Exploring reported population differences in Norway lobster (*Nephrops norvegicus*) in the Pomo Pits region of the Adriatic Sea using genome-wide markers

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The Norway lobster (*Nephrops norvegicus*) is one of the most important decapod crustacean seafood species in the Adriatic Sea. Previous research has identified significant differences in growth rates and maturation timing of *Nephrops* in the Pomo/Jabuka Pits area compared to other subpopulations in Adriatic fishing grounds. Here, we use 1,623 genome-wide single nucleotide polymorphisms (SNPs) to investigate whether the Pomo Pits subpopulation is genetically different from other sites in the Adriatic and neighbouring seas. We found no genetic differentiation among all Adriatic sites, suggesting high gene flow between Pomo Pits *Nephrops* and those of surrounding areas. We also found genetic homogeneity between the Adriatic sites and single-site samples from the Aegean and Tyrrhenian Seas. However, we detected distinct genetic differentiation between all Mediterranean sites and an Atlantic site in western Scotland, which provides evidence for a phylogenetic break between the Atlantic and the Mediterranean. Our results indicate that Pomo Pits *Nephrops* are not genetically different from others sampled in the Adriatic and that key biological parameters in Pomo Pits *Nephrops* could be driven by spatial variation in fishing pressure and/or environmental factors rather than geographic isolation.

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Abstract

The Norway lobster (*Nephrops norvegicus*) is one of the most important decapod crustacean seafood species in the Adriatic Sea. Previous research has identified significant differences in growth rates and maturation timing of *Nephrops* in the Pomo/Jabuka Pits area compared to other subpopulations in Adriatic fishing grounds. Here, we use 1,623 genome-wide single nucleotide polymorphisms (SNPs) to investigate whether the Pomo Pits subpopulation is genetically different from other sites in the Adriatic and neighbouring seas. We found no genetic differentiation among all Adriatic sites, suggesting high gene flow between Pomo Pits *Nephrops* and those of surrounding areas. We also found genetic homogeneity between the Adriatic sites and single-site samples from the Aegean and Tyrrhenian Seas. However, we detected distinct genetic differentiation between all Mediterranean sites and an Atlantic site in western Scotland, which provides evidence for a phylogenetic break between the Atlantic and the Mediterranean. Our results indicate that Pomo Pits *Nephrops* are not genetically different from others sampled in the Adriatic and that key biological parameters in Pomo Pits *Nephrops* could be driven by spatial variation in fishing pressure and/or environmental factors rather than geographic isolation.

Introduction

The Norway lobster (*Nephrops norvegicus*), hereafter *Nephrops*, is a benthic decapod crustacean found across the Mediterranean and the north-east Atlantic on the continental shelf and slope up to 800 m depth (Ungfors et al. 2013, Aguzzi et al. 2022). *Nephrops* construct and inhabit burrow systems used for shelter, usually on muddy seabed, and are not available for trawl capture when hiding in the substrate (Bell et al. 2006; Aguzzi et al 2021). The *Nephrops* fishery is extremely valuable to Europe, particularly in the Adriatic Sea and around the British Isles. In 2021, landings in the Adriatic Sea (~437 tonnes) (FAO-GFCM 2023) accounted for ~30 % of the total landings across the Mediterranean (~1,846 tonnes) (FAO-GFCM 2023), while landings in the United Kingdom and Ireland (~38,505 tonnes) accounted for ~71 % of the total global landings (FAO-GFCM 2023).

The Mediterranean is divided up into 27 geographical subareas (GSAs) established by the General Fisheries Commission for the Mediterranean (GFCM). The Adriatic Sea is split into two GSAs, GSA 17 and GSA 18 (Figure 1A). In GSA 17, a deeper area characterised by three distinct benthic depressions known as the Pomo/Jabuka Pits, hereafter Pomo Pits, located between Italy and Croatia, is known to be a valuable spawning ground for *Nephrops* (Melaku Canu et al. 2021). Research has also shown significant differences in the biology of individuals from this area compared with *Nephrops* outside of Pomo Pits, such as smaller sized animals with slower average growth rates and individuals with smaller size at the onset of first maturity (SOM), which suggests the presence of a distinctive *Nephrops* subpopulation in Pomo Pits (Froglia and Gramitto 1988; Angelini et al. 2020). In 2018, a Fisheries Restricted Area (FRA) was designated for Pomo Pits based on the GFCM/41/2017/3 recommendation (GFCM 2017), which monitors *Nephrops* fishing in the area and restricts effort, primarily via closed areas; in managed areas, restrictions include only a certain number of vessels can fish on limited days, closed seasons and days-at-sea limits (Chiarini et al. 2022).

In this study, our main goal was to investigate whether the *Nephrops* Pomo Pit subpopulation, identified based on biological differences, also exhibits genetic differences compared with those outside of Pomo Pits in GSA 17, or with GSA 18 and neighbouring GSA stocks.

Materials and Methods

Tissue sampling and DNA extraction

Samples of adult *Nephrops* were collected from ten sites, including seven from the Adriatic Sea, one from the Aegean Sea, one from the Tyrrhenian Sea, and one from the Firth of Clyde in the northeast Atlantic (Table 1, Figure 1A-B). Non-lethal tissue samples were obtained by excising two pleopods or one pereiopod. All samples were placed in 95-100 % ethanol and stored at 4°C until DNA extraction. Genomic DNA was extracted using a salting-out protocol (Jenkins et al. 2018) and the quality of each extract was assessed on a 0.8 % agarose gel. DNA purity was measured using a Nanodrop One and a Qubit 3.0 was used to quantify DNA concentration. The carapace length and sex of each animal sampled was also recorded (except for the Aegean and Tyrrhenian samples). We were able to obtain more data on carapace length and sex at each site (N_c in Table 1) because a surplus of *Nephrops* were processed during the sampling activity. These data (N_c) were used to visualise variation in carapace length at each site and by sex (Figure 1C). To statistically assess differences in size between Pomo Pits (N = 172) and other Adriatic sites (N = 135), carapace length was modelled as a function of site (a binary variable describing whether an individual was sampled in or outside of Pomo Pits) and with sex (a binary variable describing an individual as male or female).

RAD sequencing and bioinformatics

DNA extracts for each sample were sent to Floragenex (Portland, Oregon, USA) for restriction site associated DNA sequencing (RAD-seq). RAD libraries were prepared for 112 samples using the SbfI restriction enzyme and sequenced on an Illumina sequencing platform using a 2 x 100bp approach. Raw reads were trimmed using Fastp 0.20.1 (Chen et al. 2018) and further filtered using the *process_radtags* program from Stacks v2.53 (Catchen et al. 2013; Rochette et al. 2019). RAD loci were built using the Stacks *de novo* pipeline; default parameters were used for all modules, except for -m in *ustacks* which was set to 3. The *gstacks*, *tsv2bam*, and *populations* programs were subsequently re-run without these individuals by excluding them from the popmap. In addition, the following parameters were added to the *populations* command: (i) --min-samples-overall 0.75, (ii) --min-mac 5, and (iii) --write-single-snp. Using R

v4.2.0 (R Core Team, 2022), the missingno() function from poppr v2.9.4 (Kamvar et al. 2014) was used to remove any individuals with ≥ 30% missing genotypes. Functions from dartR v2.9.7 (Gruber et al. 2018; Mijangos et al. 2022) were used to filter out loci that: (i) departed from Hardy-Weinberg Equilibrium, (ii) were in linkage disequilibrium, or (iii) were monomorphic, had a minor allele count less than five, or had all missing genotypes in a single site. Lastly, OutFLANK v0.2 (Whitlock and Lotterhos 2015) was used to identify any outlier loci.

Genetic diversity and population structure

The gl.report.heterozygosity() function from dartR was used to calculate observed and expected heterozygosity (H_o/H_e). The gl.report.pa() function was used to calculate the number of private alleles per sampling site.

Genetic differentiation between sampling sites was assessed by calculating pairwise values of F_{st} (Weir and Cockerham 1984) using the genet.dist() function from hierfstat v0.5-11 (Goudet and Jombart 2022). Population structure was explored using two methods: (1) a principal components analysis (PCA), and (2) a genetic clustering analysis. Prior to analysis of population structure, missing data (NAs) were imputed with the gl.impute() function from dartR using the neighbour method. The PCA was then run using the glPca() function from adegenet v2.1.3 (Jombart and Ahmed 2011). For genetic clustering, the optimal number of genetic clusters (*K*) to use was determined by running the snapclust.choose.k() function and visualising the Akaike information criteria (AICc) for each *K*. The find.clusters() function was executed to cluster individuals into *K* groups based on k-means clustering. The snapclust() function was then run on the data set for the chosen *K*. Snapclust uses maximum-likelihood estimations to assign individual membership probabilities to each *K* cluster (Beugin et al. 2018). The resulting membership probabilities to each *K* were mean averaged per site and visualised on a projected map of the study area. Isolation-by-distance was tested by running a Mantel test on a dissimilarity matrix of pairwise genetic (F_{st}) and pairwise least-cost marine geographical distances (km) using the mantel.rtest() function from ade4 v1.7-22 (Thioulouse et al. 2018).

Effective population size

The gl.LDNe() function from dartR was used to calculate effective population size (N_e), which is a wrapper around the NeEstimator v2.1 software (Do et al. 2014). The linkage disequilibrium method was run with random mating assumed, and jackknife 95% confidence intervals were computed. Given the homogeneity of sites from the Adriatic Sea (see Results), the Adriatic sites were divided into two groups, and different minor allele frequency thresholds were tested to compare results in case of inflationary effects by rare alleles. The first group comprised all three sites from Pomo Pits (Pom1, Pom2 and Pom3), and the second group comprised of the remaining sites from the Adriatic (17I, 18II, Anc, Cgg). The rationale here was to increase the sample size in each 'population' and assess whether there was a difference in N_e between these two groups, since small sample sizes are likely to severely bias the estimation of N_e (England et al. 2006).

Results

Carapace length analysis

Median carapace length in Pomo Pits was 28 mm for males (N=95) and 24 mm for females (N=77), while outside of Pomo Pits carapace length was 46 mm for males (N=73) and 40 mm for females (N=62). The model fitted to carapace length as a function of site and sex satisfied the assumptions of a linear regression (Supplementary Material S1). This model showed that, when sex is controlled for, individuals in Pomo Pits are on average 16.98 mm (41.5 %) smaller than individuals outside Pomo Pits ($P < 0.001$). Additionally, the model showed that, when site is controlled for, males are on average 5.17 mm (12.6 %) larger than females ($P < 0.001$).

RAD loci and genetic diversity

The *de novo* pipeline assembled 259,406 catalog RAD-tag loci. Filtering of individuals and loci produced a final data set of 98 individuals genotyped at 1,623 biallelic neutral SNPs (no loci were identified as outliers). In the Mediterranean sites, 0-1 private alleles were found at each site and mean observed heterozygosity was very similar across sites, ranging from 0.089-0.108. Mean unbiased expected heterozygosity values were close to observed heterozygosity, ranging

from 0.094-0.113. In the single Atlantic site, the Firth of Clyde, nine private alleles were found, but heterozygosity was comparable to the Mediterranean sites.

Population genetic structure

Genetic differentiation was high between the Firth of Clyde and all Mediterranean sites, while differentiation amongst the Mediterranean sites was low or zero (Figure 2A). The PCA revealed two distinct groups: individuals from the Firth of Clyde (Atlantic), and all other individuals from sites in the Mediterranean (Figure 2B). This was also observed in the genetic clustering analysis, whereby K=2 was the most likely number of ancestral populations (genetic clusters) (Figure 2C), and this clearly showed Atlantic-Mediterranean separation into two distinct clusters (Figure 2D).

A PCA was run using only Mediterranean sites to check for any hierarchical structuring in the data (Figure 3A). This revealed very little evidence for hierarchical structure among our sites sampled. In addition, there was little evidence for IBD across the range covered by our Mediterranean samples (Figure 3B).

Effective population size

The N_e estimate for the Pomo Pits group was between 184 – 283 depending on the minor allele frequency threshold (Table 2). For the group representing sites outside of Pomo Pits in the Adriatic, the N_e estimate was 131 – 144. However, the upper confidence interval for all estimates was infinity, suggesting that, even with samples pooled to regional levels, sample sizes should be increased in future studies to ensure reliable estimates of N_e (Marandel et al. 2020).

Discussion

Pomo Pit and GSA 17

The carapace length results from our study closely match the findings of Angelini et al. (2020), that is, females are on average smaller than males, and both females and males in Pomo Pits are on average much smaller than animals outside of Pomo Pits. Our genetic results indicate that *Nephrops* from all sites sampled in the Adriatic Sea are panmictic and have high gene flow between them. Likewise, a recent Mediterranean-wide study of *Nephrops* did not detect any genetic differentiation within regions, including within the Adriatic (Spedicato et al. 2022). This suggests that the phenotypic differences attributed to the Pomo Pits stock, namely smaller mean sizes, slower growth rates and smaller mean SOM (Angelini et al. 2020), are not explained by random genetic drift or a lack of gene flow between neighbouring Adriatic subpopulations. Moreover, Pomo Pits *Nephrops* have no apparent differences in genetic diversity compared to *Nephrops* at surrounding sites. A recent Scientific, Technical and Economic Committee for Fisheries (STECF) report showed that average stock biomass from 1994-2020 is much higher in Pomo Pits (2,912) compared with Ancona (806) and GSA18 (1,187) and outlined that "*Ancona and GS18 [are] at relatively low biomass and Pomo/Jabuka [are] recovering to historic levels*" (STECF 2023), both points of which accord with our genetic diversity estimations.

Our finding of regional gene flow is not unique to *Nephrops* across other parts of their range, nor to other closely related decapod species of the Adriatic. Using 14 neutral microsatellite markers, Pavičić et al. (2020) found the European lobster (*Homarus gammarus*) exhibits panmixia and comparable measures of genetic diversity in sites sampled across the Adriatic. These findings suggest that, as with *Nephrops* in our study, gene flow (and/or high effective population sizes) is likely sufficient to mitigate genetic drift for both species across the sites sampled. However, connectivity modelling of *Nephrops* has revealed the potential presence of three subpopulations in the Adriatic (Melaku Canu et al. 2021). This would suggest that connectivity in the region likely follows a stepping-stone model, resulting in putative genetic homogeneity across the entire Adriatic region that is caused by adequate ocean current movement, and a sufficiently long pelagic duration (1 – 2 months; Dickey-Collas et al. 2000) for

Nephrops larval dispersal to ensure widespread admixture. Indeed, even where biophysical models strongly overestimate the realised dispersal of typical larvae, a small subset of distantly-dispersing individuals can still drive genetic homogeneity across expansive regions by inducing sufficient gene flow to offset genetic drift and effectively nullify differentiation between otherwise self-recruiting populations (Shanks, 2009; Macleod et al. 2024). Genetic connectivity at similar spatial scales has also been reported for *Nephrops* across Scandinavia in the north-east Atlantic, for which the authors likewise conclude that patterns of ocean currents and connectivity via larval drift are likely responsible for the observed genetic homogeneity (Westgaard et al. 2023).

In light of these findings, what could have driven the observed biological differences of *Nephrops* in Pomo Pits if they are not caused by genetic isolation? Other lobsters have demonstrated marked variation in SOM between discrete geographic stocks that are nevertheless highly connected genetically. Mean SOM for *H. gammarus* was estimated to be 18 mm CL larger for males and 31 mm CL larger for females in western Scotland than it was in eastern Scotland (Lizarraga-Cubedo et al. 2003), despite the stocks showing minimal genetic differences (Jenkins et al. 2019; Ellis et al. 2023), while the mean SOM of southern rock lobsters (*Jasus edwardsii*) declined markedly from 112 mm CL to just 59 mm CL along a latitudinal gradient in Tasmania (Gardner et al. 2006), despite regional genetic homogeneity (Villacorta-Rath, 2022). Indeed, the slower growth rates evidenced by Pomo Pits *Nephrops* may drive their smaller mean sizes and SOM compared to equivalent individuals in surrounding areas (Angelini et al. 2020); maturation and fecundity are often driven by age rather than size in lobsters (Gardner et al. 2006; Ellis et al. 2015), so slower growth stunts mean sizes and renders individuals smaller at a given age, reducing SOM. Environmental factors, especially temperature, are known to influence growth and SOM. Pomo Pits are considerably deeper than surrounding Adriatic areas we sampled (Table 1) and, at ~10°C, seawater in the Pomo depressions is typically cooler than shallower surrounding benthos (Artegiani et al. 1993). Yet, while the effect of lower temperatures on metabolism may explain the slower growth of *Nephrops* in Pomo Pits, this relationship is typically inversed in benthic ectotherms when it

comes to SOM; for American lobsters (*Homarus americanus*), reduced SOM is associated with increased temperatures (Le Bris et al. 2017). We do not have data with which to assess how historic fishing effort might vary among Adriatic fishing grounds, but this may be a possible driver of lower SOM among the *Nephrops* of Pomo Pits. Haarr et al. (2019) modelled potential explanatory factors against spatial variation and temporal declines in the SOM of *H. americanus*, and, while temperature and population density were poorly correlated to SOM variations, intensity of size-based harvest selection was significantly associated, suggesting that SOM decreases were an evolutionary response to fishing pressure. Likewise, in Adriatic *Nephrops*, differences in fishing pressures over time and space, local environmental conditions, and/or intraspecific competition for space and food may all play a part in driving changes in SOM (Russo et al. 2018; Angelini et al. 2020; Chiarini et al. 2022).

Intra-Mediterranean homogeneity

We sampled two Mediterranean sites outside of the Adriatic Sea, one in the Aegean Sea (Aeg) and one in the Tyrrhenian Sea (9I); both sites showed genetic similarity with each other and with the Adriatic sites. Our finding of minimal genetic divergence between Adriatic *Nephrops* and samples from adjacent sub-basins to the East and West reflects the pattern of population structure observed for the European spiny lobster (*Palinurus elephas*), for which samples from the Croatian Adriatic showed minimal differentiation to those from either Crete or the Balearic Sea via neutral SNPs (Ellis et al. 2023). Indeed, *H. gammarus* has a similar estimated window of PLD to *Nephrops*, but does show Mediterranean sub-structuring, with Adriatic samples differentiated from those of both the Aegean Sea and the Western Mediterranean (Pavičić et al. 2020; Ellis et al. 2023). Although our depiction of homogeneity between *Nephrops* of the Adriatic and adjacent areas may in part reflect a paucity of samples from these sites and other regions, our analytical methods and sample sizes for Adriatic *Nephrops* may be sufficient to explore connectivity at this scale, since previous research has detected even subtle regional differentiation with relatively low sample sizes using RAD-derived SNPs (e.g. Ellis et al. 2023).

Atlantic-Mediterranean divergence

At a basin-wide scale, the site sampled in the Atlantic (Firth of Clyde) was strongly differentiated from all Mediterranean sites (pairwise F_{st} values: 0.11 – 0.13). Although only seven individuals were genotyped from this site, this pattern of Atlantic-Mediterranean divergence has also been found in a previous study of *Nephrops* using mitochondrial D-loop variation (Gallagher et al. 2018), though homogeneity was reported using restriction fragment length polymorphisms (Stamatis et al. 2004) and allozymes (Stamatis et al. 2006), which is likely due to the lower power and resolution of these latter two molecular markers to detect genetic variation.

A pronounced phylogenetic break between Atlantic and Mediterranean populations is a characteristic common to a multitude of taxa of the north-eastern Atlantic, despite often greatly varying life history strategies (Patarnello et al. 2007), including littoral fishes (Galarza et al. 2009), benthic echinoderms (Carreras et al. 2020) and bivalve molluscs (Wenne et al. 2022). More relevantly, other wide-ranging lobsters of the region have also demonstrated hierarchical population structure, the primary feature of which is Atlantic-Mediterranean differentiation; European lobster (*H. gammarus*) and spiny lobster (*P. elehpas*) both show strong divergence across an Atlantic-Mediterranean divide (Jenkins et al. 2019; Ellis et al. 2023). This concordant pattern amongst different lobster species using putatively neutral SNP loci suggests that, at this scale, Atlantic and Mediterranean meta-populations of all three species have been sufficiently isolated for long enough to develop differing allele frequencies and accumulate new neutral mutations through genetic drift. The drivers of this isolation are likely shaped by the topographic and bathymetric enclosure of the Mediterranean, past and present oceanographic barriers, and periodic vicariance during the Pleistocene glaciations (Patarnello et al. 2007; Pascual et al. 2017; Jenkins et al. 2019). All of these factors serve to inhibit dispersal between the Atlantic and Mediterranean, limiting larval exchange and thus gene flow between populations in each basin (Ellis et al. 2023).

295 **Conclusions**

296 Our results show that, using neutral SNP loci, the *Nephrops* Pomo Pit subpopulation is not
297 genetically different from surrounding sites in the Adriatic Sea, which is likely explained by
298 connectivity and gene flow between them. In addition, we observed strong genetic
299 differentiation between the Atlantic and the Mediterranean, which supports evidence for an
300 Atlantic-Mediterranean phylogenetic break in *Nephrops* that has also been reported in many
301 other marine species.
302
303 These findings suggest that evolutionary mechanisms, such as phenotypic plasticity or
304 adaptation, are driving the phenotypic differences observed in Pomo Pits *Nephrops*, which
305 could be linked to differences in fishing pressure over time and space, to local environmental
306 conditions, and/or to intraspecific competition for space and food. The molecular mechanisms
307 underpinning these observed changes in phenotype are most likely linked to many loci under
308 strong selection pressures. As an example, using thousands of individuals and whole genome
309 sequencing, Therkildsen et al. (2019) were able to explain the polygenic mechanisms that
310 underpin rapid evolutionary change of an estuarine fish to smaller body sizes in response to
311 fishing. To explore similar questions in Pomo Pits *Nephrops*, such an approach, as well as a
312 quality reference genome, improved genomics resources building upon the *de novo*
313 transcriptome already published (Rotllant et al. 2017), and more comprehensive sample sizes,
314 would be needed.
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Data Availability Statement

Raw DNA sequence data are available from the Sequence Read Archive (BioProject: PRJNA1100511). SNP genotypes in VCF format and R code used to analyse data are available from Zenodo: https://doi.org/10.5281/zenodo.10907984.

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Author Contribution

TLJ and JRS conceived the idea in collaboration with the EU Joint Research Centre, Water and Marine Resources Unit. TLJ and JRS designed the study; MM organised fieldwork and collection of tissue samples; TLJ conducted the laboratory work; TLJ conducted the bioinformatics and data analysis; TLJ, CDE and JRS led the writing and revisions of the manuscript with support from MM. The authors have no conflicts of interest to declare.

References

Aguzzi J, Bahamon N, Doyle J, Lordan C, Tuck ID, Chiarini M, Martinelli M, Company JB (2021). Burrow emergence rhythms of *Nephrops norvegicus* by UWTV and surveying biases. *Scientific Reports*, 11, 5797.

Aguzzi J, Chatzievangelou D, Robinson NJ, Bahamon N, Berry A, Carreras M, Company JB, Costa C, del Rio Fernandez J, Falahzadeh A, Fifas S, Flögel S, Grinyó J, Jónasson JP, Jonsson P, Lordan C, Lundy M, Marini S, Martinelli M, Masmitja I, Mirimin L, Naseer A, Navarro J, Palomeras N, Picardi G, Silva C, Stefanni S, Vigo M, Vila Y, Weetman A and Doyle J (2022) Advancing fishery-independent stock assessments for the Norway lobster (*Nephrops norvegicus*) with new monitoring technologies. *Frontiers in Marine Science*, 9, 969071.

Angelini S, Martinelli M, Santojanni A, Colella S (2020). Biological evidence of the presence of different subpopulations of Norway lobster (*Nephrops norvegicus*) in the Adriatic Sea (Central Mediterranean Sea). *Fisheries Research*, 221, 105365.

Artegiani A, Gacic M, Michelato A, Kovacevic V, Russo A, Paschini E, Scarazzato P, Smircic A (1993). The Adriatic Sea hydrography and circulation in spring and autumn (1985–1987). *Deep Sea Research Part II: Topical Studies in Oceanography*, 40(6), 1143-1180.

Beugin MP, Gayet T, Pontier D, Devillard S, Jombart T (2018). A fast likelihood solution to the genetic clustering problem. *Methods in Ecology and Evolution*, 9, 1006–1016.

Carreras C, García-Cisneros A, Wangensteen OS, Ordóñez V, Palacín C, Pascual M, Turon X (2020). East is East and West is West: Population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species *Paracentrotus lividus* (Echinoidea). *Diversity and Distributions*, 26(3), 382-398.

Catchen J, Hohenlohe P, Bassham S, Amores A, Cresko W (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology*, 22, 3124-3140.

Chiarini M, Guicciard S, Zacchetti L, Domenichetti F, Canduci G, Angelini S, Belardinelli A, Croci C, Giuliani G, Scarpini P, Santojanni A, Medvešek D, Isajlovic I, Vrgoč N, Martinelli M (2022).

Looking for a simple assessment tool for a complex task: short-term evaluation of changes in fisheries management measures in the Pomo/Jabuka Pits Area (Central Adriatic Sea). *Sustainability*, 14, 7742.

Chen S, Zhou Y, Chen Y, Gu J (2018). Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34, i884-i890.

Dickey-Collas M, McQuaid N, Armstrong MJ, Allen M, Briggs RP (2000). Temperature-dependent stage durations of Irish Sea *Nephrops* larvae. *Journal of Plankton Research*, 22, 749-760.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources*, 14, 209-214.

Ellis CD, Knott H, Daniels CL, Witt MJ, Hodgson DJ (2015). Geographic and environmental drivers of fecundity in the European lobster (*Homarus gammarus*). *ICES Journal of Marine Science*, 72(s1), i91-100.

Ellis CD, MacLeod KL, Jenkins TL, Rato LD, Jézéquel Y, Pavičić M, Díaz D, Stevens JR (2023). Shared and distinct patterns of genetic structure in two sympatric large decapods. *Journal of Biogeography*, 50, 1271-1284.

England PR, Cornuet JM, Berthier P, Tallmon DA, Luikart G (2006). Estimating effective population size from linkage disequilibrium: severe bias in small samples. *Conservation Genetics*, 7, 303-308.

FAO-GFCM. (2023). Fishery and Aquaculture Statistics. GFCM capture production 1970-2021 (FishStatJ). In: FAO Fisheries and Aquaculture Division [online]. Rome. Updated 2023. www.fao.org/fishery/en/statistics/software/fishstatj.

Froglia C, Gramitto ME (1988). An estimate of growth and mortality parameters for *Nephrops norvegicus* in the central Adriatic Sea. *FAO Fisheries Report*, 394, 189-203.

385 Galarza JA, Carreras-Carbonell J, Macpherson E, Pascual M, Roques S, Turner GF, Rico C (2009).
386 The influence of oceanographic fronts and early-life-history traits on connectivity among littoral
387 fish species. *Proceedings of the National Academy of Sciences*, 106(5), 1473-1478.

388 Gallagher J, Finarelli JA, Jonasson JP, Carlsson J (2018). Mitochondrial D-loop DNA analyses of
389 Norway lobster (*Nephrops norvegicus*) reveals genetic isolation between Atlantic and East
390 Mediterranean populations. *Journal of the Marine Biological Association of the United Kingdom*,
391 99, 933-940.

392 Gardner C, Frusher SD, Barrett N, Haddon M, Buxton C (2006). Spatial variation in size at onset
393 of maturity of female southern rock lobster *Jasus edwardsii* around Tasmania, Australia.
394 *Scientia Marina*, 70 (3), 423-430.

395 GFCM (2017). General Fisheries Commission for the Mediterranean. Recommendation
396 GFCM/41/2017/3 on the Establishment of a Fisheries Restricted Area in the Jabuka/Pomo Pit in
397 the Adriatic Sea. GFCM: Roma, Italy.

398 Goudet J, Jombart T (2022). hierfstat: estimation and tests of hierarchical *F*-statistics, package
399 version 0.5-11, https://CRAN.R-project.org/package=hierfstat.

400 Gruber B, Unmack PJ, Berry OF, Georges A (2018). dartr: an r package to facilitate analysis of
401 SNP data generated from reduced representation genome sequencing. *Molecular Ecology*
402 *Resources*, 18, 691–699.

403 Haarr ML, Sainte-Marie B, Comeau M, Tremblay MJ, Rochette R (2018). Female American
404 lobster (*Homarus americanus*) size-at-maturity declined in Canada during the 20th and early
405 21st centuries. *Canadian Journal of Fisheries and Aquatic Sciences*, 75(6), 908-924.

406 Jenkins TL, Ellis CD, Stevens JR (2018). SNP discovery in European lobster (*Homarus gammarus*)
407 using RAD sequencing. *Conservation Genetics Resources*, 11, 253-257.

408 Jenkins TL, Ellis CD, Triantafyllidis A, Stevens JR (2019). Single nucleotide polymorphisms reveal
409 a genetic cline across the north-east Atlantic and enable powerful population assignment in the
410 European lobster. *Evolutionary Applications*, 12, 1881-1899.

411 Jenkins TL (2024). mapmixture: an R package and web app for spatial visualisation of admixture
412 and population structure. *Molecular Ecology Resources*, 24: e13943. DOI: 10.1111/1755-
413 0998.13943.

414 Jombart T, Ahmed I (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP
415 data. *Bioinformatics*, 27, 3070–3071.

416 Kamvar ZN, Tabima JF, Grünwald NJ. (2014) Poppr: an R package for genetic analysis of
417 populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2, e281.

418 Lizarraga-Cubedo HA, Tuck I, Bailey N, Pierce GJ, Kinnear JA (2003). Comparisons of size at
419 maturity and fecundity of two Scottish populations of the European lobster, *Homarus*
420 *gammarus. Fisheries Research*, 65(1-3), 137-152.

421 Macleod KL, Jenkins TL, Witt MJ, Stevens JR (2024). Rare, long-distance dispersal underpins
422 genetic connectivity in the pink sea fan, *Eunicella verrucosa*. *Evolutionary Applications*, 17,
423 e13649. DOI: 10.1111/eva.13649.

424 Marandel F, Charrier G, Lamy J-B, Le Cam S, Lorance P, Trenkel VM (2020). Estimating effective
425 population size using RADseq: effects of SNP selection and sample size. *Ecology and Evolution*,
426 10, 1929-1937.

427 Melaku Canu D, Laurent C, Morello EB, Querin S, Scarcella G, Vrgoc N, Froglia C, Angelini S,
428 Solidoro C (2021). *Nephrops norvegicus* in the Adriatic Sea: connectivity modelling, essential
429 fish habitats, and management area network. *Fisheries Oceanography*, 30, 349-365.

430 Mijangos JL, Gruber B, Berry O, Pacioni C, Georges A (2022). dartR v2: An accessible genetic
431 analysis platform for conservation, ecology and agriculture. *Methods in Ecology and Evolution*,
432 13, 2150-2158.

433 Pascual M, Rives B, Schunter C, Macpherson, E (2017). Impact of life history traits on gene flow:
434 A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. PLoS
435 One, 12(5), e0176419.

436 Patarnello T, Volckaert FAMJ, Castilho R (2007). Pillars of Hercules: is the Atlantic-
437 Mediterranean transition a phylogeographical break? *Molecular Ecology*, 16, 4426-4444.

438 Pavičić M, Žužul I, Matić-Skoko S, Triantafyllidis A, Grati F, Durieux ED, Celić I, Šegvić-Bubić T
439 (2020). Population genetic structure and connectivity of the European lobster *Homarus*
440 *gammarus* in the Adriatic and Mediterranean seas. *Frontiers in Genetics*, 11, 1540.

441 R Core Team (2022). R: A language and environment for statistical computing. Vienna, Austria:
442 R Foundation for Statistical Computing. Retrieved from https://www.R-project.org.

443 Rochette N, Rivera-Colón A, Catchen J (2019). Stacks 2: analytical methods for paired-end
444 sequencing improve RADseq-based population genomics. *Molecular Ecology*, 28, 4737-4754.

445 Rotllant G, Nguyen TV, Sbragaglia V, Rahi L, Dudley KJ, Hurwood D, Ventura T, Company JB,
446 Chand V, Aguzzi J, Mather PB (2017). Sex and tissue specific gene expression patterns identified
447 following de novo transcriptomic analysis of the Norway lobster, *Nephrops norvegicus*. *BMC*
448 *Genomics*, 18, 622.

449 Russo T, Morello EB, Parisi A, Scarcella G, Angelini S, Labanchi L, Martinelli M, D'Andrea L,
450 Santojanni A, Arneri E, Cataudella S (2018). A model combining landings and VMS data to
451 estimate landings by fishing ground and harbor. *Fisheries Research*, 199, 218-230.

452 Scientific, Technical and Economic Committee for Fisheries (STECF) (2023). Stock Assessments:
453 demersal stocks in Adriatic, Ionian and Aegean Seas and straits of Sicily (STECF-22-16).
454 Publications Office of the European Union, Luxembourg, JRC132157, DOI:10.2760/25344.

455 Shanks AL (2009). Pelagic larval duration and dispersal distance revisited. *The Biological*
456 *Bulletin*, 216, 373-385.

457 Spedicato MT, Cannas R, Mahé K, Morales B, Tsigenopoulos C, Zane L, Kavadas S, Maina I,
458 Scarcella G, Sartor P, Bandelj V, Russo T, Fiorentino F (2022). Study on advancing fisheries
459 assessment and management advice in the Mediterranean by aligning biological and
460 management units of priority species (MED UNITs), Final Report. European Climate,

461 Infrastructure and Environment Executive Agency, Luxembourg. ISBN 978-92-95225-33-6 DOI:
462 10.2926/909535.

463 Stamatis C, Triantafyllidis A, Moutou KA, Mamuris Z (2004). Mitochondrial DNA variation in
464 Northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*.
465 *Molecular Ecology*, 13, 1377-1390.

466 Stamatis C, Triantafyllidis A, Moutou KA, Mamuris Z (2006). Allozymic variation in Northeast
467 Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. *ICES Journal*
468 *of Marine Science*, 63, 857-882.

469 Therkildsen NO, Wilder AP, Conover DO, Munch SB, Baumann H, Palumbi SR (2019). Contrasting
470 genomic shifts underlie parallel phenotypic evolution in response to fishing. *Science*, 365, 487-
471 490.

472 Thioulouse J, Dray S, Dufour A, Siberchicot A, Jombart T, Pavoine, S (2018). Multivariate analysis
473 of ecological data with ade4. Springer, New York. https://doi.org/10.1007/978-1-4939-8850-1.

474 Villacorta-Rath C, Green BS, Gardner C, Murphy NP, Souza CA, Strugnell JM (2022).
475 Disentangling genetic from environmental effects on phenotypic variability of southern rock
476 lobster (*Jasus edwardsii*) postlarvae. *Ecology and Evolution*, 12(11), e9519.

477 Weir BS, Cockerham CC (1984). Estimating *F*-statistics for the analysis of population structure.
478 *Evolution*, 38, 1358–1370.

479 Wenne R, Zbawicka M, Prądzińska A, Kotta J, Herkül K, Gardner JP, Apostolidis AP, Poćwierz-
480 Kotus A, Rouane-Hacene O, Korrida A, Dondero F (2022). Molecular genetic differentiation of
481 native populations of Mediterranean blue mussels, *Mytilus galloprovincialis* (Lamarck, 1819),
482 and the relationship with environmental variables. *The European Zoological Journal*, 89(1), 755-
483 784.

484 Westgaard J-I, Søvik G, Johansen T (2023). Genetic population structure in Norway lobster
485 (*Nephrops norvegicus*): management regime under panmixia. *ICES Journal of Marine Science*,
486 80, 776-774.

487 Whitlock MC, Lotterhos KE (2015). Reliable detection of loci responsible for local adaptation:
488 Inference of a null model through trimming the distribution of F_{st}. *The American Naturalist*, 186,
489 S24–S36.

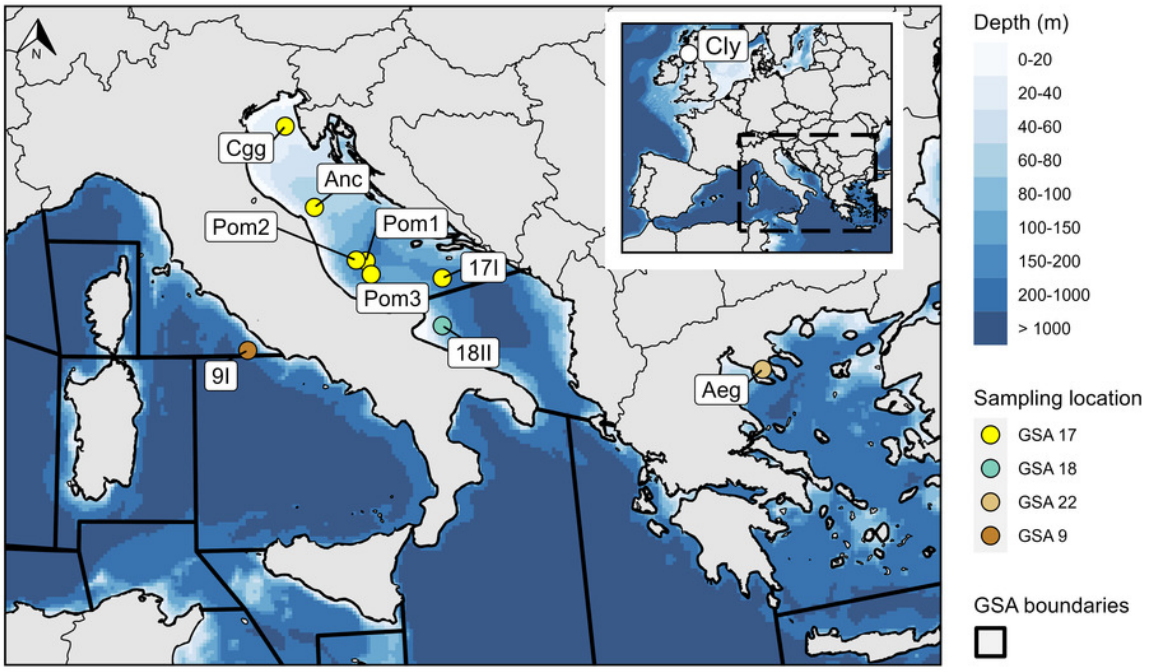
490 Ungfors A, Bell E, Johnson ML, Cowing D, Dobson NC, Bublitz R, Sandell J (2013). Chapter Seven
491 - *Nephrops* fisheries in European waters. *Advances in Marine Biology*, 64, 247-314.

Figure 1

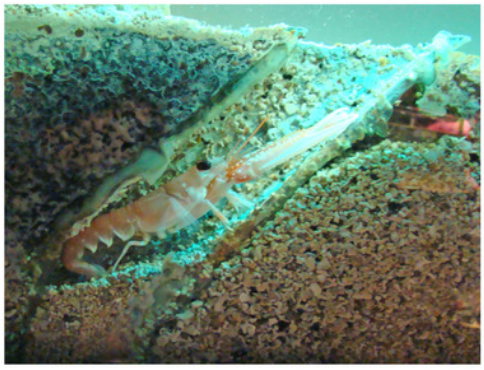
Study area, sampling information and carapace length measurements.

(A) Bathymetric map showing the sites sampled in the Adriatic Sea, the Aegean Sea and the Tyrrhenian Sea (Mediterranean), and the site sampled in the Firth of Clyde (Atlantic). The points in the Mediterranean are coloured by which geographical subarea (GSA) they are located in. (B) *Nephrops* burrow (Image: © Grand Aquarium de Saint-Malo / CC BY-SA 3.0 DEED). (C) Carapace length variation at each site sampled (except 9I and Aeg) coloured by sex. The number of individuals used to visualise carapace length for each site are denoted by N_c in Table 1.

A


Cly
Cgg
Anc
Pom2
Pom1
17I
Pom3
18II
9I
Aeg
Depth (m)
0-20
20-40
40-60
60-80
80-100
100-150
150-200
200-1000
> 1000
Sampling location
GSA 17
GSA 18
GSA 22
GSA 9
GSA boundaries

B



C

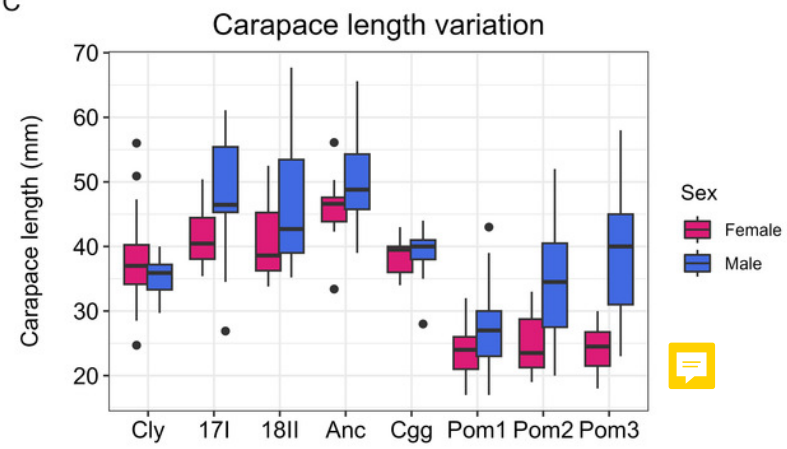

Carapace length variation
Carapace length (mm)
70
60
50
40
30
20
Cly
17I
18II
Anc
Cgg
Pom1
Pom2
Pom3
Sex
Female
Male

Figure 2

Population genetic structure results.

(A) Heatmap of pairwise F_{st} values for each site-site comparison. (B) Principal components analysis; each point represents the position an individual on axis 1 and 2 and colours correspond to sites located in the Atlantic (red) or the Mediterranean (blues). (C) The Akaike information criteria (AICc) scores for each *K* run using the snapclust algorithm; the plateau after *K*=2 suggests that two is the most likely number of ancestral populations in the data set. (D) Projected map (ESPG:3035) of the study area showing the membership proportion of individuals to each genetic cluster, averaged over each site; this map was produced using the mapmixture() function from mapmixture v1.1.0 (Jenkins 2024). The north arrow points to the north pole.

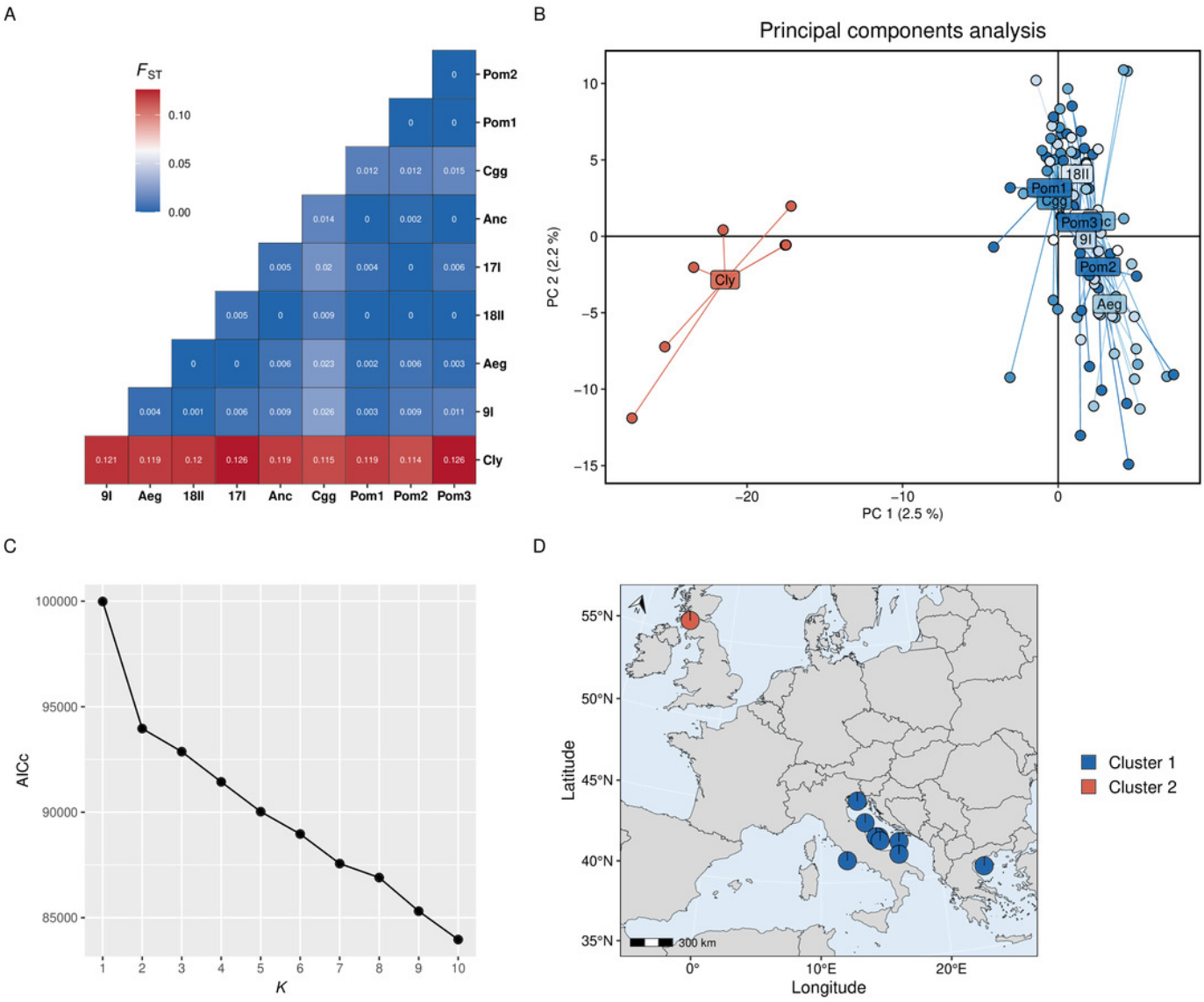

A
F_{ST}
0.10
0.05
0.00
Pom2
Pom1
Cgg
Anc
17l
18ll
Aeg
9l
Cly
9l Aeg 18ll 17l Anc Cgg Pom1 Pom2 Pom3
B
Principal components analysis
PC 2 (2.2 %)
PC 1 (2.5 %)
Cly
18ll
Pom1
Cgg
Pom3
9l
Pom2
Aeg
C
AICc
K
D
Latitude
Longitude
300 km
Cluster 1
Cluster 2

Figure 3

Hierarchical genetic structure results.

(A) Principal component analysis of Mediterranean sites only. (B) Scatter plot showing pairwise genetic (F_{st}) and geographic (km) distances between our sampling sites. The r^2 and significance result from the Mantel test is displayed in the top-left corner.

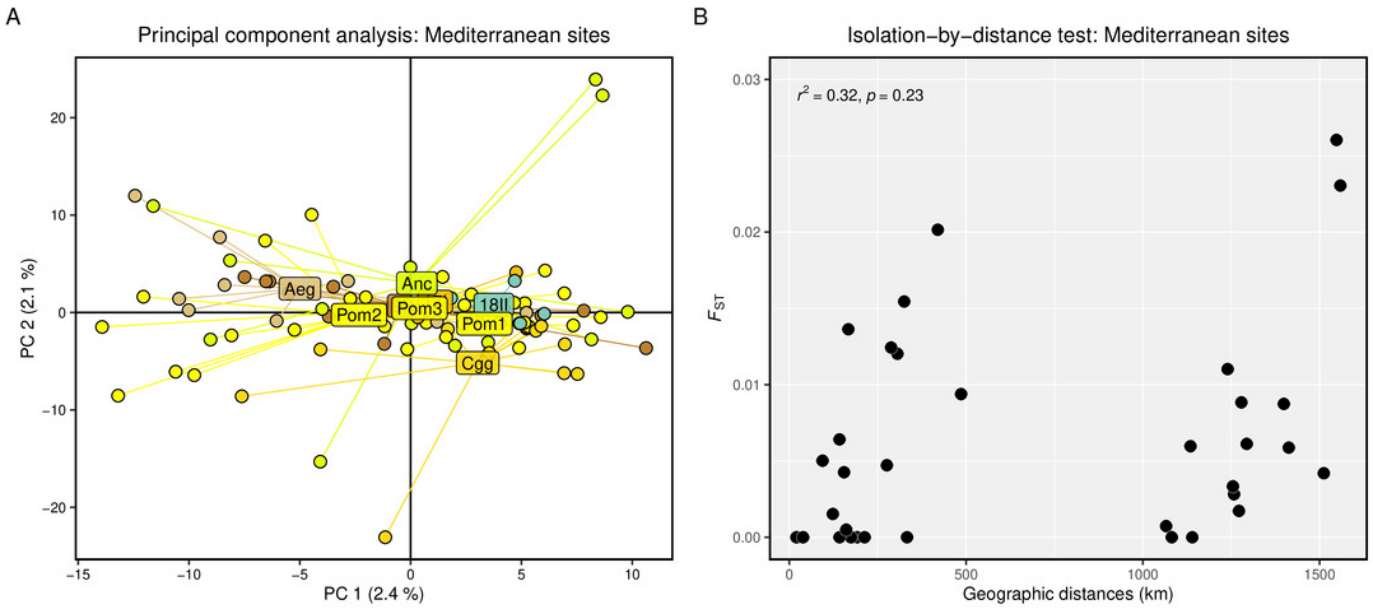


Table 1(on next page)

Sampling information and genetic diversity statistics.

Table 1 Sampling information and genetic diversity statistics.

Area	Site	Code	N_c	N_g	Depth (m)	Year	Lat	Lon	P_a	H_o	H_e
Adriatic											
GSA 17	Off Ancona	Anc	34	16	70	2019	43.78	13.85	0	0.102	0.108
GSA 17	Off Chioggia	Cgg	41	14	35-40	2019	45.18	13.24	0	0.096	0.097
GSA 17	Pomo Pits	Pom1	132	9	216	2016	42.82	14.97	0	0.089	0.094
GSA 17	Pomo Pits	Pom2	20	17	215-250	2019	42.85	14.74	1	0.108	0.111
GSA 17	Pomo Pits	Pom3	20	10	170-180	2019	42.59	15.06	0	0.096	0.099
GSA 17	17I	17I	30	5	93	2017	42.43	16.68	0	0.108	0.108
GSA 18	18II	18II	30	5	92	2017	41.63	16.59	0	0.097	0.097
Aegean											
GSA 22	Aegean Sea	Aeg	n/a	14	n/a	2019	40.17	23.54	0	0.108	0.113
Tyrrhenian											
GSA 9	9I	9I	n/a	14	530	2017	41.40	12.20	0	0.102	0.105
Atlantic	Firth of Clyde	Cly	32	8	50-75	2019	55.86	-4.90	9	0.095	0.096

GSA geographical subarea.
N_c number of individuals sampled for carapace length measurement (total = 339).
N_g number of individuals genotyped (total = 112).
P_g number of private alleles.
H_o mean observed heterozygosity.
H_e mean unbiased expected heterozygosity.

Table 2 (on next page)

Estimates of effective population size (N_e) in the Adriatic Sea.

N_e was calculated using the linkage disequilibrium method with random mating assumed, and jackknife 95% confidence intervals were computed.

Table 2 Estimates of effective population size (N_e) in the Adriatic Sea. N_e was calculated using the linkage disequilibrium method with random mating assumed, and jackknife 95% confidence intervals were computed.

	Pomo Pits	**Outside Pomo Pits**
$N_{e\ MAF\ 0.01}$	283 (70 – Inf)	131 (54 – Inf)
$N_{e\ MAF\ 0.02}$	195 (58 – Inf)	141 (53 – Inf)
$N_{e\ MAF\ 0.05}$	184 (60 – Inf)	144 (54 – Inf)

Pomo Pits includes the following sites: Pom1, Pom2, Pom3.
Outside Pomo Pits includes the following sites: 17I, 18II, Anc, Cgg.