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Genetic homogeneity in the deep-sea grenadier *Macrourus berglax* across the North Atlantic Ocean

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1 **Genetic homogeneity in the deep-sea grenadier *Macrourus berglax* across the North**
2 **Atlantic Ocean**

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20 ***Keywords***

21 **Microsatellites, mitochondrial DNA, marine, population structure, fisheries**

22 ***Abstract***

23 Paucity of data on population structure and connectivity in deep sea species remains a major
24 obstacle to their sustainable management and conservation in the face of ever increasing
25 fisheries pressure and other forms of impacts on deep sea ecosystems. The roughhead
26 grenadier *Macrourus berglax* presents all the classical characteristics of a deep sea species,
27 such as slow growth and low fecundity, which make them particularly vulnerable to
28 anthropogenic impact, due to their low resilience to change. In this study, the population
29 structure of the roughhead grenadier is investigated throughout its geographic distribution
30 using two sets of molecular markers: a partial sequence of the Control Region of
31 mitochondrial DNA and species-specific microsatellites. No evidence of significant
32 structure was found throughout the North Atlantic, with both sets of molecular markers
33 yielding the same results of overall homogeneity. We posit two non-mutually exclusive
34 scenarios that can explain such outcome: i) substantial high gene flow among locations,
35 possibly maintained by larval stages, ii) very large effective size of post-glacially expanded
36 populations. The results can inform management strategies in this by-caught species, and
37 contribute to the broader issue of biological connectivity in the deep ocean.

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44 ***Introduction***

45 Over the last few decades, it has become routine to use genetic techniques to
46 investigate population structure in marine fishes (Carvalho et al., 2016). The results have
47 led to the widespread rejection of the commonly held view that marine species are mostly
48 panmictic, due to the lack of visible barriers to larval and adult movements (Hauser and
49 Carvalho, 2008). The action of ocean circulation can in fact be two-fold: superficial or deep-
50 water currents can increase gene flow by aiding individual dispersal, especially at the larval
51 stages, but they can also act as a barrier to it, hence favouring divergence between groups.

52 The vast majority of published studies on marine fish have dealt with coastal pelagic
53 species, given their commercial value and/or the convenient sampling. Yet, the fishing
54 pressure on deep-sea stocks has been steadily increasing since the 1970s (Roberts, 2002),
55 and the depth at which fisheries operate has also been increasing at an average pace of 65.2
56 m per decade (Morato et al., 2006; Watson and Morato, 2013). Despite being increasingly
57 exploited, deep-sea fish species still suffer from a paucity of data, compared to their coastal
58 and shallow counterparts, which can have deleterious effects on their management (see
59 Clarke et al, 2015 for a quantitative discussion). The assessment of the level and range of
60 spatial structure of exploited species is pivotal for the sustainable harvest and management
61 of species, and failure to identify population structure may result in population collapse
62 (Reiss et al., 2009, Lowe and Allendorf, 2010). Given the typical life history traits of deep-
63 sea species (discrete spawning aggregations, slow growth, late maturity), any fishing
64 pressure might have serious consequences for the persistence of stocks (Baker et al., 2009).
65 Thus, it is important to gather data in order to better understand the population structure and
66 dynamics of these fish stocks, whether they are directly exploited or caught as by-catch. The
67 most recent studies on the dynamics of deep sea fish species have reported lack or very low
68 genetic structuring across wide geographical scales (*Centroscymnus crepidater* in Cunha et

69 al., 2012; *Coryphaenoides mediterraneus* in Catarino et al., 2013; *Hoplostethus atlanticus*
70 in White et al., 2009) across their vast geographic ranges, and capable of transoceanic
71 ontogenetic migrations (*Aphanopus carbo* in Longmore et al., 2014). Depth has been found
72 to represent a barrier to gene flow and promote low but significant population structuring
73 (*Haplostethus atlanticus* in Carlsson et al., 2011; *Etmopterus spinax* in Gubili et al., 2016;
74 *Coryphaenoides rupestris* and *Brosme brosme* in Knutsen et al., 2012, 2009; *Sebastes*
75 *mentella* in Shum et al., 2014, *Centroscymnus coelolepis* in Catarino et al., 2015). Although
76 the extent of pelagic habits typically influences the expected levels of genetic heterogeneity,
77 even at global scales (see Gaither et al, 2016), the deeper layers of the oceans remain less
78 understood, and no robust life-history predictors of spatial structure currently exist.

79 The roughhead grenadier *Macrourus berglax* Lacepède 1801, family Macrouridae, is
80 a benthopelagic species occurring across the northern Atlantic Ocean, between the Georges
81 Bank to the west, all the way to the Barents Sea as the easternmost edge. It dwells between
82 a depth of 100 and 1000 m, although it is especially common at depths of 300-500 m (Cohen,
83 1990). Data about its biology, population structure and dynamics are scarce, even though it
84 is an important part of the by-catch in the red fish and the Greenland halibut fisheries
85 (Garabana et al. 2016; Gonzales Costas and Murua, 2005; Gonzales costas 2010). Life
86 history traits of this species are similar to those of other deep sea fishes: it lives long (up to
87 25 years according to Lorance et al. (2008) and Drazen et al. (2012)), grows slowly and has
88 low fecundity, between 14,000 and 80,000 eggs (Devine et al., 2012; Fossen et al., 2003;
89 Murua, 2003, Drazen et al. 2012). Spawning has been documented across the species'
90 geographic distribution in late Winter/early Spring (Magnússon and Magnússon, 1995;
91 Savvatimsky, 1989), although geographical differences in time of spawning might exist
92 (Lorance et al., 2008; Garabana et al. 2016). Very little is known about the dispersal ability
93 at different life stages of *M. berglax*: spawning migration has been hypothesised (Garabana

94 et al. 2016), but not demonstrated. The other four species of the genus *Macrourus* (*M.*
95 *carinatus*, *M. whitsoni*, *M. caml* and *M. holotrachys*), are all present exclusively in the
96 southern hemisphere and have well-documented extended adult migration (Laptikhovsky
97 2011, Munster et al. 2016), while more distantly related macrourids, such as the roundnose
98 grenadier *Coryphaenoides rupestris*, have been found to have a very long pelagic phase,
99 which can last for almost a year (Lorance et al., 2008).

100 The population structure of *M. berglax* across its geographic range is also poorly
101 investigated. The only published genetic study finds low differentiation, but advocates the
102 existence of at least three units or stocks: East Greenland, West Greenland and Norwegian
103 Sea (Katsarou and Naevdal 2001). This investigation used allozyme markers, which, due to
104 their nature of being expressed by coding regions may poorly reflect patterns of neutral gene
105 flow (O'Sullivan *et al.*, 2003).

106 Concerns regarding the lack of data and the status of the stock of the roughhead
107 grenadiers in the North Atlantic were recently raised by NAFO (Northwest Atlantic
108 Fisheries Organization) and ICES (International Council for the Exploration of the Sea),
109 given the decrease in landings of grenadiers in the North Atlantic (Gonzales Costas 2010,
110 ICES 2015). In the NAFO Regulated Area (western Atlantic), this species is mainly caught
111 in subareas 3LMN, just around Flemish Cap, where it is becoming a commercially important
112 fish despite the fishery being unregulated (Gonzales Costas and Murua, 2005). In the eastern
113 Atlantic (ICES Area), it is by-caught with the roundnose grenadier *Coryphenoides rupestris*,
114 and its stock status is unknown and unmanaged (ICES 2015).

115 In this study, our aim was to fill some of these knowledge gaps by investigating the
116 population structure of the roughhead grenadier across its geographic range and over
117 multiple years, using species-specific microsatellite markers (Helyar et al., 2010) and the
118 mitochondrial DNA control region (CR). In particular, we tested whether spatial genetic

119 heterogeneity exists in *M. berglax*, and whether the three stocks suggested by Katsarou and
120 Naevdal (2001) are upheld by our data. The findings significantly enhance our
121 understanding of past and present population structure and diversification in *M. berglax*, and
122 lay the foundation for improved conservation and management of the species.

123

124 ***Materials and Methods***

125 *Sampling*

126 A total of 638 individuals were sampled at eight locations across the entire species'
127 geographic distribution, between 2000 and 2007 (Table 1), by research and commercial
128 vessels. The sampling localities are northern Norway (Bear Island), Svalbard, East and
129 South Greenland, Baffin Bay, Flemish Cap, Georges Bank and Hatton Bank (Fig. 1). Tissue
130 samples were collected and stored in absolute ethanol. Fork length was measured for each
131 individual at all except one location (Georges Bank). Finally, for all populations excluding
132 Georges Bank, anal fin length data was collected, and for Flemish Cap and South Greenland
133 individuals were sexed.

134 *Genetic analysis*

135 DNA was extracted from muscle tissue using a standard salting-out protocol (Miller et al.,
136 1988). A 1100 bp long fragment of the mtDNA Control Region (CR) was PCR amplified
137 from 124 individuals (Table 1) using primers L-Pro1 (Ostellari et al., 1996) and 12Sar-H
138 (Palumbi et al., 1991). All PCRs were carried out in a final volume of 25 μ l, containing 1X
139 PCR buffer (*Buffer BD Advantage 2 PCR* with $MgCl_2$), 0.2 mM of each dNTP, 0.2 μ M of
140 each primer, 1 μ l of template DNA, and Taq DNA polymerase (1 unit, *Taq BD Advantage™*
141 *2 Polymerase Mix*; CLONTECH-Takara). The following PCR profile was used for the
142 amplification: one cycle of 1 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 52 °C, and 70

143 s at 68 °C, and finally, one cycle of 5 min at 68 °C. PCR products were purified with an
144 ethanol/sodium acetate precipitation, and directly sequenced using the corresponding PCR
145 primers in an automated DNA sequencer (ABI PRISM 3700) using the BigDye Deoxy
146 Terminator cycle-sequencing kit (Applied Biosystems) following manufacturer's
147 instructions. Seven microsatellites (Helyar et al., 2010) were amplified for 559 individuals,
148 starting at 95°C for 15 min, followed by 35 cycles of 45 sec at 94°C, 45 sec at a set annealing
149 temperature, 45 sec at 72°C, and a final extension step of 45 min at 72°C. Markers were
150 multiplexed in 10 µl final volume using the QIAGEN Multiplex Kit as follows: 1) Mbe06,
151 Mbe02, Mbe03, Mbe04 at an annealing temperature of 57 °C; 2) Mbe01, Mbe08, Mbe10 at
152 60 °C. Both reactions were then run on an ABI3130xl genetic analyser for screening. Peaks
153 were scored using Genemapper 4.0 (Applied Biosystems).

154 *Statistical analysis*

155 Haplotype (h) and nucleotide (π) diversities and Φ_{ST} were calculated using Arlequin 3.5
156 (Excoffier and Lischer, 2010). Significance levels of all multiple statistical tests were
157 corrected using the false discovery rate (FDR) approach (Benjamini and Hochberg, 1995)
158 implemented in the QVALUE package (Dabney et al., 2010) in R (R Core Team, 2016).
159 Mitochondrial allelic richness (A_R) was estimated using the rarefaction method (El
160 Mousadik and Petit, 1996) as implemented in Contrib 1.02 (Petit and Pons, 1998). A median-
161 joining network (Bandelt et al., 1999) was calculated in PopART (<http://popart.otago.ac.nz>).
162 Mismatch analysis (Rogers and Harpending, 1992) was used to explore the demographic
163 history of the species, estimating both the raggedness index (rg, Harpending 1994) and the
164 sum of squared deviations (SSD, Schneider & Excoffier 1999) using Arlequin 3.5
165 (Excoffier and Lischer, 2010). Demographic mismatch analysis is based on the null
166 hypothesis of expansion; thus, non-significant values mean non-rejection of population

167 expansion. Initial and final θ estimates (before and after population growth or decline) and
168 τ values were calculated with ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Time of
169 inferred population expansion was determined by $T_{\text{exp}} = \tau / (2 \mu n)$, where μ is the substitution
170 rate per base and per generation, and n the number of bases of the CR fragment (Rogers &
171 Harpending 1992), assuming a generation time of 15 years (Fossen et al., 2003). Historical
172 population size changes were further investigated by calculating Tajima's D and Fu's F_s
173 (Fu, 1997; Tajima, 1989a, 1989b) in ARLEQUIN 3.5.

174 The Bayesian Skyline Plot (BSP) approach as implemented in BEAST v 1.7 (Drummond
175 et al., 2012) was employed to estimate historical changes of female effective population
176 size. The best fitting model of substitution – HKY (Hasegawa et al., 1985) - was found
177 using jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) via the Akaike
178 Information Criterion (AIC). Eight independent runs (each 10^6 generations and 10% burn-
179 in) were used to reach effective sample size value (ESS) of at least 200 as per the user's
180 manual.

181 For microsatellites, the frequency of null alleles was estimated using FreeNA (Chapuis and
182 Estoup, 2007). Marker neutrality was tested in LOSITAN (Antao et al., 2008) using the
183 approach described in Beaumont and Nichols (1996). GenAlEx v6 (Peakall and Smouse,
184 2006) was employed to estimate expected (H_E) and observed (H_O) heterozygosity indices.
185 Linkage disequilibrium, allelic richness (A_R), F_{IS} and F_{ST} were calculated in Fstat 2.9.3
186 (Goudet, 1995). Contemporary effective population size (N_e) was estimated using the
187 Linkage Disequilibrium method implemented in LDNe (Waples and Do, 2008), using 0.02
188 as minimum allowable allele frequency (P_{crit}). N_e was calculated both by location and
189 'overall', by pooling all the samples together based on the results. Population structure was
190 investigated using two approaches with different assumptions. STRUCTURE 2.3 (Falush et
191 al., 2007, 2003; Pritchard et al., 2000) was used to estimate population subdivision, allowing

192 for admixture and with both correlated and independent allele frequencies using 200,000
193 permutations following a burn-in of 50,000, with three independent runs for each value of
194 k . A second approach for estimating population structure, discriminant analysis of principal
195 components (DAPC), was implemented in the R package ADEGENET (Jombart, 2008;
196 Jombart et al., 2010). The optimal number of k clusters is chosen based on the associated
197 (lowest) Bayesian Information Criterion (BIC) calculated after 10^7 iterations. The difference
198 in these assignment tests lies in their assumptions: while STRUCTURE 2.3 groups
199 individuals in a number k of clusters by minimising Hardy-Weinberg (HWE) and linkage
200 disequilibria, DAPC groups individuals maximising the separation amongst such groups,
201 with no prior assumptions. COLONY (Jones and Wang, 2010) was used to estimate sibship
202 between sampled individuals, using Full Likelihood approach and remaining default
203 parameters. A threshold value of 0.8 was used, meaning that only pairs of individuals with
204 at least 80% probability of being fullsibs/halfsibs were considered (Bergner et al., 2014).
205 Although COLONY has proven to provide accurate results even with a low number of
206 markers (Harrison et al. 2013), given the low diversity of the present dataset the probability
207 of Type I errors cannot be disregarded (Taylor 2015). Thus, we performed the sibship
208 analysis with another package, ML-Relate (Kalinowski et al. 2006). Only the pairs identified
209 as sibs by both analyses were considered as such.

210

211 ***Results***

212 **Fork length.** Baffin Bay individuals were significantly smaller than individuals from all the
213 other populations (Fig. 5 and Fig. 3 Supplementary Material), while northern Norway
214 comprised the largest individuals. The length frequency distributions show that the only
215 population with a bi-modal trend is Svalbard.

216 **Mitochondrial Control Region.** A total of 29 haplotypes were identified in the 114
217 individuals sequenced across eight locations (GenBank accession Numbers: MG702365-
218 MG702488). All the diversity indices calculated for the mitochondrial DNA were found to
219 be lowest for Svalbard, with $h = 0.19$ and $\pi = 0.0002$. Haplotype diversity, nucleotide
220 diversity and the average number of nucleotide differences were highest for Flemish Cap,
221 being respectively 0.82, 0.0018 and 1.45. Allelic richness ranged from 1 (Svalbard) to 5.6
222 (Baffin Bay) (Table 1). The median-joining network showed haplotypes were grouped in a
223 clear star-like pattern. The most common haplotype was present in 86 individuals (75.4%
224 of the total) scattered across all sampled locations (see Fig. 1). Low but significant Φ_{ST}
225 were recorded between Svalbard and Flemish Cap (Table 2), but, given the low level of
226 genetic differentiation, demographic tests were performed for all samples pooled. Results
227 indicate that the Atlantic populations of *M. berglax* conform to both the demographic and
228 spatial expansion models ($P_{DEM} = 0.929$; $P_{SPA} = 0.796$) (Fig. 1 Supplementary Material).
229 The τ values ($\tau_{DEM} = 0.723 - 99\%CI: 0-3.639$; $\tau_{SPA} = 0.318 - 99\%CI: 0.1-4.7$) inferred were
230 used to estimate times since expansion (t) for both scenarios assuming a divergence rate of
231 11% per million years (Patarnello et al., 2007) and a generation time of 15 years (Fossen et
232 al., 2003) in the Mismatch Calculator (Schenekar and Weiss, 2011). The findings suggest
233 that the grenadier started expanding demographically 8,000 years ago (7,919, 99%CI:
234 0-39,858) and then spatially less than 4,000 years ago (3,483, 99%CI: 1,150-52,377).
235 Tajima's D (-2.32, $P \ll 0.00$) and Fu's F_S ($-\infty$, $P \ll 0.00$), both negative and highly
236 significant, further confirmed this scenario of expansion. Historical female effective
237 population size inferred with BEAST also indicated that expansion started ~4,400 years
238 ago (Fig 2).

239 **Microsatellites.** No evidence of null alleles and linkage disequilibrium was identified across
240 the microsatellite dataset. No significant departures from neutrality were detected in

241 LOSITAN when considering 99% confidence intervals. Expected heterozygosity H_E was
242 similar across locations, ranging between 0.619 (Hatton Bank) and 0.674 (Flemish Cap),
243 while the lowest H_O was recorded in Flemish Cap and Svalbard (0.53) and the highest in
244 Greenland (0.673 for South Greenland and 0.668 for Eastern Greenland). Heterozygote
245 deficiency ($H_O < H_E$) was detected in Svalbard and Flemish Cap, which indeed are the only
246 samples showing a positive and significant F_{IS} (Table 1). Pairwise F_{ST} values showed that
247 Norway was the most divergent population, with values that, despite being low, remained
248 significant after Bonferroni's correction. Other statistically significant pairwise comparisons
249 involved Svalbard against Baffin Bay and Georges Bank, and Georges Bank against South
250 Greenland (Table 2). Nevertheless, both assignment tests (STRUCTURE and ADEGENET)
251 employed failed to detect significant spatial population structure within the study area (Fig.
252 3 and Fig. 1 Supplementary Material). DAPC results did not show any significant
253 geographical pattern, even when repeated with populations grouped by year of capture (Fig.
254 3) or age class (data not shown). Effective population size was calculated both by location
255 and by pooling all the samples together, and every estimate had an infinite estimate of the
256 parameter. COLONY and ML-RELATE found 15 pairs of half-sibs and no full-sibs (Table
257 1 Supplementary Material and see Fig. 1 for a graphical representation) with no particular
258 pattern.

259 **Discussion**

260 The roughhead grenadier *Macrourus berglax* is an important part of the by-catch of halibut
261 and redfish fisheries, but is to some extent also targeted by smaller fisheries. Thus, in the
262 absence of a management plan, compounded with lack of information on the population
263 biology, any form of management strategy is hindered. The species is managed throughout
264 its range by two agencies, NAFO in the Northwest Atlantic and ICES in the Northeast
265 Atlantic. NAFO does not have specific regulations for the roughhead grenadier, and ICES

266 concludes that “no direct fishery should be allowed and that by-catch should be accounted
267 for against the TAC” (ICES, 2015).

268 In the only previously published work on population genetic structure of *M. berglax*,
269 Katsarou and Naevdal (2001) found low overall genetic diversity, but still called for each
270 subarea (West Greenland, East Greenland and Norway) to be managed as an independent
271 unit, as at least two out of the ten markers they used show heterogeneity. The authors called
272 for further investigations using more/different markers and a more intensive sampling
273 design. This has been implemented in the current study, where mitochondrial DNA and
274 nuclear hypervariable markers are simultaneously used, but still fail to support any evidence
275 of population structure. This aligns *M. berglax* with several other demersal and pelagic deep
276 sea fish species, such as the roundnose grenadier (White et al. 2010), the longnose velvet
277 dogfish (Cunha et al., 2012), the Mediterranean grenadier (Catarino et al., 2013) and the
278 black scabbard fish (Longmore et al. 2014), which were found to be largely genetically
279 homogeneous at oceanic level.

280 We used two different markers in order to investigate demographic processes at different
281 time scales: while the fast-evolving microsatellites are more suitable to untangle
282 contemporary structure (Hewitt, 2004), the mtDNA control region is usually employed to
283 unravel more ancient events on the evolutionary time scale, as well as track maternal
284 effects (being maternally inherited) (Awise, 2000). Hence, comparing and contrasting the
285 results from the two datasets can provide important insights into the processes that have
286 brought the species to be distributed as it is observed today.

287 From a management perspective, the lack of genetic differentiation at neutral markers
288 (microsatellites, markers not linked to parts of the genome under directional selection)
289 does not necessarily coincide with the existence of one single unit/stock. In marine
290 populations, low F_{ST} from neutral markers often reflect very large effective population size

291 in recently expanded populations, rather than the existence of substantial gene flow rates
292 (Cano et al., 2008), and this could be the case for the roughhead grenadier, whose
293 estimates of effective size are very large. In this scenario, even in the presence of low gene
294 flow, such large effective population size might be enough to counteract the divergence
295 caused by genetic drift, especially over a few generations, such as in the case of the slow-
296 growing *M. berglax*.

297 Comparing and contrasting the results from the two classes of markers used here, can
298 provide important insights into the processes that have shaped the genetic makeup of this
299 species. The mtDNA control region network shows a distinctive star-like shape, which is
300 an indication of demographic expansion. Two approaches with different assumptions have
301 been used to date such expansion, and both give the same time estimate: the roughhead
302 grenadier started expanding in the North Atlantic around 4,000 years ago. This
303 corresponds roughly to 200 generations, which in evolutionary scale is considered recent.
304 This timing is consistent with new habitat becoming available after the Younger Dryas
305 (~11,700 years ago). Unfortunately, based on the results, it is hard to speculate about the
306 possible location of the marine refugium which might have sourced the
307 recolonization/expansion into the North Atlantic Ocean (Kettle et al., 2011). It has been
308 hypothesised that the Atlantic Meridional Overturning Circulation (AMOC) went through
309 a strong spin-up after the last Younger Dryas glaciation and was responsible for one of the
310 most important dispersal events in the Atlantic, injecting larvae from the warmer southern
311 area, into ocean currents that led to the fastest postglacial range expansion ever recorded in
312 the deep (Henry et al., 2014).

313 The comparison between diversity indices calculated from both sets of molecular markers
314 (Table 1) allowed the determination of uneven contributions from the sampled populations
315 to the measured genetic diversity. Overall, microsatellite markers show consistent

316 homogeneity amongst locations. Mitochondrial DNA, on the other hand, shows that the
317 populations sampled at the western edge of the species distribution, Baffin Bay and Hatton
318 Bank, have the highest allelic richness and haplotypes diversity, contributing to the
319 majority of the total diversity. On the other hand, Svalbard registered the lowest values, for
320 any of the indices estimated from mtDNA (Table 2). Usually, lower genetic diversity,
321 hence lower adaptive capacity, is expected at population that originates from an expansion
322 (i.e. post-glacial) as they are subjected to a founder effect (Marko et al. 2010), compared to
323 those populations that still inhabits the refugial area (Diekmann and Serrao 2012). Yet,
324 supporting evidence is lacking (Zardi et al. 2015), so, once more, we cannot speculate on
325 the location of the refugium.

326 The F_{ST} analysis of microsatellites shows a low but statistically significant differentiation
327 of the Norwegian sample (Table 2). Having excluded the possibility of directional
328 selection acting upon these markers, we cannot conclude whether this is due to a spatial or
329 a temporal differentiation (these were the only fish caught in 2007). Nevertheless,
330 clustering analyses do not find Norway to be much different from the rest of the samples
331 (Fig. 2 Supplementary Material).

332 An interesting result unraveled by microsatellites is the presence of 15 half-sibs pairs
333 across the ocean. These are pairs of individuals born in different years that share at least
334 one parent. This could be explained by two possible causes: firstly, the roughhead
335 grenadier might have a unique spawning aggregation or secondly, and more likely, larval
336 and adult dispersal might play an important role in gene flow. Other members of the genus
337 *Macrourus* have been shown to have extended adult dispersal, and spawning migration has
338 also been hypothesized for *M. berglax* (Garabana et al. 2016). Adult dispersal and
339 migration have been shown to play an important role in ‘homogenizing’ populations, even

340 at oceanic scale (White et al. 2009), and could then explain the lack of genetic
341 differentiation detected in this study.

342 Lastly, the length distribution shows that the individuals caught in Baffin Bay are
343 significantly smaller than the ones coming from every other location (Fig. 4). This may
344 suggest that this area might be a breeding/nursery ground. Although this study can only
345 speculate about this, it would be important to consider this aspect in designing future
346 investigations, as this would be of vital importance for the management and conservation
347 of the species.

348 In conclusion, the roughhead grenadier *Macrourus berglax* shows no population structure
349 across the Atlantic Ocean. Whether this near-panmixia signal is due to a very large
350 population size or actual gene flow between locations is currently impossible to
351 disentangle. Nevertheless, caution should be taken when designing a management plan.

352 Previous studies using non-neutral markers did find significantly different units (Katsarou
353 and Naevdal, 2001), hence future work should aim at identifying markers under selection.

354 Local adaptation is now regarded as an important tool to discern populations in the marine
355 environment (Nielsen et al. 2012). Although this species is not a commercial target, it
356 makes up an important part of the bycatch, and more data should be gathered in order to
357 ensure its future conservation.

358

359 **Conflict of interest:** none

360

361 **Author contribution**

362 SM, RCa, HK and SS designed the study; IC analysed the data and wrote the manuscript
363 with input from SM and RCa; AMG, CS, SH and RCu conducted lab work; all authors
364 commented on drafts of the manuscript.

365

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610 **Table 1** Sampling locations, population codes (*ID*), year of sampling (*Year*) and genetic
611 diversity parameters inferred from mitochondrial DNA and microsatellites. *N*, number of
612 individuals screened; *N_H*, number of haplotypes; *h*, haplotype diversity; π , nucleotide
613 diversity; *A_r*, allelic richness; *N_A*, number of alleles; *H_o*, observed heterozygosity; *H_E*,
614 expected heterozygosity; *F_{IS}*, inbreeding coefficient (in bold, values that are significant;
615 $p < 0.05$).

Location	ID	Year	Mitochondrial DNA					Microsatellites					
			N	<i>N_H</i>	<i>h</i>	π	<i>A_r</i>	N	<i>N_A</i>	<i>A_r</i>	<i>H_o</i>	<i>H_E</i>	<i>F_{IS}</i>
East Greenland	EGree	2000	12	5	0.58	0.0014	2.73	95	9.86	5.14	0.668	0.665	-0.005
South Greenland	SGree	2003	25	6	0.30	0.0006	1.92	88	9.57	4.88	0.673	0.650	-0.020
George Bank	GEO	2004	12	3	0.32	0.0004	1.67	52	7.86	4.96	0.599	0.649	0.070
Svalbard	SVA	2002	20	3	0.19	0.0002	1.00	80	8.26	5.02	0.535	0.668	0.200
Hatton Bank	HAT	2000	14	7	0.76	0.0015	4.51	50	7.71	4.82	0.592	0.619	0.040
Flemish Cap	FLE	2002	11	7	0.82	0.0018	3.08	21	5.43	4.89	0.530	0.674	0.210
Baffin Sea	BAF	2001	17	10	0.79	0.0017	5.56	75	9.14	4.77	0.637	0.646	0.010
Norway	NOR	2007	13	5	0.54	0.0009	3.07	98	7.29	4.64	0.623	0.629	0.010

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618 **Table 2** Population pairwise comparisons: microsatellite-derived *F_{ST}* in the lower diagonal,
619 and mtDNA-based Φ_{ST} in the upper section. In bold, values that remain significant
620 ($p < 0.05$), and in bold and italic those that remain significant after Bonferroni's correction.

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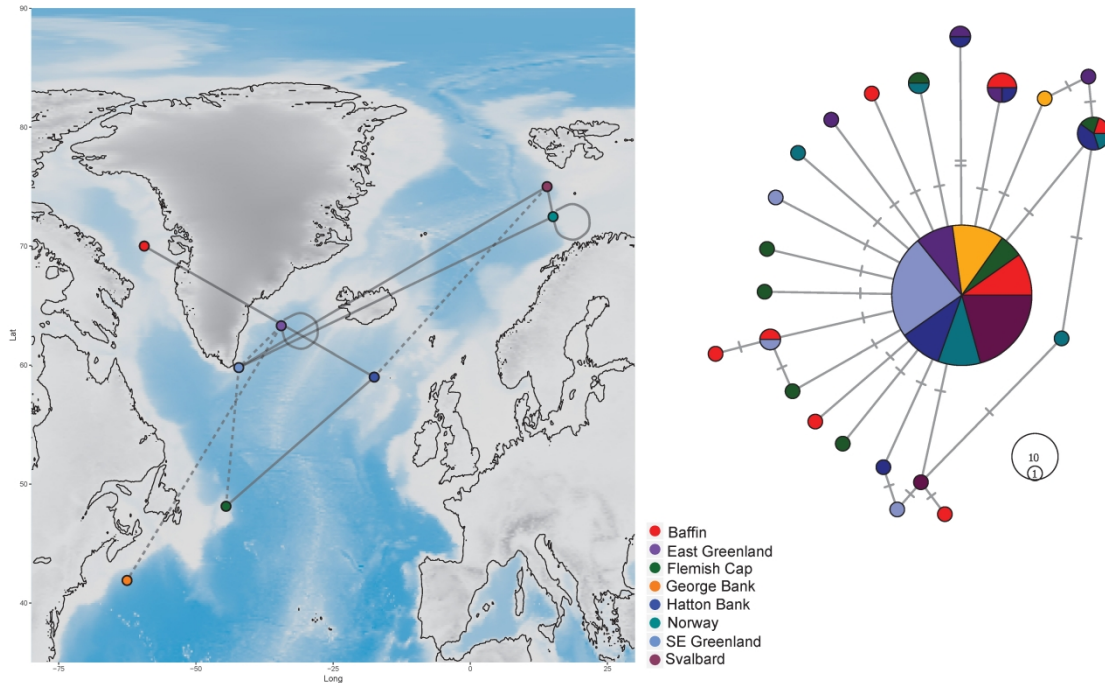
	$F_{ST} \setminus \Phi_{ST}$							
	Egree	Sgree	GEO	SVA	HAT	FLE	BAF	NOR
Egree	-	0.031	-0.02	0.035	-0.05	-0.01	-0.01	-0.01
Sgree	-0.0006	-	-0.012	-0.02	0.027	0.029	0.012	0.0307
GEO	-0.002	0.002	-	0.0091	0.009	0.057	0.0027	0.0228
SVA	0.003	0.005	0.001	-	0.0431	0.0448	0.0225	0.038
HAT	-0.007	0.001	0.0009	0.0096	-	-0.01	-0.01	-0.02
FLE	-0.002	-0.005	-0.001	-0.006	0.002	-	-0.006	-0.03
BAF	-0.003	-0.0008	-0.002	0.0045	-0.0007	-0.006	-	0.0225
NOR	0.0087	0.0068	0.0065	0.0049	0.0084	0.008	0.011	-

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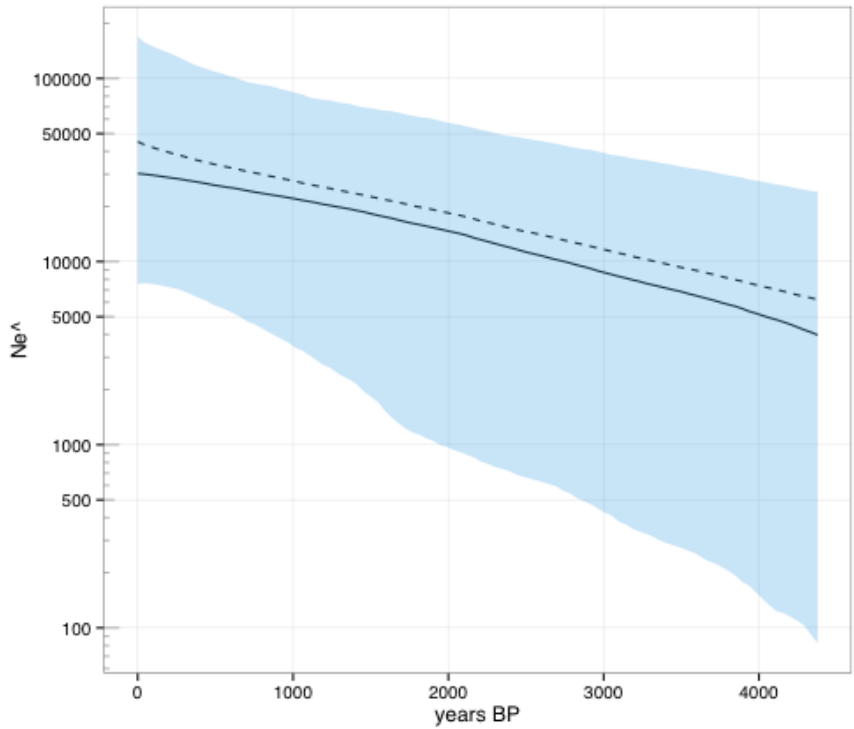


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627 **Figure 1** Map showing the colour-coded sampling locations. The lines connecting the
628 locations represent the half-sib pairs: dashed lines for two pairs between locations, whereas
629 all the other lines represent one pair (see also Table 1 supplementary material). On the right,
630 the median-joining network of mitochondrial haplotypes.

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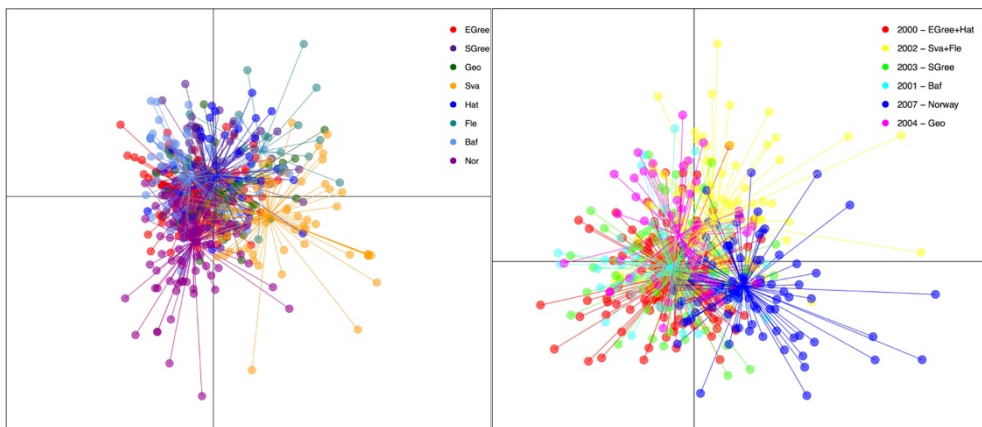
634 **Figure 2** Bayesian Skyline plot, showing the historical trend of female effective
 635 population size. The continuous and dashed lines represent the median and mean values,
 636 respectively. The shaded area represents the 95% Confidence interval.

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642 **Figure 3** DAPC scatter plots by sampling locations (*left*) and year of capture (*right*). In the
 643 latter, the corresponding locations per year of capture are indicated in the legend.

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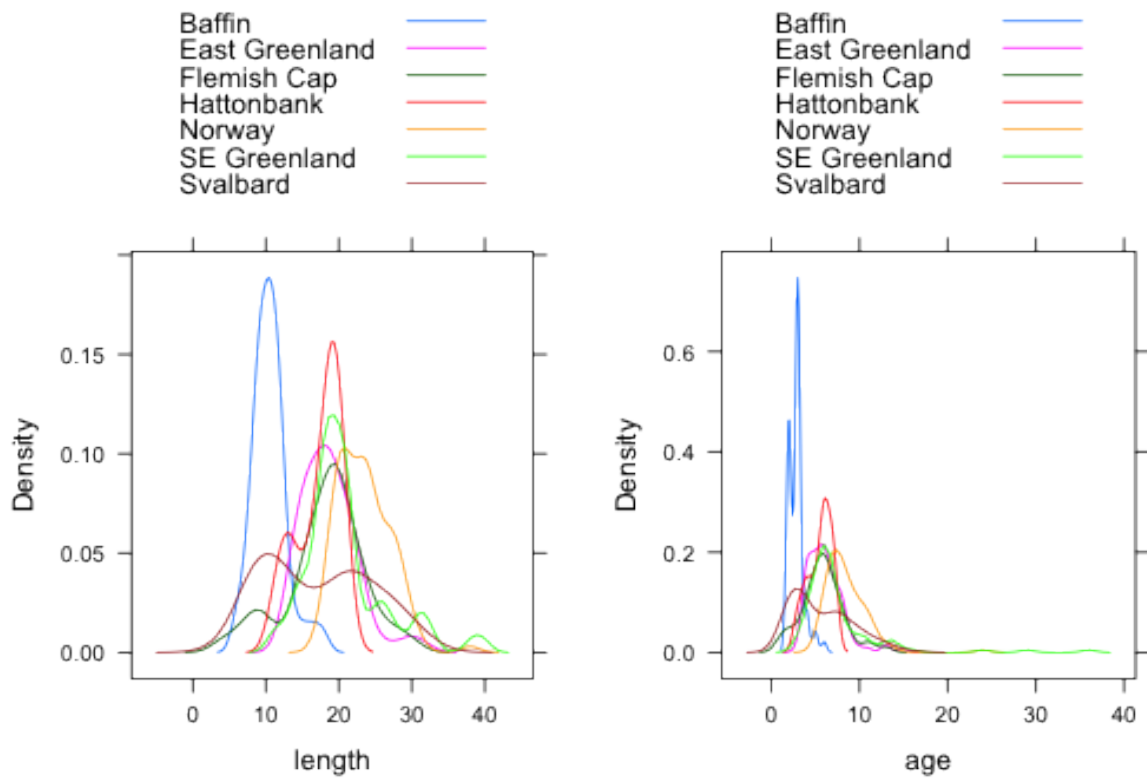
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651 **Figure 4** Length frequency distributions by location.

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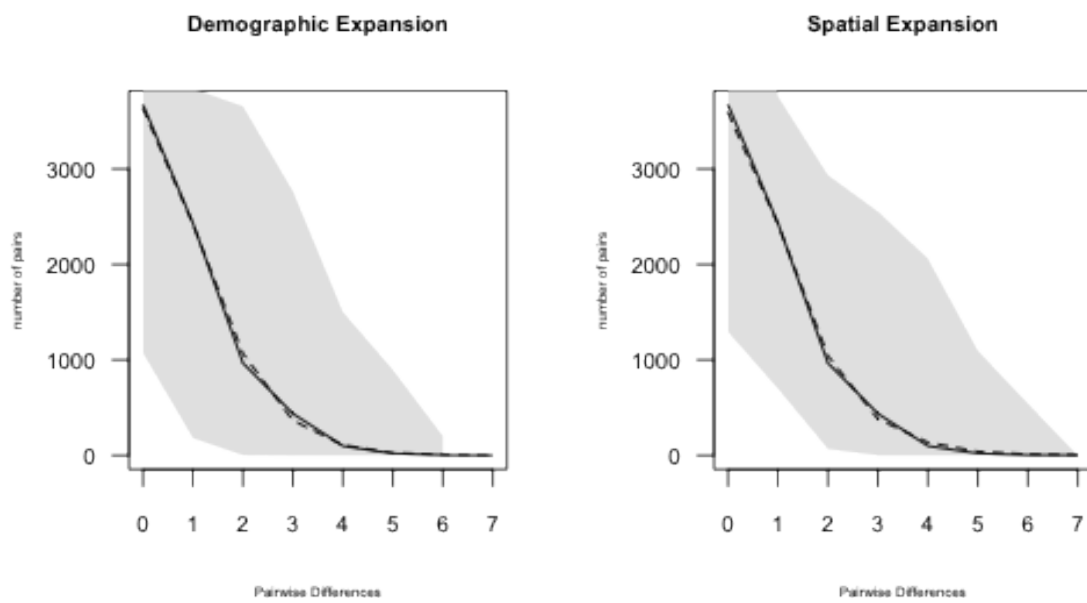
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661 **Supplementary Material**



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663 **Figure 1 Supplementary Material:** Mismatch distributions. The shaded grey area

664 represents the 95% confidence interval.

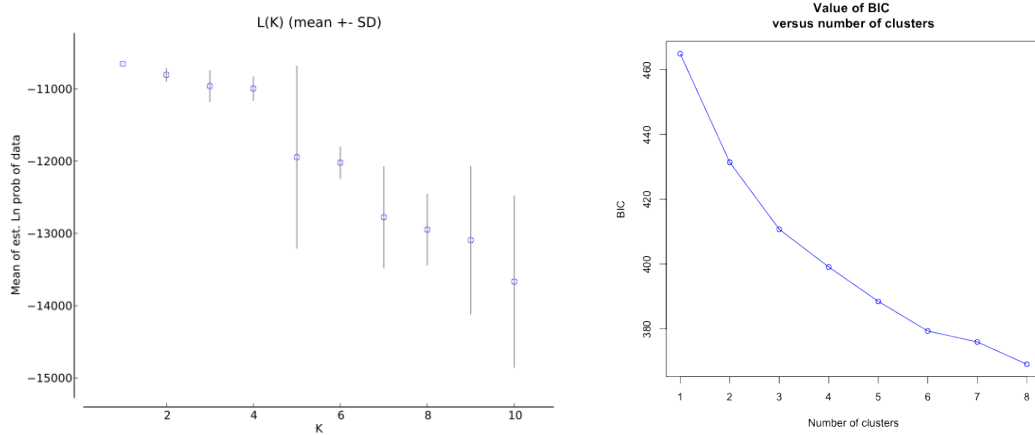
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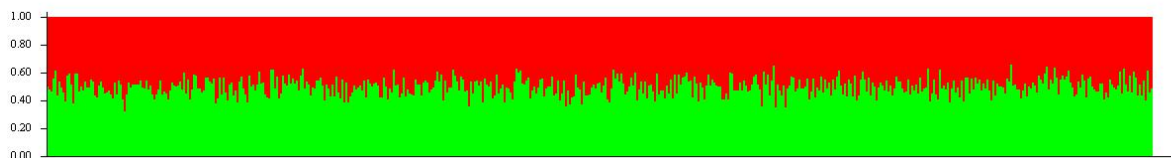
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672 **Figure 2 Supplementary Material:** *Topleft* – Mean Likelihood inferred from
673 STRUCTURE; *topright* – graphical inference of the number of genetic clusters from DAPC.
674 Both graphs show a trend that is interpreted with the absence of genetic clusters
675 (homogeneity). This is particularly evident in the barplot at the bottom (inferred from
676 STRUCTURE): on the Y-axis the Q-value, and index of admixture; on the X-axis the
677 individuals.

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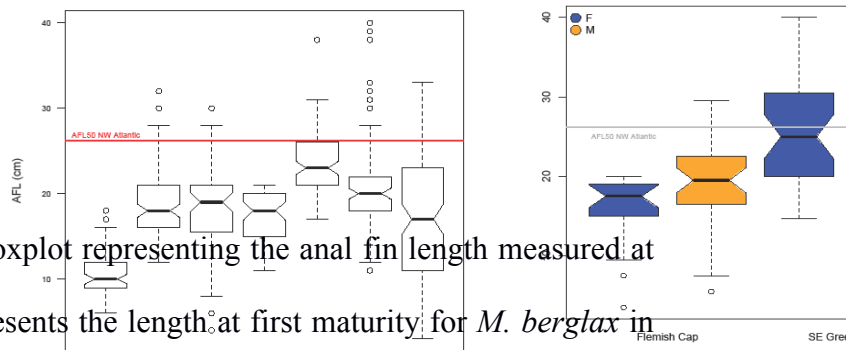


Figure 3 Supplementary Material: Boxplot representing the anal fin length measured at seven locations. *Left:* The red line represents the length at first maturity for *M. berglax* in the Atlantic. The notch in each box represents the 95% confidence interval. *Right:* length plotted by sex for two locations

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Table 1 Supplementary Material. Half-sibs pairs detected and relative likelihood inferred from COLONY. Sibship analyses results: the combination of columns Off1 and Off2 indicate the pair of individuals for which a significant relationship has been found. The third column (Colony) contains the probabilities for such relationship. Colony has found only half-sibs in the datasets. The fourth column (ML-Relate) reports the findings of the ML-

700 Relate software. The latter confirms the half-sibs relationships. The only discrepancy is for
701 the pair Nor10-Nor48, which ML-Relate identify as Parent-Offspring pair.

Off1	Off2	Colony	ML-Relate
SGre5	Fle4	0.894	Half-Sib
Sva17	Hat28	0.884	Half-Sib
Hat27	Baf61	0.872	Half-Sib
EGre73	Geo49	0.868	Half-Sib
Sva10	Nor8	0.845	Half-Sib
EGre40	Geo46	0.840	Half-Sib
Sva25	Hat15	0.839	Half-Sib
SGre78	Sva57	0.832	Half-Sib
EGre26	EGre40	0.829	Half-Sib
SGre74	Nor6	0.823	Half-Sib
EGre53	SGre54	0.813	Half-Sib
Hat24	Fle9	0.810	Half-Sib
SGre39	Fle4	0.807	Half-Sib
Nor10	Nor48	0.807	Parent-Offspring
EGre63	SGre60	0.800	Half-Sib

702