Vol. 615: 133–142, 2019
https://doi.org/10.3354/meps12929

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published April 18

Low genetic connectivity in a fouling amphipod among man-made structures in the southern North Sea

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ABSTRACT: Offshore environments are increasingly invaded by man-made structures that form hard-substrate habitats for many marine species. Examples include oil and gas platforms, wind turbines and shipwrecks. One of the hypothesised effects is an increased genetic connectivity among natural populations due to new populations growing on man-made structures that may act as stepping stones. However, few data are available on genetic connectivity among organisms inhabiting artificial offshore structures. Here, we present a study on the common fouling amphipod *Jassa herdmani* from offshore structures in the southern North Sea. Partial mitochondrial DNA sequences (cytochrome-*c*-oxidase 1, N = 514) were obtained from artificial structures at 17 locations in the southern North Sea, including 13 shipwrecks, 2 wind turbines and 2 platforms. Samples from these locations were significantly differentiated, meaning that strong population structure exists for this species in the area. Levels of intraspecific variation were consistent with stable population sizes. No evidence was found for isolation by distance. Using coalescent simulations, the oldest population subdivision events were estimated to date back to the time the study area was flooded following the Last Glacial Maximum. We therefore tentatively conclude that *J. herdmani* may have colonised man-made structures from previously existing populations on the sea floor, and that the increase in offshore installations has not led to an overall increase in genetic connectivity for this species.

KEY WORDS: Genetic structure · Connectivity · Offshore oil platform · Offshore wind farm · Amphipod · Biofouling · Gene flow

1. INTRODUCTION

Offshore man-made hard structures such as the submerged parts of oil and gas platforms and offshore wind turbines, but also navigational buoys and shipwrecks, form suitable but artificial habitat for biological hard-substrate communities (Firth et al. 2016, Bishop et al. 2017). In this way, the offshore environ-

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ment is a growing extension of naturally occurring hard bottom substrates. Very little is known to date about the extent to which species are able to disperse among these offshore structures (i.e. their connectivity) (but see Mauro et al. 2001, Atchison et al. 2008, Fauvelot et al. 2009, 2012, Sammarco et al. 2012, 2017). However, this is important knowledge for protection and management of offshore ecosystems as well as for decision-making concerning the offshore structures themselves (Duarte et al. 2013, Adams et al. 2014). The structures may function as stepping stones for dispersal of species that are otherwise unable to reach particular locations by lack of intermediate settlement opportunities (Adams et al. 2014). This may facilitate the spread of non-indigenous species as well as indigenous ones or species of conservation value (Gass & Roberts 2006, De Mesel et al. 2015).

The small tube-dwelling amphipod crustacean *Jassa herdmani* (Walker, 1893) is a common and native component of fouling communities on artificial structures in the southern North Sea together with its congener *J. marmorata* Holmes, 1905 (De Mesel et al. 2015). *J. herdmani* occurs mainly on shipwrecks and on the deeper parts of vertical structures, such as the foundations of wind turbines and platforms, where the species can reach remarkably high abundances of more than 10^6 ind. m^{-2} (e.g. Zintzen et al. 2008a, Krone et al. 2013, Coolen et al. 2018). Surprisingly, although *J. herdmani* has been reported to co-occur with *J. marmorata* and *J. falcata* (Montagu, 1808) in the inner German Bight, it was not found on the natural rock substrates in areas such as the Borkum reef grounds (near site ST0729, see Fig. 1) (Beermann 2014, Coolen et al. 2015).

Most amphipods in temperate seas exhibit high fecundities with multiple broods per year allowing for high secondary production (Sheader & Chia 1970, Sheader 1981, Highsmith & Coyle 1991). Furthermore, short generation times and a holobenthic life cycle due to the direct development of amphipod embryos facilitate successful colonisation and rapid production of dense populations in *Jassa* species (Beermann & Purz 2013, Beermann 2014). *Jassa* populations are characterized by a marked short-distance dispersal of juveniles (Franz & Mohamed 1989). However, older juveniles and adults can exhibit long-distance dispersal under certain conditions, drifting with the water surface layer, and may colonise new substrates in this way (Havermans et al. 2007). In the southern North Sea, hard-substrate habitats are predominantly restricted to anthropogenic constructions such as shipwrecks, foundations of wind turbines and oil and gas platforms, and buoy moorings. These suitable substrates for *Jassa* are surrounded by soft sediments, and *J. herdmani* populations are consequently characterized by patchy distributions.

Population structure and genetic connectivity have thus far not been studied for *J. herdmani*. The closely related *J. marmorata* was studied for 2 allozyme loci at 2 nearby (ca. 8 km apart) on-shore locations, and was found not to be differentiated (McDonald 1991). Two *Gammarus* spp. amphipds, whose life cycle and ecology resemble that of *Jassa* spp., showed population structure and reduced levels of genetic diversity consistent with postglacial demographic expansion (Krebes et al. 2011). In partial contrast to its known ability to be an effective coloniser, we hypothesised that connectivity between local *J. herdmani* populations is limited to adjacent platforms or nearby natural habitats and that we would find a signal of isolation by distance. The southern North Sea region was formed and recolonised relatively recently, after the Last Glacial Maximum (LGM). We therefore expected to find signatures of population subdivision dating from after that time.

The aim of the current project was to examine whether a common species of offshore fouling communities displays signatures of genetic connectivity among offshore man-made structures. For this purpose, we analysed DNA sequences from *J. herdmani* specimens sampled at shipwrecks, wind turbines and oil and gas platforms in the southern North Sea.

2. MATERIALS AND METHODS

2.1. Sample collection

Samples were collected in 2015 and 2016 at 22 locations by divers and during maintenance activities on wind turbine foundations, jackets of oil and gas platforms, navigational buoys and shipwrecks in the southern North Sea (Table 1). Sample depth ranged from 0 to 46 m overall, while within a location it varied between 0 and 5 m away from the depth reported in Table 1. Samples were collected opportunistically, from an area of several m^2 on shipwrecks, to samples of 100 cm^2 on some installations and from dive suits after resurfacing of divers. After collection, samples were either stored in 95% ethanol or frozen directly at −20°C. Frozen samples were stored at −80°C after transportation to the laboratory. *Jassa herdmani* occurs alongside *J. marmorata* in the study area, and the species were separated based on their DNA sequences (see Section 2.3 below).

Table 1. *Jassa herdmani* sampling locations with genetic diversity statistics in the southern North Sea. N: number of individuals genotyped; *H*: haplotype diversity; ≠: nucleotide diversity; *D*: Tajima's *D*; F_s: Fu's F_s; na: not applicable, none of the *D* or F_s values differed significantly from 0

Sample	Type	Date (dd-mm-yy)	Depth (m)	Latitude (°N)	Longitude (°E)	N	*H* (SD)	≠ (SD)	*D*	F_s
SP1033	Platform	28-06-2016	0–26	53.40	4.20	15	0.800 (0.077)	0.00605 (0.00359)	−0.767	1.053
SP0654	Platform	20-10-2015	0–30	54.85	4.69	38	0.741 (0.055)	0.00778 (0.00428)	0.640	2.813
ST0725	Turbine	23-09-2015	4	55.19	7.16	33	0.856 (0.040)	0.00550 (0.00318)	0.163	−1.228
ST0729	Turbine	29-06-2015	5	53.69	6.50	41	0.746 (0.061)	0.00639 (0.00360)	−1.043	−2.082
SW0566	Wreck	11-06-2015	30	52.77	4.21	15	0.562 (0.095)	0.00428 (0.00268)	0.537	3.888
SW0569	Wreck	11-06-2015	30	53.12	4.21	17	0.221 (0.121)	0.00168 (0.00129)	−0.820	3.034
SW0932	Wreck	11-06-2016	34	52.49	3.28	36	0.732 (0.045)	0.00390 (0.00238)	1.472	0.668
SW0933	Wreck	08-06-2016	30	51.98	3.50	24	0.714 (0.067)	0.00384 (0.00239)	0.574	1.713
SW0934	Wreck	15-06-2016	35	52.25	3.15	40	0.672 (0.051)	0.00331 (0.00208)	−0.705	1.109
SW0935	Wreck	14-06-2016	32	52.79	3.05	43	0.797 (0.042)	0.00431 (0.00257)	−0.576	−2.327
SW0936	Wreck	17-06-2016	46	51.77	2.84	41	0.795 (0.038)	0.00368 (0.00226)	0.106	0.688
SW0937	Wreck	17-06-2016	30	51.83	2.82	36	0.675 (0.081)	0.00309 (0.00197)	0.153	−1.720
SW0939	Wreck	16-06-2016	42	52.08	2.67	36	0.821 (0.036)	0.00428 (0.00257)	0.884	−0.519
SW0940	Wreck	12-06-2016	32	52.51	3.32	45	0.778 (0.043)	0.00349 (0.00216)	0.327	−0.106
SW0941	Wreck	10-06-2016	28	52.44	3.73	17	0.868 (0.068)	0.00483 (0.00294)	0.271	−2.102
SW0942	Wreck	11-06-2016	40	52.61	3.08	22	0.736 (0.060)	0.00361 (0.00228)	0.761	1.357
SW0943	Wreck	15-06-2016	32	52.25	3.04	15	0.867 (0.057)	0.00423 (0.00265)	1.051	−0.911
Godewind	Turbine	07-10-2016	0	53.99	7.06	1	na	na	na	na
SP1009	Platform	24-06-2016	4–13	53.39	4.20	5	na	na	na	na
BARD1	Turbine	27-05-2016	0	54.31	5.94	4	na	na	na	na
SP0225	Platform	12-10-2014	0–20	53.40	4.20	4	na	na	na	na
ST0727	Turbine	30-06-2015	4	53.70	6.51	1	na	na	na	na

2.2. Molecular procedures

DNA was isolated from entire *Jassa* spp. individuals using the Qiagen Tissue Kit following the manufacturer's protocol. DNA concentrations were quantified by using the Tecan Freedom Evo and qualified on 1% agarose gels. DNA was diluted to 5 ng μl^{-1} and amplified with primers jgLCO-M13F (PCR) 16-001 (5'-TGT AAA ACG ACG GCC AGT TIT CIA CIA AYC AYA ARG AYA TTG G-3') and jgHCO-M13R (PCR) 16-002 (5'-CAG GAA ACA GCT ATG ACT AIA CYT CIG GRT GIC CRA ARA AYC A-3'). PCR reactions were performed in 12 µl using One TAQ solution containing 0.1 ng μl^{-1} BSA. Initial denaturation was done at 94°C for 5 min, followed by 50 cycles of denaturation at 94°C for 45 s, annealing at 43°C for 45 s and extension at 72°C for 80 s, with a final elongation step of 72°C for 7 min. PCR products were checked on 1% agarose gels before purification using Millipore Multiscreen plates. Purified PCR product was sequenced using the M13 Forward primer M13F (5'-TGT AAA ACG ACG GCC AGT-3') and Big Dye v3.1. Sequencing reaction products were purified by precipitation with Na Ac-EDTA and 100% ethanol and dissolved in 10 µl formamide and analysed on a 48-capillary ABI fragment analyser. Sequences were analysed using the Staden package (Staden et al. 2000).

2.3. Data analyses

Sequences were aligned manually in BioEdit (Hall 1999). *J. marmorata* sequences were identified by comparing to available GenBank sequences; this could be done unequivocally because the cytochrome-*c*-oxidase I (COI) sequence difference between *J. herdmani* and *J. marmorata* is approximately 20% (Raupach et al. 2015). Haplotypes and haplotype frequencies for *J. herdmani* per sample were extracted from the alignment using a custom Python script (Luttikhuizen 2019). Amino acid translation of codons was examined using MEGA v. 7.0.21 (Kumar et al. 2016). All population genetic analyses were carried out in Arlequin v. 3.5 (Excoffier & Lischer 2010). Population structure was analysed using 1-way analysis of molecular variance (AMOVA), and pairwise levels of population differentiation among all sample pairs were estimated as pairwise Φ_{ST}. Significance levels of Φ_{ST} values were evaluated on the basis of 10 000 random permutations of the data and Bonferroni correction for multiple testing. Hierarchical AMOVAs were constructed to test for genetic differentiation between wrecks versus platforms and turbines, and for year of sampling (2015 versus 2016). A minimum spanning network among haplotypes was estimated using pairwise numbers of nucleotide

differences as a genetic distance measure. Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) were estimated to test for recent population expansion (using 10 000 permutations).

To test for isolation by distance, pairwise Φ_{ST} values were compared with linear distances between sampling stations. The latter were calculated using the package 'Fossil' version 0.3.7 in R version 3.4.3 (R Core Team 2018). Correlation between the Φ_{ST} matrix and the linear distances matrix was evaluated with a Mantel test and 10 000 permutations in R. To visualise heterogeneity among samples, a multidimensional scaling plot (MDS) was made in R.

Divergence time estimates were made by simulating population subdivision following a coalescent isolation-with-migration approach (Hey & Nielsen 2007, Sethuraman & Hey 2016). As a molecular clock, we used 2.35% sequence divergence per million years, as estimated for COI across a range of crustacean species (see Krebes et al. 2011 and references therein). Molecular clock estimates for crustaceans vary from 1.4 to 3.1% and are not different from molecular clock estimates for the broader taxonomic group of the arthropods, e.g. 2.0% for beetles (Juan et al. 1995) and 2.3% for butterflies (Brower 1994). Taking into account that *J. herdmani* has a shorter generation time than the typical 1 yr for crustaceans, and assuming it to be 3 times as short, we arrived at a mutation rate per year per 658 bp locus of 2.32×10^{-5} following the approach of Papadopoulos et al. (2005) and Luttikhuizen et al. (2008). Coalescent simulations were run using the IMa2 Markov chain Monte Carlo (MCMC) implementation with the HKY mutation model to account for heterogeneity among sites, which is crucial for mitochondrial data (Hasegawa et al. 1985), 10 heated chains with geometric heating, 5 million burn-in steps and saving 100 000 genealogies interspaced with 100 steps.

Population divergence times were estimated for a set of 3 sample pairs that had among the highest pairwise Φ_{ST} values in order to gauge what the oldest splitting times among our studied locations may have been. These pairs were: SW059–SW0933 (Φ_{ST} = 0.411), SP1033–SW0932 (Φ_{ST} = 0.338) and SW0933–SW0940 (Φ_{ST} = 0.334).

3. RESULTS

A total of 529 partial COI sequences were obtained from 22 locations and cropped to a length of 658 bp (Table 1). Among these, 44 different haplotypes were detected (GenBank accession numbers MH052599–MH052642). Five samples with fewer than 15 individuals sequenced were omitted from the analyses, leaving 42 haplotypes among 514 sequenced individuals in the final data set (Table S1 in the Supplement at www.int-res.com/articles/suppl/m615p133_supp.xlsx). Fig. 1 shows the spatial distribution of the 42 haplotypes in the study area, and Fig. 2 shows their minimum spanning network.

The 42 haplotypes totalled 27 variable sites. All except 1 of the substitutions were synonymous, and the non-synonymy of the only exception is questionable as it concerns a change from AGG to GGG in haplotypes 35 (1 individual at location ST0729) and 38 (1 individual at location SW0935), which may have a different translation in some Arthropoda than in the standard invertebrate mitochondrial code (Abascal et al. 2006). Because of this, and because none of the mutations translated to a frame shift and sequence

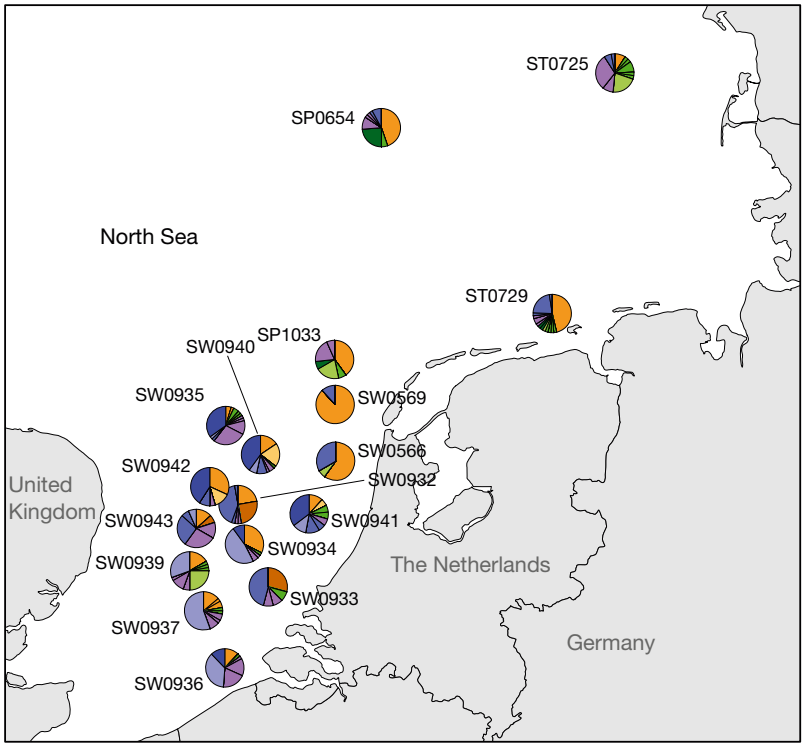

Fig. 1. Distribution of sampling locations showing spatial distribution of cytochrome-*c*-oxidase I (COI) haplotypes across the southern North Sea for *Jassa herdmani*. Details of the samples are given in Table 2. Note that only samples of sufficient size (N ≥ 15) are shown. Haplotype colours correspond to those in Fig. 2

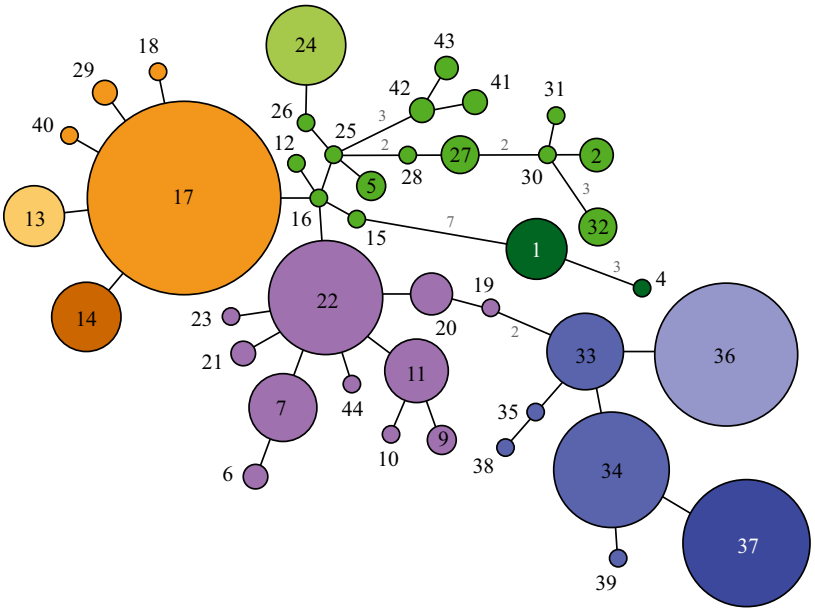

Fig. 2. Haplotype minimum spanning network among partial cytochrome-*c*-oxidase I (COI) sequences for *Jassa herdmani*. Circle area is proportional to frequency of occurrence. Numbers in black or white denote haplotype identity; branch lengths are 1 bp substitution unless otherwise indicated (in grey numbers). Haplotype colours correspond to those in Fig. 1. Colours were chosen to reflect relatedness in the haplotype network. Note that only the 42 haplotypes from samples of sufficient size (N ≥ 15) are included here, which means that haplotype numbers 3 and 8 are not shown

length was as expected, we can conclude that we did not sequence any pseudogenes.

AMOVA showed that genetic variation was significantly differentiated among sampling locations, with an overall Φ_{ST} of 0.159 ($p < 0.00001$; Table 2). Pairwise Φ_{ST} values were significantly >0 in 84 of the total of 136 comparisons (Bonferroni corrected p_{adj} = 0.00037; Table 3). A 2-level AMOVA with 2 groups as the upper level (shipwrecks versus platforms and turbines, which coincides with a north–south split) showed a significant difference associated with this upper level (Φ_{CT} = 0.0613, p = 0.0144) as well as among samples within these groups (Φ_{SC} = 0.137, p < 0.00001). A second 2-level AMOVA with sampling year (2015 versus 2016) as the upper level similarly showed a significant difference at this upper level (Φ_{CT} = 0.0398, p = 0.0315) and again also among samples within years (Φ_{SC} = 0.144, p < 0.00001). Fig. 3 shows the MDS for the *Jassa herdmani* COI sequences among the 17 sampling locations, depicting the variation associated with sampling year, latitude and substrate type.

None of the Tajima's *D* or Fu's F_s values differed significantly from 0, which is consistent with stable population sizes (Table 1). Linear distances between sampling locations did not correlate with pairwise Φ_{ST} values based on a Mantel test (Mantel r = –0.00315, ns), meaning that no evidence for an isolation-by-distance effect was seen in the data (Fig. 4).

Table 2. Analyses of molecular variance (AMOVA) for *Jassa herdmani* partial cytochrome-*c*-oxidase 1 (COI) sequences

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation index	p
A. One-level AMOVA						
Among samples	16	155.323	0.27524	15.92		
Within samples	497	722.402	1.45353	84.08		
Total	513	877.726	1.72877		Φ_{ST} = 0.159	<0.00001
B. Two-level AMOVA: shipwrecks versus platforms and turbines						
Source of variation						
Between structures	1	30.521	0.11001	6.13	Φ_{CT} = 0.0613	0.0144
Among samples within structures	15	124.803	0.23140	12.89	Φ_{SC} = 0.137	<0.00001
Within samples	497	722.402	1.45353	80.98		
Total	513	877.726	1.79493		Φ_{ST} = 0.190	0.00001
C. Two-level AMOVA: 2015 versus 2016						
Source of variation						
Between years	1	24.208	0.07036	3.98	Φ_{CT} = 0.03978	0.0315
Among samples within years	15	131.116	0.24484	13.84	Φ_{SC} = 0.144	<0.00001
Within samples	497	722.402	1.45353	82.18		
Total	513	877.726	1.76873		Φ_{ST} = 0.178	<0.00001

Table 3. Pairwise comparison of population genetic differentiation (Φ_{ST}) for *Jassa herdmani* among 17 locations in the southern North Sea. Values in **bold** are significantly different from 0 after Bonferroni correction. Details of the samples are given in Table 1

	SP1033	SP0654	ST0725	ST0729	SW0566	SW0569	SW0932	SW0933	SW0934	SW0935	SW0936	SW0937	SW0939	SW0940	SW0941	SW0942
SP1033	–															
SP0654	0.053	–														
ST0725	0.021	**0.124**	–													
ST0729	0.078	0.044	**0.113**	–												
SW0566	**0.152**	0.095	**0.206**	−0.015	–											
SW0569	**0.206**	0.137	**0.303**	0.074	0.077	–										
SW0932	**0.338**	**0.222**	**0.333**	**0.091**	0.055	**0.293**	–									
SW0933	**0.334**	**0.231**	**0.310**	**0.142**	**0.187**	**0.411**	0.089	–								
SW0934	**0.246**	**0.150**	**0.238**	**0.061**	0.100	**0.267**	**0.231**	**0.304**	–							
SW0935	**0.184**	**0.143**	**0.127**	**0.083**	**0.158**	**0.326**	**0.253**	**0.307**	**0.097**	–						
SW0936	**0.191**	**0.140**	**0.150**	**0.073**	**0.157**	**0.317**	**0.263**	**0.295**	0.043	0.015	–					
SW0937	**0.238**	**0.141**	**0.220**	0.075	**0.150**	**0.318**	**0.267**	**0.305**	−0.008	**0.083**	0.016	–				
SW0939	0.042	**0.113**	0.048	**0.082**	**0.152**	**0.271**	**0.304**	**0.306**	**0.127**	0.097	**0.085**	**0.110**	–			
SW0940	**0.347**	**0.207**	**0.320**	**0.103**	0.123	**0.343**	**0.189**	**0.334**	**0.071**	**0.117**	0.133	0.121	**0.247**	–		
SW0941	**0.272**	**0.165**	**0.249**	0.073	0.100	**0.359**	0.147	**0.242**	0.063	0.070	0.085	0.094	**0.187**	0.006	–	
SW0942	**0.282**	**0.155**	**0.283**	0.053	0.048	**0.278**	0.147	**0.311**	0.054	0.110	0.124	0.114	**0.216**	−0.019	0.010	–
SW0943	**0.138**	0.092	**0.125**	0.018	0.074	**0.289**	0.127	0.104	0.095	0.062	0.040	0.069	0.088	**0.177**	0.100	0.148

Divergence time estimates based on coalescent simulations for 3 of the most strongly differentiated sample pairs ranged from 3578 to 11 080 yr ago (Table 4). Simultaneously estimated migration rates were very low and ranged from 0.060 to 0.61 (Table 4).

4. DISCUSSION

Our results show that offshore populations of the common fouling amphipod *Jassa herdmani* on man-made structures are strongly genetically differentiated in the southern North Sea, with an overall Φ_{ST} of 0.156 (Tables 2A & 3). Our first hypothesis that gene flow among populations of *J. herdmani* is limited is thus corroborated, but the second hypothesis of isolation by distance is not. Supporting the third hypothesis, the observed population structure was indeed estimated to have been formed after the LGM. Man-made structures therefore do not appear to facilitate genetic connectivity for this species in the southern North Sea area.

Hierarchical AMOVA indicated that most of the population structure is found at the among-sample level (Table 2). In addition, small but significant levels of population structure could be attributed to a north–south difference (Table 2A, Fig. 3), a difference of shipwrecks versus platforms and turbines (Table 2B, Fig. 3) and to the 2 sampling years (Table 2C, Fig. 3). As this study was not designed to test for any of these factors (north–south, type of habitat, sampling year), we also cannot discriminate among them post hoc. This can be seen in Fig. 3: e.g. in 2015, more northerly samples were taken than in 2016, and more shipwrecks were sampled at lower latitudes. If there was a genetic north–south subdivision, this should have been reflected in an isolation-by-distance effect, which was not observed (Fig. 4). We conclude that there is no clear substructure for the study species in this region but instead most likely a mosaic pattern. Future research should employ a more rigorous sampling design that includes a north–south gradient for several types of habitats, repeated in different years, in order to discriminate among these factors.

Some of the deepest differentiation detected was estimated to trace back in time to the period soon after the LGM (Table 4). The dates of population subdivision should be interpreted with caution, because they are based on data for a single, maternally inherited genetic locus only. Future work should include data from additional independent, preferably nuclear, loci. Further uncertainty stems from the application

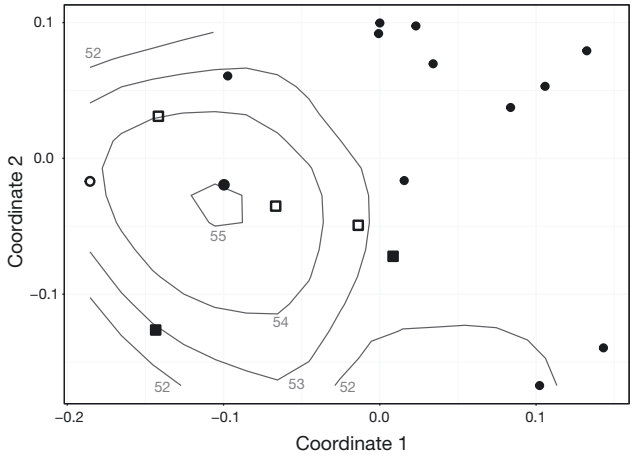

Fig. 3. Multidimensional scaling plot for *Jassa herdmani* samples consisting of partial cytochrome-*c*-oxidase 1 sequences collected at 17 offshore southern North Sea locations. Grey lines with grey numbers are latitudinal isolines; open symbols are platforms and turbines and closed symbols are shipwrecks; square symbols are samples collected in 2015 and round symbols in 2016

of a molecular clock to mitochondrial DNA and the assumptions made when using such a clock (Ballard & Whitlock 2004). The southern North Sea area was dry land during the LGM, called Doggerland (Coles 2000), connecting the British Isles with mainland Europe. Doggerland was flooded gradually, and the land connection disappeared around 8000 yr ago (Eisma et al. 1981). The dates obtained here for population subdivision in *J. herdmani* are remarkably consistent with that time: the oldest splits between populations are estimated to have happened 3500 to 11 000 yr ago (Table 4).

An alternative possibility for the observed population structure is the direct development of *J. herdmani* in combination with its high fecundity, which may lead to rapid local population turnover (Beermann & Purz 2013). The observed mosaic differences among our samples would then reflect a more recently originated structure. We deem the latter unlikely, because, while dating events using molecular clock estimates for a single gene comes with many uncertainties (Wilke et al. 2009), COI clock estimates are actually rather similar across different crustacean and even arthropod species (Brower 1994, Juan et al. 1995, Krebes et al. 2011). However, rapid local population turnover may have contributed to population divergence by essentially decreasing effective population size. We therefore tentatively conclude that at least part of the geologically recent population structure among populations of *J. herdmani* in the southern North Sea dates back to the time when the region was colonised by this species for the first time, i.e. following the flooding of Doggerland. *J. herdmani* is not able to survive on soft bottoms, which today comprise the majority of the North Sea seafloor. The present-day distribution of *J. herdmani* in the North Sea is still fragmentarily known, partly due to the former taxonomic confusion within the genus (Conlan 1990), but confirmed locations include the coasts of Britain, Norway, Denmark, Germany, the Netherlands and Belgium (see Beermann & Franke 2011 and references therein). The species may have lived on boulder fields and flat oyster beds (Sas et al. 2018), and it has probably lived on shipwrecks ever since they became available (Zintzen et al. 2006, 2008b). The scarcity of natural hard bottoms may also have contributed to genetic differentiation of *J. herdmani* populations growing on natural (mostly coastal) hard substrates before the anthropogenic transformation with artificial hard substrates.

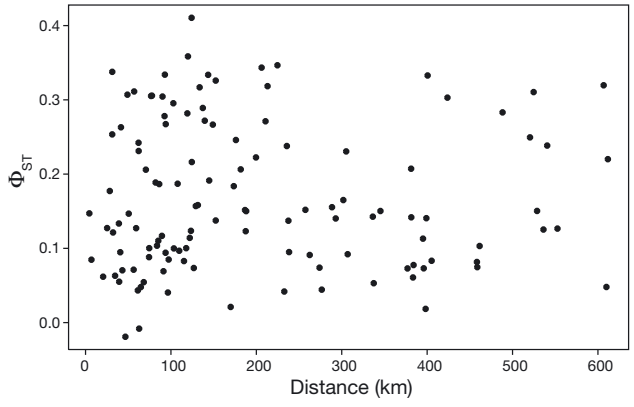

Fig. 4. Absence of isolation by distance among sampled *Jassa herdmani* locations in the southern North Sea region. Φ_{ST}: pair-wise level of population differentiation

The observation that the populations have probably been stable in size at all sampled locations (Table 1) provides further support for the idea that *J. herdmani* populations have survived in the southern North Sea ever since the habitat was formed. At an average temperature of 15°C, reproductively active females of *J. herdmani* should survive more than 3–4 mo (predation excluded), producing broods of up to 100 juveniles every 20 d and all year round (Beermann & Purz 2013, Beermann 2014). Thus, the generation time of *J. herdmani* is relatively short. As a result, individuals from the sampled locations may have originated from only few colonising individuals that built dense populations in a short time; in fact, even a single brooding female would have sufficed. However, the non-significant Tajima's *D* and Fu's F_s values (Table 1) and the large haplotypic diversity sug-

Table 4. Estimated divergence times and other parameters for *Jassa herdmani* based on coalescent isolation-with-migration simulations, carried out for 3 sample pairs that had among the highest pairwise Φ_{ST} values in order to gauge what the oldest splitting times among the studied locations may have been. Details of the samples are given in Table 1. 2Nm: number of migrants per generation

Sample 0	Sample 1	Divergence time (yr)	Migration rate 0>1 (2Nm)	Migration 1>0 (2Nm)	Population size 0	Population size 1	Ancestral population size
SW0569	SW0933	11080	0.31	0.058	5120	270	145778
SW0933	SW0940	5303	0.19	0.18	19132	6198	161946
SP1033	SW0932	3578	0.61	0.062	20748	8353	206676

gest that population sizes during such potential bottlenecks tend to be at least large enough to maintain most of the genetic variation.

The absence of pelagic larvae in this species' life cycle is consistent with our inference of low connectivity, and the dispersal potential for older *J. herdmani* (Havermans et al. 2007) apparently does not lead to an important amount of realised dispersal. The latter is the case not only for the present day but also for the longer, evolutionary time scale of several thousands of years, i.e. several tens of thousands of generations for *J. herdmani*.

In conclusion, this study adds to the few available studies on genetic connectivity among offshore man-made structures. The data presented here for the amphipod *Jassa herdmani* in the southern North Sea show that genetic connectivity among such structures is small. Future studies should focus on obtaining genetic data for more loci and on smaller spatial scales in order to identify the scale of genetic mixing.

Acknowledgements. Neptune Energy (previously ENGIE Exploration & Production Nederland BV) allowed and facilitated sampling their installations, and we are especially grateful to Ed Schmidt, Nathalie Kaarls, Ulf Sjöqvist, Maico Vrijenhoeff, Ben Waardenburg and Kees van Braak for their help arranging this cooperation. Much help in the field, e.g. collecting samples, allowing us access or performing analyses, was provided by many individuals and organisations, including the crew of the vessels 'Seamar Splendid' and 'Cdt. Fourcault', the Bluestream dive team, the Dive the North Sea Clean Foundation and its many volunteers, Roland Krone from Krone Projekte, and Bert Dibbits at Wageningen University & Research Animal Breeding and Genomics. We thank all of these individuals and organisations for their help. This work was supported by the Wageningen UR TripleP@Sea Innovation programme (grant number KB-14-007); NWO Domain Applied and Engineering Sciences (grant number 14494); the Nederlandse Aardolie Maatschappij BV, Wintershall Holding GmbH, Energiebeheer Nederland BV; and the oil and gas joint industry INSITE fund, through the RECON project.

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Editorial responsibility: Karen Miller, Hobart, Tasmania, Australia

Submitted: November 14, 2018; Accepted: March 8, 2019
Proofs received from author(s): April 12, 2019