ICES J. mar. Sci., 49: 245–249. 1992

Short Note

A comparison of the nutritional condition of herring larvae as determined by two biochemical methods – tryptic enzyme activity and RNA/DNA ratio measurements

B. Ueberschär* and C. Clemmesen*

Ueberschär, B., and Clemmesen, C. 1992. A comparison of the nutritional condition of herring larvae as determined by two biochemical methods – tryptic enzyme activity and RNA/DNA ratio measurements. – ICES J. mar. Sci., 49: 245–249.

Two biochemical methods for measuring larval fish condition – tryptic enzyme activity and RNA/DNA ratio measurement – were applied to laboratory-reared and wild-caught herring larvae. The comparison of both methods when applied to laboratory-reared herring larvae showed that tryptic enzyme activity and RNA/DNA ratio are linear and positively correlated under constant nutritional conditions. Wild-caught larvae were transferred to the laboratory and used to compare both indicators in relation to short-term changes in food availability and long-term starvation periods (13 days). In the starvation experiments with the wild-caught larvae the lowest trypsin values were obtained after 3–4 days and a significant decrease in RNA/DNA ratios was obtained after 5–6 days. Prolongation of the starvation time did not result in a further significant change in either parameter. The results of the study demonstrate the usefulness of both methods in monitoring nutritional condition of fish larvae in field samples.

Received 29 September 1991; accepted 12 November 1991.

B. Ueberschär and C. Clemmesen, Institut für Hydrobiologie und Fischereiwissenschaft, University of Hamburg, Hamburg, FRG.

Introduction

In order to investigate the nutritional condition of fish larvae, tryptic enzyme activity and the RNA/DNA ratio were found to be appropriate as indicators at the most sensitive cellular level (Buckley, 1979, 1984; Hjelmeland *et al.*, 1984; Martin *et al.*, 1985; Ueberschär, 1985, 1988; Clemmesen, 1987, 1988, 1989, 1990; Pedersen *et al.*, 1987; Pedersen and Hjelmeland, 1988).

The content of intestinal trypsin is low in starving fish larvae and a significant rise in tryptic enzyme activity can be observed following ingestion of food organisms (Hjelmeland *et al.*, 1984; Lauff and Hofer, 1984; Pedersen *et al.*, 1987). Food deprivation leads to a decrease in protein biosynthesis and finally to a decrease in RNA content, which is reflected by a fall in the RNA/DNA ratio (Henshaw *et al.*, 1971; Lied *et al.*, 1982). Due to their different position in metabolism, it is assumed that tryptic enzyme activity and RNA/DNA ratio react on different time scales in changing nutritional situations.

This paper presents a comparison of the indicators by applying them to laboratory-reared and wild-caught herring larvae from the Schlei Fjord, exposed to defined conditions in the laboratory. The specific objective of this investigation is the comparison of both indicators in response to long-term starvation periods and short-term changes in food availability.

*Present address: Institut für Meereskunde Kiel, University of Kiel, Düsternbrooker Weg 20, 2300 Kiel 1, FRG.

Materials and methods

Larval material

To compare the relationship of the two indicators, fed and starved herring larvae from a laboratory rearing experiment, 30–33 days of age post-hatch with a mean length of 13.2 mm, were used. The laboratory conditions are described in Ueberschär (1985) and Clemmesen (1987). One group was fed once a day with *Brachionus plicatilis* and *Artemia nauplii* whereas the other group was deprived of food for different intervals prior to sampling. Individual larvae were homogenized in 500 µl 0.1 M TRIS-buffer, pH 8.00, containing 0.02 M $CaCl_2 \times H_2O$. These homogenates were divided (1:1) and used for the determination of tryptic enzyme activity and RNA/DNA ratio.

The field samples were taken from the Schlei Fjord, adjacent to the Baltic Sea. The larvae were caught alive with a bait-liftnet twice in June 1989 with 1 week between the two samplings, transferred to the laboratory and fed as described above. Two starvation experiments were performed. The initial tryptic enzyme activity and the RNA/DNA ratio were determined in a subsample ("field

1054–3139/92/020245+05 $03.00/0

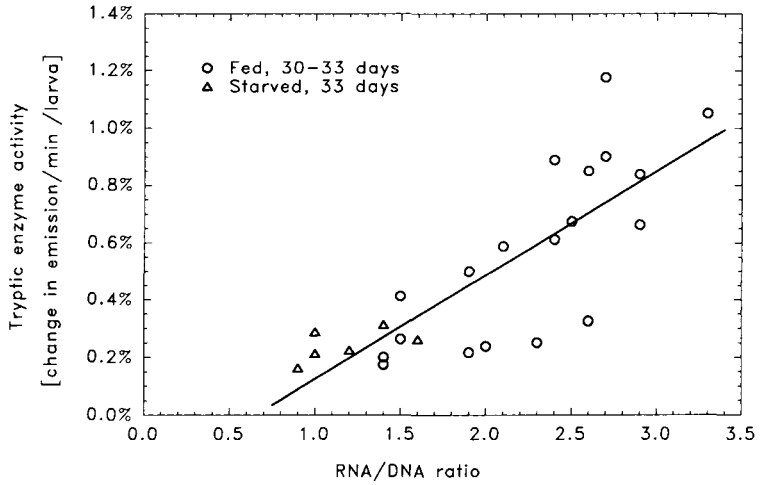

Figure 1. Relationship between tryptic enzyme activity and RNA/DNA ratio of 30–33-day-old laboratory-reared fed and starved herring larvae (mean length 13.2±2.1 mm). The starved larvae were deprived of food for 6 days. The curve was fitted by linear regression.

sample") while the other larvae were exposed to different starvation intervals. In a feeding/starving experiment larvae were fed for 7 days and deprived of food for 3 days. Feeding conditions were the same as in the laboratory rearing experiment.

Tryptic enzyme activity and RNA/DNA ratios in wild-caught herring larvae were not determined on identical homogenates but on parallel samples.

Measurement of tryptic enzyme activity

The tryptic enzyme activity measurements were performed according to the fluorescence technique described by Ueberschär (1988) with a modification: the temperature during the measuring procedure was kept constant at 30°C. The relative fluorescence enhancement was recorded every 2 min over a maximum time period of 10 min. Emission rates are given per time unit and larva.

Measurements of RNA/DNA ratios

The determination of RNA and DNA contents was performed according to the fluorescence technique described by Clemmesen (1988) with some modifications (Clemmesen, 1990). The analytical procedure involves purification of larval tissue homogenates and subsequent fluorescence-photometric measurement using a specific nucleic acid fluorescent dye – ethidium bromide (EB) – for DNA and RNA. In order to measure the DNA content of a sample, RNA was enzymatically digested by RNase and the remaining DNA was determined with EB. The methodology used is sensitive to all three kinds of RNA (mRNA, tRNA and rRNA) but the change in rRNA will be the mainly measurable one, since 85–94% of the RNA in a cell is ribosomal (Young, 1970).

Results

Comparison of the methods using identical larvae

Tryptic activity and RNA/DNA ratio were determined on identical homogenates of laboratory-reared herring larvae. Results of measurements on feeding as well as on starving larvae are given in Figure 1. Low RNA/DNA ratios as found in starving larvae are correlated with low tryptic enzyme activities. Higher RNA/DNA ratios as found in the majority of the fed larvae are correlated with high enzyme activities. This relationship is described by a significant linear regression:

$y = 0.0088 + 0.022x$, $r = 0.81$, $n = 25$.

Comparison of tryptic enzyme activity and RNA/DNA ratios of field-caught herring larvae

Tryptic enzyme activities and RNA/DNA ratios from the first starvation experiment are given in Figure 2. Three days of food deprivation led to a significant (t-test, $p \leq 0.05$) decrease of tryptic enzyme activity (10.8% of the initial value) and then showed a constant low course towards the end of the starvation experiment. With increasing starvation interval a decrease in the RNA/DNA ratio can be observed. Three days of food deprivation led to a significant (t-test, $p \leq 0.05$) decrease to 57% of the initial value. After 6 days an average of 38% was reached. The decrease in the ratio follows an asymptotic

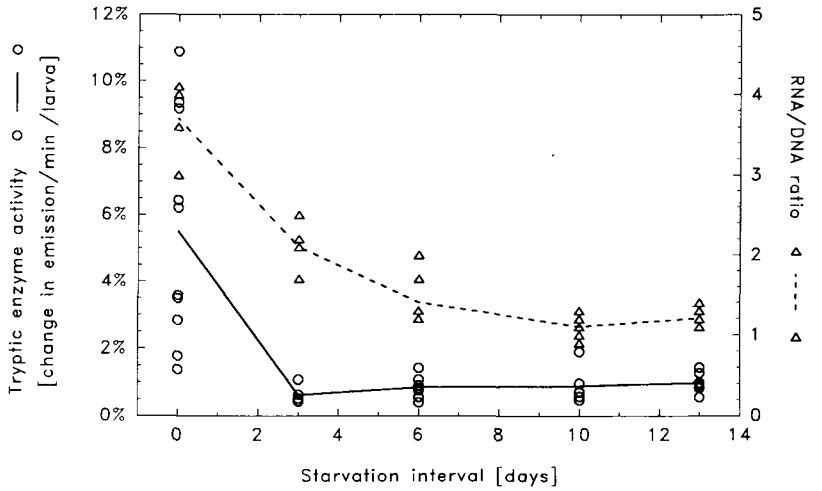

Figure 2. Comparison of tryptic enzyme activity and RNA/DNA ratio in wild-caught herring larvae in the first starvation experiment. Samples were taken after four different starvation intervals. Mean larval length for tryptic enzyme activities was 23.7 ± 2.9 mm and 23.6 ± 1.9 mm for RNA/DNA ratio measurements. Data points represent individually-measured larvae. The lines give the mean course of the individual values.

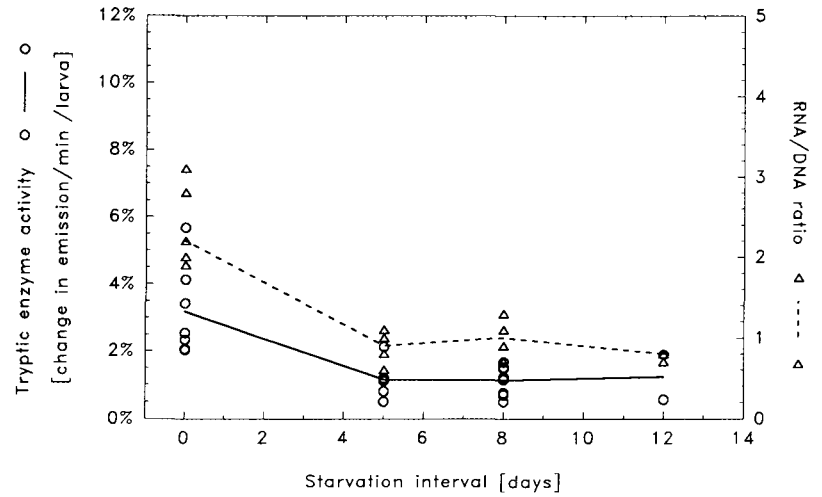

Figure 3. Comparison of tryptic enzyme activity and RNA/DNA ratio in wild-caught herring larvae in the second starvation experiment. Samples were taken after three different starvation intervals. Mean larval length for tryptic enzyme activities was 24.8 ± 2.5 mm and 24.1 ± 2.7 mm for RNA/DNA ratio measurements. Data points represent individually-measured larvae. The lines give the mean course of the individual values.

course levelling-off at a ratio of about 1.1 (30% of the average initial value).

The second field sample taken 1 week later showed significantly (t-test, $p \leq 0.05$, Fig. 3) lower trypsin values and RNA/DNA ratios. Compared to the field sample in the first experiment only 52% of the mean value for tryptic enzyme activity and 59% of the RNA/DNA value were found. However, the decrease of tryptic enzyme activity and RNA/DNA ratios during the starvation period was comparable to the first experiment (mean 18.8% for trypsin and 40.9% for RNA/DNA ratio after 5 days of food deprivation). The development of tryptic enzyme activity and RNA/DNA ratio in the feeding/starving experiment is given in Figure 4. After the first 4 days of the experiment, the tryptic enzyme activity decreased under feeding conditions to the level of the starving larvae in experiment 1 and 2, whereas the RNA/DNA ratio remained more or less constant. The larvae fed for 7 days showed an average tryptic enzyme activity which is comparable to the initial values of the field sample. The RNA/DNA ratio showed a slight increase on day 7 of this experiment compared to the field sample. Deprived of food for 3 days, the larvae reacted with a decrease in the tryptic enzyme activity as well as in the RNA/DNA ratio.

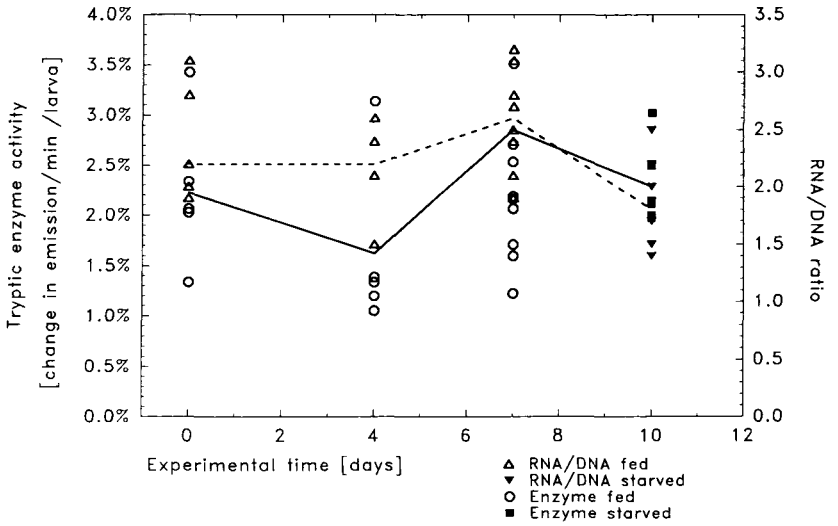

Figure 4. Comparison of tryptic enzyme activity and RNA/DNA ratio in the feeding/starving experiment with wild-caught herring larvae. Larvae were fed in the laboratory for 7 days and subsequently starved for 3 days. Three samples were taken after different feeding/starving intervals. Mean larval length for tryptic enzyme activities was 26.1 ± 1.7 mm and 24.6 ± 2.5 mm for RNA/DNA ratio measurements. Data points represent individually-measured larvae. The lines give the mean course of the individual values.

In all experiments the individual variability for both indicators decreased in the starving groups.

Discussion

To test the relationship between tryptic enzyme activity and RNA/DNA ratio as indicators for the nutritional condition of fish larvae the methods were applied to identical larval material. A significant linear correlation between both parameters was shown with laboratory-reared herring larvae kept under constant nutritional condition. When larvae experience a change in the feeding condition in the range of hours to a few days (short-term), the correlation between the two parameters will not be as good as shown in the constantly-fed laboratory-reared larvae, since both indicators react on different positions in the metabolism. The release and activation of trypsinogen is triggered by the ingestion of food (Fänge and Grove, 1979; Pedersen *et al.*, 1987) and since the analytical procedure applied for the determination of tryptic enzyme activity does not distinguish between exogenous and endogenous proteases, part of the measured activity may be attributed to trypsin-like enzymes from the ingested food organisms. These mechanisms may explain the fast reaction of tryptic enzyme activity to changing feeding regimes.

The RNA/DNA ratio does not reflect changes in food availability in the short-term range. A sudden change in food condition leads to an increase in the activity of the ribosomes but not in their numbers. Since the methodology used measures only the ribosome content, longer time periods of either food availability or deprivation are necessary to lead to a measurable change in the RNA/DNA ratio.

The assumption that both indicators react differently in the short-term range was confirmed by the results presented since the tryptic enzyme activity reacted faster to food deprivation than the RNA/DNA ratio (Fig. 2). In the long-term range both methods reflect the change in the nutritional condition in the same manner. Longer starvation periods result in a constantly low tryptic activity, as well as a low RNA/DNA ratio.

The initial values in the second field sample show larvae with lower tryptic enzyme activity and lower RNA/DNA ratios compared to the first sample 1 week earlier, indicating that food supply might have been poor for several days prior to sampling. Although the Schlei Fjord is highly eutrophic, periods with low food abundance have been described (Schnack, 1972).

The unexpected low tryptic enzyme activity in the feeding/starving experiment after 4 days of food supply (Fig. 4) might be due to handling stress (transport, change of habitat, adaption to different food organisms). The RNA/DNA ratio seems to be independent of these kind of short-term influences. The larvae which were starved for 3 days show a low tryptic enzyme activity as well as a low RNA/DNA ratio and confirm the results of the other experiments.

This study shows that both indicators are linearly correlated when long-term fed or starved larvae are compared. In the short-term range up to 3 days they differ, since the tryptic activity reacts faster to changes in food supply. In the long-term range both methods detect starving larvae and this could be useful in evaluating the

nutritional condition of fish larvae in the field. The tryptic enzyme activity can serve additionally to monitor short-term variations in food quantity and quality or feeding activity.

Acknowledgements

Part of this study was financed by the Bundesminister für Forschung und Technologie, MFE 0541/9. We are indebted to Prof. Nellen and Prof. Schnack for their support.

References

Buckley, L. J. 1979. Relationship between RNA/DNA ratio, prey density and growth rate in Atlantic Cod (*Gadus morhua*) larvae. Journal of the Fisheries Research Board of Canada, 36: 1497–1502.

Buckley, L. J. 1984. RNA/DNA ratio: an index of larval fish growth in the sea. Marine Biology, 80:291–298.

Buckley, L. J., and Lough, R. G. 1987. Recent growth, chemical composition and prey field of haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) larvae and post larvae on Georges Bank, May 1983. Canadian Journal of Fisheries and Aquatic Sciences, 44:14–25.

Clemmesen, C. 1987. Laboratory studies on RNA/DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. Journal du Conseil International pour l'Exploration de la Mer, 43:122–128.

Clemmesen, C. 1988. A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. Meeresforschung, 32:134–143.

Clemmesen, C. 1989. RNA/DNA ratios of laboratory-reared and wild herring larvae determined with a highly sensitive fluorescence method. Journal of Fish Biology 35 (Supplement A): 331–333.

Clemmesen, C. 1990. Improvements in the fluorimetric determination of the RNA and DNA content in individual marine fish larvae. ICES/CM 1990/L:98, 14 pp.

Fänge, R., and Grove, D. 1979. Digestion. *In* Fish physiology, 8. Ed. by W. S. Hoar, D. J. Randall, and J. R. Brett.Academic Press, New York, pp. 161–260.

Henshaw, E. C., Hirsch, C. A., Morton B. E., and Hiatt, B. M. 1971. Control of protein synthesis in mammalian tissue through changes in ribosome activity. Journal of Biological Chemistry, 246:436–446.

Hjelmeland, K., Huse, I., Jørgensen, T., Molvik, G., and Raa, J. 1984. Trypsin and trypsinogen as indices of growth and survival potential of cod (*Gadus morhua* L.) larvae. *In* The propagation of cod, *Gadus morhua*, Part 1, pp. 189–211. Ed. by E. Dahl, D. S. Danielssen, E. Moksness, and P. Solemdal. Institute of Marine Research, Flødevigen Biological Station.

Lauff, M., and Hofer, R. 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. Aquaculture, 37:335–346.

Lied, E., Lund, B., and von der Decken, A. 1982. Protein synthesis *in vitro* by epaxial muscle polyribosomes from cod, *Gadus morhua*. Comparative Biochemistry and Physiology, 72B:187–193.

Martin, F. D., Wright, D. A., Means, J. C., and Setzler-Hamilton, E. F. 1985. Importance of food supply to nutritional state of larval striped bass in the Potomac river estuary. Transactions of the American Fisheries Society, 114:137–145.

Pedersen, B. H., Nilssen, E. M., and Hjelmeland, K. 1987. Variations in the content of trypsin and trypsinogen in larval herring (*Clupea harengus*) digesting copepod nauplii. Marine Biology, 94:171–181.

Pedersen, B. H., and Hjelmeland, K. 1988. Fate of trypsin and assimilation efficiency in larval herring (*Clupea harengus*) following digestion of copepods. Marine Biology, 97:467–476.

Schnack, D. 1972. Nahrungsökologische Untersuchungen an Heringslarven. Ber. dt. wiss. Kommn. Meeresforsch., 22:273–343.

Ueberschär, B. 1985. Untersuchungen über die Verdauungsenzyme von Fischlarven. Diploma Thesis, Institut für Meereskunde, University of Kiel.

Ueberschär, B. 1988. Determination of the nutritional condition of individual marine fish larvae by analyzing their proteolytic enzyme activities with a highly sensitive fluorescence technique. Meeresforschung, 32:144–154.

Young, V. R. 1970. The role of skeletal and cardiac muscle in the regulation of protein metabolism. *In* Mammalian protein metabolism, pp. 585–674. Ed. by H. H. Munro. Academic Press, London.