

1 **Fouling communities and non-native species within five ports along the Bristol**
2 **Channel, South Wales, UK.**

3 Samuel Holmes^a and Ruth Callaway^a

4 ^a College of Science, Biosciences, Swansea University, Singleton Park, Swansea SA2 8PP, Wales, UK

5 Corresponding author: Samuel Holmes; sam.j.holmes1@gmail.com

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7 **Abstract**

8 Non-native species (NNS) are widely regarded to be one of the major threats to the
9 loss of biodiversity worldwide. Maritime trade is the primary pathway for the transport and
10 introduction of aquatic NNS around the world, and ports are central to this network. Our
11 knowledge of port communities and the NNS they contain is limited, with ports often
12 remaining unsurveyed for decades, which was the case within the studied region. Settlement
13 plates were deployed for 10-11 months at five commercial ports along the Bristol Channel
14 and Severn Estuary in South Wales, UK. We report unique communities in each of the ports
15 with salinity being the main driver for differences among locations. Eleven NNS were
16 identified across all ports with non-native to native species proportions ranging from 0.13 to
17 0.33 in each port. Most of these NNS are known to exist in the region and are 'established'
18 species within the UK. High variation in community structure and NNS composition among
19 all ports independent of geographic proximity highlights the importance of monitoring
20 individual ports with a view to implementing bespoke, effective NNS management strategies.

21

22 **Keywords:** Alien species; artificial harbours; maritime trade; fouling organisms; community
23 composition; biological surveys

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26 **1. Introduction**

27 Aquatic non-native species (NNS) have long been associated with the loss of
28 biodiversity and their direct impacts on native species, habitats and ecosystems (Bax et al.,
29 2003; Molnar et al., 2008; McGeoch et al., 2010; Blackburn et al., 2014). Whilst documenting
30 the impacts of NNS is vital in developing an understanding of how damaging the spread of
31 species can be, as well as raising the profile of the problem of NNS, the focus must remain
32 on managing species introductions in order to minimise impacts. Management of NNS is
33 most successful following the early detection of a species introduction to a new location, with
34 management options becoming limited as species become established (Blackburn et al.,
35 2011; Giakoumi et al., 2019). Monitoring or screening for early NNS introductions is
36 therefore an essential aspect of management (Pyšek and Richardson, 2010; Blackburn et
37 al., 2011).

38 Maritime trade is widely considered to be the primary pathway for the spread of
39 marine NNS worldwide (Ruiz et al., 1997; Katsanevakis et al., 2013; Seebens et al., 2013;
40 Williams et al., 2013; Bailey, 2015). At the centre of all maritime trade are ports, making
41 these areas particularly high risk for the introduction of NNS (Bailey, 2015). A wider concern
42 surrounding ports is the potential role that these areas may play in the invasion of species to
43 a region by serving as vectors for NNS into adjacent natural systems, following an initial
44 introduction into the port (Floerl et al., 2009). Ports are often isolated locations, where water
45 conditions can be influenced by a range of factors including freshwater input and industrial
46 port processes. Ports connected to the same water body may not therefore share the same,
47 or similar, environmental conditions or communities, including NNS.

48 Ports are often a key focus in the monitoring of NNS, with a view to identifying novel
49 species introductions early and allowing the most effective system of management to be
50 implemented (Cohen et al., 2005; Marins et al., 2010; Borrell et al., 2017; Travizi et al.,
51 2019).

52 Port fouling communities worldwide are often dominated by sessile benthic organisms,
53 included a large number of ascidians, associated with the hull fouling transport vector (e.g.
54 Cohen et al., 2005; Marins et al., 2010; Giachetti et al., 2019). However, lack of data from
55 within UK ports and the evident variation between port communities elsewhere in the world
56 highlights the importance of monitoring port fouling communities in unstudied regions.

57 Survey methodologies widely used to detect marine NNS in ports, marinas and
58 associated habitats, generally take the form of a settlement study (e.g. Arenas et al., 2006;
59 Floerl et al., 2012; Mineur et al., 2012; Marraffini et al., 2017). These surveys have been
60 used extensively around the world in various habitats and are regarded to be effective in the
61 detection and identification of NNS (Floerl et al., 2012; Marraffini et al., 2017). Ports can be
62 hostile places to carry out research, with safety being a key factor when working amongst
63 large machinery, vessels, and associated cargo. Safe locations from which to deploy
64 materials or access existing materials, necessary for conventional survey methods, can be
65 rare within ports. Many settlement studies also overlook temporal variation in communities,
66 despite succession being a key feature of fouling communities (Berntsson and Jonsson,
67 2003; Langhamer et al., 2009; South et al., 2019). With a view to detecting all NNS that may
68 be present within a fouling community, the application of a survey which takes temporal
69 variation into account may prove effective.

70 Large scale strategic monitoring of NNS in Great Britain aims, in part, to develop
71 'early detection, surveillance, monitoring and rapid response' (UK Government, 2015).
72 Efforts have so far been made to increase the involvement of various organisations and
73 incorporating citizen science with the goal of increasing the amount of data being recorded in
74 the public domain (UK Government, 2015). Ports, however, being privately owned and
75 privately run industrial areas offer several complications for this strategic monitoring effort.
76 For safety and access reasons large scale citizen science cannot be applied within ports,
77 and certain survey types may not always be suitable for use.

78 It has been noted that the spatial resolution for NNS monitoring is low across the whole of
79 Europe, with many habitats not being consistently and regularly monitored (Painting et al.,
80 2020). Whilst legislation such as the Water Framework Directive (Council Directive
81 2000/60/EC), the Marine Strategy Framework Directive (Council Directive 2008/56/EC) and
82 the EU Biodiversity Strategy (COM (2011) 0244, final) drive the notion of preserving natural
83 biodiversity and the monitoring and management of NNS, there is no clear or standardised
84 protocol to assist port operators in achieving this (Boon et al., 2020). Developing a
85 standardised survey protocol may be a step towards improving NNS monitoring within ports,
86 increasing the data flow into large scale management strategies and improving our ability to
87 minimise the risk that NNS pose to our native species and habitats, as well as coastal
88 industry.

89 The aim of this study was to understand factors influencing the fouling communities
90 in active ports, and specifically fouling of NNS. The objectives of this study were to:

- 91 1. Describe the fouling communities and detect NNS present in five ports in South
92 Wales, UK.
- 93 2. Quantify aspects of community succession (community composition, abundance,
94 coverage) over the course of an 11-month deployment of settlement plates.
- 95 3. Assess the influence abiotic variables have on determining fouling community
96 composition and the proportion of NNS within port communities.

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103 **2. Materials and methods**

104 2.1 Study area

105 Research was conducted within the ports of Newport, Cardiff, Barry, Port Talbot and
106 Swansea, each located along a 55-mile section of the Bristol Channel and Severn Estuary in
107 South Wales, UK (Fig. 1). These ports are all commercial ports owned and operated by
108 Associated British Ports (ABP) and are each connected directly to the Bristol Channel by a
109 lock. There is only one published survey from the 1950s of Swansea (Naylor, 1957) and
110 none from the remaining four ports. Water levels are maintained in all shipping areas to at
111 least 10 m depth whilst the Bristol Channel itself experiences a tidal range of up to 13 m.
112 These ports may therefore support unique subtidal communities that are influenced by a
113 range of factors including environmental conditions as well as the potential introduction of
114 species through maritime trade.

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116 Table 1. Salinity, shipping traffic and species richness of ports at the Bristol Channel (East to
117 West). Salinity range over 11 months (2019), mean shipping traffic calculated from ABP
118 logs; total number of species colonising settlement plates in 2019.

Port	Salinity	Shipping traffic (mean month ⁻¹)	Total number of species
Swansea	28 – 30	81	30
Port Talbot	0.1 - 0.3	8	6
Barry	22 – 24	22	25
Cardiff	1 – 12	42	14
Newport	8 – 16	73	13

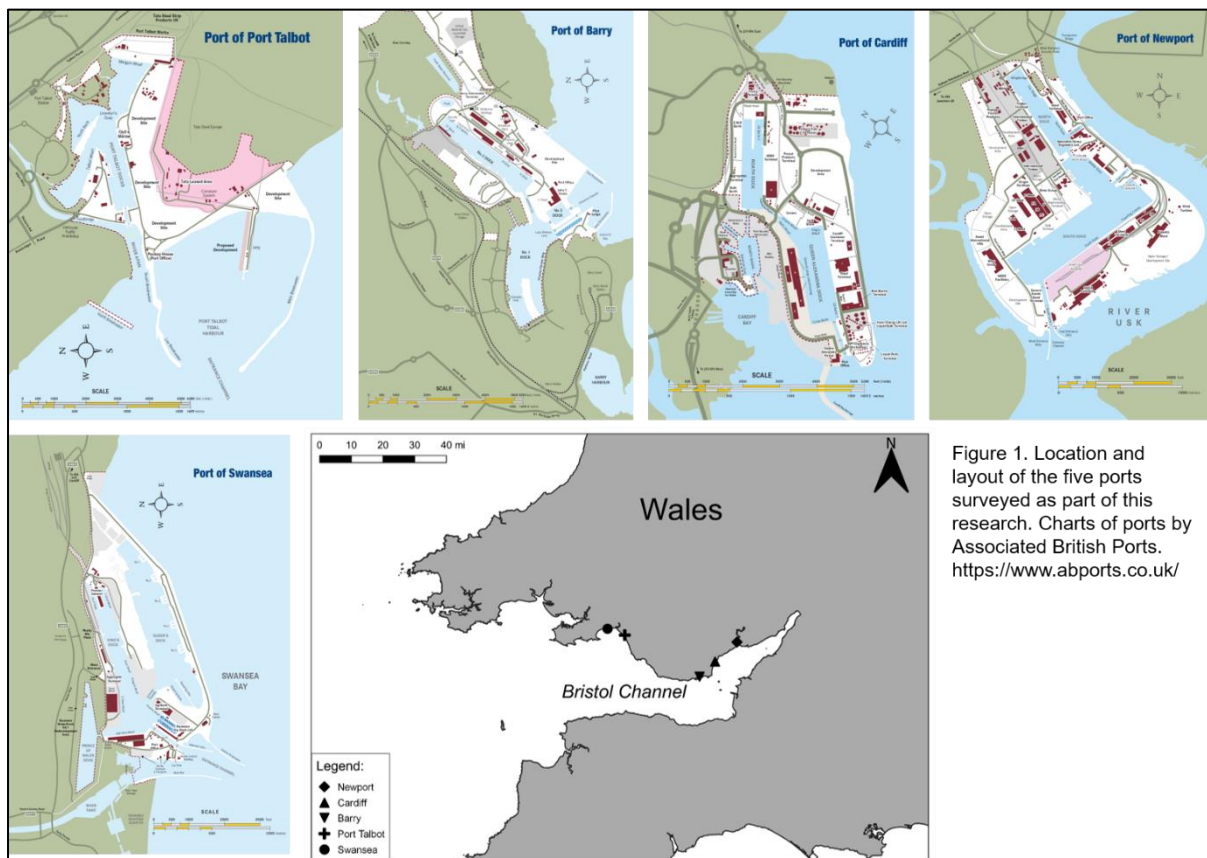
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120 Salinity levels, measured using an Aquaread AP-5000 probe, varied between ports
121 from freshwater in Port Talbot to near marine salinity in Swansea (Table 1). Barry, Cardiff
122 and Newport all occupy a range of brackish salinities, with Cardiff the only port displaying
123 any stratification whereby salinity can increase from 1 in surface water to 12 at a depth of 7
124 m.

125 The variation in salinities between ports is due to the way in which water levels are
126 maintained. Port Talbot, Cardiff and Newport all have a direct freshwater inflow from nearby
127 rivers, whilst Barry and Swansea ports rely on the pumping of water from the Bristol Channel
128 at high tide. Water in the Port of Port Talbot is consistently at least 3°C warmer than the
129 other ports due to the use of dock water for cooling within an adjacent steel works. The
130 effluent of this process is returned into the dock which results in its warming (ABP, personal
131 communication).

132 ABP provided quantitative data of the number of ships passing through port locks.
133 The data was summarised from 2018-2019, and average traffic per month was calculated
134 (Table 1). Fewer ships enter Port Talbot docks compared to the other four ports as most of
135 the trade in Port Talbot takes place outside of the docks in a tidal harbour.

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139 2.2 Survey design and data collection

140 The study was designed to record succession of ecological parameters (community
141 composition, species richness, abundance, percentage cover) over six sampling dates
142 during a 10 – 11-month deployment period in 2019. This also maximised the opportunity to
143 detect as many species as possible over almost one year. Three sites in each port were
144 chosen for the deployment of settlement plates and each of site was regarded as an
145 independent replicate for the port location.

146 Poly(methyl methacrylate) (PMMA) tiles (15 cm x 15 cm, grey in colour) were used
147 as the settlement material. 6 tiles per site were lightly sanded using 40 grit sandpaper and
148 mounted, using cable ties, vertically within an aluminium frame (Fig. 2). Each frame was
149 suspended in the water column using polypropylene rope, attached to a fixed surface
150 mounting point (e.g. a mooring bollard; Fig. 2). Frames were suspended initially to a depth of
151 approximately 4 m, although the water level in the port can vary meaning that depth did not
152 remain constant during deployment. Frames were deployed in December 2018 and retrieved
153 in October/ November 2019. One PMMA tile was removed from each frame every 6 – 8
154 weeks and returned to the laboratory for analysis, where macrofauna were visually identified
155 to the lowest possible taxon from both the front and the reverse side of the tile using guides
156 such as the *Handbook of the Marine Fauna of North-West Europe* (Hayward and Ryland,
157 2017), *British Marine Amphipoda* (Lincoln, 1979) and Linnean Society taxonomic resources.
158 Tiles were photographed in the laboratory from which percentage cover was calculated
159 using ImageJ software.

160 Temperature was measured continuously using Tinytag sensors, recording every 30
161 minutes, which were attached directly to the aluminium frame holding the settlement plates.
162 Measures of salinity, temperature, depth, pH, dissolved oxygen (DO; mg/L and %
163 saturation), and oxidation reduction potential (ORP) were taken periodically throughout the
164 survey period using an Aquaread AP-5000 probe.

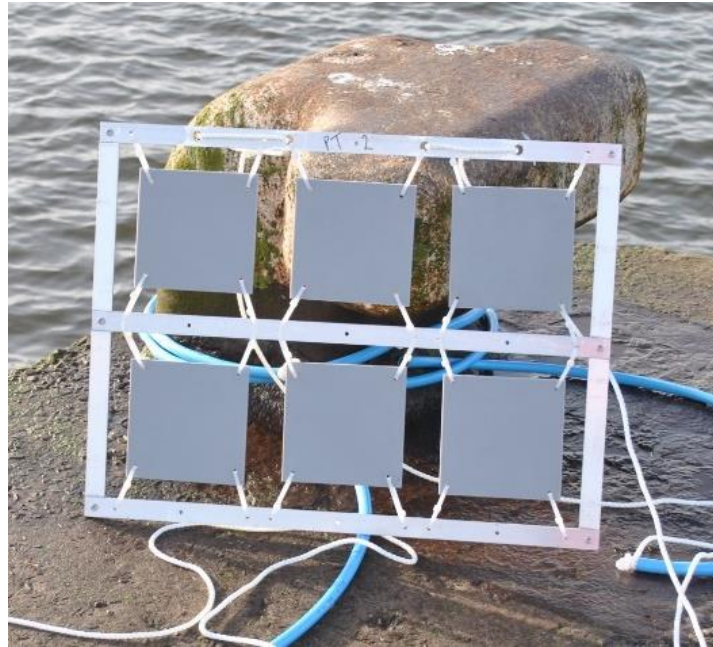


Figure 2. Survey materials prior to deployment.

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166 2.3 Data analysis

167 Whole community analysis, comparing samples collected within each port at each
168 sampling occasion, was completed using Primer 6 v.6.1.13 with PERMANOVA v.1.0.3
169 (Anderson et al., 2008). Separate analyses outlined below were conducted using square root
170 transformed abundance counts of countable species and the presence/ absence of all
171 recorded species to incorporate both countable and non-countable species.

172 All samples without colonisation were removed from multivariate analysis (January
173 and some of March 2019). A similarity matrix using a Bray Curtis similarity index was created
174 for all samples with colonising species. A non-metric multidimensional scaling (nMDS) plot
175 and PERMANOVA were completed from this matrix. A PERMANOVA test was designed with
176 the fixed factors 'Port' and 'Month' and values in the similarity matrix as response variable to
177 analyse the effect of the different ports and months on the fouling communities. The model
178 used the permutation of residuals under a reduced model with type III (partial) sum of
179 squares and 9999 permutations.

180 Pairwise PERMANOVA analyses among 'Port' groups and 'Month' groups were also
181 completed using this same model design. This design was also used to analyse the effect of
182 Port and Month as factors both on the number of non-native species and the frequency of
183 occurrence of NNS within samples based on a similarity matrix generated using Euclidian
184 distances. Inclusion of the factor 'Month' here partially addresses aim two, meaning to better
185 understand the temporal succession of colonisation.

186 Due to a high variation in community composition between ports, the factor 'Month'
187 was also analysed independently for each port, further addressing aim two. Kruskal-Wallis
188 tests were conducted to determine the effect of month on three measures of colonisation:
189 number of species, total abundance (countable species), and percentage cover (all species)
190 within each port. Pairwise Dunn's tests were used to identify significant differences in the
191 colonisation between months. These analyses were carried out within R v.3.6.2 (R Core
192 Team, 2017).

193 A distance-based linear model (DistLM) with distance-based redundancy analysis
194 plot (dbRDA) was used to quantify how much variation between samples could be attributed
195 to certain environmental and human driven variables, addressing the third aim of this study
196 to understand the importance of environmental factors in the colonisation process. Variables
197 used were temperature, salinity, pH, DO (% saturation), ORP, and ship traffic. No
198 autocorrelation between variables was detected based on correlations generated using a
199 draftsman plot in Primer 6. The DistLM was based on values in a Bray Curtis similarity matrix
200 based on square root transformed abundance data. The DistLM used a stepwise selection
201 procedure and R^2 selection criterion with 9999 permutations. When analysing the influence
202 of these variables on the number of non-native species and frequency of occurrence of NNS
203 within samples the DistLM was based on a similarity matrix created using Euclidian
204 distances.

205

206 3. Results

207 Fouling communities

208 Swansea had the highest recorded number of species, followed by Barry, Cardiff,
209 Newport, with Port Talbot having the fewest (Table 1). Fouling communities were
210 numerically dominated by different species in each port (full list of recorded taxa in the
211 Appendix). *Mytilus edulis* and *Ciona intestinalis* were the only species to feature as the most
212 abundant species in more than one port. Across all five ports, *Amphibalanus improvisus* was
213 the most abundant species, with an average of over 800 individuals recorded per 450cm²
214 sample (front and reverse side of a 15x15cm tile) from the Port of Newport.

215 The similarity in fouling communities among ports and months was visualised using
216 an nMDS (Fig. 3). Both 'Port' and 'Month' as fixed factors were found to significantly
217 influence the observed fouling communities based on presence/ absence data
218 (PERMANOVA: Port, pseudo-F = 16.372, p = 0.0001; Month, pseudo-F = 5.024, p = 0.0001),
219 as well as the abundance of countable organisms (PERMANOVA: Port, pseudo-F = 9.995, p
220 = 0.0001; Month, pseudo-F = 4.436, p = 0.0001). Communities observed within each port
221 were found to be significantly different to all other ports in a post-hoc pairwise analysis,
222 based both on the presence/ absence of all observed species and the abundance of
223 countable organisms (PERMANOVAs, p < 0.05). Communities observed between all month
224 pairs were significantly different to one another (PERMANOVAs, p < 0.05), except for March
225 and May, September and October (both for presence/ absence and abundances) and July
226 and September (abundances only).

227 A SIMPER analysis was completed to identify which species are driving the
228 dissimilarity in communities observed between ports. The dissimilarity between ports with
229 large differences in salinity and temperature, e.g. Swansea and Port Talbot, was driven by
230 species which were present in one port but absent in the other.

231 Swansea and Port Talbot had an average dissimilarity of 100% of which ca. 45% was
232 contributed by three species: *Balanus crenatus*, *Ciona intestinalis* and *Ascidiella scabra*.
233 These species were present in high abundance in the Port of Swansea (mean abundance \pm
234 SE: *B. crenatus*, 10.6 ± 2.6 450cm⁻²; *C. intestinalis*, 57.7 ± 22.8 450cm⁻²; *A. scabra*, $85.9 \pm$
235 29.1 450cm⁻²) and absent from the port of Port Talbot. Ports with similar salinity had
236 considerable overlap in the community composition and the dissimilarity between them was
237 driven by the relative abundance of species present in both ports. Cardiff and Newport, for
238 example, had an average dissimilarity of ca. 75% of which over 55% was attributed to three
239 species present in both ports with different abundances: *Amphibalanus improvisus* (mean
240 abundance \pm SE: Cardiff, 65.7 ± 26 450cm⁻²; Newport, 654.6 ± 326.7 450cm⁻²), *Ficopomatus*
241 *enigmaticus* (mean abundance \pm SE: Cardiff, 197.4 ± 91.7 450cm⁻²; Newport, 0.4 ± 0.4
242 450cm⁻²), *Gammarus zaddachi* (mean abundance \pm SE: Cardiff, 0.1 ± 0.1 450cm⁻²; Newport,
243 3.2 ± 2.5 450cm⁻²).

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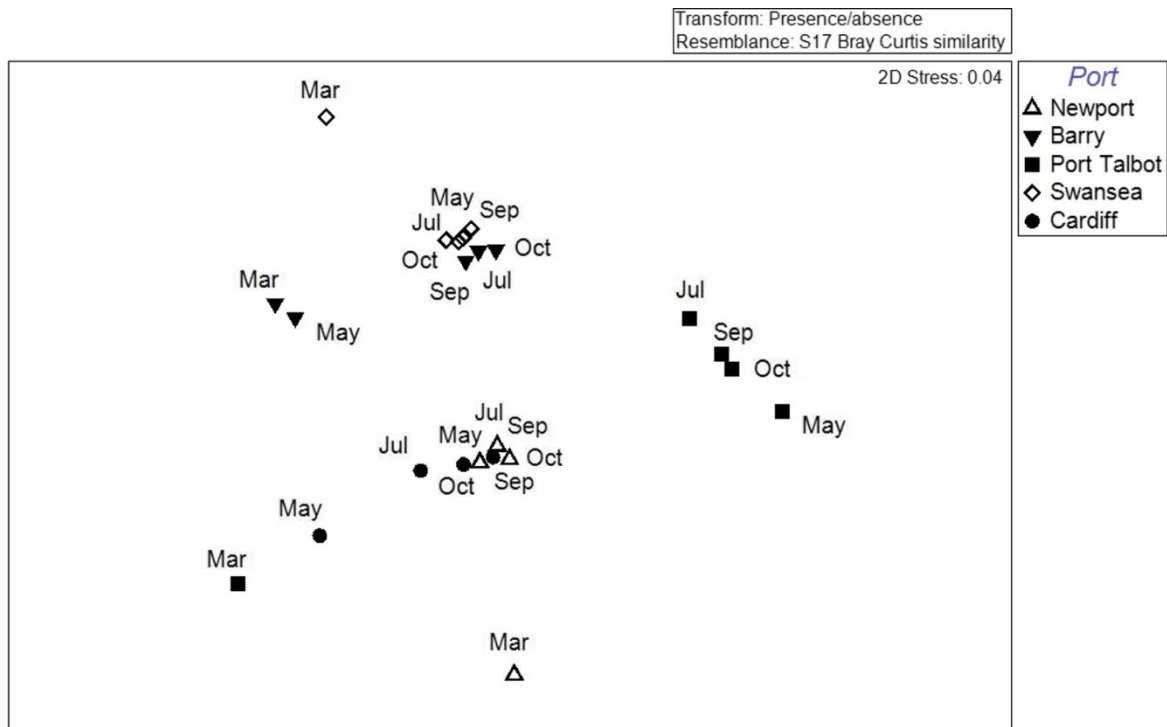


Figure 3. nMDS plot of fouling community samples collected within 5 ports at bimonthly intervals throughout 2019. Replicate measures averaged for each port per month (n= 2-3) and transformed using a presence/ absence transformation. Plot based on a resemblance matrix created using a Bray Curtis similarity index. Samples without any species were removed.

245

246 Due to the high variation in community structure among ports the influence of the
 247 factor 'month' on fouling communities was also analysed independently for each port. Whole
 248 community analysis within ports revealed 'month' to be a significant factor influencing
 249 community composition in the ports of Newport, Cardiff, Barry and Swansea
 250 (PERMANOVAs: Newport, pseudo-F = 3.9053, p = 0.0015; Cardiff, pseudo-F = 8.7874, p =
 251 0.0001; Barry, pseudo-F = 3.1573, p = 0.0001; Swansea, pseudo-F = 2.802, p = 0.0013).
 252 Three univariate measures of colonisation were analysed (species richness, total abundance
 253 of countable species and percentage cover of all species; Fig. 4) to determine the effect of
 254 succession over time ('month') on the observed communities within each port. 'Month' was a
 255 significant factor determining the observed number of species, abundance, and percentage
 256 cover in the ports of Newport, Cardiff, Barry and Swansea (Kruskal-Wallis test, p < 0.05).

257 Only percentage cover was found to be significantly influenced by the factor 'month' within
258 the port of Port Talbot (Kruskal-Wallis test, $p < 0.05$). Pairwise tests revealed that the effect
259 of month was largely influenced by differences in colonisation between early months
260 (January and March) and later months (July, September, October; Fig. 4).

261 The influence of several abiotic variables (salinity, temperature, pH, DO % saturation,
262 ORP, ship traffic) on the observed communities was analysed using a Distance-based
263 Linear Model (DistLM) and visualised using a dbRDA plot (Fig. 5). Salinity and dissolved
264 oxygen concentration (DO) together were found to explain 23% of the variation observed
265 between samples (DistLM; variable: salinity, pseudo-F = 7.733, $p = 0.0001$, prop = 0.132;
266 variable: DO, pseudo-F = 6.689, $p = 0.0001$, prop = 0.102). Temperature was found to
267 explain an additional 8% in variation (DistLM, pseudo-F = 5.543, $p = 0.001$, prop = 0.078)
268 whilst the remaining three variables included in the analysis (ship traffic, ORP, pH)
269 contributed only 9% combined. Ship traffic, ORP and pH were though all significant factors in
270 this analysis (DistLM, $p < 0.05$) despite accounting for a small percentage of the total
271 variation. In total these six variables combined explained 40% of the variation observed
272 among samples.

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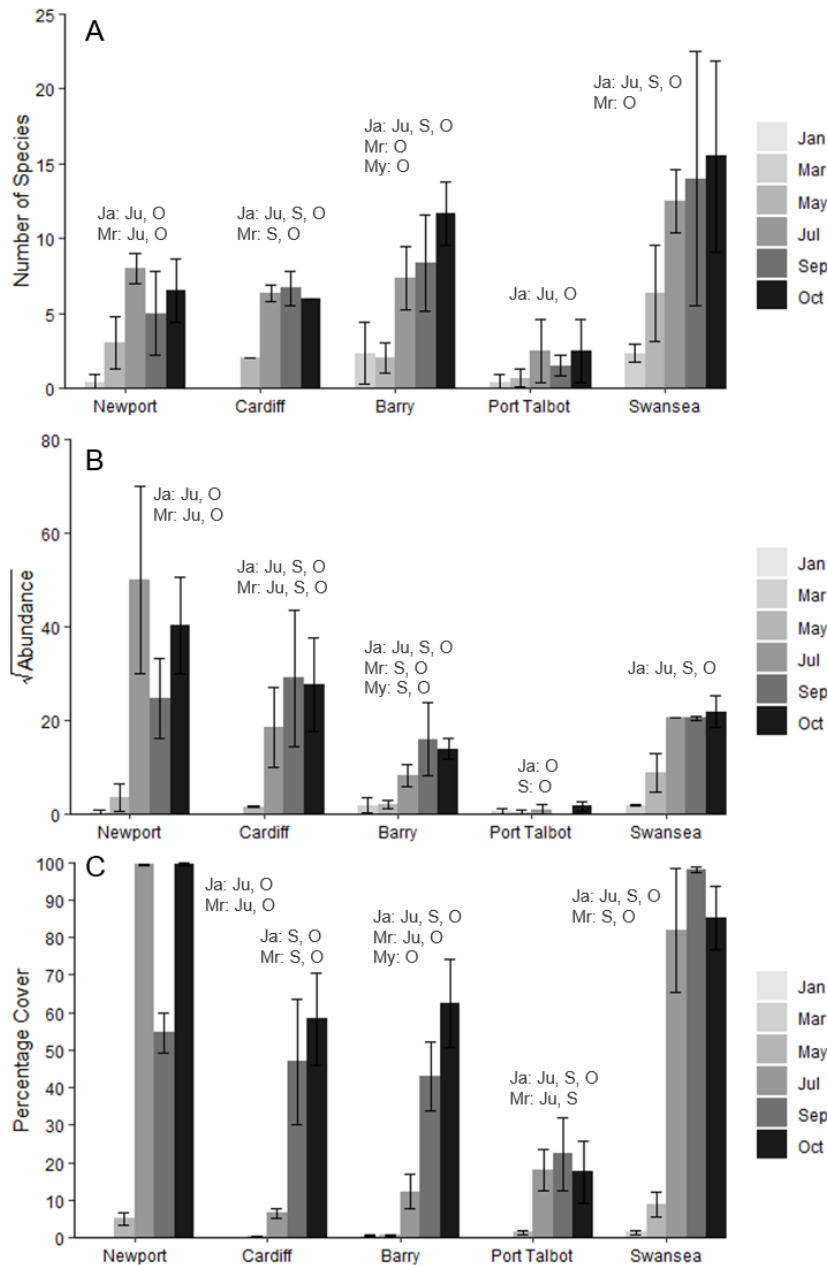


Figure 4. Colonisation of settlement plates at five ports over 10 months. Records per sampling month in the ports of Newport, Cardiff, Barry, Port Talbot and Swansea. A: Mean species richness 450cm⁻². B: mean square root abundance per sample. C: Mean percentage cover per sample. Error bars show standard deviation. n = 3 with the exception of Newport (Sep, Oct), Cardiff (Oct), Port Talbot and Swansea (Jul, Sep, Oct), where n = 2. Abundance based on countable organisms only, percentage cover based on all organisms. Results of significant pairwise Dunn's tests (p < α/2, where α = 0.05) between month groups within each port are indicated by letters above each set of bars. 'Ja: Ju, O' for example indicates a significant difference between the month pairs January and July and January and October.

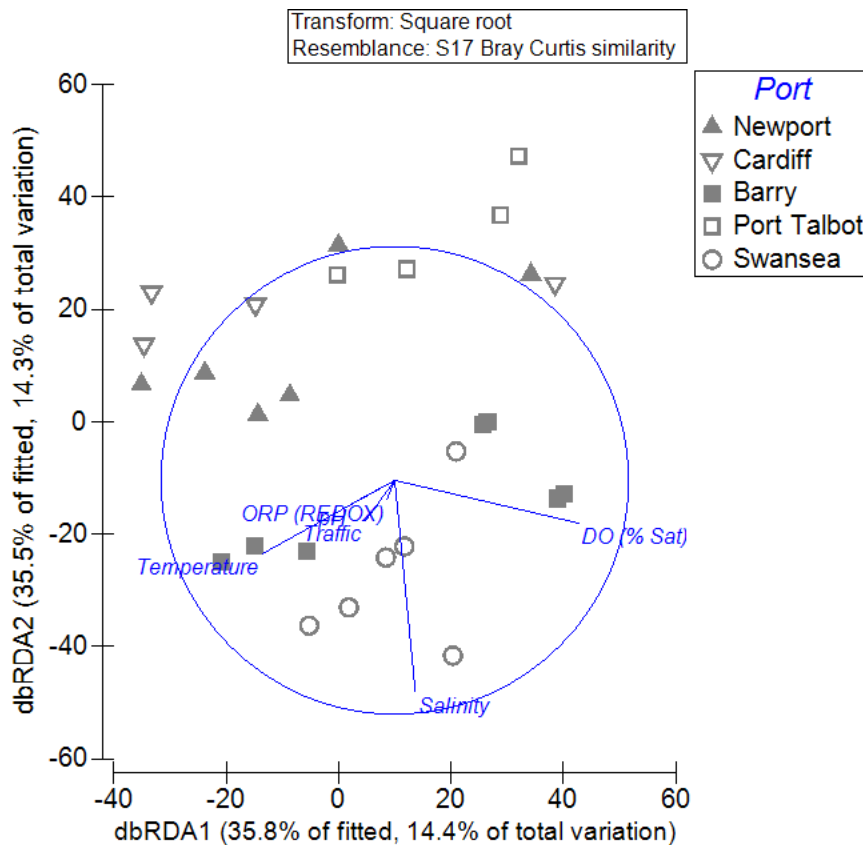


Figure 5. Distance-based redundancy analysis (dbRDA) exploring the amount of variation between communities that may be explained by six abiotic variables (salinity, temperature, pH, DO, ORP, ship traffic). Analysis completed using a similarity matrix based on a Bray-Curtis similarity index and square root transformed abundance data for countable species.

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277 Non-native species (NNS)

278 11 NNS were identified across the five ports surveyed within this study (Table 2). The

279 Port of Barry had the highest recorded number of NNS with seven species, whilst Port Talbot

280 had only two. All but one species, *Brachynotus sexdentatus*, are considered to be

281 established species within Great Britain and all but one species (*Cordylophora caspia*) have

282 been previously recorded from within the Bristol Channel area (NBN Atlas, 2020). Port

283 Talbot had the highest proportion of NNS within communities, whereby one in every three

284 species was non-native, Swansea had the lowest proportion of NNS. In descending order,

285 the proportion of NNS was Port Talbot 0.33, Barry 0.24, Cardiff 0.21, Newport 0.15,

286 Swansea 0.13.

287 The colonisation of the three most dominant NNS based on total recorded
 288 abundance (*Amphibalanus improvisus*, *Bugulina stolonifera*, *Ficopomatus enigmaticus*)
 289 varied both spatially in terms of which ports they colonise, and temporally in terms of the
 290 speed of population development and how the population was sustained thereafter (Fig. 6).

291

Table 2. Non-native species recorded in South Wales ports. GB establishment status and existing presence in the Bristol Channel based on data from NBN Atlas (NBN Atlas, 2020). * indicates species previously known to inhabit the recorded port(s) but not the Bristol Channel.

Taxa	Port(s)	# No. samples where species was present	GB establishment status	Existing Presence in Bristol Channel?
<i>Amphibalanus improvisus</i> (Darwin, 1854)	Newport, Cardiff	13	Established	Yes
<i>Austrominius modestus</i> (Darwin, 1854)	Barry, Swansea	8	Established	Yes
<i>Brachynotus sexdentatus</i> (Risso, 1827)	Swansea	1	Unknown	No *
<i>Bugulina stolonifera</i> (Ryland, 1960)	Barry, Swansea	8	Established	Yes
<i>Caprella mutica</i> (Schurin, 1935)	Barry, Swansea	11	Established	No *
<i>Cordylophora caspia</i> (Pallas, 1771)	Cardiff, Port Talbot	5	Established	No
<i>Diadumene lineata</i> (Verrill, 1869)	Barry	1	Established	Yes
<i>Ficopomatus enigmaticus</i> (Fauvel, 1823)	Newport, Cardiff	6	Established	Yes
<i>Monocorophium sextonae</i> (Crawford, 1937)	Barry	3	Established	Yes
<i>Mytilopsis leucophaeata</i> (Conrad, 1831)	Port Talbot	2	Established	Yes (in freshwater)
<i>Styela clava</i> (Herdman, 1881)	Barry	1	Established	Yes

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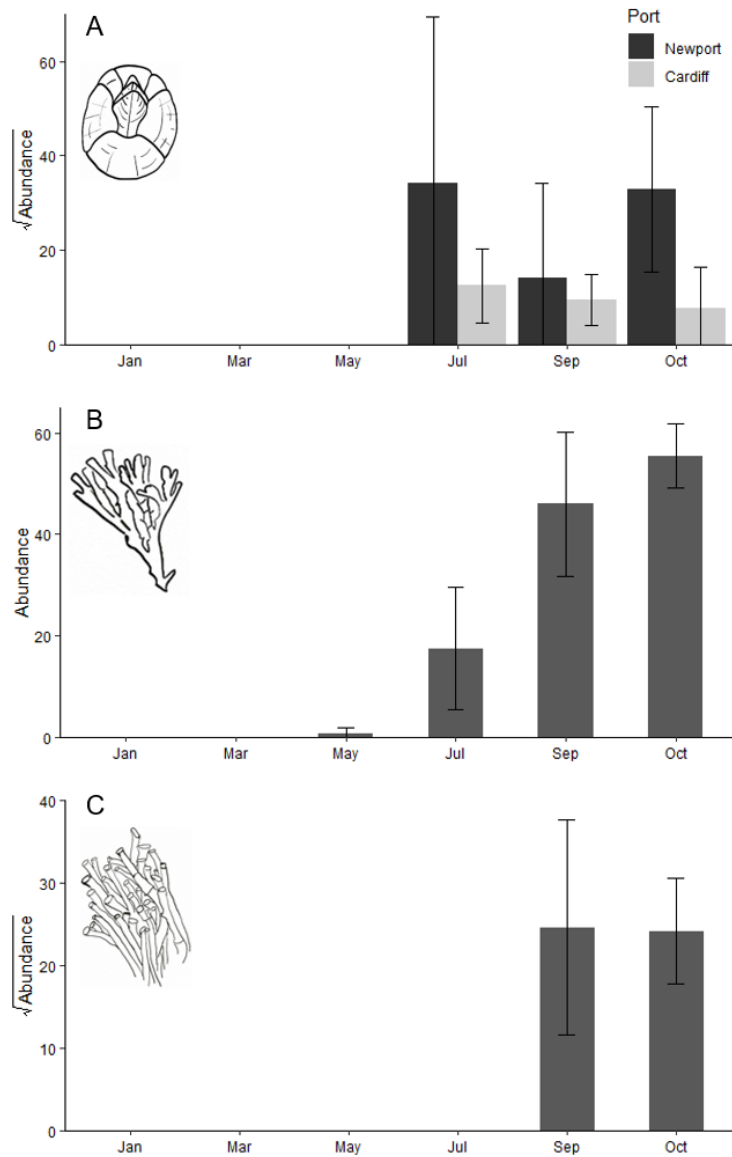


Figure 6. Spatial and temporal distribution of the three most abundant non-native species. A: *Amphibalanus improvisus* as recorded in the ports of Newport and Cardiff. B: *Bugulina stolonifera* as recorded in the port of Swansea. C: *Ficopomatus enigmaticus* as recorded in the port of Cardiff. *B. stolonifera* and *F. enigmaticus* were each present in only one sample from within the ports of Barry and Newport, respectively, and these data were omitted from the figure.

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301 Both Port and Month were found to be significant factors in determining the number
302 of NNS recorded in samples (PERMANOVA; factor: Port, pseudo-F = 2.842, p = 0.0362;
303 factor: Month, pseudo-F = 20.213, p = 0.0001). As environmental conditions and the amount
304 of shipping vary between ports and months, a Distance-based Linear Model (DistLM) was
305 used to determine whether certain environmental variables or shipping were driving the
306 observed differences in the number of NNS between ports. Temperature and salinity
307 combined accounted for over 30% of the observed variation between samples (DistLM;
308 variable: temperature, pseudo-F = 25.095, p = 0.0001, prop = 0.241; variable: salinity,
309 pseudo-F = 10.065, p = 0.0016, prop = 0.087). All other variables included in the analysis
310 (DO, ORP, pH, ship traffic) accounted for a combined < 6% of variation in the number of
311 NNS observed among samples (DistLM, p > 0.05, prop < 0.03).

312 By contrast, Port and Month as factors were found to have no significant influence on
313 the observed frequency of occurrence of NNS in samples (PERMANOVA; factor: Port,
314 pseudo-F = 1.593, p = 0.212; factor: Month, pseudo-F = 0.792, p = 0.538). Ship traffic was
315 the only significant driver of variation in the frequency of occurrence of NNS; it explained 9%
316 of the total observed variation (DistLM, pseudo-F = 5.41, p = 0.0231, prop = 0.09). All other
317 analysed variables accounted for a remaining 1.5% (DistLM, p > 0.05, prop < 0.006).
318 Combined with ship traffic all factors explained 10.6% of the variation.

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326 4. Discussion

327 The formation of fouling communities is known to be a complex process, influenced
328 by a range of abiotic and biotic factors (Sutherland and Karlson, 1977; Glasby, 1999;
329 Glasby, 2000; Nydam and Stachowicz, 2007; Giachetti et al., 2019; Leclerc et al., 2019). In
330 this study fouling communities varied significantly among five ports located along the same
331 body of water, the Bristol Channel and Severn Estuary (UK). Succession has also long been
332 recognised as a feature in the formation of fouling communities (Berntsson and Jonsson,
333 2003; Cifuentes et al., 2010; Lindeyer and Gittenberger, 2011). Despite this, most studies
334 aiming to describe fouling communities and to identify non-native species (NNS) do not
335 consider a temporal factor (e.g. Cohen et al., 2005; Arenas et al., 2006; Floerl et al., 2012;
336 Mineur et al., 2012). We identified succession of different aspects of community structure in
337 each of the five studied ports. However, the patterns of succession were not consistent in all
338 ports, with some plates being colonised rapidly early in the spring and others later in the
339 season as well as disparity in the degree of change in community composition among ports.
340 Variation in the degree of succession between communities is not uncommon (Berntsson
341 and Jonsson, 2003; Nydam and Stachowicz, 2007; Ronowicz et al., 2014), and the
342 particularly isolated nature and high variation in environmental conditions between the ports
343 studied here likely contributed to the variation we see in the formation of their respective
344 fouling communities.

345 Overall, 40% of the variation in the observed fouling communities could be explained
346 by six abiotic variables recorded and analysed within this study (salinity, temperature,
347 dissolved oxygen, pH, ORP, and ship traffic). 13% of the variation was explained by salinity
348 alone which is concurrent with previous studies highlighting salinity as a significant factor in
349 shaping fouling communities (MacGinitie, 1939; Charles et al., 2018; Pinnell and Turner,
350 2020). It appears though that other abiotic or biotic factors which were not measured within
351 this study influenced the observed succession.

352

353 Reported factors include founder effects (Lindeyer and Gittenberger, 2011; Vieira et al.,
354 2018), predation (Nydam and Stachowicz, 2007; Giachetti et al., 2019), and habitat type
355 (González-Duarte et al., 2018; Leclerc et al., 2019). The latter can be considered negligible
356 or absent from this study given that the studied ports are artificial sites with similar habitat
357 types.

358 Founder effects and predation, however, may play a significant role in the succession
359 and formation of the observed fouling communities. The effects of predation varied between
360 studies, with some reporting a reduced species diversity in the presence of macropredators,
361 while other studies found no significant effect (Nydam and Stachowicz, 2007; Giachetti et al.,
362 2019; Leclerc et al., 2019). Founder effects may have influenced the succession and
363 composition of the fouling communities. Solitary ascidians, found in high abundance in the
364 ports of Barry and Swansea, may have served as founder species in community
365 development (Lindeyer and Gittenberger, 2011). *Ascidrella scabra* has indeed been linked
366 with succession in fouling communities within the Port of Swansea in a previous study
367 (Holmes and Callaway, 2020). Habitat complexity is known to influence the species diversity
368 of a community (Sueiro et al., 2011; Mendez et al., 2015). Solitary ascidians can colonise
369 rapidly, forming complex 3-dimensional habitats capable of supporting a wide range of other
370 taxa (Svane and Gröndahl, 1988). By contrast, barnacles, such as *Amphibalanus*
371 *improvisus*, recorded in high abundance in the Port of Newport, form dense mats with little
372 habitat complexity in comparison to solitary ascidians.

373 It follows then that the observed lower total abundance and higher species diversity
374 in the ports of Barry and Swansea were possibly facilitated by the abundant solitary
375 ascidians present. However, these biotic variables are inherently linked to environmental
376 conditions within each port, particularly salinity. For example, whilst *A. improvisus* would be
377 able to thrive in the environmental conditions in the Port of Swansea (Dineen Jr. and Hines,
378 1992) and may have been spatially outcompeted by ascidians, *A. scabra* would likely not
379 tolerate the lower salinity environment in the Port of Newport (Hiscock, 2006).

380 Brackish environments also tend to support lower diversity in general (Cognetti and
381 Maltagliati, 2000), which likely contributed to the lower observed diversity in the brackish
382 ports of Newport and Cardiff. It seems plausible that whilst founder effects may be crucial in
383 forming the observed communities within each port, salinity remains the underpinning
384 variable in determining community composition and explaining the variation in communities
385 between these ports.

386 A major concern regarding ports is the role they may play in the spread of NNS by
387 serving as vectors into a region (Floerl et al., 2009). Eight of the 11 NNS recorded across the
388 five ports have an existing presence within the Bristol Channel and Severn Estuary, the
389 immediately adjacent water body to each port (NBN Atlas, 2020). Known effects of
390 propagule pressure in aquatic invasion biology (Lockwood et al., 2005; Colautti et al., 2006;
391 Wilson et al., 2008; Bacon et al., 2014) indicate a likelihood that the studied ports contributed
392 to the introduction and establishment of NNS in this region, although it is impossible to
393 quantify the role they played in this process due to a lack of historical data. The focus here
394 will be on the three NNS that have to date not been recorded outside of the ports within this
395 region: *Brachynotus sexdentatus*, *Caprella mutica* and *Cordylophora caspia*. Based on the
396 environmental tolerances of each of these species, *B. sexdentatus* and *C. caspia* are at a
397 low risk of spreading out of the ports where temperatures can range from <5°C to 23°C and
398 salinities from 17 to >30 (Henderson et al., 2012). *B. sexdentatus* is native to the
399 Mediterranean Sea and is thought to have become resident within the Port of Swansea
400 whilst artificial warming was taking place within the port, which ceased in the 1970s (Arenas
401 et al., 2006). It is likely that *B. sexdentatus* has become adapted to the cooler water
402 temperatures over time, and therefore may be able to tolerate the temperature ranges within
403 the Bristol Channel, however it remains to be seen whether this is the case since *B.*
404 *sexdentatus* has not been recorded from outside of the ports to date. It may be that the
405 salinity range within the Bristol Channel is not favourable for this species, or that the low
406 abundance within the port is limiting the potential for dispersal.

407 *C. caspia* is a freshwater/ brackish species of hydroid, capable of tolerating salinities below
408 16 (Folino-Rorem and Indelicato, 2005), therefore unlikely to survive in the considerably
409 higher salinities of the Bristol Channel.

410 In contrast, *C. mutica* has the environmental tolerance to survive in these conditions
411 (Ashton et al., 2007a). *C. mutica* has a strong link to human activity in the marine
412 environment, and particularly the maritime trade industry, having regularly been recorded
413 within ports and marinas worldwide and rarely within natural habitats (Willis et al., 2004;
414 Ashton et al., 2007b). This suggests that *C. mutica* is not successful at dispersing beyond
415 enclosed environments, perhaps due to the a lack of a larval stage in Caprellid amphipods
416 (Thiel, 1998; Cook et al., 2007). It may also be due to the relatively recent dispersal of this
417 species around the world, and the spread of populations out of ports may accelerate over
418 time. A lack of records from outside of ports and marinas should therefore not be used to
419 downplay the risk of spread. The relatively low abundances recorded for *C. mutica* across
420 the five surveyed ports (total abundance of 50) does though reduce the risk of wider
421 dispersal as recruitment is most likely low within the ports.

422 When considering the abiotic variables which drive the number of NNS within ports,
423 temperature and salinity alone accounted for over 40% of the observed variation between
424 samples. In general, the number of NNS was greater in samples collected from
425 environments with higher salinity and temperature. Most aquatic NNS tend to be marine
426 species, owing to the necessity to survive interactions with marine bodies of water in most
427 ship transits (Carlton, 1996; Seebens et al., 2013; Bailey, 2015). This may contribute to the
428 increased number of NNS in ports of near marine salinity, as observed within this study. The
429 amount of ship traffic was not found to be a significant factor in determining the number of
430 NNS, yet was a significant factor in determining the frequency of NNS within a samples.

431

432 We believe this to be driven by the high proportion of NNS found in Port Talbot, where one in
433 every three species was non-native, coupled with the considerably lower ship traffic by
434 comparison with the four other ports. This does nevertheless highlight the importance of
435 monitoring a range of port types and not focussing on the ones with the busiest traffic.

436 Surveying within these challenging environments limited the number of settlement
437 plates that could be deployed, and therefore the number of replicates obtained. It also led to
438 loss of some replicates during deployment. It should be noted that the low number of
439 replicates here may have played a role in the observed statistical variation both spatially
440 among ports and temporally between month groups. The use of frames with six settlement
441 tiles was intended to accommodate increased total replicates as well as allowing for a
442 temporal factor in the study. Such a survey design does however introduce potential pseudo-
443 replication when comparing between month groups in individual ports, since settlement tiles
444 are housed in close proximity within a single frame, thus reducing their independence due to
445 possible interaction between species of individual tiles. The limited number of safely
446 accessible deployment sites limited survey design option, and we feel that on balance the
447 importance of surveying for temporal variation justified the less than desirable survey design.
448 Results should though be viewed with caution and, for example, standard variations should
449 not be over-interpreted, due to the limitations in terms of replication and sampling design.

450 In terms of management of NNS in ports, the findings of this study suggest that short-
451 term monitoring efforts and resources could be focussed on ports which are more likely to
452 support a larger number of NNS based on the environmental conditions. However, with a
453 view to documenting all NNS within a region and therefore developing the most effective
454 management strategies, long-term monitoring must not focus on any one port. Whilst larger
455 and more active ports may be more at risk to novel introductions based on the amount of
456 ship traffic they experience (Seebens et al., 2013, 2016; Sardain et al., 2019), smaller and
457 historically active ports are likely harbouring NNS and may therefore pose a similar risk to
458 the neighbouring natural systems.

459 Developing an understanding of the species present within these ports may prove decisive in
460 understanding if, how, and over what timescale NNS spread from a port into a natural
461 habitat.

462

463 **Conclusion**

464 The high degree of spatial variation in the observed communities between ports
465 showed that it cannot be assumed that any two ports, even in close proximity, will be
466 colonised by similar fouling communities. Whilst environmental conditions can provide an
467 indication as to the potential diversity within a port community, the high level of complexity
468 surrounding the formation of fouling communities limits our ability to make assumptions
469 based on environmental conditions alone. This is of importance when considering the
470 monitoring of non-native species (NNS) within ports or similar isolated environments. The
471 high degree of variation in the NNS recorded among ports highlights the importance of
472 monitoring individual ports, with the aim of documenting the introduction of NNS and
473 minimising the risk of spread. Increasing the spatial and temporal resolution of community
474 data from within the port environment could prove essential in implementing the most
475 effective NNS management strategies.

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483 **Acknowledgements**

484 We acknowledge the contributions of Associated British Ports in granting access to
485 the studied ports and in facilitating and assisting field surveys and the technical contribution
486 of Phil Hopkins in the construction of survey materials. This research was financially
487 supported by the Knowledge Economy Skills Scholarships (KESS 2) which is part funded by
488 the European Social Fund (ESF) via the Welsh Government. The funding sources had no
489 involvement in any aspect of this research.

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491 **Data availability statement**

492 Data for this manuscript are available on request from the corresponding author.

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Appendix. Abundance count or measure of coverage (cm²) for each species recorded within each of the five studied ports: Newport, Cardiff, Barry, Port Talbot, Swansea. * denotes species recorded as area covered when abundance counts were not possible. The status of each species within the UK is provided as either native (N), introduced non-native (I) or cryptogenic (C).

Species	Status	Newport	Cardiff	Barry	Port Talbot	Swansea	Species	Status	Newport	Cardiff	Barry	Port Talbot	Swansea
Porifera							<i>Harmothoe impar</i> (Johnston, 1839)	N	-	-	1	-	-
<i>Axinella damicornis</i> *	C	-	-	-	-	3	<i>Lepidonotus squamatus</i> (Linnaeus, 1758)	C	-	-	3	-	27
<i>Grantia compressa</i> (Fabricius, 1780)	N	-	-	-	-	36	<i>Nereimyra punctata</i> (Müller, 1788)	N	-	-	2	-	6
<i>Stelligera rigida</i> *	N	-	-	10	-	-	<i>Nereis zonata</i> (Malmgren, 1867)	C	-	-	1	-	2
Cnidaria							<i>Platynereis dumerilii</i> (Audouin & Milne Edwards, 1833)	C	30	19	5	-	3
<i>Cordylophora caspia</i> *	I	-	0.5	-	18.5	-	<i>Spirobranchus lamarcki</i> (Quatrefages, 1866)	N	-	-	16	-	-
<i>Diadumene lineata</i> (Verrill, 1869)	I	-	-	1	-	-	<i>Syllis cornuta</i> (Rathke, 1843)	N	-	-	-	-	2
<i>Laomedea flexuosa</i> *	N	1.5	-	0.5	-	0.5	Echinodermata						
<i>Obelia longissima</i> *	N	-	1	0.5	-	2.5	<i>Amphipholis squamata</i> (Delle Chiaje, 1828)	N	-	-	-	-	62
Platyhelminthes							Arthropoda						
<i>Prostheceraeus vittatus</i> (Montagu, 1815)	N	-	-	-	-	1	<i>Allomelita pellucida</i> (Sars, 1882)	N	128	-	-	-	-
Annelida							<i>Amphibalanus improvisus</i> (Darwin, 1854)	I	10474	1117	-	-	-
<i>Exogone naidina</i> (Örsted, 1845)	N	-	-	-	-	1	<i>Athanas nitescens</i> (Leach, 1813)	N	-	-	-	-	8
<i>Ficopomatus enigmaticus</i> (Fauvel, 1823)	I	7	3355	-	-	-	<i>Austrominius modestus</i> (Darwin, 1854)	I	-	-	23	-	12

Species	Status	Newport	Cardiff	Barry	Port Talbot	Swansea	Species	Status	Newport	Cardiff	Barry	Port Talbot	Swansea
<i>Balanus crenatus</i> (Bruguière, 1789)	N	-	-	57	-	159	<i>Monocorophium acherusicum</i> (Costa, 1853)	C	522	3	51	-	8
<i>Brachynotus sexdentatus</i> (Risso, 1827)	I	-	-	-	-	1	<i>Monocorophium sextonae</i> (Crawford, 1937)	I	-	-	6	-	-
Caddisfly larvae	N	-	-	-	1	-	Xanthidae sp.		-	-	-	-	1
<i>Caprella mutica</i> (Schurin, 1935)	I	-	-	37	-	13	<i>Sphaeroma serratum</i> (J.C. Fabricius, 1787)	N	3	8	-	-	-
<i>Crangonyx subterraneus</i> (Spence Bate, 1859)	N	-	-	-	6	-	Mollusca						
<i>Crassicorophium bonellii</i> (H. Milne Edwards, 1830)	N	-	-	-	-	4	<i>Cerastoderma edule</i> (Linnaeus, 1758)	N	3	-	-	-	-
<i>Dexamine spinosa</i> (Montagu, 1813)	N	-	-	-	-	6	<i>Ecrobia ventrosa</i> (Montagu, 1803)	N	-	20	-	-	-
<i>Endeis spinosa</i> (Montagu, 1808)	N	-	-	-	-	1	<i>Mytilus edulis</i> (Linnaeus, 1758)	N	1745	1189	1083	-	80
<i>Gammarus duebeni</i> (Lilljeborg, 1852)	N	-	26	-	1	-	<i>Mytilopsis leucophaeata</i> (Conrad, 1831)	I	-	-	-	4	-
<i>Gammarus salinus</i> (Spooner, 1947)	N	-	-	6	-	-	Bryozoa						
<i>Gammarus zaddachi</i> (Sexton, 1912)	N	51	2	-	-	-	<i>Bugulina stolonifera</i> (Ryland, 1960)	I	-	-	1	-	240
<i>Jaera nordmanni</i> (Rathke, 1837)	N	-	7	-	-	-	<i>Cryptosula pallasiana</i> *	N	15	-	-	-	4.5
<i>Lembos websteri</i> (Bate, 1857)	N	-	-	14	-	21	<i>Electra Pilosa</i> *	C	152	174.5	-	-	-
<i>Maera grossimana</i> (Montagu, 1808)	N	-	1	-	-	-	<i>Fredericella sultana</i> *	C	-	-	-	513	-
<i>Microdeutopus Gryllotalpa</i> (Costa, 1853)	N	-	-	36	-	42							

Species	Status	Newport	Cardiff	Barry	Port Talbot	Swansea	Species	Status	Newport	Cardiff	Barry	Port Talbot	Swansea
Chordata							<i>Diplosoma listerianum</i> *	N	-	-	-	-	13
<i>Asciella scabra</i> (Müller, 1776)	N	-	-	123	-	1288	<i>Molgula socialis</i> (De Kay, 1843)	N	8	-	93	-	-
<i>Botryllus schlosseri</i> *	N	-	-	180.5	-	7	<i>Styela clava</i> (Herdman, 1881)	I	-	-	1	-	-
<i>Ciona intestinalis</i> (Linnaeus, 1767)	N	-	-	162	-	865							