Argopecten nucleus is a pectinid species endemic of the Caribbean Sea that forms very sparse populations in nature, to the extent that no natural beds have been described in literature (Díaz & Puyana, 1994; Lodeiros, Freites, Núñez, & Himmelman, 1993). Therefore, the only source of wild live specimens to date has been the obtention of seeds using artificial collectors suspended in the sea (Castellanos & Campos, 2007; Lodeiros et al., 1993). This species inhabits seagrass beds and sand bottoms, particularly those located in coral
Argopecten nucleus is a species with a short life span (1–2 years), characterized by early sexual maturity (at 3 months) (Lodeiros & Freites, 2008; Velasco & Barros, 2008) and spawning activity occurring throughout the year (Lodeiros et al., 1993; Velasco, Barros, & Acosta, 2007). This species is also a simultaneous hermaphrodite with external fertilization that is able to release gametes of one sex first, usually male, before releasing other sex gametes, with a time lapse of approximately 15 min between them (Velasco et al., 2007).

Studies on population genetic structure of marine species attract considerable interest, since they allow us to assess the state of conservation of natural populations (Piñero, Caballero-Mellado, Cabrera-Toledo, & Zuñiga, 2008; Smith, 1996), to adopt proper management practices in their fisheries, and to develop aquaculture strategies to reduce the risk of inbreeding depression and fitness loss (Gjedrem & Baranski, 2009), thus contributing to sustainable production over time (Petersen, Baerwald, Ibarra, & May, 2012; Wang, Fu, & Xia, 2013). To date, there is no information about the genetic structure of wild populations of A. nucleus, but the low population densities usually found for this species suggest the existence of high levels of genetic drift and inbreeding, as previously reported for other mollusks such as Haliotis iris (Smith & Conroy, 1992). This situation could be further exacerbated due to the effects of spontaneous self-fertilization, which has been estimated at 12% ± 1% on average for reproduction in hatchery (Barros, Velasco, & Winkler, 2018; Barros, Winkler, & Velasco, 2018). In Argopecten irradians irradians, it has been estimated that self-fertilization might be able to reduce the genetic diversity of the population in 10%–40% in just one generation (Zheng, Zhang, Guo, & Liu, 2008). However, although similar or even higher self-fertilization rates have been observed in A. purpuratus (Winkler & Estévez, 2003), no significant deviations from the Hardy–Weinberg (H-W) equilibrium for allozyme loci have been observed in wild populations, and when present, deviations only affect some loci, but not all of them (von Brand & Kijima, 1990; Galleguillos & Troncoso, 1991; Moraga et al., 2001).

The use of selectively neutral genetic markers allows to estimate genetic diversity and obtain information about the genetic structure of populations, which can be useful to infer the occurrence of phenomena such as genetic drift and inbreeding (Blouin, 2003; Qin, Liu, Zhang, Zhang, & Guo, 2007; Wu, Feng, Ma, Pan, & Liu, 2015). Furthermore, they are valuable to understand the factors regulating population dynamics (Bert et al., 2014). Among these markers, microsatellites (SSR, Simple Sequence Repeats) are particularly convenient because they usually display Mendelian inheritance and codominance. SSRs are short DNA sequences of 2–6 base pairs that can repeat in tandem a variable number of times in the genome and are frequently flanked by sequences with unique copies in the genome, which make possible to design specific primers and to amplify them using polymerase chain reaction (PCR; Liu & Cordes, 2004; Tagu & Moussard, 2003). They exhibit high polymorphism and allele number per locus, and since they are noncoding sequences, their variations usually are not affected by natural selection (Liu & Cordes, 2004). These markers have been very useful for paternity analysis in bivalves (Taris, Baron, Sharbel, Sauvage, & Boudry, 2005), for the assessment the diversity and population genetic structure (Wang et al., 2013), and for studies of linkage and genetic maps building (Petersen et al., 2012; Qin et al., 2007).

Although most microsatellite loci show random recombination, deviations from Mendelian proportions have been reported in experimental crosses for different species associated with the presence of null alleles (Baranski, Rourke, Loughnan, Austin, & Robinson, 2006; De Meeûs, 2018; Hedgecock, Li, Hubert, Bucklin, & Ribes, 2004; Li, Park, Kobayashi, & Klima, 2003; Zhan et al., 2007). Null alleles appear when a mutation affects one of the union sites of the primers with the flanking zone of the microsatellite and impedes that it amplifies, and heterozygotes for them are then genotyped erroneously as homozygotes (Lewin, 2004). The existence of null alleles can lead to errors in the estimation of allele frequencies, thus overestimating homozygosis (Becquet, Lanneluc, Bouhet, & Garcia, 2009; Pereira, Arias, Méndez, Insua, & Freire, 2009; Weetman, Hauser, Shaw, & Bayes, 2005) and the inbreeding levels of a population (Jones, Stockwell, Walker, & Avise, 1998; Xu et al., 2001). It also affects the reliability of the use of microsatellite markers for other applications, such as paternity testing, estimation of the degree of relatedness between individuals, and decisions about species management, among others (Ardren, Borer, Thrower, Joyce, & Kapuscinski, 1999).

In order to correct the bias that the presence of null alleles can introduce in population genetic analysis, their frequency can be inferred using different theoretical models (Jones et al., 1998; van Oosterhout, Weetman, & Hutchison, 2006; Xu et al., 2001). For such purposes, statistical packages based on maximum likelihood algorithms use the differences between the observed and the expected heterozygosity in a sample to estimate their frequencies (Brookfield, 1996; Chakraborty, de Andrade, Daiger, & Budowle, 1992; van Oosterhout, Hutchison, Wills, & Shipley, 2004). Software packages such as Micro-checker indicate the presence of null alleles if the combined probability of Fisher test shows a significant, general excess of homozygotes that are evenly distributed between the homozygotes classes (van Oosterhout et al., 2004). The presence of null alleles has been observed in different aquatic species, such as the bivalves Donax trunculus (Rico et al., 2017), Pinna nobilis (González-Wangüemert et al., 2015), Pinctada margaritifera (Lemer, Rochel, & Planes, 2011) and Crassostrea gigas (Hedgecock et al., 2004), and the flatfish Scophthalmus maximus (Borrell et al., 2004), among others. These estimations, however, do not allow to correct the index of genetic diversity, and the precision and confidence interval of the estimations can be very variable depending on the real frequency of the null alleles and other factors such as the sample size, the number of sampled generations, the level and duration of the genetic bottlenecks of the population, nonrandom mating, changes of density or migration.
rates (Dabrowski et al., 2014). In addition, the frequencies of null alleles could be overestimated due to the occurrence of inbreeding (Chybicki & Burczyk, 2009).

An alternative method to estimate the actual frequency of null alleles is the use of segregation analysis, a method that allows to verify the presence of recessive alleles in the genotype of individuals with dominant phenotype, based on data from families (Elston, 1981). Under an appropriated crossing design, it can be used to verify the genotype of a putatively homozygous individual for a dominant gene, based on the genotypes of their progenies, and the expected frequencies with respect to the corresponding Mendelian proportions (Zhan et al., 2007). This kind of analysis has been used to test the presence of null alleles in the Pacific oyster (Crassostrea virginica; Reece, Ribeiro, Gaffney, Carnegie, & Allen, 2004), rainbow trout (Oncorhynchus mykiss; Holm, Loeschcke, & Bendixen, 2001), the Coleoptera Pissodes strobi (Liewlaksaneeyananwim, Ritland, & El-Kassaby, 2002), hop (Humulus lupulus; Vašek et al., 2010) and coffee (Coffea liberica; López, Rutherford, & Moncada, 2014), among other species. Therefore, the segregation analysis allows to correct the biases generated by the presence of null alleles on microsatellite markers, thus making the estimations of genetic diversity more reliable when such alleles are present in high frequencies in a population.

At present, there are no microsatellite markers described for A. nucleus, and hence, this tool is not currently available to study relevant genetic aspects of its populations. In addition, there is no information on the genetic diversity of this scallop species in natural populations. Therefore, the purpose of the present study is to identify and characterize the first set of primers for microsatellite loci in A. nucleus and use them to estimate the genetic diversity in a wild population from Bahía Neguanje, Santa Marta (Colombia), as well as to assess the effect of the presence of null alleles on the estimations of genetic diversity in a species with self-fertilization and very low population densities in the wild.

2 | MATERIALS AND METHODS

2.1 | Experimental design

To evaluate the levels of genetic diversity in A. nucleus, primers for 8 SSR loci were developed. At the same time, scallops from a wild population were collected and conditioned in captivity. They were later induced to spawn under controlled conditions, performing cross-fertilization following a nested design. Observed self-fertilization rates were registered during spawning process for each individual used as dam. The progeny of each full-sib family was cultured separately until they reach adulthood. Tissue samples were extracted from each parent and their progenies for the microsatellite loci genotyping. The genotype of each parent was confirmed after the segregation analysis of the SSR alleles in their progenies.

2.2 | Microsatellites obtaining

2.2.1 | Obtention of biological material and DNA

In order to develop and standardize the microsatellites, samples of approximately 0.1 cm³ were obtained from the adductor muscle of 10 adults of A. nucleus (43.6 ± 4.3 mm shell length), which were produced by cross-fertilization in the Laboratorio de Moluscos y Microalgas of Universidad del Magdalena, and cultured in suspended systems in Taganga Bay (Santa Marta) (11°16′03″N, 74°11′24″W). Tissue samples were preserved in ethanol 99% at room temperature until their analysis. DNA was extracted using the phenol-chloroform technique (Gardes & Bruns, 1993), until obtaining 20 µl with a concentration of 50 ng/µl of DNA. DNA integrity was confirmed using electrophoresis in agarose gel 1.2% and stained with SYBR Green. The amount of DNA was quantified in an Epoch equipment, and its purity was verified by the absorbance ratio 260/280 nm, using DNA samples with 1.8–2.0. The DNA was sent to the OMICS-Solutions company, which provided a genomic library enriched with 300 microsatellites (SSRs) and their respective flanking regions. The microsatellite reads were characterized based on the number of nucleotides of their motif, structure, and the number of repeats.

2.2.2 | Microsatellite standardization

From the library, 10 microsatellite loci were selected based on (a) the repetition motif composed of 3 no degenerate bases; (b) the expected products of the PCR amplification in a range between 150 and 350 base pairs (bp); and (c) the expected annealing temperature in a range between 55 and 60°C. Pairs of primers flanking microsatellite sequences (F and R) were designed using the Primer3web v4.1.0 software (Kõressaar et al., 2018) and tested. Microsatellite loci with clearly distinct amplification products and consistent amplification were selected for the study. The conditions of PCR amplification were standardized for the 10 loci, testing different annealing temperatures. An initial denaturation temperature of 95°C for 5 min was used, followed by 30 denaturation cycles at 94°C for 45 s, an annealing gradient (Ta) of 46 to 56°C for 30 s (Table 1), extension at 72°C for 45 s, and finally 5 min at 72°C (Orozco & Narváez, 2014). These reactions were performed in a gradient thermocycler ESCO-SWIF MaxPro, with a final volume of 10 µl of reaction mixture: 1.5 µl DNA with a concentration of 5 ng/µl, 2 µl reaction buffer (20 mM Tris-HCl; pH 8; 50 mM KCl, final), 0.3 µl MgCl₂ (1.5 mM), 0.2 µl dNTPs (0.2 mM), 0.2 µl of each primer (0.2 µM), and 1 µl Taq polymerase (0.5 U, Biolase©). PCR products were separated by capillary electrophoresis in a QIAxcel Advanced equipment, QIAGEN, using the High Resolution Kit QIAGEN and a ladder of 50–800 bp (v2.0 QIAGEN). These general conditions were used for PCR in the following amplifications, with adjustments of annealing temperature for each locus, as shown in Table 1.


### TABLE 1

Characteristics of the 8 microsatellite loci isolated in *Argopecten nucleus* from Neguanje Bay, Colombia

| Locus   | Repeated motif | Primers sequences Forward (F) and Reverse (R) | Ta (°C) | Size range (bp) | PIC  |
|---------|----------------|-----------------------------------------------|---------|-----------------|------|
| An001   | (ATA)$_n$      | F: ATTCCTATGTACGTCACTGC R: CATGAAAGTATACCTCTTACAG | 50.3    | 119–134         | 0.68 |
| An002   | (AAC)$_n$      | F: CTGTATGTAAAGACAGATG R: ACCGACTTAAGATGTATGTC  | 50.3    | 173–188         | 0.63 |
| An003   | (ATA)$_n$      | F: ATTCCTATGTACGTCACTGC R: CATGAAAGTACCTTCAACAG  | 50.3    | 119–131         | 0.69 |
| An004   | (ATC)$_n$      | F: GAATATTAACAGACAGAG R: CAACAGTACTTACATGTTCG   | 50.3    | 162–174         | 0.50 |
| An005   | (TAA)$_n$      | F: ATTCCTATGTACGTCACTGC R: CATGAAAGTACCTTCAACAG  | 50.3    | 172–190         | 0.70 |
| An006   | (ATA)$_n$      | F: CCAGTAGTTTCTGAAGAAAA R: CATGAAAGTACCTTCAACAG  | 52.3    | 128–149         | 0.75 |
| An007   | (ATG)$_n$      | F: ACATCTCTTGTACCCCTTACAC R: AGACTTTCAAGAGAACACTCTC | 50.3    | 125–134         | 0.64 |
| An008   | (TTG)$_n$      | F: TCTAACCTGTACCATATGC; R: GACAAGTACACGGTACCAC  | 51.0    | 153–159         | 0.44 |

Abbreviations: PIC, polymorphic information content; Ta, annealing temperature.

#### 2.3 Segregation analysis

From crosses of 48 wild scallops (41.2 ± 1.8 mm shell length) collected from Neguanje Bay, Santa Marta (Lat. 11°20′03″N, Long. 74°09′24″W), a total of 1,010 individuals were obtained from 38 full-sib and 10 half-sib families. Brooders were obtained as seed from artificial collectors suspended in the bay area, which were provided by the Instituto de Investigaciones Marinas y Costeras (INVEMAR, Santa Marta). Families were built as described by Barros, Velasco, et al. (2018); Barros, Winkler, et al. (2018); and Velasco et al. (2007). Crosses were performed using a nested design, according to which the sperm of one scallop used as male (sire) was used to separately fertilize the oocytes of 2–4 individuals (dams). Thus, each dam was crossed with only one male and no scallop was simultaneously used as mother and father. The self-fertilization rate was estimated as the frequency of nonfertilized oocytes that spontaneously initiate embryonic development within 4 hr after spawning in a sample (10 ml) from each individual used as female (Barros, Velasco, et al., 2018; Barros, Winkler, et al., 2018; Winkler & Estévez, 2003).

When the progenies were 9 months old, the scallops were removed from the culture system and samples of the mantle tissue (aprox. 0.05 cm$^3$) were collected (60 scallops per FS-family). Similar mantle tissue samples were obtained by biopsy from the 48 available brooders. Samples were individually fixed and preserved in ethanol 99% at room temperature until DNA extraction. DNA was extracted following the methods of Lopera-Barrero et al. (2008), replacing sodium chloride for ammonium acetate. DNA integrity was confirmed using agarose gel (0.8%) electrophoresis (80 V, 40 min) stained with GelRed, and visualized in a Biodoc UVP LLC transilluminator.

To estimate the genetic diversity of the population of Neguanje Bay, the genotype from each individual was assessed for the microsatellite loci previously standardized. The putative genotype of each parent was confirmed based on the genotypes observed in the offspring of each mating. The existence of full-sib and half-sib families allowed to confirm the genotype of each father in two or more crosses with different mothers, thus reducing the uncertainty in determining genotypes that can be introduced by the potential occurrence of self-fertilization. An individual was considered to be the result of self-fertilization when its genotype for 2 or more loci analyzed was only possible to arise if both alleles came from the individual used as mother, and the genotype for the remaining loci did not contradict this hypothesis. For this analysis, absence of self-fertilization in the fathers was assumed. Therefore, in crosses between two putative homozygotes for different alleles that progenies presented three genotypes, both parents were assumed to be heterozygous for a null allele, regardless of the genotypic proportions in the progeny of that cross.

#### 2.4 Data analysis

The size of each amplification product from individuals used as broodstock, and their progeny were determined with the software QIAGEN ScreenGel QIAxcel v1.0. The polymorphic information content (PIC) was estimated with the software CERVUS 3.0.7. For the wild brooders, the null allele frequencies were estimated using the models of van Oosterhout (Girard & Angers, 2008), Brookfield 1 (Brookfield, 1996), and Brookfield 2 (Girard & Angers, 2008) methods using the Micro-Checker 2.2.3 package. The allelic frequencies, as well as the number of alleles (Na), observed (Ho) and expected (He) according to the H-W equilibrium heterozygosities,
were similar to the range of frequencies obtained using the software Micro-Checker 2.2.3 and from the segregation analysis with similar standard deviations of allele frequencies using both methods (Table 2). The frequencies of null alleles obtained from the segregation analysis ranged from 0.10 to 0.41 (Table 2), and the estimations inferred using Micro-Checker 2.2.3 were, on average, 11.5% ± 9.8% higher than those estimated based on the segregation analysis (p < .05, Table 2). No significant correlation across loci was observed between both methods for the estimation of null allele frequencies (Figure 1) (r < .3114; p > .05). There were no statistical differences between null allele frequencies estimated using different models (van Oosterhout, Brookfield 1 and 2) (p > .05). The observed heterozygosity (H0) ranged from 0 and 0.79, depending on the locus and estimation method for allele frequencies, while the expected heterozygosity (He) ranged from 0.53 and 0.80, with higher values obtained based on the segregation analysis (p < .05, Table 3). The values of FIS ranged between −0.01 and 1.00, with lower values estimated based on the segregation analysis (p < .01, Table 3).

4 | DISCUSSION

The present study reports the first 8 microsatellite markers for A. nucleus, which were designed, standardized, and used to estimate the genetic diversity in a wild population of this species. This population exhibited relatively high values of genetic diversity, although a considerable excess of homozygotes with respect to Hardy–Weinberg equilibrium was observed. The segregation analysis shows that Micro-Checker 2.2.3 tends to overestimate the null allele frequencies in comparison with the segregation analysis, thus generating a bias that was not consistent across loci.

The intrapopulation genetic diversity of wild organisms for neutral loci, such as microsatellites, is mainly driven by the counteracting effects of genetic drift and inbreeding, which tend to reduce it, and the effects of mutation and migration, which are able to increase it (Gjedrem & Baranski, 2009). The wild population of A. nucleus from Neguanje Bay exhibited high levels of genetic diversity, in terms of polymorphism and number of alleles per locus. These results are similar to those encountered in wild populations of other species of bivalve mollusks using this kind of markers, such as M. chilensis (Larrain et al., 2015), M. galloprovincialis (Li, Liang, Sui, Gao, & He, 2011), Cerastoderma edule (Martínez, Arias, Méndez, Insua, & Freire, 2009) and Pinna nobilis (González-Wangüemert et al., 2015), and also similar to other pectinids such as A. purpuratus (Marín, Fujimoto, & Araí, 2013), C. nobilis (Hui et al., 2006), Patinopecten yessoensis (Li, Qi, Nie, Kong, & Yu, 2016), and N. subnodosus (Ibarra, Petersen, Fumula, & May, 2006).

The deviations from Hardy–Weinberg equilibrium observed for all loci on the studied wild population shows a considerable excess of homozygotes, which can be attributed to several factors. One of those factors is the presence of null alleles, which are caused by a failure in the microsatellites amplification during PCR (Callen et al., 1993; Stadhouders et al., 2010; Wattier, Engel, Saumitou-Laprade, 2011).
Table 2: Comparison of allelic frequencies in 8 microsatellite loci in wild (= parental) individuals of Argopecten nucleus from Neguanje Bay, Colombia, estimated using Micro-Checker 2.2.3 software (St) and segregation analysis (Se). 0: null allele.

| Loci  | Allelic frequencies | Brookfield1 (Used in this study) | Oosterhout | Brookfield2 | SD |
|-------|---------------------|----------------------------------|------------|-------------|----|
|       | N                   | St                               | Se         |             |    |
| An001 |                     | 119 122 125 128 131 134          | 0          | 0           | 0  |
| St    | 40                  | 0.072 0.242 0.108 0.176 0.015 0.005 | 0.382      | 0.421       | 0.382 0.097 |
| Se    | 40                  | 0.125 0.188 0.138 0.263 0.025 0.013 | 0.250      |             |    |
| An002 |                     | 173 176 179 182 185 188          | 0          |             |    |
| St    | 40                  | 0.010 0.020 0.169 0.266 0.118 0.020 | 0.397      | 0.441       | 0.396 0.095 |
| Se    | 40                  | 0.013 0.038 0.250 0.288 0.150 0.025 | 0.238      |             |    |
| An003 |                     | 119 122 125 128 131              | 0          |             |    |
| St    | 30                  | 0.109 0.203 0.089 0.169 0.015    | 0.415      | 0.451       | 0.414 0.111 |
| Se    | 30                  | 0.250 0.250 0.100 0.167 0.050    | 0.180      |             |    |
| An005 |                     | 162 165 168 171 174              | 0          |             |    |
| St    | 38                  | 0.034 0.169 0.405 0.039 0.017    | 0.336      | 0.393       | 0.351 0.095 |
| Se    | 38                  | 0.053 0.211 0.434 0.079 0.039    | 0.184      |             |    |
| An006 |                     | 175 178 181 184 187 190          | 0          |             |    |
| St    | 36                  | 0.029 0.133 0.268 0.059 0.059 0.039 | 0.414      | 0.423       | 0.622 0.101 |
| Se    | 36                  | 0.056 0.208 0.333 0.083 0.069 0.042 | 0.167      |             |    |
| An007 |                     | 128 131 134 137 140 143 146 149  | 0          |             |    |
| St    | 42                  | 0.008 0.288 0.242 0.030 0.061 0.121 0.106 0.038 | 0.106      | 0.121       | 0.102 0.099 |
| Se    | 42                  | 0.150 0.313 0.284 0.045 0.104 0.030 0.149 0.060 0.119 | 0.119      |             |    |
| An009 |                     | 125 128 131 134                  | 0          |             |    |
| St    | 29                  | 0.186 0.128 0.226 0.49           | 0.411      | 0.453       | 0.431 0.092 |
| Se    | 29                  | 0.259 0.190 0.121 0.138          | 0.290      |             |    |
| An010 |                     | 153 156 159                      | 0          |             |    |
| St    | 18                  | 0.271 0.346 0.033                | 0.350      | 0.421       | 0.377 0.138 |
| Se    | 18                  | 0.242 0.242 0.121                | 0.394      |             |    |

Abbreviations: N, number of individuals analyzed; SD, standard deviation of allelic frequencies per locus.
Brookfield2
Brookfield1
Oosterhout

FIGURE 1 Association between the null allele frequency estimations using segregation analysis (Se) and Micro-Checker 2.2.3 software (St) applying three different models

| Locus  | Ho  | He  | Fis  |
|--------|-----|-----|------|
|        | St  | Se  | St   | Se   | St  | Se   |
| An001  | 0.07| 0.55| 0.73 | 0.80 | 0.90*| 0.31 |
| An002  | 0.02| 0.50| 0.69 | 0.77 | 0.98*| 0.35 |
| An003  | 0.02| 0.40| 0.74 | 0.80 | 0.98*| 0.50 |
| An005  | 0.04| 0.42| 0.53 | 0.72 | 0.93*| 0.42 |
| An006  | 0.02| 0.36| 0.62 | 0.80 | 0.96*| 0.55 |
| An007  | 0.60| 0.79| 0.79 | 0.78 | 0.23*| -0.01|
| An009  | 0.00| 0.59| 0.70 | 0.78 | 1.00*| 0.25 |
| An010  | 0.00| 0.72| 0.54 | 0.71 | 1.00*| -0.01|
| Media  | 0.10b| 0.54a| 0.68b| 0.77*| 0.87*| 0.29b|

Note: Different super indexes indicate significant differences between both methods (p < .05).

Abbreviations: Ho, Observed heterozygosity; He, Expected heterozygosity; Fis, Homozygosity Index.

*H-W p < .01.

& Valero, 1998). They are particularly frequent in marine invertebrates (Hare, Karl, & Avise, 1996; Hedgecock et al., 2004), and their occurrence can result in the incorrect classification of heterozygote individuals for these alleles as homozygotes for the dominant alleles (Dakin & Avise, 2004; Lemer et al., 2011; Pompanon, 2005). Other factors that can further reduce the observed frequency of heterozygotes in a population are high levels of inbreeding (Chapuis & Estoup, 2007) and the Wahlund effect (Wahlund, 1928).

The use of statistical tools and the segregation analysis evidenced the presence of null alleles in all the examined microsatellite loci in Argopecten nucleus, with similarly high accuracy, which can significantly influence the estimated values of heterozygosity and inbreeding in this species. The frequency of null alleles per locus was high (39%–45%), with the exception of locus An007 (12%). Their frequency estimated using statistical methods, however, was higher than those obtained from the segregation analysis, and no significant correlation between the results using both methods was observed. The segregation analysis of alleles allows to infer the parental genotype based on the parental phenotype and the genotypes observed in their offspring (Reece et al., 2004). Although this analysis is methodologically more laborious, it is a direct and reliable technique to estimate the frequency of null alleles in a population. However, if both parents are phenotypically homozygotes for the same dominant allele, it is not possible to infer with certainty if one or both parents are heterozygotes for a null allele with the same genotype. Thus, the frequency of the null alleles estimated using this method could be slightly underestimated. The mating of one male with several females reduces this risk by increasing the certainty about the genotypes of the males and improving the reliability of the inferences about the mother's genotype. However, it does not completely prevent the difficulties that arise from determining the genotype of the fathers, as it has been observed in individuals (20%) whose genotype could not be confirmed for some loci using this method in the present study. In addition, if the individuals of A. nucleus used as parents were able to contribute to the offspring by self-fertilization, this technique could overestimate the frequencies of null alleles.

The estimation of the frequency of null alleles using statistical methods, such as those included in softwares like Micro-Checker v.2.2.3 (van Oosterhout et al., 2004; Shipley, 2003), and GENEPOP v.3.4 (Raymond & Rousset, 1995), are based on the assumptions that the presence of null alleles produces an excess of homozygotes in the population dataset, in comparison with the expected Hardy–Weinberg equilibrium (van Oosterhout et al., 2004). Both inbreeding and the Wahlund effect can cause a consistent increase of homozygosity across the genome, unlike the effect of the null alleles, whose influence varies among loci depending on the frequencies of null alleles present in them (Girard & Angers, 2008; Walples, 2015). Nonetheless, the consequences of null alleles, inbreeding or the Wahlund effect are rather complex to distinguish solely based on the application of statistical analysis strategies. Recently, different tools for statistical inference have been proposed to distinguish between the presence of null alleles and the Wahlund effect in studies on population genetics (De Meeûs, 2018; Walples, 2015, 2018; Zhivotovsky, 2015), but their application requires the use of specific sampling designs (De Meeûs, 2018; Walples, 2018) and does not consider the simultaneous occurrence of inbreeding. The estimations of null allele frequencies in the wild population of A. nucleus using statistical methods were much higher than those obtained from the segregation analysis, and no significant correlation was found between them. These results are not in agreement with those reported in the study by Oddou-Muratorio, Vendramin, Buiteveld, and Fady (2009), in which no significant differences were found between the use of segregation analysis and statistical methods for the tree species Fagus sylvatica, suggesting that frequency estimations for null alleles with both methods could strongly depend on the studied species, probably as a consequence of its reproductive strategy and population structure. Thus, the reproductive strategy and population structure must be considered when defining the experimental design for analyzing the genetic diversity in species similar.
to *A. nucleus*. The use of alternative methods to statistical analysis for estimating null alleles frequency in those populations is highly recommended, as well as increasing the number of studied loci and eliminating those that present significantly high frequencies of null alleles (Bürkli, Sieber, Seppälä, & Jokela, 2017; De Sousa, Finkeldey, & Gailing, 2005; Estoup, Jarne, & Cornuet, 2002; Oddou-Muratorio et al., 2009; Stadhouwers et al., 2010); redesigning primers to avoid the presence of mutations affecting the primer pairing with flanking zones (Holm et al., 2001; Reece et al., 2004); and, whenever possible, performing a segregation analysis to infer the parental genotypes. Another option is the use of SNPs markers, since they are easier to identify, show Mendelian segregation, and exhibit few null alleles at controlled crosses, compared to microsatellites (Harney et al., 2018).

Argopecten nucleus is a functional hermaphrodite that forms sparse, low-density populations in nature. To date, the only source of live wild individuals has been seeds obtained from artificial collectors suspended in the sea (0–19 seed m⁻² collector) (Díaz & Puyana, 1994; Lodeiros et al., 1993; Valero et al., 2000; Velasco & Barros, 2009). The data registered during the controlled spawning process indicate that 0%–25% of zygotes per family began spontaneous embryonic development after the gametes release, with an average of 12%, presumably due to self-fertilization. Such levels of selfing are similar to those reported for other species of hermaphroditic mollusks, like *A. purpuratus* (Toro, Montoya, Martínez, Gutiérrez, & Vergara, 2010; Winkler & Estévez, 2003) and *Radix bathica* (7%–20%; Bürkli et al., 2017) under similar experimental conditions, although in *A. purpuratus* the selfing rates can be very variable among different females (Concha, Figueroa, & Winkler, 2011; Toro et al., 2010; Winkler & Estévez, 2003). In addition, the occurrence of self-fertilization has been verified by molecular analysis of massive spawns in farmed *A. irradians* (Li & Li, 2011) and *N. subnodosus* (Petersen, Ibarra, Ramirez, & May, 2008). Winkler and Estévez (2003) have hypothesized that self-fertilization in *A. purpuratus* takes place in the nephridia during the release of gametes, since all possible precautions to avoid self-fertilization of the released oocytes were considered in hatchery operations. The same situation seems to occur in *A. nucleus*.

The adults of *A. nucleus* exhibit a limited capability of displacement, but its larvae spend 11–15 days in the plankton. In the particular geographic area of this study, planktonic larvae are continuously exposed to diverging marine currents, such as the Caribbean current that flows toward the west and the Darien countercurrent that flows in direction to north (CIOH, 2018). In addition, low salinity barriers (0–38.3 ppt) existing in the mouths of rivers Magdalena, Canal del Dique, Sinú and Atrato (Vivas-Aguas, Espinoza, Sánchez, Cadavid, & Ibarra, 2012) can act as natural barriers to larval dispersion near to the coast, since low salinities are lethal for this pectinid. As a consequence, it is possible that self-fertilization might be the rule rather than the exception for *A. nucleus*, due to the occurrence of self-fertilization during the gametes release, and considering that low densities of natural populations could imply a low chance of encounter between gametes released by different individuals. It has been estimated that oocytes must be fertilized within 2 hr after release, and for sperm, the time lapse would be 4 hr as maximum (Velasco, 2008). Thus, functional hermaphroditism and self-fertilization could have been evolved as an efficient reproductive strategy for a species with extremely low population densities. As a result, the offspring of different crossing events would exhibit high levels of individual inbreeding, but allelic frequencies would remain quite diverse between crosses. Oceanographic factors, population structure, and reproductive strategy could generate conditions favoring high levels of homozygosis, which is in agreement with what has been observed in the population of Neguanje Bay, as a result of the added influence of inbreeding and Wahlund effect.

A singular aspect of the present results is the high frequency of null alleles in most of the loci. Kimura and Ohta (1969) inferred that the number of generations required for the fixation by chance of a new selectively neutral mutation in a finite population depends on the effective population size. As a consequence, the combination of low densities populations and selfing in *A. nucleus* could favor the accumulation of selectively neutral mutations in a particular population, as it occurs when the population size decreases (Kimura, 1979).

Therefore, it can be inferred that if a set of new independent microsatellite markers is developed for this species, the frequency of null alleles per locus will likely be similar to those observed in this study.

Assuming that *A. nucleus* have populations with very low density and high rates of self-fertilization, as the results of this study suggest, a remarkable feature is their high levels of polymorphism and allele richness. Genetic evidences suggest that individuals of *A. purpuratus* exhibiting more inbreeding have higher mortality rates than their less inbred sibs (Toro et al., 2010; Winkler et al., 2009). The same phenomenon could be occurring in *A. nucleus*, thus contributing to preserve the genetic variability in wild populations. On the other hand, in a hermaphroditic species with high fecundity and low chance of cross reproduction, the effective population size in time will tend to be one. This implies that within-population genetic variability can be low, but total genetic variability could remain unaffected (Falconer & Mackay, 2006), although out of the H-W equilibrium due to the Wahlund effect and inbreeding. Aquaculture usually induces changes in the populations genetic structure and diversity due to founder effects, low effective number (Ne) of brooders, differences in genetic contribution of brooders in the reproductive process and domestic selection (Hedgecock & Sly, 1990; Li, Shu, Yu, & Tian, 2007; Liu, Zeng, Du, & Rao, 2011; Praipue, Klinbunga, & Jarayabhand, 2010; Rhode et al., 2012; Verspoor, 1988, among others). As a consequence, the genetic pool of wild populations might be negatively affected when populations generated through aquaculture are used for restocking wild populations, or gametes admixture occurs due to both wild and aquaculture populations share the same environment (Beaumont, 2006; Harada, Yokota, & Iizuka, 1998; Ryman, Jorde, & Laikre, 1995; Ryman & Laikre, 1991; Waples, Hindar, Karlsson, & Hard, 2016). However, the present results in *A. nucleus* seem to represent a paradox in this sense, because even when aquaculture populations and artificial reproduction can reduce the inbreeding and increase the Ne in comparison with wild populations, both factors can cause genetic loss in wild populations.
subject to supportive breeding or exposed to genetic introgression from cultured populations. To minimize the potential genetic impact of hatchery-produced scallops on wild populations, there are different alternatives, including the systematic use of wild brooders (Yokota, Harada, & lizuka, 2003), the use of completely genealogized brooders to ensure low inbreeding during a controlled reproduction process (Evans, Bartlett, Sweijd, Cook, & Elliott, 2004), the use of genetic markers to avoid inbred crosses (Liu et al., 2011), and the culture of triploids to prevent reproduction and genetic introgression in wild populations (Piferrer et al., 2009). However, this last method is not completely safe if the triploidization is not complete or if the triploids are not completely sterile (Winkler, Concha, & Concha, 2019).

In summary, the first 8 microsatellites designed and standardized for A. nucleus are reported, all of which were polymorphic with 4–9 alleles per locus. The segregation analysis evidenced an overestimation of the null alleles frequencies using regular statistical tools, and a lack of correlation between this data and the direct estimation of segregating null alleles, suggesting that the use of both methods can introduce an important bias in the estimations of null allele frequencies in populations that are highly polymorphic but have very low observed heterozygosity. The microsatellite markers exhibited high levels of genetic diversity in the A. nucleus population of Neguanje Bay (Santa Marta, Colombia), but also indicated a high homozygosity, suggesting the occurrence of the self-fertilization associated with the low population densities reported for the species.

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CONFLICT OF INTEREST
Authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
The author's contributions to this study were as follows: J. Barros performed the experiments, analyzed the data, and wrote this manuscript. L.A. Velasco provided the original idea, advised the experiments, and wrote this manuscript. F.M. Winkler advised the experimental design and analyses as well as wrote this manuscript.

ETHICAL APPROVAL
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

DATA AVAILABILITY STATEMENT
Supporting information of microsatellite genotypes in Appendix S1: Dryad: https://doi.org/10.5061/dryad.h9w0vt4f0.

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REFERENCES
Ardren, W. R., Borer, S., Thrower, F., Joyce, J. E., & Kapuscinski, A. R. (1999). Inheritance of 12 microsatellite loci in Oncorhynchus mykiss. Journal of Heredity, 90, 529–536. https://doi.org/10.1093/jhered/90.5.529
Baranski, M., Rourke, M., Loughnan, S., Austin, C., & Robinson, N. (2006). Isolation and characterization of 125 microsatellite DNA markers in the blacklip abalone, Haliotis rubra. Molecular Ecology Notes, 6, 740–746. https://doi.org/10.1111/j.1471-8286.2006.01327.x
Barros, J., Velasco, L. A., & Winkler, F. M. (2018). Heritability, genetic correlations and genotype by environment interactions in productive traits of the Caribbean scallop, Argopecten nucleus (Mollusca: Bivalvia). Aquaculture, 488, 39–48. https://doi.org/10.1016/j.aquaculture.2018.01.011
Barros, J., Winkler, F. M., & Velasco, L. A. (2018). Heritability, genetic correlations and genotype-environment interactions for growth and survival of larvae and post-larvae of the Caribbean scallop, Argopecten nucleus (Mollusca: Bivalvia). Aquaculture, 495, 948–954. https://doi.org/10.1016/j.aquaculture.2018.06.047
Beaumont, A. (2006). Genetics. In S. E. Shumway, & G. J. Parsons (Eds.), Scallops: Biology and Ecology (pp. 543–594). New York, NY: Elsevier.
Becquet, V., Laneluc, I., Bouhet, B. S., & García, P. (2009). Microsatellite markers for the Baltic clam, Macoma balthica (Linne 1758), a key species concerned by changing southern limit, in exploited littoral ecosystems. Conservation Genetics Resources, 1, 265–267. https://doi.org/10.1007/s12686-009-9065-0
Bert, T. M., Arnold, W. S., Wilbur, A. E., Seyoum, S., ... Yarbro, L. (2014). Florida gulf bay scallop (Argopecten irradians concentricus) population genetic structure: Form, variation, and influential factors. Journal of Shellfish Research, 33, 99–136. https://doi.org/10.2983/035.033.0112
Blouin, M. S. (2003). DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends in Ecology & Evolution, 18, 503–511. https://doi.org/10.1016/S0169-5347(03)00225-8
Borrell, Y. J., Álvarez, J., Vázquez, E., Pató, C. F., Tapia, C. M., Sánchez, J. A., & Blanco, G. (2004). Applying microsatellites to the management of farmed turbot stocks (Scophthalmus maximus L.) in hatcheries. Aquaculture, 241, 133–150. https://doi.org/10.1016/j.aquaculture.2004.08.021
Brookfield, J. F. Y. (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. Molecular Ecology, 5, 453–455. https://doi.org/10.1046/j.1365-294X.1996.00098.x
Bürkli, A., Sieber, N., Seppälä, K., & Jokela, J. (2017). Comparing direct and indirect selfing rate estimates: When are population-structure estimates reliable? Hereditas, 118, 525–533. https://doi.org/10.1038/hdy.2017.1
Callen, D. F., Thompson, A. D., Shen, Y., Phillips, H. A., Richards, R. I., Mulley, J. C., & Sutherland, G. R. (1993). Incidence and origin of “null” alleles in the (AC)n microsatellite markers. American Journal of Human Genetics, 52, 922–927.
Castellanos, C., & Campos, N. H. (2007). Variación espacial y temporal de juveniles de Pinctada imbricata (Röding, 1798) y Argopecten nucleus (Born, 1778) en la región norte del Caribe colombiano. Boletín De Investigaciones Marinas y Costeras, 36, 209–227.
Lemer, S., Rochel, E., & Planes, S. (2011). Correction method for null alleles in species with variable microsatellite flanking regions, a case study of the black-lipped pearl oyster Pinctada margaritifera. *Journal of Heredity*, 102, 243–246. https://doi.org/10.1093/jhered/esq123

Lewin, B. (2004). Genes VIII. London, UK: Pearson Prentice Hall.

Li, H., Liang, Y., Sue, L., Gao, X., & He, C. (2011). Characterization of 10 polymorphic microsatellite markers for Mediterranean blue mussel Mytilus galloprovincialis by EST database mining and cross-species amplification. *Journal of Genetics*, 90, 30–33. https://doi.org/10.1007/s12041-011-0049-y

Li, Q., Park, C., Kobayashi, T., & Klima, A. (2003). Inheritance of microsatellite DNA markers in the Pacific Abalone *Haliotis discus hannai*. *Marine Biotechnology*, 5, 331–338. https://doi.org/10.1007/s10126-002-0116-8

Li, Q., Qi, M., Nie, H., Kong, L., & Yu, H. (2016). Microsatellite-centromere mapping in Japanese scallop *Patinopexy yessoensis* through half-tetrad analysis in gynogenetic diploid families. *Journal of Ocean University of China*, 15(3), 541–548. https://doi.org/10.1007/s11802-016-2904-1

Li, Q., Shu, J., Yu, R., & Tian, C. (2007). Genetic variability of cultured populations of the Pacific abalone *Haliotis discus hannai* in China based on microsatellites. *Aquaculture Research*, 38, 981–990. https://doi.org/10.1111/j.1365-2109.2007.01764.x

Li, R., & Li, Q. (2011). Mating systems and reproductive success in hermaphroditic bay scallop, *Argopecten irradians* irradians (Lamarck 1819), inferred by microsatellite-based parentage analysis. *Journal of the World Aquaculture Society*, 42, 888–898. https://doi.org/10.1111/j.1749-7345.2011.00521.x

Liewlaksaneeyanawin, C., Ritland, C. E., & El-Kassaby, Y. A. (2002). Development of twelve polymorphic microsatellite markers in the Pacific Abalone *Patinopecten yessoensis* (Bivalvia: Cardiidae). *Aquaculture*, 203, 19267–19275. https://doi.org/10.1016/S0044-8486(02)00254-3

Lodéiros, C., & Freites, L. (2008). Estado actual y perspectivas del cultivo de la pulpastra *Bivalvia: Veneridae*. *Conservation Genetics Resources*, 1, 107–109. https://doi.org/10.1007/s12686-009-9134-4

López, G. A., Rutherford, S., & Moncada, M. P. (2014). Segregation analysis of molecular markers in a population derived from *Coffea liberica* Hier x C. *eugenoides* L. *Acta Agronomica*, 63, 153–163. https://doi.org/10.15446/acag.v63n2.39879

Marín, A., Fujimoto, T., & Arai, K. (2013). Genetic structure of the Peruvian scallop *Argopecten purpuratus* inferred from mitochondrial and nuclear DNA variation. *Marine Genomics*, 9, 1–8. https://doi.org/10.1016/j.margen.2012.04.007

Martínez, L., Arias, A., Méndez, J., Insua, A., & Freire, R. (2009). Development of twelve polymorphic microsatellite markers in the edible cockle *Cerastoderma edule* (Bivalvia: Cardiidae). *Conservation Genetics Resources*, 1, 107–109.

Mora, D., Avendaño, M., Peña, J., Le Pennec, M., Tanguy, A., & Baron, J. (2001). Genetic and morphological differentiation between two pec-tinid populations of *Argopecten purpuratus* from the northern Chilean coast. *Estudios Oceanológicos*, 20, 51–60.

Nei, M. (1975). *Molecular population genetics and evolution*. Oxford, UK: North-Holland Publishing Company Amsterdam.

Oddou-Muratorio, S., Vendramin, G. G., Buitvedl, J., & Fady, B. (2009). Population estimators or progeny tests: What is the best method to assess null allele frequencies at SSR loci? *Conservation Genetics*, 10, 1343–1347. https://doi.org/10.1007/s10592-008-9648-4

Orroco, G., & Narváez, J. C. (2014). Genetic diversity and population structure of bocachoco *Prochilodus magdalenae* (Pisces, Prochilodontidae) in the Magdalena River basin and its tributaries. *Colombia. Genetics and Molecular Biology*, 37(1), 37–45.

Peakall, R., & Smouse, P. (2009). GenAlEx Tutorials-Part 5 Advanced features including data import and export based on material provided at the national graduate workshop Genetic Analysis for Populations Studies offered by Rod Peakall and Peter Smouse at the Australian National University. Canberra, Australia.

Pereira, S. M., Arias, A., Méndez, J., Insua, A., & Freire, R. (2009). Isolation of twelve microsatellite markers in the pullet carpet shell *Venerupis pullastra* (Bivalvia: Veneridae). *Conservation Genetics Resources*, 2, 201–203. https://doi.org/10.1007/s12686-009-9134-4

Petersen, J. L., Baerwald, M. R., Ibarra, A. M., & May, B. (2012). A first-generation linkage map of the Pacific lion-paw scallop (*Nodopenpecten subnodosus*): Initial evidence of QTL for size traits and markers linked to orange shell color. *Aquaculture*, 350–353, 200–209. https://doi.org/10.1016/j.aquaculture.2012.03.039

Petersen, J. L., Ibarra, A. M., Ramírez, J. L., & May, B. (2008). An induced mass spawn of the hermaphroditic lion-paw scallop, *Nodopenpecten subnodosus*: Genetic assignment of maternal and paternal parentage. *Journal of Heredity*, 99, 337–348. https://doi.org/10.1093/jhered/esn012

Piñero, D., Caballero-Mellado, J., Cabrera-Toledo, D., & Zúñiga, G. (2008). La diversidad genética como instrumento para la conservación y el aprovechamiento de la biodiversidad: Estudios en especies mexicanas. In D. Piñero (Ed.), *Capítulo natural de México, vol 1: Conocimiento actual de la biodiversidad* (pp. 437-494). México: Conabio.

Pomponio, F., Bonin, A., Bellemain, E., & Taberlet, P. (2005). Genotyping errors: Causes, consequences and solutions. *Nature Reviews Genetics*, 6, 847–859. https://doi.org/10.1038/nrg1707

Prăipău, P., Klinbunga, S., & Jarayabhand, P. (2010). Genetic diversity of wild and domesticated stocks of Thai abalone, *Haliotis asinina* (Haliotidae), analyzed by single-strand conformational polymorphism of AFLP-derived markers. *Genetics and Molecular Research*, 9(2), 1136–1152. https://doi.org/10.4238/vol9-2gm088

Qin, Y., Liu, X., Zhang, H., Zhang, G., & Guo, X. (2007). Genetic mapping of size-related quantitative trait loci (QTL) in the bay scallop (*Argopecten irradians*) using AFLP and microsatellite markers. *Aquaculture*, 272, 281–290. https://doi.org/10.1016/j.aquaculture.2007.07.214

Raymond, M., & Rousset, R. (1995). *GENEPOP* version 12: Population genetics software for exact tests and ecumesis. *Journal of Heredity*, 86, 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Reece, K. S., Ribeiro, W. L., Gaffney, P. M., Carnegie, R. B., & Allen, K. Jr (2004). Microsatellite marker development and analysis in the Eastern Oyster (*Crassostrea virginica*): Confirmation of null alleles and non-Mendelian segregation ratios. *Heredity*, 93, 346–352. https://doi.org/10.1038/jhered.es058
Rhode, C., Hepple, J., Jansen, S., Davis, T., Vervalle, J., Bester-van der Merwe, A. E., & Roodt-Wilding, R. (2012). A genetic analysis of abalone domestication events in South Africa: Implications for the management of the abalone resource. *Aquaculture*, 356–357, 235–242. https://doi.org/10.1016/j.aquaculture.2012.05.012

Rico, C., Cuesta, J. A., Drake, P., Macpherson, E., Bernatchez, L., & Marie, A. D. (2017). Null alleles are ubiquitous at microsatellite loci in the Wedge Clam (*Donax trunculus*). *PeerJ*, 5, e3188. https://doi.org/10.7717/peerj.3188

Ryman, N., Jorde, P. E., & Laikre, L. (1995). Supportive breeding and variance effective population size. *Conservation Biology*, 9, 1619–1628. https://doi.org/10.1046/j.1523-1739.1995.09061619.x

Ryman, N., & Laikre, L. (1991). Effects of supportive breeding on the genetically effective population size. *Conservation Biology*, 5, 325–329. https://doi.org/10.1046/j.1523-1739.1991.tb00144.x

Shipley, P. (2003). MICRO-CHECKER ver 2.2.3. Hull, UK: University of Hull.

Smith, J. P. (1996). *La diversidad genética de los recursos pesqueros marinos: Posibles repercusiones de la pesca*. Documento Técnico de Pesca No 344. Roma, Italy: FAO.

Smith, P. J., & Conroy, A. M. (1992). Loss of genetic variation in hatchery populations of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, 45, 1686–1690. https://doi.org/10.1139/f88-199

Vivas-Aguas, L. J., Espinoza, L., Sánchez, J., Cadavid, B., & Ibarra, K. (2012). Diagnóstico y Evaluación de la Calidad Ambiental Marina en el Caribe y Pacifico Colombiano. *Red de Vigilancia para la Conservación y Protección de las Aguas Marinas y Costeras de Colombia-REDCAM*. Informe Técnico 2012. Santa Marta, Colombia: Invermap.

von Brand, E., & Kijima, A. (1990). Comparison of genetic markers between the Chilean scallop *Argopecten purpuratus* and the Japanese scallop *Patinopecten yoiesoensis*. *Tohoku Journal of Agricultural Research*, 41, 25–35.

Wahlund, S. (1928). Zusammensetzung von population und korrelationsscheinung vom stand-punkt der vererbungslehre aus betrachtet. *Hereditas*, 11, 65–106 [English translation. In: Weiss KM, Ballonoff PA, Editors 1975]. https://doi.org/10.1111/j.1600-2252.1928.tb02483.x

Wang, Y., Fu, D., & Xia, J. (2013). The genetic diversity of the noble scallop (*Chlamys nobilis*, Reeve 1852) in China assessed using five microsatellite markers. *Marine Genomics*, 9, 63–67. https://doi.org/10.1016/j.margen.2012.06.003

Waples, R. S. (2015). Testing for Hardy-Weinberg proportions: Have we lost the plot? *Journal of Heredity*, 106, 1–19. https://doi.org/10.1093/jhered/esu062

Waples, R. S. (2018). Null alleles and $F_{IS}$ correlations. *Journal of Heredity*, 109, 457–461. https://doi.org/10.1093/jhered/esu062

Waples, R. S., Hindar, K., Karlsson, S., & Hard, J. J. (2016). Evaluating the Ryman-Laikre effect for marine stock enhancement and aquaculture. *Current Zoology*, 62, 617–627. https://doi.org/10.1093/czoz/ow060

Wattier, R., Engel, R., Saumitou-Laprade, P., & Valero, M. (1998). Short allele dominance as a source of heterozygote deficiency at microsatellite loci: Experimental evidence at the dinucleotide locus Gvc1T in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology*, 7, 1569–1573. https://doi.org/10.1046/j.1365-294x.1998.00477.x

Weetman, D., Hauser, L., Shaw, P. W., & Bayes, M. (2005). Microsatellite markers for the whelk *Buccinum undatum*. *Molecular Ecology Notes*, 5, 361–362. https://doi.org/10.1111/j.1471-8286.2005.00926.x

Winker, F. M., Concha, M., & Concha, C. (2019). Modeling the reproductive impact of partially sterile triploids on conspecific diploid populations. *Aquaculture Environment Interactions*, 11, 205–211. https://doi.org/10.3354/aei00308

Winker, F. M., & Estévez, B. F. (2003). Effects of self-fertilization on growth and survival of larvae and juvenile of the scallop *Argopecten purpuratus*. *Journal of Experimental Marine Biology and Ecology*, 292, 93–102. https://doi.org/10.1016/S0022-0981(03)00147-3

Winker, F. M., Mettifogo, L., Pérez, M. A., Callejas, C., Brokordt, K. B., & Martínez, V. (2009). Effects self fertilization on allelloses loci heterozygosidad in juveniles of the hermaphroditic scallop *Argopecten purpuratus*. 17th. International Pectinid Workshop. Santiago de Compostela, Spain. April 22–28, 2009. Abstract.

Wu, X., Feng, Y., Ma, H., Pan, Y., & Liu, X. (2015). Characterization of new microsatellite loci from the razor clam (*Sinonovacula constricta*) and transferability to related species. *Biochemical Systematics and Ecology*, 61, 175–178. https://doi.org/10.1016/j.bse.2015.06.016
Xu, Z., Primavera, J. H., de la Pena, L. D., Pettita, P., Belaka, J., & Alcivar-Warren, A. (2001). Genetic diversity of wild and cultured Black Tiger Shrimp (Peneaus monodon) in the Philippines using microsatellites. *Aquaculture, 199*, 13–40. https://doi.org/10.1016/S0044-8486(00)00535-4

Yokota, M., Harada, Y., & Iizuka, M. (2003). Genetic drift in a hatchery and the maintenance of genetic diversity in hatchery-wild systems. *Fisheries Science, 69*, 101–109. https://doi.org/10.1046/j.1444-2906.2003.00593.x

Zar, J. (1999). *Biostatistical analysis* (4th ed.). Upper Saddle River, NJ: Prentice Hall.

Zhan, A. B., Bao, Z. M., Hui, M., Wang, M. L., Zhao, H. B., Lu, W., ... Hu, J. J. (2007). Inheritance pattern of TST-SSRs in self-fertilized larvae of the bay scallop Argopecten irradians. *Annales Zoologici Fennici, 44*, 259–268.

Zheng, H., Zhang, G., Guo, X., & Liu, X. (2008). Inbreeding depression for various traits in two cultured populations of the American bay scallop, Argopecten irradians irradians Lamarck (1819) introduced into China. *Journal of Experimental Marine Biology and Ecology, 364*, 42–47. https://doi.org/10.1016/j.jembe.2008.06.027

Zhivotovsky, L. A. (2015). Relationships between Wright’s $F_{ST}$ and $F_{IS}$ statistics in a context of Wahlund effect. *Journal of Heredity, 106*, 306–309. https://doi.org/10.1093/jhered/esv019

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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