THE USE OF CONDITION INDICES IN LARVAL FISH

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Abstract

Mortality of larval fish and invertebrates may potentially be estimated from indices of larval condition or health, on the assumption that larvae in poor condition grow slower and are subject to the cumulative effects of starvation, predation or disease. Larval condition integrates feeding success of the previous few days and, to the extent this influences growth and mortality, may ultimately determine not only recruitment but also the structure of adult assemblages. This paper briefly reviews morphological, biochemical, otolith and histological condition indices, with specific reference to a comparison of dry weight, lipid and otolith indices of pelagic juvenile cod (*Gadus morhua*). Morphological indices such as body depth or dry weight are simple, but are often insensitive, and require shrinkage correction for the early larvae. Biochemical indices such as RNA/DNA ratio appear to be equivalent across species, but require sorting of the larvae at sea before rapid freezing. The peripheral increment widths of the otolith are a simple measure of recent growth, while histological measurements of the gut epithelium or muscle cell diameter represent a direct index of starvation mortality.

Validation of indices is necessary, but caution is advised when extrapolating from reared larvae to the field. The within-larva correlation of many indices is low or not significant probably due to varying temporal responses to star- vation of each index. In general, condition indices must be tailored to the species and the size (or stage). There are very few Australasian studies of larval condition. The relative high diversity and low density of Australian fish larvae, requires indices to be equivalent across species to assess the impact of oceanographic features or events.

Introduction

Starvation has been proposed as a major source of larval mortality, but is rarely observed in the ocean (e.g. Strasburg 1959). Indices of larval condition - such as health and growth - are a relatively new technique to objectively assess the impact of oceanographic conditions (or aquaculture techniques) on prey distribution and abundance, and ultimately on potential survival. Since the initial studies of larval condition by Ehrlich *et al.* (1976), and O'Connell (1976; 1980), who showed evidence that up to 8% of wild clupeid larvae were in starving condition, many studies have since confirmed that food, or feeding opportunity, is a limiting factor for larval survival (Setzler-Hamilton *et al.* 1987). Consequently the recruitment limitation hypotheses in fisheries that invoke larval survival as determining either year-class strength or adult community structure (Doherty and Williams 1988) may now be tested. Such an approach is attractive given that obtaining robust estimates

of larval mortality in the field is highly unlikely (Taggart and Leggett 1987). Most condition indices probably reflect the feeding environment over scales of 2-7 days.

Condition indices involve the assessment of a starvation sensitive variable, which then must be standardised by a starvation insensitive variable (Table 1). The morphometric variables indicate a heavier or fatter larva per unit length. Ash weight may increase in larvae in poor condition due to relative increase in skeleton and skin, and osmotic influx of salts (Ehrlich 1974), but other studies have shown ash to decrease due to decreasing ossification of the skeleton. The amount of RNA is directly proportional to the amount of protein synthesis, which is standardised by the number of cells (DNA content). The triacylglycerol (TAG) index indicates the amount of storage fat standardised by the amount of structural fat (cholesterol is a component of cell membranes). The otolith index shows the amount of recent (or peripheral) otolith growth relative to larval length. Finally the histological index may be a summed score estimated from a number of target tissues, or simply a measurement of cell size (either gut epithelium or muscle cell diameter).

A major assumption is that an index is positively correlated with larval survival, such that larvae in good condition will continue to feed well, and be less susceptible to predation and disease due to spending less time in the larval phase (e.g. Shepherd and Cushing 1980). Condition indices cannot account for unfavourable advection which could operate regardless of larval condition.

The uses and limitations of condition indices are still being explored, and some major questions in this field are as follows:

1) are the various indices correlated?;

2) are condition indices comparable between different developmental stages?;

3) is variation within an individual larva greater than between individuals?;

4) and what are the limitations of the various indices?

In this report I wish to address these issues from published information on larval condition, and illustrate my case with data on pelagic juvenile cod >12 mm standard length (*Gadus morhua*) from the N.W. Atlantic (Suthers *et al.* 1992). The data are unique because 3 different indices of condition (morphological, biochemical and otolith) are compared from the same individual.

Materials and methods

Pelagic juvenile cod were collected off southwestern Nova Scotia in the northwest Atlantic during May and early June of 1987 (cruises 87-1, 87-2 respectively). Each larva was freezedried (DWT), had the otoliths extracted (OTO), and then was lipid extracted (TAG). Details may be found in Suthers *et al.* (in press). The outer 14 daily growth increments and otolith radius were measured using an image analysis system (Campana 1987; Suthers *et al.* 1989). These growth increments were assumed to be a conservative index of recent growth, reflecting the environmental conditions at capture. During 1985, measurements were made to a resolution of ± 0.04 mm; anal body depth (ABD), pectoral body depth (PBD), eye diameter (EYED), and head length (HL), after Koslow *et al.* (1985). Condition indices were then calculated as residuals of univariate regressions of the starvation dependent variables on SL. Also, for each cruise a principal component analysis of the starvation dependent and independent variables was performed.

Results and discussion

1. Correlation of TAG, DWT and OTO.
After discarding the first (size) component, the plots of principal components PC2, PC3, and PC4 show that OTO varied differently relative to the other variables (Figure 1a). PC2 (<7% of

the total variance) was a contrast between OTO and all other variables, while PC3 (<4%) represented a contrast between TAG and SL. PC4 contrasted CHOL and DWT with TAG and SL (<1% of total variance).

Similarly for cruise 85-1,2, PC2 (5%) also contrasted OTO with all other variables and PC3 (1%) contrasted the 2 body depths with head length (Figure 1b). PC4 contrasted head length with eye diameter - a result found in morphometric studies of herring (Ehrlich *et al.* 1976; McGurk 1985a).

Not surprisingly therefore, from 16 pairwise index comparisons (including a variety of cruises), the correlation of OTO index with either TAG or morphometric indices was often zero, or not significant (Suthers *et al.* in press). The DWT and ABD indices were usually significantly correlated and particularly the TAG and DWT indices.

Why is OTO telling a different story? The answer may lie in the fact that otolith growth is generally correlated with somatic growth, which is fundamental to larval survival. It is unlikely therefore that pelagic juveniles will store excess TAG in preference to growth (Ehrlich 1974). Note however that these conclusions are species specific - for example cod have approximately triple the TAG content of capelin which may swamp any variation in lipid levels. Some larval bivalves and crustaceans need a lipid reserve for settlement or metamorphosis, and TAG versus CHOL may be a useful index in these species (Fraser 1989). The conclusions may also be size specific - the importance of TAG as a condition index in larval cod is unknown. Finally, note that otolith growth tends to be very conservative relative to body growth, and under poor growth the otolith will still lay down a narrower daily growth increment, ultimately producing a heavier or larger otolith relative to fish size (Reznick *et al.* 1989; Secor and Dean 1989). This problem is ameliorated, as in this study, by considering only short (14 d) time intervals.

Martin *et al.* (1985) compared the condition of striped bass larvae from samples taken over 8 weeks using 4 measures of condition, each derived from separate larvae. The histological score (of a number of target tissues) and RNA/DNA ratio fluctuated similarly (no correlation statistic provided), and a morphological score showed a roughly similar pattern (Figure 2). Content of free fatty acids declined through the sampling period, and appeared to be an unsatisfactory index (free fatty acids are the physiologically mobile lipids, and are the breakdown product of TAG and phospholipids). As low correlations between indices occur even when values are derived from the same individual, it appears that each index, for each species, at each size, may operate at a different response rate to the starvation event (or not at all!). Martin and Wright (1987) concluded that RNA/DNA ratios respond the most rapidly (1-2 d), morphology the most slowly (5-7 d) and histology at an intermediate rate.

2) Condition in larval vs. juvenile fish
It is generally believed that pelagic juveniles are less susceptible to starvation than larvae, presumably due to the inertia of having larger body reserves and liver, and due to selection against slow growing larvae. In general, pre-flexion larvae do more frequently exhibit emaciation than post-flexion larvae, or are more easily discriminated as starved or moderately starved from both laboratory and field studies (Martin *et al.* 1985; Powell and Chester 1985; Grover and Olla 1986; Yin and Blaxter 1986; Clemmesen 1989). In particular, Theilacker (1986) showed the proportion of dying/starving larvae changed dramatically from <3.5 mm larvae to 3.5-4.0 mm larvae. However, authors who have examined condition in pelagic juveniles in the wild have also found evidence of food limitation. Bailey (1989) found that 50-60 mm walleye pollock grew significantly faster at a station with ten times the biomass of zooplankton,

based on the outer ten daily growth increments of the otolith. Karakiri *et al.* (1989) found that variation in the width of the peripheral ten daily increments in juvenile plaice (10-40 mm), was attributed to food supply. Peterman (1987) concluded for Pacific salmon that most of the density dependent component of mortality occurred in the first 12-18 mo (during the initial marine phase, and not in the natal streams), and that the *zooplankton prey abundance was the proximal* factor. Rearing larvae to the pelagic juvenile stage in the laboratory for validation of condition indices is difficult, but it would seem that starvation mortality in even the later stages occurs but is often overlooked.

3) Is variation within larvae greater than between?

Despite the surprisingly low correlation of indices from the same larva, variation in condition at large spatial scales appears to be the dominant signal. To address this, four nearshore and four offshore stations were randomly selected from the 1985 data set (Suthers *et al.* 1992), and four larvae were selected from within each sample which had DWT, ABD and OTO measured. The studentised residuals (to homogenise the variance) of each index were nested within fish (as replicates), in a fully nested 3 factor ANOVA (Underwood 1981; Table 2). There were large significant differences in condition between the inshore and offshore (due to the low zooplankton biomass inshore, Suthers *et al.* 1989), but of importance to this issue - the condition of larvae within a sample were not significantly different (and the MS of the error term was the smallest).

4) Limitations of each index?

Morphometric indices are simple, and require no special preservation but the results are often insensitive - particularly for larvae - unless the effects of shrinkage due to time since death and preservation are taken into account (Theilacker 1986). This entails careful laboratory calibration, and dedicated short plankton tows. Multivariate measurements may moderate the shrinkage problem (particularly the apparent contrast between EYED and HL in cod and herring). Pelagic juveniles >10 mm are less susceptible to this shrinkage, and ABD seems to be a simple technique. All such measures are species specific.

Biochemical indices require rapid freezing of the larvae at sea, which poses difficulties in trying to identify larvae and separate them from the zooplankton. The main advantage of the RNA/DNA method is that it appears to give a consistent relationship across species (Buckley 1984; Clemmesen 1987). Protein growth rate may therefore be calculated from the ratio of RNA/DNA and water temperature. Recently these relationships have been questioned and in fact exhibit considerable subdaily variation (A. Ferron, pers. comm.). For larvae >3 mg DWT (approximately 12 mm), RNA/DNA ratios also increase with size, but this has not been examined in detail. The persistent use of ratios in this technique is disturbing as a large ratio may be due to a large numerator, or a small denominator. The TAG/sterol ratio appears to successfully identify nutrient-stressed anchovy larvae (Håkenson 1989a; b) and pollutant stressed crab, lobster, and herring larvae (Fraser 1989).

The otolith index was first used by Methot (1981), in comparing the otolith growth of larval anchovy with a myctophid from 13 samples off Oregon, covering 4° of latitude. Remarkably there were no spatial trends in recent growth, and nor did the 2 species co-vary, possibly due to uniform environmental conditions (range 1°C), and to the method of analysis. Of particular concern with this technique is the fidelity of the otolith in recording daily changes in growth. Recent studies show that the metabolic lag between a starvation event and its record in the otolith may be of the order of 1-4 days (Govoni *et al.* 1985; Bailey and Stehr 1988; Maillet and Checkley 1989).

By examining the width of the outer daily growth increments (e.g. 7-14), one can circumvent the effect of larger otoliths in slower growing fish (e.g. Secor and Dean 1989; Reznick *et al.* 1989). A great advantage is to back-calculate periods of 7d growth - up to 4 week precapture - as a natural tag of the pelagic juvenile's past feeding environment (Suthers *et al.* 1989). There can be considerable effort to obtain these data - unless one is ageing the larvae anyway.

Histological indices are laborious, requiring special preservation procedures and short tow duration, and required a specialist to consistently score the target tissues. In response, Theilacker and Watanabe (1989) developed a robust technique to measure epithelial gut cell height. The histochemical determination of liver glycogen (O'Connell and Paloma 1981) supported previous histological work (O'Connell 1980), but was not recommended as a simple technique. The advantage of histological examination is the ability to discriminate larvae in increasing condition versus decreasing condition (Theilacker 1986), and it is truly an index of starvation mortality rather than growth rate.

Conclusion

Larval feeding studies often make the valid point that feeding rates could not have a significant impact on their prey density - or larvae may not compete for food (e.g. Jenkins 1987) - thereby implying that food levels are not limiting survival. However it is not the absolute food abundance but the relative food abundance that is limiting (*sensu* Andrewartha and Birch 1954, p. 489); the availability of food relative to the larva's vision and mobility. Only recently have we become aware of small-scale turbulence and contact rates contributing to feeding incidence (Rothschild and Osborn 1988; Sundby and Fossum 1990). Persistent suggestions by a few members at this workshop, that starvation may be a minor component of larval and pelagic juvenile mortality, are ignoring a solid body of

evidence that shows declining condition with declining prey abundance. The Australasian data are sparse in this area, and we should aim to fill this void, particularly as the Australian marine environment is relatively nutrient-poor. If larval food limitation can limit recruitment and ultimately alter adult community assemblages, then the Australian marine environment should provide examples.

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Table 1. The various types of condition indices

Type of index	Starvation dependent variable	Starvation independent variable
Morphometric	dry/ash weight body depth	standard length
Biochemical	ribonucleic acid (RNA) triacylglycerol (TAG)	deoxyribonucleic acid (DNA) sterol/cholesterol standard length
	tripsen content	
Otolith	peripheral daily increment widths	standard length otolith radius
Histological	target tissues e.g. gut, liver muscle	–
	cell height/diameter	?

Table 2. Results of a nested 3 factor ANOVA, using the studentised residuals of DWT, ABD and OTO as within fish replicates, to determine if variation in condition index within larvae is greater than that between larvae

Data collected in May 1985 off southwestern Nova Scotia, Canada, and re-analysed from Suthers *et al.* (in press). **, $p < 0.01$; *, $0.01 < p < 0.05$

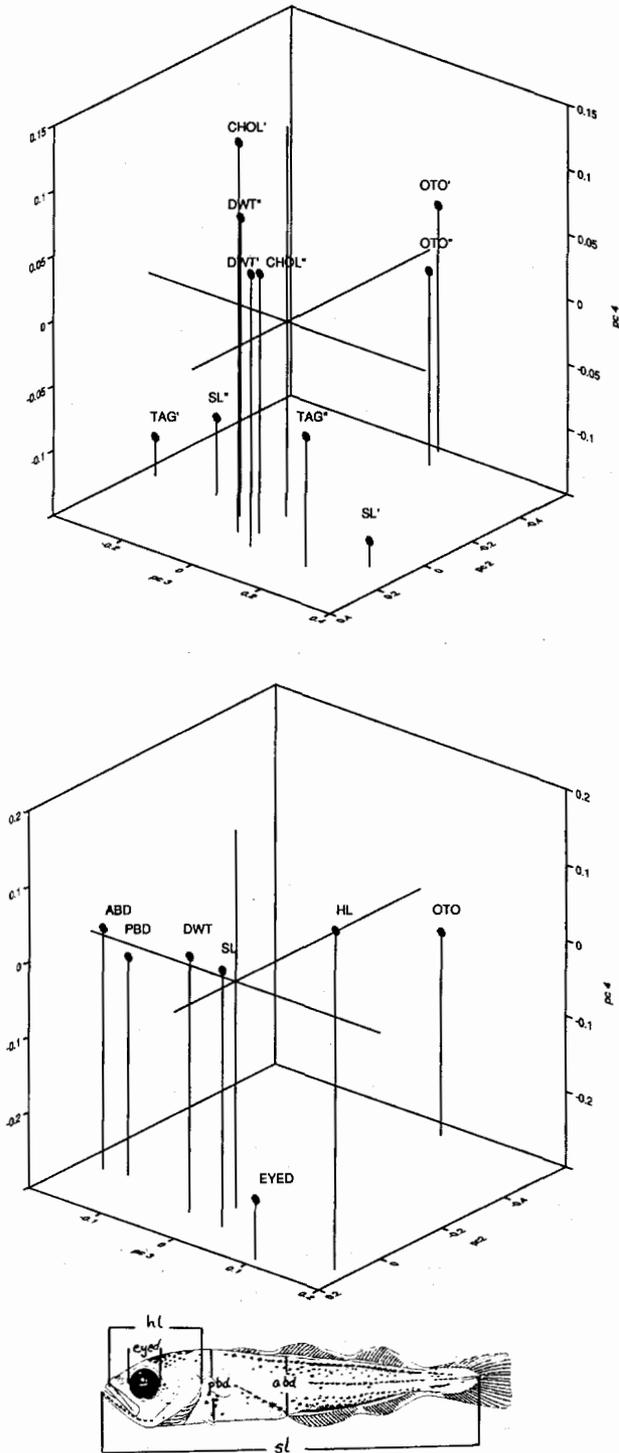| Source	df	SS	MS	F	P
Inshore/ offshore	1	27.17	27.17	11.49	**
Sample	6	14.19	2.37	2.68	*
Larva	24	21.17	0.88	1.62	-
Error	64	34.83	0.54		

Figure 1. Three dimensional plot of principal components 2, 3 and 4 of a) cruise 87-1 (') and 2 (") combined, and b) cruise 85-1. CHOL, cholesterol; DWT, dry weight; OTO, peripheral width of daily growth increments; SL, standard length; TAG, triacylglycerol. Note how both plots show the otolith condition index to load alone on PC2, and how TAG is contrasted with SL on PC 3 in a), and ABD is contrasted with SL in b). From Suthers *et al*. in press.
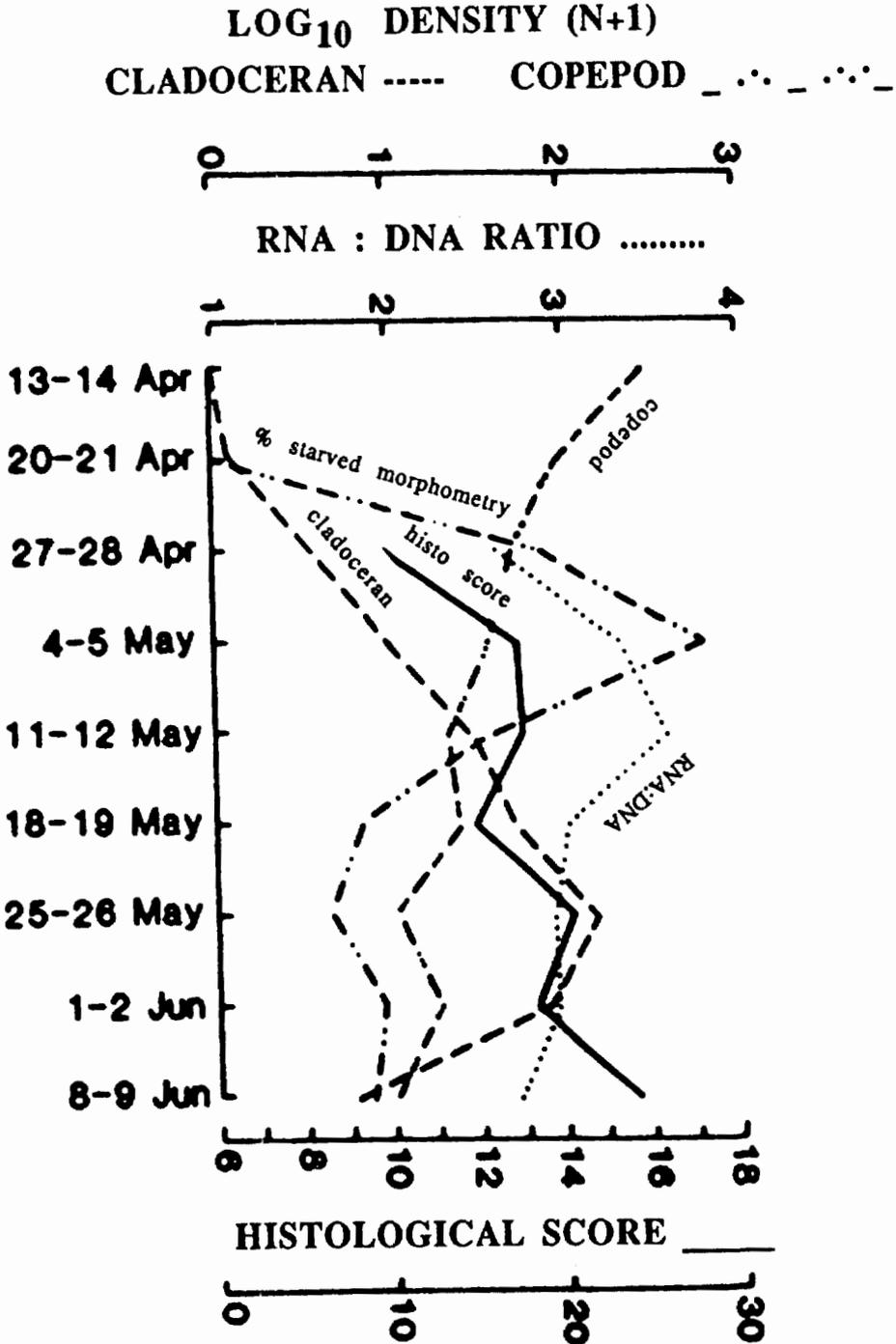
LOG$_{10}$ DENSITY (N+1)

CLADOCERAN -----   COPEPOD _ ·· _ ···_

0   1   2   3

RNA : DNA RATIO ....

1   2   3   4

13-14 Apr
20-21 Apr
27-28 Apr
4-5 May
11-12 May
18-19 May
25-26 May
1-2 Jun
8-9 Jun

% starved morphometry

copepod

cladoceran

histo score

RNA:DNA

6   8   10   12   14   16   18

HISTOLOGICAL SCORE ____

0   10   20   30

% with STARVED MORPHOMETRY --··--··

Figure 2. Comparison of RNA/DNA ratio, histological score, and percent starving striped bass larvae from the Potomac River, with their primary food resource - copepod and cladoceran density (from Martin *et al.* 1985).