Understanding extinction risk in aquatic invertebrates using range size and genetic diversity

by

Surya Deepika Garugu

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science in Wildlife Sciences

Auburn, Alabama
August 5, 2023

Keywords:
Genetic diversity; IUCN; range size; Mollusca; Arthropoda

Approved by

Dr. Willoughby, Chair, Assistant Professor, College of Forestry, Wildlife and Environment
Dr. Ditchkoff, Professor, College of Forestry, Wildlife and Environment
Dr. Christopher A. Lepczyk, Professor, College of Forestry, Wildlife and Environment
Dr. Steury, Associate Professor, College of Forestry, Wildlife and Environment

Abstract

Genetic diversity is not only necessary for evolutionary processes but also for ecosystem functioning and population stability. This is particularly true for ecosystem engineers that contribute broadly to function and food webs in the habitats in which they are found. In this study, we assessed the global patterns of extinction risk, range size, and genetic diversity, specifically focusing on two large invertebrate clades: Mollusca and Arthropoda. We collected data from the International Union for Conservation of Nature (IUCN) and also conducted a systematic, quantitative review of existing Mollusca and Arthropoda nuclear and mitochondrial data. In total we considered 4075 species evaluated by the IUCN as well as 2452 populations with genetic data. We found that range size is significantly smaller in critically endangered species compared to least concern species, but that genetic diversity did not vary significantly between extinction risk categories. However, when we considered the variability in genetic diversity between populations within a species, we found that mollusks are significantly more variable than arthropods in the nuclear genome but not the mitochondrial genome. Combined, this suggests that future efforts aimed at identifying extinction risk using genetic data will need to consider taxonomic biases when applying population-level assessments to species-level determinations.

Acknowledgments

I would like to express my gratitude to my advisor Dr. Janna Willoughby for her awesome mentorship and guidance. I would like to thank my committee members Dr. Ditchkoff, Dr. Lepczyk, and Dr. Steury, for all their help and guidance. I would like to thank those who have extended their great support in collecting data. I would like to thank my colleagues in the Conservation and Genomics Lab for all their help and support. I would also like to thank the Auburn College of Forestry, Wildlife and Environment. Finally, thank you to my parents, family, and friends for their endless support in my pursuit of higher education.

Table of Contents

List of Tables

List of Figures

Understanding extinction risk in aquatic invertebrates using range size and genetic diversity

Abstract

Genetic diversity is not only necessary for evolutionary processes but also for ecosystem functioning and population stability. However, the broad patterns of baseline genetic diversity across many species, particularly invertebrates, with varied extinction risks remain unknown because summarizing across these species has not yet occurred. In this study, we assessed the global patterns of extinction risk, range size, and genetic diversity, specifically focusing on two large invertebrate clades: Mollusca and Arthropoda. We collected data from the International Union for Conservation of Nature (IUCN) and also conducted a systematic, quantitative review of existing Mollusca and Arthropoda nuclear and mitochondrial data. In total we considered 4,075 species evaluated by the IUCN as well as 2,452 populations with genetic data. Range size was significantly smaller in critically endangered species compared to least concern species, but genetic diversity did not vary significantly between extinction risk categories. We also found that mollusks are significantly more variable than arthropods in the nuclear genome but not the mitochondrial genome when evaluating variability in genetic diversity between populations within a species. Combined, our results suggests that future efforts aimed at identifying extinction risk using genetic data will need to consider taxonomic biases when applying population-level assessments to species-level determinations.

Introduction

Conserving biodiversity requires consideration of ecosystems, species, and genetic diversity to achieve a sustainable pattern of use of our natural resources (Convention on Biodiversity 2004). Consideration of ecosystem diversity requires protecting both terrestrial and aquatic habitats as well as the abiotic properties associated with various habitats, whereas conservation species diversity focuses on the number and function of species that live in particular ecosystems (Pimm et al, 1995). Because genetic variety across the genome supplies the raw materials for adaptation to shifting environmental conditions, genetic diversity conservation is crucial for enabling evolutionary change. Increasing mobility (e.g., by creating movement corridors or translocations) and reducing threats to population size can conserve genetic diversity in threatened species by halting the loss of genetic variation in the present population (Meffe 1996; Prior et al. 1997). Importantly, these three facets of biodiversity often change in tandem. For example, when an ecosystem loses critical functions, this may lead to the loss of some species outright and may also cause populations of some species to shrink, likely reducing genetic diversity. For example, some algae depend on substrate provided by mussels so, when these mussel populations are disturbed, dependent algae decrease, and the entire ecosystem can collapse (Collier et al. 2016). Because of the linkage across these areas of biodiversity, conservation across multiple facets is typically required in effective conservation efforts (Bangert et al., 2004).

Globally, Mollusca and Arthropoda are the largest and most specious invertebrate phyla. Mollusca, which include such species as octopuses, mussels, squid, and snails. Mollusks are found in a variety of marine habitats, including intertidal zones, rocky shores, coral reefs, and deep-sea environments (Ruppert et al., 2004) and they also inhabit freshwater habitats, including rivers, lakes, and wetlands (Dillon R.T 2000). Likewise, Arthropoda occupy a similar breadth of habitats, and many are found across diverse aquatic habitats, although the majority of these aquatic species are free-living, detritus-feeding species (Dehghani R et al, 2015). In both groups, approximately half of evaluated species are considered threatened by the International Union for Conservation of Nature (IUCN 2021), largely due to habitat loss (Collen et al., 2014).

Mollusk and arthropod conservation is important because these species often have greater than average effects on ecosystems and serve important indicator roles. For example, community structure is strongly influenced by these species, as they have the capacity to create, maintain, modify, or destroy their habitats (Jones et al., 1994). In particular, mollusks such as snails and clams play a significant role in nutrient cycling and sedimentation processes, while others, such as octopuses and squid, are important predators in marine food webs (Ruppert et al., 2004) (Guay and Himmelman, 2004; Gutiérrez et al., 2003; Hubbard, 2016; Hill C, 1991; Kraeuter et al., 2003). For example, mussel beds change the structure of the environment (Jones et al., 1994), providing important habitat for algae that are usually unable to attach to rocks (Albrecht and Reise 1994, Buschbaum 2001). These species are also valuable as bioindicators. For example, ghost shrimp accumulate contaminants over the course of their life and can be analyzed and used as an indicator of habitat contamination (*Amoozadeh E* et al. 2014). As an assemblage, mollusks and arthropods alter the aquatic ecosystems in ways that are important to survival of other species and provide critical insight into ecosystem function as bioindicators (McGeoch 1998, Weisser and Siemann 2004).

The IUCN employs an array of metrics for understanding species extinction risk and assigning conservation status, including population and species-specific data, number of mature adults alive in a given time frame, the extent of occurrence or area of occupancy, and estimates of population viability (IUCN 2021). Although the IUCN criteria were designed to support consistent assessment of species, many are unsuitable for assessing extinction risk in species that are difficult to sample, including mollusks and arthropods (Cardoso et al. 2011). As a result, invertebrate species assessment groups that are responsible for determining extinction risk rely extensively on criteria related to the extent of occurrence and area of occupancy, as these can often be assessed without the extensive time and resources required to repeatedly collect specimens. However, it is not yet known to what extent the efficiency of conserving biodiversity in these clades and other taxonomic groups is limited by reliance on range size alone (Possingham at al., 1999).

In addition to currently recognized IUCN determinants for assessing extinction risk, many conservationists agree that maintaining genetic diversity is of high conservation concern (DeWoody et al. 2021, García Dorado and Caballero, 2021). Across taxa generally, a decrease in population size is related to a decrease in genetic diversity and fitness (Allendorf et al, 2012). Typically, the reduced fitness occurs when inbreeding, which increases in frequency when population size decreases in size, results in the expression of recessive deleterious alleles (Reed et al. 2003). In addition, genetically diverse populations can tolerate environmental changes more effectively than those populations that are genetically depauperate because the increased genetic variability at protein-coding genes helps populations evolve more quickly (Hughes and Stachowicz, 2004). Because genetic diversity can be closely related to survival probability and population adaptability, maintaining genetic diversity is important, and identifying populations with very little genetic diversity can be a conservation priority (Willoughby et al. 2015, Kardos et al. 2021). For example, molecular data suggests that restoring corridors across black-tailed prairie dog (*Cynomys ludovicianus*) habitats will support the persistence of these populations even in the face of on-going disease threats (Sackett et al. 2012). Because the behavior of this species changes the landscape substantially, black-tailed prairie dog extirpation is likely to result in the loss of other species that are adapted to the prairie-dog modified landscape (Baker et al. 2013). Similarly, in aquatic habitats, heavy anthropogenic pressures have resulted in the rapid extirpation of aquatic invertebrates (Maehr et al., 2006), many of which were important contributors to ecosystem function (Commito et al. 2005).

Despite the importance of genetic diversity in maintaining population stability in many systems, predicting how genetic diversity is distributed across populations and species can be difficult. For example, increasing conservation need can be related to species genetic diversity when population sizes across the species are small or declining, but some endangered species do not show reduced genetic variation (Doyle et al. 2015). This variability may be because the current methods for identifying and ranking conservation need neither include genetic diversity nor systematically target species with reduced genetic diversity (Willoughby et al. 2015). Additionally, some species' phylogenetic history may mean that whole clades have reduced genetic variation with no observable effect on fitness (Wiens et al. 2015), further complicating the relationship among conservation need/population trends and-genetic diversity (González et al. 2018). Across aquatic invertebrate species specifically, the baseline pattern of genetic diversity has not been summarized. This lack of information has the potential to significantly hinder management options because it is unclear the extent to which populations and species require intervention related to reduced genetic variability, and how best to supply that intervention.

Given the problems with applying IUCN criteria to taxa, our goal is to understand how extinction risk and range size of aquatic invertebrate species interacts with patterns of nuclear and mitochondrial genetic diversity. To address this goal, we sought to test the following hypotheses. First, we hypothesized that species more at risk for extinction would have smaller range sizes than species less at risk of extinction. Second, we hypothesized that species classified as increasing would have larger range sizes than species classified as decreasing because smaller populations tend to have less genetic diversity. Third, we hypothesized that species more at risk for extinction would have more nuclear diversity than species less at risk of extinction because high extinction risk species typically have small populations. Fourth, we hypothesized that Mollusca would be more variable than Arthropoda due to their reliance on host species for breeding, which we expect would sometimes decrease population sizes. Testing these hypotheses will provide a baseline understanding of underlying global patterns that are important for informing future conservation and management in these declining taxa.

Materials and Methods

Data collection

To understand how extinction risk, range size, and genetic diversity are related in aquatic invertebrates, we focused on invertebrate taxonomic groups that had have the most extinction risk evaluations completed by IUCN: Mollusca and Arthropoda. We started our analyses by downloading and combining the IUCN's spatial range size and extinction risk data and identified 5,887 unique species with complete records (i.e., these species had range maps available and had IUCN records; IUCN 2021). We used these data to calculate the percentage of these invertebrate taxa from the IUCN database that were listed under the following extinction risk categories:

Critically Endangered (CR), Endangered (EN), and Vulnerable (VU), all of which are considered threatened by the IUCN, as well as Near Threatened (NT), and Least Concern. We also extracted the IUCN-determined population stability category value, which includes declining, increasing, or stable for each species that has been evaluated in this way.

To supplement the IUCN data, we collected genetic diversity estimates from the primary literature. We identified relevant literature using the ISI (Institute of Scientific Information) Web of Science and Google Scholar using two different search approaches in April 2021. First, we conducted a systematic literature review using the search terms “gen* diversity” and “Mollusca,” as well as using “gen* diversity” and “Arthropoda” as search terms. In our second search, we supplemented these searches using species names and “gen* diversity” or “SNP” or “microsat” or “genome” or “gene” or “allozyme” for species that were not found in the original sets of records but were included in our IUCN data. All searches were limited to published and peer reviewed literature. We identified articles that focused on at least one population and contained either nuclear or mitochondrial genetic data. Following the initial screening, we noted the genus and specific epithet of the population under study, the number of genotyped loci, and the number of individuals for the population under study.

We also recorded genetic diversity estimates generated in each of the studies we identified. Microsatellites are non-coding and neutral nuclear markers and are the most common types of markers used to quantify a population’s genetic diversity (Coltman and Slate, 2003). However, mitochondrial loci are also informative as these can provide more insight into historical demographic events that alter population structure and evolution (Jenyfer Fernandez-Perez et al., 2017). Because of these differences in marker types and data insights, we collected both microsatellite assessments of population genetic diversity and mitochondrial sequence data for mollusks and arthropods. From each study and for each population included in the study, we recorded measures of genetic diversity, type and number of genetic markers used, and depending on the type of genetic assessment conducted, we recorded observed heterozygosity, expected heterozygosity and number of alleles (for microsatellite assessments) as well as nucleotide diversity and haplotype diversity (for mitochondrial sequencing assessments).

Statistical analyses

In our first model, we used a linear regression to understand how range size is distributed amongst extinction risk categories in Mollusca and Arthropoda. Specifically, we used the log transformed range size estimate as the response variable and IUCN extinction risk category as the predictor variable. We interpreted non-overlapping 95% confidence intervals of the coefficient estimates as indicating significant differences in range sizes between extinction risk groups and analyzed mollusks and arthropods independently.

In our second regression model, we were interested in how range sizes differed between species with different population trajectories (decreasing, increasing, or stable) in Mollusca and Arthropoda. We tested whether population trend status predicted range size, using log-transformed range size as the response variable and a means parametrized regression model. Again, mollusks and arthropods were analyzed in two different regression analyses.

To address our hypothesis related to extinction risk and genetic diversity, we used a phylogenetic least square regression (PGLS). We used nuclear and mitochondrial genetic metrics as response variables and IUCN extinction risk and the taxonomic relationships between species, estimated using Class, Order, Family, and Genus and setting all branch lengths to one, were used as predictors. We created our phylogenetic object in R using the multi2di function in the ape package (Paradis E et al., 2019) and ran our regression model using the "gls" function available in the "nlme" package (Pinheiro J et al., 2022). We assumed Brownian evolutionary patterns and fit our means parameterized model using maximum likelihood. We considered our estimates of genetic diversity (heterozygosity, number of alleles, nucleotide diversity, haplotype diversity) as response variables in independent models, where estimates from multiple populations were averaged to generate a single estimate for each species/genetic diversity metric combination. We also compared the 95% confidence intervals around the coefficient estimates from this model between the IUCN extinction risk groups to understand how genetic diversity varied between these groups.

Finally, we considered how variation among populations (but within a species) was different between mollusks and arthropods. For species with more than one population-level estimate of genetic diversity, we calculated the coefficient of variation (standard deviation/mean) for each of the genetic diversity estimates we considered. We then calculated the mean coefficient of variation, and 95% confidence interval around this mean, for each of the genetic variation metrics within Mollusca and Arthropoda. We assessed significant differences between these taxonomic groups by comparing the 95% confidence intervals.

Results

We identified 4,075 species where extinction risk and range size had been evaluated by the IUCN. These species included 1,685 Arthropods, all from the order Decapoda and 2,390 Mollusks from 15 different orders: Allogastropoda (17 species), Architaenioglossa (88 species), Arcoida (4 species), Cycloneritida (49 species), Ellobiida (24 species), Hygrophila (236 species), Littorinimorpha (236 species), Mytiloida (3 species), Neogastropoda (546 species), Ostreoida (1 species), Pylopulmonata (4 species), Sorbeoconcha (179 species), Unionida (266 species) and Yenerida (129 species). Despite having somewhat large group sizes, these data were not distributed equally over extinction risk categories. For example, 70% percent of arthropods and 60% of mollusks included in this dataset were listed as least concern (Table 1).

Table 1. Number of Arthropoda and Mollusca species for each IUCN extinction risk category. All of these species also had range size estimates generated by the IUCN.

IUCN extinction risk	Arthropoda species	Mollusca species
Least concern	1,186	1,431
Near threatened	66	182
Vulnerable	216	323
Endangered	120	199
Critically endangered	91	232

We found limited information about population stability for arthropod species (1 species noted as stable) as well as mollusk species (14 decreasing, 4 increasing, and 10 stable species). Although we report our comparisons between these population trend categories for mollusks, we caution against over interpretation of this set of data due to this limited dataset size (Table 2).

Table 2. Number of Arthropoda and Mollusca species for each of the IUCN determined categories of population trajectory. All of these species also had range size estimates generated by the IUCN.

Population trajectory	Arthropoda species	Mollusca species
Decreasing	55	257
Increasing	3	18
Stable	365	275
Unknown	1,262	1,840

We used the set of IUCN-evaluated species as targets for our genetic diversity data collection efforts and supplemented this list with species not yet evaluated by the IUCN. In total, we collected genetic diversity data from 2,452 populations (365 Arthropoda and 2390 Mollusca populations) that included 242 species (32 Arthropoda and 210 Mollusca). We found similar biases in these data as we did in the larger, IUCN evaluated list of species: genetic diversity data were non-uniformly distributed over species in different extinction risk categories, such that 100% of the genetic diversity estimates collected from arthropods evaluated by IUCN was from least concern species, as was 66% of genetic diversity estimates collected for mollusks.

Table 3. Number of Arthropoda and Mollusca populations for which we collected genetic diversity data. The number of species is noted in parentheses.

IUCN extinction risk	Arthropoda species	Mollusca species
Least concern	56 (5)	365 (32)
Near threatened	0 (0)	31 (1)
Vulnerable	0 (0)	62 (5)
Endangered	0 (0)	50 (5)
Critically endangered	0 (0)	46 (3)
Not evaluated	309 (27)	1,533 (164)

In our first regression analysis, we found that as extinction risk increased, range size significantly decreased in arthropods and mollusks (Table 4, Figure 1). For example, the 95% confidence interval for the range size coefficient in least concern arthropods was significantly greater than all extinction risk groups, and the coefficient for critically endangered arthropod species was significantly less than all of the other extinction risk groups. This same pattern was observed in Mollusks (Table 4). However, in arthropods and mollusks range size coefficient confidence intervals overlapped in the vulnerable and endangered species groups, suggesting that there are important characteristics of mid-range sized species that are important predictors of extinction risk in these groups. Furthermore, there is substantial overlap in the range size values (Figure 1), again indicating that factors other than range size are important to understanding extinction risks.

Table 4. Coefficient (beta) estimates and 95% confidence interval (CI) around these estimates where range size was compared between IUCN categories.

	IUCN Categories	Estimate (95% CI)
Mollusca	Least concern	3.45 (3.32, 3.58)
	Near Threatened	-0.23 (-0.61, 0.13)
	Vulnerable	-0.99 (-1.27, -0.17)
	Endangered	-1.32 (-1.67, -0.96)
	Critically Endangered	-2.47 (-2.80, -2.13)
Arthropoda	Least concern	2.28 (2.16, 2.41)
	Near Threatened	0.44 (-0.08, 0.98)
	Vulnerable	-0.85 (-1.15, -0.56)
	Endangered	-0.89 (-1.29, -0.50)
	Critically Endangered	-2.00 (-2.46, -1.54)

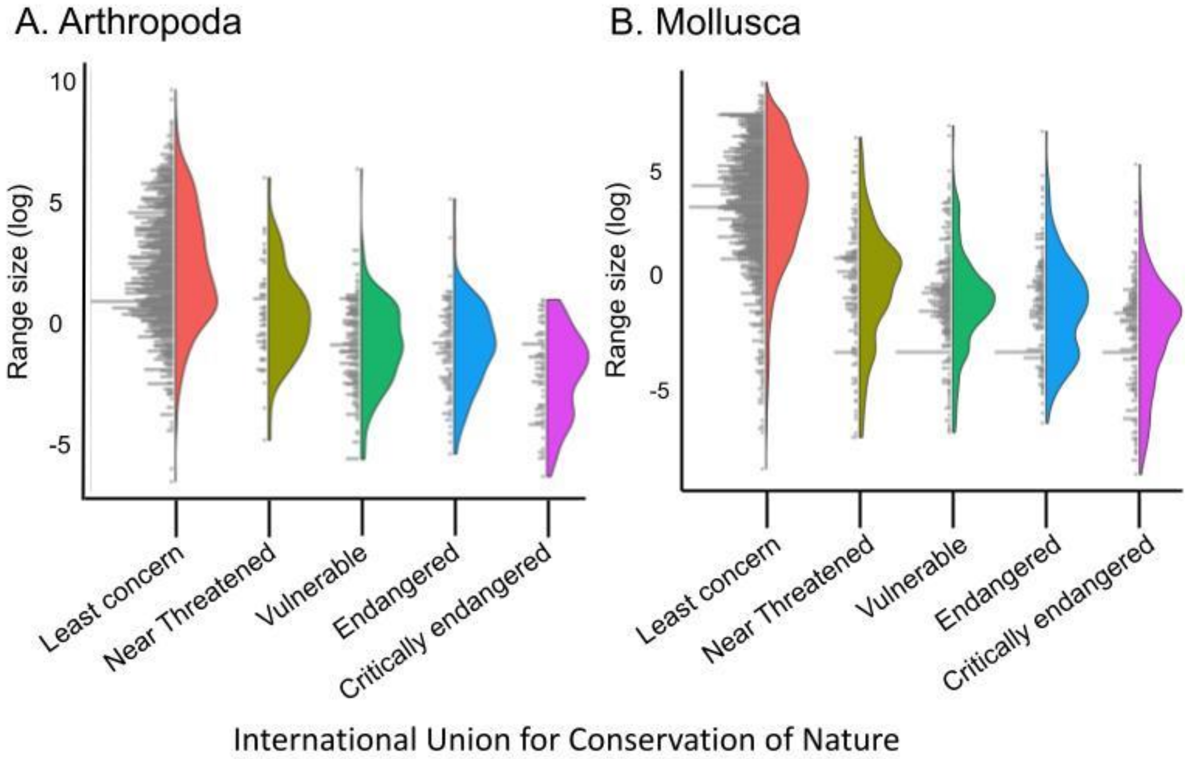

Figure 1. The distribution of range size, shown here as the log of range size, varies significantly between extinction risk categories in Arthropoda (A) and Mollusca (B). (See Table 4 for regression results). Range size estimates were made in square kilometers, and each dot represents a different species. As shown in Table 1, there is a signficant decrease in range size as extinction risk increases, and this trend exists for all category comparisons other than vulnerable and endangered where range size is equal.

In terms of how population stability trends may be a predictor of species range size we found that in arthropods range size was not significantly different between species with decreasing, increasing, or stable populations (Figure 2, Table 5). However, for Mollusca species, we found that stable populations have significantly smaller range sizes compared to species with increasing populations (Figure 2, Table 5). Considering both Arthropods and Mollusks, we also found that there was less variation in range size in stable species compared to either decreasing or increasing population size species. However, our sample size in these comparisons is limited so this may, at least partially, explain this outcome (Table 5).

Table 5. Coefficient estimates and 95% confidence interval (CI) around these estimates where range size was compared between population trend categories.

	Population trends	Estimate	p-value
Mollusca	Decreasing	3.76 (2.68, 4.84)	<0.001
	Increasing	5.24 (3.80, 6.68)	<0.001
	Stable	2.79 (2.40, 3.18)	<0.001
Arthropoda	Decreasing	2.08 (0.69, 3.48)	0.003
	Increasing	4.51 (2.23, 6.78)	<0.001
	Stable	2.83 (2.62, 3.04)	<0.001

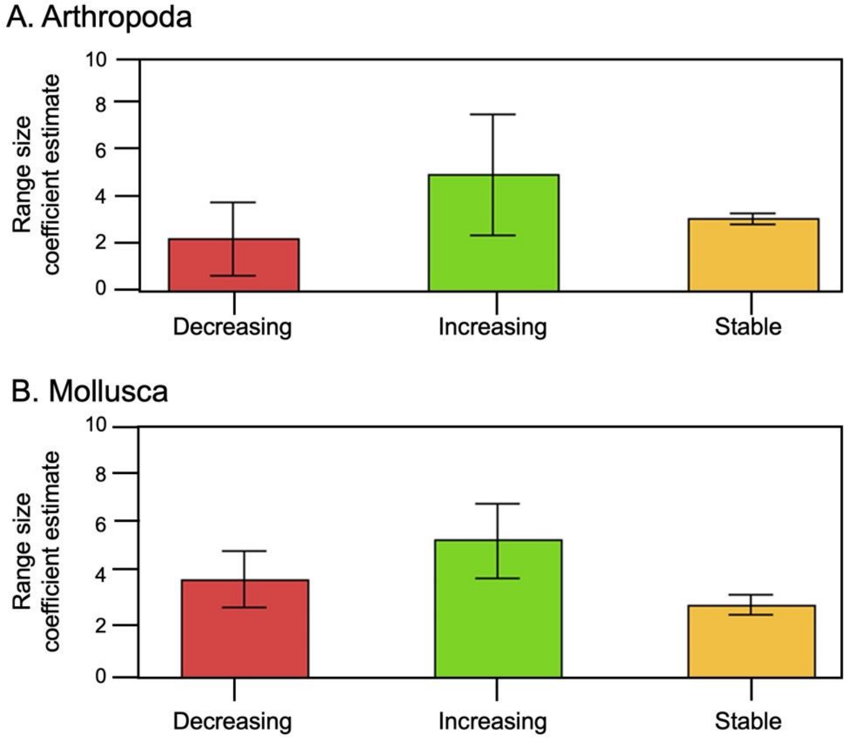

Figure 2. Population stability is largely unrelated to range size in Arthropods (A) and Mollusks (B). We used a linear regression where the log of range size was our response variable and the categorical population trend variable was our predictor. Within each Phylum, all population trend groupings had overlapping 95% confidence intervals, displayed by the error bars, except for the comparison between increasing and stable populations Mollusca (B).

In terms of how extinction risk relates to genetic diversity measures our PGLS analyses suggested that that genetic diversity was significantly correlated with taxonomy; nuclear genetic diversity estimate correlations averaged 0.84 (observed heterozygosity = 0.82, expected heterozygosity = 0.86, number of alleles = 0.88) and mitochondrial genetic diversity estimate correlations averaged 0.55 (nucleotide diversity = 0.49 and haplotype diversity = 0.61). When we included taxonomic relationships in our analyses, we found no significant differences in nuclear (Figure 3, Table 6) or mitochondrial (Figure 4) genetic diversity estimates between any extinction risk categories. This lack of differences suggests that future efforts to understand extinction risks related to genetic diversity should consider taxonomic variables explicitly since there may be evolution-related causes of high or low genetic diversity that may be unrelated to population stability and viability.

Table 6. Coefficient estimates and 95% confidence intervals around these estimates of species genetic diversity for each IUCN extinction risk category. These were estimated using a phylogenetic least squares regression using Mollusca (denoted as M) and Arthropoda (denoted as A) species.

Genetic metric	IUCN categories	Coefficients	p-value
Observed Heterozygosity	Least concern (M)	0.52 (0.44,0.59)	<0.001
	Near threatened (M)		
	Vulnerable (M)	0.54 (0.38, 0.70)	<0.001
	Endangered (M)	0.36 (0.18, 0.54)	<0.001
	Critically endangered (M)	0.57 (0.38, 0.75)	<0.001
	Least Concern (A)	0.49 (0.29, 0.69)	<0.001
Expected Heterozygosity	Least Concern (M)	0.60 (0.52, 0.68)	<0.001
	Near threatened (M)		<0.001
	Vulnerable (M)	0.54 (0.38, 0.70)	<0.001
	Endangered (M)	0.40 (0.22, 0.58)	<0.001
	Critically endangered (M)	0.66 (0.47, 0.80)	<0.001
	Least concern (A)	0.58 (0.37, 0.50)	<0.001
Number of alleles	Least concern (M)	7.90 (5.86, 9.94)	<0.001
	Near Threatened (M)		
	Vulnerable (M)	3.68 (-1.73, 9.11)	0.180
	Endangered (M)	5.08 (0.69, 9.47)	0.023
	Critically Endangered (M)	11.76 (6.09, 17.43)	<0.001
Nucleotide diversity	Least concern (M)	0.004 (0.003, 0.005)	<0.001
	Near threatened (M)	0.001 (-0.001, 0.004)	0.443
	Vulnerable (M)	0.003 (0.0001, 0.006)	0.004
	Endangered (M)	0.003 (-0.0005, 0.005)	0.105
	Critically endangered (M)	0.001 (-0.001, 0.003)	0.261
	Least Concern (A)	0.007 (0.004, 0.001)	<0.001
Haplotype diversity	Least concern (M)	0.58 (0.53, 0.63)	<0.001
	Near Threatened (M)	0.47 (0.33,0.62)	<0.001
	Vulnerable (M)	0.71 (0.45, 0.97)	<0.001
	Endangered (M)	0.45 (0.28, 0.62)	<0.001
	Critically endangered (M)	0.41 (0.29,0.53)	<0.001
	Least concern (A)	0.93 (0.76, 1.09)	<0.001

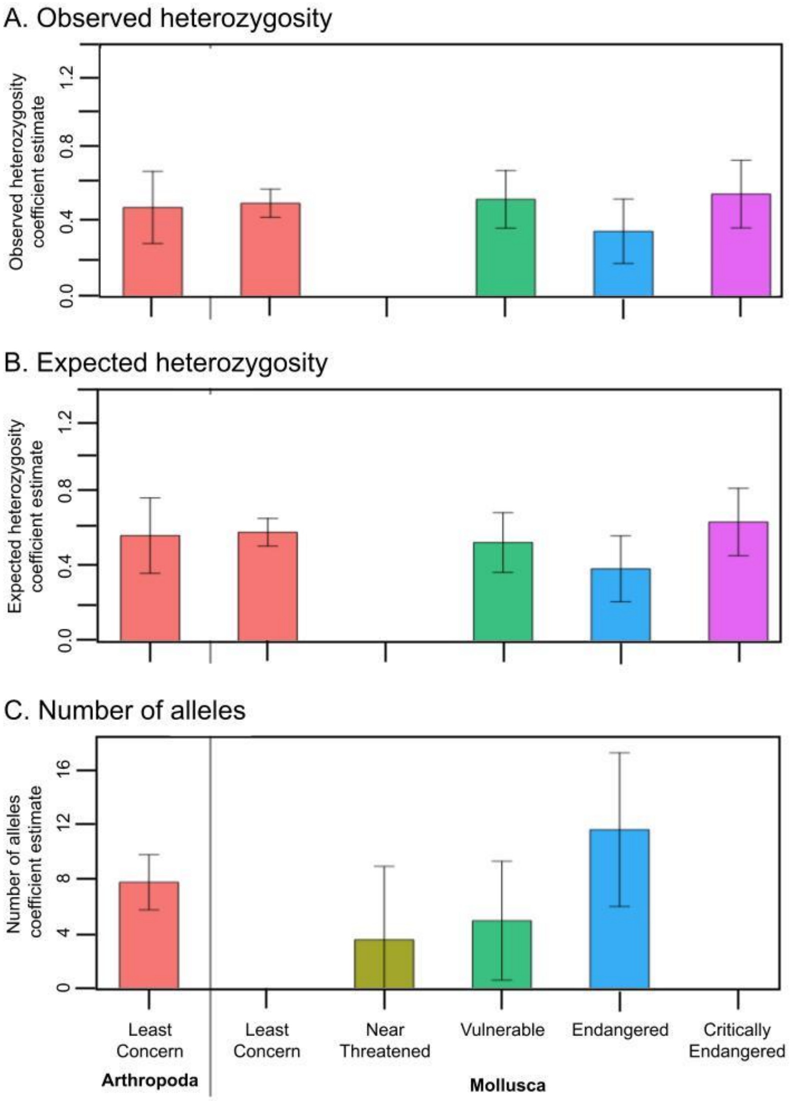

Figure 3. When including taxonomic information, there is no significant difference in the nuclear genetic diversity across different extinction risk categories. We specifically compared observed heterozygosity (A), expected heterozygosity (B), and the number of alleles (C) in Arthropods and Mollusks.

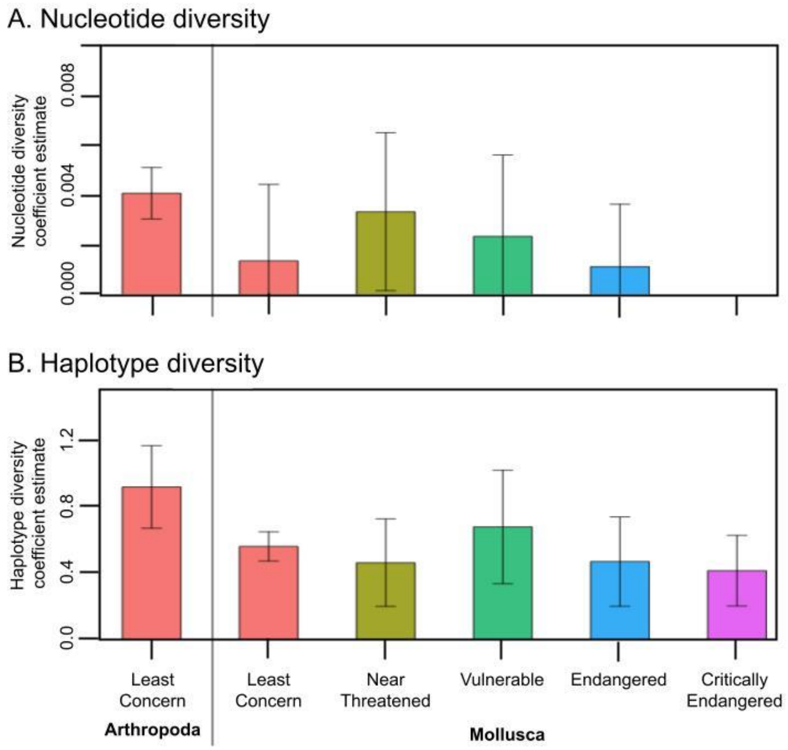

Figure 4. When including taxonomic information, there is no significant difference in the mitochondrial genetic diversity across different extinction risk categories. We specifically compared nucleotide diversity (A) and haplotype diversity (B) in arthropods and mollusks.

When considering genetic diversity in the nuclear genome, we found that Arthropods always had significantly smaller variation in measures of heterozygosity (observed and expected) and number of alleles compared to Mollusks (Figure 5, Table 7). However, this was not the case for mitochondrial diversity; we found that Arthropods had significantly more variation in measures of nucleotide diversity and both taxa had similar measures of variation in haplotype diversity (Figure 5, Table 7). This suggests that nuclear estimates of genetic diversity made for some populations of Arthropods may be more accurate indicators of genetic diversity in other populations than similar inferences made for mollusks. This is particularly relevant to considerations for using genetic diversity when considering extinction risks in these species.

Table 7. Comparison of the variability of Mollusca and Arthropoda populations, within species. The coefficient of variation for each genetic diversity estimate calculated across same species populations was compared between Mollusca and Arthropoda using the 95% confidence interval is defined by the Lower and Upper limit columns.

	Genetic metric	Mean	Lower limit	Upper limit
Mollusca	Observed heterozygosity	0.294	0.239	0.349
	Expected heterozygosity	0.289	0.2304	0.348
	Number of alleles	0.245	0.212	0.278
	Nucleotide diversity	0.723	0.673	0.772
	Haplotype diversity	0.408	0.365	0.452
Arthropoda	Observed heterozygosity	0.154	0.104	0.204
	Expected heterozygosity	0.113	0.0671	0.159
	Number of alleles	0.106	0.0894	0.123
	Nucleotide diversity	1.173	0.903	1.443
	Haplotype diversity	0.282	0.182	0.382

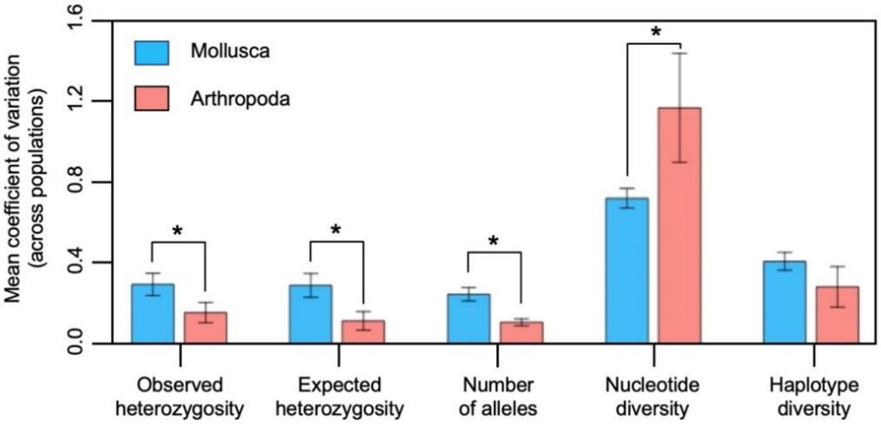

Figure 5. Variation of genetic diversity within Mollusca and Arthropoda populations, within species. Coefficient of variations are calculated from means of nuclear and mitochondrial diversity and bars are the standard deviations of the estimates. In the nuclear genome (estimated via heterozygosity and number of alleles), Mollusca species are more variable between populations compared to Arthropoda populations. However, this was not true for the mitochondrial genome, where nucleotide diversity was more variable between Arthropoda populations and haplotype diversity was equal between these groups.

Discussion

In this study, we combined assessments of extinction risk, range size estimates, and genetic diversity data to broadly understand the various threats to aquatic invertebrate population persistence. Our findings supported the initial hypothesis that species classified as critically endangered exhibited considerably smaller range sizes compared to all other categories defined by the IUCN. Contrary to our hypothesis, our results revealed that species with stable population trends exhibited larger range sizes compared to those with increased population trends. Our results did not support the hypothesis that species with higher extinction risk exhibit lower genetic diversity, despite our examination of available genetic diversity measures across a large number of species. However, we did find that the variation in genetic diversity among Mollusca populations (within species) was greater than the variation among Arthropoda populations.

Range size assessments

We assessed the differences in range size for species in the different IUCN extinction risk categories (Figure 1, Table 4). Because determining extinction risk in insects can be limited by data availability, many species specialist groups rely heavily on range size to make these determinations (Cardoso et al. 2011). Accordingly, we expected to find a steadily decreasing range size as extinction risk increased. We found support for the first hypothesis that critically endangered species had significantly smaller range sizes than any other IUCN category, which supports the idea that small ranges and high regional endemism make species with poor dispersal ability especially vulnerable to extinction risk. For instance, when lineages with limited dispersal have greater speciation rates and these give rise to species with naturally smaller geographical ranges, dispersal may affect range sizes and gene flow between populations and consequently speciation rates (Lester et al., 2007), potentially decreasing species' abilities to adapt to new environmental conditions.

Small range sizes may also lead to small population sizes that increase the strength of genetic drift and decrease heterozygosity through inbreeding, leading to increased extinction risk (Vandewoestijne et al. 2008). Generally, low genetic diversity increases the risk of extinction due to the accumulation of genetic load (DeWoody et al. 2021), increasing the probability of extinction by decreases in reproduction and survival (Frankham 1995, Bijlsma et al. 1999). This greater local extinction risk from smaller-ranged species might contribute to shifts in species composition and the scale-dependent patterns of genetic diversity change that other studies report with long-term data (Boakes et al., 2018). This emphasizes the significance of targeted conservation efforts for these species and the ecosystems they occupy because ecosystem services that influence health and well-being of human populations can also be affected by changes in species composition.

Large-ranged species may also have a greater chance of colonizing effectively and systematically displacing small-ranged species if species with narrow ranges are preferentially declining (Pelletier et al. 2017). For example, the distribution and abundance of mayfly species (*Ephemeroptera*) are impacted by the size of their ranges; mayfly species with small range sizes are more constrained in their distribution and abundance compared to those with larger range sizes that are more numerous and extensive throughout a single stream network (Boyero et al 2012). This pattern illustrates how aquatic invertebrate species with larger range sizes may be better able to colonize and persist in degraded areas, which may result in the systematic displacement of species with smaller range sizes that are more vulnerable to habitat degradation.

For mollusks and arthropods specifically, this supports considering range size as an important predictor of extinction risk.

We found that species in the least concern category had significantly larger range sizes than species in the other IUCN categories, but that species in the near threatened, vulnerable, and endangered groups had similar range sizes (Figure 1). Finding overlapping ranges was unexpected because range size is relied upon heavily when making extinction risk determinations in insects (Cardoso et al. 2011). Despite this reliance, variables other than range size that affect the target species are also important to understanding threat status. For example, for unionid mussels that rely on host species for dispersal, movement restrictions on host fishes such as those imposed by dams can alter their distributions and abundances (Harding Mann 2000; Schwalb et al. 2011). This pattern can be seen in the mandatory and host-specific parasitic stage of *Margartifera margaritifera* (Salonen and Taskinen 2017) that live in the gills of Atlantic salmon (*Salmo salar*) or brown trout (*S. trutta*) (Salonen et al. 2017). This reliance means threats to these hosts will also have negative effects on the symbiotic marine invertebrates that depend on these hosts (Stampar et al. 2010;Wood et al. 2012). However, it is not clear how current IUCN risk assessment categories can incorporate these peculiarities into the extinction risk categories systematically.

In contrast to populations of mussels that only use salmon as hosts, Geist et al. (2018) discovered that populations of mussels that solely use trout as hosts had extremely high levels of genetic divergence, suggesting that host species can influence and alter genetic diversity patterns. Studies of adaptations by local freshwater mussels to their fish hosts by Douda et al. (2017) highlighted the significance of geographical scale and the potential for host counter-adaptation.

Therefore, the effectiveness of conservation activities using mussels may be significantly impacted by local adaptation and population-level changes in freshwater mussel host compatibility with regard to fish host populations. To improve the success of freshwater mussel conservation, these characteristics should be further studied in order to account for any association-specific changes.

Direct genetic diversity assessments must be used in conjunction with well-validated proxies or indicators of genetic variability in order to determine a species' susceptibility to different factors that are affecting genetic diversity (Hanson et al. 2017; Hoban et al. 2020). We assessed the predictability of range size using the three population trend categories used by IUCN (increasing, decreasing, or stable population size; Figure 2, Table 5). Trends in species' populations, whether increasing, stable, or declining, are a key factor in assessing extinction risk. We found no significant differences in range size between Arthropoda categorized into the different population trend groups. However, our sample sizes for this analysis were somewhat limited and so these results may require additional investigation.

Genetic diversity assessments

We evaluated genetic diversity of Mollusca species as potential indicators of IUCN extinction using a phylogenetic approach because these methods can reveal the patterns and processes of speciation as well as the critical natural-history related predictors of data trends (Martinez et al. 2018). For example, the mode of reproduction in mollusks means that mitochondrial information can be passed from female and male parents, influencing patterns of mitochondrial diversity in these species. Another factor that can influence genetic diversity in these groups is their history of geographic and ecological isolation over evolutionary time scales (Avice 2000). In the case of the marine snail genus *Nerita*, current patterns of genetic variation were influenced by historical barriers, resulting in extreme divergence within the group even though similar levels of genetic diversity might be expected (Klunzinger et al. 2020). Importantly, these historic events can interact with contemporary events, such as habitat fragmentation, leading to newer patterns of genetic diversity loss through genetic drift (Vanhove et al. 2020). Interpreting these patterns responsibly requires phylogenetic inferences for understanding how evolutionary processes and contemporary influences have resulted in the patterns of genetic diversity observed, which is important because understanding evolution is critical for understanding ecology.

While we considered genetic diversity measures that were available in a large number of species we found no relationships between these measures and extinction risk. We predicted that species with increased extinction risk would have lesser genetic diversity due to bottleneck effects, where rapid decreases in population size results in loss of genetic variants due to sampling errors. However, these effects can be absent in some cases when species have very large initial population size or long generation times because these natural history characteristics will buffer against the loss of alleles (Frankham et al. 2014). Accordingly, some threatened and endangered species will not necessarily have less genetic diversity than non-threatened species due to their evolutionary histories (Frankham 2010). Species with large historical population sizes are more likely to have higher genetic diversity, even if they have recent declines in populations, because the large ancestral population has contributed to a diverse gene pool (Allendorf et al., 2012). The findings of our study indicate that low genetic diversity should not be automatically assumed as the primary constraint for mollusk species recovery. Species that exhibit genetic diversity levels outside the typical range observed should be carefully considered, taking into account additional factors and considerations (see below). Furthermore, identifying genetic factors that accurately predict extinction in some species, or that could serve as early warnings signs of extinction risk, would be ideal for proactively mitigating the effects of stressors on various species.

Although our data were largely focused on assessments of a few to tens of loci per population, increases in the use of genome-wide data may support conservation endeavors in the future (DeWoody et al. 2022). We surveyed results primarily generated from microsatellite markers, as these types of data represent most of the population genetic datasets. While higher density, whole-genome data are becoming more popular in conservation genetics studies, there are not yet enough data points to test broad conclusions for those data types. However, we know that whole-genome data from a population can give us insights into recent demographic changes in that population, and for species or populations of concern, these higher-density types of data may be better suited for determining extinction risk than microsatellite or other lower-density data. For example, genome-wide measures of genetic diversity may be more sensitive to population declines, and variation in coding regions may provide predictive insights, particularly when considering the effects of selection due to habitat changes (Gomulkiewicz and Holt 1997). This is because multi-marker tests of association can be used to maximize power to understand species associations with different demographic and environmental factors that are reflective of their populations at the gene or regional level (Wang et al. 2015). Because genome-wide studies might provide us with different perspective of population history, targeting these kinds of data in the future across a diversity of taxa will allow us to assess the viability of these data for this kind of extinction risk assessment.

Genetic metrics that provide insight into genetic diversity changes over time, such as genetic erosion, may provide utility in assessing conservation risk (Schmidt et al. 2023). For example, in general, identifying long runs of homozygosity in genome data suggests that there has been widespread inbreeding in recent generations and, when these long runs of homozygosity are common, indicates that widespread genetic diversity losses are occurring and additional conservation protections may be warranted (Brüniche-Olsen et al. 2018). Combining these genetic insights with other known data may also be important. For example, populations with large estimates of genetic diversity (or a large effective population size, Garner et al. 2020), but small populations sizes, are likely to be highly affected by genetic drift, resulting in lost genetic variants over short time periods (Peart et al. 2020). Since low genetic diversity or high rates of genetic diversity loss can be mitigated by increasing population size and gene flow, relating genetic diversity estimates and demographic information to extinction risk provides an opportunity to prevent extinction (Vitorino et al. 2019). By relating genetic diversity estimates and demographic information to extinction risk, we can identify species that are at risk of extinction and develop strategies to prevent genetic diversity loss and increase population size (Frankham 2010). This outcome could be accomplished by increasing connectivity, because fragmented populations tend to lose more genetic variants (Ralls et al. 2020).

One alternative to considering whole genome diversity is focusing on variation at specific loci that affect fitness when assessing extinction risk and management of vulnerable populations. This method is based on the idea that these loci are vital for defining an organism's ability to survive and reproduce in a changing environment. For instance, a study on the freshwater mussel *Elliptio complanata* found that variation in genes associated to heat tolerance greatly influenced both individual fitness and population persistence (Fritts et al. 2018). Concentrating on important loci can also help with the creation of targeted management plans for populations of aquatic invertebrate species that are at risk. However, breeding with respect to a single genetic variant or phenotype will result in loss of genetic diversity elsewhere in the genome, meaning that this strategy can have unintended effects (Willoughby et al. 2017). Hence, to manage and protect vulnerable aquatic invertebrate populations, a thorough strategy that takes into account both specific loci and whole genome diversity is advised, and locus-specific approaches should be used sparingly.

Species-specific trends and recommendations

The relationship between species' genetic diversity and extinction risk can be masked by variation in response to local and contemporaneous environmental changes. Species with historically low abundance or small ranges may have low genetic diversity compared to larger populations, but this difference will not substantially affect population fitness or stability (Charlesworth et al. 2010). For example, some populations of *Nodularia douglasiae*, a species of least concern, have low mitochondrial diversity compared to other least concern species in our dataset (Table 4). While not the only explanation of this trend, lower than expected genetic diversity suggests that this species may be subject to pressures that are limiting the population. This species is found in a relatively large range that includes parts of China, Japan, Korea, Mongolia, Russia, Taiwan, and Vietnam, suggesting that habitat destruction or fragmentation may be limiting factors because overall range size is large. For many aquatic species, habitat loss and pollution can lead to population isolation, increasing the rate that alleles are lost via genetic drift and inbreeding, and reducing long-term population viability (Vandewoestijne et al. 2008). Therefore, considering species data across taxa can aid identification of species on the brink, even when they are not identified by current IUCN methodology. Although we have not assessed the natural history and background of each species, here we identify species listed as least concern but that have genetic diversity estimates in the lower 5% of all population estimates in our database (Table 8). We suggest that these species may warrant additional investigations to understand these contradicting trends.

Table 8. Species identified with the lowest genetic diversity compared to other species in our database despite being at low risk of extinction. These species are listed as Least Concern for extinction by the IUCN but that fell in the lowest 5% of all population-level estimates of genetic diversity. We suggest that these species may warrant further investigation and, potentially, increased conservation concern and protections.

Genetic diversity metric	5% cutoff value	Species
Nuclear genome		
Observed heterozygosity	0.044	*Sepia officinalis*
Expected heterozygosity	0.1052	*Pseudosuccinea columella, Sepia officinalis*
Number of alleles	1.545	*Sepia officinalis*
Mitochondrial genome		
Nucleotide diversity	0.0005925	*Anodonta anatine, Amphioctopus fangsiao, Amphioctopus neglectus, Cistopus indicus, Galba truncatula, Haliotis asinine, Nodularia douglasiae, Radix auricularia,*
Haplotype diversity	0.2444	*Amphioctopus fangsiao, Amphioctopus neglectus, Anodonta anatina, Cistopus indicus, Galba truncatula, Haliotis asinina, Nodularia douglasiae, Radix auricularia*

Generally, populations with a restricted distribution or populations that are small and isolated tend to have low levels of genetic diversity due to the effects of inbreeding and drift (Frankham 2005). However, we identified some species with higher-than-expected genetic diversity. For example, the endangered species *Unio foucauldianus* has shown high variation in nuclear genetic diversity when compared to other species. This high genetic diversity is likely due to a combination of factors, such as historical population fragmentation, poor dispersion capacity, and the existence of several refugia throughout climatic variations (Lopes-Lima et al. 2017).

Generally speaking, genetic diversity is important because it can enhance a species' capacity for adaptation, enabling it to respond to environmental challenges more effectively and increase persistence probability. Moreover, we suggest that *Unio foucauldianus* and other species that have larger than expected genetic diversity might be used as a model species to comprehend the genetic processes driving species resilience. Research on this species and others with higher than expected genetic diversity (Table 9) could provide insight into how genetic diversity is maintained in isolated and fragmented populations.

Table 9. Species identified with the highest genetic diversity compared to other species in our database despite being at high risk of extinction. Species are listed as Critically Endangered for extinction by the IUCN but that fell in the highest 5% of all population-level estimates of genetic diversity. We suggest that these species may provide insight into species characteristics that result in retention of genetic diversity in wild populations despite demographic indicators of extinction risk.

Genetic diversity metric	5% cutoff value	Species
Nuclear genome		
Observed heterozygosity	0.833	*Bulimulus reibischi, Pinna nobilis, Posidonia oceanica, Unio foucauldianus*
Expected heterozygosity	0.898	*Bulimulus reibischi, Pinna nobilis, Posidonia oceanica, Unio foucauldianus*
Number of alleles	18.265	*Bulimulus reibischi, Pinna nobilis*
Mitochondrial genome		
Nucleotide diversity	0.106	*Posidonia oceanica, Unio foucauldianus*
Haplotype diversity	0.999	*Posidonia oceanica, Unio foucauldianus*

Future use of genetic diversity in Mollusca and Arthropoda assessments

We advocate for continued assessment of the use of genomic data in understanding extinction risk across different taxonomic groups, and our data suggest that there are important taxon-level differences to consider when doing so. We compared, within species, the variation in genetic diversity among populations and found that Mollusca populations tend to be more variable than

Arthropoda populations (Figure 5; Table 7). This difference means that population-level assessments of nuclear genome diversity patterns in Mollusca may be less likely to be representative of species-level genome patterns compared to population-level assessments in Arthropoda. As habitats change, we predict that these differences between populations in a species will increase due to differential ability for populations to adapt to these new conditions and increased isolation of some populations (Mori and Takamura 2000). These variations in genetic diversity could have been due to factors like habitat fragmentation and host dependency. For example, the endangered freshwater pearl mussel (*Margaritifera margaritifera*) requires brown trout or Atlantic salmon as a host to complete its life cycle, and trout-mussel populations have significantly more genetic diversity than the salmon-mussel populations (Karlsson et al. 2014). This suggests that population-specific factors are important considerations when interpreting genetic diversity in these species. Taken together, this means that collecting estimates of genome diversity in multiple populations may be warranted when using genetic data to predict extinction risks for an entire species.

Although microsatellite and mitochondrial sequences do not seem very well suited for assessing extinction risk in mollusks and arthropods, understanding recent population size change through genetic diversity is appealing for some species due to its relative ease and increased information content compared to other approaches. In particular, Hoban et al. (2020) suggest using estimates of effective population size, which reflects the rate at which genetic diversity is expected to be lost (Hare et al. 2011), when developing conservation action plans. This approach suggests comparing the number of populations within a species that have effective population sizes above 500 to those who have effective population sizes below 500 to gauge species-wide concerns relative to population-specific concerns. There are several ways that effective population size can be estimated, but new and efficient methods involve analyzing whole-genome sequencing data for populations. While this effort is likely to be costly compared to microsatellite analyses, sequencing costs are constantly declining and the ability to assess these populations on the brink of extinction would certainly, in our view, warrant this level of financial investment.

Conclusions

We found that least concern species have significantly larger range size which suggests that these species are more widespread, and this may provide important ecological buffers to these species. We also found that critically endangered species have significantly smaller range sizes compared to other IUCN categories, which supports additional conservation protections since these species may be more vulnerable to stochastic environmental events. Range size did not relate to genetic diversity and genetic diversity was similar across all IUCN rankings. Combined, this suggests that even small-ranged species may have the necessary genetic variants to support on-going adaptation recovery of these species. However, we found that population-wide genetic diversity compared among mollusk populations (within species) is significantly larger than the same measure in arthropods. Making species-level decisions from data originating from a single population may be viable in some aquatic invertebrates but inadvisable in others. Rapid habitat change will present species with new hurdles as anthropogenic impacts continue to occur. While we focused on putatively neutral genetic markers here, genome-wide genetic diversity measures may provide greater insight into the survival of populations confronting environmental change.

References

Albrecht, A., & Reise, K. (1994). Effects of Fucus esiculosus covering intertidal mussel beds in the Wadden Sea. Helgolander Meeresunters, 48, 243-256.

Allendorf, F. W., Luikart, G. H., & Aitken, S. N. (2022). Conservation and the genetics of populations. John Wiley & Sons.

Bangert, R. K., Turek, R. J., Martinsen, G. D., Wimp, G. M., Bailey, J. K., & Whitam, T. G. (2004). Benefits of Conservation of Plant Genetic Diversity to Arthropod Diversity. Conservation Biology, 19, 379-390.

Boakes, E. H., Isaac, N. J. B., Fuller, R. A., Mace, G. M., & McGowan, P. J. K. (2018). Examining the relationship between local extinction risk and position in range. Conservation Biology, 32, 229-239.

Boyero, L., Pearson, R. G., Dudgeon, D., Grava, M. A. S., Gessner, M. O., Albari, R. J., & Yule, C. M. (2012). Global patterns of stream detritivore distribution: implications for biodiversity loss in changing climates. Global Ecology and Biogeography, 21, 134-141.

Brüniche-Olsen, A., Kellner, K. F., Anderson, C. J., & DeWoody, J. A. (2018). Runs of homozygosity have utility in mammalian conservation and evolutionary studies. Conservation Genetics, 19, 1295-1307.

Buschbaum, C., Saier, B. (2001). Growth of the mussel Mytilus edulis L. in the Wadden Sea affected by tidal emergence and barnacle epibionts. Journal of Sea Research., 45, 27-36.

Charlesworth, B., & Charlesworth, D. (2010). Elements of evolutionary genetics. Roberts & Company Publishers, Greenwood Village, Colorado, USA.

Collen, B., Whitton, E. E., Dyer, J. E. M., Baillie, N. C., Cumberlidge, W. R. T., Darwall, T., & Böhm M. (2014). Global patterns of freshwater species diversity, threat and endemism. Global Ecology and Biogeography, 23, 40-51.

Collier, K. J., Probert, K. P., & Michael, J. (2016). Conservation of aquatic invertebrates: concerns, challenges, and conundrums. Marine and Freshwater Ecosystems, 26, 817–837.

Coltman DW, Slate J. (2003). Microsatellite measures of inbreeding: a meta-analysis. Evolution, 57, 971-983.

Commito, J. A., Celano, E. A., Celico, H. J., Como, S., & Johnson, C. P. (2005). Mussels matter: postlarval dispersal dynamics altered by a spatially complex ecosystem engineer. Journal of Experimental Marine Biology and Ecology, 316, 133-147.

Cornman, R. S., Robertson, L. S., Galbraith, H., Blakeslee, C. (2014). Transcriptomic analysis of the mussel *Elliptio complanate* Identifies candidate stress-response genes and an abundance of novel or noncoding transcripts. PLoSOne. 9, e112420.

Dehghani R., Zarghi I., Aboutalebi M., Barzegari Z., Ghanbari M., (2015). Fauna and habitat of aquatic arthropods of Kashan in 2010. Bangladesh Journal of medical science. 13, 306-310.

DeWoody, J. A., Harder, A. M., Mathur, S., & Willoughby, J. R. (2021). The long-standing significance of genetic diversity in conservation. Molecular Ecology, 30, 4147–4154.

DeWoody, J. Jeon, J.Y. Bickham, J.W. Willoughby, J.R. (2022). The Threatened Species Imperative: Conservation assessments would benefit from population genomic insights. Proceedings of the National Academy of Sciences. U.S.A. 119, e2210685119.

Doyle, M. J., Hacking, C. C., Willoughby, J. R., Sundaram, M., DeWoody, J. A. (2015). Mammalian genetic diversity as a function of habitat, body size, trophic class, and conservation status. Journal of Mammalogy, 96, 564–572.

Douda, K., Janssen, J., Weiperth, A. (2017). Geographical size and the potential for host counter-adaptation affect local adaptation in a freshwater mussel. Journal of Evolutionary Biology, 30, 1241-1252.

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: a review. Genetical Research, 66, 95-107.

Frankham, R. (2005). Genetics and extinction. Biological Conservation, 126, 131-140.

Frankham, R. (2010). Challenges and opportunities of genetic approaches to biological conservation. Biological Conservation, 143, 1919-1927.

Frankham, R., Bradshaw, C. J. A., & Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation, 170, 56-63.

Garner, B.A., Hoban, S. Luikart, G. (2020) IUCN Red List and the value of integrating genetics. Conservation genetics 21, 795–801.

Geist, J., Kuehn, R., Haase, P. (2018). Local adaptation in a host–parasite system: The role of host traits, parasite diversity, and environmental characteristics. Ecology and Evolution, 8, 312.

García Dorado A., Caballero A., (2021). Neutral genetic diversity as a useful tool for conservation biology. Conservation Genetics,21, 541-545 .

Gomulkiewicz R., Holt R. (1997). How does immigration influence local adaptation? A reexamination of a familiar paradigm. The American Naturalist, 149, 563-572.

González. A. L., Régis. C., Olivier. D., Vinicius. F. F., Céline. L., Barbara. A. R., Michael. J. R., Gustavo.Q. R., Diane, S. S. (2018). Ecological mechanisms and phylogeny shape invertebrate stoichiometry: A test using detritus-based communities across Central and South America. Functional Ecology, 32, 2448–2463.

Guay, J.A., Himmelman, J.H. (2004). Reproduction and recruitment of the green sea urchin (*Strongylocentrotus droebachiensis*) in a cold coastal ecosystem. Marine Biology, 145, 277-288.

Hanson, J. O., Rhodes, J. R., Riginos, C., Fuller, R. A. (2017). Environmental and geographic variables are effective surrogates for genetic variation in conservation planning. Proceedings of the National Academy of Sciences. 114(48): 201711009.

Harding J. M., Mann R. (2000). Estimates of naked goby (*Gobiosoma bosc*), striped blenny (*Chasmodes bosquianus*) and eastern oyster (*Crassostrea virginica*) larval production around a restored Chesapeake bay oyster reef. Bulletin of Marine Science, 66, 29–45.

Hoban, S., Bruford, M., Durban Jackson, J., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P. A., Paz-Vinas, I., Sjögren-Gulve, P., Segelbacher, G., Vernesi, C., Aitken, S., Bertola, L. D., Bloomer, P., Breed, M., Rodríguez-Correa, H., Funk, W. C., Grueber, C. E., Hunter, M. E., Jaffe, R., Liggins, L., Mergeay, J., Moharrek, F., O'Brien, D., Ogden, R., Palma-Silva, C., Pierson, J., Ramakrishnan, U., Simo-Droissart, M., Tani, N., Waits, L., Laikre, L. (2020). Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. Biological Conservation. 248: 108654.

Hughes, A. R, Stachowicz, J. J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proceedings of the National Academy of Sciences.101, 8998-9002.

Hill C., (1991). Is the activity of benthic suspension feeders a factor controlling water quality in the Bay of Brest? Marine Ecology. 69, 179–180.

Hubbard W.A., (2016). Benthic studies in upper Buzzards Bay, Massachusetts: 2011-12 as compared to 1955. Marine Ecology. 37, 532-542.

IUCN (2021). The IUCN Red List of Threatened Species. Version 2021-1. https://www.iucnredlist.org. Downloaded on 04/06/2021.

Jones, Clive & Lawton, John & Schachak, Moshe. (1994). Jones CG, Lawton JH, Shachak M.. Organisms as ecosystem engineers. Oikos 69: 373-386. Oikos. 69. 373-386. 10.2307/3545850.

Kardos, M.; Armstrong, E.E.; Fitzpatrick, S.W.; Hauser, S.; Hedrick, P.W.; Miller, J.M.; Tallmon, D.A.; Funk, W.C. The crucial role of genome-wide genetic variation in conservation. Proceedings of the National Academy of Sciences. USA 2021, 118, e2104642118.

Karlsson, S., Larsen, B. M., Hindar, K. (2014). Host-dependent genetic variation in freshwater pearl mussel (*Margaritifera margaritifera*). Hydrobiologia 735, 179–190.

Klunzinger, M. W., Holman, L. E., Stow, A. J., (2020). Phylogeographic structure and historical demography of the intertidal snail *Nerita atramentosa* (Gastropoda: Neritidae) in southern Australia. Marine Biology. 167, 163.

Kraeuter, J. N., Kennish, M. J., Dobarro, J., Fegley, S. R., Flimlin, G. E., (2003). Rehabilitation of the northern quahog (hard clam) (*Mercenaria mercnaria*) habitats by shelling – 11 years in Barnegat Bay, New Jersey. Journal of Shellfish Research. 22, 61-67.

Lopes-Lima, M.; Teixeira, A.; Froufe, E.; Lopes, A.; Varandas, S.; Sousa, R. Biology and conservation of freshwater bivalves: Past, present and future perspectives. Hydrobiologia 2014, 735, 1–13.

Maehr, D. S., Crowley, P. , Cox, J. J., Larkin, J. L., Hoctor, T. S., Harris, L. D. (2006). Of cats and haruspices: genetic intervention in the Florida panther. Animal Conservation. 9, 127-132.

Martinez, A.S., Willoughby, J.R., Christie, M.R. (2018). Genetic diversity in fishes is influenced by habitat type and life-history variation. Ecology and Evolution. 8, 12022–12031.

McGeoch, M. A (1998). The selection, testing and application of terrestrial insects as bioindicators. Biological Review. 73:181-201.

Meffe, G. K., (1996). Conserving genetic diversity in natural systems. *In* R. C. Szaro, D. W. Johnston, editors. Biodiversity in managed landscapes. Oxford University Press, Oxford, United Kingdom. Pp. 41–76.

Mori, S., Takamura, K. (2000). Present status and future conservation of three-spined sticklebacks in Japan. *In*: Mori S (ed) Logics and Practices in Environmental Conservation. Tokyo, Sinzansha Saitekku, Tokyo. Pp. 22–44.

Paradis, E., Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics. 35, 526-528.

Peart, C. R., Tusso, S., Pophaly, S. D. (2020). Determinants of genetic variation across eco-evolutionary scales in pinnipeds. Nature Ecology and Evolution. 4, 1095–1104

Pedro, C., Paulo, A. V., Borges, K. A., Triantis, M., A., Ferrández, J. L., Martín. (2011). Adapting the IUCN Red List criteria for invertebrates. Biological Conservation, 114.

Pelletier, T. A., Carstens, B. C. (2017). Data from: Geographic range size and latitude predict population genetic structure in a global survey. Dryad Digital Repository.

Possingham, H.., Andelman, S. J. (1999). Conservation Biology for the Biodiversity Crisis: A Freshwater Example. Biological Conservation. 93, 247-255.

Prior, K. A., Gibbs, H. L., Weatherhead, P. J. (1997). Population genetic structure in the black rat snake: implications for management. Conservation Biology 11, 1147–1158.

Ralls, K. , Sunnucks, P., Lacy, R.C., Frankham, R. (2020). Genetic rescue: a critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation. Biological Conservation, 251.

Reed, D.H., Frankham R. (2003). Correlation between fitness and genetic diversity. Conservation Biology. 17, 230–237.

Repetto, M., Griffen, B. D. (2012). Physiological consequences of parasite infection in the burrowing mud shrimp, *Upogebia pugettensis*, a widespread ecosystem engineer. Marine and Freshwater Research. 63: 60–67.

Ruppert, E. E., Fox, R. S., Barnes, R. D. (2004). Invertebrate Zoology: A Functional Evolutionary Approach. 7th edition. Brooks/Cole Thomson Learning. Pp. 523-555.

Sackett, L. C., Cross, T. B., Jones, R. T., Johnson, W. C., Ballare, K., Ray, C., Collinge, S. K., Martin, A. P. (2012). Connectivity of prairie dog colonies in an altered landscape: inferences from analysis of microsatellite DNA variation. Conservation Genetics. 13, 407–418.

Salonen, J. K., Luhta, P.-L., Moilanen, E., Oulasvirta, P., Turunen, J., Taskinen, J. (2017). Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) differ in their suitability as a host for the endangered freshwater pearl mussel (*Margaritifera margaritifera*) in northern Fennoscandian rivers. Freshwater Biology. 62, 1346–1358.

Salonen, J. K., Taskinen, J. (2017). Electrofishing as a new method to search for unknown populations of the endangered freshwater pearl mussel *Margaritifera margaritifera*. Aquatic Conservation: Marine and Freshwater Ecosystems. 27, 115-127

Schwalb, A. Cottenie, K. Poos, M. Ackerman, Josef. (2011). Dispersal limitation of unionid mussels and implications for their recovery. Freshwater Biology. 56. 1509 - 1518.

Schmidt C, Hoban S, Hunter M, Paz-Vinas I, Garroway CJ. Genetic diversity and IUCN Red List status. Conservation Biology. 2023, e14064.

Stampar, S. N., Emig, C. C., Morandini, A. C., Kodja, G., Balboni, A. P., Da Silveira, F. L. (2010). Is there any risk in a symbiotic species associating with an endangered one? A case of a phoronid worm growing on a Ceriantheomorphetube. Cahiers de Biologie Marine. 51, 205–211.

Vandewoestijne, S., Schtickzelle, N., Baguette, M. (2008). Positive correlation between genetic diversity and fitness in a large, well-connected metapopulation. BMC Biology. 6, 46

Moodley Y, Russo IM, Robovský J, Dalton DL, Kotzé A, Smith S, Stejskal J, Ryder OA, Hermes R, Walzer C, Bruford MW. Contrasting evolutionary history, anthropogenic declines and genetic contact in the northern and southern white rhinoceros (Ceratotherium simum). Proceedings Biological Science. 2018 ;285(1890):20181567.

Wang, X., Biernacka, J. M. (2015). Assessing the effects of multiple markers in genetic association studies. Frontier Genetics. 24, 6-66.

Wiens, J. J. (2015). Explaining patterns of vertebrate diversity. Biology Letters. 11, 505-506.

Willoughby, J. R., Sundaram, M., Wijayawardena, B .M., Kimble, S. J. A., Ji, Y., Fernandez, N. B., Antonides, J. D., Lamb, M. C., Marra, N. J., DeWoody, J .A. (2015). The reduction of genetic diversity in threatened vertebrates and new recommendations regarding IUCN conservation rankings. Biological Conservation. 191, 495- 503.

Willoughby J. R., Ivy, J. A., Lacy, R. C., Doyle, J. M., DeWoody, J. A. (2017). Inbreeding and selection shape genomic diversity in captive populations: implications for the conservation of endangered species. PLoS One. 12, e0175996.

Willoughby, J. R., Sundaram, M., Wijayawardena, B .M., Kimble, S. J. A., Ji, Y., Fernandez, N. B., Antonides, J. D., Lamb, M. C., Marra, N. J., DeWoody, J .A. (2017). Biome and migratory behavior significantly influence vertebrate genetic diversity. Biological Journal of the Linnean Society. 121, 446–457

Wood, A. C.,L., Probert, P. K., Rowden, A. A., Smith, A. M. (2012). Complex habitat generated by marine bryozoans: a review of its distribution, structure, diversity, threats and conservation. Aquatic Conservation: Marine and Freshwater Ecosystems. 22, 547–56.