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).

Experimental set up

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 plate 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 the cord. The foam floats allowed to find experimental units on the bottom and pick them up from the boat.

The experimental units were divided into three groups: “Me-dominated”, “Mt-dominated” and “Mixed”. In two experiments conducted in 2017 on each plate from the first group we placed 100 mussels sampled in Pop1. On the plates of the second group we placed 100 mussesls collected in Pop2. Finally on the plates of the third group we placed 50 mussels from Pop1 and 50 mussels from Pop2. In 2018 the design of experimental set up was the same but we placed only 60 mussels on each plate (30+30 in the case of “Mixed” units). 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 ceramic plates or on the walls around it but inside the unit, 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 unit was approximately 1 m.

Experimental units were exposed for 61 (experiment 1 in 2017), 121 (experiment 2 in 2017) and 113 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.

Dry shells (eaten and uneaten) were measured (maximum distance from the umbo to the opposite side of the shell) using an electronic caliper with an accuracy of 1 mm. The morphotype of each shell was assessed according to Khaitov et al. (2021). This data will further denoted as “Experimental” data set.

Field samples for assessment of sea stars influence on Mt-Me composition

In August 2019 mass crowd of sea stars attacked shallow water mussel populations in the upper part of the Kandalaksha bay (our personal observation). Such massive invasions periodically occur in the area (++++). We used this event to sample mussels in three types patches. The patches of first type were intact mussel settlement presented in the starfish crowding area but not attacked still. The patches of the second type - dense starfish group feeding on the mussel patch. And the third type included patches of dead shells visually presented on the bottom surface. Since all three types of patches were situated close to each other (the maximal distance between them was several meters) we suppose that these patch’s types represent the temporal sequence: before starfishes attack - in the period of the attack - after it.

We sampled patches of each type in two sites (+++ km apart). In both sites snorkel diver recognized the suitable place orienting on sea star abundance. When appropriate place was found 10 quantitative samples on each type of patches were taken by using a round core (++ mm diameter). Thus 30 samplings were made in each of two site.

After the samples were washed through sew-screen (mesh size +++ mm) they were sorted. When sorting the samples we took into account alive mussels and their dead shells (we sampled shell with length exceeded 10 mm) and sea stars. Sea stars were counted and weighted. Dead shells were counted (each valve was counted separately). Alive mussels were boiled, their soft tissues remooved and shells dried. On dried mussel shells we evaluated mussel’s morphotype.

Each sample was characterized by values as follow. The proportion of dead shells was estimated as the ratio of the doubled number of dead shells to the sum of this value and the number of live shellfish in the sample. The proportion of T-morphotype (P\_T) - as the ratio of number of mussel with T-morphotype to total number of alive mussels in the sample. Finally total starfish biomass was assessed. This data will be denoted further as “Observation” data set.

Statistic processing

All statistical processing and data visualizations were conducted with functions of statistical programming language R 4.05 (R core team, 2021).

Data from experimental data set was analyzed by the means of logistic generalized linear mixed model construction (binomial distribution with logit link-function). The probability to be eaten was considered as a dependent variable in this analysis. Each individual mussel was codded as “1” if it was eaten by sea star and “0” otherwise. The total amount of analyzed mussels was +++.

The predictor set for the fixed part of the model included six covaries. No interaction between predictor described bellow was included in the model.

The probability to be identified as Mt by the means of mussel’s morphotype (P\_Mt). We evaluated this probability for mussels collected in Pop 1 as P\_Mt = 1 - P(ME|E) = 1 - 0.96 = 0.04 for mussels of E- morphotype and P\_Mt = P(Mt|T) = 0.63 for mussels of T-morphotype. For mussels collected in Pop2 corresponding values were assessed as P\_Mt = P(Mt|T) = 0.94 for mussels of T-morphotype and P\_Mt = 1 - P(Me|E) = 0.54 for E-morphotype. This continuous predictor was used instead just a discrete factor “Morpotype” since taxonomic signal of morphotype is highly dependent on species prevalence in the area of samples (Khaitov et al., 2021). The interpretation of this values is obvious: more P\_Mt - more probability that the concrete mussel is Mt.

Number of “conspecifics” (N\_consp). For each mussels included in the analysis we calculated the number of mussels of the same morphotype presented in the experimental unit where this mussel was placed. We interpret this predictor as follow: when this value low the mussel of given morphotype is surrounded mostly by mussels likely belong to another species, otherwise when N\_consp is high a mussel is surrounded by conspecifics. This value will be in average high for mussels placed into “Mt-dominated” or “Me-dominated” units and low for mussels placed in “Mixed” units.

Proportion of mussels of T-morphotype (P\_T). This value describes the taxonomic structure of a mixed population. The lowest value of this predictor is in the “E-dominated” units, the higher one - in “Mixed” and the highest values presented by “T-dominated” units.

Mussel size (L) and sea stars biomass (B\_aster) are self-evident predictors. Total amount of mussel (N\_tot) was used as a technical covariate which is needed since different amount of mussels was placed on experimental units in 2017 and 2018.

All predictors were scaled by subtracting the mean and dividing by the standard deviation. The scaling of the predictors allowed us to compare the regression coefficients directly.

The random part of the mixed model described the variation of model intercept. Two random factors was included in the model: The factor "Experimental unit" was hierarchically nested within the "Experiment" factor .

The model was fitted by using glmer() function from lme4 package (+++). The validity of model fitted was checked by means of residual plots. No violations of logistic regression analysis was found.

The second, “observation” data set was analyzed by the means of generalized additive model construction (GAM, beta distribution with logit link-function). The proportion of mussel of T-morphotype among alive mussel (PT\_alive) was considered as dependent variable. As a main predictor in the model we considered the discrete factor “PatchType” with three levels describe above. The biomass of sea stars was included in the model as covariate being fitted as a smoother. The factor “Site” was included in the model as a random effect smoother (Pedersen at al, 2019). The GAM was fitted by gam() function from the mgcv package (+++).

The GAM construction was followed by pairwise comparisons of model predictions for three levels of “Patch type” predictor. The For the comparison Tukey test was applied by using glht() function from the “multcomp” package (+++).

Results

“Experimental” data set

Since all predictors in the model constructed (Table ++) were continuous and scaled we can directly compare the power of their influence on dependent variable. The most powerful predictor from those included in the model (Table ++) was sea star biomass. The high positive coefficient denotes increase probability to be eaten when starfish biomass increased (Fig. ++).

The second most influential predictor was probability to be Mt (P\_Mt). The positive value of corresponding regression coefficient (Table +) can be interpreted as higher probability to be eaten for mussels of T-morphotype (maximal value of P\_Mt) in comparison with mussels of E-morphotype (minimal value of P\_Mt).

The third most powerful predictor (N\_total) was associated with negative regression coefficient. It means that probability to be eaten was lesser in more abundant mussel settlement (Fig +).

The next predictor, mussel size (L), again negatively correlated with probability to be eaten. Thus starfish consumed more actively smaller mussels than larger one.

The last predictor describing abundance of mussels of the same morphotype for a given mussel (N\_consp) was negatively correlated with dependent variable (Table +, Fig. ++). Thus if on an experimental plate there were negligible number of specimens taxonomicaly similar to given mussels the probability to be eaten for this mussel was higher then for a mussel surrounded by high amount of conspecifics.

No significant associations between probability to be eaten and proportion of T-morphotype (P\_T) was revealed (Table +., Fig. +).

“Observation” data set

The biomass of sea stars was differ on pathches of differing types showing minimal value on the intact mussels and maximal one on crowding starfish (Fig + ). The proportion of dead shells was minimal on alive mussel patches and maximal on dead shell’s patches (Fig +).

The proportion of T-morphotype gradually decreased from intact patches through crowding of starfish to patches of dead shells (Fig +). The regression model constructed (Table +) revealed significant dependence of P\_T on all predictors and smoothers included. Pairwise post-hoc comparison revealed significantly higher proportion of P\_T in intact mussel patch than in patches surrounded by sea stars and in patches of dead shell. The difference between last two groups was not significant (Fig +).

Discussion

In this study we provide the first evidence that *Asterias rubens* can discriminate between sympatric Mytilus species in natural conditions. All previous attempt to determine whether *M. edulis* or *M. trossulus* is more susceptible to predation by *A. rubens* were performed in closed aquarium set up in laboratory or in natural biotopes (Loven et al., 2013; Khaitov et al., 2018; Kautsky et al., 1990). Such closed microcosms may provide conditions shifted in comparison with natural one. In our experiments we offered sea stars infesting the natural habitats a free choice between artificially constructed settlements of mussels differing in their taxonomic composition. This approach was made possible by using a low-cost method of identifying mussel morphotypes followed by estimating the probability of belonging to one of the two cryptic mussel species, as was proposed in Khaitov et al. (2021). Due to this approach, we were able to form several dozen experimental units, on which several thousand mussels were landed.

The second advantage of our approach was that we included many covariates potentially affecting the probability of mussel mortality in a single analysis. The influence of some of these covariates was self-evident. For example, we have shown that the greatest influence on the probability of being eaten is the abundance (biomass) of sea stars attacking experimental units (Table +). It seems to be a trivial and expected correlation: more predators, more chance that the victim will die.

The another trivial dependency revealed in our analysis was that the probability to be eaten was higher for smaller mussels than for larger one (Table +). The fact that sea stars avoid larger mussels has long been known (O’Neill et al., 1998; Hummel et al., 2011; Khaitov et al., 2018). This is related to that the delicate walls of the predator's stomach, embedding between the valves of the victim, suffer less mechanical damage if the smaller individuals are attacked (Hummel et al., 2011). However, the value of the regression coefficient (Table + ) associated with prey size was one of the lowesin the model (recall that all predictor values were standardized, which allows us to directly compare their influence). Thus the probability of being eaten depends only to a small extent on the size of the victim.

The another covary which expected to be associated with probability of being eaten was total amount of mussels presented in the experimental unit (Table ++). The negative coefficient indicates that the more mussels in the experimental unit, the less probability of being eaten. This is the dependence to be expected for predators showing functional response of Type II (+++), which is typical for sea stars (+++). In the case of such functional response, when the prey density increases the proportion of prey consumed per predator per unit time should decrease (+++).

What was more interesting was that the one of the highest values among the coefficients in the model was the coefficient associated with the taxonomic status of prey. In this work, we evaluated the belonging of a specimen to one or another species basing on the assessment of mussel morphotype. By its nature, morphotype is a semi-diagnostic trait (+++) that allows identifying the species only with a certain probability, which depends, in turn, on the ratio of Mt and Me in the mixed settlement. Using empirical models constructed on the basis of genotyped specimens (+++), we estimated the taxonomic status of mussels as the probability of being Mt (P\_Mt). The positive value of the regression coefficient indicates that the probability of being eaten increases as P\_Mt values increase. Indeed, in total, combining results of all three experiments (Fig. +) we can see that among Mt-like mussels (P\_Mt = 0.94) 34% was eaten whereas 22 % was eaten among Me-like mussels (P\_Mt = 0.04). It is noticeable that mussels with intermediate values of P\_Mt tending to possess intermediate proportion of eaten (Fig. ++). Thus, our study confirms that even under conditions as close to natural as possible, sea stars attack Mt with greater preference than Me, as has been shown in aquarium conditions (Khaitov et al., 2018). At present there is no unequivocal answer as to why this is so. On the one hand, it is known that Mt have a lower weight of soft tissues, and hence a lower energy value than Me (Penney et al., 2008). On the other hand, sea stars in its prey choice is guided either by tactile or chemical signals from the prey and these signals hardly directly associated with energetic status of mussels.

It is possible that the reason lies in the different flexibility of the shells of species: Mt have a thinner and more flexible shell than Me (Beaumont et al., 2008). This may reduce the handling time of the prey and make Mt more preferable.

Chemical signals may also play a role. In experiments conducted by Lowen et al (2008) sea stars, having no tactile contact with Mt and Me seating in different parts of aquarium, nevertheless, tended to those part which was occupied by Mt. The nature of the chemical signals is unclear, but there is an obvious candidate for this role: the glycoprotein KEYSTONEin, whose release stimulates sea stars to attack mussels (Zimmer et al., 2016, 2017). Considering that this glycoprotein is localized in the epidermis, extrapallial fluid and shell periostracum (Zimmer at al., 2017) and the fact of difference in Mt and Me shell thickness (Beaumont et al., +++) it is highly possible that mussels of two species produce KEYSTONEin in different quantity (or different, spesies specific KEYSTONEins).

Initially, our hypothesis was that experimental units dominated by Mt (i.e., T-morphotype dominated settlements) would be hit the hardest by starfish attacks. Surprisingly but despite the high vulnerability of Mt that was considered abowe, we did not observe a higher probability of being eaten for mussels from experimental units that were dominated by mussels of T-morphotype. The probability of being eaten was not dependent significantly on taxonomic structure of settlement (Table +). We suppose that a possible resolution of this paradox may be related to the last of the predictors included in our model, the number of conspecifics (N\_consp) in the experimental unit.

The abundance of conspecifics is high for a mussel let it be the individual of T-morphotype if it presents in a settlement dominated by individuals of the same morphotype. Similarly, this value will be high for any E-morphotype mussel in a settlement dominated by E-morphotype. The value of this predictor decrease if a mussel of any morphotype if it presents in mixed settlement. However the smallest value would reach if mussel of given morphotype situates in a settlement dominated by mussels of another morphotype. Thus, a negative coefficient for this predictor means that the minimum probability of being eaten will be observed if the mussel exists among its conspecifics. the highest probability of being killed by a predator will be for alone mussels living among aliens. The pattern observed may testify to some cooperation between individuals of the same species, as a result of which mortality may decrease. For example, it is known that Me, forming aggregations, more often combine itself with individuals of their own species (+++). It is possible that alone aliens will end up in the periphery of aggregations or outside them, becoming more available prey for sea stars.

If the pattern described really exists, then the "pure" settlements should be more protected against sea stars. This, apparently, explains the absence of a clear dependence of the probability of being eaten on the taxonomic composition of mussel's settlements. At the same time, one would expect a higher mortality of mollusks in the mixed settlements. However, we did not observe a clear increase in the probability of being eaten for mussels in settlements with average P\_T values (Fig. ++).