



An evaluation of the genetic connectivity and temporal stability of the blue and red shrimp *Aristeus antennatus*: a case study of spawning females' grounds in the Western Mediterranean Sea

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Abstract The blue and red shrimp *Aristeus antennatus* has been intensively exploited by trawling fishery in the Western Mediterranean Sea. Implementation of an adequate management plan needs complete genetic data of the non-spawning and spawning grounds of the species. During the reproductive period, the species forms spawning aggregations in the middle slope, mainly constituted of mature females carrying spermatophores. Seven spawning females' grounds in the Western Mediterranean Sea from Gulf of Lions (France) to Cabo de Palos (Spain) were sampled during two consecutive years, 2016 and 2017. This study assessed for the first time the levels of genetic diversity within spawning grounds, the genetic divergence among them and estimated the degree of temporal genetic stability using multilocus

genotyping. Our results showed a high connectivity of the spawning females' grounds that remains stable during the two analysed years, explained by passive larval dispersal, together with juveniles and adults' migration. We advise that a future management plan for *A. antennatus* fishery should consider the Mediterranean Subarea GSA6 as a single unit, but it should be combined with local monitoring in order to ensure the sustainable exploitation of the populations.

Keywords *Aristeus antennatus* · Deep-sea shrimp · Gene flow · Microsatellite loci · Geo-temporal genetic analysis · Spawning females · Mediterranean Sea

Introduction

In 2002, the Council of the European Union emphasized the need of conducting a sustainable exploitation of fishery resources in order to ensure their long-term viability (European Council, 2002). The inclusion of genetic results in management advice is crucial to achieve that goal, especially when it is combined with life-traits and ecological information (Allendorf et al., 2008; Waples et al., 2008; Waples & Naish, 2009). Indeed, the exploitation of a fishery resource can generate, among others, a loss of genetic diversity within subpopulations, and a modification of fine-scale genetic structure and gene flow. Those genetic changes could lead to a reduction in the recovery of the population or increase extinction risks,

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produce a decrease in biomass and a reduction of the sizes at first maturity (Allendorf et al., 2008). Genetic markers assist in identifying the species' conservation units, in other words the populations, and to estimate their structure, connectivity and genetic diversity (Hauser & Carvalho, 2008). In addition, genetic studies allow to improve the assessment or development of management strategies to ensure the sustainability of exploited marine resources (Ward, 2000).

Furthermore, the population structure of a marine species could be temporally unstable due to several factors, such as processes that modify the ecosystem, sweepstakes reproductive success or the individuals' behaviour (Pilling et al., 2008; Waples & Naish, 2009). Hence, an accurate research about the identification of the genetic structure in marine species should include temporal replicates (Waples & Naish, 2009). In addition to molecular data, it is also important to collect other biological data, such as life-history traits (e.g. the size), in order to obtain integrative information on the species (Carvalho & Hauser, 1994). It has been pointed out that a fishery should not show a tendency in the annual variation in size composition of catches so that it could be considered sustainable, since a reduction of individuals' size over time has been indicated as a possible consequence of overfishing (Leitão, 2019).

Aristeus antennatus (Risso, 1816) (Crustacea, Aristeidae) (En—Blue and red shrimp; Fr—Crevette rouge; Sp—Gamba roja) is distributed across the whole Mediterranean Sea, although it increases westwards (Sardà et al., 2004; Fernández et al., 2011). In response to the overexploitation of *A. antennatus* in the Western Mediterranean, the General Fisheries Commission for the Mediterranean Sea decided to include this species into the priority list for action (FAO, 2006), urging to a reduction of the fishing effort in the Balearic Islands and the Eastern coast of Spain (Mediterranean Subareas GSA5 and GSA6, respectively). In fact, even though *A. antennatus* in these areas has been considered one of the main marine resources where it has been intensively exploited by trawling fishery (Massutí et al., 2008), a management plan specific for the species which integrates genetic information with traditional methods has not been developed yet. Nowadays, the fishery of the species is carried out following general guidelines for trawling fisheries (European Council, 2019). To implement an adequate management plan for *A.*

antennatus, it is necessary to identify the population structure with appropriate spatial and temporal replicates (Hauser & Carvalho, 2008; Waples & Naish, 2009), thus ensuring that genetic patterns are stable over time and not caused by sampling artefacts (Waples, 1998).

The blue and red shrimp is the most eurybathic species in the Mediterranean Sea, inhabiting the muddy bottoms from 80 m to nearly 3000 m of depth (Sardà et al., 2004). In the GSA6, the highest abundances have been found in Palamós, Blanes, Dénia and Santa Pola (Esteban, 2018). Adult females are more abundant at depths shallower than 1000 m, whereas the proportion of adult males and juveniles increase in deeper layers (below 1000 m) (Sardà et al., 2003, 2004). The spawning period takes place from late May to late September, with maximum values between June and August (Demestre, 1995). Spawning aggregations are formed in the middle slope (600–800 m depth) during that period, mainly composed of spawning females carrying one or more spermatophores (Sardà et al., 1997; Gorelli et al., 2014; Planella et al., 2019). Since fishery is performed between depths of 400 and 800 m (Carbonell et al., 1999; Maynou, 2008), catches are essentially constituted of females (Gorelli et al., 2014). *A. antennatus* presents sex dimorphism, where females show higher size than males, estimated by the cephalothorax length (CL) (Sardà et al., 2004; Deval & Kápiris, 2016). Sexual maturity in females is indicated according to the stage of gonad maturity, and 26 mm CL is the size at first maturity, which corresponds to age-1 individuals (Demestre & Martín, 1993; Carbonell et al., 2008).

A recent study using microsatellite markers in *A. antennatus* indicated a high level of genetic connectivity among fishing grounds along the Mediterranean Subarea GSA6 (from Gulf of Lions, France, to Cabo de Palos, Spain), supported mainly by the low level of population differentiation (i.e. low F_{ST} values) and the high migration rates (Agulló et al., 2020). In that study, seven fishing grounds (Port de la Selva, Roses, Palamós, Blanes, Vilanova i la Geltrú, Dénia and Santa Pola) were sampled during winter seasons of 2016 and 2017, when captures are mostly composed of small-sized specimens (16–34 mm CL, cohorts 1+ and 2+) and the sex ratio is 1:1 (Carbonell et al., 1999; Agulló et al., 2020). A small but significant regional structure was suggested among 2017

samples, and also local temporal genetic fluctuations between 2016 and 2017, mainly due to the oceanographic singularity at the Blanes fishing ground.

Performing studies during spawning season in addition to non-breeding season is critical because the maximum integrity of the stocks appears during the reproductive period (Reiss et al., 2009). During the spawning period, stocks are likely to present higher genetic differences among them than during non-spawning period, even over short geographical distances (Hauser & Carvalho, 2008 and references therein). Several marine species have been analysed during the spawning period, such as the short-finned squid *Illex argentinus* (Castellanos, 1960) (Roldán et al., 2014), the white-streaked grouper *Epinephelus ongus* (Bloch, 1790) (Nanami et al., 2017) and the deep-water velvet shrimp *Metapenaeopsis sibogae* (De Man, 1907) (Rahman & Ohtomi, 2017). In *A. antennatus*, spawning aggregations are formed during summer, involving females from 1+ to older than 5+ cohorts (Carbonell, 2005) so it would be relevant to evaluate the population genetics during that season.

Here, we expand our former study focussing our attention on summer spawning females. The genetic diversity and divergence of the spawning grounds of the blue and red shrimp were assessed using multilocus genotyping. The studied area covered the Mediterranean Subarea GSA6 by sampling two consecutive years, 2016 and 2017. A multilocus study sampling spawning females would allow to test the population structure and the stability of the genetic pattern in the same years as Agulló et al. (2020). If the genetic pattern was not maintained, it could be due to several reasons, such as sex-biased dispersal and mesoscale features. Finally, the integration of our outcomes into future management plans could improve the species' fishery, making it more sustainable, especially in the medium and long term.

Materials and methods

Sampling

A total of 1400 adult females of *A. antennatus* were obtained from 7 Western Mediterranean locations (GSA6: Port de la Selva, Roses, Palamós, Blanes, Vilanova i la Geltrú, Dénia and Santa Pola), with a North–South distance of 719 km (Fig. 1) in two

consecutive years (2016 and 2017). All samples were captured by commercial trawling vessels during July, and were provided by local fishermen on the date of capture (Table 1). For each individual, CL was measured from the orbital margin to the mid-posterior edge of the carapace, using a digital vernier calliper. Spermatophores were recorded on the thelycum of almost every captured female, which were also sexually mature according to the macroscopic external coloration of the ovary (Demestre & Fortuño, 1992). Analysis of variance (ANOVA) and Tukey post hoc tests were performed to compare the individuals' CL among samples using the GraphPad Prism version 8.4.1 (GraphPad Software, San Diego, CA, USA). Besides, an unpaired two-sided Student's *t* test was performed to compare all the individuals' CL between the two temporal collections.

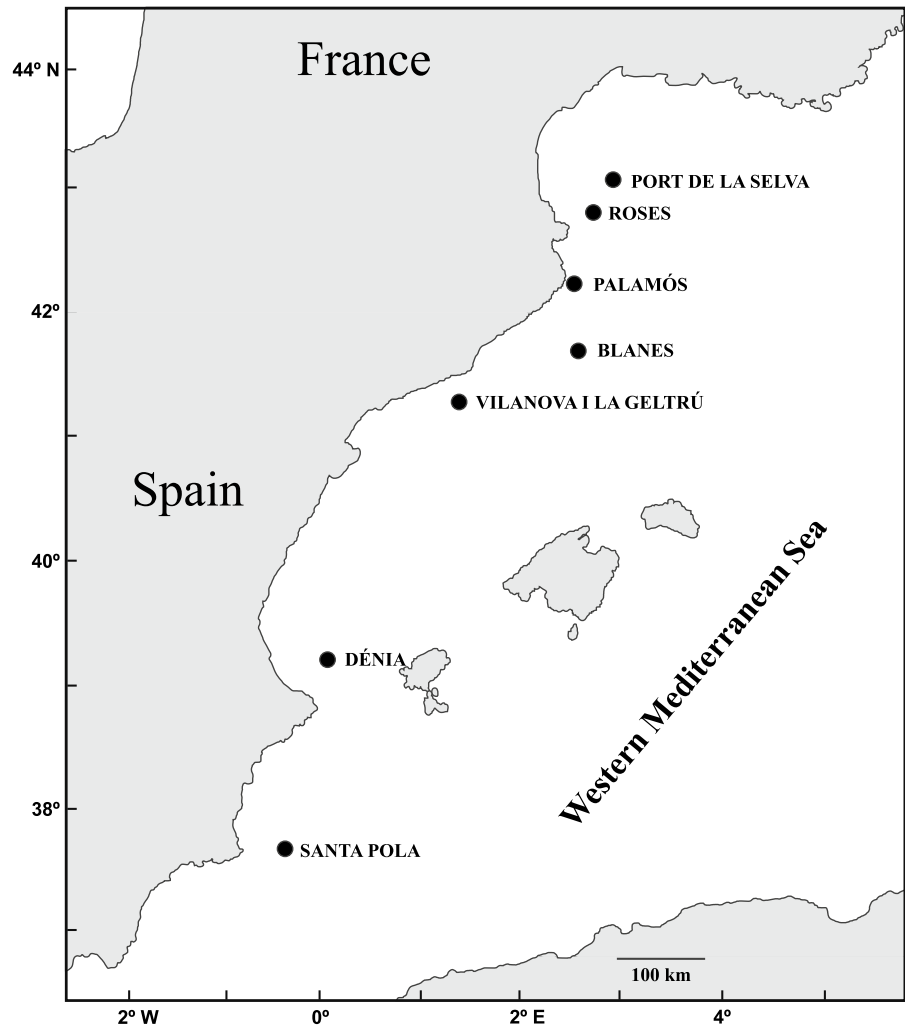
DNA extraction, microsatellite amplification and genotyping

A portion of muscle tissue from each specimen was stored in 70% ethanol. Total genomic DNA extraction was performed according to the phenol: chloroform procedure outlined in Fernández et al. (2013). After isolation, DNA extractions were resuspended in ddH₂O and stored at −20 °C. Twelve previously reported microsatellite loci (*Aa123*, *Aa1255*, *Aa138*, *Aa1444*, *Aa496b*, *Aa667*, *Aa681*, *Aa751*, *Aa956*, *Aa1061*, *Aa1195* and *Aa818*; Heras et al., 2016) were amplified with three PCR multiplex and one PCR singleplex, following the conditions published in Planella et al. (2019). PCR products were visualized in an automatic sequencer (ABI PRISM 3730xl, Applied Biosystems, Foster City, CA) at the sequencing unit of the University of Santiago de Compostela (Campus Lugo, Spain). All genotypes were obtained by allele scoring with GeneMapper 4.0 software (Applied Biosystems, Foster City, CA). GeneScan 500 LIZ Dye Size Standard (Applied Biosystems) was used as internal standard. Individuals genotyped for < 8 loci were excluded from further consideration, leaving a total of 1343 individuals (95.9% of sampled specimens) suitable for genetic statistical analyses.

Genetic data analysis

The software Genepop v. 4.6 (Rousset, 2008) was used to estimate allele frequencies, observed (H_o) and

Fig. 1 Sampling locations of *Aristeus antennatus* in the Western Mediterranean Sea. Further details are given in Table 1



expected (H_e) heterozygosities, inbreeding coefficient (F_{IS}) (Weir & Cockerham, 1984), to test for deviations from Hardy–Weinberg equilibrium (HWE), and for linkage disequilibrium between pairs of loci. The last two tests were performed with 10,000 dememorizations, 100 batches and 5000 iterations per batch, and P values were adjusted for multiple comparisons using the Bonferroni correction (Rice, 1989). Number of alleles per locus (N_A) and allelic richness (A_R) were computed using FSTAT v. 2.9.3 (Goudet, 2001). Micro-Checker v. 2.2.3 (Van Oosterhout et al., 2004) was performed to evaluate the frequency of possible null alleles with 1000 randomizations.

Pairwise F_{ST} values among all locations and hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) were performed using Arlequin

v. 3.5 (Excoffier & Lischer, 2010). The significance of F -statistics was assessed by permutation tests with 10,000 replicates. FreeNA (Chapuis & Estoup, 2007) was carried out to correct pairwise F_{ST} values for null alleles. Hierarchical models of AMOVA were used to assess the partitioning of genetic diversity within and among spawning grounds in 2016 and 2017, as well as the temporal component (2016 vs 2017). Additionally, we calculated pairwise F_{ST} between sampling campaigns (winter 2016, summer 2016, winter 2017 and summer 2017) for each fishing ground to assess the seasonal component using winter female data from Agulló et al. (2020).

The effective population size (N_e) was calculated for the fourteen samples using the bias-corrected version of the method based on linkage disequilibrium

Table 1 Details of the fourteen analysed *Aristeus antennatus* samples, with geographical coordinates, sampled year, depth and sample size (*N*)

Location	Sample code	Sampled year	<i>N</i>	Fishing ground	Geographical coordinates	Depth (metres)
Port de la Selva	PDS16	2016	100	Avión	42°35′32″N 3°46′04″E	555
	PDS17	2017	100			
Roses	ROS16	2016	100	La Trencada	42°23′40″N 3°32′11″E	600
	ROS17	2017	100			
Palamós	PAL16	2016	100	Sant Sebastià	41°53′06″N 3°16′08″E	545
	PAL17	2017	100			
Blanes	BLA16	2016	100	La Barana	41°24′04″N 3°19′05″E	534
	BLA17	2017	100			
Vilanova i la Geltrú	VIG16	2016	100	Can Pere Negre	41°03′71″N 2°01′87″E	750
	VIG17	2017	100			
Dénia	DEN16	2016	100	Dénia's fishing ground	39°04′N 0°22′E	666
	DEN17	2017	100			
Santa Pola	SPO16	2016	100	Playa Nueva	37°35′07″N 0°13′01″W	658
	SPO17	2017	100			

(Hill, 1981; Waples, 2006) as implemented in *NeEstimator* v. 2.1 (Do et al., 2014), and 95% confidence intervals were determined using the non-parametric Jack-knife method. Given the minimum sample size of 77 (VIG16 in Table 2), a critical allele frequency (P_{crit}) of 0.01 was applied to ensure that $P_{crit} > 1/2 N$, where N represents the total sample size, in order to avoid single copy alleles in the analysis.

Pearson's chi-square and Fisher exact tests were carried out in the software POWSIM v. 4.1 (Ryman &

Palm, 2006) to assess the statistical power of our set of loci to detect significant genetic differentiation for the number of loci, the allele frequencies, and sample sizes (1000 dememorizations, 100 batches and 1000 iterations) and 1000 replicates. The power of the tests for detecting the defined level of genetic divergence was interpreted by the percentage of significant outcomes. Different values for F_{ST} were explored by changing the number of generations of drift (t).

Table 2 Estimates of genetic variability for 12 microsatellite loci

	Sample code	n	N_A	A_R	H_o	H_e	F_{IS}	N_e	CI for N_e
Number of genotyped individuals (≥ 8 genotyped loci) (n); mean number of alleles detected (N_A); mean allele richness (A_R); mean observed heterozygosity (H_o); mean expected heterozygosity (H_e); inbreeding coefficient (F_{IS}); effective population size (N_e); confidence interval (Jack-knife method) (CI). Sample codes as in Table 1	PDS16	89	8.8	8.3	0.465	0.613	0.241*	6007.0	224.6-infinite
	PDS17	100	8.8	8.6	0.464	0.632	0.266*	Infinite	348.6-infinite
	ROS16	96	8.3	7.7	0.427	0.635	0.327*	386.9	161.7-infinite
	ROS17	100	8.8	8.5	0.475	0.628	0.244*	2848.0	348.2-infinite
	PAL16	99	9.1	8.5	0.473	0.623	0.241*	1142.3	213.9-infinite
	PAL17	99	8.4	8.2	0.479	0.628	0.237*	631.6	211.8-infinite
	BLA16	96	9.7	8.9	0.491	0.623	0.211*	2109.8	220.6-infinite
	BLA17	100	9.8	9.4	0.479	0.633	0.243*	481.9	169.7-infinite
	VIG16	77	8.5	8.2	0.434	0.643	0.325*	Infinite	388.3-infinite
	VIG17	100	9.8	9.4	0.489	0.644	0.240*	Infinite	299.2-infinite
	DEN16	93	8.4	7.8	0.447	0.631	0.292*	Infinite	427.6-infinite
	DEN17	94	8.9	8.8	0.492	0.642	0.234*	Infinite	425.3-infinite
	SPO16	100	9.0	8.3	0.487	0.629	0.225*	4858.2	243.6-infinite
	SPO17	100	9.3	9.1	0.477	0.637	0.251*	928.7	200.6-infinite
	Average		9.0	8.6	0.470	0.632	0.256		

*Significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction

Isolation by distance (IBD) among samples within temporal collection was evaluated testing the correlation between genetic (linearized as $F_{ST}/(1-F_{ST})$; Rousset, 1997) and geographical distances (measured as the shortest coastline between two locations in kilometres using Google Earth) with a Mantel test with 1000 permutations by NTSYSpc v. 2.1 (Rohlf, 1993).

We investigated the direction and magnitude of relative migration between samples within temporal collections with div-Migrate online (Sundqvist et al., 2016). For each temporal collection, a relative migration network was generated using the effective number of migrants (N_m) estimator. A filter threshold was set to 0.4 in both 2016 and 2017.

Results

Statistical analysis of the cephalothorax length

Despite all individuals were considered to belong to the medium-sized commercial category by the fishermen, the total CL's range varied from 24.63 mm (in SPO16) to 47.18 mm (in PAL17) (Fig. 2). The Tukey's post hoc test indicated significant differentiation in 71 out of 91 pairwise comparisons (Supplementary Table S1). Furthermore, the unpaired t test performed

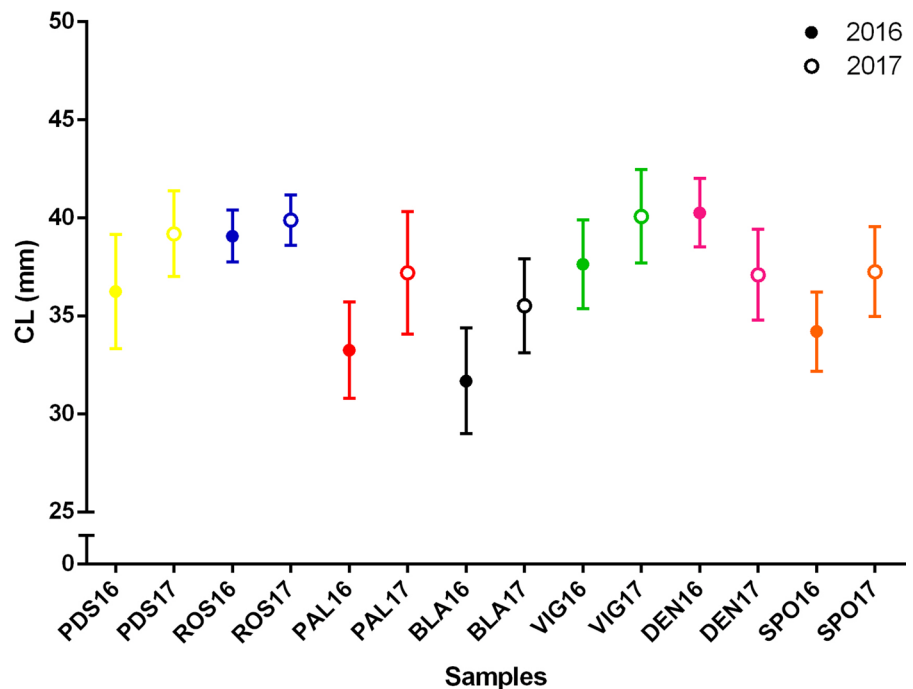
between the two temporal 2016 and 2017 collections showed a significant differentiation ($t=11.29$, degree of freedom = 1398, P value < 0.0001).

Diversity within locations

The number of alleles per locus (N_A) ranged between 2 and 23 in 2016 samples and from 2 to 24 in 2017 ones (Supplementary Table S2). The average N_A was 8.8 in 2016 and 9.1 in 2017 (Table 2). The mean allelic richness (A_R) was 8.2 in 2016 and 8.9 in 2017. The mean observed heterozygosity (H_o) was 0.461 in 2016, whereas in 2017 it was 0.479. The mean expected heterozygosity (H_e) was 0.628 in 2016 and 0.635 in 2017 (Table 2). Significant linkage disequilibrium was detected in the pair of loci *Aa1444* and *Aa681* in SPO17 after Bonferroni correction ($P < 0.0001$), but not in the other 13 samples.

Only three out of twelve loci (*Aa138*, *Aa496b* and *Aa1195*) showed no departure from the Hardy–Weinberg equilibrium (HWE) in any sample (Supplementary Table S2). The fourteen samples presented a significant deviation from HWE after the Bonferroni correction due to heterozygote deficit, as indicated by the observed positive F_{IS} values (Table 2). This fact could be a consequence of null alleles. Estimated allele frequencies for null alleles were generally low

Fig. 2 Mean cephalothorax length (CL) per sample, with standard deviation. Each colour represents a location. Sample codes as in Table 1



and they were not consistent in all loci and across samples (Supplementary Table S2). Only the locus *Aa818* was discarded from all subsequent analyses because it showed null alleles frequency higher than 0.2 in four samples. Additionally, significant departures from HWE might result from pooling specimens from distinct cohorts.

Geographical and temporal variation

Five out of the fourteen samples had an estimated infinite N_e , whereas in three it was close to 500 (Table 2), so $N_e = 500$ and $N_e = 10,000$ were selected to assess the statistical power of our loci to detect

genetic differentiation. The evaluation of the statistical power estimated a high probability (> 99%) of detecting structure for 0.001–0.003 F_{ST} values, suggesting that our test would detect a real population structure if true estimates of F_{ST} were at this level. Nevertheless, AMOVA results did not detect significant genetic divergence either in the 2016 or in 2017 ($F_{ST} = 0.0013$, $P > 0.05$ and $F_{ST} = 0.0003$, $P > 0.05$, respectively) (Table 3). Likewise, analyses of pairwise genetic differentiation between each pair of samples within each temporal collection showed no significant F_{ST} values ranging from 0.0000 to 0.0028 in both collections (Table 4). In addition, there were

Table 3 Hierarchical analysis of molecular variance (AMOVA) with P values (P)

Hypothesis	Source of variation	Sum of squares	Variance components	% Variation	F -statistic	P
2016 Unstructured	Among fishing grounds	25.25	0.004	0.128	$F_{ST} = 0.0013$	0.535
	Within samples	4287.30	3.432	99.872		
2017 Unstructured	Among fishing grounds	22.18	0.001	0.033	$F_{ST} = 0.0003$	0.920
	Within samples	4723.37	3.477	99.967		
2016 vs 2017	Between years	4.85	0.001	0.020	$F_{CT} = 0.0002$	0.164
	Among fishing grounds within year	47.43	0.003	0.078	$F_{SC} = 0.0008$	0.855
	Within samples	9010.67	3.456	99.902	$F_{ST} = 0.0010$	0.806

Table 4 Pairwise F_{ST} values between the seven samples (below the diagonal) and P values (above the diagonal) for the two temporal collections. Sample codes are given in Table 1

2016	PDS16	ROS16	PAL16	BLA16	VIG16	DEN16	SPO16
PDS16	–	0.194	0.828	0.986	0.984	0.992	0.996
ROS16	0.0028	–	0.326	0.239	0.505	0.551	0.382
PAL16	0.0000	0.0018	–	0.163	0.995	0.263	0.427
BLA16	0.0000	0.0022	0.0024	–	0.738	0.971	0.860
VIG16	0.0000	0.0013	0.0000	0.0000	–	0.874	0.953
DEN16	0.0000	0.0010	0.0021	0.0000	0.0000	–	0.988
SPO16	0.0000	0.0015	0.0011	0.0000	0.0000	0.0000	–
2017	PDS17	ROS17	PAL17	BLA17	VIG17	DEN17	SPO17
PDS17	–	0.692	0.417	0.787	0.979	0.962	0.630
ROS17	0.0002	–	0.218	0.133	0.548	0.507	0.467
PAL17	0.0013	0.0022	–	0.663	0.835	0.955	0.757
BLA17	0.0000	0.0028	0.0003	–	0.685	0.948	0.784
VIG17	0.0000	0.0008	0.0000	0.0003	–	0.999	0.695
DEN17	0.0000	0.0008	0.0000	0.0000	0.0000	–	0.775
SPO17	0.0000	0.0011	0.0000	0.0000	0.0002	0.0000	–

Table 5 Pairwise F_{ST} and P values between sampling campaigns (winter 2016, summer 2016, winter 2017 and summer 2017) at each fishing ground

	Port de la Selva	Roses	Palamós	Blanes	Vilanova i la Geltrú	Dénia	Santa Pola
2016 W vs S	F_{ST} value P	0.0030 0.354	0.0039 0.147	– 0.0020 0.938	– 0.0005 0.808	– 0.0007 0.904	– 0.0005 0.814
2016 S vs 2017 W	F_{ST} value P	– 0.0033 0.999	0.0028 0.298	0.0032 0.252	– 0.0012 0.873	0.0053 0.053	– 0.0017 0.981
2017 W vs S	F_{ST} value P	0.0006 0.595	0.0015 0.504	0.0067 0.060	– 0.0012 0.895	– 0.0009 0.893	0.0002 0.722
2016 W vs 2017 S (whole studied period)	F_{ST} value P	– 0.0009 0.905	0.0026 0.294	– 0.0014 0.916	– 0.0007 0.839	– 0.0006 0.874	0.0005 0.684

Winter females from Agulló et al. (2020), Winter (W), Summer (S)

no substantial differences in estimated F_{ST} values correcting, or not, for the presence of null alleles.

The AMOVA performed to compare the two temporal collections revealed no significant variation between them ($F_{CT}=0.0002$, $P>0.05$) (Table 3). Furthermore, no pairwise F_{ST} differed significantly from zero when comparing the two temporal samples of each location ($P>0.05$) (Supplementary Table S3). On the other hand, none of the F_{ST} between consecutive sampling campaigns (winter 2016, summer 2016, winter 2017 and summer 2017) at each of the seven fishing grounds was significant (Table 5) and no significant genetic differences were observed throughout the entire study period (winter 2016 vs. summer 2017).

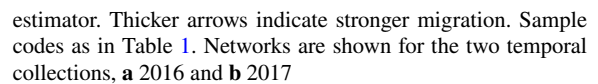
No association between genetic differentiation and geographical distance was detected either in 2016 ($r=-0.1925$, $P=0.228$) or in 2017 samples ($r=-0.2861$, $P=0.138$). Results of migration indicated a high level of genetic connectivity among all samples at the two summer seasons, without a pattern relating the level of connectivity with geographical proximity (Fig. 3).

Discussion

Diversity within locations

The mean observed and expected heterozygosities are in accordance with the previous studies using the same set of microsatellite loci and the same species ($H_o=0.470$, $H_e=0.632$, Table 2) ($H_o=0.458$, $H_e=0.628$, Heras et al., 2019; $H_o=0.443$, $H_e=0.611$ recalculated excluding *Aa421*, Planella et al., 2019; $H_o=0.468$, $H_e=0.633$, Agulló et al., 2020). The mean number of alleles per locus ($N_A=9.0$, Table 2) was slightly higher than the values obtained in Heras et al. (2019) ($N_A=7.8$) and Planella et al. (2019) ($N_A=7.9$ recalculated excluding *Aa421*) due to the higher sample size of the present study ($N=494$ in Heras et al., 2019; $N=111$ in Planella et al., 2019). Indeed, the mean N_A was almost the same when analysing similar sample size in winter season ($N=1386$, $N_A=9.1$, Agulló et al., 2020).

The heterozygote deficit (i.e. F_{IS} positive values), which is responsible for the deviations from HWE found across the fourteen samples, is a common feature on penaeids (Heras et al., 2016 and references



samples was 37.04 ± 3.42 , which confirms the pooling of different cohorts in our samples.

The main objective of this study was to assess for the first time the divergence of the spawning females' grounds of *A. antennatus* during the reproductive period in the Mediterranean Subarea GSA6. Our results showed an overall regional genetic homogeneity confirming a high genetic connectivity among females between spawning aggregations (Fig. 3, Tables 3 and 4), as recently observed from samples captured during the winter season at the same fishing grounds, when smaller males and females contributed to the fishery (Agulló et al., 2020, but see below for local divergences). Our findings also agree with the previous studies indicating high genetic homogeneity among *A. antennatus* populations in the Western and Central Mediterranean Sea applying different genetic markers such as allozymes (Sardà et al., 1998), mitochondrial DNA (Maggio et al., 2009; Roldán et al.,

2009; Sardà et al., 2010; Fernández et al., 2011; Marra et al., 2015) and microsatellites (Cannas et al., 2012; Heras et al., 2019).

The high level of genetic connectivity that it has been reported in the species is probably due to passive dispersal by oceanographic currents and active migration. In the Northwestern Mediterranean Sea, the Northern Current (Fernández et al., 2005) could be considered as a dispersal mechanism for *A. antennatus*. Thus, blue and red shrimp larval stages have been found in the upper water layers (Carreton et al., 2019 and references therein), which means that they are likely dispersed by this surface current. Furthermore, juvenile and/or adult migration may contribute to genetic connectivity. Indeed, it has been proven that adults have the capability of movement, as they do seasonal displacements between the open slope and submarine canyons, mainly related to the spawning period (Sardà et al., 1997, 2004). Females and males could present a different degree of dispersal, as several genetic studies using microsatellite loci have indicated. Planella et al. (2019) found that Palamós' females mated with both males from the same location and males from other fishing grounds, suggesting a migration of *A. antennatus*' adult males from one location to another. Recently, Abras et al. (2021) detected significant juvenile males' incomes at the Palamós' fishing ground from neighbouring ones.

On the other hand, the temporal analyses showed that the regional patterns of population genetic structure remain stable for females not only between years (Table 3 and Supplementary Table S3) but also between seasons within years (Table 5) despite changes in the relative abundance of cohorts during winter and summer collections (Carbonell et al., 1999). By contrast, a small but significant differentiation was found between 2016 and 2017 winter collections, when sex ratios were close to 1:1 (Agulló et al., 2020). Thus, specific patterns of males' migration beyond passive larval dispersal could be the main reason for the temporal differentiation found in Agulló et al. (2020) at some of the studied fishing grounds. Cannas et al. (2012) had already pointed out a sex-biased dispersal favouring of *A. antennatus* females in the Central Mediterranean Sea.

Management implications

Fisheries management must take into account the conservation of genetic diversity to ensure the perpetuation of natural populations, avoiding their over-exploitation (Waples & Naish, 2009). Since variations in the genetic population structure, including a decrease in genetic variability, can occur in a short period of time, a temporal monitoring is recommended; both at levels of genetic diversity within the population and at the levels of connectivity, to study long-term changes (Carvalho & Hauser, 1994; Allendorf et al., 2008).

The effective population size (N_e) is a crucial parameter to draw inferences on the adaptive potential of the population and its value should not be lower than 1000 to ensure population sustainability and evolutionary potential, thus minimizing extinction risk (Frankham et al., 2014). *A. antennatus* fishing grounds in the GSA6 displayed moderate and high N_e values (Table 2), which means that the blue and red shrimp fishery would maintain the genetic variability in the long term.

On the other hand, even though our temporal analysis includes four sampling campaigns in two years, 2016 and 2017, more than 2000 female individuals were genotyped. The genetic information obtained is a forceful estimation of the genetic connectivity and the temporal stability of the blue and red shrimp populations in the GSA6.

The low and non-significant differentiation detected among females from distant *A. antennatus* fishing grounds indicates a high gene flow that would limit local adaptation (Hauser & Carvalho, 2008). Therefore, we advise considering *A. antennatus* in the GSA6 as a single management unit, although the boundaries could be expanded beyond the limits of GSA6 if genetic homogeneity was detected in adjacent Mediterranean Subareas. Nevertheless, because of genetic instability indicated at some fishing grounds between consecutive winter samples by Agulló et al. (2020), when males are present (Carbonell et al., 1999), and females show increased dispersal (Cannas et al., 2012), keeping each fishing ground under local monitoring is needed for a sustainable exploitation of *A. antennatus*. Thus, the establishment of a fishery closure for the blue and red shrimp during winter in the GSA6 as declared for this and other Spanish demersal fisheries (Boletín Oficial

del Estado, 2020) is likely to improve male recruitment and reproduction in the next spawning season. Finally, a future management plan of the blue and red shrimp fishery integrating genetic data, biological information and hydrodynamic modelling would allow the sustainable exploitation of the fishing grounds (Ward, 2000; Reiss et al., 2009).

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Data and material availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent to participate Not applicable.

Consent for publication Not applicable.

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