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Responsiveness of selected condition measures of herring, *Clupea harengus*, larvae to starvation in relation to ontogeny and temperature

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Synopsis

A laboratory experiment was performed to study the responsiveness of selected condition measures to starvation in herring, *Clupea harengus*, larvae in relation to temperature and ontogeny. The larvae at two intervals of development, i.e. 'stage 1' larvae with initial exogenous feeding and 'stage 2' larvae with established feeding prior to notochord flexion, were reared at three temperatures (5, 8 and 11°C) and subjected to sub-lethal durations of starvation. Temporal changes in standard length, dry weight, DNA concentration (% of dry weight), RNA concentration (% of dry weight), Fulton's condition factor (CF) and RNA/DNA were assessed and compared with fed controls. Starvation led to decreases in dry weight, CF, RNA concentration and RNA/DNA, while it led to an increase in DNA concentration. Higher responsiveness to starvation was observed at higher temperatures, and the magnitude of the changes was higher in stage 2 larvae. The shortest latency in starvation response was found with respect to RNA/DNA which was length independent in the size range studied. RNA/DNA was also significantly related to average DNA growth rate, and the model for DNA growth rate was, $SGR_{DNA} = 4.49\ RNA/DNA + 7.14\ T - 0.42\ T^2 - 37.5$; $n = 32$, $r^2 = 0.85$, $p < 0.001$). While the model seemed to adequately represent the average temperature dependent DNA growth, a relatively low classification success made it unsuitable for depicting individually starving larvae. Critical levels in DNA concentration can be used (2.2% for stage 1 larvae, 2.9% for stage 2 larvae) to differentiate starving larvae (after 3 – 5 days) from feeding larvae. RNA/DNA was the most sensitive and suitable condition index studied in detecting early starvation of herring larvae.

Introduction

Many biological and physical factors can interact in affecting the growth and survival of young fish during their first year (Fogarty et al. 1991). Starvation has long been considered an important mortality factor during this period of fish life (Hjort 1914). The vulnerability of fish larvae to predation may be increased by starvation either through prolonged duration within a life interval due to reduced growth, or directly through poor nutritional condition (Cushing 1972, Hunter 1984). Identifying the condition of larvae at an early time of starvation is very important in the attempt to understand the

recruitment variability (Clemmesen 1994). Many methods have been developed to characterize the condition and growth of fish larvae (Buckley 1979, Theilacker 1986, Clemmesen 1988, 1993, Hovenkamp & Witte 1991), but the methods involving the assessment of biochemical changes at the cellular level are considered among the most sensitive (Robinson & Ware 1988, Ferron & Leggett 1994). The use of nucleic acids as growth and condition indices is a relatively recent approach which has been investigated by many authors (Haines 1973, Buckley 1979, Robinson & Ware 1988, Clemmesen 1994, Bisbal & Bengtson 1995).

Studies on fish larvae have indicated that changes in factors which affect growth are reflected in RNA levels as well as in RNA/DNA ratios (Buckley 1979, 1984). Temperature is the environmental variable most frequently linked to the recruitment variability of temperate marine fishes (Sissenwine 1984). It affects the physiological processes of the individual fish, and its effect on biochemical indices of fish needs further investigations (Clemmesen 1996).

The present study investigates the effects of food deprivation on some selected condition measures of herring, *Clupea harengus*, with special consideration on the responsiveness of RNA/DNA. Larvae were reared at three temperatures, and two ontogenetic intervals were considered, 'stage 1' larvae (Doyle 1977) (initial exogenous feeding) and 'stage 2' larvae (Doyle 1977) (established feeding prior to notochord flexion).

Materials and methods

Adult Norwegian Spring spawning herring, *Clupea harengus*, caught from two locations off the western coast of Norway were used to obtain eggs and sperm, and the fertilized eggs were incubated in filtered sea water (salinity 31–32‰) at 8°C (± 0.1 sd) and 12°C (± 0.3 sd) in the laboratory. Three batches of herring embryos hatched in three incubation tanks on 10 April, 16 April, and 3 May 1995 were counted and collected separately. Three fibreglass rearing tanks (1 × 1 × 0.6 m) filled with filtered sea water (500 l) were supplemented each with an inner rearing cage of 335 µm mesh (75 × 75 × 50 cm). Eight hundred embryos from each batch were transferred into the rearing cage of each tank. Larvae originating from 8°C incubations were subsequently reared at 5°C and 8°C while the group incubated at 12°C was reared at 11°C. The larvae were fed in excess from 3–4 days after hatching with natural live zooplankton obtained using 80–250 µm sieve size (mainly nauplii and copepodite stages of copepods, adult cyclopoides, calanoides and harpacticoides, rotifers and cladocerans).

The mean rearing temperatures based on daily measurements during the experimental period (°C ± sd) were 5.3 ± 0.3 (n = 10), 8.3 ± 0.2 (n = 27) and 11.3 ± 0.4 (n = 18), and salinity was in the range of 31.9–32.6. Dissolved oxygen in water was maintained above 80% of saturation level by means of continuous aeration, and water renewal was attained by the daily addition of water with zooplankton into the tanks. The programmable light system (Lysstyr [10], Norway, Hansen 1990) in the laboratory simulated the natural light conditions in Bergen, Norway (60° 23′ N, 5° 20′ E).

Experimental design and sampling

Two cages (25 × 35 × 45 cm, 65 µm mesh size; hereafter called experimental cages) were installed beside each of the three rearing cages, and the small mesh size of these cages prevented the exchange of available prey. Two groups of larvae (75 or 100) from each rearing cage were transferred into the two respective experimental cages 5 days after hatching at 8°C and 11°C, and 10 days after hatching at 5°C (stage 1b and 1c larvae, Doyle 1977). One experimental cage was maintained deprived of food while the other cage was maintained with a food density similar to the respective rearing cage.

About 15–20 herring larvae were taken as an initial sample from the rearing cage on day 0, and subsequently from both fed and starved groups on day 1, day 3, day 5, and day 7 (only in 8°C unit) after initiation of starvation. On the last sampling day (day 5 or 7), another sample of larvae from the rearing cage was taken, and is hereafter called the reference sample. A second round of experimentation was carried out with well established feeding larvae

prior to notochord flexion (except for the 5°C unit due to a technical problem). The majority of the larvae were in stage 2b at the onset of the experiment. All the sampling was carried out at a similar time of the day (17:00 h). Each sampled larva was length measured (in mm) alive under a dissecting microscope using a calibrated micrometer. The developmental interval was determined according to Doyle (1977). Afterwards, the larvae were placed individually in reaction vials (Eppendorf) and shock-killed in liquid nitrogen and subsequently stored in an ultra freezer (– 80°C) until nucleic acid analysis.

Prior to the analysis of nucleic acids, individual larvae were dried at – 67°C overnight using a freeze dryer (VirTis, VirTis company Inc.). The freeze-dried weight of each larva was measured to the nearest µg using an electronic micro-balance (Sartorius Micro M3P, Sartorius GmbH) after keeping them in a desiccator with silica gel for about 30 min. A sub-sample of 15 larvae from each sample was used in the analysis for nucleic acids, but 2.1% of them were lost or excluded during the analysis leaving a total number of 645 larvae analysed.

Analysis for nucleic acids

The determination of DNA content and RNA content of individual larvae were carried out by the fluorometric method described by Le Pecq & Paoletti (1966) and further improved by Clemmesen (1993), excluding the prior purification of the larval homogenate. All chemicals used in the procedure were of analytical grade. Tris-NaCl buffer was prepared (0.05 M Tris (hydroxymethyl) aminomethane, 0.1 M NaCl, 0.01 M EDTA; Merck) with pH adjusted to 8.0 with concentrated HCl, and this buffer was used to prepare all the working reagents. Pulverized Ribonuclease A (RNase-Type 1-AS from bovine pancreas; Sigma Chemical Co.) in a final concentration of 0.2 mg ml^{-1} buffer, and Ethidium Bromide (EB) (Sigma) with a final concentration of 10 µg ml^{-1} buffer was used.

Each larva was homogenized with Tris-NaCl buffer for 10 sec using an ultrasonic cell disrupter (25 W, Virsonic 50, VirTis Company Inc.), and the final volume of the homogenate was set to 250 µl. Any increase in the temperature of the homogenate was minimized by carrying out all the preparatory steps while keeping the vials with larval tissue on ice. The homogenates were centrifuged using a refrigerated centrifuge (Hermle ZK380) for 5 min (8000 rpm or 6082 g) at 0°C, and the supernatant was used in the fluorometric determination of the nucleic acids. Two portions of 100 µl of the homogenate were pipetted into new reaction vials (2.2 ml). For one portion, 1.4 ml of EB was added, and the fluorescence measurement for DNA and RNA (total fluorescence) was determined by exciting at 360 nm and reading the emission at 590 nm using a luminescence spectrometer (LS 30 Perkin-Elmer). In the other 100 µl sample, RNA was enzymatically digested at 37°C over 30 minutes after adding 5 µl of RNase, and the measured fluorescence was assumed to be only due to DNA (Clemmesen 1988).

The difference between the total fluorescence and the DNA fluorescence was corrected to determine the RNA fluorescence, based on the calibrations made by Le Pecq & Paoletti (1966) [RNA fluorescence = (total fluorescence – DNA fluorescence) × 2.2]. The fluorescence measurements for DNA and RNA were directly used to calculate the RNA/DNA of larvae. Linear calibration curves were obtained using known amounts of DNA (DNA-Sodium Salt, Type XIV from herring testes; Sigma) for each series of measurements, and these were subsequently used to calculate absolute DNA and RNA contents.

Growth and condition measures

Mean daily growth rate was calculated as $(L_2 - L_1)/(t_2-t_1)$, where L is the mean standard length at respective time periods t_1 and t_2 (days) (Westerman & Holt 1994). The daily growth rate in terms of dry weight and DNA (% day^{-1}) was reported as the specific growth rate (SGR) (Malloy & Targett 1991) using the equation:

$$SGR\ (\%\ day^{-1}) = (e^{G} - 1) \cdot 100,$$

where $G = \ln(W_2) - \ln(W_1) / (t_2-t_1)$, with W as mean dry weight or mean DNA content (µg) at respective

days t_1 and t_2. The ordinary condition factor (Fulton's condition factor) (Ehrlich et al. 1976) was calculated as CF = 1000 · dry weight (mg)/ length^3 (mm).

Statistical analysis

All the raw data (length, weight, DNA, RNA) were checked for normality. Statistical analyses and regressions were performed for the two ontogenetic intervals separately. Differences between the means of morphological or biochemical parameters of fed and starved herring larvae on given days were tested by using Student's t-test. The regression slopes between fed and starved data of morphological and biochemical variables were compared using analysis of covariance (ANCOVA). One way analysis of variance (ANOVA) was performed separately for fed and starved larvae of each of the two 'stages' to test the differences among starving days (excluding day zero), followed by Tukey's honestly significant difference (HSD) test (Sokal & Rohlf 1981) to identify different means. The level of significance used in all the statistical testing and regression analyses was 0.05 (Sokal & Rohlf 1981). Stepwise multiple regression was used to model growth of herring larvae, in terms of dry weight and DNA using mean growth values of larvae from different temperatures, stages and feeding regimes. Principal component analysis was performed to group correlated variables and thereby to identify uncorrelated factors (McGurk 1985) using the inputs of all the age or size dependent variables together with growth and condition measures. All the data presentations and statistical analyses were carried out using Statistica® for Windows (version 5.0).

Results

Growth and survival of herring larvae

Fed larvae in the experimental cages generally had higher growth rates than the respective starved larvae (Table 1). However, the growth of these fed larvae seemed to be affected compared to the fed larvae in the respective rearing tanks. The herring larvae from the rearing tanks had specific growth rates of 2.3, 7.1% and 6.6% day^{-1} respectively at 5°C, 8°C and 11°C. In general, the specific growth rate of the fed larvae in experimental cages at 5°C was lower than that at the other two temperatures. Among the starved larvae, the lowest growth rate was found at 11°C. The average daily mortality rate in the rearing tank, after excluding sampled larvae, was highest at

Table 1. Summary of the growth rates of herring larvae in the experimental cages.

Temp. (°C)	Stage*	Age range (day)	Feeding status	Growth rate			
				Length (mm day^{-1})	Dry weight (% day^{-1})	DNA (% day^{-1})	Protein (% day^{-1})
5	1	10–15	fed	0.20	1.3	0.5	0.3
	1	10–15	starved	0.14	−1.3	0.5	−1.4
8	1	5–12	fed	0.23	1.7	7.1	3.6
	1	5–12	starved	0.17	−2.0	3.4	0.3
	2	20–27	fed	0.28	4.4	7.1	4.1
	2	20–27	starved	0.05	−3.7	2.8	−1.9
11	1	5–10	fed	0.23	8.3	0.8	4.4
	1	5–10	starved	−0.01	−1.7	−3.3	−0.3
	2	16–21	fed	0.09	1.2	−1.4	5.8
	2	16–21	starved	0.07	−4.4	−1.4	−0.7

The daily growth rates based on SL were calculated by the linear model. SGR (% day^{-1}) of dry weight and DNA were estimated using the exponential model and expressed as $(e^G - 1)\cdot 100$. Mean protein growth rates were based on the equation by Buckley (1984) using the values at the final day of experimentation at respective temperatures and ontogenetic stages.

* Ontogenetic stage designations are according to Doyle (1977).

8°C (2.1% day^{-1}) compared to 1.7% day^{-1} at 5°C and 1.2% day^{-1} at 11°C.

Morphometrics and condition measures

The effects of starvation on the different condition indices varied (Table 2). Standard length (SL) of fed and starved larvae broadly overlapped in most of the groups. No significant decreases in SL were observed in starved larvae while SL of fed larvae increased by 1.9%, 2.0% and 2.2% day^{-1}, respectively, at 5°C, 8°C and 11°C among stage 1 larvae. The corresponding daily increases were 1.9% and 0.5% respectively at 8°C and 11°C among stage 2 larvae. Starvation effects on dry weight were more recognisable. Higher reductions in dry weight of starved larvae occurred at 8°C (2.6% day^{-1}) and 11°C (2.2% day^{-1}) than at 5°C (1.6% day^{-1}) among stage 1 larvae, and the decreases were 1.3% day^{-1} at 8°C and 2.2% day^{-1} at 11°C among stage 2 larvae. Significant differences in dry weight between fed and starved larvae (t-test, $p < 0.05$) were observed earlier at 11°C than at the other two temperatures.

The calculated mean condition factor (Fulton's CF) of fed and starved herring larvae broadly overlapped in most of the groups during the first 1–3 days after the onset of starvation. It decreased in starved larvae during the first 5 days from 0.21 to 0.16, from 0.19 to 0.10 and from 0.17 to 0.15, respectively, at 5°C, 8°C and 11°C. CF decreased significantly also in fed groups during the first 1–3 days but increased afterwards (ANOVA, $p < 0.05$). No general trends in manifestation of starvation effects on CF related to temperature were observed (Table 2), and no characteristic values could be extracted to describe starving condition.

The absolute DNA content increased in feeding larvae in the rearing cages, except on one occasion where an unexpected growth retardation occurred (at 11°C). In general, no consistent trends were observed for DNA content of herring larvae in the experimental cages during the starvation periods. DNA content of fed larvae increased with time only at 8°C in both ontogenetic stages, and lower DNA contents were found in starved larvae than in the fed ones only after 5–7 days (t-test, $p < 0.05$) (Table 2). No significant decrease in DNA content with time was observed in starved larvae except at 11°C among the stage 1 larvae (ANOVA, $p < 0.05$), and no differences in DNA content between fed and starved stage 2 larvae were observed at 11°C (t-test, $p > 0.05$).

Generally, DNA concentration (% dry weight) was higher in starved larvae than in the fed larvae (Figure 1). Although variable, it generally increased with time in starved larvae. DNA concentration in fed larvae apparently decreased with time except at 8°C. Values of DNA concentration higher than 2.2% seemed characteristic of starving larvae after about 3 days of starvation in the first ontogenetic stage, while values higher than 2.9% were characteristic of starving stage 2 larvae.

Absolute RNA content of feeding herring larvae

Table 2. Number of days of starvation elapsed before significant differences ($p < 0.05$) between fed and starved herring larvae were observed with respect to various variables. The parentheses, where present, indicate the occurrence of significant differences between fed and starved groups on that single day and not on the subsequent sampling day.

		Growth and condition measures							
Temp. (°C)	Stage*	SL	DW	CF	DNA	DNA conc.	RNA	RNA conc.	RNA/DNA
5	1	(3)	5	(3)	(3)	5	(1) 5	(1)	(1) 5
8	1	ns	5	5	7	(5)	5	7	5
	2	5	5	5	5	5	3	1	1
11	1	3	3	3	3	3	1	1	1
	2	(3)	3	5	ns	3	3	1	1

SL = standard length, DW = dry weight, conc. = concentration, ns = no significant difference during the study period.
* Ontogenetic stage designations are according to Doyle (1977).

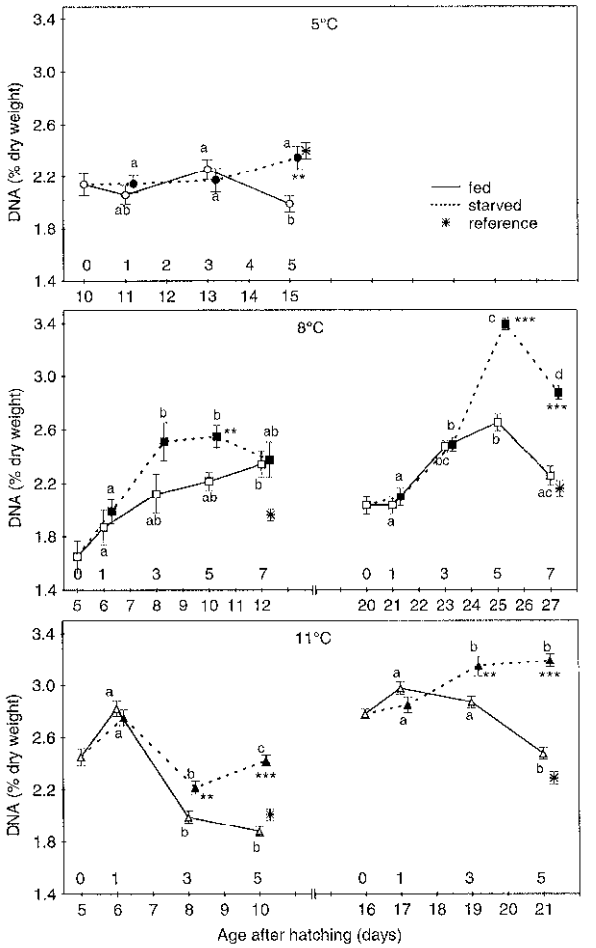

Figure 1. Effects of starvation on DNA concentration of herring larvae at the three experimental temperatures (mean ± standard error of mean, n = 13–15). The numbers written above the x-axis indicate the starvation duration in days. The asterisks indicate differences (*p < 0.05, **p < 0.01, ***p < 0.001) between fed and starved larvae on each day of starvation. Differences between days are indicated by the absence of any common letters within respective stage and feeding category (p < 0.05, Tukey's HSD test). Open and filled symbols represent fed and starved larvae respectively, and the double-cross represent the reference sample for each of the stages taken from the respective rearing tanks on the final sampling day. The x-axis is offset to right by 0.2 for starved data and by 0.3 for reference data for clarity.

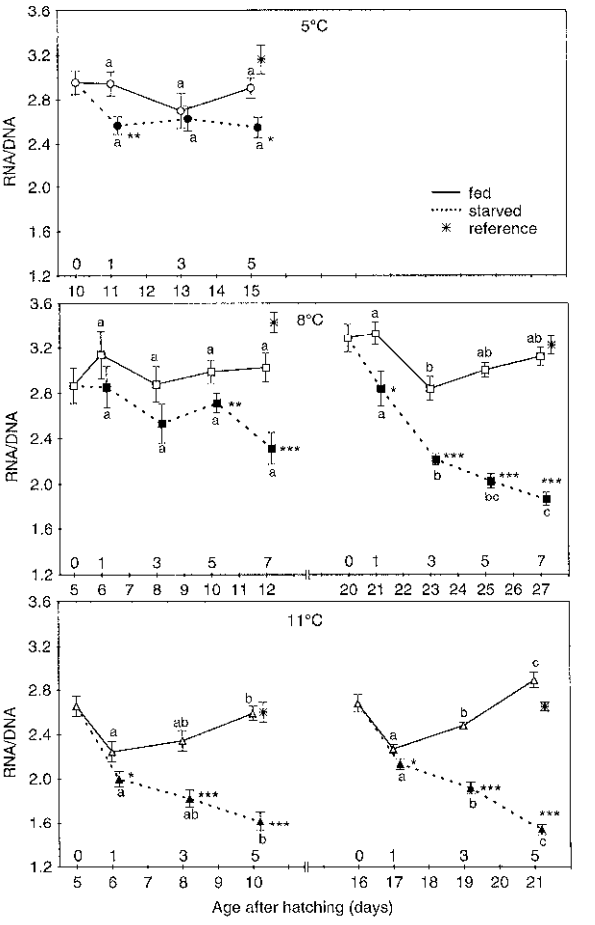

Figure 2. Effects of starvation on RNA/DNA of herring larvae at the three experimental temperatures. Symbols are as in Figure 1.

increased with time at all three temperatures. Starvation led to a decrease in RNA content of herring larvae in the experimental cages by 2.7%, 0.3% and 9% day^{-1}, respectively, at 5°C, 8°C and 11°C in stage 1 larvae, and by 4.9% at 8°C and 9% day^{-1} at 11°C in stage 2 larvae. The starvation effects were statistically recognisable as early as 1 day after onset of starvation in one of the groups (Table 2).

In general, starved larvae had lower RNA concentration (% dry weight) than fed larvae on a given day. A general decreasing trend in this measure with time was observed in starved stage 1 larvae except at 8°C and among the stage 2 larvae. High variation in RNA concentration was observed among the fed larvae, and there were no consistent trends with time. Although starvation effects on RNA concentration were recognisable already after one day of food deprivation (t-test, p < 0.05) (Table 2), no general values were definable for characterizing starving condition of the larvae.

Comparison of the RNA/DNA of starved and fed larvae in the experimental cages showed that deprivation of food led to an immediate decrease in the

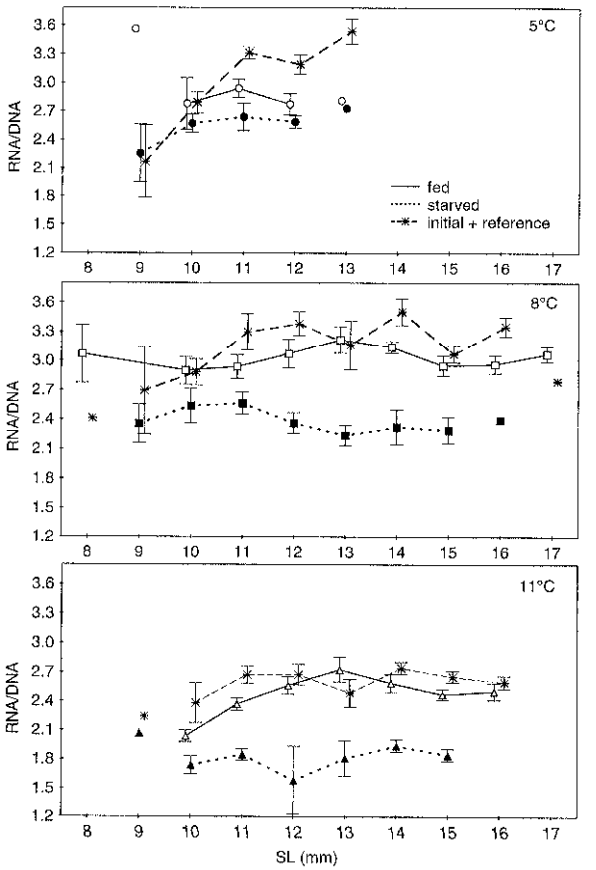

Figure 3. Trends of mean RNA/DNA-at-length of herring larvae of fed (open symbols), starved (filled) and rearing (initial + reference; double-cross) groups. The x-axis is offset by 0.1 to left for fed data and to right for rearing data for clarity. Single data points are not connected.

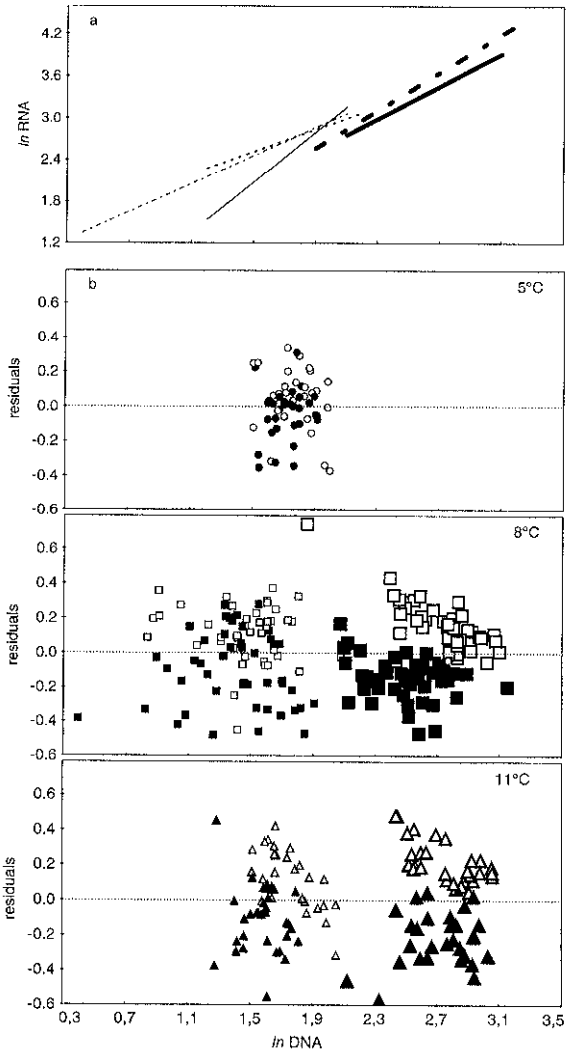

Figure 4. a – Common linear regressions between *ln* RNA and *ln* DNA for herring larvae in experimental cages after three or more days from the onset of starvation at 5°C (-----), 8°C (– – –) and 11°C (——) for ontogenetic stages 1 (thin lines) and 2 (thick lines). b – Corresponding residual distributions for fed (open symbols) and starved larvae (filled symbols) at the three temperatures. Similar symbols of small and large fonts represent ontogenetic stages 1 and 2 respectively.

RNA/DNA in all groups (Figure 2). Increase in the starvation interval resulted in a further decrease in the ratio and the changes seemed more dramatic at higher temperatures. A weak inverse relation between temperature (T, independent variable) and RNA/DNA was found in both fed ($y = 3.54 - 0.085$ T, $r^2 = 0.13$, $p < 0.001$) and starved larvae ($y = 3.41 - 0.133$ T, $r^2 = 0.26$, $p < 0.001$).

No significant length dependency of the RNA/DNA of herring larvae in the experimental cages was found except in one group (stage 1 fed larvae at 11°C) (ANCOVA, $p < 0.05$). Moreover, the trends of mean RNA/DNA with SL of herring larvae of fed, starved and rearing categories tended to remain at a constant level over the size range studied with some variations at 5°C (Figure 3). The starving larvae generally had lower RNA/DNA-at-length, and the index apparently decreased with increasing temperature. Furthermore, lower RNA/DNA was found in starving stage 2 larvae than in stage 1 larvae (t-test, $p < 0.05$).

DNA content and RNA content were significantly correlated with both dry weight and standard length of larvae (all but SL log transformed) at all

three temperatures and with temperature groups combined (overall combined correlations $r > 0.9$, $p < 0.001$). Residual analysis of the common regression between DNA content and RNA content (log transformed) showed a clear separation between fed and starved larvae after 3 or more days from the onset of starvation (Figure 4). The negative residuals represented the individuals starved or below an average condition, while the positive residuals indicated the individuals fed or above an average condition. The separation was much clearer among stage 2 larvae (Table 3). A critical level of RNA/DNA of 2.5 characterized starving larvae of all groups even though the classification success with respect to starving larvae was rather poor at 5°C where a broad overlap between fed and starved larvae existed. Considerable classification success was also observed with respect to DNA concentration and Gpi (Buckley 1984) while SGR_{DNA} index calculated for individual larvae using the derived model (see below) showed a highly variable classification success of starved larvae at different temperatures.

Growth relations

Average specific growth rates in terms of dry weight (SGR_{DW}) were positively related with RNA/DNA in the following model generated by step-wise regression:

$$SGR_{DW} = 7.58\ RNA/DNA - 5.48\ T + 0.41\ T^2 - 3.30\ (n = 32, r^2 = 0.66, p < 0.001),$$

where T is the mean temperature. The corresponding partial correlations of the included predictor variables and the response variable were 0.80, – 0.54 and 0.62, respectively. Average specific DNA growth rates (% day^{-1}), designated as SGR_{DNA}, were also related with the temperature and RNA/DNA of herring larvae in a step-wise regression:

$$SGR_{DNA} = 4.49\ RNA/DNA + 7.14\ T - 0.42\ T^2 - 37.5\ (n = 32, r^2 = 0.85, p < 0.001),$$

where T is the mean temperature. The corresponding partial correlations of the included predictor variables and the response variable were 0.81, 0.82 and – 0.81, respectively. The data for the stage 2 lar-

Table 3. Classification success of fed and starved larvae (as % of total in each category) in the experimental cages using various classification indices. Only data from three or more days after the onset of starvation are included.

Temp. (°C)	Stage**	Feeding status	Index*				
			RNA/DNA	DNA conc.	Regression residuals	Gpi	SGR_{DNA} index
5	1	fed	76	64	66	24	55
	1	starved	38	44	53	93	80
8	1	fed	83	55	74	83	98
	1	starved	53	62	62	53	13
	2	fed	95	98	88	95	100
	2	starved	100	52	86	100	18
11	1	fed	50	62	70	100	47
	1	starved	100	84	77	73	100
	2	fed	72	83	100	100	59
	2	starved	100	86	90	69	100

* RNA/DNA below 2.5 characterizes starving larvae. DNA concentration of ≥ 2.2% for stage 1 larvae and ≥ 2.9% for stage 2 larvae characterize starving larvae. Negative and positive residuals from the *ln* RNA versus *ln* DNA regression represent starving and fed larvae respectively. Gpi was calculated from Buckley (1984) equation for protein growth, and Gpi of <1% represents starving larvae. SGR_{DNA} of <1% represent starving larvae.

** Ontogenetic stage designations are according to Doyle (1977).

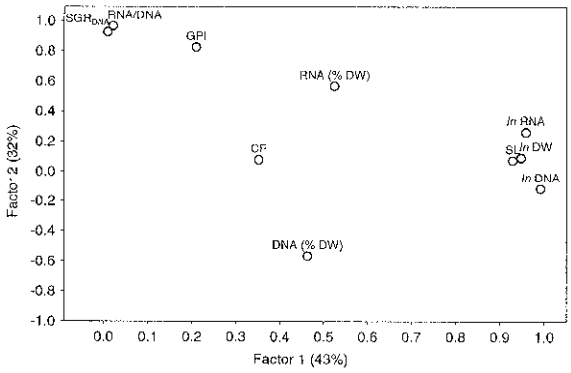

Figure 5. Factor loadings plot of size and condition measures used in Principal Component Analysis with Varimax normalized rotation. The numbers within parentheses indicate the proportion of the total variability in the data explained by respective factors (SL = standard length, DW = dry weight, SGR_{DNA} = individual DNA growth rates calculated using the derived model, GPI = protein growth rates by Buckley (1984) equation).

vae at 11°C were excluded in both models due to the unexplainable poor growth performance.

Results of the Principal Component Analysis showed that three uncorrelated factors could be extracted from all the ten variables used as inputs (Figure 5). Factor one which may be interpreted as a size factor explained 43% of the total variability in the data, while the second factor (comprised of RNA/DNA, Gpi, SGR_{DNA}, RNA concentration to a lesser extent) which may be interpreted as a growth and condition factor, explained 32% of the variability. The third factor which was positively correlated with the Fulton's condition factor (CF) and negatively correlated with DNA concentration and RNA concentration explained 14% of the total variability. The size measures along the first axis were generally uncorrelated with the growth and condition measures along the second axis.

Discussion

Growth and survival of herring larvae

Growth and survival of herring larvae in the rearing tanks were comparable to other studies (Ehrlich et al. 1976, Øiestad & Moksness 1981, Folkvord et al. 1996). The somewhat lower growth in the experimental cages may be attributed to the stress associated with handling and confinement. Older larvae in good condition appeared relatively more vulnerable to handling than the smaller larvae. The second ontogenetic group at 11°C experienced the lowest growth. The larvae in question were observed sinking out of the water column after about 3 days in the experimental cage, indicating that they were not feeding by that time (McGurk 1984), and there were no surviving larvae left after 5 days of the experiment.

Average DNA growth rate of the larvae was related with the mean RNA/DNA in the present study (partial correlation of 0.81), and the model derived seemed to adequately represent the temperature dependent growth, with 85% of the variability in DNA growth rates explained. RNA/DNA based growth indices have been useful in characterizing growth and condition of fish larvae (Buckley 1984, Bulow 1987, Martin & Wright 1987, Folkvord et al. 1996). Individual DNA growth rates estimated by using the SGR_{DNA} model in the present study were used to classify starved and fed larvae, and values below 1% daily DNA growth rates seemed characteristic of starving larvae or larvae with poor growth, except at 8°C. The DNA growth rates can supplement other average growth measures (e.g. dry weight based growth) obtained in the laboratory and in the field, and provide added information about to what extent the observed growth is due to an increase in cell size or cell numbers in the larvae.

Starvation effects in relation to temperature

Changes in DNA content in fed larvae were similar to what has been observed in various studies (Buckley 1980, Raae et al. 1988, Richard et al. 1991). The starved larvae had lower DNA contents as well as generally lower DNA growth rates compared to that of fed larvae except at 5°C. High variability in DNA content of cod larvae during the first two weeks after hatching has been observed by Raae et al. (1988). Similar to the trends in the RNA content observed in many species (Buckley 1979, 1980, Clemmesen 1987, Westerman & Holt 1994), RNA content decreased significantly during starvation. The responsiveness of RNA content to starvation

was higher at higher temperatures and higher in the second ontogenetic stage. However, varying dynamics of starvation effects were found with respect to RNA concentration which was lower in starved larvae than in fed ones. Similar trends have been observed in herring and turbot larvae by Clemmesen (1987), and in cod and winter flounder larvae by Buckley (1981).

RNA/DNA was the most sensitive measure to starvation among the examined indices. Increasing DNA concentration and decreasing RNA concentration in starved larvae, suggests that DNA was conserved while RNA was metabolised relative to other body constituents during starvation (Buckley 1981), leading to a sharp decrease in RNA/DNA within a short period of food deprivation. Inter-species variability in the values of RNA/DNA as well as the sensitivity of the ratio to various food levels are frequently observed phenomena (Bulow 1970, Haines 1973, Buckley 1981). The fact that all the samples were taken at the same time of the day excludes any effects of diel variations of RNA/DNA (Rooker & Holt 1996).

Expression of starvation effects on dry weight, RNA and RNA/DNA increased as the temperature increased, probably as a result of temperature dependent metabolism rates. The growth of fish larvae is mainly affected by food levels and temperature (Heath 1992). Similar to Buckley's (1982) finding, a weak negative relation between RNA content and temperature was found. Higher RNA contents at lower temperatures have been attributed to compensatory mechanism in many studies (Goolish et al. 1984, Houlihan 1991). Miglavs & Jobling (1989) suggested that the rates of change in RNA concentration with a new nutritional level are probably determined by such factors as size of the animal and temperature. The rate of the decrease of RNA content in starved larvae of the stage 1 larvae was 2.7% per day at 5°C, and 9% per day at 11°C in the present study, suggesting temperature dependency. No significant decrease in RNA was observed at 8°C due to the possible presence of yolksac in the initial days.

Larvae of the second ontogenetic stage showed higher growth rate, higher DNA concentration as well as higher RNA concentration compared to those of the first ontogenetic stage. It has been noted that biochemical composition of fish depends on developmental interval (Ehrlich 1974a, b), and such chemical changes generally are more closely linked to larval size than to age (Ehrlich 1974a, Buckley 1981). Although variable, starvation effects on dry weight, RNA content and RNA/DNA seemed more dramatic in stage 2 larvae compared to stage 1 larvae. However, in a study on sole larvae, Richard et al. (1991) concluded that a rapid decrease in RNA/DNA occurred after few days of food deprivation in young larvae but not in the older metamorphosed larvae, and there are more studies which are in agreement with this observation (Clemmesen 1987, 1994). Bisbal & Bengtson (1995) found in summer flounder that RNA/DNA is sensitive to starvation only in the juvenile period. The delayed response to starvation in smaller larvae in the present study can be explained to some extent by the possible dependence of some of the larvae upon their endogenous food supply during the first days of the experiment. The fact that the older larvae seemed more vulnerable to handling, and showed a higher confinement effect from the holding net than for the smaller larvae may have caused an added stress for the older starving larvae. Varying observations between the larvae of the two ontogenetic stages in the present study substantiate the suggestions made in other studies (Richard et al. 1991, Westerman & Holt 1988) that the comparisons with respect to nucleic acid ratio should only be done among larvae of the same developmental interval. Even though it was found that the responsiveness of the RNA/DNA to starvation is stage-dependent, there were no significant differences in RNA/DNA between the feeding larvae of the two ontogenetic stages studied implying that the RNA/DNA ratio of feeding herring larvae with good condition is independent of developmental interval in the age range studied.

The trends in RNA/DNA-at-length were apparently constant with some minor fluctuations at constant temperature, and higher values were generally observed at lower temperatures. However, a size dependent RNA/DNA relation in fish larvae has been suggested by several authors (Buckley 1982, Buckley et al. 1984, Buckley & Lough 1987, Clem-

mesen 1994). The principal component analysis in which the size factor is well separated from the growth or condition factor, clearly showed that there was no overall size dependence of the condition factors of the larvae in the size range studied. However, the lack of a size effect in this study could also be due to the relatively narrow size range studied.

Condition indices

Since it is important to detect early starvation, condition indices with the shortest latency would be most valuable in the field. Latency is defined as the time required for a given change in food availability to be reflected as a significant change in the index of condition used (Ferron & Leggett 1994). Regarding the latency and dynamics of the condition measures in the present study, the shortest latency was observed with respect to RNA/DNA, and it clearly depended upon temperature and developmental interval. Therefore, it is important to be concerned about the ambient temperature and the interval when interpreting the results. The present results are in agreement with the findings of Wright & Martin (1985), even though the determination of the changes within hours was not attempted in the present study. The rate of decrease (or 'deterioration dynamic') of the RNA/DNA ranged from 0.11 to 0.36 per day between day 0 and day 3, and from 0.04 to 0.19 per day in the subsequent 2 or 4 days depending on temperature and size, in the present study. The rate of decrease in RNA/DNA apparently followed the model of declining decay proposed by Ferron & Leggett (1994) for the time response of biochemical indices of condition.

The critical level of RNA/DNA suggested in the present study characterized the starving larvae well, especially among stage 2 larvae. The same critical level of RNA/DNA was suggested by Clemmesen (1989) for the herring larvae. No herring larvae had RNA/DNA values below 1.0 in the present study, and this supports the suggestion by Clemmesen (1994) that a minimum RNA/DNA value around 1.0 seems necessary for survival in herring larvae.

The present study found that the residuals of the regression between DNA and RNA may be used as a condition measure since it separates the starved and fed larvae to a considerable amount. The residuals from this regression can be interpreted as deviations from an average condition (Folkvord et al. 1996, Suthers et al. 1996). The separation of fed and starved larvae using these residuals was better at 8°C and 11°C than at 5°C, especially in the second ontogenetic stage. However, the presence of fed larvae with DNA contents which are beyond those of the respective starved larvae may have caused the slopes of the regression lines to be artificially steep, and hence may have effects on the residual distribution. Furthermore, several studies have reported the difficulty in finding starved larvae at sea (Buckley & Lough 1987, Robinson & Ware 1988). Therefore, this condition measure in the field may erroneously classify larvae in good condition as starving if starving larvae are underrepresented in the sample, because the regression represents the best fit line for the available data.

Starvation effects were indicated in the DNA concentration and RNA concentration by an increase and a decrease respectively. Very high variability in the RNA concentration made it difficult to use this index to characterize starving larvae. Relatively high variability also in the trends of DNA concentration was observed between experimental groups, and a higher level of this index was present at 11°C than at 8°C in the second ontogenetic stage group. On the contrary, Richard et al. (1991) found that the percentage of DNA relative to dry weight is much more stable in fed sole larvae than the RNA/DNA, and hence suggested the use of it as a better and simpler index of nutritional status than the RNA/DNA. Although this index was not sensitive enough to express starvation effects immediately like the RNA/DNA, the results of the present study were useful in suggesting some critical levels to differentiate starved herring larvae from the fed ones. For stage 1 larvae, a level of 2.2% of DNA concentration may differentiate herring larvae starved for 3 or 5 days depending on the temperature while a value of 2.9% may be useful in identifying stage 2 larvae after 3 or 5 days of starvation. In general, the present study supports the use of the index of DNA

concentration (% of dry weight) as an index of nutritional condition in herring larvae.

Protein growth index (Gpi) calculated using Buckley (1984) equation did not result in any better classification of fed and starved larvae than the RNA/DNA ratio. The larvae with Gpi less than 1% were considered characterizing poor growing or starving condition, and the slow growing fed stage 2 larvae from the 11°C group was characterized as being in good condition. However, the direct use of Buckley's equation to calculate protein growth rates without any inter-methodological calibrations may have contributed to added variability to the calculated values, and the estimated growth rates should thus be interpreted with caution (Canino et al. 1991, Canino & Caldarone 1995).

Findings of this study support the suitability of RNA/DNA as an index of nutritional condition to detect early starvation. RNA/DNA was independent of length in the size range studied, and this absence of correlation with size is important especially in field studies. The responsiveness of RNA/DNA and other indices to starvation is temperature and stage dependent. The study also showed that the applicability of indices like DNA concentration cannot be rejected and that further studies regarding the sensitivity of these indices are needed. Since RNA/DNA enables to detect short time-scale changes in nutritional status, it provides a sensitive tool in characterizing individual condition of fish larvae in the field. Although RNA/DNA based growth index derived in the present study adequately represented the average temperature dependent DNA growth rate of herring larvae, inconsistent classification success and poor recognition of starving larvae in some groups suggest that it may not satisfactorily depict the individual growth of starving larvae.

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