Effects of prey concentration, light regime, and parental origin on growth and survival of herring larvae under controlled experimental conditions

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Experiments were undertaken to study the combined effects of environmental (prey concentration and photoperiod) and genetic (parental spawning season) factors on growth and survival of herring larvae. During the experimental period, constant prey concentrations were maintained at one of the two nominal levels, under either a spring or an autumn light regime. Eggs of one autumn-spawning North Sea Buchan female herring were fertilized either with cryopreserved sperm from three Norwegian spring-spawning males or with fresh sperm from three Buchan males. Larvae of the two groups (the hybrids marked with alizarin) were mixed in replicated treatment tanks, thus ensuring identical environmental conditions. Mean larval growth rates were mostly influenced by food availability, but the hybrids grew significantly faster than the pure autumn-spawned offspring. In addition, the hybrids experienced a survival advantage at low prey concentrations. Hybrid survival was also somewhat better at high prey concentrations under a spring photoperiod than the corresponding group under an autumn photoperiod, suggesting a possible genetic adaptation to seasonal light conditions. The experiment documents the viability beyond first-feeding of offspring from parents with different spawning periods. The results are discussed in relation to herring metapopulation structure.

Keywords: *Clupea harengus*, genetic effect, hybrid, metapopulation, viability.

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Introduction

Food availability and predation are crucial factors in determining growth and survival of early life stages, and hence variations in recruitment (Bailey and Houde, 1989). The dependence of larvae on available food during the transition from endogenous to exogenous feeding was hypothesized to be the critical element almost a century ago (Hjort, 1914). Subsequent studies have shown the importance of the timing of seasonal plankton production and its consequences for larval survival (Cushing, 1990). In a review of Cushing's "match–mismatch" concept, and using herring (*Clupea harengus*) as an example, Sinclair and Tremblay (1984) questioned the importance of the timing of plankton production because it had been shown that different herring populations may spawn virtually year around, not only just before the peak of spring production. The herring is indeed a versatile species (Blaxter, 1985), with spring-spawning and autumn- or winter-spawning populations occurring together in the same areas (Fossum and Moksness, 1993; Bjørke and Sætre, 1994; Brophy *et al.*, 2006).

Because the environment facing spring-spawned larvae will be quite different from the one facing autumn- or winter-spawned larvae, the question is raised whether there are special adaptations to these spawning times. Gamble *et al.* (1985) studied the offspring from two North Sea populations that spawned at different times of the year. They suggested that the development of the offspring of the autumn spawners was delayed relative to that of the spring spawners, so preventing metamorphosis during winter. Their experiments were carried out during the respective spawning seasons of the two populations, and confounding environmental factors influencing growth and development could not be ruled out. Johannessen *et al.* (2000) investigated the role of seasonal differences in light regime and found that the spring light regime promoted faster growth of larvae reared under similar experimental conditions and at the same temperature. They also suggested that, with decreasing photoperiod, autumn-spawned larvae maximize the storage of surplus energy, so increasing their fitness for winter survival. These experiments were prone to confounding genetic differences as well as unintentional environmental differences because they were carried out with genetically different material at different seasons.

The genetic structure of herring populations has been investigated intensively, and the prevailing notion is that different spawning populations are characterized by relatively low genetic divergence (Safford and Booke, 1992; Mariani *et al.*, 2005). This could be indicative of a larger metapopulation complex within which genetic material is exchanged extensively among subunits (Brophy *et al.*, 2006). However, other examples exist of closely situated populations along the Norwegian coast that are

genetically distinct, indicating that little mixing is taking place (Jørstad *et al.*, 1991).

One barrier to mixing among populations is the difference between the spawning times of co-occurring herring. Indeed, many examples exist of autumn-spawned herring being present in schools of spring spawners, and vice versa, but whether they actually spawn together is a question yet to be resolved. The seasonal origin of individuals can be determined by the use of otolith microstructure analysis (Clausen *et al.*, 2007), and the presence of ripe adults among schools spawning at a different time of year than their own origin would predict has also been documented (Brophy *et al.*, 2006). McQuinn (1997) discussed the concept of year-class twinning, whereby sympatric populations produce good year classes sequentially (e.g. a good year class of autumn spawners in one year is followed by a good year class of spring spawners in the following year). This could be attributable to excellent feeding conditions promoting growth and survival of both groups of larvae during spring, or to a large proportion of spring spawners joining the autumn spawners during spawning. If the latter is a common phenomenon, the existence of a barrier to genetic mixing would be questionable. However, despite many examples of co-occurring autumn- and spring-spawned larvae, as well as adults, cross fertilization between herring originating from different spawning seasons in nature has not been documented (Husebø *et al.*, 2005), nor have differences in the viability of their offspring past first-feeding. Successful cross fertilization involving gametes of adults from different spawning periods was described by Blaxter (1953), but these experiments did not proceed beyond the first-feeding stage.

Preliminary investigations by Johannessen *et al.* (2000) suggested that growth and survival of herring larvae are determined by both genetic and environmental factors. We attempt to elucidate whether genetic factors are more influential on early larval growth and survival than environmental factors. Our hypothesis was that hybrid offspring should be better adapted to the seasonal spring light regime than the pure offspring of autumn spawners. Conversely, under an autumn light regime, the pure offspring should be better adapted for growth and survival than the hybrid offspring, especially when feeding conditions are relatively poor. As we were unable to cryopreserve herring eggs successfully but had available cryopreserved sperm from a spring-spawning population, the hypothesis was tested using an experimental approach that involved cross fertilizing the gonad products of spring-spawning males and an autumn-spawning female, and comparing the growth and survival of the hybrid offspring with those of co-reared pure autumn-spawner offspring. Two feeding levels and two light regimes were established to determine whether the two groups responded differently to these external conditions.

Material and methods

Fresh gonads from one female and three male ripe-running autumn-spawning herring, caught by purse-seine off Peterhead (northeast Scotland; 57°29′N 01°40′W; 13 August 1998), were stored on dry ice in individual sample vials and transported to Bergen by aircraft. Testes dissected from three mature male herring, caught in spring (April 1998) off the southwest coast of Norway (59–60°N 05°W), had already been placed separately in 10-ml vials with a dilutant of 10% dimethylsulphoxide (Stoss and Refstie, 1983), stirred, and stored in liquid nitrogen (−196°C) for later use. Eggs from the single autumn-spawning female were stripped onto glass plates and fertilized artificially with sperm from three males, originating from either the autumn-spawning stock or the spring-spawning stock, on 13 August 1998 (23:00–23:50 h). The motility of the cryopreserved sperm after thawing was checked microscopically and found to be similar to the fresh sperm. Only one female was used to avoid non-genetic maternal effects (Chambers and Leggett, 1996; Høie *et al.*, 1999), but the sperm of multiple males were used to secure genetic diversity and high fertilization success (89–95%) for both groups. The fertilized eggs were incubated at 10°C. At ∼2 d before expected hatching, the hybrids were subjected to immersion marking with an alizarin complexone (AZ; Sigma Chemical Co.; 100 mg l^{-1}) for 11 h in darkness (Folkvord *et al.*, 2004). Hatching (50%) occurred on 25 August, which was designated as 0-d post-hatch (dph).

The experimental set-up involved two different light regimes, simulating a natural spring light regime (S) and a natural autumn light regime (A), with gradually increasing and decreasing daylength, respectively. Within each light regime, two food regimes were tested: one with low (L: 40 prey ml^{-1}) and the other with high food concentrations (H: 1200 prey ml^{-1}). All treatments (AL, AH, SL, SH) were replicated twice in eight 180-l rearing tanks. Three hundred larvae, 150 from each of the two groups, were placed together in each tank to provide them with identical conditions of food availability, temperature, and light regime. The AZ-marked hybrids could be readily identified during subsequent analyses. In addition, two replicates of 100 unfed (U) larvae from each group were kept in 5-l buckets with filtered seawater for 14 dph under both light regimes (AU and SU) and used as controls for initial growth and survival (∼90% to 14 dph).

The environmental conditions in the rearing tanks were maintained at 10 ± 0.1°C, 35.0 psu salinity, and an oxygen content above 80% saturation level. The autumn and spring light regimes were simulated according to the periods 25 August–20 October and 21 April–16 June, respectively, for Bergen (60°23′N 05°20′E). During the experiment, the hours of light were increased from 17 to 21 h for the spring light regime and decreased from 17 to 12 h for the autumn light regime, following a sinusoidal pattern in intensity, with a maximum at the surface of nearly 18 $\mu E\ m^{-2}\ s^{-1}$ at midday. From 2 dph onwards, 1–2 l of phytoplankton culture were added to the tanks daily, as well as (from 3 dph onwards) live natural zooplankton (mainly rotifers and nauplii) in a concentration that matched the predefined nominal concentrations (L and H). For further details, see Folkvord *et al.* (2000).

Twenty larvae were sampled (during daylight) from each rearing tank at 7, 14, 21, 28, 35, 42, 49, and 56 dph. The larvae that remained after sampling on day 56 were counted. One AL replicate was emptied (and counted) on day 49 because there were too few remaining larvae. Daily average mortality rate up to day 56 was estimated by using the initial and final numbers of larvae in each tank and correcting for the numbers removed when sampling. This censoring procedure provided the true survival rate of larvae as experienced up to the time of sampling. Twenty larvae were also sampled from the unfed controls at 0, 7, and 14 dph. In total, 1500 larvae were sampled for biological characteristics in this experiment (Table 1). Additional larvae were sampled at 35 dph for other purposes, the results of which are not reported in this study. The set-up of the experiment and procedures involved was approved by the Norwegian Animal Research Authority (NARA).

Standard length (SL) and developmental stage (Doyle, 1977) were determined shortly after sampling. Each larva was then

Table 1. Sampling design and numbers of larvae removed during the experiment: larvae from pure autumn strains (A × A) and hybrids (S × A) were kept in separate units as unfed controls (U), while equal numbers of the two groups (150 + 150) were mixed (Mix) in two replicate tanks for each treatment of prey concentration (L: low; H: high) and light regime (S: spring; A: autumn).

	Spring light regime				Autumn light regime				
dph	SU (A × A)	SU (S × A)	SL (Mix)	SH (Mix)	AU (A × A)	AU (S × A)	AL (Mix)	AH (Mix)	Total
0	20	20	0	0	20	20	0	0	80
7	20	20	20 + 20	20 + 20	20	20	20 + 20	20 + 20	240
14	20	20	20 + 20	20 + 20	20	20	20 + 20	20 + 20	240
21	0	0	20 + 20	20 + 20	0	0	20 + 20	20 + 20	160
28	0	0	20 + 20	20 + 20	0	0	20 + 20	20 + 20	160
35[a]	0	0	20 + 20	20 + 20	0	0	20 + 20	20 + 20	160
42	0	0	20 + 20	20 + 20	0	0	20 + 20	20 + 20	160
49	0	0	20 + 20	20 + 20	0	0	20 + 20	20 + 20	160
56	0	0	20 + 20	20 + 20	0	0	20 + 0[b]	20 + 20	140
Total	60	60	160 + 160	160 + 160	60	60	160 + 140	160 + 160	1500

[a]Additional 68 larvae from high-prey-concentration tanks were taken out for other analyses.
[b]One replicate terminated on day 49 because too few larvae were surviving.

cryopreserved individually in liquid nitrogen (N_2) and subsequently kept in a biofreezer (−80°C). Dry weight (DW) was determined to the nearest microgramme (μg) after 24 h of freeze-drying. After weighing, larvae were rehydrated in Tris EDTA buffer, and both sagittal otoliths were removed and mounted on glass slides, according to Folkvord *et al.* (2004). The larvae were subsequently sonicated in the buffer and analysed for RNA and DNA content by fluorometric techniques, using ethidium bromide as described by Belchier *et al.* (2004). All biochemical analyses of larvae reported in this study were completed within 1 year.

Average daily growth rates in length (mm d^{-1}) were calculated as $(SL_t - SL_{t'})/(t - t')$, where t and t' represent dph at the current and preceding sampling dates, respectively. Daily specific growth rate (%) was estimated as $100 \cdot (e^g - 1)$, where the instantaneous growth rate g is estimated from $g = (\ln DW_t - \ln DW_{t'})/(t - t')$.

Otolith radii were measured from the core to the outer edge along the longest radius, using a light microscope as described in Folkvord *et al.* (2004). Alizarin marking was identified as a clear fluorescent mark in the core of one or both of the extracted otoliths when viewed under a fluorescence microscope. In total, 1312 larvae were identified to the respective group. The remaining larvae were either lost or damaged during processing. Apart from 28 larvae from the high-prey-concentration treatments, which were removed at 35 dph and excluded because of delay in biochemical analysis, the remaining larvae were evenly distributed across ages and groups.

Size-at-age was analysed with R statistical software using a mixed-effects ANOVA model, with age, prey concentration, light regime, and parental group as fixed effects, and tank as random effect nested within prey concentration and light regime. The selection of variables for inclusion in the mixed-effects model was determined by a stepwise removal of higher-order non-significant factors. Variance was stabilized using the varPower option as a function of age (Pinheiro and Bates, 2000). Significance was determined at the 0.05 level in all analyses.

Results

Survival

Estimated daily mortality (Table 2) was considerably greater in the low-prey-concentration treatments (>1.3%) than in the high-prey-concentration treatments (<0.8%). The proportion of hybrids was generally close to 50% during the first 3 weeks (overall 48.8%, $p > 0.05$ in seven out of eight tank-specific tests). The proportion increased thereafter in the low-prey-concentration tanks and averaged 67.0% during the last 3 weeks (binomial test: $p < 0.001$). No trend was apparent in the high-prey-concentration groups, with an overall proportion of hybrids of 49.9%. The proportion of hybrids was somewhat larger in the spring-light-regime tanks than in the autumn-light-regime tanks, but the difference just failed to be significant (54.3 vs. 45.8%; binomial test: $p = 0.052$).

Table 2. Total number of larvae sampled (n), larvae left after the experiment was terminated (N), estimated survival to day 56 (Surv), daily mortality rate (M), and proportion of hybrids (S × A) in sampled larvae (First, first 3 weeks of the experiment, last, last 3 weeks; total) by experimental tank (cf. Table 1).

					S×A (%)		
Tank	n	N	Surv (%)	M (%)	First	Last	Total
AH1	228[a]	11	69.0	0.66	46.3	44.2	47.5
AH2	228[a]	2	64.1	0.79	53.7	40.0	44.2
AL1	160	17	44.1	1.45	59.1	63.2	61.3
AL2	140	4[b]	26.5	2.34	32.8	87.2	61.1
SH1	228[a]	34	81.3	0.37	50.0	62.0	55.1
SH2	228[a]	37	82.6	0.34	59.2	54.7	53.5
SL1	160	25	48.1	1.30	46.8	65.4	60.6
SL2	160	13	41.7	1.55	45.1	57.7	56.8

[a]Including an additional 68 larvae taken out for other purposes.
[b]Terminated on day 49.

Growth and development

A significant difference in length and DW (Figure 1) between the prey-concentration treatments was observed from 7 dph onwards, and this difference was enhanced during the first half of the experiment and maintained subsequently, with differences in prey concentration representing the primary environmental variable affecting growth (nested ANOVAs: $p < 0.001$). Genetic effects were also significant from first sampling, at 7 dph, onwards and were maintained during the experiment ($p < 0.01$). The effect of light regime was generally not significant at any given age ($p > 0.07$), but from 49 dph onwards, larvae reared under the spring light regime tended to be longer than those reared under the autumn light regime (see below). On most sampling dates, a

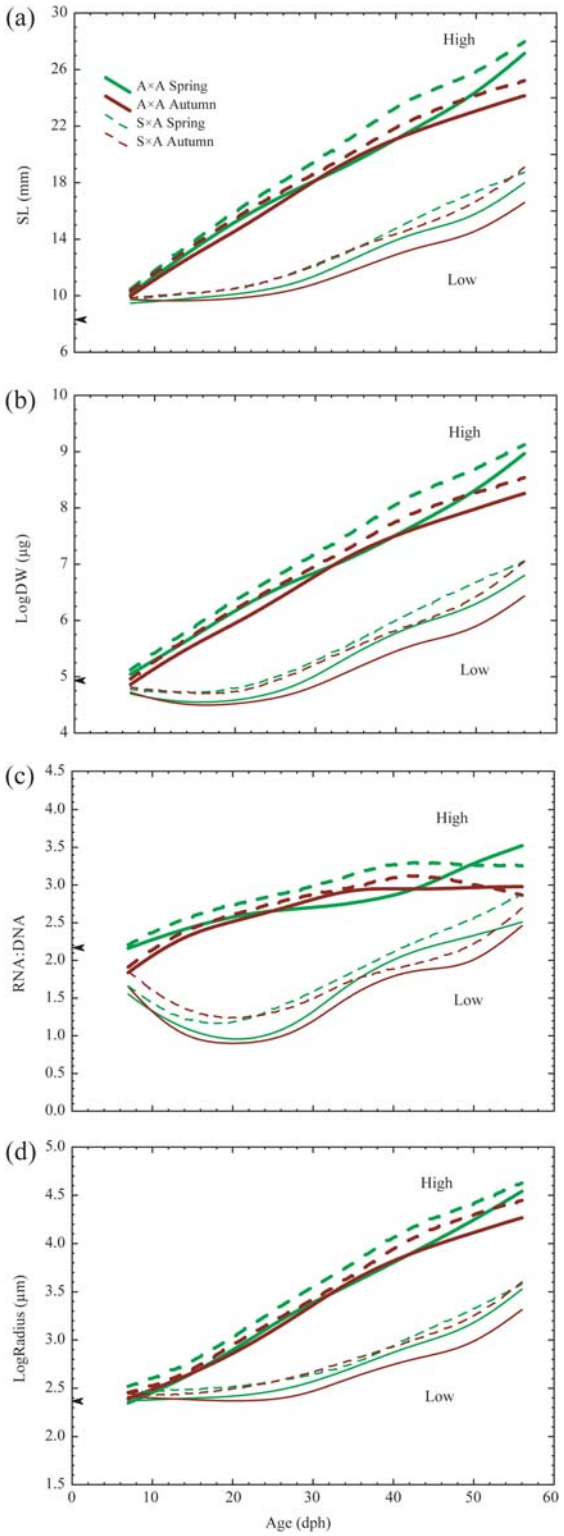

Figure 1. Trends (distance-weighted least-squares fits) in morphometric characteristics of pure autumn strain (A × A) and hybrid (S × A) larvae feeding under a spring or an autumn light regime at low (L: thin lines) or high (H: thick lines) prey concentrations: (a) SL; (b) dry weight (logDW); (c) RNA:DNA ratio; and (d) otolith radius (logRadius). Arrows on *y*-scale indicate mean value on day of hatching.

small but significant ($p < 0.05$ and $F < 7.6$) tank effect was observed, but always the tank effect was minor relative to the other significant effects.

Growth in length of larvae in the high-prey-concentration treatments was initially almost linear, averaging 0.35 mm d^{-1} during the first half of the experiment and declining somewhat thereafter, while the average daily growth in DW was 9.3% d^{-1} and, in terms of DNA and RNA, 7.9 and 9.9% d^{-1}, respectively (Figure 1). The low-prey-concentration treatments resulted in minimal growth in length from 7 to 14 dph, and the larvae grew only marginally more than the unfed controls (Figure 2; 0.05 vs. 0.02 mm d^{-1}). During the second half of the experiment, when larval concentrations had become reduced as a result of sampling and mortality, the growth rate increased and became similar to those observed initially in the high-prey-concentration treatments (Figure 1).

Between 7 and 14 dph, the DW of larvae in the unfed and low-prey-concentration treatments declined by an average of 4.6 and 2.0% d^{-1}, respectively. In the unfed controls (Figure 2), the rate of decline did not differ between the hybrids and the pure autumn strain, but larvae reared under the autumn light regime were 4.5% heavier than those under the spring light regime (ANCOVA: $p < 0.001$). The contribution of DNA was slightly lower (1.3%) among hybrids than among the pure autumn strain (ANOVA: $p < 0.05$), and the otolith size of the hybrids after 7 dph was 5% smaller (ANOVA: $p < 0.01$).

The mixed-effects model, including the data of fed larvae from all sampling dates, confirmed the main findings of the size-at-age analysis. In addition to significant prey-concentration and parental-origin effects, second-order age effects and interactions among these variables describing the curvature of the increase in length in the different treatments were also significant (Table 3). The age × prey-concentration interaction reflects the increase in growth in the low-prey-concentration treatments during the second half of the experiment ($p < 0.001$), while the age × light-regime interaction reflects the relatively faster growth of larvae under the spring light regime, compared with those under the autumn light regime, during the second half of the experiment ($p = 0.001$). Mixed-effects models (not shown) for the growth patterns of the different groups in terms of log(DW), log(otolith radius), log(DNA), and log(RNA) contained the same terms as for log(SL) and gave broadly similar results. For log(otolith radius) and RNA:DNA, the age × parental-origin interaction was not significant, indicating less divergence with age between hybrids and the pure autumn strain when using these variables.

The RNA:DNA ratio in individual larvae confirmed its potential as an indicator of the large difference in growth rate observed between larvae from the two prey-concentration treatments (Figure 1c). The ratio generally increased with size, and high-prey-concentration treatments resulted in significantly higher ratios than the low-prey-concentration treatments at any given age from 7 dph onwards (nested ANOVAs: $p < 0.05$). Notwithstanding individual variations, hybrids were characterized on average by higher ratios than the pure autumn strain from 14 through to 49 dph (except 35 dph; nested ANOVAs: $p < 0.05$). The larvae reared under the spring light regime generally had higher RNA:DNA ratios than those reared under the autumn light regime (Figure 1), but the difference was not significant except at 49 dph (nested ANOVA: $p < 0.05$).

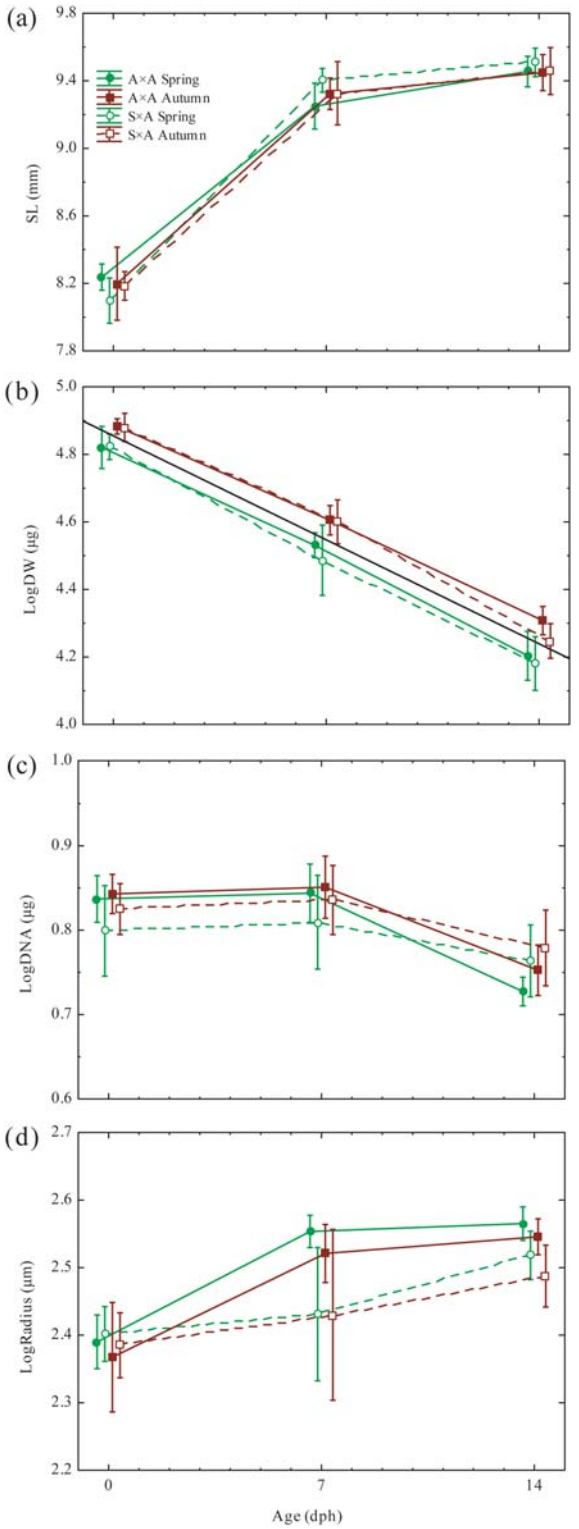

Figure 2. Morphometric characteristics of pure autumn strain (A × A) and hybrid (S × A) larvae in unfed controls kept under spring and autumn light regimes until 14 dph: (a) SL; (b) dry weight (logDW); (c) total DNA content (logDNA); and (d) otolith radius (logRadius). Whiskers indicate 95% confidence intervals. Points shifted along *x*-axis for graphical purposes.

Table 3. Results of the mixed-effects model treating log(SL) as a dependent variable, tank (replicate) as a random variable, and age and age^2 as continuous variables, and others as fixed variables.

Variable	numDF	denDF	*F*-value	*p*-value
Intercept	1	1 241	88 222.2	<0.0001
Age	1	1 241	11 274.2	<0.0001
Prey concentration	1	6	223.1	<0.0001
Parental origin	2	1 241	32.5	<0.0001
Age^2	1	1 241	15.2	0.0001
Age × prey concentration	1	1 241	979.6	<0.0001
Age × light regime	1	1 241	17.1	<0.0001
Age × parental origin	2	1 241	9.7	0.0001
Prey concentration × age^2	1	1 241	520.8	<0.0001

The s.d. of the random variable (tank) was 0.023 and the residual s.d. was 0.016; numDF and denDF represent the numerator and denominator degrees of freedom in the test of respective factor.

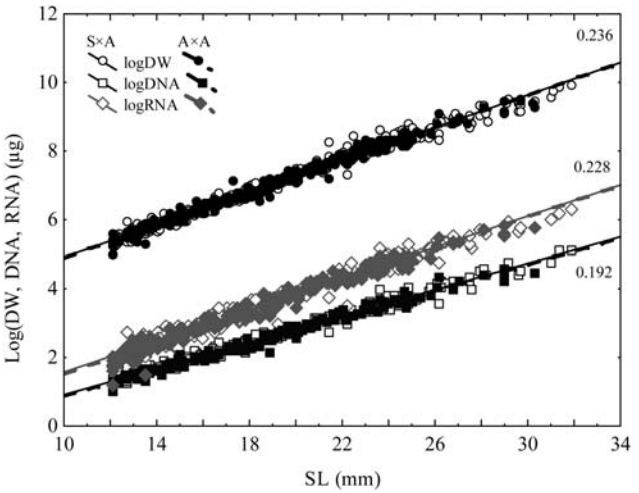

Figure 3. Relationship between logDW (circles), logDNA (squares), and logRNA (diamonds) vs. SL for hybrids (open symbols and dashed lines) and pure autumn strains (filled symbols and solid lines). The estimated common slopes are indicated.

Among fed larvae >12 mm, the SL vs. log(DW) relationship was linear and indistinguishable between the hybrids and pure autumn strain (Figure 3; ANCOVA: $p > 0.4$). The same applies to the SL vs. DNA and SL vs. RNA relationships, but hybrids contained 3.9% more DNA and 4.8% more RNA at a given length than the pure autumn strain (ANCOVAs: $p < 0.05$). All slopes were highly significant (ANCOVA: $p < 0.001$). The slope for log(DW) was higher than that for DNA (0.24 vs. 0.19), indicating a general increase in DW per unit DNA with increasing SL for both groups.

Towards the end of the experiment, the hybrids were slightly more advanced in terms of development-at-age than the pure autumn strain (Figure 4), but no differences were observed in length at a given development stage between larvae of different parental origin (nested ANOVAs: $p > 0.05$). However, slower-growing larvae from low-prey-concentration treatments were smaller throughout stage 2 (Figure 4, S2) than those from high-prey-concentration treatments (nested ANOVAs: $p < 0.05$).

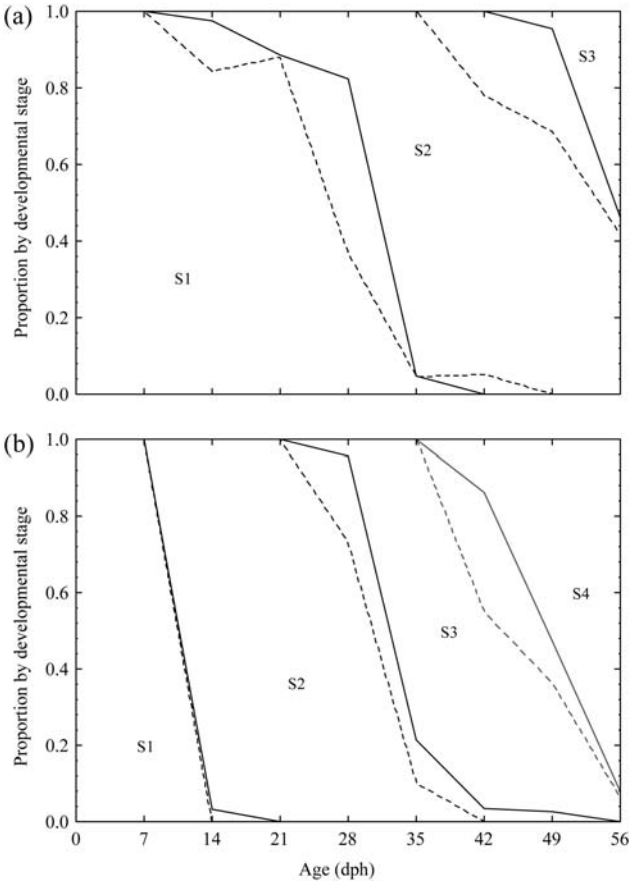

Figure 4. Proportion of larvae at different stages of development (S1–S4 refer to stages 1–4 according to Doyle, 1977) at age for the pure autumn strain (solid lines) and hybrids (dashed lines) for: (a) low-prey-concentration treatments; and (b) high-prey-concentration treatments.

Discussion

Genetic effects

The hybrids generally exhibited as good or better growth and survival than the pure autumn strain, and the experiments confirmed first-generation hybrid viability for the first 60 d of the larval stage. The results indicate that paternal origin in respect of spawning season sets no restrictions on larval survival. Therefore, offspring produced by twinning and switching of adults between spawning periods (McQuinn, 1997; Brophy *et al.*, 2006) are likely to contribute to future recruitment of the populations involved. If twinning is a common feature, a metapopulation approach to managing herring populations that spawn in different seasons may be warranted (Brophy *et al.*, 2006).

This conclusion is in line with the view that the North Sea herring populations must be considered to represent one genetically homogenous unit, with the Norwegian spring-spawning herring population being a somewhat more divergent unit (Mariani *et al.*, 2005). The lack of inherent barriers to cross fertilization between adults of different seasonal origin may also be reflected in the rather low genetic diversity between regionally and seasonally distinct spawning populations. However, there are exceptions to this pattern (Jørstad *et al.* 1991), and further studies are warranted to understand how genetic diversity is maintained in these cases.

Contrary to our expectation, the pure autumn strain did not perform better than the hybrids under the autumn light regime. This could be due to a first-generation "hybrid-vigour" effect, caused by the production of offspring with a relatively larger proportion of heterozygote genotypes (Falconer and Mackay, 1996). This effect will probably diminish in a field situation as a result of dilution in future generations, but should help to reduce the genetic discreteness of the original populations. However, the larvae with a spring-spawning paternal background tended to perform better under a spring light regime at high prey concentrations, and this may be indicative of a genetic effect being superimposed on the "hybrid-vigour" effect. Such a genetic effect is to be expected, according to the studies by Gamble *et al.* (1985) and Johannessen *et al.* (2000), in which spring-spawned larvae eventually outperformed autumn-spawned larvae under similar experimental conditions. However, the results of these previous experiments have been confounded by non-controlled environmental and maternal genetic and non-genetic effects, such as egg-size differences between the two groups (Gamble *et al.*, 1985). The use of eggs from only one female in our experiments minimized any confounding maternal egg-size effects (Chambers and Leggett, 1996), and the genetic differences between the groups were of paternal origin only. Indications of a paternal genetic effect during early development could be seen in DNA and otolith size-at-age differences in the unfed controls, where the hybrids developed at a slower rate than the pure autumn strain. The adaptive significance of this difference in developmental rate remains unclear.

The results of our experiments did not indicate delayed development in larvae from autumn-spawning herring compared with spring-spawning herring, as was suggested by Gamble *et al.* (1985). Indeed, the length–weight relationship revealed hardly any differences between the two groups, while the hybrids contained somewhat greater amounts of nucleic acids at a given length. This is in line with the observation that the hybrids were slightly more advanced developmentally at a given age than the pure autumn strain. However, most of the variability in development-at-age was directly related to the variability in larval size, indirectly caused by differences in prey level.

The proportion of hybrids increased in all low-prey-concentration tanks from the beginning until towards the end of the experiment. The finding that the survival of the pure autumn strain was significantly lower at low prey concentrations is somewhat surprising because autumn- and winter-spawned larvae should be adapted to survive long periods of relatively low light and food availability (Johannessen *et al.*, 2000). Although this might also be explained by a first-generation "hybrid-vigour" effect, one would expect the selection value of the ability of autumn-spawned larvae to cope with temporary food shortage to be high. Alternatively, the lower survival may be caused by a slightly higher size-selective mortality in the pure autumn strain. However, the RNA:DNA ratio, which to some extent reflects instantaneous growth capacity (Clemmesen, 1996), was systematically higher among hybrids from first sampling onwards and throughout the experimental period, even before starvation mortality started to occur in the unfed groups. Therefore, it seems unlikely that selective starvation mortality can explain the apparent better performance of hybrids under these conditions. However, some caution should be exercised regarding the conclusions about the effects of genetic differences

from this study, because the genetic diversity from the respective parental groups was limited. Further studies involving a wider selection of parental fish are required to validate these conclusions.

Environmental effects

The most important factor influencing growth and survival in the experiment was prey concentration. This is not surprising because the effect of prey availability has been shown in several other studies (Kiørboe and Munk, 1986; Folkvord *et al.*, 2000). Herring are capable of maintaining a relatively high larval survival at low prey concentrations and low growth rates (Kiørboe and Munk, 1986), a feature that may contribute to their success as measured by absolute abundance (Blaxter, 1985). The lowest nominal prey concentration (40 prey l^{-1}) was clearly insufficient to support good growth and survival, at least during the first part of the experiment, when larval concentration was still high. With an initial larval concentration of $<2\ l^{-1}$, an average of 20 prey was available per larva daily. This prey availability may not seem low compared with field studies (Kiørboe and Munk, 1986), but comparable prey concentrations, used in the laboratory and observed in the field, have generally not resulted in comparable growth rates (MacKenzie *et al.*, 1990). During the second half of the experiment, the same nominal prey concentrations were sufficient to promote growth rates that were comparable with those observed when the larvae were exposed to high prey concentrations. During the course of the experiment, the concentration of the remaining plankton in the low-prey-concentration tanks just before the daily re-establishment of nominal prey concentration increased from ca. 20 to 30 prey l^{-1}, presumably because of the reduction in grazing pressure as larval concentrations decreased. In addition, the improved search behaviour of more advanced larvae suggests that their growth may be less susceptible to the limits of food availability in late autumn or winter situations.

The light regimes had little direct effect on larval growth, although, over time, the spring light regime seemed to promote faster growth than the autumn light regime. A similar photoperiod effect, documented in Johannessen *et al.* (2000), was also related to an age vs. light-regime interaction, reflecting that larvae in the spring light regime eventually grew faster than those in the autumn light regime. Several factors may have contributed to the seemingly lesser effect of the light regime: (i) the number of hours available for feeding may not have been sufficiently different (Urtizberea *et al.* 2008), and the effect might have been greater if the experiment had lasted longer; and (ii) the prey concentrations may have been sufficiently high to ensure the ingestion of a daily ration during daylight. In a field situation with shorter daylengths during winter, low prey availability will not permit sufficiently high ingestion rates (Munk *et al.*, 1991, Fiksen and Folkvord, 1999). Also, seasonal light patterns in nature are associated with seasonal temperature patterns (Munk *et al.*, 1991), a factor that we held constant. Larval growth is markedly temperature-dependent (Folkvord *et al.*, 2004), and the combination of high temperature and low prey availability appears to be detrimental (Fiksen and Folkvord, 1999). Further experiments that incorporate seasonal temperature pattern in addition to seasonal light regime may clarify any interactive effects of these two factors.

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