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ORIGINAL ARTICLE

Temporal changes in allele frequencies but stable genetic diversity over the past 40 years in the Irish Sea population of thornback ray, *Raja clavata*

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Rays and skates are an unavoidable part of the by-catch in demersal fisheries. Over the past 40 years, the thornback ray (*Raja clavata*) has decreased in numbers and even disappeared in some areas, leading to concerns about genetic risk. For this reason, the effective population size (N_e), the migration rate (m) and temporal changes in the genetic diversity were estimated for the population of thornback rays in the Irish Sea and Bristol Channel. Using genotyped, archived and contemporary samples (1965 and 2003–2004), N_e was estimated at 283 individuals (95% CI = 145–857), m at 0.1 (95% CI = 0.03–0.25) and the N_e/N ratio between 9×10^{-5} and 6×10^{-4}. Although these results must be treated with caution, due to the small sample sizes, this is the first attempt to estimate N_e in an elasmobranch species. The low N_e/N ratio suggests that relatively few individuals contribute to the next generation. The combined effect of sex bias, inbreeding, fluctuations in population size and, perhaps most important, the variance in reproductive success may explain the low N_e/N ratio. In addition, the relatively high gene flow between Irish Sea population and other source populations is likely to have had an impact on our estimate, which may be more relevant at the metapopulation scale. No significant loss of genetic diversity was found over the 40-year timeframe and long-term maintenance of the genetic diversity could be due to gene flow.
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Keywords: effective population size; census population size; genetic diversity; elasmobranchs; thornback ray; *Raja clavata*

Introduction

Preventing loss of genetic diversity is one of the priorities for conservation and has long been acknowledged to be important for the evolutionary potential of a species (Frankham, 1995; Franklin and Frankham, 1998). In an isolated population, the level of genetic diversity is the result of equilibrium between mutations, which generate genetic variation; and genetic drift, which decreases it. In small populations, the probabilities of losing rare alleles and increasing the frequency of deleterious alleles are higher as the effect of genetic drift is stronger (Lande, 1995; Franklin and Frankham, 1998). This can reduce population fitness and increase the risk of population extinction. Genetic diversity is coupled with the effective population size (N_e), which refers to the size of an ideal population with the same rate of genetic drift as the studied population (Wright, 1931). In a review of 192 studies, Frankham (1995) established that, on average, N_e is nine times smaller than the census population size (N). In marine teleosts this ratio can be much smaller, generally estimated at about 10^{-5} (Hauser *et al.*, 2002; Hutchinson *et al.*, 2003; Hoarau *et al.*, 2005; Poulsen *et al.*, 2006), though Turner *et al.* (2002) estimated 10^{-3} in red drum *Sciaenops ocellatus*. Hence, N_e is an important parameter in population risk assessment.

Direct estimates of N_e are difficult unless good estimates of the mating system and long-term population demography are known. An alternative indirect approach for estimating N_e is to examine temporal changes in allelic frequencies, under the assumption that they are explained by genetic drift (that is, by assuming the effects of mutation, migration and selection are negligible, or by allowing for them; Waples, 1989; Wang and Whitlock, 2003; Wang, 2005). This temporal method has been found to be more reliable than estimates based on demographic data (Frankham *et al.*, 2002).

Skates (Rajidae) are particularly vulnerable to exploitation because of their life history traits: slow growth rate, late maturity, low fecundity and large size at hatching (Heist, 1999; Dulvy *et al.*, 2000). Female thornback rays, *Raja clavata* L., mature between 9 and 12 years of age (Nottage and Perkins, 1983) and produce 38–150 eggs per female per year (Holden, 1975; Ryland and Ajayi, 1984; Ellis and Shackley, 1995; Chevolot *et al.*, 2007).

Many skate species are taken as by-catch in mixed demersal fisheries, and some are also taken in directed fisheries and recreational fisheries. Unfortunately,

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long-term data on landings are not available on a species-specific basis (Dulvy *et al*., 2000; Ellis *et al*., 2005), so that even the disappearance of the common skate *Dipturus batis* (Brander, 1981; (Dulvy *et al*., 2000) or the decline of the white skate (*Rostroraja alba*) Dulvy *et al*., 2000) went relatively unnoticed. Thornback rays are targeted in inshore longline and gillnet fisheries in coastal waters of the British Isles, and are also an important component of the mixed demersal trawl fisheries. Despite being one of the most commercially valuable rays, the economic value of their total catch is small in comparison to other demersal species (for example, sole and plaice), consequently skates have not been the focus of fisheries management. They are now a high priority for assessment because they are thought to have declined by about 45% in the Irish Sea (Dulvy *et al*., 2000; Rogers and Ellis, 2000) and nearly 80% in the North Sea (Walker and Heessen, 1996). Although thornback ray is locally abundant in the south-western North Sea, its decline in other parts of the North Sea has prompted further concern about its sustainability (Walker and Heessen, 1996; ICES, 2007), and ICES (ICES, 2006) advised a zero quota for North Sea skates and rays if they continue to be managed under a common TAC (total allowable catch). For all of these reasons, skates and elasmobranchs in general are now a high priority for stock assessment (Heessen, 2004; Ellis *et al*., 2005).

In the North Sea, tagging studies indicated that most individuals forage within 20 nautical miles (nmi; Walker *et al*., 1997). This 'home range' has since been extended to a maximum travelling distance of 70 nmi from studies using data storage tags (Hunter *et al*., 2005). In the Irish Sea and Bristol Channel, tagging studies showed that few individuals were recaptured from outside the Irish Sea/Bristol Channel area (Cefas, unpublished data). These results suggested that *R. clavata* populations are moderately isolated.

The aims of this study were to estimate whether there has been a loss in genetic diversity in *R. clavata* in the Irish Sea/Bristol Channel over the past 40 years, and to estimate N_e, the effective population size, by comparing archived vertebrae collected in 1965 with contemporary samples collected in 2003–2004.

Materials and methods

Sampling

Archived samples of vertebrae collected in 1965 from the Irish Sea were obtained from the CEFAS (Centre for Environment, Fisheries and Aquaculture Science) archives in Lowestoft, UK (Figure 1; Table 1). Contemporary samples were collected in 2003 and 2004 and were part of a previous study on *R. clavata* (Chevolot *et al*., 2006). From this previous study, five locations sampled in the Irish Sea/Bristol Channel were reanalysed (Figure 1; Table 1), as well as five other locations sampled in the North Sea and English Channel.

DNA extraction and genotyping

Archived vertebrae were preserved in ethanol, and DNA was extracted using a specialized SDS-based protocol (Hutchinson *et al*., 1999). All extractions of archived samples were performed in a DNA-free laboratory using filter tips for pipettes to avoid contamination and with negative controls at all steps. Contemporary samples (muscle tissue) were extracted using a silica-based protocol (Elphinstone *et al*., 2003).

Samples were genotyped for five unlinked microsatellite loci as described in Chevolot *et al*. (2005). PCR products were separated on a 6% polyacrylamide gel and

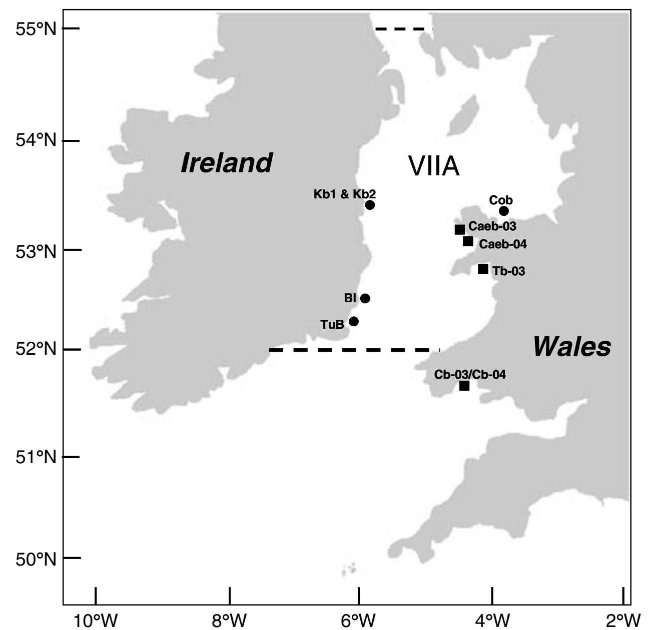

Figure 1 Sampling locations of *Raja clavata*. Black dashed lines delimit the sampling location named VIIA (see Table 1), which corresponds to the VIIa ICES rectangle. Circles denote 1965 samples; squares, 2003–2004 samples. Putative source populations are not shown. For the complete sampling map see Chevolot *et al*. (2006).

Table 1 Sampling information for *Raja clavata*

Location	*Code*	N	*Life stage*	*Tissue*	*Sampling date*
Blackwater Bay	Bl	23	Adults	Vertebrae	May 1965
Conwy Bay	CoB	10	Adults	Vertebrae	October 1965
Kish Bank 1	Kb1	24	Adults	Vertebrae	October 1965
Kish Bank 2	Kb2	20	Adults	Vertebrae	July 1965
Tuskaw Bay	TuB	26	Adults	Vertebrae	May 1965
ICES area VIIA	VIIA	45	Adults	Vertebrae	July 1965
Carmarthen Bay 03	Cb-03	16	Juveniles	Muscle	September 2003
Carmarthen Bay 04	Cb-04	33	Juveniles	Muscle	September 2004
Caernarfon Bay 03	Caeb-03	18	Juveniles	Muscle	September 2003
Caernarfon Bay 04	Caeb-04	16	Juveniles	Muscle	November 2004
Tremadog Bay 03	Tb-03	18	Juveniles	Muscle	September 2003
Thames estuary 1	Te1-03	35	Juveniles	Muscle	February 2003
Thames estuary 2	Te2-04	21	Juveniles	Muscle	August 2004
English Channel 2	Ec2-02	19	Juveniles	Muscle	April 2002
Lyme Bay	LyB-03	18	Juveniles	Muscle	September 2003
East English Channel	Eec-04	21	Juveniles	Muscle	September 2003

visualized with an ABI Prism-377 automatic sequencer (Applied Biosystems). Allele size was determined using an internal lane standard (GENESCAN 350 Rox) and GENESCAN software (Applied Biosystems).

Data analysis

The software MICRO-CHECKER 2.2.1 (Van Oosterhout *et al.*, 2004) was used to check for null alleles, stuttering and large allele drop out. For all samples, GENETIX 4.05 software (Belkhir *et al.*, 2004) was used to estimate the following: (1) the unbiased expected heterozygosity (H_{exp}) (Nei, 1978) and observed heterozygosity (H_{obs}); (2) the number of alleles (N_a) for each locus and location; (3) the single and multilocus F_{IS} estimate using Weir and Cockerham *f* estimator and (4) global and pairwise F_{ST} to test for population differentiation using Weir and Cockerham θ estimator (Weir and Cockerham, 1984). Significance was tested against 10 000 permutations and corrected using the sequential Bonferroni method when necessary (Rice, 1989).

The effective population size, N_e, was estimated alone and jointly with the migration rate, *m*. In both cases, we used a pseudo-likelihood method implemented in the software MLNE 2.0 (Wang and Whitlock, 2003). The idea is to estimate the N_e based on the fluctuations in allelic frequencies between at least two temporal samples separated by a known number of generations (Waples, 1989; Wang and Whitlock, 2003). When N_e is estimated alone, it is assumed that the population is isolated. When N_e and *m* are jointly estimated, the assumption is that migrants are coming from an infinitely large source population and migration rate is constant. However, the method to estimate N_e and *m* jointly is robust to deviations from the assumption of an infinitely large source population (Wang and Whitlock, 2003). Our definition of the source population was based on results from a previous study in Chevolot *et al*. (2006). Locations Te1-03, Te2-04, Ec2-02, LyB-03, Eec-04 (see Figure 1 in Chevolot *et al*., 2006), which showed no significant differentiation with contemporary samples from the Irish Sea and Bristol Channel (Cb-03, Cb-04, CaeB-03, CaeB-04,

Table 2 Summary statistics of genetic variability for five microsatellite loci for locations sampled for *Raja clavata*

	Location											
Locus	*Bl*	*Cob*	*Kb1*	*Kb2*	*TuB*	*VIIA*	*Cb-03*	*Cb-04*	*Caeb-03*	*Caeb-04*	*Tb-03*	*Mean N_a per locus*
Rc-B3												
N	20	8	17	22	24	43	14	22	17	12	15	
N_a	6	4	3	4	5	7	5	5	4	3	5	4.6
H_{exp}	0.66	0.711	0.642	0.744	0.661	0.761	0.622	0.584	0.635	0.632	0.595	
H_{obs}	0.435	0.800	0.632	0.792	0.600	0.533	0.429	0.438	0.556	0.600	0.530	
f	**0.320***	−0.076	0.017	−0.050	**0.382***	**0.335***	−0.014	**0.356***	0.106	−0.029	0.141	
Rc-B4												
N	20	8	17	22	24	43	14	22	17	12	15	
N_a	17	11	15	19	20	22	14	22	20	15	17	17.5
H_{exp}	0.936	0.942	0.928	0.941	0.937	0.946	0.938	0.9531	0.962	0.934	0.951	
H_{obs}	0.909	0.900	1.000	1.000	0.885	0.932	0.875	0.933	0.944	0.938	0.833	
f	−0.011	0.08	−0.08	−0.057	0.065	0.016	0.009	0.043	0.023	−0.004	**0.092***	
Rc-B6												
N	20	8	17	22	24	43	14	22	17	12	15	
N_a	18	11	18	16	24	25	21	21	21	16	18	19
H_{exp}	0.943	0.942	0.935	0.896	0.937	0.937	0.938	0.899	0.925	0.899	0.95	
H_{obs}	0.955	1.000	0.790	0.957	0.923	1.000	0.938	0.9394	1.000	0.938	1.000	
f	−0.011	−0.066	**0.131***	−0.059	−0.016	−0.066	0.003	−0.091	−0.075	−0.109	−0.047	
Rc-G2												
N	20	8	17	22	24	43	14	22	17	12	15	
N_a	5	5	6	5	4	7	4	5	5	3	6	5
H_{exp}	0.585	0.511	0.612	0.667	0.580	0.584	0.635	0.637	0.674	0.394	0.544	
H_{obs}	0.565	0.400	0.550	0.708	0.560	0.556	0.625	0.540	0.647	0.462	0.625	
f	0.034	**0.451***	0.010	−0.072	0.029	0.075	0.091	0.146	0.041	−0.118	−0.125	
Rc-E9												
N	20	8	17	22	24	43	14	22	17	12	15	
N_a	4	3	6	4	7	5	3	6	3	5	6	4.7
H_{exp}	0.392	0.451	0.293	0.576	0.499	0.448	0.502	0.617	0.418	0.587	0.638	
H_{obs}	0.364	0.444	0.263	0.522	0.520	0.422	0.438	0.606	0.389	0.625	0.833	
f	0.059	0.125	0.096	0.000	−0.029	0.073	0.197	−0.039	0.059	−0.146	−0.254	
Mean N_a	10	7	9.6	9.6	12,0	13,2	8.8	11.8	10.4	8.4	10.4	
Multilocus H_{exp}	0.703	0.711	0.682	0.765	0.723	0.735	0.726	0.738	0.723	0.689	0.735	
Multilocus f	0.084	0.003	0.050	−0.042	0.090	0.064	0.09	0.030	0.020	−0.030	−0.040	

N is number of individuals; N_a, number of alleles; H_{exp}, non-biased expected heterozygosity; H_{obs}, observed heterozygosity; *f*, inbreeding coefficient (Weir and Cockerham, 1984).
Significant values are in bold after Bonferroni corrections (* denotes $P < 0.05$).

Tb-03) were pooled to form the source population. Based on an estimated generation time of 10 years for *R. clavata* (Maxwell and Jennings, 2005), it was assumed that four generations separated the two temporal samples.

To compare genetic diversity between archived and contemporary samples and to test for loss of genetic diversity, we estimated the allelic richness for each temporal sample using the mean number of alleles per locus (N_a) and the unbiased expected heterozygosity, H_{exp} (Nei, 1978). As the number of alleles and the expected heterozygosity are strongly dependent on sample size, GENCLONE (Arnaud-Haond and Belkhir, 2007) was used to correct for uneven sample sizes with a resampling procedure. Mean allelic diversity and the non-biased expected heterozygosity were calculated across all resampling procedures. The allelic diversity was estimated with 1000 resampling procedures and corrected for the smallest sample size. Differences in allelic richness and expected heterozygosity between archived and contemporary samples were tested using a Wilcox rank sum test (Wilcoxon, 1945).

Results

Data quality

Evidence for null alleles was found in only one locus, Rc-B3 at VIIA, TuB and Bl locations collected in 1965. For the other microsatellite loci and sampling locations, no evidence for null alleles, stuttering and large allele drop out was observed. Deviations from Hardy–Weinberg equilibrium were observed for the locus Rc-B3 at VIIA, TuB, Bl and Cb-04 locations; for Rc-B4 at Tb location; for Rc-B6 at Kb1 location and Rc-G2 at Cob location. For all locations, the multilocus f was not significant after Bonferroni corrections ($P>0.05$) (Table 2).

Population differentiation

The global genetic differentiation among locations sampled in 1965 was extremely low ($\theta=0.004$) and not significant ($P=0.1$). Removal of locus Rc-B3 from the analysis (which has significant null alleles) did not affect the results. Similarly, no significant genetic differentiation between 2003–2004 samples was observed ($\theta=0.005$; $P=0.15$). Without Bonferroni corrections, pairwise multilocus θ estimates among the 11 locations showed that location Kb2 was significantly different from 7 locations; 2 of them being historical sampling sites. Kb1 was significantly differentiated from 4 locations; three were contemporary sampling sites (Table 3). Following Bonferroni corrections, none of the pairwise comparisons remained significant. Thus, samples from 1965 were pooled (excluding Kb2) into a single population. All 2003–2004 samples were pooled into a single population. Genetic differentiation between the two temporal samples was low ($\theta=0.007$) but in this case highly significant ($P<0.001$).

Estimation of effective population size

The joint estimation of N_e and m by the pseudo-maximum likelihood method, based on five loci, was 283 individuals (95% CI = 145–857) and 0.1 (95% CI = 0.03–0.25), respectively. The estimation of N_e alone gave an estimation of 512 (95% CI = 259–2320). Based on annual ground fish surveys, adult census population size in the Irish Sea and Bristol Channel (ICES Divisions VIIa, f) can be estimated to be in the order of 0.5–3 million mature individuals (JR Ellis, unpublished data). Thus, the N_e/N ratio ranges approximately between 9×10^{-5} and 6×10^{-4} (with migration), or 1.8×10^{-4} and 10^{-3} (without migration).

Temporal patterns in genetic diversity

The number of alleles per locus varied from 7 (Rc-B3) to 45 (Rc-B6) among all locations in 1965 and from 6 (Rc-B3) to 41 (Rc-B6) in the contemporary samples (Table 3). Following correction for sample size with GENCLONE, mean expected heterozygosity was 0.733 and 0.719 for

Table 4 Comparison of the genetic diversity at five microsatellite loci in *Raja clavata* population sampled in 1965 and 2003–2004

Locus	*1965*					*2004*		
	N_a	H_{obs}	H_{exp}	N_c	H_{expc}	N_a	H_{obs}	H_{exp}
Rc-B3	7	0.523	0.708	6.81	0.711	6	0.541	0.601
Rc-B4	27	0.928	0.941	26.21	0.942	27	0.908	0.943
Rc-B6	45	0.955	0.943	42.47	0.947	41	0.960	0.927
Rc-G2	8	0.523	0.557	8.62	0.583	6	0.620	0.621
Rc-E9	8	0.414	0.435	8.41	0.461	8	0.528	0.520
Mean	19	0.669	0.716	19.2	0.733	17.6	0.705	0.719

N_a is number of alleles; H_{exp}, non-biased expected heterozygosity (Nei, 1978); N_c, number of alleles corrected for sample size; H_{expc}, non-biased expected heterozygosity (Nei, 1978) corrected for sample size.
As contemporary population is the smallest, no correction for sample size was done (see Materials and methods).

Table 3 Pairwise F_{ST} comparisons among the 11 locations

Locations	*Bl*	*Cob*	*Kb1*	*Kb2*	*TuB*	*VIIA*	*Cb-03*	*Cb-04*	*Caeb-03*	*Caeb-04*	*Tb-03*
Bl	—	−0.016	0.013	**0.016***	−0.004	0.001	−0.003	0.011	0.005	0.002	−0.011
Cob		—	−0.008	0.014	−0.015	−0.012	−0.009	0.001	−0.002	−0.010	−0.020
Kb1			—	**0.031***	0.002	0.010	**0.029***	**0.043****	**0.025***	0.012	0.002
Kb2				—	0.009	0.002	**0.022***	**0.025****	**0.019***	**0.017***	**0.026***
TuB					—	−0.001	0.003	**0.010**	0.002	−0.007	−0.005
VIIA						—	0.006	0.018	0.006	0.004	0.004
Cb-03							—		0.001	−0.000	−0.007
Cb-04								—	0.011	0.012	0.006
Caeb-03									—	0.007	0.004
Caeb-04										—	0.001
Tb-03											—

Significant P-values are in bold, with * when $P<0.05$ and ** when $P<0.01$. None of the pairwise comparisons remain significant after Bonferroni corrections (corrected $P=0.0009$).

past and present samples, respectively (Table 4). The number of alleles per locus and expected heterozygosity were not significantly different ($P > 0.20$) between the two temporal samples, with or without Locus Rc-B3.

Discussion

Effective population size

The results presented here are the first attempt to estimate N_e and to follow genetic diversity through time in an elasmobranch. The estimated N_e for *R. clavata* is 283 (95% CI = 145–857) when jointly estimated with m and 512 when estimated alone (95% CI = 259–2320). Simulation studies suggest that an N_e of 50 individuals represents the absolute minimum threshold below which genetic erosion will occur on the short term and that of 500 individuals represents the minimal threshold for long-term maintenance of the genetic diversity (Frankham, 1995; Franklin and Frankham, 1998). Although our estimated N_e is at the borderline, suggesting the need for a long-term monitoring of thornback rays, these results must be interpreted with caution due to the small sample size and number of markers.

Factors influencing the estimate of N_e

Violations in underlying assumptions can affect the accuracy of the estimate of N_e (N_{est}). Cohort sampling effects and the underlying life history model (discrete generations) are some of these factors. In 1965, only adults were sampled, whereas in 2003–04 only juveniles were sampled. As age cohorts might be genetically differentiated, N_{est} could, in principle, be underestimated. However, the results of Chevolot *et al*. (2006) suggested high migration rates among juveniles independent of length class. Secondly, the model used to estimate the effective population size assumes discrete generations. Waples and Yokota (2007) reported that particular caution should be taken when estimating N_e in species with overlapping generations. They found that estimation of N_e could be biased and the bias was dependent upon the type of survival curve of the species. *R. clavata* has a type II survivorship curve as mortality is assumed to be constant throughout the cohorts. Thus, N_{est} is likely to have been underestimated (Waples and Yokota, 2007). To get a better N_{est} for *R. clavata*, larger sample sizes and greater temporal separation would be desirable. However, as is the case for most species, including *R. clavata*, archived samples are rare and usually restricted to small areas and relatively short periods of time.

Gene flow can have an impact on N_{est}. The estimated migration rate found here is 0.1 migrant per generation. Although there are few records of skates tagged in the Irish Sea and Bristol Channel being recaptured from outside this area (Pawson and Nichols, 1994), this estimated m supports the weak, though significant, genetic differentiation found in British waters (Chevolot *et al*., 2006). Therefore, the Irish Sea/Bristol Channel population is not completely isolated and the temporal changes are not only due to genetic drift but also due to gene flow. According to Wang and Whitlock (2003), the effect of gene flow should cause underestimation of N_{est} in the short term, if migration is ignored. This is because the change in allele frequencies is larger than expected by genetic drift alone. However, as reported by Fraser *et al*. (2007), the bias on N_{est} strongly depends on the extent of genetic differentiation between the source and the receiving populations. In cases where genetic differentiation between populations is low, as is the case here for thornback rays (global θ in British waters = 0.013; see Chevolot *et al*., 2006), N_{est} could be overestimated if migration is ignored, as migration might counterbalance the effect of genetic drift. Here, N_e estimated with genetic drift alone is slightly higher than when it is jointly estimated with migration rate (512 vs 287), and so is slightly overestimated. However, both estimates are within the confidence intervals and thus are in the same range. Furthermore, because of the low genetic differentiation between our target and the source populations, the most relevant scale for our N_{est} is probably the metapopulation scale rather than the discrete population (Fraser *et al*., 2007).

Factors influencing N_e

Several factors influence N_e and thus the N_e/N ratio. These include unequal sex ratio, fluctuations in population size, inbreeding, overlapping generations and variance in reproductive success (Caballero, 1994; Frankham *et al*., 2002; Hedrick, 2005). Sex specific fisheries can play a role in some species and catch records have occasionally reported female-sex bias in *R. clavata* due to the earlier inshore migration of females to the spawning grounds (Ryland and Ajayi, 1984). Likewise, multiple paternity and polyandry (Chevolot *et al*., 2007) could, in principle, affect sex ratios. In the case of *R. clavata*, however, bias in sex ratio alone is extremely improbable to explain the low N_e/N ratio as it will require a sex ratio of approximately 1.5×10^{-5} of one sex, based on the formula $N_e = 4N_{ef} \times N_{em}/(N_{em} + N_{ef})$ (with N_{ef} the effective number of females and N_{em}, the effective number of males; Wright, 1931). The influence of population size fluctuations on the N_e/N ratio is more difficult to assess because there are no long-term reports on fishing effort in relation to population size in *R. clavata*. However, in the timeframe of this study (1965–2004, that is, four generations), fluctuations in population size alone would be unlikely to have a large effect on N_e. Furthermore, the long lifespan, which includes overlapping generations would tend to buffer fluctuations in population size (Jorde and Ryman, 1995; Waite and Parker, 1996). Inbreeding is also unlikely to explain the low N_e/N ratio as the population would have to be almost fully inbred in order to explain the ratio based on $N_e = N/(1+F)$ (with N the census population size and F the inbreeding coefficient; Wright, 1931); and no high f was found in any of the locations studied. Finally, the variance in reproductive success is estimated between 2350 and 80 000 individuals based on $N_e = (4N-2)/(V_k+2)$ (with N the census population size and V_k the variance in reproductive success; Wright, 1938). This kind of variance seems high for a species with low fecundity and high juvenile survival. In comparison with marine species expected to have a high variance in reproductive success (high fecundity and low juvenile survival), the variance is between 57 000 and 1 000 000 for the North Sea plaice (*Pleuronectes platessa*), between 18 000 and 165 000 for the New Zealand red snapper (*Pagrus auratus*) (Hauser *et al*., 2002), between 30 000 and 240 000 for the North Sea cod

(*Gadus morhua*) (Hutchinson *et al*., 2003) and 4500 for the red drum (*S. ocellatus*; Turner *et al*., 2002). The estimated variance in reproductive success for thornback ray is, therefore, in the same range as these species. Although, complex courtship behaviours have been observed in some batoids (Yano *et al*., 1999; Chapman *et al*., 2003), which could lead to male variance in reproductive success, to have a variance in *R. clavata* in about the same range as the North Sea plaice, cod, New Zealand red snapper and the red drum is improbable.

Thus, all of these parameters taken individually do not explain the low N_e/N ratio. As the effective population size is a summarized parameter of many demographic parameters (Caballero, 1994), the synergistic effect of these factors may have reduced the N_e over N, as each one affects the other. In addition to all of these parameters, the effect of gene flow on our N_{est} may further reduce the estimated N_e/N ratio.

Comparison with other marine species

For most marine species, the estimated N_e/N ratio is between three to five orders of magnitude smaller than the census population size (Hauser *et al*., 2002; Planes and Lenfant, 2002; Turner *et al*., 2002; Hutchinson *et al*., 2003; Hoarau *et al*., 2005; Gomez-Uchida and Banks, 2006; Poulsen *et al*., 2006; Laurent and Planes, 2007; Ovenden *et al*., 2007). All these species are highly fecund species with a typical type III survival curve (that is, high fecundity and high juvenile mortality). In this case, variance in reproductive success is thought to be the primary factor reducing N_e (Hedrick, 2005) due to sweepstake recruitment (Hedgecock, 1994). In this model, young-of-the-year may come from a very small number of successful breeders as a consequence of matched breeding time and local oceanographic conditions. Surprisingly, the estimated N_e/N ratio for *R. clavata* (with a Type II survivorship) is the same order of magnitude. Although caution has to be taken with our estimates, this may suggest that biological characteristics, such as fecundity, mortality rate through cohorts and age of maturity, may not be the best predictors of N_e.

Genetic diversity

The finding of no apparent loss in genetic diversity in *R. clavata* is a good sign, although this estimate spans only the past 40 years and does not predate exploitation by major fisheries. In the absence of samples predating the 1960s, we cannot exclude the possibility that some genetic diversity has, in fact, been lost. The estimated migration rate is similar to that found in Chevolot *et al*. (2006). Coupled with weak genetic differentiation these results suggest that the Irish Sea population exchanges alleles with more distant populations. Therefore, genetic diversity could have been maintained, at least quantitatively, through gene flow during this past 40 years. Likewise, no loss of genetic diversity was found in the Baltic and Moray Firth cod populations (Poulsen *et al*., 2006). However, Hutchinson *et al*. (2003) found a loss of genetic diversity in the North Sea cod between 1954 and 1970 and a subsequent recovery of the level of genetic diversity between 1970 and 1988, thanks to gene flow from neighbourhood populations.

Given the estimated population size of mature *R. clavata* in the Irish Sea and Bristol Channel—within the broad range of between 500 000 and 3 000 000 individuals—long-term monitoring of the abundance and genetic diversity of *R. clavata* in the Irish Sea is certainly warranted. *Raja clavata* has undergone a more severe decline in the North Sea, though it is uncertain if there has been a decrease in genetic diversity as historical samples are not available for this area. This and earlier studies (for example, Hauser *et al*., 2002; Hutchinson *et al*., 2003) highlight the usefulness of properly archived samples for examining temporal trends in the genetic diversity of exploited fish species.

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