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Divergent origins of sympatric herring population components determined using genetic mixture analysis

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ABSTRACT: The origin and reproductive interactions of sympatric, spatially separated spawning components of Atlantic herring *Clupea harengus* have received long-standing interest. In the western Baltic most herring spawn in spring, with smaller components spawning in winter. We used microsatellite DNA analysis and a novel Bayesian genetic mixture analysis approach to compare the genetic relationships of 2 western Baltic winter-spawning aggregations with those of their sympatric spring-spawning components, and combined information for genetic markers and morphological traits (otolith-determined hatching time and growth relationships) to test alternative hypotheses for the origin of winter spawners. We show that genetic relationships between sympatric components differ greatly between the 2 locations; the results indicate that winter spawning has arisen via 2 fundamentally different processes: (1) as a result of 'spawning-time switching' in a local spring-spawning component and (2) via 1 or more founder events from an extant winter-spawning population into an area otherwise dominated by spring spawners.

KEY WORDS: Sympatric spawning · *Clupea harengus* · Microsatellite DNA · Spawning-time switching · Life history · Founder event · Assignment analysis

INTRODUCTION

Atlantic herring *Clupea harengus* are renowned both for their migratory and colonising capabilities and for large variations in morphology and life-history traits among stocks (Hay et al. 2001). Stock differences have been ascribed to a range of effects, spanning from phenotypic plasticity and transient subdivisions in an otherwise genetically panmictic species, to reproductively isolated, locally adapted population components (reviewed in McQuinn 1997a). Knowledge of demographic sub-structure has improved by replacing otolith macro- with otolith micro-structure analysis (McQuinn 1997b building on Messieh 1972 vs. Brophy & Danilowicz 2002 building on Mosegaard & Madsen 1996). Moreover, recent studies demonstrate low but significant genetic structure among Atlantic herring spawning components (McPherson et al. 2001, 2004, Bekkevold et al. 2005, Jørgensen et al. 2005, Mariani et al. 2005), rejecting panmixia and corroborating that, at least across large scales (e.g. seas, but also see Jørstad et al. 2004 for an example of structure on local scales),

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individual spawning components represent distinct populations. Genetic stratification is likely determined by mechanisms of natal homing, larval retention and possibly natural selection (e.g. Bekkevold et al. 2005).

Analyses of mixed-feeding and wintering schools show that spawning time often varies among stocks (e.g. in the Norwegian Sea: Husebø et al. 2005; the North Sea: Cushing 1967, Rosenberg & Palmén 1981, Hulme 1995; west of the British Isles: Brophy & Danilowicz 2002, 2003; and Gulf of St Lawrence: McQuinn 1997a). Individuals maturing in different seasons may even spawn at the same locations, and this phenomenon has been termed sympatric spawning with seasonal segregation (Winters et al. 1986). Two fundamentally different hypotheses can be invoked to explain the origin of sympatric components with divergent spawning times. The first, which has been coined 'year-class twinning' (McQuinn 1997b), entails a scenario by which juvenile growth coupled to variation in environmental conditions in some years causes fractions of individuals to mature and spawn in an earlier or later season than that in which they themselves were spawned (and hatched). Once individuals have switched they are expected to continue spawning in that season throughout their lives. In this case, seasonally separated sympatric components thus share population origin, and spawning time reflects a plastic response to external cues, operating under alternative reproductive strategies (cf. Gross & Repka 1998). In the second scenario, temporally divergent spawning components arise through founding events from populations exhibiting a different, be it genetically or environmentally determined, spawning season. In this scenario, sympatric components have different evolutionary origins and are expected to display genetic differentiation. The 2 hypotheses for establishment of spatially sympatric, temporally separated spawning components are thus testable, as distinguishing between them can be based on analyses of allele frequency differences.

Here, we determine genetic relationships and infer the most likely origin of 2 western Baltic winter-spawning components that occur sympatrically with larger spring-spawning components. We use microsatellite DNA analysis in conjunction with previously obtained data for major spawning components in the North Sea–Baltic Sea area and a novel Bayesian genetic mixture estimation approach by Pella & Masuda (2006). Their method provides a means for partitioning samples of individuals into baseline populations and putatively unsampled populations based on allele frequency information. Compared to other approaches the method has been shown to produce superior results under a range of scenarios (Pella & Masuda 2006), and the approach is ideal for our purpose as it allows us to test for the 2 aforementioned hypotheses (year-class twinning and immigration by founding event), as well as estimating the probability that the samples originated from 1 or more unsampled populations. Genetic results are compared with growth trajectories for individual spawning components, and results indicate that the examined spawning components likely arose via different processes.

MATERIALS AND METHODS

Sampling. Samples of herring *Clupea harengus* were collected in winter from 2 locations where temporally separated, sympatric spawning occurs. At Lillebælt, in inner-Danish waters, samples were collected in 2002 and 2003, and at Rügen, in the western Baltic, a sample was collected in 2004 (Fig. 1, Table 1). Among herring populations in the Northeast Atlantic, temporal separation in spawning time is observed among a large component in the English Channel spawning in winter (December and January), components in the western North Sea spawning in autumn (August to November) and components in the eastern North Sea, the Norwegian Sea, the Skagerrak, Kattegat, inner-Danish waters and the Baltic mainly spawning in spring (February to May) (ICES 1991). At both locations sampled in the present study, spawning thus otherwise mainly takes place in spring. While winter spawning is reported to occur regularly in both areas, the demography and temporal stability of winter-spawning components are not well described (Biester 1979). Although winter and spring spawners at Lille-

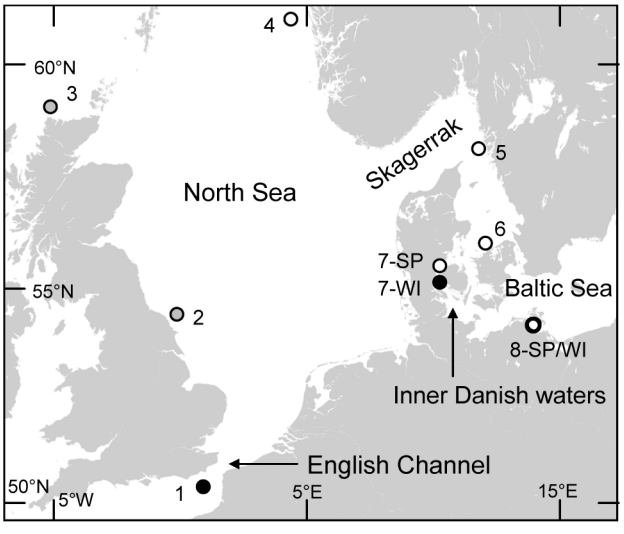

Fig. 1. *Clupea harengus* sampling locations (sample numbers refer to Table 1) (open, black and grey symbols: spring, winter and autumn spawners, respectively)

Table 1. *Clupea harengus.* Samples used in the analysis. Age (mean ± SE) and sample size (N) are given with spawning and otolith-inferred hatching seasons. Sample locations, refer to Fig. 1. Details about Samples 1 to 3 are given in Mariani et al. (2005) and about Samples 4 to 6, 7-SP and 8-SP in Bekkevold et al. (2005). SP: spring; WI: winter

Location	Sample no.	Spawning season	Hatching season	Sampling date	N	Age
English Channel	1	Winter	Winter	20 Nov 2003	99	3.91 ± 0.12
Flamborough	2	Autumn	Autumn	17 Sep 2003	96	3.39 ± 0.15
Cape Wrath	3	Autumn	Autumn	23 Aug 2003	112	3.38 ± 0.11
Møre	4	Spring	Spring	17 Feb 2003	78	7.74 ± 0.20
Skagerrak—Flatbrotten	5	Spring	Spring	19 Mar 2003	100	3.95 ± 0.11
Kattegat	6	Spring	Spring	3 Apr 2003	100	4.40 ± 0.11
Lillebælt	7-WI02	Winter[a]	Winter	11 Nov 2002	77	3.95 ± 0.14
	7-WI03	Winter[a]	Winter	23 Nov 2003	77	3.14 ± 0.06
	7-SP	Spring	Spring	7 Apr 2003	100	5.79 ± 0.15
Rügen	8-WI	Winter	Spring	7 Dec 2004	100	4.18 ± 0.11
	8-SP	Spring	Spring	24 Apr 2003	100	5.72 ± 0.14

[a]Inferred from maturity stage at time of sampling

bælt were collected from locations separated by 30 km, the location sampled in winter also acts as a spawning location for spring-spawning components, assumedly representing the same population as the analysed spring spawners. The maturity stage of each fish was recorded using a standard maturation scale (ICES 1962), and the hatching season (the season in which the fish was born) was determined from otoliths using a visual inspection procedure that has been shown to perform reliably across samples and readers (Clausen et al. 2007). The latter examinations revealed that the Lillebælt collections consisted of fish with different hatching times (winter, spring and autumn). However, in both years, the majority of sampled herring was winter spawned (70 and 68% in 2002 and 2003, respectively). Furthermore, as the spring- and autumn-spawned herring were less mature, they were expected to represent migrants rather than spawners and were therefore excluded from the analyses. Standard length was recorded for each fish, and the number of otolith winter rings was determined using the procedure described in ICES (2003) and entered as a proxy for age.

Molecular analysis. DNA was isolated from fin tissue using a Chelex technique (Walsh et al. 1991). A suite of 9 tetranucleotide microsatellites, corresponding with those analysed by Bekkevold et al. (2005) and Mariani et al. (2005), were PCR amplified, and fragment sizes were screened using a BaseStation 51 fragment analyser (MJ Research) in conjunction with the software Cartographer 1.2.6 (MJ Geneworks) under the conditions described in Bekkevold et al. (2005).

Baseline samples. Baselines used to determine the most likely population origin of samples were collected and analysed in connection with the genetic studies reported in Bekkevold et al. (2005) and Mariani et al. (2005). Between these 2 studies, herring were collected from a total of 18 spawning locations spanning the North Sea, Skagerrak, inner-Danish waters and the western Baltic Sea. Samples analysed in these studies represent the area's major populations, and were previously shown to exhibit genetic relationships that were temporally stable and to conform to an isolation-by-distance model. Genetic differentiation varies within and among seas, with spawning components in the North Sea and English Channel exhibiting close genetic relationships (F_{st} estimated at 0.001; Mariani et al. 2005) and components spanning the North Sea–Baltic Sea transition zone exhibiting higher differentiation (F_{st} estimated at 0.008; Bekkevold et al. 2005). Genetic information for populations of spring-spawning herring from Lillebælt and Rügen (Bekkevold et al. 2005) and for the winter-spawning component from the English Channel (Mariani et al. 2005) was compared to the western Baltic winter-spawning components sampled for the present study. Moreover, in order to examine the genetic resolution in the data, we used genotype information for populations at Møre, Cape Wrath and Flamborough (representing, respectively, eastern, western and central North Sea populations), in the Skagerrak and Kattegat and in inner-Danish waters (Table 1). These samples provided good representation of the area's population genetic structure (Bekkevold et al. 2005, Mariani et al. 2005) and cover populations potentially occurring in the western Baltic (see below). Genotype data were calibrated among all samples and were comparable over the full geographic scale (Ruzzante et al. 2006). To ensure consistency in scoring of microsatellite fragment sizes between studies, the same set of standard individuals was run on all gels.

Analysis of genetic variation. Overall heterozygosity and allelic richness were estimated for the 3 winter-spawning samples and compared with estimates for

the baseline samples previously reported in Bekkevold et al. (2005) and Mariani et al. (2005). Allelic richness was estimated using a rarefaction method, implemented in the software FSTAT (Goudet 2001). To illustrate genetic relationships among samples, F_{st} was estimated by θ for all sample pairs following Weir & Cockerham (1984), and statistical significances were evaluated by permutation tests using FSTAT run with 10 000 replicates. Genetic relationships were visualised applying multidimensional scaling (MDS) analysis, implemented in ViSta (Young 1996), of the matrix of pair-wise F_{st} estimates.

Genetic mixture analysis. To examine genetic relationships of the winter samples with those of the major spawning components in the area, we used a novel Bayesian Monte-Carlo Markov Chain (MCMC) method developed by Pella & Masuda (2006) and implemented in the software HWLER. The approach uses genotype information to partition samples of unknown origin into subsets of individuals from known (baseline) populations and from unknown (extra-baseline) populations by grouping individuals so that Hardy-Weinberg and linkage disequilibrium conditions are satisfied. The probability that individuals of unknown origin represent 1 or more genetically distinct populations not included in the baseline is gauged, along with the most likely population origin of each individual. Analyses were carried out for each of the 3 samples (Rügen 2004 and Lillebælt 2002 and 2003), following recommendations in the HWLER manual. To assess the statistical robustness of the HWLER approach, we carried out an additional analysis, in which Kattegat individuals (Sample 6 in Table 1) were entered as having unknown origin and tested against a baseline comprising the remaining 7 populations. This population was chosen as its genetic differentiation from Lillebælt spring spawners was estimated at roughly the same magnitude as that between English Channel and Lillebælt spring spawners (see below). Finally, we carried out 2 analyses in which either the Lillebælt 2002 or 2003 sample was used as an additional, separate population sample in the baseline, and the Lillebælt 2003 or 2002 sample was entered, respectively, as having unknown origin.

For each analysis, HWLER was run for 420 000 MCMC partitions thinned by 4; the second halves of chains were used to assess κ, the posterior number of populations represented among samples of unknown origin. The convergence of chains after burn-in was assessed by checking consistency in binary trees based on, respectively, the first and second halves of chains obtained after burn-in, using PartitionView (available at www.univ-montp2.fr/~genetix/partition/partition.htm) in conjunction with NJplot (available at http://pbil.univ-lyon1.fr/software/njplot.html). HWLER was also used to estimate the probability of each individual originating in each of the baseline populations or 1 or more of the potential unsampled populations.

Growth analysis. To assess growth patterns for the different components, length-at-age was compared between sympatric samples using ANCOVA of log-transformed total body length on log-transformed age estimates. In these analyses information for Lillebælt winter spawners was combined for the 2 sampling years, as the 2003 sample mainly represented a single year class.

RESULTS

Maturity and otolith analyses

All individuals in the Rügen sample were ripe-and-running spawners, whereas both Lillebælt samples consisted of mature (Stage 5) individuals that were not yet spawning, but could be assumed to do so within <1 to 2 mo (see 'Discussion'). All Lillebælt individuals in the analysis were winter hatched and thus showed correspondence between hatching and spawning seasons. In contrast, the Rügen sample consisted entirely of spring-hatched individuals, and thus represented individuals that spawned in a season different from that of their parents.

Sample genetic differentiation

High genotyping success was observed in the 3 winter-spawning samples, as scoring success across 9 loci in 254 individuals was 99.15%. Observed heterozygosity and allelic richness, along with pair-wise F_{st} values, are given for samples in Table 2, and an MDS plot illustrating genetic relationships is shown in Fig. 2. The MDS analysis indicated that the Rügen winter-spawning sample grouped with their sympatric spring spawners (and with Lillebælt spring spawners), whereas both Lillebælt winter-spawning samples grouped with the geographically distant English Channel population, and not with their sympatric spring-spawning Lillebælt component. The 2 Lillebælt samples exhibited low differentiation that was statistically significant prior to correction for multiple tests and were therefore analysed both separately and as a pooled sample in the genetic mixture analyses.

Genetic mixture analyses

In both analyses involving the Lillebælt winter-spawning samples, HWLER returned low probabilities for the samples originating from 1 or more unsampled

Table 2. *Clupea harengus*. Sample heterozygosity (H_o) and allelic richness (r) across 9 microsatellite loci, and pair-wise genetic differentiation estimated by F_{st} (above diagonal); p-values for tests for differentiation are given below diagonal. Significance following sequential Bonferroni correction (k = 55) is shown by asterisks. $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$. Comparisons between sympatric components are underlined

Sample (sample no.)	H_o	r	(1)	(2)	(3)	(4)	(5)	(6)	(7-SP)	(7-WI02)	(7-WI03)	(8-SP)	(8-WI)
English Channel (1)	0.812	15.31		0.0018	0.0024	0.0021	0.0025	0.0147^{***}	0.0157^{***}	0.0025	0.0055^{***}	0.0169^{***}	0.0143^{***}
Flamborough (2)	0.820	14.75	0.3520		0.0000	0.0022	0.0039	0.0179^{***}	0.0177^{***}	0.0051	0.0066^{*}	0.0174^{***}	0.0134^{***}
Cape Wrath (3)	0.801	14.70	0.1160	0.5853		0.0014	0.0038^{**}	0.0164^{***}	0.0157^{***}	0.0034	0.0050^{***}	0.0161^{***}	0.0127^{***}
Møre (4)	0.842	14.28	0.3998	0.0514	0.1229		0.0048^{**}	0.0193^{***}	0.0182^{***}	0.0051^{*}	0.0077^{***}	0.0183^{***}	0.0141^{***}
Skagerrak (5)	0.811	14.98	0.0097	0.0303	0.0002	<0.0001		0.0116^{***}	0.0090^{***}	0.0032^{*}	0.0054^{***}	0.0120^{***}	0.0105^{***}
Kattegat (6)	0.810	12.99	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.0021	0.0125^{***}	0.0172^{***}	0.0039^{***}	0.0047^{***}
Lillebælt spring (7-SP)	0.832	13.30	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0051		$\underline{0.0125^{***}}$	$\underline{0.0172^{***}}$	0.0020^{*}	0.0017
Lillebælt winter 2002 (7-WI02)	0.787	15.50	0.0293	0.0192	0.0181	0.0006	0.0021	<0.0001	<0.0001		0.0047	0.0109^{***}	0.0114^{***}
Lillebælt winter 2003 (7-WI03)	0.805	14.51	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0088		0.0194^{***}	0.0174^{***}
Rügen spring (8-SP)	0.793	12.96	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0061	<0.0001	<0.0001	$\underline{0.0003}$	
Rügen winter (8-WI)	0.817	13.16	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0337	<0.0001	<0.0001	0.1888	

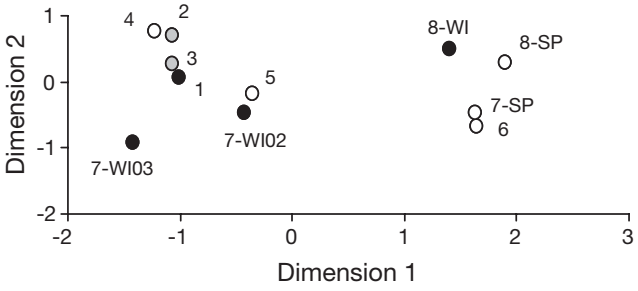

Fig. 2. *Clupea harengus*. Multidimensional scaling plot of the first 2 dimensions (describing, respectively, 70 and 9% of variation; stress = 0.071) for pair-wise F_{st} values for western Baltic winter-spawning and baseline samples. Sample numbers, refer to Table 1 (open, black and grey symbols: spring, winter and autumn spawners, respectively)

baseline populations (p[κ > 0] = 0.11 and 0.02, for 2002 and 2003 samples, respectively), indicating that all individuals likely originated from one of the baseline samples. The assignment analyses indicated that individuals sampled in 2002 were genetically similar to English Channel winter spawners, as 51 of 77 fish assigned to this population at an average probability of 0.56. Nineteen fish assigned to the North Sea population Cape Wrath (at average p = 0.55) and the remaining 7 were assigned among baseline samples Møre, Flatbrotten, Lillebælt and Rügen at fairly low probabilities (average p = 0.39). All individuals in the 2003 sample were assigned to the English Channel at p > 0.93. When Lillebælt 2002 and 2003 samples were pooled and entered as a single unknown sample, results were consistent, as p[κ > 0] = 0.06. Again, individuals most likely originated in the English Channel (at average p = 0.79, with only 1 of 155 multi-locus genotypes being slightly more likely in the Cape Wrath sample compared to English Channel [p = 0.51 and 0.44, respectively]).

HWLER did not return evidence that individuals in the Rügen sample originated from a distinct, unsampled population either, as p[κ > 0] = 0.06, but in contrast to Lillebælt, Rügen winter spawners assigned to their sympatric population (100 fish at average p = 0.66, with individuals alternatively assigned to the Lillebælt spring-spawning population at average p = 0.33). When Kattegat fish were entered as having unknown origin, the algorithm successfully recognised the presence of 1 or more unsampled populations, with highest probabilities for 2 or 3 unsampled populations (p[κ = 0] = 0.01, p[κ = 1] = 0.08, p[κ = 2] = 0.45, p[κ = 3] = 0.35, p[κ >3] = 0.11).

When Lillebælt winter spawners from 2002 were included as an additional baseline population and Lillebælt 2003 individuals represented the unknown sample, the algorithm produced ambiguous results.

The posterior probability was highest for a single unknown population (p[κ > 0] = 81%, with p[κ = 1] = 75%). However, in the individual assignment analyses, only 3 fish most likely originated in the suggested unsampled population (assigned at average p = 0.81), and the remaining fish most likely originated in the Lillebælt winter-spawning 2002 sample (58 fish at average probability 0.76), the English Channel population (10 fish at average p = 0.52), or in other populations (6 fish at average p = 0.41). In contrast, when the 2003 Lillebælt sample was included in the baseline and run against individuals sampled in 2002, results indicated low probability for 1 or more unsampled baselines (p[κ > 0] = 0.12, with p[κ = 1] = 0.11), and that 2002 individuals most likely originated in the English Channel, Cape Wrath, or Lillebælt 2003 (36, 32 and 31% at average probabilities of 0.57, 0.43 and 0.56, respectively), demonstrating low power for distinguishing among individual origins in these 3 baseline samples.

Table 3. *Clupea harengus*. ANCOVA results for differences in intercepts and slopes of (log-log transformed) length-at-age in samples

	Intercept			Slope		
	F	df	p	*F*	df	p
Lillebælt winter vs. spring						
	84 821.1	247	<0.001	0.753	246	0.387
Lillebælt winter vs. English Channel						
	50 324.9	245	<0.001	5.329	244	0.022
Rügen winter vs. spring						
	42 470.0	194	<0.001	0.201	193	0.655

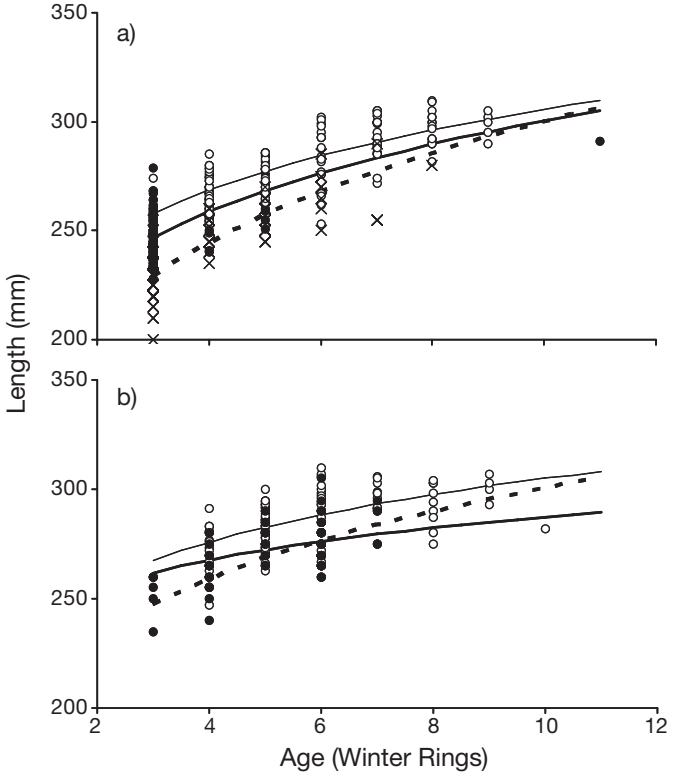

Fig. 3. *Clupea harengus*. Length-at-age relationships and estimated regression lines (for log-log transformed data). (a) Lillebælt winter 2002/2003 (●, bold line), Lillebælt spring (○, fine line) and English Channel (×, dashed line). (b) Rügen winter (●, bold line) and Rügen spring (○, fine line). The regression line for Lillebælt winter 2002/2003 is also shown for comparison (dashed line)

Population-specific growth patterns

All length-at-age comparisons showed highly significant differences in intercepts for the estimated relationships, but only Lillebælt winter spawners and English Channel winter spawners exhibited significant differences in estimated slopes (Table 3). In all other comparisons, slopes were not significantly different, indicating that growth patterns were similar for winter- and spring-spawning components (Fig. 3).

DISCUSSION

Using a Bayesian approach for assessing genetic relationships in samples of unknown population origin, we demonstrated that 2 western Baltic herring *Clupea harengus* winter-spawning components from an area of predominant spring spawning exhibited fundamentally different genetic relationships with their sympatric components. Rügen winter spawners, which themselves were spring hatched, closely resembled their sympatric spring-spawning component with respect to both allele frequencies and growth patterns. The combined genetic and morphological analyses thus indicated that the Rügen winter-spawning component had arisen through spawning-time switching in local spring-spawned herring, corroborating that spawning time is not genetically predetermined. Several otolith-based analyses have shown that individuals hatched in one season may spawn in another (e.g. Aneer 1985, McQuinn 1997b, Brophy et al. 2006), but also that spawning-time fidelity is prevalent for East Atlantic herring (Husebø et al. 2005, Brophy et al. 2006, Clausen et al. 2007). Although juvenile growth rate is known to influence age-at-maturity, the general consequences for spawning season are not clear (compare McQuinn 1997b with Brophy & Danilowicz 2003), and proximate determinants of spawning-season switching have not been identified. Contrary to expectations under a hypothesis of spawning time deter-

mined by growth conditions (e.g. McQuinn 1997b), growth trajectories were similar for spring and winter spawners, suggesting that individuals experienced similar environmental conditions and/or growth responses during (parts of) their life cycles. It has not yet been possible to determine whether winter spawning is recurrent in the area, or thus whether the sampled winter-spawning component is transient or ecologically stable over time.

In Lillebælt, analyses of temporally repeated samples from a winter-spawning component demonstrated lack of genetic correspondence between sympatric spring and winter spawners. Whereas Lillebælt spring spawners show a genetic relationship with neighbouring spring-spawning populations in the western Baltic (Bekkevold et al. 2005), winter spawners in contrast exhibited a close genetic relationship with the English Channel component. Winter-spawning components also occur west of the British Isles, but English Channel herring represent the only major winter-spawning component likely to be present in the western Baltic (ICES 1991). Following hatching, North Sea autumn-spawned and English Channel winter-spawned larvae drift into the Skagerrak, where they feed for 1 to 2 yr before migrating back to spawn in natal areas. Munk & Christensen (1990) showed that autumn-spawned larvae drift east and enter the Skagerrak–Kattegat area 4 to 5 mo post-hatching. The drift pattern for English Channel herring correspondingly describes a north-easterly direction (Bückmann & Hempel 1958), but whether larvae can drift as far as the western Baltic has to our knowledge not been investigated. We were not able to directly determine whether the Lillebælt winter spawners were born locally or elsewhere, drifting or actively migrating into the area. However, we found evidence that different year classes dominated in successive years (Table 1), indicating that their presence was a recurrent phenomenon. All Lillebælt winter spawners were Stage 5 and none was ripe-and-running (Stage 6) at the time of sampling. Though herring may retain maturity Stage 5 for several months depending on temperature, body size and condition (Bowers & Holliday 1961, Iles 1964, Lambert 1987), typical behaviour is to congregate at spawning locations days to weeks prior to spawning (Haegele & Schweigert 1985). Individuals were collected in a known winter-spawning area (Jensen 1949), approximately 1500 km (shortest water-way distance) from the English Channel, during that population's spawning season. Herring in Stage 5 in autumn (November) are, moreover, likely to display a short maturation cycle and spawn shortly after reaching full maturity (Bradford & Stephenson 1992). It thus seems reasonable to assume that individuals eventually spawn at the location, although timing only could be determined to within 1 or 2 mo (i.e. December and January). Moreover, the fact that their growth rates (Fig. 3) differed from those of English Channel spawners, but not from sympatric spring spawners, indicated that the Lillebælt winter spawners had experienced environmental conditions similar to those of sympatric spawning components and dissimilar from those of English Channel spawners.

The 2 Lillebælt samples exhibited marginally significant differentiation in allele frequencies between the 2 sampling years, and, although both samples grouped with the English Channel in the MDS analysis (Fig. 2), the 2003 sample exhibited statistically significant differentiation from this population (Table 2). Moreover, the HWLER analyses indicated that, whereas the 2002 sample was overall similar to the English Channel population, the 2003 sample tended to be more closely related to the Lillebælt 2002 sample. A possible explanation for this initially puzzling result is related to error associated with sampling a limited number of year classes (Jorde & Ryman 1995). Whereas the 2002 sample, which was not differentiated from its inferred population of origin, represented 3 main year classes, 92% of the individuals in the 2003 sample represented a single year class. Together with the very high resolution obtained with the chosen set of microsatellite markers (cf. Ryman et al. 2006), this unequal sampling of age classes may have led to the signal of significant allele frequency differentiation between temporal samples. Post hoc tests of pair-wise differentiation among year classes in the 2 samples returned no significant results (results not shown). The signal of differentiation between temporal samples is thus likely to be a combined result of sampling effects and of the high statistical power for detecting allele frequency differences, rather than evidence for different populations being sampled, or for the 2003 sample being affected by genetic drift. This was also suggested by the estimates of genetic variability, as all samples exhibited high levels of heterozygosity, and allelic richness corresponded between samples of presumed similar population origin (Table 2).

Herring spawning locations are widely distributed in the Northeast Atlantic (ICES 1991), and the applied baseline did not represent exhaustive sampling of components. However, the aim of the analysis was to test the hypothesis that sympatric, temporally separated spawning components are genetically related, rather than to examine the potential for assigning individuals to populations. Using mixed-stock simulations based on genotype information from the studies by Bekkevold et al. (2005) and Mariani et al. (2005), Bekkevold et al. (unpubl. data) examined the statistical power in mixed-stock analysis and effects of including or excluding multiple weakly differentiated baseline

population samples. They found that the probability for distinguishing among contributions from local (sub-) populations was low, and only on larger geographic scales (e.g. among seas) was statistical resolution adequate for partitioning stock contributions. They also showed that analyses incorporating baseline information for 9 vs. 15 populations generated consistent estimates of mixed-stock proportions, albeit confidence intervals narrowed when baseline sample numbers increased. Individual assignment procedures exhibit low statistical power under weak population differentiation (reviewed by Manel et al. 2005), and the approach is sub-optimal for determining mixed-stock proportions under most scenarios (Koljonen et al. 2006). In the present study, population differentiation was weak among North Sea and English Channel components, and between Rügen and Lillebælt spring spawners. This was directly reflected in the difficulty of assigning Lillebælt winter spawners to English Channel and North Sea baselines, and Rügen winter spawners to Rügen and Lillebælt spring-spawning baselines. Although individual assignment results should thus be interpreted with caution, their main merit was 2-fold. First, individuals collected together, in most cases, could be assigned to a common known baseline population and not an extra-baseline population, and, second, the most likely population of origin constituted that predicted from otolith data. The low power for distinguishing among origins in weakly differentiated populations meant that Rügen winter spawners potentially could represent immigrants from an unsampled weakly differentiated population and that Lillebælt winter spawners could have originated from North Sea autumn spawners that switched to winter spawning, although these present less parsimonious scenarios. Moreover, the analysis in which Kattegat fish were entered as an unknown sample unambiguously identified the presence of 1 or more genetically divergent components, although the algorithm, in line with results of other genetic approaches for estimating numbers of populations (reviewed by Waples & Gaggiotti 2006), exhibited low success in determining the actual number of extra-baseline populations. Nonetheless, these combined results showed that Rügen and Lillebælt winter spawners originated from populations that were genetically and presumably geographically close to, respectively, Rügen and English Channel herring.

The indication of a founder event from the English Channel (or western North Sea) to the Lillebælt raises questions about the frequency and ecological stability of immigrations. Range expansions and changes in the use of spawning locations have been reported for several herring stocks in the North Atlantic following fisheries-induced population collapses (reviewed in Corten 2001). Corten (2001) suggested that migratory changes are likely to occur when a year class recruits in the absence of older year classes, e.g. following fisheries depletion. In Corten's scenario, migratory routes are socially transmitted from older to younger fish. In the absence of the former, traditional routes are not transmitted to naïve recruits, which, as a result, may end up in novel spawning locations. Although the Lillebælt 2003 winter spawners were mainly from a single year class, the pooled Lillebælt samples collected over 2 consecutive years comprised several year classes, and it is therefore unlikely that they represented naïve recruits in line with Corten's scenario. The winter spawners may, however, be descendents of strayers that for some reason, be it related to effects of social learning, divergent growth, hydrographic features, or other factors, failed to home with other English Channel herring.

An important caveat when predicting effects of migratory behaviour on population structure is that observed frequencies of straying between populations need not reflect levels of reproductive isolation and gene flow. Hence, immigration success ultimately depends on selection pressures in the novel environment, as divergent local selection pressures may impede or prevent reproductive success in strayers (e.g. Rundle 2000). Immigrants and their descendents may reproduce successfully in some years, but show lack of persistence over ecological or evolutionary time scales. The spatially explicit genetic structure of herring populations in the North Sea–Baltic Sea area (Bekkevold et al. 2005, Jørgensen et al. 2005, Ruzzante et al. 2006) provides direct evidence that a significant degree of reproductive isolation is maintained over ecological and evolutionary time scales, although levels of gene flow vary across geographic scales. Within both the North and Baltic Seas population differentiation is, for instance, of comparatively lower magnitude than between the 2 seas and among populations in the transition zone. The 2 seas differ greatly in environmental conditions. The North Sea is a temperature-stable, saline (ca. 34) environment, whereas the Baltic Sea and the transition zone have more variable temperatures and are brackish, with salinities decreasing from 34 in the Skagerrak to almost zero in the Northeast Baltic. Proximate mechanisms restricting gene flow among herring populations have not been resolved, but patterns of genetic differentiation covary with salinity and temperature parameters (Bekkevold et al. 2005, Jørgensen et al. 2005), suggesting that local adaptation to these (or associated) environmental variables may be a factor. The observation that several fishes and other marine organisms in the North Sea–Baltic Sea area show population structuring at similar geographic levels (reviewed in Johan-

nesson & André 2006) further suggests a role for adaptive diversification in response to local salinity and/or temperature conditions across species. In connection with this, it is interesting that Lillebælt winter spawners seemingly spawn at much lower salinities (ca. 16) than English Channel herring (ca. 35)—their presumed population origin. Low salinity is known to reduce reproductive success in Pacific herring *Clupea pallasi* (Griffin et al. 1998), but it is yet unknown to which extent, e.g., English Channel herring would be reproductively impaired in a brackish spawning environment.

In conclusion, our analyses demonstrated that sympatric spawning herring components can exhibit divergent genetic origins and that combining genetic and morphological trait information presents a valuable means of determining the most likely origins of individual spawning components. Overall, our results yield a complex picture of previously not fully recognised biological diversity. In conjunction with recent demonstrations of spatially explicit stable population differentiation in Northeast Atlantic herring, it is however indicated that, although the observed life-history variation and plastic migratory behaviour lead to dynamic demographics and a high potential for gene flow, herring spawning components uphold significant levels of reproductive isolation, possibly affected by selective differences among spawning and/or larval habitats.

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