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Genetic studies on species composition and population structure of sand eels (Genera: *Ammodytes*, *Hyperoplus* and *Gymnammodytes*) in Norwegian waters

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Summary

Five species of sand eels (Ammodytidae) are regularly found in the north-east Atlantic. Some of these species (i.e. *Ammodytes tobianus* L. and *A. marinus* Raitt) are difficult to identify by morphological criteria. The aims of the study reported were (a) to reveal unambiguous genetic traits for species classification and (b) to study possible population structure of the more common species in Norwegian waters. In total more than 900 specimens were analysed for inter- and intraspecific allozyme variation. Reference samples were obtained from Scotland and Denmark. By combining the patterns at different loci, all five species could be unambiguously defined. In Norwegian waters *A. marinus* was found together with minor numbers of *Hyperoplus lanceolatus* (Le Sauvage). As expected, *Gymnammodytes semisquamatus* (Jourdain) and *H. immaculatus* (Corbin) were not found, but not finding *A. tobianus* in these waters was unexpected. Distribution of alleles at three polymorphic loci did not indicate any structure of *A. marinus* in Norwegian waters.

Introduction

In the north-east Atlantic the Lesser sand eels (*A. tobianus* L. and *A. marinus* Raitt), Greater sand eel (*Hyperoplus lanceolatus* (Le Sauvage)), Corbin's sand eel (*H. immaculatus* (Corbin)) and Smooth sand eel (*Gymnammodytes semisquamatus* (Jourdain)) are regularly found. In Norway the first three occur regularly, although distinction between species may be difficult; their distributions are therefore not clear (Pethon 1994). Sand eels are important food fish for cod, *Gadus morhua* L. and coalfish, *Pollachius virens* (L.), and are exploited in the reduction fishery. Sand eels are also principal prey of many seabirds and therefore are a very valuable ecological and commercial resource.

Some of the sand eel species, especially *A. marinus* and *A. tobianus*, are morphologically very similar and therefore difficult to identify. However, it has been shown that diagnostic allozyme traits exist for all five species (Simonsen 1986; Donaghy et al. 1995; Fehervari and Nævdal 1995). These authors have also described polymorphic traits within all species. Likewise Mitchell et al. (1998) have used mitochondrial DNA variation for species identification. Nævdal et al. (1996) provided an overview for samples of *A. marinus* from five main regions: Danish coastal waters, the North Sea (central and north), Iceland, Faeroe Islands and the Shetland Islands.

The aims of the study are to develop further and describe simple traits to be used for species identification to describe unequivocally the sand eel species composition along the Norwegian coast and adjacent waters (North Sea and the Barents Sea: hereafter called Norwegian waters). Likewise another goal of this work was to use the polymorphic traits to look for evidence of the existence of separate gene pools of the more common species, *A. marinus*.

Materials and methods

Samples were obtained from commercial fisheries (1994 and 1995) and research cruises in charge of the Institute of Marine Research, Bergen (1997 and 1998). Locations and main characteristics for each sample of Lesser sand eels are listed in Table 1. At position 57°04′N, 07°48′E, large sand eels assumed to be *H. lanceolatus* were sampled from a trawl haul where this species occurred in high numbers. Reference samples were obtained from Scottish (four species) and from Danish waters (three species). After capture the specimens were immediately frozen whole and later stored at –80 °C in an ultra-freezer until analysis. Alternatively, a piece of skeletal muscle was cut off and placed into the wells of microtiter plates with an equal volume of distilled water before freezing.

Nine enzymes were examined by starch gel electrophoresis (Murphy et al. 1990): aspartate aminotransferase (AAT), creatine kinase (CK), esterase (EST, a-naphthyl acetate specific), glucose-6-phosphate isomerase (GPI), isocitrate dehydrogenase (IDHP), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and α-glycerophosphate dehydrogenase (GPD). After initial screening using different buffers, histidine buffer, pH 7.0 (Harris and Hopkinson 1976), was used for AAT, IDHP, GPD and MDH, and TCB-buffer, pH 8.6 (Murphy et al. 1990), was used for CK, PGM and PGI in routine analyses. EST and LDH were found to be non-informative and not analysed routinely.

The staining recipes generally followed (Harris and Hopkinson 1976), and the nomenclature and designations of the alleles followed the conventions laid out by Shaklee et al. (1990).

Homogeneity of genotype distributions (Table 2) was tested by Monte Carlo simulations as suggested by Roff and Bentzen (1990) and programmed by Zaykin and Pudovkin (1993). For multiple tests of the same hypothesis the sequential Bonferroni correction of significance level was applied (Holm 1979).

Results and discussion

Species diversity

MDH displayed patterns with three main components (probably produced by two loci with interlocus heterodimers) which

Table 1
Account of samples of *A. marinus* from Norwegian waters together with observed allele frequencies for three polymorphic enzymes

Sample	Position	Month	Sample size	*PGI-1**			*PGM**					*IDHP**		
				p_{10}	p_{150}	p_{170}	p_{30}	p_{70}	p_{100}	p_{150}	p_{170}	p_{70}	p_{100}	p_{130}
1 North Sea	57.00, 06.00	July 1994	165	0.73	0.26	0.01	0.01	0.01	0.74	0.24	0.01	0.01	0.94	0.05
2 North Sea	60.40, 02.00	June 1995	161	0.71	0.29	–	–	0.003	0.73	0.27	–	–	0.98	0.02
3 North Sea	56.47, 03.56	July 1997	45	0.72	0.28	–	–	–	0.68	0.32	–	–	1.00	–
4 North Sea	57.16, 04.50	July 1997	60	0.78	0.22	–	–	–	0.72	0.28	–	–	0.95	0.05
5 North Sea	57.37, 04.15	July 1997	95	0.71	0.29	–	–	–	0.68	0.27	0.01	0.01	0.95	0.04
6 North Sea	57.43, 04.20	July 1997	120	0.70	0.30	–	–	0.01	0.65	0.34	–	–	0.95	0.05
7 Karmøy	59.15, 05.03	July 1997	71	0.75	0.25	–	–	–	0.73	0.27	–	–	0.96	0.04
8 Varanger	68.20, 41.01	August 1997	59	0.72	0.28	–	0.01	0.01	0.78	0.25	–	–	0.99	0.01
9 Barents Sea	72.59, 19.29	August 1997	25	0.82	0.18	–	–	–	0.82	0.18	–	–	0.92	0.08
10 North Sea	57.10, 05.32	January 1998	77	0.74	0.26	–	–	–	0.67	0.33	–	–	0.95	0.05

Table 2
Summary of homogeneity tests (Monte Carlo simulations) for samples of *A. marinus* from Norwegian waters

	All samples			Southern samples			Northern samples			Northern versus southern		
	χ^2	d.f.	P	χ^2	d.f.	P	χ^2	d.f.	P	χ^2	d.f.	P
*PGM**	59.38	54	0.27	45.74	42	0.32	3.31	4	0.59	9.94	6	0.13
*PGI-1**	17.62	36	0.99	14.08	28	0.99	1.68	2	0.46	0.86	4	0.81
*IDHP**	23.16	18	0.18	19.69	14	0.13	6.55	1	0.025[a]	0.70	2	0.76
All loci	100.16	108	> 0.05	79.51	84	> 0.05	11.53	7	> 0.05	11.40	12	> 0.05

[a]Not significant when applying Bonferroni corrections.

in *G. semisquamatus* occurred much closer than that in the other species and thus displayed a clear diagnostic pattern. In the other species only insignificant differences were seen. In *A. marinus* low frequency heterozygotes were observed, probably the same system as described by Donaghy et al. (1995), but too infrequently for studies on population structure of this species.

GPD displayed monomorphic patterns except in *A. marinus* where two different heterozygotes occurred, although too infrequently to be used in population studies. The main GPD component of *A. tobianus* displayed clearly lower anodic mobility than for any of the other species, while *H. immaculatus* likewise displayed intermediate mobilities making this enzyme a suitable diagnostic tool for these two species compared to the other three.

Two zones of AAT activity were seen that were presumably controlled by separate loci (called *AAT-1** and *AAT-2**), both of which appeared to be polymorphic. The components controlled by the *AAT-1** locus migrated toward the cathode in *A. marinus*, *H. immaculatus* and *G. semisquamatus*, but towards the anode in *A. tobianus* and *H. lanceolatus*, making this enzyme useful for distinguishing between the two species of Lesser sand eels. Due to somewhat blurred patterns, unequivocal classification of the genotypes was only possible for locus *AAT-1** of *A. tobianus* and for locus *AAT-2** of *G. semisquamatus*.

Two, or possibly three, groups of IDHP isoenzymes were similarly observed as described by Donaghy et al. (1995). The middle group, assumed to be controlled by a locus called *IDHP-2**, occurred with one common allele, *IDHP-2*100*, shared by all species. In *A. marinus* two additional alleles, *IDHP-2*70* and *IDHP-2*130* (called *IDHP-2*118* by Fehervari and Nævdal 1995), were observed. Thus IDHP could be used for population studies of *A. marinus*, but not for species identification.

Two groups of GPI isoenzymes, probably representing two loci called *GPI-1** and *GPI-2**, both showed extensive variation. Similar patterns of variation were described by Donaghy et al. (1995) and Simonsen (1986). Three alleles at the *GPI-1** locus were found in *A. marinus*. *H. lanceolatus* did not share any allele with the other species, while the common alleles in *A. tobianus*, *G. semisquamatus* and *H. immaculatus* were difficult to separate from one another and from the allele *GPI-1*150* of *A. marinus*. The *GPI-2** locus was usually expressed as diffuse bands which could not be used for identification of species or for population studies.

Five alleles occurred in PGM of *A. marinus*. *A. tobianaus* and *H. lanceolatus* displayed similar monomorphic patterns which were diagnostic due to much higher anodic mobility compared to the other species. The invariable components of *H. immaculatus* and of *G. semisquamatus* could not be distinguished, respectively, from the **100* and **150* allele of *A. marinus*.

CK displayed somewhat blurred patterns consisting of weak and moderately strong cathodic and anodic components. The main anodic component appeared to be polymorphic in *A. marinus*, although not clearly enough for accurate classification of the individuals. *A. tobianus* displayed a faster moving anodic invariable component compared to all other species.

In summary, no single enzyme was found to be diagnostic for *A. marinus*; however, the sand eel species may be classified by eliminating specimens of *G. semisquamatus* by their diagnostic MDH pattern, *A. tobianus* by their diagnostic GPD and CK patterns, *H. immaculatus* by their GPD pattern and *H. lanceolatus* by their specific GPI pattern. In addition, AAT and PGM patterns can be used to distinguish *A. tobianus* and *H. lanceolatus* from the other species. The results reported here support similar studies by Donaghy et al. (1995) and Simonsen (1986) with respect to identification of

sand eel species by simple genetic markers. Mitchell et al. (1998) obtained similar results by using RFLP analyses of mtDNA.

Only *A. marinus* and *H. lanceolatus* were found in Norwegian waters. The latter was found in low numbers together with *A. marinus* in some samples off the Norwegian coast and at Karmøy, indicating that this species may occur regularly on the Norwegian coast but in low numbers compared to *A. marinus*. The analyses of the sample collected at 57°04′N, 07°48′E confirmed that all 96 individuals were of the *H. lanceolatus* species.

Unexpectedly, not a single specimen of *A. tobianus* was seen in the samples from Norwegian waters. According to previous descriptions such as that by Pethon (1994), this species should be very common all along the Norwegian coast and, together with *A. marinus*, contribute to the fisheries. In the present study no samples were collected in the inner parts of the coast or in the fjords where *A. tobianus* might be found, but on the outer coast and in adjacent coastal waters it seems to be greatly outnumbered by *A. marinus*, if present at all.

Population studies of *A. marinus*

Three polymorphic loci were chosen for studies on the population structure of *A. marinus*. These loci, *IDHP-2** (three alleles), *PGI-1** (three alleles) and *PGM** (five alleles), all displayed clear phenotypes and no significant discrepancies were found between observed and expected Hardy–Weinberg distributions when applying the Bonferroni correction of significance level. Allele frequencies are presented in Table 1.

The allele frequencies appeared similar in all samples. When testing the genotype distributions and applying the Bonferroni correction of significance level for each loci and for all loci taken together, significant intersample variations were neither found among the total samples (general homogeneity) nor among samples from the northern and southern localities, respectively, or when comparing the southern versus the northern samples (tests not shown). This is in accordance with the results from the previous report (Nævdal et al. 1996) where only samples from Iceland deviated from samples from the more eastern localities. Thus no indication of a structuring of the total population of *A. marinus* in Norwegian waters was found based on the present polymorphic traits. The southern sampling sites are located rather close to each other and are separated by relatively shallow water; the two northern sites are situated a long distance from the others. It would be expected that long distances are significant for sand eel structuring since they have specific ecological requirements (e.g. sandy substrates and shallow waters) and are not known to make long migratory journeys (Reay 1986). Further studies using other markers as well as finer sampling grids may support or reject the results of the present study.

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