Genetic and environmental factors underlying phenotypic traits in the Mediterranean mussel Mytilus galloprovincialis Lamarck, 1819

Grbin, Dorotea

University of Zagreb

FACULTY OF SCIENCE

DEPARTMENT OF BIOLOGY

Dorotea Grbin

Genetic and environmental factors underlying phenotypic traits in the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819

DOCTORAL THESIS

Zagreb, 2019

Sveučilište u Zagrebu

PRIRODOSLOVNO-MATEMATIČKI

BIOLOŠKI ODSJEK

Dorotea Grbin

Utjecaj genetičkih i okolišnih čimbenika na fenotipska obilježja mediteranske dagnje *Mytilus galloprovincialis* Lamarck, 1819

DOKTORSKI RAD

Zagreb, 2019

This doctoral thesis was made in Department of Biology, Faculty of Science, University of Zagreb, under the supervision of Asst. Prof. Anamaria Štambuk and in one part in Department of Animal and Plant Sciences, University of Sheffield, under the supervision of Patrik Nosil, PhD. The doctoral thesis was made in the University doctoral study in Biology, Faculty of Science, University of Zagreb.

Asst. Prof. Dr. Anamaria Štambuk is a professor of Evolutionary Ecology and Zoology at the University of Zagreb, Faculty of Science. She obtained her PhD in 2011 at the University of Zagreb, working on genetics of Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) populations. Professor Štambuk has worked for 11 years in the field of Ecotoxicology as a research and teaching assistant, at Department of Biology, University of Zagreb.

She shifted her main research interest to evolutionary genomics and evolutionary ecology in 2012, and now she is a leader of the Evolutionary Ecology group at Faculty of Science.

She is the head of two international projects studying contemporary genomic adaptations (HRZZ, Interreg) and have published over 30 scientific papers in the international scientific journals. She is a reviewer in many international scientific journals with high impact factor.

Dr. Patrik Nosil is an ERC Principal Investigator, at the CRNS**,** Montpellier. His main research interests are centered on understanding the origin and maintenance of biological diversity, with a special focus on the processes which drive and constrain the formation of new species. His work specifically tests the role of natural selection and genomic architecture in evolutionary divergences. Currently, his research is focused on dynamics of the eco-evolutionary interactions.

Dr. Patrik Nosil received 14 Academic awards among which are those for best science/engineering thesis in Canada and the one for young evolutionary biologist awarded annually by the Society for the Study of Evolution. By now he got granted by 12 scientific projects. He is an author of the book Ecological speciation (2012) and editor of two special volumes (Genomic divergence during speciation, 2012; Genome evolution and speciation, 2013).

He published over 100 scientific papers in the international scientific journals with high impact factor such as *Science*, *Nature*, *Molecular Ecology*, *Nature Ecology and Evolution*, *Ecology Letters,* PNAS etc.

Dr. Nosil reviewed 152 Manuscripts for 32 different peer-reviewed journals including Nature, Science, Ecology Letters, Evolution and many others.

ZAHVALE

Hvala mojoj mentorici, doc. dr. sc. Anamariji Štambuk na ukazanom povjerenju i pruženim prilikama za učenje na radionicama i kongresima. Najviše hvala na maloj-velikoj školi genomike, evolucije i ekologije koja je bila svakodnevna inspiracija. Zahvalna sam što me toliko puta bacila u vatru i uvijek dočekala s osmjehom, a time gradila moju samostalnost i vjeru u sebe!
Hvala mom mentoru, dr. Patriku Nosilu jer mi je otvorio vrata svijeta predivne znanosti. Zahvalna sam što je, unatoč tome što je toliko velik, uvijek bio pozitivan prema svemu što sam radila, vjerovao kad ja nisam, i vidio što ja nisam. Hvala na svim sugestijama i komentarima.

Veliko hvala mom suborcu Ivi jer je bila samnom od prvog dana. Ive, hvala ti jer si samnom patila kad smo uhodavale protokole, tjerale GST da pada, borile se s R-om, kad su nam eksplodirale epice….

Hvala izv. prof. dr. sc. Sandri Radić Brkanac na spektrofotometaru, i jer je uvijek bila pri ruci za pitanja. Hvala prof. dr. sc. Zlatku Liberu na homogenizatoru. Hvala djelatnicima Instituta Ruđer Bošković u Zagrebu koji su odradili analize teških metala.
Zahvaljujem Aquarium-u Pula, Institutu Ruđer Bošković s postajama u Rovinju i Šibeniku, nacionalnom parku Mljet, dr. Bojanu Hameru i Mileni Miličić za dostupnost njihovih laboratorija i opreme.

Hvala Stuartu i Victoru jer su odradili najveći dio posla vezan uz genomske analize i Romainu na pomoći sa scriptama. Hvala svima u NCGR-u koji su doprinjeli razvoju moje ljubavi prema bioinformatici.

Hvala Vidu koji nikada nije bio moja konkurencija, već najveći prijatelj. Hvala Maji jer je uvijek bila dobri duh ureda. Hvala Aleksandru jer je brojao mrtve dagnje i postrugao na stotine smrdljivih ljuštura. Hvala svim volonterima koji su prošli kroz naš labos i doprinijeli postojanju mojih rezultata.

Hvala mojim prijateljima koji su bili svakodnevna garda podrške!

Hvala Toniju na strpljenju od samih početaka i motivaciji onda kad bih ja odustala. Hvala mom predivnom sinu za sve prospavane noći kad sam mogla raditi i pisati. Nadam se da ćeš jednog dana ponosno čitati što sam ja pisala dok si ti učio hodati i pričati.

Hvala mojim roditeljima. na svemu što ste me naučili da bih danas bila ovdje. Hvala vam jer ste bili strogi kad je trebalo, a danas strpljivi, hrabri, ponosni i najveća podrška.

Za kraj, hvala onoj koja je bila moje djetinjstvo, koja bi bila najponosnija, onoj koja nije kraj mene ali će uvijek biti dio mene. Za moju baku.

University of Zagreb
Faculty of Science
Department of Biology

Doctoral thesis

GENETIC AND ENVIRONMENTAL FACTORS UNDERLYING PHENOTYPIC TRAITS IN THE MEDITERRANEAN MUSSEL *Mytilus galloprovincialis* LAMARCK, 1819

DOROTEA GRBIN
University of Zagreb

Phenotypic diversity is multifactorial and understanding how it arises within and among species is a long-term goal of evolutionary biology. This study tested the association between environment, phenotype and genotype of *Mytilus galloprovincialis* populations along the eastern Adriatic coast. The analyses of environmental variables, mussel morphology, genetic architecture, biomarkers, and induced mortality were implemented on native populations and in transplant experiments. Core results underline that phenotypic variability exists between mussel's populations and is driven by numerous environmental factors. Results of the transplant experiment led to a conclusion that biomarker status varies among Adriatic regions dependent upon the environmental variables, and between clean and polluted sites depending on metal concentrations. Mesocosm experiment showed population effect on survival and biomarker response. Estimates of mussel's genetic architecture pointed to conclusion that analysed morphological traits are polygenic and moderately heritable. This study promotes the importance of combining quantitative genetics with experimental approaches to obtain insightful data on phenotypic plasticity and adaptive responses.

(155 pages, 33 figures, 6 tables, 404 references, original in english)
Keywords: *Mytilus galloprovincialis,* environmental factors, phenotype, biomarkers, genetic architecture, GWAS
Supervisor 1: Anamaria Štambuk, Assistant Professor
Supervisor 2: Patrik Nosil, research fellow
Reviewers: 1. Mirjana Pavlica, Full Professor
2. Sandra Radić Brkanac, Associate Professor
3. Joann Mudge, Senior Research Scientist

Sveučilište u Zagrebu Doktorski rad
Prirodoslovno-matematički fakultet
Biološki odsjek

UTJECAJ GENETIČKIH I OKOLIŠNIH ČIMBENIKA NA FENOTIPSKA OBILJEŽJA MEDITERANSKE DAGNJE *Mytilus galloprovincialis* LAMARCK, 1819

DOROTEA GRBIN
Sveučilište u Zagrebu

Fenotipska raznolikost određena je nizom čimbenika, te je njeno razumijevanje unutar i između vrsta jedna od glavnih zadaća evolucijske biologije. Cilj ovog rada bio je testirati povezanost između okolišnih varijabli, fenotipa i genetičkih karakteristika populacija dagnje *Mytilus galloprovincialis,* duž istočne obale Jadrana. U tu svrhu provedene su analize okolišnih varijabli, morfoloških karakteristika i genetičke strukture dagnji, biomarkera, transplant eksperimenata, i inducirane smrtnosti u prirodnim populacijama i transplant eksperimentima. Ključni rezultati ističu da fenotipska raznolikost postoji između populacija dagnji, te je određena brojnim okolišnim čimbenicima. Rezultati transplant eksperimenta pokazali su da aktivnost biomarkera varira između Jadranskih regija ovisno o okolišnim čimbenicima, te između čistih i onečišćenih istraživanih postaja ovisno o akumuliranoj koncentraciji metala u tkivu dagnje. Mezokozmos eksperiment pokazao je populacijski efekt s obzirom na aktivnost biomarkera. Kvantitativna procjena genetičke strukture dagnj ističe poligenska i slabo nasljedna svojstva analiziranih morfoloških karakteristika. Ovaj rad ističe važnost kombiniranja eksperimentalnih pristupa s kvantnom genetikom u svrhu procjene fenotipkse plastičnosti i adaptivnih odgovora.
(155 pages, 33 figures, 6 tables, 404 references, original in english)
Keywords: *Mytilus galloprovincialis,* okolišne varijable, fenotip, biomarkeri, genetička arhitektura, GWAS
Supervisor 1: Doc. dr. sc. Anamaria Štambuk
Supervisor 2: Dr. Patrik Nosil
Reviewers: 1. Prof. dr. sc. Mirjana Pavlica
2. Izv. prof. dr. sc. Sandra Radić Brkanac
3. Dr. Joann Mudge

1. INTRODUCTION

Recent changes in ecosystems (especially aquatic ones) are becoming widespread in alarming proportions, being distractedly induced by anthropogenic influence. The effects of growing anthropogenic pollution can be observed across all levels of biological organization, and early detection of disturbances in organism homeostasis is reasonably desirable. It facilitates not only understanding, but also prediction and prevention of impacts that environmental alteration can exert at population and ecosystem level.

Environmentally caused stress is a common phenomenon, especially during the establishment and spread of a species in a non-native environment (Reznick and Endler 1982, Hendry et al., 2000, Huey et al., 2000, Carroll et al., 2001, Koskinen et al., 2002, Lee et al., 2003, Bossdorf et al., 2005, Calsbeek et al., 2011, Matesanz et al., 2012, Sultan et al., 2013, Lucek et al., 2014). It can arise from both biotic (parasites, pathogens, predators, intra and interspecific competition) and abiotic (light, oxygen deficiency, deficit of mineral substances, the presence of heavy metals, salinity, temperature, mechanical activity; waves, sea currents, pollutants) factors (Hoffman and Parsons, 1991). As a response to changing environment, phenotypic traits can vary at different levels, such as morphology, biochemistry, behavior, life history (e.g. longevity, age and size at first reproduction, number and size of offspring), physiological change in metabolism and functional diversity. Moreover, biological systems are continuously influenced by seasonally and spatially variable natural environmental factors (e.g. temperature, salinity, food availability), which are in further complex interactions with biological endogenous factors (e.g. sex, age, reproductive status). The described complexity makes somewhat difficult to pinpoint phenotypic responses toward specific environmental alterations, including pollution. Still, scientists are actively improving their knowledge in this respect, whereas interactions between the organism's phenotype and environment are drivers of the eco-evolutionary dynamics. Phenotypic variation could represent the effect of phenotypic plasticity (capacity of a single genotype to produce a range of phenotypes), selection, or both. Phenotypic plasticity facilitates colonization of different habitats by genetically similar or identical individuals and sometimes impedes genetic differentiation between ecologically distinct populations (Wund, 2012, Huang et al., 2015). On the other hand, environments can favor individual phenotypes with the highest fitness through natural selection (Levins, 1968, Endler, 1986), and sorting of the preexisting alleles can lead to

adaptive and heritable phenotypic differentiation (Nosil, 2012). Natural selection can act upon a variety of environmental changes driven by natural or anthropogenic environmental modifications and lead to evolution. However, demonstrating the evidence for natural selection in promoting the evolution is difficult and technically challenging. It requires several conditions to be met in the system of interest: (i) phenotypic variation among the traits that results in different survival and reproduction (i.e. fitness) (ii) additive genetic variation among traits (Endler, 1986, Hoffmann and Sgrò, 2011). A trait's genetic architecture (i.e. mapping of its genotype to its phenotype) provides a description of how many loci underlie traits, and the effect size of each locus – that is, the proportion of phenotypic variance each locus controls, patterns of pleiotropy, dominance and epistasis (Flint and Mackay, 2009). Furthermore, it can provide insight into how evolutionary change might proceed in specific traits, as the genetic architecture of a trait can be a major determinant of its evolutionary potential (Hansen, 2006). There are two main ways in which researchers can map the genetic architecture of a trait. Linkage mapping allows researchers to pinpoint the genetic loci that co-vary with phenotypic variation, as well as estimating the effect size of each locus, using linkage disequilibrium (LD) resulting from genetic crosses (Mackay, 2001, Slate, 2005). Genome-wide association studies (GWAS) provide similar information, yet they rely on mating, to cause admixture/recombination in populations; because all the alleles in the population are tested at the same time, multiple alleles at each locus can be compared.

Main aim of this research was to address many environmental variables (including pollution) as the evolutionary forces in marine ecosystems. By combining evolutionary and eco-toxicological approaches with the latest genomic technologies (i.e. 'next-generation-sequencing' NGS) and computational biology, aim was to test how environment affects the evolution, ecology and genetic characteristics of *Mytilus galloprovincialis* populations (Figure 1) along the Croatian eastern Adriatic coast.

Figure 1. Native population of *Mytilus galloprovincialis* Lamarck, 1819

Bivalves provide a good model system to understand the value of phenotypic diversity. There is well documented great phenotypic variation, both inter- and intraspecific (Seed, 1968). Bivalves are sessile, intertidal filter-feeding organisms, owing the ability to transmit large amounts of water through the mantle cavity. They are capable to accumulate and tolerate high concentrations of many organic and inorganic pollutants in their tissues (Livingstone, 1991), which makes the state of oxidative stress sort of norm rather than an exception. These organisms fulfill the requirements which make them useful bioindicators of chemical pollution. Bivalves have a wide geographical distribution in brackish and sea water environments, are ecologically relevant, easy to collect and simple to retrieve with a facile access to the gametes. They are suitable for caging experiments in field sites (Livingstone, 1993, Hamza-Chaffai, 2014, Rossi et al., 2016).

Due to their global distribution and the commercial use, bivalves are frequently studied organisms, in particular, mussels belonging to the genus *Mytilus* (Alcapán et al., 2007, Zieritz and Aldridge, 2009, Zieritz et al., 2010, Brown et al., 2011,).

Shell morphology is a central tenet of bivalve biology in fields such as taxonomy, evolution, and functional anatomy (Márquez et al., 2010, Fassatoui et al., 2014). However, little is known about the heritability of their variation within particular species, and specific effects of phenotypic plasticity and phenotypic selection have not been successfully disentangled so far.

1.1. Objective and hypothesis

Objective of this research is to estimate the associations between genotype, phenotype and environment that underlie phenotypic diversity of *Mytilus galloprovincialis*, using a combination of transplant experiments and genome wide association mapping. With many factors affecting phenotype (which in this research is consisted of morphology and biochemical and cellular biomarkers) disentangling the genotypic and environmentally induced effects may provide insights into the evolutionary processes. Combining information's on both the genetic architecture and natural history of traits can help estimate theoretical predictions of the genetics of adaptation. This study also promotes the importance of combining quantitative genetics with experimental approaches to obtain insightful data on both phenotypic plasticity and adaptive responses.

To accomplish the objective of this research, three specific hypotheses were tested (patterns and experiments used to support each of them are explained in the next paragraph):

H1: Substantial phenotypic variation exists between and within mussel populations and is driven by numerous environmental factors.

H2: Environment affects mussel's phenotypic variation both through the phenotypic plasticity and natural selection in the face of high gene flow.

H3: Genetic architecture of morphological traits in Mediterranean mussel is highly polygenic.

1.2. Methods

Fifteen native populations of Mediterranean mussel (*Mytilus galloprovincialis)* were sampled along the Eastern Adriatic coast, in two seasons, in order to test first hypothesis (H1), and gain insight into pollution-driven population's biomarker responses. First, biochemical and cellular change between and within mussel populations was assessed by sampling 100 individuals per population (15 populations collected in fall 2013, 1400 mussels analysed in total), and analysing 15 morphometric traits related to shell shape and position and size of retractor and adductor muscles. Standard tools for geometric morphometry were used based on landmark data to analyze morphological traits. Digital photographs of inner shell side were taken for each individual under standard light conditions. From these standardized images we collected most of the phenotypic measurements using the software Image J (v. 1.48).

Further, the role of specific environmental factors and metals accumulated in mussel's tissue in expressed morphological variability was examined. In that regard, Partial least squares regression (PLS-R2) analysis was ran on 15 native populations, with the aim to determine how, and to what extent, the response variables (morphological traits) vary as a function of changes in the predictor variables (here set of environmental variables and set of bioaccumulated metals). To do so, bioclimatic variables and bioaccumulated pollutants were analysed as proxy for environmental conditions that could all contribute to morphological differences. Data for bioclimatic variables were compiled from Bio-Oracle, online database.

Considering that most of the morphological traits were measured on both shells, additional attention was given to determination of fluctuating asymmetry (FA), measure of developmental stability promoted as a useful bioindicator of stressors in habitats.

Seven biomarkers, indicators of oxidative stress (catalase, glutathione reductase, glutathione S-transferase, content of malondialdehyde and carbonyls), genotoxicity (Comet assay), and neurotoxicity (acetylcholinesterase) were analysed to get an insight into populations responses on molecular and cellular level toward differing environmental conditions. Biomarkers were analysed in 15 native populations sampled from polluted and reference ("clean") habitats in two seasons (fall and spring) upon life – long in situ exposure at sites characterised for various environmental variables.

Specifically, to test how mussel's biomarker status in different seasons depends on the pollution status, the multivariate biomarker activity data and their covariation were examined, using the phenotypic trajectory analysis (PTA). Transplant experiments both in wild and in mesocosm were done to test the second hypothesis (H2). To do so, 1) one population was exposed for four weeks in transplant experiment (polluted vs. clean sites in three geographic regions); 2) two source populations were exposed to contrasted environments (clean vs. polluted) in one week mesocosm study. First, mussel's plasticity in biomarker response was assessed toward differing environmental conditions by evaluating biomarker response in transplant experiment conducted in wild (under realistic environmental conditions). Second, population effect of morphological and stress responses was estimated by comparing morphological traits and biomarker responses of two different source populations in controlled mesocosm study. To concisely determine biomarker status of populations in both experiments, biomarkers were analysed through integrated biomarker response index (IBR), which combines and summarizes them in the form of a multivariate dataset. Additionally, the role of specific environmental factors and metals accumulated in mussel's tissue regarding the biomarker response variability was examined. Aiming to do so, as for the morphological traits, Partial least squares regression (PLS-R2) analysis was done.

By further measuring survival on air of individuals from mesocosm and transplant experiment in 'stress on stress' experiment, an estimation of individuals fitness under the exposure to severe stress was set.

In order to test the third hypothesis (H3) and unravel the genetic architecture of morphologic traits in Mediterranean mussel, the tool of multilocus genome-wide association study (GWAS) was implemented. GWAS was implemented using the genotyping by sequencing approach (GBS) on five data sets; Gruž population used in mesocosm experiment (394 individuals, 19129 SNPs), Marina population used in mesocosm experiment (377 individuals, 19129 SNPs), Marina population used in transplant experiment (883 individuals, 18850 SNPs), a large-scale pool of Marina individuals used in both experiments (1258 individuals, 18728 SNPs) and on 15 native populations (288 individuals, 18655 SNPs).

The phenotypic and genotypic data were processed in the software GEMMA (Genome-wide Efficient Mixed Model Association; Zhou et al., 2013) configured to use Bayesian sparse linear mixed models and Markov chain Monte Carlo. GEMMA estimates three hyperparameters

describing the genetic architecture of the phenotypes measured: the total phenotypic variation explained by all SNPs in the model (PVE), the proportion of the variation that is explained by 'sparse effect' SNPs (PGE), and the number of 'sparse effect' SNPs (n – SNP). By identifying number of loci that influence phenotypic variation and the strength of their effects, we tested the third hypothesis (H3) – that morphological traits in *M. galloprovincialis* are highly polygenic.

2. LITERATURE OVERVIEW

2.1. Source of phenotypic variation

2.1.1. Phenotypic plasticity

Beneficial phenotypes may be expressed through phenotypic plasticity, capacity of a genotype to produce different phenotypes in response to diversity of multiple environmental variables (Price et al., 2003, Pfennig et al., 2010, Matesanz et al., 2012). The set of phenotypes into which single genotype can be mapped, as the environment varies, is described by reaction norms - the property of a genotype. As such, by providing information about the magnitude of trait plasticity and the presence of genotype × environment interactions on the phenotypic expression of a given trait (de Jong, 2005), norms of reaction have great potential to increase our understanding of the ability of genotypes, and ultimately populations and species, to respond adaptively to natural and human-induced environmental variability, including climate change (Visser, 2008).

Plasticity is physiological process, but can be manifested as changes in morphology, biochemistry, physiology, behavior, or life history. It is a key mechanism with which organisms can confront a changing climate, as it allows individuals to respond to variations within their lifetime (Gienapp et al., 2008, Hendry et al., 2008, Merila, 2012). For instance, Teplitsky et al. (2008) provided evidence that climate-driven plastic decreases in the body size of red-billed gulls (*Larus novaehollandiae*) were likely the result of environmental stress, rather than genetic adaptive responses. This is thought to be particularly important for species with long generation times, as evolutionary responses via natural selection may not produce change fast enough to mitigate the current effects of a climate change.

One of the theories behind phenotypic plasticity is that it is more beneficial to sessile organisms, as those that migrate can behaviorally avoid non-optimum conditions (Gregorius and Kleinschmit, 1999). After the pelagic larvae stage, mussels become sessile and have relatively little ability to migrate from their initial attachment site. Therefore, morphological plasticity can ameliorate the effects of some abiotic or biotic factors (e.g. wave exposure, predators' pressure).

Early scientific quests were focused on traits believed to be unaffected by the environment. Even more, environmentally affected phenotypes were considered less important because of their 'apparent' lack of a genetic basis. Today, evolutionary biologists rejected this assumption, because phenotypic plasticity often has a genetic basis (Agrawal et al., 2001), and it has been promoted not only as a product, but also a co-driver of genetic evolution (West-Eberhard, 2003, Ghalambor et al., 2007, Pfennig et al., 2010, Wennersten and Forsman 2012, Wund, 2012). It is generally not plasticity itself that is the key to differentiation. The basic idea is that new phenotypes first appear as a result of environmental induction and once expressing multiple phenotypes, plasticity may reach new adaptive peaks through 'genetic assimilation' (Grether, 2013) or can be fixed via 'genetic accommodation' (Kopp and Matuszewski, 2014). Genetic assimilation is a phenomena where a phenotype created by an environmental cue is refined through quantitative genetic changes into an adaptive phenotype that becomes "inherited" (i.e., canalized) after a number of generations of exposure to the environmental stimulus (Pfennig et al., 2010). Genetic accommodation is a more general 'fine-tuning' of the novel phenotype via changes in allele frequencies, potentially facilitated by a release of hidden genetic variation (Hermisson and Wagner 2004, West-Eberhard, 2005, Crispo, 2007, Ghalambor et al., 2007, Moczek, 2007). Plasticity leading to ecological success in a novel habitat is a simple concept; however, the prospect of evolutionary divergence in novel habitats due to plasticity is not as straightforward (Agrawal, 2001). Relatively little is known about the developmental mechanisms that produce phenotypic plasticity or how it is related with ontogeny (Nijhout, 2003, Boege and Marquis, 2005, Hoverman and Relyea, 2007). The most common approaches to studying phenotypic plasticity are controlled experimental conditions, yielding the information on the phenotypes produced by a given genotype under certain conditions. Such experiments are the most effective for inbred lines or clones, because a single genotype can be examined in multiple environments (Hendry, 2016).

Examples of phenotypic plasticity include monarch butterflies, which develop increased wing melanisation in low temperatures (Davis et al., 2005), and swallowtail butterflies, whose larvae are significantly darker when reared in autumnal conditions rather than midsummer conditions (Hazel, 2002). The latter species responds to both temperature and photoperiod. Freshwater mussels (Unionoida) show high intraspecific morphological variability, and some shell traits are believed to be associated with habitat conditions. It was not known whether and which of these eco-phenotypic differences reflect underlying genetic differentiation or are the result of phenotypic plasticity. Using 103 amplified fragment length polymorphism (AFLP) markers, Zieritz et al., (2010) studied population genetics of three paired *Unio pictorum* populations sampled from two different habitat types (marina and river) along the River Thames. They found genetic differences along the Thames which were consistent with a pattern of isolation by distance and probably reflected limited dispersal via host fish species upon which unionoid larvae are obligate parasites. No consistent genetic differences were found between the two eco-morphs inhabiting different habitat types, suggesting that morphological differences in the degree of shell elongation and the shape of dorso-posterior margin are caused by phenotypic plasticity.

2.1.2. Genetic adaptation

Through the process of natural selection, phenotypes exhibiting sub-optimal, or maladapted phenotypes, will be selected against. A central parameter in estimating responses to selection and summarizing the proportion of variance due to genetics is heritability (Wright, 1920, Falconer and Mackay, 1996, Lynch and Walsh 1998, Hill, 2010). Two different terms shall be distinguished: broad sense heritability and narrow sense heritability. Broad sense heritability (H^2) is defined as the proportion of trait variance that is due to all genetic factors including dominance and gene-gene interactions. Narrow sense heritability (h^2) is defined as the proportion of trait variance that is due to additive genetic factors. Both kinds of heritability are highly complex to estimate and to interpret. An estimate of the heritability of a trait is specific to population and environment, and it can change over time as circumstances change. Heritability estimates range from zero to one. Being close to zero indicates that almost all of the variability in a trait among individuals is due to environmental factors, with very little influence from genetic differences. Heritability closer to one indicates that most of the phenotypic variance is attributable to a

variance in genetic background. Genomic-based estimates of heritability, together with the ability to collect genome - scale polymorphism data can make precise estimates of heritability, practical even for natural populations of long-lived non-model species. Such estimates may be valuable for understanding evolution in natural populations and predicting population responses to environmental perturbations including ongoing climate change (Lavergne et al., 2010, Shaw and Etterson, 2012). In case of marine bivalves, many studies reported fairly high values of h^2 for body mass and size (Lannan, 1972, Longwell and Stiles, 1973, Newkirk et al., 1977, Mallet et al., 1986, Toro and Newkirk, 1990, Toro and al., 1995, Toro and Paredes, 1996). Depending on the strength of selection and the heritability of the trait, a population can rapidly adapt to new environmental conditions if the trait is oligogenic.

Distinguishing genetic responses to natural selection from those of other evolutionary forces can be challenging, because selection does not frequently leave distinguishable footprints in the genome. Adaptation can be locally impeded or even offset by gene flow (i.e. 'gene swamping', Lenormand, 2002). Gene flow is any movement of individuals, and/or the genetic material they carry, from one population to another. When gene versions are carried to a population where they previously did not exist, gene flow can be a very important source of genetic variation. Selection processes may be particularly effective in marine invasive species, which generally display large population's size and a high level of genetic diversity (e.g. Simon-Bouhet et al., 2006). Large population sizes and dispersive phases of many marine species mean that populations are connected by high gene flow, opposing local adaptation (Nielsen et al., 2009). Most marine species have therefore traditionally been viewed as a collection of demographically open populations that are interconnected by high gene flow. This expectation followed from the apparent lack of dispersal barriers in marine systems and the fact that most marine invertebrates and fishes have planktonic larvae that spend days to months in the water column (Grosberg and Cunningham, 2001). However, this paradigm of well-mixed marine populations has changed considerably in recent decades (Palumbi, 2004, Levin, 2006). Recent theoretical and empirical studies have shown that even in the face of considerable gene flow and no differentiation at neutral loci, selection from environmental heterogeneity can still result in adaptation (Nosil et al., 2009, Michel et al., 2010, Yeaman and Whitlock, 2011, Feder et al., 2012). This is because different regions across the genome will show variability, where some genomic regions are more affected by genetic drift and gene flow, and less by selection, while other regions (or regions

linked by linkage disequilibrium) are more strongly influenced by selection (Nosil et al., 2009, DeFaveri et al., 2013). Selection acting on a few large effect genes can make them rapidly increase in frequency in the population, which can further boost divergence in the face of gene flow (Nosil et al., 2009, Comeault et al., 2014). Luttikhuizen et al. (2003) aimed to use a quantitative approach to test to what extent additive genetic variance contributed to observed shell shape variation for the bivalve *Macoma baltica*. Through a common garden experiment, and molecular variance they deduced that gene flow was on-going. This would lead to the assumption that the shell variation was due to phenotypic plasticity. However that hypothesis had to be rejected on the grounds that shell shape has shown a genetic component and those ecotypes were genetically different (heritability estimated at 23%). Supporting this, the offspring with distinct morphs when reared in a common garden maintained the shell shape exhibited by their parents. This highlights that even with on-going gene flow and high levels of dispersal, genetic variations among habitats exist. It also promotes the importance of combining quantitative methods with morphometric analyses to obtain insightful data on phenotypic plasticity and evolutionary mechanisms.

Disentangling and simultaneous quantification of the relative contributions of plasticity and genetic differentiation have been studied a lot recently, especially from the point of adaptation to climate change. Experimental approaches can provide powerful tests of local adaptation. These approaches generally take two forms: “common garden” experiments in the laboratory, and reciprocal transplant experiments in the field.

Assessing the association between genotype, phenotype and environment can help disentangle the relative effects of genetics and environment, which is important because biological invasions that lead to the formation of distinct ecotypes can sometimes lead to ecologically differentiated species (Adams and Huntingford, 2004) and even to adaptive radiations (Simpson, 1953, Schluter, 2000, Yoder et al., 2010, Lucek et al., 2014).

2.2. *Mytilus galloprovincialis* Lamarck, 1819

species*: Mytilus galloprovincialis* Lamarck, 1819
genus: Mytilidae
order: Mytilioida
class: Mollusca

The Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819, is one of the three commercially and ecologically important sibling species in the *M. edulis* species complex; together with *M. edulis* Linnaeus, 1758, and *M. trossulus* Gould, 1850. Based on Me 15/16 locus as a genetic marker, Hamer et al., (2012) showed that *M. galloprovincialis* is the most common mussel species in the Adriatic sea. As inhabitants of the mediolitoral zone, these organisms endure extreme environmental conditions, such as occasional drought, great differences in temperature and strong wave influence (Petricioli, 2007). Being marine broadcast spawners, reproduction involves gametes releasing directly into the water column, where they are exposed to turbulent environment. On such occasions, a sexually mature female can release over 25 million eggs (Ceccherelli and Rossi, 1984), from which, upon fertilization, planktonic larvae develop and freely float in the water column. This occurrence is important in many ways, in particular because of the species dispersal. Larval transport of the Mediterranean mussel can be manifested via ballast water, ship hull fouling, and, as it is commonly cultured, through aquaculture activities. It is traditionally grown in aquaculture throughout the Mediterranean, and more recently in the other parts of the world. Native to the Mediterranean Sea, *M. galloprovincialis* has also been introduced to the southern hemisphere (New Zealand, Australia, South Africa, Chile), the Northwest Pacific Ocean (Russia, Japan, Korea, and China), and the Northeast Pacific Ocean (British Columbia to Baja California, Mexico, with the apparent exception of Oregon and perhaps northernmost California) (Fofonoff et al., 2016).

Morphologically, they are characterized by the presence of a triangular, dark blue, brown or black bivalve shell, filtrating gills, no differentiated head, and a lack of radula. Other anatomical features such as adult byssal attachment and mantle fusion also play an important role in their adaptation as filter feeders and burrowers, respectively (Murgarella et al., 2016). Individual size is greatly affected by the characteristics of the biotope itself. The average height of the shell is 5-8 cm, but some individuals can grow up to 15 cm.

Some bivalves, as does *M. galloprovincialis*, show an atypical double uniparental inheritance (DUI) of mitochondria. In these species, all progeny inherit one mitochondrial genome from the mother (F-type), while males also receive a mitochondrial genome from their father (M-type). This DUI, initially described in *M. edulis* (Skibinski et al., 1994), has been extensively studied in the genus *Mytilus* (Zouros, 2000, Breton et al., 2006).

Despite the commercial and scientific interest in mussels in biology and aquaculture, the number of genomic resources available in public databases for these organisms is quite limited, and usually restricted to their transcriptomes. However, a draft genome is available for the *M. galloprovincialis* (Murgarella et al., 2016), as well as a transcriptome (Moreira et al., 2015).
Murgarella et al., (2016) carried out a whole-genome sequencing study and shed some light onto the genome complexity and (partial) gene repertoire of *M. galloprovincialis*. Mediterranean mussel *de novo* genome can be used to provide first insights into the composition and structure of genomes in non-model organisms. Authors estimated the genome size to be 1.6 Gb from the k-mer count data, but discrepancies between genome sizes estimated from sequencing and experimental data have been previously reported (Elliott and Gregory, 2015). Using flow cytometry, *M. galloprovincialis* was proposed to have a genome size of either 1.4 Gb (Ieyama et al., 1994) or 1.9 Gb (Rodríguez-Juíz et al., 1996). The genome size of *M. galloprovincialis* is only comparable with *Aplysia californica* genome, while those of *Pinctada fucata, Crassostrea gigas* and *Lottia gigantea* are 33, 66 and 75% smaller, respectively (Murgarella et al., 2016). The comparative analyses of the genomic features observed in *M. galloprovincialis* with other marine molluscs have shown that an important part of the genome in these organisms contains a large number of repetitive sequences (~30% of the genome), a feature that is also shared with many other marine molluscs. A comparative analysis with other molluscs further revealed a gene enrichment of gene ontology categories related to multixenobiotic resistance, glutamate biosynthetic process, and the maintenance of ciliary structures. Another notable characteristic is their natural resistance to diseases. The immune system of bivalves is solely based on innate defences, which play a prominent role in protecting these animals against invading microorganisms (Murgarella et al., 2016).

2.3. Environmental influence on morphology and internal anatomy

The calcitic and/or aragonitic shell of species in phylum Mollusca is an important characteristic as it protects against predators, parasites and environmental stress. It is a substratum for attachment of epibionts and transport of solutes and particles in the benthic environment. Shells also play a systemic role in the metabolism of molluscs, participating in the capture and deposition of respiratory CO_2 in the shell mineral (Wilbur, 1964, Wheeler, 1992) and in buffering of extracellular pH during environmental anaerobiosis (Crenshaw, 1972). The shells are produced by specialized epithelial cells of the mantle with the assistance of $CaCO_3$-transporting hemocytes (blood cells) (Wheeler, 1992). They consist of 3 major layers: the outermost proteinaceous layer called periostracum, and 2 mineralized layers called ostracum (middle layer) and hypostracum (inner layer), composed primarily of $CaCO_3$ crystals (Wheeler, 1992).

A few specific shell characteristics have been extensively studied (McDonald et al., 1991), such as thickness (Zieritz et al., 2010), width, length, height or their ratios (Blythe et al., 2008, McDonald et al., 1991, Zieritz et al., 2010). The internal anatomy of bivalves is also subjected to environmental variation, especially the ligament, and position and size of adductor and retractor muscles (Innes and Bates, 1999, Freeman, 2007). Ligament connects the separated shell plates and the adductor muscles control the opening and closing of the shell plates. In the planktonic veliger larva, the adductor muscle typically appears in two parts (the anterior and posterior adductor muscle) and is retained in post-metamorphic stage, although, in some species, one of the adductor muscles is lost after settlement (Baker and Mann, 1997). Anterior and posterior pedal retractors are the muscles mainly responsible for movement of the foot. They retract the foot and effect back-and-forth movements. It is known that mussels living in the subtidal zones have thicker shells and a wider posterior muscle than the mussels living in intertidal environments (Beadman et al., 2003, Savoya, 2012).

There are numerous environmental factors leading to hypothesis that mussels shell size and shape (together with mentioned muscles) are only partially heritable. Usually in nature, not only one of them changes and affects phenotype, but they rather alter simoultaniously. However, thanks to many explorations regarding mussel's phenotypic variability, literature history appoints to many specific environment – phenotype relations.

2.3.1. Ocean acidification

Seawater has substantial buffering capacity. However, variation in seawater chemistry due to factors such as elevated carbon dioxide (CO_2) levels caused by biological activity, freshwater inputs, and runoff from acidic soils, leads to shifts of seawater pH. Previous studies have shown significant effects of seawater acidification on genetic expression, changes in physiological responses, reduction of metabolic rate, as well as mortality of larvae (Hiebenthal et al., 2011, Byne, 2011, Melzner et al., 2012). An increase in the CO_2 concentration in seawater can impair shell deposition and increase shell dissolution rates, weakening the shells and affecting their functional properties in bivalves (Orr et al., 2005, Ries et al., 2009). Moreover, the energy costs of biomineralization may contribute to the basal metabolic costs of marine calcifiers, especially when $CaCO_3$ is lost due to erosion in acidic seawater (Wood et al., 2008). Beniash et al. (2010) demonstrated that the increase in CO_2 partial pressure (pCO_2) in seawater, and associated decrease in pH, have negative effects on physiology, rates of shell deposition and mechanical properties of the shells of eastern oysters *Crassostrea virginica* (Gmelin). High CO_2 levels (pH ~7.5, pCO2 ~3500 μatm) inhibited both shell and soft-body growth compared to the control conditions (pH ~8.2, pCO2 ~380 μatm). The high CO_2 conditions also led to changes in the ultrastructure and mechanical properties of shells, including increased thickness of the calcite laths within the hypostracum and reduced hardness and fracture toughness of the shells. These data strongly suggest that the rise in CO_2 can impact physiology and biomineralization in marine calcifiers such as eastern oysters, threatening their survival and potentially leading to profound ecological and economic impacts in ecosystems.

2.3.2. Predators

Predator–prey interactions are one of the most important biotic ecological features in shaping biologic diversity (Liew and Schilthuizen, 2014). Substantial work has been undertaken to understand inducible predator defences in adult bivalves (Freeman, 2007, Caro et al., 2008, Freeman et al., 2009, Brown et al., 2011). Mussels respond to predators foraging strategy with specific morphological defences (Freeman et al., 2009). There are a specific sets of responses to predator that have been observed in detail, including thickening of the shell, increased adductor

muscle mass, aggregation behaviors and the increased production of byssus threads (Caro et al., 2008).

In order to maximize their rate of energy intake, foraging predators must select prey that yields the maximum amount of energy for the minimum amount of time taken to search for and handle (MacArthur and Pianka, 1966, Krebs, 1978). 'Handling time' for prey of a certain size or morphology, and consequently, 'optimal prey size/morphology' will differ depending on the type of foraging technique used by a given predator (Zieritz et al., 2012). Generally, the studies focus on a single predator interaction (Freeman and Byers, 2006, Brown et al., 2011, Eschweiler and Christensen, 2011) and search for induced responses. However, in natural world, single predator environments are uncommon, unless the organism is near the apex of the trophic web. Therefore, it is important to understand what the induced responses would be to multiple predators at the same time. Freeman et al., (2009) investigated the induced response of *M. edulis* to multiple predator effluents simultaneously. The result of this study showed poor phenotypic integration, which is indicative of a trade-off in predator resistance (DeWitt et al., 2000), and not an inability to recognise cues. Moreover, the volume of previous studies on single predator recognition and defence serves in support of this hypothesis. Specifically, Freeman and colleagues (2009) presented *M. edulis* with multiple potential predators in pairwise combinations and obtained data on shell thickness adductor muscle mass and behaviours. In response to the crab *Carcinus spp.* alone mussels developed thicker shells, whereas when alone with the sea star *Asterias spp*. they developed larger adductor muscles. During simultaneous exposure to both predators, thickening of the shell was not observed; even when functionally similar groups such as other species of crab, like *Cancer spp.* were in combination. This counterintuitive find of functional groups suggested to the authors that the inducible defences are species dependent and often lead to poorly integrated responses to combinations of predators. One method that may be more beneficial to mussels is not to devote energy into specific shell defences that only protect against one predator, but to grow bigger. If an organism achieves size refuge then the morphological defences are not required (Hoverman et al., 2014). It is likely that with more than one species of predator present in natural communities mussels invest in the more likely predator and become phenotypically specialist. They may also attempt to reduce likelihood of predation through attaining a size protection. Either way using quantitative and molecular techniques could shed light on the processes on evolution at work.

2.3.3. Community structure and food availability

Mussel's sedentary filter-feeding life style allows them to feed on a wide spectrum of planktonic organisms; phytoplankton, zooplankton, bacteria, and dissolved organic matter (Gavrilović et al., 2011). The growth rate of *M. galloprovincialis* depends on intra-specific competition due to the density of animals within the mussel bed. It is shown that density is an important environmental factor for the genus *Mytilus* (Seed, 1968), whereby the higher population density and the smaller quantity of available food lead to narrower and elongated shells compared to those growing in conditions of low density. Additionally, it is likely that during strong pCO_2 stress coupled to food limited conditions, energy is allocated to more vital processes (e.g. somatic mass maintenance) over inner shell surface integrity (Melzner et al., 2011).. The extension of the shell can also provide a more favorable position of siphon with regard to food access (Senechal et al., 2008) which is also considered to be adaptation to food competition at high population density (Alunno-Bruscia et al., 2001). High-density mussels can be stretched to the edge of the population where there are less restrictions for opening the valves (Lauzon-Guay et al., 2005).

2.3.4. Wave exposure

In communities that inhabit the tidal zone, there hydrodynamic changes caused by waves are constantly present (Gaylord et al., 1994). Waves are not only moving organisms, but also regulate food supply and pathogen delivery, and play a key role in shaping the structure and dynamics of life communities (Paine and Levin, 1981). Therefore, wave force has been reported as another factor influencing the characteristics of the shell shape (Bell and Gosline, 1997). Akester and Martel (2000) determined striking differences in shell morphology between *M. trossulus* collected from wave-exposed and sheltered sites. *M. trossulus* shells tended to be thicker and have lower shell height / shell width ratio at wave-exposed sites, perhaps reducing the effect of hydrodynamic forces (Akester and Martel, 2000, Steffani and Branch, 2003). Mussels from wave-exposed sites had a thicker hinge ligament as well (Akester and Martel, 2000). These observations suggest that wave exposure could be the cause of the observed phenotypic plasticity in both shell morphology and ligament thickness.

2.3.5. Salinity and temperature

Seawater salinity and temperature are the most important environmental factors for organisms distribution in the rocky coastline (Hiebenthal et al., 2012). Hamer et al., (2010) showed that the salinity is relativelyconstant in the open waters of the Adriatic sea, but varied in intertidal zones, estuaries, locations close to under-sea freshwater springs and during rainy days in closed lagoons on different locations along the coast. Salinity changes are affecting organism's size, age, phenotypic, and genetic structure and geographical distribution (Shurova, 2001). Researchers showed that lower salinity reduces shell stability (Blythe and Lea 2008), probably due to lower availability of calcium and carbonate for biomineralization (Bayne 1976, Shields et al., 2008). More specifically, carbon (δ13C) in mussel's shell might be used as an indicator of environmental salinity and hypo-osmotic stress (Hamer et al., 2010). Furthermore, the morphometric shell variability has shown a correlation with the gradient of salinity, according to which elongated specimens are found in the area of lower salinity (Valladares et al., 2010). Temperature is another factor influencing physiological and biochemical processes at seawater organisms (Petes et al., 2007). Seasonal decline in population may be related to temperature, i.e. thermal stress as a cause of mortality in mussels (Shields et al., 2008).

2.3.6. Pollution

Due to their non-selective filter-feeding nature and accumulation of chemical contaminants from a large quantity of seawater, environmental quality is a key factor in the growth and development of the mussels. The concentration of chemicals in their tissue (organic and inorganic substances, heavy metals such as Cu, Zn and Hg; Steinert et al., 1998) can reach 1000 times the seawater concentrations.

Metals represent one of the most studied groups of molecules. Metal contamination is a matter of huge concern, especially in marine environments, due to their abundance, persistence and subsequent bioaccumulation (Barlas et al., 2005, Khedher et al., 2014). They can either be accumulated and persist in the sediments, and/or be released from sediments, acting as a back source to the overlying water during natural or anthropogenic disturbance (Chalghmi et al., 2016). Furthermore, it is also important to understand the interactions between metals and their spatio-temporal variation in coastal environments. At a cellular level, metal toxicity mainly

involves the generation of oxidative stress, leading to reactive oxygen species (ROS) generation, which can cause adverse cellular effects such as DNA damage, protein oxidation and/or lipid peroxidation. The sources of heavy metal pollution are the anti-fouling colors, communal waters of urban areas, industrial waste water, and natural rock wear. Today, copper and zinc are used as active ingredients in biocides (Chen et al., 2011). Such chemicals also have a toxic effect on organisms, inhibiting the Krebs cycle, inducing oxidative stress and related mutations and affecting the proper functioning of the reproductive system (Fitridge et al., 2012). There is currently no convention to regulate the entry of these heavy metals into the marine environment. However, exposure to contaminants for a prolonged period can lead to acclimatization (phenotypic change during a lifetime of given genotype) and some level of adaptation (refers to change over several generations - evolutionary process - within a populations or species). Thanks to acclimatization, individuals in the polluted environment are more physiologically tolerant and have longer lasting survival in the air than individuals collected in non-polluted areas (Koukouzika and Dimitriadis, 2005). For example, mussels from polluted sites show elevated values of LT50 (Koukouzika and Dimitriadis, 2005, Hiebenthal et al., 2012), a fact that supports the assumption that some degree of adaptation to pollution can be developed in mussels.

2.4. Morphometry

Shell shape is routinely used for morphological recognition in the taxonomy of bivalves. It is particularly useful in those cases when genetic studies cannot be performed, as happens with fossil and many archaeological records (Gardner, 2004). Shell shape is a key morphological characteristic reflecting phylogenetic history, function and life habit (Crampton and Maxwell, 2000) and has been used for discrimination among species of genus *Mytilus* (McDonald et al., 1991, Innes and Bates, 1999, Gardner, 2004, Krapivka et al., 2007, Beaumont et al., 2008, Valladares et al., 2010). Variations in shell shape have been examined using ratios of shell length, height and width (Seed, 1968, Beaumont et al., 1989). Direct analysis of bivalve shell shape, based on a digitized outline, has been developed using elliptic Fourier analysis (Ferson et al., 1985, Crampton, 1995), which analyses complex outlines with little loss of shape information (Rohlf and Archie 1984, McLellan and Endler, 1998). Innes and Bates (1999), for instance, found morphological differences between *Mytilus edulis* and *Mytilus trossulus* from a sympatric

population, proving the existence of differentiation in shell morphology related to the mussels genotype of the mussels even under similar environmental conditions.

2.4.1. Traditional morphometry

Traditional morphometry applies multivariate statistical methods (e.g., principal components analysis, canonical variety analysis, discriminant function analysis, or multivariate analysis of variance) to a set of traits measured on each individual (Parsons et al., 2003). In many instances, these traits are linear distances measured between pairs of landmarks on the body, or body parts. The measurements are usually taken with a floating point or calliper, a hand-held measuring instrument with a precision of less than one millimeter. The results are mostly expressed numerically and graphically in terms of linear combinations of the measured variables.

Increased computing power drove the development of traditional morphometrics in the 1960s and 1970s to the point that permitted simultaneous analysis of multiple traits, which was an obvious improvement over univariate approaches (e.g., Jolicoeur 1963, Parsons et al., 2003). However, limitations relating to these traditional methods became increasingly obvious (e.g. linear lengths are strongly positively related to body size, the same set of lengths measures could be obtained from two different shapes because the location of where the lengths were made relative to one another was not included in the data.). These issues may be overcomed using a geometric morphometric method, which allows analysis of the overall shape of the individual, independently of its size (Rohlf and Marcus, 1993, Bookstein, 1996, Adams et al., 2004).

2.4.2. Geometric morphometry

Geometric morphometric methods focus on the geometry of form estimated using the relative locations of landmarks (and sometimes outlines) rather than on linear measurements taken between landmarks. In a review Rohlf and Marcus (1993) emphasized applications of geometric morphometric to exploratory studies in taxonomy and evolution. Data are recorded to capture the geometry of the structure being studied. This is in the form of two dimensional (2-D) or three-dimensional (3-D) coordinates of morphological landmark points. One can check their adequacy in covering the structures of interest by a visual evaluation of a graphical display of the landmarks. Rather than just reporting that the shape is different, one can report that certain

structures have moved relative to others. If one is interested in the overall outline or surface of a structure (or of just parts of a structure between landmarks in 2-D or a surface in 3-D), then this can be captured by a sequence of points along the outline or over a surface.

Geometric methods still require the same set of homologous landmarks on all specimens. Unfortunately, specimens can be missing landmarks if they are broken, poorly preserved, if structures are articulated differently, or landmarks found on one taxa are not present on another. Options are limited in these cases. Variant landmarks are either eliminated from the analysis (effectively reducing shape information), or damaged specimens missing landmarks are eliminated from the data set when rare, or missing landmarks are estimated using sample means (Adams et al., 2004). Despite these problems, proponents of the geometric methods have claimed significant progress at solving many of the limitations of traditional morphometric methods (Rohlf and Marcus, 1993, Adams et al., 2004).

2.4.3. Fluctuating asymmetry (FA)

Phenotypic variation of a species can be examined at different organizational levels: (i) among populations; (ii) among individuals within a population; and (iii) within an individual. Most studies take place at the first level, i.e., comparing populations described by the mean values of morphological characters. The third level - variation within an individual - expresses differences between an individual's symmetrical structures, i.e., as fluctuating asymmetry (FA), the random non-directional deviations from perfect symmetry (Van Valen, 1962). FA has been examined in a variety of plants and animals, and promoted as a useful bioindicator of exogenous stressors in habitats, whether of natural or anthropogenic origin (Allenbach, 2011). The homeostatic control of morphological development, or developmental stability (DS), may be perturbed when naturally-occurring or anthropogenic stressors are experienced during development. Consequently, development does not follow its pre-programmed trajectory, and aberrant phenotypes, including deviations of bilateral asymmetry, may result. While no bilateral structure is perfectly symmetrical, the inference is that greater degrees of asymmetry arise when organisms are exposed to exogenous environmental stressors during development (Allenbach, 2011). FA has generated interest among population biologists because it potentially reflects one of the components of fitness - developmental stability, i.e., the ability of an organism to

consistently produce an 'ideal' phenotype in a given environment. Although an association between FA and fitness is not always observed in empirical studies, recent reviews concluded that, overall, FA can be considered a useful tool for assessing a population's average fitness (Allenbach, 2011, Graham et al., 2010). A number of reviews examine the relationship between FA and environmental stressors across broad phylogenetic scales (e.g., Leary and Allendorf, 1989, Graham et al., 1993b, Møller, 1997, Moller and Thornhill, 1998, Hoffmann and Woods, 2003, Graham et al., 2010).

2.5. Oxidative stress

In polluted environments and especially in coastal waters, living organisms are often exposed to complex mixtures of chemical contaminants. Because of the diversity and variability of the chemical threat, defense mechanisms exhibit considerable versatility and adaptability. Among the strategies that have been developed by organisms at the cellular level to protect themselves from the toxic effects of anorganic or organic compounds, the major ones are the antioxidant defense systems. Excessive production of reactive oxygen species (ROS), caused by environmental stress or large amounts of xenobiotics, can trigger a change in the balance between oxidants and antioxidants, resulting in oxidative stress. Oxidative stress therefore delineates an imbalance between the production of ROS and the organism's antioxidant defence (Betteridge, 2000). ROS are unstable atoms or molecules that contain an unbalanced electron in the outer shell. In order to become more stable, they can take electrons from other molecules, causing the formation of new radicals and oxidation chains (Halliwell and Gutteridge, 1984). ROS naturally occur during the cellular aerobic metabolism as a result of partial oxygen reduction to water, or as a by-product during the certain xenobiotics metabolism (Livingstone et al., 1990). The main reactive oxygen species, formed by the metabolism or contaminants, are superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$), peroxyl radicals ($ROO^{\cdot}$), alkoxyl radicals ($RO^{\cdot}$) and peroxynitrite ($OONO^{-}$) (Camus et al., 2004). Low levels of free radicals are necessary for maintenance of the cell homeostasis (Ames et al., 1993), signaling mechanisms and regulation of various cellular functions such as secretion, growth and gene expression (Halliwell and Gutterigde, 1997). However, longer exposure leads to oxidative damage on DNA, lipids and proteins (Kaloyianni et al., 2009). In that case, ROS can induce tissue damage, change physiological and chemical properties of cell membranes and disrupt vital

organs (Manduzio et al., 2005). A complex defence system, consisted of non-enzymatic components and antioxidative enzymes, provides a cell protection from the free radical toxicity (Regoli, 1998). Specifically, in the mussels, the antioxidative defense system contains enzymes such as catalase, glutathione S-transferase, superoxide dismutase, glutathione reductase, and non-enzymatic glutathione molecule (Livingstone, 2001). Many studies have shown a positive correlation between the degree of antioxidative defense and the presence of xenobiotics in the organism (Orbea et al., 2002). Measurements of oxidative damage, such as lipid peroxidation, protein carbonylation, and antioxidative response are often used as biomarkers in ecotoxicological researches and considered a good method for analyzing the various environmental impacts on the individuals (Vidal-Liñán et al., 2010).

2.5.1. Oxidative stress biomarkers

Biological threat of the high number of chemicals and their complex mixtures can only be partially monitored through chemical methods (Muir and Howard, 2006), because they do not provide a true indication of the toxic effects on the marine ecosystems (Livingstone, 2001). Concentration of contaminants in the organism's tissues does not provide explicit information of its biological significance or exact harmful effects. In order to overcome this limitation, biological responses must be used in monitoring programs in addition to chemical analyses (Ices, 2008). To achieve this, many biological monitoring methods have been developed, ranging from the biological feedback on the sub-cellular level, to the whole organism responses. Biomarkers reveal environmental stress caused by chemical contaminants, as well as other environmental variables. Thus, integration of biomarkers and chemical analysis is essential in order to establish links between stress and pollution (Galloway et al., 2004). Biomarkers may be defined as quantitative measurements of changes occurring at cellular, biochemical, molecular, or physiological levels, that can be measured in cells, body fluids, tissues or organs and that may be indicative of xenobiotic exposure and/or effect (e.g. McCarthy and Shugart, 1990, Lam and Gray, 2003, Allen and Moore, 2004).

The main function of the biomarkers is to give early alert signals to significant biological changes, as it is considered that responses at lower levels of organisms come before those at higher levels of biological organization (e.g. population, community, or ecosystem). The

biomarker techniques are further complicated by a range of natural environmental and biological factors and processes (e.g. seasonality, reproductive cycle, body mass, quality of available food, etc.) potentially interfering with the effects of contaminants on the biological responses of monitored organisms (Viarengo et al., 1991, Astley et al., 1999, Shaw et al., 2004, Lesser, 2006). Most studies on biomarkers have been carried out on fish (Lemaire and Livingstone, 1993, Rodriguez-Ariza et al., 1993, Fenet et al., 1996, Van der Oost et al., 1996, Eufemia et al., 1997) and marine invertebrates (Livingstone et al., 1990, Porte et al., 1991, Ribera et al., 1991, Livingstone et al., 1992, 1995, Regoli and Principato, 1995, Labrot et al., 1996, Fitzpatrick et al., 1997, Telli Karakoc et al., 1997).

A battery of biomarkers, including both enzymatic and molecular parameters, is used in environmental risk assessment.

Catalase (CAT) is a commonly studied antioxidant enzyme involved in the initial antioxidative mechanism and widely used as a biomarker in mussel (Cajaraville et al., 2000, Khessiba et al., 2001, Nasci et al., 2002, Lau and Wong, 2003, Roméo et al., 2003). It reduces H_2O_2, originated from the superoxide dismutase enzyme (SOD), to produce water and oxygen. This enzyme can also act as peroxidase, for which several organic substances, especially ethanol, can act as a hydrogen donor. It occurs in almost all aerobically respiring organisms and is localized in peroxisomes (Jourmi et al., 2015).

The glutathione-S-transferases (GST) are a group of quantitatively the most important biotransformation enzymes, involved in the metabolism of lipophilic organic contaminants (Fitzpatrick et al., 1997). These enzymes also play a role in protection against oxidative stress by catalyzing a selenium-independent glutathione-peroxidase activity (Prohaska, 1980). They catalyse conjugation reaction of glutathione with various organic compounds including PAH.

Glutathione reductase (GR) does not play a direct role in the elimination of oxygen radicals. However, it is considered as an essential antioxidant enzyme because it reduces oxidative glutathione (GSSG) and maintains the balance of GSSG / GSH that is necessary for homeostasis and other enzymes activity (Winston and Di Giulio, 1991). Cell redox status is generally determined by the ratio of reduced (GSH) and oxidized glutathione. In that sense, GR and

NADPH maintain this ratio in favor of GSH (Schafer and Buettner, 2001). If the ratio is more in favor of the GSSG, apoptosis may occur (Matés and Sánchez-Jiménez, 2000).

Malondialdehydes (MDA) are a naturally occurring products of lipid peroxidation and prostaglandin biosynthesis, that are mutagenic and carcinogenic (Marnett, 1999). They react with DNA to form adducts (Marnett, 1999). Increasing amount of MDA in the tissues can be associated with environmental degradation and decreased water quality (Charissou et al., 2004). Research has shown that lipid peroxidation is a relevant index of biochemical damage induced by toxins (Pedrajas et al., 1995). They are considered useful biomarkers for measuring the level of oxidative stress (Del Rio et al., 2005).

The effect of oxidative damage on proteins is the formation of carbonyl groups (Stadtman and Berlett, 1998, Zusterzeel et al., 2001). Exposure to ROS can cause irreversible modifications of protein's aminoacid side chains (mostly lysine, arginine, proline and histidine) into aldehyde and ketone groups (carbonylation), which can lead to aggregation, inactivation or degradation of proteins (Levine et al., 1990). One such modification is formation of carbonyl moieties (-C=O) at amino acid side chains. Carbonyl groups can be introduced in proteins by a number of different pathways, predominantly via metal catalysed oxidation (MCO) but also via adduction of oxidized lipids or sugars containing carbonyls (Requena et al., 2003). Protein carbonyls can also form via secondary mechanisms resulting from reactions of free radicals with other cellular constituents, such as lipids, carbohydrates and nucleic acids (Grune, 2000). An increase in the number of carbonyl groups correlates well with protein damage caused by oxidative stress (Shacter et al., 1994). The formation of carbonyl derivatives is non-reversible, causing conformational changes, decreased catalytic activity in enzymes and ultimately resulting in breakdown of proteins by proteases due to increased susceptibility (Almroth et al., 2005).

2.5.2. Neurotoxicity biomarker - Acetylcholinesterase (AChE)

Acetylcholinesterase (AChE) is an essential enzyme for the correct transmission of nerve impulses since it catalyzes the degradation of acetylcholine, the most important neurotransmitter in the nervous system of many animals (Bocquené and Galgani, 1991). AChE is commonly found as a transmembrane protein in various cell membranes of the invertebrates, such as

membrane glands and digestive glands and hemolymph. Since AChE is susceptible to neurotoxic substances, measurement of its activity is widely used as a sensitive neurotoxicity biomarker in mollusks (Valbonesi et al., 2003, Rickwood and Galloway, 2004). Inhibition of AChE is directly related to the toxic effects of organophosphate, carbamate pesticides (Galgani and Bocquené, 1989) and some metals and hydrocarbons (Jebali et al., 2006, Banni et al., 2010).

2.5.3. Genotoxicity biomarker – DNA damage

In a process of determining stress caused by contaminants in living organisms assessment of DNA damage is of great importance. In order to monitor genotoxicity in marine systems, the single-cell gel electrophoresis assay method (comet assay) can be used. The comet-assay is a method based on the mobility of damaged DNA portions, in the electrical field, resulting in their separation from nucleoids that are immobilized in agarose gel. It is possible to detect cumulatively various forms of DNA damage in particulate cells, in many organisms and various cell types. Comet-assay is capable of detecting single-stranded and double-stranded DNA fractures, DNA-proteins or DNA- DNA cross-linking and lysine-sensitive sites (apurin or apurimidine sites), depending on the pH buffer during the denaturation and electrophoresis of DNA (Rojas et al., 1999, Tice et al., 2000).

2.6. Survival as the proxy for fitness

Within evolutionary biology, fitness can be interpreted as the quantitative representation of natural and sexual selection (Williams, 1996) because it merges selection related concepts (e.g. survival, reproduction) into one idea. Selection tends to make alleles underlying traits that confer higher fitness more common over time, resulting in Darwinian evolution. Term fitness is also used to describe how good a particular genotype is at leaving offspring in the next generation - 'Survival of the fittest' (Eertmann and de Zwaan, 1994). Therefore, the fittest individual is not necessarily the strongest, fastest, or biggest. A genotype's fitness includes its ability to survive, find a partner, produce offspring — and ultimately leave its genes in the next generation. It might be tempting to think of natural selection acting exclusively on survival ability — but, as the concept of fitness shows, that anyhow is a half of the story (https://evolution.berkeley.edu/evolibrary/article/evo_27). While the reproductive success of

mussels cannot be directly measured, many studies use the estimates of growth and survival as proxies for fitness (Gardner and Skibinski 1990, Gardner et al., 1993, Arnold, 1997). Survival time in air can indicate the general health of marine organisms (Viarengo et al., 2007). Species from genus *Mytilus* are able to survive aerial exposure for many days, but under sustained aerial exposure the mussels will eventually die. The 'Stress on stress' (SOS) test is a physiological biomarker used to evaluate mussel resistance to air exposure (Eertman et al., 1993). Various studies have demonstrated that bivalves exposed to contaminants have reduced tolerance to anoxia. Stress on stress response can therefore be proposed as an index of general stress at the organism level which can be applied as a monitoring tool for the assessment of contaminated coastal areas (Viarengo et al., 1995*).* The SOS response was first experimentally tested in mussels by Veldhuizen-Tsoerkan et al. (1991). Short term laboratory exposure to cadmium and long term exposure to PCBs resulted in a significantly reduced tolerance to aerial exposure. The first application of the "Survival in air" response of mussels following field exposure produced significant inverse correlations between tissue contaminant concentrations and tolerance to aerial exposure (Smaal et al., 1991) confirming the potential of this parameter as stress indicator in coastal waters (Eertman et al., 1993).

2.7. Genetic architecture

Ecology and conservation biology have developed greatly in recent decades through the use of genetic markers in investigating organisms and evaluating the effect of environmental disturbances (Narum et al., 2013). However, many of these studies have been limited to narrow regions of the genome, making it difficult to generalize about the organisms and their evolutionary history. Advances in sequencing technology (i.e. next-generation sequencing; NGS) have enabled to sample the genome much more densely, and observe the patterns of genetic variation that results from the full range of evolutionary processes acting across the genome (Allendorf et al. 2010, Stapley et al. 2010). Yet, uncovering the evidence of genetic adaptation is almost always hampered by the effects of evolutionary phenomena such as genetic drift, phenotypic plasticity, complex demographic history and complex genetic architecture (Villemereuil and Gaggiotti, 2015).

Genetic architecture refers to the underlying genetic basis of a phenotypic trait and its variation (Hansen, 2006). A description of genetic architecture may include statements about gene and allele number, the distribution of allelic and mutational effects, patterns of pleiotropy, dominance, and epistasis. Despite the obvious complexity of the developmental processes that underlie the genetic architecture, it is necessary to understand it for many biological questions, including speciation, the survival of small populations, inbreeding, understanding diseases, understanding the processes and genetics of adaptation and population differentiation. Because it describes or determines the phenotypic traits variations, and thus their evolutionary potential, understanding the evolution itself depends upon understanding the evolution of genetic architecture. R. A. Fisher's (1930) geometric theory was one of the first into explaining how genetic architecture is shaped by – and can shape – adaptive evolution. He mathematically reasoned that many genes of small effect were likely to control traits (Agrawal et al., 2001). On the other hand, it is thought that mutations in large-effect loci play an important role in allowing populations, which are far from their phenotypic optimum, to rapidly adapt (Orr, 1998). Because of this, large-effect loci are thought to be important during initial stages of adaptation to a new environment; these traits will later be tweaked to an optimum state by changes at small-effect loci (Nadeau and Jiggins, 2010). Large-effect loci might also play an important role during divergence with gene flow. If the effect of a locus on fitness has a magnitude greater than the rate of gene flow, then adaptive divergence can occur with greater ease (Slatkin, 1987).

2.7.1. Genotyping-by-sequencing (GBS)

Understanding the genetics basis has been limited by the high cost of *de novo* genotyping of species with limited marker data. Non-resource-prohibitive methods that overcome the limitation of genotyping are now available. The ability to screen genome polymorphism data through genotyping such as RAD-tag, multiplexed shotgun genotyping or genotype-by-sequencing (GBS) (Baird et al., 2008, Andolfatto et al., 2011, Elshire et al., 2011), allows estimates of heritability even for natural populations of non-model species. Genotyping-by-sequencing (GBS) has been developed as a rapid and robust approach for sequencing of samples that combines genome-wide molecular marker discovery and genotyping (Poland and Rife, 2012). The flexibility and low cost of GBS makes this an excellent tool for many applications and research

questions. It can offer the screening of thousands of polymorphisms throughout the genome. Single nucleotide polymorphism (SNP) is a variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population. Such 'variable' SNPs are particularly valuable for quantitative genetic and evolutionary studies, because they represent the most abundant class of genetic variations in eukaryotic genomes and have a great potential for quickly identifying causal genes responsible for either complex traits or adaptive evolution (Jiao et al., 2014). However, SNP markers have been insufficiently developed for molluscs in comparison with well-studied model organisms.

There is increasing number of studies utilizing genome scans to search for potentially adaptive genetic variation in a population genomics context, as well as to estimate demographic parameters. Various species of plants, marine invertebrates, marine and freshwater fish, and small mammals are included, making novel inferences regarding selection in natural populations using genetic markers (Catchen et al., 2013, Corander et al., 2013, De Wit and Palumbi, 2013, Hyma and Fay, 2013, Keller et al., 2013, Reitzel et al., 2013, Roda et al., 2013). Multiple papers demonstrate the utility of GBS for phylogenetic reconstruction across species (Jones et al., 2013, Keller et al., 2013, Ogden et al., 2013, Roda et al. 2013). Additionally, some papers take advantage of GBS to identify genomic regions involved in hybridization (Hohenlohe et al., 2013), speciation (Jones et al., 2013) and divergent adaptation (Keller et al., 2013). GBS has also been shown as useful to reveal how heterogeneous recombination rates can modulate consequences of selection and influence outlier tests for positive selection in stickleback populations (Roesti et al., 2013).

2.7.2. Genome – wide association study (GWAS)

Linking underlying genetic architecture to phenotypic variation is a key component to understanding the evolutionary responses. Identifying genetic basis of a trait can answer the question whether traits are largely controlled by many loci of small effect (polygenic genetic architecture), or by few loci of large effect (oligogenic architecture). Fortuitously, methods to estimate quantitative genetic parameters in natural populations have evolved rapidly during the last 10 years in parallel with advances in genomic technology. Two main approaches are used to disentangle relative contribution of genotype and environment on a phenotype; quantitative trait

loci (QTL), and genome wide association (GWA) studies. QTL analyses are accomplished by scanning recombinant mapping created from controlled (e.g., laboratory) genetic crosses for genetic regions that are associated with phenotypic variation (Barton and Keightley, 2002, Slate, 2005, Comeault et al., 2014). Although QTL studies have benefits, they require either a detailed population genealogy or controlled crosses (Slate, 2005), often lack sufficient recombination for fine-scale mapping (Buerkle and Lexer, 2008), and characterize genetic variation that is not necessarily representative of that found in natural populations (Rockman, 2012).

Genome-wide association study (GWAS) is a powerful way to estimate the genetic architecture of morphological traits and search for statistical associations between genotypes at specific loci in natural populations (Hirschhorn and Daly, 2005). This method identifies numerous genetic variants (e.g., SNPs), associated with traits. A substantial fraction of these identified loci often display association with more than one trait — a phenomenon known as pleiotropy (Solovieff et al., 2013). GWAS takes advantage of potentially lower levels of linkage disequilibrium (LD) due to longer histories of recombination existing within natural populations than in controlled crosses (e.g. Cho et al., 2009, Brachi et al., 2010, Fournier-Level et al., 2011). It has been primarily carried out in model genetic systems and employed to understand the genetic underpinnings of complex human diseases, although studies of non-model species are rapidly accumulating. Now, with the advent of RADseq and GBS it is technically feasible in any system (Kingston, 2017) and can be achieved in a large number of individuals (e.g., Gompert et al., 2010, Hohenlohe et al., 2010, Elshire et al., 2011, Andolfatto et al., 2011). GWAS in *Arabidopsis thaliana* provided some of the best examples of the genetic architecture of complex traits in nature and it has been shown that numerous loci of minor effect underlie traits variation (Brachi et al., 2010, Fournier-Level et al., 2011). Berg and Coop (2014) have further combined knowledge from GWAS with robust population genetic modeling to identify human traits that show putative signals of local adaptation. Comeault and colleagues (2014) described the genetic architecture of traits that are subject of differential selection between host plant species in stick insect *Timema cristinae*, to better understand the evolution of adaptive traits and how trait divergence between natural populations on different hosts occurs in the genome. They assert that employing the GWAS is a powerful way to estimate the genetic architecture of complex traits controlled by many loci with minor phenotypic effects, as exemplified also by recent GWAS in model genetic systems. GWAS are now routinely applied in a range of model organisms and to

non-model systems; Arabidopsis (Atwell et al., 2010), mouse (Flint and Eskin, 2012), crops (Wang et al., 2012), cattle (Olsen, 2011). SNPs associated with disease resistance, heat tolerance, head size, and hypoxia tolerance were reported in catfish (Geng et al., 2016, Jin et al., 2017, Wang et al., 2017, Zhou et al., 2017), and SNPs associated with propensity to migrate, survival under thermal stress, and bacterial cold water disease resistance were reported in trout (Hecht et al., 2013, Narum et al., 2013); similar researches were carried out in Atlantic salmon (Ayllon et al., 2015, Tsai et al., 2015). However, there are only a few papers discussing genetic components affecting bivalve's morphology. Already discussed example, by Luttikhuizen and colleagues (2003), used a quantitative approach to test if genetic background contributed to observed shell shape variation in the bivalve *Macoma balthica* in presence of high gene flow. They have concluded that these morphological variants originate at least partly due to divergent phenotypic selection and that intraspecific adaptive genetic differentiation in marine broadcast spawners is apparently not constrained by a high gene flow. Jones and colleagues (2013) investigated the genetic architecture of complex pearl quality traits in the pearl oyster, *Pinctada maxima* and presented quantitative trait loci (QTL) and genetic association for these traits. The results provided strong evidence that pearl quality traits have a low to moderate additive genetic component (h^2 from 0.14 to 0.34), and also supported previous quantitative genetic studies that these traits are polygenic in nature. Kingston et al., (2017) used GWAS on *Mytilus edulis* and *M. trossulus*, native to the Gulf of Maine (GOM). Aim of their study was to reveal the genetic basis of a trait predicted to be under strong, multifarious selection in the next 100 years - the net rate of calcification. They used predictions from the global circulation models under high emissions scenarios to guide simulated physical and biological conditions likely to occur in the Gulf of Maine (GOM) by the year 2100. Authors expected natural selection to maximize net calcification (calcification minus any $CaCO_3$ lost through dissolution) under increasing environmental stress. They found that under projected climate stress from multiple variables, blue mussels from the (GOM) exhibit extensive variability in calcification rate phenotype, and this variation is linked to a handful of loci of moderate effect. Estimates of narrow-sense heritability for this key trait were on the order of 30% – indicating that substantial genetic variation for calcification under climate stress exists within these populations.

A potential limitation of using GWAS in new systems or traits is the statistical power to detect QTL with potentially small effects. A working assumption is that most organisms are well-

adapted to long term, stable conditions; however, there may be rare alleles segregating in the population that will be acted upon by selection as conditions change. The power to detect loci of moderate effects with a GWAS will increase when the phenotypic variance is maximal. Kingston et al. (2017) have shown that the phenotypic response under multivariate climate stress was significantly more variable than under more ideal control conditions. Related to this increased variance under stress, environmental changes can uncover novel genetically determined phenotypes for selection to act upon (Waddington, 1956).

3. MATERIALS AND METHODS

3.1. Sampling design

In October 2013 and March 2014 native populations of Mediterranean mussels *Mytilus galloprovincialis* were collected at 14 and 15 sites respectively, along the Eastern Adriatic coast (Figure 2). Sampling sites were chosen to cover wide range of geographical locations with different pollution intensity, characterized as clean or polluted, based on the historical and literature data (Petrović et al., 2004, Klobučar et al., 2008, Štambuk et al., 2013).

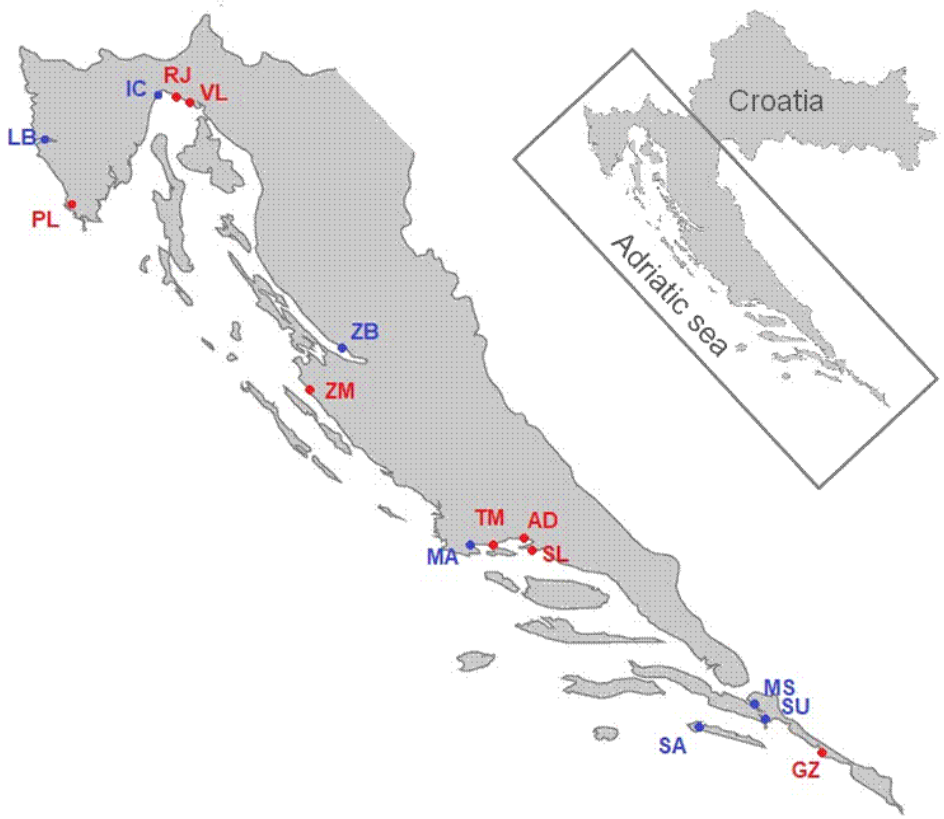

Figure 2. A map of the study populations and location of sampling sites.A map of the study populations and location of sampling site:clean sites are marked by blue color – Lim Bay (LB), Ičići (IC), Zadar Seline (ZB), Marina (MA), Ston (SU), Mali Ston (MS), Babine kuće (SA, National park Mljet); polluted sites are marked by red color – Rijeka (RJ), Viktor Lenac (VL), Pula (PL), Zadar marina (ZM), Trogir (TM), Adriavinil (AD), Split (SL), Gruž (GZ).

Reference sites were mainly represented by native populations sampled at aquaculture sites, as those are regularly monitored for pollutant occurrence - Lim Bay (LB), Zadar Seline (ZB), Marina (MA), Ston (SU, sampled only in spring), marine protected areas (national parks and special reserve) - Babine kuće (SA, National park Mljet), and small villages without any industrial plants - Ičići (IC), Mali Ston (MS). Those sites are further through text referred as "clean". Polluted sites were represented by populations sampled at heavily trafficked harbours and marinas with high boat maintenance activities - Pula (PL), Rijeka (RJ), Zadar marina (ZM), Trogir (TM), Split (SL), Gruž (GZ), big shipyard - Viktor Lenac (VL) and polluted industrial area Adriavinil (AD). Most of those sites have previously been characterised as polluted or pinpointed as the pollution hotspots in Adriatic (Petrović et al., 2004, Klobučar et al., 2008, Kljaković-Gaspić et al., 2010, Štambuk et al., 2013). Mussels were collected from 0.5 to 1 m depth at each site using metal clutch. Ten individuals per population were sampled for all biomarkers analyses at each site, in both seasons, and 290 native mussels were analysed in total. First, hemolymph was taken by syringe from the posterior adductor muscle of the animals. They were dissected, and digestive glands were frozen in liquid nitrogen and stored at -80 °C for subsequent assessment of biomarkers activity. The digestive gland was selected because it is considered the target organ in environmental pollution assessment. Additional 15 individuals from each population were dissected and their wet soft tissues were used to determine the concentration of certain metals and metalloids. For GWA analysis 20 individuals per population collected in fall, and 20 individuals from SU (collected only in spring) were sampled (300 individuals in total) by taking the hemolymph for DNA isolation. Further mussels were sampled for GWAS during the transplant and mesocosm experiment (please see below). To assess larger scale phenotypic variation (between and within mussel populations) through analysing morphometric traits, 100 individuals per population were sampled in fall, and 1400 mussel's shells were analysed in total.

3.1.1. Sampling sites description

Lim Bay (LB) is a semi-enclosed embayment. It is located on the west side of the Istrian peninsula in the north-eastern Adriatic, protected and proclaimed a special marine reserve park from 1979. Mussels and fish farming are present in the inner and middle parts of the Bay (Krajnović-Ozretić et al., 2001), also known for providing a good spawning ground, as well as a hiding place for many commercial fish (Huljev and Strohal, 1983). According to the data collected by Kuzmanović (1985) the water exchange within the bay is rapid. Comparison of the physico-chemical properties and phytoplankton dynamics between Lim Bay and other locations in the middle Adriatic Sea have indicated moderate eutrophication in Lim Bay (Bosak et al., 2009). Petrović et al., 2004 affirmed that mussels from referent sites situated in the Lim bay are in good physiological condition, could easily cope with natural stressors and preserve the integrity and stability of lysosomal membranes, exhibiting small oscillations throughout year.

Aquaculture Zadar Seline (ZB) is located in the south-eastern part of the Velebit Channel. This site is about 40 meters away from the coast, without significant anthropogenic pollution. Sea depth of the area is about 10 meters or more. An important condition for mussel farming in this area is the freshwater inflow from Novsko Ždrilo that brings nutrients and decrease salinity of seawater. More than that, significant changes in salinity can occur during the activity of freshwater springs, however, in relatively limited sphere. Hence the whole area has balanced salinity of 37-38 ‰.

Aquaculture Marina (MA) is located 12 kilometers west of town Trogir, on the inner part of the Marinski Bay. Physico-chemical parameters (seawater temperature, salinity, dissolved oxygen), microbiological quality, biotoxins and heavy metals (Cd, Hg, Pb, Cu, As) did not show measurable anthropogenic influence. Apart from mussels, there is a breeding ground for white fish (European bass and Gilt-head seabream).

Babine kuće (SA) is a site located in the area of the National Park Mljet. Due to the absence of any sources of pollution, the site is considered as a reference (“clean”) site.

Ston (SU) is located within the 28 km long Malostonian Gulf, with the maximum depth of 29 meters. The exterior and middle parts of the bay are periodically under the stronger influence of the river's fresh water, and therefore ecological conditions are more affected by the land and less by the open sea. The hydrophysical and ecological relationships of the inner part are more

affected by the strong fresh underwater runoff. According to the nutrient concentration and the phytoplankton amount, the bay can be qualified as a moderately eutrophicated system. Due to the very low population density in the surrounding area, the bay is not exposed to a stronger anthropogenic eutrophication. In the production area mussels and oysters are grown, such as *Venus verrucosa*, *Arca noe*, and *Ruditapes decussatus*.

Mali Ston (MS) is a small village with dozens of berths for local boats. It has an anthropogenic impact, though it is very low. However, there are no known sources of greater pollution on this station, so it is considered a reference site.

Ičići (IC) is a small place on the Opatija Riviera. Low intensity of anthropological and sea traffic activities exists because it has ACI marina and a small harbor for local boats. The Wastewater Treatment Facility was constructed as part of the Adriatic Project, providing the first stage of wastewater purification.

Pula (PL) is the largest city in the Istrian peninsula, notable for shipyard Uljanik Pula and mechanical engineering Uljanik Strojogradnja, whose releases are poured out into the sea. Moreover, Pula has its own big port (Luka Pula), whose traffic contributes to pollution.

Rijeka (RJ) is the largest Croatian port with an annual turnover of more than 6 million tons. In the area of the city, refinery INA Rafinerija Mlaka and the industry of grease and bitumen are pouring their releases into the sea, and their waste waters are purified with only a first stage of purification.

Zadar marina (ZM) is located in the city of Zadar, one of the largest ferry ports in the central Adriatic. There is also a transport company Tankerska plovidba d.d. with 15 tankers and dry cargo ships. The marina itself, with 300 berths, is a site with unconcerned level of pollution. Colors used for antifouling coatings contain copper components and other organic bioactive substances. Waste waters are purified through two wastewater treatment plants - Borik (pre-purification and I degree of purification) and Centar (pre-purification and II degree purification). In this research the mussels were collected directly below the raft in the center of the marina, where the berths are blue from the washed over antifouling colours.

The site Trogir (TM) is located at the nautical port in city of Trogir. Since there are more than 200 berths in the marina, it is considered to be a contaminated site. Additional pollution is connected with the immediate vicinity of the Trogir shipyard.

Split (ST) is the second largest city in Croatia, and the third port in the Mediterranean by passenger traffic. There are still several water outlets in the Split area, of which the Katalinića Brig discharge has a mechanical purification plant, and it drains to 1300 meters from the shore, while the smaller discharge in the port and the Lora discharge does not have purification facilities.

The Gruž (GZ) site is located in Dubrovnik, in the port of Gruž, that has a role of acceptance of passenger ships (ferry services, yachts, special purpose vessels), and an increasing number of cruisers. In 2014, it exceeded 1 300 000 passengers and was declared tenth the busiest cruising pinch of the world in 2008. In the wider Dubrovnik area, municipal waste waters are poured out, passing only through the process of mechanical purification. Measurements of average mass of heavy metals (Cd, Pb, Cu, Zn, Cr and Hg) in mussels' tissue for the period 2000 - 2009 were above average values (Initial Assessment of Marine Condition and Stress Croatian part of Adriatic Sea 2012).

Viktor Lenac (VL) is a shipyard, established in 1896 and was one of the first in the world to deal with ship's upgrading and extension. It is also one of the largest Croatian shipyards with already known negative impacts on the marine environment, and therefore considered a polluted site.

Site Adriavinil (AD) is located in the Kaštelan Gulf, near the factory of polyvinylchloride masses Adriachem, whose drainage is nearby. In the period from 1949 to 1990 there was another plant in the area, Adriavinil (formerly Jugovinil), and it is estimated that during that decade about 200 t of mercury has passed through Kaštelan Gulf (Zvonarić, 1991).

3.2. Experiments

3.2.1. Transplant experiment

In transplant experiment (April 2014), native mussels originating from the same reference site, Marina (MA), were exposed to 6 realistic environmental conditions using paired block design (polluted vs. clean sites in three geographic regions) (Figure 3). Sites were selected according to their environmental quality status. Lim Bay (LBT), Zadar Seline (ZBT) and Ston (SUT) were considered as "clean", Pula (PLT), Zadar marina (ZMT) and Gruž (GZT) were considered polluted sites.

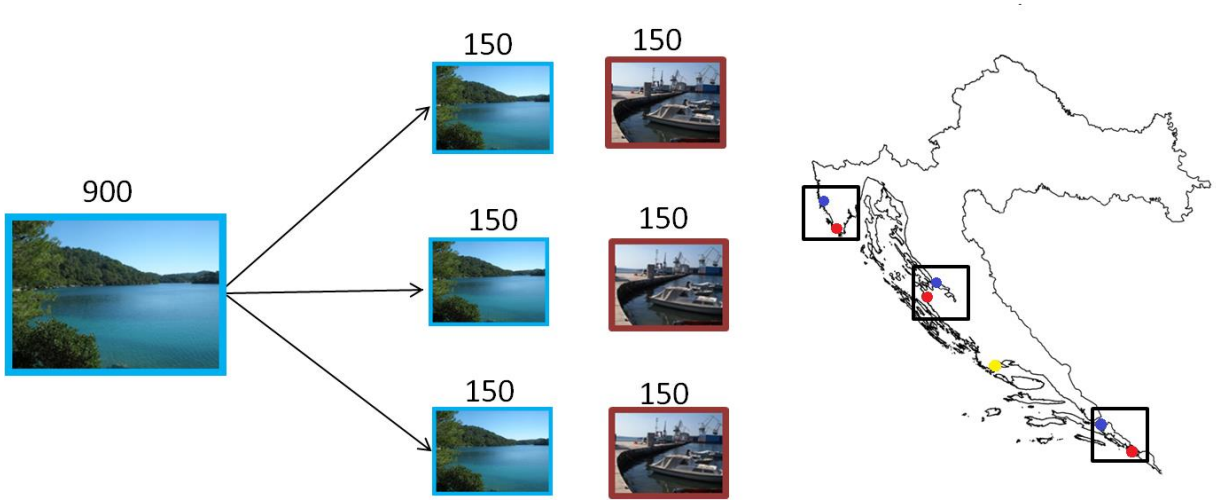

Figure 3. Transplant experiment scheme and exposure sites. Yellow dot on the map represent source population Marina– aquaculture site, from which mussels were exposed to 6 realistic environmental conditions using paired block design - polluted (PLT, ZMT, GZT) vs. clean (LBT, ZBT, SUT) sites in three geographic regions (North, Middle, South Adriatic).

Mussels were transported in cold boxes from the source reference site Marina and, after the initial sorting, divided into groups (of about 200 individuals each), placed in 50x50m cages constituted of polyethylene netting, immersed at 1 - 1.5 m depth and secured by anchor and rope at each site. Animals were collected after 4 weeks of exposure, brought on ice to the laboratory in each of the regions, where haemolymph was taken from the posterior adductor muscle and digestive glands (N = 10 per site) were dissected and immediately frozen in liquid nitrogen and stored at -80 °C.

3.2.2. Mesocosm experiment

To evaluate population effect of phenotypic stress responses, 800 mussels in total were collected in April 2014 from two source populations; Marina (MA) –aquaculture area representing clean site, and Gruž (GZ) harbour, representing polluted site (Figure 4).

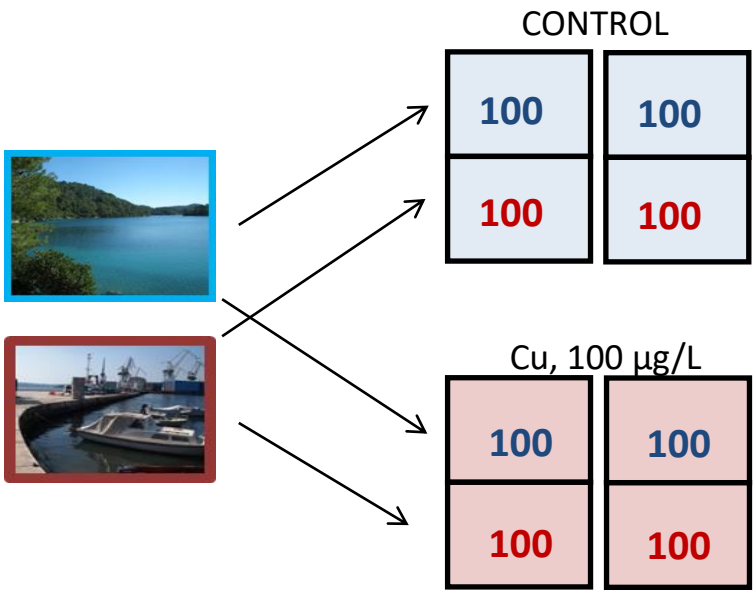

Figure 4. Mesocosm experiment scheme. Mussels were collected in April 2014 from two source populations (400 individuals per population); Marina (MA) – representing a clean site, and Gruž (GZ) harbour - representing polluted site. After acclimation, 100 mussels from each source population were exposed to either copper (Cu) or clean seawater, in two replicates per population.

Mussels were acclimated during 4 weeks in tanks containing 150 L of natural seawater. Seawater was constantly aerated, and half of it replaced with fresh quantity daily. Water quality was analysed daily by measuring salinity (34 ± 0.1), temperature (16.1 ± 0.4 ◦C) and pH (7.9 ± 0.34). Mussels were fed with 1.5 ml of a concentrated algal paste (Shellfish Diet 1800, Reed Mariculture Co., USA) daily. After acclimation, 100 mussels, separated by a partition in same tank, were exposed to daily dose of 100 μgL^{-1} copper or clean seawater in two replicates per population/exposure (N=200 per population per treatmant). One half of the total seawater volume (75 L) was replaced with fresh quantity and copper was re-administered daily. Exposure experiments were conducted in controlled conditions under 12h : 12h light/dark cycles. Seawater quality was analysed daily by measuring salinity (35 ± 0.07), temperature (15.8 ± 0.5 °C) and pH (8.07 ± 0.1). Every day, mussels were fed with the same concentrated algal paste as was used during the acclimatization period. After 8 days of exposure, haemolymph was taken from the posterior adductor muscle for Comet assay and digestive glands were dissected for each

population (N=10; 5 per replica) and treatment. Digestive glands were immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

3.2.3. ‘Stress on stress’ response

The survival in air (stress on stress - SOS) test was performed on mussels from mesocosm (N=800) and transplant experiment (N=900). After exposure period, mussels shells were scratched from periphyton, washed in ethanol and labeled with Brother TZe-221 Label Tape, 6mm (0.25") Black on White using Brother P-Touch PT-H75 Labelmaker. The labeled mussels were placed on ice in portable fridges and transferred to aquarium in Pula where they were left in the air (constant room temperature of 18 ± 1 °C) on wet filter paper (re-soaked daily). Survival was checked every 24h until 100% mortality was reached (Figure 5). Mussels were considered dead when the valves gaped and an external stimulus (squeezing of valves) did not show any vital response.

Figure 5. Stress on stress (SOS) experiment. After exposure period in transplant and mesocosm experiments, mussels were left in the air on wet filter paper where survival was checked daily.

3.3. Extract preparation and biomarkers activity measurements

For protein extraction, digestive glands were homogenized in Tissue Lyser MM300 (Qiagen-Retsch) in 1.2 mL of 50 mM potassium phosphate buffer (pH 7.0) with 0.1 mM EDTA. The homogenate was centrifuged at 10000 × g for 12 min at 4 °C. Supernatant was collected and used for the following assays.

For enzyme assays supernatants were diluted with extraction buffer 1:5 (v/v). Catalase (CAT) activity was assayed by measuring the decrease in absorbance at 240 nm (ε = 36 $mM^{-1}cm^{-1}$) according to Aebi (1984) with minor modifications. Glutathione reductase (GR) activity was determined by the oxidation of NADPH at 340 nm (ε = 6,22 $mM^{-1}cm^{-1}$) according to Ramos-Martinez et al., (1983). Glutathione S-transferase (GST) activity was assayed by measuring the decrease in absorbance at 340 nm (ε = 9,6 $mM^{-1}cm^{-1}$) according to Habig et al., (1974). Acetylcholinesterase (AChE) activity was assayed by measuring the decrease in absorbance at 412 nm (ε = 0,07 $mM^{-1}cm^{-1}$), according to Ellman et al., (1961). For carbonyl quantification, dinitrophenylhydrazine (DNPH) reaction was used as described by Levine et al., (1994). The level of lipid peroxidation was determined indirectly as the formation of malondialdehyde (MDA) in a reaction with thiobarbituric acid (TBA), according to Buege and Aust (1978). Total protein content was determined by Bradford method (Bradford 1976).

To perform the alkaline Comet assay (single cell gel electrophoresis assay), 200 µL of hemolymph was taken by subcutaneous injection needle from the adductor muscle of 10 individuals per population. Immediately after extraction, hemolymph was transferred to labelled microcentrifuge tubes on the ice, and the comet assay was performed according to Štambuk et al. (2013). Prior to examination, the slides were rehydrated and stained with 10 μgmL^{-1} ethidium bromide and examined using a Zeiss Axioplan epifluorescence microscope. At least 100 cells were examined per single slide. The extent of DNA migration was determined as a percentage of DNA in the tail (% tDNA) using an image analysis system Komet 5, Kinetic Imaging Ltd.

3.4. Geometric morphometrics (GM)

For geometric morphometrics (GM) the right shells of 20 individuals per population sampled in fall (N=280) were analysed. 800 individuals from mesocosm and 900 from transplant experiment were used for both GM and FA analyses (both shells were measured, right shell analysef for GM and both for FA for these 1700 individuals).

All individuals were photographed using the Olympus digital camera 7.2V (model NO. E-PL1, lens M. ZUIKO DIGITAL 14-22 mm). The inner side of both shells was photographed, with clearly visible imprints of the adductor and retractor muscles, pallial line and ligament. To ensure

consistent quality and uniformity of the photographs, darkroom lighting was used, dark background, color calibration tape and millimeter paper, placed for scaling.

These digital images were utilized to obtain landmarks using the software Image J (v. 1.48) (Figure 6). Seventeen landmarks were placed along the shell and muscles outline and assigned as x,y coordinates. The coordinates of two specific landmarks were used for calculating the distance between them, which denotes the given traits.

Figure 6. Geometric morphometrics. Digital images of the inner side of mussel's shells were utilized to obtain landmark coordinates in software Image J

Twelve shell characters measured by landmark-based GM approach were (Figure 7): distance between umbo and posterior end of the ligament - LIG, distance between pallial line and ventral shell margin midway along shell – PAL, distance between ventral muscle scar and ventral shell margin – PADV, length of posterior adductor muscle scar – PAD, distance between anterior edge of posterior adductor muscle scar and posterior shell margin – PADP, distance between posterior edge of posterior adductor muscle scar and posterior shell margin – PPAD, length of posterior

retractor muscle scar – LPR, width of posterior retractor muscle scar - WPR, distance between ventral edge of posterior retractor muscle scar and dorsal shell margin – VPR, distance between the anterior end of posterior retractor muscle scar and dorsal shell margin – DPR, shell height – H and shell length – L (used to standardize the variables for size and FA analysis).

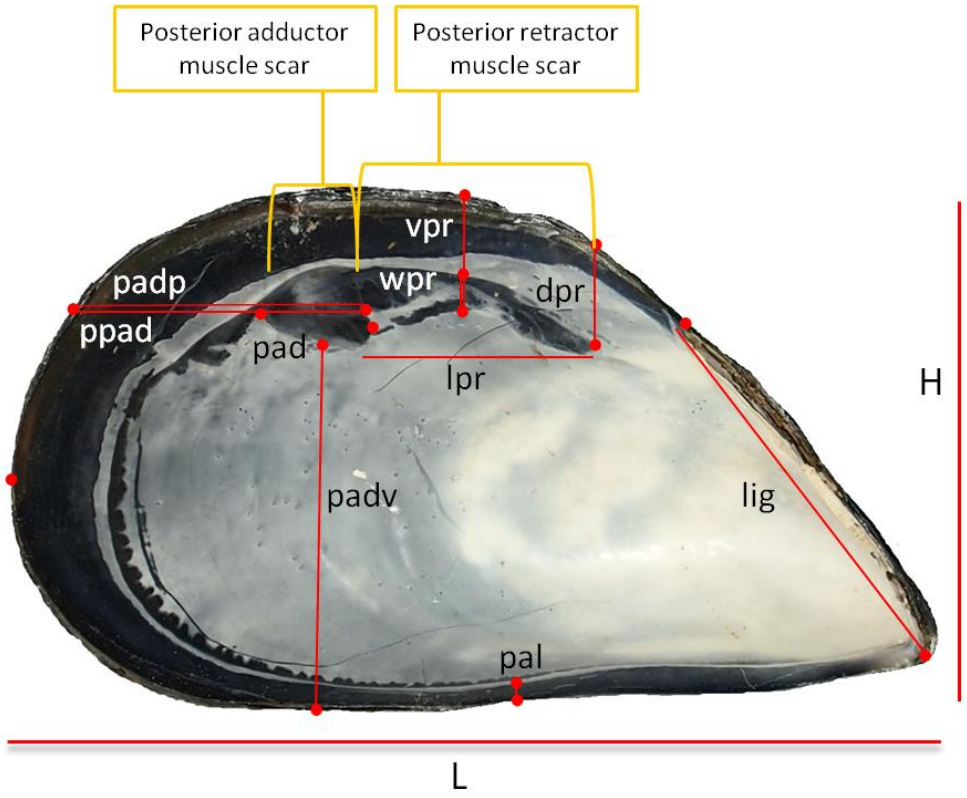

Figure 7. Shell morphological traits measured by landmark-based geometric morfometrics approach: distance between umbo and posterior end of the ligament - LIG, distance between pallial line and ventral shell margin midway along shell – PAL, distance between ventral muscle scar and ventral shell margin – PADV, length of posterior adductor muscle scar – PAD, distance between anterior edge of posterior adductor muscle scar and posterior shell margin – PADP, distance between posterior edge of posterior adductor muscle scar and posterior shell margin – PPAD, length of posterior retractor muscle scar – LPR, width of posterior retractor muscle scar - WPR, distance between ventral edge of posterior retractor muscle scar and dorsal shell margin – VPR, distance between the anterior end of posterior retractor muscle scar and dorsal shell margin – DPR, shell height – H and shell length – L (used to standardize the variables for size and FA analysis).

All variables were log-transformed and standardized to shell length, as proxy for individuals size. Additionally, three morphological characteristics were *hand-measured*; both shells were weighted (MASS) (data was standardized as described), width was measured with vernier calipers (±0.01 mm), log-transformed and standardized for size (WL) and height (WH) and shell volume was calculated using formula: V = log((4/3*π)*shell height*width*lenght)/log (shell length) (Shields et al., 2008). Applying the measured values of 13 morphological characteristics (WH, WL, V not included), the subtraction between left and right shell for each morphological characteristic was calculated, and obtained absolute value to estimate the level of fluctuating asymmetry.

3.5. Environmental variables assemble

Quantitative environmental data were collected from Bio–Oracle (Tyberghein et al., 2012) online database. Bio–Oracle is a set of GIS rasters providing geophysical, biotic and environmental data for surface and benthic marine realms, based on monthly averages in the time period between 2000 and 2014, at a spatial resolution of 5 arcmin (approximately 9.2 km at the equator).
Variables considered in our study were: currents - current velocity (mean at min depth), light - light at bottom (mean at min depth), SST - sea surface temperature (mean), T_max - sea water temperature (maximum at min depth), salinity - sea water salinity (mean at min depth), Chl_a - chlorophyll concentration (mean), O_2 - dissolved oxygen concentration (mean), silicates - silicate concentration (mean at min depth), phosphates - phosphate concentration (mean), nitrates - nitrate concentration (mean).

3.6. Metals and metalloids determination

In order to determine the concentration of certain metals and metalloids, a pool of the wet soft tissues of 5 mussels per sample site (triplicates for all, N=15) were digested in a flask with 10 ml of Aqua regia, a mixture of nitric acid and hydrochloric acid in optimal molar ratio of 1:3, and placed in a microwave (Multiwave 3000, Anton Paar, Graz, Austria). After digestion samples were diluted with Mili-Q water and Indium was added (1 μgL^{-1}) as a standard for inductively coupled plasma mass spectrometry (ICPMS) measuring (instrument Element2, Thermo, Bremen, Deutschland). In order to eliminate spectral interference, specific isotopes were measured in

three different resolutions (R, ability to distinguish two peaks of slightly different mass-to-charge ratios, in a mass spectrum): low (^{7}Li, ^{107}Ag, ^{111}Cd, ^{120}Sn, ^{208}Pb, ^{209}Bi), medium (^{51}V, ^{52}Cr, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{121}Sb) and high (^{27}Al, ^{39}K, ^{56}Fe), where R = 300, 4000, and 10000, respectively.

3.7. DNA isolation

In order to isolate genomic DNA, 500 µl of hemolymph was collected by syringe from the posterior adductor muscle of the animals and mixed with an equal volume of 96% EtOH into 1.5 mL micro-tubes. Suspension was centrifuged at 10000xg for 2 min (at 4 ◦C). Supernatants were pipetted out before the resulting pellets were frozen with liquid nitrogen, crushed with scissors and handled for DNA isolation using a kit of DNA isolation reagents (GenEluteTM Mammalian Genomic DNA Miniprep Kit, Sigma-Aldrich) according to the instructions. Isolated DNA was preserved in micro-tubes at 4 ◦C.

Concentration and purity of the DNA was measured spectrophotometrically on a Nanodrop (NanoDrom(TM) 2000 c Thermo Scientific). The concentration of DNA in all samples was over 50 ng/µL. Purity of DNA was defined according to calculated A260 / A280 values (range 1.6-1.9 means that DNA is pure), and all tested samples were satisfied for purity.

3.8. Genotype-by-sequencing (GBS) library preparation

To generate genome-wide SNP data, reduced complexity genomic libraries were sequenced for 1700 individuals from experiments and 300 native individuals that were scored for phenotypic traits. The library preparation protocol of Parchman et al. (2012) that is designed for Illumina sequencing chemistry was used.

Genomic DNA was digested with the restriction endonucleases *Mse*I and *Eco*RI (New England Biolabs). Adaptor sequences and their reverse complements that allowed for ligation to the restriction sites were annealed to each other by incubating at 95 ° C for five minutes and slow cooling to room temperature. The restriction digests were incubated with T4 DNA ligase (New England Biolabs) and oligonucleotides containing the first Illumina adaptor sequence followed by eight, nine, or 10 bases of barcode sequence, and the *Eco*R1 cut site and oligonucleotides containing the second Illumina adaptor and the *Mse*I cut site. Restriction and ligation were accomplished simultaneously to 12 hours of incubation, followed by dilution with 189 µ L

0.1×TE buffer. Fragments were then amplified via polymerase chain reaction (PCR; 30 total cycles) using standard Illumina primers (Illumina, Inc.);

Illpcr1(Forward): A*A*TGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Selective Illpcr2 (Reverse): C*A*AGCAGAAGACGGCATACGAGCTCTTCCGATCTGTAAG

PCR amplicons were checked on gel, and barcoded PCR products were pooled to be sequenced per lane. In total, 2000 individuals were sequenced across 9 lanes. Sequencing was accomplished on at the National Center for Genome Resources (NCGR) in Santa Fe, NM. Quality control, alignment, variant detection and population differentiation were done by project collaborators at University of Sheffield, UK (Table S1, Figures S12 and S13, Supplementary materials)

3.9. Genetic architecture of *Mytilus galloprovincialis* morphological traits estimated using GWAS

To describe the genetic architecture of mussel's morphological traits, GWAS was performed on different data sets (including only SNPs with minor allele frequencies ≥0.05): 1) population effect has been addressed by comparing genetic architecture in two populations inhabiting contrasting environments, using individuals from Gruž (394 individuals, 19129 SNPs) and Marina population (377 individuals, 19129 SNPs) in mesocosm experiment; 2) great-scale subset has been performed on Marina population used in transplant experiment (883 individuals, 18850 SNPs), and 3) a large-scale pool of Marina individuals used in both experiments (1258 individuals, 18728 SNPs); 4) population effect has been further addressed in a sample of 15 native populations inhabiting various environments (288 individuals, 18655 SNPs). Genome-wide SNP data was implemented to test for associations with mussel's traits, related to shell height and width, shell shape and position and size of retractor and adductor muscles.

To describe the genetic architecture of traits multi-locus Bayesian sparse linear mixed models (BSLMMs) was used. It was implemented in the software package *gemma* (Zhou and Stephens, 2012, Zhou et al., 2013). BSLMMs allow for multi-SNP mapping and was used to estimate three hyperparameters that describe aspects of the genetic architecture of a given trait (Zhou and Stephens, 2012, Zhou et al., 2013). First, the model estimates the proportion of variance (proportion of phenotypic variation explained; PVE) explained by all the SNPs (both 'sparse (i.e., detectable) and SNPs with minor effects (i.e., infinitesimal and undetectable) included in

the model. Second, *gemma* estimates the proportion of the total phenotypic variation that can be explained only by 'large-effect' SNPs (proportion of genetically-explained variation; PGE). Third, *gemma* estimates the number of SNPs (n-SNP) that have non-zero effects on phenotypic variation (i.e. the number for which the relationship between genotype and phenotype is greater than zero). In addition to the hyperparameters described above, *gemma* provides the posterior inclusion probability (PIP; γ parameter in the BSLMMs) of each SNP that is identified to have a non-zero effect on phenotypic variation. This is the proportion of MCMC steps that a SNP is retained as being trait associasted, i.e., having a detetable or sparse effect. SNPs that are more strongly associated with phenotypic variation will have larger PIPs and these SNPs are the strongest candidates of being linked to the functional variant(s) underlying phenotypic variation.

For each trait BSLMMs were implemented in *gemma* with 10 independent Markov-chain Monte Carlo (MCMC) chains, ran for 20 million steps with an initial burn-in period of 5 million steps. All additional options in *gemma* remained at default values. Prediction analyses were carried out to test the strength of the genetic signal in our data set to accurately estimate hyperparameters. A permutation test was conducted using GWA mapping in *gemma* as described above with Marina_pool data, generated by randomly permuting phenotypic scores for each individual.

3.9.1. Single-SNP GWA mapping

To validate results from BSLMM analyses, we also carried out the EIGENSRAT method in the R package GENABEL v1.8.0 (Aulchenko et al., 2007) to perform single locus GWA mapping analyses. Briefly, genotype probabilities were recoded into genotype values accepted by GENABEL using a custom Perl script. Transformed genetic probabilities were filtered using GENABEL quality control function. SNPs with MAF inferior or equal to 1%, were excluded from analysis. Individuals with extreme heterozygosity at a false discovery rate <1% and with too high an identity by state (hereafter IBS>=0.95, calculated on a subset of 2000 SNPs), were discarded from analysis. Analyses were run both controlling for population structure (using the GENABEL egscore function (Price et al., 2006)) and not controlling for population structure (using the GenABEL qtscore function). The egscore function extracts principal components of a kinship matrix (here IBS indices) calculated using a subset of 2000 SNPs. The principal components are then used as covariates in the GWA linear models.

3.9.2. Cross validation (predictive power of the models)

To quantified the predictability of the models, cross validation was performed on the largest data set – Marina_pool, using the genomic prediction function in *GEMMA*. Cross validation was based on results from 10 independent MCMC chains, ran for 20 million steps with an initial burn-in period of 5 million steps.

3.10. Statistical analysis

3.10.1. Morphological multivariate analysis

All results were obtained and plotted using R v. 3.2.0. A threshold of $p < 0.05$ is considered as significant in all analysis.

Multivariate analyses of the morphometric data were carried out using principal component analysis (PCA) and linear discriminant analysis (LDA). PCA was applied for the interpretation of data variability (Reid and Spencer, 2009). It is widely used to rotate and project data into subspace of variants of reduced dimensionality. Reducing the data to dominant components or factors is achieved by suppressing parts of the total variance in the data and results in a more interpretable output for exploratory purposes. Significant principal components were determined by the broken stick method (Farinas-Franco et al., 2016) of the scree plot (components plotted against eigenvalues). In addition, linear discriminant analysis (LDA) was used to evaluate the influence of the sites and regions on the grouping of data into classes. This analysis computes a linear projection for one or more predictors and yields a new set of transformed data for grouping them according to classes (Wang and Mizaikoff, 2008) without dimensional reduction. A jackknife-based classification (i.e. leave-one-out cross-valdation) was applied to estimate the accuracy of the discrimination between sampling sites and regions. Finally, we calculated the canonical scores (also known as canonical discriminant function coefficients; Zuur et al., 2007) to better interpret the relationship between group discrimination and morphological variation.

Further packages were used in R: MASS, ggplot2, scales, ggpubr, ggfortify, gridExtra, mvtnorm, Momocs. To test for significance ANOVA on principal component scores of morphological traits was performed. Significant difference for 15 morphological traits between Marina and Gruž populations, exposed to copper in mesocosm experiment, was obtain with post hoc Tukey test (using “agricolae” package) and indicated by asterix above represented plots.

The Partial Least Squares Regression approach (PLS-R2) was used in order to analyze the effects of linear combinations of environmental factors and several metals and metalloids (predictors - X) on morphological data and biomarkers (response - Y). Analysis was performed using the "plsdepot" package in statistical software R according to (Sanchez, 2012). The PLS scores associated with the first two PLS components, generated in the model, are new variables summarizing the X variables. Scores contain the information about the objects and their similarity (Wold et al., 2001) and were therefore used for the interpretation of the PLS-R2 model. We performed glm analysis fitted with aov function on PLS scores to test for the significance of status and regions specifics in 'response-predictor' relation.

3.10.2. Biomarkers

PTA was performed according to (Adam and Collyer, 2009). Here, it was conducted by using PC scores derived from Principal component analysis (PCA) on the centred and scaled biomarkers data set. The centroid averages of the PC scores were plotted for each of pollution status (clean vs. polluted), in each season (fall and spring). The benefit of using PC scores lies in the simplified visual interpretation (Dennis et al., 2010). Assessment of trajectories is calculated based on the multidimensional properties of the entire dataset simultaneously and is supported statistically by permuting the residuals of a simplified model to estimate the probability of fitting the same trajectory by chance. Analysis was conducted using R v. 3.2.0. For plotting the results "ggbiplot" package was used.

Integrated biomarker response (IBR) analysis was based on major steps described in (Beliaeff et al., 2002), and modified according to (Pain-Devin et al., 2014). It provides a numeric value that integrates all responses at once, following a prior step of biomarker responses standardization and creation of circular permutations of *k* biomarkers. The IBR is the sum of the area defined by the k biomarkers (arranged in a radar diagram). It results in a $(k-1)!$ matrix of IBR values that allows the calculation of median IBR for a site and prioritization of IBR values among sites. Here, a battery of six biomarkers were analysed in total (CAT, GR, GST, ACHE, MDA and Carbonyls) which resulted on a matrix of 120 values for all six biomarkers. All the possible circular permutations of biomarkers and therefore all possible IBR values, were calculated according to (Beliaeff et al., 2002) using "permute" and "graphic" packages in R v. 3.2.0. In

order to compare the results and test for significance among various sites, pollution status, region or treatment (depending on the data set), generalized linear model (glm) analysis was performed (using basic R "stats" package). The models were fitted with aov function ("stats" package), and analysed with post hoc Tukey test (using "agricolae" package). The results of the IBR are represented as boxplots (using "ggplot2" package) with different letters indicating between-site differences.

3.10.3. Survival analyses

Mussel's fitness was evaluated by measuring the number of death individuals over a period of time spent on the air. The data were analyzed using the survival analysis in R (package "survival") and visualized through Kaplan-Meier survival estimator, a non-parametric statistic that allows us to estimate the survival functions. The lengths of the horizontal lines along the X-axis represent the survival duration for that interval, where the horizontal gap means that it took longer for one group to experience a certain fraction of deaths. The interval is terminated by the occurrence of the event of interest. Longer vertical gap means that at a specific time point, one group had a greater fraction of subjects surviving. Therefore, the vertical distances between horizontals are important because they illustrate the change in cumulative probability of surviving as the curve advances. The non-continuous nature of the Kaplan-Meier curve emphasizes that they are not smooth functions, but rather step-wise estimates.

4. RESULTS

4.1. Phenotypic variation

4.1.1. Phenotypic variation between native populations

PCA analysis on phenotypic data of 15 native populations revealed that the first two principal components of the entire data set explained 42.42% of the total variance, where first one explained 24.2% and the second one 17.22% of the total variation (Figure 8a). Scree plot analysis indicated PC's 1-3 should be considered (whenever possible) for interpreting the results (PC3 accounted for 13.3% of the total variation).

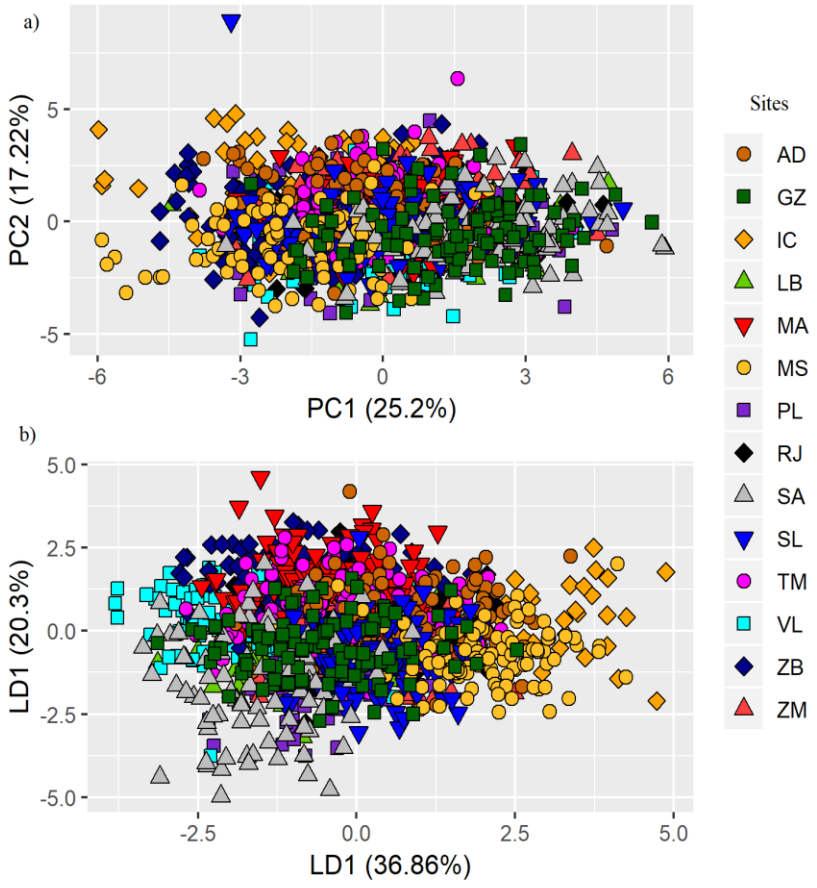

Figure 8. PCA (a) and LDA (b) plots on morphological traits of 15 native populations, analyzed per sampling sites. Plots are showing the first two principal components and discriminant scores obtained in analysis, explaining 42.4% and 57.16% of the variation, respectively.

The plot of PC1 against PC2 showed that specimens that were separating the most belong to Ičići (IC) and Mali Ston (MS), as being considerably smaller regarding their age. This indicated that shell morphometric characteristics are highly influenced by the individuals size and, accordingly, their age. ANOVA on PC scores showed that traits significantly differed between sampling sites, pollution status and Adriatic regions. PC1 was positively correlated with almost all observed traits (except WH). PC2 can be considered a shape axis as it was positively correlated with HL, and negatively with WH, WL and V. PC loadings on first three PC's showed that populations mostly split up according to the traits related to shell shape; HL, WL, WH, V and the position of two muscles; PADP, PPAD, DPR, VPR **(**Figure 9, Table 1**)**.

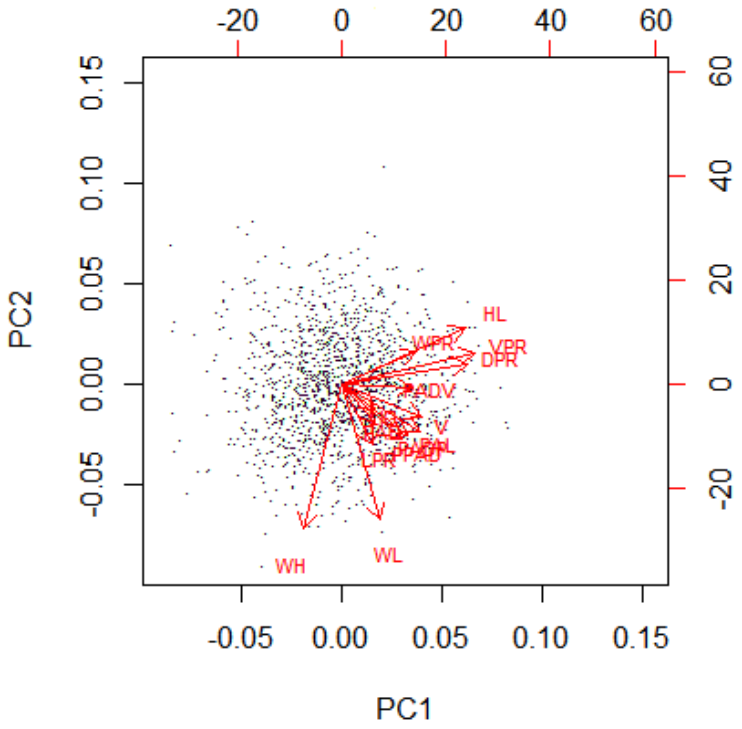

Figure 9. PCA biplot on morphological traits of 15 native populations. Biplot shows the first two principal components obtained in analysis, explaining 42.42% of the variation.

LDA analysis on morphological traits revealed that the first two discriminant scores of the entire data set explained 57.16% of the total variance, where first explained 36.86% and the second one 20.3% of the variation among individuals (Figure 8b). LDA also showed greatest separation for Ičići (IC), Mali Ston (MS) and additionally Babine kuće (SA), which are all clean sites. Jackknife-based correct classification accuracy (Table 2) varied from 4.85% (TM) to 64.42%

(VL), and was overall 39.39%. Misclassifications were mostly higher for individuals belonging to same region (e.g. between MA, TM, AD, SL) or pollution status (e.g. PL-ZM, GZ-ZM, TM-VL). The contribution of each variable to the model is showed by standardized canonical discriminant function coefficients (Table 1), allowing to compare variables measured on different scales. Coefficients with large absolute values correspond to variables with greater discriminating ability. Results showed the greatest discriminating ability for the traits related to shell shape; HL, WL, WH, V and trait related to position of the posterior adductor muscle; PADV.

Table 1. Principal component loadings (PC1, PC2 and PC3) and standardized canonical discriminant function coefficients (F1, F2 and F3) on morphological traits of 15 native populations. Table is showing first three principal components and standardized canonical discriminant function coefficients for each trait.

	PC1	PC2	PC3	F1	F2	F3
Standard deviation	*1.88*	*1.55*	*1.37*			
Proportion of Variance	*0.25*	*0.17*	*0.13*			
Cumulative Proportion	*0.25*	*0.42*	*0.56*			
HL	0.42	0.24	-0.15	-1.53	0.14	-0.51
WL	0.13	-0.56	-0.23	-1.43	-0.89	0.01
WH	-0.13	-0.59	-0.11	-0.29	0.52	-0.09
V	0.27	-0.13	-0.35	2.44	1.02	0.20
LIG	0.11	-0.10	-0.39	0.20	-0.20	-0.23
PAL	0.26	-0.19	0.22	-0.33	0.39	-0.23
PADV	0.24	-0.01	0.10	-0.21	-0.36	0.69
PAD	0.11	-0.15	-0.15	0.19	0.02	-0.03
PADP	0.22	-0.21	0.47	0.00	0.26	0.26
PPAD	0.21	-0.22	0.54	0.24	0.38	0.15
LPR	0.10	-0.24	-0.09	0.25	0.16	0.22
WPR	0.25	0.14	-0.18	0.17	-0.45	-0.34
VPR	0.45	0.13	-0.03	-0.36	0.00	-0.04
DPR	0.43	0.09	0.01	0.01	0.10	-0.27

Table 2. Jackknife-based classification, comparing field samples (rows) and the group assigned by the linear discriminant function (columns). The proportion of correct classification accuracy is provided in the last column.

	AD	GZ	IC	LB	MA	MS	PL	RJ	SA	SL	TM	VL	ZB	ZM	**% corr**
AD	23	3	12	2	19	3	2	5	1	19	2	4	1	13	21.10%
GZ	4	16	0	6	10	1	3	2	8	12	3	16	4	17	15.69%
IC	6	0	22	1	1	0	0	1	1	0	2	1	2	3	55.00%
LB	5	7	5	7	3	1	6	6	9	22	5	12	5	5	7.14%
MA	11	0	1	1	64	0	3	2	5	3	8	5	9	3	55.65%
MS	6	2	9	1	1	62	2	5	0	9	0	8	0	3	57.41%
PL	2	1	1	1	3	3	36	2	7	13	1	6	2	26	34.62%
RJ	7	6	9	2	10	2	5	16	6	4	6	11	5	14	15.53%
SA	1	6	2	1	2	1	6	1	57	2	0	19	2	0	57.00%
SL	9	4	2	4	1	1	7	5	2	56	2	4	5	9	50.45%
TM	5	2	4	1	23	2	3	8	1	3	5	17	9	20	4.85%
VL	0	2	1	2	2	0	5	2	4	4	1	67	10	4	64.42%
ZB	1	0	1	2	10	1	1	2	2	2	4	17	61	3	57.01%
ZM	3	2	4	0	3	0	12	2	0	9	2	3	2	63	60.00%

4.1.2. Population effect of phenotypic variation (mesocosm experiment)

To evaluate population effect, morphological traits of two source populations (MA and GZ) from contrasting environments were compared, using large scale of 400 individuals per population. Upon testing for normal distribution, ANOVA's posthoc Tukey test determined that these populations diverged according to most of the traits, excluding PAD and PPAD for which no significant difference was recorded (Figure 10). PCA analysis revealed that the first two principal components of the entire data set explained 46.3% of the total variance, where first one explained 28.7% and the second component 17.6% of the variation (Figure 11**).** PCA scores of morphological traits showed that two populations have mostly split up according to the traits related to shell shape; HL, WH, WL, V and the position of posterior adductor muscle; PPAD and PADP (Table 3). Tukey test determined that GZ and MA don't differ according to PPAD, but PCA analysis revealed that this trait has a very low value of 0.05 for the first loading, and its strength pops-up toward third loading (0.54). This implies the importance of using different analysis in revealing the signal.

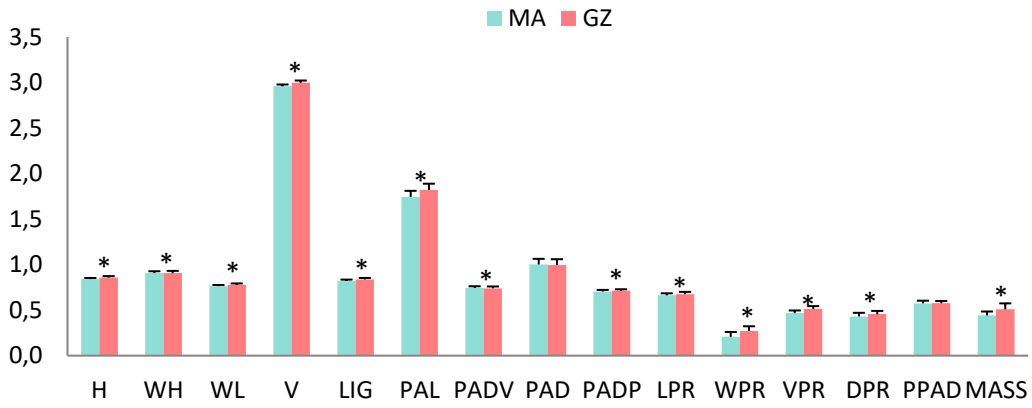

Figure 10. Plot on 15 morphological traits of Marina and Gruž populations, collected for mesocosm experiment. Significant difference between populations for each trait is indicated by asterix above plots.

Figure 11. PCA biplot on morphological traits of Marina and Gruž populations. Plot is showing the first two principal components obtained in analysis, explaining 46.4% of the variation. Populations were grouped by 95% confidence interval ellipses around centroids of each sampling locations. Two populations are significantly different ($p<0.0001$).

Table 3. Principal Component Analysis (PCA) on morphological traits of two populations – Marina (MA) and Gruž (GZ), used in mesocosm experiment. Table is showing first three principal components for each trait.

	PC1	PC2	PC3
Standard deviation	*2.12*	*1.57*	*1.39*
Proportion of Variance	*0.30*	*0.16*	*0.13*
Cumulative Proportion	*0.30*	*0.46*	*0.59*
HL	-0.41	-0.26	0.03
WL	-0.25	0.35	-0.30
WH	0.11	0.50	-0.29
V	-0.40	-0.04	-0.22
LIG	-0.26	0.10	-0.23
PAL	-0.23	-0.23	-0.10
PADV	-0.12	-0.26	0.19
PAD	0.05	0.38	0.24
PADP	-0.16	0.22	0.54
PPAD	-0.05	0.18	0.54
LPR	-0.13	0.23	-0.08
WPR	-0.32	0.09	0.04
VPR	-0.39	-0.01	0.09
DPR	-0.30	-0.10	0.11
MASS	-0.27	0.37	0.03

4.2. Partial least square analysis on morphological traits of native populations

4.2.1. Environmental variables and metals contributing to morphological differences

Environmental variables collected from Bio–Oracle online database, used for the analysis, are shown in Table S2 (Supplementary materials). Most environmental variables used in this research showed gradient data range, depending on Adriatic regions (Figure 12). Currents, nitrates, salinity and sea surface temperature (SST) exhibited an increase toward south. Contrary, light, O_2, silicates and phosphates exhibited a decrease toward southern sites. Maximum sea water temperature (T_max) was highest in LB, PL and GZ, and it varied between the rests of the sites. Similar was recorded for chlorophyll a (Chl_a), with the highest concentrations in LB and PL. PCA analysis on

environmental data revealed that the first two principal components of the entire data set explain 90.2% of the total variance, where first one explained 69.7% and the second one 20.5%. ANOVA on PC scores showed that environmental variables significantly differed between sampling sites and between Adriatic regions (Figure 13), but not according to pollution status **(**Table 4**).**

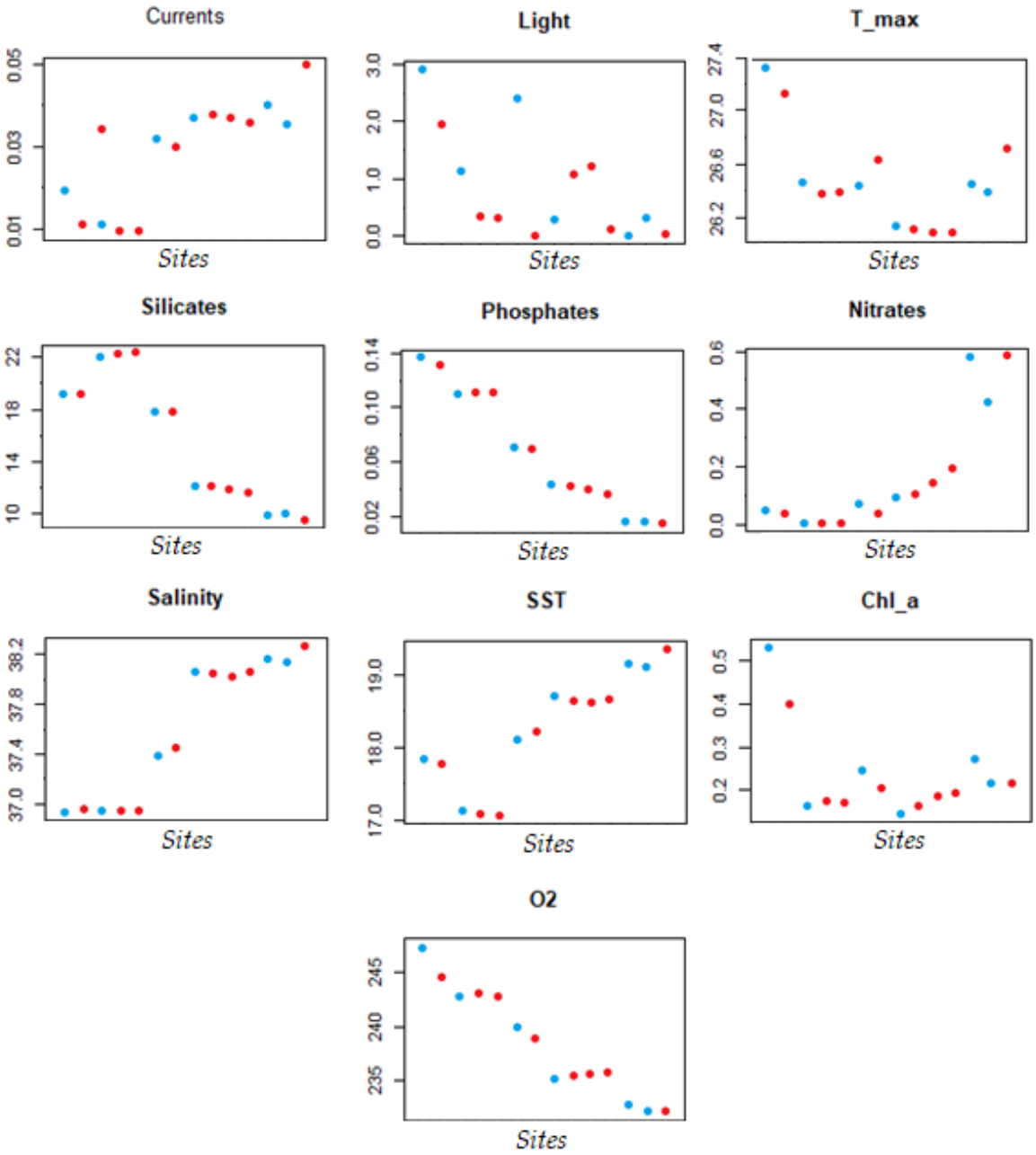

Figure 12. Environmental variables collected from Bio–Oracle online database, based on monthly averages in the time period between 2000 and 2014. Variables are distributed per 15 sample sites, shown north to south. Clean sites are marked as blue versus polluted sites which are marked red.

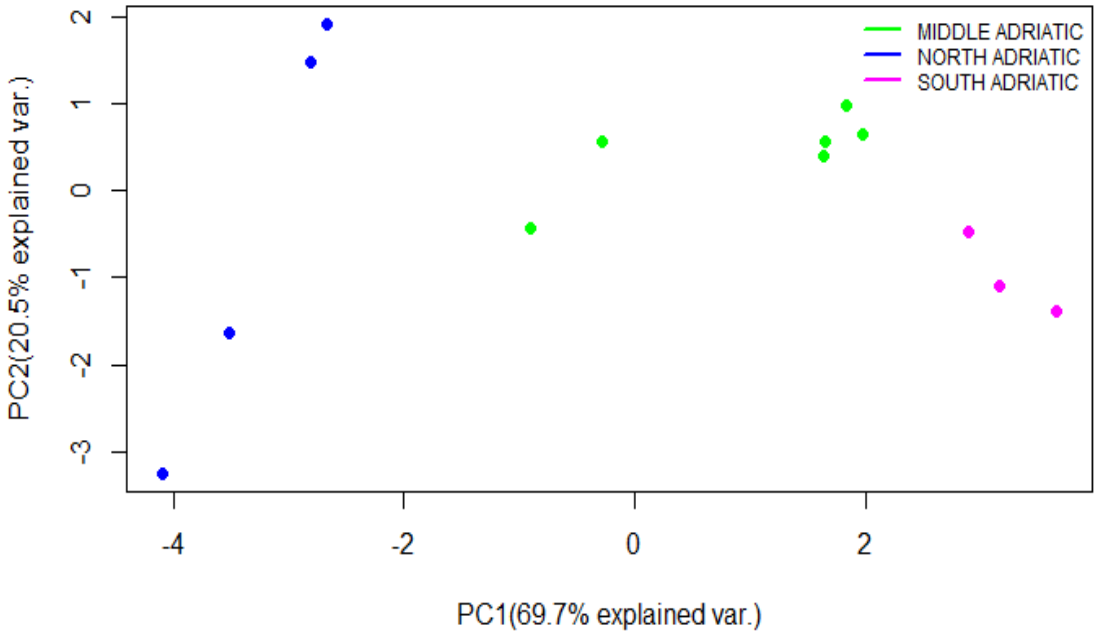

Figure 13. PCA plot on environmental variables significantly separated between Adriatic regions (2000 - 2014). Plot is showing the first two principal components obtained in analysis, explaining 90.2% of the variation. Environmental variables significantly differed between sampling sites and according to Adriatic regions ($p < 0.0001$).

Table 4. ANOVA on principal component scores of environmental variables (ENV.VAR) and metals. Table is showing significance for sampling sites, different contamination status and Adriatic regions.

ANOVA significance	**p(ENV.VAR)**	**p(METALS)**
SITE	< 0.0001	< 0.0001
STATUS	0.8	< 0.0001
REGION	< 0.0001	0.4

Metals and metalloids determined from the mussel's tissue, collected at the research sites in spring 2014, are shown in Table S3 (Supplementary materials). Concentrations were highest on sites previously described as contaminated. The highest antimony concentrations were found in GZ, and silver in RJ and ZM. Zadar Marina had also dominant concentrations of lead, bismuth, tin, zinc and copper. High concentrations of lead were determined in VL and PL, tin and zinc in VL, silver in RJ, chromium in VL, cadmium in MS and VL and nickel in VL. Concentrations of metals

and metals that appear naturally as suspended particles were generally higher on sites previously determined as clean. The highest concentrations of molybdenum were in ZB and SA, where the highest concentration of selenium was also recorded. Concentrations of cobalt, lithium, iron, arsenic, rubidium, strontium and uranium were not found in higher concentrations at sites with strong anthropogenic influences (ports, marinas), but a bit higher values of cobalt, lithium and iron were recorded in VL. Manganese and aluminum had higher values in all clean sites. Titanium concentrations were highest at ZM and GZ, and the concentration of vanadium was dominant in PL. PCA analysis on metal concentration from the mussel's tissue revealed that the first two principal components of the entire data set explain 44.2% of the total variance, where first one explained 27.9% and the second one 16.3% (Figure 14). Triplicates are grouped for each sampling site. ANOVA on PC scores showed that metals concentrations significantly differed between sampling sites and between contamination status, but not between Adriatic regions **(**Table 4**)**.

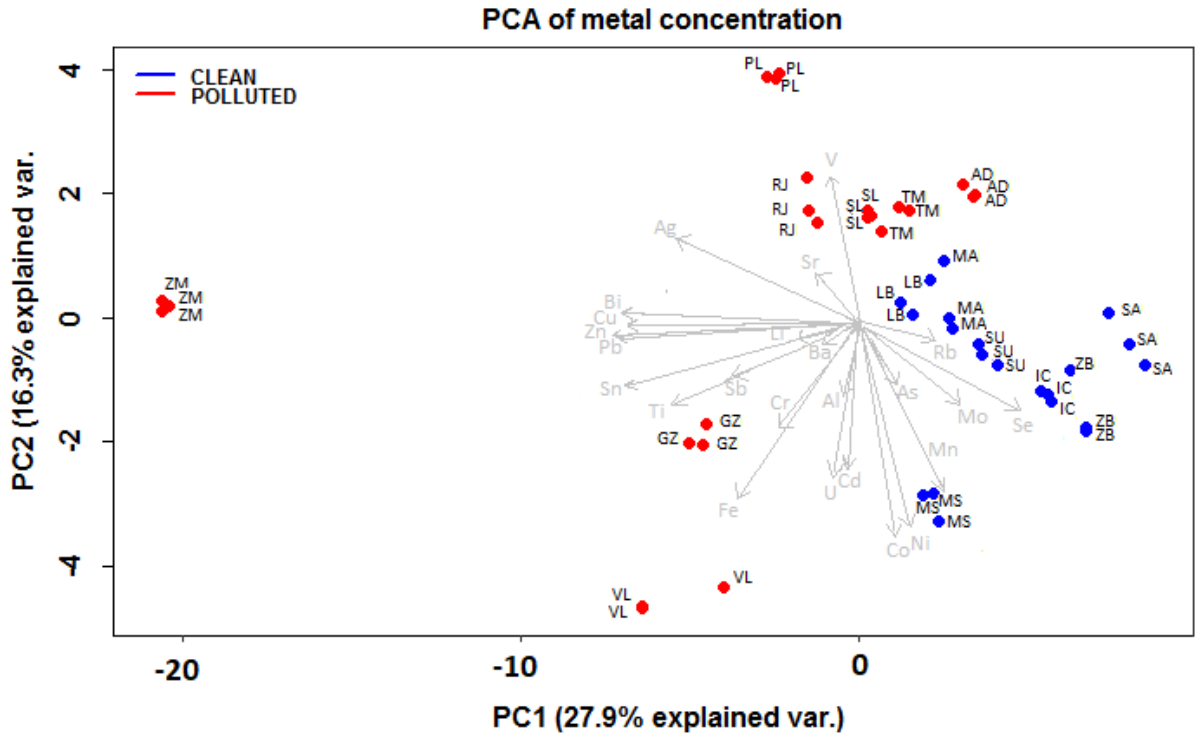

Figure 14. PCA biplot on metal concentrations accumulated in mussel's tissue. Metals are grouped in triplic ates for each sampling site. Plot is showing the first two principal components obtained in analysis, explaining 44.2% of the variation. Metals significantly differed between sampling sites and according to pollution status ($p < 0.0001$).

4.2.1. Relationship between the morphological traits and two blocks of predictors

Using PLS-R2 multivariate technique the relationship between the morphological traits and two blocks of predictors - environmental variables and metals has been determined.

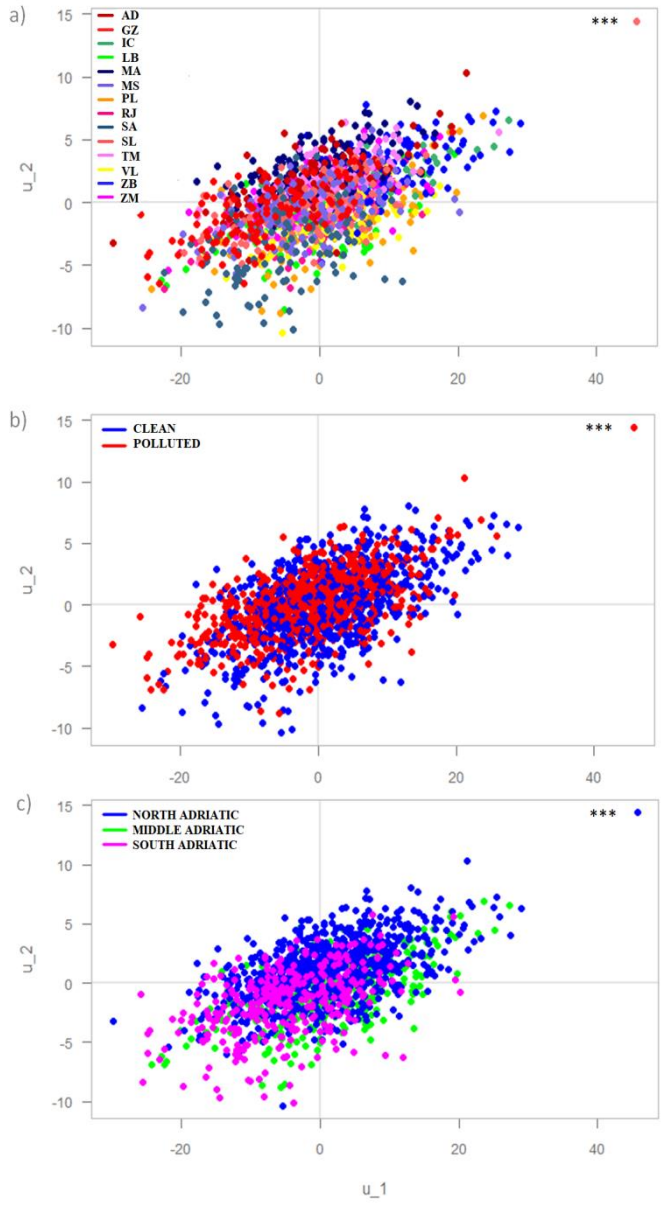

Figure 15. PLS-R2 score plots of native populations morphometric data, based on *y* components (u1 and u2). Plots are representing relationship between response variables (morphological traits) and predictors (environmental variables) towards sample sites (a), pollution status (clean vs. polluted sites – b) and spatial distribution (Adriatic regions – c).

ANOVA test on PLS-R2 scores showed that morphological traits significantly differed between sampling sites, pollution status and Adriatic regions depending on both blocks of predictors (Figures 15 and 16).

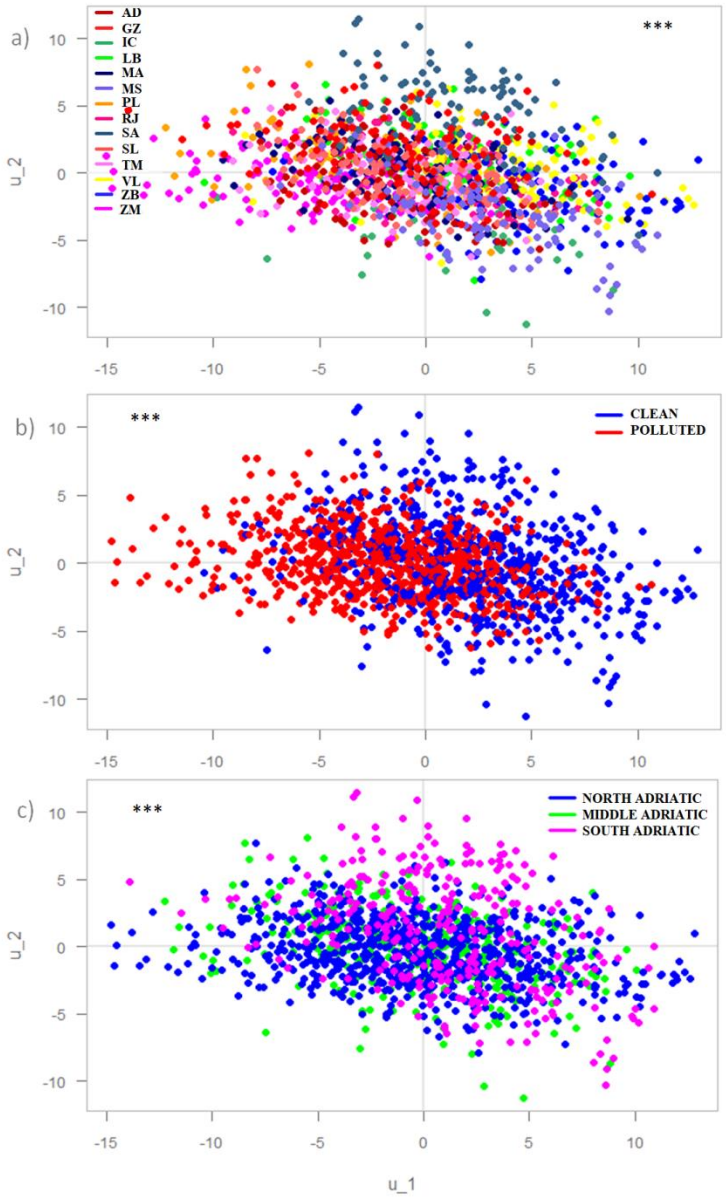

Figure 16. PLS-R2 score plots of native populations morphometric data, based on *y* components (u1 and u2). Plots are representing relationship between response variables (morphological traits) and predictors (metals) towards sample sites (a), pollution status (clean vs. polluted sites – b) and spatial distribution (Adriatic regions – c).

Variable importance for the projection - VIP plots (allow to quickly identify which environmental variables contribute the most to the model) and standardized coefficients (show how increases of predictors affects response variables) are presented in Supplementary materials (Figures S1 and S2, respectevely). Validation model of the morphological traits vs. environmental variables relationship shows R^2 and Q^2 values of a given model. R^2 is used to measure predictive power of the data, where R^2 = 100% indicates perfect description of the data by the model. Q^2 measures the global goodness of fit and the predictive quality of the model. Q^2 = 100% indicates perfect predictability, whereas low percentages suggests that the quality of the fit varies a lot. Environmental variables showed higher descriptive power than metals (86.4% for environmental variables, 53.7% for metals) (Figure 17). Nevertheless, despite generally very low predictive quality for both sets of variables, metal data showed somewhat higher predictability (1% environmental variables, 8% for metals).

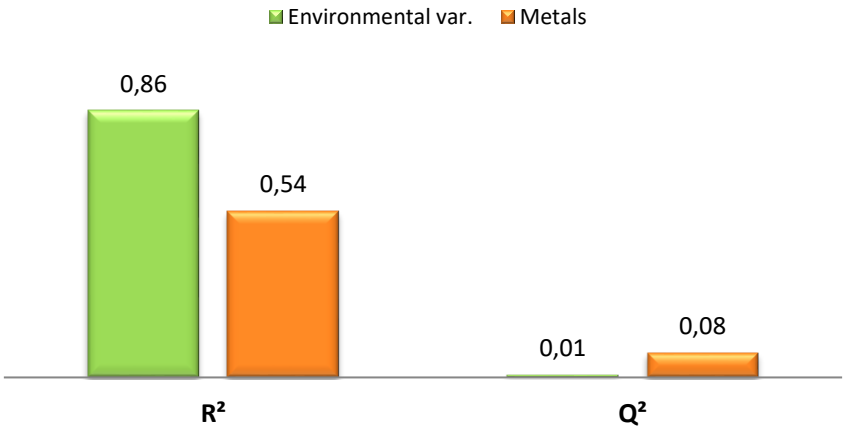

Figure 17. Validation model of the morphological traits vs. environmental variables (green)/metals (orange) relationship using PLS-R2. The R^2 value of a given model is used to measure descriptive power of the data, and the Q^2 value of the model is used to assess the predictive power of the model. R^2 = 100% indicates perfect description of the data by the model, whereas Q^2 =100% indicates perfect predictability.

4.2. Fluctuating asymmetry

4.2.1. Transplant

Comparing the differences of the left and right shell morphological characteristics (on the sample of 900 individuals in Marina population) measures of fluctuating asymmetry (FA) were obtained. The highest asymmetry values were observed for MASS, PAL and WPR (Figure 18). The lowest asymmetry is characterized by LIG and H.

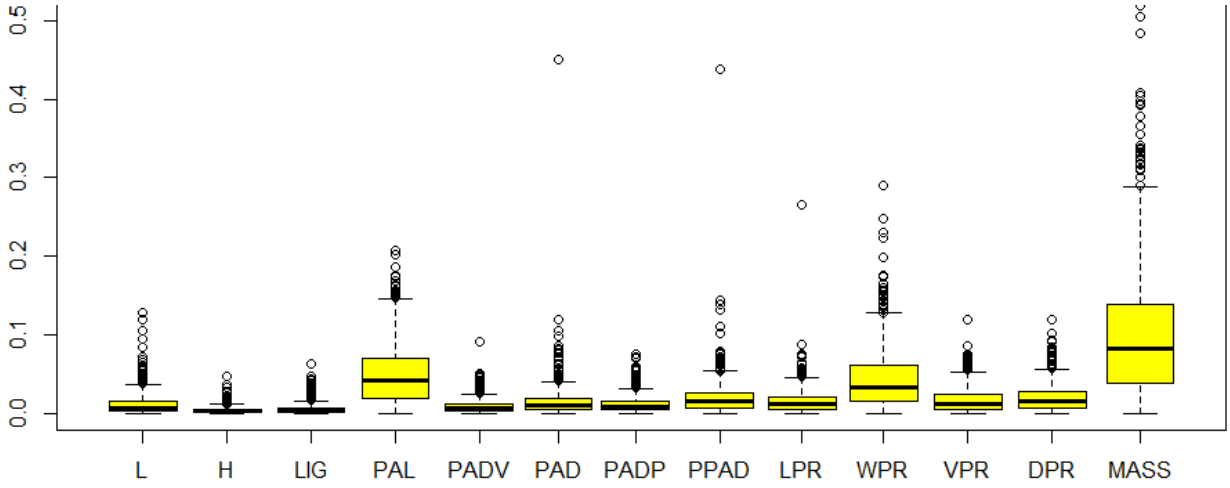

Figure 18. Fluctuating asymmetry of 13 morphological traits, measured on one, large scale population of 900 individuals (Marina, exposed in transplant experiment).

4.2.2. Mesocosm

Additionally, FA on the samples of 800 individuals from two populations (Marina and Gruž) was obtained. Results showed a similar FA patterns for particulate traits for both observed populations. The highest asymmetry values for both populations were observed for PAL, PAD, WPR and L (Figure 19). These traits also have the greatest standard deviation. The lowest asymmetry for both populations is characterized by LIG, H and MASS. Overall, Marina population showed wider distribution of FA values among individuals, and somewhat higher FA values.

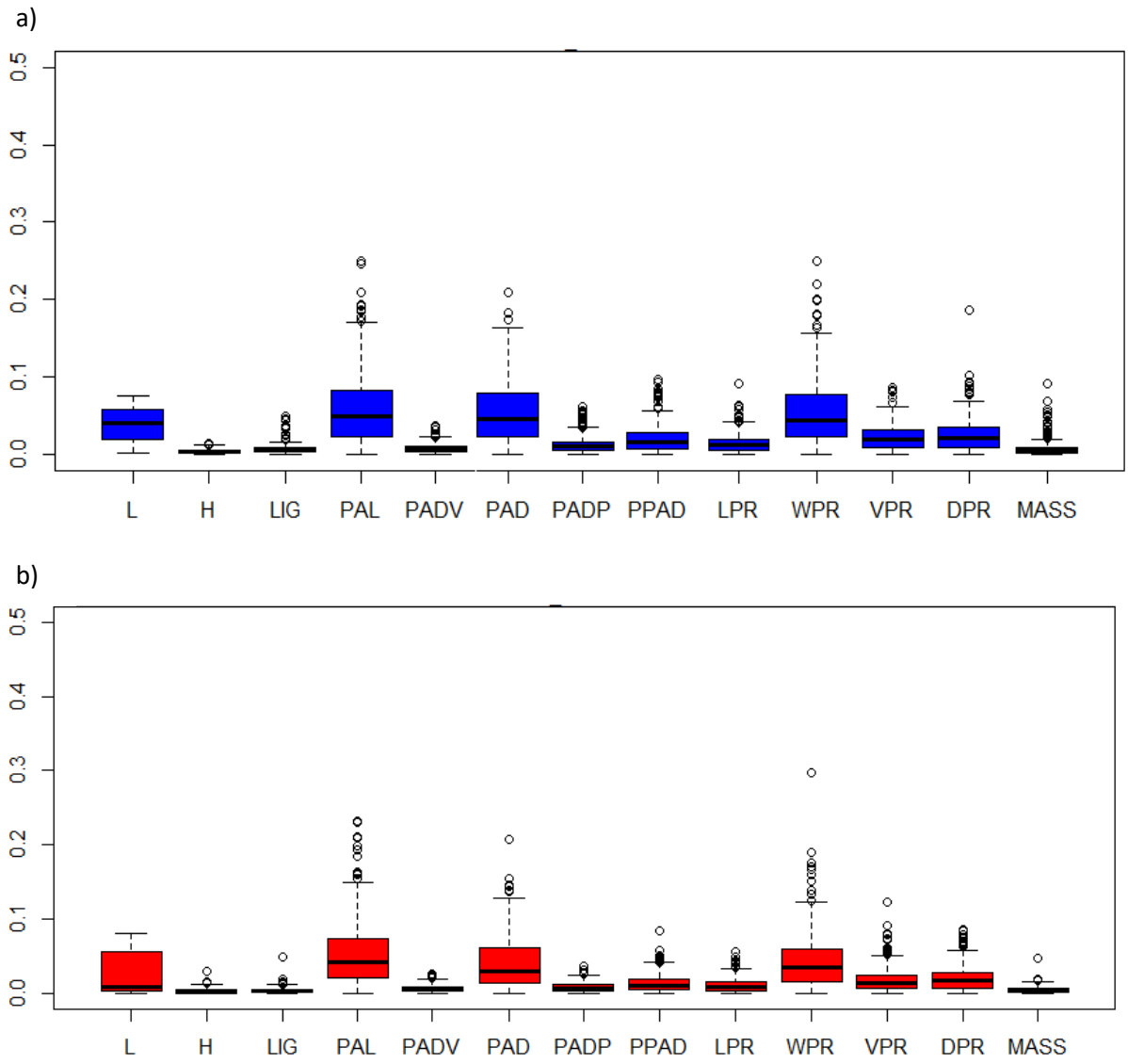

Figure 19. Fluctuating asymmetry of 13 morphological traits, measured on two populations (400 individuals each) – a) Marina and b) Gruž.

4.3. Biomarkers

4.3.1. Seasonality in pollution-depended biomarker status

PCA analysis on natural populations biomarker data, conducted to perform PTA, revealed that the first two principal components of the entire data set explained 43.6% of the total variance, where first one explained 25.8% and the second one 17.8% (Figure 20). The trajectories representing two seasons didn't exhibit significant amounts of biochemical and cellular change ($p=0.059$).

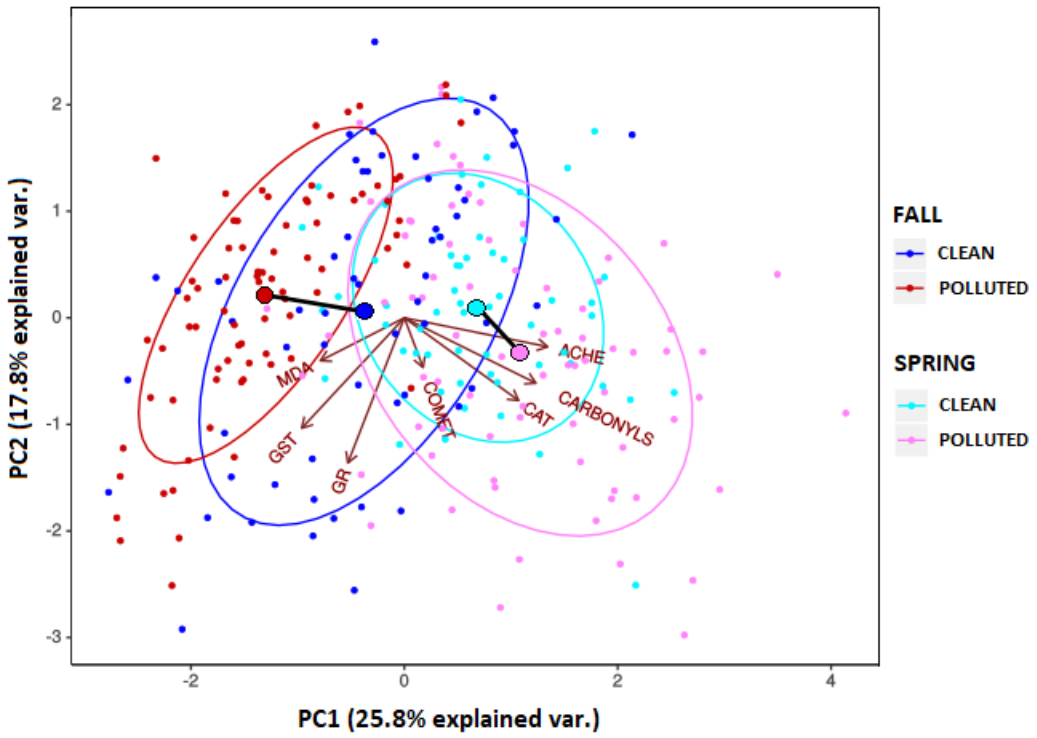

Figure 20. PCA plot on natural populations biomarker data, showing how biomarker status in different seasons depends on the pollution status. There are two trajectories plotted, one for each sampling event (season; fall is the longer trajectory, indicated with darker shades while spring is shorter one, indicated with brighter shades). Each trajectory joins the middle of the "clean sites data" (blue and turquoise shades) to the "polluted sites data" (red and pink shades). Trajectory ends are centers of group ellipses. Plot is also showing the relationship between biomarkers (labelled).

However, centroids of a data for clean and polluted sites move in opposite directions ($p=0.046$) along PC1 depending on the sampling season. More than that - clean sites exhibit significantly more similarity in biomarker response between seasons, than polluted sites ($p=0.001$). Moreover, centroids of the pollution status data move in the similar direction, between two seasons, indicating similar direction of the seasonal effect.

4.3.2. Biomarker response capacities toward pollution status

Results of generalized linear model (glm) (Table 5) on paired block design showed significant differences between mussel's exposed to clean and polluted sites in each region ($p < 0.001$), where populations exposed to polluted sites consistently exhibit higher IBR values (Figure 21a). Moreover, biomarker status also significantly differed between three Adriatic regions ($p < 0.001$), showing persistent decrease in IBR values from north to south. Additionally, Tukey's post hoc test revealed differences between all sites of exposure ($p < 0.05$) except between ZBT and GZT. The result of Tukey's post hoc test on mesocosm experiment highlighted significant difference ($p < 0.05$) between individuals originating from GZ exposed to control or copper, while MA population didn't demonstrate an effect upon exposure to copper (Figure 21b). The results of glm (Table 5) revealed population effect of biomarker response between GZ and MA populations ($p < 0.001$) with generally higher IBR in GZ, which decreased upon exposure to copper.

Table 5. Generalized linear model fitted with aov() on IBR data. Df = degrees of freedom, Sum Sq = sum of squares, Mean Sq = mean squares. Means of all tested group comparisons, in both experiments, are significantly different; $p < 0.001$***

Transplant

	Df	Sum Sq	Mean Sq	F value	*p* value
SITE	5	4785	957	1988	<2e-16 ***
STATUS (Clean/Polluted)	1	1832	1832	3804.8	<2e-16 ***
REGION (North/Middle/South)	2	2490.2	1245.1	2585.9	<2e-16 ***
STATUS:REGION	2	462.8	231.4	480.6	<2e-16 ***
Residuals	714	343.8	0.5		

Mesocosm

	Df	Sum Sq	Mean Sq	F value	*P* value
TREATMENT (Control vs. Cu)	1	23.17	23.17	38.39	1.26e-09 ***
POPULATION (Gz/Ma)	1	247.97	247.97	410.84	<2e-16 ***
TREATMEMT:POPULATION	1	23.7	23.7	39.27	8.28e-10 ***
Residuals	476	287.3	0.6		

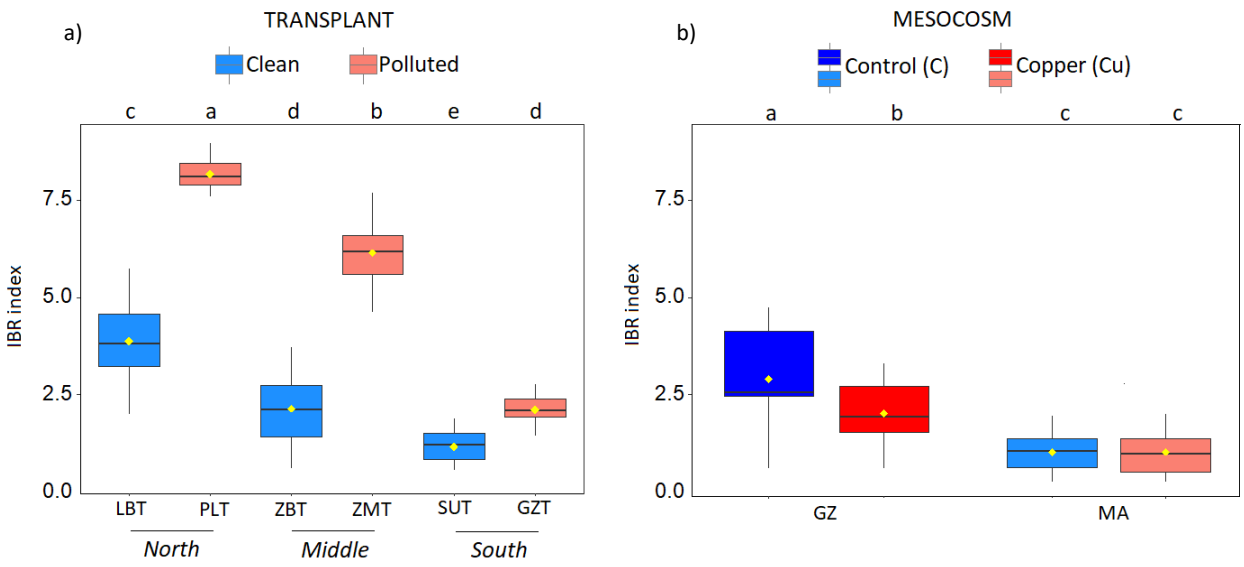

Figure 21. Boxplots for the calculated IBR index in a - transplant and b - mesocosm experiment.The yellow square stands for mean, bold line stands for median, the box represents quartiles and whiskers stand for minimum and maximum. Different letters indicate between-site differences, which were analysed with ANOVA's post hoc Tukey test.

4.3.3. The roles of environmental factors and metals in expressed biomarker status variability

Biomarker status of mussels in paired block designed transplant experiment significantly differed between regions when predictor were environmental variables (Figure 22b), and between sites of different pollution status when predictor were metals accumulated in mussel's tissue (Figure 22c). We didn't observe reverse significance (Figures 22a i d). Significance representing the p value < 0.001 is indicated by *** on score plots obtained by PLS-R2 analysis.

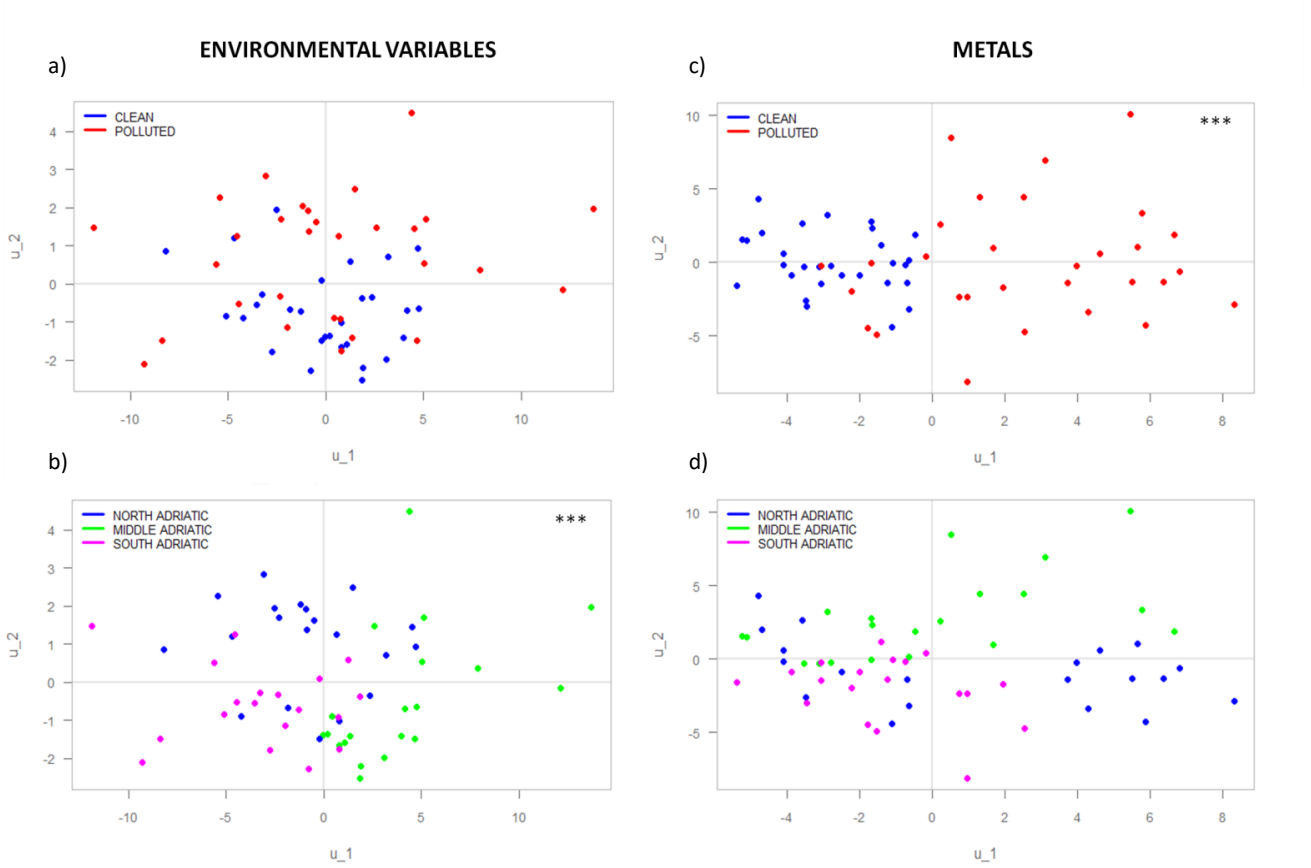

Figure 22. PLS-R2 score plots of transplant data, based on *y* components (u1 and u2). Plots are representing relationship between response variables (biomarkers) and predictors (environmental variables – a,b; metals – c,d) towards pollution status (clean vs. polluted sites – a,c) and spatial distribution (Adriatic regions – b,d). ANOVA test on PLS-R2 scores shows the significance of status and regions specifics in 'response-predictor' relation, where *** represents significant

Variable importance for the projection and standardized coefficients are presented in Supplementary materials (Figures S3 and S4, respectevely). Environmental variables showed higher descriptive power than metals (94.5% for environmental variables, 63% for metals) (Figure 23). Nevertheless, despite higher explanation by environmental data, metal data showed higher predictability (3.7% environmental variables, 18.5% for metals).

Additionally, we ran the PLS-R2 analysis on native populations, to compare it with the results from transplant experiment (Figures S5-11, Supplementary materials).

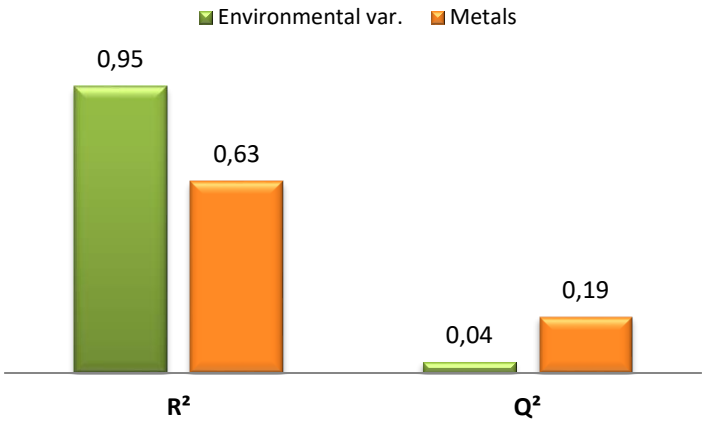

Figure 23. Validation model of the biomarkers vs. environmental variables/metals relationship using PLS-R2. The R^2 value of a given model is used to measure descriptive power of the data, and the Q^2 value of the model is used to assess the predictive power of the model. R^2 = 100% indicates perfect description of the data by the model, whereas Q^2 =100% indicates perfect predictability. Environmental variables have higher descriptive power than metals – 94.5% for environmental variables, 63% for metals, with Q^2 - 3.7% and 18.5%, respectively.

4.4. Stress on stress experiment

After they have been pre-exposed to certain source of stress (polluted environment in transplant, Cu in mesocosm experiment), mussels from both experiments were left on air, and mortality was checked daily.

4.4.1. Transplant

Individuals exposed to polluted site Pula (PLT) had the longest survival time, with maximum of 12 days (Figure 24a). This population is followed by individals pre-exposed to another polluted site - ZMT, with maximum survival time of 10 days. All the others populations (ZBT, GZT, LBT and SUT) had the survival time of 9 days, among which SUT had the lowest survival probability.

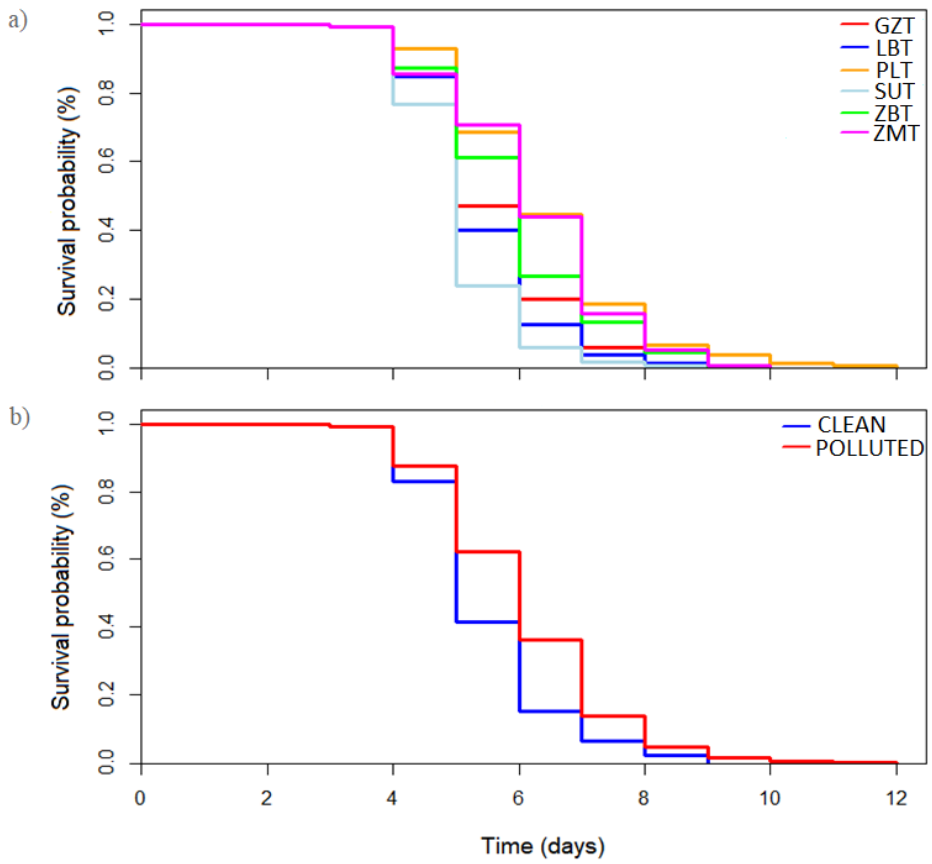

Figure 24. Kaplan-Meier's *stress on stress* survival curves – transplant experiment. Plots are showing survival duration of mussels pre-exposed to six realistic environmental conditions (a), using paired block design - polluted vs. clean sites (b) in transplant experiment, and left on air before all individuals experienced mortality. Longer horizontal gap means that it took longer for one group to experience a certain fraction of deaths. The interval is terminated by the occurrence of mortality. Longer vertical gap means that at a specific time point, one group had a greater fraction of subjects surviving.

Based on the pollution status (Figure 24b), populations pre-exposed to polluted environment have generally longer survival time and higher survival probability, that is, induced higher fitness. This pattern is repeated in each Adriatic region (Figure 25).

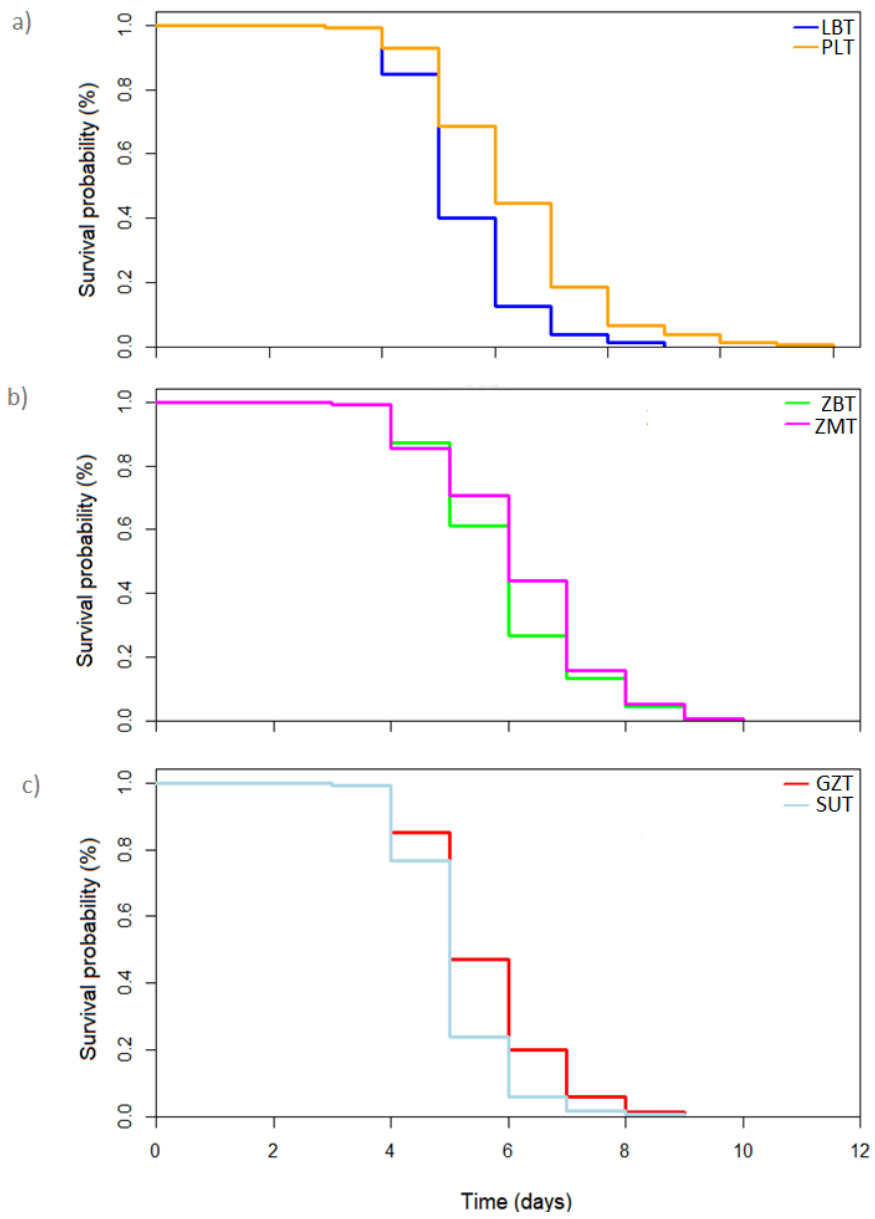

Figure 25. Kaplan-Meier's *stress on stress* survival curves – transplant experiment. Plots are sžž howing survival duration of mussels pre-exposed to six realistic environmental conditions using paired block design three geographic regions (a – North, b – Middle, c – South) in transplant experiment, and left on air before all individuals experienced mortality. Longer horizontal gap means that it took longer for one group to experience a certain fraction of deaths. The interval is terminated by the occurrence of mortality. Longer vertical gap means that at a specific time point, one group had a greater fraction of subjects surviving.

4.4.2. Mesocosm

Source population Gruž exhibited the longest survival time for specimens in control group and those pre-exposed to copper, where control group had the longest survival time of 15 days, and highest survival probability (Figure 26a). Individuals from Gruž pre-exposed to copper lived maximum 12 days. Source population Marina showed the same pattern as Gruž, where exposure to toxicant decreased the fitness.

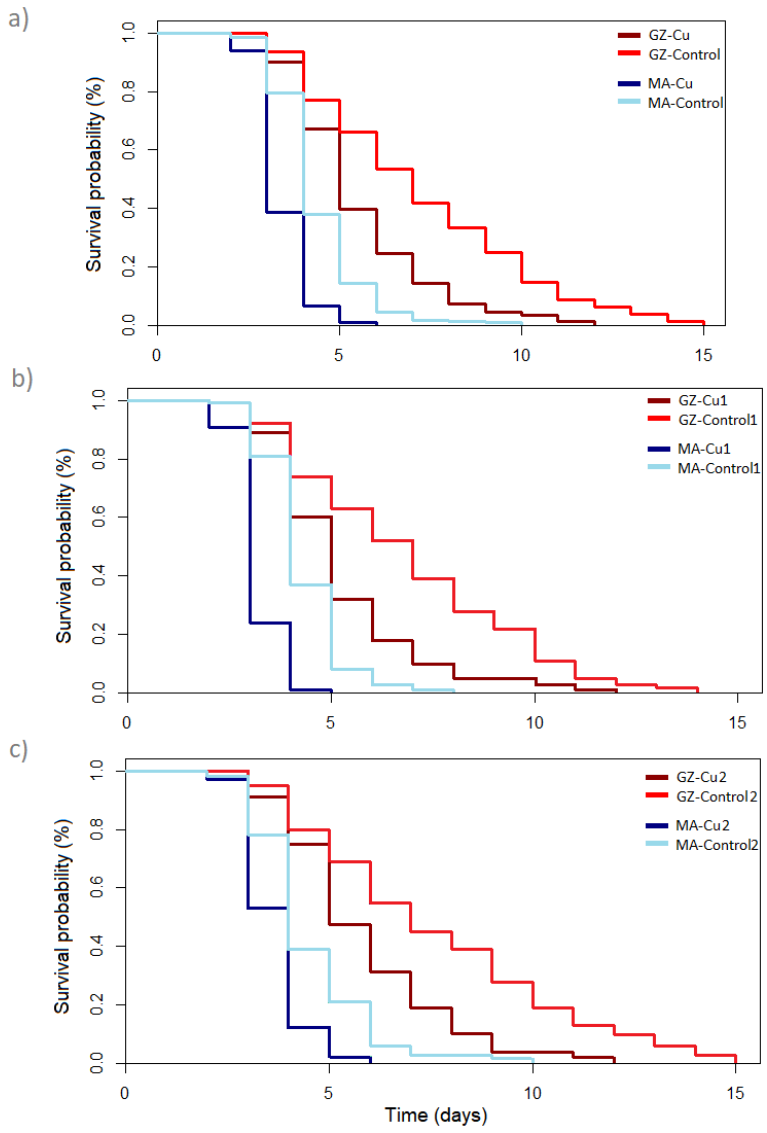

Figure 26. Kaplan-Meier's *stress on stress* survival curves – mesocosm experiment. Plots are showing survival duration of two mussel's populations (Marina – MA and Gruž - GZ) pre-exposed to copper or clean seawater (a), in two replicates per population (b and c), and left on air before all individuals experienced mortality. Longer horizontal gap means that it took longer for one group to experience a certain fraction of deaths. The interval is terminated by the occurrence of mortality. Longer vertical gap means that at a specific time point, one group had a greater fraction of subjects surviving.

Control group had longer survival time (maximum 10 days) than group pre-exposed to copper (maximum 6 days). We achieved the mesocom experiment in two replicas, and both exibited the same pattern (Fig 30b, c).

4.5. Genetic architecture of *Mytilus galloprovincialis* morphological traits estimated using GWAS

4.5.1. Hyperparameters on five *Mytilus galloprovincialis* data sets

We described the genetic architecture of mussel's morphological traits using five data sets, with minor allele frequency (MAF) greater than 0.05 for GWA mapping analyses. Here we report the median, lower and higher 95% confidence interval (95% equal tail posterior probability intervals [95% ETPIs]) for the proportion of the total phenotypic variation (i.e. PVE), proportion of the phenotypic variation that can be explained by 'large-effect' SNPs alone (i.e. PGE) and number of SNPs (n_SNP) that have non-zero effects on phenotypic variation for each data set and comparisons. We also report the priors h and rho, used to estimate the proportion of variance explained by the model and conditional prior probability that defines the sparsity of the model, respectively (Tables S4 – S8, Supplementary materials).

In Gruž_meso dataset (394 individuals, population Gruž, 19129 SNPs) total phenotypic variation being explained by genotype (PVE) varied between 8.4% (WPR) and 56.3% (PAD) (Figure 27). The proportion of the total phenotypic variation that can be explained only by 'large-effect' SNPs (PGE) varied between 18% (PAL) and 57% (VPR), being due to 13 (WH) – 44 (PAL) SNPs with measurable phenotypic effects (median estimates).

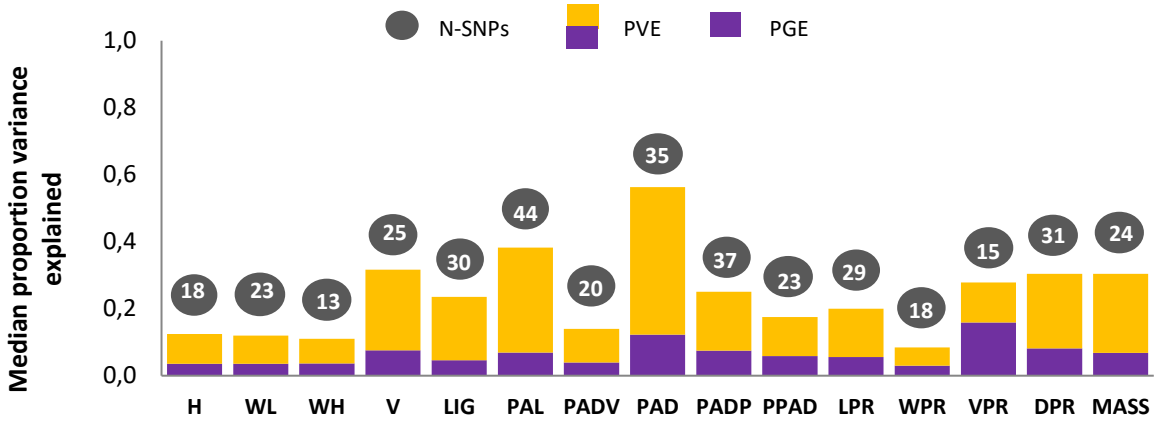

Figure 27. Hyper-parameter estimates of Gruž population's genetic architecture (Gruž_meso dataset), 394 individuals used in mesocosm experiment. Results were obtained by *Gemma* (Zhou and Stephens 2012, Zhou et al., 2013). Plot is showing proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'large-effect' SNPs alone (PGE) and number of SNPs (N-SNPs) that have non-zero effects on phenotypic variation.

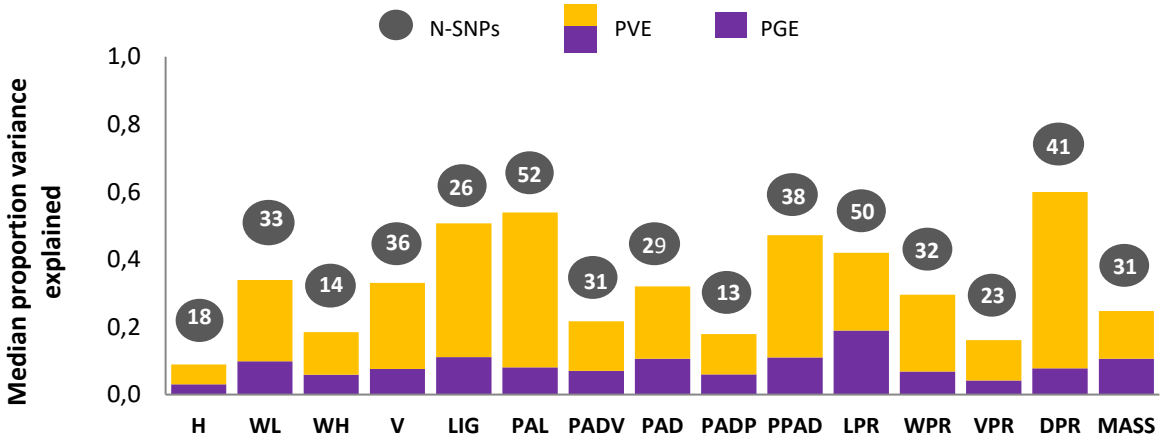

Figure 28. Hyper-parameter estimates of Marina population's genetic architecture (Marina_meso dataset), 377 individuals used in mesocosm experiment. Results were obtained by *Gemma* (Zhou and Stephens 2012, Zhou et al., 2013). Plot is showing proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'large-effect' SNPs alone (PGE) and number of SNPs (N-SNPs) that have non-zero effects on phenotypic variation.

Marina_meso dataset (377 individuals, population Marina, 19129 SNPs) had larger PVEs, that varied between 9% (H) and 60% (DPR) with PGE varied between 15% (PAL) and 45% (LPR), and being due to 13 (PADP) – 52 (PAL) SNPs with large phenotypic effects (median estimates) (Figure 28).

Marina_trans dataset (883 individuals, population Marina, 18850 SNPs) had generally lower PVE and PGE values than Marina_meso for most of the traits, except WL and WH, with PVEs between 6% (H) and 48.9% (WH) and PGE between 12.3% (WL) and 61% (PPAD), being due to 8 (PPAD) – 88 (WH) SNPs with measurable phenotypic effects (median estimates) (Figure 29). Also, Marina_trans had somewhat narrower PVE ETPIs than mesocosm populations. Marina_pool (1258 individuals, 18728 SNPs) showed PVEs between 10% (PADP) and 44.4% (PAL) (Figure 30). The proportion of the total phenotypic variation that can be explained only by 'large-effect' SNPs varied between 2.3% (WH) and 25.4% (PADP) with n_SNPs between 7 (WH) – 43 (WPR) (median estimates).

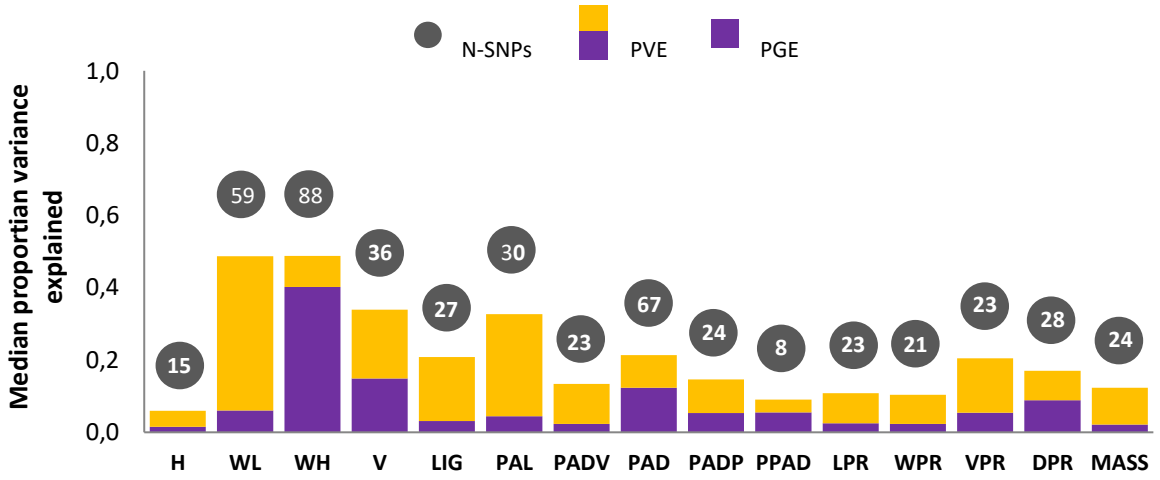

Figure 29. Hyper-parameter estimates of Marina population's genetic architecture (Marina_ trans dataset), 883 individuals used in transplant experiment. Results were obtained by *Gemma* (Zhou and Stephens 2012, Zhou et al., 2013). Plot is showing proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'large-effect' SNPs alone (PGE) and number of SNPs (N-SNPs) that have non-zero effects on phenotypic variation.

General trend is quite similar when the pool of individuals is compared to separated data sets of Marina_meso and Marina_trans. ETPIs are lower and a bit narrower for PVEs and PGEs compared to other data sets (Marina_meso and Gruž_meso showed the highest ETPIs span for PVEs among other data sets). The most of the lower ETPIs for PGE were firmly on zero, except for VPR - 1.8% - Gruž population, volume - 1.5% - Marina transplant, LIG – 1% - Marina pool.

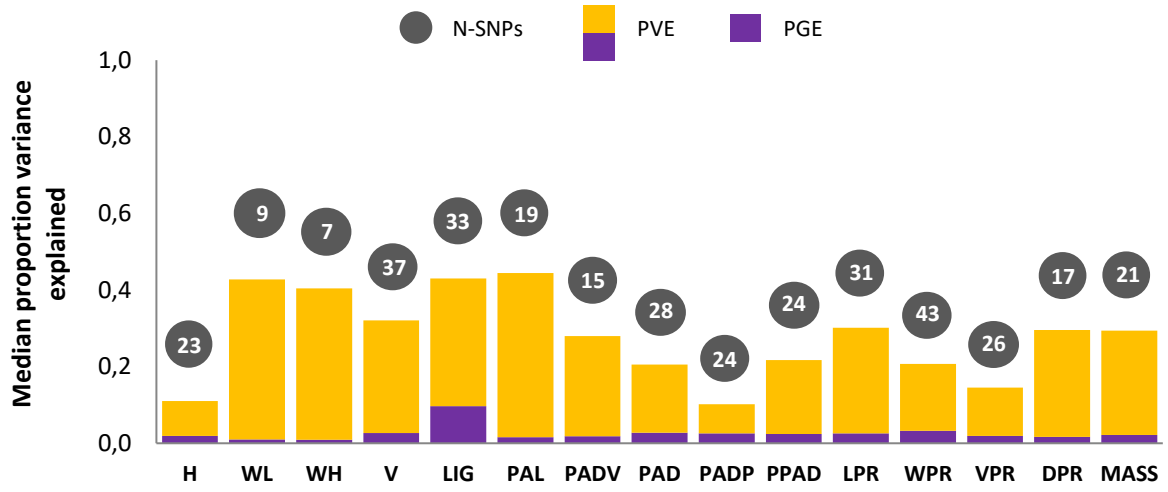

Figure 30. Hyper-parameter estimates of genetic architecture analysed for all individuals of Marina population (Marina_pool dataset), 1258 individuals exposed in mesocosm and transplant experiment. Results were obtained by *Gemma* (Zhou and Stephens 2012, Zhou et al., 2013). Plot is showing proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'large-effect' SNPs alone (PGE) and number of SNPs (N-SNPs) that have non-zero effects on phenotypic variation.

Exceptionally, data set composed of 15 native populations (288 individuals, 18655 SNPs) showed surprisingly high PVE values (Figure 31). This data set contains the lowest number of analyzed individuals among all data sets, which were in addition sampled from number of populations exerting phenotypic divergence). Native populations had the highest PVEs, between 44.3% (H) and 98.5% (MASS). Results for the other hyperparameters (PGE, n-SNPs) remained consistent in showing small PGEs and small number of SNPs with measurable effect with the proportion of the total phenotypic variation that can be explained only by non zero effect SNPs

between 8% (WPR) and 35.5% (H). Number of SNPs with measurable phenotypic effects was between 20 (DPR) – 69 (PAD) (median estimates).

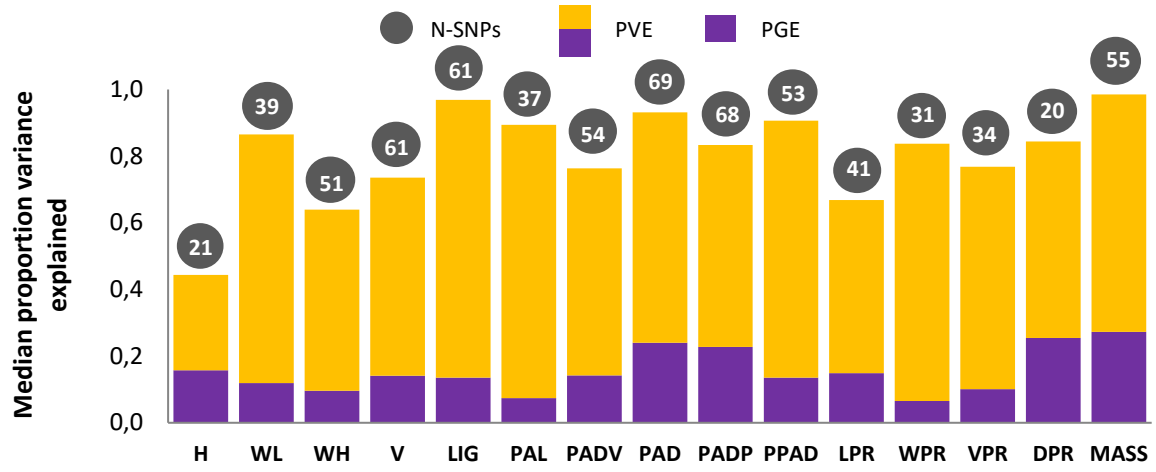

Figure 31. Hyper-parameter estimates of native populations (288 individuals) genetic architecture (Native_pops dataset). Results were obtained by *Gemma* (Zhou and Stephens 2012, Zhou et al., 2013). Plot is showing proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'large-effect' SNPs alone (PGE) and number of SNPs (N-SNPs) that have non-zero effects on phenotypic variation.

Correlation of median hyperparameter estimates between the different data sets were not observed for most of the traits. Lower ETPIs for PVE in all data sets, for most of the traits, do tend to be above zero (Figure 32). Thereforer results on PVE continue to point to a modestly heritable basis at best.

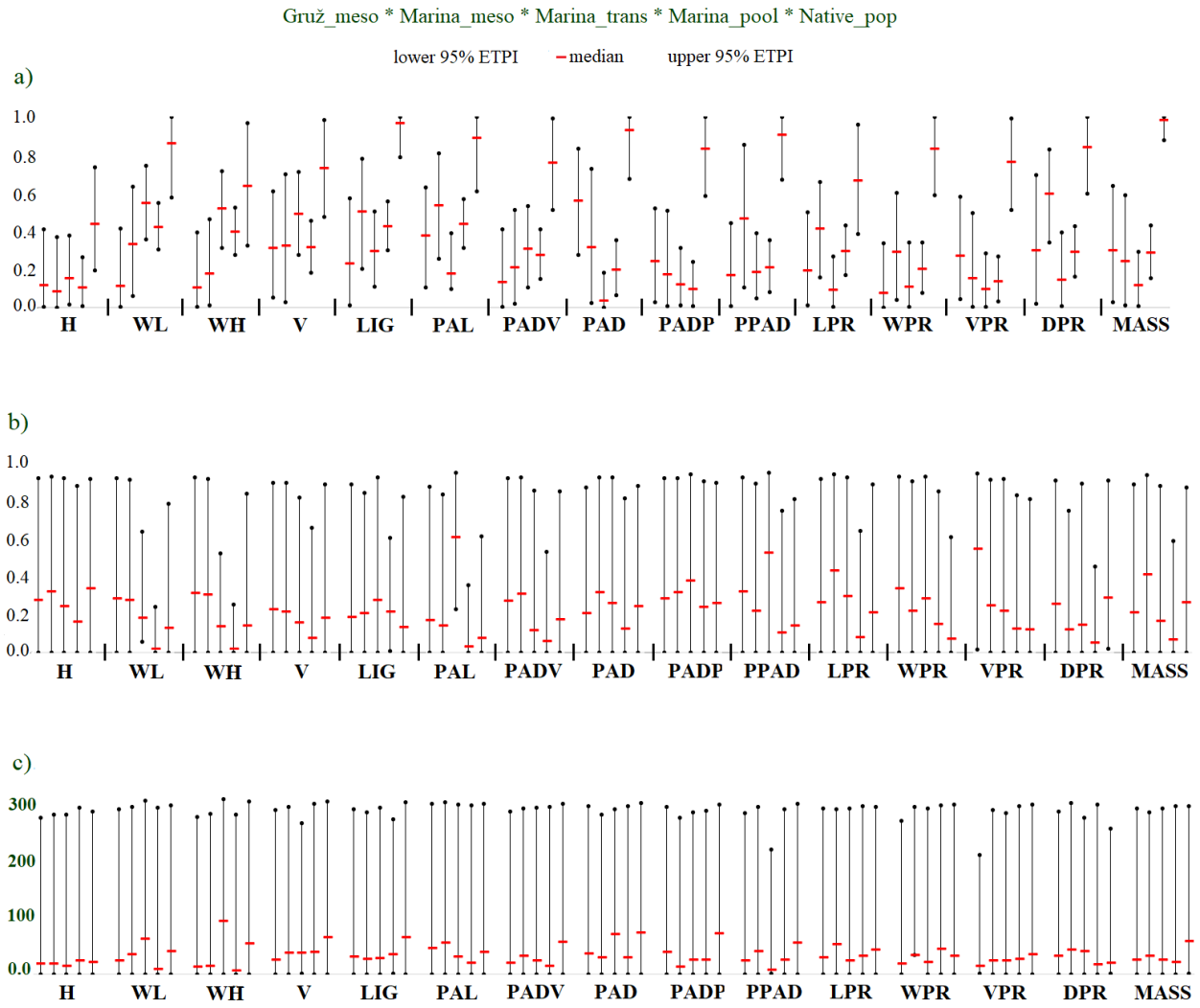

Figure 32. Comparison of the ETPI's estimation (a) PVE, b) PGE, c) N-SNPs) between the datasets (Gruž_meso, Marina_meso, Marina_trans, Marina_pool, Native_pop; respectively). Lower and upper 95% ETPIs are represented with a black dot, median values are represented with a red horizontal line. Results were obtained by *Gemma* (Zhou and Stephens 2012, Zhou et al., 2013).

For each data set top 1% SNPs (Figures S14 - S18, Supplementary materials) and number of SNPs with posterior inclusion probability (PIP) greater than 0.01 (SNPs that are more strongly associated with phenotypic variation will have larger PIPs) were calculated. Finally, we

examined the number of shared $top_{1\%}$ and $PIP_{0.01}$ SNPs for each trait, between data sets (Table S9 – Supplementary materials).

The number of overlapping SNPs was very low between most of the data sets. Number of shared SNPs was higher between subsets Marina_meso and Marina_pool, where number of shared $top_{1\%}$ SNPs between these two data sets was between 25 (V and WH) and 48 (DPR), and the number of shared $PIP_{0.01}$ SNPs was in range from 2 (WH) – 36 (LPR). PAD exerted high number of shared $PIP_{0.01}$ SNPs (36) between Marina_trans and Native populations.

The results are in accordance with overall low PIP values. Somewhat higher PIP values have volume in Marina_trans (max 0.8), and MASS (max 0.4) and DPR (max 0.7) in Native populations.

4.5.2. Single SNP analysis

Results on single SNP analysis with controlling for population structure didn't showed associated SNPs at genome-wide significance. Without controlling for the population structure most of the traits in native populations had at least few associated SNPs, but none of them was shared with any other data set (Table 6). There were just few associated SNPs in Marina_pool and Marina_trans, and these SNPs are mainly shared between mentioned data sets, for PAL and PPAD. There were few associated SNPs in mesocosm data sets, and only one of them in Gruž_meso was shared with Marina_pool (for PAD).

Table 6. Associated SNPs shared between data sets. Results were obtained within single SNP analysis using R package GenABEL v1.8.0, without controlling for population structure.

	N of associated SNPs			
Trait	**GRUZ_MESO**	**MARINA_TRANS**	**MARINA_POOL**	Shared SNPs
pal	0	7	5	4
ppad	0	1	2	1
pad	1	0	1	1

4.5.3. Cross validation (predictive power of the models)

Cross - validation results on Marina_pool dataset showed that the models have modest predictive power, that ranges for different traits between 0.03 (PADP) to 0.29 (LIG). Null prediction accuracy was observed only for H. Predictive ability was positively correlated with the PVE values (higher PVE is, the higher predicting power), but the opposite goes for PGE (Figure 33).

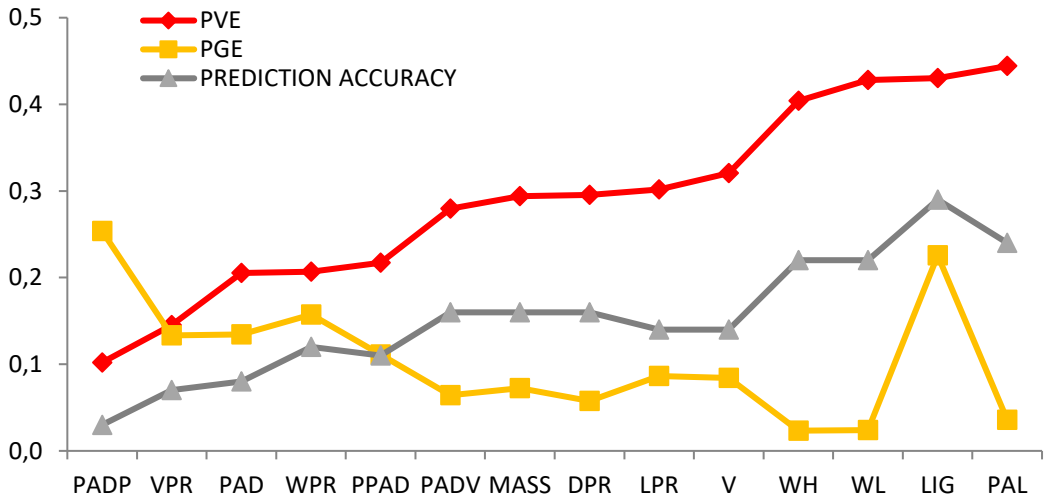

Figure 33. Relation between PVE, PGE and prediction accuracy (Marina_pool dataset) for 14 morphological traits of *M. galloprovincialis*. H was not included due to lack of predicting power.

5. DISCUSSION

5.1. Phenotypic variation

It is known that shell morphometry is a good taxonomic tool, used to discriminate among species of genus *Mytilus* (McDonald et al., 1991, Sarver et al., 1993, Innes and Bates, 1999, Gardner, 2004, Krapivka et al., 2007, Beaumont et al., 2008, Valladares et al., 2010). For example, McDonalds et al. (1991) analyzed individuals of mussels from locations for which allozyme characters indicated the presence of only a single species. They managed to distinguish *M. galloprovincialis* and *M. edulis* based on morphological traits and proved length of the anterior adductor muscle scar and length of the hinge plate to be useful for distinguishing these *Mytilus* species. The most informative morphological characters for distinguishing between *M. galloprovincialis* and *M. trossulus* (Sarver et al., 1993) were byssal retractor muscle scar width, posterior adductor muscle scar length, and byssal retractor muscle scar length. Assuming they allow distinction of species, these morphometric traits could be genetically conditioned to some extent. However, many authors also showed intraspecific phenotypic variations regarding shell morphological traits (discussed below).

Results of this study are indicating very high genetic connectivity among studied populations of *M. galloprovincialis* on a relatively large geographical scale (over 500 km of maritime distances). This pattern of broad-scale panmixia is consistent with the hypothesis of high gene flow (caused by the long lived larval pelagic state), which in the eastern Adriatic basin seems to be strong enough to counteract neutral genetic differentiation caused by the genetic drift. However, prediction of significant intraspecific morphological variability among the *M. galloprovincialis* populations is confirmed (H1), not only related to the origin of samples, but also to pollution status and to a longitude as well (three geographic regions along the eastern Adriatic coast). Both environmental variables and metals contributed to that. Krapivka et al. (2007) showed a highly significant morphological variation between the *Mytilus chilensis* populations using a Fourier elliptical analysis on shell outline shapes. Chilean blue mussel was examined in eight populations covering the totality of the southeastern Pacific distribution range, which represents over 1800 km of its latitudinal gradient. These authors found significant differences in the convexity of the shell ventral margin, umbo shape and shell elongation (characters that were not included in this study). Karakousis et al. (1993) found a significant

degree of variation investigated at the morphological level, within and among eight populations of *M. galloprovincialis*, from different coasts of the Northern and Central Aegean Sea. Additionally, the results of their investigation indicate that morphological variation does not correlate with genetic variation and that the overall genetic differentiation among the populations is rather low. Populations of *M. galloprovincialis* along the Adriatic Sea were mainly distinguished by traits related to shell shape (HL, WL, WH, V) and position of posterior adductor and retractor muscles (PADP, PPAD, PADV, VPR, DPR). Comparing large samples from the two source populations (MA and GZ) representing contrasting environments introduced significant difference between populations for almost all morphological traits. Here, comparison between higher number of individuals provide a clearer picture of morphological disjunction, highlighting a few traits that are contributing the most to the variation, and emphasizing the importance of shell shape and position of both posterior muscles, especially adductor. Shell length, height, and width are measures that describe the morphology of the mussel body in three dimensions (Seed, 1968). Those dimensions change because of an incremental growth from shell deposition, which is a labile contemporary factor (Blythe and Lea, 2008). Because such increments culminate over time, shell dimension traits are an obvious first place to look for long term morphological pattern in responses to changing environment. Results of this research point to that. Other authors have already recognized these traits as subjected to environmentally induced variation. *M. californianus* shell height and width varied at different locations along a mussel bed, corresponding to intertidal height (Kopp, 1979). Measurements of pollution also have association with the height over the width (H/W) of the mussels *M. edulis* (Lobel et al., 1991) and *M. californianus* (Lares et al., 2005). To round up the story, question can be address to the functional role of these phenotypic variations. Blythe and Lea (2008) hypothesized that the utility of height and width dimensions might change in response to parasites, predators and toxin bio-accumulation. In addition, the shell width is hypothesized to contribute to basal metabolism for a variety of reasons, and wider mussels have more tissue that confers metabolic cost (Blythe and Lea, 2008). Growth-related traits (i.e. associated to shell size), are of major interest for mollusc farming, and spotlight them as the object of separation between population may contribute to future research perspectives for improving aquaculture yields in an increasingly changing world.

Not to be ignored, except HL, WL, WH and V, this research also marked traits related to position of posterior muscles as responsible for phenotypic variability. Can those characters be related with the ones previously discussed (shell shape traits)? Freeman et al. (2009) experimentally compared the inducible defenses of the *M. edulis* from pairwise combinations of three predators. Predators were represented by the sea star, *Asterias vulgaris*, and the crabs *Carcinus maenas* and *Cancer irroratus*. As a response to predators, mussels did not simultaneously increase shell growth and adductor muscle growth, which might be suggesting that these induced traits require an energetic tradeoff, are phenotypically incompatible, and won't be induced easily together. However, the relation between shell shape traits and the position of posterior muscles can be alternatively explained by the process of shell accretion. Accretion occurs in the mussel's extrapallial space (near the shell margin), and progresses more rapidly at the shell margins than near the shell center (Wilbur and Saleuddin, 1983). As a mussel shell grows, the adductor and retractor muscles must migrate away from the shell hinge, toward the posterior shell margin. This highlights how enentually shift in position of posterior muscles can appear together with induced changes in shell shape.

Although morphological variation in bivalve molluscs has been addressed in several studies dealing with changes in shell morphology, few studies have related exact factors that impact morphological patterns in *M. galloprovincialis.*

This research highlighted environmental variables having a higher descriptive power than metals (used here as proxy for environmental pollution burden). Most important traits for population's variability were highly related to nitrates, Chl_a, T_max, light and anthropogenic heavy metals. Environmental variables that contributed the most to phenotypic variability, in general, were nutrients, light, O2, salinity and sea surface temperature. Most of these environmental factors are in direct relationship with phytoplankton contribution in the water column, and food availability affects the growth rate of mussels (Dahlhoff and Menge, 1996). Under nutrient-saturated conditions, temperature and light are the key factors in controlling phytoplankton productivity, but e.g. after algal blooms, the nutrient supply is low and determines the total algal biomass (Sakshaug and Holm-Hansen, 1986, Graneli, 1987). For phytoplankton, light changes may cause variations in the photosynthesis and the respiration rate (Verity, 1982, Harris, 1986). Light itself

is generally important environmental feature and shell width dimension would increase the rate of light absorbance, because this increases the surface area that is exposed to normally incident solar radiation (Blythe and Lea, 2008). Price and Lakshmi (2014) found that the mussel's growth along the Oregon coast is more affected by the average sea temperature than the amount of food. Morán at al. (2018) highlighted the phenotypic plasticity of *Ameghinomya antiqua* as a possible response to different environmental conditions, where shells morphometric differences could be linked to variations in wave action, tidal influences, predation pressure and/or sea surface temperature substrate, which all potentially modify the shape and size of this species. Variations in salinity have widespread effects on aquatic organisms and can influence the geographical distribution of mussels (*M. californianus*) (Young, 1941) and its genetic structure. As shown by Shurova (2001), variations in salinity can modify size, age, sex and phenotypic structures of mussel populations, a fact that can be considered as an adaptive strategy. According to Krapivka et al. (2007), more elongated specimens are found in lower salinity environments.

Anthropogenic metals such as Mn, Co, Ni, Cu, Zn, Se, Sr, Pb were highlighted as most contributing to the phenotypic variability in this study. Additionally, Cd, Mn and As were shown to be negatively related to VPR, DPR, HL, PADP, PPAD, V; and Ni was positively related with PADV, PPAD, PADP and WL. In Jordaens et al., (2006) Zn concentration was negatively correlated with shell strength, shell thickness, shell dry weight and shell volume. Several researches found a negative correlation of mussel size with iron and copper concentrations (Boyden 1977, Cossa et al., 1980, Popham and D'Auria 1983, Riget et al., 1996). Metal bioaccumulation is influenced by numerous environmental (salinity, temperature, dissolved oxygen, pH, dissolved organic carbon) and biological factors (size, seasonal growth cycle, gender, sexual maturity, reproductive stage) (Rainbow and Phillips, 1993). As a result, the relationship between the size and concentration of metal often depends on the locality from which the mussels are sampled (Giusti et al., 1999). Along the Adriatic coast, metal concentration from mussels tissue significantly varied by sampled sites and pollution status, while Adriatic regions didn't have significant influence. Correlations of morphometric traits with some metals probably point to correlation with the general state or type of environment which is then manifested through correlation with some of these parameters. This does not necessarily mean that the concentrations of particular metals directly affect the measured morphometric characteristics.

5.1.1. Fluctuating asymmetry

Fluctuating asymmetry (FA) is commonly used to estimate environmentally caused stress, whose aftermaths can be marked as minor developmental accidents. These instabilities and susceptability to them differ between individuals. Scalici et al. (2017) studied how marine pollution affects the valve morphological alterations in the mussel *M. galloprovincialis*. Investigations on asymmetries interpreted deviations from perfect bilateral symmetry as environmental changes induced developmental instability. Since morphological abnormalities increase with pollution, deformations may be considered indicators of the organism exposition to pollution. Authors noted that the individual asymmetry scores (IAS) significantly varied among the investigated sites, where IAS showed higher values in disturbed areas than those of undisturbed ones. Their results are demonstrating some detrimental effects of chemicals on organism's development, although the investigated morphological marker did not discriminate the actual source of disturbance. Ghemari et al. (2018) studied asymmetry exhibited by a species of woodlouse, *Porcellio laevis*, sampled from 15 sites belonging to Tunisian industrialized areas. Contrary to their expectations and hypothesis, the results showed that individuals from contaminated sites have a low FA level, whereas those from uncontaminated sites have a high FA level.

Our results, however, showed quite consistent results between two mussels populations from contrasted environment (i.e. Gruž vs. Marina). We did detected FA for the same traits related to different features in both populations (e.g. shell length, adductor length - PAD, retractor width – WPR, PAL), which cannot be associated with pollution status of sampling sites, because we didn't observed significant differences. Interestingly, traits that were previously discussed as most contributing to phenotypic variation between Adriatic populations of *M. galloprovincialis* appear to be more stable regarding FA.

5.3. Biomarkers

In response to oxidative stress, mussel's antioxidant enzyme activities exhibit seasonal variations (Sheehan and Power, 1999) related to individual and environmental factors, such as reproductive status, genetic background, food availability, temperature and oxygen consumption (Regoli and Orlando, 1994, Bocchetti and Regoli, 2006). In addition to the highly seasonal natural processes,

stress in mussels can be further induced by the occurring pollutants (i.e. Manduzio et al., 2004, Pain-Devin et al., 2014, Jimenez et al., 2015, González-Fernández et al., 2016). Therefore, our aim in this study was to elucidate the effect of pollution status on mussel's biomarker response in different seasons. Pollution status of sites was confirmed by metals concentration in mussel's tissue, showing separations between clean and polluted sites, with higher variability among polluted sites. Furthermore, lower variability of biomarker state between seasons (spring and autumn) was observed for groups of mussels from clean than from polluted sites. This can be either due to different nature of pollution in respect to seasons, or due to inferences of seasonal natural processes and pollution. This indicates that pollution-exposed, and therefore stress challenged mussels, show higher temporal fluctuations of biomarker response. Similarly, mussels in the west coast of Algeria showed more pronounced difference in biomarker response between seasons at the impacted/polluted than at the reference/cleaner sites (Benali et al., 2015). For a long period of time, the comparison of organism's biomarker status between seasons has not been straightforward because individual biomarkers tell little about the impact of mixed spatial and temporal variations on mussel populations (Marcogliese et al., 2005, Isaksson 2010, Gassó et al., 2016). Therefore, a multivariate analysis, as provided here, supplies a synthetic illustration improving the diagnostic of mussel's biochemical and cellular change and determination of the extent to which it is affected by seasons or pollution (Guerlet et al., 2007, Benali et al., 2015).

Pollution represents environmental pressure whose effect can be compensated through local genetic adaptation and/or through phenotypic plasticity. Previous study on the same mussel populations revealed the lack of significant genome wide population structure in the eastern Adriatic Sea (Štambuk et al., 2013), but we have no knowledge on the existence of local adaptation involving specific genomic regions. Here, we specifically assessed mussel's biomarker response capacities (i.e. phenotypic plasticity) toward differing environmental conditions, and tested for population effect using experimental setups with one and two source populations (H2). Transplant experiments have been already successfully employed to reveal changes in biomarker responses of marine organisms, including mussels (Hollander and Butlin 2010, Mayfield et al., 2012, Burford et al., 2014, Ramajo et al., 2016). Those studies pointed to a differential reaction norm depending on mussel's exposure to different environmental factors, but also depending on different population. Our results, assembled from IBR analysis on

transplant experiment, demonstrated significant differences in biomarker response between mussels from the same population exposed to different levels of pollution, confirming the effects of phenotypic plasticity. Caged exposed mussels in transplant experiment uniformly exhibited higher responses on impacted sites in each of the regions. Similarly, IBR index in eight populations of zebra mussels also revealed higher biomarker response in more contaminated sites (Pain-Devin et al., 2014). Such response tends to arise when organisms are pushed towards stressful conditions (Abele et al., 2002, Oliveira et al., 2005, Heise 2006, Buttermer et al., 2010, Jimenez et al., 2015). In this study we identified not only a response towards pollution status, but also towards differing environments in respect to three geographic regions in the Adriatic Sea. The shallow northern part of the Adriatic Sea receives significant outflow of the Po river, providing over 50% of the freshwater input and accounting for about 50% of the total nutrients transported into the basin (Degobbis 1986, Degobbis and Gilmartin 1990, Viličić et al., 2002) thus impacting the productivity in this area. Salinity of the southern part is 38 ‰ and decreases towards the north, but in the north salinity varies through seasons due to periodical advections of high salinity water from the south (Viličić et al., 2002). Besides that, northern Adriatic shows typical shallow water characteristics affected by seasonal temperature variability and higher sea tide changes (up to 0.8 m) than the southern part, influencing biological characteristics of the system (Franco and Michelato, 1992). Our results are in accordance with decreasing variability toward oligotrophic middle and southern Adriatic offshore, showing persistent decrease in IBR values from north to south. To test for population effect, we implemented mesocosm experiment where two source populations, polluted (GZ) and clean site (MA), were exposed to common marine traffic pollutant – copper, after 4 weeks of acclimatization. Results confirmed population effect of biomarker status between GZ and MA with generally higher IBR and higher within-group variability for GZ. Although individuals from GZ inhabited a copper rich environment (Carić et al., 2014), our data don't suggest their acclimatization to the presence of high concentrations of metals in their natural habitat, but rather a pronounced response to it. This population had a higher basal activity in biochemical and cellular response than population from reference site (MA) after 4 weeks of acclimatization. Although copper didn't influence low, baseline biomarker activity of MA originating mussels, it seemed to decrease it in the GZ mussels, which might be result of copper inhibitory capacities towards enzymes activity (Company et al., 2004). In respect to these mesocosm results, the origin of mussels must be taken

into consideration when studying the biochemical responses of mussels experimentally exposed to chemical pollutants.

Concentrations of particular pollutants can be readily revealed by chemical analyses, and environmental physicochemical factors can be recorded, but it is often difficult to disentangle the influence of xenobiotics from natural environmental factors in shaping the mussel's biomarker status (Sheehan and A. Power 1999, Camus et al., 2004, Manduzio et al., 2004, Durou et al., 2007). Importantly, it is also very difficult to clearly separate the anthropogenic and natural contribution to a variation of many environmental factors, including some naturally occurring metals, whose environmental concentrations can additionally anthropogenically increased.

With aim to do so, we analysed individuals from the transplant experiment - because such experimental design was shown to be relevant both in evaluating the biomarker responses when coping with natural environmental factors (Osores et al., 2017) and anthropogenic pollutants (Marigómez et al., 2013). In dependence on the chosen set of environmental variables, biomarker status significantly differed among Adriatic regions, but not among the sites of different pollution status. Using the same experimental design and IBR approach we already pointed out biomarker response divergence toward differing environments in respect to the three geographic regions of the Adriatic, where Northern Adriatic exhibited highest values of biomarker activity. Results on PLS-R2 analysis thus confirm variability in biomarker response in relation to geographic area, reflecting the impact of different ecological conditions other than metal pollution. Equally, and not less expected, in dependence on the metals accumulated in mussel's tissue, biomarker status of transplanted mussels significantly differed between clean and polluted sites, and not among the regions. PLS-analysis further confirmed higher variability among individuals transplanted to polluted sites, than for the ones on clean sites. The measurement of the biological effects of accumulated metals should therefore be taken into consideration as important screening tool for distinguishing clean versus polluted environment, as well as for the assessment of the environmental quality per se. Transplant experiment was shown to be useful in disentangling the effects of other environmental variables vs. metals, and in that sense, it shall be considered as discerning tool for defining the relative role of these variables in expressed biomarker response variability toward pollution status and natural ecological pressures.

5.4. Survival as the proxy for fitness (SOS)

Several studies of *Mytilus* spp. have shown that environmental effects are large determinants of both growth and survival (Dickie et al., 1984, Mallet and Carver, 1989, Johannesson et al., 1990, Stirling and Okumus, 1994). Eertman et al. (1993) and Viarengo et al. (1995) have shown that mussels exposed to pollutants use a large amount of energy for the detoxification process and have less tolerance to anoxic conditions. Despite the fact that previously field studies revealed decrease in survival in air caused by exposure to pollution, there are results showing that survival time after aerial exposure doesn't need to be totally dependent on pollution. As it has been proposed by Thomas et al. (1999), mussels exposed to significantly higher pollutant concentrations didn't show significantly reduced survival times compared to the reference groups. The SOS method on both experiments in this research performed on *M. galloprovincialis* showed that mussels pre-exposed to polluted environment (transplant) or originated from polluted environment (mesocosm) had longer survival time and higher survival probability. Mussels pre-exposed to polluted site Pula (PLT), which is a traffic harbor, influenced by poorly cleaned communal, industrial and shipwreck wastewater, had the longest survival time of 12 days, among all individuals from transplant experiment. In case of individuals inhabiting the contaminated habitat, with presence of heavy metal contamination (e.g. Cu, Zn, Cd, Hg), an antioxidant defense system will be already activated, as opposed to individuals from clean habitats that do not have this defense capability (Viarengo, 1989). This can lead to two possible outlines. The contamination of pollutants can strongly impact the mussel health status and cause reduced survival ability in air (Pampanin et al., 2005). As it has been showed in Biomarkers section, in biomarker response of Gruž population, pollution can cause even a greater sensitivity to it. In other scenario, the mussel exposure to pollutants over a long period of time can lead to some level of pollution adaptation or acclimatization, increasing antioxidant capacity in both cases. Mussels sampled from polluted sites may be more tolerant to contamination than those collected in non-polluted areas and as a result they show elevated values of LT50, increased physical tolerance and longer lasting survival in the air (Koukouzika and Dimitriadis, 2005). On the other hand, species that have evolved under highly stable conditions are expected to be the most sensitive to environmental change and stress (Overgaard et al. 2011). This is consistent with performed results, in both experiments. Source population Gruž from mesocosm experiment

exhibit longer survival time for both the control group and individuals pre-exposed to copper, where control group had longer survival time of 15 days, and higher survival probability. Same pattern was recorded within Marina populations, and repeated in two replicates. Contrary to what they expected, Koukouzika and Dimitriadis (2005) found that mussels from polluted stations are more resistant to aerial exposure with higher LT50 values than mussels from the reference area. They confer that the survival in air can show a direct dependence on concentration of pollutants only in mussels exposed for a short time in laboratory conditions, while exposure of mussels to pollutants for a long time may result in some level of acclimatisation to pollution. Kamel et al., (2014) examined decreased resistance in survival on air in particular in mussels from more polluted site. However, they additionally revealed decreased resistance in survival in August, compared to May, which is pointing to a seasonal effect and specific environmental variables contribution. It is therefore possible that temperature, water currents, the availability of food, as well as some other ecological factors affect the response of mussels to pollutants and conceal differences in biomarker response.

5.5. Genetic architecture

Genetic architecture describes the characteristics of genetic variation responsible for heritable phenotypic variability. It depends on the number of genetic variants affecting a trait, their frequencies in the population, the magnitude of their effects and their interactions with each other and the environment. Genetic architecture is often described as falling along a continuum ranging from monogenic, to oligogenic to polygenic, meaning that one, few or many genetic variants contribute to phenotypic variability, respectively. GWAS use genome-wide genotyping arrays to measure genetic variation, and they are the standard platform to test the association of a phenotype with common genetic variants. The statistical power to detect associations between DNA variants and a trait depends on the experimental sample size, the distribution of effect sizes of (unknown) causal genetic variants that are segregating in the population, the frequency of those variants, and the LD between observed genotyped DNA variants and the unknown causal variants.

In this research GWAS provided quantitative estimates of the *M. galloprovincialis* genetic architecture of already discussed morphological traits (H3). The fact that many traits had modest

PVEs and most had large ETPIs, and the fact that the number of large effect SNPs (n - SNP) often had a lower ETPI of zero, all pointed to predictions (both within and among data sets) that analysed traits are polygenic and weakly heritable (any heritable effects are likely due to many loci with infinitesimal effects). The recent development of SNP arrays for Pacific oyster (*Crassostrea gigas*) raised the opportunity to test genomic selection strategies for polygenic traits in that species. In study of Gutierrez et al., (2018), a population of 820 oysters (comprising 23 full-sibling families) were genotyped using a medium density SNP array, and the genetic architecture of growth-related traits - shell height, shell length, and wet weight was evaluated. Heritability was estimated to be moderate for all three traits (0.26 ± 0.06 for height, 0.23 ± 0.06 for length and 0.35 ± 0.05 for weight), and results of a GWAS indicated that the underlying genetic architecture was polygenic.

For complex traits (derived from any combination of multiple genetic factors, environmental factors and their interactions), association signals tend to be spread across most of the genome (Boyle et al., 2017). As the number of genes grows very large, the contribution of each gene becomes correspondingly smaller, leading to Fisher's ''infinitesimal model'', named by the limit of a model of Mendelian inheritance (Barton et al., 2016). Even the most important loci in the genome have small effect sizes and the significant hits only explain a modest fraction of the predicted genetic variance. This has been referred to the ''missing heritability'' (Manolio et al., 2009). The mystery of ''missing heritability'' has been partially resolved by analyses showing that common single-nucleotide polymorphisms (SNPs) with effect sizes well below genome-wide statistical significance, rare alleles and epigenetic effects account for most of the ''missing heritability'' of many traits (Yang et al., 2010, Shi et al., 2016). A reasonable argument for some weak heritability in this study lies in a fact that lower ETPIs for PVE tend to be above zero. Additionally, indicating significantly greater predictive power than zero, cross-validation point estimates suggested that shell morphological traits of *M. galloprovincialis* were at least modestly heritable. Nevertheless, all the lower ETPIs for PGE are firmly on zero. Accordingly, when the traits are actually so polygenic and there is no strong support for having detectable effect SNPs, lack of shared SNPs with detectable effects across data sets is expected. Anyhow, some shared SNPs are retained in the model and their effects are still rather small.

Though the conclusions remain comparable, results are moving a bit around among data sets. Inconstant results between the data sets could be the effect of the differing sample size. Bigger dataset (Marina_pool – 1258 individuals) is giving more reliable insights comparing to the other data sets. Marina_pool had lower and a bit narrower ETPIs for PVE and PGE comparing to Marina_meso (377 ind.) and Marina_trans (883 ind.) datasets. In this regard, the results on native populations are rather dubious. Kingston et al. (2017) did the simulation of GWAS power regarding the sample size using *M. galloprovincialis* as a model. Sample size of approximately 118 individuals (due to incomplete genotype matrix), had low power, only 13.7% (at $a < 4 \times 10^{-7}$ significance level), to detect loci with rare alleles. As discussed above, rare alleles could contribute to real heritable variation and be the part of the explanation for the missing heritability. To attain 50% of power, approximately 310 individuals (effective size) needed to be genotyped and phenotyped; an effective sample size of 900 allowed for 90% power with a significance level. They noted that for sufficient power to detect individual loci with intermediate effect sizes (0.1 - 0.2) and rare alleles, one needs to use fairly large sample sizes, on the order of hundreds to even thousands of individuals (similarly was discussed by Spencer et al., 2009). Additionally, despite the fact that genome wide population structure is weak, by using different populations we may be suffering from effects of cryptic population structure that can be a confounding factor for the results of GWAS. The kinship matrix is meant to 'control' for family structure (which can help show effects of overall relatedness when individual SNPs don't contribute strongly). Moreover, the power to detect loci of moderate effects with a GWAS will increase when the phenotypic variance is maximal (Kingston, 2017). We already confirmed that there is a significant morphological variation in observed traits among native populations. The fact that we could capture higher amount of infinitesimal effect loci in native populations might be reason for high PVE, but somewhat similar PGE values as within other data sets.

5. CONCLUSIONS

H1) *Substantial phenotypic variation exists between and within mussel populations and is driven by numerous environmental factors*.

The present study shows that despite high genetic connectivity, significant morphological and biochemical and cellular variability exists among the *M. galloprovincialis* populations along the eastern Adriatic coast. *M. galloprovincialis* populations were mainly distinguished by traits related to shell shape and position of posterior adductor and retractor muscles. The study demonstrates interactions between environmental pollution status and seasonality in their effects on biomarker state of native *M. galloprovincialis*.

H2) *Environment affects mussel's phenotypic variation both through the phenotypic plasticity and natural selection in the face of high gene flow*.

Mussel's morphological variation between sampling sites, pollution status and Adriatic regions is shaped in response to both environmental variables and metals. Substantial morphological differentiation is revealed among populations, especially when using larger datasets. Mesocosm experiment showed diverse survival and biomarker response between two populations of different origin when exposed to common conditions, revealing population effect toward single stressor. Disentangling the effect of environmental variables and metals on mussel's biomarker response by using paired block transplant experiment, led to a conclusion that biomarker status significantly differs between Adriatic regions depending on the set of environmental variables. Further on, biomarker activity significantly differs between sites of different pollution status depending on metals accumulated in mussel's tissue. Environmental variables are highlighted as having a higher descriptive power on phenotypic variability than metals.

The 'Stress on stress' method on transplant and mesocosm experiments showed that mussels pre-exposed to polluted environment (transplant) or originated from polluted environment (mesocosm) had longer survival time and higher survival probability. Those mussels may be more tolerant to contamination than mussels collected in non-polluted areas.

H3) *Genetic architecture of morphological traits in Mediterranean mussel is highly polygenic.*

GWAS provided quantitative estimates of the *M. galloprovincialis* genetic architecture and pointed to three core conclusions: (i) analysed traits are polygenic and weakly heritable (ii) any

heritable effects are likely due to many loci with infinitesimal, not large effects (iii) strong environmental effects are possible.
Data set compiled of the largest number of individuals gives narrower, therefore more reliable hyperparameters describing the genetic architecture of the phenotypes measured.

Main advantage of this thesis is implementation of several multivariate data analyses in defining mussel's biomarker status, morphological variability, and its underlying genetic architecture in highly complex marine intertidal system. Valuating a multivariate description of biomarkers activity and application of specific experiments allowed gaining a comprehensive insight in the mussel's biomarker response to seasonality, natural environmental factors and pollution status. Such type of data analysis enables to characterize the response as a strategy rather than a single, self-contained event development.

7. REFERENCES

1. Abele D, Heise K, Pörtner HO, Puntarulo S (2002) Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam Mya arenaria. J. Exp. Biol., vol. 205, no. Pt 13, pp. 1831–1841.

2. Adams C, Huntingford FA (2004) Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic charr. Biological Journal of the Linnean Society, 81(4), 611-618.

3. Adams DC, Collyer ML (2009) A general framework for the analysis of phenotypic trajectories in evolutionary studies. Evolution (N. Y), vol. 63, no. 5, pp. 1143–1154.

4. Adams DC, Rohlf FJ, Slice DE (2004) Geometric morphometrics: ten years of progress following the 'revolution'. Italian Journal of Zoology, 71(1), 5-16.

5. Aebi H (1984) Oxygen Radicals in Biological Systems. Methods Enzymol., vol. 105, no. 1947, pp. 121–126.

6. Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science, 294(5541), 321-326.

7. Akester RJ, Martel AL (2000) Shell shape, dysodont tooth morphology, and hinge-ligament thickness in the bay mussel *Mytilus trossulus* correlate with wave exposure. Canadian Journal of Zoology, 78(2), 240-253.

8. Alcapán AC, Nespolo RF, Toro JE (2007) Heritability of body size in the Chilean blue mussel (*Mytilus chilensis* Hupe 1854): effects of environment and ageing. Aquaculture Research, 38(3), 313-320.

9. Allen JI, Moore MN (2004) Environmental prognostics: Is the current use of biomarkers appropriate for environmental risk evaluation? Mar. Environ. Res. 58, 227–232.

10. Allenbach DM (2011) Fluctuating asymmetry and exogenous stress in fishes: a review. Reviews in Fish Biology and Fisheries, 21(3), 355-376.

11. Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. Nature reviews genetics, 11(10), 697.

12. Almroth BC, Sturve J, Berglund Å, Förlin L (2005) Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. Aquatic Toxicology, *73*(2), 171-180.

13. Alunno-Bruscia M, Bourget E, Fréchette M (2001) Shell allometry and length-mass-density relationship for *Mytilus edulis* in an experimental food-regulated situation. Marine Ecology Progress Series, 219, 177-188.

14. Ames BN, Shigenaga MK, Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences, 90(17), 7915-7922.

15. Andolfatto P, Davison D, Erezyilmaz D, Hu TT, Mast J, Sunayama-Morita T, Stern DL (2011) Multiplexed shotgun genotyping for rapid and efficient genetic mapping. Genome research, gr-115402.

16. Arnold ML (1997) Natural hybridization and evolution. Oxford University Press, Oxford

17. Astley KN, Meigh HC, Glegg GA, Braven J, Depledge MH (1999) Multi-variate analysis of biomarker responses in *Mytilus edulis* and *Carcinus maenas* from the Tees Estuary (UK). Marine Pollution Bulletin, 39(1-12), 145-154.

18. Atwell S, Huang YS, Vilhjálmsson BJ, Willems G, Horton M, Li Y, Jiang R (2010) Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. Nature, 465(7298), 627.

19. Aulchenko YS, Ripke S, Isaacs A, Van Duijn CM (2007) GenABEL: an R library for genome-wide association analysis. Bioinformatics, 23(10), 1294-1296.

20. Ayllon F, Kjærner-Semb E, Furmanek T, Wennevik V, Solberg MF, Dahle G, Edvardsen RB (2015) The vgll3 locus controls age at maturity in wild and domesticated Atlantic salmon (*Salmo salar* L.) males. PLoS genetics, 11(11), e1005628.

21. Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Johnson EA (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. PloS one, 3(10), e3376.

22. Baker P, Mann R (1997) The postlarval phase of bivalve mollusks: a review of functional ecology and new records of postlarval drifting of Chesapeake Bay bivalves. Bulletin of Marine Science, 61(2), 409-430.

23. Bakran-Petricioli T, Vacelet J, Zibrowius H, Petricioli D, Chevaldonné P, Rađa T (2007) New data on the distribution of the 'deep-sea'sponges *Asbestopluma hypogea* and *Oopsacas minuta* in the Mediterranean Sea. Marine Ecology, 28, 10-23.

24. Banni M, Negri A, Dagnino A, Jebali J, Ameur S, Boussetta H (2010) Acute effects of benzo [a] pyrene on digestive gland enzymatic biomarkers and DNA damage on mussel *Mytilus galloprovincialis*. Ecotoxicology and Environmental Safety, 73(5), 842-848.

25. Barlas N, Akbulut N, Aydoğan M (2005) Assessment of heavy metal residues in the sediment and water samples of Uluabat Lake, Turkey. Bulletin of environmental contamination and toxicology, 74(2), 286-293.

26. Barton NH, Keightley PD (2002) Multifactorial genetics: understanding quantitative genetic variation. Nature Reviews Genetics, 3(1), 11.

27. Barton NH, Etheridge AM, Véber A (2016) The infinitesimal model. bioRxiv, 039768.

28. Bayne BL, Bayne CJ, Carefoot TC, Thompson RJ (1976) The physiological ecology of *Mytilus californianus* Conrad. Oecologia, 22(3), 229-250.

29. Beliaeff B, Burgeot TN (2002) Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem., vol. 21, no. 6, pp. 1316–1322.

30. Beadman H, Caldow R, Kaiser M, Willows R (2003) How to toughen up your mussels: using mussel shell morphological plasticity to reduce predation losses. Marine Biology, 142(3), 487-494.

31. Beaumont AR, Hawkins MP, Doig FL, Davies IM, Snow M (2008) Three species of *Mytilus* and their hybrids identified in a Scottish Loch: natives, relicts and invaders? Journal of Experimental Marine Biology and Ecology, 367(2), 100-110.

32. Beaumont AR, Seed R, Garcia-Martinez P (1989) Electrophoretic and morphometric criteria for the identification of the mussels *Mytilus edulis* and *M. galloprovincialis*. Reproduction, genetics and distributions of marine organisms, 251-258.

33. Bell EC, Gosline JM (1997) Strategies for life in flow: tenacity, morphometry, and probability of dislodgment of two *Mytilus* species. Marine Ecology Progress Series, 159, 197-208.

34. Benali I, Boutiba Z, Merabet A, Chèvre N (2015) Integrated use of biomarkers and condition indices in mussels (*Mytilus galloprovincialis*) for monitoring pollution and development of biomarker index to assess the potential toxic of coastal sites. Mar. Pollut. Bull., vol. 95, no. 1, pp. 385–394.

35. Beniash E, Ivanina A, Lieb NS, Kurochkin I, Sokolova IM (2010) Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. Marine Ecology Progress Series, 419, 95-108.

36. Ben-Khedher S, Jebali J, Houas Z, Nawéli H, Jrad A, Banni M, Boussetta H (2014) Metals bioaccumulation and histopathological biomarkers in *Carcinus maenas* crab from Bizerta lagoon, Tunisia. Environmental Science and Pollution Research, 21(6), 4343-4357.

37. Berg JJ, Coop G (2014) A population genetic signal of polygenic adaptation. PLoS genetics, 10(8), e1004412.

38. Betteridge DJ (2000) What is oxidative stress? Metabolism, 49(2), 3-8.

39. Bhatia R (2013) Matrix analysis (Vol. 169). Springer Science & Business Media.

40. Blythe JN, Lea DW (2008) Functions of height and width dimensions in the intertidal mussel, *Mytilus californianus*. Journal of Shellfish Research, 2008, 27.2: 385-392.

41. Bocchetti R, Regoli F (2006) Seasonal variability of oxidative biomarkers, lysosomal parameters, metallothioneins and peroxisomal enzymes in the Mediterranean mussel *Mytilus galloprovincialis* from Adriatic Sea. Chemosphere, vol. 65, no. 6, pp. 913–921, 2006.

42. Bocquené G, Galgani F (1991) Acetylcholinesterase activity in the common prawn (*Palaemon serratus*) contaminated by carbaryl and phosalone: Choice of a method for detection of effects. Ecotoxicology and environmental safety, 22(3), 337-344.

43. Boege K, Marquis R.J (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends in ecology and evolution, 20(8), 441-448.

44. Bookstein FL (1996) Combining the tools of geometric morphometrics. In Advances in morphometrics (pp. 131-151). Springer, Boston, MA.

45. Bosak S, Burić Z, Djakovac T, Viličić D (2009) Seasonal distribution of plankton diatoms in Lim Bay, northeastern Adriatic Sea. Acta Botanica Croatica, 68(2), 351-365.

46. Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11.

47. Boyden CR (1977) Effect of size upon metal content of shellfish. Journal of the Marine Biological Association of the United Kingdom, 57(3), 675-714.

48. Boyle EA, Li YI, Pritchard JK (2017) An expanded view of complex traits: from polygenic to omnigenic. Cell, 169(7), 1177-1186.

49. Brachi B, Faure N, Horton M, Flahauw E, Vazquez A, Nordborg M, Roux F (2010) Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. PLoS genetics, 6(5), e1000940.

50. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., vol. 72, no. 1–2, pp. 248–254.

51. 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.

52. Brown KM, Aronhime B, Wang X (2011) Predatory blue crabs induce byssal thread production in hooked mussels. Invertebrate biology, 130(1), 43-48.

53. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods in enzymology (Vol. 52, pp. 302-310). Academic Press.

54. Buerkle CA, Lexer C (2008) Admixture as the basis for genetic mapping. Trends in ecology and evolution, 23(12), 686-694.

55. Burford MO, Scarpa J, Cook BJ, Hare MP (2014) Local adaptation of a marine invertebrate with a high dispersal potential: Evidence from a reciprocal transplant experiment of the eastern oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser., vol. 505, pp. 161–175.

56. Buttemer WA, Abele D, Costantini D (2010) From bivalves to birds: Oxidative stress and longevity. Funct. Ecol., vol. 24, no. 5, pp. 971–983.

57. Byrne M (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. Oceanogr Mar Biol Annu Rev 49(49):1–42

58. Cajaraville MP, Bebianno MJ, Blasco J, Porte C, Sarasquete C, Viarengo A (2000) The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. *Science of the Total Environment*, *247*(2-3), 295-311.

59. Calsbeek B, Lavergne S, Patel M, Molofsky J (2011) Comparing the genetic architecture and potential response to selection of invasive and native populations of reed canary grass. Evol. Appl. 4:726–735.

60. Camus L, Pampanin DM, Volpato E, Delaney E, Sanni S, Nasci C (2004) Total oxyradical scavenging capacity responses in *Mytilus galloprovincialis* transplanted into the Venice lagoon (Italy) to measure the biological impact of anthropogenic activities. Mar. Pollut. Bull., vol. 49, no. 9–10, pp. 801–808.

61. Camus V, Kraehenbühl H, Preisig M, Büla CJ, Waeber G (2004) Geriatric depression and vascular diseases: what are the links?. Journal of Affective Disorders, 81(1), 1-16.

62. Carić H, Klobučar G, Štambuk A, (2016) Ecotoxicological risk assessment of antifouling emissions in a cruise ship port. Journal of cleaner production 121: 159-168.

63. Caro AU, Escobar J, Bozinovic F, Navarrete SA, Castilla JC (2008). Phenotypic variability in byssus thread production of intertidal mussels induced by predators with different feeding strategies. Marine Ecology Progress Series, 372, 127-134.

64. Carroll SP, Dingle H, Famula TR, Fox CW (2001) Genetic architecture of adaptive differentiation in evolving host races of the soapberry bug, *Jadera haematoloma*. Genetica 112–113:257–272.

65. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. Molecular ecology, 22(11), 3124-3140.

66. Ceccherelli VU, Rossi R (1984) Settlement, growth and production of the mussel *Mytilus galloprovincialis.* Marine ecology progress series. Oldendorf, 16(1), 173-184.

67. Chalghmi H, Zrafi I, Gourves PY, Bourdineaud JP, Saidane-Mosbahi D (2016) Combined effects of metal contamination and abiotic parameters on biomarker responses in clam *Ruditapes decussatus* gills: an integrated approach in biomonitoring of Tunis lagoon. Environmental Science: Processes & Impacts, 18(7), 895-907.

68. Charissou AM, Cossu-Leguille C, Vasseur P (2004) Relationship between two oxidative stress biomarkers, malondialdehyde and 8-oxo-7, 8-dihydro-2′-deoxyguanosine, in the freshwater bivalve *Unio tumidus*. Science of the total environment, *322*(1-3), 109-122.

69. Chen X, Chen G, Chen L, Chen Y, Lehmann J, McBride MB, Hay AG (2011) Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. Bioresource technology, 102(19), 8877-8884.

70. Cho WS, Kim S, Han BS, Son WC, Jeong J (2009) Comparison of gene expression profiles in mice liver following intravenous injection of 4 and 100 nm-sized PEG-coated gold nanoparticles. Toxicology letters, 191(1), 96-102.

71. Comeault AA, Soria-Carrasco V, Gompert Z, Farkas TE, Buerkle CA, Parchman TL, Nosil P (2014) Genome-wide association mapping of phenotypic traits subject to a range of intensities of natural selection in *Timema cristinae*. The American Naturalist, 183(5), 711-727.

72. Company R, Serafim A, Bebianno MJ, Cosson R (2004) Effect of cadmium , copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. Mar. Environ. Res., vol. 58, pp. 377–381, 2004.

73. Corander J, Majander K, Cheng L, Merilä J (2013) High degree of cryptic population differentiation in the Baltic Sea herring *Clupea harengus*. Molecular ecology, 22(11), 2931-2940.

74. Cossa D, Bourget E, Pouliot D, Piuze J, Chanut JP (1980) Geographical and seasonal variations in the relationship between trace metal content and body weight in *Mytilus edulis*. Marine Biology, 58(1), 7-14.

75. Crampton JS (1995) Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. Lethaia, 28(2), 179-186.

76. Crampton JS, Maxwell PA (2000) Size: all it's shaped up to be? Evolution of shape through the lifespan of the Cenozoic bivalve Spissatella (Crassatellidae). Geological Society, London, Special Publications, 177(1), 399-423.

77. Crenshaw MA (1972) The inorganic composition of molluscan extrapallial fluid. The Biological Bulletin, *143*(3), 506-512.

78. Crispo E. (2007) The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. Evolution: International Journal of Organic Evolution, 61(11), 2469-2479.

79. Gassó et al., (2016) Oxidative stress in wild boars naturally and experimentally infected with *Mycobacterium bovis*, PLoS One, vol. Under Revi, pp. 1–17.

80. Dahlhoff EP, Menge BA (1996) Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. Marine ecology Progress series, 144, 97-107.

81. Davis AK, Farrey BD, Altizer S (2005) Variation in thermally induced melanism in monarch butterflies (Lepidoptera: Nymphalidae) from three North American populations. *Journal* of Thermal Biology, 30(5), 410-421.

82. De Wit P, Palumbi SR (2013) Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. Molecular Ecology, 22(11), 2884-2897.

83. DeFaveri J, Jonsson PR., Merilä J (2013) Heterogeneous genomic differentiation in marine threespine sticklebacks: adaptation along an environmental gradient. Evolution, 67(9), 2530-2546.

84. Degobbis D, Gilmartin M (1990) Nitrogen, phosphorus, and biogenic silicon budgets for the northern Adriatic Sea. Oceanol. Acta, 13, 31–45.

85. Degobbis D, Gilmartin M, Revelante N (1986) An annotated nitrogen budget calculation for the northern Adriatic Sea. Marine Chemistry, 20(2), 159-177.

86. de Jong G (2005) Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. New Phytol., 166:101–117.

87. Del Rio D, Stewart AJ, Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, metabolism and cardiovascular diseases, 15(4), 316-328.

88. Dennis SR, Carter MJ, Hentley WT, Beckerman AP (2010) Phenotypic convergence along a gradient of predation risk. Proceedings of the Royal Society B: Biological Sciences, 278(1712), 1687-1696

89. De Oliveira AS, Da Rosa A, Belló-Klein A, Da Silva RSM, Kucharski LC (2005) Effects of environmental anoxia and different periods of reoxygenation on oxidative balance in gills of the estuarine crab *Chasmagnathus granulata*," Comp. Biochem. Physiol. - B Biochem. Mol. Biol., vol. 140, no. 1, pp. 51–57.

90. DeWitt TJ, Robinson BW, Wilson DS (2000) Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evolutionary Ecology Research, 2(2), 129-148.

91. Dickie LM, Boudreau PR, Freeman KR (1984) Influences of stock and site on growth and mortality in the blue mussel (*Mytilus edulis*). Canadian Journal of Fisheries and Aquatic Sciences, 41(1), 134-140.

92. Durou et al., (2007) Biomonitoring in a clean and a multi-contaminated estuary based on biomarkers and chemical analyses in the endobenthic worm *Nereis diversicolor*. Environ. Pollut., vol. 148, no. 2, pp. 445–458.

93. Eertman RH, Wagenvoort AJ, Hummel H, Smaal AC (1993) "Survival in air" of the blue mussel *Mytilus edulis* L. as a sensitive response to pollution-induced environmental stress. Journal of Experimental Marine Biology and Ecology, 170(2), 179-195.

94. Eertmann RHM, de Zwaan A (1994) Survival of the fittest: Resistance of mussels to aerial exposure. In K. J. M. Kramer (Ed.), Biomonitoring of coastalwaters and estuaries (pp. 269–282). Boca Raton: CRC.

95. El Jourmi L, Amine A, Boutaleb N, Abouakil N, Lazar S, El Antri S (2015) The use of biomarkers (catalase and malondialdehyde) in marine pollution monitoring: Spatial variability. J. Mater. Environ. Sci, 6, 1592-1595.

96. Elliott TA, Gregory TR (2015) What's in a genome? The C-value enigma and the evolution of eukaryotic genome content. Phil. Trans. R. Soc. B, 370(1678), 20140331.

97. Ellman GL, Courtney KD, Andres V, and Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., vol. 7, no. 2, pp. 88–95.

98. Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS one, 6(5), e19379.

99. Endler JA (1986) Natural selection in the wild (No. 21). Princeton University Press

100. Eschweiler N, Christensen HT (2011) Trade-off between increased survival and reduced growth for blue mussels living on Pacific oyster reefs. Journal of Experimental Marine Biology and Ecology, 403(1-2), 90-95.

101. Eufemia NA, Collier TK, Stein JE. Watson DE, Di Giulio RT (1997) Biochemical responses to sediment-associated contaminants in brown bullhead (*Ameriurus nebulosus*) from the Niagara River ecosystem. Ecotoxicology, 6(1), 13-34.

102. Falconer DS, Mackay TF (1996) Introduction to quantitative genetics, 82-86.

103. Fariñas-Franco JM, Sanderson WG, Roberts D (2016) Phenotypic differences may limit the potential for habitat restoration involving species translocation: a case study of shape ecophenotypes in different populations of *Modiolus modiolus* (Mollusca: Bivalvia). Aquatic Conservation: Marine and Freshwater Ecosystems, 26(1), 76-94.

104. Fassatoui C, Ben Rejeb Jenhani A, Romdhane MS (2014) Geographic pattern of shell morphology in the endemic freshwater mussel *Unio ravoisieri* (Bivalvia: Unionidae) from northern Tunisia. Journal of Molluscan Studies, 81(1), 152-160.

105. Feder JL, Egan SP, Nosil P (2012) The genomics of speciation-with-gene-flow. Trends in genetics, 28(7), 342-350.

106. Fenet H, Casellas C, Bontoux J (1996) Hepatic enzymatic activities of the European eel *Anguilla anguilla* as a tool for biomonitoring freshwater streams: laboratory and field caging studies. Water Science and Technology, 33(6), 321-329.

107. Ferson S, Rohlf FJ, Koehn RK (1985) Measuring shape variation of two-dimensional outlines. Systematic Biology, 34(1), 59-68.

108. Fisher RA (1930).The genetical theory ofnatural selection. Clarendon, Oxford.

109. Fitridge I, Dempster T, Guenther J, de Nys R (2012) The impact and control of biofouling in marine aquaculture: a review. Biofouling, 28(7), 649-669.

110. Fitzpatrick PJ, O'Halloran J, Sheehan D, Walsh AR (1997) Assessment of a glutathione S-transferase and related proteins in the gill and digestive gland of *Mytilus edulis* (L.), as potential organic pollution biomarkers. Biomarkers, 2(1), 51-56.

111. Flint J, Eskin E (2012) Genome-wide association studies in mice. Nature Reviews Genetics, 13(11), 807.

112. Flint J, Mackay TFC (2009) Genetic architecture of quanti-tative trait in mice, flies, and humans. Genome Research 19:723– 733

113. Fofonoff, P. W., Ruiz, G. M., Steves, B., & Carlton, J. T. (2003). National exotic marine and estuarine species information system. *Web publication< http://invasions. si. edu/nemesis*.

114. Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics 180.2:977-993.

115. Fournier-Level A, Korte A, Cooper MD, NordborgM, Schmitt J, Wilczek AM (2011) A map of local adaptation in *Arabidopsis thaliana*. Science, 334(6052), 86-89.

116. Franco P, Michelato A (1992): Northern Adriatic Sea: oceanography of the basin proper and the western coastal zone. Sci. Total Environ. (Suppl.) 35–62.

117. Freeman AS (2007) Specificity of induced defenses in Mytilus edulis and asymmetrical predator deterrence. Marine Ecology Progress Series, 334, 145-153.

118. Freeman AS, Byers JE (2006) Divergent induced responses to an invasive predator in marine mussel populations. Science, 313(5788), 831-833.

119. Freeman, AS, Meszaros J, Byers JE (2009) Poor phenotypic integration of blue mussel inducible defenses in environments with multiple predators. Oikos, *118*(5), 758-766.

120. Galgani F, Bocquene G (1989) A method for routine detection of organophosphates and carbamates in sea water. Environmental Technology, 10(3), 311-322.

121. Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Karl DM (2004) Nitrogen cycles: past, present, and future. Biogeochemistry, 70(2), 153-226.

122. Gardner JP (2004) A historical perspective of the genus *Mytilus* (Bivalvia: Mollusca) in New Zealand: multivariate morphometric analyses of fossil, midden and contemporary blue mussels. Biological Journal of the Linnean Society, 82(3), 329-344.

123. Gardner JPA, Skibinski DOF (1990) Genotype-dependent fecundity and temporal variation of spawning in hybrid mussel (*Mytilus*) populations. Marine Biology, 105(1), 153-162.

124. Gardner JPA, Skibinski DOF, Bajdik CD (1993) Shell growth and viability differences between the marine mussels *Mytilus edulis* (L.), *Mytilus galloprovincialis* (Lmk.), and their hybrids from two sympatric populations in SW England. The Biological Bulletin, 185(3), 405-416.

125. Gavrilovic A, Jug-Dujakovic J, Bonacic AM, Conides A, Bonacic K, Ljubicic A, Van Gorder S (2011) The influence of environmental parameters on the growth and meat quality of the Mediterranean mussel *Mytilus galloprovincialis* (Mollusca: Bivalvia). Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux), 4(5).

126. Gaylord B, Blanchette CA, Denny MW (1994) Mechanical consequences of size in wave-swept algae. Ecological monographs, *64*(3), 287-313.

127. Geng X, Liu S, Yao J, Bao L, Zhang J, Li C, Liu Z (2016) A genome wide association study identifies multiple regions associated with head size in catfish. *G3:* genes, genomes, genetics, g3-116.

128. Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional ecology, 21(3), 394-407.

129. Ghemari C, Ayari A, Hamdi N, Waterlot C, Douay F, Nasri-Ammar K (2018) Measure of environmental stress on *Porcellio laevis* Latreille, 1804 sampled near active Tunisian industrial areas. Ecotoxicology, 1-13.

130. Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. Molecular ecology, 17(1), 167-178.

131. Giusti L, Williamson AC, Mistry A (1999) Biologically available trace metals in *Mytilus edulis* from the coast of northeast England. Environment international, 25(8), 969-981.

132. Gompert Z, Forister ML, Fordyce JA, Nice CC, Williamson RJ, Alex Buerkle C (2010) Bayesian analysis of molecular variance in pyrosequences quantifies population genetic structure across the genome of Lycaeides butterflies. Molecular ecology, 19(12), 2455-2473.

133. Gompert Z, Lucas LK, Buerkle CA, Forister ML, Fordyce JA, Nice CC (2014) Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. Molecular Ecology,23(18), 4555-4573.

134. González-Fernández C, Albentosa M, Campillo JA, Viñas L, Franco A, Bellas J (2016) Effect of mussel reproductive status on biomarker responses to PAHs: Implications for large-scale monitoring programs. Aquat. Toxicol., vol. 177, pp. 380–394.

135. Graham AL, Hayward AD, Watt KA, Pilkington JG, Pemberton JM, Nussey DH (2010) Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. Science, 330(6004), 662-665.

136. Graham JH, Emlen JM, Freeman DC (1993) Developmental stability and its applications in ecotoxicology. Ecotoxicology, 2(3), 175-184.

137. Granéli E (1987) Nutrient limitation of phytoplankton biomass in a brackish water bay highly influenced by river discharge. Estuarine, Coastal and Shelf Science, 25(5), 555-565.

138. Gregorius HR, Kleinschmit JRG (1999) The environmental dichotomy of adaptation and the role of genetic diversity. Silvae Genetica, 48, 193-198.

139. Grether JM, Mathys NA (2013) The pollution terms of trade and its five components. Journal of Development Economics, 100(1), 19-31.

140. Grosberg R, Cunningham CW (2001) Genetic structure in the sea. Marine community ecology. Sinauer, Sunderland, MA, 61-84.

141. Grune T (2000) Oxidative stress, aging and the proteasomal system. Biogerontology, 1(1), 31-40.

142. Guerlet E, Ledy K, Meyer A, Giambérini L (2007) Towards a validation of a cellular biomarker suite in native and transplanted zebra mussels: A 2-year integrative field study of seasonal and pollution-induced variations. Aquat. Toxicol., vol. 81, no. 4, pp. 377–388.

143. Gutierrez AP, Bean TP, Hooper C, Stenton CA, Sanders MB, Paley RK, Houston RD (2018) A genome-wide association study for host resistance to Ostreid Herpesvirus in Pacific oysters (*Crassostrea gigas*)." G3: Genes, Genomes, Genetics 8.4: 1273-1280.

144. Habig WH, Michel RH, McGovern PE (1974) Glutathione S-Transferases," J. Biol. Chem., vol. 249, no. 8, pp. 7130–7139.

145. Halliwell B, Gutteridge J (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. Biochemical journal, 219(1), 1.

146. Halliwell B, Gutteridge JM (1997) Lipid peroxidation in brain homogenates: the role of iron and hydroxyl radicals. Journal of neurochemistry, 69(3), 1330-1330.

147. Hamer B, Korlević M, Durmiši E, Nerlović V, Bierne N (2012) Nuclear marker Me 15/16 analyses of *Mytilus galloprovincialis* populations along the eastern Adriatic coast. Cahiers de Biologie Marine 53: 35-44.

148. Hamer B, Medaković D, Pavičić-Hamer D, Jakšić Ž, Štifanić M, Nerlović V, Travizi A, Precali R, Kanduč T (2010) Estimation of freshwater influx along eastern Adriatic coast as a possible source of stress for marine organisms" Acta Adriatica 51(2): 181-194.

149. Hamza-Chaffai A (2014) Usefulness of bioindicators and biomarkers in pollution biomonitoring. International Journal of Biotechnology for Wellness Industries, 3(1), 19-26.

150. Hansen J, Sato M, Ruedy R, Lo K, Lea DW, Medina-Elizade M (2006) Global temperature change. Proceedings of the National Academy of Sciences, 103(39), 14288-14293.

151. Hansen TF (2006) The evolution of genetic architecture. Annu. Rev. Ecol. Evol. Syst., 37, 123-157.

152. Harris GP (1980) Temporal and spatial scales in phytoplankton ecology. Mechanisms, methods, models, and management. Canadian Journal of Fisheries and Aquatic Sciences, 37(5), 877-900.

153. Hazel WN (2002) The environmental and genetic control of seasonal polyphenism in larval color and its adaptive significance in a swallowtail butterfly. Evolution, 56(2), 342-348.

154. Hecht BC, Campbell NR, Holecek DE, Narum SR (2013) Genome-wide association reveals genetic basis for the propensity to migrate in wild populations of rainbow and steelhead trout. Molecular ecology, 22(11), 3061-3076.

155. Heinemann A, Hiebenthal C, Fietzke J, Eisenhauer A, Wahl M (2011) Disentangling the biological and environmental control of *M. edulis* shell chemistry. Geochemistry, Geophysics, Geosystems, 12(3).

156. Heise K, Puntarulo S, Nikinmaa M, Abele D, Pörtner HO (2006) Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus* L. Journal of experimental biology, 209(2), 353-363.

157. Hendry AP (2016) Eco-evolutionary dynamics. Princeton university press.

158. Hendry AP, Farrugia TJ, Kinnison MT (2008) Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology, 17(1), 20-29.

159. Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290:516–519.

160. Hermisson J, Wagner GP (2004) The population genetic theory of hidden variation and genetic robustness. Genetics, 168(4), 2271-2284.

161. Hiebenthal C, Philipp EER, Eisenhauer A, Wahl M (2012) Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis* and *Arctica islandica*. Aquatic Biology, 14(3), 289-298.

162. Hill WG (2010) Understanding and using quantitative genetic variation. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 365(1537), 73-85.

163. Hirschhorn JN, Daly MJ (2005) Genome-wide association studies for common diseases and complex traits. Nature Reviews Genetics, 6(2), 95.

164. Hoffman AA, Parsons PA (1994) Evolutionary Genetics and Environmental StressOxford University PressOxford, UK

165. Hoffmann AA, Woods RE (2003) Associating environmental stress with developmental stability: problems and patterns. Developmental instability: causes and consequences, 387-401.

166. Hoffmann AA, Sgro CM (2011) Climate change and evolutionary adaptation. Nature, 470, 479–485.

167. Hohenlohe PA, Amish SJ, Catchen JM, Allendorf FW, Luikart G (2011) Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. Molecular ecology resources, 11, 117-122.

168. Hohenlohe PA, Day MD, Amish SJ, Miller MR, Kamps-Hughes N, Boyer MC, Luikart G (2013) Genomic patterns of introgression in rainbow and westslope cutthroat trout illuminated by overlapping paired-end RAD sequencing. Molecular ecology, 22(11), 3002-3013.

169. Hollander J, Butlin RK (2010) The adaptive value of phenotypic plasticity in two ecotypes of a marine gastropod. BMC Evol. Biol., vol. 10, no. 1, p. 333.

170. Hoverman JT, Relyea RA (2007) How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. *Ecology, 88*(3), 693-705.

171. Hoverman JT, Cothran RD, Relyea RA (2014) Generalist versus specialist strategies of plasticity: snail responses to predators with different foraging modes. Freshwater biology, 59(5), 1101-1112.

172. Huang R, Zheng Z, Wang Q, Zhao X, Deng Y, Jiao Y, Du X (2015) Mantle branch-specific RNA sequences of moon scallop Amusium pleuronectes to identify shell color-associated genes. PLoS One 10(10):e0141390

173. Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. Genetics 132: 583–589.

174. Huey R, Gilchrist GW, Carlson M Berrigan D, Serra L (2000) Rapid evolution of a geographic cline in size in an introduced fly. Science 287:308–309.

175. Huljev D, Strohal P (1983) Physico-chemical processes of humic acid-trace element interactions. Marine Biology, 73(3), 243-246.

176. Hyma KE, Fay JC (2013) Mixing of vineyard and oak-tree ecotypes of *Saccharomyces cerevisiae* in North American vineyards. Molecular ecology, 22(11), 2917-2930.

177. ICES (2008) Report of the Fourth ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMON IV). ICES Document CM 2008/ACOM:49 Ref. MHC, OSPAR.

178. Ieyama H, Kameoka O, Tan T, Yamasaki J (1994) Chromosomes and nuclear DNA contents of some species in Mytilidae. Venus (Japanese Journal of Malacology), 53(4), 327-331.

179. Innes DJ, Bates JA (1999) Morphological variation of *Mytilus edulis* and *Mytilus trossulus* in eastern Newfoundland. Marine Biology, 133(4), 691-699.

180. Isaksson C (2010) Pollution and its impact on wild animals: A meta-analysis on oxidative stress. Ecohealth, vol. 7, no. 3, pp. 342–350.

181. Jebali J, Banni M, Guerbej H, Almeida EA, Bannaoui A, Boussetta H (2006) Effects of malathion and cadmium on acetylcholinesterase activity and metallothionein levels in the fish *Seriola dumerilli*. Fish Physiology and Biochemistry, 32(1), 93.

182. Jiao W, Fu X, Li J, Li L, Feng L, Lv J, Bao Z (2014) Large-scale development of gene-associated single-nucleotide polymorphism markers for molluscan population genomic, comparative genomic, and genome-wide association studies. DNA Research, 21(2), 183–193.

183. Jin Y, Zhou T, Geng X, Liu S, Chen A, Yao J, Liu Z (2017) A genome-wide association study of heat stress-associated SNP s in catfish. Animal genetics, 48(2), 233-236.

184. Jimenez AG, Jayawardene S, Alves S, Dallmer J, Dowd WW (2015) Micro-scale environmental variation amplifies physiological variation among individual mussels. Proceedings of the Royal Society B: Biological Sciences, 282(1820), 20152273.

185. Johannesson K, Kautsky N, Tedengren M (1990) Genotypic and phenotypic differences between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal transplantations. II. Genetic variation. Marine ecology progress series, 211-219.

186. Jolicoeur P (1963) 193. Note: the multivariate generalization of the allometry equation. Biometrics, 19(3), 497-499.

187. Jones JC, Shaohua F, Franchini P, Schartl M, Meyer A (2013) Hybridization and speciation in Xiphophorus fish: a genome wide approach using RAD sequencing. Molecular Ecology, 22(11), 2986-3001.

188. Jordaens K, De Wolf H, Vandecasteele B, Blust R, Backeljau T (2006) Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis* (Gastropoda, Helicidae). Science of the Total Environment, 363(1-3), 285-293.

189. Kaloyianni M, Dailianis S, Chrisikopoulou, E, Zannou A, Koutsogiannaki S, Alamdari DH, Dimitriadis VK (2009) Oxidative effects of inorganic and organic contaminants on haemolymph of mussels. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 149(4), 631-639.

190. Kamel N, Burgeot T, Banni M, Chalghaf M, Devin S, Minier C, Boussetta H (2014) Effects of increasing temperatures on biomarker responses and accumulation of hazardous substances in rope mussels (*Mytilus galloprovincialis*) from Bizerte lagoon. Environmental Science and Pollution Research, 21(9), 6108-6123.

191. Karakousis Y, Spandou E, Sophronidis K, Triantaphyllidis C (1993) Morphological and allozymic variation in populations of *Mytilus galloprovincialis* from the Aegean Sea. Journal of molluscan studies, 59(2), 165-173.

192. Keller I, Wagner CE, Greuter L, Mwaiko S, Selz OM. Sivasundar A, Seehausen O (2013) Population genomic signatures of divergent adaptation, gene flow and hybrid speciation in the rapid radiation of Lake Victoria cichlid fishes. Molecular Ecology, 22(11), 2848-2863.

193. Khessiba A, Hoarau P, Gnassia-Barelli M, Aissa P, Roméo M (2001) Biochemical response of the mussel *Mytilus galloprovincialis* from Bizerta (Tunisia) to chemical pollutant exposure. Archives of environmental contamination and toxicology, 40(2), 222-229.

194. Kingston SE, Parchman TL, Gompert Z, Buerkle CA, Braun MJ (2017) Heterogeneity and concordance in locus-specific differentiation and introgression between species of towhees. Journal of evolutionary biology, 30(3), 474-485.

195. Klobučar GI., Štambuk A, Hylland K, Pavlica M (2008) Detection of DNA damage in haemocytes of *Mytilus galloprovincialis* in the coastal ecosystems of Kastela and Trogir Bays, Croatia. Sci. Total Environ. 405, 330–337.

196. Kljaković-Gaspić Z, Herceg-Romanić S, Kozul D, Veza J (2010) Biomonitoring of organochlorine compounds and trace metals along the Eastern Adriatic coast (Croatia) using *Mytilus galloprovincialis*. Mar. Pollut. Bull. 60 (10), 1879—1889.

197. Kopp M, Matuszewski S (2014) Rapid evolution of quantitative traits: theoretical perspectives. Evolutionary Applications, *7*(1), 169-191.

198. Kopp JC (1979) Growth and the inter-tidal gradient in the sea mussel *Mytilus-californianus* conrad, 1837 (mollusca, bivalvia, mytilidae). Veliger, 22(1), 51-56.

199. Koskinen MT, Haugen TO, Primmer CR (2002) Contemporary fisherian life-history evolution in small salmonid populations. Nature 419:826–830.

200. Koukouzika N, Dimitriadis VK (2005) Multiple biomarker comparison in *Mytilus galloprovincialis* from the Greece coast: lysosomal membrane stability, neutral red retention, micronucleus frequency and stress on stress. Ecotoxicology, 14(4), 449-463.

201. Krajnović-Ozretić M, Ozretić B, Petrović S, Nikolić T (2001) Seasonal variations of some blood parameters in farmed sea bass (*Dicentrarchus labrax* L.). Periodicum Biologorum, 103(1), 67-75.

202. Krapivka S, Toro JE, Alcapán AC, Astorga M, Presa P, Pérez M, Guiñez R (2007) Shell-shape variation along the latitudinal range of the Chilean blue mussel *Mytilus chilensis* (Hupe 1854). Aquaculture Research, 38(16), 1770-1777.

203. Krebs CJ (1978) A review of the Chitty hypothesis of population regulation. Canadian journal of zoology, 56(12), 2463-2480.

204. Kuzmanovic N (1985) Preliminarna istrazivanja dinamike vodenih masa Limskog kanala (Zavrsni izvjetstaj). Rep Institut Ruder Boškovic, OOUR Centar za istrazivanje mora, Rovinj, 1-27.

205. Labrot F, Ribera D, Denis MS, Narbonne JF (1996) In vitro and in vivo studies of potential biomarkers of lead and uranium contamination: lipid peroxidation, acetylcholinesterase, catalase and glutathione peroxidase activities in three non-mammalian species. Biomarkers, 1(1), 21-28.

206. Lam PK, Gray JS (2003) The use of biomarkers in environmental monitoring programmes. Marine Pollution Bulletin, 46(2), 182-186.

207. Lannan JE (1972) Estimating heritability and predicting response to selection for the Pacific oyster, *Crassostrea gigas*. In Proc Natl Shellfish Assoc (Vol. 62, pp. 62-66).

208. Lares ML, Rivero LE, Huerta-Diaz MA (2005) Cd concentration in the soft tissue vs. the nacreous layer of *Mytilus californianus*. Marine pollution bulletin, 50(11), 1373-1381.

209. Lau PS, Wong HL (2003) Effect of size, tissue parts and location on six biochemical markers in the green-lipped mussel, *Perna viridis*. Marine pollution bulletin, 46(12), 1563-1572.

210. Lauzon-Guay JS, Dionne M, Barbeau MA, Hamilton DJ (2005) Effects of seed size and density on growth, tissue-to-shell ratio and survival of cultivated mussels (*Mytilus edulis*) in Prince Edward Island, Canada. Aquaculture, 250(3-4), 652-665.

211. Lavergne S, Mouquet N, Thuiller W, Ronce O (2010) Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. Annual review of ecology, evolution, and systematics, 41, 321-350.

212. Leary RF, Allendorf FW (1989) Fluctuating asymmetry as an indicator of stress: implications for conservation biology. Trends in Ecology & Evolution, 4(7), 214-217.

213. Lee CE, JL Remfert, Gelembiuk G 2003 Evolution of physiological tolerance and performance during freshwater invasions. Integr. Comp. Biol. 43:439–449.

214. Lemaire P, Livingstone DR (1993) Pro-oxidant/antioxidant processes and organic xenobiotic interactions in marine organisms, in particular the flounder *Platichthys flesus* and the mussel *Mytilus edulis*. Trends Comp. Biochem. Physiol, 1, 1119-1150.

215. Lenormand T (2002) Gene flow and the limits to natural selection. Trends in Ecology & Evolution, 17(4), 183-189.

216. Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol., 68, 253-278.

217. Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions. Integrative and comparative biology, 46(3), 282-297.

218. Levine RL (1994) Carbonyl assay for determination of oxidatively modified proteins. Methods. Enzymol., 233, 246-257.

219. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. In Methods in enzymology (Vol. 186, pp. 464-478). Academic Press.

220. Levins R (1968) Evolution in changing environments: Some theoretical expectations. Monogr Popul Biol. eds. Levin SA, Horn HS Princeton University Press: Princeton, New Jersey, USA.

221. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics, 25(14), 1754-1760.

222. Liew TS, Schilthuizen M (2014) Association between shell morphology of micro-land snails (genus *Plectostoma*) and their predator's predatory behaviour. *PeerJ, 2*, e329.

223. Livingstone DR (1991) Organic xenobiotic metabolism in marine invertebrates. In Advances in comparative and environmental physiology (pp. 45-185). Springer, Berlin, Heidelberg.

224. Livingstone DR (1993) Biotechnology and pollution monitoring: use of molecular biomarkers in the aquatic environment. Journal of Chemical Technology & Biotechnology, 57(3), 195-211.

225. Livingstone DR (2001) Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine pollution bulletin, 42(8), 656-666.

226. Livingstone DR, Lemaire P, Matthews A, Peters LD, Porte C, Fitzpatrick PJ, Goldfarb P (1995) Assessment of the impact of organic pollutants on goby (*Zosterisessor ophiocephalus*) and mussel (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy: biochemical studies. Marine Environmental Research, 39(1-4), 235-240.

227. Livingstone DR, Lips F, Martinez PG, Pipe RK (1992) Antioxidant enzymes in the digestive gland of the common mussel *Mytilus edulis*. Marine Biology, 112(2), 265-276.

228. Livingstone D, Martinez PG, Michel X, Narbonne JF, O'hara S, Ribera D, Winston GW (1990) Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel, *Mytilus edulis* L., and other molluscs. Functional Ecology, 415-424.

229. Lobel PB, Bajdik CD, Belkhode SP, Jackson SE, Longerich HP (1991) Improved protocol for collecting mussel watch specimens taking into account sex, size, condition, shell shape, and chronological age. Archives of Environmental Contamination and Toxicology, 21(3), 409-414.

230. Longwell AC, Stiles SS (1973) Oyster genetics and the probable future role of genetics in aquaculture. Malacological Review, (2).

231. Lucek K, Sivasundar A, Seehausen O (2014) Disentangling the role of phenotypic plasticity and genetic divergence in contemporary ecotype formation during a biological invasion. Evolution, 68(9), 2619–2632.

232. Luttikhuizen PC, Drent J, Baker AJ (2003) Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. Molecular Ecology, 12(8), 2215-2229.

233. Luttikhuizen PC, Drent J, Van Delden W, Piersma T (2003) Spatially structured genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. Journal of Evolutionary Biology, 16(2), 260-272.

234. Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits (Vol. 1, pp. 535-557). Sunderland, MA: Sinauer.

235. MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. The American Naturalist, 100(916), 603-609.

236. Mackey E (2005) House of difference: Cultural politics and national identity in Canada. Routledge.

237. Mallet AL, Carver CEA (1989) Growth, mortality, and secondary production in natural populations of the blue mussel, *Mytilus edulis*. Canadian Journal of Fisheries and Aquatic Sciences, 46(7), 1154-1159.

238. Mallet AL, Zouros E, Gartner-Kepay KE, Freeman KR (1986) Genetics of growth in blue mussels: family and enzyme-heterozygosity effects. Marine Biology, 92(4), 475-482.

239. Manduzio H, Rocher B, Durand F, Galap C, Leboulenger F (2005) The point about oxidative stress in molluscs. *ISJ*, *2*(2), 91-104.

240. Manduzio H, Monsinjon T, Galap C, Leboulenger F, Rocher B (2004) Seasonal variations in antioxidant defences in blue mussels *Mytilus edulis* collected from a polluted area: Major contributions in gills of an inducible isoform of Cu/Zn-superoxide dismutase and of glutathione S-transferase. Aquat. Toxicol., vol. 70, no. 1, pp. 83–93

241. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, Cho JH (2009) Finding the missing heritability of complex diseases. Nature, 461(7265), 747.

242. Marcogliese DJ, Brambilla LG, Gagné F, Gendron AD (2005) Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch *Perca flavescens*. vol. 63, pp. 77–84, 2005.

243. Marigómez I, Garmendia L, Soto M, Orbea A, Izagirre U, Cajaraville M (2013) Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige oil spill "Mussel Watch". Ecotoxicology 22:486–505.

244. Marnett LJ (1999) Lipid peroxidation—DNA damage by malondialdehyde. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 424(1), 83-95.

245. Márquez F, Amoroso R, Sainz MFG, Van der Molen S (2010) Shell morphology changes in the scallop *Aequipecten tehuelchus* during its life span: a geometric morphometric approach. Aquatic Biology, 11(2), 149-155.

246. Matés JM, Sánchez-Jiménez FM (2000) Role of reactive oxygen species in apoptosis: implications for cancer therapy. The international journal of biochemistry & cell biology, 32(2), 157-170.

247. Matesanz S, Horgan-Kobelski T, Sultan SE (2012) Phenotypic plasticity and population differentiation in an ongoing species invasion. PLoS One, 7(9), e44955.

248. Mayfield AB, Chan PH, Putnam HM, Chen CS, Fan TY (2012) The effects of a variable temperature regime on the physiology of the reef-building coral *Seriatopora hystrix*: results from a laboratory-based reciprocal transplant. J. Exp. Biol., vol. 215, no. 23, pp. 4183–4195.

249. McCarthy JF, Shugart LR (1990) Biomarkers of environmental contamination. United states

250. McDonald JH, Seed R, Koehn RK (1991) Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Marine Biology, 111(3), 323-333.

251. McLellan T, Endler JA (1998) The relative success of some methods for measuring and describing the shape of complex objects. Systematic Biology, 47(2), 264-281.

252. Melzner F, Stange P, Trübenbach K, Thomsen J, Casties I, Panknin U, Gutowska MA (2011) Food supply and seawater pCO2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. PloS one, 6(9), e24223

253. Merilä J (2012) Evolution in response to climate change: in pursuit of the missing evidence. BioEssays, 34(9), 811-818.

254. Michel AP et al. (2010) Widespread genomic divergence during sympatric speciation. Proc. Natl. Acad. Sci. U.S.A. 107, 9724–9729

255. Moczek AP (2007) Developmental capacitance, genetic accommodation, and adaptive evolution. *Evolution & development*, *9*(3), 299-305.

256. Møller AP, Swaddle JP (1997) Asymmetry, developmental stability and evolution. Oxford University Press, UK.

257. Møller AP, Thornhill R (1998) Bilateral symmetry and sexual selection: a meta-analysis. The American Naturalist, 151(2), 174-192.

258. Morán GA, Martínez JJ, Boretto GM, Gordillo S, Boidi FJ (2018) Shell morphometric variation of *Ameghinomya antiqua* (Mollusca, Bivalvia) during the late quaternary reflects environmental changes in North Patagonia, Argentina. Quaternary International.

259. Moreira R, Pereiro P, Canchaya C, Posada D, Figueras A, Novoa B (2015) RNA-Seq in *Mytilus galloprovincialis*: comparative transcriptomics and expression profiles among different tissues. BMC genomics, 16(1), 728.

260. Muir DC, Howard PH (2006) Are there other persistent organic pollutants? A challenge for environmental chemists. Environmental science & technology, 40(23), 7157-7166.

261. Murgarella M, Puiu D, Novoa B, Figueras A, Posada D, Canchaya C (2016) A first insight into the genome of the filter-feeder mussel *Mytilus galloprovincialis*. PLoS One, 11(3), e0151561.

262. Nadeau NJ, Jiggins CD (2010) A golden age for evolutionary genetics? Genomic studies of adaptation in natural populations. Trends in Genetics, 26(11), 484-492.

263. Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA (2013) Genotyping-by-sequencing in ecological and conservation genomics. Molecular ecology, 22(11), 2841-2847.

264. Nasci C, Nesto N, Monteduro RA, Da Ros L (2002) Field application of biochemical markers and a physiological index in the mussel, *Mytilus galloprovincialis*: transplantation and biomonitoring studies in the lagoon of Venice (NE Italy). Marine environmental research, 54(3-5), 811-816.

265. Newkirk GF, Haley, LE, Waugh DL, Doyle R (1977) Genetics of larvae and spat growth rate in the oyster *Crassostrea virginica*. Marine biology, 41(1), 49-52.

266. Nielsen TD, Jensen FV (2009) Bayesian networks and decision graphs. Springer Science & Business Media.

267. Nijhout HF (2003). The control of body size in insects. Developmental biology, 261(1), 1-9.

268. Nosil P (2012) Ecological speciation. 1st ed. Oxford Univ. Press, Oxford, U.K.

269. Nosil P, Funk DJ, Ortiz-barrientos, D (2009). Divergent selection and heterogeneous genomic divergence. Molecular ecology, 18(3), 375-402.

270. Ogden R, Gharbi K, Mugue N, Martinsohn J, Senn H, Davey JW, Sergeev A (2013) Sturgeon conservation genomics: SNP discovery and validation using RAD sequencing. Molecular Ecology, 22(11), 3112-3123.

271. Olsen HG, Hayes BJ, Kent MP, Nome T, Svendsen M, Larsgard AG, Lien S (2011) Genome-wide association mapping in Norwegian Red cattle identifies quantitative trait loci for fertility and milk production on BTA12. Animal genetics, 42(5), 466-474.

272. Orbea A, Ortiz-Zarragoitia M, Solé M, Porte C, Cajaraville MP (2002) Antioxidant enzymes and peroxisome proliferation in relation to contaminant body burdens of PAHs and PCBs in bivalve molluscs, crabs and fish from the Urdaibai and Plentzia estuaries (Bay of Biscay). Aquatic toxicology, 58(1-2), 75-98.

273. Orr HA (1998) The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. Evolution, 52(4), 935-949.

274. Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Key RM (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature, 437(7059), 681.

275. Osores et al., (2017) Plasticity and inter-population variability in physiological and life-history traits of the mussel *Mytilus chilensis*: A reciprocal transplant experiment. J. Exp. Mar. Bio. Ecol., vol. 490, pp. 1–12.

276. Overgaard CE, Daugherty BL, Mitchell LA, Koval M (2011) Claudins: control of barrier function and regulation in response to oxidant stress. Antioxidants & redox signaling, 15(5), 1179-1193.

277. Pain-Devin S, Cossu-Leguille C, Geffard A, Giambérini L, Jouenne T, Minguez L, Tarnowska K (2014) Towards a better understanding of biomarker response in field survey: a case study in eight populations of zebra mussels. Aquatic toxicology, 155, 52-61.

278. Paine RT, Levin SA (1981) Intertidal landscapes: disturbance and the dynamics of pattern. Ecological monographs, 51(2), 145-178.

279. Palumbi SR (2004) Marine reserves and ocean neighborhoods: the spatial scale of marine populations and their management. Annu. Rev. Environ. Resour., 29, 31-68.

280. Pampanin DM, Volpato E, Marangon I, Nasci C (2005) Physiological measurements from native and transplanted mussel (*Mytilus galloprovincialis*) in the canals of Venice. Survival in air and condition index. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 140(1), 41-52.

281. Parchman T, Gompert Z, Benkman C, Schilkey F, Mudge J & Buerkle CA (2012) Genome wide association mapping of an adaptive trait in lodgepole pine. Mol. Ecol., 21, 2991–3005.

282. Parsons KJ, Robinson BW, Hrbek T (2003) Getting into shape: an empirical comparison of traditional truss-based morphometric methods with a newer geometric method applied to New World cichlids. Environmental Biology of Fishes, 67(4), 417-431.

283. Pedrajas JR, Peinado J, Lopez-Barea J (1995) Oxidative stress in fish exposed to model xenobiotics. Oxidatively modified forms of Cu, Zn-superoxide dismutase as potential biomarkers. Chemico-Biological Interactions, 98(3), 267-282.

284. Petes LE, Menge BA, Murphy GD (2007) Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. Journal of Experimental Marine Biology and Ecology, 351(1-2), 83-91.

285. Petrović S, Semenčić L, Ozretić B, Ozretić M, (2004). Seasonal variations of physiological and cellular biomarkers and their use in the biomonitoring of north Adriatic coastal waters (Croatia). Marine Pollution Bulletin 49, 713–720.

286. Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. Trends in ecology & evolution, 25(8), 459-467.

287. Poland JA, Rife TW (2012) Genotyping-by-sequencing for plant breeding and genetics. The Plant Genome, 5(3), 92-102.

288. Popham JD, d'Auria JM (1983) Combined effect of body size, season, and location on trace element levels in mussels (*Mytilus edulis*). Archives of environmental contamination and toxicology, 12(1), 1-14.

289. Porte C, Sole M, Albaiges J, Livingstone DR (1991) Responses of mixed-function oxygenase and antioxidase enzyme system of *Mytilus* sp. to organic pollution. Comparative biochemistry and physiology. C, Comparative pharmacology and toxicology, 100(1-2), 183-186.

290. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nature genetics, 38(8), 904.

291. Price JR, Lakshmi V (2014) Growth studies of *Mytilus Californianus* using satellite surface temperatures and chlorophyll data for coastal Oregon. Remote Sensing of the Terrestrial Water Cycle, 206, 427.

292. Price TD, Qvarnström A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. Proc. R. Soc. Lond. B 270:1433– 1440.

293. Prohaska JR (1980) The glutathione peroxidase activity of glutathione S-transferases. Biochimica et Biophysica Acta (BBA)-Enzymology, 611(1), 87-98.

294. Rainbow PS, Phillips DJ (1993) Cosmopolitan biomonitors of trace metals. Marine pollution bulletin, 26(11), 593-601.

295. Ramajo L, Prado L, Rodriguez-Navarro AB, Lardies MA, Duarte CM, Lagos NA, (2016) Plasticity and trade-offs in physiological traits of intertidal mussels subjected to freshwater-induced environmental variation. Mar. Ecol. Prog. Ser., vol. 553, pp. 93–109.

296. Ramos-Martinez JI, Bartolomé TR, Pernas RV (1983) Purification and properties of glutathione reductase from hepatopancreas of *Mytilus edulis* L. Comp. Biochem. Physiol. Part B Comp. Biochem., vol. 75, no. 4, pp. 689–692.

297. Regoli F (2000) Total oxyradical scavenging capacity (TOSC) in polluted and translocated mussels: a predictive biomarker of oxidative stress. Aquatic Toxicology, 50(4), 351-361.

298. Regoli F Principato G (1995) Glutathione, glutathione-dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers. Aquatic Toxicology, 31(2), 143-164.

299. Regoli F, Orlando E (1994) Seasonal variation of trace metal concentrations in the digestive gland of the Mediterranean mussel *Mytilus galloprovincialis*: comparison between a polluted and a non-polluted site. Arch. Environ. Contam. Toxicol. 7 (1), 36—43.

300. Reid MK, Spencer KL (2009) Use of principal components analysis (PCA) on estuarine sediment datasets: the effect of data pre-treatment. Environmental Pollution, 157(8-9), 2275-2281.

301. Reitzel AM, Herrera S, Layden MJ, Martindale MQ, Shank TM (2013Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Molecular ecology, 22(11), 2953-2970.

302. Requena JR, Levine RL, Stadtman ER (2003) Recent advances in the analysis of oxidized proteins. Amino acids, 25(3-4), 221-226.

303. Reznick DN, Ghalambor CK (2001) The population ecology of contemporary adaptations: what empirical studies reveal about the con- ditions that promote adaptive evolution. Genetica 112:183–198.

304. Ribera D, Narbonne JF, Michel X, Livingstone DR, O'hara S (1991) Responses of antioxidants and lipid peroxidation in mussels to oxidative damage exposure. Comparative biochemistry and physiology. C, Comparative pharmacology and toxicology, 100(1-2), 177-181.

305. Rickwood CJ, Galloway TS (2004) Acetylcholinesterase inhibition as a biomarker of adverse effect: a study of *Mytilus edulis* exposed to the priority pollutant chlorfenvinphos. Aquatic Toxicology, 67(1), 45-56.

306. Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification. Geology, 37(12), 1131-1134.

307. Riget F, Johansen P, Asmund G (1996) Influence of length on element concentrations in blue mussels (*Mytilus edulis*). Marine Pollution Bulletin, 32(10), 745-751.

308. Rockman MVM (2012) The QTN program and the alleles that matter for evolution: all that's gold does not glitter. Evolution: International Journal of Organic Evolution, 66(1), 1-17.

309. Roda F, Liu H, Wilkinson MJ, Walter GM, James ME, Bernal DM, Ortiz-Barrientos D (2013) Convergence and divergence during the adaptation to similar environments by an Australian groundsel. Evolution, 67(9), 2515-2529.

310. Rodriguez-Ariza A, Peinado J, Pueyo C, Lopez-Barea J (1993) Biochemical indicators of oxidative stress in fish from polluted littoral areas. Canadian Journal of Fisheries and Aquatic Sciences, 50(12), 2568-2573.

311. Rodríguez-Juíz AM, Torrado M, Méndez J (1996) Genome-size variation in bivalve molluscs determined by flow cytometry. Marine Biology, 126(3), 489-497.

312. Roesti M, Moser D, Berner D (2013) Recombination in the threespine stickleback genome—patterns and consequences. Molecular Ecology, 22(11), 3014-3027.

313. Rohlf FJ, Archie JW (1984) A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). Systematic Zoology, 33(3), 302-317.

314. Rohlf FJ, Marcus LF (1993) A revolution morphometrics. Trends in ecology & evolution, 8(4), 129-132.

315. Rojas E, Lopez MC, Valverde M (1999) Single cell gel electrophoresis assay: methodology and applications. Journal of Chromatography B: Biomedical Sciences and Applications, 722(1-2), 225-254.

316. Roméo M, Hoarau P, Garello G, Gnassia-Barelli M, Girard JP (2003) Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. Environmental Pollution, 122(3), 369-378.

317. Rossi SL, Tesh RB, Azar SR, Muruato AE, Hanley KA, Auguste AJ, Weaver SC (2016) Characterization of a novel murine model to study Zika virus. The American journal of tropical medicine and hygiene, 94(6), 1362-1369.

318. Sanchez G, (2012) R package plsdepot PLS Regression 1. pp. 1–13.

319. Sakshaug E, Holm-Hansen O, (1986) Photoadaptation in Antarctic phytopfankton: variations in growth rate, chemical composition and P versus I curves. Journal of Plankton Research, 8(3), 459-473.

320. Sarver SK, Foltz DW (1993) Genetic population structure of a species' complex of blue mussels (*Mytilus* spp.). Marine Biology, 117(1), 105-112.

321. Savoya V, Otero JG, Schwindt E (2013) Toward a better understanding of the native–nonnative status of *Mytilus* mussels in the southwestern Atlantic: comparing pre-European middens and modern populations. Journal of Coastal Research, 31(3), 742-748.

322. Scalici M, Traversetti L, Spani F, Malafoglia V, Colamartino M, Persichini T, Colasanti M (2017) Shell fluctuating asymmetry in the sea-dwelling benthic bivalve *Mytilus galloprovincialis* (Lamarck, 1819) as morphological markers to detect environmental chemical contamination. Ecotoxicology, 26(3), 396-404.

323. Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free radical biology and medicine, 30(11), 1191-1212.

324. Schluter D (2000). *The ecology of adaptive radiation*. OUP Oxford.

325. Seed R (1968) Factors influencing shell shape in the mussel *Mytilus edulis*. Journal of the Marine Biological Association of the United Kingdom, 48(3), 561-584.

326. Sénéchal J, Grant J, Archambault MC (2008) Experimental manipulation of suspended culture socks: growth and behavior of juvenile mussels (*Mytilus* spp.). Journal of Shellfish Research, 27(4), 811-826.

327. Shacter E, Williams JA, Lim M, Levine RL (1994) Differential susceptibility of plasma proteins to oxidative modification: examination by western blot immunoassay. Free Radical Biology and Medicine, 17(5), 429-437.

328. Shaw M, Tibbetts IR, Müller JF (2004) Monitoring PAHs in the Brisbane River and Moreton Bay, Australia, using semipermeable membrane devices and EROD activity in yellowfin bream, *Acanthopagrus australis*. Chemosphere, 56(3), 237-246.

329. Shaw RG, Etterson JR (2012) Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. New Phytologist, 195(4), 752-765.

330. Sheehan D, Power A (1999) Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve molluscs. Comp. Biochem. Physiol. - C Pharmacol. Toxicol. Endocrinol., vol. 123, no. 3, pp. 193–199.

331. Shi H, Kichaev G, Pasaniuc B (2016) Contrasting the genetic architecture of 30 complex traits from summary association data. The American Journal of Human Genetics, 99(1), 139-153.

332. Shields JL, Barnes P, Heath DD (2008) Growth and survival differences among native, introduced and hybrid blue mussels (*Mytilus* spp.): genotype, environment and interaction effects. Marine Biology, 154(5), 919.

333. Shurova NM (2001) Influence of salinity on the structure and the state of bivalve *Mytilus galloprovincialis* populations. Russian Journal of Marine Biology, 27(3), 151-155.

334. Simon-bouhet B, Garcia-Meunier P, Viard F (2006) Multiple introductions promote range expansion of the mollusc *Cyclope neritea* (Nassariidae) in France: evidence from mitochondrial sequence data. Molecular Ecology, 15(6), 1699-1711.

335. Simpson GG (1953) The baldwin effect. Evolution, 7(2), 110-117.

336. Skibinski DO, Gallagher C, Beynon CM (1994) Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. Genetics, 138(3), 801-809.

337. Slate JON (2005) Invited review: Quantitative trait locus mapping in natural populations: progress, caveats and future directions. Molecular Ecology, 14(2), 363-379.

338. Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science, 236(4803), 787-792.

339. Smaal AC, Wagenvoort A, Hemelraad J, Akkerman I (1991) Response to stress of mussels (*Mytilus edulis*) exposed in Dutch coastal waters. Comp. Biochem. Physiol. C 100, 197–200.

340. Smital T, Sauerborn R, Pivčević B, Krča S, Kurelec B (2000) Interspecies differences in P-glycoprotein mediated activity of multixenobiotic resistance mechanism in several marine and freshwater invertebrates. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 126(2), 175-186.

341. Solovieff N, Cotsapas C, Lee PH, Purcell SM, Smoller JW (2013) Pleiotropy in complex traits: challenges and strategies. Nature Reviews Genetics, 14(7), 483.

342. Soria-Carrasco V, Gompert Z, Comeault AA, Farkas TE, Parchman TL, Johnston JS, Egan SP (2014) Stick insect genomes reveal natural selection's role in parallel speciation. Science, 344(6185), 738-742.

343. Spencer CC, Su Z, Donnelly P, Marchini J (2009) Designing genome-wide association studies: sample size, power, imputation, and the choice of genotyping chip. PLoS genetics, 5(5), e1000477.

344. Stadtman ER, Berlett BS (1998) Reactive oxygen-mediated protein oxidation in aging and disease. Drug metabolism reviews, 30(2), 225-243.

345. Stapley J, Reger J, Feulner PG, Smadja C, Galindo J, Ekblom R, Slate J (2010) Adaptation genomics: the next generation. Trends in ecology & evolution, 25(12), 705-712.

346. Steffani, C. N., & Branch, G. M. (2003). Growth rate, condition, and shell shape of Mytilus galloprovincialis: responses to wave exposure. *Marine Ecology Progress Series*, *246*, 197-209.

347. Steinert SA, Montee RS, Sastre MP (1998) Influence of sunlight on DNA damage in mussels exposed to polycyclic aromatic hydrocarbons. Marine Environmental Research, 46(1-5), 355-358.

348. Stirling HP, Okumuş İ (1994) Growth, mortality and shell morphology of cultivated mussel (*Mytilus edulis*) stocks cross-planted between two Scottish sea lochs. Marine Biology, 119(1), 115-123.

349. Sultan SE, Horgan-Kobelski T, Nichols LM, Riggs CE, RK Waples (2013) A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. Evol. Appl. 6:266–278.

350. Štambuk A, Šrut M, Šatović Z, Tkalec M, GIV Klobučar (2013) Gene flow vs. pollution pressure: genetic diversity of *Mytilus galloprovincialis* in eastern Adriatic. Aquat. Toxicol., vol. 136–137, pp. 22–31

351. Telli Karakoc F, Hewer A, Phillips DH, Gaines AF, Yuregir G (1997) Biomarkers of marine pollution observed in species of mullet living in two eastern Mediterranean harbours. Biomarkers, 2(5), 303-309.

352. Teplitsky C, Mills JA, Alho JS, Yarrall JW, Merilä J (2008) Bergmann's rule and climate change revisited: Disentangling environmental and genetic responses in a wild bird population. Proceedings of the National Academy of Sciences, 105(36), 13492-13496.

353. Thomas RE, Harris PM, Rice SD (1999) Survival in air of *Mytilus trossulus* following long-term exposure to spilled Exxon Valdez crude oil in Prince William Sound. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 122(1), 147-152.

354. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environmental and molecular mutagenesis, 35(3), 206-221.

355. Toro JE, Newkirk GF (1991) Response to artificial selection and realized heritability estimate for shell height in the Chilean oyster *Ostrea chilensis*. Aquatic Living Resources, 4(2), 101-108.

356. Toro JE, Paredes LI (1996) Heritability estimates of larval shell length in the Chilean blue mussel *Mytilus chilensis*, under different food densities. Aquatic living resources, 9(4), 347-350.

357. Toro JE, Sanhueza MA, Winter JE, Aguila P, Vergara AM (1995) Selection response and heritability estimates for growth in the Chilean oyster *Ostrea chilensis* (Philippi 1845). Journal of Shellfish Research, 14(1), 87-92.

358. Tsai HY, Hamilton A, Tinch AE, Guy DR, Gharbi K, Stear MJ, Houston RD (2015) Genome wide association and genomic prediction for growth traits in juvenile farmed Atlantic salmon using a high density SNP array. BMC genomics, 16(1), 969.

359. Tyberghein L, Verbruggen H, Pauly K, Troupin C, Mineur F, De Clerck O (2012) Bio–ORACLE: a global environmental dataset for marine species distribution modelling. Global Ecology and Biogeography 21: 272–281.

360. Valbonesi P, Sartor G, Fabbri E (2003) Characterization of cholinesterase activity in three bivalves inhabiting the North Adriatic sea and their possible use as sentinel organisms for biosurveillance programmes. Science of the Total Environment, 312(1-3), 79-88.

361. Valladares A, Manríquez G, Suárez-Isla BA (2010) Shell shape variation in populations of *Mytilus chilensis* (Hupe 1854) from southern Chile: a geometric morphometric approach. Marine biology, 157(12), 2731-2738.

362. Van der Oost R, Goksøyr A, Celander M, Heida H, Vermeulen NP (1996) Biomonitoring of aquatic pollution with feral eel (*Anguilla anguilla*) II. Biomarkers: pollution-induced biochemical responses. Aquatic Toxicology, 36(3-4), 189-222.

363. Van Valen L. (1962) A study of fluctuating asymmetry. Evolution, 16(2), 125-142.

364. Veldhuizen-Tsoerkan MB, Holwerda DA, De Bont AMT, Smaal AC, Zandee DI (1991) A field study on stress indices in the sea mussel, *Mytilus edulis*: application of the "stress approach" in biomonitoring. Archives of environmental contamination and toxicology, 21(4), 497-504.

365. Verity PG (1982) Effects of temperature, irradiance, and daylength on the marine diatom *Leptocylindrus danicus* Cleve. III. Dark respiration. Journal of Experimental Marine Biology and Ecology, 60(2-3), 197-207.

366. Viarengo A (1989) Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. Rev. Aquat. Sci, 1(2), 295-317.

367. Viarengo A, Canesi L, Pertica M, Livingstone DR (1991) Seasonal variations in the antioxidant defence systems and lipid peroxidation of the digestive gland of mussels. Comparative biochemistry and physiology. C, Comparative pharmacology and toxicology, 100(1-2), 187-190.

368. Viarengo A, Canesi L, Pertica M, Mancinelli G, Accomando R, Smaal AC, Orunesu M (1995) Stress on stress response: a simple monitoring tool in the assessment of a general stress syndrome in mussels. Marine Environmental Research, 39(1-4), 245-248.

369. Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007) The use of biomarkers in biomonitoring: a 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 146(3), 281-300.

370. Vidal-Liñán L, Bellas J, Campillo JA, Beiras R (2010) Integrated use of antioxidant enzymes in mussels, *Mytilus galloprovincialis*, for monitoring pollution in highly productive coastal areas of Galicia (NW Spain). Chemosphere, 78(3), 265-272.

371. Vilicic D, Marasovic I, Miokovic D (2002) Checklist of phytoplankton in the eastern Adriatic Sea. Acta Bot. Croat. 61, 57–91.

372. Villemereuil P, Gaggiotti OE (2015) A new FST-based method to uncover local adaptation using environmental variables. Methods in Ecology and Evolution, 6(11), 1248-1258.

373. Visser ME (2008) Keeping up with a warming world; assessing the rate of adaptation to climate change. Proc R Soc B., 275:649–659.

374. Waddington CH (1956) Genetic assimilation of the bithorax phenotype. Evolution, 10(1), 1-13.

375. Wang J, Wang E, Yang X, Zhang F, Yin H (2012) Increased yield potential of wheat-maize cropping system in the North China Plain by climate change adaptation. Climatic Change, 113(3-4), 825-840.

376. Wang L, Mizaikoff (2008) Application of multivariate data-analysis techniques to biomedical diagnostics based on mid-infrared spectroscopy. Analytical and bioanalytical chemistry, 391(5), 1641-1654.

377. Wang X, Liu S, Jiang C, Geng X, Zhou T, Li N, Zhong X (2017) Multiple across-strain and within-strain QTLs suggest highly complex genetic architecture for hypoxia tolerance in channel catfish. Molecular Genetics and Genomics, 292(1), 63-76.

378. Wennersten L, Forsman A (2012) Population-level consequences of polymorphism, plasticity and randomized phenotype switching: a review of predictions. Biological Reviews, 87(3), 756-767.

379. West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press.

380. West-Eberhard MJ (2005) Developmental plasticity and the origin of species differences. Proceedings of the National Academy of Sciences, 102(suppl 1), 6543-6549.

381. Wheeler AP (1992) Mechanisms of molluscan shell formation. Calcification in biological systems, 179-216.

382. Wilbur KM (1964) Shell formation and regeneration. Physiology of Mollusca. 243-282.

383. Wilbur KM, Saleuddin ASM (1983) Shell formation. In The Mollusca, Volume 4 (pp. 235-287).

384. Williams GC (1996) Adaptation and natural selection: A critique of some current evolutionary thought (Vol. 61). Princeton university press.

385. Winston GW, Di Giulio RT (1991) Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic toxicology, 19(2), 137-161.

386. Wold S, Sjöström M, Eriksson L (2001) PLS-regression: A basic tool of chemometrics. Chemom. Intell. Lab. Syst., vol. 58, no. 2, pp. 109–130.

387. Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. Proceedings of the Royal Society of London B: Biological Sciences, 275(1644), 1767-1773.

388. Wright S (1920) The relative importance of heredity and environment in determining the piebald pattern of guinea-pigs. Proceedings of the National Academy of Sciences, 6(6), 320-332.

389. Wund MA (2012) Assessing the impacts of phenotypic plasticity on evolution. Integr Comp Biol 52: 5–15.

390. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, Goddard ME (2010) Common SNPs explain a large proportion of the heritability for human height. Nature genetics, 4*2*(7), 565.

391. Yeaman S, Whitlock MC (2011) The genetic architecture of adaptation under migration–selection balance. Evolution: International Journal of Organic Evolution, 65(7), 1897-1911.

392. Yoder JB, Clancey E, Des Roches S, Eastman JM, Gentry L, Godsoe W, Sarver BA J (2010) Ecological opportunity and the origin of adaptive radiations. Journal of evolutionary biology, 23(8), 1581-1596.

393. Young RT (1941) The distribution of the mussel (*Mytilus californianus*) in relation to the salinity of its environment. Ecology, 22(4), 379-386.

394. Zakharov VM (1993) Appearance, fixation and stabilisation of environmentally induced phenotypic changes as a microevolutionary event. Genetica, 89(1-3), 227.

395. Zhou T, Liu S, Geng X, Jin Y, Jiang C, Bao L, Wang X (2017) GWAS analysis of QTL for enteric septicemia of catfish and their involved genes suggest evolutionary conservation of a molecular mechanism of disease resistance. Molecular Genetics and Genomics, 292(1), 231-242.

396. Zhou X, Stephens M (2012) Genome-wide efficient mixed-model analysis for association studies. Nature genetics, 44(7), 821.

397. Zhou X, Carbonetto P, Stephens M (2013) Polygenic modeling with Bayesian sparse linear mixed models. PLoS genetics, 9(2), e1003264.

398. Zieritz A, Aldridge DC (2009) Identification of ecophenotypic trends within three European freshwater mussel species (Bivalvia: Unionoida) using traditional and modern morphometric techniques. Biological Journal of the Linnean Society, 98(4), 814-825.

399. Zieritz A, Gum B, Kuehn R, Geist J (2012) Identifying freshwater mussels (Unionoida) and parasitic glochidia larvae from host fish gills: a molecular key to the North and Central European species. Ecology and Evolution, *2*(4), 740-750.

400. Zieritz A, Hoffman JI, Amos W, Aldridge DC (2010) Phenotypic plasticity and genetic isolation-by-distance in the freshwater mussel *Unio pictorum* (Mollusca: Unionoida). Evolutionary Ecology, 24(4), 923-938.

401. Zouros E (2000) The exceptional mitochondrial DNA system of the mussel family Mytilidae. Genes & genetic systems, 75(6), 313-318.

402. Zusterzeel PLM, Rütten H, Roelofs HMJ, Peters WHM, Steegers EAP (2001) Protein carbonyls in decidua and placenta of pre-eclamptic women as markers for oxidative stress. Placenta, 22(2), 213-219.

403. Zuur A, Ieno EN, Smith GM (2007) Analyzing ecological data. Springer Science & Business Media.

404. Zvonaric T (1991) The cycling of mercury through the marine environment of Kastela Bay. MAP technical reports series. Athens.

8. SUPPLEMENTARY DATA

Quality control

Initial quality control included removing reads with greater than 5% N's or with evidence of polyA regions, reads where 20% or more of the calls were considered low quality bases, adaptor polluted reads, overlapping reads, and duplicated reads. After removing reads containing contaminant sequences, 1,309,592,331 reads retained (650k/sample - 90.5% mapped) for analysis.

Alignment and variant detection

Reads were aligned to a *de novo* genome of the *M. galloprovincialis* sequenced by Murgarella et al., 2016.

BWA-backtrack algorithm was implemented in bwa 0.7.5a-r405 (H. Li and R. Durbin, 2009) to align sequences from each individual to the *Mytilus* genome scaffolds. Bases were discarded with quality scores less than 10, allowed a maximum edit distance of 4 between the read and reference sequences, and only placed reads with a unique best match. We used a 20 bp seed with a maximum edit distance of two to increase the speed of the alignment method. We used custom Perl scripts along with bcftools and samtools (H. Li and R. Durbin, 2009) to call variant sites in the assembled contigs. samtools processes input BAM files (a compressed file format for storing assembly data), computes the probability of the data given each possible genotype and stores the probabilities in the BCF format. bcftools then executes the calling of variant sites based on a Bayesian model that accounts for uncertainty in the data. We defined a site as variable if the probability of the data under the null hypothesis (no variation at the site) was less than 0.01 using the full prior with F = 0.001. We required data for 85% of individuals to designate a variable locus, and identified variable loci separately for each mapping family. Single nucleotide variants were identified as follows in **Table S1**:

Table S1. Single nucleotide variants identified per data sets.

Data set	N of individuals	SNPs
Gruž_meso	381	72758
Marina_meso	394	72730
Marina_trans	883	71534
Native_pops	288	83375

Population differentiation

Genome-wide genetic differentiation was quantified between 15 mussel populations by estimating Hudson's F_{ST} (Hudson et al., 1992, Bhatia et al., 2013), as a measure of structure in natural populations. It was calculated according to Soria-Carrasco et al. (2014). Genetic structure was assessed across populations using the ENTROPY algorithm, a hierarchical Bayesian model, that takes genotype likelihoods from variant calling via SAMtools/BCFtools as the starting point and provides a clustering solution. This model was used according to Gompert et al. (2014) for 15 native populations (k=15). Signatures of diversifying selection were analyzed between populations by identifying F_{ST}-outlier SNPs, using BAYESCAN (Foll and Gaggiotti, 2008). This program calculates locus-specific pairwise F_{ST} between each population and a common gene pool of all populations. These F_{ST} coefficients are then decomposed into two components: α-component, which is locus specific and shared by all populations considered, and β-component, which is population-specific and shared by all loci. If the α-component significantly differs from zero for a particular locus, this implies that selection is necessary to explain the population differentiation at this locus. Positive values of α-component indicate diversifying selection, while negative values indicate balancing or purifying selection (Foll and Gaggiotti 2008). Significance is based on FDR-corrected q-values (<0.05).

Table S2. Quantitative environmental data collected from Bio–Oracle online database, based on monthly averages in the time period between 2000 and 2014.

Description	ID	Unit	LB	PL	IC	RJ	VL	ZB	ZM	MA	TM	AD	SL	MS	SA	GZ
Current velocity (mean at min depth)	Currents	m/s	0.02	0.01	0.01	0.01	0.01	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.05
Light at bottom (mean at min depth)	Light	mol/m/s	2.94	1.95	1.10	0.31	0.31	2.41	0.01	0.26	1.08	1.24	0.13	0.00	0.31	0.04
Sea water temp. (max. at min depth)	T_max	°C	27.33	27.14	26.47	26.38	26.38	26.44	26.63	26.14	26.11	26.07	26.10	26.46	26.39	26.72
Sea water salinity (mean at min depth)	Salinity	PSS	36.93	36.95	36.93	36.93	36.93	37.39	37.44	38.05	38.06	38.06	38.07	38.18	38.15	38.29
Silicate conc. (mean at min depth)	Silicates	mol/m^3	19.19	19.06	22.22	22.39	22.39	17.74	17.88	12.09	12.00	11.91	11.66	10.00	10.17	9.41
Sea surface temp. (mean)	SST	°C	17.85	17.77	17.10	17.05	17.05	18.12	18.25	18.68	18.66	18.63	18.66	19.14	19.10	19.38
Chlorophyll conc. (mean)	Chl_a	mg/m^3	0.53	0.40	0.17	0.17	0.17	0.25	0.21	0.14	0.16	0.18	0.20	0.28	0.22	0.22
Dissolved O2 conc. (mean)	O2	mol/m^3	247.5	244.5	242.9	242.8	242.8	239.9	238.8	235.1	235.6	235.9	236	232.9	231.9	232.3
Phosphate conc. (mean)	Phosphate	mol/m^3	0.14	0.13	0.11	0.11	0.11	0.07	0.07	0.04	0.04	0.04	0.04	0.02	0.02	0.01
Nitrate conc. (mean)	Nitrates	mol/m^3	0.05	0.04	0.00	0.00	0.00	0.07	0.05	0.09	0.10	0.14	0.20	0.58	0.43	0.59

Table S3. Heavy metals concentrations determinated from mussels *M. galloprovincialis* (Lamarck, 1819) tissue, by using high resolution mass spectrometry. Mussels were collected on sampling sites in spring 2014. Concentrations are expressed in mg/kg.

	LB	PL	IC	RJ	VL	ZB	ZM	MA	TM	AD	SL	MS	SA	GZ
Li	1.52	1.20	1.45	1.28	1.00	1.37	1.86	1.78	1.40	1.22	1.26	1.55	1.44	1.53
Rb	5.95	5.97	6.08	5.59	5.96	6.74	5.61	6.70	6.99	6.25	5.16	5.78	6.02	6.12
Mo	0.99	1.25	5.01	1.00	2.69	7.21	3.11	2.42	1.49	1.33	1.52	1.79	9.23	1.26
Ag	0.03	0.07	0.01	0.24	0.03	0.04	0.25	0.01	0.11	0.01	0.07	0.01	0.01	0.04
Cd	0.72	0.64	0.96	1.02	1.30	0.88	0.95	0.69	0.70	0.94	0.86	1.96	0.72	0.65
Sn	0.07	0.45	0.12	0.22	1.70	0.06	2.39	0.08	0.29	0.22	0.19	0.21	0.05	0.40
Sb	0.03	0.07	0.03	0.05	0.07	0.02	0.05	0.02	0.03	0.03	0.03	0.04	0.03	0.13
Pb	0.74	8.41	1.03	5.10	11.23	0.96	14.05	1.12	2.10	2.71	3.27	2.28	0.55	6.04
Bi	0.01	0.03	0.02	0.02	0.03	0.01	0.13	0.02	0.02	0.02	0.04	0.02	0.01	0.04
U	0.10	0.11	0.13	0.13	0.14	0.14	0.14	0.11	0.09	0.10	0.12	0.18	0.17	0.19
Al	619.5	184.6	342.3	242.2	318.0	408.4	328.4	453.5	410.2	186.5	169.0	293.7	151.5	507.7
Ti	32.40	13.10	21.50	16.40	23.10	22.60	54.20	28.70	24.00	10.10	10.70	23.40	9.10	36.50
V	3.23	18.13	2.42	1.01	2.12	2.54	1.77	2.78	1.74	1.40	1.25	1.52	1.83	2.65
Cr	1.44	2.48	1.61	1.82	5.17	1.26	3.13	2.26	1.66	1.72	1.70	2.77	0.81	2.90
Mn	9.19	5.15	8.48	6.48	10.27	17.32	6.33	9.05	8.81	6.77	6.64	17.13	6.63	8.96
Fe	425.3	235.0	274.6	264.1	661.6	284.5	386.9	304.0	280.0	179.4	207.6	345.6	143.3	388.8
Co	0.75	0.44	1.03	0.52	1.27	0.71	0.67	0.82	0.64	0.68	0.69	1.05	0.98	0.75
Ni	1.49	1.13	1.77	1.52	2.42	1.78	1.30	1.39	1.53	1.26	1.55	1.73	2.03	1.97
Cu	4.76	16.21	5.88	19.28	46.65	5.24	312.98	5.08	55.82	7.49	9.78	13.31	5.20	43.99
Zn	107.7	176.9	86.7	152.9	278.1	64.2	410.9	111.8	102.0	148.0	209.2	126.2	72.5	172.1
Sr	33.30	65.90	47.70	56.10	56.00	36.10	80.50	80.80	84.70	60.70	55.50	65.30	95.60	55.20
Ba	3.46	11.16	2.66	1.98	8.93	6.68	5.36	16.39	6.10	9.36	4.54	5.06	7.31	18.69
As	24.00	23.79	31.70	27.30	27.50	32.39	24.82	29.23	23.62	26.33	27.13	23.09	22.19	27.24

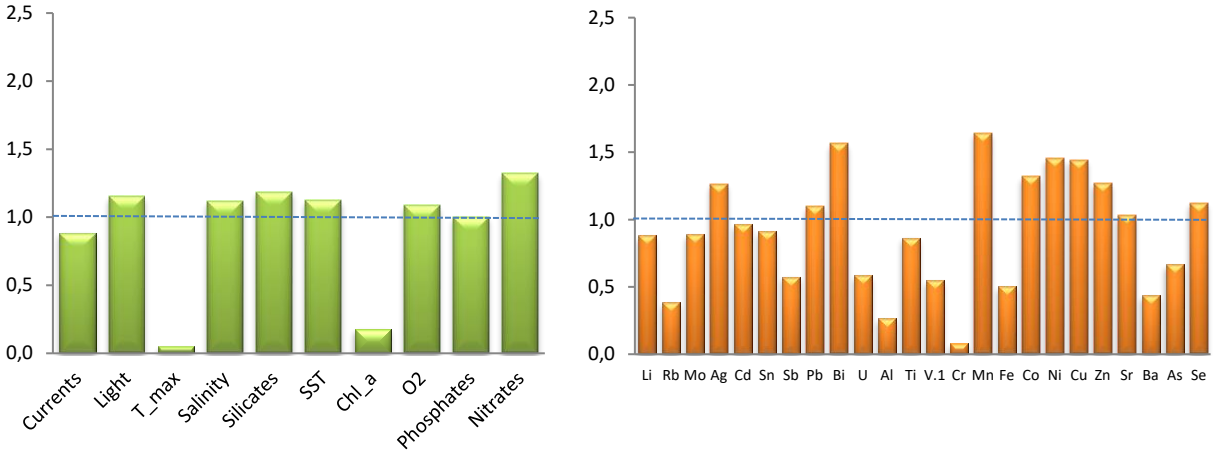

Figure S1. Variable importance for the projection (VIP), modeled on first component (t1) of native populations. Plots are giving a way to classify the predictors (green – environmental variables, orange - metals) in terms of their explanatory power of morphological traits. The predictors with a VIP > 1 are considered to be the most relevant to the construction of morphological traits.

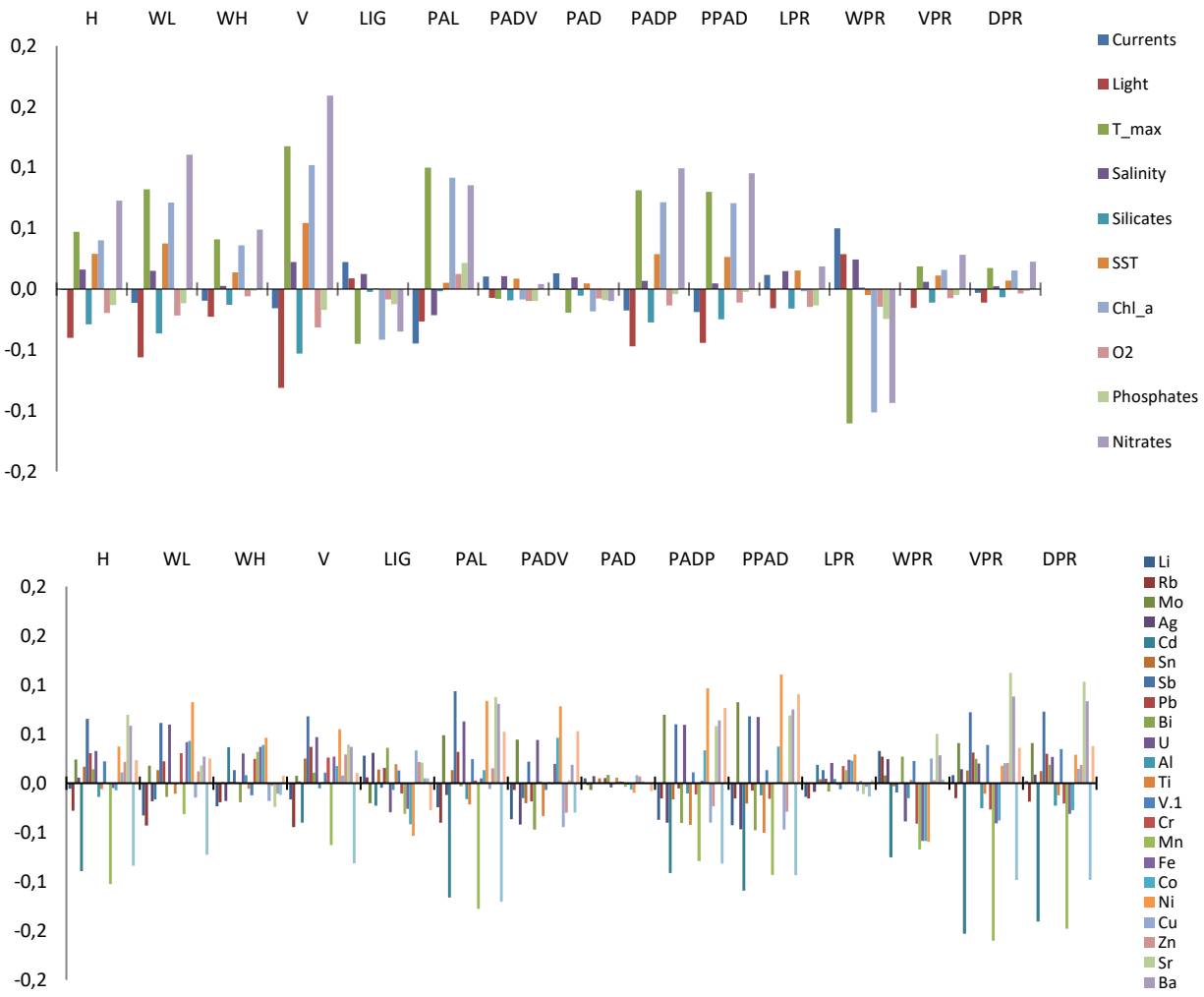

Figure S2. Standardized coefficients of native populations. Plot shows how increases of predictors (environmental variables, metals) affects response variables (morphological traits). The closer to the absolute value of 1 the coefficient is, the stronger the effect of that predictor on the response variable (controlling for other variables in the equation).

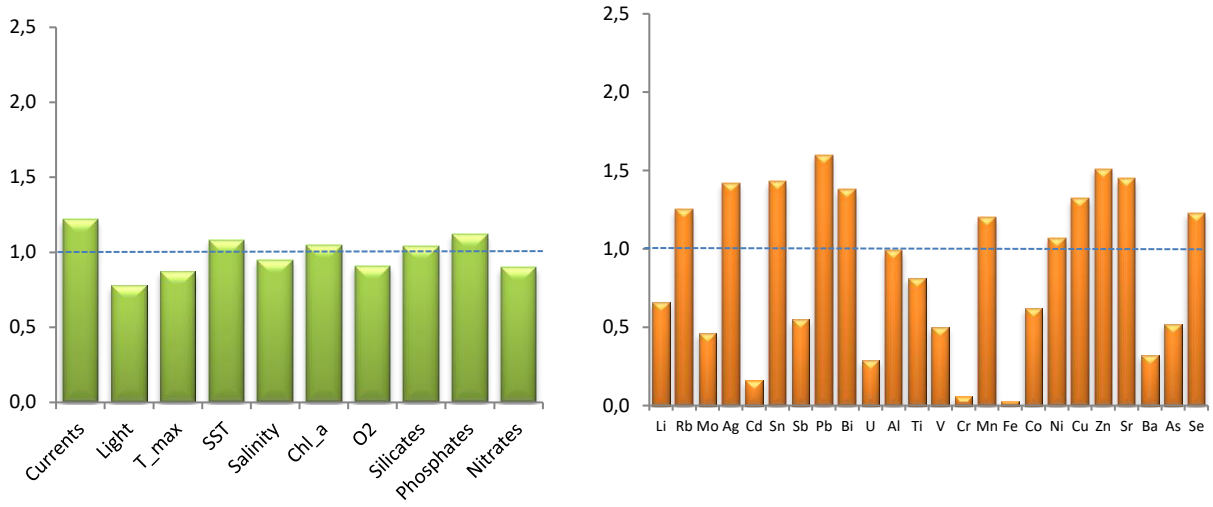

Figure S3. Variable importance for the projection (VIP), modeled on first component (t1) in transplant experiment. Plots are giving a way to classify the predictors (gree n – environmental variables, orange - metals) in terms of their explanatory power of biomarkers. The predictors with a VIP > 1 are considered to be the most relevant to the construction of biomarkers.

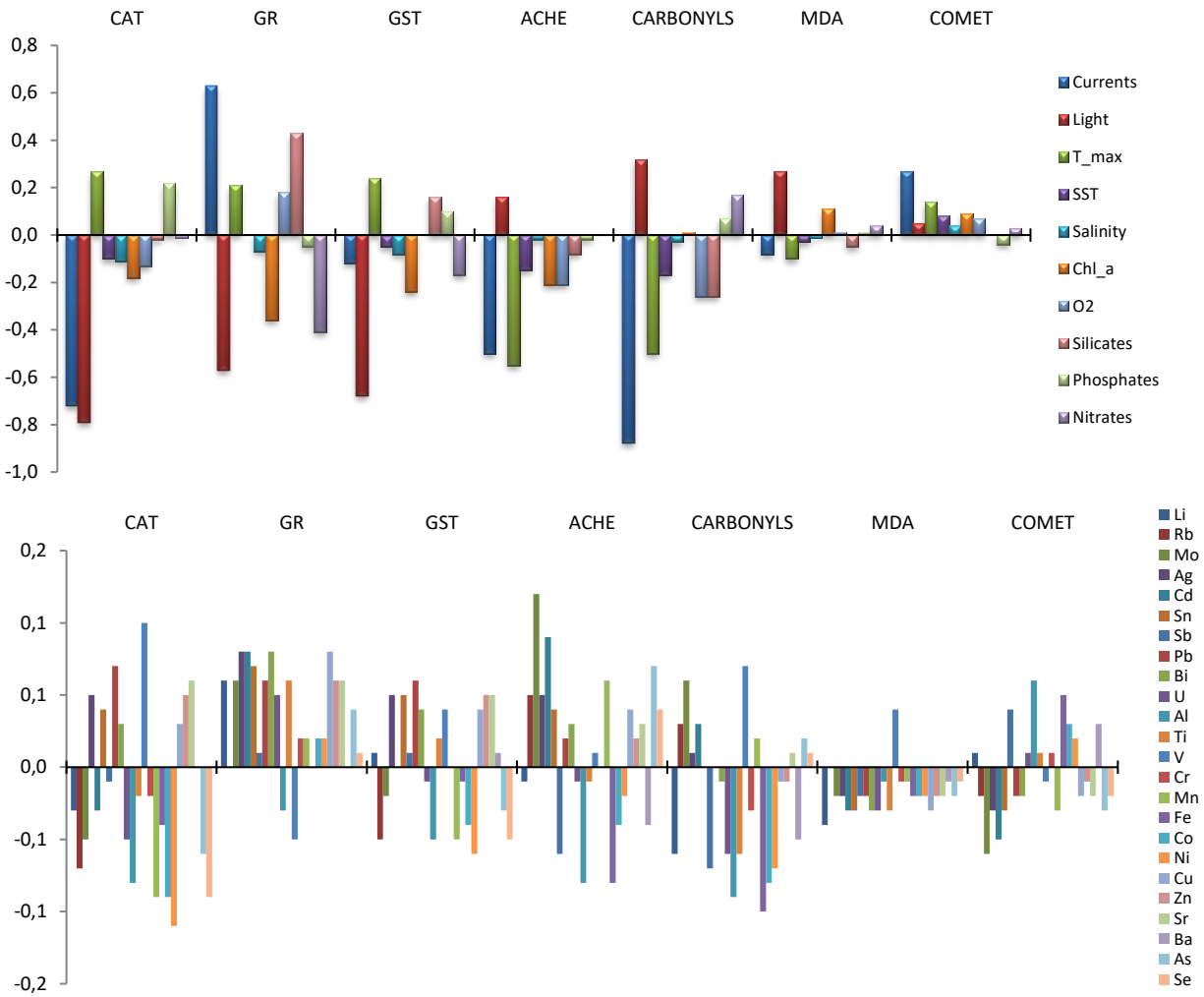

Figure S4. Standardized coefficients of transplant data. Table shows how increases of predictors (environmental variables, metals) affects response variables (biomarkers). The closer to the absolute value of 1 the coefficient is, the stronger the effect of that predictor on the response variable (controlling for other variables in the equation).

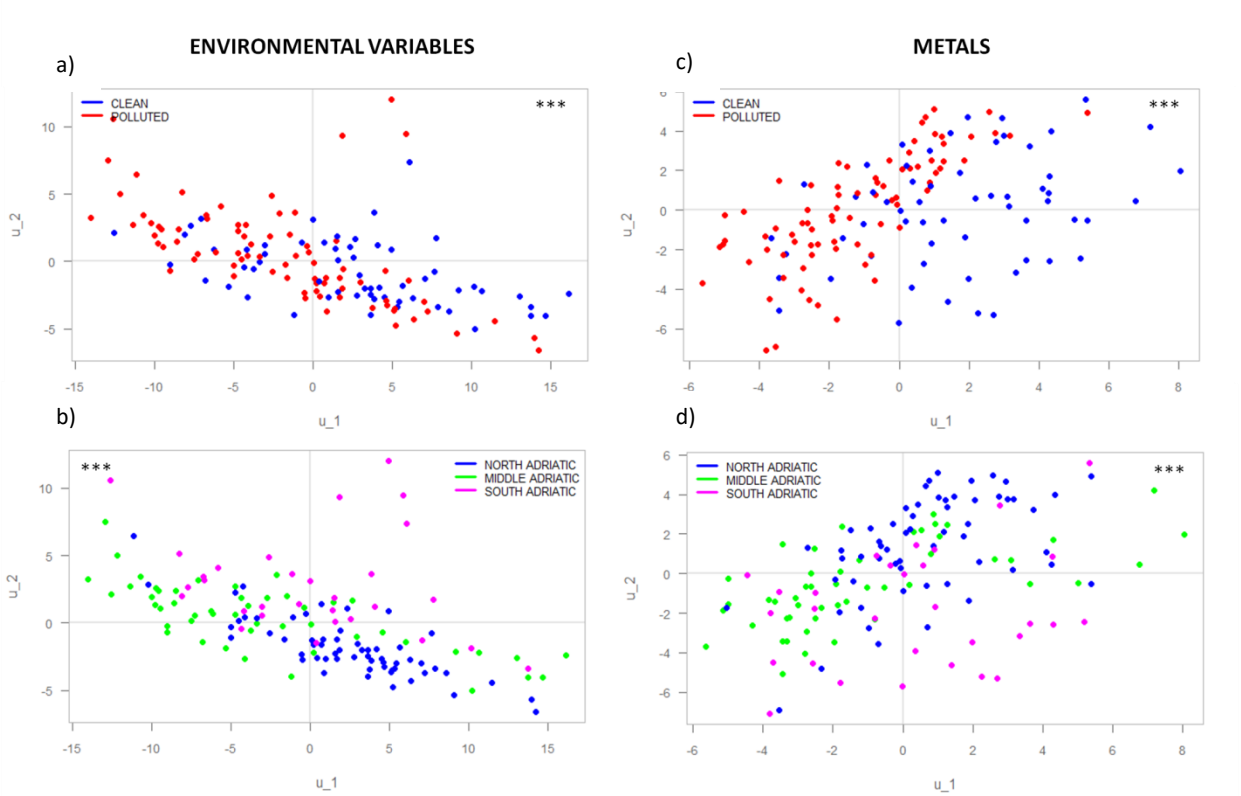

Figure S5. PLS-R2 score plots, native populations sampled in fall. Plots are representing relationship between response variables (biomarkers) and predictors (environmental variables – a,b; metals – c,d) towards pollution status (clean vs. polluted sites – a,c) and spatial distribution (Adriatic regions – b,d). ANOVA test on PLS-R2 scores shows the significance of status and regions specifics in 'response-predictor' relation, where *** represents significant effect.

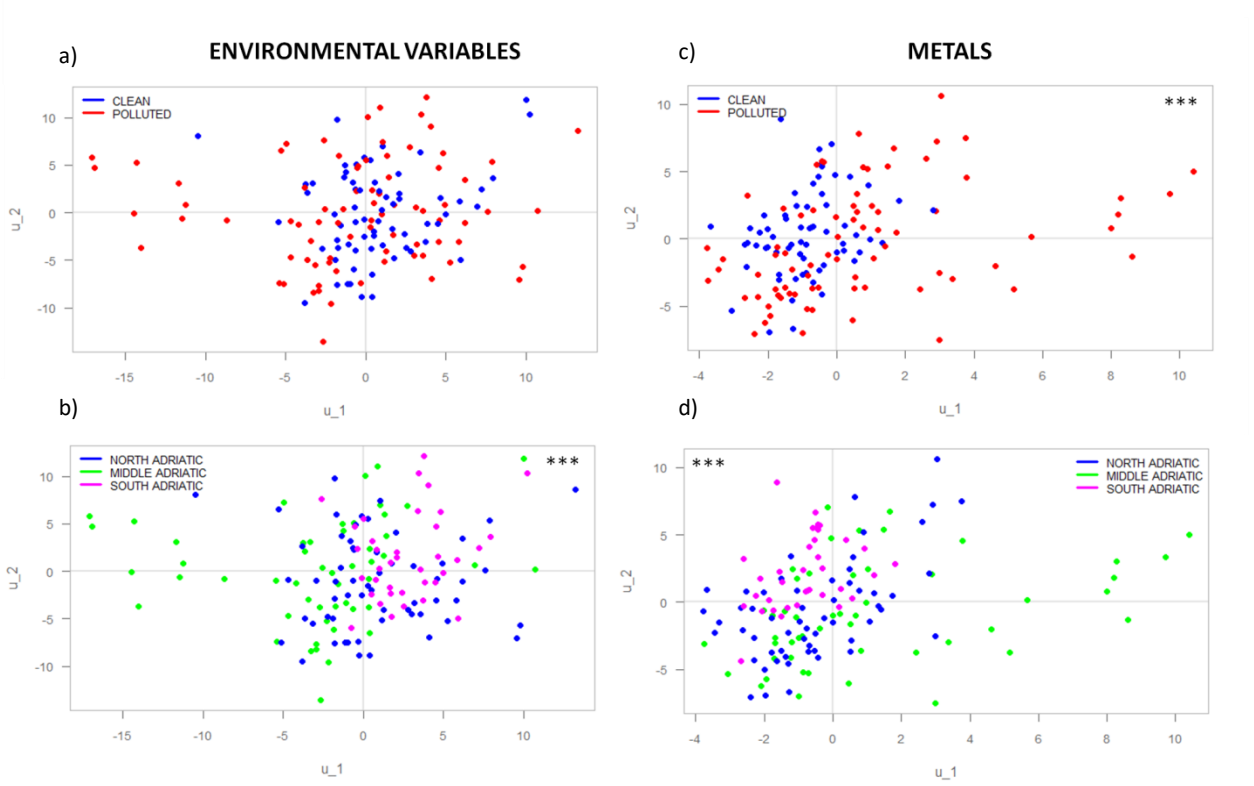

Figure S6. PLS-R2 score plots, native populations sampled in spring. Plots are representing relationship between response variables (biomarkers) and predictors (environmental variables – a,b; metals – c,d) towards pollution status (clean vs. polluted sites – a,c) and spatial distribution (Adriatic regions – b,d). ANOVA test on PLS-R2 scores shows the significance of status and regions specifics in 'response-predictor' relation, where *** represents significant effect.

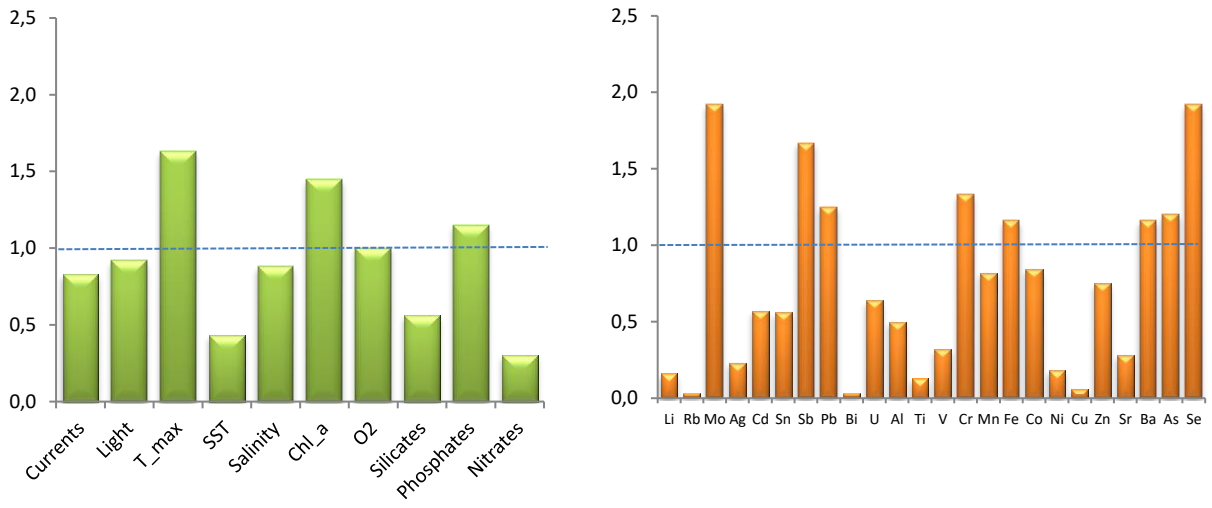

Figure S7. Variable importance for the projection (VIP), modeled on first component, of native populations data, sampled in fall. Table gives a way to classify the predictors (green – environmental variables, orange - metals) in terms of their explanatory power of biomarkers. Those predictors with a VIP > 1 are considered to be the most relevant to the construction of biomarkers.

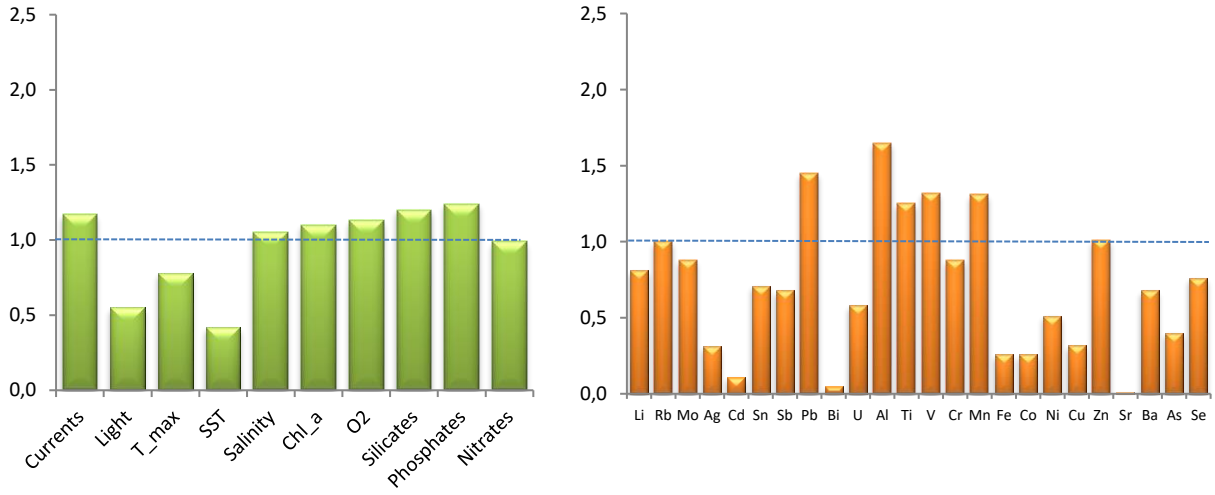

Figure S8. Variable importance for the projection (VIP), modeled on first component (t1) of native populations data, sampled in spring. Table gives a way to classify the predictors (a – environmental variables, b - metals) in terms of their explanatory power of biomarkers. Those predictors with a VIP > 1 are considered to be the most relevant to the construction of biomarkers.

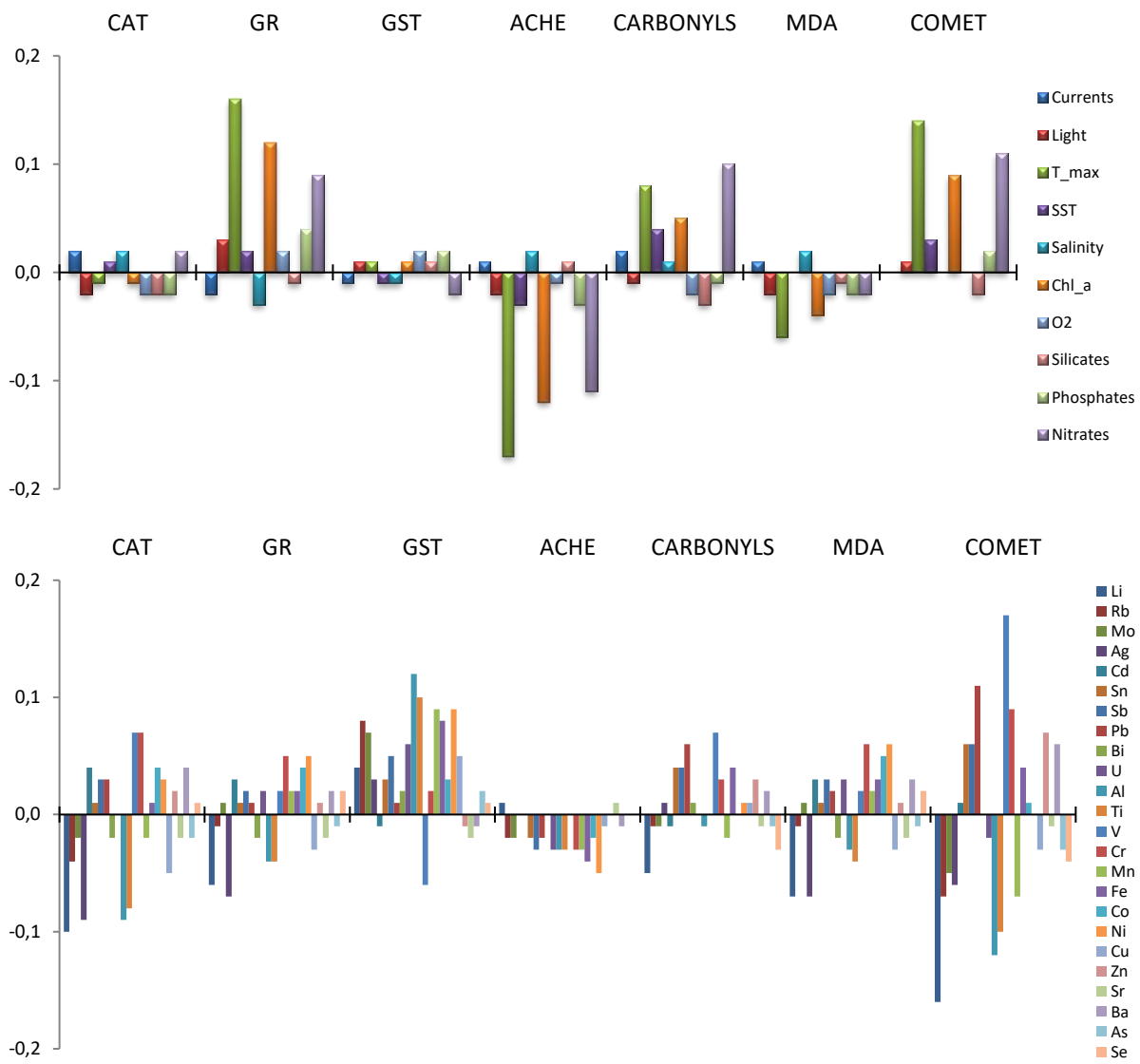

Figure S9. Standardized coefficients of native populations sampled in fall. Table shows how increases of predictors (a – environmental variables, b - metals) affects response variables (biomarkers). The closer to the absolute value of 1 the coefficient is, the stronger the effect of that predictor on the response variable (controlling for other variables in the equation).

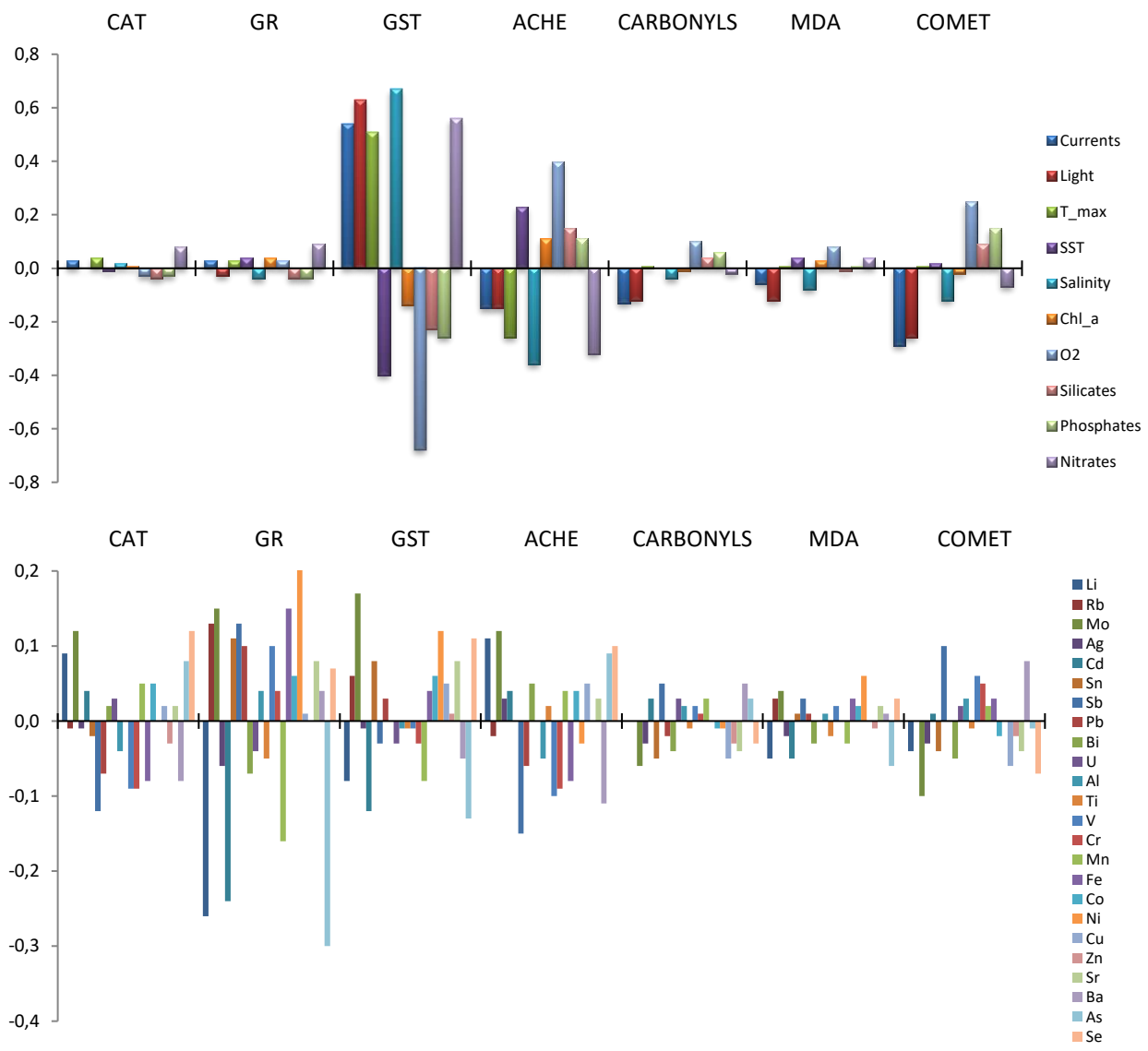

Figure S10. Standardized coefficients of native populations sampled in spring. Table shows how increases of predictors (environmental variables, metals) affects response variables (biomarkers). The closer to the absolute value of 1 the coefficient is, the stronger the effect of that predictor on the response variable (controlling for other variables in the equation).

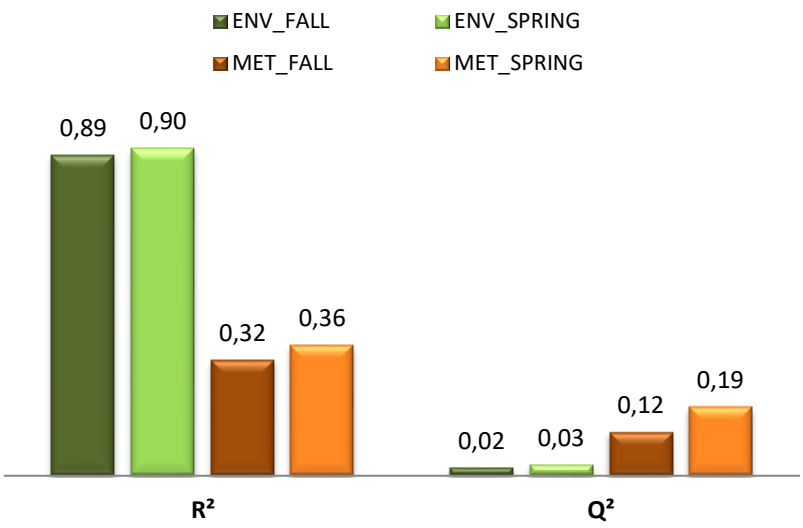

Figure S11. Validation model of the biomarkers vs. environmental variables/metals relationship using PLS-R2. The R^2 value of a given model is used to measure descriptive power of the data, and the Q^2 value of the model is used to assess the predictive power of the model. R^2 = 100% indicates perfect description of the data by the model, whereas Q^2 =100% indicates perfect predictability. Environmental variables had higher degree of fitting the data (88.5% - fall, 90.2% - spring) than metals (32%– fall, 36%– spring), with Q^2 - 2.2% - fall, 3% - spring and 12% - fall, 19% - spring, respectively.

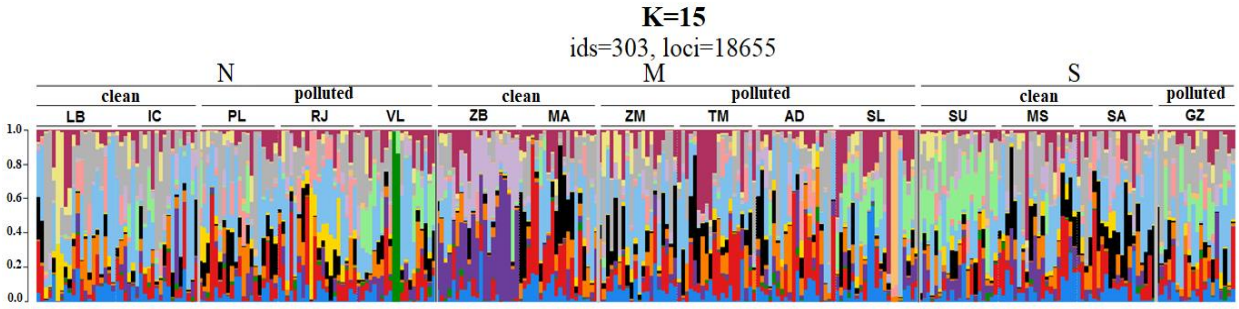

Figure S12. Admixture proportion estimates from the hierarchical Bayesian model implemented in ENTROPY. Each vertical bar represents an individual, and bars are colored to reflect the posterior medians of each individual's admixture proportions, for each of k=15 clusters. Population names, as well as regions and pollution status are indicated on the top, along the abscissa.

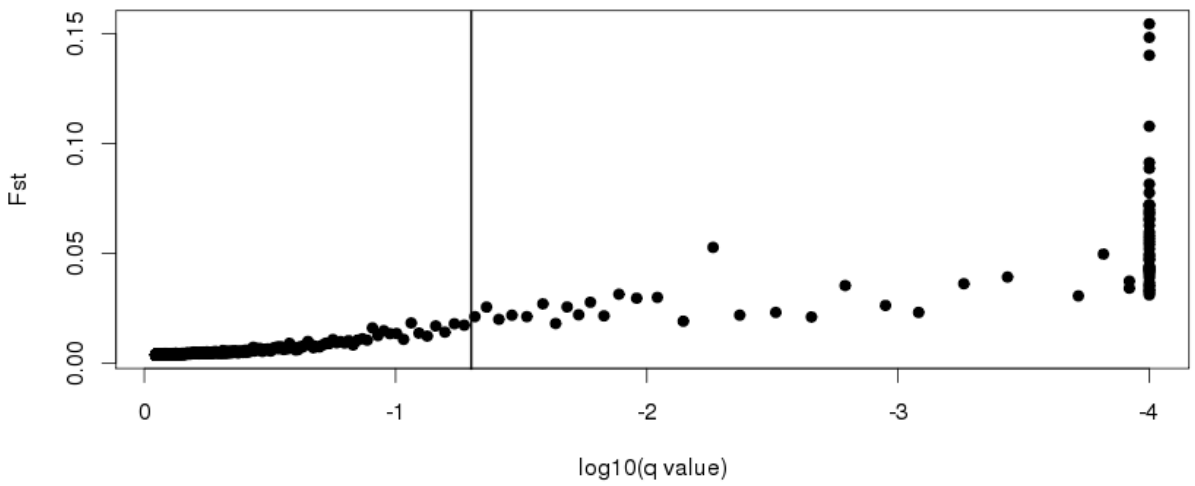

Figure S13. Outlier SNPs inferred in BAYESCAN analysis. The vertical axis represents values of locus-specific F_{ST} coefficient, and the horizontal axis indicates the logarithm of q-values. The vertical line corresponds to a threshold q-value assumed in each analysis. Dots correspond to SNPs.

Table S4. Hyper-parameter estimates of genetic architecture on Gruž population. Table shows the median, lower and higher 95% credible interval (ETPIs) of 15 traits for the prior h proportion of variance explained by the model, conditional prior probability that defines the sparsity of the model (rho), proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'measurable-effect' SNPs alone (PGE) and number of SNPs (N-SNP) that have non-zero effects on phenotypic variation.

trait	estimate	h	rho	PVE	PGE	N-SNPs
	median	0.147	0.440	0.124	0.291	18
H	lower 95% ETPI	0.007	0.020	0.006	0	0
	upper 95% ETPI	0.527	0.968	0.410	0.952	256
	median	0.142	0.442	0.120	0.297	23
WL	lower 95% ETPI	0.007	0.020	0.005	0	0
	upper 95% ETPI	0.523	0.969	0.415	0.954	270
	median	0.135	0.481	0.110	0.329	13
WH	lower 95% ETPI	0.006	0.023	0.005	0	0
	upper 95% ETPI	0.524	0.974	0.396	0.957	258
	median	0.345	0.361	0.317	0.238	25
V	lower 95% ETPI	0.066	0.017	0.057	0	0
	upper 95% ETPI	0.692	0.939	0.609	0.926	269
	median	0.262	0.329	0.235	0.198	30
LIG	lower 95% ETPI	0.020	0.014	0.016	0	0
	upper 95% ETPI	0.668	0.942	0.574	0.920	271
	median	0.400	0.290	0.382	0.180	44
PAL	lower 95% ETPI	0.116	0.012	0.107	0	0
	upper 95% ETPI	0.712	0.918	0.632	0.908	280
	median	0.164	0.435	0.140	0.285	20
PADV	lower 95% ETPI	0.010	0.020	0.007	0.000	0
	upper 95% ETPI	0.534	0.967	0.413	0.951	267
	median	0.578	0.309	0.563	0.218	35
PAD	lower 95% ETPI	0.281	0.014	0.278	0	0
	upper 95% ETPI	0.864	0.909	0.833	0.904	276
	median	0.269	0.406	0.250	0.299	37
PADP	lower 95% ETPI	0.037	0.018	0.032	0	0
	upper 95% ETPI	0.602	0.959	0.519	0.954	275
	median	0.198	0.460	0.176	0.336	23
PPAD	lower 95% ETPI	0.015	0.022	0.012	0	0
	upper 95% ETPI	0.542	0.968	0.443	0.959	264
	median	0.224	0.405	0.200	0.279	29
LPR	lower 95% ETPI	0.016	0.018	0.013	0	0
	upper 95% ETPI	0.592	0.961	0.500	0.949	272
	median	0.105	0.506	0.084	0.352	18
WPR	lower 95% ETPI	0.004	0.025	0.003	0	0
	upper 95% ETPI	0.456	0.977	0.339	0.963	251
	median	0.310	0.636	0.278	0.570	15
VPR	lower 95% ETPI	0.052	0.082	0.048	0.018	1
	upper 95% ETPI	0.649	0.982	0.583	0.979	196
	median	0.332	0.380	0.304	0.269	31
DPR	lower 95% ETPI	0.030	0.017	0.024	0	0
	upper 95% ETPI	0.753	0.952	0.696	0.941	267
	median	0.339	0.356	0.304	0.223	24
MASS	lower 95% ETPI	0.040	0.016	0.033	0	0
	upper 95% ETPI	0.718	0.941	0.637	0.920	272

Table S5. Hyper-parameter estimates of genetic architecture on Marina population – mesocosm experiment. Table shows the median, lower and higher 95% credible interval (ETPIs) of 15 traits for the prior h - used to estimate the proportion of variance explained by the model, conditional prior probability that defines the sparsity of the model (rho), proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'measurable-effect' SNPs alone (PGE) and number of SNPs (N-SNP) that have non-zero effects on phenotypic variation.

trait	estimate	h	rho	PVE	PGE	N-SNPs
H	median	0.111	0.488	0.090	0.337	18
	lower 95% ETPI	0.005	0.024	0.003	0	0
	upper 95% ETPI	0.489	0.976	0.372	0.960	262
WL	median	0.363	0.398	0.339	0.291	33
	lower 95% ETPI	0.072	0.020	0.064	0	0
	upper 95% ETPI	0.699	0.951	0.633	0.945	275
WH	median	0.214	0.449	0.184	0.320	14
	lower 95% ETPI	0.018	0.022	0.015	0	0
	upper 95% ETPI	0.579	0.964	0.466	0.949	263
V	median	0.356	0.337	0.331	0.229	36
	lower 95% ETPI	0.040	0.014	0.033	0	0
	upper 95% ETPI	0.759	0.940	0.701	0.929	274
LIG	median	0.525	0.302	0.507	0.220	26
	lower 95% ETPI	0.211	0.018	0.207	0	0
	upper 95% ETPI	0.819	0.885	0.779	0.873	266
PAL	median	0.551	0.240	0.539	0.150	52
	lower 95% ETPI	0.259	0.010	0.258	0	
	upper 95% ETPI	0.847	0.873	0.807	0.862	282
PADV	median	0.239	0.441	0.217	0.325	31
	lower 95% ETPI	0.026	0.021	0.021	0	0
	upper 95% ETPI	0.595	0.965	0.512	0.958	272
PAD	median	0.349	0.435	0.320	0.333	29
	lower 95% ETPI	0.032	0.024	0.027	0	0
	upper 95% ETPI	0.776	0.962	0.729	0.955	262
PADP	median	0.214	0.478	0.179	0.332	13
	lower 95% ETPI	0.013	0.024	0.010	0	0
	upper 95% ETPI	0.615	0.969	0.508	0.954	257
PPAD	median	0.499	0.337	0.472	0.233	38
	lower 95% ETPI	0.122	0.016	0.110	0	0
	upper 95% ETPI	0.884	0.931	0.855	0.923	275
LPR	median	0.427	0.498	0.419	0.452	50
	lower 95% ETPI	0.164	0.028	0.162	0	0
	upper 95% ETPI	0.697	0.972	0.658	0.973	270
WPR	median	0.317	0.347	0.296	0.231	32
	lower 95% ETPI	0.051	0.015	0.044	0	
	upper 95% ETPI	0.682	0.943	0.602	0.934	274
VPR	median	0.187	0.404	0.161	0.259	23
	lower 95% ETPI	0.010	0.018	0.008	0	0
	upper 95% ETPI	0.599	0.962	0.495	0.945	269
DPR	median	0.607	0.216	0.599	0.130	41
	lower 95% ETPI	0.340	0.009	0.343	0	0
	upper 95% ETPI	0.865	0.812	0.829	0.776	281
MASS	median	0.266	0.502	0.247	0.430	31
	lower 95% ETPI	0.020	0.029	0.016	0	0
	upper 95% ETPI	0.637	0.972	0.588	0.969	265

Table S6. Hyper-parameter estimates of genetic architecture on Marina population – transplant experiment. Table shows the median, lower and higher 95% credible interval (ETPIs) of 15 traits for the prior h - used to estimate the proportion of variance explained by the model, conditional prior probability that defines the sparsity of the model (rho), proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'measurable-effect' SNPs alone (PGE) and number of SNPs (N-SNP) that have non-zero effects on phenotypic variation.

trait	estimate	h	rho	PVE	PGE	N-SNPs
H	median	0.078	0.455	0.060	0.248	15
	lower 95% ETPI	0.004	0.021	0.003	0	0
	upper 95% ETPI	0.419	0.974	0.223	0.944	262
WL	median	0.493	0.217	0.487	0.123	59
	lower 95% ETPI	0.330	0.010	0.335	0	0
	upper 95% ETPI	0.735	0.765	0.633	0.683	284
WH	median	0.492	0.254	0.489	0.176	88
	lower 95% ETPI	0.323	0.011	0.331	0	0
	upper 95% ETPI	0.727	0.826	0.641	0.800	287
V	median	0.358	0.500	0.339	0.440	36
	lower 95% ETPI	0.163	0.061	0.160	0.015	1
	upper 95% ETPI	0.575	0.949	0.526	0.945	248
LIG	median	0.230	0.298	0.208	0.148	27
	lower 95% ETPI	0.057	0.012	0.049	0	0
	upper 95% ETPI	0.577	0.916	0.388	0.877	273
PAL	median	0.344	0.269	0.327	0.137	30
	lower 95% ETPI	0.177	0.011	0.163	0	0
	upper 95% ETPI	0.644	0.866	0.484	0.808	278
PADV	median	0.157	0.348	0.134	0.173	23
	lower 95% ETPI	0.015	0.014	0.012	0.000	0
	upper 95% ETPI	0.516	0.948	0.319	0.911	273
PAD	median	0.222	0.610	0.214	0.578	67
	lower 95% ETPI	0.044	0.051	0.039	0	0
	upper 95% ETPI	0.443	0.980	0.399	0.979	270
PADP	median	0.164	0.467	0.146	0.362	24
	lower 95% ETPI	0.025	0.026	0.025	0	0
	upper 95% ETPI	0.460	0.967	0.343	0.960	266
PPAD	median	0.109	0.672	0.091	0.610	8
	lower 95% ETPI	0.019	0.097	0.025	0.023	1
	upper 95% ETPI	0.356	0.986	0.246	0.981	204
LPR	median	0.129	0.398	0.108	0.228	23
	lower 95% ETPI	0.009	0.017	0.007	0	0
	upper 95% ETPI	0.467	0.961	0.290	0.934	272
WPR	median	0.126	0.398	0.104	0.227	21
	lower 95% ETPI	0.008	0.018	0.006	0	0
	upper 95% ETPI	0.471	0.961	0.302	0.932	272
VPR	median	0.227	0.377	0.205	0.266	23
	lower 95% ETPI	0.044	0.023	0.039	0.000	0
	upper 95% ETPI	0.525	0.937	0.414	0.918	264
DPR	median	0.183	0.573	0.170	0.523	38
	lower 95% ETPI	0.031	0.053	0.028	0	0
	upper 95% ETPI	0.421	0.977	0.364	0.975	256
MASS	median	0.144	0.349	0.123	0.176	24
	lower 95% ETPI	0.013	0.015	0.010	0	0
	upper 95% ETPI	0.500	0.948	0.294	0.912	272

Table S7. Hyper-parameter estimates of genetic architecture on the Marina_pool. Table shows the median, lower and higher 95% credible interval (ETPIs) of 15 traits for the prior h - used to estimate the proportion of variance explained by the model, conditional prior probability that defines the sparsity of the model (rho), proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'measurable-effect' SNPs alone (PGE) and number of SNPs (N-SNP) that have non-zero effects on phenotypic variation.

trait	estimate	h	rho	PVE	PGE	N-SNPs
H	median	0.130	0.351	0.111	0.171	23
	lower 95% ETPI	0.012	0.015	0.009	0	0
	upper 95% ETPI	0.489	0.950	0.264	0.908	273
WL	median	0.449	0.116	0.428	0.024	9
	lower 95% ETPI	0.306	0.004	0.305	0	0
	upper 95% ETPI	0.781	0.794	0.548	0.251	273
WH	median	0.428	0.122	0.404	0.023	7
	lower 95% ETPI	0.282	0.004	0.278	0	0
	upper 95% ETPI	0.773	0.804	0.527	0.263	262
V	median	0.338	0.203	0.321	0.084	37
	lower 95% ETPI	0.188	0.008	0.184	0	0
	upper 95% ETPI	0.675	0.823	0.457	0.683	279
LIG	median	0.433	0.273	0.430	0.226	33
	lower 95% ETPI	0.296	0.042	0.303	0.010	1
	upper 95% ETPI	0.594	0.668	0.555	0.627	254
PAL	median	0.459	0.131	0.444	0.036	19
	lower 95% ETPI	0.313	0.005	0.314	0	0
	upper 95% ETPI	0.771	0.766	0.570	0.368	277
PADV	median	0.305	0.202	0.279	0.064	15
	lower 95% ETPI	0.161	0.008	0.152	0	0
	upper 95% ETPI	0.668	0.828	0.411	0.551	274
PAD	median	0.225	0.272	0.205	0.135	28
	lower 95% ETPI	0.072	0.012	0.066	0	0
	upper 95% ETPI	0.576	0.898	0.357	0.844	276
PADP	median	0.121	0.423	0.102	0.254	24
	lower 95% ETPI	0.013	0.021	0.010	0	0
	upper 95% ETPI	0.423	0.961	0.242	0.938	268
PPAD	median	0.238	0.251	0.217	0.111	24
	lower 95% ETPI	0.093	0.011	0.085	0	0
	upper 95% ETPI	0.588	0.877	0.357	0.773	270
LPR	median	0.317	0.208	0.302	0.086	31
	lower 95% ETPI	0.177	0.009	0.173	0	0
	upper 95% ETPI	0.654	0.820	0.432	0.664	276
WPR	median	0.224	0.303	0.207	0.157	43
	lower 95% ETPI	0.087	0.013	0.081	0	0
	upper 95% ETPI	0.555	0.913	0.343	0.882	277
VPR	median	0.163	0.298	0.145	0.133	26
	lower 95% ETPI	0.042	0.012	0.037	0	0
	upper 95% ETPI	0.527	0.920	0.270	0.859	276
DPR	median	0.317	0.169	0.295	0.057	17
	lower 95% ETPI	0.172	0.007	0.165	0	0
	upper 95% ETPI	0.673	0.806	0.427	0.472	278
MASS	median	0.316	0.195	0.294	0.073	21
	lower 95% ETPI	0.163	0.008	0.156	0	0
	upper 95% ETPI	0.667	0.432	0.432	0.610	276

Table S8. Hyper-parameter estimates of genetic architecture on 15 native populations. Table shows the median, lower and higher 95% credible interval (ETPIs) of 15 traits for the prior h - used to estimate the proportion of variance explained by the model, conditional prior probability that defines the sparsity of the model (rho), proportion of the total phenotypic variation (PVE), proportion of the phenotypic variation that can be explained by 'measurable-effect' SNPs alone (PGE) and number of SNPs (N-SNP) that have non-zero effects on phenotypic variation.

trait	estimate	h	rho	PVE	PGE	N-SNPs
	median	0.451	0.408	0.443	0.355	21
H	lower 95% ETPI	0.173	0.024	0.196	0	0
	upper 95% ETPI	0.778	0.946	0.735	0.947	267
	median	0.867	0.229	0.865	0.138	39
WL	lower 95% ETPI	0.563	0.010	0.579		0
	upper 95% ETPI	0.999	0.831	0.999	0.815	277
	median	0.650	0.241	0.640	0.150	51
WH	lower 95% ETPI	0.323	0.010	0.327	0	0
	upper 95% ETPI	0.972	0.875	0.968	0.867	283
	median	0.733	0.283	0.735	0.192	61
V	lower 95% ETPI	0.454	0.012	0.478	0	0
	upper 95% ETPI	0.985	0.914	0.984	0.918	283
	median	0.966	0.229	0.969	0.140	61
LIG	lower 95% ETPI	0.766	0.009	0.790		0
	upper 95% ETPI	1.000	0.847	1.000	0.852	282
	median	0.897	0.169	0.894	0.083	37
PAL	lower 95% ETPI	0.596	0.007	0.609	0	0
	upper 95% ETPI	1.000	0.724	1.000	0.636	279
	median	0.761	0.275	0.763	0.186	54
PADV	lower 95% ETPI	0.492	0.012	0.514	0	0
	upper 95% ETPI	0.990	0.881	0.989	0.883	280
	median	0.930	0.322	0.931	0.258	69
PAD	lower 95% ETPI	0.656	0.016	0.674	0	0
	upper 95% ETPI	1.000	0.908	1.000	0.912	281
	median	0.829	0.338	0.834	0.273	68
PADP	lower 95% ETPI	0.558	0.016	0.585	0	0
	upper 95% ETPI	0.998	0.922	0.998	0.929	278
	median	0.905	0.240	0.907	0.150	53
PPAD	lower 95% ETPI	0.645	0.010	0.669	0	0
	upper 95% ETPI	1.000	0.842	1.000	0.839	280
	median	0.675	0.320	0.668	0.223	41
LPR	lower 95% ETPI	0.378	0.015	0.389	0	0
	upper 95% ETPI	0.964	0.917	0.958	0.918	274
	median	0.834	0.167	0.837	0.079	31
WPR	lower 95% ETPI	0.562	0.007	0.590	0	0
	upper 95% ETPI	0.998	0.722	0.998	0.630	278
	median	0.761	0.226	0.768	0.131	34
VPR	lower 95% ETPI	0.480	0.009	0.511	0	0
	upper 95% ETPI	0.992	0.840	0.992	0.837	278
	median	0.849	0.417	0.844	0.302	20
DPR	lower 95% ETPI	0.572	0.057	0.596	0.022	1
	upper 95% ETPI	0.999	0.934	0.999	0.940	239
	median	0.983	0.350	0.985	0.277	55
MASS	lower 95% ETPI	0.852	0.026	0.875	0.001	1
	upper 95% ETPI	1.000	0.883	1.000	0.901	276

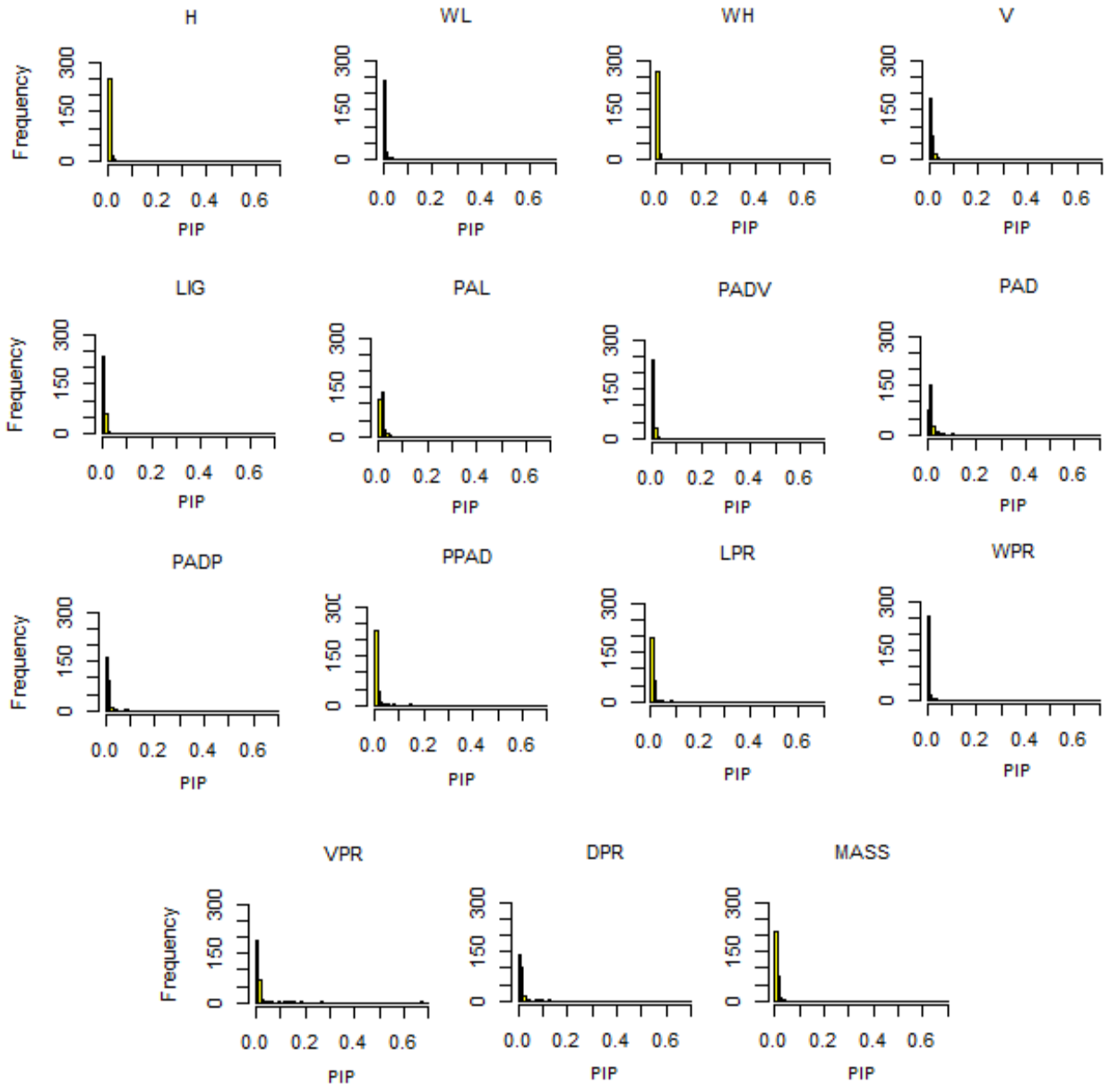

Figure S14. Posterior inclusion probability of the top 1% SNPs, in Gruž population.

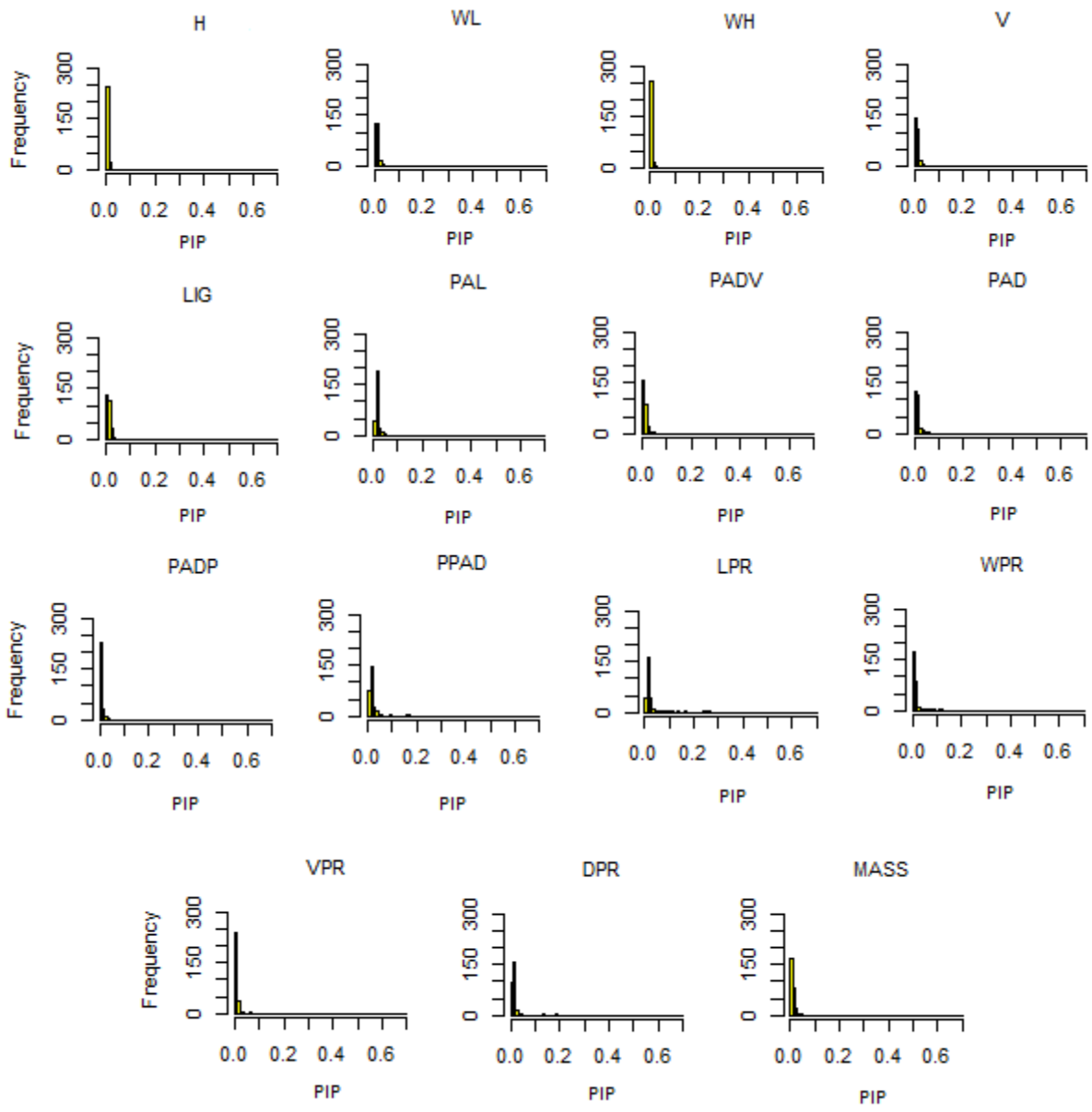

Figure S15. Posterior inclusion probability of the top 1% SNPs, in Marina population, exposed in mesocosm experiment.

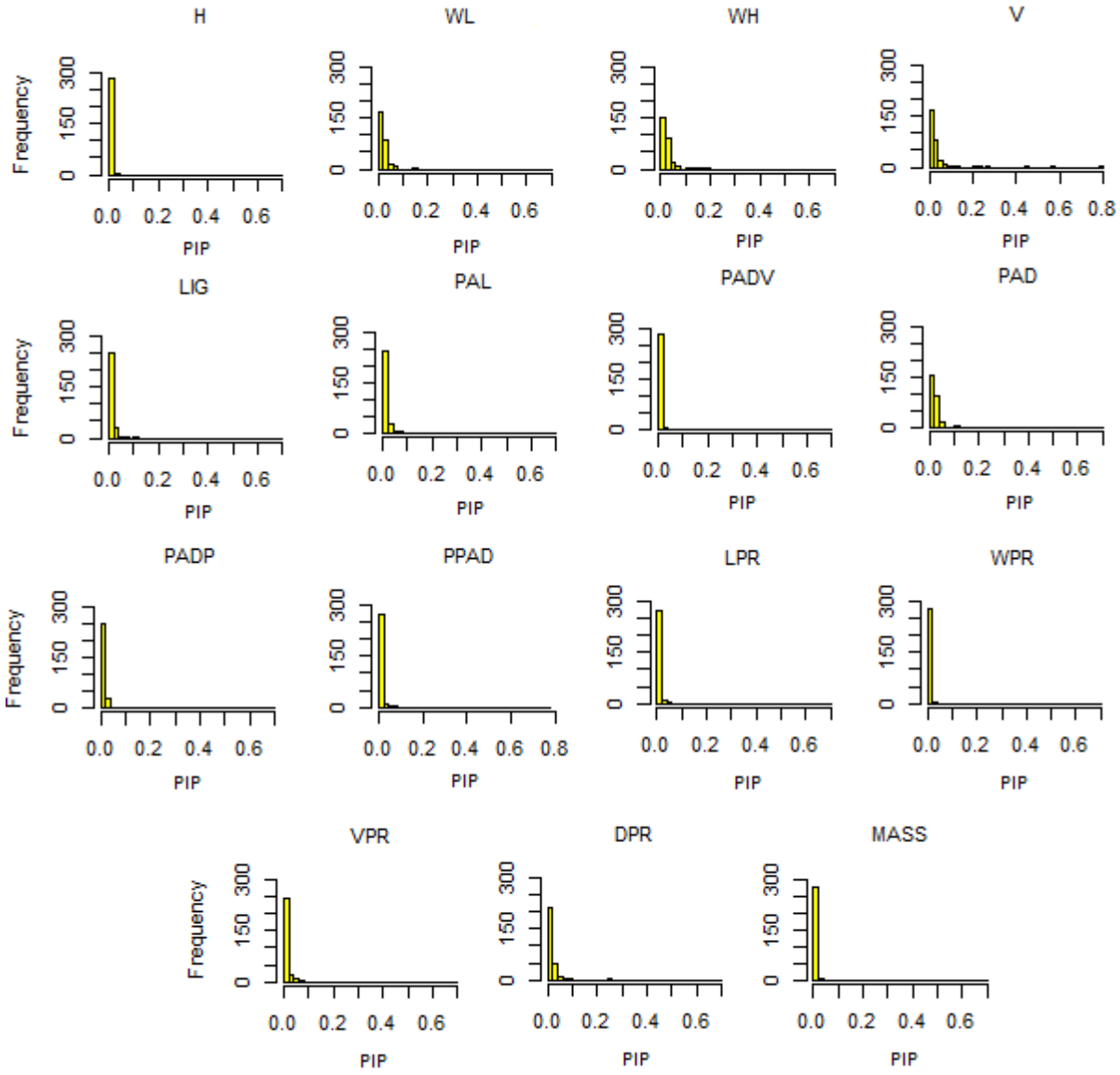

Figure S16. Posterior inclusion probability of the top 1% SNPs, in Marina population, exposed in transplant experiment.

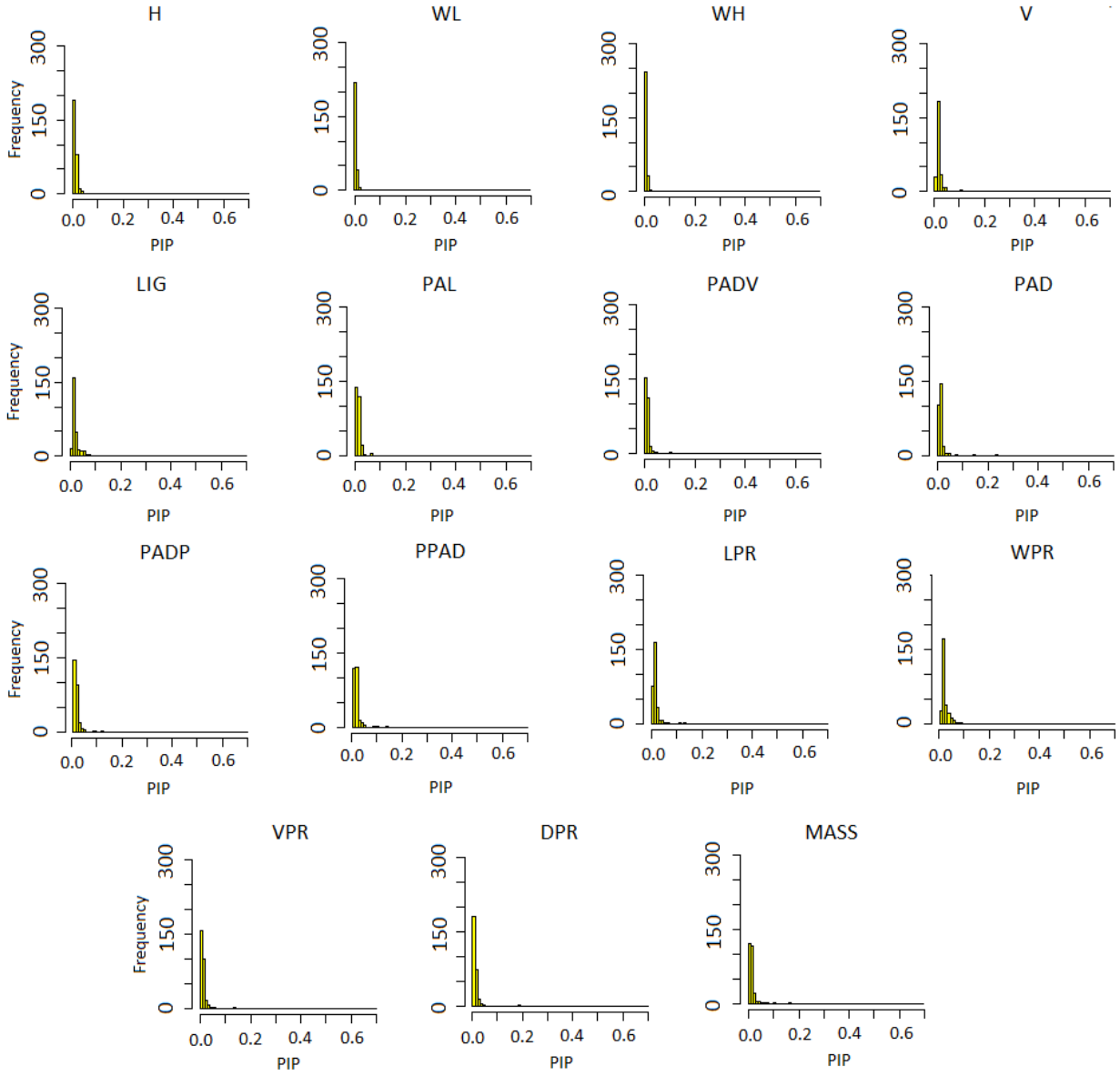

Figure S17. Posterior inclusion probability of the top 1% SNPs, for all individuals of Marina population, exposed in mesocosm and transplant experiment.

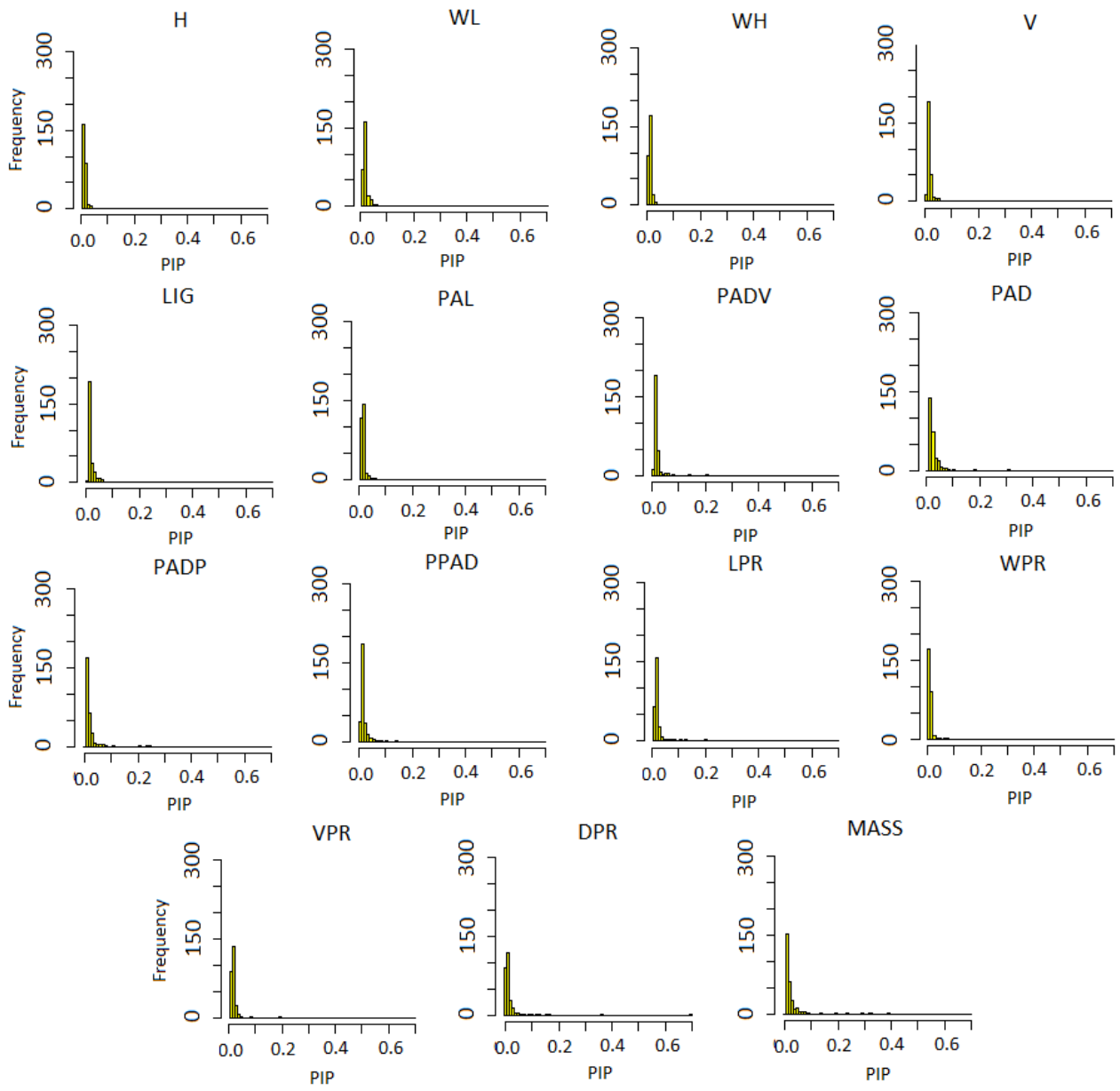

Figure S18. Posterior inclusion probability of the top 1% SNPs, for native populations.

Table S9. Matrix of top 1% (upper panel) and PIP > 0.01 (lower pannel) shared SNP. Number of shared SNPs is shown for each trait and between all data sets (Marina meso – MM, Gruž meso – GM, Marina trans – MT, Marina pool – MP, native populations –N)

	MM	7	2	29	2		**MM**	5	2	38	4
	0	**GM**	0	4	5		0	**GM**	0	3	5
H	0	0	**MT**	7	2	**PADP**	0	0	**MT**	6	5
	3	0	1	**MP**	3		7	1	2	**MP**	4
	0	0	0	0	**N**		0	5	3	3	**N**
	MM	5	3	25	4		**MM**	5	4	46	4
	0	**GM**	0	1	1		2	**GM**	0	3	4
WL	2	0	**MT**	7	2	**PPAD**	1	0	**MT**	2	2
	4	0	4	**MP**	6		25	0	4	**MP**	2
	3	0	4	1	**N**		1	2	0	0	**N**
	MM	4	4	27	5		**MM**	3	4	32	5
	0	**GM**	0	2	3		0	**GM**	0	4	7
WH	0	0	**MT**	9	4	**LPR**	2	0	**MT**	5	8
	2	0	2	**MP**	2		36	2	0	**MP**	4
	0	0	4	0	**N**		7	3	3	5	**N**
	MM	4	3	25	2		**MM**	2	6	44	4
	0	**GM**	0	4	5		0	**GM**	0	2	9
V	2	0	**MT**	4	4	**WPR**	0	0	**MT**	5	3
	18	1	5	**MP**	2		23	0	0	**MP**	1
	2	4	4	8	**N**		1	0	1	0	**N**
	MM	3	4	35	2		**MM**	1	5	26	4
	0	**GM**	0	6	2		0	**GM**	0	5	6
LIG	1	0	**MT**	3	4	**VPR**	1	0	**MT**	4	1
	24	1	2	**MP**	2		4	0	2	**MP**	1
	4	2	2	6	**N**		0	3	1	1	**N**
	MM	6	0	40	6		**MM**	8	5	48	6
	4	**GM**	0	5	7		2	**GM**	0	5	1
PAL	1	0	**MT**	8	2	**DPR**	1	0	**MT**	1	1
	30	3	3	**MP**	3		21	1	1	**MP**	5
	4	7	2	0	**N**		5	2	2	1	**N**
	MM	1	6	46	2		**MM**	5	0	30	5
	0	**GM**	0	4	6		2	**GM**	0	6	3
PADV	2	0	**MT**	6	2	**MASS**	0	0	**MT**	5	4
	12	0	0	**MP**	1		9	3	0	**MP**	2
	1	1	2	4	**N**		2	4	0	3	**N**
				MM	11	3	32	5			
				7	**GM**	0	4	1			
			PAD	5	0	**MT**	3	9			
				16	4	7	**MP**	7			
				2	3	36	10	**N**			

9. CURRICULUM VITAE

Dorotea Grbin was born on 9th February 1989, in Karlovac. She started undergraduate studies in Experimental Biology at Faculty of Science, University of Zagreb in 2008. She finished the graduate studies in Ecology and Nature preservation at Faculty of Science, University of Zagreb with a Master's thesis "Diversity of phytoplankton in aquatorium NP Telašćica" in 2013.

From 2014 she works as a research and teaching assistant at Faculty of Science, University of Zagreb within the UKF project "The effects of pollution on rapid evolution and ecological change in the Mediterranean mussel (*Mytilus galloprovincialis*)". She was a teaching assistant in Ecotoxicology (2015) and Biotests (2016). She was assistant supervisor of three Master's thesis at Faculty of Science, University of Zagreb.

In 2015 she did a brief professional training – bioinformatic intership - for one month, in National Centre for Genome Research (NCGR) New Mexico, Santa Fe. She was a part of several international scientific conferences and workshops with oral and poster presentations. Dorotea Grbin is an author on two scientific papers (one of which is in the submission process);

Grbin D, Pfannkuchen M, Babić I, Mejdandžić M, Mihanović H, Marić Pfannkuchen D, Godrijan J, Peharec Štefanić P, Olujić G, Ljubešić Z (2017) Multigene phylogeny and morphology of newly isolated strain of *Pseudo-nitzschia mannii* Amato and Montresor (Adriatic Sea). Diatom research 32, 1; 127-13

Grbin D, Sabolić I, Klobučar G, Dennis SR, Šrut M, Bakarić R, Baković V, Radić Brkanac S, Nosil P, Štambuk A (2019) Biomarker response of mussel's regarding environmental conditions, pollution impact and seasonal effects; *submitted* (*STOTEN-D-19-05764*)