

Being abundant is not enough: a decrease in effective population size over eight generations in a Norwegian population of the seaweed, *Fucus serratus*

James A. Coyer^{1,*}, Galice Hoarau¹,
Kjersti Sjøtun² and Jeanine L. Olsen¹

¹Department of Marine Benthic Ecology and Evolution, Centre for Ecological and Evolutionary Studies, Biological Centre, University of Groningen, 9750 AA, Haren, The Netherlands

²Department of Biology, University of Bergen, PO Box 7800, 5020 Bergen, Norway

*Author for correspondence (j.a.coyer@rug.nl).

The brown alga *Fucus serratus* is a key foundation species on rocky intertidal shores of northern Europe. We sampled the same population off the coast of southern Norway in 2000 and 2008, and using 26 microsatellite loci, we estimated the changes in genetic diversity and effective population size (N_e). The unexpectedly low N_e (73–386) and N_e/N ratio (10^{-3} – 10^{-4}), in combination with a significant decrease (14%) in allelic richness over the 8-year period, suggests an increased local extinction risk. If small N_e proves to be a common feature of *F. serratus*, then being abundant may not be enough for the species to weather future environmental changes.

Keywords: effective population size; *Fucus serratus*; genetic diversity; N_e/N

1. INTRODUCTION

Several recent experiments have demonstrated that the genetic diversity of a population is positively correlated with its ability to maintain fitness in the face of environmental changes (e.g. Ehlers *et al.* 2008). Genetic diversity is a function of a population's effective size (N_e , the size of an ideal population undergoing the same amount of genetic drift as the real population or, roughly, the number of individuals that successfully contribute to the next generation). Natural populations, however, are rarely ideal and N_e is usually smaller than N (see Palstra & Ruzzante 2008). Consequently, even populations with a large census size may face a loss of genetic diversity. Clearly, small values of N_e can constrain a population's evolutionary response to environmental change much more than the actual census size (Frankham *et al.* 2002).

Classically, temporal measurements of demographic parameters (growth, sex ratio and mating system) have been used to estimate N_e (Frankham 1995), but

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2008.0403> or via <http://journals.royalsociety.org>.

each parameter is difficult to measure directly in natural populations. An alternative approach uses genetic data to assess temporal changes in allele frequency within a given population (Waples 1989). A DNA-based temporal method has been used for several animal species, but for only a very few plant species (see Palstra & Ruzzante 2008), and to our knowledge, for no species of marine macroalgae.

Intertidal shores throughout the North Atlantic are characterized by *Fucus* spp., with *Fucus serratus* dominant from the mid-intertidal to lower subtidal (Lüning 1990). The species is dioecious, reproduces annually with a generation time of 1–2 years (Coyer *et al.* 2007), and microsatellite analyses suggest a panmictic unit of 0.5–2 km (Coyer *et al.* 2003). We sampled the same population of *F. serratus* off the coast of southern Norway in 2000 and 2008, and using 26 microsatellite loci, we estimated N_e and temporal changes in genetic diversity.

2. MATERIAL AND METHODS

(a) Sampling and DNA extraction

Slåttholmen is a small islet directly offshore from the Espeland Marine Biological Station (University of Bergen), Norway. Census size (N) of the *F. serratus* population was obtained in May 2008 using a systematic sampling approach: a 50 m transect line was established at the upper extent of *F. serratus*, along which 3–5, 0.25 m² quadrats were placed perpendicular and contiguously (to the lowest extent) at 1.5 m intervals (175 quadrats). Three patches separated from Slåttholmen by approximately 250, 500 and 1000 m were censused in the same manner with transects varying from 30 to 50 m (depending upon topology); mean densities of the four areas were then combined for a grand mean density. The total census size of *F. serratus* was estimated by determining the range of suitable habitat within a 1 km radius of Slåttholmen using aerial maps and the software IMAGEJ (Abramoff *et al.* 2004), assuming a width of 2 m (determined from the contiguous quadrats) and using the grand mean density.

Tissue samples were collected from 64 individuals spaced at 1 m intervals along a linear transect. Exactly the same area was sampled in June 2000 and April 2008. Tissue was blotted dry and placed in silica crystals for dehydration and storage; DNA was extracted and purified as described by Hoarau *et al.* (2007) and modified by Coyer *et al.* (in press).

(b) Microsatellite genotyping

A total of 26 microsatellite loci were genotyped: FsA198, FsB113, FsB128, FsE6, FsF4 (Coyer *et al.* 2002); L20, L38, L58, L94 (Engel *et al.* 2003); and F9, F12, F14, F17, F19, F21, F22, F36, F37, F45, F47, F49, F50, F58, F65, F69, F72 (Coyer *et al.* in press). The genotypes were visualized on an ABI 3730 automatic sequencer (Applied Biosystems) and analysed with GENEMAPPER v. 4.0 software (Applied Biosystems).

(c) Data analysis

The average number of alleles per locus (α), H_{exp} (non-biased; Nei 1978) and θ estimator of F_{ST} (2000 replications; Weir & Cockerham 1984) were estimated with GENETIX v. 4.02 (Belkhir *et al.* 2001). N_e was estimated using a 1- and 2-yr generation time with the software MLNe (Wang & Whitlock 2003). As the model assumes no selection, we also tested for neutrality using the F_{ST} -outlier test (Beaumont & Nichols 1996), which revealed locus F65 as the only significant outlier ($p < 0.01$). Therefore, we estimated N_e with and without locus F65. An estimate of temporal changes in N_e was obtained using the software TMVP, which estimates N_e at the time of the oldest (2000) and most recent sample (2008), assuming a model of exponential growth or decline during the interval (Beaumont 2003).

3. RESULTS AND DISCUSSION

Our estimate of N_e was between 73 and 386 (table 1) comparable to the average N_e of 260 obtained from temporal studies of 65 diverse species (Palstra & Ruzzante 2008). The N_e/N ratio was unexpectedly low, 1.8×10^{-3} – 3.5×10^{-4} , based on an estimated

Table 1. Estimation of N_e in a population of *F. serratus* collected in 2000 and 2008. (N_e was estimated (95% CI) with the software MLNe over a generation time interval of 1 or 2 yrs using all 26 loci and without the EST-derived microsatellite locus F65 a significant outlier, see §2c).

	N_e
<i>generation time = 2 yrs</i>	
all loci	99 (73–193)
without F65	125 (87–200)
<i>generation time = 1 yr</i>	
all loci	188 (136–279)
without F65	241 (168–386)

census size of 208 000 individuals within a 1 km radius of Slåttholmen (grand mean density (s.d.) from four areas = 10.13 m^{-2} (3.86); potential *Fucus* habitat = $10.28 \times 0.002 \text{ km}$). For land plants, N_e/N ratios of approximately 10^{-1} have been estimated with temporal analyses (see Siol *et al.* 2007). The low N_e/N ratio for *F. serratus*, therefore, appears to be more similar to the 10^{-3} – 10^{-6} differences observed among species with a type III survivorship curve (high fecundity and high juvenile mortality) such as bony fishes (e.g. Hoarau *et al.* 2005) or shrimp (Ovenden *et al.* 2007). In *F. serratus*, female fecundity is also very high (approx. 10^6 eggs per female; Knight & Parke 1950; J. A. Coyer, G. Hoarau, W. T. Stam & J. L. Olsen, unpublished data) and high juvenile mortality is probable given the low dispersal of gametes (1–2 m; Serrão *et al.* 1997) and the relatively low density of adults compared with gamete production. Similar to fishes and shrimp, *F. serratus* appears to have a high variance in individual reproductive success, which probably leads to the low N_e/N ratio we estimated.

Gene flow can significantly bias estimates of N_e (Fraser *et al.* 2007). Immigration from highly differentiated populations will lead to changes in allele frequencies larger than expected by genetic drift alone and a subsequent underestimation of N_e . This scenario is very unlikely in *F. serratus*, as gene flow is very restricted (Coyer *et al.* 2003) and such immigrants would have highly divergent genotypes, a pattern not revealed using a factorial correspondence analysis (electronic supplementary material). Immigration from weakly differentiated neighbouring populations cannot be excluded, although it would result in an overestimation of N_e (Fraser *et al.* 2007). Additionally, sampling bias due to small-scale family structure can be excluded as no kinship was detected within 1–100 m (encompassing our sampling scale; Coyer *et al.* 2003). Thus, our estimate of N_e is likely to be reasonably unbiased.

Populations with small N_e may suffer from inbreeding depression and loss of genetic diversity, potentially leading to a reduced capacity to respond to changing environmental conditions and to increased extinction risk (Frankham 2005). An N_e of at least 500 appears necessary to minimize inbreeding depression and to maintain gene diversity (Frankham *et al.* 2002). Consequently, populations with small N_e and limited

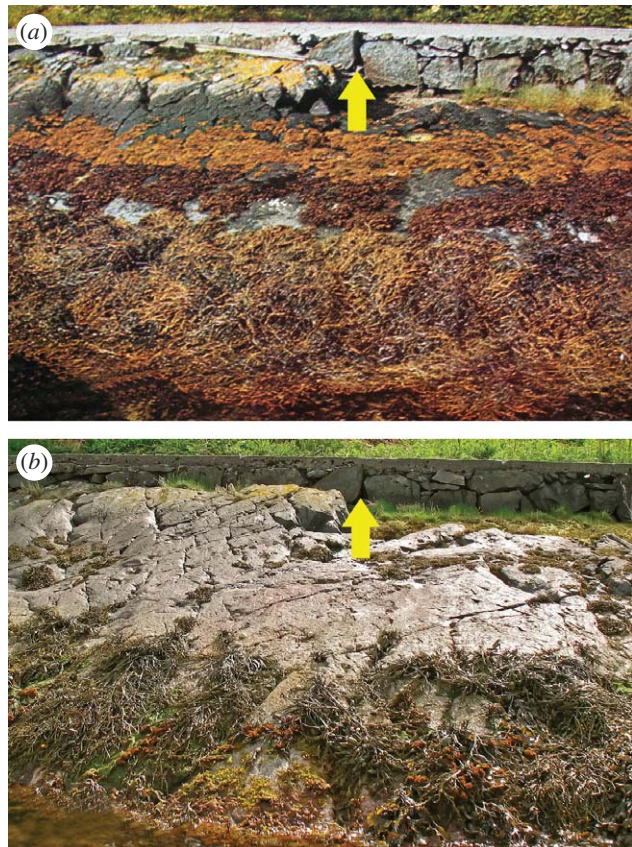


Figure 1. Photograph of the Slåttholmen site in (a) 1968 (Lewis 1968) and (b) 2008. Arrows indicate a common location.

dispersal, such as *F. serratus*, could face localized extinction in the face of any environmental perturbation that reduces census size and/or constrains the population.

Considerable differences in fucoid abundance are noted when comparing photographs taken at Slåttholmen in 1968 (Lewis 1968) and 2008: two species (*Pelvetia canaliculata* and *Fucus spiralis*) were absent and another (*Ascophyllum nodosum*) was significantly reduced in 2008 (figure 1). *Fucus serratus* also has declined (as it has in the general area; K. Sjøtun 2004–2008, unpublished data), although it still remains widespread. Observed changes may be due to the increased frequency of exceptionally warm summers/mild winters observed in southern Norway during the past decade (Husa *et al.* 2007) and/or the 10-fold increase of herbivores relative to nearby areas (E. Heggøy 2001–2007, personal communication). Genetic data also revealed that from 2000 to 2008, N_e declined nearly threefold, allelic richness decreased significantly (14%; $p < 0.05$, 10 000 permutations, FSTAT; Goudet 2002) and pairwise F_{ST} was highly significant (table 2).

The Slåttholmen population may be at risk and its decline will lead to significant changes in the intertidal community structure. If the very small N_e and N_e/N ratio estimated here are more generally characteristic of *F. serratus* (and possibly other fucoids), then the species may be less resilient to environmental change than assumed. Being abundant does not guarantee local survival in the long term.

Table 2. Genetic changes in a population of *F. serratus* over eight generations. (Abbreviations: average number of alleles^{-locus} (α), H_{exp} (non-biased), θ estimator of F_{ST} (reflecting temporal differences in allelic frequencies between 2000 and 2008) and estimates of temporal changes in N_e . Parenthetical values of N_e represent estimations without locus F65 (see table 1 legend); n.s., non-significant; * $p < 0.05$; ** $p < 0.001$.)

	2000	2008
α^*	5.7143	4.8929
$H_{exp}^{n.s.}$	0.4602	0.4570
N_e	437 (392)	152 (230)
θ^{**}		0.017

This study was supported by NWO-ALW 2006 (contract number 815.01.011 to J.A.C. and 863.05.008 to G.H.), Schure-Beijerinck-Popping 2008-22 and the Directorate for Nature Management (Norway).

- Abramoff, M. D., Magelhaes, P. J. & Ram, S. J. 2004 Image processing with image. *J. Biophoton. Int.* **11**, 36–42.
- Beaumont, M. A. 2003 Estimation of population growth or decline in genetically monitored populations. *Genetics* **164**, 1139–1160.
- Beaumont, M. A. & Nichols, R. A. 1996 Evaluating loci for use in the genetic analysis of population structure. *Proc. R. Soc. B* **263**, 1619–1626. (doi:10.1098/rspb.1996.0237)
- Belkhir, K., Borsa, P., Goudet, J., Raufaste, N. & Bonhomme, F. 2001 *GENETIX, logiciel sous Windows pour la genétique des populations*. Montpellier, France: Laboratoire Genome et populations.
- Coyer, J. A., Veldsink, J. H., Stam, W. T., Serrão, E. A., Stam, W. T. & Olsen, J. L. 2002 Characterization of microsatellite loci in the marine rockweeds, *Fucus serratus* and *F. evanescens* (Heterokontophyta; Fucaceae). *Mol. Ecol. Notes* **2**, 35–37. (doi:10.1046/j.1471-8286.2002.00138.x)
- Coyer, J. A., Peters, A. F., Stam, W. T. & Olsen, J. L. 2003 Post-ice age recolonization and differentiation of *Fucus serratus* L. (Fucaceae: Phaeophyta) populations in northern Europe. *Mol. Ecol.* **12**, 1817–1829. (doi:10.1046/j.1365-294X.2003.01850.x)
- Coyer, J. A., Hoarau, G., Stam, W. T. & Olsen, J. L. 2007 Hybridization and introgression in a mixed population of the intertidal seaweeds *Fucus distichus* and *F. serratus*. *J. Evol. Biol.* **16**, 3606–3616.
- Coyer, J. A. et al. In press. Expressed sequence tag derived polymorphic SSR markers for *Fucus serratus* and amplification in other species of *Fucus*. *Mol. Ecol. Resour.*
- Ehlers, A., Worm, B. & Reusch, T. B. H. 2008 Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to climate warming. *Mar. Ecol. Prog. Ser.* **355**, 1–7. (doi:10.3354/meps07369)
- Engel, C. R., Brawley, S., Edwards, K. J. & Serrão, E. 2003 Isolation and cross-species amplification of microsatellite loci from the fucoid seaweeds *Fucus vesiculosus*, *F. serratus*, and *Ascophyllum nodosum* (Heterokontophyta, Fucaceae). *Mol. Ecol. Notes* **3**, 180–182. (doi:10.1046/j.1471-8286.2003.00390.x)
- Frankham, R. 1995 Effective population size-adult population size ratios in wildlife: a review. *Genet. Res.* **66**, 95–107.
- Frankham, R. 2005 Genetics and extinction. *Biol. Conserv.* **126**, 131–140. (doi:10.1016/j.biocon.2005.05.002)
- Frankham, R., Ballou, J. D. & Briscoe, D. A. 2002 *Introduction to conservation genetics*. Cambridge, UK: Cambridge University Press.
- Fraser, D. J., Hansen, M. M., Østergaard, S., Tessier, N., Legault, M. & Bernatchez, L. 2007 Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. *Mol. Ecol.* **16**, 3866–3889. (doi:10.1111/j.1365-294X.2007.03453.x)
- Goudet, J. 2002 FSTAT 2.9.3.2, a program to estimate and test gene diversities and fixation indices. See <http://www2.unil.ch/popgen/softwares/fstat.htm>.
- Hoarau, G., Boon, E., Jongma, D. N., Ferber, S., Palsson, J., Van der Veer, H. W., Rijnsdorp, A. D., Stam, W. T. & Olsen, J. L. 2005 Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proc. R. Soc. B* **272**, 497–503. (doi:10.1098/rspb.2004.2963)
- Hoarau, G., Coyer, J. A., Stam, W. T. & Olsen, J. L. 2007 A fast and inexpensive DNA extraction/purification method for brown macroalgae. *Mol. Ecol. Notes* **7**, 191–193. (doi:10.1111/j.1471-8286.2006.01587.x)
- Husa, V., Steen, H. & Åsen, P. A. 2007 Hvordan vil makroalgemiljøene langs norskekysten påvirkes av økt sjøtemperatur? In *Kyst og havbruk 2007, Fisken og havet, særnr. 2-2007* (eds E. Dahl, P. K. Hansen, T. Haug & Ø. Karlsen). Institute of Marine Research, see <http://www.imr.no/produkter/publikasjoner/havbruk/srapport/2007>.
- Knight, M. & Parke, M. 1950 A biological study of *Fucus vesiculosus* L. and *F. serratus* L. *J. Mar. Biol. Assoc. UK* **29**, 439–514.
- Lewis, J. R. 1968 Water movements and their role in rocky shore ecology. 2nd European Symposium on Marine Biology. *Sarsia* **34**, 13–26.
- Lüning, K. 1990 *Seaweeds: their environment, biogeography and ecophysiology*. New York, NY: J. Wiley.
- Nei, M. 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.
- Ovenden, J. R., Peel, D., Street, R., Courtney, A., Hoyle, S., Peel, S. & Podlich, H. 2007 The genetic effective and adult census size of an Australian population of tiger prawns (*Penaeus esculentus*). *Mol. Ecol.* **16**, 127–138. (doi:10.1111/j.1365-294X.2006.03132.x)
- Palstra, F. & Ruzzante, D. E. 2008 Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Mol. Ecol.* **15**, 3428–3447. (doi:10.1111/j.1365-294X.2008.03842.x)
- Serrão, E. A., Kautsky, L., Lifvergren, T. & Brawley, S. H. 1997 Gamete dispersal and pre-recruitment mortality in Baltic *Fucus vesiculosus*. *Phycologia Suppl.* **36**, 101–102.
- Siol, M., Bonnin, I., Olivieri, I., Prosperi, J. M. & Ronfort, J. 2007 Effective population size associated with self-fertilization: lessons from temporal changes in allele frequencies in the selfing annual *Medicago truncatula*. *J. Evol. Biol.* **20**, 2349–2360. (doi:10.1111/j.1420-9101.2007.01409.x)
- Wang, J. L. & Whitlock, M. C. 2003 Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* **161**, 429–446.
- Waples, R. S. 1989 A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**, 379–391.
- Weir, B. S. & Cockerham, C. C. 1984 Estimating F -statistics for the analysis of population structure. *Evolution* **38**, 1358–1370. (doi:10.2307/2408641)