

Article

Mate Choice in Molluscs of the Genus *Littorina* (Gastropoda: Littorinidae) from White Sea

Eugene V. Kozminsky^{1,*}, Elena A. Serbina² and Alexey O. Smurov¹

¹ Zoological Institute of the Russian Academy of Sciences, Universitetskaya nab., 1, St. Petersburg 199034, Russia

² Institute of Systematics and Ecology of Animals of the Siberian Branch of the Russian Academy of Sciences, Frunze Str., 11, Novosibirsk 630091, Russia

* Correspondence: ekozminsky@gmail.com

Abstract: We studied the reproductive behaviour associated with mate choice by size in three species of White Sea molluscs of the genus *Littorina*—*L. saxatilis*, *L. obtusata* and *L. fabalis*. Three behavioural patterns were revealed after a comparative analysis of the behaviour of the male snails. The males of *L. obtusata*, regardless of their size, could mate with all females but preferred to mate with larger ones. The males of *L. fabalis* chose females of their own size or larger. The males of *L. saxatilis* mated with females of approximately their own size. In the first case (*L. obtusata*), there is a free exchange of genes between all the size groups in the population. In the second case (*L. fabalis*), the gene transfer from small males to females of any size is free but the gene transfer from large males to small females is limited. In the third case (*L. saxatilis*), the gene flow is limited between groups of individuals of dramatically different size. Thus, in the case of *L. saxatilis* and *L. fabalis*, to varying degrees restriction of random mating associated with size assortativity in choosing a mating partner was expressed, which under certain circumstances (divergence of individuals in different ecotopes, the presence of ecotypes differing in size, lack of stronger natural selection in the other direction) can favour sympatric speciation. The males of all the three periwinkles' species preferred larger females but sexual selection in favour of larger females was found only in *L. obtusata*. In the other two species, there were no statistically significant size differences between copulating and non-copulating females. This appears to be due to the occurrence of size-assortative mating in these species, which limits the ability of males to choose larger female partners.

Keywords: *Littorina*; White Sea; reproductive behaviour; mate choice; size-assortative mating; sexual selection



Citation: Kozminsky, E.V.; Serbina, E.A.; Smurov, A.O. Mate Choice in Molluscs of the Genus *Littorina* (Gastropoda: Littorinidae) from White Sea. *Diversity* **2023**, *15*, 297. <https://doi.org/10.3390/d15020297>

Academic Editors: Dimitry Schepetov and Michael Wink

Received: 29 December 2022

Revised: 10 February 2023

Accepted: 13 February 2023

Published: 17 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Mate choice is a key element of various microevolutionary processes. The ability to choose a mate is fundamental for most animal species because it may underlie prezygotic reproductive isolation and directly affect speciation [1]. From the point of view of an individual, an effective mate-searching strategy makes it possible to avoid futile energy expenditure and reduced viability or even sterility of the offspring [2,3].

A crucial aspect of reproductive behaviour, characteristic of many animals, is the ability to choose a mate of a certain size [4–7]. If a species occupies various ecotopes and if environmental conditions (e.g., the level of wave impact) favour divergent selection on size, ecotypes differing in size may arise. Size differences may affect reproductive behaviour, resulting in the emergence of assortative mating, imposing limits on random mating and gradually leading to the reproductive isolation of size groups within a species.

Snails of the genus *Littorina* are promising objects for studies of various microevolutionary processes owing to their diverse ecology, a complex demographic structure of their populations and a high intrapopulation diversity by several morphological characters. Various aspects of their reproductive biology have received considerable attention in recent

years [8–12]. For Littorinidae, the possibility of sympatric speciation associated with size assortativity in choosing a mating partner was shown. In particular, several ecotypes differing in size have been described in *L. saxatilis* and *L. fabalis*, and in some cases assortative mating between them has been shown [2,13–16]. It is established that assortative mating between size ecotypes of *L. saxatilis* results in a considerable decrease in gene flow between them in the co-occurrence zones [2,17,18]. At the same time, it is shown that the males of many periwinkle species prefer to mate with larger females, which results in sexual selection on size [19–21].

The most important features of the reproductive behaviour of the main “model” species of periwinkles (*L. saxatilis*, *L. littorea*, *L. fabalis*) were identified in the study of populations located in the center of their species areal. Features of the reproductive biology of periwinkles at the boundaries of the area and, in particular, in the northern seas of Russia (the White Sea and the Barents Sea) are practically not studied. Some aspects of reproductive behaviour of *L. saxatilis*, *L. obtusata* and *L. littorea* at the White Sea were observed by Kaufman [22]. Sergievsky [23] analysed mate choice related with the size and the shell colouration in *L. obtusata* at the White Sea and provided some data on the possibility of mating between the species. Unfortunately, *L. fabalis* was not identified in the northern Russian seas at that time and Sergievsky’s observations, based on the mixture of *L. obtusata* and *L. fabalis*, have lost much of their value. In the recently published study, the effectiveness of reproductive isolation at the mating partner choice stage in the Barents Sea periwinkles, including *Littorina saxatilis*, *L. obtusata*, and *L. fabalis*, was examined [24]. This work also provides limited data on the size-assortative mating of individuals for the studied species.

The aim of the present study was to examine the features of the reproductive behaviour associated with the mate choice by size in three species of periwinkles from the White Sea (*Littorina saxatilis* (Olivier, 1792), *L. obtusata* (Linnaeus, 1758) and *L. fabalis* (W. Turton, 1825)). The fourth periwinkle occurring at the White Sea, *L. littorea* (Linnaeus, 1758), was left out of the analysis because of the low density of its populations in the study area and the prohibitive methodological difficulties associated with the search for its copulating pairs.

2. Materials and Methods

Periwinkles are common intertidal snails. *L. obtusata* and *L. fabalis* have a globular shell, while *L. saxatilis* has an ovate-conical shell. The maximum shell size of the White Sea periwinkles is about 10 mm. Periwinkles are gonochoristic, and fertilization is internal. *L. saxatilis* is ovoviviparous, while *L. obtusata* and *L. fabalis* lay eggs. In the White Sea, *L. saxatilis* release young snails throughout the warm season, with the peak in August–September. *L. obtusata* and *L. fabalis* have a limited reproductive period, laying eggs from late May to late June [25] (unpublished data). Periwinkles mostly occur on stony and rocky shores. *L. obtusata* and *L. fabalis* are strictly associated with brown algae from the family Fucaaceae, using them as food, habitat and the substrate for egg-laying [26–29]. In the White Sea, *L. saxatilis* mostly occurs at the upper and the middle level of the intertidal, *L. obtusata*, at the middle and the lower level, while *L. fabalis* is found in the upper subtidal below the zero tidal datum.

The material for this study was collected in the Chupa Inlet of the White Sea in 2016 (samples of *L. fabalis*) and in 2017 (samples of *L. obtusata* and *L. saxatilis*). Copulating pairs of *L. fabalis* were collected at two sites: in the Levaya Bay (66°20′16.7″ N 33°39′36.3″ E) and near the “Kartesh” White Sea Biological Station (66°20′09.6″ N 33°38′39.4″ E). Copulating pairs of *L. obtusata* and *L. saxatilis* were collected in the Levaya Bay.

We looked for copulating pairs in late May–June at low tide. Copulating pairs were recognised by their characteristic position and then tested for copulation, which was considered as established if the penis of the male was inserted into the mantle cavity of the female mate. Each mating pair was placed into a separate tube.

To identify the parameters of the demographic structure of *L. fabalis* and *L. obtusata* populations and to test the hypothesis about the presence of aggregations of snails of the

same size, two series of quantitative samples were taken in the Levaya Bay: at the zero tidal datum (*L. fabalis*) and at the middle level of the intertidal (*L. obtusata*). Each of the series consisted of four plots, each with an area of 1/20 m². For *L. saxatilis*, eight quantitative samples, each with an area of 1/40 m², were taken at the upper intertidal level.

Copulating pairs and quantitative samples were transported to the laboratory and processed there. The maximum shell diameter of all the snails was measured. After that, the snails were dissected under MBS-10 binocular microscope, and their species was ascertained based on the reproductive system structure. Their sex, the state of the reproductive system and the presence of trematode parthenitae were also recorded.

In the assessment of size-assortative mating, Pearson correlation coefficient (R) was applied to the shell diameter of snails in copulating pairs. The statistical significance of the correlations was assessed with the help of classical parametric tests [30]. Heteroscedasticity index was used to identify the type of assortative mating (“true” or “apparent”) [31]. Since Pearson correlation coefficient may be misleading when applied to aggregations of similarly sized individuals [32,33], we tested our material for the presence of such aggregations by comparing, with the help of the F-test, the ratio of standard deviations of the shell diameter in the snails involved in reproduction at separate plots and in the snails involved in reproduction in the population in general. This ratio should be equal to one if there are no aggregations of similarly sized individuals and equal to zero if all individuals at a plot are uniform in size.

For comparison of binomial frequencies, we used standard methods based on normal approximation [30] or, in case of small samples, exact procedures as described in [34]. Sexual selection on size was measured with the help of the Sexual Selection Intensity index (S.S.I) [2].

3. Results

In total, we analysed 493 copulating pairs and 733 individual snails in quantitative samples. In this study, we used the data on 370 mating male-female pairs (143 pairs of *L. fabalis*, 145 pairs of *L. obtusata* and 82 pairs of *L. saxatilis*). In some of them (60 pairs), at least one of the partners was infected with trematode parthenitae. The corresponding data were recorded in the analysis: according to our observations, the infected partner was almost always the female, whereas the individuals of the active sex, the males, did not distinguish between infected and uninfected female mates. We left out of the analysis 102 homosexual and 21 interspecies copulating pairs.

The least shell diameter of copulating males and females of *L. obtusata* with a normal reproductive system was 5.4 and 6.3 mm, respectively. The males of *L. fabalis* were involved in mating starting with a shell diameter of 4.5 mm, and the females, starting with 5.0 mm. The smallest copulating males and females of *L. saxatilis* with a fully formed reproductive system had a shell diameter of 3.3 mm (It should be noted that we also found one copulating *L. saxatilis* female with a shell diameter of 2.9 mm, which, had an underdeveloped reproductive system. Since this female was involved in copulation, we left her in the analysis).

The ratio between the sizes of mates varied in a broad range for all the three periwinkle species under study (Figure 1). In *L. obtusata* there were no size constraints on the formation of copulating pairs ($R = 0.036$, $\alpha = 0.665$). In the other two species, we found a statistically significant correlation between the sizes of mates in copulating pairs ($R = 0.287$, $\alpha = 0.014$ and $R = 0.578$, $\alpha < 0.001$, *L. fabalis* and *L. saxatilis*, respectively). The distribution of residues around the regression line in all the species corresponded to the normal distribution ($\alpha > 0.200$), but in case of *L. obtusata* had a truncated upper border, being limited by the maximum possible shell diameter in females. Heteroscedasticity index did not statistically significantly differ from zero in any of the cases, which means that the size of the females in copulating pairs did not increase statistically significantly with the increasing size of the males. The index was +0.07, −0.17 and −0.20 in *L. obtusata* ($\alpha = 0.421$), *L. fabalis* ($\alpha = 0.155$) and *L. saxatilis* ($\alpha = 0.070$), respectively.

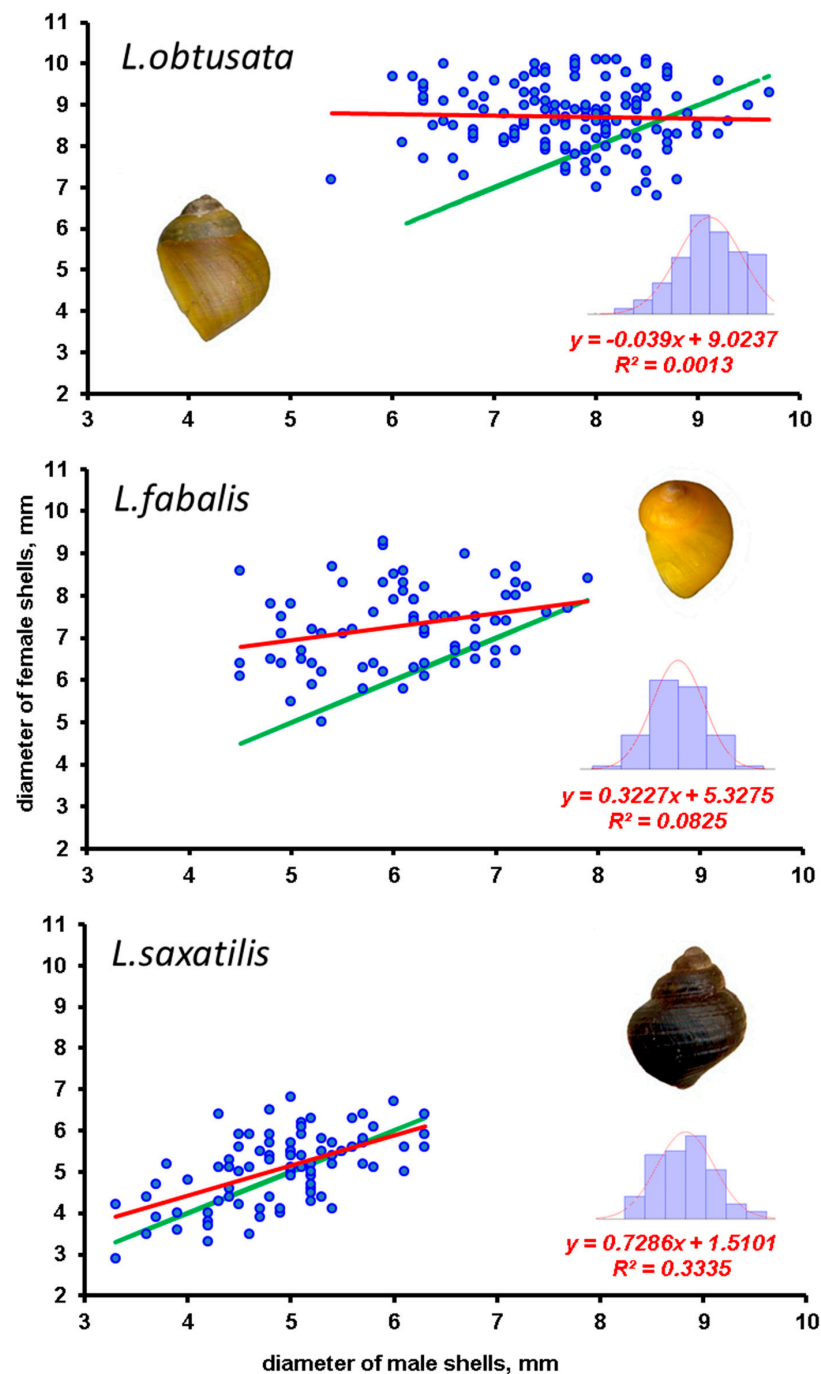


Figure 1. Correlation between the size of males and that of females in copulating pairs. The regression line is shown in red, equal-size line is shown in green. Regression equation and the histogram of distribution of residues around the regression line are shown.

The analysis of the regression lines (Figure 1) pointed to the considerable differences in the reproductive behaviour of the studied species. In case of *L. obtusata*, the regression line was almost parallel to the X-axis and passed at an angle of 45° to the line of equal size of males and females. This indicates that the males of this species may mate with females of any size. In *L. saxatilis*, the regression line and the equal-size line almost coincided, though the corresponding regression coefficients (0.729 and 1.000) were statistically significantly different ($\alpha = 0.021$). This means that the males of *L. saxatilis* mate with females of approximately their own size. In *L. fabalis*, the lower border of the point cloud roughly corresponded to the equal-size line, while its upper border corresponded to the maximum

size of the females. The regression line occupied an intermediate position. This means that the males of *L. fabalis* prefer females of approximately their own or larger size.

A comparison of standard deviations of the shell diameter of the snails involved in reproduction at separate sites and in the population in general revealed statistically significant indications of aggregation ($\alpha = 0.031$) only at one out of the eight sites where *L. saxatilis* was sampled. In the other two periwinkles, no indications of the existence of aggregations of similarly sized individuals were found. Thus, the correlation between the sizes of mates found in *L. saxatilis* and *L. fabalis* cannot be explained by a non-random distribution of individuals in the population.

The shell diameter of females in copulating pairs was, on the average, larger than that of the males (Figure 2). This effect was especially pronounced in *L. fabalis* and *L. obtusata*. The average difference between shell diameters of the mates made up 1.1 mm, 1.0 mm and 0.2 mm in, respectively, *L. fabalis*, *L. obtusata* and *L. saxatilis*.

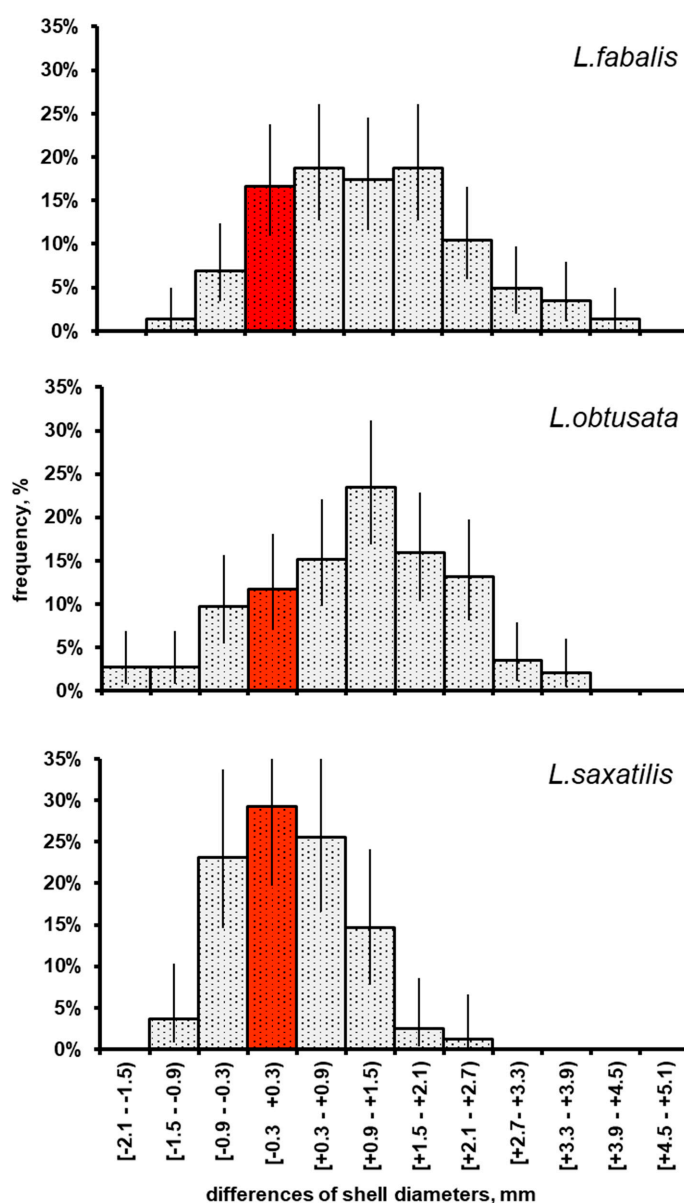


Figure 2. Distribution of differences of shell diameter of females and males in copulating pairs. Deviations corresponding to the upper and the lower border of the 95% exact interval are given [34]. The interval corresponding to the equal size of the partners is highlighted in colour.

The average sizes of males and females in the sexually mature part of the population and in copulating pairs, and the corresponding values of the S.S.I. index, are given in Table 1. The size characteristics were different only in case of *L. obtusata*: copulating females were statistically significantly ($\alpha < 0.001$) larger than all reproductively active females in the population. In case of *L. fabalis* and *L. saxatilis*, there were no statistically significant differences in the size of the females in copulating pairs and the sexually mature part of the population. The shell diameter of males in copulating pairs and in the sexually mature part of the population showed no statistically significant differences in any of the three species under study.

Table 1. Sexual selection intensity on size in males and females of the three species of periwinkles: mean sizes, standard deviations and sample sizes for mating and non-mating individuals.

		Copulating Snails	Non-Copulating Snails	S.S.I.	S.L.
<i>Littorina fabalis</i>					
males	Mean	6.1	5.8	0.34	$\alpha = 0.0545$
	S.D.	0.84	0.96		
	N	72	49		
females	Mean	7.3	7.4	−0.10	do not differ
	S.D.	0.94	0.94		
	N	72	28		
<i>Littorina obtusata</i>					
males	Mean	7.8	7.8	0.00	do not differ
	S.D.	0.78	1.01		
	N	145	37		
females	Mean	8.7	8.0	0.71	$\alpha < 0.001$
	S.D.	0.85	1.06		
	N	145	40		
<i>Littorina saxatilis</i>					
males	Mean	4.9	4.8	−0.07	do not differ
	S.D.	0.69	0.59		
	N	82	25		
females	Mean	5.1	5.1	−0.01	do not differ
	S.D.	0.87	0.71		
	N	82	40		

Mean—mean size; S.D.—standard deviation; N—sample size; S.S.I. is the sexual selection intensity (mean size of mating snails−mean size of all snails/standard deviation of all snails); S.L. is the level of significance.

4. Discussion

Two statistically independent components are identified in the reproductive behaviour: sexual selection and sexual isolation. Each component may have various causes and, which is even more important, various evolutionary implications. Sexual selection leads to a change in the frequency of genes in the population; sexual isolation does not alter genetic frequencies but leads to an isolation of different groups of individuals. Sexual selection is estimated by comparing the frequency of mating individuals with that of non-mating ones in the population. Sexual isolation is estimated based on the mating pattern of copulating individuals or, more specifically, based on the occurrence of size-assortative mating. The relationships between sexual selection and sexual isolation may be fairly complex because they have different mechanisms and are affected by different factors. Therefore, these two components should be clearly partitioned during the research [2].

In littorinid snails, males are the active sex, while females are usually passive [8,15,24] and only reject partners in some rare cases [8,21,35]. Therefore, it is the ability of males to choose mates that is the driving force behind the emergence of sexual selection and, in many cases, of size-assortative mating in littorinids [14,21,36].

Physical constraints on mating associated with the structure of the reproductive system seem to be an indispensable prerequisite of size-assortative mating [2,37]. Since the males of littorinid snails prefer to mate with larger females [2,14,21,38,39], size-assortative mating, if any, is also closely associated with the ability to choose a mate.

Arnqvist et al. [31] introduced the terms of “true” and “apparent” size-assortative mating and suggested the heteroscedasticity index for their separation. “True” assortative mating is described by a linear dependence between the size of the males and that of the females, with observations being symmetrically distributed around the regression line. It corresponds to the situation when the gene exchange between contrasting size groups within a population is limited. In the case of “apparent” assortative mating, a positive regression is the result of decreasing dispersion of the size of the females with the increasing size of the males. This corresponds to the situation when small males may mate with any females, while large males only mate with large females. In such a case, the gene exchange between different size groups in the population occurs, though less intensively than in case of random mating.

Size-assortative mating was revealed in several littorinid species [2,14,15,21,38] (and others). It was established that a significant correlation between the sizes of the mates was observed not in all the populations, even within a species. Whether or not size-assortative mating occurs seems to depend on the direction of the strongest natural selection in a given habitat [2,21]. It was shown based on the evidence from *L. saxatilis* that the true size-assortative mating promotes the establishment of a strong reproductive barrier between groups of individuals of different sizes [17,18,40]. In case of apparent size-assortative mating in *L. fabalis* [15], the gene exchange is also somewhat limited but not as strongly as in *L. saxatilis* [41].

Our data indicate that the males of the three periwinkle species under study demonstrate three different patterns of behaviour. In case of *L. obtusata*, the regression line and the point cloud are almost parallel to the X-axis. This means that the males of this species, regardless of their size, may mate with females of any size but prefer (as follows from the histogram) larger ones (pattern 1). In case of *L. fabalis*, the point cloud is limited by the equal-size line from below and by the maximum possible shell diameter of the females (about 9 mm) from above; the regression line occupies an intermediate position. This means that the males of *L. fabalis* prefer to mate with females of approximately their own or larger size (pattern 2). In case of *L. saxatilis*, the regression line and the equal-size line almost coincide. This means that the males of *L. saxatilis* mate with females of approximately their own size (pattern 3).

Our results are in good agreement with the observations of Maltseva et al. [24]. In both cases, the ratio of partner sizes in copulating pairs in *L. obtusata*, *L. fabalis*, and *L. saxatilis* varied widely. Additionally, as in our case, according to Maltseva et al. [24] (Supplement 15), males of the Barents Sea *L. obtusata*, without reference to of their size, could mate with all females. *L. fabalis* males chose females of the same size or larger, while a significant correlation in the size of partners in copulating pairs was observed only in the case of *L. saxatilis*.

Several hypotheses have been proposed to explain the correlation between the sizes of mates in copulating pairs: (1) non-random distribution of individuals of different sizes in the habitat (2) mechanical constraints on mating due to the mismatch of the genitals' size between individuals of different size and (3) the choice of a mate of a certain size combined with competition between individuals of the choosing sex.

In our study, some indications of the presence of aggregations of similarly sized individuals were found only in *L. saxatilis*, and the input of this factor into the assortative mating was inconsiderable. In the case of *L. fabalis*, no indications of aggregations of individuals of the same size were found. This means that non-random distribution of individuals in the population could be disregarded in both cases.

According to several authors, the existence of mechanical constraints on mating (which related to genital size) is unlikely [8,21,38], since it poorly agrees with a high variability

of the size ratio of mates shown for many littorinids [2,8,21,37] (this study). However, in our opinion, the existence of such limitations is in good agreement with our data and can explain, at least in part, the observed correlation between the sizes of partners in copulating pairs in *L. fabalis* and *L. saxatilis*. As compared with the other two species, *L. obtusata* males have the most massive erected penis, and females have the largest copulatory bursa. The genital organs of males and females of *L. fabalis* and *L. saxatilis* are significantly smaller in size [29,42] (own observations), so they should have the smallest size enabling normal mating. This size range must be species-specific; the narrower it is, the more pronounced size-assortative mating would be. Our data indicate that this range is the broadest in *L. obtusata*, intermediate in *L. fabalis* and the narrowest in *L. saxatilis*. The high variability in partner size ratios that we observed can be explained by the high elasticity of the genital organs in molluscs. In the case of *L. saxatilis*, the conical shape of the shell may also play a role in the appearance of size assortativity.

Size-assortative mating in littorinid snails is thought to be associated with the ability to choose the mate combined with competition between individuals of the choosing sex [8,21,38]. It is obvious that alongside with the structural features of the reproductive system, these are apparently the key factors resulting in the emergence of size-assortative mating in our case, since males are known to choose the mate in both *L. saxatilis* and *L. fabalis* [13–15].

In all the three periwinkle species in this study, females in copulating pairs were on the average large than males (Figure 2). Preference given by males to larger females seems to be a common phenomenon in littorinids [14,15,21,38,39,43]. This behaviour is usually explained by a positive correlation between the fecundity and the size of the female [15,19–21,44–47] allowing the males to leave more offspring. Similar data were obtained by Maltseva et al. [24] when studying the Barents Sea periwinkles. As in our case, the females of *L. saxatilis* and *L. fabalis* in copulating pairs were slightly larger than males. At the same time, Maltseva et al. [24] did not find a pronounced excess in the size of *L. obtusata* females relative to males, which we revealed in White Sea molluscs. Apparently, this is due to differences in the used dimensional characteristics—diameter (this work) and height [24] of the shell. *L. saxatilis* have oval-conical shell while for the *L. obtusata* and *L. fabalis* it is spherical-oval. In the latter case, the shell diameter increases faster with age than height. Accordingly, for *L. obtusata* and *L. fabalis*, the shell diameter is a more sensitive characteristic in terms of detecting sexual selection.

A larger size of females in copulating pairs may be due to the sexual dimorphism, a varying duration of mating in pairs of different size composition and the choice of a larger mate [21]. Sexual size dimorphism in *L. obtusata* and *L. fabalis* is poorly expressed: according to our observations, in the former species, females are larger than males of the same age only by 3%, and in the latter species, by 12%. Copulating pairs with larger females may indeed occur somewhat more often, since such females are more easily involved into mating and the mating with them usually lasts longer [8,21,35,38,39]. Finally, it has been shown by several authors that male periwinkles of the genus *Littorina* indeed choose larger partners [14,15,19,21,35,36]. This preference seems to be the main reason of the larger size of the females in copulation pairs revealed in our study.

Sexual selection in favour of larger females is not uncommon in littorinid snails [21,38,39]. The choice of larger female mates at the level of individual copulating pairs could be seen in all the species under study. At the same time, a statistically significantly larger average size of females in copulating pairs than in the population in general, indicating the sexual selection on size, was found only in *L. obtusata*. In the other two species, there were no noticeable differences between the size of mating and non-mating females. This seems to be the result of size-assortative mating in these species, limiting the possibilities of the choice of larger mates and preventing selection in this direction. The emergence of sexual selection in favour of larger females is explained as mentioned by a positive correlation between their size and fecundity, ensuring competitive advantage for the males [19–21,35,36,48].

Sexual selection (as well as size-assortative mating) is manifested not in all the populations even in the same periwinkle species [21]. Moreover, the characteristics of the sexual selection are not necessarily determined by mate choice but may be due to the impact of a stronger selection in a given habitat. For instance, in several Swedish populations of *L. saxatilis* copulating males and females of the S-morph were much larger than non-copulating ones, whereas males and females of the E-morph were smaller. These differences are probably due to ecological reasons: small E-morphs, less affected by the wave impact, have an advantage in exposed habitats, while larger S-morphs, less affected by predation by crabs, do better in sheltered habitats [2].

No sexual selection in periwinkle males was found in our study, which seems to be the rule for the littorinid snails [21,38,39]. Exceptions to this rule seem to be associated with the cases when sexual selection in males is due not to mate choice but to the impact of a stronger selection in a particular habitat [2].

In the study, a noticeable size assortativity was revealed during mating in *L. saxatilis* and *L. fabalis*. Our observations have a good agreement with the data of other authors [2,15,24]. Since size assortativity was found in different, geographically distant, populations within the species areal, it should be considered as a feature of reproductive behaviour inherent in this species, which, under certain circumstances, can be involved in the process of sympatric speciation. Maltseva et al. [24] after studying the effectivity of reproductive isolation mechanisms at the mating partner selection stage in the Barents Sea periwinkles concluded that the ability for assortative mating played an important role in reproductive isolation during speciation in periwinkles, representatives of the subgenus *Neritrema*. However, the divergence of molluscs into different ecological niches was primary.

5. Conclusions

In the White Sea periwinkles, we found three models of reproductive behaviour that differ in the degree of expression of size assortativeness and its possible consequences in terms of sympatric speciation. In the case of *L. saxatilis* (model 3), there is a significance size-assortative mating with limited gene flow between groups of individuals that differ greatly in size. When implementing model 2 (*L. fabalis*), free gene transfer from small males to females of any size is possible, but gene transfer from large males to small females is limited. In the population of *L. obtusata* (model 1), there is a free exchange of genes between all size groups. Thus, in the case of *L. saxatilis* and *L. fabalis*, there is an expressed to varying degrees restriction of random mating associated with size assortativity in choosing a mating partner, which under certain circumstances (divergence of individuals in different eoniches, the presence of ecotypes differing in size, lack of stronger natural selection in the other direction) can favour sympatric speciation.

Author Contributions: E.V.K.—conceptualization, methodology, resources, investigation, formal analysis, writing—original draft, review and editing. E.A.S.—formal analysis, editing, project administration. A.O.S.—original draft preparation, editing, visualization. All authors have read and agreed to the published version of the manuscript.

Funding: This work was carried out as part of the State Tasks (state registration numbers AAAA-A19-119022690122-5, AAAA-A19-119020690091-0), and Federal Fundamental Scientific Research Program for 2021-2025, project number 1021051703269-9-1.6.12.

Institutional Review Board Statement: Our work conforms to the legal requirements and guidelines in the Russian Federation as well as to international ethical standards. The species from our study is not included in the 'Threatened' category of the IUCN Red List of Threatened Species.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

- Coyne, J.A. Genetics and speciation. *Nature* **1992**, *355*, 511–515. [[CrossRef](#)] [[PubMed](#)]
- Erlandsson, J.; Rolán-Alvarez, E. Sexual selection and assortative mating by size and their roles in the maintenance of a polymorphism in Swedish *Littorina saxatilis* populations. *Hydrobiologia* **1998**, *378*, 59–69. [[CrossRef](#)]
- Ng, T.P.T.; Davies, M.S.; Stafford, R.; Williams, G.A. Mucus trail following as a mate-searching strategy in mangrove littorinid snails. *Anim. Behav.* **2011**, *82*, 459–465. [[CrossRef](#)]
- Nagel, L.; Schluter, D. Body size, natural selection, and speciation in sticklebacks. *Evolution* **1998**, *52*, 209–218. [[CrossRef](#)]
- Ratcliffe, L.M.; Grant, P.R. Species recognition in Darwin's finches (*Geospiza*, Gould) I. Discrimination by morphological cues. *Anim. Behav.* **1983**, *31*, 1139–1153. [[CrossRef](#)]
- Richmond, J.Q.; Reeder, T.W. Evidence for parallel ecological speciation in scincid lizards of the *Eumeces skiltonianus* species group (squamata: Scincidae). *Evolution* **2002**, *56*, 1498–1513. [[CrossRef](#)]
- Jones, A.G.; Moore, G.I.; Kvarnemo, C.; Walker, W.D.; Avise, J.C. Sympatric speciation as a consequence of male pregnancy in seahorses. *Proc. Nat. Acad. Sci. USA* **2003**, *100*, 6598–6603. [[CrossRef](#)] [[PubMed](#)]
- Ng, T.P.T.; Williams, G.A. Size-dependent male mate preference and its association with size-assortative mating in a mangrove snail, *Littoraria arduiniana*. *Ethology* **2014**, *120*, 995–1002. [[CrossRef](#)]
- Rolán-Alvarez, E.; Austin, C.J.; Boulding, E.G. The contribution of the genus *Littorina* to the field of evolutionary ecology. *Oceanogr. Mar. Biol. Annu. Rev.* **2015**, *53*, 157–214.
- Carvalho, J.; Sotelo, G.; Galindo, J.; Faria, R. Genetic characterization of flat periwinkles (Littorinidae) from the Iberian Peninsula reveals interspecific hybridization and different degrees of differentiation. *Biol. J. Linn. Soc.* **2016**, *118*, 503–519. [[CrossRef](#)]
- Johannesson, K.; Saltin, S.H.; Charrier, G.; Ring, A.-K.; Kvarnemo, C.; André, C.; Panova, M. Non-random paternity of offspring in a highly promiscuous marine snail suggests postcopulatory sexual selection. *Behav. Ecol. Sociobiol.* **2016**, *70*, 1357–1366. [[CrossRef](#)]
- Estévez, D.; Kozminsky, E.; Carvajal-Rodríguez, A.; Caballero, A.; Faria, R.; Galindo, J.; Rolán-Alvarez, E. Mate choice contributes to the maintenance of shell color polymorphism in a marine snail via frequency-dependent sexual selection. *Front. Mar. Sci.* **2020**, *7*, 614237. [[CrossRef](#)]
- Hull, S.L. Does size assortative mating maintain a direct polymorphism between two distinct populations of *Littorina saxatilis* (Olivi) on the north-east coast of England. *Hydrobiologia* **1998**, *378*, 79–88. [[CrossRef](#)]
- Johnson, L.J. Size assortative mating in the marine snail *Littorina neglecta*. *J. Mar. Biol. Assoc. U. K.* **1999**, *79*, 1131–1132. [[CrossRef](#)]
- Saltin, S.H.; Schade, H.; Johannesson, K. Preference of males for large females causes a partial mating barrier between a large and a small ecotype of *Littorina fabalis* (W. Turton, 1825). *J. Molluscan Stud.* **2013**, *79*, 128–132. [[CrossRef](#)]
- Galindo, J.; Grahame, J.W. Ecological speciation and the intertidal snail *Littorina saxatilis*. *Adv. Ecol.* **2014**, *2014*, 239251. [[CrossRef](#)]
- Panova, M.; Hollander, J.; Johannesson, K. Site-specific genetic divergence in parallel hybrid zones suggests nonallopatric evolution of reproductive barriers. *Mol. Ecol.* **2006**, *15*, 4021–4031. [[CrossRef](#)]
- Johannesson, K.; Havenhand, J.N.; Jonsson, P.R.; Lindgarth, M.; Sundin, A.; Hollander, J. Male discrimination of female mucous trails permits assortative mating in a marine snail species. *Evolution* **2008**, *62*, 3178–3184. [[CrossRef](#)]
- Hughes, R.N.; Answer, P. Growth, spawning and trematode infection of *Littorina littorea* (L.) from an exposed shore in north Wales. *J. Molluscan Stud.* **1982**, *48*, 321–330. [[CrossRef](#)]
- Janson, K. Variation in the occurrence of abnormal embryos in females of the intertidal Gastropod *Littorina saxatilis* Olivi. *J. Molluscan Stud.* **1985**, *51*, 64–68. [[CrossRef](#)]
- Erlandsson, J.; Johannesson, K. Sexual selection on female size in a marine snail, *Littorina littorea* (L.). *J. Exp. Mar. Biol. Ecol.* **1994**, *181*, 145–157. [[CrossRef](#)]
- Kaufman, Z.S. *Osobennosti Polovykh Tsiklov Belomorskikh Bespozvonochnykh (Features of Sexual Cycles of White Sea Invertebrates)*; Nauka: Leningrad, Russia, 1977; pp. 1–264. (In Russian)
- Sergievsy, S.O. *Vybor Partnera Dlya Sparivaniya v Populyatsiyakh Litoal'nogo Mollyuska Littorina obtusata (L.) (Gastropoda: Prosobranchia) (The Choice of a Partner for Mating in Populations of the Intertidal Mollusc Littorina obtusata (L.) (Gastropoda: Prosobranchia))*; Vestnik Leningrad State University: Saint Petersburg, Russia, 1983; Volume 21, pp. 22–27. (In Russian)
- Maltseva, A.L.; Varfolomeeva, M.A.; Gafarova, E.R.; Panova, M.A.; Mikhailova, N.A.; Granovitch, A.I. Divergence together with microbes: A comparative study of the associated microbiomes in the closely related *Littorina* species. *PLoS ONE* **2021**, *16*, e0260792. [[CrossRef](#)]
- Kozminsky, E.V. *Opredelenie Vozrasta u Littorina obtusata (Gastropoda, Prosobranchia) (Determination of age in Littorina obtusata (Gastropoda, Prosobranchia))*. *Zool. Zhurnal* **2006**, *85*, 146–157. (In Russian)
- Kuznetsov, V.V. *Beloe More I Biologicheskie Osobennosti Yego Flory I Fauny (White Sea and Biological Features of Its Flora and Fauna)*; AS USSR Publishing: Moscow, Russia, 1960. (In Russian)
- Beskupskaja, T.P. *Pitanie Nekotorykh Obychnykh Litoral'nykh Bespozvonochnykh Belogo Moray (Nutrition of Some Common Littoral Invertebrates in the White Sea)*. *Proc. Kandalaksha Reserve* **1963**, *4*, 114–135. (In Russian)
- Matveeva, T.A. *Ecologia I Zhiznennyye Tsikly Obychnykh Vidov Mokkyusk Barentseva I Belogo Morey (Ecology and Life Cycles of Common Gastropod Species in the Barents and the White Seas)*. In *Seasonal Phenomena in the Life of the White and the Barents Seas*; Byhovskiy, B.E., Ed.; Nauka: Leningrad, Russia, 1974; pp. 65–190. (In Russian)
- Reid, D.G. *Systematics and evolution of Littorina*; The Ray Society: London, UK, 1996; 463p.
- Glantz, S. *Medico-Biologicheskaya Statistika (Primers of Biostatistics)*; Praktika: Moscow, Russia, 1998; pp. 1–469. (In Russian)

31. Arnqvist, G.; Rowe, L.; Krupa, J.J.; Sih, A. Assortative mating by size: A meta-analysis of mating pattern in water striders. *Evol. Ecology* **1996**, *10*, 265–284. [[CrossRef](#)]
32. Johannesson, K.; Rolán-Alvarez, E.; Eken Dahl, A. Incipient reproductive isolation between two sympatric morphs of the intertidal snail *Littorina saxatilis*. *Evolution* **1995**, *49*, 1180–1190. [[CrossRef](#)] [[PubMed](#)]
33. Fernandez-Meirama, M.; Estevez, D.; Ng, T.P.; Williams, G.A.; Carvajal-Rodriguez, A.; Rolan-Alvarez, E. A novel method for estimating the strength of positive mating preference by similarity in the wild. *Ecol. Evol.* **2017**, *9*, 2883–2893. [[CrossRef](#)]
34. Lloyd, E.; Lederman, W. *Spravochnik po Prikladnoy Statistike (Handbook of Applicable Statistic)*; Finances and Statistics: Moscow, Russia, 1989; Volume 1, pp. 1–511. (In Russian)
35. Saur, M. Mate discrimination in *Littorina littorea* (L.) and *L. saxatilis* (Olivi) (Mollusca: Prosobranchia). *Hydrobiologia* **1990**, *193*, 261–270. [[CrossRef](#)]
36. Zahradnik, T.D.; Lemay, M.A.; Boulding, E.G. Choosy males in a littorinid gastropod: Male *Littorina subrotundata* prefer large and virgin females. *J. Molluscan Stud.* **2008**, *74*, 245–251. [[CrossRef](#)]
37. Yu, T.L.; Wang, L.M. Mate choice and mechanical constraint on size-assortative pairing success in a simultaneous hermaphroditic pond snail *Radix lagotis* (Gastropoda: Pulmonata) on the Tibetan Plateau. *Ethology* **2013**, *119*, 738–744. [[CrossRef](#)]
38. Cardoso, R.S.; Costa, D.S.; Loureiro, V.F. Mating behaviour of the marine snail *Littoraria flava* (Mollusca: Caenogastropoda) on a boulder shore of south-east Brazil. *J. Mar. Biol. Assoc. U. K.* **2007**, *87*, 947–952. [[CrossRef](#)]
39. Ng, T.P.T.; Rolan-Alvarez, E.; Dahlen, S.S.; Davies, M.S.; Estevez, D.; Stafford, R.; Williams, G.A. The causal relationship between sexual selection and sexual size dimorphism in marine gastropods. *Anim. Behaviour* **2019**, *148*, 53–62. [[CrossRef](#)]
40. Grahame, J.W.; Wilding, C.S.; Butlin, R.K. Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. *Evolution* **2006**, *60*, 268–278. [[CrossRef](#)]
41. Tatarenkov, A.; Johannesson, K. Evidence of a reproductive barrier between two forms of the marine periwinkle *Littorina fabalis* (Gastropoda). *Biol. J. Linn. Soc.* **1998**, *63*, 349–365. [[CrossRef](#)]
42. Granovich, A.I.; Mikhailova, N.A.; Znamenskaya, O.; Petrova, Y.A. Species complex of mollusks of the genus *Littorina* (Gastropoda, Prosobranchia) from the eastern murman coast. *Zool. Zhurnal* **2004**, *83*, 1305–1317. (In Russian)
43. Ito, A.; Wada, S. Intrasexual copulation and mate discrimination in a population of *Nodilittorina radiata* (Gastropoda: Littorinidae). *J. Ethol.* **2006**, *4*, 45–49. [[CrossRef](#)]
44. Dewitt, T.J. Gender contests in a simultaneous hermaphrodite snail: A size advantage model for behaviour. *Anim. Behav.* **1996**, *51*, 345–351. [[CrossRef](#)]
45. Yusa, Y. The effects of body size on mating features in a field population of the hermaphroditic sea hare *Aplysia kurodai* Baba, 1937 (Gastropoda: Opisthobranchia). *J. Molluscan Stud.* **1996**, *62*, 381–386. [[CrossRef](#)]
46. Angeloni, L. Sexual selection in a simultaneous hermaphrodite with hypodermic insemination: Body size, allocation to sexual roles and paternity. *Anim. Behav.* **2003**, *66*, 417–426. [[CrossRef](#)]
47. Ng, T.P.T.; Williams, G.A. Contrasting reproductive traits in two species of mangrove-dwelling littorinid snails in a seasonal tropical habitat. *Invertebr. Biol.* **2012**, *131*, 177–186. [[CrossRef](#)]
48. Rolán-Alvarez, E.; Zapata, C.; Alvarez, G. Multilocus heterozygosity and sexual selection in a natural population of the marine snail *Littorina mariae* (Gastropoda: Prosobranchia). *Heredity* **1995**, *75*, 17–25. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.