INVERTEBRATE REPRODUCTION & DEVELOPMENT
https://doi.org/10.1080/07924259.2023.2298687

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The mytilid bivalve *Mytilus trossulus* exhibits area-specific proportions of heteromorphic spermatozoa in the Sea of Japan

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ABSTRACT

The present study is devoted to a comparison of the structure and quantitative ratio of heterogeneous spermatozoa of native wild mussels *Mytilus trossulus* Gould, 1850, collected in four areas of the Peter the Great Bay of the Sea of Japan (the northwestern part of the Pacific Ocean). The mussels were identified as *M. trossulus* by shell appearance, and the species was confirmed by sequencing the mitochondrial cytochrome *c* oxidase subunit I gene (COI). Heteromorphic spermatozoa were found; namely, a total of eight morphs (SPERM1–SPERM8) in *M. trossulus*. Surprisingly, some of the detected sperm morphs overlap morphologically with the sperms of other mytilids such as *M. edulis*, *Crenomytilus* (*M.) grayanus* and *M. coruscus*. Possible reasons for this phenomenon are discussed. In each geographic area, the 'quantitative proportions of heterogeneous spermatozoa' (QPHS) were unique. It has been suggested that the QPHS score can be considered in the context of its applicability as a biological marker for finding optimal mussel rearing sites.

ARTICLE HISTORY
Received 10 May 2023
Accepted 12 December 2023

KEYWORDS
Sea of Japan; *Mytilus trossulus*; COI DNA barcoding; shells; sperm heteromorphism

Introduction

The mytilid bivalve *Mytilus trossulus* (Gould, 1850) is widely distributed in the northern hemisphere, where it is a common member of benthic communities in the northern part of the Pacific Ocean, the northern part of the Atlantic Ocean, and the Baltic Sea (Gosling 1992; Chichvarkhin et al. 2000; Evseev et al. 2011; Vainola and Strelkov 2011; Keiji et al. 2017). In various regions of the world, *M. trossulus* is being developed through aquaculture (Pring-Ham 2018; Sokołowski et al. 2022). To carry out effective aquaculture, it is necessary to identify the regions most favourable for mussels with the help of biomarkers (Buer et al. 2020).

Recently, 'quantitative proportions of heterogeneous spermatozoa' (QPHS) have begun to be considered in the context of the applicability of this parameter in the search for areas representing favourable habitat for bivalves. The principle of operation of this criterion is demonstrated by the example of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793), which has six sperm morphs (Reunov et al. 2018), the mactrid bivalve *Mactra chinensis* (Philippi, 1846), which has four sperm morphs (Reunov et al. 2021), and the venerid bivalve *Ruditapes philippinarum* (Adams, Reeve, 1850), the heterogeneous sperm set of which includes six morphs (Reunov et al. 2023). For these three species, sperm plasticity has been suggested to be an adaptation to the unpredictable environment in the context of external fertilization, which can be influenced by wave intensity, water turbulence, temperature and salinity variability, and anthropogenic pollution. It has been proposed that the expression of additional sperm types is induced epigenetically to increase the likelihood of reproductive success, the chance of which increases if there is a sperm type most suitable for certain fertilization conditions. The number of possible sperm variants is not always represented in a set, and the largest number of sperm samples is usually found in molluscs living in the most stressful conditions, while in populations living in optimal conditions the number of morphs is small. At the same time, in different habitats, different variants of sperm may dominate, corresponding to the main environmental factors. Considering that the types of sperms and their quantity correlate with the environmental conditions of collection sites, it is proposed to use the QPHS as marker to determine the geographical origin of bivalves, as well as to identify both environmentally stressful and favourable sites (Reunov et al. 2018, 2021, 2023).

Plasticity in the shape of spermatozoa was noted in *M. trossulus* (Vekhova et al. 2013). However, it is still unclear whether the proportions of sperm morphs of

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this species can vary across different areas and, therefore, it is unclear whether QPHS can be used to more accurately identify aquaculture sites for this species.

The aim of the present study was to test whether spermatozoa are heterogeneous and whether the proportions of heterogeneous spermatozoa can be specific in the Pacific mussel *M. trossulus* living in different areas of the Peter the Great Bay of the Sea of Japan (North Pacific Ocean). To do this, samples of *M. trossulus* were collected from four different areas. Genetic verification of the samples was carried out using the analysis of subunit I of the cytochrome *c* oxidase (COI) gene. The ultrastructure and external morphology of spermatozoa were studied, and the proportions of spermatozoa were calculated. The results were discussed in the context of the idea of using QPHS to select optimal sites for mussel aquaculture.

Materials and methods

Sample collection

Samples of the Pacific mussel *M. trossulus* Gould, 1850 were collected during the spawning season, which usually occurs in May for this species. In May 2019, samples were taken at a depth of 0.5–2 m by the diving service of the A. V. Zhirmunsky National Scientific Center of Marine Biology (Vladivostok, Russia) from four areas of Peter the Great Bay (the Sea of Japan): collection site 1, Amursky Bay (43°10′07.05″N, 131°53′49.33″E), collection site 2, Eastern Bosphorus Strait (43°04′39.9″N, 131°51′10.74″E), collection site 3, Ussuriisky Bay (43°18′83.65″N, 132°11′23.44″E), and collection site 4, Vostok Bay (42°53′37.22″N, 132° 43′33.14″E) (Figure 1).

Upon arrival at the laboratory, the males were identified by observing the spermatozoa under a light microscope. The collected samples were immediately processed by fixing in alcohol for genetic analysis, as well as fixing in glutaraldehyde for both transmission electron microscopy and scanning electron microscopy. The shells were dried and photographed. Dry shell valves of each specimen are stored in the personal collection of Dr. E. Vekhova at the A. V. Zhirmunsky National Scientific Center of Marine Biology (Vladivostok, Russia).

Genetic analyses

Three male *M. trossulus* from each of four collection sites were processed for genetic analysis at the Biotechnology Laboratory of the Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS (Vladivostok, Russia). Total DNA was isolated from mantle pieces (3–5 mm^3) according to a published protocol (Kiselev et al. 2015). Partial sequences of mitochondrial cytochrome *c* oxidase subunit I gene (COI) were amplified and sequenced using the universal invertebrate primer pairs: LCO1490: 5′GGT CAA CAA ATC ATA AAG ATA TTG G and HCO2198: 5′TAA ACT TCA GGG TGA CCA AAA AAT CA (Folmer et al. 1994). PCR amplification was performed in a 25 µl reaction volume. Amplification products were applied as templates for sequencing, using the same primers as for PCR and 'Big Dye Terminator Cycle Sequencing Kit' v. 3.1 (Applied Biosystems, USA) following the manufacturer's protocol. Sequencing reaction products were purified by ethanol precipitation and analysed using an ABI-3130 Genetic Analyzer (Applied Biosystems, ABI, USA). The 12 COI sequences were deposited in GenBank.

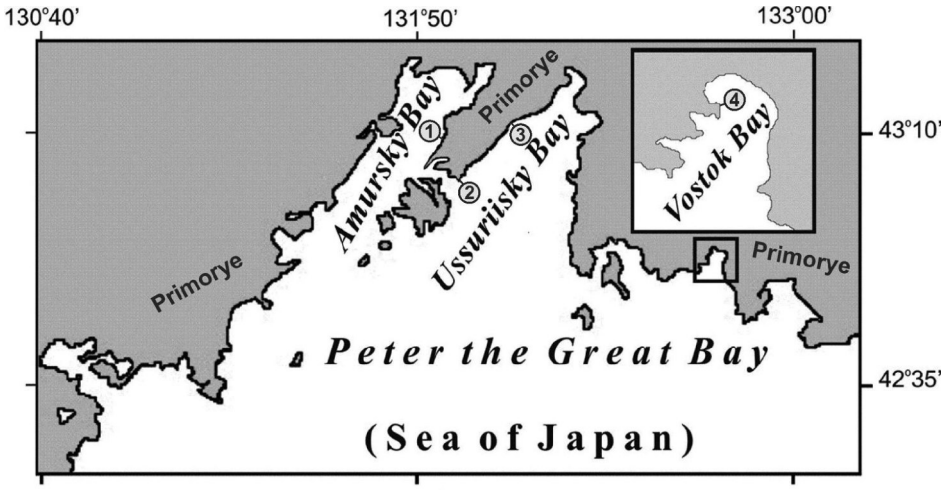

Figure 1. Map of collection sites for the Pacific mussel *Mytilus trossulus* in Peter the Great Bay (the Sea of Japan, Russia). 1 — Amursky Bay; 2 — Eastern Bosphorus Strait; 3 — Ussuriisky Bay; 4 — Vostok Bay.

Transmission electron microscopy (TEM)

The male gonads were dissected, cut into small pieces (1–2 mm) and fixed overnight in primary fixator containing 2.5% glutaraldehyde in a 0.1 M cacodylate buffer (pH 7.4) with osmolarity 1100 mOsm adjusted by sodium chloride. Fixed tissues were washed in the same buffer, postfixed in a 2% OsO4 in sea water, rinsed in a 0.1 M cacodylate buffer and distilled water, dehydrated in an ethanol series, infiltrated and embedded in Spurr's resin. Ultra-thin sections were mounted on slot grids that were coated with formvar film stabilized with carbon. Sections were stained with 2% alcoholic uranyl acetate and Reynolds lead citrate and were examined with a transmission electron microscope Zeiss Libra 120 (A Carl Zeiss SMT AG Company, Oberkochen, Germany) and Philips 410 Transmission Electron Microscope (Philips 123 Electronics, Eindhoven, The Netherlands).

Scanning electron microscopy (SEM)

The male gonads were dissected, cut into small pieces (1–2 mm) and fixed overnight in primary fixator containing 2.5% glutaraldehyde in a 0.1 M cacodylate buffer (pH 7.4) with osmolarity 1100 mOsm adjusted by sodium chloride. Fixed tissues were washed in the same buffer and postfixed in a 2% OsO4 in sea water. Fixed samples were rinsed in buffer. Sperm suspension was prepared by crushing pieces of fixed materials. The sperm suspension was pipetted onto a Termanox Plastic Coverslips (Cat. № 174942, USA) and allowed to settle for 30 min. It was then washed for 15 min in the cacodylate buffer, and dehydrated in a series of alcohol solutions with increasing concentrations, gradually bringing to pure acetone. Coverslips with attached sperm cells were transferred to acetone and were finally critical-point dried in carbon dioxide in a Bal-Tec 030 critical point dryer, then placed on the surface of aluminium stubs, and sputter-coated with chromium using a Q 150T ES vacuum sputter coater for thin membranes. Then the morphological features of the obtained spermatozoa of *M. trossulus* were examined under a Zeiss Sigma 300 VP scanning electron microscope.

Quantitative analysis of sperm morph amounts

Three genetically identified male samples were taken from each collection. Five hundred sperm cells of each samples were evaluated, for a total of 6000 sperm cells altogether. Sperm phenotypes were identified by scanning electron microscopy and frequency of each phenotype was calculated. All values are expressed as means with standard error of the mean (SEM). Differences between groups were assessed using Student's t-test. P-value < 0.05 was considered statistically significant.

Results

Species validity identification by analysis of the COI gene

Samples collected at four collection sites (Figure 1) were subjected to genetic testing. A phylogenetic tree was constructed using sequences obtained from our samples and sequences selected for the current study from the GenBank database (NCBI) (Figure 2). This tree shows that all *M. trossulus* sequences belong to the same clade. Moreover, using BLAST (Basic Local Alignment Search Tool, NCBI; http://blast.ncbi.nlm.nih.gov/Blast.cgi), we compared sequences of MW033214, MW033212, MW033220, MW033209, MW033210, MW033213, MW033218, MW033216, MW033219, MW033215, MW033217, MW033211 with other sequences from GenBank. These sequences matched previously deposited sequences of *M. trossulus* (Identity 99.6–100%). Geographically, the sequences are distributed as follows: Amursky Bay (MW033209, MW033210, MW033211), Eastern Bosphorus Strait (MW033215, MW033216, MW033217), Ussuriisky Bay (MW033218, MW033219, MW033220), Vostok Bay (MW033212, MW033213, MW033214). Based on genetic analysis, all mussel specimens belong to the species *M. trossulus*.

Shell appearance and geographic distribution of genetically tested mussels

Geographically and genetically, mussel shells (Figure 3) are distributed as follows: Amursky Bay (MW033209, MW033210, MW033211) (Figure 3a–c), Eastern Bosphorus Strait (MW033215, MW033216, MW033217) (Figure 3d–f), Ussuriisky Bay (MW033218, MW033219, MW033220) (Figure 3g–i), Vostok Bay (MW033212, MW033213, MW033214) (Figure 3j–l). In each of the four collection sites, the shells of mussels have a typical triangular-oval shape. The periostracum on the outer surface of the shells is black-brown. The anterior and ventral parts of the shell are usually brown. No clear geographical specificity of shell parameters was found.

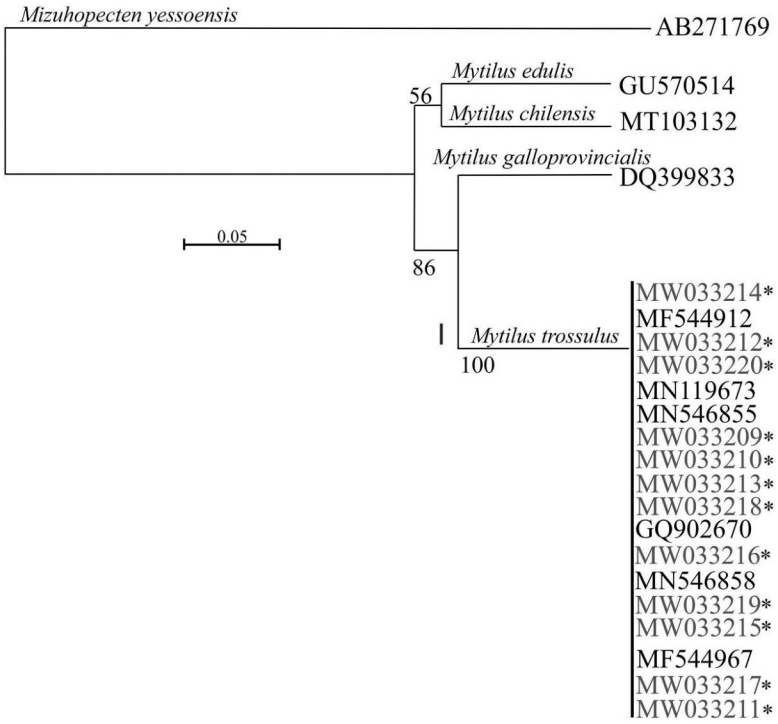

Figure 2. Neighbor-joining (NJ) phylogram generated from COI data for the Pacific mussel *Mytilus trossulus*. Note: the length of sequences in the first dataset (I) is 661 bp. The branches are bootstrap values of the NJ analysis. Note that all tested specimens marked with an asterisk belong to the same clade *M. trossulus*.

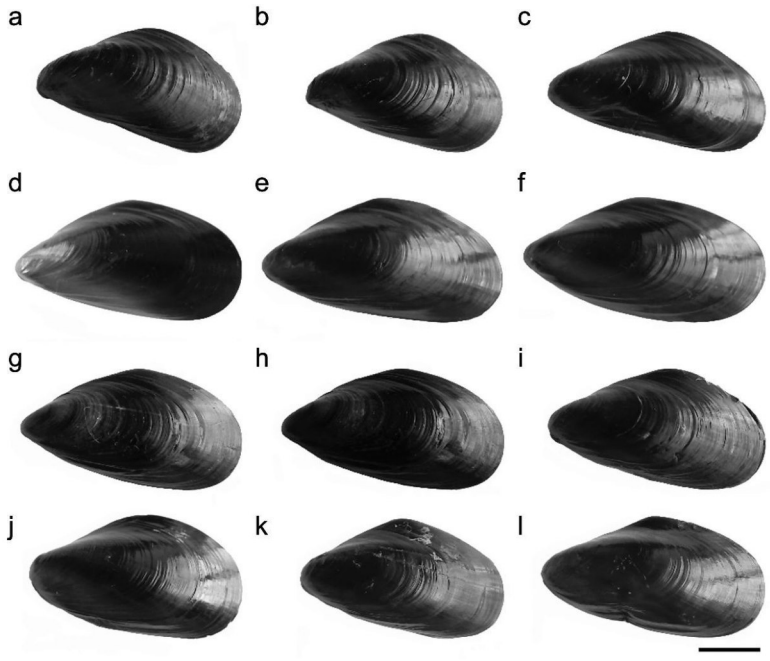

Figure 3. Right shell valves of the genetically identified Pacific mussel *Mytilus trossulus*, corresponding to the samples collected in Amursky Bay (MW033209, MW033210, MW033211) (a–c), Eastern Bosphorus Strait (MW033215, MW033216, MW033217) (d–f), Ussuriisky Bay (MW033218, MW033219, MW033220) (G–I), Vostok Bay (MW033212, MW033213, MW033214) (j-l). Scale −1 cm.

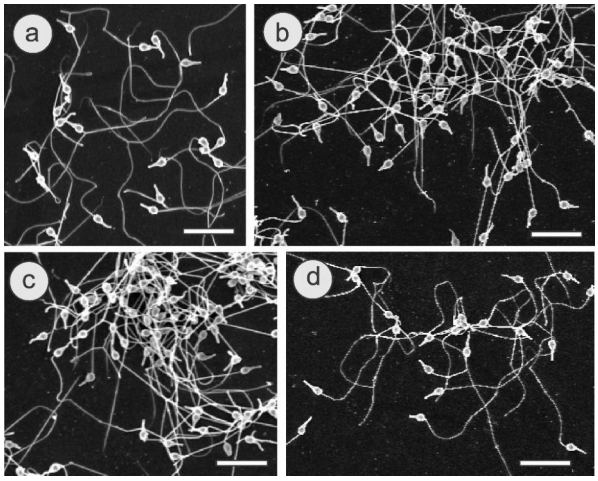

Figure 4. Low magnification SEM images showing spermatozoa of the Pacific mussel *Mytilus trossulus* collected at four locations in Peter the Great Bay, such as Amursky Bay (a), Eastern Bosphorus Strait (b), Ussuriisky Bay (c) and Vostok Bay (d). Scale –30 µm.

General sperm morphology by SEM

At low magnification, spermatozoa of mussels collected in four different areas appear similar, with compact heads surmounted by pointed acrosomes and thin flagella on the other side of the spermatozoa (Figure 4a–d).

Sperm ultrastructure using TEM

The sperm head consists of a barrel-shaped nucleus filled with electron-dense chromatin (Figure 5a). The acrosome is funnel-shaped and contacts the nucleus with its wide base. The acrosome cavity contains an axial rod (Figure 5a). The axial rod passes through the nuclear channel down to the septum formed by the nuclear membrane (not shown). The two centrioles are perpendicular to each other and are located under the basal part of the nucleus. The distal centriole serves as the basal body of the flagellum, which has a typical arrangement of axonemal microtubules (9 + 2) (not shown). The centriolar apparatus is surrounded by round mitochondria (Figure 5a), which form a ring of five organelles (not shown). It was possible to identify four models of spermatozoa according to their acrosome types. These were spermatozoa with a long acrosome (Figure 5b), spermatozoa with medium length acrosomes, which could be relatively straight (Figure 5c) or strongly curved (Figure 5d). Spermatozoa with a short acrosome were also found (Figure 5e).

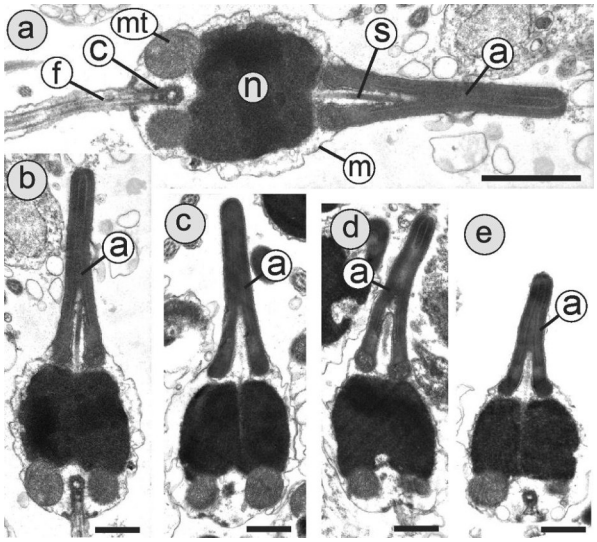

Figure 5. Spermatozoa of the Pacific mussel *Mytilus trossulus* by the TEM. General view of the sperm head (a); sperm head with long acrosome (b); sperm heads with an acrosome of medium length, which can be straight (c) or curved (d); sperm head with a short acrosome (e). Scale – 1 µm (A), 0.5 µm (b-e).

External morphology of spermatozoa by SEM

In total, eight types of spermatozoa were identified (Figure 6). Pairs that are morphologically close have been identified. The first pair includes a morph with an oval nucleus and a straight acrosome SPERM1 (Figure 6a) and a morph with an oval nucleus and a curved acrosome SPERM2 (Figure 6b). The second pair includes two morphs that have a similar trapezoidal nucleus, but differ in the shape of the acrosome. In the first morph, the acrosome has an expanded base continued by a straight tip, SPERM3 (Figure 6c), while in the second morph, the expanded base of the acrosome continues with an elongated and curved tip, SPERM4 (Figure 6d). The third pair includes morphs with an oval nucleus and a straight, long acrosome with an expanded base, SPERM 5 (Figure 6e), and an asymmetrically inclined long acrosome with an asymmetrically expanded base, SPERM6 (Figure 6f). The fourth pair includes two morphs with an oval nucleus and a short acrosome. One morph has a short and straight acrosome, SPERM7 (Figure 6g), and the other morph has a short, asymmetrically inclined acrosome, SPERM8 (Figure 6h).

Quantitative analysis showed that the proportions of spermatozoa in mussels differ depending on their habitat. Samples found at collection sites 1–3 had SPERM1–SPERM6 and no SPERM7 and SPERM8. In collection site 1, the proportions of SPERM1–SPERM6 correspond to 49%, 19%, 13%, 2%, 2%, 15% (Figure 7a). In collection site 2 SPERM1–SPERM6 correspond to 45%, 23%, 12%, 4%, 4%, 12% (Figure 7b). In collection site 3, SPERM1–SPERM6

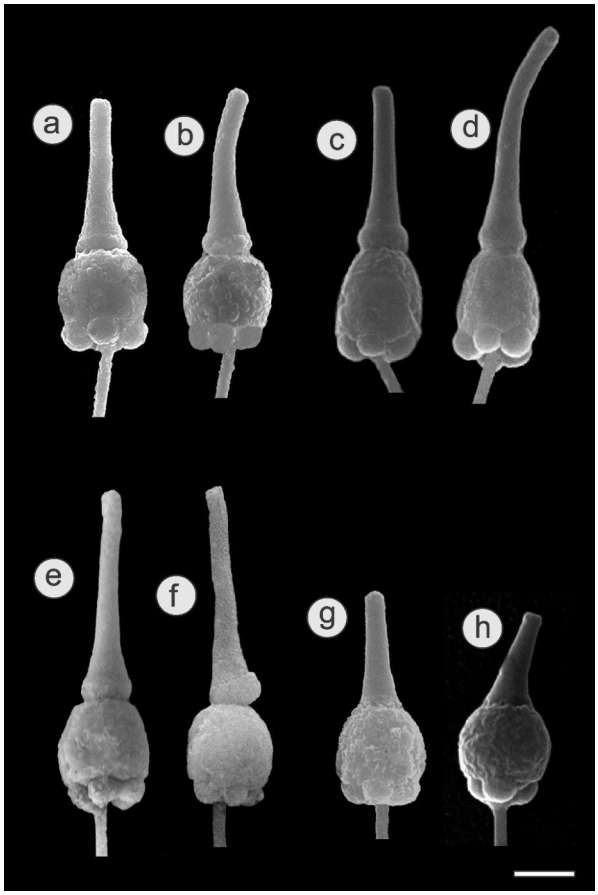

Figure 6. Spermatozoa types identified by SEM in the Pacific mussel *Mytilus trossulus*. SPERM1 (a); SPERM2 (b); SPERM3 (c); SPERM4 (d); SPERM5 (e); SPERM6 (f); SPERM7 (g); SPERM8 (h).

represented 47%, 27%, 10%, 4%, 2%, 10%, respectively (Figure 7c). At collection site 4, the samples had eight sperm morphs, and the proportions belonging to SPERM1–SPERM8 were 33%, 13%, 11%, 5%, 5%, 4%, 27%, and 2% (Figure 7d).

Discussion

In the Sea of Japan, *M. trossulus* coexists with the Mediterranean mussel *M. galloprovincialis*, which invaded around 1930–1935 (Ivanova and Lutaenko 1998; Skurikhina et al. 2001; Wilkins et al. 2008; Brannock et al. 2009; Vainola and Strelkov 2011). Since *M. trossulus* is a mytilid species whose shell characteristics are very similar to those of other mussels belonging to the *M. edulis* species complex, identification of this species by shell colour and morphology is problematic (Katolikova et al. 2016). Therefore, despite the fact that we identified mussels by shell parameters to target *M. trossulus*, we assumed that genetic testing of the specimens was necessary. Indeed, this species was discovered in the 1980s using allozyme analysis and was one of the first taxa discovered by molecular genetic methods (Koehn et al. 1984; McDonald and Koehn 1988; Varvio et al. 1988; Gosling 1992). We therefore confirmed identification using COI analysis known as a reliable method for species identification of bivalves (Feng et al. 2011; Layton et al. 2016). We are confident that we were dealing with *M. trossulus*.

TEM studies have shown that the spermatozoa of *M. trossulus* at the ultrastructural level correspond to the description made earlier by Drozdov and Reunov (1997). However, extended analysis by SEM showed that this sperm model is the dominant but not the only one in the *M. trossulus* sperm set. It has been established that the Pacific *M. trossulus* is characterized by heteromorphism of spermatozoa with eight regularly repeating morphs.

Interestingly, some sperm morphs found in the Pacific *M. trossulus* overlap morphologically with the spermatozoa of other mytilids. SPERM3 of the Pacific *M. trossulus* definitely corresponds to the spermatozoa of *Crenomytilus (M.) grayanus* living in the Vostok Bay of the Sea of Japan (Drozdov and Reunov 1997), and information about this similarity was published by us earlier (Reunov et al. 2012). Also, SPERM5 of the Pacific *M. trossulus* morphologically coincides with the spermatozoa of *M. coruscus* living in the Vostok Bay of the Sea of Japan (Reunov and Drozdov 1987; Drozdov and Reunov 1997) and in the coastal waters of Gyeokpo, in western Korea (Kim et al. 2010). Moreover, SPERM7 of the Pacific *M. trossulus* is very similar to the spermatozoa of *M. edulis* collected by Hodgson and Bernard (1986a, 1986b) in the North Atlantic near Plymouth (Great Britain) and *M. edulis* living in the White Sea (Drozdov and Reunov 1987; Inoue et al. 1997).

When analysing the phenomenon of interspecific overlap of sperm morphs, it seems appropriate to mention interspecific hybridization. Indeed, hybridization is known between pairs of species within mytilids belonging to the *M. edulis* complex, which includes species such as *M. edulis*, *M. trossulus*, and *M. galloprovincialis* (Breton et al. 2006). Between mussels, mtDNA recombination occurs between F-type (maternally inherited F genome) and M-type (paternally inherited M genome), the so-called MF recombination (Breton et al. 2006). In the Baltic populations, the original F and M mt genomes of *M. trossulus* were replaced by *M. edulis* mtDNA (Zbawicka et al. 2007). In the Sea of Japan, hybridization also occurs between mussels (Inoue et al. 1997; Chichvarkhin et al. 2000; Vainola and Strelkov 2011; Kartavtsev et al. 2014).

Regarding the consequences of hybridization, it is known that the morphological parameters of mussels can change. For example, in Scotland, in aquacultured

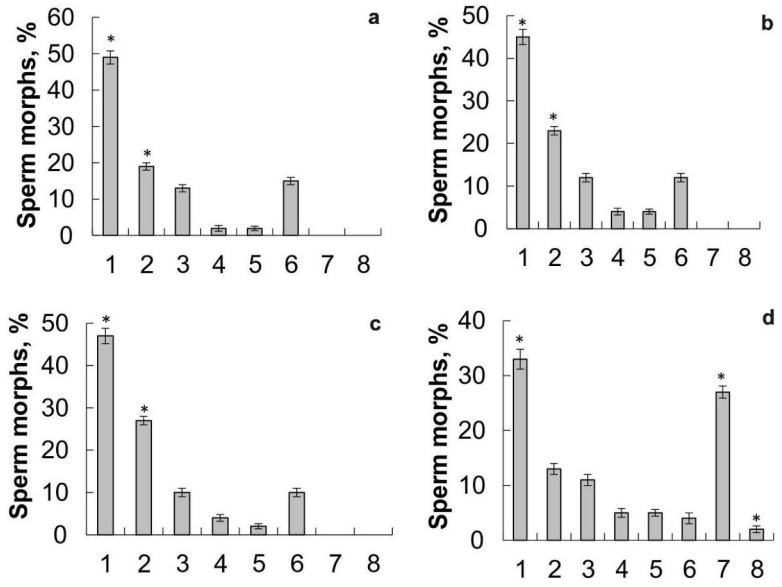

Figure 7. Ratio of sperm morphs of the Pacific mussel *Mytilus trossulus* collected from four areas in Peter the Great Bay, such as Amursky Bay (a), East Bosphorus Strait (b), Ussuriisky Bay (c), and Vostok Bay (d). Number 1 through 8 correspond to sperm types SPERM1-SPERM8. Data shown as mean ±SEM for each sperm morphs. Morphs quantities are statistically different (*$P < .001$).

M. edulis and *M. galloprovincialis*, introgression of *M. trossulus* genes was found to result in commercially unfavourable morphological traits (Michalek et al. 2021). However, there is still no evidence that sperm morphotypes are inherited as a result of gene introgression that occurs during hybridization. To clarify this issue, targeted studies involving genetically confirmed hybrids are needed.

In addition, with regard to the overlap of sperm morphs, the monophyly of mytilids should be noted. Indeed, the obtained genetic data definitely indicate the monophyly of the family Mytilidae with the most confirmed monophyly of the subfamily Mytilinae, which includes such species as *M. edulis*, *M. trossulus*, *M. galloprovincialis*, *M. coruscus*, *M. chilensis*, *M. californianus*, *C. (M.) grayanus* (Kartavtsev et al. 2018). It can be speculated that the interspecific overlap in sperm structure found in the heteromorphic set of sperm of the Pacific *M. trossulus* may be somehow related to the close relationship between the Mytilinae species. In any case, to contribute more knowledge to this interesting problem, apparently concerning the reproductive evolution of mytilids, more comparative studies between different mussel species are needed.

Interestingly, SPERM2, SPERM4, SPERM6, and SPERM8 have no known analogues among mytilid bivalves and are reported for the first time here in a heterogeneous set of spermatozoa from the Pacific *M. trossulus*. It is likely that SPERM2, SPERM4, SPERM6, and SPERM8 are heterogeneous derivatives of the corresponding paired morphs. Based on morphological similarities, these morphs correspond to morphs such as SPERM1, SPERM3, SPERM5 and SPERM7, to form pairs such as SPERM1–SPERM2, SPERM3–SPERM4, SPERM5–SPERM6 and SPERM7–SPERM8 which may be treated as *M. trossulus* – like sperm pair, *C. (M.) grayanus* – like sperm pair, *M. coruscus* – like sperm pair, and *M. edulis* – like sperm pair. More research is needed to find out if SPERM2, SPERM4, SPERM6 and SPERM8 can be found among the mytilid species mentioned above.

In this study of sperms from *M. trossulus* collected from four sites, quantitative differences were found and therefore QPHS could be analysed. Only two abundant sperm morphs (SPERM1 and SPERM2) were found among the six sperm morphs of the samples collected at site 3 – Ussuriisky Bay. Assuming that a lower number of abundant sperm morphs is associated with the presence of relatively favourable reproductive conditions (Reunov et al. 2018), we assume that out of the four areas considered in this study, collection site 3 is the best habitat for mussels. Sites 1 and 2 – Amursky Bay and the Eastern Bosphorus Strait also have six sperm morphs, but have four abundant sperm morphs, which in both cases fall on SPERM1–SPERM3 and SPERM6. Assuming that a greater number of abundant sperm morphs indicates a more stressful reproductive environment (Reunov et al. 2018), it can be assumed that sites 1 and 2 are less favourable for *M. trossulus* reproduction. At site 4 – Vostok Bay, *M. trossulus* also has four abundant sperm morphs that belong to SPERM1–SPERM3 as well as SPERM7, but shows a higher total number of sperm morphs (eight). According to our hypothesis, the large total number

of sperm morphs may be associated with the presence of a large gradient of the stress factors (Reunov et al. 2018), and indeed, Vostok Bay is known for its variable salinity, which fluctuates greatly from 0.5 to 30 ‰ (Grigoryeva and Kashenko 2010). Thus, based on the QPHS analysis, we consider site 4 to be the most unfavourable for mussel habitat and reproduction.

In summary, recognizing that the mechanisms of sperm heteromorphy still need to be elucidated for *M. trossulus*, especially in terms of possible interspecific hybridization, it seems likely that environmental factors may control QPHS in this species, as they do in the Pacific oyster *C. gigas*, surf clam *M. chinensis* and Manila clam *R. philippinarum*, in which hybridization has never been detected (Reunov et al. 2018, 2021, 2023). Given the uniqueness of QPHS in each *M. trossulus* habitat, we are proposing that QPHS analysis can be used to determine the best conditions for *M. trossulus* aquaculture.

Conclusion

The structure of spermatozoa was studied in the Pacific *M. trossulus* collected in four different areas of Peter the Great Bay (Sea of Japan). Spermatozoa exhibited heteromorphism, and eight morphs were identified. Some of the detected sperm morphs overlap morphologically with the sperms of other mytilids such as *M. edulis*, *Crenomytilus* (*M.) grayanus* and *M. coruscus*. The sperm cells were found to exhibit geographically unique 'quantitative proportions of heterogeneous spermatozoa' (QPHS). It is suggested that QPHS can be used as a sensitive biomarker to identify optimal locations for the aquaculture of *M. trossulus*.

Acknowledgments

The authors are grateful to colleagues from the A. V. Zhirmunsky National Scientific Centre of Marine Biology FEB RAS (Vladivostok, Russia), who helped to carry on this project such as the Director of the scuba diving service A.G. Goloseev and Director of the Collective Use Microscopy Unit D.V. Fomin. Part of the study was conducted at the Microscopy Facility Unit at St. Francis Xavier University (Antigonish, Nova Scotia, Canada).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Author contributions

All authors contributed to the study. EV conceived, designed the study, analysed the data, performed the routine work with scanning electron microscopy, wrote the paper. KK obtained the COI sequences, wrote the genetic protocol description, analysed the genetic data. YA and AA performed routine work using transmission electron microscopy. YR participated in work using scanning electron microscopy and performed statistical analyses. AR analysed the data, submitted important topics for discussion, and edited the article. All authors read and approved the final version of the manuscript.

References

Brannock PM, Wethey DS, Hilbish TJ. 2009. Extensive hybridization with minimal introgression in *Mytilus galloprovincialis* and *M. trossulus* in Hokkaido, Japan. Mar Ecol Prog Ser. 383:161–171. doi: 10.3354/meps07995.

Breton S, Burger G, Stewart DT, Blier PU. 2006. Comparative analysis of gender-associated complete mitochondrial genomes in marine mussels (*Mytilus* spp.). Genetics. 172 (2):1107–1119. doi: 10.1534/genetics.105.047159.

Buer A-L, Maar M, Nepf M, Ritzenhofen L, Dahlke S, Friedland R, Krost P, Peine F, Schernewski G. 2020. Potential and feasibility of *Mytilus* spp. farming along a salinity gradient. Front Mar Sci. 7:371. doi: 10.3389/fmars.2020.00371.

Chichvarkhin A, Kartavtsev Y, Kafanov AI. 2000. Genetic relationships among some species of Mytilidae (Mollusca: Bivalvia) from the northern Pacific Ocean. Russ J Genet. 36 (9):1003–1016.

Drozdov AL, Reunov AA. 1987. The morphology of gametes of the blue mussel *Mytilus edulis* from the White and Japan seas and Avacha Bay. Biol Morya. 4:52–55.

Drozdov AL, Reunov AA. 1997. Sperm morphology in mytilid bivalves. Russ J Mar Biol. 23(3):136–142.

Evseev GA, Kolotukhina NK, Kulikova VA. 2011. Morphological structures of the shell of *Mytilus trossulus* and *Crenomytilus grayanus* in early ontogenesis and their importance in the taxonomy of Mytilinae (Bivalvia: Mytilidae). J Mar Biolog Assoc UK. 91(8):1655–1664. doi: 10.1017/S0025315410000949.

Feng Y, Li Q, Kong L, Zheng X-D. 2011. COI-based DNA barcoding of Arcoida species (Bivalvia: Pteriomorphia) along the coast of China. Mol Ecol Resour. 11(3):435–41. doi: 10.1111/j.1755-0998.2010.02975.x.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech. 3:294–299.

Gosling EM. 1992. Developments in aquaculture and fisheries science. In: Gosling E, editor. The mussel *Mytilus*: ecology, physiology, genetics and culture. Amsterdam; New York: Elsevier; p. 25–589.

Grigoryeva NI, Kashenko SD. 2010. Study on interannual and seasonal variations of thermohaline conditions in the Vostok Bay (Peter the Great Bay, Japan Sea). Izvestiya TINRO. 162:242–255.

Hodgson AN, Bernard RTF. 1986a. Observations on the ultrastructure of the spermatozoon of two mytilids from the south-west coast of England. J Mar Biolog Assoc UK. 6 (2):385–390. doi: 10.1017/S0025315400043010.

Hodgson AN, Bernard RTF. 1986b. Ultrastructure of the sperm and spermatogenesis of three species of Mytilidae (Mollusca, Bivalvia). Gamete Res. 15(2):123–135. doi: 10.1002/mrd.1120150204.

Inoue K, Odo S, Noda T, Nakao S, Takeyama S, Yamaha E, Yamazaki F, Harayama S. 1997. A possible hybrid zone in

the *Mytilus edulis* complex in Japan revealed by PCR markers. Mar Biol. 128(1):91–95. doi: 10.1007/s002270050072.

Ivanova MB, Lutaenko KA. 1998. On the Distribution of *Mytilus galloprovincialis* Lamarck, 1819 (Bivalvia, Mytilidae) in Russian Far Eastern Seas. Bull Inst Malacol Tokyo. 3(5):67–71.

Kartavtsev YP, Katolikova MV, Sharina SN, Chichvarkhina OV, Masalkova NA. 2014. A population genetic study of the hybrid zone of *Mytilus trossulus* Gould, 1850 and an introduced species, *M. galloprovincialis* Lamarck, 1819, (Bivalvia: Mytilidae) in Peter the Great Bay in the Sea of Japan. Russ J Mar Biol. 40(3):208–216. doi: 10.1134/S1063074014030055.

Kartavtsev YP, Sharina SN, Chichvarkhin A, Chichvarkhina OV, Masalkova NA, Lutaenko KA, Oliveira C. 2018. Genetic Divergence of Mussels (Mollusca, Mytilidae) based on the 28S rRNA, 18S rRNA, and H3 nuclear gene sequences. Russ Genet. 54(6):652–669. doi: 10.1134/S1022795418060078.

Katolikova M, Khaitov V, Väinölä R, Gantsevich M, Strelkov P, Colgan DJ. 2016. Genetic, ecological and morphological distinctness of the blue mussels *Mytilus trossulus* Gould and *M. edulis* L. in the White Sea. PLoS One. 11(4):1–25. e0152963. doi: 10.1371/journal.pone.0152963.

Keiji I, Soh I, Takashi B, Yasuhiro K. 2017. Records of *Mytilus trossulus* (Bivalvia: Mytilidae) and its possible distribution along the northern and central Japan Sea coast of Honshu Island. Venus (J Malacolog Soc Japan). 75(1–4):67–81.

Kim JH, Chung EY, Choi KH, Park KH, Park SW. 2010. Ultrastructure of germ cells during spermatogenesis and some characteristics of sperm morphology in male *Mytilus coruscus* (Bivalvia: Mytilidae) on the west coast of Korea. Korean J Malacol. 26(1):33–43.

Kiselev KV, Dubrovina AS, Tyunin AP. 2015. The methylation status of plant genomic DNA influences PCR efficiency. J Plant Physiol. 175(175):59–67. doi: 10.1016/j.jplph.2014.10.017.

Koehn RK, Hall JG, Innes DJ, Zera A. 1984. Genetic differentiation of *Mytilus edulis* in eastern North America. Mar Biol. 79 (2):117–126. doi: 10.1007/BF00951820.

Layton KKS, Martel AL, Hebert PDN. 2016. Geographic patterns of genetic diversity in two species complexes of Canadian marine bivalves. J Molluscan Stud. 82(2):282–291. doi: 10.1093/mollus/eyv056.

McDonald JH, Koehn RK. 1988. The mussels *Mytilus galloprovincialis* and *Mytilus trossulus* on the Pacific coast of North America. Mar Biol. 99(1):111–118. doi: 10.1007/BF00644984.

Michalek K, Vendrami DLJ, Bekaert M, Green DH, Last KS, Telesca L, Wilding TA, Hoffman JI. 2021. *Mytilus trossulus* introgression and consequences for shell traits in longline cultivated mussels. Evol Appl. 14:1830–1843. doi: 10.1111/eva.13245.

Pring-Ham C 2018. Insight into aquatic farming of shellfish and aquatic plants in Alaska//Sustainable AG conference, November 6, 2018, Anchorage, AK (https://www.adfg.alaska.gov/static).

Reunov AA, Drozdov AL. 1987. Spermatogenesis and ultrastructure of spermatozoa of the mussel *Mytilus coruscus*. Tsitologiia. 29:260–266.

Reunov AA, Lutaenko K, Vekhova E, Zhang J, Zakharov E, Sharina S, Alexandrova Y, Reunova Y, Akhmadieva A, Adrianov A. 2021. In the Asia-Pacific region, the COI DNA test revealed the divergence of the bivalve mollusc *Mactra chinensis* into three species; can these species be distinguished using shell coloration and sperm structure? Helgol Mar Res. 75(7). doi: 10.1186/s10152-021-00553-0.

Reunov AA, Vekhova EE, Alexandrova YN, Reunova YA, Adrianov AV. 2012. Intraspecific male gamete diversity in the mussels *Crenomytilus grayanus* and *Mytilus trossulus*; phenomenon of sperm pattern overlap. In: Lutaenko K, editor. Proceedings the Russia-China Bilateral symposium on "Marine ecosystems under the Global Change in the Northwest Pacific"; Oct 8–9 Vladivostok, Russia. Vladivostok: Dalnauka; p. 111–114.

Reunov A, Vekhova E, Milani L, Piccinini G, Iannello M, Alexandrova Y, Reunova Y, Zakharov E, Akhmadieva A, Pimenova E. 2023. In the Sea of Japan and the Adriatic Sea, a COX1 DNA test revealed genetic similarity of Manila clams, although shell parameters, spermatogenesis patterns, and sperm characteristics are area specific. Biologia. 78 (10):2803–2817. doi: 10.1007/s11756-023-01414-6.

Reunov A, Vekhova E, Zakharov E, Reunova Y, Alexandrova Y, Sharina S, Adrianov A. 2018. Variation of sperm morphology in Pacific oyster precludes its use as a species marker but enables intraspecific geo-authentification and aquatic monitoring. Helgol Mar Res. 72(8). doi: 10.1186/s10152-018-0510-x.

Skurikhina LA, Kartavtsev Y, Chichvarkhin A, Pan'kova MV. 2001. Study of two species of mussels, *Mytilus trossulus* and *Mytilus galloprovincialis* (Bivalvia, Mytilidae), and their hybrids in Peter the Great Bay of the Sea of Japan with the use of PCR markers. Russ J Genet. 37(12):1448–1451. doi: 10.1023/A:1013264400526.

Sokołowski A, Lasota R, Alias I, Piłczyńska J, Wołowicz M. 2022. Prospects and opportunities for mussel *Mytilus trossulus* farming in the southern Baltic Sea (the Gulf of Gdańsk). Oceanol Hydrobiol Stud. 51(1):53–73. doi: 10.26881/oahs.2022.1.06.

Vainola R, Strelkov P. 2011. *Mytilus trossulus* in Northern Europe. Mar Biol. 158:817–833. doi: 10.1007/s00227-010-1609-z.

Varvio SL, Koehn RK, Väinölä R. 1988. Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. Mar Biol. 98(1):51–60. doi: 10.1007/BF00392658.

Vekhova EE, Reunov AA, Alexandrova Y, Reunova Y, Adrianov AV. 2013. First data on sperm heteromorphism in the mussels *Crenomytilus grayanus* and *Mytilus trossulus* (Bivalvia) from the Sea of Japan. Dokl Biol Sci. 453 (1):358–361. doi: 10.1134/S0012496613060021.

Wilkins NP, Fujino K, Gosling E. 2008. The Mediterranean mussel *Mytilus galloprovincialis* Lmk in Japan. Biol J Linn Soc. 20 (4):365–374. doi: 10.1111/j.1095-8312.1983.tb01597.x.

Zbawicka M, Burzyński A, Wenne R. 2007. Complete sequences of mitochondrial genomes from the Baltic mussel *Mytilus trossulus*. Gene. 406(1–2):191–198. doi: 10.1016/j.gene.2007.10.003.