Lipid class composition as a measure of nutritional condition in individual larval Atlantic cod (*Gadus morhua*)

Steve E. Lochmann, Gary L. Maillet, Kenneth T. Frank, and Christopher T. Taggart

Abstract: Atlantic cod (*Gadus morhua*) larvae were reared under various feeding environments to assess their lipid class composition and survival during development. Lipids were assessed in individual larvae. Triacylglycerol (TAG), phospholipids (PL), and defatted dry weight (DDW) all fell during yolk absorption. TAG increased after initiation of exogenous feeding in different feeding treatments but did not increase in starved larvae. The percentage of individuals greater than 8 d old with high TAG or PL increased with increasing prey concentration. Survivorship was low in all feeding trials, but groups with a larger percentage of individuals in poor condition tended to exhibit a higher risk of death. We suggest a condition index based on a discriminant function using TAG, PL, and DDW. We assessed the condition of field-collected larvae based on this index.

Résumé : Des larves de morue de l'Atlantique (*Gadus morhua*) ont été élevées sous divers régimes alimentaires afin d'évaluer leur composition par classes de lipides et leur taux de survie pendant la croissance. L'analyse des lipides a été faite de façon distincte chez chaque larve. Les teneurs de triacylglycérol (TAG) et de phospholipides (PL) et le poids sec après dégraissage (PSD) ont diminué après résorption du vitellus. La teneur de TAG a augmenté après le début des divers essais d'alimentation, mais non chez les larves privées de nourriture. Le pourcentage d'individus de plus de 8 jours, à teneurs élevées de TAG ou de PL, a augmenté en fonction de la concentration des proies. La survie a été faible au cours de tous les essais d'alimentation, mais les groupes comprenant un pourcentage plus élevé d'individus en mauvaise condition avaient tendance à présenter un taux de mortalité plus élevé. Nous présentons un indice de condition reposant sur une fonction discriminante de TAG, PL et PSD. Nous avons appliqué cet indice à la détermination de la condition de larves recueillies en mer.

[Traduit par la Rédaction]

Introduction

Marine fish populations with planktonic larval phases often exhibit high interannual variability in abundance, show weak relationships between spawning stock and recruits, and pose difficulties to sustainable management (Houde 1987; Graham and Sherman 1987; Getz et al. 1987). Early and accurate estimates of year-class size should serve to reduce some of the uncertainties in the evaluation of future fish production. However, attempts to predict year-class strength from surveys of the early life history stages have been generally unsuccessful (Smith 1981; Saville and Schnack 1981; Sissenwine 1984). Predictions based on abundance indices alone assume that all larvae have the same probability of avoiding death and recruiting to a fishery. This may be an erroneous assumption given that individuals represent a unique summation of genotype and life experiences and, hence, can be expected to differ accordingly (see DeAngelis and Gross (1992)). It follows that individuals in poor physiological condition may have a lower probability of survival than individuals in good condition. This notion has motivated research into the assessment of condition in early life history stages. The goal is to improve correlations between larval abundance indices and year-class strength by correcting for the inequality of larvae.

There are numerous physical and biological factors that affect recruitment (see Houde 1987 for review). Larval condition integrates these effects. For example, larval haddock (*Melanogrammus aeglefinus*) in poor condition, based on a morphometric index, were generally nearer the surface,

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putting them at greater risk to wind-forced advection from nursery areas (Frank and McRuer 1989). Condition may affect probability of predation because larvae in poor health are less likely to evade predators (Folkvard and Hunter 1986). However, a clear link between condition and survival probability has not been established for larval fish, although this link was recently established for shrimp larvae (Ouellet et al. 1992).

A variety of condition indices are available, including morphological, histological, and biochemical (see Ferron and Leggett 1994 for review). The sensitivity and objectivity of these indices vary. Setzler-Hamilton et al. (1987) compared morphometric, histological, and biochemical indices of condition in populations of striped bass (*Morone saxatilis*) larvae. Some indices disagreed completely. On the basis of total fatty acids, all the larvae from one sample were classified in poor nutritional condition, whereas none of these individuals were classified in poor condition using a morphometric index.

Recent work by Hakanson (1989*a*, 1989*b*) and Fraser et al. (1987) indicated that lipid class composition was a reasonable measure of nutritional condition. Concentration of the energy storage product triacylglycerol (TAG) declined during starvation in anchovy (*Engraulis mordax*) (Hakanson 1989*a*), herring *Clupea harengus*) (Tocher 1985), and Atlantic cod (*Gadus morhua*) (Fraser et al. 1988). Concentrations of other lipid classes such as phospholipids (PL) and sterols (ST) also responded to food deprivation.

A lipid-based condition index has several strengths. Lipid classes, especially TAG, adjust quickly to changes in feeding. Morphometric or histological methods reflect longer term changes. Other biochemical measures, such as RNA and DNA content, can exhibit diel periodicities that can complicate condition assessment (Ferron 1991). Although a diel periodicity in feeding has been demonstrated for cod (Kane 1984) a diel periodicity in lipid class composition has not been found. The lipid technique is also nondestructive. Defatted dry weight (DDW) and otoliths are available from a larva after lipid extraction. A condition index that incorporates TAG content might also indicate ability of the larvae to handle future food shortages, since TAG is an energy storage product.

Before lipid class composition can be used as a condition index in field-collected larvae, laboratory calibrations are necessary. There are problems relating condition of laboratory-reared and field-collected larvae owing to tank effects and exposure to diets composed of unnatural prey. Hakanson (1989*a*) showed that anchovy reared in the laboratory had much higher lipid content than field-collected individuals. As larger larvae generally have higher absolute concentrations of lipids, Fraser et al. (1988) proposed a ratio of TAG to ST as a means of eliminating the size-dependence of the TAG measure. Since ST are a structural class of lipids, the absolute content of ST could be an adequate proxy for body size.

The objective of our study was to calibrate a condition index based on lipid class composition for assessment of Scotian Shelf cod larvae. We assessed lipid class composition using the Iatroscan TLC–FID (thin-layer chromatography – flame ionization detection) technique (Ackman et al. 1990). We addressed four questions. Can lipid class composition be quantified in individual cod larvae? Do lipid contents correlate with the feeding environment? What is the best combination of lipid class measures for assessing condition in cod larvae? Can lipids be linked with probability of survival? To further assess the applicability of a lipid-based condition index we compared lipid contents from laboratory-reared and field-collected larvae.

Materials and methods

Brood stock and rearing conditions

Atlantic cod eggs were obtained from three different captive breeding stocks maintained in 1500- to 5000-L seawater tanks. Adults were collected from the Scotian and Newfoundland Shelf regions. Atlantic cod eggs used in feeding trials were either fertilized eggs collected in tanks following natural spawning and (or) stripped for artificial fertilization. Fertilized eggs were treated with penicillin-G (15 $mg \cdot L^{-1}$) and streptomycin sulphate (20 $mg \cdot L^{-1}$) to retard bacterial and fungal growth. Eggs and larvae were incubated at 4–6°C in 120- to 1500-L tanks with overhead fluorescent lighting providing a surface irradiance of 0.86 ± 0.27 $\mu E \cdot m^{-2} \cdot s^{-1}$ (mean ± SD) (1 $\mu E \cdot m^{-2} \cdot s^{-1} = 0.217\ W \cdot m^{-2}$) on a 12-h light : 12-h dark photoperiod. The experimental tanks were circular, with black interiors, insulated and covered with clear perspex. Each 120-L tank had a continuous flowthrough of 0.5 $mL \cdot min^{-1}$ degassed, 1-μm filtered seawater. Larvae and their prey were retained by 53-μm Nitex mesh screened columns in the centre of each tank. The 1500-L experimental tank had no flowthrough. Tanks were aerated from middepth with compressed air to gently mix the tanks and facilitate high contact rates between larvae and microzooplankton prey. Dead larvae and settled plankton were removed from the bottom every 1–3 d. Water temperature was measured hourly during trials using an Electronic Controls Design model 50 datalogger (resolution ±0.1°C). The average salinity over all feeding trials was maintained at 30.74 ± 0.22 parts per thousand. Prey concentrations were monitored every 2 or 3 d using a Coulter Multisizer-II particle analyzer fitted with an 800-μm aperture tube. First-feeding larvae were offered a natural assemblage of microzooplankton (50–400 μm equivalent spherical diameter) collected from Bedford Basin, Nova Scotia, and maintained in 1500-L tanks. Collections of microzooplankton were made at weekly intervals during feeding trials. Additions of cultured algae (*Thalassiosira pseudonana*, *T. weisflogii*, and T. *Isochrysis galbana*, 10^6 $cells \cdot mL^{-1}$) were made biweekly to provide food for the microzooplankton.

Experimental procedures: feeding trials

In trial 1 (Table 1), cod larvae were starved for 12 d following the modal hatch date (4–8 d after yolk-sac resorption), and were then fed concentrations of microzooplankton at 219 ± 9.7 $prey \cdot L^{-1}$. A total of 2500 cod eggs were placed in three 120-L tanks. Hatching duration was 7 d at an incubation temperature of 5.0 ± 2.4°C. Hatching commenced at 12 d, and peaked at 17–18 d after fertilization. Thirty late-stage eggs and a total of 500 larvae (20–90 larvae every 1–3 d) were sampled from 2 to 18 d after modal hatch date. All eggs and larvae were frozen

Table 1. Summary of laboratory trials investigating lipid content and survival of larval Atlantic cod.

Number	Date	Brood stock origin	Mating design	Duration (days after modal hatching)	Temperature	Feeding treatments	Prey concentration (prey·L^{-1})
1	Feb.–Mar. 1991	SS	2 male × 7 female full- and half-sib crosses	23	4.5 (1.7)	Starved 6 d post-yolk sac absorption then fed	219
2	June–July 1992	NC	Unknown	20	4.1 (0.7)	Starved continuously	0
						Low food	100
						High food	300
3	Dec. 1992	SS	2 male × 7 female	16	5.7 (1.4)	Fed continuously	1000

[a]SS, Scotian Shelf cod; NC, northern cod.
[b]Values are means with standard deviations given in parentheses.

in liquid nitrogen immediately on collection and were subsequently extracted for lipids, dried at 60°C to constant weight, and weighed to the nearest 0.1 μg with a Cahn Gram Electrobalance (model G, Ventron Corp, Paramount, Calif.).

In trial 2, lipid composition and survival of cod larvae were evaluated at different feeding levels. Two treatment groups consisting of 100 ± 100 prey·L^{-1}, and 300 ± 140 prey·L^{-1} were randomly assigned in quadruplicate to experimental tanks. A control treatment of continuously starved individuals was assigned to a single tank. A total of 1500 eggs were counted and placed into each of nine 120-L tanks. Hatching duration was 5 d at an incubation temperature of 5.1 ± 0.8°C. Hatching commenced at 19 d and peaked at 21–22 d after fertilization. A total of 870 larvae (20 larvae·tank^{-1} every 4 d) were sampled from 1 to 16 d after the modal hatch date. Dead larvae were removed every 1–4 d and counts recorded for survival analysis. The number of unaccounted larvae at the end of the feeding trial ranged from 21 to 46%. Mortality of these larvae was assumed to have been proportional to observed mortalities. To assign unobserved mortalities to specific time intervals, the proportion of the total mortality occurring during a time interval was multiplied by the total number of unobserved mortalities. Collection and processing of larvae were identical to trial 1 except that gut contents were removed prior to lipid analysis.

In trial 3, lipid composition of cod larvae was evaluated at a single prey concentration of 1000 ± 400 prey·L^{-1}. A total of 15 000 eggs were placed in a single 1500-L tank. Hatching duration was 4 d at an incubation temperature of 5.2 ± 0.9°C. Hatching commenced at 16 d and peaked 18 d after fertilization. A total of 287 larvae were sampled from 0 to 15 d after modal hatch date. Collection and processing of larvae were identical to trial 2.

Lipid class determination

Cod larvae were removed from liquid nitrogen and immersed in an ice bath to prevent lipid degradation. A single larva was placed into a borosilicate glass tube containing 1 mL of dichloromethane–methanol solvents (2:1 vol/vol, HPLC grade) and extracted for ca. 24 h. Five micrograms of nonadecane (internal standard) was added to each tube to estimate recovery efficiency. Samples were maintained on ice prior to evaporation under purified nitrogen gas and reconstituted twice in 10 μL of extraction solvent.

Lipid class measurements were made using a Chromarod-Iatroscan Mark V (IATRON, Inc., Tokyo, Japan) TLC–FID. The flame ionization detector was operated at a hydrogen flow rate of 160 mL·min^{-1} and an air flow rate of 2000 mL·min^{-1}. The S-III Chromarods (IATRON, Inc., Tokyo, Japan) were calibrated every 2 or 3 weeks using synthetic standards that were weighed to the nearest 0.1 μg. Standards were treated in the same manner as the larval cod samples and were used immediately or stored at −20°C under a nitrogen atmosphere for a maximum of 48 h before use. Loading ranges of 0.05–100 μg were prepared for standards to encompass the expected range of variation in different lipid classes for different age/size classes of larvae.

The power model $y = ax^b$ was used to regress a known quantity of standard on area under chromatogram peaks.

Although the relationship between known quantity and area is strongly linear over much of the range examined, at quantities <1.0 μg, the power model accounted for a greater proportion of the variance than a linear model. The lipid classes (and synthetic standards) examined in larval cod were sterol esters (cholesteryl palmitate), TAG (tripalmitin), free fatty acids (palmitic acid), diglycerols (dipalmitin), ST (cholesterol), monoglycerols (1-monopalmitoyl-rac-glycerol), and PL (L-*a*-phosphatidylcholine).

Chromarods were first developed in hexane – diethyl ether – formic acid (67.9:2.1:0.04, vol/vol/vol) for 33 min. Rods were then dried for 2 min at 60°C and partially scanned to detect internal standard and sterol esters. Chromarods were then developed in hexane – diethyl ether – formic acid (47.9:21.2:0.06, vol/vol/vol) for 33 min. After drying, Chromarods were partially scanned for neutral lipid classes (mono- and di-acylglycerides, TAG, free fatty acids, and ST). The Chromarods were last developed in dichloromethane–methanol–water (42.2:25.3:2.5, vol/vol/vol) for 33 min. After drying, the entire rod was scanned to detect total PL.

Chromarods were cleaned and reactivated between samples and stored in a desiccator when not in use. Baseline noise levels were maintained at <0.5 mV peak height differential. We identified unknown lipid compounds from individual larvae by the position and the peak shape of the compound compared with known standards in identical solvent development conditions. To calculate quantities of lipid classes present in larval cod, integrated area under chromatogram peaks was first corrected for recovery efficiency.

Statistical analysis

We applied event analysis (Chambers and Leggett 1989) to compare patterns of larval survival among the different feeding treatments in trial 2. The survival function is defined as

$$[1] \quad S(t) = \text{prob(an individual survives} > t)$$

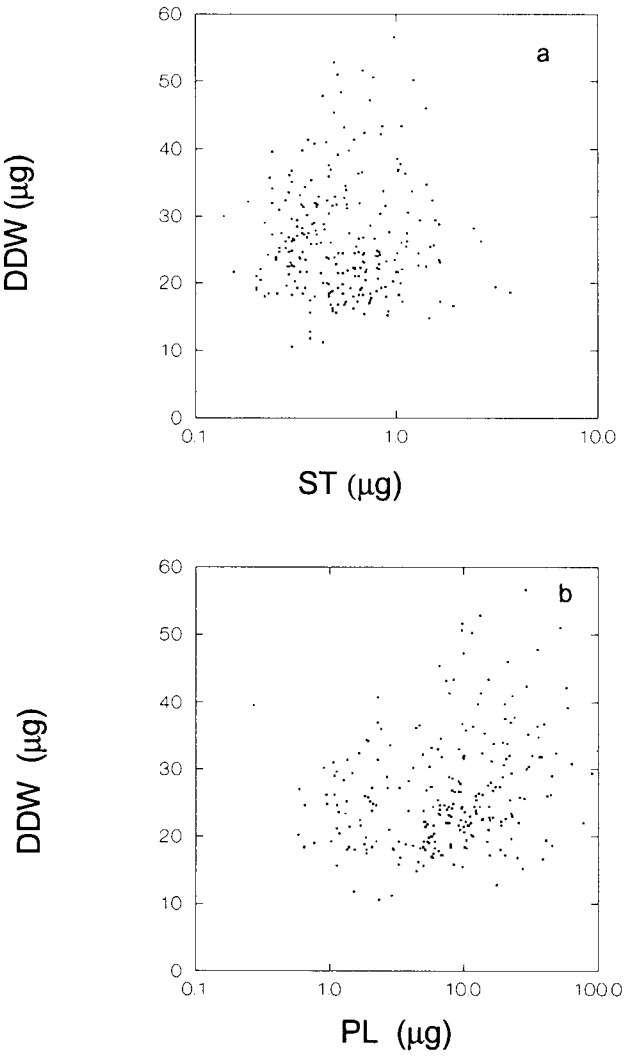
where $S(t)$ is the cumulative frequency distribution of a survival event during a time interval t, and the hazard function is defined as

$$[2] \quad H(t) = -\ln(S(t))$$

where $H(t)$ is the age-specific conditional probability of mortality. We computed survival and hazard functions according to equations provided in Ouellet et al. (1992). A risk of death index was computed from linear regression of log-transformed $H(t)$ on age and related the slope of the relationship to the proportion of larvae in poor condition in each feeding treatment.

We evaluated the relationship among TAG, PL, and DDW in a canonical discriminant function analysis (SAS Institute Inc. 1985) to investigate the utility of lipid and gravimetric measures to discriminate individuals from different feeding trials. We selected individuals older than 8 d (after modal hatch date) because the majority of larvae older than 8 d had depleted yolk-sac reserves and initiated exogenous feeding. The data were log transformed because this transformation facilitated greater discrimination than an analysis based on the untransformed data or a nonparametric discriminant function. Ten individuals from each of the treatments were randomly excluded from the data set to test the ability of the discriminant function with observations not included in the analysis. Data from field-collected larvae were included for comparison with laboratory observations.

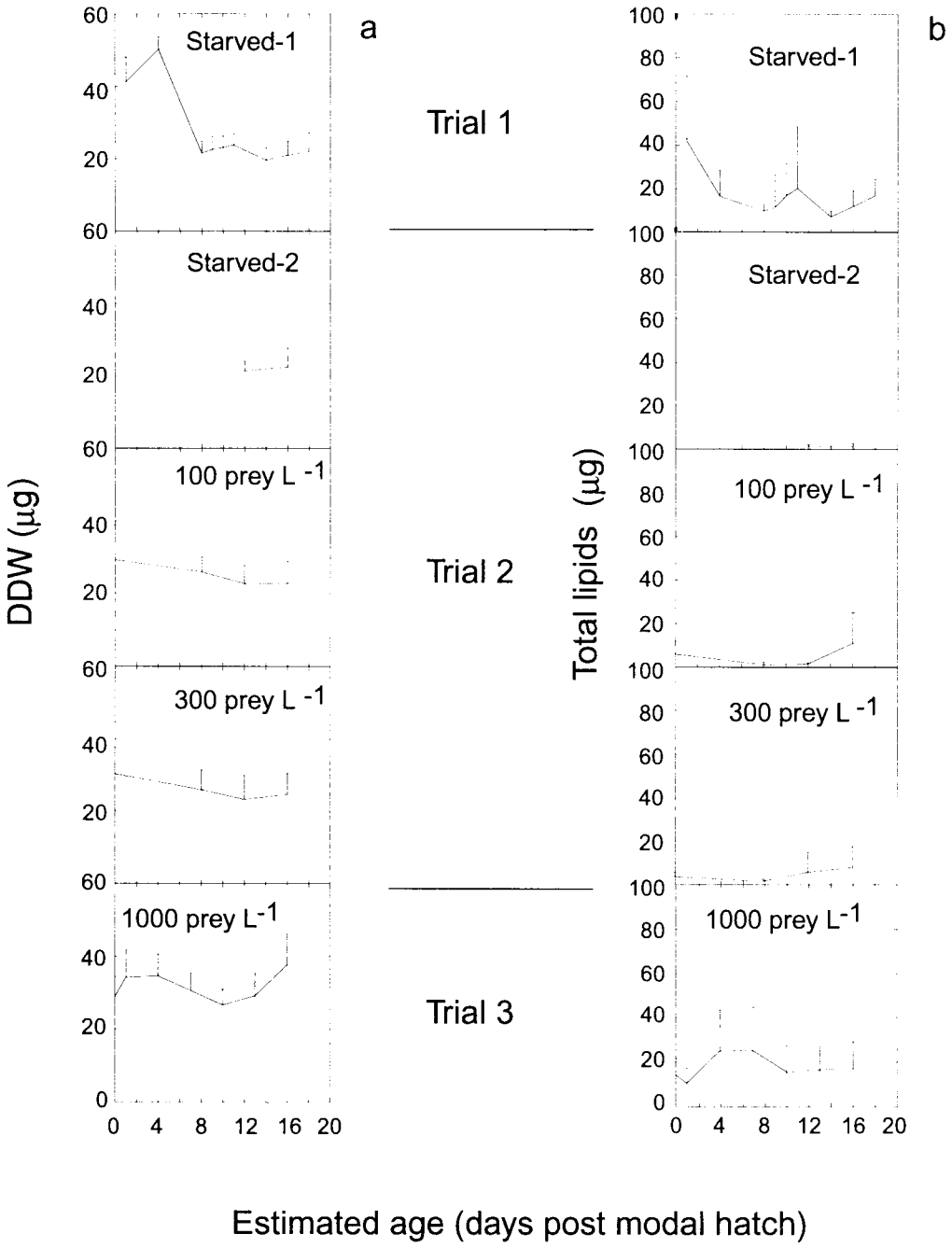
Fig. 1. Relationship between DDW and structural lipid classes. Quantity of ST and PL were plotted against DDW of individual larvae.



Results

Average initial DDW of cod larvae ranged from 29 μg in trial 3 to 41 μg in trial 1. Average initial total lipid contents varied from 4 μg in trial 2 to 43 μg in trial 1. The lipid content of cod larvae was size dependent. Larger larvae had higher absolute concentrations of most lipid classes. To compare the lipid class composition among larvae of different sizes, a method of standardization was necessary. One method of adjusting for size dependence is to standardize TAG measures by a lipid class related to body size. ST and PL are involved in membrane synthesis and previous studies showed high correlations between these two lipid classes and dry weight. In this study, ST and PL were poor predictors of DDW (Fig. 1). Therefore, we corrected for larval size by dividing lipid class measures by DDW of a larva.

Fig. 2. Time responses of mean (+SD) (*a*) DDW and (*b*) total lipids of cod larvae from starved, 100, 300, and 1000 prey·L^{-1} treatments. Food was not offered until 12 d in trial 1, but was offered continuously in trials 2 and 3.

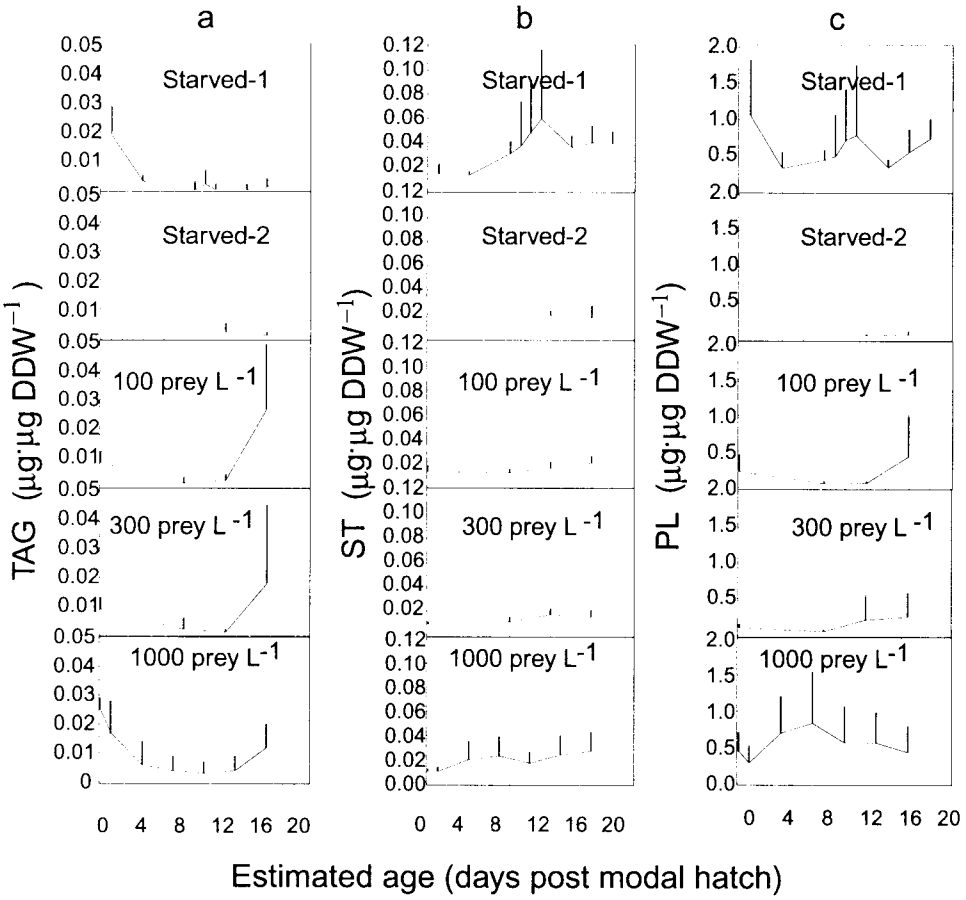


Trial 1

Average DDW and lipid content of starved cod larvae decreased between 1 and 8 d (Figs. 2*a* and 2*b*). The decrease in average lipid content between 1 and 4 d was followed by a decline in average dry weight between 4 and 8 d. Forty-two percent of the larvae were feeding by 14 d (food was offered at 12 d) and this fraction increased to 60% by 16 d. Dry weight remained at prefeeding levels even after prey were consumed. Average lipid content fluctuated around 10 µg for the duration of the trial. In this trial, lipids were extracted from larvae without gut removal and the lipids from the undigested prey items may explain part of the variance in lipid content. Therefore, all subsequent lipid extractions were conducted after the guts were removed.

TAG content diminished to a minimum at 9 d coincident with the end of the yolk sac stage and remained low for the duration of the trial (Fig. 3*a*). ST increased during the trial, but PL fluctuated without showing much overall change. ST and PL were most variable between 9 and 11 d, just at the end of endogenous feeding (Figs. 3*b* and 3*c*).

Fig. 3. Time responses of mean (+SD) (*a*) TAG, (*b*) ST, and (*c*) PL of cod larvae from starved, 100, 300, and 1000 prey·L^{-1} treatments. All lipid classes are presented as μg·μg DDW^{-1}.



Ninety-five percent of larvae greater than 8 d old had TAG below 0.004 μg·μg DDW^{-1} (Fig. 4*a*). The proportional distributions of PL and ST concentrations were more normal (Figs. 4*b* and 4*c*). Sixty-seven percent of the larvae had ST content lower than the mode (0.04 μg·μg DDW^{-1}) and 50% had PL content less than the mode (0.4 μg·μg DDW^{-1}). The width of size groups for all frequency histograms were chosen to best separate entire distributions. The exact concentration of any lipid class below which an individual may be considered in poor condition could not be clearly established.

Trial 2

Dry weight did not drop significantly during endogenous feeding for the two fed treatments (Fig. 2*a*). Data during this period from the starved treatment were lost due to machine malfunctions. Lipid content decreased slightly and then increased during the course of the trial in the two fed treatments (Fig. 2*b*). Lipid contents were near zero at 12 and 16 d in the starved treatment.

TAG content fell to a minimum between 8 and 12 d, but rose dramatically by 16 d in the two fed treatments (Fig. 3*a*). TAG content fell to 0.001 μg·μg DDW^{-1} between 12 and 16 d in the starved treatment. PL decreased initially, and then increased between 8 and 16 d in the two fed treatments, but did not increase between 12 and 16 d in the starved treatment. ST fluctuated little during the course of the trial in all three treatments.

The distribution of TAG in larvae older than 8 d indicated that between 81 and 86% of the population had less than 0.004 μg·μg DDW^{-1} in fed treatments (Fig. 4*a*). All individuals from fed treatments had ST concentrations less than 0.04 μg·μg DDW^{-1} (Fig. 4*b*). Eighty-eight to 91% of larvae had PL lower than 0.4 μg·μg DDW^{-1} (Fig. 4*c*). Lipid class distributions were not plotted for the starved treatment owing to low sample size (high mortality).

Mortality was measured only in trial 2. Probability of survival was high during the first 8 d in all cases except one high food treatment (Fig. 5*a*). Risk of death increased dramatically after yolk-sac absorption (Fig. 5*b*). In general, risk of death did not vary between treatments (ANOVA, $p = 0.3594$).

Trial 3

Dry weight remained relatively stable early in trial 3, but increased slightly between 10 and 16 d (Fig. 2*a*). Lipid contents showed minor changes throughout the course of the trial. When food was offered continuously from the start of the trial, 20% of the larvae were feeding by 7 d (Fig. 2*a*).

TAG fell to a minimum at 10 d, but was five times higher by 16 d (Fig. 3*a*). PL fluctuated during the trial, but beginning and ending values varied by only 5%.

Fig. 4. Relative frequency histograms of microgram quantities of (*a*) TAG, (*b*) ST, and (*c*) PL for 8- to 16-d-old Atlantic cod larvae exposed to different feeding treatments.

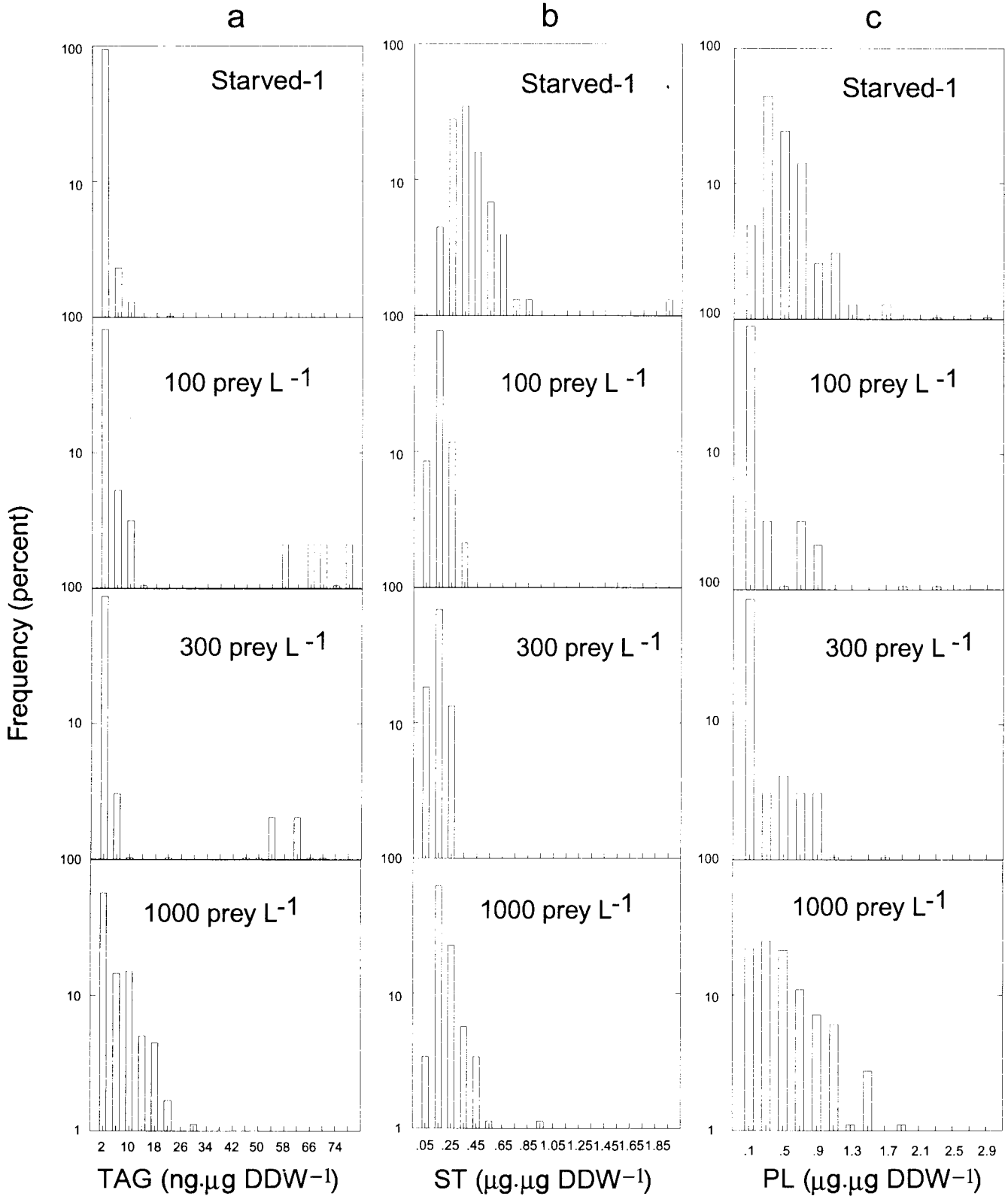
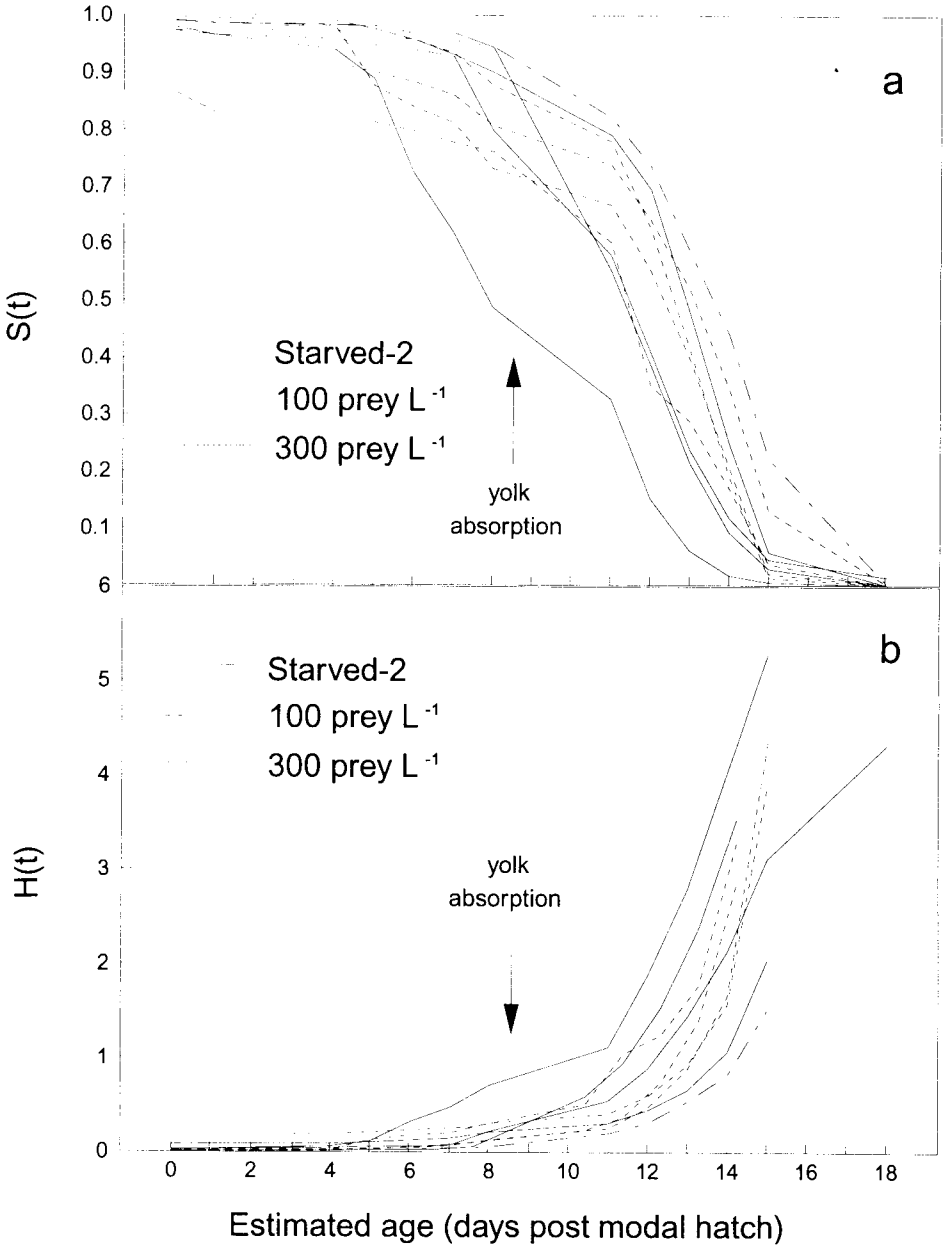


Fig. 5. (*a*) Survival, $S(t)$, and (*b*) hazard, $H(t)$, functions of cod larvae versus age after modal hatch date. $S(t)$ is the probability of survival and $H(t)$ is the conditional probability of death of cod larvae exposed to different feeding treatments.



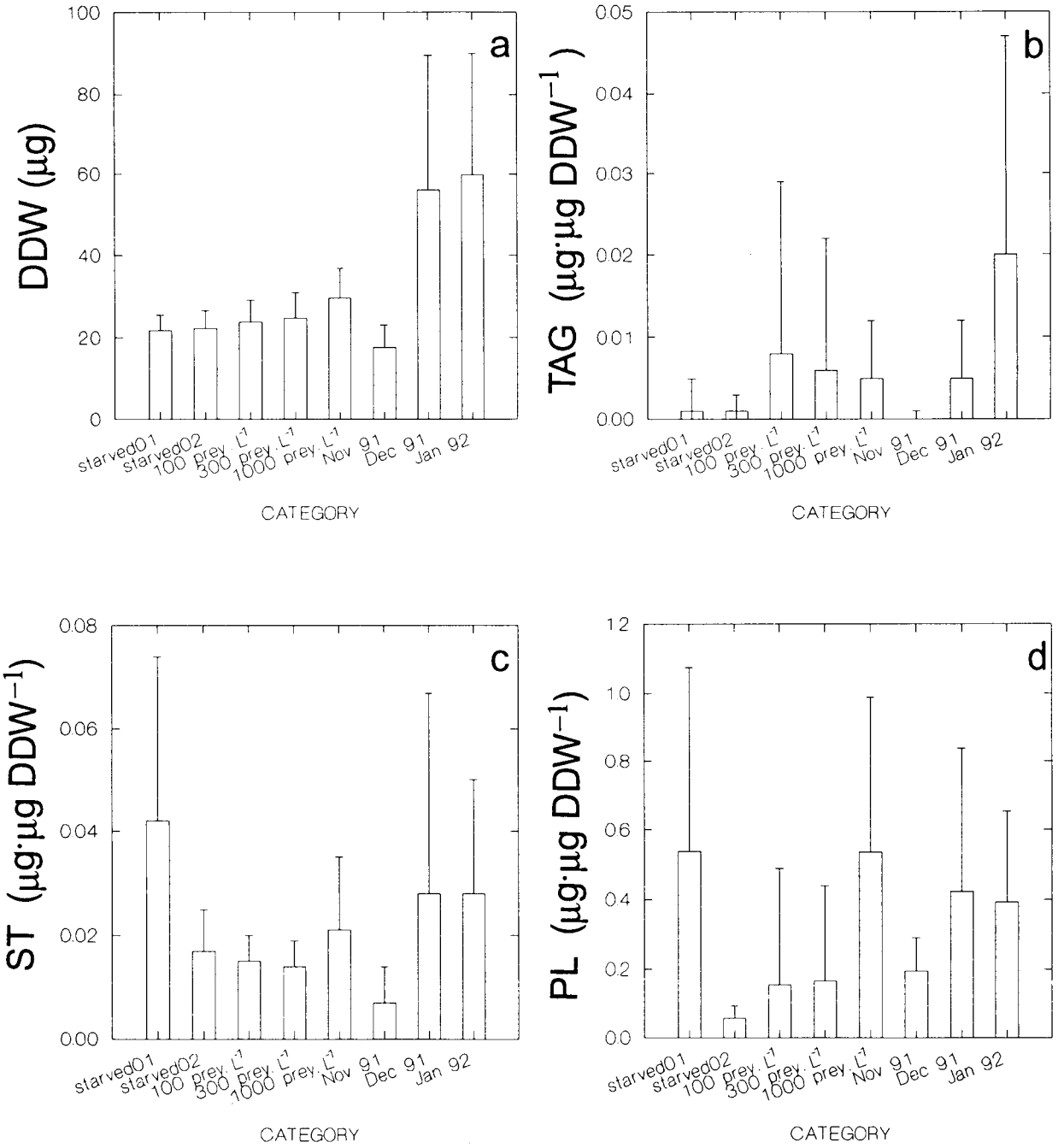
ST content was relatively stable and showed a slight increase over the course of the trial.

Only 57% of the population had TAG concentrations below 0.004 μg·μg DDW^{-1} (Fig. 3*a*), 93% had ST contents less than 0.04 μg·μg DDW^{-1}, and 48% of the population had PL contents lower than the mode (0.4 μg·μg DDW^{-1}).

Field and laboratory comparisons

We compared DDW, TAG, ST, and PL of 8- to 16-d-old laboratory-reared post-yolk-sac larvae with larvae of approximately the same age/size collected on the Scotian Shelf during monthly ichthyoplankton surveys, November and December 1991 and January 1992 (Fig. 6). Ages of field-collected larvae were estimated using age–length relationships for Georges Bank cod with size at hatching adjusted to reflect Scotian Shelf cod (Bolz and Lough 1983). Larvae collected in December and January weighed more than larvae from our feeding trials, but November larvae weighed less. December larvae had TAG contents similar to larvae from fed treatments, but January larvae had more than twice as much TAG as any feeding trial. November larvae had less TAG than larvae from starved treatments. Except for trial 1, larvae showed a pattern of increasing PL with improved feeding environment. December and January larvae had PL concentrations similar to larvae from the 1000 prey·L^{-1} treatment. November larvae had PL concentrations similar to the 100 and 300 prey·L^{-1} treatments. Variability in ST between laboratory treatments was

Fig. 6. Mean (+SD) quantities of (*a*) DDW, (*b*) TAG, (*c*) ST, and (*d*) PL of 8- to 16-d-old post-yolk-sac larvae from feeding trials and Scotian Shelf collections from November and December 1991 and January 1992.

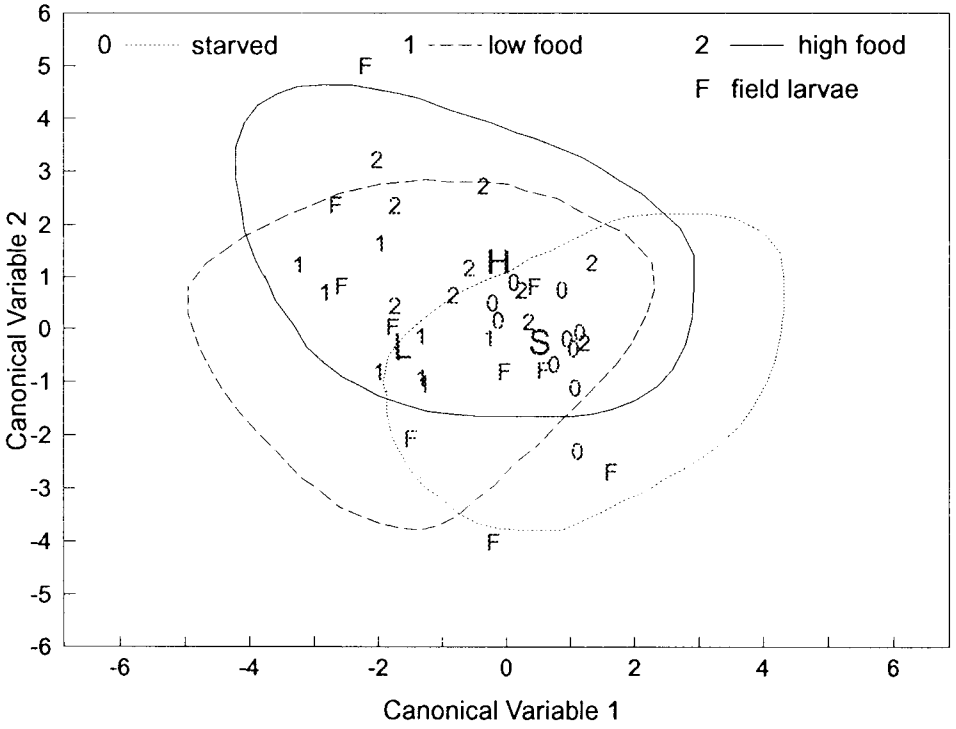


minimal and interpretation of the differences in ST between months is difficult. Lipid class compositions were similar between laboratory-reared and field-collected cod larvae. Defatted dry weight, PL, and TAG measures suggested that January larvae were in good condition relative to November larvae, with December larvae intermediate.

Lipid-based condition indices

A condition index based on a single lipid class obviously does not take into account the information available from assessment of other lipid classes. We, therefore, used TAG, PL, and DDW in a discriminant function analysis to test whether these variables could be used to classify larvae according to feeding treatment. The frequency distributions for ST showed little difference among treatments (Fig. 4*b*) so ST was not included in the discriminant function analysis. We combined trial 1 individuals with individuals from trial 2 starved treatment. We also combined individuals from the 100 and 300 prey·L^{-1} treatments from trial 2 because there were no significant differences in average PL, TAG, or DDW between the two treatments. Following the discriminant function analysis, we tested

Fig. 7. Discriminant function analysis using gravimetric and lipid measures of laboratory-reared cod larvae. The irregular curves are distributions of individuals greater than 8 d old from each of the trial treatments. The overlap in distributions is caused by the high proportion of larvae in all treatments that appeared to be in poor condition. Numbers represent starved (0), 100 and 300 prey·L^{-1} (1), and 1000 prey·L^{-1} (3) larvae initially withheld from the analysis. F designates field-collected cod larvae. S (starved), L (100 and 300 prey·L^{-1}), and H (1000 prey·L^{-1}) denote distribution centroids.



for differences in the centroids (group means of each canonical variate) using ANOVA. For each canonical variate, the mean from any group was significantly different from the others (ANOVA, $p = 0.0001$).

We randomly excluded 10 individuals from each group to test the ability of the discriminant function to correctly classify larvae by group. All 10 individuals from the starved treatments were classified as starved. Four individuals from the 100 and 300 prey·L^{-1} group were misclassified as starved. The remaining six larvae from this group were correctly classified. Five of the individuals from the 1000 prey·L^{-1} treatment were incorrectly classified as starved. The other five were correctly classified.

The distribution of starved individuals was limited to a small area in canonical variate space (Fig. 7). Distributional area increased as prey concentration of the treatments increased, but there was considerable overlap between all groups. We plotted the individuals originally withheld from the discriminant function analysis and individuals randomly selected from the field in canonical variate space. The misclassified larvae from fed treatments fell within the distribution of starved individuals, suggesting that despite the availability of prey, these particular larvae were not in good condition. The mean values for canonical variates one and two from the starved treatments are the coordinates of the centroid of their distribution. The coordinates are (0.538, −0.358). We propose that the relative condition of any larva can be determined by its euclidean distance from this centroid in canonical variate space. The formula for condition (C) would be

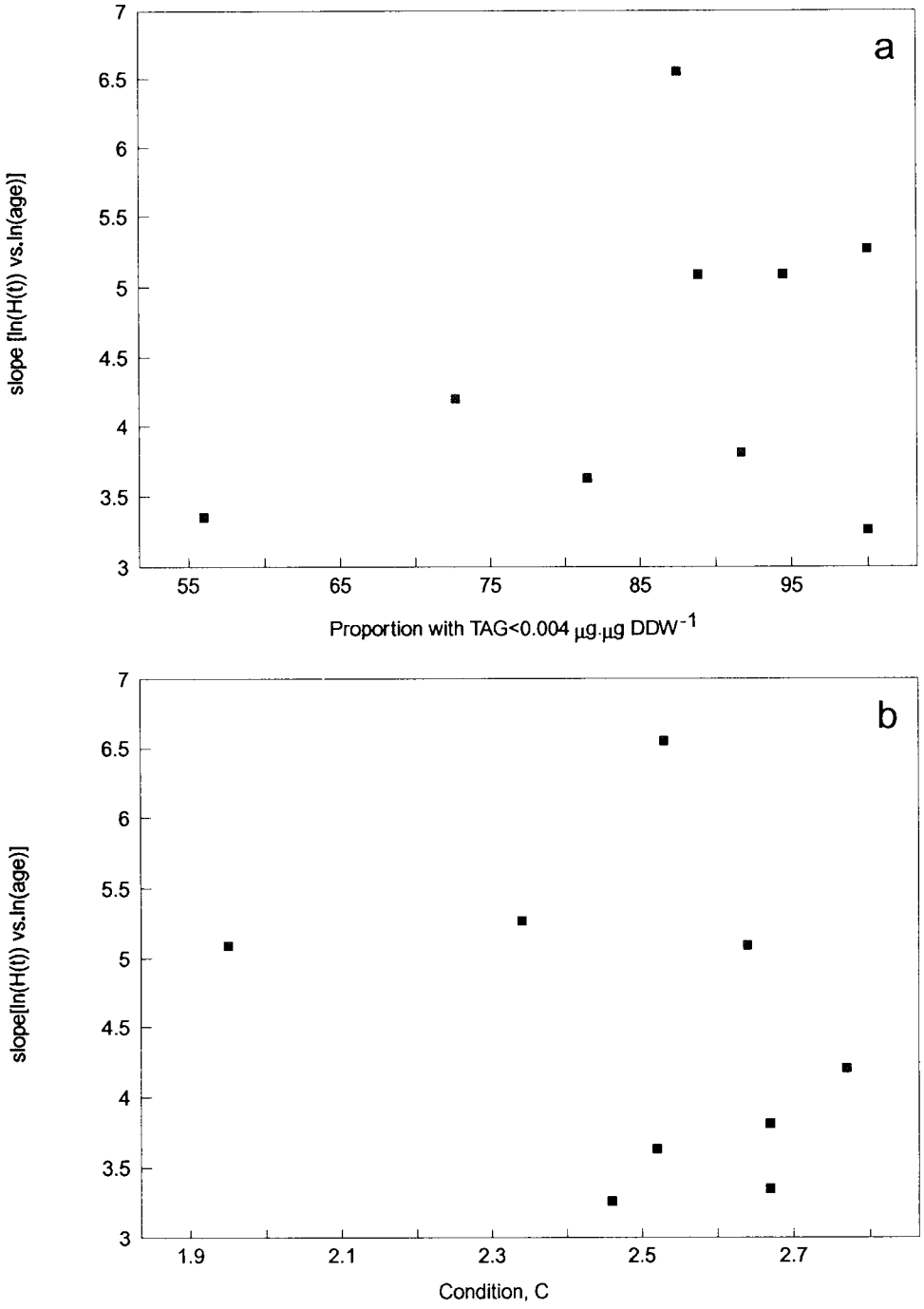
$$[3] \quad C = \sqrt{(0.538 - \text{can1})^2 + (-0.358 - \text{can2})^2}$$

The average C's for 9- to 16-d-old larvae from starved, 100 and 300, and 1000 prey·L^{-1} treatments were 1.04, 2.54, and 1.99 respectively. This analysis indicated that we had some ability to classify larvae according to treatment. But more importantly, the condition of an individual larva could be assessed, relative to larvae stressed by starvation beyond their ability to recover, based on its TAG, PL, and DDW.

Condition and survival

The slope of the cumulative hazard function is a risk of death index, or a measure of how rapidly the population is dying. A slope of zero would indicate a population that suffered no mortality. A slope of infinity indicates a population that suffered instantaneous extinction. Risk of death was related to the proportion of each treatment group with low TAG. Individuals greater than 8 d old with TAG concentrations <0.004 μg·μg DDW^{-1} were classified in poor condition. In general, groups with a low percentage of starved individuals were at low risk of death (Fig. 8). Groups with more than 85% of the population in poor condition showed varying levels of risk of death. We also

Fig. 8. Slope of log-transformed hazard function (conditional probability of death) versus time plotted against (*a*) proportion of 8- to 16-d-old cod larvae with TAG <0.004 μg·μg DDW^{-1} and (*b*) mean condition.



related risk of death to *C* (Eq. 3). When condition was high ($C > 2.6$), larvae were at a lower risk of death. When condition was low, risk of death varied. This partially supports the hypothesis that cohorts with a lower proportion of individuals in poor condition are likely to exhibit higher survival and to produce better year classes. It appears that factors other than condition are contributing to the observed mortality patterns in trials with high proportions of individuals in poor condition.

Discussion

Our studies have shown that lipid class composition can be quantified in individual cod larvae. The small size of young cod larvae did not preclude assessment of their lipid composition. The Iatroscan MK-5 limit of detection is approximately 0.03 μg. Our work suggested that TAG concentrations of ca. 0.004 μg·μg DDW^{-1} was a reasonable threshold below which individuals can be considered in

poor condition (Fig. 5*a*). Larvae weighing as little as 10 μg and having as much as 0.04 μg TAG would still be considered in poor condition. Therefore, the level of detection did not interfere with assessment of condition. Our coefficients of variation at the quantification limit (0.03 μg) compare favourably with Parrish and Ackman (1985) and Sebedio and Juaneda (1991).

Lipid contents correlated with the feeding environment to varying degrees. We used various prey concentrations as treatments to emphasize the relationship between food and lipid content in cod larvae. All larvae exhibited a decrease in TAG during yolk absorption, but fed larvae exhibited a subsequent increase following the initiation of exogenous feeding. Larvae stressed by starvation did not exhibit an increase in TAG, even after initiation of feeding. Furthermore, the percentage of the individuals having low TAG or PL decreased as the prey concentration increased.

However, it is apparent that individuals from low food treatments frequently had higher lipid concentrations than individuals from high food treatments. Sixteen-day-old larvae from the 100 and 300 prey·L^{-1} treatments (trial 2) had higher average TAG than larvae from the 1000 prey·L^{-1} treatment (trial 3). The variability in the 100 and 300 prey·L^{-1} treatments at 16 d was considerable. Much of the population was in moderate condition at 100 prey·L^{-1}, but some individuals were in good condition. Because of the influence of outliers on average values there is a danger in basing decisions concerning the overall health of a population on mean values alone. The distributions of characteristics used to determine condition are also important.

Hakanson (1989*b*) noted that in his rearing experiments "the top 10% of the larvae were doing very well" regardless of prey composition or concentration. In our highest prey concentration only 43% of the individuals were above the poor condition TAG threshold (0.004 μg·μg DDW^{-1}). This suggests factors other than prey concentration (such as genotype, container effects, or prey quality) play roles in determining condition of laboratory-reared cod larvae.

The best combination of lipid class measures for assessing condition in individual cod larvae was also assessed. Fraser (1989) proposed a ratio of TAG to ST content as a means of assessing condition in larval fish and aquatic invertebrates. He demonstrated a strong correlation of both ST and PL, with dry weight. These two lipid classes are components of cell membranes and might, therefore, be related to body size. The correlations were generated from pooled samples of between 140 and 500 larvae. Pooling samples could make the correlations appear artificially high. We demonstrated that for individual cod larvae less than 100 μg DDW, there was a poor relationship between ST or PL and DDW. We suggest that DDW is a better proxy than ST for size in cod larvae.

Hakanson (1993) defined larvae in poor condition to be those with a TAG/ST ratio less than 0.2 and a PL content less than 80% of the mean for its size class. His criteria were based on TAG, ST, and PL concentrations. We proposed an index based on two lipid class measures and DDW. We chose to include PL and not ST for the following reasons: (*i*) ST and PL were correlated ($\rho = 0.7$, $p < 0.0001$); (*ii*) ST varied little through time during trials 2 and 3; and (*iii*) the relative frequency distribution of ST changed little over a two order of magnitude range in prey concentration (Fig. 3*c*). We included DDW because it increased with age in trial 3, consistent with the expectation for growth of good condition larvae. We included TAG because it increased in fed post-yolk-sac larvae, but did not respond in severely starved post-yolk-sac larvae. The index was generated by transformation of lipid and gravimetric measures to canonical variates. Martin and Wright (1987) also used a discriminant function analysis based on morphometric data to distinguish between starved and fed striped bass larvae.

In canonical variate space the centroid of the distribution of starved cod larvae was taken as a base line. The distance between this point and a point with coordinates denoted by the first and second canonical variables from the discriminant function are a relative measure of condition. This concept is supported by the location of starved individuals, withheld from the original discriminant function analysis, within the distribution of starved larvae in canonical variate space. Individuals from fed treatments and field collections were located at varying distances from the base-line centroid. Not all field-collected larvae were in good condition. Several were in poor condition according to their location in variate space and *C* values (Fig. 8). This index is objective and can be assigned to any larva without knowing the mean PL content for its size class.

Can lipids be linked with probability of survival? Poor condition can be translated into poor survival by several mechanisms. Hakanson (1993) found that anchovy larvae with the fastest growth rates were those which had high lipid content. In general, fast growing larvae will be larger, have higher swimming speeds, and search greater volumes for food than slow growing larvae. Folkvard and Hunter (1986) demonstrated that fish larvae in poor condition were more susceptible to predation than larvae in good condition. However, faster growing larvae may not always survive better (Litvack and Leggett 1992).

The relationship between survival and condition might be strengthened in two ways. First, a wider range of feeding environments would generate larger differences in survivorship. We used varying concentrations of natural zooplankton to generate lipid differences among treatments. This resulted in rearing of cod larvae with lipid profiles similar to field-collected larvae. However, the varying concentrations did not generate large differences in survivorship curves (Fig. 5*a*). Ouellet et al. (1992) used artificial diets to maximize differences in lipid composition and survivorship. Second, the lack of predators in the trials allowed larvae in poor condition to survive longer than they might under natural conditions. The link between likelihood of survival and a lipid-based condition index has been demonstrated for shrimp larvae (Ouellet et al. 1992). To our knowledge, ours is the first attempt to demonstrate such a relationship for fish larvae. Our results suggest that when condition is high, risk of death is low, but when condition is low, risk of death is variable. Our results provide support for the notion that larval abundance estimates, prorated according to the condition of the population, may better reflect future year-class strength than unadjusted estimates.

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