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Application of RNA/DNA ratio and tryptic enzyme activity on laboratory-reared and wild-caught herring larvae - Short communication -

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Abstract

RNA/DNA ratio and tryptic enzyme activity were shown to be appropriate indicators to determine starving herring larvae or herring larvae in bad nutritional condition in laboratory rearing experiments. Based on the laboratory results, the methods were applied to evaluate the nutritional condition of larvae from a field sample and to determine the proportion of starving larvae.

Keywords: herring, larvae, nutritional condition, starvation, RNA/DNA ratio, tryptic enzyme activity

Introduction

For studies on the recruitment process of fish larvae and in aquaculture, indicators are needed for the determination of the nutritional condition. RNA/DNA ratios and tryptic enzyme activity measurements were found to be appropriate indicators to describe the nutritional condition (Buckley 1979, Clemmesen 1987, Hjelmeland et al. 1984, Martin et al. 1985, Pedersen et al. 1987, Pedersen and Hjelmeland 1988, Ueberschär 1985). Highly sensitive fluorescence techniques were developed to measure both indicators in individual marine fish larvae (Clemmesen 1988, Ueberschär 1988). Before these methods can be applied to fish larvae with unknown nutritional condition, they have to be calibrated with fish larvae reared under defined conditions in the laboratory.

The paper presents an application of RNA/DNA ratio and tryptic enzyme activity determinations on laboratory-reared fed and starved herring larvae and gives an example for the evaluation of the nutritional condition of herring larvae from a field sample based on the laboratory results.

Materials and methods

Larval fish. Herring *(Clupea harengus)* larvae aged 9-65 days for the RNA/DNA ratio measurements and aged 1-45 days for the tryptic enzyme activity determinations were reared under laboratory condition as described by Ueberschär (1985) and Clemmesen (1987). Larvae were fed with *Brachionus plicatilis* (5/ml) and *Artemia* nauplii (1/mL) or deprived of food for intervals of 6-9 days (RNA/DNA ratios) or 2-6 days (tryptic enzyme activity measurements).

Herring larvae were sampled in the English Channel in January 1986 by using a MOCNESS- system (0.3mm mesh size, Wiebe et al. 1976). Fish larvae were individually collected from the sample, shock frozen in liquid nitrogen and stored at -74°C.

Measurement of RNA/DNA ratio and tryptic enzyme activity. RNA and DNA contents of the larvae were determined by using the fluorescence technique by Clemmesen (1988) with some modifications (Clemmesen 1990). The tryptic enzyme activity measurements were performed according to the fluorescence technique described by Ueberschär (1988).

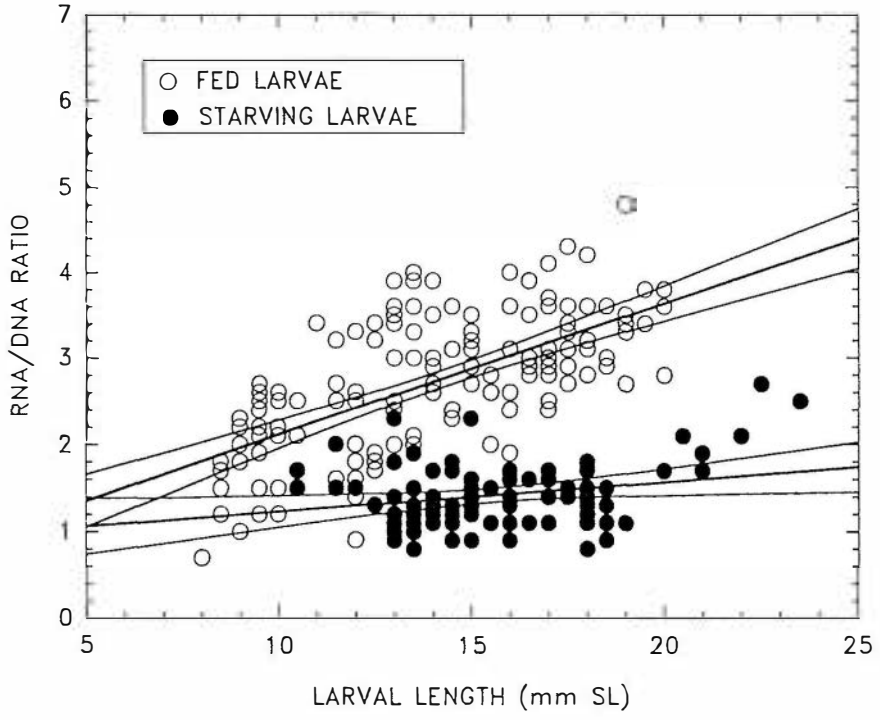

Fig. 1. RNA/DNA ratios of laboratory-reared fed and 6-9 days starved herring larvae. Data points represent 5 to 15 larvae measured individually. Lines were fitted by linear regression analysis with: $y = 0.591 + 0.152x$, $r = 0.62$ for fed larvae, and $y = 0.888 + 0.034x$, $r = 0.25$ for starving larvae. In addition, the 95% confidence level of the regression is presented.

Results

A comparison of the RNA/DNA ratios of laboratory-reared fed and starving herring larvae is shown in Fig. 1. Tryptic enzyme activity measurements of laboratory-reared larvae are given in Fig. 2. The RNA/DNA ratios as well as the tryptic enzyme activities were significantly lower in the starving larvae than in fed larvae. RNA/DNA ratios and tryptic enzyme activities of both fed and starving larvae showed a linear increase with larval length, but the slopes of the regression lines fitted to the data were different between fed and starving larvae.

Based on the results of RNA/DNA ratios and tryptic enzyme activities determined on starving larvae from the laboratory, an estimation of the nutritional condition of field-caught herring larvae was performed. For both indicators, twice the standard deviation (2s) of the regression for laboratory-reared starving larvae was defined as the critical level for determination of larvae as starving, including 95% of the values found in the

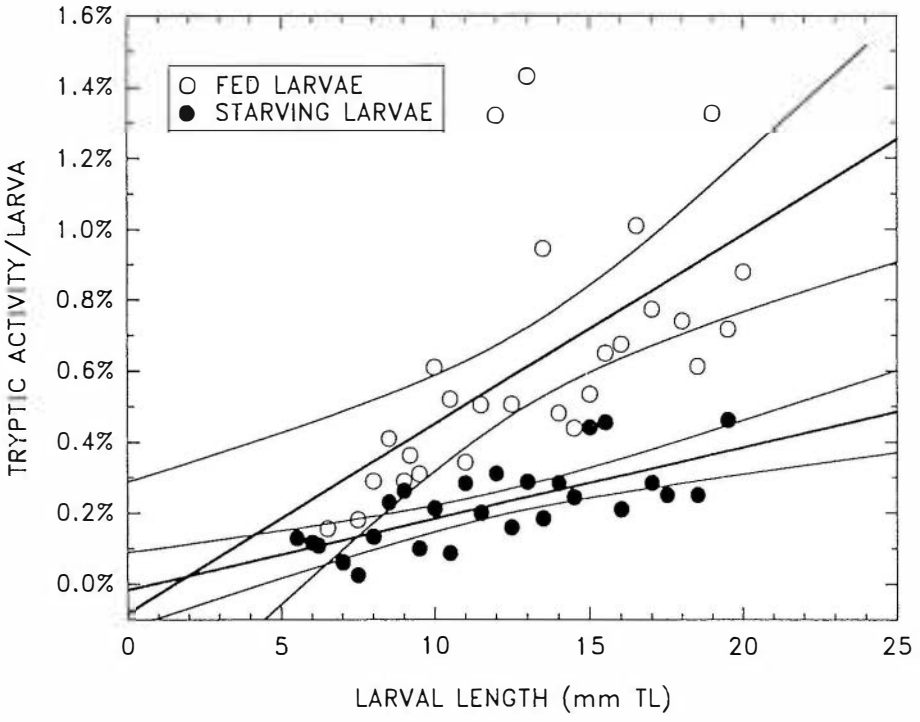

Fig. 2. Tryptic enzyme activity of laboratory reared and 2-6 days starved herring larvae. Data points are means of 5 to 15 larvae measured individually. Lines were fitted by linear regression analysis with: $y = -0.081 + 0.053x$, $r = 0.62$ for fed larvae, and $y = -0.037 + 0.025x$, $r = 0.61$ for starving larvae. In addition, the 95% confidence level of the regression is presented.

starving group. Based on their RNA/DNA ratios, 12.5% of the larvae from the field sample were starving (Fig. 3) compared to 16.0% as determined by their tryptic enzyme activity values (Fig. 4).

Discussion

The present study shows that both indicators, the RNA/DNA ratio and the tryptic enzyme activity, are appropriate to distinguish between feeding and starving fish larvae. The laboratory rearing results clearly demonstrate significantly lower values in starving larvae for both indicators. Since the RNA/DNA ratios and tryptic enzyme activity values in starving larvae showed a lower coefficient of variance compared to fed larvae, they were used for the evaluation of the nutritional condition of field larvae.

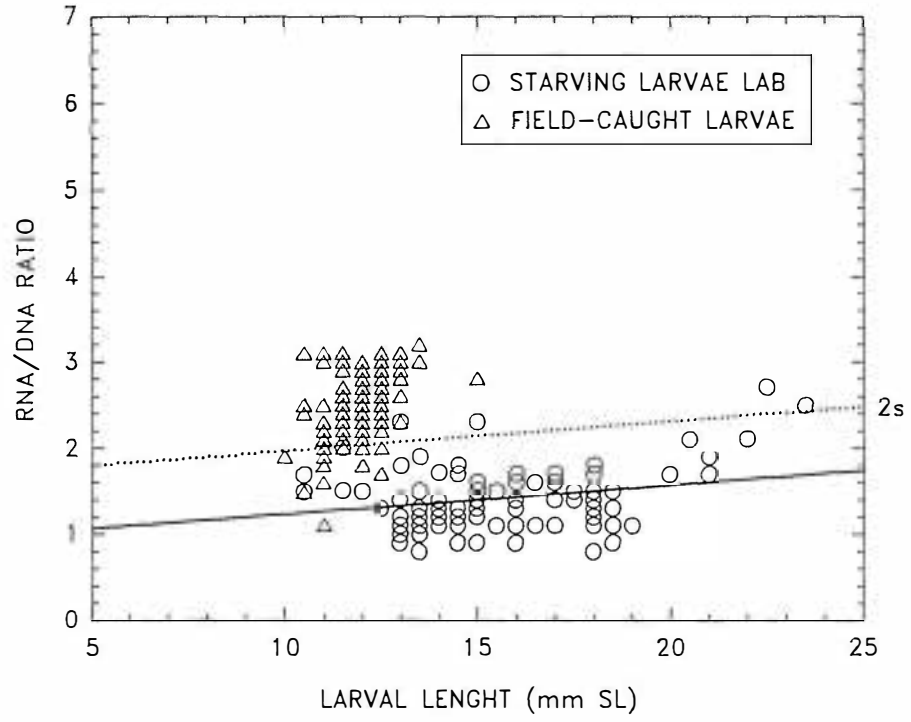

Fig. 3. Evaluation of the nutritional condition of herring larvae sampled from the English Channel based on RNA/DNA ratios of laboratory reared starving herring larvae. Data points represent 5 to 15 larvae measured individually in relation to their length. The solid line shows the linear regression fitted to the data of laboratory reared starving larvae (see Fig. 1). The dotted line represents twice the standard deviation (2s) of the regression and gives the critical levels below which larvae were determined as starving.

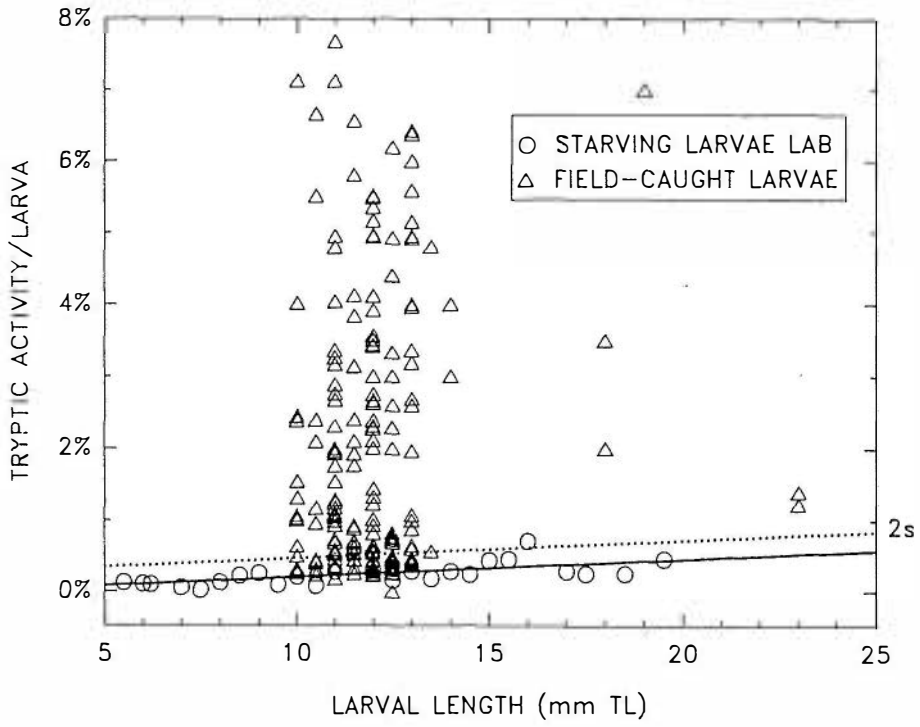

Fig. 4. Evaluation of the nutritional condition of herring larvae sampled from the English Channel based on tryptic enzyme activity measurements of laboratory reared starving herring larvae. Data points represent 5 to 15 larvae measured individually in relation to their length. The solid line shows the linear regression fitted to the data of laboratory reared starving larvae (see Fig. 2). The dotted line represents twice the standard deviation (2s) of the regression and gives the critical levels below which larvae were determined as starving.

The actual values of the RNA/DNA ratios and tryptic enzyme activity measurements might differ between species, therefore, at present, it seems advisable to use laboratory results on starving larvae only to determine the nutritional condition of larvae from field samples of the same species.

The study shows that both indicators are able to detect larvae, which were exposed to starvation intervals in the range of 4 - 9 days. When estimating the proportion of starving larvae from a field sample, both methods correspond well. In the short-term range, the tryptic enzyme activity reacts faster to food deprivation than the RNA/DNA ratio, as shown by Ueberschär and Clemmesen (1992). The tryptic enzyme activity was already significantly lower in starving larvae after 2 days of food deprivation, if compared to fed larvae, whereas the RNA/DNA ratio needed a starvation period of 3 - 4 days to result in significantly lower values compared to fed larvae. In this study as well, starvation intervals from 2 - 6 days already lead to significantly lower tryptic enzyme activity values in starving larvae, whereas the RNA/DNA ratio is more conservative and is more

useful for evaluation of longer starvation intervals in the range of 6 - 9 days. In future experiments, the effect of temperature on the RNA/DNA ratio and tryptic enzyme activity in relation to different starvation periods will have to be evaluated. Higher temperatures might lead to a faster decrease of both indicators. The values for RNA/DNA ratios and tryptic enzyme activity given here might, therefore, be reached in shorter time intervals. The actual values for both indicators from larvae suffering from long-term starvation influences should not be affected by temperature.

The results of this study demonstrate the usefulness of RNA/DNA ratios and tryptic enzyme activity for the detection of starving larvae in the field. It is assumed that these methods are valuable for assessment of rearing conditions in aquacultural situations including optimum food density, quality of the food, growth rates and feeding activity.

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