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The influence of different parental combinations and incubation temperature on the RNA and DNA content of herring larvae at hatching: a pilot study

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In four half-sib pairings, herring mothers affected the standard length, dry weight, and RNA and DNA content of their progeny, while fathers affected larval RNA and DNA content. The amounts of RNA and DNA in offspring from one male were also influenced by temperature in that the highest RNA contents were found at the lowest temperature and highest DNA contents at the highest temperature. The results indicate that both environmental and genetic factors influence nucleic acid contents of young herring larvae, and this may limit the suitability of RNA : DNA ratio as a condition measure of newly hatched herring larvae.

Key words: *Clupea harengus*; larvae; nucleic acids; temperature; parental effects; development.

INTRODUCTION

The traits of larval fish at hatching show large variation between species, between populations of the same species and between individuals of the same population (Chambers, 1997). The origin of variation in traits is due to genetic variation from the parents, variance due to the environment, variance due to interaction between the genotype and the environment (phenotypic plasticity) and the covariance between the environment and genotype (Chambers & Leggett, 1993). One special and indirect type of environmental influence is the maternal effects that Reznick (1991) defined as non-genetic contributions of a female to its offspring. These non-genetic effects result from environmental influences on the female leading to differences in female status such as size and condition, and act early in life on its offspring (Chambers & Leggett, 1993; Chambers, 1997; Trippel *et al.*, 1997). Paternal effects on marine larval traits are not examined as often as maternal effects, but have been found in herring larvae *Clupea harengus* L. (Panagiotaki & Geffen, 1992; Evans & Geffen, 1998).

Measurements of larval deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are used to define larval condition and growth rate by the RNA : DNA ratio (Bergeron, 1997). The RNA : DNA ratio just before the larvae start feeding is suggested to represent a minimum threshold ratio which is necessary for normal protein synthesis (Westerman & Holt, 1994). Several studies have documented the influence of temperature on growth and nucleic acid content in larval herring (Blaxter, 1992; Clemmesen, 1996), but the relation between incubation temperature and larval nucleic acid contents have not been examined

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as in other species (Buckley *et al.*, 1990; Canino, 1994). Also, the magnitude of environmentally induced variation in larval nucleic acid contents compared with variation caused by different parents is not known. Therefore this study was designed to examine the effect of temperature on herring larval size and nucleic acid content at hatching, and to compare temperature-induced variation with variation caused by offspring of different parental combinations. Two males and two females were crossed in a factorial design and their eggs incubated under three different temperatures. Maternal genotype and maternal environmental effects cannot be separated in this study (Reznick, 1991), so the maternal effect is considered as an unknown combination of these effects. Also, this work should be considered as a pilot study since the small number of parental fish precludes analysis of any functional relationship between condition of parental fish and the traits of their offspring.

MATERIALS AND METHODS

Brood-fish of the Norwegian spring spawning herring stock were captured west of Karmøy, Norway (59°13′46″ N, 5°8′ E) in late March 1995. Running ripe males and females were kept live and transported to Bergen High Technology Centre, University of Bergen, where the experiment was carried out.

Eggs from one large and one medium-sized female were stripped onto plastic sheets and fertilized with sperm from one large and one medium-sized male, resulting in four different parental combinations. Each of these combinations were incubated at three different temperature regimes (± S.D.): 4·0 ± 0·2, 8·1 ± 0·2 and 12·0 ± 0·6° C. Light was regulated automatically to a normal light regime for Bergen, latitude 60° N, using the computer program Lysstyr 2.00 (Hansen, 1990). The parental fish had the following weights and lengths: male 1, 438 g and 35·5 cm; male 2, 342 g and 35·0 cm; female 1, 460 g and 38·0 cm; and female 2, 352 g and 35·5 cm. They were all aged 12 years. Further information on the experimental set-up is presented in Høie *et al.* (1999). All larvae within each parental combination were sampled on the first day of the hatching period for each temperature group. Standard length of the larvae (15 from each parental combination at each temperature) were recorded to the nearest 0·04 mm on the day of hatching. Then the larvae were put immediately into Eppendorf vials and frozen in liquid nitrogen before transferring them to a − 80° C freezer for storage. Before RNA and DNA analysis (summer 1997) the larvae were freeze-dried at − 67° C for *c.* 18 h and thereafter weighed on a Sartorious Micro M3P microbalance to the nearest µg. The RNA and DNA measurements were carried out by the fluorometric method described in Suneetha *et al.* (1999), with the exceptions of centrifuging the homogenates at 6000 rpm for 8 min and using half the volume of the reagents (50 µl homogenate, 5 µl RNAse and 0·7 ml Ethidium bromide).

The data were examined by analysis of variance (ANOVA) where different incubation temperatures, mothers and fathers were fixed effects. Homogeneity of variance was examined by Levene's F test, and Newman–Keuls *post hoc* tests were used following the ANOVA. The intensity of association between the measured variables was estimated by partial correlation. All statistical analyses and data presentations were carried out using Statistica® for Windows (StatSoft Inc., 1995).

RESULTS

All larvae from the four different parental combinations reared at 12 and 8° C hatched on the same day, 10 and 15 days after fertilization, respectively. At 4° C, all larvae hatched 29 days after fertilization except for larvae from parental combination male 2 and female 2, that hatched 2 days later.

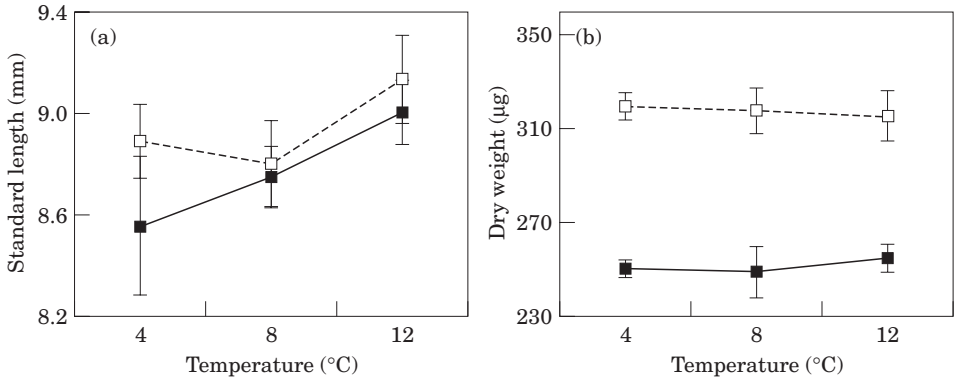

FIG. 1. (a) Mean larval standard length, and (b) mean larval dry weight at different incubation temperatures for females 1 (– –□– –) and 2 (—■—). Offspring from male 1 and 2 are pooled. Vertical bars indicate ± 2 S.E.M.

Maternal ($P<0{\cdot}001$) and temperature effects ($P<0{\cdot}05$), but no paternal effects ($P>0{\cdot}05$) were found on standard length [three-way ANOVA; Fig. 1(a)]. The larvae from the 12° C group were longer than those from the other temperature groups but with no significant differences between larvae incubated at 4 and 8° C. Offspring from female 1 were generally longer than offspring from female 2. A maternal effect was also evident on larval dry weight ($P<0{\cdot}001$) where offspring from female 1 had 27·7% to 23·6% higher dry weights than offspring from female 2 in the three temperature groups [Fig. 1(b)]. No temperature ($P>0{\cdot}8$) or paternal ($P>0{\cdot}2$) effects were found (three-way ANOVA). The temperature effect on larval DNA and RNA content was different for offspring from the two males (male–temperature interaction effects; Table I). Two-way ANOVAs with temperature and female as effects were therefore performed on offspring from each male separately.

Offspring from male 1 had higher amounts of DNA at 12° C compared with 4 and 8° C (Fig. 2, $P<0{\cdot}001$), and more RNA at 4° C than at 8 and 12° C (Fig. 3, $P<0{\cdot}001$). No temperature effects were evident on either RNA or DNA content of larvae from male 2 ($P>0{\cdot}1$). Larvae from female 1 contained generally more RNA and DNA than larvae from female 2 in combination with both males ($P<0{\cdot}001$). The largest differences in RNA and DNA content caused by different females were 20·6% (at 4° C) and 11·6% (at 8° C) respectively. Offspring from male 1 had higher RNA content at 4° C than at 8 and 12° C and higher DNA content at 12° C than at 8 and 4° C (Fig. 4), while offspring from male 2 had lower RNA content at 4° C than at 8 and 12° C and slightly higher DNA content at 4° C than at 8 and 12° C. The largest differences in RNA and DNA contents caused by different males were 15·2% (at 4° C) and 35·5% (at 12° C) respectively.

The RNA : DNA ratios varied between 2·75 and 3·8 (Fig. 5), and there were significant interactions between all effects (three-way ANOVA interaction effects; Table I).

Partial correlations were performed on larval standard length, dry weight, RNA and DNA contents for offspring from each male separately, but temperature and female groups were pooled (Table II). Offspring from male 1 showed

TABLE I. Three-way ANOVA with incubation temperature, female and male as fixed effects on DNA and RNA content, and RNA : DNA ratio

	d.f.	MS effect	d.f. error	MS error	*F*	*P*
Larval DNA content						
T	2	1·003	163	0·072	13·88	<0·001
F	1	1·962	163	0·072	27·13	<0·001
M	1	6·417	163	0·072	88·75	<0·001
TF	2	0·041	163	0·072	0·56	0·570
TM	2	1·585	163	0·072	21·92	<0·001
FM	1	0·155	163	0·072	2·15	0·145
TFM	2	0·043	163	0·072	0·60	0·551
Larval RNA content						
T	2	0·592	162	0·608	0·97	0·380
F	1	35·797	162	0·608	58·85	<0·001
M	1	1·350	162	0·608	2·22	0·138
TF	2	1·149	162	0·608	1·89	0·154
TM	2	4·626	162	0·608	7·60	<0·001
FM	1	0·627	162	0·608	1·03	0·312
TFM	2	0·276	162	0·608	0·45	0·636
Larval RNA : DNA ratio						
T	2	1·092	161	0·095	11·49	<0·001
F	1	0·260	161	0·095	2·74	0·100
M	1	9·022	161	0·095	94·94	<0·001
TF	2	0·437	161	0·095	4·60	0·011
TM	2	4·181	161	0·095	43·99	<0·001
FM	1	0·501	161	0·095	5·27	0·023
TFM	2	0·300	161	0·095	3·16	0·045

T, Temperature; F, female; M, male; d.f., degrees of freedom; MS, mean square.

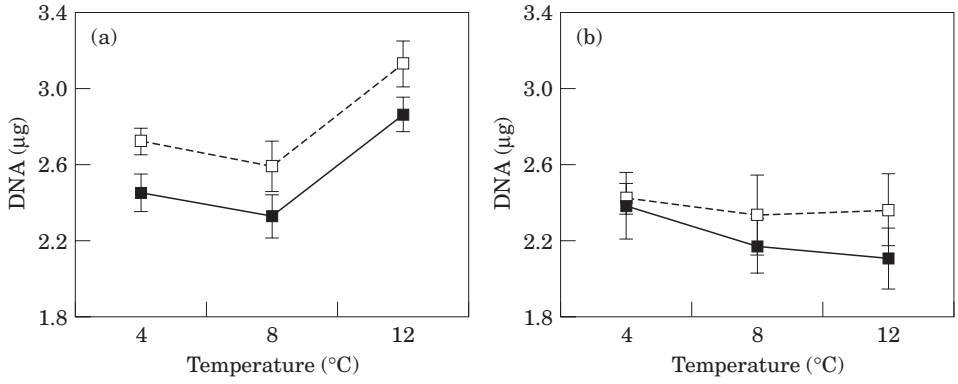

FIG. 2. Mean DNA content at different incubation temperatures of larvae from female combinations with (a) male 1 and (b) male 2. – –□– –, Female 1; —■—, female 2. Vertical bars indicate ± 2 S.E.M.

negative correlation between standard length and dry weight, but positive correlation between RNA and DNA contents. Both standard length and dry weight were correlated positively with RNA content, but not with DNA content. The pattern was somewhat different for offspring from male 2. No significant

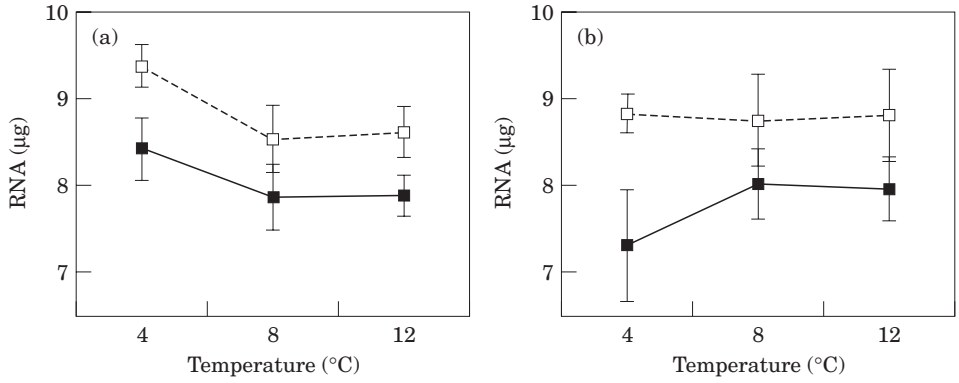

FIG. 3. Mean RNA content at different incubation temperatures of larvae from female combinations with (a) male 1 and (b) male 2. – –□– –, Female 1; —■—, female 2. Vertical bars indicate ± 2 S.E.M.

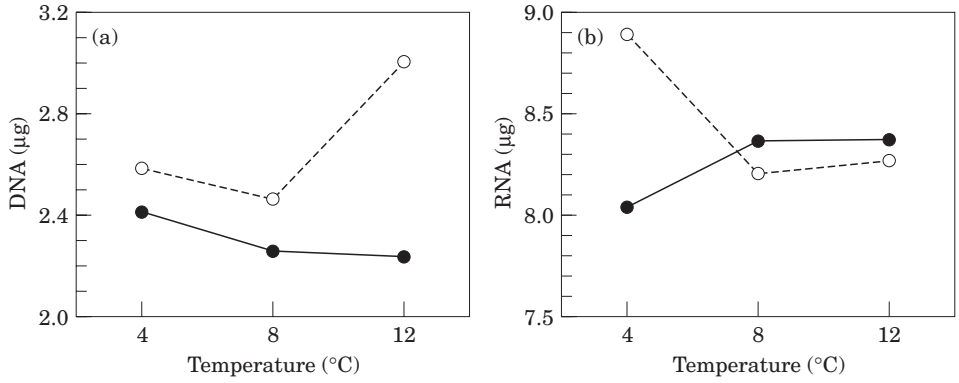

FIG. 4. Plot of interaction between the influence of incubation temperature and male parent on (a) mean larval DNA content and (b) mean larval RNA content. – –○– –, Male 1; —●—, male 2. Values from females 1 and 2 are pooled.

correlations were found between standard length and dry weight, nor between RNA and DNA contents. However, both standard length and dry weight were correlated positively with both RNA and DNA contents.

DISCUSSION

Surprisingly, larval standard length and dry weight were unaffected by incubation temperature except for larger lengths at 12° C. The larval material used in this study was of the same origin as used in Høie *et al.* (1999), where larval standard length was related inversely to temperature and larval dry weight was higher in the 8 and 12° C groups than in the 4° C group. However, larvae used in Høie *et al.* (1999) hatched 1–5 days later than in this study, possibly since the eggs were incubated inside wells in Nunc plates and had therefore slightly different environmental history. When the two data sets were combined, incubation time was correlated positively with standard length at hatching at 4 and

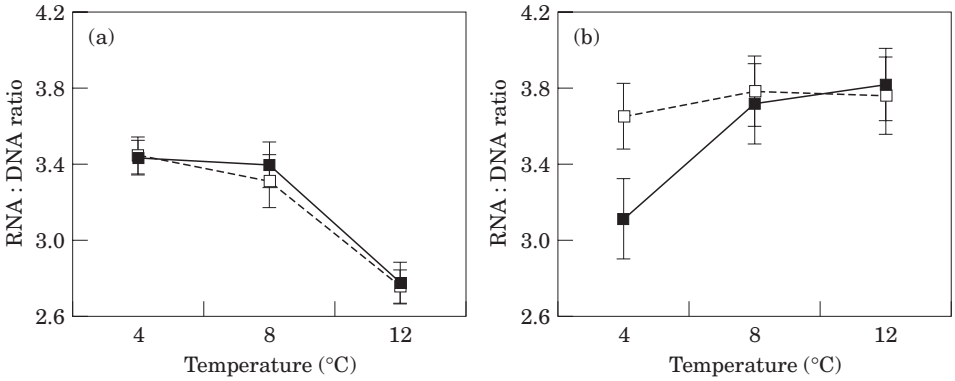

FIG. 5. Mean RNA : DNA ratios at different incubation temperatures of larvae from female combinations with (a) male 1 and (b) male 2. – –□– –, Female 1; —■—, female 2. Vertical bars indicate ± 2 S.E.M.

TABLE II. Partial correlations between larval dry weight, standard length, RNA and DNA content on offspring from males 1 and 2

	Dry weight	DNA	RNA
Male 1			
Standard length	− 0·43**	0·11	0·69***
Dry weight		0·08	0·58***
DNA			0·25**
Male 2			
Standard length	− 0·13	0·54***	0·22*
Dry weight		0·29**	0·43***
DNA			0·10

The temperature and maternal groups are pooled. *P<0·05, **P<0·01, ***P<0·001.

8° C but correlated negatively at 12° C (unpublished data). Therefore sampling the larvae later in the hatching period would result in smaller larvae at 12° C and longer larvae at 8 and 4° C than found in this study.

An inverse relationship between incubation temperature and larval length and RNA content at hatching has been found in winter flounder *Pseudopleuronectes americanus* and walleye pollock *Theragra chalcogramma* (Pallas) (Buckley *et al.*, 1990; Canino, 1994), but in both studies there was no effect of incubation temperature on larval DNA content. In this study partial correlation revealed a negative relationship between dry weight and standard length for offspring from one of the males, which probably reflected conversion of yolk to body mass as the larvae developed. However, there was no significant correlation between larval size (dry weight and standard length) and DNA content in the same larvae. Offspring from the other male showed a positive correlation between larval size and DNA content, but no significant relation between standard length and dry weight. Therefore it seems that DNA content does not always reflect larval size

at hatching, so other factors must be involved. One possible explanation of varying DNA content at a given larval size might be differences in cell size, through the relative contribution of growth caused by increased size of muscle fibres (hypertrophy) *v.* growth caused by new fibre production (hyperplasia) (Goss, 1966; Johnston *et al.*, 1998). Higher rearing temperatures for herring embryos resulted in more numerous, but smaller muscle fibres (Vieira & Johnston, 1992; Johnston, 1993). Atlantic salmon *Salmo salar* L. larvae showed the opposite pattern with fewer, but larger, cell sizes, and fewer nuclei at higher incubation temperatures (Stickland *et al.*, 1988; Usher *et al.*, 1994). Also, there are probably critical stages during embryogenesis when cell division is particularly temperature dependent. Atlantic salmon embryos incubated at different temperatures contained the same number and size of muscle fibres at pre-hatching stages while larvae incubated at high temperatures had fewer, but larger, muscle fibres at hatching than those incubated at lower temperatures (Stickland *et al.*, 1988; Usher *et al.*, 1994). This can also explain the paternal-dependent temperature effect of DNA content at hatching in this study. Genetic differences regulating developmental rate between offspring from the two males could produce larvae of similar standard length and dry weight but with different amounts of DNA through different levels of hypertrophy. Interaction between the genotype and temperature has been shown to influence larval growth in Atlantic salmon (Johnston & McLay, 1997), and Valente *et al.* (1998) on finding differences in DNA concentration between two rainbow trout *Oncorhynchus mykiss* (Walbaum) strains suggested these to be of genetic origin. Therefore the paternal effects resemble genotype effects, while the maternal differences, found in this study, appear to be caused by different environmental conditions experienced by the females (Trippel *et al.*, 1997).

The RNA : DNA ratio of herring larvae decreases from hatching to onset of exogenous feeding due to a reduction of RNA and an increase of DNA content (Clemmesen, 1994). This has also been observed for other species and is referred to as nucleic acid metabolism (Westerman & Holt, 1994). This rapid change in nucleic acid content occurs at a very important stage for fish larvae just prior to the onset of exogenous feeding. Fish larvae seem to give higher priority to increased cell numbers at the expense of protein synthesis, at a stage when feeding is vital for survival, although the underlying dynamics of the phenomenon still remain unclear. The mortality rate in larval fish is highest at the early stages (Bradford, 1992). Therefore it is of special interest to quantify individual larval condition in very young cohorts. However, the use of RNA : DNA ratios as a measure of condition for young larvae can be less informative in the absence of more knowledge about the parentally imposed variation in nucleic acid content among larvae. Also it is important to know the persistence of such parental effects to be able to separate them from direct environmental effects on early developmental stages. Larvae were sampled and analysed for nucleic acid contents only at hatching in this study, but starved larvae from the same groups were also sampled at yolk depletion for other purposes (Høie *et al.*, 1999). The starved larvae still exhibited differences in standard length and dry weight among offspring from different females. Therefore variations in nucleic acid content of herring larvae at the time of first feeding due to maternal effects may be expected also. However, Nathanailides *et al.* (1994) found differences in densities of nuclei

in the muscles of Atlantic salmon larvae at hatching when reared at different temperatures, but not at the first-feeding stage.

Young herring larvae fed *ad libitum* are capable of increasing their DNA content by more than 10% day^{-1} (Folkvord *et al.*, 1996). The largest observed differences in mean larval DNA content in this study caused by different mothers and fathers were 11·6 and 35·5%, respectively, which corresponded to 1·2 and 3·2 days of growth given an estimated daily DNA growth rate of 10%. Therefore smaller larvae can outgrow larger larvae in a few days if given favourable conditions and thereby smooth out any parental effects.

Considering the findings of this study, we recommend that care should be taken when using RNA : DNA ratio as a condition measure of newly hatched herring larvae. Further studies should be undertaken to examine for how long parental effects persist and significantly influence variability in nucleic acid contents among feeding larvae.

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References

Bergeron, J.-P. (1997). Nucleic acids in ichthyoplankton ecology: a review, with emphasis on recent advances for new perspectives. *Journal of Fish Biology* **51** (Suppl. A), 284–302.

Blaxter, J. H. S. (1992). The effect of temperature on larval fishes. *Netherlands Journal of Zoology* **42,** 336–357.

Bradford, M. J. (1992). Precision of recruitment predictions from early life stages of marine fishes. *Fishery Bulletin* **90,** 439–453.

Buckley, L. J., Smigielski, A. S., Halavik, T. A. & Laurence, G. C. (1990). Effects of water temperature on size and biochemical composition of winter flounder *Pseudopleuronectes americanus* at hatching and feeding initiation. *Fishery Bulletin* **88,** 419–428.

Canino, M. F. (1994). Effects of temperature and food availability on growth and RNA/DNA ratios of walleye pollock *Theragra chalcogramma* (Pallas) eggs and larvae. *Journal of Experimental Marine Biology and Ecology* **175,** 1–16.

Chambers, R. C. (1997). Environmental influences on egg and propagule sizes in marine fishes. In *Early Life History and Recruitment in Fish Populations* (Chambers, R. C. & Trippel, E. A., eds), pp. 63–102. London: Chapman & Hall.

Chambers, R. C. & Leggett, W. C. (1993). Phenotypic variability in fish populations and its representation in individual-based models. *Transactions of the American Fisheries Society* **122,** 404–414.

Clemmesen, C. (1994). The effect of food availability, age or the size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. *Marine Biology* **118,** 377–382.

Clemmesen, C. (1996). Importance and limits of RNA : DNA ratios as a measure of nutritional condition in fish larvae. In *Survival Strategies in Early Life Stages of Marine Resources* (Watanabe, Y., Yamashita, Y. & Oozeki, Y., eds), pp. 67–82. Rotterdam: A. A. Balkema.

Evans, J. P. & Geffen, A. J. (1998). Male characteristics, sperm traits, and reproductive success in winter-spawning Celtic Sea Atlantic herring, *Clupea harengus*. *Marine Biology* **132,** 179–186.

Folkvord, A., Ystanes, L., Johannessen, A. & Moksness, E. (1996). RNA : DNA ratios and growth of herring (*Clupea harengus* L.) larvae reared in mesocosms. *Marine Biology* **126,** 591–602.

Goss, R. J. (1966). Hypertrophy versus hyperplasia. *Science* **153,** 1615–1620.
Hansen, E. H. (1990). Bruk av kunstig lys og lysmanipulering for styrt produksjon av laksefisk (Use of artificial light and light manipulation in controlled production of salmonids). Ph.D. thesis, University of Trondheim (NTH), Norway.
Høie, H., Folkvord, A. & Johannessen, A. (1999). Maternal, paternal and temperature effects on otolith size of young herring (*Clupea harengus* L.) larvae. *Journal of Experimental Marine Biology and Ecology* **234,** 167–184.
Johnston, I. A. (1993). Temperature influences muscle differentiation and the relative timing of organogenesis in herring (*Clupea harengus*) larvae. *Marine Biology* **116,** 363–379.
Johnston, I. A. & McLay, H. A. (1997). Temperature and family effects on muscle cellularity at hatch and first feeding in Atlantic salmon (*Salmo salar* L.). *Canadian Journal of Fisheries and Aquatic Sciences* **75,** 64–74.
Johnston, I. A., Cole, N. J., Abercromby, M. & Vieira, V. L. A. (1998). Embryonic temperature modulates muscle growth characteristics in larval and juvenile herring. *Journal of Experimental Biology* **201,** 623–646.
Nathanailides, C., Lopez-Albors, O. & Stickland, N. C. (1994). Influence of prehatch temperature on the development of muscle cellularity in posthatch Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **52,** 675–680.
Panagiotaki, P. & Geffen, A. J. (1992). Parental effects on size variation in fish larvae. *Journal of Fish Biology* **41,** 37–42.
Reznick, D. N. (1991). Maternal effects in fish life histories. *The Unity of Evolutionary Biology: Proceedings of the Fourth ICSEB* (Dudley, E. C., ed.), pp. 780–793. Oregon, Portland: Dioscorides Press.
StatSoft Inc. (1995). *Statistica for Windows*. Tulsa, Oklahoma: Statsoft Inc.
Stickland, N. C., White, R. N., Mescall, P. E., Crook, A. R. & Thorpe, J. E. (1988). The effect of temperature on myogenesis in embryonic development of the Atlantic salmon (*Salmo salar* L.). *Anatomy and Embryology* **178,** 253–257.
Suneetha, K-B., Folkvord, A. & Johannessen, A. (1999). Responsiveness of selected condition measures of herring, *Clupea harengus*, larvae to starvation in relation to ontogeny and temperature. *Environmental Biology of Fishes* **54,** 191–204.
Trippel, E. A., Kjesbu, O. S. & Solemdal, P. (1991). Effects of adult age and size structure on reproductive output in marine fishes. In *Early Life History and Recruitment in Fish Populations* (Chambers, R. C. & Trippel, E. A., eds), pp. 31–62. London: Chapman & Hall.
Usher, M. L., Stickland, N. C. & Thorpe, J. E. (1994). Muscle development in Atlantic salmon (*Salmo salar*) embryos and the effect of temperature on muscle cellularity. *Journal of Fish Biology* **44,** 953–964.
Valente, L. M. P., Gomes, E. F. S. & Fauconneau, B. (1998). Biochemical growth characterization of fast- and slow-growing rainbow trout strains: effect of cell proliferation size. *Fish Physiology and Biochemistry* **18,** 213–224.
Vieira, V. L. A. & Johnston, I. A. (1992). Influence of temperature on muscle-fibre development in larvae of the herring *Clupea harengus*. *Marine Biology* **112,** 333–341.
Westerman, M. & Holt, G. J. (1994). RNA : DNA ratio during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. *Marine Biology* **121,** 1–9.