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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 ship wrecks. 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, very little data is available on genetic connectivity among artificial offshore structures. Here, we present 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 17 locations in the southern North Sea, all artificial structures: 13 ship wrecks, two wind turbines and two platforms. Samples from these locations were found to be 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.

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 ship wrecks, form suitable but artificial habitat for biological hard-substrate communities (Firth et al. 2016, Bishop et al. 2017). The offshore environment is in this way 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). *Jassa herdmani* occurs mainly on ship wrecks 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 a million individuals per 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 ST0729 in Figure 1) (Beermann 2014, Coolen et al. 2015).

63 Most amphipods in temperate seas exhibit high fecundities with multiple broods per year allowing
64 for high secondary production (Sheader & Chia 1970, Sheader 1981, Highsmith & Coyle 1991).
65 Furthermore, short generation times and a holobenthic life cycle due to the direct development of
66 amphipod embryos facilitate successful colonisation and rapid production of dense populations in
67 *Jassa* species (Beermann & Purz 2013, Beermann 2014). *Jassa* populations are characterized by a
68 marked short-distance dispersal of juveniles (Franz & Mohamed 1989). However, older juveniles and
69 adults can exhibit long-distance dispersal under certain conditions, drifting with the water surface
70 layer and may colonize new substrates in that way (Havermans et al. 2007). In the southern North
71 Sea, the hard-substrate habitats are predominantly restricted to anthropogenic constructions such as
72 shipwrecks, foundations of wind turbines and oil and gas platforms, and buoy moorings. These
73 suitable substrates for *Jassa* are surrounded by soft sediments and *J. herdmani* populations are
74 consequently characterized by patchy distributions.
75
76 Population structure and genetic connectivity have thus far not been studied for *Jassa herdmani*. The
77 closely related *J. marmorata* was studied for two allozyme loci at two nearby (approx. 8 km apart)
78 on-shore locations, which were found not to be differentiated (McDonald 1991). Two amphipods
79 *Gammarus* spp., whose life cycle and ecology resemble that of *Jassa* spp., were found to show
80 population structure, and reduced levels of genetic diversity consistent with postglacial demographic
81 expansion (Krebes et al. 2011). In partial contrast to its known ability to be an effective colonizer, we
82 hypothesise that connectivity between local *J. herdmani* populations is limited to adjacent platforms
83 or nearby natural habitats and that we will find a signal of isolation-by-distance. The southern North
84 Sea region was formed and recolonized relatively recently, after the Last Glacial Maximum. We
85 therefore expect to find signatures of population subdivision dating from after that time.
86
87 The aim of the current project was to examine whether a common species of offshore fouling
88 communities displays signatures of genetic connectivity among offshore man-made structures. For

this purpose, we analysed DNA sequences from *J. herdmani* specimens sampled at ship wrecks, wind turbines and oil and gas platforms in the southern North Sea.

2. 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 meters overall, while within a location it varied between 0 and 5m 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 on 95% ethanol or frozen directly at -20^{o}C. Frozen samples were stored at -80^{o}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 sequence (see below).

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 5ng/µl and amplified with primers jgLCO-M13F (PCR) 16-001 (5'-TGTAAAACGACGGCCAGTTITCIACIAAYCAYAARGAYATTGG-3') and jgHCO-M13R (PCR) 16-002 ('5-CAGGAAACAGCTATGACTAIACYTCIGGRTGICCRAARAAYCA-3'). PCR reaction was performed in 12 µl using One TAQ solution containing 0.1 ng/µl 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-TGTAAAACGACGGCCAGT-3') and Big Dye v3.1.

115 Sequencing reaction products were purified by precipitation with Na Ac-EDTA and 100% ethanol and
116 dissolved in 10ul formamide and analysed on a 48 capillary ABI fragment analyser. Sequences were
117 analysed using the Staden package (Staden et al. 2000).
118
119 **2.3. Data analyses**
120 Sequences were aligned manually in BioEdit (Hall 1999). *Jassa marmorata* sequences were identified
121 by comparing to available Genbank sequences; this could be done unequivocally because the COI
122 sequence difference between *J. herdmani* and *J. marmorata* is approximately 20% (Raupach et al.
123 2015). Haplotypes and haplotype frequencies for *J. herdmani* per sample were extracted from the
124 alignment using custom Python script (Luttikhuizen 2019). Amino acid translation of codons was
125 examined using MEGA v. 7.0.21 (Kumar et al. 2016). All population genetic analyses were carried out
126 in Arlequin v. 3.5 (Excoffier & Lischer 2010). Population structure was analysed using one-way
127 Analysis of Molecular Variance (AMOVA) and pairwise levels of population differentiation among all
128 sample pairs was estimated as pairwise Φ_{ST}. Significance levels of Φ_{ST} values were evaluated on the
129 basis of 10,000 random permutations of the data and Bonferroni correction for multiple testing.
130 Hierarchical AMOVAs were constructed to test for genetic differentiation between wrecks versus
131 platforms and turbines, and for year of sampling (2015 versus 2016). A minimum spanning network
132 among haplotypes was estimated using pairwise numbers of nucleotide differences as genetic
133 distance measure. Tajima's D (Tajima 1989) and Fu's F_s (Fu 1996) were estimated to test for recent
134 population expansion (using 10,000 permutations).
135
136 To test for isolation by distance, pairwise Φ_{ST} values were compared with linear distances between
137 sampling stations. The latter were calculated using the package 'Fossil' version 0.3.7 in R version 3.4.3
138 (R Core Team 2018). Correlation between the Φ_{ST} matrix and the linear distances matrix was
139 evaluated with a Mantel test and 10,000 permutations in R. To visualise heterogeneity among
140 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, which are, 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 one year for crustaceans, and assuming it to be three times as short, we arrived at a mutation rate per year per 658 bp locus of $2.32 \cdot 10^{-5}$ following the approach by Papadopoulos *et al.*, (2005) and Luttikhuizen *et al.*, (2008). Coalescent simulations were run using the IMa2 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, five million burnin steps and saving 100,000 genealogies interspaced with 100 steps.

Population divergence times were estimated for a set of three 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 ($\Phi_{ST} = 0.411$), SP1033-SW0932 ($\Phi_{ST} = 0.338$) and SW0933-SW0940 ($\Phi_{ST} = 0.334$).

3. RESULTS

A total of 529 partial COI sequences were obtained from 22 locations and cropped to a length of 658 base pairs (Table 1). Among these, 44 different haplotypes were detected (Genbank accession numbers MH052599-MH052642). Five samples with less than 15 individuals sequenced were omitted from the analyses, leaving 42 haplotypes among 514 sequenced individuals in the final data set (Table S1). Figure 2 shows the minimum spanning network among the 42 haplotypes, and Figure 1

shows their spatial distribution in the study area. The colours of haplotypes in Figure 1 corresponds to those in Figure 2.

The 42 haplotypes totalled 27 variable sites. All except one 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 (one individual at location ST0729) and 38 (one 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 length was as expected, we can conclude that we did not sequence any pseudogenes.

Analysis of molecular variance (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}'s were significantly larger than zero in 84 of the total of 136 comparisons (Bonferroni corrected $p_{adj} = 0.00037$; Table 3). A two-level AMOVA with two groups as upper level (shipwrecks versus platforms and turbines, which coincides with a north-south split) showed that there is a significant difference associated with this upper level ($\Phi_{CT} = 0.0613$, $p = 0.0144$) as well as among samples within these groups ($\Phi_{SC} = 0.137$, $p < 0.00001$). A second two-level AMOVA with sampling year (2015 versus 2016) as upper level similarly also shows a significant difference at this upper level ($\Phi_{CT} = 0.0398$, $p = 0.0315$) and again also among samples within years ($\Phi_{SC} = 0.144$, $p < 0.00001$). Figure 3 is a multidimensional scaling plot (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 zero, which is consistent with stable population sizes (Table 1). Linear distance between sampling locations did not correlate with

192 pairwise Φ_{ST} values based on a Mantel test (Mantel *r* = -0.00315, n.s.), meaning that no evidence for
193 an isolation-by-distance effect was seen in the data (Figure 4).
194
195 Divergence time estimates based on coalescent simulations for three of the most strongly
196 differentiated sample pairs ranged from 3,578 to 11,080 years ago (Table 4). Simultaneously
197 estimated migration rates were very low and ranged from 0.060 to 0.61 (Table 4).
198

199 ## 4. DISCUSSION

200 Our results show that offshore populations on man-made structures of the common fouling
201 amphipod *Jassa herdmani* are strongly genetically differentiated in the southern North Sea with an
202 overall Φ_{ST} of 0.156 (Table 3 and Table 2A). Our first hypothesis that gene flow among populations of
203 *J. herdmani* is limited is thus corroborated, but the second one of isolation-by-distance is not.
204 Supporting the third hypothesis, the observed population structure was indeed estimated to have
205 been formed after the last glacial maximum. Man-made structures therefore do not appear to
206 facilitate genetic connectivity for this species in the southern North Sea area.
207
208 Hierarchical analyses of molecular variance (AMOVA) indicated that most of the population structure
209 is found at the among-sample level (Table 2). In addition, small but significant levels of population
210 structure could be be attributed to a north-south difference (Table 2A, Figure 3), a difference of
211 shipwrecks versus platforms and turbines (Table 2B, Figure 3), and to the two sampling years (Table
212 2C, Figure 3). As this study was not designed to test for any of these factors (north-south, type of
213 habitat, sampling year) we also cannot discriminate among them post-hoc. This can be seen in Figure
214 3: e.g., in 2015 more northerly samples were taken than in 2016, and more shipwrecks were sampled
215 at lower latitudes. If there was a genetic north-south subdivision, this should have been reflected in
216 an isolation-by-distance, which was not observed (Figure 4). We conclude that there is no clear
217 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 Last Glacial Maximum (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 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 years ago (Eisma et al. 2009). 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 3.5 to 11 thousand years 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 colonized by this species for the first time, i.e. following the flooding of Doggerland. *Jassa herdmani* is not able to survive on soft bottoms, which today comprises the majority of the North Sea seafloor. The present-day distribution of *J. herdmani*

244 in the North Sea is still fragmentarily known, partly due to the former taxonomic confusion within the
245 genus (Conlan 1990), but confirmed locations include the coasts of Britain, Norway, Denmark,
246 Germany, the Netherlands and Belgium (see Beermann and Franke, 2011 and references therein).
247 The species may have lived on bolder fields and flat oyster beds (Sas et al. 2018) and it has probably
248 lived on ship wrecks ever since they were around (Zintzen et al. 2006, 2008b). The scarcity of natural
249 hard bottoms may also have contributed to genetic differentiation of *J. herdmani* populations
250 growing on natural (mostly coastal) hard substrates before the anthropogenic transformation with
251 artificial hard substrates.
252
253 The observation that the populations have probably been stable in size at all sampled locations
254 (Table 1) provides further support for the idea that *Jassa herdmani* populations have survived in the
255 southern North Sea ever since the habitat was formed. At an average temperature of 15°C,
256 reproductively active females of *J. herdmani* should survive more than 3-4 months (predation
257 excluded), producing broods of up to 100 juveniles every 20 days and all year round (Beermann &
258 Purz 2013, Beermann 2014). Thus, the generation time of *J. herdmani* is relatively short. As a result,
259 individuals from the sampled locations may have originated from only few colonizing individuals that
260 built dense populations in a short time; in fact, even a single brooding female would have sufficed.
261 However, the non-significant Tajima's D and Fu's F_s's (Table 1) and the large haplotypic diversity
262 suggest that population sizes during such potential bottlenecks tend to be at least large enough to
263 maintain most of the genetic variation.
264
265 The absence of pelagic larvae in this species' life cycle is consistent with our inference of low
266 connectivity, and the dispersal potential for older *J. herdmani* (Havermans et al. 2007) apparently
267 does not lead to an important amount of realised dispersal. The latter is the case not only for the
268 present day but also for the longer, evolutionary time scale of several thousands of years - which
269 means 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.

Data accessibility

Table S1. Haplotype frequencies for 658 base pair cytochrome c oxidase I sequences for N = 514 *Jassa herdmani* individuals at 17 locations in the southern North Sea.

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Table 1. *Jassa herdmani* sampling locations with genetic diversity statistics in the southern North Sea; N = number of individuals genotyped; H = haplotype diversity; π = nucleotide diversity; s.d. = standard deviation; D = Tajima's D; F_s = Fu's F_s; n.a. = not applicable; none of the D or F_s values differ significantly from zero.

Sample	Type	Date	Depth (m)	Latitude (°N)	Longitude (°E)	N	H (s.d.)	π (s.d.)	D	F_s
SP1033	platform	28-06-2016	0-26	53.402722	4.2013444	15	0.800 (0.077)	0.00605 (0.00359)	-0.767	1.053
SP0654	platform	20-10-2015	0-30	54.852894	4.6949389	38	0.741 (0.055)	0.00778 (0.00428)	0.640	2.813
ST0725	turbine	23-09-2015	4	55.195	7.1583333	33	0.856 (0.040)	0.00550 (0.00318)	0.163	-1.228
ST0729	turbine	29-06-2015	5	53.69	6.498	41	0.746 (0.061)	0.00639 (0.00360)	-1.043	-2.082
SW0566	wreck	11-06-2015	30	52.766283	4.2129833	15	0.562 (0.095)	0.00428 (0.00268)	0.537	3.888
SW0569	wreck	11-06-2015	30	53.121	4.2071667	17	0.221 (0.121)	0.00168 (0.00129)	-0.820	3.034
SW0932	wreck	11-06-2016	34	52.494966	3.282189	36	0.732 (0.045)	0.00390 (0.00238)	1.472	0.668
SW0933	wreck	08-06-2016	30	51.979767	3.5012	24	0.714 (0.067)	0.00384 (0.00239)	0.574	1.713
SW0934	wreck	15-06-2016	35	52.246933	3.1502833	40	0.672 (0.051)	0.00331 (0.00208)	-0.705	1.109
SW0935	wreck	14-06-2016	32	52.789183	3.0528333	43	0.797 (0.042)	0.00431 (0.00257)	-0.576	-2.327
SW0936	wreck	17-06-2016	46	51.773183	2.8429667	41	0.795 (0.038)	0.00368 (0.00226)	0.106	0.688
SW0937	wreck	17-06-2016	30	51.83	2.8183333	36	0.675 (0.081)	0.00309 (0.00197)	0.153	-1.720
SW0939	wreck	16-06-2016	42	52.0801	2.6723833	36	0.821 (0.036)	0.00428 (0.00257)	0.884	-0.519
SW0940	wreck	12-06-2016	32	52.507883	3.3196667	45	0.778 (0.043)	0.00349 (0.00216)	0.327	-0.106
SW0941	wreck	10-06-2016	28	52.436683	3.7328333	17	0.868 (0.068)	0.00483 (0.00294)	0.271	-2.102
SW0942	wreck	11-06-2016	40	52.606833	3.0845167	22	0.736 (0.060)	0.00361 (0.00228)	0.761	1.357
SW0943	wreck	15-06-2016	32	52.2454	3.0425833	15	0.867 (0.057)	0.00423 (0.00265)	1.051	-0.911
Godewind	turbine	07-10-2016	0	53.98833	7.063333	1	n.a.	n.a.	n.a.	n.a.
SP1009	platform	24-06-2016	4-13	53.39321	4.201086	5	n.a.	n.a.	n.a.	n.a.
BARD1	turbine	27-05-2016	0	54.31048	5.939418	4	n.a.	n.a.	n.a.	n.a.
SP0225	platform	12-10-2014	0-20	53.4	4.2	4	n.a.	n.a.	n.a.	n.a.
ST0727	turbine	30-06-2015	4	53.69767	6.512167	1	n.a.	n.a.	n.a.	n.a.

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

A. One-level AMOVA					
Source of variation	*d.f.*	*Sum of squares*	*Variance components*	*Percentage of variation*	*Fixation index*
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 (p < 0.00001)
B. Two-level AMOVA: ship wrecks versus platforms and turbines					
Source of variation	*d.f.*	*Sum of squares*	*Variance components*	*Percentage of variation*	*Fixation index*
Between structures	1	30.521	0.11001	6.13	Φ_{CT} = 0.0613 (p = 0.0144)
Among samples within structures	15	124.803	0.23140	12.89	Φ_{SC} = 0.137 (p < 0.00001)
Within samples	497	722.402	1.45353	80.98	
Total	513	877.726	1.79493		Φ_{ST} = 0.190 (p < 0.00001)
C. Two-level AMOVA: 2015 versus 2016					
Source of variation	*d.f.*	*Sum of squares*	*Variance components*	*Percentage of variation*	*Fixation index*
Between years	1	24.208	0.07036	3.98	Φ_{CT} = 0.03978 (p = 0.0315)
Among samples within years	15	131.116	0.24484	13.84	Φ_{SC} = 0.144 (p < 0.00001)
Within samples	497	722.402	1.45353	82.18	
Total	513	877.726	1.76873		Φ_{ST} = 0.178 (p < 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 zero after Bonferroni correction.

	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

Table 4. Estimated divergence times and other parameters for *Jassa herdmani* based on coalescent isolation-with-migration simulations, carried out for three 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.

Sample 0	Sample 1	Divergence time (years)	Migration rate 0>1 (2Nm)	Migration 1>0 (2Nm)	Population size 0	Population size 1	Ancestral population size
SW0569	SW0933	11,080	0.31	0.058	5,120	270	145,778
SW0933	SW0940	5,303	0.19	0.18	19,132	6,198	161,946
SP1033	SW0932	3,578	0.61	0.062	20,748	8,353	206,676

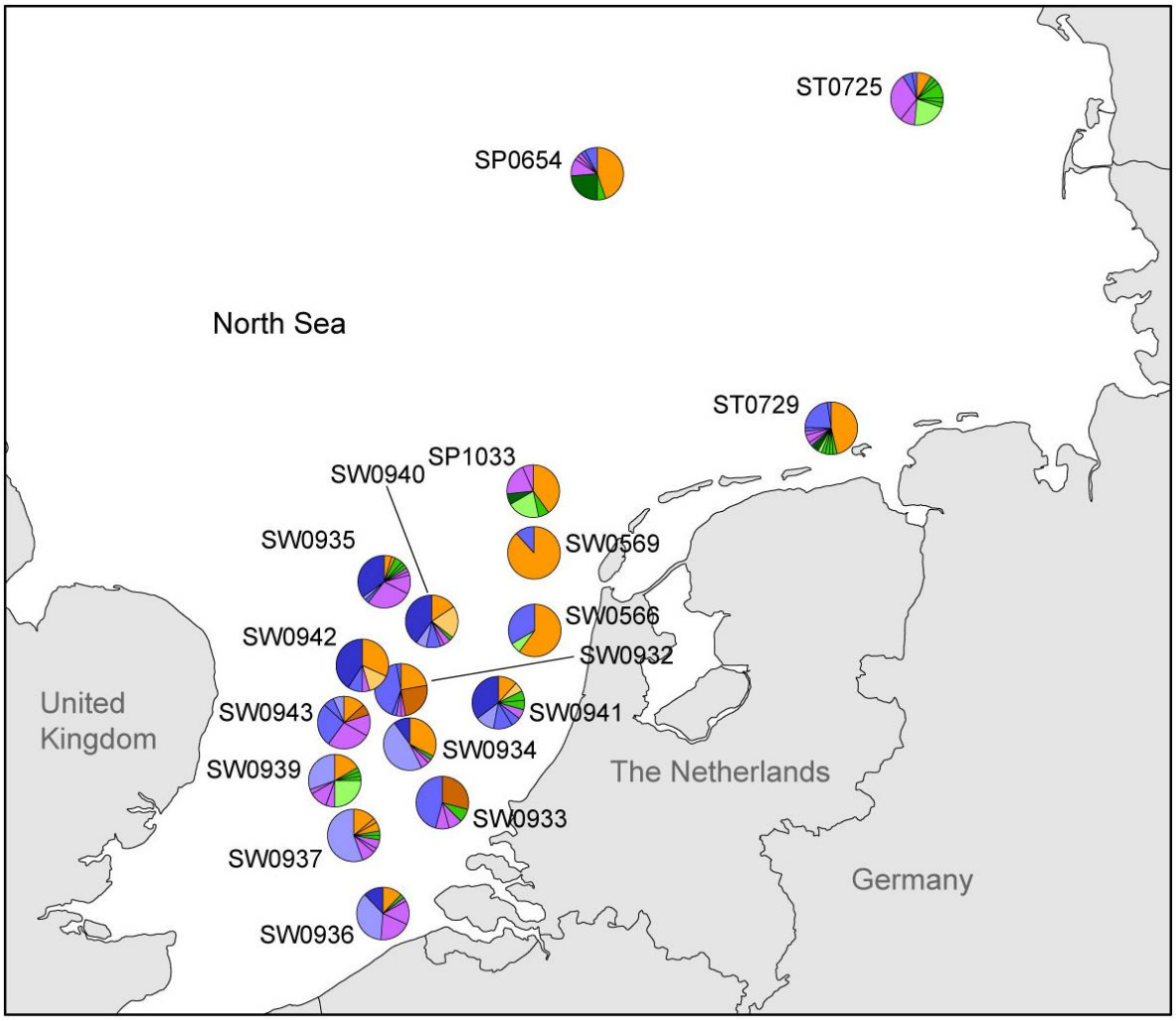

Figure 1. Distribution of sampling locations showing spatial distribution of COI haplotypes across the southern North Sea for *Jassa herdmani*. Note that only samples of sufficient size (N >= 15) are shown. Haplotype colours correspond to those in Figure 2.

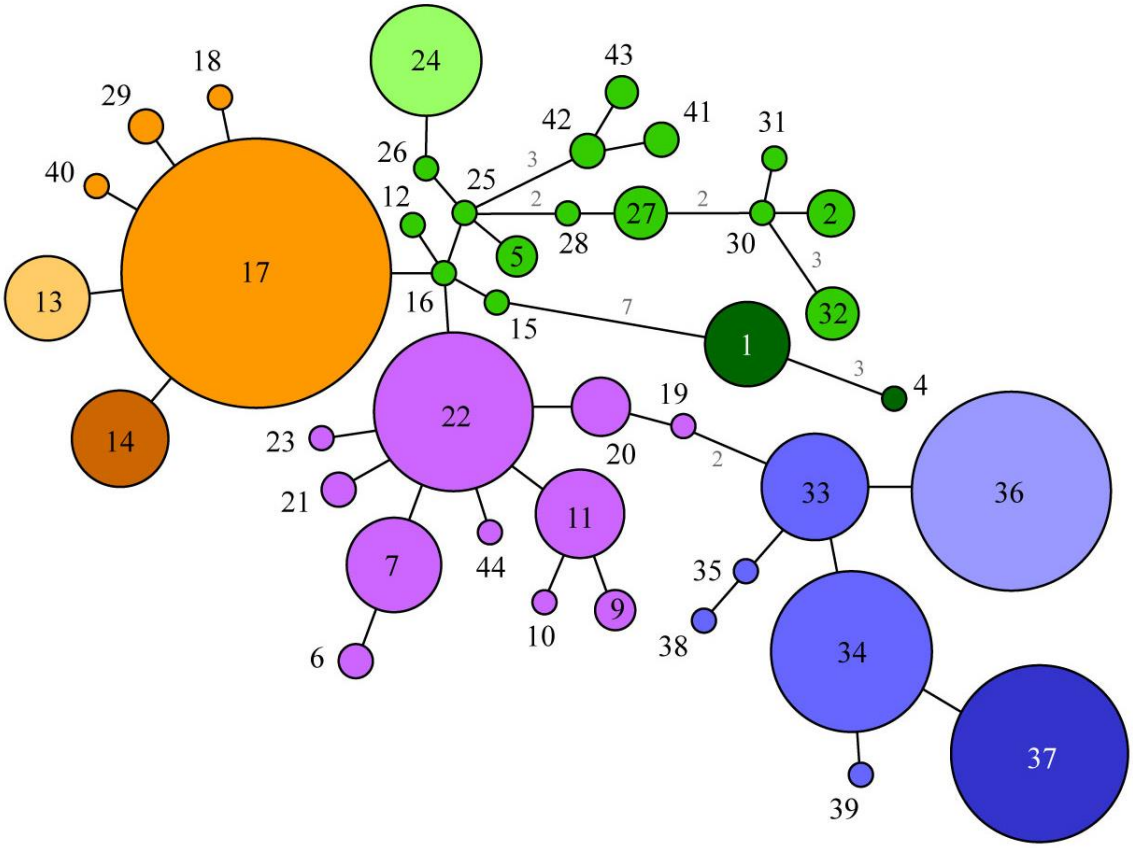

Figure 2. Haplotype minimum spanning network among partial cytochrome c oxidase I (COI) sequences for *Jassa herdmani*. Circle area is proportional to frequency of occurrence. Numbers in black or white denote haplotype identity; branch lengths are one base pair substitution unless otherwise indicated (in grey numbers). Haplotype colours correspond to those in Figure 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 numbers 3 and 8 are not shown.

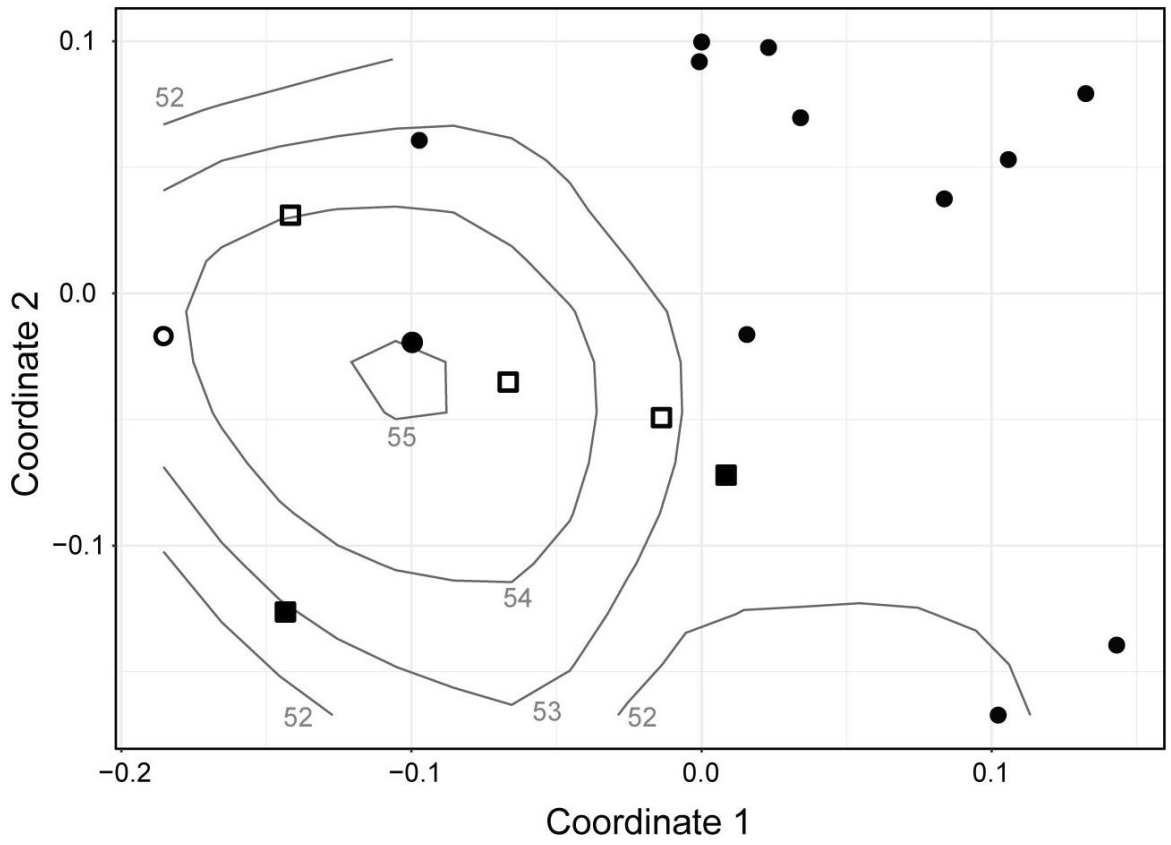

Figure 3. Multidimensional scaling plot (MDS) for *Jassa herdmani* samples consisting of partial cytochrome-*c*-oxidase 1 sequences collected at 17 offshore southern North Sea locations. Grey lines with grey numbers are latitudinal isolines; open symbols are platforms and turbines and closed symbols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

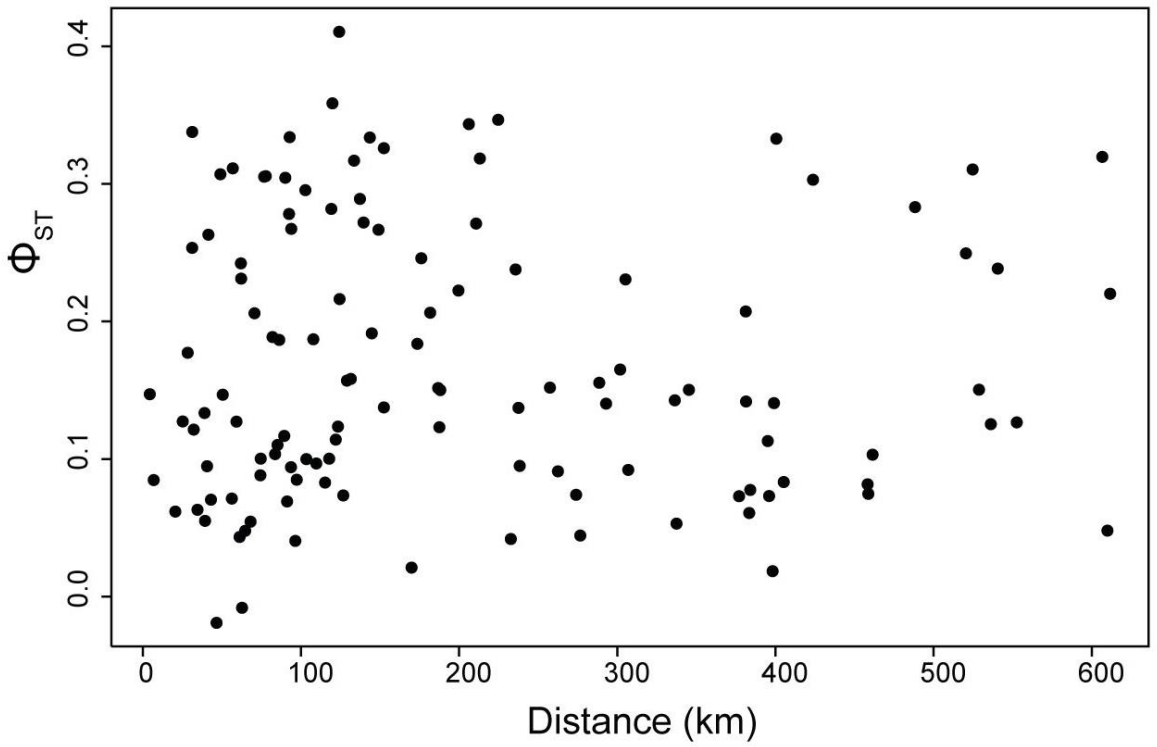

Figure 4. Graph showing absence of isolation-by-distance among sampled *Jassa herdmani* locations in the southern North Sea region. Φ_{ST} = pairwise level of population differentiation.

The following supplement accompanies the article

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

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haplotype	L10A	SP0654	ST0725	ST0729	SW0566	SW0569	SW0932	SW0933	SW0934	SW0935	SW0936	SW0937	SW0939	SW0940	SW0941	SW0942	SW0943
1	1	9	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
7	3	4	0	1	0	0	0	0	1	1	1	2	2	0	0	0	2
9	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0
10	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
11	1	0	3	2	0	0	0	0	0	1	6	1	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	9	1	3	0
14	0	0	0	0	0	0	9	7	0	0	0	0	0	0	0	0	1
15	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
17	6	17	3	19	9	15	8	0	13	2	5	5	6	7	2	7	2
18	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
19	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	5	0	0	0	1	0	0	0
21	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
22	0	1	10	1	0	0	0	0	2	12	8	3	4	0	1	0	4
23	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
24	3	0	7	1	1	0	0	0	0	0	0	0	9	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
27	0	0	3	1	0	0	0	0	0	1	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
30	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
33	0	1	2	1	0	0	1	11	0	0	0	0	0	0	1	0	4
34	0	3	1	9	5	2	15	0	0	0	0	0	0	4	2	2	1
35	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	19	1	15	20	11	3	2	0	1
37	0	0	0	0	0	0	0	0	4	15	5	0	0	18	6	9	0
38	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
39	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
43	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
44	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0