Australian Journal of Ecology (1998) **23**, 265–273

Bigger? Fatter? Or is faster growth better? Considerations on condition in larval and juvenile coral-reef fish

IAIN M. SUTHERS
School of Biological Science, University of New South Wales, Sydney, NSW 2052, Australia

Abstract Analyses of condition data are conspicuous by their paucity in the extensive tropical reef-fish literature. Researchers typically quantify abundance at settlement, with little regard for the demonstrably variable quality of newly settled fishes. Condition may be functionally classified by indices of growth (e.g. the RNA–DNA index or peripheral growth increments of the otolith), starvation (e.g. height of midgut mucosal cells), storage (e.g. lipid content), or morphometry (e.g. dry weight/length^3), all of which are variably correlated with each other. At present all indices are species-, stage-, technique- and therefore often investigation-specific, as laboratory-reared larvae for calibrating field-collected condition indices are often specific to the rearing procedure. RNA indices are particularly appropriate for estimating larval condition. In pelagic juveniles, or in recently settled juveniles, the width of peripheral growth increments of the otolith estimates average growth rate in length or dry weight during the previous few days, which discerns increasing from decreasing condition. Increment width changes in otoliths are particularly responsive to starvation events, and are correlated with RNA indices. Growth indices have great potential for determining which individuals were growing faster, thereby reducing their pelagic duration, and thus increasing their survival potential. The recent debate regarding whether bigger larvae have better survival could be re-addressed, by determining if larvae with faster growth indices have relatively enhanced survival.

Key words: condition, coral reefs, fish, growth, health, larvae, otolith growth.

INTRODUCTION

Many critical issues in the management and ecology of coral-reef fishes involve the larval and pelagic juvenile stages (Doherty & Williams 1988). Yearly or monthly variation in larval abundance and settlement is of prime concern to many reef-fish ecologists, but few recognize that the quality, health or condition of larvae can significantly vary in time and space. Condition of individual larvae can provide an objective means to assess the relative effect of environmental conditions such as prey abundance (Theilacker *et al.* 1996). Food limitation is not only a problem for larvae. Pelagic juvenile gadoids (Bailey 1989; Suthers *et al.* 1989, 1992), plaice (Karakiri *et al.* 1989) and juvenile Pacific salmon (Peterman 1987) all exhibit variable growth in response to food supply. In one of the few studies of condition in tropical fishes, Kerrigan (1996) reported that two pomacentrids at settlement had a biochemical composition coefficient of variation (CV) of > 30%, while the CV for size and age were only 5 and 7%, respectively. McCormick and Molony (1993) observed that the lipid content of pre-settlement goatfish (*Upeneus tragula*) can vary by ± 30% within a sample, and by ± 50% between samples. The cause, or significance of this variation in larval quality is uncertain, but demonstrates that condition is not constant in time and space.

Larval condition has been suggested as an important covariate to pro-rate larval abundance data to improve predictions of recruitment (Frank & McRuer 1989; Theilacker *et al.* 1996), although explicit tests of larval condition influencing survival potential are rare (e.g. Neilson *et al.* 1986). Nevertheless, differential growth affects the duration of the larval–pelagic juvenile stage, and thus the time when individuals are especially vulnerable to starvation and predation (Shepherd & Cushing 1980; Houde 1987). Therefore, larvae with faster growth probably contribute proportionally more to recruitment than those with slower growth (e.g. Setzler-Hamilton *et al.* 1987; Bailey *et al.* 1995; Meekan & Fortier 1996; Theilacker *et al.* 1996).

Unfortunately, few researchers have applied techniques of larval condition to the early life-history stages and recruitment of tropical reef fishes. For example, differential condition and its effects on survival of larvae

Accepted for publication June 1997.

could generate cross-Great Barrier Reef patterns in adult abundance (Williams 1991), as systematic cross-shelf variation in larval and pelagic juvenile condition has been observed off California (Theilacker 1986) and Nova Scotia (Suthers *et al.* 1989). Therefore, spatial variation in the abundance of new recruits might be altered if settlers in better condition had greater survival during the first few months.

In this paper, my aim is to outline the use and pitfalls of larval condition indices, and thereby to encourage reef-fish ecologists to consider the quality, or condition, of larvae as well as their quantity in studies of settlement and relative survival. Techniques available to assess larval-fish condition (reviewed by Ferron and Leggett 1994) are briefly grouped below according to the condition process, rather than technique, along with some cautionary notes. Growth indices, such as the popular RNA:DNA index and peripheral otolith growth, are examined in detail as possible measures of survival potential. Many reef-fish ecologists may be more comfortable with the otolith technique, because it has a considerable supporting literature. The difficulty of calibrating condition indices using laboratory-reared larvae is explored. I conclude with a discussion of how larval condition, as a proxy for growth and potential survival, can provide a focus in coral-reef fish studies.

SOME CAUTIONARY ISSUES

The use of morphometric indices illustrates four issues that are relevant to condition indices in general. Morphometric indices simply involve measuring starvation-sensitive variables (e.g. body depth, dry weight), which are standardized for size by less starvation-sensitive variables (e.g. eye diameter, length). These indices have been used successfully to compare larval condition between years and seasons, but have been criticized for having low sensitivity to short-term events (e.g. less than a week), such as upwelling or river plumes (Ferron & Leggett 1994).

Effect of body size

Condition indices must be independent of body size. This can be achieved by a ratio of the starvation-sensitive to the less-sensitive variable. However, not all ratios are independent of body size, because of the allometric relationships of morphologies (or biochemical content). Ratios, such as Fulton's K ($100 \times \text{weight}/\text{length}^3$), are often still variably correlated with size and the actual exponent should be determined (rather than assuming 3). Because of allometry, a number of studies have suggested careful analysis before using ratios to remove the effect of size (e.g. Reist 1985; Packard & Boardman 1987; Hare & Cowen 1995; Suthers *et al.* 1996).

Size-independent indices can be achieved using residuals derived from a regression of the two variables, providing that the regression slope is not significantly different among regions or treatments. Size-independence of residuals, or ratios, may be confounded by ontogenetic changes in body proportions during flexion. These problems can be easily determined and solved, or obviated by using smaller size ranges. Another robust approach is to generate size-independent condition indices by a principal component analysis of a number of morphometric (or biochemical) variables (McGurk 1985), and discarding the first component, which is usually highly correlated with size.

Specificity of each index

Indices are species- and stage-specific, and cannot be easily compared between studies because of the variable allometry noted earlier. Morphometric condition indices are also technique-specific, because of the shrinkage that can occur during capture in the net, and the type of preservation (see Theilacker 1986 for a comprehensive treatment of the assessment and correction of shrinkage). Many studies assume that differential shrinkage of length and body depth is consistent among individuals within a study. Shrinkage tends to be less of a problem in post-flexion fish as their vertebrae ossify. This species- or stage-specificity of condition is particularly problematic in speciose waters, where finding sufficient quantities of the same species for comparison between environments can be difficult (e.g. Rissik & Suthers 1996).

Temporal response of indices

The temporal response of an index to a starvation period is not precisely known. This 'latency' (Ferron & Leggett 1994) is probably size-, species-, temperature- and certainly technique-dependent (see below), varying from hours to as much as weeks. Latency is one reason why condition indices derived from the same individual are poorly correlated (Suthers *et al.* 1992), and why most indices are unable to distinguish between increasing or decreasing condition. For example, an individual in below-average condition may have been advected into and subsequently caught in zooplankton-rich waters, but its condition may not yet reflect its new environment.

Calibration of indices

Relative condition indices of ocean-caught larvae should be calibrated with the condition of known, laboratory-reared larval condition (Ehrlich 1975; Theilacker 1978, 1986; McGurk 1985; Martin & Wright 1987; Håkanson 1989). After all, what does a

condition index actually mean in terms of starvation period, or growth rate? However, care should be taken when extrapolating the attributes of laboratory-reared larvae to ocean-caught larvae (see Ferron & Leggett 1994 for details). For example, larvae reared in the laboratory are usually fatter (herring: Balbontin *et al.* 1973; anchovy: Håkanson 1989 and references therein), they exhibit less shrinkage, have less histological variation, less RNA and DNA relative to length (Ferron & Leggett 1994), and may require prey concentrations 2–3 orders of magnitude greater than in the field to survive. Laboratory-reared anchovy larvae had hearts 40% longer than ocean-caught larvae of the same size (9 mm), using the same preservation procedure (Arthur 1980). The size of the container (10 L *vs* 100 L) used for rearing also had a significant effect on the size and histological condition of jack mackerel larvae (Theilacker 1980). In summary, it is virtually impossible to simulate the ocean environment in the laboratory to realistically calibrate condition indices, although the trends observed in the laboratory are obviously a useful start. Calibration of indices in outdoor mesocosms (> 10 m^3) may solve some of these concerns.

STARVATION INDICES

Histological indices are among the least exploited and potentially most powerful as they can indicate increasing and decreasing condition, and they represent the only true starvation index. The technique entails scoring histological sections of various tissues (gut, liver, muscle and brain), relative to experimentally reared material, which requires an experienced observer (Theilacker 1986; Margulies 1993). Height of the midgut mucosal cells obviates the need for specialist interpretation and is highly sensitive (responding in 1–2 days), and these cells are least susceptible to autolysis (Theilacker 1986; Theilacker & Watanabe 1989). This technique was developed using a straight section through the non-coiled larval anchovy gut. Attempts to apply this technique to reared, first-feeding Australian bass larvae (*Macquaria novemaculeata*) were confounded by a coiled gut, requiring sections to be reliably cut relative to a characteristic axis (Nock & Suthers, unpubl. data).

STORAGE INDICES

Measurement of carbohydrate, protein or lipid content in individual larvae is now reasonably routine, revealing biochemical responses to dietary shifts or ontogenetic habitat changes. The analyses of fish during starvation indicate inverse relationships between the body and viscera in protein, lipid and carbohydrate content (Molony 1993a,b), as well as among total lipid, protein and water content (Kerrigan 1996). A simple histological measure of energy storage is the area of selectively stained glycogen granules or lipid globules (O'Connell & Paloma 1981).

Lipid indices generally relate the 'storage lipid' (triacylglycerol; TAG) to the 'structural lipid' (such as various sterols) to account for size (Håkanson 1993). Whole-fish extracts of TAG may vary in different species (e.g. cod have 2–3 times the amount of TAG per unit dry weight than capelin; Suthers *et al.* 1992). Lipid analyses have been successfully applied to coral-reef settlement-stage fish (e.g. McCormick & Molony 1993; Kerrigan 1996), temperate larvae (e.g. Håkanson 1993) and invertebrate larvae (e.g. Fraser 1989; Ouellet *et al.* 1995). These biochemical analyses require larvae to be quickly sorted from the plankton at sea, and stored at < −80°C until analysis. One concern with total lipid analyses is the contribution of gut content lipid which can be as high as 95% of total lipid (Lochman *et al.* 1996). This discrepancy may also explain the lack of correlation previously observed between morphometric, otolith and lipid indices in pelagic juvenile cod, sampled over a large range in zooplankton-prey biomass (Suthers *et al.* 1992). On the other hand, lipid analyses can provide additional information on food chains in frontal and stratified habitats, by identifying flagellate- or diatom-specific lipid biomarkers (St John & Lund 1996).

GROWTH INDICES: RELATIVE RNA CONTENT

RNA indices are generally the most favoured of condition indices (Ferron & Leggett 1994), and are well correlated with independently measured growth rates (Westerman & Holt 1994). The RNA index estimates total protein synthesis varying in proportion to the total amount of RNA, standardized for size by total DNA (indicative of the total number of cells). The RNA:DNA index is well suited to use with early larvae, and its response to food or starvation is, in general, consistent with other indices of larval growth (Hovenkamp 1990; Ferron & Leggett 1994; Clemmesen 1996; Suthers 1996). Furthermore, it is relatively cost-effective. With the advent of highly sensitive fluorescent dyes for nucleic acid, 80–90 individual larvae (> 10 μg dry weight) can be assessed, per person per day (Caldarone & Buckley 1991; Canino & Caldarone 1995).

There are, however, potential problems with the use of RNA indices, as outlined earlier for the morphometric indices. For example the RNA:DNA ratio may not be independent of body size (e.g. Buckley *et al.* 1984; Robinson & Ware 1988; Clemmesen 1994; Westerman & Holt 1994), as DNA content cannot

account for other aspects of size or weight such as bone and lipid, especially in older larvae. Therefore total RNA may indeed be greater relative to DNA in larger (and growing) larvae, or the index may be confounded if there are any body size differences between treatments or oceanographic regions. Analysis of a consistent size range is one solution.

RNA concentration is obtained by either subtracting DNA fluorescence from total nucleic acid (TNA) fluorescence (e.g. Richard *et al.* 1991), or by sequentially digesting TNA with RNAase and DNAase (e.g. Canino *et al.* 1991; Clemmesen 1994). As RNA (the numerator) is not derived independently from DNA (the denominator), any errors in the measurement of DNA and TNA will be magnified in the final ratio (Packard & Boardman 1987; Hovenkamp & Witte 1991; Suthers *et al.* 1996). As an example, RNA:DNA condition data of a larval wrasse, *Tautoga onitis* (E. Caldarone, unpubl. data), determined using flow injection analysis and ethidium bromide (Caldarone & Buckley 1991) is examined. Using this data set, the RNA:DNA ratio is correlated with size between 10 and 30 mg dry weight ($r = 0.73$), and independent of size > 30 mg, indicating that trends in the ratio may reflect dry weight rather than condition. Consequently, the ratio shows a monotonic increase and decline for the fed and starved treatments, respectively (until Day 7, Fig. 1a). On the other hand, the residual indices (RNA on dry weight, Fig. 1b) show a horizontal trend for the fed treatment, while in the starved treatment, the mean residual indices show a monotonic decline until a rapid rise on the penultimate day, which was not observed with the ratio. The mean residuals have larger error bars (Fig. 1b) but show an entirely different relationship with respect to time compared with the ratio. Potentially informative variation in the nucleic acid content is being presumably absorbed by the ratio. ANCOVA is used to determine homogeneity of slopes between treatments, and then an overall regression produces the residuals for an individual index. This index is thus size controlled, and specific to the technique and species (e.g. Bailey *et al.* 1995). Comparisons between studies can be made concisely with the regression, which can explicitly incorporate covariates such as temperature (Suthers *et al.* 1996).

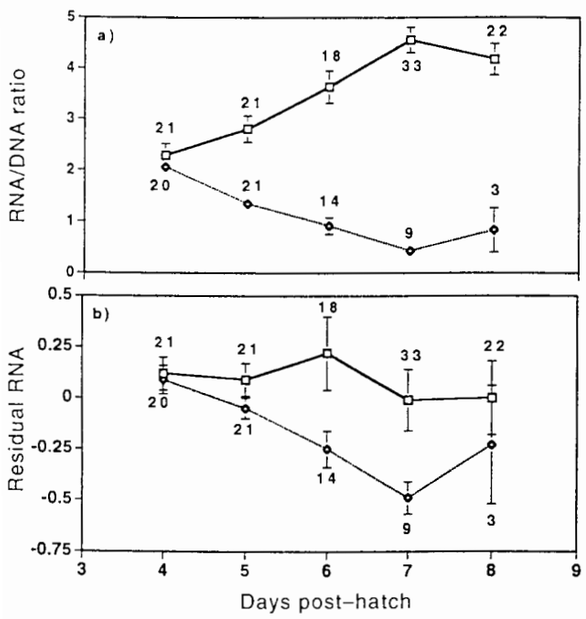

Fig. 1. Fed (-□-) and starved (···○···)tautog larvae (Labridae) from Day 4 to Day 8 post hatch. Larvae were reared in the laboratory and on Day 4 post-hatch, larvae were split into a fed and starved group, and sampled until Day 8 post-hatch. The Fed tank received rotifers at a concentration of 4 per ml, and the larvae grew at approximately 5 $mg.d^{-1}$. (a) RNA:DNA ratio (R/D), and (b) residual of RNA on dry weight ($RNA = -1.09 + 0.12*DW$, $n = 182$, $r^2 = 0.85$). Sample size (n). Error bars are standard error. The ratio trends are misleading, as the ratio is highly correlated with dry weight ($r = 0.73$). Data from E. Caldarone (unpubl.).

Relative RNA content drops after a starvation event (1–2 days after, Martin & Wright 1987; 3–4 days, Clemmesen 1996), although a diel cycle in RNA content is apparent in some studies (Rooker & Holt 1996 and references therein). The RNA:DNA ratio in 20-day-old red drum larvae (*Sciaenops ocellatus*) fluctuated over a 48 h test period by about 50%, declining between midnight and 04.00 h. If this is a common phenomenon, then it has important implications for assessing condition between night and day sampling.

The biochemical extraction methods of RNA have a large effect on yields. Recent research has shown that whole-body extracts of RNA (or of lipid) can differ substantially compared to extracts from muscle tissue alone. RNA:DNA ratios of whole-fish samples of *S. ocellatus* were 30–35% of values from white muscle tissue alone (Rooker & Holt 1996). The effect of gut content RNA on total RNA is minimal (of the order of < 5%, Clemmesen 1996). In sum, it seems we must carefully distinguish between condition indices based on assays of whole-body versus muscle samples, and where possible use one tissue type (e.g. the muscular tail).

Yields of RNA (and presumably other biochemical yields) can also vary significantly with regard to other aspects of the extraction method. Therefore ratios or regressions are only relevant within methods, and should not be compared across studies unless methodologies are standardized. For example yields will be enhanced by the addition of centrifugation and biochemical washes, or with the addition of detergents. The type and quality of RNA and DNA standards used to convert dye fluorescence units to actual nucleic acid concentrations can influence the slope of standard curves (Caldarone & Buckley 1991). In general, baker's yeast RNA and calf thymus DNA are the usual standards, but the potential use of fish RNA and DNA

standards may alter apparent yields. Furthermore, juvenile fish tend to have higher endogenous fluorescence than larval fish, highlighting the necessity of blanks. In sum, a unified protocol is obviously required, such as the methods of Canino and Caldarone (1995).

The existing RNA–DNA techniques are quite varied. Therefore, application of Buckley's (1984) generalized equation, which converted RNA:DNA ratios to daily protein growth rates in eight species (based on absorbance rather than fluorescence), is not recommended without inter-calibration (Canino & Caldarone 1995). No study has updated Buckley's (1984) multi-species equations, or established if protein growth rates under similar conditions are comparable among species. Development of a generalized protein growth-rate equation, for the species-rich Australian waters would be a tremendous advance.

GROWTH INDICES: PERIPHERAL OTOLITH GROWTH

Peripheral daily growth increments of otoliths have great potential as a growth index, and have obvious appeal when used in conjunction with other condition indices. First, it may be possible to determine average recent growth estimates in mm d^{-1} or mg d^{-1} (i.e. it is not an index). Second, the recent growth history can indicate increasing or decreasing condition. Most requirements of the method (e.g. fish size–otolith size) can be routinely assessed as part of any ageing study. Since the discovery of daily growth increments 25 years ago, more than 170 studies on greater than 300 species (Secor *et al.* 1992) have critically examined the techniques, interpretation and validation of otolith increments. The same level of scrutiny is presently being focused on RNA–DNA indices.

Two prime concerns with the use of this recent growth measure as a condition index are the temporal response of increment widths to a starvation event and, in particular, the relationship of the otolith increment width to somatic growth. Some studies have detected a decrease in increment width within 1–3 days of starvation (e.g. Govoni *et al.* 1985; Eckmann & Rey 1987; Bailey & Stehr 1988; Maillet & Checkley 1990; Suthers & Sundby 1993; Peterson 1996). However, other studies report a 1–2 week time lag between otolith growth and somatic growth (120-day-old ambassid, Molony & Choat 1990; 40-day-old monocanthid, Milicich & Choat 1992). Back-calculating change in fish size over the previous week may be confounded by potential decoupling of the fish size–otolith size relationship (e.g. Secor & Dean 1989; Hoff & Fuiman 1993). For example, non-linear growth curves may occur close to settlement. While otolith decoupling may be species specific, or even laboratory specific, it is possible that it is less likely to persist in the ocean, where predation may selectively remove slow-growing individuals.

The decoupling issue can be addressed by use of the biological intercept method of back-calculation over the peripheral increment widths, using each individual fish's body size–otolith size relationship (Campana 1990; Campana & Jones 1992). Also, potential errors in back-calculation are vastly reduced over the proportionally small interval such as the outer periphery of the otolith. The actual growth over a selected time interval can be back-calculated, provided there is a known (preferably linear) otolith size–body length relationship (Campana 1990). Similarly, changes in dry weight over the 14 days before capture have also been back-calculated (Suthers & Sundby 1996) by using a ln(radius)–ln(dry weight) relationship. Allometric changes in the fish size–otolith size proportionality, from yolk-sac to pelagic juvenile stages (Hare & Cowan 1995), may potentially confound this method. Therefore, where these sorts of comparisons are made they should be within an ontogenetic stage (e.g. post-flexion).

Peripheral otolith growth has been examined in at least 12 studies of 11 species, to determine the effect of environmental variation and hydrographic features on growth (Table 1). These studies have confirmed that while water temperature is a fundamental variable controlling larval fish growth (e.g. Campana & Hurley 1989), prey abundance or dissolved oxygen can also limit increment widths (Table 1). This technique is only feasible for post-larval stages, when increments are at least 3–6 μm. As for other condition indices, back-calculated change in recent growth may be compared among individuals (by residuals) or among regions (by ANCOVA), using standard length or otolith radius as the independent variable.

USES AND IMPLICATIONS OF PERIPHERAL OTOLITH GROWTH AND RNA INDICES

The use of two independent indices (Ferron & Leggett 1994), preferably from the same individual is recommended (e.g. Clemmesen & Doan 1996). The RNA–DNA index and 1–3 day peripheral otolith growth were shown to be positively correlated, although indices were derived from separate fish in the same sample (Hovenkamp 1990; Hovenkamp & Witte 1991; Suthers 1996). Clemmesen (1996) found that the RNA:DNA ratio had the highest correlation ($r = 0.51$) with the width of the two most recent otolith increments in larval *Engraulis anchoita*, supporting the 1–2 day RNA–DNA latency described by Martin and Wright (1987). Future studies should further compare RNA–DNA indices estimated using the tail region, and peripheral otolith growth from the same individual (Clemmesen & Doan 1996).

Table 1. Summary of field studies using peripheral otolith increments

Species	Size range (mm)	*n*	Increment precapt.	Variable	Source
European smelt (*Osmerus eperlanus*)	5–20	158	2	Dissolved oxygen	Sepúlveda 1994
Atlantic menhaden (*Brevoortia tyrannus*)	5–25	>1000	(1)	Storms/temperature	Maillet & Checkley 1991
Pilchard (*Sardinops ocellatus*)	10–45	220	5	Temperature[a]	Thomas 1986
Anchovy (*Engraulis capensis*)	10–40	294	5	Temperature	Thomas 1986
Anchovy (*Engraulis mordax*)	5–18	367	3	None	Methot 1981
Myctophid (*Stenobrachius leucopsarus*)	5–12	141	3	None	Methot 1981
Myctophid (*Diaphus kapalae*)	11–15	95	2	Prey	Suthers 1996
Walleye pollock (*Theragra chalcogramma*)	50–60	93	10	Prey	Bailey 1989
Atlantic cod (*Gadus morhua*)	7–32	489	7, 14	Prey	Suthers *et al.* 1989
	17–48	159	7, 14	Prey/temperature	Suthers & Sundby 1993; 1996
Spot (*Leiostomus xanthurus*)	<9	98	1, 2, 3	Prey	Govoni *et al.* 1985
Plaice (*Pleuronectes platessa*)	15–35	50	10, 20	Prey	Karakiri *et al.* 1989
	9–13	99	5	Prey/temperature	Hovenkamp 1990

n, sample size; increment precapt., number of peripheral increments used in the width measurement; variable, environmental factor ascribed to explain variation in recent growth; [a], prey not significant.

Ferron and Leggett (1994) concluded 'that the initial, and still central purpose of measuring condition is to predict reliable survival probabilities under given food regimes in order that observed larval abundances can be used as early predictors of recruitment' (p. 283). Larvae in better condition should contribute proportionally more to recruitment than those in poor condition and, indeed, recruitment studies have shown that years with larvae exhibiting faster growth produced stronger year classes (Setzler-Hamilton *et al.* 1987; Bailey *et al.* 1995; Meekan & Fortier 1996). However, those studies that have examined survival of larvae in good and poor condition in the laboratory have yet to demonstrate an effect on survival potential (e.g. Neilson *et al.* 1986; McCormick & Molony 1993). On a related issue, Leggett and Deblois (1994) have recently questioned the dogma that 'bigger is better', whereby bigger larvae are presumed to have a better survival potential. Furthermore, Pepin (1991) and other authors (refer Leggett & Deblois 1994) have also shown that while species with larger larvae tend to be better swimmers, mortality rates in general are not lower as would be expected.

A solution to this debate may be found in the use of growth indices as predictors of survival potential. For example back-calculated growth reveals that larger larvae at similar ages have higher survival probabilities (e.g. Campana 1996; Meekan & Fortier 1996). Houde (1989) illustrated how large recruitment variability could be generated by subtle variations in mortality (M) and growth (G) schedules. He contrasted how the effect of a single large advective loss of larvae (unfavourable transport of < 80% of larvae) can be exceeded by a 2–3% day^{-1} change in M or G during a 50–70 day early life period. Growth indices such as relative RNA content (as an index of protein growth) and peripheral otolith growth can determine which individuals exhibit the faster growth, and have the greater potential to survive. It is ironic that although coral-reef researchers are no strangers to the techniques of age and growth measurements, few have examined peripheral otolith growth as a measure of quality and survival potential (but see Thorrold & Williams 1989).

Bigger larvae may not experience better survival, but faster growing larvae will, in general (Houde 1987, 1989). A bigger larva is only better if it has experienced faster growth, to survive getting big! A fatter (or better condition larvae) may experience better survival, but only if is correlated with faster growth.

In conclusion, there is no 'best' condition index, as indices should be tailored to (and be specific for) the species/stage (larvae/pelagic juvenile) and time frame of the study (days, years; Ferron & Leggett 1994). Relative RNA content and peripheral otolith growth are complementary analyses, each being more appropriate for larvae and pelagic juveniles, respectively. In pursuing these techniques we should not lose sight of the purpose—to determine what are survival-limiting processes. The difficulties of laboratory calibrations point to coral-reef lagoons as natural laboratories for manipulative survival experiments using fish with variable growth or condition indices (e.g. Kerrigan 1996). These issues are important; the techniques are available and the otoliths are often already collected, waiting to test the hypothesis that faster growth is better.

ACKNOWLEDGEMENTS

While any flaws in fact or logic are mine alone, I am grateful for discussions with many people, including Catriona Clemmesen and especially Elaine Caldarone

(who provided the unpublished data). I thank Mark McCormick for encouraging me to attend Reefish '95 and to put these ideas on paper, and am grateful for the commentaries from Julian Caley, Jeff Leis, Tony Miskiewicz, Dave Booth and my anonymous referees.

REFERENCES

Arthur D. K. (1980) Differences in heart size between ocean-caught and laboratory-grown larvae of the northern anchovy, *Engraulis mordax* Girard. *J. Exp. Mar. Biol. Ecol.* **43**, 99–106.

Bailey K. M. (1989) Interaction between the vertical distribution of juvenile walleye pollock *Theregra chalcogramma* in the eastern Bering Sea, and cannibalism. *Mar. Ecol. Prog. Ser.* **53**, 205–13.

Bailey K. M., Canino M. F., Napp J. M., Spring S. M. & 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. *Mar. Ecol. Prog. Ser.* **119**, 11–23.

Bailey K. M. & Stehr C. L. (1988) The effects of feeding periodicity and ration on the rate of increment formation in otoliths of larval walleye pollock *Theragra chalcogramma* (Pallas). *J. Exp. Mar. Biol. Ecol.* **122**, 147–61.

Balbontin F., de Silva S. S. & Ehrlich K. F. (1973) A comparative study of anatomical and chemical characteristics of reared and wild herring. *Aquaculture* **2**, 217–40.

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

Buckley L. J., Turner S. I., Halavik T. A., Smigielski A. S., Drew G. G. & Lawrence S. M. (1984) Effects of temperature and food availability on growth, survival, and RNA–DNA ratio of larval sand lance (*Ammodytes americanus*). *Mar. Ecol. Prog. Ser.* **15**, 91–7.

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

Campana S. E. (1990) How reliable are growth back-calculations based on otoliths? *Can. J. Fish. Aquat. Sci.* **47**, 2219–27.

Campana S. E. (1996) Year-class strength and growth rate in young Atlantic cod *Gadus morhua*. *Mar. Ecol. Prog. Ser.* **135**, 21–6.

Campana S. E. & Hurley P. C. F. (1989) An age- and temperature-mediated growth model for cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) larvae in the Gulf of Maine. *Can. J. Fish. Aquat. Sci.* **46**, 603–13.

Campana S. E. & Jones C. M. (1992) Analysis of otolith microstructure data. In: *Otolith Microstructure Examination and Analysis. Canadian Special Publication of Fisheries and Aquatic Sciences 117* (eds D. K. Stevenson & S. E. Campana) pp. 73–100. Department of Fisheries and Oceans, Ottawa.

Canino M. F., Bailey K. M. & Incze L. S. (1991) Temporal and geographic differences in feeding and nutritional condition of walleye pollock larvae *Theregra chalcogramma* in Shelikof Strait, Gulf of Alaska. *Mar. Ecol. Prog. Ser.* **79**, 27–35.

Canino M. F. & Caldarone E. M. (1995) Modification and comparison of two fluorometric techniques for determining nucleic acid contents of fish larvae. *Fish. Bull. US* **93**, 158–65.

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

Clemmesen C. (1996) Importance and limits of RNA/DNA ratios as a measure of nutritional condition in fish larvae. In: *Survival Strategies in Early Life Stages of Marine Resources* (eds Y. Watanabe, Y. Yamashita & Y. Oozeki) pp. 67–82. A. A. Balkema, Rotterdam.

Clemmesen C. & Doan T. (1996) Does otolith structure reflect the nutritional condition of a fish larva? Comparison of otolith structure and biochemical index (RNA/DNA ratio) determined on cod larvae. *Mar. Ecol. Prog. Ser.* **138**, 33–9.

Doherty P. J. & Williams D. McB. (1988) The replenishment of coral reef fish populations. *Oceanogr. Mar. Biol. Ann. Rev.* **26**, 487–551.

Eckmann R. & Rey P. (1987) Daily increments on the otoliths of larval and juvenile *Coregonus* spp. and their modification by environmental factors. *Hydrobiologia* **148**, 137–43.

Ehrlich K. F. (1975) A preliminary study of the chemical composition of sea-caught larval herring and plaice. *Comp. Biochem. Physiol.* **51B**, 25–8.

Ferron A. & Leggett W. C. (1994) An appraisal of condition measures for marine fish larvae. *Adv. Mar. Biol.* **30**, 217–303.

Frank K. T. & McRuer J. K. (1989) Nutritional status of field-collected haddock (*Melanogrammus aeglefinus*) larvae from southwestern Nova Scotia: an assessment based on morphometric and vertical distribution data. *Can. J. Fish. Aquat. Sci.* (Suppl. 1) **46**, 125–33.

Fraser A. J. (1989) Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. *Can. J. Fish. Aquat. Sci.* **46**, 1868–73.

Govoni J. J., Chester A. J., Hoss D. E. & Ortner P. B. (1985) An observation of episodic feeding and growth of larval *Leiostomus xanthurus* in the northern Gulf of Mexico. *J. Plank. Res.* **7**, 137–46.

Håkanson J. L. (1989) Analysis of lipid components for determining the condition of anchovy larvae, *Engraulis mordax*. *Mar. Biol.* **102**, 143–51.

Håkanson J. L. (1993) Nutritional condition and growth rate of anchovy larvae (*Engraulis mordax*) in the California Current: two contrasting years. *Mar. Biol.* **115**, 309–16.

Hare J. A. & Cowen R. K. (1995) Effect of age, growth rate, and ontogeny on the otolith size-fish size relationship in bluefish, *Pomatomus saltatrix*, and the implications for back-calculation of size in fish early life history stages. *Can. J. Fish. Aquat. Sci.* **52**, 1909–22.

Hoff G. R. & Fuiman L. A. (1993) Morphometry and composition of red drum otoliths: changes associated with temperature, somatic growth rate and age. *Comp. Biochem. Physiol.* **106A**, 209–19.

Houde E. D. (1987) Fish early life dynamics and recruitment variability. *Am. Fish. Soc. Symp.* **2**, 17–29.

Houde E. D. (1989) Subtleties and episodes in the early life of fishes. *J. Fish. Biol. Suppl.* **35**, 29–38.

Hovenkamp F. (1990) Growth differences in larval plaice *Pleuronectes platessa* in the southern Bight of the North Sea as indicated by otolith increments and RNA/DNA ratios. *Mar. Ecol. Prog. Ser.* **58**, 205–15.

Hovenkamp F. & Witte J. I. J. (1991) Growth, otolith growth and RNA/DNA ratios of larval plaice *Pleuronectes platessa* in the North Sea 1987–1989. *Mar. Ecol. Prog. Ser.* **70**, 105–16.

Karakiri M., Berghahn R. & von Westernhagen H. (1989) Growth differences in 0-group plaice *Pleuronectes platessa* as revealed by otolith microstructure analysis. *Mar. Ecol. Prog. Ser.* **55**, 15–22.

Kerrigan B. A. (1996) Temporal patterns in size and condition at settlement in two tropical reef fishes (Pomacentridae: *Pomacentrus amboinensis* and, *P. nagasakiensis*). *Mar. Ecol. Prog. Ser.* **135**, 27–41.

Leggett W. C. & Deblois E. (1994) Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? *Netherlands J. Sea Res.* **32**, 119–34.

Lochmann S. E., Maillet G. L., Taggart C. T. & Frank K. T. (1996) Effect of gut contents and lipid degradation on condition measures in larval fish. *Mar. Ecol. Prog. Ser.* **134**, 27–35.

Maillet G. L. & Checkley D. M. (1990) Effects of starvation on the frequency of formation and width of growth increments in sagittae of laboratory-reared Atlantic menhaden *Brevoortia tyrannus* larvae. *Fish. Bull. US* **88**, 155–65.

Maillet G. L. & Checkley D. M. (1991) Storm-related variation in the growth rate of otoliths of larval Atlantic menhaden *Brevoortia tyrannus*: a time series analysis of biological and physical variables and implications for larva growth and mortality. *Mar. Ecol. Prog. Ser.* **79**, 1–16.

Margulies D. (1993) Assessment of the nutritional condition of larval and early juvenile tuna and Spanish mackerel (Pisces: Scombridae) in the Panama Bight. *Mar. Biol.* **115**, 317–30.

Martin F. D. & Wright D. A. (1987) Nutritional state analysis and its use in predicting striped bass recruitment: laboratory calibration. *Am. Fish. Soc. Symp.* **2**, 109–14.

McCormick M. I. & Molony B. W. (1993) Quality of the reef fish *Upeneus tragula* (Mullidae) at settlement: is size a good indicator of condition? *Mar. Ecol. Prog. Ser.* **98**, 45–54.

McGurk M. D. (1985) Multivariate analysis of morphometry and dry weight of Pacific herring larvae. *Mar. Biol.* **86**, 1–11.

Meekan M. G. & Fortier L. (1996) Selection for fast growth during the planktonic life of Atlantic cod *Gadus morhua* on the Scotian Shelf. *Mar. Ecol. Prog. Ser.* **137**, 25–37.

Methot R. D. (1981) Spatial covariation of daily growth rates of larval northern anchovy, *Engraulis mordax*, and northern lampfish, *Stenobrachius leucopsarus*. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer* **178**, 424–31.

Milicich M. J. & Choat J. H. (1992) Do otoliths record changes in somatic growth rate? Conflicting evidence from a laboratory and field study of a temperate reef fish, *Parika scaber*. *Aust. J. Mar. Freshwat. Res.* **43**, 1203–14.

Molony B. W. (1993a) Effects of feeding history on mobilisation and deposition of body constituents and on growth in juvenile *Ambassis vachelli* (Pisces: Chandidae). *Mar. Biol.* **116**, 389–97.

Molony B. W. (1993b) The use of multiple indices in the description of growth in the tropical estuarine fish *Ambassis vachelli*, Richardson (Pisces: Chandidae), with reference to the effects of feeding history. PhD thesis, James Cook University of North Queensland, Townsville.

Molony B. W. & Choat J. H. (1990) Otolith increment widths and somatic growth rate: the presence of a time-lag. *J. Fish. Biol.* **37**, 541–51.

Neilson J. D., Perry R. I., Valerio P. & Waiwood K. G. (1986) Condition of Atlantic cod *Gadus morhua* larvae after the transition to exogenous feeding: morphometrics, buoyancy and predator avoidance. *Mar. Ecol. Prog. Ser.* **32**, 229–35.

O'Connell C. P. & Paloma P. A. (1981) Histochemical indications of liver glycogen in samples of emaciated and robust larvae of the northern anchovy, *Engraulis mordax*. *Fish. Bull. US* **78**, 806–12.

Ouellet P., Taggart C. T. & Frank K. T. (1995) Early growth, lipid composition, and survival expectations of shrimp *Pandalus borealis* larvae in the northern Gulf of St. Lawrence. *Mar. Ecol. Prog. Ser.* **126**, 163–75.

Packard G. C. & 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 & R. B. Huey) pp. 216–35. Cambridge University Press, Cambridge, MA.

Pepin P. (1991) Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Can. J. Fish. Aquat. Sci.* **48**, 503–18.

Peterman R. M. (1987) Review of the components of recruitment of Pacific salmon. *Am. Fish. Soc. Symp.* **1**, 417–29.

Peterson K. (1996) Effect of prey abundance and temperature on the otolith microstructure of larval fish. Honours thesis, Centre for Marine Science, University of New South Wales, Sydney.

Reist J. D. (1985) An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. *Can. J. Zool.* **64**, 1363–8.

Richard P., Bergeron, J-P., Boulhic M., Galois R. & Ruyet J. P. (1991) Effect of starvation on RNA, DNA and protein content of laboratory-reared larvae and juveniles of *Solea solea*. *Mar. Ecol. Prog. Ser.* **72**, 69–77.

Rissik D. & Suthers I. (1996) Feeding in a larval fish assemblage: the nutritional significance of an estuarine plume front. *Mar. Biol.* **126**, 233–40.

Robinson S. M. C. & 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. *Can. J. Fish. Aquat. Sci.* **45**, 1422–9.

Rooker J. R. & Holt G. J. (1996) Application of RNA:DNA ratios to evaluate the condition and growth of larval and juvenile red drum (*Sciaenops ocellatus*). *Mar. Freshwat. Res* **47**, 283–90.

Secor D. H. & Dean J. M. (1989) Somatic growth effects on the otolith–fish size relationship in young pond-reared striped bass, *Morone saxatilis*. *Can. J. Fish. Aquat. Sci.* **46**, 113–21.

Secor D. H., Dean J. M. & Laban E. H. (1992) Otolith removal and preparation for microstructural examination. In: *Otolith Microstructure Examination and Analysis. Canadian Special Publication of Fisheries and Aquatic Sciences 117* (eds D. K. Stevenson & S. E. Campana) pp. 19–57. Department of Fisheries and Oceans, Ottawa.

Sepúlveda A. (1994) Daily growth increments in the otoliths of European smelt *Osmerus eperlanus* larvae. *Mar. Ecol. Prog. Ser.* **108**, 33–42.

Setzler-Hamilton E. M., Wright D. A., Martin F. D., Millsaps C. V. & Whitlow S. I. (1987) Analysis of nutritional condition and its use in predicting striped bass recruitment: field studies. *Am. Fish. Soc. Symp.* **2**, 115–28.

Shepherd J. G. & Cushing D. H. (1980) A mechanism for density-dependent survival of larval fish as the basis of a stock–recruitment relationship. *J. Cons. Int. Explor. Mer* **39**, 160–7.

St John M. A. & Lund T. (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. *Mar. Ecol. Prog. Ser.* **131**, 75–85.

Suthers I. M. (1996) Spatial variability of recent otolith growth and RNA indices in pelagic juvenile *Diaphus kapalae* (Myctophidae): an effect of flow disturbance near an island? *Mar. Freshw. Res* **47**, 273–82.

Suthers I. M., Cleary J. J., Battaglene S. C. & Evans R. (1996) Relative RNA content as a measure of condition in larval and juvenile fish. *Mar. Freshwat. Res* **47**, 301–7.

Suthers I. M., Frank K. T. & Campana S. C. (1989) Spatial comparison of recent growth in post-larval cod (*Gadus morhua*) off southwestern Nova Scotia: inferior growth in a presumed nursery area. *Can. J. Fish. Aquat. Sci.* (Suppl. 1) **46**, 13–24.

Suthers I. M., Fraser A. & Frank K. T. (1992) Comparison of lipid, otolith and morphometric condition indices of pelagic juvenile cod (*Gadus morhua*) from the Canadian Atlantic. *Mar. Ecol. Prog. Ser.* **84**, 31–40.

Suthers I. M. & Sundby S. (1993) Dispersal and growth of pelagic juvenile Arcto-Norwegian cod (*Gadus morhua*), inferred from otolith microstructure and water temperature. *ICES J. Mar. Sci.* **50**, 261–70.

Suthers I. M. & Sundby S. (1996) Role of the midnight sun: comparative growth of pelagic juvenile cod (*Gadus morhua*) from the Arcto-Norwegian and a Nova Scotian stock. *ICES J. Mar. Sci.* **53**, 827–36.

Theilacker G. H. (1978) Effect of starvation on the histological and morphological characteristics of jack' mackerel *Trachurus symmetricus* larvae. *Fish. Bull. US* **76**, 403–14.

Theilacker G. H. (1980) Rearing container size affects morphology and nutritional condition of larval jack mackerel, *Trachurus symmetricus*. *Fish. Bull. US* **78**, 789–91.

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

Theilacker G. H., Bailey K. M., Canino M. F. & Porter S. M. (1996) Variations in larval walleye polloc feeding and condition: a synthesis. *Fish. Oceanogr.* **5** (Suppl. 1), 112–23.

Theilacker G. H. & Watanabe Y. (1989) Midgut cell height defines nutritional status of laboratory raised larval northern anchovy, *Engraulis moradax*. *Fish. Bull. US* **87**, 457–69.

Thomas R. M. (1986) Growth of larval pelagic fish in the southeast Atlantic from daily otolith rings in 1982/83 and 1983/84. *S. Afr. J. Mar. Sci.* **4**, 61–77.

Thorrold S. R. & Williams D. McB. (1989) Analysis of otolith microstructure to determine growth histories in larval cohorts of a tropical herring (*Herklotsichthys castelnaui*). *Can. J. Fish. Aquat. Sci.* **46**, 1615–24.

Westerman M. E. & Holt G. J. (1994) RNA:DNA ratio during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. *Mar. Biol.* **121**, 1–9.

Williams D. McB. (1991) Patterns and processes in the distribution of coral reef fishes. In: *The Ecology of Fishes On Coral Reefs* (ed. P. F. Sale) pp. 437–74. Academic Press, San Diego.