Introduction

Mussels of several cryptic species, known as “Mytilus edulis” complex (Riginos and Cunningham, 2005), are widely distributed around North hemisphere. Different species of this complex frequently coexist sympatricaly as do, for instance, M. edulis (Me) and M. trossulus (Mt) along both coasts of the North Atlantic (Riginos and Cunningham, 2005; Väinölä and Strelkov, 2011 and references therein). The proportion of both species in mixed populations vary in broad limits (Katolikova et al. +++) but factors regulating species composition in locality is poorly understood.

The most considered factors influencing Mt-Me composition in mixed populations are abiotic ones: temperature and its correlates (Popovic & Riginos, 2019; Wenne et al., 2020), salinity (Riginos & Cunningham, 2005; Ridgway & Naewdal, 2004; Kijewski et al., 2019; Ridgway & Naevdal, 2004), surf effects (Tam, Scrosati, 2014; Comesaña et al., 1999; Bates, Innes, 1995; Innes and Bates, 1999 Проверить в каком Innes что говорится). Only few attempts were made to assess the role of biotic interactions in regulation of Mt-Me proportion. It was shown that proportion of Mt is significantly higher in mussel’s settlements on fucoid’s tally in comparison with surface of ambient ground where Me predominate (Katolikova et al. 2016). In this case, however, the main factor is probably not fucoids as biotic substrate but the force of the surf again. The fucoid's thallae work as shock absorbers, saving Mt possessing thinner shells (Katolikova et al. 2016).

The only described true biotic interaction playing the role in regulating of Mt-Me proportion is starfish pressure. Starfish let to prey on the Baltic mussels (Mt) and on the North Sea ones (Me) preferably attacked Mt (Kautsky et al., 1990). In experiments with Mt and Me from Canadian hybride zone sea stars attacked more actively on Mt than on Me defence reactions of which were generally stronger (Lowen et al., 2013).

In practice, the analysis of ecological interactions require numerous samples with high specimens amount. Identification of mussel species by using of expensive and exhausting genotyping do not facilitate it. The use of semi-diagnostic morphological markers, which give the ability to identify species with a high (but not 100%) probability, can facilitate the solution of ecological tasks (Khaitov et al. 2021). The pattern of nacre deposit on mussel shells was suggested as a possible semi-diagnostic marker for probabilistic species identification (Khaitov et al. 2021). Accordingly to this trait, two discrete morphotypes (T and E) were recognized in different seas of the world. These morphtypes are corresponded well to Mt and Me, respectively (Khaitov et al. 2021).

The ecological analysis of the Mt-Me hybride zone in the White Sea has been significantly enhanced by the use of the semi-diagnostic marker mentioned (Katolikova et al., 2016). In particular, the use of morphotypes allowed a much larger number of mussels to be involved in experiments for assessment mussel-stearfish interaction and to obtain more pronounced results (Khaitov et al. 20++). Choice experiments conducted in the White Sea confirmed results from other areas - starfish feel the difference between Mt and Me and prefer to consume the former (Khaitov et al. 20++).

These findings, however, being revealed in cage experiments, do not indicate the role of starfish in regulation of Mt-Me composition in natural conditions. Up to date we do not know if sea stars can change proportions of species in mixed populations in situ. In present investigation we conducted field experiments and observations to answer three questions. (1) Will sea stars attack Mt with higher probability (as was shown in cage experiments) but in conditions close to natural? (2) Would the mussel settlements dominated by Mt more preferable foraging place than those dominated by Me, as it could be expected from the fact that Mt is more preferable prey. (3) Does the Mt-Me proportion change in natural populations after attack of starfish crowd?

Material and methods

Mussel identification

This investigation was based on the indirect species identification by using morphotypes as semi-diagnostic markers. We used approach proposed in Khaitov et al. (2021) which, in short, can be described as follows. We assigned mussels to T-morphotype if nacre was undeveloped in the zone approached to ligament nympha. A thin stripe of prismatic layer uncovered by nacre could be seen in this shell region. In contrast mussels were assigned to E-morfotype if nacreous layer came closely to ligament nympha, no uncovered prismatic layer was recognized in this region. The trait considered could be seen well both on alive mussels after their dissection and on dead shells (including killed by sea stars) collected in the field.

The proportion of Mt in a population (*Ptros*) is highly correlated with proportion of T-morphotype (*PT*) in the White Sea (Khaitov et al., 2021) and can be recalculated using the equation as follows:

(Eq 1).

However for probabilistic species identification of individual mussels, which is necessary for experiments, the information on Mt-Me proportion in the site of mussel collection is needed (Khaitov et al, 2021). For mussels of T-morphotype sampled from population with known Ptros the probaility to be Mt could be assessed be the Eq 2. For mussels of E-morphotype originated from population with known Ptros the probability to be Me can be assessed by Eq 3.

(Eq 2)

(Eq 3)

Samples of mussel for experiments

To increase the probability of species identification of mussels used in field experiments we sampled material from two populations contrasting by their Ptros. The first population (Pop1) was located on mussel bed in the Voronya Bay (N, E). According to genetic survey (Katolikova et al 2016), the average Ptros in this area equal to 0.11 (see S1 table in Katolikova et al.2016, populations # 24-27). It is close to assessment of Ptros = 0.10 obtained from Eq 1, using the proportion of T-morphotype in this population as PT = 0.03 (the proportion of T-morphotype between mussels sampled in Pop1 and used in experiments). Accordingly to these assessments, the probability to be Me for any specimens of E-morphotype sampled from Pop1 can be assessed as P(Me|E) = 0.96, but specimens of T-morphtype sampled in Pop1 could be identified as Mt with lower probability P(Mt|T) = 0.63. Thus any randomly taken mussels with E-morphotype sampled in Pop1 can be considered as Me with high probability but degree of taxonomic uncertainty for mussels with T-morphotype sampled in Pop1 is high enough.

The second population (Pop2) was located on mussel bed situated between Telachiy and Oleny islands (N, E). No direct assessment of Ptros was made in this area however knowing the proportion of mussel with T-morphotype in this locality (PT = 0.69) we can calculate the proportion of Mt in Pop2 using Eq 1: Ptros = 0.79. This value is close to average Ptros calculated for genotyped samples, located closely (populations #18-23, see S1 table in Katolikova et al 2016): Ptros = 0.78. Using this data for mussels sampled from Pop2 we can assess P(Me|E)= 0.46 and P(Mt|T) = 0.94 . Thus any randomly taken mussel with T-morphotype sampled in Pop2 can be considered as Mt with very high probability. However degree of taxonomic uncertainty for mussels with E-morphotype sampled in Pop2 is very high (such mussels could be with equal probability assigned both to Mt and to Me).

Mussels sampled in Pop1 and Pop2 were sampled +++ 2017 (experiment 1 and 2) and +++ 2018 (experiment 3). Mussels were washed and cleaned from overgrowing organisms. Only mussels with shell length ranged in ++ - ++ mm were used for further manipulations. Both samples were placed separately in mesh bags and kept in sea water by being suspended from the pier. After several days of adaptations each mussel was labeled by color tag marking their origin (Pop1 or Pop2).

We constructed +++ experimental units consisted of ceramic plate (++ x ++ mm) surrounded by a plastic barrier (++ mm hight) around the perimeter. This barrier prevented the mussel movement outside the experimental unit but allowed sea stars to crawl inside. On the corner of the experimental plat we fastened four ropes which were knot together (appr 30 cm above a plate) and a cord (50 cm length) with foam float was tied to these ropes.

The experimental units were divided into three groups. In two experiments conducted in 2017 on each plate from the first group we placed ++ mussels sampled in Pop1. On the plates of the second group we placed ++ mussesls collected in Pop2. Finally on the plates of the third group we placed ++ mussels from Pop1 and +++ mussels from Pop2. In 2018 the design of experimental set up was the same but we placed only ++ mussels on each plate. When mussels were set up on experimental units the later were placed on the bottom of an intertidal pool which never visited by sea stars. After two tidal cycles all mussels fast themself on the surface of plates, only few marked specimens were washed out. After the processing described all experimental units were submerged on the bottom for the depth about 3 m in the starfish infested area. Spatial distribution of units from different groups was random and the distance to the nearest neighbour was approximately 1 m.

Experimental units were exposed for ++ (experiment 1 in 2017), ++ (experiment 2 in 2017) and ++ hours (experiment 3 in 2018). After exposition period all units were picked up and transposed to the laboratory. Starfish found on each plate were counted and weighted. Dead mussels (all of them were lack soft tissues which indicated they were eaten by sea stars) were dried. Alive mussels were boiled their soft tissues were removed and shells were dried.