shore, fig. 2). Transects "MoN" and "MoS" were placed in the mouth of the Inlet, "MidN" in its central part, "SN" and "SS" – from the opposite coasts across the seaside border of the Bed and "BS" and "SS"– across the central part of the Bed. Mussels were also sampled from the most riverside littoral settlement (locality R+05) (Fig. 2). Each quantitative sample (in total 43 samples) at transects were taken by the same manner as in the observation period I.

***Mussel processing***

Mussels from each sample were counted and weighted. All mollusks were used in the following analyses. The shell length was measured as maximal anterior-to-posterior length up to 0.1 mm with calipers or, for small mussels, with a binocular microscope equipped with a micrometer. Age of mussels was determined by counting “winter rings” - marks of winter growth delays on shells (for description of the method see Sukhotin et al. 2007 and references therein). For medium-aged mussels (4-7 years old) shell morphotypes (E-morphotype, more characteristic of ME or T-morphotype, more characteristic of MT) were identified as in Khaitov et al 2021. Only medium-aged mussels were used in the taxonomic analysis in order to avoid the possible bias generated by non-random association between size and morphotypes in conspecific mussels (see Khaitov et al 2021 and below). +аллозимы?

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**Environmental, demographic and taxonomic parameters of mussel settlements**

The next environmental characteristics of settlements were considered:*Depth* **-** depth/height from the chart datum, m; *Slope* - slope of the shore, degrees; *Dist* - distance from R+0.5 in the top of the Inlet, m; *Sal* - predicted salinity, ppt (see below); *Width* - vertical width of the zone populated by mussels, m; *Exp* - north or south shore; *Ground* – type of bottom ground, boulders, rocks or sand, *Kelp* - presence/absence of kelps; and *Cov -* cover abundance of macrophytes, points.

The environmental characteristics were assessed as follow. The variable “Depth” was assessed as the level of sampling position on the transect (see above). The degree of bottom incline at the sampling point (“Slope”) was estimated as the value of slope for the tangent at this point. The distance from the river mouth was measured as euclidean distance from a given sampling point to R+05 which was considered as a reference point. Salinity (“Sal”) was calculated accordingly to model describing the dependence between ++ and +++ (see bellow). Exposition of transect (“Exp”) reflected the orientation of a transect (North vs South). Vertical width of the zone populated by mussels was measured as +++ and denoted as “Width”. The ground type (“Ground”) was considered as categorical variable with three levels: boulders, rocks or sand. We assessed the presence of Laminaria in the sample (“Kelp”) as binary variable: present vs absent. Finally we roughly assessed the cover of bottom surface by macrophytes (“Cov”) as a percentage of area of 1 sq.m in the sampling point covered by any species of algae.

For details of calculation of *Slope*, *Dist* and *Sal* see Extended materials and Methods in ESM ?. In brief, salinity was monitored through one tidal cycle in 25-26 July 2009 at different depth horizons (0 m, +1 m, +2 m) at transects BN, MidN, MoS and R +0.5 locality. The angle between the tangent at this point and the vertical profile of the transect …Cover abundance was rated on a point scale: 1 - <5%, 2 - 5-25%, 3 - 25-50%, 4 - 50-75%, 5 - >75% cover.

The next biological characteristics of mussel settlements were considered: *B* – biomass, g\*m-2; *N* – total density, ind\*m-2; *N2-3*, *N4-6*, *N7-9*, *N9+* – densities of mussels 2-3, 4-6, 7-9 and over 9 years old, respectively; *Lmax* - maximal size (the length of the largest mussel), *SAI* - the mussel size-at-age index, *Ptros* – proportion of *M. trossulus*-like mussels (i.e. purebreds and hybrids … ) predicted by frequency of T-morphotypes. One years old mussels were not considered as a separate group (but were considered in calculation of N) because of their patchy distribution which is difficult to account for in a limited sampling. SAI = log (L∞ \* K), where L∞ and K – parameters of the von Bertalanffy equation calculated – КАК? - from the average values of the lengths of animals of different ages over 2 years. This index is used in ecophysiology to account to the rate of organism size increase during the lifetime and names «overall growth performance» (Brey, 2001 and references therein). Here, SAI is used as an indirect measure of (adult) mussel growth conditions in settlements.

For calculations, individual samples (from 2004-2005) or pooled (*Lmax*, *Ptros*) or averaged (other characteristics) data on multiple samples from individual settlements (other years) were used.

Khaitov et al. 2021 provided separate formulas to predict the proportion of M. trossulus (Ptros) basing on proportion of T-morphotypes (PT) in samples from low- (<30 ppt) and high- (≥30 ppt) saline localities. Since mussels in Tuyva experience salinities around 30 ppt (see the Results) we clarified the relationship between Ptros and PT for local settlements . Empirical relationship between PT and Ptros was derived using a regression approach (see Khaitov et al. 2021 for details) using 9 collection samples of shells of genotyped mussels from studies of Bufalova et al., 2005, VS 2011 and Khaitov et al. 2021 and 6 new samples from 2018 (see ESM table?). The age of mussels was identified and mussels 4-7 years old were used in the analysis.

**Statistical analyses**

Солёность – там хитрая модель, надо, чтобы ВМ кратко написал

Калькулятор

Структура поселений по данным 2009-2010

Пункт про динамику

**Mussel stocks assessment**

**Анализ исторической литературы**