

Nearshore fish (*Pholis gunnellus*) persists across the North Atlantic through multiple glacial episodes

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Abstract

The intertidal biota of the North Atlantic is characterized by two disjunct communities (North American and European) exposed to different climatic regimes during the Pleistocene and in the Holocene. We collect multilocus DNA sequence data from the nearshore fish *Pholis gunnellus* to help uncover processes determining biogeographical persistence during periodic coastal glaciations. Coalescent-based estimates from the multilocus DNA sequence data suggest that *P. gunnellus* persisted on both sides of the North Atlantic throughout the last two glacial maxima (> 202 000 years) with little trans-Atlantic gene flow since divergence, very little structure among populations within Europe ($\Phi_{ST} < 0.05$) and some structure within the North American coastline ($\Phi_{ST} = 0.0–0.21$). Although the ecological flexibility and high local migration of *P. gunnellus* could have enhanced this species' survival across the Atlantic, logistic regression did not find a significant determinant of trans-Atlantic persistence when considering 12 other North Atlantic phylogeographical studies from the literature.

Keywords: coalescence, intertidal, North Atlantic, phylogeography, Pleistocene, refugia

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Introduction

In spite of a rich understanding of the physical and ecological processes shaping community structure in northern hemisphere temperate intertidal area (Paine 1994; Bertness 2001), our picture of the long-term persistence, stability, and historical assembly of these communities is far from complete (Valentine & Jablonski 1993; Menge & Branch 2001). Although the physical landscapes hosting present intertidal communities have experienced drastic alterations during the Pleistocene, some extant intertidal species are likely to have originated before the Pleistocene era. The physical landscape hosting this community has been subjected to repeated glaciations, shifts in sea level, and glacial scouring causing a net increase in rocky substrate (Imbrie *et al.* 1993; Jacobs *et al.* 2004). Given this historical context, a central puzzle regarding the persistence and assembly of expanding and contracting temperate intertidal

communities is what determines community membership against the backdrop of climate change. On the one hand, dispersal and colonization ability could be the most important factor in community membership (MacArthur & Wilson 1967; Dynesius & Jansson 2000; Hubbell 2001). Alternatively, temperate rocky-shore communities could be more strictly 'niche-assembled', where community membership and co-existence is determined by avoidance of competition and built through tens of thousands of years of colonization, extinction, and evolutionary change among interacting species (Diamond 1975; Walker & Valentine 1984; Pandolfi 1996).

Two periods of profound climate change make the North Atlantic intertidal community an excellent setting for testing hypotheses of co-existence. First, 80% of the Pleistocene has been dominated by colder glacial epochs (Lambeck *et al.* 2002), such that the present North Atlantic intertidal community could be dominated by species with long-term niche-assembled associations that repeatedly expand their ranges from glacial refugia during the shorter warm periods, such as today's Holocene epoch. Alternatively, this community could be dominated by ephemeral associations among both geographically persistent species

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and colonists that repeatedly colonize North America from European populations during warmer periods (Valentine & Jablonski 1993; Dynesius & Jansson 2000). Secondly, the North Atlantic intertidal presently consists of hundreds of taxa that are thought to have colonized from the North Pacific following the opening of the Bering Strait (Durham & MacNeil 1967; Vermeij 1991). This ice-free trans-Arctic connection is thought to have started approximately 3.5 million years ago (Ma) during a time of pronounced global warming, and phylogeographical and phylogenetic data can help test if trans-Arctic colonization was a dispersal limited process or determined by niche-driven community assembly (Cunningham & Collins 1998).

Instead of a community responding to climate change as a cohesive unit, phylogeographical and palaeontological studies so far suggest that temperate intertidal species respond idiosyncratically and possibly according to their ecological requirements and flexibility (Valentine & Jablonski 1993; Wares & Cunningham 2001; Hickerson & Cunningham 2005). However, neither molecular nor palaeontological approaches have yielded unambiguous inferences of rocky intertidal community history.

In the present study, we collect and analyse multilocus genetic data from *Pholis gunnellus* (Linnaeus 1758) to help unravel the community history of the North Atlantic intertidal. *Pholis gunnellus* is a North Atlantic nearshore fish found both subtidally and intertidally (i.e. regularly found above the lowtide mark) and is most commonly found along rocky shorelines on both sides of the North Atlantic (Scott & Scott 1989). This association with rocky substrate could have made *P. gunnellus* more vulnerable to extinction on the North American side because this region had substantially less unglaciated rocky coastline during the last glacial maximum (LGM) approximately 15 000–20 000 years BP (Shackleton *et al.* 1984). However, its present North American distribution includes areas with predominantly nonrocky substrate (Robins & Ray 1986; Scott & Scott 1989). This could have either enhanced trans-Atlantic persistence or be the result of an adaptive habitat switch during glacial maxima as rocky substrate became less common. Another characteristic possibly influencing the trans-Atlantic persistence of *P. gunnellus* is the different vertical depth exhibited within North American populations. Specifically, North American *P. gunnellus* is only intertidal during the warmer months, whereas *P. gunnellus* retains its intertidal distribution throughout the year along most of the European coastline (Sawyer 1967; Moring 1990), with the exception of Icelandic populations (Ingólfsson, personal communication).

To test hypotheses of trans-Atlantic persistence and postglacial colonization, we collected multilocus genetic data (mtDNA control region plus two tRNA's, nuclear α -enolase, and nuclear α -tropomyosin) from 64 to 86 individuals and seven populations of *P. gunnellus* on the North

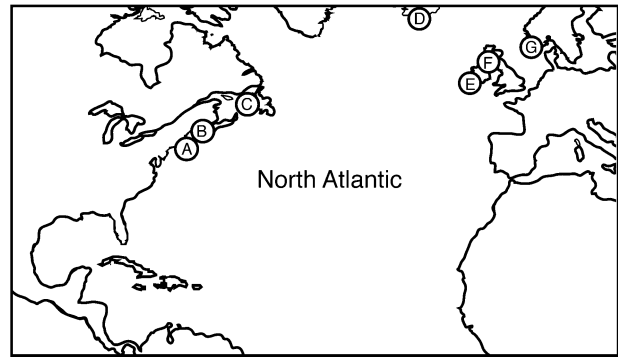


Fig. 1 Sampling sites of *Pholis gunnellus*. A: Rockport, USA (Massachusetts); B: Winter Harbor, USA (Maine); C: Bonavista Bay, Canada (Newfoundland); D: Reykjavik, Iceland; E: Sherkin Island, Ireland; F: Isle of Cumbrae, Scotland; G: Bergen, Norway.

Table 1 Sample sizes, number of alleles, number of unique alleles and haplotype diversities of the sampled populations of *Pholis gunnellus*. The allelic numbers and haplotype diversities are calculated before recombinant intron sites were removed

Gene	# alleles	# unique alleles	Sample size	Sample locality
Control region	21	10	6	Rockport, MA
			11	Maine
			9	Newfoundland
			9	Iceland
			10	Scotland
			11	Ireland
			3	Norway
α -enolase	8	2	10	Rockport, MA
			8	Maine
			12	Newfoundland
			16	Iceland
			16	Scotland
			18	Ireland
α -tropomyosin	5	0	6	Norway
			6	Rockport, MA
			12	Maine
			12	Newfoundland
			10	Iceland
			8	Scotland
			10	Ireland
6	Norway			

American and European coastlines (Fig. 1; Table 1). Given these multilocus data, we use coalescent models that incorporate pre-isolation gene coalescence and post-isolation migration to estimate minimum trans-Atlantic divergence times (Hey & Nielsen 2004). The results of this study will help illuminate how climate change may result in shifting ecological interactions and if ecological flexibility is associated with persistence during glaciation of rocky intertidal habitat. In order to report divergence time estimates in

units of years, we estimate the rate of substitution at these three loci by assuming that *P. gunnellus* diverged from its phylogenetically closest North Pacific *Pholis* relative during the first major trans-Arctic invasion 3.5 Ma. (Durham & MacNeil 1967; Vermeij 1991).

Materials and methods

Geographical sampling, and DNA sequence markers

The specimens of *Pholis gunnellus* were collected from three localities on the North American coast (Rockport, MA, Winter Harbor ME, and Bonavista Bay, NF) and four localities on the European coast (Iceland, southwestern Ireland, southwestern Scotland, and southern Norway) in the fall of 2002 (Fig. 1). These localities correspond to where *P. gunnellus* is most common, yet this species is less commonly found south of the English Channel and between Cape Cod and Delaware Bay (Scott & Scott 1989). Specimens of other *Pholis* taxa used for the DNA substitution rate estimates (*P. dolichogaster*, *P. picta*, *P. nebulosa*, and *P. crassispina*, *P. ornata*, *P. laeta*) were collected between 2000 and 2002 from localities in Friday Harbor, WA (USA) and Hokkaido (Japan). These North Pacific *Pholis* taxa that are restricted to either the Northwest Pacific (*P. dolichogaster*, *P. picta*, *P. nebulosa*, and *P. crassispina*) or Northeastern Pacific (*P. laeta* and *P. ornata*), with no overlapping ranges (Hart 1973; Lindberg & Krasnyukova 1989; Mecklenburg *et al.* 2002). Fin clips were taken from individuals and stored in 100% ethanol.

For population genetic analyses, DNA sequence data was collected from one mitochondrial locus and two nuclear loci. An approximately 515-bp fragment of the 5' end of the mitochondrial control region was amplified using A-PRO (5'-TTCCACCTCTAACTCCCAAAGCTAG-3') and (3'-TATGCTTTAGTTAAGGCTACG-3'); (Lee *et al.* 1995). In all individuals, this also included the entire tRNA-Pro and 14 bases of the tRNA-Thr gene. An approximately 448-bp fragment partly containing the α -enolase intron I was amplified using CCAGGCACCCAGTCTACCTG as the 5' primer and TGGACTTCAAATCCCCGATGATCCAGC as the 3' primer (Friesen 1997). An approximately 505-bp fragment of the 5' of α -tropomyosin intron I was amplified using GAGTTGGATCGCGCTCAGGAGCG as the 5' primer and CGGTCAGCCTCCTCAGCAATGTGCTT as the 3' primer (Friesen *et al.* 1999). These intron primers also amplified 13–20 bp of flanking exon sequences.

For the phylogenetic analyses, we added the mitochondrial 16S rRNA gene from at least one individual of each *Pholis* species. This was accomplished by amplifying an approximately 600-bp fragment using the 16Sar-L and 16Sbr-H 'universal' primers (Palumbi *et al.* 1990). DNA was extracted using a QIAGEN DNA extraction kit following the manufacturer's protocol.

Polymerase chain reaction (PCR) amplifications of the mtDNA (control region plus tRNA's) fragments were carried out in 50- μ L reactions: 50 mM Tris-HCl pH 9.0, 20 mM ammonium sulphate, 1.5 mM MgCl₂, 250 mM each dNTP, 500 nM each primer, 0.1–1.0 μ g of genomic DNA, and 0.5 U of *Taq* DNA polymerase. PCR conditions for the amplification of α -enolase intron and the α -tropomyosin intron were similar except for being 2.5 mM MgCl₂ in the case of α -enolase intron and 1.25 mM dimethyl sulphoxide (DMSO) in the case of the α -tropomyosin intron. Specific thermocycling parameters were optimized for each gene amplified. Reaction components without genomic DNA were included in every amplification series to screen for possible foreign-DNA contamination. Each amplified product was extracted using a QIAquick gel extraction kit following the manufacturer's guidelines. The extracted double-stranded DNA was cycle-sequenced by the dideoxy chain termination method using an ABI PRISM BigDye Terminator Cycle Sequencing ready reaction kit. Excess dye terminators were removed by ethanol precipitation. Single-stranded dideoxy chain-terminated products were sequenced with an automated sequencer in both directions (Applied Biosystems model 373 A). In order to discern both nuclear alleles from individuals observed to have more than one multiple peaks in direct sequences of PCR products (both directions), heterozygous products were TOPO TA cloned (Invitrogen) and multiple clones were sequenced until the cis/trans-position of polymorphisms in both alleles were discerned.

The DNA sequences of *P. gunnellus* were submitted to GenBank using the following accession nos: (i) mtDNA control region plus tRNA's, AY554491–AY554549; (ii) α -enolase, AY554704–AY554776; and (iii) α -tropomyosin, AY554906–AY554965. The mtDNA control region plus tRNA sequences for the five North Pacific *Pholis* species were submitted to GenBank using the following accession nos: (i) *P. ornata*, AY554555; (ii) *P. laeta*, AY554556; (iii) *P. picta*, AY554553; (iv) *P. nebulosa*, AY554554; and (v) *P. crassispina*, AY554550–AY554552. The α -enolase sequences for the five North Pacific *Pholis* species were submitted to GenBank using the following accession nos: (i) *P. ornata*, AY554695; (ii) *P. laeta*, AY554696; (iii) *P. picta*, AY554698; (iv) *P. nebulosa*, AY554697; and (v) *P. crassispina*, AY554699–AY554703. The α -tropomyosin sequences for the five North Pacific *Pholis* species were submitted to GenBank using the following accession nos: (i) *P. ornata*, AY554902; (ii) *P. laeta*, AY554901; (iii) *P. picta*, AY554900; (iv) *P. nebulosa*, AY554903; and (v) *P. crassispina*, AY554899.

Phylogenetic analysis

Population genetic models used for the inference of population history usually assume that the genealogical history of each locus is bifurcating, such that intralocus recombination is negligible. To detect potential recombination

and identify nonrecombinant blocks in the two intron loci, we employed SITES (Hey & Wakeley 1997) and verified this using Hudson's 'four gamete test' (Hudson 1985). All subsequent analyses on these intron loci were based on the largest identified nonrecombinant blocks, such that each nonrecombinant block consisted of sequence spanning adjacent nonrecombinant sites, and were bounded by the midpoint between adjacent pairs of recombinant sites.

In order to estimate intraspecific gene tree networks for each of the three loci of *P. gunnellus*, sequences were aligned and an unrooted parsimony network was constructed by comparing a pool of most parsimonious trees obtained by maximum parsimony to a maximum likelihood phylogeny constructed using the best-fit model of evolution (Cunningham *et al.* 1998) obtained with PAUP*4.0 (Swofford 1999) and MODELTEST (Posada & Crandall 1998). In all cases, heuristic searches were performed with stepwise addition, tree-bisection-reconnection (TBR) branch swapping and zero-length branches collapsed. We used PAUP to compute tree-to-tree distances between each of the most parsimonious trees and the maximum likelihood tree, and for each locus we present a gene tree network that is based on the maximum parsimony tree that is least distant to the maximum likelihood tree.

Estimates of substitution rates

We use the three single-locus phylogenies of each locus to estimate rates of DNA mutation at each of the loci. These phylogenies included eight *Pholis* taxa (*P. dolichogaster*, *P. ornata*, *P. laeta*, *P. picta*, *P. nebulosa*, *P. crassispina*, North American *P. gunnellus* and European *P. gunnellus*) and *Xererpes fucorum* as an outgroup taxa that is in the same family as *Pholis* (Pholidae; Yatsu 1985). To be consistent with the intraspecific *P. gunnellus* analysis, we restricted the phylogenetic analysis of *Pholis* to the same nonrecombinant blocks identified by SITES given the intraspecific data.

In the case of the mtDNA data, we added an approximately 600-bp fragment of mitochondrial 16S mtDNA gene sequences from each taxon. The validity of phylogenetic groupings in each tree was assessed with 100 bootstrap replicates.

As with many other temperate marine taxa, the centre of *Pholis* diversity is in the North Pacific and morphological data suggests that *P. gunnellus* is phylogenetically nested within its Pacific congeners (Yatsu 1985; Mecklenburg 2003). This phylogenetic pattern and Miocene fossils of ancestral *Pholis* found in Sakhalin (north of Japan) suggest the ancestor of *P. gunnellus* migrated from the Pacific (Nazarkin 2002) during the trans-Arctic interchange 2.4–3.5 Ma (Durham & MacNeil 1967; Vermeij 1991). By assuming that the 3.5 Ma date for the first major trans-Arctic invasion was the earliest opportunity for Pacific *Pholis* to colonize the North Atlantic coastlines, we obtain each rate estimate from the average net pairwise differ-

ences between *P. gunnellus* and their closest phylogenetic congener in the North Pacific. The rates of molecular evolution of the three loci used in this study were estimated from net trans-Arctic genetic divergences between *P. gunnellus* and its closest phylogenetic congener determined from the three phylogenetic reconstructions and by assuming that trans-Arctic colonization occurred 3.5 Ma. In order to correct for ancestral polymorphism, we subtract the mean pairwise divergence found within North American and European samples of *P. gunnellus* from the average pairwise distance between *P. gunnellus* and the closest North Pacific relative (Nei & Li 1979). Our rate estimates incorporated maximum likelihood genetic distances between all sampled individuals using the best-fit model of DNA substitution (Cunningham *et al.* 1998) as determined by MODELTEST (Posada & Crandall 1998). Each trans-Arctic divergence was obtained by calculating the average trans-Arctic pairwise distance under the best-fit model of evolution. Because there is great uncertainty in these molecular rate estimates, such as if trans-Arctic gene flow occurred later than 3.5 Ma or if the Bering Strait opened at a much earlier date (Nakashima 2002), we always consider what rates would change our overall conclusions.

Genetic diversity, selective neutrality, and population structure

Haplotype diversity for each region (North America and Europe) and locus was calculated in the program DNASP (Rozas *et al.* 2003), version 4.10.4. To investigate if selection or population expansion is acting upon any of the three loci in *P. gunnellus*, the Tajima's *D* (Tajima 1989) statistic was calculated to test the null hypothesis of selective neutrality for each regional subsample (North America and Europe). Significance of Tajima's *D* was calculated by comparing with a simulated null distribution of this test statistic within the program DNASP. Significantly, negative values at all three loci would indicate that they shared a common history demographic expansion. On the other hand, if one locus showed a markedly different signature than the other, this may suggest a history of positive selection at a linkage group associated with one of the genes. As an alternative, we gauged the magnitude of demographic expansion (or selection) from estimates of the exponential growth parameter obtained from Markov chain Monte Carlo (MCMC) likelihood estimates obtained in the program FLUCTUATE on the same regional subsamples (Kuhner *et al.* 1998). Conditions for this analysis include 10 short chains with 1000 steps, and five long chains with 20 000 steps. Significant regional growth (or selection) was determined in cases when a no-growth model could be rejected in a log-likelihood ratio test (Huelsenbeck & Rannala 1997).

To determine patterns of population subdivision, we used ARLEQUIN 2001 (Schneider *et al.* 1997) to calculate

overall Φ_{ST} values, as well as pairwise Φ_{ST} values among all pairs of sampled populations at each of the three loci. These Φ_{ST} values were tested for significance by nonparametric permutation in ARLEQUIN 2001.

Estimates of population divergence times and migration

To estimate minimum trans-Atlantic divergence times while accounting for the possibility of ongoing migration, we used a coalescent MCMC approach that jointly estimates these two potentially confounding parameters [isolation with migration (IM); Hey and Nielsen 2004]. To assure that IM converged on consistent estimates, we used it under conditions recommended in the most recent documentation. Specifically we used 10 coupled chains with a 'burn in' time of at least 200 000 steps. To assure that estimates are not biased by excessive autocorrelation, we made sure that the effective sample size for divergence times was at least 100 and that all parameters and genealogies had update rates and swap rates between chains of > 20%. The analysis was conducted on just the single mtDNA data set as well as on the full three locus data set. To report divergence time estimates in years, we applied our DNA substitution rate estimates obtained from the trans-Arctic divergence in *Pholis*. Although IM assumes a two-population model, we must violate this assumption if our Φ_{ST} analysis identifies > 2 panmictic populations. In this case, we will report trans-Atlantic IM estimates among the largest pooled panmictic samples identified in our Φ_{ST} analysis.

Tests of ecological predictors of trans-Atlantic persistence

To investigate whether particular ecological traits could statistically predict trans-Atlantic persistence across past phylogeographical studies, we reviewed the literature for studies involving intertidal taxa sampled on both sides of the North Atlantic. We then tested whether a particular trait is associated with persistence by using a method of logistic regression. This method is used when the predictor variable (having a trait) and the dependent variable (persistence) is dichotomous. This test is evaluated by performing a likelihood ratio test (Hosner & Lemeshow 1989).

Results

Phylogenetic analysis at the population level

The unrooted parsimony networks depicting the three *Pholis gunnellus* genealogies are shown in Fig. 2. In the approximately 500 bp control region of the mtDNA, there were 17 variable sites. The α -enolase and α -tropomyosin intron regions had a total of six and four variable sites, respectively. These included a single-base insertion/deletion

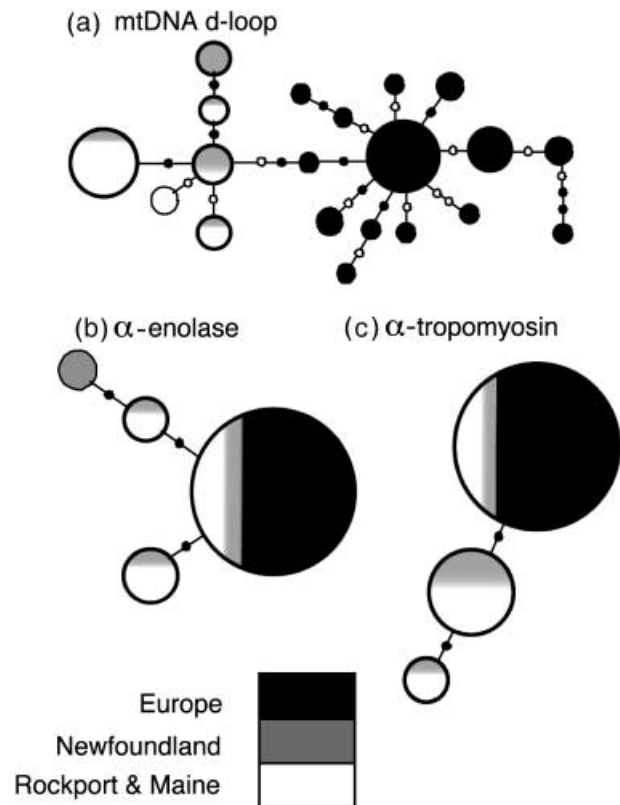


Fig. 2 Exemplar maximum parsimony networks depicting genealogical relationships of three loci collected from *Pholis gunnellus* (a: mtDNA control region, b: α -enolase intron, and c: α -tropomyosin intron). The intron networks are based on the inferred non-recombinant blocks determined from SITES. The control region network was selected from a pool of 286 most parsimonious trees after calculating the tree-to-tree distances between these trees and the maximum likelihood phylogeny obtained under the best-fit model of evolution using MODELTEST. By doing these comparisons, we report the maximum parsimony tree that is closest to the maximum likelihood phylogeny. The alleles are depicted as circles and the size of each circle is proportional to the allele's frequency. The shading of the alleles is based on three regions from which the samples were collected; black (Europe); grey (Newfoundland); and white (USA; Maine and Rockport). Alleles that are found in multiple regions are depicted with multiple shadings that are proportional to where each allele is found. The inferred mutations between alleles are depicted as black and white dots, where the former are unambiguous (consistency index = 1.0) and the latter are homoplastic (consistency index < 1.0).

in each locus. The largest nonrecombinant segments identified with SITES included three and two variable sites in these two intron loci, respectively. At the mtDNA control region locus, the North American and European populations were distinguished by two fixed differences. At both nuclear loci, one common allele was found in the majority of individuals and on both sides of the Atlantic. In both of these cases, European samples were fixed for

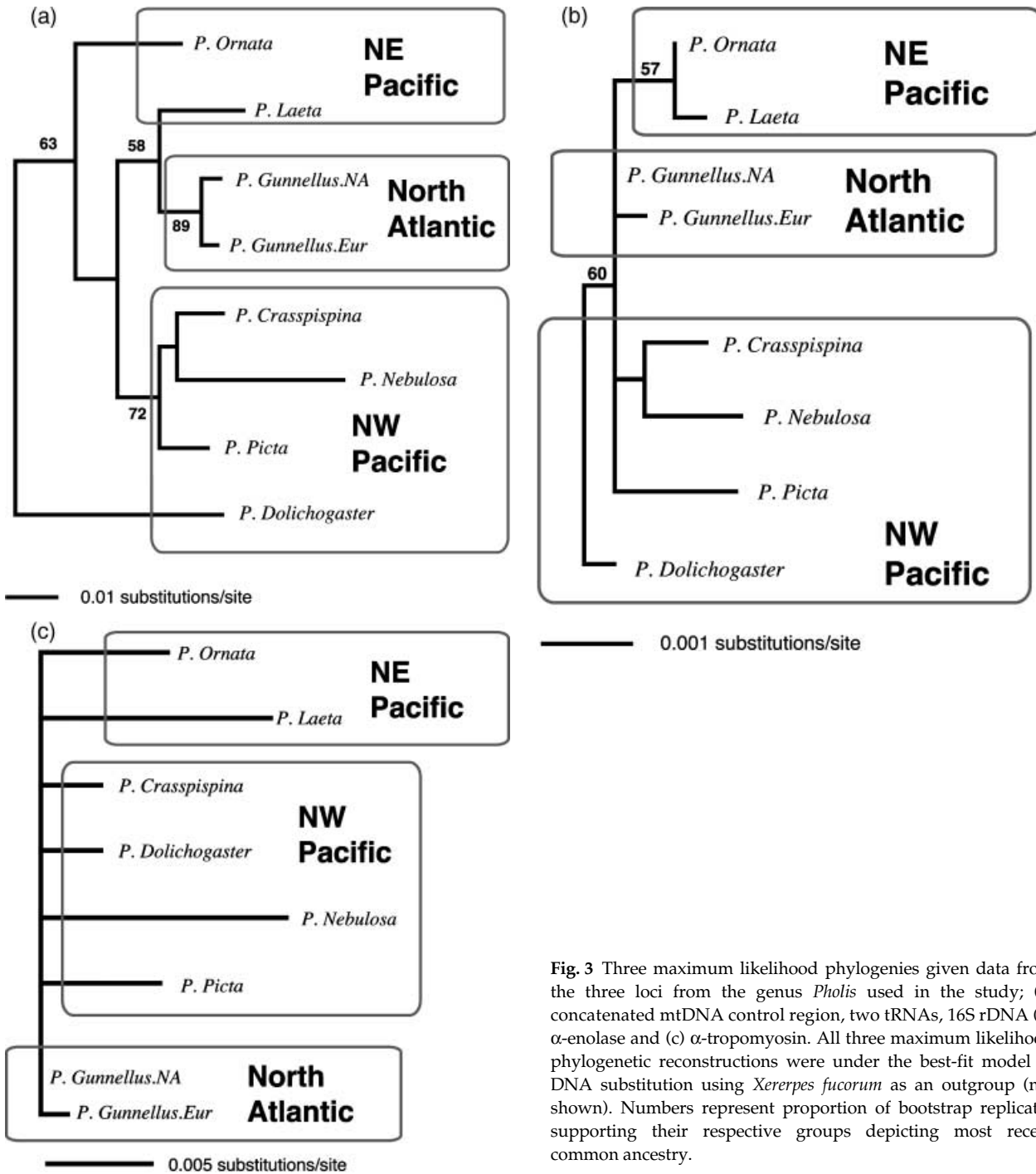


Fig. 3 Three maximum likelihood phylogenies given data from the three loci from the genus *Pholis* used in the study; (a) concatenated mtDNA control region, two tRNAs, 16S rDNA (b) α -enolase and (c) α -tropomyosin. All three maximum likelihood phylogenetic reconstructions were under the best-fit model of DNA substitution using *Xererpes fucorum* as an outgroup (not shown). Numbers represent proportion of bootstrap replicates supporting their respective groups depicting most recent common ancestry.

a single trans-Atlantic allele, while North American samples included these trans-Atlantic alleles as well as five other intron alleles not found in the European samples. The proportion of these North American alleles that were unique to a population sample (private alleles) was 32.26%, 2.33%, and 0% for the mtDNA locus, α -enolase, and α -tropomyosin, respectively (Fig. 2).

Given the mtDNA data set, the maximum likelihood phylogenetic reconstruction of *Pholis* revealed three geographically restricted clades with high or moderate bootstrap support (excluded *Pholis dolichogaster* Fig. 3a) under the best-fit model of DNA substitution [Hasegawa-Kishino-Yano (HKY) plus gamma and invariant sites] (Hasegawa *et al.* 1985). This included North American and

Table 2 Population genetic summary statistics calculated on data from *Pholis gunnellus*. N/A denote cases where lack of variation prevented analysis. Intron data was analysed after recombinant sites were removed. (A) Haplotype diversity per regional grouping. Standard deviation is denoted by \pm (B) Tajima's D statistic (1989) from regional subsamples (North America and Europe). None of the values are significant (all $P > 0.05$). (C) Regional estimates of the exponential growth parameter from FLUCTUATE. A bold value with an asterisk denotes cases in which a no-growth could be rejected in a log-likelihood ratio test (D) Overall Φ_{ST} . Statistical significance was tested using 10 000 Monte Carlo permutations of the data set. (E) Pairwise Φ_{ST} in the lower diagonals and corresponding levels of significance in upper diagonals

Locus:	mtDNA control region	α -enolase	α -tropomyosin
(A) Haplotype Diversity (H)			
North America	0.587 \pm 0.131	0.647 \pm 0.069	0.601 \pm 0.001
Europe	0.828 \pm 0.058	N/A	N/A
(B) Tajima's D			
North America	$D = 0.477$ ($P > 0.10$)	$D = 0.770$ ($P > 0.10$)	$D = 0.411$ ($P > 0.10$)
Europe	$D = -1.648$ ($P = 0.07$)	N/A	N/A
(C) Exponential growth parameter? (FLUCTUATE)			
North America	241.531	-60.521	445.642
Europe	1990.785*	N/A	N/A

European *P. gunnellus* restricted to the North Atlantic, *Pholis ornata*, *Pholis laeta* restricted to the Northeast Pacific, and *Pholis picta*, *Pholis nebulosa*, and *Pholis crassispina* being restricted to the Northwest Pacific. The position of *P. dolichogaster* as sister to the other *Pholis* species is consistent with morphological phylogenetic analysis (Yatsu 1985; Mecklenburg 2003). The nuclear data however, did not reveal highly resolved gene trees, an unsurprising result given the low number of base pairs used. As expected from coalescent theory, the gene trees are not expected to agree with each other (Maddison 1997).

Estimates of substitution rates

In two out of three loci, *P. gunnellus* was most closely related to *P. laeta*, and therefore we use this trans-Arctic divergence under the best-fit model of evolution and correcting for ancestral polymorphism to estimate the rates of molecular evolution. For the mtDNA region used in the population genetic analyses (control region and two tRNA's), this yielded a mutation rate of 0.73% per million years (Myr) under the maximum likelihood model of evolution (one half the commonly used 'divergence rate'). For the largest nonrecombinant blocks within the α -enolase, and α -tropomyosin loci, the rates were 0.14% per Myr and 0.15% per Myr, respectively. Because there is great uncertainty in these molecular rate estimates, we always ask what rate estimates would change our overall conclusions.

Genetic diversity, selective neutrality, and population structure

European samples of *P. gunnellus* had higher mtDNA haplotype diversity than North American samples, whereas

the opposite pattern was found at the intron loci (Table 2A). Although the standard deviations of these haplotype diversities given the control region locus did not overlap, these discordant diversity patterns may have arisen by stochastic variance and/or different sample sizes. None of the Tajima's D values were found to be significant (Table 2B), although Tajima's D from the European control region sample was quite negative ($D = -1.648$; $P = 0.07$). Consistent with negative Tajima's D , the FLUCTUATE estimate of the growth parameter was significantly greater than 0.0 for this control region locus given the European data (Table 2C). Significantly high overall Φ_{ST} values were found at all three loci ($\Phi_{ST} = 0.211$ – 0.626 ; $P = 0.00$), and pairwise Φ_{ST} values indicated that the population structure within Europe is effectively panmictic, including Iceland (Tables 3, $P = 0.18$ – 0.99). On the North American coastline, the pairwise Φ_{ST} 's indicate that the Rockport and Maine population samples to be highly connected by gene flow or recent divergence (Table 3; $\Phi_{ST} = 0.03$ – 0.11 ; $P = 0.11$ – 0.21), while Newfoundland was more isolated from these two North American populations (Tables 3, $P = 0.00$ – 0.31).

Trans-Atlantic divergence and migration

If one assumes that net divergence corresponds to population divergence time after correcting for ancestral polymorphism (Nei & Li 1979), the two fixed mtDNA haplotype differences between the North American and European coastlines are consistent with a divergence time of $> 200\ 000$ years ago when deploying our rate estimate. The multilocus and mtDNA IM estimates of divergence time between pooled European and Rockport/Maine population samples also yield minimum divergence times of 293 590 and 202 984 years ago, respectively (Fig. 4a, b).

Table 3 Pairwise Φ_{ST} in the lower diagonals and corresponding levels of significance in upper diagonals. Statistical significance was tested using 10 000 Monte Carlo permutations of the data set. Significant P values that are < 0.05 indicated in bold

mtDNA control region							
	Rockport	Maine	NF	Iceland	Scotland	Ireland	Norway
Rockport		0.11	0.03	0.00	0.00	0.00	0.00
Maine	0.11		0.00	0.00	0.00	0.00	0.00
NF	0.32	0.56		0.00	0.00	0.00	0.02
Iceland	0.61	0.72	0.65		0.68	0.63	0.59
Scotland	0.63	0.73	0.65	0.00		0.18	0.76
Ireland	0.70	0.77	0.72	0.00	0.04		0.24
Norway	0.60	0.74	0.65	0.00	0.00	0.00	

α -enolase							
	Rockport	Maine	NF	Iceland	Scotland	Ireland	Norway
Rockport		0.14	0.01	0.27	0.06	0.00	0.14
Maine	0.10		0.31	0.00	0.00	0.00	0.07
NF	0.25	0.01		0.00	0.01	0.00	0.04
Iceland	0.41	0.36	0.38		0.99	0.99	0.99
Scotland	0.41	0.36	0.38	0.00		0.99	0.99
Ireland	0.44	0.38	0.40	0.00	0.00		0.99
Norway	0.25	0.17	0.22	0.00	0.00	0.00	

α -tropomyosin							
	Rockport	Maine	NF	Iceland	Scotland	Ireland	Norway
Rockport		0.21	0.00	0.10	0.24	0.67	0.54
Maine	0.03		0.00	0.06	0.06	0.04	0.06
NF	0.99	0.99		0.00	0.00	0.00	0.00
Iceland	0.12	0.18	0.99		0.99	0.60	0.99
Scotland	0.05	0.17	0.99	0.00		0.83	0.93
Ireland	0.01	0.22	0.99	0.01	0.00		0.99
Norway	0.00	0.14	0.99	0.00	0.00	0.00	

Trans-Atlantic migration subsequent to divergence was estimated by IM to be either nonexistent or very low depending on which loci were used in the analysis. Using the mtDNA data alone resulted in maximum posterior migration rate estimates of 0.0 genes per generation across the Atlantic in either direction. When combining the multilocus data, maximum posterior migration rate estimates yielded moderate westward (from Europe to North America) migration, and no gene flow in the opposite direction (0.0 individuals per generation; Fig. 4).

Discussion

Trans-Atlantic isolation and persistence

The genetic data we collected from *Pholis gunnellus* does not support a history involving trans-Atlantic colonization

subsequent to the LGM and instead favours a history involving persistence on both sides of the ocean through at least the last two glacial periods spanning approximately 200 000 years. To begin, the reciprocal monophyly seen in the mtDNA data (Fig. 2a) is not expected in only 21 000 years. The posterior probability density from the multilocus Bayesian method IM gives very little support in a history of colonization post-dating the LGM (Fig. 4b). While these divergence time estimates are based on our trans-Arctic calibrations, even substitution rates an order of magnitude larger would not change the conclusion of trans-Atlantic persistence pre-dating the LGM. For example, an mtDNA rate of 7.5% per Myr (instead of the 0.73% per Myr rate we report) would be required for the trans-Atlantic divergence time estimate to be $<$ the LGM.

Another result of interloci discordance is the pattern of local genetic diversity. Although the mtDNA data had

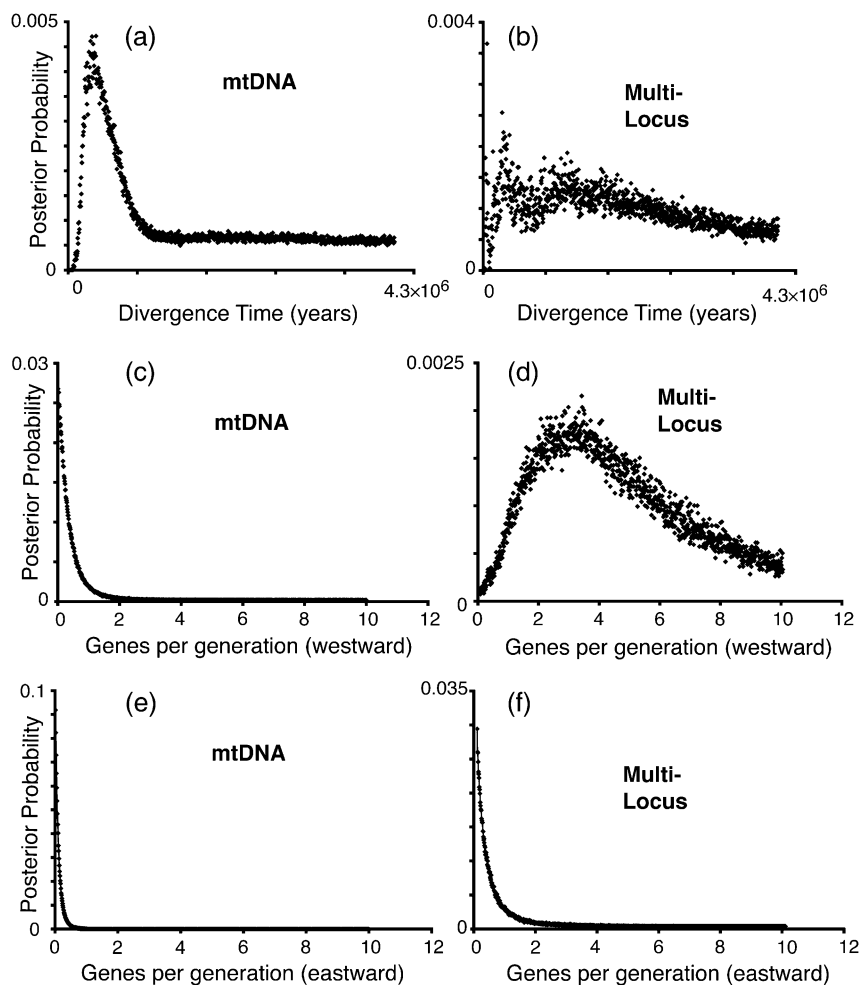


Fig. 4 Marginal posterior probability distributions of trans-Atlantic divergence times (a & b) and migration rates (genes per generation; c–f) between pooled Rockport/Maine and European samples of *Pholis gunnellus*. The single mtDNA control region estimates are depicted in (a) (c), and (e) and the multilocus estimate are depicted in (b) (d), and (f). The east to west migration rate estimates are depicted in (c) and (d), and the other direction in (e) and (f).

comparable levels of haplotype diversity on both sides of the Atlantic, there was a lack of allelic diversity at the intron loci in the European population samples. Although this may suggest postglacial colonization of Europe from North America (Hewitt 2000), these data are more consistent with other histories including trans-Atlantic persistence with localized bottlenecks and lower mutation rates at these two loci (Lessa *et al.* 2003). In this case, genetic divergence across a geographical barrier is a more consistent gauge of biogeographical persistence, and our multilocus IM estimates strongly suggest trans-Atlantic persistence greatly pre-dating the LGM and very little ongoing trans-Atlantic migration.

Although most of the IM co-estimates of migration yielded gene flow values that were sufficiently low for allopatric divergence to arise (Slatkin 1987), data from the two nuclear intron loci yielded estimates of moderate westward trans-Atlantic migration subsequent to divergence (Fig. 4d). Although this discordant pattern could have resulted from a pure isolation history with incomplete lineage sorting at the nuclear loci due to a fourfold

higher effective population size (Hudson & Turelli 2003; Rosenberg 2003), the posterior densities from IM which incorporates these differences in effective population sizes are consistent with a history involving some migration subsequent to divergence. This discordance in migration among different portions of the genome could have arisen stochastically, from gender biased dispersal, or geographical differences in selection. The higher within-coastline gene-flow estimates derived from Φ_{ST} values that suggest European panmixia are tentative without further geographically sampling, sampling more individuals and collecting more loci (Felsenstein 2006). If populations within the European coastline are consistent with a metapopulation, the resulting genetic patterns would conform to an unstructured population with regards to the coalescent (Wakeley 2004).

Although this study suggests that *P. gunnellus* has been evolving independently into separate North American and European lineages through peaks of coastal glaciation, it is uncertain whether these two lineages are becoming reproductively isolated species, and it would be premature to

propose a new species based on mitochondrial reciprocal monophyly. Even if these two lineages of *P. gunnellus* are on the road to speciation, it is probably rather early in this process in the absence of divergent selection (Gavrilets 2004).

A trait that could be undergoing ecologically divergent selection in *P. gunnellus* is geographical differences in vertical depth. North American populations shift from the intertidal to the subtidal during the colder months, whereas individuals in the rest of the European populations can be found above the low tide mark throughout the year (Sawyer 1967; Moring 1990). Divergence in vertical depth has been shown to be associated with species diversification in other groups of fishes (Turner 1999; Schluter 2000; Ruber *et al.* 2003; Herler & Patzner 2005). However, two observations make divergence in depth unlikely. First, most of the other *Pholis* species show substantial intraspecific variation in seasonal vertical range (Lindberg & Krasnyukova 1989; Mecklenburg *et al.* 2002; Sheiko 2005). Secondly, not only North American but Icelandic populations of *P. gunnellus* also exhibit this seasonal variation (Ingólfsson, personal communication). Our data show that Icelandic populations are genetically much closer to the other European populations at all three loci. Given these two observations, we cannot distinguish between the exclusive intertidal behaviour in most European populations of *P. gunnellus* being a phenotypically plastic or alternatively a heritable and recently derived trait in the European populations.

Community history of the North Atlantic rocky intertidal

To consider the historical and contemporary processes that influence the inference of the ecological history of the North Atlantic rocky intertidal, our phylogeographical study is best interpreted within the context of other phylogeographical studies involving taxa that geographically overlap with *P. gunnellus*. Potential determinants for intertidal persistence in the North Atlantic include the availability of and dependency on rocky substrate, ocean currents, sea-surface temperatures and the history of glaciation. Although *P. gunnellus* is most strongly associated with rocky-shore habitats, its occasional association with nonrocky areas on the North American coastline could suggest substrate flexibility.

It is unsurprising to find persistence on the European coastline because rocky habitat stretches further south than the maximum glacial advance at the LGM. In contrast, most of the available rocky habitat on the North American coastline is north of Long Island. Although most of this area was covered by over 1 km of ice at the LGM (Pratt & Schlee 1969; Shackleton *et al.* 1984; Dyke *et al.* 2002), the Grand Banks area proximal to Nova Scotia and Newfoundland most likely remained unglaciated, thereby

suggesting northern rocky intertidal refugia (Dyke & Prest 1987; Pflaumann *et al.* 2003). It is more likely that North American persistence of *P. gunnellus* was enhanced by its depth and substrate flexibility. It is occasionally found in nonrocky areas as far south as Delaware Bay (Scott & Scott 1989), and seasonally becomes entirely subtidal during the colder months in North American populations (Sawyer 1967; Moring 1990). This ecological flexibility could have buffered it against the perils of coastal sea ice and lower sea surface temperatures. In order to consider whether such traits allowed trans-Atlantic persistence in other North Atlantic rocky intertidal taxa, we briefly review previous studies.

These studies include a broad array of North Atlantic intertidal taxa including arthropods (Brown & Rand 2001; Wares 2001b; Wares 2001c; Wares & Cunningham 2001), mollusks (Dahlgren *et al.* 2000; Riginos *et al.* 2004), red algae (Chopin *et al.* 1996), seagrasses (Olsen *et al.* 2004), mammals (Stanley *et al.* 1996), polychaetes (Breton *et al.* 2003), and echinoderms (Wares 2001a). While none of these species exhibit the seasonal variation in vertical depth found in *P. gunnellus*, most of these species can be dichotomized with respect to habitat substrate preferences (generalist vs. rocky obligate) and dispersal tendencies (low vs. high). Summarized on Table 4, neither of these traits emerge as factors that significantly predict biogeographical response to Pleistocene glaciations if we use logistic regression; $P = 0.41$ (Hosner & Lemeshow 1989). It is worth noting that correctly finding a significant ecological predictor of biogeographical persistence is obscured if high ongoing migration lowers statistical power in correctly rejecting a history of recent colonization given phylogeographical data (Kalinowski 2002). However, even if the high dispersal species on Table 4 were all persistent across the Atlantic, logistic regression is still unable to uncover a significant predictor of biogeographical persistence ($P = 1.00$).

Our study circumstantially suggests that substrate generality and seasonal migration can facilitate persistence of temperate intertidal species and this result can become a hypothesis to further test with integrative comparative phylogeographical approaches (Arbogast & Kenagy 2001). These approaches will have potential to better test community hypotheses by making use of distributional niche models, palaeoclimatic reconstructions (Graham *et al.* 2004) and flexible simulation-based population genetic methodologies. These approaches in conjunction with broadening the scope of North Atlantic taxa will help identify relevant ecological determinants of community assembly and thereby be used to test niche-assembly and dispersal-limited assembly biogeographical hypotheses (Hubbell 2001) as well as identify the key biogeographical determinants across taxa within a community.

Table 4 North Atlantic temperate intertidal taxa that have been sampled in phylogeographical studies. The columns delineate dispersal ability, substrate preference, seasonal migration tendencies and if the phylogeographical data indicate persistence in refugia on both sides of the North Atlantic during through the LGM (last glacial maximum). *, indicates difficulty in correctly rejecting contraction hypothesis due to high dispersal potentially obscuring genetic signature of North Atlantic persistence. **, original authors do not conclude North Atlantic persistence

Taxon	Dispersal	Substrate preference	Seasonal migration?	North Atlantic persistence through LGM?	Reference
Plantae					
<i>Zostera marina</i>	High	Generalist	No	No*	Olsen <i>et al.</i> (2004)
Rhodophyta					
<i>Chondrus crispus</i>	High	Generalist	No	Yes	Chopin <i>et al.</i> (1996)
Crustacea					
<i>Semibalanus balanoides</i>	High	Obligate Rocky	No	Yes	Wares & Cunningham (2001) Brown <i>et al.</i> (2001)
<i>Idotea baltica</i>	Low	Generalist	No	Yes	Wares (2001b)
Bivalvia					
<i>Mytilus edulis</i>	High	Generalist	No	Yes	Riginos <i>et al.</i> (2004)
<i>Arctica islandica</i>	High	Generalist	No	Yes**	Dahlgren <i>et al.</i> (2000)
Gastropoda					
<i>Nucella lapillus</i>	Low	Obligate Rocky	No	No	Wares & Cunningham (2001)
<i>Littorina obtusata</i>	Low	Obligate Rocky	No	No	Wares & Cunningham (2001)
Polychaeta					
<i>Hediste diversicolor</i>	Low	Generalist	No	Yes	Breton <i>et al.</i> (2003)
<i>Neanthes virens</i>	High	Generalist	No	No*	Breton <i>et al.</i> (2003)
Asteroidea					
<i>Asterias rubens</i>	High	Generalist	No	No*	Wares & Cunningham (2001)
Mammalia					
<i>Phoca vitulina</i>	High	Generalist	Yes	Yes	Stanley <i>et al.</i> (1996)
Actinopterygii					
<i>Pholis gunnellus</i>	High	Generalist	Yes	Yes	present study

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