

RNA-DNA RATIO IN EXTRACTS OF FISH SCALES

CAN INDICATE FEEDING CONDITION

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

The ratio of RNA to DNA (R/D) has been used as an index of growth and feeding condition of fish (review: Bulow 1987), and R/D in extracts of whole larval or early juvenile fish have been used to examine variability in growth and condition in individuals (review: Buckley *et al.* 1999). These approaches typically require sacrificing the fish, which precludes repeated sampling.

However, a method using teleosts scales, which are covered with live tissue (Kardong 1998), offers a new approach. In the present study we measured the nucleic acid content of extracts of scales removed from juvenile cod (*Gadus morhua*). Our hypothesis was that R/D in scale extracts would provide a non-lethal, non-surgical method for estimating the recent feeding condition and growth of live fish, which could be performed repeatedly on the same individuals. We performed experiments in which juvenile cod were fed either different quantities, or different types, of food. Scales were periodically removed and assayed for nucleic acids to calculate R/D.

Methods and Results

Assay of RNA and DNA

Juvenile cod reared from eggs (Buckley *et al.* 2000) were anaesthetized with MS-222 (75 mg/L, Sigma), measured (total length) and weighed. Scales or epidermal scrapings were removed from the left side of each fish just above the pectoral fin. Samples were stored in extraction buffer (1 % n-lauroylsarcosine, 5 mM TRIS, 0.5 mM EDTA, pH 7.5) at -80°C.

Total RNA and DNA were quantified using the fluorescent dye ethidium bromide (EB) as previously described (Wagner *et al.* 1998). Briefly, nucleic acids were extracted from tissue with extraction buffer by shaking for 1 hour at room temperature. The samples were centrifuged and the supernatant was retained. EB was added to RNA standards, DNA standards and samples. RNA was estimated from the difference between total fluorescence (prior to an RNase treatment) and fluorescence after treatment with RNase (DNA fluorescence).

Statistical tests

T-tests, and repeated measures analysis of variance (RM-ANOVA) were performed with StatView 4.51. Significant differences within each sampling period were analyzed with the Bonferroni post-hoc test. Statistical tests were considered significant at $P < 0.05$.

Experiment I

Extracts from scales of juvenile cod (mean length 18.8 cm) exhibited no significant endogenous or residual fluorescence when compared to the RNA and DNA standards. We concluded that in our samples extracted from scales of juvenile cod, endogenous and residual fluorescence were not a significant source of error in the quantitation of RNA and DNA.

Experiment II

Juvenile cod (mean length 18.8 cm) were either starved, or fed (2.5% to 7% body weight/day; BW/D). Two replicate tanks were used for starved fish; four for the fed fish, with nine fish in each tank. The fish were sampled every two weeks. After the fourth inventory (day 42), the tanks were divided in half, with four fish on one side and five on the other. One half of the tank was maintained at the original treatment, while in the other half the treatment was reversed. So for one week some of the originally starved fish were then fed ("Starved/Fed"), and some of the originally fed fish were starved ("Fed/Starved").

Length of the fed and starved groups was significantly different only on day 42 (by RM-ANOVA and Bonferroni post-hoc test). The mean R/D of fed fish was significantly higher than that of the starved fish on days 15, 28 and 42 (Figure 1). On day 49, mean R/D in the fed treatment was significantly greater than in the Fed/Starved treatment (Figure 1).

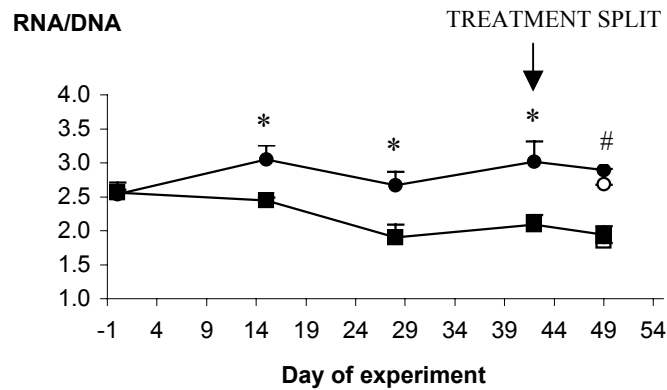


Figure 1. Mean R/D (\pm sd) of fish in Experiment I. Fed (filled circles) and Starved (filled squares) on days 0, 15, 28 & 42 were compared by RM-ANOVA, followed by the Bonferroni post-hoc test; *=significant difference, $P < 0.05$. Comparisons of Fed vs. Fed/Starved (open circle), and Starved vs. Starved/Fed (open square) on day 49 were performed by t-test; #=significant difference, $p < 0.05$.

Experiment III

Sixty fish (mean length 2.8 cm) were divided among six tanks: three fed a commercial diet at 5% BW/D, and three fed a diet of mixed copepods at 5% BW/D. Inspection of these fish with a dissecting microscope revealed no discernable scales.

Mean weight and total length were significantly greater for the fish fed the commercial diet (by t-test; Figure 2A & 2B). R/D in tissue scrapings also differed, with fish fed the commercial diet exhibiting a significantly higher mean R/D than the fish fed copepods (Figure 2C).

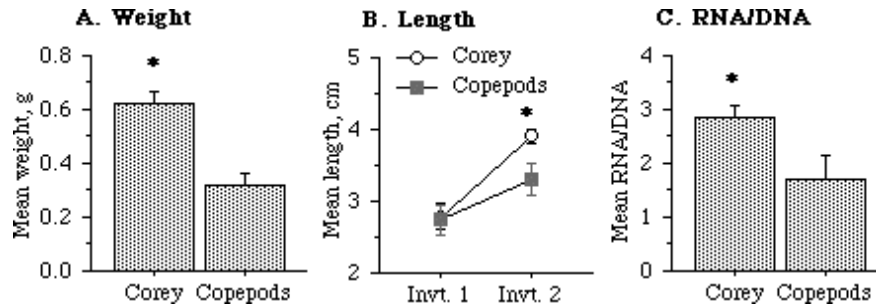


Figure 2. Size and R/D of juvenile cod with no apparent scales: mean weight (A), mean length (B), and mean R/D of epidermal scrapings (C) at inventory 2. Fish were fed a commercial diet or wild-caught plankton; inventory 2 (Invt. 2) was 21 d after inventory 1 (Invt. 1). Means represent 3 tanks (\pm 1 sd). The treatments were compared by t-test; *=significant difference, $P < 0.05$

Conclusions

Our data indicate that RNA and DNA in scale extracts can be measured and that the R/D of scale tissue is a responsive measure of feeding condition and growth. R/D of scale extracts was more sensitive to a change in feeding condition than measurements of fish weight or length. We also calculated R/D in surface scrapings from juvenile cod that had no scales. The R/D from this tissue reflected differences in diet, indicating that the method is not limited to fish with discernable scales.

Applying this fluorescence-based assay of R/D to samples extracted from scales would be a useful tool for fisheries research, especially in studies that require repeated sampling of the same individual. Samples can be obtained quickly from individual animals, and sample collection is non-lethal and non-surgical.

References

- Buckley, L., E. Caldarone, and T-L. Ong. 1999. RNA-DNA ratio and other nucleic-acid-based indicators for growth and condition of marine

fishes. *Hydrobiologia* 401:265-277.

- Buckley, L.J., T.M. Bradley, and J Allen (2000) Production, quality and low temperature incubation of eggs in Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). *Journal of the World Aquaculture Society* 31:22-29.
- Bulow, F.J. 1987. RNA-DNA ratios as indicators of growth in fish. Pages 45-64 *in* Summerfelt, R.C. and G.E. Hall editors. *The Age and Growth of Fish*. Iowa State University Press, Iowa.
- Kardong, K.V. 1998. *Vertebrates: Comparative Anatomy, Function, Evolution*, 2nd edition. WCB/McGraw-Hill, Massachusetts.
- Wagner, M., E. Durbin and L. Buckley. 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. *Marine Ecology Progress Series* 162:173-181.

