Invertebrate Biology x(x): 1–8.

DOI: 10.1111/ivb.12183

Does peripheral dislodgement contribute to heterozygote deficiencies in blue mussels?

Eleanor Q. Daniels and Marian K. Litvaitis[a]

Department of Natural Resources and the Environment, University of New Hampshire, Durham, New Hampshire 03824, USA

Abstract. Differential loss of heterozygous individuals that move to the periphery of mussel aggregations, where they are at greater risk for dislodgement, has been proposed as an explanation for observed heterozygote deficiencies in blue mussels. To test the dislodgement hypothesis, correlations between heterozygosity and mussel motility, as well as characteristics of byssogenesis and byssal thread attachment strengths were determined in a wild and a farmed population of blue mussels *(Mytilus edulis)* from New Hampshire, USA. Although both populations exhibited a heterozygote deficit as measured by three microsatellite loci, no relationship was found between heterozygosity and increased motility in either population. Similarly, no relationship was found between heterozygosity and byssogenesis or attachment strength. Hence, differential dislodgement is highly unlikely as a possible contributor to the loss of heterozygous individuals.

Additional key words: *Mytilus edulis*, mussel movement, byssal thread attachment, heterozygosity, microsatellites

Not only is increased heterozygosity in blue mussels (*Mytilus edulis* Linnaeus 1758) positively correlated with higher growth rates (Diehl & Koehn 1985; Diehl et al. 1986) but it also confers an increased survival during environmentally stressful conditions, most notably elevated temperatures and air exposure (Tremblay et al. 1998; Myrand et al. 2002; LeBlanc et al. 2008). Furthermore, heterozygosity has been correlated with increased fecundity (Rodhouse et al. 1986) and potentially increased immune function (Carissan-Lloyd et al. 2004). Despite such apparent positive relationships, both natural and farmed mussel populations reveal significant heterozygote deficiencies (Zouros & Foltz 1984; Tremblay et al. 1998; Myrand et al. 2002, 2009a; Addison et al. 2008; Diz & Presa 2008; LeBlanc et al. 2008; Kijewski et al. 2009). Potential explanations for the observed deficit have been based on hypotheses of selection acting on the examined loci, inbreeding, null alleles, aneuploidy, and spatial and temporal Wahlund effects, among others (Zouros & Foltz 1984; Gaffney et al. 1990; Beaumont 1991; Plutchak et al. 2006; Silva & Skibinski 2009). However, to date, no consensus explanation has been reached (Wei et al. 2013).

[a]Author for correspondence.
E-mail: m.litvaitis@unh.edu

An as-yet untested dislodgement hypothesis (Myrand et al. 2009b) posits that heterozygous individuals are more motile, and move to the periphery of a long-line culture to gain a competitive feeding advantage (Fréchette et al. 1992, 1996). As a consequence, the more motile mussels are lost at a disproportionate rate because they are at greater risk for fall-off or predation (Lachance et al. 2008; Myrand et al. 2009b). Mussel fall-off is not trivial, as evidenced by studies on blue mussels in the Gulf of St. Lawrence (Lachance et al. 2011) and on greenshell mussels *(Perna canaliculis* Gmelin 1791) in New Zealand (Inglis & Gust 2003). Although amounts vary considerably over time and location, fall-off does not affect individuals randomly. If heterozygous mussels are indeed more motile and thus are more likely to move to the periphery of long-lines, then their differential loss will affect heterozygosity of the suspension culture. Such findings can have a negative impact on mussel yield because remaining homozygous individuals are characterized by lower growth and fecundity (Diehl & Koehn 1985; Diehl et al. 1986; Rodhouse et al. 1986). Furthermore, in light of changing water temperatures, long-term survival is compromised because homozygous individuals are less tolerant of stressful conditions (Tremblay et al. 1998; Myrand et al. 2002; LeBlanc et al. 2008).

	I V B	12183	Wiley	Dispatch: 27.5.17	CE: Stella
	Journal Code	Manuscript No.		No. of pages: 8	PE: Kamatchi E.

A similar dislodgement hypothesis has been invoked for wild mussels in intertidal mussel beds, resulting in selection against heterozygotes. Suspected silt-avoidance behavior and increased access to food lead to the vertical stratification of a mussel bed, with heterozygous mussels moving toward the surface of the bed (Schneider et al. 2005). Consequently, there is a trade-off between increased food availability, which may result in faster growth, and increased risk to predation or to dislodgement by wave action, current velocity, or turbulence (O'Donnell 2008; de Jager et al. 2011) for individuals positioned at the periphery of any mussel aggregation (e.g., mussel bed and rope culture).

To prevent dislodgement, mussels secrete numerous, collagenous byssal threads, each anchoring the mussel to the substrate via a small adhesive plaque at the distal end of the thread (Brown 1952). Byssal threads are not permanent structures, though; they are continuously reformed (Lee et al. 1990; Wiegemann 2005), allowing individuals to spatially reorganize within a mussel aggregate. When byssal threads are severed, the adhesive plaques remain on the substrate and provide evidence for mussel movement (Wiegemann 2005). In some instances, entire byssal bundles are abandoned which have been shown to provide an effective estimate of mussel movement (Ishida & Iwasaki 2003; Garner & Litvaitis 2013a, 2016). Various biotic and abiotic factors have been shown to affect the number and thickness of byssal threads produced and thus attachment strength (Young 1985; Hunt & Scheibling 2001, 2002; Carrington 2002; Moeser et al. 2006; Lachance et al. 2008; Garner & Litvaitis 2013a,b).

Because dislodgement is dependent on byssal thread attachment strength (Bell & Gosline 1997), identifying a relationship between genetic diversity and byssal thread numbers and attachment strength may provide insights into the heterozygote deficit observed in blue mussel populations. A direct comparison of motility and byssal thread attachment strength among homozygous and heterozygous individuals will help assess the validity of the dislodgement hypothesis. Traditionally, mytilid heterozygosity has been determined using multiple allozyme loci, but these loci clearly are under selection and thus can affect measures of genetic diversity (Karl & Avise 1992; Riginios et al. 2002). Neutral markers on the other hand have no effect on fitness. Microsatellites are commonly used neutral markers, and although some heterozygote deficiencies have been found for microsatellites too, they can be attributed to null alleles and genotyping errors rather than to selection acting specifically on the loci (Diz & Presa 2008; Lallias et al. 2009).

The overall goal of our study was to determine whether heterozygous mussels also showed increased motility. In support of the dislodgement hypothesis, we expected a positive correlation between heterozygosity and motility, as well as a negative relationship between heterozygosity and byssal thread attachment strength. Using microsatellites, our specific objectives were to: a) determine the genetic diversity of a wild and a farmed population of blue mussels, b) determine whether a heterozygosity deficit exists in either or both populations, c) evaluate the relationship between heterozygosity and motility, and d) compare heterozygosity with byssogenesis and byssal thread attachment strength.

Methods

Sample collection

Wild *Mytilus edulis* (300 individuals; shell length 44.4–61.6 mm, mean 52.41±3.51 mm SD) were collected from a series of floating docks at the Wentworth by the Sea Marina, located on the northwestern shore of Little Harbor in New Castle, New Hampshire (43.057°N, 70.726°W). Farmed *M. edulis* (150 individuals; length 30.0–47.6 mm, mean 36.1±3.47 mm SD) were collected from the surface of a single line of a grow-out culture located beneath the UNH Marine Research Pier at the Judd Gregg Marine Science Center on the northern shore of Fort Constitution Point in New Castle, New Hampshire (43.072°N, 70.712°W). Wild spat was used to seed the long-line cultures. Prior to any testing, all existing byssal threads were trimmed and morphometric measurements of shell length, width, and height (mm) were recorded using a digital caliper (General Tools and Instruments, New York, NY, USA; precision ±0.01 mm). It was assumed that growth throughout the study period (June–September 2015) was negligible; hence, morphometric measurements were only recorded once.

To assure comparable selective pressures between wild-collected and long-line farmed mussels, the wild individuals were collected from a series of floating docks rather than an intertidal, benthic mussel bed. However, this choice severely limited our potential collecting sites and hence, the ability to obtain equivalent size classes between the two populations. As a consequence, size was relatively uniform within each population, but larger in wild-collected individuals.

Motility determination

Motility of 274 wild and 150 farmed blue mussels was quantified as the number of abandoned plaques over 132 h. Although movement can be inferred

from the number of abandoned byssal bundles (Ishida & Iwasaki 2003; Garner & Litvaitis 2013a, 2016), mussels in our setups preferentially abandoned plaques rather than byssal bundles. Consequently, we used the number of abandoned plaques as a proxy for mussel movement. To this end, individual mussels were maintained in labeled 10 cm diameter glass dishes that were covered with mesh netting to prevent escape. Dishes were submerged randomly in a shallow seawater flow-through table to provide food and maintain uniform temperature for 132 h. Ambient temperature over the duration of the trials ranged from 10.4°C (June 2015) to 15.4°C (September 2015). After 132 h, the number of attached (functional) byssal threads and abandoned plaques were recorded for each individual. Total byssal thread production (byssogenesis) was calculated as the sum of functional threads and abandoned plaques for each individual over 132 h.

Byssal thread attachment strength determination

Byssal thread attachment strength was determined by measuring the force required to dislodge each mussel from its substrate after it had been allowed to secrete byssal threads for 132 h. To this end, a monofilament line knotted into a two-loop pattern was used, in which one loop was secured around each shell end and the central knot was attached to a dual-range force sensor (Vernier Software and Technology, Beaverton, Oregon, USA). Steady normal force was applied until failure of all attached byssal threads. Total maximum force of thread failure was recorded in newtons (N). All force readings were visualized on LoggerLite software (Vernier Software and Technology). Force per byssal thread was calculated by dividing the total force required for dislodgement by the number of functional byssal threads. Mussels (130 wild; 15 farmed) were excluded from further analysis if they escaped from the culture dishes (despite mesh netting secured over the dishes), attached to neighboring dishes, released their byssal threads before attachment strength testing, or if the force sensor failed to record.

Genomic DNA extraction and heterozygosity determination

Adductor muscles of 144 wild and 135 farmed mussels were severed with a clam-shucking knife, and gill and mantle tissue was dissected using a scalpel. Other tissues were not extracted to reduce the possibility of contamination from stomach contents. Tissue samples were stored at −20°C until DNA extraction. Genomic DNA was extracted following the DNeasy Blood and Tissue Kit protocol (Qiagen Inc., Valencia, CA, USA), and was stored at −20°C.

Thirteen microsatellite loci (Presa et al. 2002; Gardeström et al. 2008; Lallias et al. 2009; Ye et al. 2014) had been selected to assess heterozygosity. However, 10 loci either could not be amplified at all or resulted in products in only a small subset of samples. Thus, they were excluded from further analysis. Consequently, heterozygosity determination was based on three loci (two *M. edulis*-specific loci [Med367, Med397] from Lallias et al. 2009; one locus [Mgμ3] derived from *Mytilus galloprovincialis* Lamarck 1819 from Presa et al. 2002), which amplified successfully (total wild mussels genotyped=110; total farmed mussels genotyped=63). Genomic DNA dilutions of 1:50 were used in 12.5 μL PCRs for Med367 and Mgμ3; Med397 was amplified from undiluted DNA because of low template concentrations. PCR and cycling conditions followed the Type-It optimized cycling protocol for multiplex amplification of microsatellites (Qiagen Inc.). A negative control for Genome Analysis (Yale School of Medicine, Orange, CT, USA) for fragment analysis and alleles were scored using Geneious vers. 6.8.1 (Kearse et al. 2012). Microchecker vers. 2.2.3 was used to determine the probability of null alleles (van Oosterhout et al. 2004). For all three loci, 10% of samples were re-typed to ensure sample-reading consistency.

Observed and expected heterozygosities and an exact probability test for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were calculated using the web-interface version of GenePop vers. 4.2 (Raymond & Rousset 1995; Rousset 2008). For correlative analyses, individual mussels with ≥1 heterozygous locus were considered heterozygotes.

Statistical analyses

All analyses were run in JMP Pro vers. 12 (SAS Institute Inc 1989-2010). Shapiro–Wilk tests were used to determine whether variable frequencies (i.e., byssogenesis, functional threads, plaque abandonment, and total attachment strength) were normally distributed. Non-parametric Wilcoxon/Kruskal–Wallis rank sum tests were used to compare different measures of byssal thread attachment strength and byssogenesis with heterozygosity, as a consequence of non-normal distribution of all variables.

Results

Genetic diversity of mussels

Observed and expected heterozygosities were significantly different at the three loci. An exact probability test revealed significant deviations from Hardy–Weinberg equilibrium ($p<0.0001$) for two or three loci in farmed versus wild mussels, respectively (Table 1). Although all three loci were polymorphic, the two loci developed for *Mytilus edulis* (Med367, Med397) revealed much larger numbers of alleles (N_A) than the one derived from *M. galloprovincialis* (Mgμ3). Similarly, gene diversity (H_E) also was high for these same loci. There was no evidence of linkage disequilibrium for the three loci. Null alleles (F_{Null}) were found at all three loci in wild mussels and at two loci in mussels from the farmed population (Table 1). Even though null alleles generally are attributed to primer mismatch, the two *M. edulis*-specific loci had a higher frequency of null alleles than the locus designed for *M. galloprovincialis*. Presence of null alleles was based on observed homozygote excess (van Oosterhout et al. 2004); there was no evidence of scoring error due to stuttering. Relatively high inbreeding coefficients (F_{IS}) were found at all three loci for both populations (Table 1).

Motility, byssogenesis, and byssal thread attachment strength

Heterozygosity did not explain differences in plaque abandonment, and hence motility of either wild or farmed mussels (Fig. 1, Table 2). In both wild-collected and farmed mussels, heterozygosity also did not significantly affect byssogenesis, number of functional threads, or individual or total byssal thread attachment strength (Table 2).

Table 1. Gene diversity of 110 wild and 63 farmed blue mussels from New Hampshire. N_A, mean number of observed alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, Weir and Cockerham inbreeding coefficient; p-values for exact tests of HWE fit; F_{Null}, frequency of null alleles.

Locus	N_A	H_O	H_E	F_{IS}	p	F_{Null}
Wild mussels						
Med367	18	0.473	0.826	0.429	***	0.213
Med397	46	0.245	0.975	0.300	***	0.372
Mgμ3	5	0.109	0.156	0.749	***	0.096
Farmed mussels						
Med367	18	0.823	0.898	0.085	***	0.036
Med397	32	0.387	0.962	0.035	***	0.295
Mgμ3	6	0.274	0.265	0.600	0.271	−0.022

***Significant deviation from Hardy–Weinberg equilibrium ($p<0.0001$).

Discussion

In this study, we tested the dislodgement (drop-off) hypothesis (Schneider et al. 2005; Lachance et al. 2008; Myrand et al. 2009b), which postulates

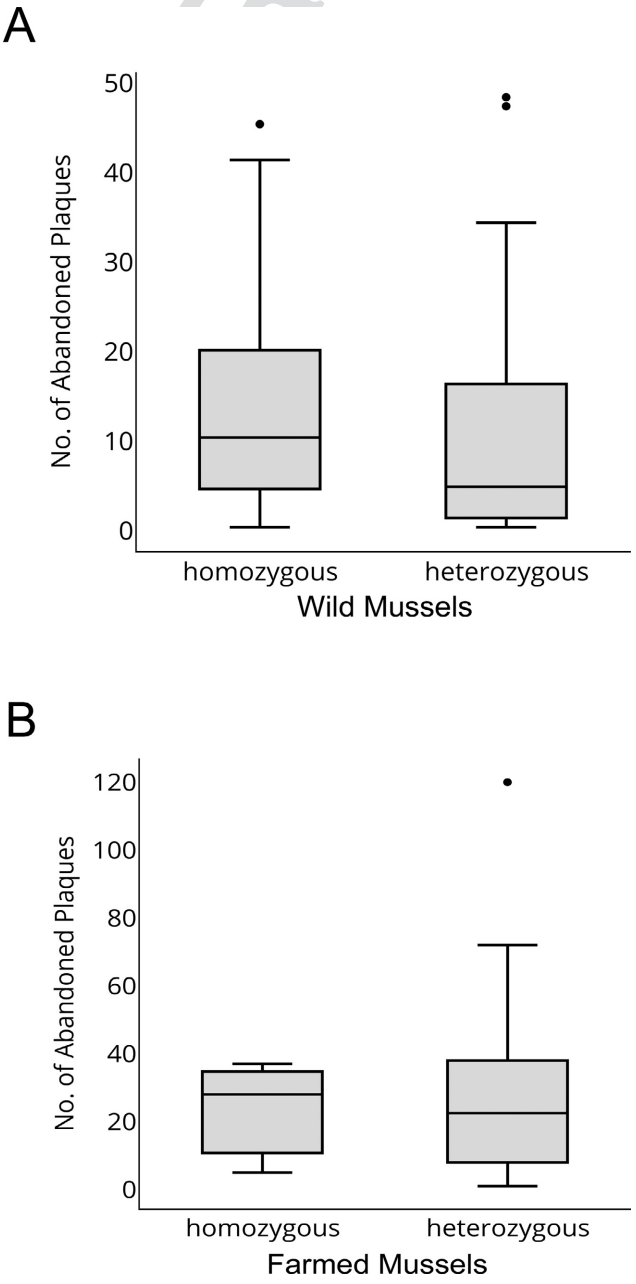

Fig. 1. Effects of heterozygosity on plaque abandonment in wild (**A**) and farmed (**B**) mussels.

Table 2. Results of Wilcoxon/Kruskal–Wallis tests (rank sums) for one-way test (chi-squared approximation) of association between heterozygosity and five response variables including motility (as measured by plaque abandonment), byssogenesis, or byssal thread attachment strength in wild and farmed blue mussels from New Hampshire.

	x^2	p
Wild mussels		
No. of abandoned plaques	2.8930	0.089
Byssogenesis	0.2683	0.605
No. of functional threads	2.6030	0.107
Individual thread strength (*N*)	0.1044	0.747
Total attachment strength (*N*)	1.1881	0.276
Farmed mussels		
No. of abandoned plaques	0.0051	0.943
Byssogenesis	1.4358	0.231
No. of functional threads	2.4758	0.116
Individual thread strength (*N*)	0.5111	0.475
Total attachment strength (*N*)	1.2274	0.268

the differential loss of heterozygous individuals that increasingly move to the periphery of mussel aggregations (mussel beds and long-line cultures), where they are at greater risk of dislodgement. To this end, we determined correlations between mussel motility and heterozygosity, and to avoid bias of genetic diversity estimates introduced by loci under natural selection, we employed a set of neutral markers to determine heterozygosity. We expected a positive correlation between heterozygosity and motility, and a negative relationship between heterozygosity and attachment strength. Because we found no relationship between heterozygosity and other variables (increased motility, number of abandoned plaques, byssogenesis, or byssal thread attachment strength) in either mussel population, we conclude that differential dislodgement is highly unlikely as a possible contributor to the loss of heterozygous individuals. Based on the observation that heterozygous individuals have lower maintenance metabolism (Myrand et al. 2002; LeBlanc et al. 2008) and, thus, potentially could reallocate energy resources into other functions, Lachance et al. (2011) attempted to link increased byssogenesis to mussel energy reserves and heterozygosity. However, they too found no correlation between heterozygosity and byssal thread numbers.

Despite using microsatellites to assess heterozygosity, we found that the two mussel populations in our study also deviated from Hardy–Weinberg expectations, revealing significant heterozygote deficiencies, except at one locus in the farmed population. Generally, selection on microsatellites is considered non-existent (Ellgren 2004). However, molluscs are known for an unusually high frequency of null alleles (Presa et al. 2002; Hedgecock et al. 2004; Lallias et al. 2009). Null alleles are the result of primer mismatch, with subsequent non-amplification of an allele. Not surprisingly then, N_A of *Mytilus edulis*-specific loci (Med367, Med 397) was greater than that of the locus developed for *M. galloprovincialis*. Primer mismatch not only contributes to low cross-species amplification but it may also result in low intraspecific and even intra-population amplification as was evident in our study from frequently failed PCRs using three *M. edulis*-specific primers. As a consequence, genetic diversity is underestimated. The mismatch can be attributed to a high rate of molecular evolution. In fact, Zhang & Guo (2010) estimated one single nucleotide polymorphism (SNP) for every 20 base pairs in the oyster *Crassostrea virginica* Gmelin 1791. High substitution rates in turn, can be due to high fecundity, a characteristic of bivalve reproduction.

Both population samples revealed large positive F_{IS} values at all three loci. This is best explained by the reproductive mode of blue mussels. Positive F_{IS} values are commonly observed in marine, broadcast-spawning species, like blue mussels, despite the assumption of open populations (Addison & Hart 2005). Such species are characterized by very high fecundity and also very high early mortality (Type III survivorship), leading to a sweepstakes-like probability of reproductive success, known as the Hedgecock effect (Waples 1998; Hedrick 2005). This variance is due to the fact that within any generation, only a few individuals are actually breeding successfully (i.e., high variance in family size). Variance in reproductive success can significantly affect effective population size (N_e), resulting in a substantial reduction of the N_e/N ratio (Hedgecock 1994; Hedrick 2005). Thus, a large population of blue mussels with highly stochastic reproductive success will ultimately have a very low effective population, in which genetic drift can reduce genetic variation considerably. It is likely, then, that a combination of rapid evolutionary rates resulting in a high frequency of null alleles, and a greatly reduced effective population size are responsible for the observed heterozygote deficiencies in blue mussels. Our findings of high frequencies of null alleles and high F_{IS} values provide support for such explanations.

Our findings have implications for the shellfish industry. First, although mussel drop-off does affect overall yield, we conclude that peripheral dislodgement does not contribute to differential loss of

heterozygotes. Such selection appears to occur at a much earlier life history stage (i.e., zygote and larva). Second, current sleeving techniques used to prevent loss while mussels are young and more motile are highly appropriate, because they allow mussels to knit themselves to the culture ropes more securely until they reach the size class at which byssogenesis and attachment strength are maximized. Finally, surface water temperatures in the Gulf of Maine are increasing faster than in any other oceans (Pershing et al. 2015). As previously noted, increased heterozygosity confers increased survival during elevated temperatures in blue mussels (Tremblay et al. 1998; Myrand et al. 2002; LeBlanc et al. 2008). Hence, the loss of heterozygous individuals, regardless of the stage at which it occurs, may be of concern under future climate predictions. We recommend genotyping shellfish stock to ensure that ropes are seeded with genetically diverse spat.

Acknowledgments. We thank Dr. Michael Chambers (UNH, Cooperative Extension) for supplying us with farmed mussels and Nate Rennels for providing facilities at the Judd Gregg Marine Science Center. We also gratefully acknowledge Wentworth by the Sea (Marriott International, Inc.) for allowing access to their marina. We thank Dr. Rich Smith (UNH) for help with statistical analyses, Sarah Clements (UNH) for help with genotyping, and Dr. Yvette Garner (University of West Georgia) for technical support and constructive input. Partial funding was provided by the New Hampshire Agricultural Experiment Station. This is Scientific Contribution Number 2726. This work was supported by the USDA National Institute of Food and Agriculture Hatch Project 222760.

References

Addison JA & Hart MW 2005. Spawning, copulation and inbreeding coefficients in marine invertebrates. Biol. Lett. 1: 450–453.

Addison JA, Ort BS, Mesa KA, & Pogson GH 2008. Range-wide genetic homogeneity in the California sea mussel (*Mytilus californianus):* a comparison of allozyme, nuclear DNA markers, and mitochondrial DNA sequences. Mol. Ecol. 17: 4222–4232.

Beaumont A 1991. Genetic studies of laboratory reared mussels, *Mytilus edulis:* heterozygote deficiencies, heterozygosity and growth. Biol. J. Linn. Soc. 44: 273–285.

Bell EC & Gosline JM 1997. Strategies for life in flow: tenacity, morphometry, and probability of dislodgment of two *Mytilus* species. Mar. Ecol. Prog. Ser. 159: 197–208.

Brown C 1952. Some structural proteins of *Mytilus edulis*. Quart. J. Microsc. Sci. 3: 487–502.

Carissan-Lloyd FMM, Pipe RK, & Beaumont AR 2004. Immunocompetence and heterozygosity in the mussel *Mytilus edulis*. J. Mar. Biol. Assoc. U.K. 84: 377–382.

Carrington E 2002. Seasonal variation in the attachment strength of blue mussels: causes and consequences. Limnol. Oceanogr. 47: 1723–1733.

Diehl WJ & Koehn RK 1985. Multiple-locus heterozygosity, mortality, and growth in a cohort of *Mytilus edulis*. Mar. Biol. 88: 265–271.

Diehl WJ, Gaffney PM, & Koehn RK 1986. Physiological and genetic aspects of growth in the mussel *Mytilus edulis*. I. Oxygen consumption, growth, and weight loss. Physiol. Zool. 59: 210–211.

Diz AP & Presa P 2008. Regional patterns of microsatellite variation in *Mytilus galloprovincialis* from the Iberian Peninsula. Mar. Biol. 154: 277–286.

Ellgren H 2004. Microsatellites: simple sequences with complex evolution. Nature Rev. Genetics 5: 435–445.

Fréchette M, Aitken AE, & Page L 1992. Interdependence for food and space limitation of a benthic suspension feeder using self-thinning relationships. Mar. Ecol. Prog. Ser. 65: 15–23.

Fréchette M, Bergeron P, & Gagnon P 1996. On the use of self-thinning relationships in stocking experiments. Aquaculture 145: 91–112.

Gaffney PM, Scott TM, Koehn RK, & Diehl WJ 1990. Interrelationships of heterozygosity, growth rate and heterozygote deficiencies in the coot clam, *Mulinia lateralis*. Genetics 124: 687–699.

Gardeström J, Pereyra RT, & André C 2008. Characterization of six microsatellite loci in the Baltic blue mussel *Mytilus trossulus* and cross-species amplification in North Sea *Mytilus edulis*. Conserv. Genet. 9: 1003–1005.

Garner YL & Litvaitis MK 2013a. Effects of conspecifics and predators on byssogenesis, attachment strength and movement in the blue mussel, *Mytilus edulis*. J. Exp. Mar. Biol. Ecol. 448: 136–140.

——— 2013b. Effects of wave exposure, temperature and epibiont fouling on byssal thread production and growth in the blue mussel, *Mytilus edulis,* in the Gulf of Maine. J. Exp. Mar. Biol. Ecol. 446: 52–56.

——— 2016. Effects of artificial epibionts on byssogenesis, attachment strength, and movement in two size classes of the blue mussel, *Mytilus edulis*. Invert. Biol. ???: ???–???. https://doi.org/10.1111/ivb.12144.

Hedgecock D 1994. Does variance in reproductive success limit effective population size of marine organisms? In: Genetics and Evolution of Aquatic Organisms. Beaumont A, ed., pp. 122–134. Chapman and Hall, London.

Hedgecock D, Li G, Hubert S, Bucklin K, & Ribes V 2004. Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. J. Shellfish Res. 23: 379–385.

Hedrick P 2005. Large variance in reproductive success and the N_e/N ratio. Evolution 59: 1596–1599.

Hunt HL & Scheibling RE 2001. Predicting wave dislodgement of mussels: variation in attachment strength with body size, habitat and season. Mar. Ecol. Prog. Ser. 213: 157–164.

——— 2002. Movement and wave dislodgment of mussels on a wave-exposed rocky shore. Veliger 45: 273–277.

Inglis GJ & Gust N 2003. Potential indirect effects of shellfish culture on the reproductive success of benthic predators. J. Anim. Ecol. 40: 1077–1089.

Ishida S & Iwasaki K 2003. Reduced byssal thread production and movement by the intertidal mussel *Hormomya mutabilis* in response to effluent from predators. J. Ethol. 21: 117–122.

de Jager M, Weissing FJ, Herman PM, Nolet BA, & van de Koppel J 2011. Lévy walks evolve through interaction between movement and environmental complexity. Science 332: 1551–1553.

Karl SA & Avise JC 1992. Balancing selection at allozyme loci in the oyster: implications from nuclear RFLPs. Science 256: 100–102.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, & Drummond A 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649.

Kijewski T, Wijsman JWM, Hummel H, & Wenne R 2009. Genetic composition of cultured and wild mussels *Mytilus* from The Netherlands and transfers from Ireland and Great Britain. Aquaculture 287: 292–296.

Lachance AA, Myrand B, Tremblay R, Koutitonsky V, & Carringon E 2008. Biotic and abiotic factors influencing attachment strength of blue mussels *Mytilus edulis* in suspension culture. Aquatic Biol. 2: 119–129.

Lachance AA, Hennebicq RH, Myrand B, Sévigny J-M, Kraffe E, Marty Y, Marcotte I, & Tremblay R 2011. Biochemical and genetic characteristics of suspension-cultured mussels *(Mytilus edulis)* in relation to byssal thread production and losses by fall-off. Aquat. Living Res. 24: 283–293.

Lallias D, Stockdale R, Boudry P, LaPègue S, & Beaumont AR 2009. Characterization of ten microsatellite loci in the blue mussel *Mytilus edulis*. J. Shellfish Res. 28: 547–551.

LeBlanc N, Tremblay R, Davidson J, Landry T, & McNiven M 2008. The effect of selection treatments on *Mytilus edulis,* modifications of genetic and physiological characteristics. Mar. Biol. 153: 1141–1152.

Lee CY, Lim SSL, & Owen MD 1990. The rate and strength of byssal reattachment by blue mussels *(Mytilus edulis* L.). Can. J. Zool. 68: 2005–2009.

Moeser GM, Leba H, & Carrington E 2006. Seasonal influence of wave action on thread production in *Mytilus edulis*. J. Exp. Biol. 209: 881–890.

Myrand B, Tremblay R, & Sévigny J-M 2002. Selection against blue mussels *(Mytilus edulis* L.*)* homozygotes under various stressful conditions. J. Heredity 93: 238–248.

——— 2009a. Impact of suspension culture using mesh sleeves on genetic characteristics of *Mytilus edulis* L. in Canada. Aquaculture 291: 147–153.

——— 2009b. Decrease in multi-locus heterozygosity in suspension-cultured mussels *(Mytilus edulis)* through loss of the more heterozygous individuals. Aquaculture 295: 188–194.

O'Donnell MJ 2008. Reduction of wave forces within bare patches in mussel beds. Mar. Ecol. Prog. Ser. 362: 157–167.

van Oosterhout C, Hutchinson WF, Wills WPM, & Shipley P 2004. MicroChecker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4: 535–538.

Pershing AJ, Alexander MA, Hernandez CM, Kerr LA, Le Bris A, Mills KE, Nye JA, Record NR, Scannell HA, Scott JD, Sherwood GD, & Thomas AC 2015. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. Science 350: 809–812.

Plutchak LL, Simmons RE, & Woodruff DS 2006. Multilocus allozyme heterozygote deficiencies in *Crepidula onyx:* geographic and temporal patterns among adult snails in Mission Bay, California. J. Mollusc. Stud. 72: 337–348.

Presa P, Pérez M, & Diz AP 2002. Polymorphic microsatellite markers for blue mussels (*Mytilus* spp.). Conserv. Genet. 3: 441–443.

Raymond M & Rousset F 1995. Genepop (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86: 248–249.

Riginios C, Sukhdeo K, & Cunningham CW 2002. Evidence for selection at multiple allozyme loci across a mussel hybrid zone. Mol. Biol. Evol. 19: 347–351.

Rodhouse PG, McDonald JH, Newell RIE, & Koehn RK 1986. Gamete production, somatic growth and multiple-locus enzyme heterozygosity in *Mytilus edulis*. Mar. Biol. 90: 209–214.

Rousset F 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Res. 8: 103–106.

SAS Institute Inc 1989-2010. Using JMP Pro Version 12. SAS Institute, Cary, North Carolina, USA.

Schneider KR, Wethy DS, Helmuth BST, & Hilbish TJ 2005. Implications of movement behavior on mussel dislodgement: exogenous selection in a *Mytilus* spp. hybrid zone. Mar. Biol. 146: 333–343.

Silva EP & Skibinski DOF 2009. Allozymes and nDNA markers show different levels of population differentiation in the mussel *Mytilus edulis* on British coasts. Hydrobiologia 620: 25–33.

Tremblay R, Myrand B, Sevigny J-M, Blier P, & Guderley H 1998. Bioenergetic and genetic parameters in relation to susceptibility of blue mussels, *Mytilus edulis* (L.) to summer mortality. J. Exp. Mar. Biol. Ecol. 221: 27–58.

Waples RS 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J. Hered. 89: 438–450.

Wei K, Wood AR, & Gardner JPA 2013. Population genetic variation in the New Zealand greenshell mussel: locus-dependent conflicting signals of weak structure and high gene flow balanced against pronounced structure and high self-recruitment. Mar. Biol. 160: 931–949.

Wiegemann M 2005. Adhesion in blue mussels *(Mytilus edulis)* and barnacles (genus *Balanus*): mechanisms and technical applications. Aquat. Sci. 67: 166–176.

Ye Y, Wu C, Guo B, Zhu A, & Lv Z 2014. Isolation and characterization of ten polymorphic microsatellite markers for the blue mussel *(Mytilus edulis)*. Biochem. Syst. Ecol. 54: 5–7.

Young GA 1985. Byssus-thread formation by the mussel, *Mytilus edulis:* effects of environmental factors. Mar. Ecol. Prog. Ser. 24: 261–271.

Zhang LS & Guo XM 2010. Development and validation of single nucleotide polymorphism markers in the eastern oyster *Crassostrea virginica* Gmelin by mining ESTs and resequencing. Aquaculture 302: 124–129.

Zouros E & Foltz DW 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia 25: 583–591.