Mar. Freshwater Res., 1996, **47**, 301–7

Relative RNA Content as a Measure of Condition in Larval and Juvenile Fish

Iain M. Suthers[A], Jennifer J. Cleary[A,B], Stephen C. Battaglene[B,C] and Rachael Evans[A]

[A]*School of Biological Science, University of New South Wales, Sydney, NSW 2052, Australia.*
[B]*New South Wales Fisheries, Port Stephens Research Centre, Salamander Bay, NSW 2301, Australia.*
[C]*Present address: ICLARM, PO Box 438, Honiara, Solomon Islands.*

Abstract. The RNA:DNA ratio for first-feeding larvae (12 days after hatching, 4–6 mm standard length, SL) of Australian bass (Percichthyidae, *Macquaria novemaculeata*) exposed to four different feeding regimes over 8 days was found to be insensitive to the level of starvation. An alternative condition index based on residuals derived from the regression of RNA on SL showed significant differences over the course of the experiment; these reflected the four feeding treatments. Field collections of juvenile monacanthids (*Paramonacanthus otisensis*, 10–30 mm SL) from a local estuary revealed no significant difference in RNA:DNA ratio at three sites over six weeks. A residual-based index (RNA on dry weight) showed parallel fluctuations at all sites; they were positively correlated with water temperature. The RNA:DNA ratio depends on the difference in fluorescence between total nucleic acids (TNA, using thiazole orange) and DNA (using Hoechst 33258) to calculate RNA, as there is no RNA-specific fluorescent dye. The numerator is thus dependent on the denominator, and measurement error may be compounded in the ratio, exacerbating potential variability in the index. Ratios may also be variably correlated with age or size and consequently may erroneously indicate condition or growth in larger and faster-growing fish.

Introduction

Accurate assessment of larval condition or health has widespread application in the fields of fisheries management, aquaculture and fish ecology, as an indicator of sub-lethal effects. Many indices have been used to determine nutritional condition in larval fish, involving morphological (e.g. Ehrlich *et al.* 1976; Wright and Martin 1985), histological (e.g. Ehrlich *et al.* 1976; Theilacker 1986; Martin and Wright 1987; Bisbal and Bengston 1995) and biochemical techniques (e.g. Buckley 1984; review in Ferron and Leggett 1994).

The RNA:DNA ratio is based on the assumption that the concentration of RNA changes as a function of the amount of protein synthesis occurring in the cell (Bulow 1970; Regnault and Luquet 1974; Buckley 1981, 1984). The amount of DNA in a cell is relatively constant for all somatic cells for a given species (Buckley 1980, 1984), and so indicates the number of cells in an organism. Therefore, the RNA:DNA ratio is considered a measure of protein synthetic activity per cell (Raae *et al.* 1988). Larvae in good condition tend to have a higher RNA:DNA ratio than those in poorer condition (e.g. Robinson and Ware 1988; Clemmesen 1993). It is reported that the ratio can respond to changes in environmental conditions within 1–3 days (Martin and Wright 1987), and the ratio has been used to give a measure of instantaneous growth in the field, avoiding periodic measurements (Buckley 1981; Clarke *et al.* 1989).

Recent studies have advocated caution in the use of RNA:DNA ratios on the basis of techniques (extraction method, McGurk and Kusser 1992; type and quality of RNA and DNA standards, Caldarone and Buckley 1991; endogenous fluorescence in juvenile fish, Canino and Caldarone 1994), or lack of sensitivity (Bergeron *et al.* 1991; Richard *et al.* 1991; Mathers *et al.* 1994). Other workers have advocated caution in the use of ratios in removal of the allometric effect of size (e.g. Reist 1985; Packard and Boardman 1987), but rarely in the context of a widely used technique such as the RNA:DNA ratio (Hovenkamp and Witte 1991). Here, the numerator and/or the denominator can vary to produce a similar ratio.

The present study examined the ability of the RNA:DNA ratio to assess nutritional condition on the basis of whole homogenates of larvae from controlled and field conditions. Condition of Australian bass larvae *(Macquaria novemaculeata)* was altered in four feeding treatments in the laboratory, and recently settled monacanthid juveniles (*Paramonacanthus otisensis*) were studied in a large estuary. The aims were firstly to compare the ratio to a residual-based index derived from RNA content and an independently determined variable such as standard length or dry weight, and secondly to evaluate a relatively new and

1323-1650/96/020301$05.00

sensitive dye, thiazole orange, by means of a simplified extraction technique (after Berdalet and Dortch 1991).

Materials and Methods

Laboratory Study

Specimens of adult *M. novemaculeata*, a catadromous percichthyid, were captured from Seaham Weir, Williams River, New South Wales, in July 1992, and twelve pairs were induced to spawn. Four 60-L aerated flow-through glass aquaria were set up in a room at constant temperature (19±1°C) with a 12-h light–dark cycle. Water temperatures varied between 18° and 19°C, and salinities varied between 33 and 36.

On the first day after hatching (Day 1), larvae were stocked into four tanks at a density of 750 ± 250 larvae. During final absorption of the yolk-sac and oil globule, larvae were fed rotifers (*Brachionus plicatilis*) once daily at 10 mL^{-1} (Talbot *et al.* 1990) from Day 8 until most of the larvae were feeding (Day 12). After Day 12, the feeding regimes were altered as follows. *(1)* FF: fully fed; fed approximately 15 rotifers mL^{-1} per day. *(2)* FS: fully starved; no food was given from Day 13. *(3)* S1: starved 1 day, then fed from Day 14 at 15 rotifers mL^{-1}. *(4)* S3: starved 3 days, then fed from Day 16 at 15 rotifers mL^{-1}.

The experiment continued until 100% mortality occurred in the FS tank on Day 20. A sample of 3–12 larvae from each treatment was taken daily with a dip-net before feeding. For logistical reasons, there was only one tank per treatment, and consequently our conclusions are tank-specific and are assumed to be in response to the starvation treatment. Standard lengths (SLs) were measured under a binocular microscope before the individual larvae were stored in liquid nitrogen for total nucleic acids (TNA) and DNA analysis. Indices were compared across treatments on Days 14, 16, 18 and 20 with separate 1-factor analyses of variance (ANOVAs) (significance level at $P < 0.05$). There was no significant heterogeneity of the data (Bartlett's test). Treatments were compared within each day by Tukey's test adjusted for unbalanced data.

Field Study

Botany Bay is a large (56 km^2) embayment in south-eastern Sydney, New South Wales, fed by the Cooks and Georges rivers. The bay is shallow, with a maximum depth of 19 m in the dredged shipping channel. Three shallow seagrass and sand sites, separated by 6–12 km, were sampled with an epibenthic sled (40 × 80 cm mouth opening, 500 μm mesh) on 1, 18 and 27 June and 17 July 1993. At each site, three–four 5-min tows were conducted over a broad area to avoid sampled regions, with water temperature being recorded at the surface and near bottom at the beginning and end of sampling. The dominant species was the dusky leatherjacket, *Paramonacanthus otisensis* (Monacanthidae). Modifications to the determination of RNA and DNA used in the laboratory study are shown in Fig. 1, with the major difference being to freeze dry the larvae before they were homogenized. Ratios and residual indices were compared among sites on each sampling date, with a separate ANOVA for each date because fish did not occur at all sites on all dates. Then, indices were compared at Site 3 across the four dates in a separate ANOVA.

Analytical Procedure

The technique involved the use of thiazole orange (TO) to determine TNA concentration (Coulter Electronics T-1376) and Hoechst 33258 (HO) for DNA determinations (Sigma), in conjunction with Tris–Ca^{2+} buffer (Fig. 1, adapted from the double-fluorochrome method for phytoplankton developed by Berdalet and Dortch 1991). Larvae were thoroughly homogenized by hand in 10-mL Halfu homogenizing glass tubes with 1 mL ice-cold Tris–Ca^{2+} buffer, and were then sonicated for 30 s before being combined with the dyes. The concentrations of HO and TO used (Fig. 1) were appropriate for the larvae (Cleary 1992) and juveniles (Evans 1993) used in this study. All materials were maintained on crushed ice.

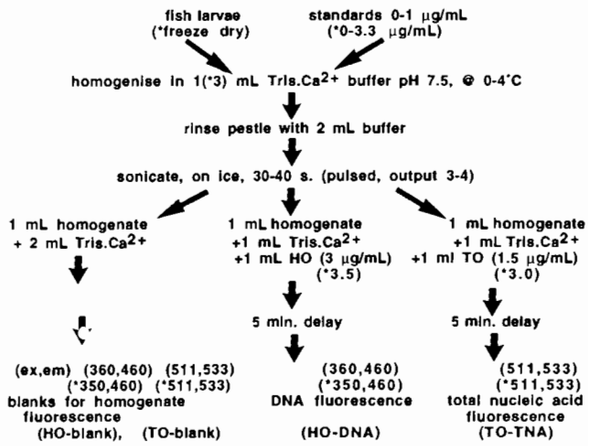

Fig. 1. Flow chart of analytical procedure (after Berdalet and Dortch 1991): em, emission wavelength; ex, excitation wavelength; HO, Hoechst 33258; TO, thiazole orange; TNA, total nucleic acid; *modification for dusky leatherjacket *Paramonacanthus otisensis*.

Standard nucleic acid solutions were made from single-stranded calf-thymus DNA Type I (Bioscientific D8899) and baker's yeast RNA Type III (Bioscientific R7125). Three standard curves were run daily (DNA–HO; DNA–TO; RNA–TO). RNA and DNA concentrations were calculated from the three standard curves and the fluorescence values obtained for each sample. A Perkin Elmer fluorescence spectrophotometer measured the nucleic acid–dye complex fluorescence for the larval fish and standard curves. This machine was run on the DC mode with the slit widths of both the excitation and emission wavelengths set at 10 nm. Spike retrievals of RNA and DNA usually averaged 90% and 91% respectively. The freeze-drying procedure for the field-caught fish had no significant effect on RNA or DNA content of replicate homogenates.

Residual Indices

An index of condition was derived from the residuals of an overall, simple linear regression of ln(RNA+1) on SL (laboratory) or dry weight (field), once an ANCOVA had established that there was no significant difference among treatments ($P > 0.05$).

The ANCOVA was used only to test for homogeneity of slopes; it was not completed to compare intercepts because each treatment included fish sampled on Day 13–20 and we wished to obtain an individual index for each fish to compare to the ratio.

Results

Laboratory Study

The size of the larvae ranged from approximately 4·3 mm to 5·6 mm during the course of the experiment (Fig 2). Fully fed fish grew on average 0·11 mm day^{-1} from Day 13 to 20, while fully starved grew on average 0·01 mm day^{-1}. The S1 and S3 fish were intermediate, growing 0·07 and 0·04 mm day^{-1} respectively (Fig. 2). FF larvae were significantly larger than S3 and FS larvae on Day 20, but not on Day 18, 16 or 14.

The amount of RNA and DNA increased with SL, with DNA showing a curvilinear response to SL (Fig. 3). From

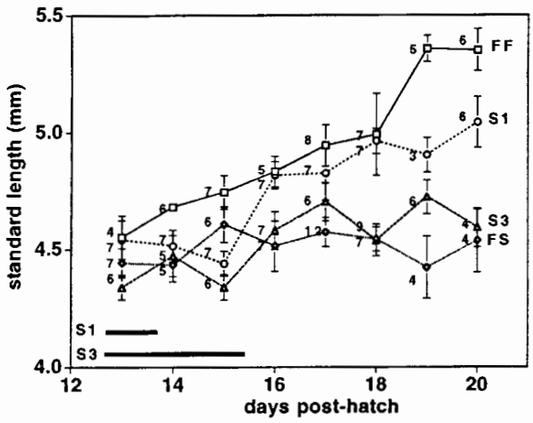

Fig. 2. Change in average standard length of *M. novemaculeata* larvae over the four feeding treatments: FF (□), fully fed through entire experiment; S1 (○), starved on Day 13 then fed; S3 (△), starved on Days 13, 14 and 15 then fed; and FS(◇), fully starved. Solid bars show periods of starvation for S1 and S3. Error bars are standard error.

the relationship of ln(RNA+1) on ln(SL), residual indices were calculated. Residual plots show no remaining trends or non-linearity.

The mean RNA:DNA ratios for each of the four treatments for each day show no systematic trend across days or among the treatments (Fig. 4*a*).

The mean residual condition (RNA–SL, Fig. 4*b*) shows a trend of enhanced condition for the FF and S1 treatments over S3 and FS. On Day 16 and Day 18 the mean FF residual indices were significantly greater than FS and S3 (Tukey's $P < 0{\cdot}02$), but FF was never significantly different to S1. By Day 20, S3 and FS indices increased and none of the treatments were significantly different (Fig. 4*b*). The FS treatment showed a rapid rise in apparent condition 2 days before death, due to a relative increase in RNA with respect to SL.

Field Study

Between 6 and 24 *Paramonacanthus otisensis* were caught at each site on each date, ranging between 10 and 42 mm SL (1–250 mg dry weight), although fish >30 mm SL (120 mg) were excluded from biochemical analysis (n = 110

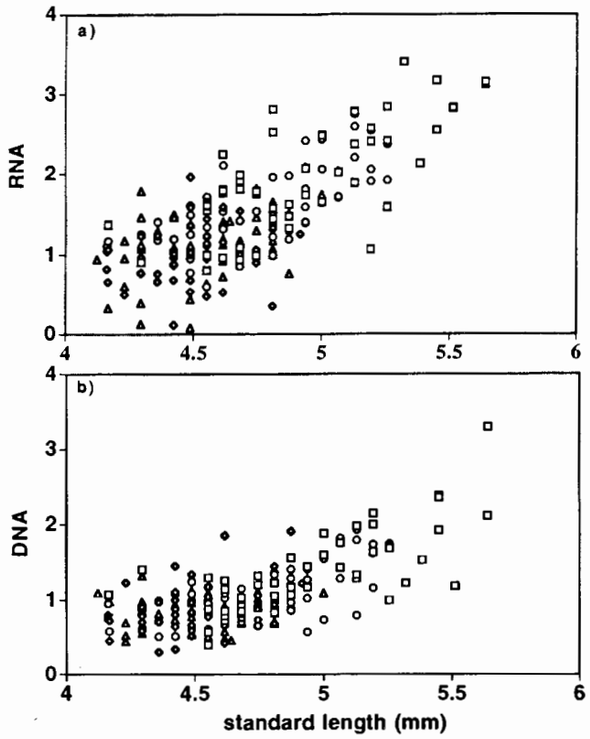

Fig. 3. Relationship of *(a)* RNA (μg) and *(b)* DNA (μg) on standard length (mm, SL) of *M. novemaculeata*. FF (□), fully fed through entire experiment; S1 (○), starved on Day 13 then fed; S3 (△), starved on Days 13, 14 and 15 then fed; and FS(◇), fully starved. ln(RNA + 1) = −3·17 + 2·61*ln(SL), (n = 203, r^2 = 0·46), ln(DNA + 1) = −2·21 + 1·89 * ln(SL), (n = 203, r^2 = 0·42).

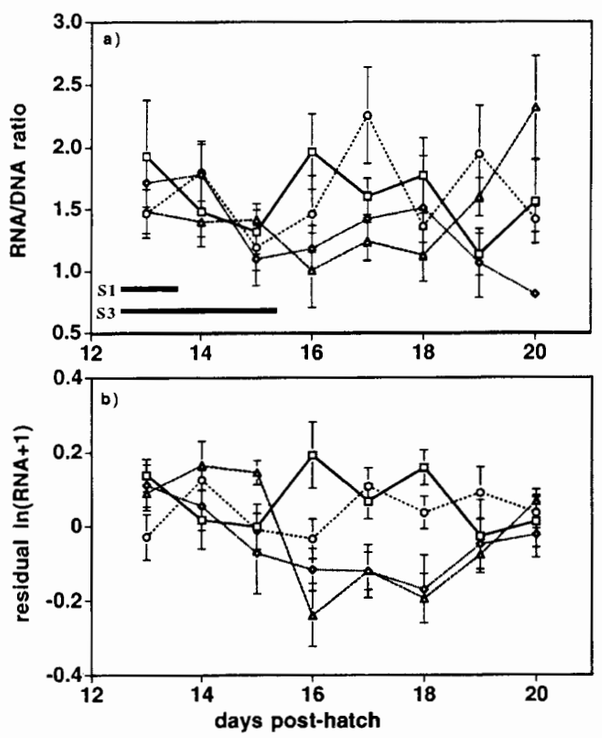

Fig. 4. Change in *(a)* average RNA:DNA ratio and *(b)* average residual of ln(RNA + 1) on ln(SL), of *M. novemaculeata* larvae over the four feeding treatments. FF (□), fully fed through entire experiment; S1 (○), starved on Day 13 then fed; S3 (△), starved on Days 13, 14 and 15 then fed; and FS(◇), fully starved. Solid bars show periods of starvation for S1 and S3. Error bars are standard error.

for nucleic acid analysis). If it is assumed that the same population was being sampled, growth at Site 1 was slight (0·11 mm day^{-1}) and then declined. At Sites 2 and 3 average SL increased at approximately 0·40 and 0·37 mm day^{-1} respectively, but by mid July growth declined or ceased (0·18 and –0·04 mm day^{-1} respectively, Fig. 5).

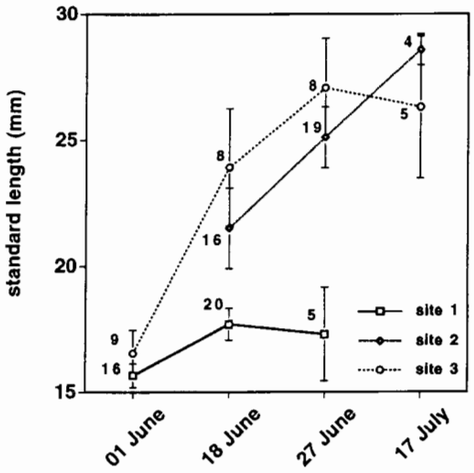

Fig. 5. Change in average SL of *P. otisensis* over the three sites and four dates in 1993. Error bars are standard error. Sample size is indicated.

RNA and DNA were linearly related to dry weight (Fig. 6), with no pattern in the residuals and there was no significant difference in the slopes among sites or dates (ANCOVA $P > 0·3$). Consequently, a common regression was fitted to calculate individual residual condition indices.

Average RNA:DNA ratios showed no significant difference among sites on each date (four 1-way ANOVAs, $P > 0·1$) and no significant difference or trends among dates at Site 3 (Fig. 7*a*). The RNA–DW residuals also showed no significant differences among sites or dates (Fig. 7*b*) but did exhibit parallel trends across all sites. The ratio showed no causal relationship with temperature (Fig. 8*a*), whereas the residuals from RNA–DW were significantly correlated with water temperature ($r^2 = 0·61$, Fig. 8*b*).

Discussion

RNA:DNA Ratio and Residual Indices

The RNA:DNA ratio derived from the technique in Fig. 1 proved unsuitable as a diagnostic tool of larval condition in Australian bass (Fig. 4*a*), despite starvation of the larvae under controlled laboratory conditions. In the field, RNA:DNA ratios of dusky leatherjacket did fluctuate synchronously at three sites over six weeks, although there was no relationship with the 4°C range in water temperature (Fig. 8*a*).

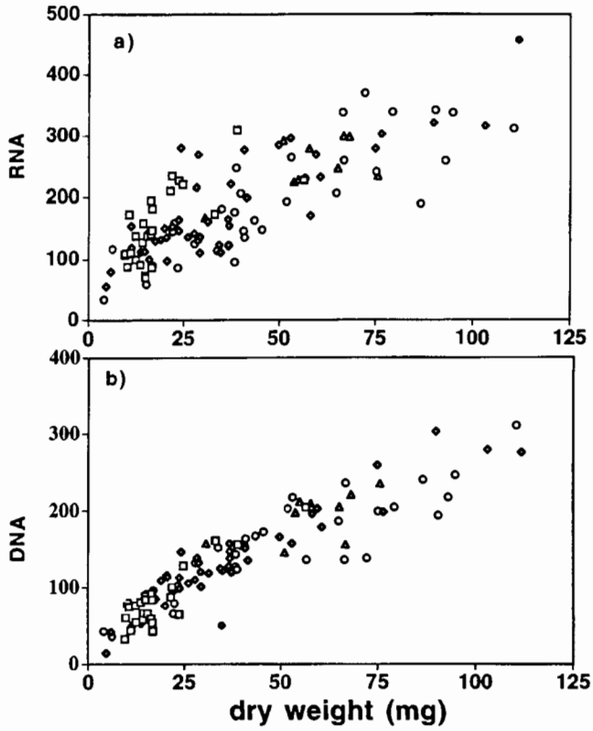

Fig. 6. Relationship of *(a)* RNA (μg) and *(b)* DNA (μg) on dry weight (mg) for *P. otisensis*. Symbols as in Fig. 3. RNA = 94·39 + 2·46*DW (n = 112, $r^2 = 0·60$), DNA = 48·24 + 2·26*DW (n = 112, $r^2 = 0·82$)

In contrast, the use of residual indices did reveal significant effects of starvation in the laboratory. In the field, the residuals revealed a general decline in condition on the third sampling date (27 June) at one site, and all sites exhibited parallel fluctuations over 6 weeks that were correlated with water temperature (Fib. 8*b*). With other biochemical techniques, however, RNA:DNA ratios are normally correlated with temperature (e.g. Buckley 1982). Although the ratio was correlated with the residual index overall (*M. novemaculeata*, $r = 0·52$; *P. otisensis*, $r = 0·65$), these results illustrate where possible errors in the calculation of DNA and TNA are magnified in the ratio. As RNA (the numerator) is derived from DNA (the denominator), any errors in the measurement of DNA and TNA are magnified in the final ratio (Packard and Boardman 1987; Hovenkamp and Witte 1991).

Another potential source of error arises if the ratio is correlated with size (e.g. Buckley *et al.* 1984; Robinson and Ware 1988; Westerman and Holt 1994; Clemmesen 1994), although we did not find the correlation of the ratio with either SL or dry weight that had been observed in other studies (e.g. Buckley *et al.* 1984; Buckley and Lough 1987). However, some studies have shown an allometric effect of the RNA:DNA ratio (Westerman and Holt 1988; Canino *et*

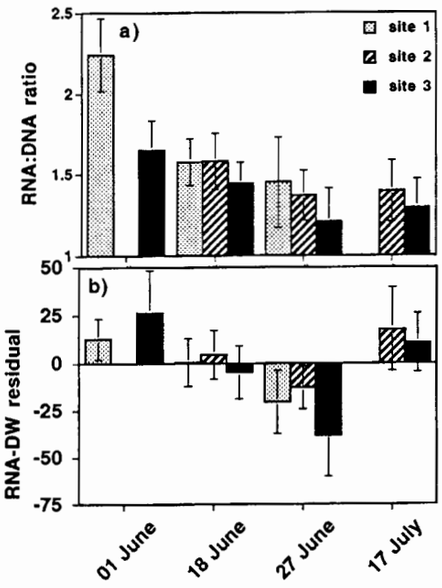

Fig. 7. *(a)* Average RNA:DNA ratio for *P. otisensis* across the four sampling occasions and three sites. Site 2 was not sampled on 1 June. Error bars are standard error. *(b)* Average residual of RNA–DW.

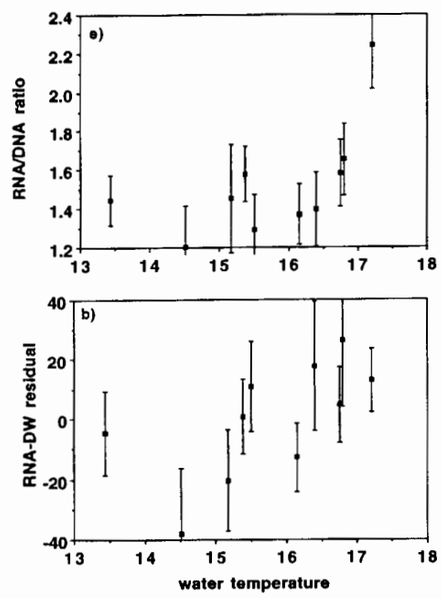

Fig. 8. *(a)* Average RNA : DNA ratio and *(b)* average RNA–DW residual of *P. otisensis* at each site and date with respect to average water temperature.

al. 1991; Clemmesen 1994), where presumably DNA does not account for increasing body size. Naturally, the RNA:DNA ratio will be higher in those fish that are growing, and those fish will be bigger. Nevertheless, the effect of size is still incorporated in the ratio and may confound studies with unknown (field) conditions. In the field, Robinson and Ware (1988) observed over time that larger larvae had a higher RNA:DNA ratio, but they were careful to note that generally within a day there was no significant correlation between ratio and SL. Alternatively, the ratio itself can be de-trended (e.g. Canino *et al.* 1991).

Residuals from a simple linear regression explicitly remove the effect of size and provide a powerful way around ratios because linear models can be built to incorporate the effect of temperature, for example. The ANCOVA is a complementary analysis by comparing slopes and treatments (e.g. Bailey *et al.* 1995), and it can provide an individual index such as a residual. Different techniques and species can differ in their yields of nucleic acid (Caldarone and Buckley 1991; McGurk and Kusser 1992), so residuals are solely relative within any one study and the inter-comparison of ratios is not valid. Similarly, the conversion of RNA:DNA ratios to daily protein growth rates using Buckley's (1984) generalized equation is not recommended without running an intercalibration (Canino and Caldarone 1994).

In the present study, because DNA is used in the calculation of RNA, standard length (e.g. Bailey *et al.* 1995) or dry weight were used as independent variables to standardize RNA. In juvenile fish, other components such as lipid can influence size not accounted for by the number of cells and by DNA. By using least-squares regression (rather than geometric-mean regression), we assumed that the measurement error of dry weight (or SL) is small relative to that of RNA.

The laboratory study produced a surprising result in the residual analysis for the FS treatment, with the proportion of RNA increasing markedly two days before death (Fig. 4*b*). A similar trend in the RNA–DW residual was evident in an independent data-set for larval wrasse (E. Caldarone, unpublished data). These larvae may be resorting to cannibalism, but such a trend is not evident in the ratio (Fig. 4*a*). This appears to be the result of physiological stress, and such fish are morphologically distinctive and would presumably be removed by predation in the field.

The laboratory study also presented difficulties in generating residuals, not encountered with field larvae, because the starved treatments had a smaller size range of larvae, increasing the possibility of non-homogeneity of slopes between treatments. In our analyses from the laboratory and field, the ANCOVA was used solely to ensure homogeneity of slopes, because the larvae came from samples on different dates. We assumed that slopes were similar among days. Ideally, the ANCOVA should incorporate day and treatment effects, but sample size was insufficient on each day to confidently produce a regression. The laboratory example had only one tank per feeding

treatment because of logistic constraints and, although the trends observed could be tank effects, we assumed that they represented the feeding treatments.

Thiazole Orange and RNA Residuals

Berdalet and Dortch (1991) found fluorescence yield of TO was about 20 fold that of ethidium bromide, and this was evident in our trials (Cleary 1992). Our extraction procedure was particularly simple, involving only homogenization and sonication (Berdalet and Dortch 1991). More-complex extraction procedures, however, may result in higher RNA and DNA yields than do simpler ones (McGurk and Kusser 1992). Simple additions to the technique which may result in increased yields include the addition of heparin or other proteases (e.g. Karsten and Wollenberger 1977), centrifugation steps and more complex washes (e.g. Clemmesen 1987) and detergents (sarcosyl, Caldarone and Buckley 1991). For phytoplankton, Berdalet and Dortch (1991) found that the best recovery of TNA was obtained with Tris–Ca^{2+} buffer, without the use of proteases, lysozymes, or detergents, although this has yet to be investigated in larval and juvenile fish.

RNA:DNA ratios are useful in detecting the occurrence of starvation in the ocean, on the basis of laboratory calibration (e.g. Buckley and Lough 1987; Canino *et al.* 1991). However, ratios have the potential either to produce spurious variation due to correlated errors or to size, or to absorb meaningful variation, causing the ratio to appear insensitive (Bergeron *et al.* 1991; Richard *et al.* 1991; Mathers *et al.* 1994). Use of ANCOVA to determine homogeneity of slopes, and then generation of residuals from an overall regression, provides a relative, individual index that is size controlled and specific to the technique and species. Comparisons between studies can be made concisely with the regression.

Acknowledgments

We gratefully acknowledge NSW Fisheries Port Stephens Research Centre, Salamander Bay, for the use of the hatchery facilities and for the support provided. This study was supported in part by a small ARC grant to I.M.S. We thank B. Talbot, P. Beevers and M. Lockhart for their technical assistance and support. We are also grateful to B. Sherwin, B. Houlden, P. J. Cleary, E. Berdalet and Bonnie Chan for their advice, and in particular to E. Caldarone and our referees.

References

Bailey, K. M., Canino, M. F., Napp, J. M., Spring, S. M., and Brown, A. L. (1995). Contrasting years of prey levels, feeding conditions and mortality of larval walleye pollock *Theragra chalcogramma* in the western Gulf of Alaska. *Marine Ecology Progress Series* **119**, 11–23.

Berdalet, E., and Dortch, Q. (1991). New double-staining technique for RNA and DNA measurement in marine phytoplankton. *Marine Ecology Progress Series* **73**, 295–305.

Bergeron, J-P., Boulhic, M., and Galois, R. (1991). Effet de la privation de nourriture sur la teneur en ADN de la larve de sole (*Solea solea* L.). *ICES Journal of Marine Science* **48**, 127–34.

Bisbal, G. A., and Bengston, D. A. (1995). Description of the starving condition in summer flounder, *Paralichthys dentatus*, early life history stages. *Fisheries Bulletin (US)* **93**, 217–30.

Buckley, L. J. (1980). Changes in ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and effect of starvation. *Fisheries Bulletin (US)* **77**, 703–8.

Buckley, L. J. (1981). Biochemical changes during ontogenesis of cod *(Gadus morhua L.)* and winter flounder *(Pseudopleuronectes americanus)* larvae. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer* **178**, 547–52.

Buckley, L. J. (1982). Effects of temperature on growth and biochemical composition of larval winter flounder (*Pseudopleuronectes americanus*). *Marine Ecology Progress Series* **8**, 181–6.

Buckley, L. J. (1984). RNA–DNA ratio: an index of larval fish growth in the sea. *Marine Biology* **80**, 291–8.

Buckley, L. J., and Lough, R. G. (1987). Recent growth, biochemical composition, and prey field of larval haddock *Melanogrammus aeglefinus* and Atlantic cod *Gadus morhua*, on Georges Bank. *Canadian Journal of Fisheries and Aquatic Science* **44**, 14–25.

Buckley, L. J., Turner, S. I., Halavik, T. A., Smigielski, A. S., Drew, S. M., and Laurence, G. C. (1984). Effects of temperature and food availability on growth, survival, and RNA–DNA ratio of larval sand lance (*Ammodytes americanus*). *Marine Ecology Progress Series* **15**, 91–7.

Bulow, F. J. (1970). RNA–DNA ratios as indicators of recent growth rates of a fish. *Journal of the Fisheries Research Board of Canada* **27**, 2343–9.

Caldarone, E. M., and Buckley, L. J. (1991). Quantitation of DNA and RNA in crude tissue extracts by flow injection analysis. *Analytical Biochemistry* **199**, 137–41.

Canino, M. F., Bailey, K. M., and Incze, L. S. (1991). Temporal and geographic differences in feeding and nutritional condition of walleye pollock larvae *Theragra chalcogramma* in Shelikof Strait, Gulf of Alaska. *Marine Ecology Progress Series* **79**, 27–35.

Canino, M. F., and Caldarone, E. M. (1994). Modification and comparison of two fluorometric techniques for determining nucleic acid contents of fish larvae. *Fishery Bulletin (US)* **93**, 158–65.

Clarke, A., Rodhouse, P. G., Holmes, L. J., and Pascoe, P. L. (1989). Growth rate and nucleic acid ratio in cultured cuttlefish *Sepia officinalis* (Mollusca:Cephalopoda 1). *Journal of Experimental Marine Biology and Ecology* **133**, 229–40.

Cleary, J. (1992). RNA and DNA indices of condition in larval fish. B. Sc. (Honours) Thesis, University of New South Wales. 112 pp.

Clemmesen, C. M. (1987). Laboratory studies on RNA/DNA ratios of starved and fed herring *(Clupea harengus)* and turbot *(Scophthalmus maximus)* larvae. *Journal du Conseil, Conseil International de l'Exploration de la Mer* **43**, 122–8.

Clemmesen, C. M. (1993). Improvements in the fluorimetric determination of the RNA and DNA content of individual marine fish larvae. *Marine Ecology Progress Series* **100**, 177–83.

Clemmesen, C. (1994). The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. *Marine Biology* **118**, 377–82.

Ehrlich, K. F., Blaxter, J. H. S., and Pemberton, R. (1976). Morphological and histological changes during the growth and starvation of herring and plaice larvae. *Marine Biology* **35**, 105–18.

Evans, R. (1993). Comparison of juvenile fish condition within Botany Bay. B.Sc. (Honours) Thesis, University of New South Wales. 63 pp.

Ferron, A., and Leggett, W. C. (1994). An appraisal of condition measures for marine fish larvae. *Advances in Marine Biology* **30**, 217–303.

Hovenkamp, F., and Witte, J. I. J. (1991). Growth, otolith growth and RNA/DNA ratios of larval plaice *Pleuronectes platessa* in the North Sea 1987 to 1989. *Marine Ecology Progress Series* **70**, 105–16.

Karsten, U., and Wollenberger, A. (1977). Improvements in the ethidium bromide method for direct fluorometric estimation of DNA and RNA in cell and tissue homogenates. *Analytical Biochemistry* **77**, 464–70.

Mathers, E. M., Houlihan, D. F., and Burren, L. J. (1994). RNA, DNA and protein concentrations in fed and starved herring *Clupea harengus* larvae. *Marine Ecology Progress Series* **107**, 223–31.

Martin, F. D., and Wright, D. A. (1987). Nutritional state analysis and its use in predicting striped bass recruitment: laboratory calibration. *American Fisheries Society Symposium* **2**, 109–14.

McGurk, M. D., and Kusser, W. C. (1992). Comparison of three methods of measuring RNA and DNA concentrations of individual Pacific herring, *Clupea pallasi*, larvae. *Canadian Journal of Fisheries and Aquatic Science* **49**, 967–74.

Packard, G. C., and Boardman,T. J. (1987). The misuse of ratios to scale physiological data that vary allometrically with body size. In 'New Directions in Ecological Physiology'. (Eds M. E. Feder, A. F. Bennett, W. W. Burggren and R. B. Huey.) pp. 216–35. (Cambridge University Press: Cambridge.)

Raae, A. J., Opstad, I., Kvenseth, P., and Walther, B.T. (1988). RNA, DNA and protein during early development in feeding and starved cod *(Gadus morhua* **L.**) larvae. *Aquaculture* **73**, 246–59.

Reist, J. D. (1985). An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. *Canadian Journal of Zoology* **63**, 1429–39.

Regnault, M., and Luquet, P. (1974). Study by evolution of nucleic acid content of prepuberal growth in the shrimp, *Crangon vulgaris*. *Marine Biology* **25**, 291–8.

Richard, P., Bergeron, J. P., Boulhic, M., Galois, R., and Le Ruyet, J. (1991). Effect of starvation on RNA, DNA and protein content of laboratory-reared larvae and juveniles of *Solea solea*. *Marine Ecology Progress Series* **72**, 69–77.

Robinson, S. M. C., and Ware, D. M. (1988). Ontogenetic development of growth rates in larval Pacific herring, *Clupea harengus pallasi*, measured with RNA–DNA ratios in the Strait of Georgia, British Columbia. *Canadian Journal of Fisheries and Aquatic Science* **45**, 1422–9.

Talbot, B., Smith, I., and Piddington, J. (1990). Mass production of the rotifer *Brachionus plicatilis*. NSW Agriculture and Fisheries Agdex 486/00 No. 2/90. 4 pp.

Theilacker, G. H. (1986). Starvation induced mortality of young sea-caught jack mackerel *Trachurus symmetricus*, determined with histological and morphological methods. *Fisheries Bulletin (US)* **84**, 1–17.

Westerman, M. E., and Holt, G. J. (1988). The RNA–DNA ratio: measurement of nucleic acids in larval *Sciaenops ocellatus*. *Contributions in Marine Science* **30**(Suppl.), 117–24.

Westerman, M., and 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.

Wright, D. A., and Martin, F. D. (1985). The effect of starvation on RNA:DNA and growth of larval striped bass, *Morone saxatalis*. *Journal of Fish Biology* **27**, 479–85.

Manuscript received 13 October 1995; revised and accepted 19 April 1996