INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

An appraisal of condition measures for marine fish larvae with particular emphasis on maternal contribution, circadian periodicity, and the time response of nucleic acids and proteins.

André Ferron

Department of Biology

McGill University

June 2000

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

©André Ferron
2000

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-69876-9

Canada

ABSTRACT

The physical and physiological condition of larval fishes throughout their development is believed to influence their survival, and ultimately to contribute to recruitment to the adult population. The study of larval condition is therefore central to larval fish ecology. While various indices used to measure larval fish condition have been championed through time, and while the tendency for sophistication of these measures continues to increase, there has, to date, been no systematic evaluation of their relative effectiveness. Indeed, for many of the measures used, there has been no assessment of the assumptions underlying the methods.

In this thesis I sought to 1) carry out an appraisal of the characteristics and the reliability of condition measures now being used, 2) assess the importance of maternal contribution to the nutritional status of larval fish, 3) evaluate the possibility that diel variability in metabolism could lead to serious biasing of the interpretation of condition measures obtained over time, and 4) assess the time course of the condition of larval fishes subjected to periods of intermittent feeding.

A comprehensive review of the literature on the use of condition measures to assess the nutritional status of larval fish (chapter 1) led to the identification of seven broad categories of indices and eleven different attributes of these. The categories include: morphological indices, histological scores, histological cell heights, nucleic acids and proteins, lipids, digestive enzymes, and metabolic enzymes. Most of these indices reliably detect condition changes related to poor feeding, with the exception of indices based on lipids and digestive enzymes which can be influenced by gut content. Several potential sources of variability (both biotic and abiotic factors) other than nutrition have been identified for these indices, however few have been seriously investigated. Genetic factors, time of the day, and diet composition were among the most commonly cited biotic factors.

To more effectively classify the response of condition to changes in food availability, we proposed three new attributes: sensitivity, latency and dynamics. We defined sensitivity as the minimum change in food ration that can be detected by a particular index of condition and developed a bioenergetic model which links condition to food concentration using literature data from field and laboratory studies. The time response (latency and dynamics) of condition to changes in feeding is poorly known. This greatly limits the use of condition indices in field studies. We defined latency as the time lag between a change in food availability and a significant change in condition. Dynamics was defined as the rate of change in condition over time after a response has been detected. Models of response dynamics were developed from published data and were further tested in laboratory experiments (chapter 4). Our review revealed that sensitivity, latency, and dynamics of most indices are poorly known. However, we found a wide range of variability (up to tenfold) in these attributes across the different categories of indices reviewed.

Laboratory versus field differences in condition measures were found to be large. This was especially true for indices based on morphological and lipid data. This problem must be addressed if laboratory calibrations are to be used in the interpretation of field data. Similarly, size, age and species differences have important effects on indices based on morphological measurements, histological scores and digestive enzymes. Processing time, costs and requirements are the last three attributes investigated from the literature, and were found to vary greatly depending on the condition measure used.

The experiments described in chapter 2 were designed 1) to assess the impact of female nutritional status on the quality of the eggs and larvae they produced, 2) to determine which of a series of nucleic acid and protein measurements were most responsive to post-hatching starvation, and 3) to determine whether the starvation dynamics of those measures was affected by female source. No significant correlation could be found between any of the maternal traits studied and eggs and

larval measures. The results did show, however, that egg size was more variable between-clutches than within-clutch, was independent of embryonic developmental rate, but was positively related to larval size. Substrate indicators, such as dry weight and quantity of protein per larva, were the most affected by starvation but their dynamics was affected by their female source. Rate indicators such as RNA/DNA ratio were slightly less affected by starvation, but their dynamics were independent of female source.

The existence and ontogeny of circadian (24 hrs.) and ultradian (<24 hrs.) oscillations in the nucleic acids and protein content of larval capelin was investigated in the laboratory experiments outlined in chapter 3. The most obvious long-terms trends occurred during the embryonic period when DNA and RNA content increased constantly, and during the post-yolk-sac period when RNA and protein decreased following sub-optimal feeding. The most conspicuous periodicity identified were 3 d cycles in the protein content of developing embryos, and ultradian oscillations (6 hr) in the protein content and the protein/DNA ratio of newly-hatched and post-yolk-sac larvae.

The objectives of the study described in chapter 4 were threefold, 1) to determine which of a series of nucleic acid and protein measurements were affected by intermittent (delayed-fed and delayed-starved) feeding conditions in capelin larvae, 2) to determine the dynamics and shape of the time response, and 3) to determine whether the empirical data gathered were consistent with models developed as a consequence of the review of the literature (chapter 1). Only the dry weight, and the quantity of DNA, RNA, and Protein per dry weight differed significantly between starvation and *ad libitum* feeding controls. The divergence between starvation and feeding controls was less than predicted, probably as a consequence of sub-optimal feeding conditions in the *ad libitum* feeding controls. However, recovery dynamics were inconsistent with the predictions especially for the DNA and RNA/dry weight indices. Starvation dynamics were less consistent with

predictions. Of the indices investigated, the dynamics of the quantity of DNA and RNA per dry weight were the most consistent dynamics with model predictions. Significant between-replicate (tank) differences were found in *ad libitum* feeding controls and delayed-fed treatments, which suggest difficulties in replicating optimal feeding. A large data scatter around the fitted lines in those same treatments, exemplified the large inter-individual variability in condition which typically characterize feeding larvae.

RÉSUMÉ

On croit que la condition physique et physiologique des larves de poissons pendant leur développement influence leur survie, et peut ultimement contribuer à faire varier le recrutement chez la population adulte. La condition larvaire est donc un outil essentiel à l'étude de l'écologie des larves de poissons. Bien que plusieurs indices aient été utilisés et prônés pour mesurer la condition nutritionnelle des larves de poissons, et que la tendance actuelle soit à une sophistication accrue des méthodes de mesure; Il n'existe, à l'heure actuelle, aucune évaluation systématique de leur performance respective. En fait, aucune vérification des présomptions sous-jacentes n'a été faite pour plusieurs méthodes présentement utilisées.

Dans la présente thèse je tente 1) par l'entremise d'une révision complète de leur caractéristiques de faire une évaluation de la fiabilité des indices de condition présentement utilisés, 2) d'évaluer l'importance de la contribution maternelle à l'état nutritionnel des larves de poissons, 3) d'évaluer la possibilité que des variations journalières dans le métabolisme puissent induire un biais sérieux dans l'interprétation des données sur la condition larvaire, et 4) de suivre l'évolution et de décrire les changements dans la condition de larves soumises à des périodes d'alimentation intermittentes.

Une revue exhaustive de la littérature traitant de l'utilisation des mesures de condition pour évaluer la condition nutritionnelle des larves (chapitre 1) a permise d'identifier sept grandes catégories d'indices et onze attributs les caractérisant. Ces catégories comprenaient: Les indices morphométriques, les indices histologiques, les mesures cellulaires histologiques, les acides nucléiques et protéines, les lipides, les enzymes digestifs et finalement les enzymes métaboliques. La plupart de ces indices parvenaient avec fiabilité à détecter des changements dans la condition en rapport avec une alimentation pauvre, sauf exception des indices basés sur les mesures de lipides et d'enzymes digestifs qui semblent encore trop influencés par

le contenu digestif. Plusieurs sources de variabilité (à la fois des facteurs biotiques et abiotiques) autre que la nutrition ont également put être identifiés, même si peu de ces facteurs aient été sérieusement étudiés. Parmi les facteurs les plus souvent cités, notons les facteurs génétiques, le temps de la journée et la composition de la diète.

De façon à permettre une meilleure classification des changements de condition suite à des changements dans la disponibilité de nourriture, nous proposons trois nouveaux paramètres soit: La sensibilité, le temps de latence, et la dynamique. La sensibilité est définie comme étant la variation minimale dans la ration alimentaire requise pour susciter un changement dans un indice de condition donné. Comme première approximation pour calculer la sensibilité d'un indice de condition, nous avons développé un modèle bioénergétique à partir de données publiées permettant de lier la condition des larves à des concentrations variables de nourriture. Le temps de réponse (mesuré à partir du temps de latence et de la dynamique) de la condition suivant des changement dans l'alimentation est très mal connue à l'heure actuelle et ceci limite grandement l'utilisation des indices de condition sur le terrain. Nous définissons le temps de latence comme la période séparant le moment où un changement dans la disponibilité de nourriture se produit et le moment où un changement significatif de condition est détecté. La dynamique par ailleurs est définie comme le taux de variation de condition par rapport au temps aussitôt qu'une réponse à été détectée. Des modèles de dynamique ont été développés à partir de données publiées pour être ensuite testés expérimentalement (chapitre 4). Notre revue de la littérature a démontrée que la sensibilité, le temps de latence, et la dynamique de la plupart des indices sont très peu connus. Cependant, nous avons put remarquer une grande gamme de variabilité (de l'ordre de 10) dans ces facteurs pour les différentes catégories d'indices.

La revue de la littérature à aussi montrée que des différences marquées ont été signalées à plusieurs reprises entre des mesures de condition prises sur des spécimens élevés en laboratoire et ceux prélevés dans leur milieu naturel. Ces différences étaient surtout évidentes pour des indices basés sur des données de type morphométrique et lipidique. Ce problème doit être réglé avant d'utiliser des calibrations établies en laboratoire pour interpréter des données provenants d'échantillonnage sur le terrain. De plus, toutes différences d'âge, de taille et d'espèce entre les spécimens analysés semblaient induire des variations indésirables de condition, surtout pour des indices utilisant des données morphométriques, histologiques ou des enzymes digestifs. Le temps requis pour le traitement des échantillons, les coûts associés, et les besoins spécifiques avant analyses, sont d'autres considérations soulignées par plusieurs auteurs, qui variaient considérablement selon le type de mesure de condition utilisé.

Les expériences décrites à l'intérieur du chapitre 2 avaient comme objectifs 1) d'évaluer l'impact de l'état nutritionnel de la femelle sur la qualité des œufs et des larves produites, 2) de déterminer à partir d'une séries de mesures d'acides nucléiques et de protéine, lesquels étaient les plus affectés par le jeûne après l'éclosion, et 3) pour déterminer si le taux de dégradation (dynamique) de ces mesures était affecté par l'origine maternelle des larves. Aucune corrélation significative n'a été obtenue entre une séries de mesures décrivant l'état nutritionnel de la femelle et la condition des œufs et des larves produits. Les résultats ont cependant démontrés que la taille des œufs était plus variable entre progénitures provenant de différentes femelles qu'à l'intérieur de la progéniture d'une seule femelle. La taille des œufs était corrélée positivement avec la taille des larves à l'éclosion, mais n'avait aucune influence significative sur le taux de développement embryonnaire. Parmi l'ensemble des mesures de condition, ceux référant à la quantité de substrat disponible (poids sec et contenu en protéines) étaient d'avantage affectés par le jeûne mais leur dynamique (taux de dégradation) était fonction de leur origine maternelle. Les indices référant aux taux de dégradation

(rapport ARN/ADN) étaient un peu moins affectés par le jeûne, par contre leur dynamique était indépendante de leur origine.

Les expériences décrites dans le chapitre 3 ont été menées pour confirmer l'existence et le temps d'apparition d'oscillations circadiennes (24 hrs.) et ultradiennes (<24 hrs.) dans le contenu en acides nucléiques et en protéines de larves de poissons. Les tendances de longue période les plus évidentes chez le capelan se sont produites pendant la période du développement embryonnaire où les quantités d'ADN et d'ARN ont augmentées continuellement, et pendant la période suivant la résorption du sac vitellin où les quantités d'ARN et de protéines ont chutés progressivement suivant une alimentation sub-optimale. Les périodicités les plus marquées étaient des cycles de 3 jours dans le contenu protéique des embryons, et des variations ultradiennes (6 hr) dans le contenu protéique et le rapport protéine/ADN des larves nouvellement écloses, de même que chez celles débutant leur alimentation exogène.

Pour ce qui est des expériences décrites dans le chapitre 4, celles-ci avaient trois objectifs principaux: 1) déterminer lesquels d'une séries de mesures d'acides nucléiques et de protéines étaient les plus affectés par une alimentation intermittente chez des larves de capelan, 2) déterminer la dynamique et l'allure des réponses par rapport au temps, et 3) évaluer si les données empiriques étaient conformes avec les modèles développés à partir de la revue de la littérature. Seuls le poids sec et la quantité d'ADN, d'ARN et de protéines par poids sec étaient significativement différent entre les individus des contrôles soumis au jeûne et ceux exposés à une alimentation *ad libitum*. La divergence des courbes obtenues entre ces deux contrôles était moins accentuée que prévue, conséquence probable d'une alimentation sub-optimale provenant des diètes *ad libitum*. Pendant que la dynamique du jeûne était conforme avec les prédictions, celle de la dynamique de récupération ne l'était pas. De tous les indices compilés, la dynamique de la quantité d'ADN et d'ARN par poids sec était sans doute ceux les plus conforment

avec les prédictions du modèle. Des différences significatives entre réplicats (bassins) ont été détectées dans les contrôles d'alimentation *ad libitum* de même qu'à l'intérieur des traitements (bassins) où la nourriture était introduite suivant un jeûne plus ou moins prolongé, ce qui suggère une difficulté à reproduire des conditions d'alimentation optimale. Une grande variation de la condition entres individus à l'intérieur de ces même bassins suggère également une variabilité dans les patrons d'alimentation.

TABLE OF CONTENTS

CHAPTER 4

THE TIME RESPONSE OF CHANGES IN THE PROTEIN AND NUCLEIC ACID COMPOSITION OF LABORATORY-REARED CAPELIN *(Mallotus villosus)* LARVAE FOLLOWING INTERMITTENT FEEDING REGIMES 174

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: **AN APPRAISAL OF CONDITION MEASURES FOR MARINE FISH LARVAE**

CHAPTER 2: **FEMALE CONTRIBUTIONS TO EGG AND HATCHLING SIZE ANDTO THE BIOCHEMICAL CHANGES ASSOCIATED WITH STARVATION IN CAPELIN (Mallotus villosus) LARVAE**

PREFACE

The faculty of Graduate Studies and Research requires the following statement to be reprinted in all Ph D theses:

"The candidate has the option, subject to the approval of their Department, of including as part of the thesis the text, duplicated published text, of an original paper or papers. Manuscript-style theses must still conform to all other requirements explained in the guidelines concerning thesis preparation. Additional material (procedures and design data as well as descriptions of equipment) must be provided in sufficient detail (e.g. in appendices) to allow clear and precise judgement to be made of the importance and originality of the research reported. The thesis should be more than a mere collection of manuscripts published or to be published. It must include a general abstract, a full introduction and literature review and a final overall conclusion. Connecting texts which provide logical bridges between different manuscripts are usually desirable in the interest of cohesion.

It is acceptable for theses to include, as chapters, authentic copies of papers already published, provided these are duplicated clearly and bound as an integral part of the thesis. In such instances, connecting texts are mandatory and supplementary material is always necessary. Photographs or other materials which do not duplicate well must be included in their original form. While the inclusion of manuscripts co-authored by the candidate and others is acceptable, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent, and supervisors must attest to the accuracy of the claims at the Ph.D. Oral Defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make the responsibilities of authors perfectly clear."

In accordance with the above I offer the following information. The general introduction as well as Chapter 1 was co-authored by my supervisor Dr. W.C. Leggett and has been published (Ferron, A. and W.C. Leggett. 1994. An appraisal of condition measures for marine fish larvae. *Advances in Marine Biology,* 30: 217-303). For Chapter 2, Dr. R.C. Chambers a PDF in Dr. Leggett's laboratory assisted in sampling, and shared some of the data obtained. He also offered advice on the experimental design and data analysis. Dr. Leggett offered constructive criticism on the analysis, the interpretation and the editing of all chapters in addition to providing necessary human and physical resources. The contributions of Dr. Leggett to this thesis fall within the normal bounds of a Ph D student supervision. In all chapters the pronoun "we" is used to reflect this participation.

CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

1. I provide a first and needed comprehensive review of the use of condition measures for establishing the nutritional status of marine fish larvae. The recommendations included in that review will be of value to researchers who may now select indicators on the basis of a knowledge of their characteristics, sensitivity, time response, species and size-specificity, processing time, and costs.

2. Using a bioenergetic approach, I provide a conceptual framework for the study of the time response of condition measures to intermittent feeding. The terminology developed is now being used by several researchers working in the area of larval fish ecology.

3. I provide evidence that egg size variability in capelin can be very small within-family as opposed to between-female differences. Large eggs are produced at the cost of lower relative fecundity for this species.

4. I demonstrate that at the family level in capelin, egg size is related to larval size at hatch but unrelated to embryonic developmental rate which seems to be maternally-driven. The consequence is that a constant proportion of egg yolk is used for embryonic development. There is evidence that this might also hold for other fish species.

5. I provide some evidence that energy substrates in starving fish larvae decrease at the rate which is dependent on their female origin, and that egg size may not affect the total period of time a larvae can withstand starvation, but rather the condition of the fish when they first encounter food.

6. I confirm the existence of ultradian rhythms in protein content of capelin larvae which had previously been identified in other species, and I provide evidence

that circadian cycles probably develop as a result of a switch between activity and growth in exogenously feeding larvae.

7. I provide the first evidence that individual variability in larval fish condition greatly limit the generalization of time response curves under given food regimes.

8. I demonstrate that larval condition is very sensitive to intermittent feeding environment, but because it integrates a series of various processes it is very hard to accurately replicate even under strictly controlled experimental settings.

9. I establish that given current methodological and experimental limitations as well as the bias introduced by maternal effects and circadian rhythms, the DNA/dry weight and RNA/dry weight are the two most reliable indices amongst nucleic acid/protein based measurements.

ACKNOWLEDGEMENTS

Several peoples contributed in important ways to this work. First and foremost I wish to thank Dr. William Leggett who provided guidance, assistance and a stimulating working environment. I thank Drs. Christopher Chambers, Matthew Litvak and Tom Miller for their assistance and advice, especially on experimental design and statistical analysis. My supervisory committee, composed of Dr. Joe Rassmussen, Dr. Greg Brown, Dr, Martin Lechowicz must also be acknowledged.

Special thanks go to Martha Krohn, Heidi Kirschner, Christèle du Souich, Paula Boutis, Jackie Elliott and Kathy Tallon for their help in the laboratory and field work. Thanks too to my labmates Brian McKenzie, Elizabeth Deblois, Alfred Appenzeller, Doug Bertram, Matthew Sclafani, Joel Elliot, John Downer, and Tom Herra for providing constructive criticism through informal discussions and laboratory meetings.

Financial support for this work was provided by strategic and operating grants from the Natural Sciences and Engineering Research Council of Canada (NSERC), and from Fonds pour la formation des Chercheurs et l'Aide à la Recherche (FCAR) to W.C. Leggett, and from OPEN (the Ocean Production Enhancement Network), one of 15 Centres of Excellence supported by the Government of Canada through NSERC. Personal support was provided by doctoral fellowships from NSERC and from GIROQ (Groupe Interuniversitaire de Recherches Océanographiques du Québec).

My wife Lucie made many sacrifices to accommodate my life as a student. Her understanding and efforts allowed me to pursue this research. "Merci de tout coeur Lucie".

GENERAL INTRODUCTION

Recruitment, usually defined as the number of young fish from a single year-class that enter the fishery, might be expected to relate to the total biomass of the spawners comprising a stock. However, the typical dome-shaped or asymptotic stock-recruitment relationships reported for most marine fish stocks are characterized by high variance, with "outliers" reflecting the presence of exceptionally large or small year-classes (Sissenwine, 1984; Rothschild, 1986; Fogarty et al., 1991; Koslow, 1992). The low predictive power of such relationships, and the difficulties associated with predicting recruitment with accuracy, has been a central problem in fisheries management (Sissenwine, 1984; Rothschild, 1986; Koslow, 1992). Since very few of the many eggs produced by marine fish species eventually give rise to adults (typically less than 0.01 %), it is believed that small changes in the mortality rates of the young stages can lead to the observed high variability in recruitment (Underwood and Fairweather, 1989; Sale, 1990; Fogarty et al., 1991; Koslow, 1992; Fogarty, 1993). Although there has been substantial controversy over the ontogenetic stage at which recruitment is determined (Peterman et al., 1988; Bradford, 1992), the larval stage is still considered important in this respect (Smith, 1985; Sundby et al., 1989; Bailey and Spring, 1992).

Many biological and physical factors can interact to affect the growth and mortality of young fish during their first year (Fogarty et al., 1991). Starvation and predation, acting independently or together with the modifying impact of abiotic factors (physical processes), have often been suggested as the main sources of mortality (Hjort, 1914; Cushing, 1972; Hunter, 1976, 1984; Lasker, 1981; Rothschild and Rooth, 1982; Sissenwine, 1984; Hewitt et al., 1985; Rothschild, 1986; Rice et al., 1987; Sinclair, 1988; Owen et al., 1989). Differential growth may also be important because it determines larval stage duration and thus the length of time the larvae are susceptible to any mortality source (Buckley and Lough, 1987; Buckley et al., 1987). The potential for growth rate and stage

duration influences on survival has been illustrated by Chambers and Leggett (1987), Houde (1987), Pepin (1991), and Rice et al. (1993). The extent to which these potential effects are realized is, however, the subject of debate (Litvak and Leggett, 1992; Pepin et al., 1992). While predation may be the ultimate cause of mortality, the measurement of its direct impact in the field is exceptionally difficult and has yet to be conclusively demonstrated (Hunter, 1984; Bailey and Houde, 1989). Potential or realized starvation mortality, in contrast, can be estimated directly from the measurement of the nutritional condition of the larval fish, provided that the threshold for irreversible starvation (often referred to as the point-of-no-return or PNR) can be reliably established (O'Connell, 1980; Theilacker, 1986; Owen et al., 1989; Theilacker and Watanabe, 1989).

Hunter (1984) proposed to quantify predation mortality by measuring the incidence of starvation at sea and subtracting it from the total mortality. This approach was used by Hewitt et al. (1985), and Theilacker (1986), who showed that starvation mortality could be equal to total mortality during the early larval stages of jack mackerel *(Trachurus symmetricus)*. While other approaches have been adopted to measure predation (Bailey and Houde, 1989; Purcell and Grover, 1990), the assessment of the physiological condition of larval fishes at sea, in order to evaluate their vulnerability to death due to food limitation and predation, continues to be a major focus of research (Buckley and Lough, 1987; Robinson and Ware, 1988; Frank and McRuer, 1989; Håkanson, 1989b; Owen et al., 1989; Hovenkamp, 1990; Powell et al., 1990; Canino et al., 1991; Hovenkamp and Witte, 1991; McGurk et al., 1992; Sieg, 1992a; Suthers et al., 1992; Margulies, 1993; McGurk et al., 1993).

Larval condition can be assessed from measurements taken at three organizational levels: organismal, tissue, and cellular. At the organism level, interest focuses on detecting changes in external body shape, which relate to condition. These morphological changes of shape are usually depicted as ratios or multivariate vectors of body measurements. At the tissue level, changes in

condition are detected through analyses of modifications in the appearance of the cells and their arrangement in different tissues of the animal. This is made possible because the tissue histology of starved fish larvae often differs from that of well-fed specimens. Finally, biochemical condition can be assessed by quantifying chemical constituents used as energy substrates or by measuring physiological rate indicators that are known to vary at the cellular level in relation to the nutritional status of the animal.

We review in the first chapter, the results of studies which have used various methods to assess larval fish condition and the reliability that have been associated to each. We then define and investigate, using existing data, the sensitivity of larval condition to changes in the food availability, and I examine the rate of change (dynamics) and the latency of condition following starvation and feeding delays. These aspects dealing with the time response of condition to varying in situ food concentrations are very important to consider when sampling plankton at sea, but have received little attention. The later part of chapter one focuses on differences in condition seen between laboratory-reared and field-collected fish larvae, on the confounding effects of age and size on condition estimates, and on the species-specificity of various condition measures. Practical aspects related to the processing time, costs and requirements associated with the use of different methods are finally reviewed, and general recommendations provided.

Since starvation has long been taught as being the main biotic factor affecting the survival of first-feeding marine fish larvae (reviewed in May, 1974; Leggett, 1986), the effect of nutrition on larval condition was the main focus of laboratory and field studies in marine larval fish ecology until recently. However, we realized from a comprehensive review of the literature, that several other factors also known to affect larval fish condition had been identified, without being systematically studied. For biochemical measurements, biotic factors such as the maternal origin of the larvae and diel variations in condition were identified.

chapters two, three and four of this thesis address some of these issues raised in chapter one. They describe three separate experiments conducted using laboratory-reared capelin *(Mallotus villosus)* larvae, which were designed to investigate: 1) the maternal contribution to larval fish condition, 2) the diel variability in condition, and 3) the time response of larval condition following intermittent feeding.

In chapter 2, the importance of maternal source on the nucleic acid and protein content of capelin larvae is assessed by sampling larvae from half-sib families during starvation. After examining the amount and composition of egg yolk between and within clutches, the focus is put on correlations between egg and larval size and between egg and larval biochemical composition. The last part of chapter 2 identifies, from a series of biochemical measures, the ones that are most sensitive to starvation, and those for which the rate of biochemical deterioration during starvation is affected by their female source. Chapter 3 describes a study that was conducted to detect for the presence of trends, discontinuities and periodicities in the nucleic acid and protein composition of developing capelin embryos and larvae. Spectral analysis techniques are used to detect for trends, and to identify during ontogeny, periodicities which had been documented before and are suspected of contributing significantly to the variance of biochemical condition measures. Causal factors are proposed for the appearance of periodicities, based on the time during ontogeny at which they are observed, and on the developmental events to which they correspond. In the experiments described in chapter 4, post-yolk-sac capelin larvae were exposed to various starvation and feeding delay duration's in order to measure the time response of biochemical measures of condition to intermittent feeding. The shape of the responses is assess for different feeding treatments and the results compared with time response models established from existing data and proposed in chapter 1.

CHAPTER 1

AN APPRAISAL OF CONDITION MEASURES FOR MARINE FISH LARVAE

1. INTRODUCTION

Notwithstanding the growing number and use of condition indices in the study of fish growth and survival, no comprehensive review of the topic is available. In this chapter, we define important considerations in the use of condition indices as applied to marine fish larvae, and review the present state of knowledge concerning each index. An appraisal of their relative merits is then attempted by comparing the strengths and limitations of each category. Finally we provide suggestions for the use of condition indices in different situations and identify topics requiring further research.

2. RELIABILITY OF CONDITION INDICES

2.1 Morphometric Indices

The first person to use the term "condition" to describe the nutritional status of larval fish was Shelbourne (1957) whose qualitative classification of condition in larval plaice *(Pleuronectes platessa)* was based on an impression that larvae in poor condition were typically leaner for a given length than were healthier larvae. He established five categories of robustness based on a subjective assessment of the proportion of soft parts (gut and muscular axis) to hard parts (head structure). Such shape differences were later quantified using dry weight/length^3 (also known as the Fulton-K index and previously applied to adult fish by LeCren (1951)). This isometric index was applied to larvae of herring *(Clupea harengus)* sampled at sea (Hempel and Blaxter, 1963; Blaxter, 1971; Vilela and Zijlstra, 1971), and to fed and starved grunion *(Leuresthes tenuis)* larvae reared in the laboratory (May, 1971). The reliability of the Fulton-K index as applied to larval fishes was soon challenged by Ehrlich (1974a), who noted problems associated with the concurrent loss of both length and weight during starvation of larvae of plaice and the allometric growth and ossification of the skeleton in older individuals.

Several other morphological ratios have since been proposed. Wyatt (1972) obtained significant correlations between length, height (at pectorals), and height/length ratios of plaice larvae and the food densities offered to these larvae in the laboratory. The correlation between ration and the height/length ratio was greater than that for either length or height taken separately. Ehrlich *et al.* (1976) showed that the pectoral angle (angle made by the ventral body contour and the pectoral girdle), the body height of herring and plaice larvae, and the eye height/head height ratio of herring larvae, were all affected by starvation. However, problems associated with size and species-specificity, and with between-individual variability, restricted the broad application of these ratios. Yin and Blaxter (1986), working with larvae of cod *(Gadus morhua)* and flounder *(Platichthys flesus)*, reported that the ratio of gut height/myotome height was useful for discriminating fed and starved specimens shortly before the point of irreversible starvation. They also reported the total length/body height and eye height/head height ratios to be effective in categorizing nutritional status.

Theilacker (1978) first suggested the use of ratios in which the numerator alone was sensitive to starvation to allow the detection of starving specimens. However, the finding that few of these morphological measurements proved to be indicative of starvation in laboratory-reared jack mackerel larvae led her to propose the application of multivariate stepwise discriminant analysis (SWDA). SWDA conducted with eleven different morphometric variables (including some ratios), showed five to have significant discriminating power. These were, in order, (1) anal body depth/standard length, (2) anal body depth/head length, (3) pectoral body depth/head length, (4) eye diameter/standard length, and (5) pectoral body depth. When applied to the same data-set, 87% of the fed and 94% of the starved specimens could be correctly assigned to their feeding treatment (Table 1). Powell and Chester (1985) re-analyzed Theilacker's (1978) measurements for laboratory-reared spot *(Leiostomus xanthurus)* larvae and noted that anal body depth and pectoral body depth declined faster than any

TABLE 1. Condition of larvae of *(Trachurus symmetricus)* fed or starved for 1, 2, or 3 d (column 1). Each larvae was classified using morphometric (column 2) and histological (column 3) methods. The number of larvae correctly classified by each method, and by both methods, is indicated. The numbers in brackets are percentages. Asterisks* refer to the number of larvae classified similarly by the two methods (reproduced from Theilacker, 1978).

(1) experimental conditions	(2) MORPHOMETRIC ANALYSIS		(3) HISTOLOGICAL ANALYSIS		
			healthy	Intermediate	starved
fed	Fed	53 (82.8)	44 (68.8)*	8	1
	Starved 1-2 d	10 (15.6)	8	2	0
	Starved 3 d	1 (1.6)	1	0	0
	TOTAL	64	53 (82.8)	10 (15.6)	1 (1.6)
starved 1-2 d	Fed	1 (3.7)	0	1	0
	Starved 1-2 d	26 (96.3)	5	11 (40.7)*	10
	Starved 3 d	0	0	0	0
	TOTAL	27	5 (18.5)	12 (44.5)	10 (37.0)
starved 3 d	Fed	0	0	0	0
	Starved 1-2 d	4 (22.2)	1	1	2
	Starved 3 d	14 (77.8)	1	0	13 (92.9)*
	TOTAL	18	2 (11.1)	1 (5.6)	15 (83.3)

other measurements during starvation. However, a multivariate analysis of variance (MANOVA) showed all measurements to be significantly different between fed and starved larvae. Canonical discriminant functions calculated from these data showed pectoral and anal body depths to have the highest discriminating power, leading to 84% of the fed and 83% of the starved larvae being correctly classified.

Martin and Wright (1987) applied Theilacker's measurements to delayed-starved and delayed-fed striped bass *(Morone saxatilis)* larvae in the laboratory. A stepwise discriminant analysis retained eight ratios with F-values higher than 4.0, from which head length/eye diameter, notochord length/eye diameter, head length/anal body depth and notochord length/anal body depth had the highest discriminating power. However, overlapping canonical centroids prevented the distinction between larvae of different delayed-feeding and starved treatments. This failure was attributed to the fact that most computed ratios shared size information (r values between 0.86 and 0.98 for regressions of ratios against standard length). Koslow *et al.* (1985) computed linear regressions relating body measures of cod larvae believed to be sensitive to starvation (body height at the pectoral fins and at the anus, inter-orbital distance and total dry weight) with body measures believed to be insensitive to starvation (total length, eye diameter and head length), and used the residuals as inputs to a principal component analysis. They concluded that the multivariate approach offered no apparent advantage over a bivariate analysis.

McGurk (1985b) recorded six morphological measurements (head width, pectoral and anal body depth, eye diameter and standard and total length) from laboratory starved and fed herring larvae. By contrasting the results obtained from a stepwise discriminant analysis (SWDA) of ratios, residuals, and principal components, he found that several ratios and residuals were correlated, but that principal components based on these measures were not. He determined that ratios and residuals were correlated because they shared size information, which

lead to biased estimates of classification. However, estimates based on the principal components were not biased, since shape differences between starved and fed animals could be obtained from the second principal component after size effects had been removed with the first principal component. A SWDA using the second principal component of the six variables measured successfully classified 95.8% of the starved larvae. McGurk's (1985a, 1985b) approach was justified on the basis of arguments that it conformed to the three essential elements of the ideal morphometric condition factor, (1) size-independence, (2) biological meaning, and (3) orthogonality. These conditions appear to be met rarely in bivariate analyses.

An important confounding factor associated with the use of morphometric condition indices is the presence of rapid morphological changes which are more related to important developmental events such as the transition to exogenous feeding (Theilacker, 1978; Neilson *et al.*, 1986) than to starvation. Farbridge and Leatherland (1987) also suggest that growth in length and mass may be out of phase in young fish, such that an increase in mass is preceded by an increase in length. This, too, can potentially influence the accuracy of morphometric indices.

2.2 Histological Indices

Histological changes in the digestive tract, liver, pancreas, and inter-muscular tissues associated with starvation in laboratory culture were first described for larvae of yellowtail *(Seriola quinqueradiata)* by Umeda and Ochiai (1975) and northern anchovy *(Engraulis mordax)* by O'Connell (1976) cultured in the laboratory. O'Connell (1976) employed a 10 variables SWDA to demonstrate that pancreas condition, notochord shrinkage, muscle fibre separation, inter-muscular tissue and liver cytoplasm, in that order, were effective for distinguishing robust, severely and moderately starved larvae. Over 90% of the larvae surveyed were correctly classified using these variables. The number and size of hindgut inclusions had a high discriminating power, but this variable was later discarded

because it was believed to be more related to feeding status than condition. Ehrlich *et al.* (1976) examined the histology of herring and plaice larvae following starvation. They reported that head shrinkage in herring, in response to starvation, was demonstrated in the histological sections by a reduction of the inter-orbital distance. A contraction of the gut (in length and diameter) and a reduction in the area of the liver in plaice larvae was also observed. In starving black bullhead *(Ictalurus melas)* adults, Kayes (1978) measured the largest weight loss in the liver, followed by intestine, stomach and eviscerated carcass. The liver was more responsive to starvation in the older fish because of its greater energy storage efficiency as compared with fish larvae; the atrophy of the gut was, however, as responsive to starvation as in larval fish.

Theilacker (1978) recorded 12 different histological characteristics associated with the brain, liver, pancreas, digestive tract and musculature of jack mackerel larvae starved in the laboratory. SWDA revealed that two of these variables (acinar arrangement of the pancreatic cells and the presence of midgut mucosal cells sloughed in the lumen) allowed correct classification of 83% of the fed animals into a healthy class, and 83% of larvae starved for 3d into a starved class. However, only 44% of the larvae starved for 1-2 d were correctly classified into an intermediate class (Table 1). Inclusion of the 10 remaining variables yielded no improvement in group classification. Cousin *et al.* (1986) reported that the first histological anomalies observed in turbot *(Scophthalmus maximus)* larvae after 6 d of starvation in the laboratory were atrophy of the digestive tract and/or a desquamation of the intestinal mucosa. Concurrent with these changes these authors also observed hyperplasia (high rate of cell division) of the cardiac muscle and of the gut epithelium, especially in the hindgut area. Some degeneration of the muscle tissue, but not of the pancreas, was also observed. Several older moribund individuals (10-14 d post-hatching) collected on the bottom of the tanks, showed guts full of prey while some areas of the gut epithelium were degenerated. There is reason to believe that these larvae had

passed the point of irreversible starvation and were unable to digest the food ingested.

Oozeki *et al.* (1989) studied the effect of starvation on the histology of sea-caught and laboratory-reared larvae of stone flounder *(Kareius bicoloratus)*. They found that the epithelial cell heights of the anterior, mid, and posterior parts of the gut, and the heights of the liver and pancreatic cells decreased during starvation. They noted that these quantitative measurements were easy to obtain and did not require interpretation by experienced workers. Theilacker and Watanabe (1989) evaluated the utility of the size of the midgut mucosal cells as an index of starvation in northern anchovy larvae. These cells were selected for study because they were not affected, as were other tissue cells, by autolysis which occurred 3-4 min after death. The size of the midgut mucosal cells was strongly correlated with other histological scores, but was a better predictor of recent feeding history in larvae for which first-feeding had been delayed. This index correctly classified 95% of the fed, 77% of the severely-starved, and 74% of the moderately-starved larvae.

The process of scoring histological changes related to starvation is typically qualitative. Hence, the quality of the data is strongly coupled to the experience of the observer. O'Connell and Paloma (1981), who conducted a histochemical study of liver glycogen content in larvae of northern anchovy, reported that two experienced readers disagreed on 12% of the samples in three categories (low, medium and high) based on the degree of PAS (Periodic Acid-Schiff) coloration. These differences were reconciled only after the sections had been re-examined and the different ratings discussed. (Similar calibration problems are faced by those who employ otoliths to determine fish ages. Here, strict protocols and inter-laboratory exchanges have been instituted to overcome the bias that could result. We are not aware of similar protocols having been initiated by those using histological methods to assess condition in larval fish, and feel that such procedures should be instituted.)

The results of the histologically based studies reported to date suggest that severely emaciated larvae would die of starvation even if food became available because of the permanent loss of digestive abilities following the degeneration of the digestive tract and its associated organs. For this reason, histological examination is likely to be the most reliable method now available with which to identify correctly the point of irreversible starvation or point-of-no-return (PNR) in starving larvae.

2.3 Biochemical Indices

Condition is linked to the quantity of energy reserves available to an organism. However, the storage of energy after feeding, or its mobilization following starvation, is very complex when measured at the cellular level, because of the numerous energy substrates and metabolic pathways available. This reality was highlighted by Frolov and Pankov (1992), who studied the effect of starvation on the biochemical composition of rotifers *(Brachionus plicatilis)*. They therefore subdivided major biomolecules into three main classes: (1) substances which are quickly and readily mobilized following starvation, (2) substances forming the bulk of energy reserves, and (3) molecules which are mobilized only during severe starvation and which elicit profound metabolic reorganizations. Frolov and Pankov (1992) suggested that the pattern of mobilization of biomolecules could differ between species. Given what is currently known about biochemical processes during starvation in fishes, small molecules such as glucose, free fatty acids and free amino acids are likely to be included in the first category; larger molecules such as glycogen, lipids, and small soluble proteins in the second; and some lipids and large non-soluble structural proteins in the third (Love, 1970, 1980).

The sequence of mobilization of primary energy stores in adult cod following starvation and re-feeding was studied by Black and Love (1986). In these fishes

which have less than 3% muscle fat, and which have the majority of their lipids stored in the liver, liver lipids, white muscle and liver glycogen were the first to be mobilized. Liver lipids were also the first to be exhausted about 10 weeks after the onset of starvation, just before liver and white muscle glycogen were completely depleted. Red and white muscle protein and red muscle glycogen begin to decrease 9 weeks after the onset of starvation. Red muscle glycogen was depleted after 22 weeks leaving red and white muscle proteins as the only energy source. When cod were re-fed after 11 weeks of starvation, liver glycogen, red and white muscle glycogen, and plasmatic fatty acids all increased rapidly to above pre-starvation levels. The pattern of mobilization described by Black and Love (1986) was also reported for starved barred sand bass *(Paralabrax nebulifer)* by Lowery *et al.* (1987), and was paralleled by a decrease in the activity of glycolytic enzymes. In fish which have high lipid content (>20% muscle fat), such as herring or eels, lipids occur in abundance not only in the liver but also in the muscle tissues, and the pattern of utilization seems to differ slightly. Moon (1983) showed that in American eels *(Anguilla rostrata)* no changes in muscle glycogen occurred when they were starved for 6 months; there was, however, a decrease in muscle protein and an increase in muscle lipids over this interval.

No comprehensive study of the sequence of mobilization of the energy stores following starvation has yet been reported for larval fishes. Early attempts to correlate chemical composition with larval condition employed measurements of proximal components such as carbon, hydrogen, nitrogen and ash (May, 1971; Ehrlich, 1974a, 1974b; Ehrlich, 1975). The carbon/nitrogen ratio, an index believed to reflect the quantity of lipid relative to the quantity of protein, was derived from these measurements. Proteins have a C/N ratio near 3.0, while lipids are carbon-rich molecules. Therefore, when lipids are present, the ratio increases above a value of 3.0 (Harris *et al.*, 1986). In grunion larvae (May, 1971), the C/N ratio decreased following starvation due to fat catabolism; well-fed larvae, however, also exhibited a decrease in C/N ratios with age as a

consequence of protein deposition. Increasing trends in the Fulton-K index and decreasing trends of the C/N ratio with age led May (1971) to conclude that the C/N ratio was an unreliable index of condition. Ehrlich (1974a, 1974b) reached a similar conclusion, noting no consistent pattern of change in the C/N ratio during starvation in plaice larvae. Both authors measured the quantity of nitrogen (as an index of proteins), carbohydrates and neutral fats, and found that these constituents exhibited a more or less gradual decline until the larvae died of starvation. Kiørboe *et al.* (1987) reported that in laboratory-reared larvae of herring, a higher C/N ratio (4.54) in yolk-sac larvae than in post-yolk sac larvae (3.8) was a result of a lower carbon and a higher nitrogen content in the latter. There was also no significant difference in the C/N ratio of starved larvae and larvae fed at different food rations. Moreover, the C/N ratio measured in the larvae reflected the same value as in the copepods used as food. The authors therefore concluded that herring yolk-sac larvae metabolize lipids preferentially and utilize protein for growth. The post-yolk sac larvae, on the other hand, metabolized body constituents or utilized ingested protein and lipids in proportion to their occurrence in the diet, and did not form significant lipid energy stores but rather channeled the ingested matter into protein and growth.

Several factors might explain the lack of sequential mobilization expected from nitrogen, carbon, and carbohydrates measurements during starvation. The most obvious (also problematic in the evolution of the C/N ratio during starvation) is that nitrogen is associated not only with protein but also with several other molecules (Pfeila and Luna, 1984) including lipoproteins and phospholipids. Hence, protein estimates derived from the nitrogen content are likely to be overestimated. Second, sequential mobilization may be masked by integration of tissue and individual differences resulting from groups of whole larvae being homogenized. Finally it is possible that a decrease in the quantity of one component such as protein (nitrogen) could be compensated for by *de novo* synthesis from non-protein precursors such as carbohydrates or lipids which contribute to the carbon value. Such a continuous flow of molecules through

different metabolic pathways complicates, and may confound, analyses of the sequential mobilization of various metabolic compounds during starvation and may, in fact, require simultaneous measurement of all major substrates in order to assess condition accurately.

Ehrlich (1974a, 1974b) computed the quantity of energy obtained from the catabolism of the main chemical components available to starving larvae of herring and plaice prior to the point-of-no-return. The majority of this energy was obtained from the catabolism of proteins (74.3%), followed by triglycerides (20.7%) and carbohydrates (5.0%). This corresponds with the order of importance attributed to food reserves by Smith (1957) and Lasker (1962). Ehrlich (1974a, 1974b) also observed that the very limited lipid and carbohydrate reserves possessed by post- yolk-sac larvae caused protein catabolism to commence almost immediately following the onset of starvation. Nitrogen and carbohydrates were laid down faster than were neutral fat stores (triglycerides) in well fed first-feeding plaice (Ehrlich, 1974a) and herring (Ehrlich, 1974b) larvae. This finding was interpreted as evidence that food was converted to growth (protein), rather than to energy stores in larval fish which are believed to operate on a tighter energy budget than adults.

Govoni (1980) observed that high carbohydrate storage (in the form of hepatic glycogen) in the larvae of spot had a sparing effect on protein catabolism. He also determined, from histological sections, that these carbohydrate stores were readily metabolized to meet short-term energy needs. O'Connell and Paloma (1981) also reported liver glycogen to be the first line energy reserves in northern anchovy larvae. However changes in liver glycogen were less effective in reflecting the degree of energy deprivation than were other histological characteristics. Margulies (1993) observed that liver glycogen was the first energy reserve to be mobilized at the onset of starvation in wild pre-flexion scombridids (*Scomberomorus siera, Euthynnus lineatus, Auxis* spp.) larvae sampled in the Panama Bight.

Free amino acids are believed to be important energy substrates in developing embryos and in first-feeding larvae (Fyhn and Serigstad, 1987; Fyhn, 1989; Rønnestad *et al.*, 1992a, b). These amino acids are obtained from the egg yolk during embryonic development, and a small proportion of these available egg reserves are incorporated into the embryo body protein. When the yolk is exhausted after hatching, catabolism of body protein becomes the main source of amino acids prior to first-feeding (Fyhn and Serigstad, 1987; Fyhn, 1989). Species which possess oil globule(s) in their eggs are less dependent on amino acids immediately after hatching since they can rely on lipid reserves for a longer interval (Eldridge *et al.*, 1982; Vetter *et al.*, 1983; Rønnestad *et al.*, 1992a). Lipid reserves are, however, relatively small in larval fish, and appear to increase significantly only in the later juvenile stages (Marshall *et al.*, 1937; Balbontin *et. al.*, 1973; Ehrlich, 1974a, 1974b, 1975; Fukuda *et al.*, 1986, Suthers *et al*, 1992), at which time they provide a good buffer against starvation (Love, 1980). This is true of species having high and low lipid stores

The overall patterns of energy mobilization and storage in fish larvae seem to be similar to those documented for adult fish. Glycogen, free amino acids and fatty acids are utilized as short-term energy stores, and are rapidly depleted following starvation. Lipids yield some energy after glycogen reserves are consumed, but are also rapidly exhausted because of their low quantity in the liver which is not well developed as an efficient storage organ in fish larvae. Structural proteins thus become the ultimate energy substrate sustaining larval fishes during starvation. As in adult fish, it is expected that these proteins are the last to be utilized and the first to be replenished when feeding is resumed.

2.3.1 Proteins

Unlike higher vertebrates, in which muscle protein is protected from being metabolized during starvation (Stryer, 1981), fish are well adapted to mobilize

these body constituents as fuel for survival (Love, 1970). Because fish contain more than 50 % protein by weight, increases in fish biomass are accomplished primarily through protein synthesis (Love, 1970; Bulow, 1970, 1987; Lied and Roselund, 1984). It has been estimated that in adult fish under optimal feeding conditions, 20% of the protein synthesis is allocated to maintenance metabolism while the remaining 80% is incorporated into muscle growth (Roselund *et al.*, 1983). In fish larvae, these proportions are likely to differ because, as Kiørboe (1989) suggested, larvae have a lower rate of protein turnover than adults, and are able to retain a higher proportion of synthesized protein which is laid down as growth.

Lasker (1962), working with sardine *(Sardinops caerulea)* larvae, observed an energy deficit prior to complete yolk-sac resorption. This deficit was evident in sardine larvae (average dry weight of 40 mg) by a resorption of muscle tissue and a loss of dry weight at a rate approaching 2 mg d^{-1}. Ehrlich (1974a, 1974b), Zeitoun *et al.* (1977), Buckley (1979, 1980), Clemmesen (1987) and Buckley *et al.* (1990) all concluded that protein is an important energy source during the period immediately following hatching. In rainbow trout *(Oncorhynchus mykiss)*, for example, 19.4% of the protein present in unfertilized eggs had been metabolized or lost at the time of hatching and up to 50% by the time the yolk-sac was fully absorbed (Zeitoun *et al.*, 1977). In starved cod larvae, absolute protein content decreased by 45% between 2 and 11 d post-hatching although relative protein content, expressed as a percentage of dry weight, did not change (Buckley, 1979). A large part of the weight loss was therefore attributed to a net loss of protein. In winter flounder *(Pleuronectes americanus)* larvae, 45% of the protein present at hatching was lost prior to initiation of feeding 7 d later (Buckley, 1980). Powell and Chester (1985), employing some of Houde and Schekter's (1983) bioenergetic calculations to determine the amount of energy provided by the yolk and oil globule of spot larvae, concluded that these larvae could survive in a food-limited environment only if they catabolized existing tissue (mainly composed of protein). Pedersen *et al.* (1990) demonstrated a decrease

in protein content between yolk absorption (4 d post-hatch) and 14 d after hatching, in groups of larval herring fed at low food levels (15 copepod nauplii/larva). Herring larvae fed high rations (80 nauplii/larva) showed no significant difference in their protein content during the same time interval.

The mobilization of muscle protein following months of starvation in adult cod is known to produce an increase in intercellular space and water content which leads to a negative relationship between protein content and the water content of muscle tissue (Love, 1970). Starved fish are able to undergo considerable degeneration in the cells of their body musculature and still survive, but their buoyancy changes significantly under such conditions. In larval fish, an increase in water content and buoyancy is often observed concurrent with muscle protein catabolism during the interval between hatching and the beginning of exogenous feeding This has been reported both in laboratory (Blaxter and Ehrlich, 1974; Neilson *et al.*, 1986; Yin and Blaxter, 1987; Mathers *et al.*, 1993) and in wild larvae (Frank and McRuer, 1989). Blaxter and Ehrlich (1974), who examined the buoyancy forces acting on larvae of plaice and herring, determined that buoyancy resulted from the balance between lift forces provided by water and fat content and sinking forces exerted by proteins. Increased buoyancy following starvation was caused primarily by a significant decrease in protein content. Neilson *et al.* (1986) concluded that for larvae of a given age and size, buoyancy was a better predictor of condition than were morphometric indices.

2.3.2 Lipids

Total fat content was related to condition and growth in adult (Olivier *et al.*, 1979; Wicker and Johnson, 1987) and juvenile fish (Keast and Eadie, 1985). However, no survival value could be assigned to lipid levels. The fat content of larval fish has been measured (Balbontin *et al.*, 1973; Ehrlich, 1974a, 1974b; Fukuda *et al.*, 1986) but has rarely been related to condition because of its high rate of turnover. Although lipids are typically found in small quantities in first-

feeding larvae (Ehrlich, 1974a, 1974b), some lipid classes appear to be preferentially used or retained at given periods of fish development (Tocher *et al.*, 1985a, 1985b). Tocher *et al.* (1985a) and Fraser *et al.* (1987) reported a predominance of triacylglycerols (TAG) over other lipid classes in yolk-sac herring larvae. TAG were also preferentially stored in feeding larvae that were able to meet their basic lipid metabolic requirements from dietary sources. Håkanson (1989a) also reported that TAGs were used preferentially for short-term energy needs by starving northern anchovy larvae, and that cholesterol and polar lipids, which are cell membrane constituents, were conserved except in cases of severe starvation. This phenomenon has been observed in several species of bivalve larvae, in which high TAG levels have been correlated with high growth rates and higher rates of survival to metamorphosis (Gallager *et al.*, 1986). Delauney *et al.* (1992) found no such correlation in scallops (*Pecten maximus).*

The level of TAG in larval fish was first proposed as an index of condition by Fraser *et al.* (1987). However, high variability in absolute TAG levels, which were unrelated to diet in first-feeding larvae, led these authors to modify the index to the ratio of TAG/sterol. This is because sterols, which are important membrane components, are believed to be correlated with the biomass of the animal, and therefore to be unaffected by nutritional status. They are also easily obtained from TLC/FID (thin layer chromatography-flame ionization detection) chromatograms using a non-polar solvent (Fraser *et al.*, 1985; Fraser, 1989). However, Håkanson (1989a), who reared northern anchovy larvae in the laboratory under different starvation and feeding regimes, found that both polar (including sterols) and neutral lipids varied with starvation. Cholesterol levels, in contrast, were unaffected by starvation. Polar lipid - standard length relationships were also more variable than were cholesterol - standard length relationships. He concluded that a ratio of TAG/cholesterol would be superior to a TAG/sterol ratio as an index of condition. TAG/cholesterol ratios smaller than 0.2 were determined as being indicative of poor nutritional condition in anchovy larvae

kept in the laboratory (Håkanson, 1989a). Using this criteria, Håkanson (1989b) estimated that from 8 to 27% of the anchovy larvae sampled over a large grid of stations in the Southern California Bight were in poor nutritional condition. He also reported important between-station differences in the proportion of starving larvae as indicated by their TAG/cholesterol ratio.

Relatively few studies have yet employed TAG-sterol or TAG-cholesterol ratios as indices of condition. Problems associated with their use have, however, been encountered due to the small quantities of lipids present in individual marine fish larvae and the difficulty of quantifying these levels. Levels of these constituents are often close to, or below, the detection limits of the chromatography techniques used. For example, Gatten *et al.* (1983) could detect TAG in herring larvae only 10 d after hatching, when they had begin to feed. McClatchie *et al.* (pers. comm.), who used TLC/FID techniques, reported no detectable quantities of TAG in 90% of cod larvae that had been feeding for 6 d in the laboratory. Fluorescence techniques, which are more sensitive and require less time and equipment (Gleeson and Maughan, 1986; Nemeth *et al.*, 1986), and the availability of numerous fluorescent binding probes specific for different classes of lipids (Haugland, 1992), might help to resolve these detection difficulties.

Exogenous lipids are lacking in starving larvae and hence endogenous lipids become increasingly important. Under these conditions, it appears that amino acids released through protein breakdown provide most of the energy required to prolong life (Ehrlich, 1974a, b). For adult fish, Love (1970) commented that: "...while a steady depletion of lipid characterizes subsistence without food in almost all cases, the lipid content cannot necessarily be taken as a reliable index of the nutritional status of the fish." Because lipids, like carbohydrates, are often utilized first during starvation, but can be obtained from dietary sources, the TAG-cholesterol ratio of individual larvae can provide a reasonable indication of the time since the last ingestion of planktonic prey. However, it gives only a poor

indication of the ability of the animal to sustain a longer period of starvation and to recover from it. It is possible for instance, that a larvae could be judged in good condition on the basis of a high protein level, and yet exhibit a low TAG/cholesterol ratio simply because these two indices respond on different time scales. We shall return to this question when comparing the sensitivity, and the time response, of different indices.

The composition of fatty acids in larval fish has also been proposed as an indicator of the quality and the quantity of food intake (Tocher *et al.*, 1985b; Fraser *et al.*, 1987; Klungsøyr *et al.*, 1989; Navarro and Sargent, 1992). Soivio *et al.* (1989) showed that for whitefish *(Coregonus muksun)* larvae, the fatty acid composition of the body lipids often resembles that of their food. Fatty acid composition may also reflect variation in the quantity of non-essential relative to essential fatty acids which are thought to be preferentially conserved during starvation. However, the variety of fatty acids types and the numerous transformation they undergo (desaturation and saturation) renders their dynamics difficult to follow. Further, similar fatty acids can occur in different lipid classes, which, in turn, are differentially mobilized during starvation. For example, Frolov and Pankov (1992) measured the fatty acid composition of polar and neutral lipids in starving rotifers *(Brachionus plicatilis).* They observed that the composition of these fatty acids was rather stable, in spite of a decrease of polyunsaturated acids in neutral lipids and an increase in polar lipids. We conclude from the available data that the potential for the use of fatty acid composition as an index of condition in larval fish is low. However, the potential for the use of fatty acids as guides to the composition of the diet, or as biomarkers for studying food webs, may be real.

We now turn to indicators of the rate of synthesis or degradation of some of the energy substrates discussed above. These include nucleic acids as indicators of the rate of protein synthesis, and enzymes which have been suggested as indicators of aerobic and anaerobic metabolism. Digestive

enzymes, which are released in the gut of fish larvae when food is ingested, have been used as indicators of the feeding status of fish larvae and will also be reviewed.

2.3.3 Nucleic Acids

The quantity of DNA per cell is believed to be a species-constant. In contrast, the quantity of RNA (primarily associated with ribosomes), varies with the rate of protein synthesis. Since growth in fish is mainly accomplished through protein synthesis, the RNA-DNA ratio has been proposed as a short-term (Bulow, 1970) and as a long-term (Haines, 1973) index of growth in adult fish.

Buckley (1979, 1980) found that in the larvae of cod and winter flounder reared in the laboratory, the RNA/DNA ratio was positively correlated to food densities offered and to growth rates achieved. He therefore proposed its use as an index of the nutritional condition of larval fishes. In the larvae of winter flounder, the RNA/DNA ratio more consistently tracked changes in food availability than did individual measures of RNA, DNA, protein content or dry weight (Buckley, 1980). Clemmesen (1987) confirmed these findings for larvae of herring and turbot. All four species (Buckley, 1979, 1980, 1981; Clemmesen, 1987) also exhibited a decrease in protein content following hatching, and a decrease in RNA and protein content at first-feeding, if food was not available. Although first used as an index of nutritional condition for fish larvae, it soon became evident that the RNA/DNA ratio was also an index of larval growth, since good condition is a prerequisite for sustained exponential growth. Significant correlations have now been reported between growth rate, expressed as the increase in protein quantity per unit time, and the RNA-DNA ratio of several species of phytoplankton, mollusks, crustaceans, echinoderms, fish larvae and juveniles (Frantzis *et al.*, 1993).

It is well known that growth of fish larvae is mainly affected by food levels and temperatures (Heath, 1992). The relationship between protein growth rate and the RNA/DNA ratio in larval winter flounder and sand lance *(Ammodytes* spp.) was evaluated at different temperatures by Buckley (1982), and Buckley *et al.* (1984). Under constant food levels, protein growth rates of larval winter flounder were higher at higher temperatures, even though RNA/DNA ratios remained constant (Buckley, 1982). In larval sand lance, food levels had a greater impact on growth than did temperature or the interaction of temperature and food levels. Here too, the RNA/DNA ratio was less variable than was growth in response to rearing temperatures. The addition of temperature as an independent variable in regressions relating RNA/DNA ratios to protein growth rates significantly reduced the unexplained variance in growth rates. Buckley (1984) reported that protein growth rate in larvae of eight different marine species was related to the RNA/DNA ratio and to temperature as follows:

$$G_p = 0.93\ T + 4.75\ \text{RNA/DNA} - 18.18 \quad (r^2=0.92) \qquad (1)$$

where G_p = protein growth rate, and T = temperature. Goolish *et al.* (1984) reported a similar relationship for adult carp *(Cyprinus carpio)*:

$$G_p = (0.065\ T + \text{RNA/DNA}) / 0.707 \quad (r^2=0.76) \qquad (2)$$

The terms of these two relationships differ substantially because fish larvae tend to have much higher RNA/DNA ratios at a given growth rate and temperature than do older fish (Mathers *et al.*, 1993). When all temperatures were analyzed simultaneously for adult carp, the RNA/DNA ratio was more highly correlated to growth rate than was RNA/protein or RNA/tissue. However, at a given temperature, all three correlation coefficients had the same magnitude (Goolish *et al.*, 1984). At lower temperatures a higher quantity of RNA was required to achieve a given growth rate (compensation mechanism), emphasizing the fact that RNA activity was lower and, therefore temperature

dependent. This was also confirmed by Ferguson and Danzmann (1990) who worked on juveniles of rainbow trout, and by Foster *et al.* (1992, 1993) who worked on juveniles of cod. Mathers *et al.* (1993) measured the RNA/DNA ratio and the protein growth rate of rainbow trout fry of different ages (21, 30, 58 and 65 d posthatch) maintained at different temperatures in the laboratory. They found no significant differences in RNA concentration between fry maintained at 5, 10 and 15 C. They explained the absence of RNA compensation mechanisms at lower temperatures by the fact that larvae already possess very high RNA levels relative to juveniles, and that compensation at lower temperature could only be achieved through increased RNA activity. They also computed the RNA/protein ratio, an index of RNA efficiency, and showed that the ratio doubled between 5 and 10 °C indicating that the rate of protein synthesis does vary independently of RNA concentrations at different temperatures. Temperature effects must therefore be assessed, if estimates of protein growth rates are to be obtained from the RNA/DNA ratio of field-collected fish larvae.

If the protein growth rate of fish larvae is assigned a value of zero in Buckley's (1984) model, the critical RNA/DNA ratio can be calculated after rearranging Eq. (1):

$$RNA/DNA_{crit.} = (18.18-0.93T) / 4.75 \quad (3)$$

This critical ratio, which typically ranges from 1.0 to 3.0 depending on temperature, was used by Robinson and Ware (1988), Hovenkamp (1990) and McGurk *et al.* (1992) to calculate the proportion of animals showing negative growth rates. Robinson and Ware (1988) reported that the critical RNA/DNA ratio corresponded closely to RNA/DNA ratios exhibited by cultured larval Pacific herring *(Clupea pallasi)* at the point of irreversible starvation. They also reported a decrease in standard length and a net catabolism of protein (as indicated by predicted negative protein growth rates) soon after complete yolk absorption. Buckley and Lough (1987) and Robinson and Ware (1988) were the first to report

RNA/DNA ratios for field-collected larvae. In both cases, the frequency distribution of the RNA/DNA ratio was log-normal and truncated on the left side, suggesting growth-selective (or condition-selective) mortality in larvae characterized by RNA/DNA values below the critical ratio. Hovenkamp (1990) estimated the growth of larval plaice sampled in the North Sea using three different methods, (1) width of otolith growth increments, (2) size-at-age data, and (3) RNA/DNA ratios based on Buckley's (1984) model (Eq.(1)). The results obtained from the three methods were consistent. However, more recently, Bergeron and Boulhic (1994) evaluated Buckley's (1984) growth model (Eq. (1)) using laboratory-reared sole *(Solea solea)* and found no direct relationship between observed and predicted specific protein growth rates. They concluded that extreme caution should be used when employing Buckley's model to predict growth rates of larval fishes, for species other than those used to develop the model. Canino *et al.* (1991) also employed this model to calculate the protein growth rate of field-collected larvae of walleye pollock *(Theragra chalcogramma),* and found that most specimens sampled on two different cruises had negative growth rates despite normal ingestion rates. They suggested that intercalibration studies of RNA/DNA ratios should be conducted to reconcile the results obtained using different standards, buffers and extraction techniques.

One major limitation of the methods used by Buckley (1979, 1980, 1981, 1982, 1984), Buckley *et al.* (1984) and Buckley and Lough (1987), is the large quantity of material (>800 μg) required for accurate nucleic acid determination. Such quantities can only be obtained by pooling many individuals when marine fish larvae are assayed. To overcome this limitation, Clemmesen (1988) proposed new methods for determining nucleic acids in individual fish larvae. Individual nucleic acid determinations have, however, revealed a large and unexplained inter-individual variability in the RNA content and in the RNA/DNA ratios of fed larvae reared under identical conditions (Clemmesen, 1988, 1989, 1992; Robinson and Ware, 1988; Westerman and Holt, 1988; McGurk and Kusser, 1992; Ueberschär and Clemmesen, 1992; Clemmesen and Ueberschär,

1993; Mathers *et al.*, 1993). Westerman and Holt (1988) suggested that this variability between individuals might be caused by periods of cell proliferation and growth during the development of organs. These findings led Bergeron and Bouhlic (1994) to question the reliability of RNA/DNA ratios as indices of the condition or growth rate of individual larval fish.

The rationale underlying the use of the RNA/DNA ratio dictates that DNA should increase during development since DNA content per cell is constant and the total number of cells increases as the animal grows. However, Clarke *et al.* (1989) found no significant relationship between DNA content and cuttlefish *(Sepia officinalis)* (Mollusca:Cephalopoda) size. It should be noted, however, that their reports of some negative RNA values suggests that their DNA values may have been overestimated. We have experienced as did Clarke *et al.* (1989) the problems of negative RNA values when using Hoechst 33258 (the dye recommended in Clemmesen (1988) protocol), because this sensitive dye can yield high fluorescence in the presence of SDS (a strong detergent used to disrupt the cells) or other extraction solvents. We found it necessary to extract buffer blanks in a manner identical to that used for samples in order to correct for this effect (Ferron *et al.* pers. data). The rationale underlying the use of the RNA/DNA ratio also dictates that DNA should not vary with starvation. However, exceptionally high DNA values (μg/larvae) have been reported in starved post-yolk-sac larvae of cod reared in the laboratory and in an outdoor pond (Raae *et al.*, 1988). This was argued to occur as a result of rapid unscheduled DNA synthesis following a collapse of cellular control mechanisms resulting from inadequate nutrition. While the possibility of a link between high DNA synthesis and hyperplasia of the digestive tract epithelium and liver in starving larvae, as proposed by Cousin *et al.* (1986), cannot be excluded, the possibility of non-rigorous methodological procedures should first be addressed. Several recent studies have confirmed that the observed variability in the RNA/DNA ratio results primarily from variation in RNA levels (Clemmesen, 1988, 1989, 1992; Ferron, 1991; Bergeron and Boulhic, 1994). Recent work by Clemmesen (1988, 1993)

and McGurk and Kusser (1992) have also clearly demonstrated that purification and extraction of nucleic acids is an essential step in the accurate determination of RNA/DNA ratios in larval fishes. This purification was not a component of the standard protocol used prior to Clemmesen's (1988) study, and conclusions based on these earlier analyses should therefore be interpreted with caution. When highly purified material and a DNA- specific dye such as Hoechst 33258 are employed, DNA variability (coefficient of variation) can be reduced to about 8.4% (Clemmesen, 1988). This contrasts with variability as high as 35.4% when RNAse digestion and non-specific dyes such as ethidium bromide are employed (Clemmesen, 1988). Most recently, Clemmesen (1993) has recommended the use of ethidium bromide rather than Hoescht 33258, because of quenching and self-fluorescence problems associated with the latter. We have been successful in overcoming self-fluorescence problems associated with Hoechst 33258 by washing the extracted homogenates with diethyl ether to remove traces of phenol and chloroform, and by reading the fluorescence at a low temperature (1°C) to reduce background noise (see chapter 2).

Several studies in which RNA/DNA ratios were employed have reported an increase in DNA content (expressed as a percentage of dry weight) concurrent with the decrease in the RNA/DNA ratio associated with starvation in fish larvae (Buckley, 1979; Clemmesen, 1987; Raae *et al.*, 1988; Richard *et al.*, 1991). This appears to occur because starved animals lose weight while DNA is conserved. Hence, a ratio of DNA to dry weight (DNA/DW) has potential as an indicator of starvation. Bergeron *et al.* (1991) and Richard *et al.* (1991) reported that fed sole larvae exhibited lower between-individual variability in DNA/DW ratios than in RNA/DW or RNA/DNA ratios. They therefore suggested the use of the DNA-DW ratio in studies of larval fish condition because it appears to reflect more accurately condition changes related to starvation. The approach has the added advantage that it is easier to obtain reliable DNA values in isolation than it is to assess DNA and RNA simultaneously. However, the DNA/DW ratio does not allow evaluation of the growth potential, and of the ability of the larvae to recover

from starvation at the time of sampling, as does the RNA/DNA ratio. Moreover, reduction in individual variability should not be seen as an end in itself, because there is no reason to expect individual larvae to respond similarly in a common feeding environment. Given the reliability of protocols assessed to date, simultaneous evaluation of DNA, RNA, protein and total dry weights of individual larvae can easily be achieved. This allows various ratios (RNA/DNA, DNA/DW, RNA/DW, RNA/prot, prot/DNA) to be calculated and used in order to investigate details of growth response and condition (Lone and Ince, 1983). Multivariate approaches to the analysis of such data, similar to those now applied to morphometric variables, should also be explored (Farber-Lorda, 1991). Buckley and McNamara (1993) have recently used nucleic acid probes to examine changes in 18S rRNA, and actin and myosin mRNA in fed and starved larvae of cod. The use of these molecular techniques to assess larval fish condition, however, remains in its infancy and it is premature to assess the significance of these results.

2.3.4 Digestive enzymes

In an effort to assess the nutritional status of fish larvae, some workers have quantified the content and activity of various digestive enzymes. These enzymes, which are known to be produced and secreted when food is ingested, have activity levels that should reflect the extent to which fish larvae have been feeding. Because most early stage larval fish are stomachless and possess only a functional intestine, trypsin-like enzymes are the main proteolytic enzymes produced (Vu, 1983; Hjelmeland *et al.*, 1984; Govoni *et al.*, 1986). However, their activity can arise from two sources, endogenous (produced by the larvae) or exogenous (obtained from the guts of ingested food items). Repeated studies have shown that these two sources can contribute equally to the total enzyme activity measured (Dabrowski and Glogowski, 1977; Dabrowski, 1982; Munilla-Moran *et al.*, 1990). Dabrowski (1982) measured pepsin and trypsin-like activity

in starving pollan *(Coregonus pollan)* and rainbow trout larvae and found that the enzymes were at their lowest level at the point of irreversible starvation.

Three problems confront the measurement of trypsin-like enzyme levels: (1) the presence of proteolytic enzymes other than trypsin can react with synthetic substrates, (2) the presence of trypsin inhibitors in homogenates of fish larvae can cause underestimation of enzyme activity, and (3) the presence of trypsinogen, the inactive proform of trypsin, can contribute to the determination. Hjelmeland and Jϕrgensen (1985) proposed a radio-immunoassay method to overcome these problems. Using these methods, Pedersen *et al.* (1987) reported that intestinal tryptic enzyme levels in larval herring were positively related to the number of planktonic prey in the gut, at values up to five prey. Beyond that prey level, an asymptote in enzyme activity was reached. They also estimated the enzyme/substrate ratio to be between 1 and 4. However, Hjelmeland *et al.* (1988) found no significant correlation between trypsin activity and the number of prey ingested as a result of large variations observed between individuals. Hjelmeland *et al.* (1984) observed that only growing cod larvae produced trypsin and its precursor trypsinogen. They suggested that the level of these compounds could be used as a growth index and as an indicator of nutritional condition. Their results were, however, confounded by distinct phases of enzyme activity related to specific developmental stages.

Ueberschär (1988) developed a fluorometric method to measure the tryptic enzyme content of individual fish larvae. The method is equal in sensitivity to radioimmunoassays, but is less trypsin-specific because 7% of the proteolytic activity measured is attributable to proteolytic enzymes other than trypsin. Fluorescence determination (FL) and radioimmunoassay (RIA) do, however, give similar results and significant ($P<0.05$) correlations have been obtained between (RIA) quantity and (FL) activity of trypsin in all tissues of larval herring (Ueberschär *et al.*, 1992), except the pancreas in which trypsinogen leads to overestimated trypsin content when assessed by RIA methods.

Adult fish muscle is rich in proteolytic enzymes and this apparently facilitates the catabolism of body tissues when energy is required after long periods of starvation (Love, 1980). Larval fish, too, contain endogenous proteolytic enzymes which are probably produced under the same starvation situations. However, to date, the relative contribution of such endogenous sources to the total proteolytic enzyme activity in a larvae has not been adequately assessed. As is the case with triglycerides, proteolytic enzymes are greatly affected by exogenous sources of food, hence their activity is likely to be more indicative of short-term feeding status than longer-term condition and susceptibility to starvation.

2.3.5 Metabolic enzymes

Quantification of the activity levels of key enzymes involved in different metabolic pathways has also been suggested as a means of assessing condition in fish. The activity of various metabolic enzymes has provided good information on the rate of synthesis or degradation of major energy substrates (Moon and Johnston, 1980; Moon, 1983) as well as the organs affected during starvation in juvenile and adult fish (Goolish and Adelman, 1987, 1988; Lowery *et al.*, 1987; Lowery and Somero, 1990). Citrate synthase (a Krebs cycle enzyme) and cytochrome c oxidase (an enzyme of the oxidative phosphorylation) are two enzymes for which activity is believed to be related to feeding-induced increases in aerobic metabolism. In contrast, lactate dehydrogenase (an enzyme of the Cori cycle) activity is thought to vary with the rate of feeding, anaerobic metabolism and locomotory performance (Sullivan and Somero, 1980). Mathers *et al.* (1992) reported significant correlations between individual growth rate and the activity of citrate synthase, cytochrome oxidase, lactate dehydrogenase and the amount of RNA in the muscle of adult saithe *(Pollachius virens)*, supporting the hypothesis of a link between growth rate and aerobic and anaerobic capacity.

For larval fish, the interest in metabolic enzymes as tools for assessing condition is relatively new, and has been restricted to the use of two major

enzymes, citrate synthase (CS) and lactate dehydrogenase (LDH). Clarke *et al.* (1992) measured LDH and CS activity in fed and starved larvae of red drum *(Sciaenops ocellatus)* and lane snapper *(Lutjanus synagris)*. Although LDH and CS activities changed markedly with age, independently of food densities, the LDH activities were significantly influenced by the quantity and the quality of food offered. The enzyme activities were also found to be affected by temperature, although this effect was not quantified. Mathers *et al.* (1993) reported no significant correlation between CS and LDH activity and RNA concentration and protein growth rate in fry of rainbow trout. Despite this, the activity of the two enzymes differed significantly between fed and starved individuals, but not between individuals fed at different rations. It seems from the limited data available, that metabolic enzymes can be affected by the feeding status of larval fish. However, it is important to realize that metabolic enzyme activity provides no more than a "snapshot" of the current rate of metabolism of the animal. Such measures will need to be combined with some estimates of the quantity of various substrates involved if an assessment of condition in response to past food deprivation is to be attempted.

2.4 Sources of variability other than nutrition

The main source of variability evaluated in studies dealing with the measurement of condition in larval fish has been food availability, since starvation was long believed to be the predominant source of mortality for first-feeding larval fish (reviewed in May, 1974; Leggett, 1986). However, when studying the impact of food availability on the condition of larval fish, it is vital that other potential sources of variability are identified and controlled. Such sources have been identified and are briefly detailed below.

For morphological measurements, Blaxter and Hempel (1963) and Hempel and Blaxter (1963) demonstrated that length, height and weight of herring larvae are influenced by the spawning stock from which the specimens originated.

Variability in the morphology of individual fish larvae, often originating from egg size variation, is also known to have maternal sources (Wilson and Millemann, 1969; Beacham and Murray, 1985; Knutsen and Tilseth, 1985; Marsh, 1986; Bengtson *et al.*, 1987; Meffe, 1987, 1990; Hislop, 1988; Chambers *et al.*, 1989; Zastrow *et al.*, 1989; Buckley *et al.*, 1991b; De March, 1991; McEvoy and McEvoy, 1991; Panagiotaki and Geffen, 1992). Martin and Wright (1987) also reported between-year differences in the morphometric condition of laboratory-reared larvae of striped bass *(Morone saxatilis)*, which they assigned to genetic differences between broodstocks.

When grading histologically fed and starved anchovy larvae, O'Connell (1976) noticed the presence of several moderately and severely emaciated specimens in the fed group. He concluded that food availability may not always be the underlying cause of declining condition. When examining the condition of larvae collected at sea, O'Connell (1980, 1981) noted that three-quarters of the emaciated larvae were concentrated in 4 of the 64 net tows. However, numerous emaciated larvae were found to occur within samples characterized by larvae in good condition that were collected in areas offering good feeding conditions. O'Connell attributed this low condition to genetic factors, accident, or chance failure to capture the available food. Changes in the glycogen content of hepatocytes seems to be under the influence of rhythmical (diel) feeding periods related to time of the day (O'Connell and Paloma, 1981; Segner and Möller, 1984; Sieg, 1992b; Sieg, 1993). Rather than accounting for this source of variability, it has been suggested that other histological criteria insensitive to diel changes be used. However, three additional histological traits (the number of inclusions present in hindgut mucosal cells, the number of midgut mucosal cells and the inflation of the swimbladder) were also found to vary on a diel basis in jack mackerel larvae sampled at sea (Theilacker, 1986). Increased hindgut inclusions, a lower rate of mitosis of midgut mucosal cells, and a deflation of the swimbladder during daytime were also reported to be associated with feeding activity. At night, the empty gut produced an inflation of the swimbladder, a

disappearance of hindgut inclusions and a higher rate of midgut mucosal cell mitosis (Theilacker, 1986).

Among the different sources of environmental variability shown to influence condition in fish, the largest number has been associated with biochemical indices. For nucleic acids and protein measurements, these include biotic sources such as maternal origin (Buckley *et al.*, 1990, 1991a, 1991b; Ferron *et al.* pers. data), diel variations (Ferron, 1991; Mugiya and Oka, 1991) and parasitism (Steinhart and Eckmann, 1992), all of which can produce strong confounding effects. Several abiotic sources of variability have also been identified. These include water temperature (Buckley, 1982; Buckley *et al.*, 1984, Jürss *et al.*, 1987), salinity (Jürss *et al.*, 1986), toxicant levels (Rosenthal and Alderdice, 1976; Barron and Adelman, 1984, 1985), oxygen levels (Peterson and Brown-Peterson, 1992) and lunar cycles (Farbridge and Leatherland, 1987). Variability in lipid-based indices are suspected to result from diet composition (Gatten *et al.*, 1983; Klungsøyr *et al.*, 1989), diel periodicity in lipid metabolism and deposition (De Vlaming *et al.*, 1975; Farbridge and Leatherland, 1987; Boujard and Leatherland, 1992), maternal source (Tocher and Sargent, 1984; Fraser *et al.*, 1988; Zastrow *et al.*, 1989) and pollution stress (Fraser, 1989). Tryptic enzyme levels are affected by time of day (Pedersen *et al.*, 1987), higher values occurring in the afternoon relative to morning. Variability in trypsin activity levels in larval herring has also been attributed to inter-stock differences (Pedersen *et al.*, 1987). In the balance of this review, we focus on food availability as the main source of environmental variability, because it is the only source for which different aspects of condition have been documented.

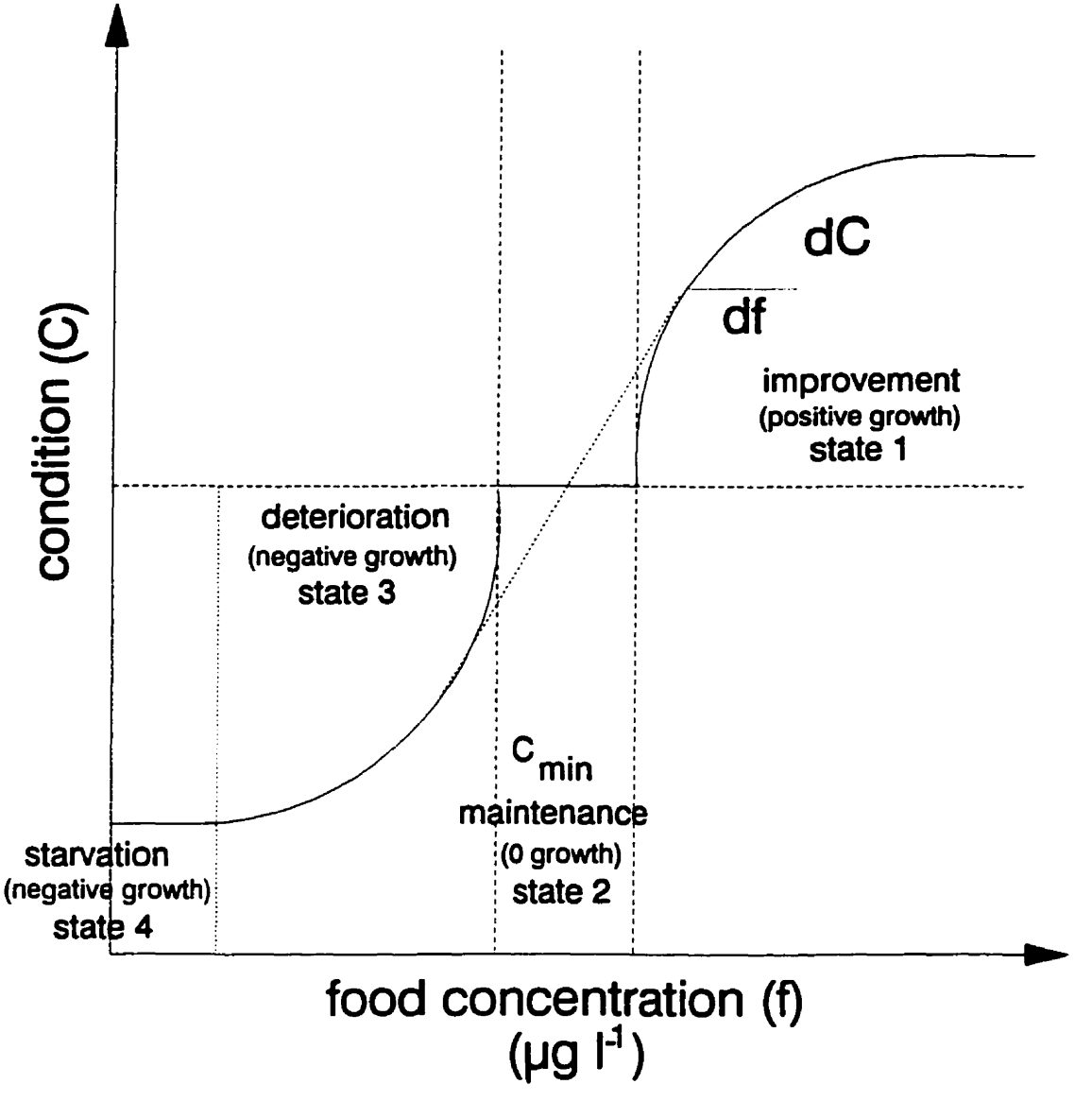
3. SENSITIVITY

Variability in the ocean's physical forcing occurs throughout a spectrum of space and time scales ranging from seconds to years, and from centimeters to hundreds of kilometers (Harris, 1980; Denman and Powell, 1984; Horne and

Platt, 1984). Plankton patchiness is produced through biological responses to such forcing, and as a consequence of interactions between the numerous species comprising the plankton (Platt and Denman, 1975; Steele, 1978; Mackas *et al.*, 1985). Marine fish larvae thus experience during their planktonic life, a dynamic, diverse and patchy feeding environment. It is therefore necessary to consider the time response of larval condition to varying food levels, if the dynamic nature of processes relating food availability to condition and survival are to be studied effectively.

We define sensitivity as the minimum environmental change (eg. food ration) that can be detected by a particular index of condition (Figure 1). The sensitivity of the condition index employed is of primary importance, particularly in situations where small changes in environmental conditions lead to significant short-time scale changes in growth rate and/or survival probabilities. The term sensitivity has often been used to express the time duration of a particular set of environmental conditions necessary to induce changes in a given condition index (which we define below as latency) or to express the rate of change in the index of condition after a response has been elicited (which we define as dynamics). In reality, sensitivity is likely to be a more useful measure than latency or dynamics when applied to field studies, since a total lack of food is likely to be improbable, and temporal changes in food availability are more likely to be commonly experienced by wild larvae (Vlymen, 1977; Lasker and Zweifel, 1978; Hunter, 1981; Rothschild and Rooth, 1982). To measure sensitivity effectively and to quantify the extent to which individual indices differ in their sensitivity, it is necessary that their response be evaluated against a common source of variability (eg. food ration) and over a range of conditions that produce positive responses. Below, we propose a bioenergetic framework within which the sensitivity of condition indices to changes in the rate of food ingestion may be assessed.

FIGURE 1. Expected relationship between condition (C) and food concentration (f) based on bioenergetic models proposed by Kamler (1992). The sensitivity (S) of a particular index of condition is defined by (S=dC/df), where dC=changes in condition and df=changes in prey concentration. Note that sensitivity is low (small dC/df) at or near high and low food concentrations and at food concentrations yielding near maintenance rations.


condition (C)
dC
df
improvement
(positive growth)
state 1
deterioration
(negative growth)
state 3
C min
maintenance
(0 growth)
state 2
starvation
(negative growth)
state 4
food concentration (f)
(µg l-1)

Bioenergetic models initially developed for adult fish, and subsequently applied to younger life stages, indicate that all the energy assimilated from ingested food by larvae is used for growth, metabolism and excretion (Laurence, 1977; Houde and Schekter, 1983; Checkley, 1984; Kiørboe *et al.*, 1987; Houde, 1989; Kiørboe, 1989; Yamashita and Bailey, 1989). A standard bioenergetic model (see Kamler, 1992) describing this relationship is:

$$C=R+P+F+U \qquad (4)$$

where C is consumption (energy assimilated from ingested food), R is respiration (energy losses through aerobic and anaerobic metabolism), P is production (energy used for growth), F is faeces (energy losses through faeces production), and U is nonfaecal excretion (energy losses through osmoregulation and urine production). Kamler (1992) identified four different feeding states based on Eq. (4) which are directly relevant to the consideration of larval fish condition. In state 1, $C > 0$, $C > C_{min}$ (C_{min} = maintenance consumption), growth rate increases with consumption to an asymptote defined by the maximum rate at which food can be processed. In state 2, $C > 0$, $C = C_{min}$, the energy budget becomes C=R+F+U because growth is zero across some finite range of consumption rates, the width of this range being a function of variable maintenance costs associated with the animal's ability to regulate metabolism through behavioral or physiological means. In state 3, $C > 0$, $C < C_{min}$, negative growth (catabolism) increases as consumption declines toward zero, the energy budget being then expressed by C+P=R+F+U. Finally, state 4 relates to total deprivation of food C=0, F=0, the energy budget is then given by P=R+U, negative growth reaches an asymptote defined by the maximum rate at which stored energy (protein/fat) can be catabolized at that time.

If, as a first approximation, we consider condition to be a reasonable correlate of growth, condition should then be related to the energy status of the larvae. Moreover, detectable changes in condition should occur only at levels of prey

consumption intermediate between maintenance requirements and the positive and negative asymptotes at which the rate of change in condition (dC) relative to the rate of change in consumption (ingestion) (df) differs from zero ($dC/df \neq 0$), as illustrated in Figure 1. The sensitivity of a given condition index will thus be related to both the minimum value of di required to elicit a measurable change in dC, and to the particular position of an individual larva on the consumption continuum. Moreover, it is expected that sensitivity will be maximal at consumption levels immediately above and below maintenance, and minimal or insensitive within the range of consumption values satisfying maintenance requirements, or near the upper and lower asymptotes, characterized by starvation or *ad libitum* regimes (Figure 1). The width of the maintenance range is likely to differ depending on the condition measure used (narrower for short-term and wider for long-term energy substrates) and the size of the animal (narrow for small and wider for large organisms)

3.1 Laboratory studies

Until recently, the influence of starvation on larval survival was the primary focus of research in larval fish ecology, and most laboratory experiments were therefore designed to evaluate the effect of total food deprivation on growth and condition. Relatively few studies specifically examined the sensitivity of condition indices to sustained or intermittent differences in food levels. As a consequence, knowledge of the sensitivity of commonly used condition indices to food availability is weak, unsystematic, and frequently confused. We review below the most reliable of these, with the goal of (1) assessing the extent to which they support or reject the existence of the relationship depicted in Figure 1, and (2) attempting to quantify the relationship if it is supported.

Wyatt (1972), reported linear relationships between the logarithm of body height/length against time for plaice larvae fed for 7 d in the laboratory. The slope of these relationships increased in a non-linear fashion as food densities

increased (50, 100, 200, 500, and 1000 *Artemia* nauplii l^{-1}) and approached an asymptote at the highest prey concentration. Approximately 78 % of the variability in mean height/length could be predicted from food levels offered in these experiments. The observed non-linear increase in condition is consistent with that predicted for ration levels above maintenance (Figure 1). The point at which $C=C_{min}$ cannot be defined, because improvement of condition (positive growth) was recorded at all food concentrations. Therefore under the conditions experienced in these experiments, maintenance requirements were probably near or below 50 nauplii l^{-1}, the lowest food level provided. Using a mean weight of 1.85 µg for an *Artemia* nauplius (table 1 of MacKenzie *et al.*, 1990 used for weight conversions), the maintenance food density under the conditions experienced was likely to be $\leq$92.5 µg l^{-1}, and the asymptotic value $\geq$1850 µg l^{-1}.

Laurence (1974) provided laboratory-reared haddock *(Melanogrammus aeglefinus)* larvae with natural plankton at densities of 10, 100, 500, 1000 and 3000 plankters (pl) l^{-1}. Mortality was 100% after 2 and 3 weeks at densities of 10 and 100 pl l^{-1}. Assuming rearing conditions were adequate in other regards, these prey levels may be assumed to result in consumption rates $C<C_{min}$. Unfortunately, the relationship between condition and maintenance feeding rates cannot be deduced from these experiments because condition was not reported for food levels in the range 10-100 pl l^{-1}. Standard length, the dry weight and the Fulton-K index (weight/length^3) increased at rations of 500, 1000, and 3000 pl l^{-1}. Growth rates, expressed as changes in both standard length and dry weight, exhibited a consistent increase with increasing food density These results, too, are consistent with the predicted non-linear response of condition to changes in consumption (prey density). The relatively small differences in the slope of standard length and dry weight at prey densities of 1000 and 3000 pl l^{-1} suggest that the upper asymptote occurs within this range. C_{min} appears to have occurred at prey densities of 100-500 pl l^{-1}. The plankton offered to the larvae was dominated by nauplii of *Acartia clausi, Centopages hamatus* and *Balanus balanoides*. Based on the average weight of an *Acartia tonsa* nauplius (0.26µg),

the maintenance concentration under the conditions employed in these experiments is estimated to be in the range of 26-130 μg l^{-1}, and the upper asymptote value >260 μg l^{-1}.

Buckley (1979) reported that the RNA/DNA ratio of larvae of cod, 7-11 d posthatch, was positively correlated with the concentrations of natural plankton (0, 200, 1000 prey l^{-1}) offered in laboratory rearings. The fact that larvae maintained at prey densities of 200 prey l^{-1} exhibited a RNA/DNA ratio typical of starved larvae, suggests that 200 prey l^{-1} may be near the maintenance level for larvae of this species and age under the rearing conditions employed. Buckley *et al.*, (1984) examined the effect of three temperatures (5, 7, and 9°C) and three rotifer *(Brachionis plicatilis)* densities (200, 500 and 1000 pl l^{-1}) on the RNA/DNA ratio of laboratory-reared larvae of sand lance *(Ammodytes* spp.). A two-way analysis of variance indicated that RNA content and RNA/DNA ratios were significantly ($P<0.01$) affected by rotifer concentrations and the age of the larvae. The multiple linear regression,

$$\text{RNA/DNA} = 1.20 \text{ rotifers ml}^{-1} + 0.03 \text{ Age} + 1.40 \quad (r^2 = 0.76) \qquad (5)$$

explained 76 % of the variability in the RNA/DNA ratio. Instantaneous protein growth rates for all treatments ranged from -6.7 % to 12.2 % d^{-1}, and were found to be between -3.0 and 3.0% d^{-1} for the larvae fed at densities of 200 pl l^{-1}. Assuming that all energy above maintenance requirements was channeled into protein growth, it is reasonable to conclude that C_{min} occurred near the prey density of 200 prey l^{-1} which yielded positive and negative protein growth rates. Given a mean weight of 0.16 μg for a rotifer nauplius, this corresponds to 32 μg l^{-1}.

Pedersen *et al.* (1990) measured the effect of two different daily rations (high = 80 and low = 15 prey $larva^{-1}$ d^{-1}) on the growth and digestive capacity of three groups of larval herring. *Acartia tonsa* nauplii were fed during the first week after

first-feeding (4-11 d posthatch) and a mixture of nauplii and copepodites thereafter. The first group, exposed to a high ration for 31 d after yolk absorption, had higher trypsin and trypsinogen content than did a second group fed at low ration for 10 d and then switched to high ration, and a third group fed at low ration throughout the experiment. Between 4 and 14 d posthatch, all the three groups increased in length but decreased in protein content, indicating a decoupling of growth in length and weight. Between 14 and 35 d posthatch, the first group fed throughout at high ration grew at the highest rate and the second group (switched from low to high ration) showed compensatory growth in both standard length and protein content when compared with the high ration group. However, the trypsin activity of the second group failed to reach the high levels found in animals exposed continuously to high ration. The third group, which was fed a low ration throughout, exhibited reduced growth rate (3.81 μg protein d^{-1}, characteristic of slightly above maintenance level), as opposed to approximately 12 μg protein d^{-1} in the other two groups. These findings suggest that under the experimental conditions employed, trypsin and trypsinogen levels, and growth in length and protein content, were sensitive to ration differences in the range of 65 prey $larva^{-1}$ d^{-1}. Given the larval stocking densities and the container size used, the high and low rations corresponded to food concentrations of 100 and 536 prey l^{-1}, or 26 and 140 μg l^{-1} (assuming a mean weight of 0.26 μg for *Acartia tonsa* nauplius). However, the actual prey concentrations were, likely to be much higher than these, because copepodites were provided in addition to nauplii.

The results of the above laboratory studies suggest that prey concentrations of 25-35 μg l^{-1} are sufficient to meet maintenance requirements and that the shape of the curve relating condition to prey levels at prey densities above maintenance values will be as predicted in Figure 1. The results also suggest that the upper asymptote will be reached at prey levels of 140-1850 μg l^{-1}. Values reported in the range of concentrations leading to below maintenance rations level (negative growth) were inadequate to assess the relationship between ration and condition. Therefore, the shape of the relationship between condition

and prey concentration at below maintenance ration levels remains hypothetical. While the data are presently too limited to provide adequate resolution of its shape throughout the full prey concentration continuum, the curve depicted in Figure 1 can probably be approximated to a sigmoid curve, given the narrow range of prey concentrations yielding near maintenance rations. The uncertainty in the above estimates derives primarily from the small number of prey concentrations surveyed, and the large variance typically associated with individual estimates of food density in such experiments. Other possible sources of uncertainty include the inherent variability in ingestion rates of individual larvae at a given prey density (Kiørboe and Munk, 1986), and the variable calorific intake achieved from different prey types. It must be noted, too, that the application of these laboratory findings to field situations should be approached with caution (see MacKenzie *et al.*, 1990; Mackenzie and Leggett, 1991).

3.2 Field studies

The problem of resolving small-scale patchiness renders the quantification of food levels to which larvae are exposed in the field, far more difficult than is the case in laboratory experiments (Rothschild and Rooth, 1982; Houde, 1982). In addition, recent studies of the relationship between prey densities, microscale turbulence levels and prey ingestion rates (MacKenzie and Leggett, 1991) indicate that prey densities alone may be a poor predictor of feeding rates in larval fishes. As a consequence, only a very small number of studies provide insights into the shape and quantitative character of the relationship between larval condition and measures of food availability, and of the sensitivity of the various indices that have commonly been applied in field studies. Shelbourne (1957), O'Connell (1980), O'Connell and Paloma (1981), Koslow *et al.* (1985), Buckley and Lough (1987), Håkanson (1989b) and Canino *et al.* (1991), were successful; and Owen *et al.* (1989), Powell *et al.* (1990), Ferron (1991) and McGurk *et al.* (1992, 1993) unsuccessful in detecting a positive relationship between larval condition (assessed by different means) and various indices of *in*

situ food concentrations. Below, we report the results from some of these studies which provide some insight into the sensitivity of condition indices to prey concentrations measured at sea.

O'Connell and Paloma (1981) reported that the liver glycogen content of field-collected northern anchovy larvae, was more sensitive to changes in food levels than were histological scores. The authors concluded that glycogen represents the first line of energy reserves in the larvae, whereas histological scores are more representative of the extent of food deprivation and hence of the magnitude of the emaciation suffered. These conclusions are consistent with the expected higher sensitivity (and probably narrower maintenance range) of a short-term energy source such as glycogen, relative to longer-term energy stores such as protein and lipids which underlie changes in histological scores.

Powell *et al.* (1990) assessed morphological condition, as described in Powell and Chester (1985), on spot larvae sampled along a transect across the Mississippi river plume front, in order to determine their nutritional condition in relation to local food availability. Despite large intra-station variability in condition, these authors reported a weak negative relationship between the proportion of larvae classified to be in starving condition and the proportion of larvae with food in their gut. This was believed to result from the fact that measures of gut fullness and condition respond on different time scales: gut content on a scale of hours, and morphometric condition on a scale of days. In this study then, condition was independent of gut content, as measured at the time of sampling, notwithstanding the statistical relationship between the two. This finding emphasizes the importance of considering the time response of condition indices (to be discussed in next section) in addition to sensitivity, if the relationship between feeding success and condition is to be assessed reliably.

Suthers *et al.* (1992) used otolith indices (a measure of recent growth), triglyceride levels, dry weight, standard length and anal body depth of juvenile

cod sampled on the Scotian Shelf to generate condition indices based on residuals of univariate regressions of these values on standard length. When these indices were compared with estimates of zooplankton biomass (mg m^{-3}) sampled coincident with the fish, triglyceride and morphometric condition indices were found to be insensitive to the food levels experienced, despite an order of magnitude variability in food abundance. The authors concluded that the animals sampled were capable of integrating small-scale variability in prey abundance. An alternative explanation could be that the maintenance range (Figure 1) was wider than the variability in the food levels encountered. This is plausible since larger and older fish have a higher rate of turnover of the main energy substrates (including triglycerides), and a greater capability of adjusting their metabolism to the quantity of energy available (Laurence, 1977), therefore minimizing any growth or condition changes resulting from differences in prey densities encountered. The greater specialization of storage organs such as the liver in older fish is also known to contribute to greater access to, and stability of, short-term energy sources such as glycogen (Margulies, 1993).

Canino *et al.* (1991) measured the RNA/DNA ratio of walleye pollock larvae in relation to prey concentrations and ingestion at four stations and on two different cruises in Shelikof Strait (Gulf of Alaska). *Pseudocalanus* spp. nauplii and eggs comprised the bulk of the diets, and occurred at concentrations of 12.5-23.1 nauplii l^{-1} (8.8-16.2 µg l^{-1}). Computing the critical RNA/DNA ratio Eq. (3) indicated that all larvae on one cruise, and 89-92% on the other cruise, exhibited negative protein growth rates indicative of starvation. Daily rations estimated for the same two cruises ranged from 7.2 to 27.9 prey $larva^{-1}$ d^{-1}. While only average gut fullness were reported for each of the four sampling sites, average gut fullness was positively related to food availability (nauplii l^{-1}), and to the RNA/DNA ratio of the fish larvae collected at the same site. Based on laboratory bioenergetic calculations provided by Yamashita and Bailey (1989), Canino *et al.* (1991) also determined that a 5.5 mm pollock larvae would require 69 nauplii d^{-1} (≈25.5 µg d^{-1}) to obtain the weight-specific ration of 21.5-29% of body dry weight d^{-1} required

for maintenance and growth. Considering a search volume of 4.57 l d^{-1}, a daily feeding time of 16h, and a capture efficiency of 15.2 %, Canino *et al.* (1991) determined that pollock larvae would require prey concentrations of 190 prey l^{-1} or 70 µg l^{-1} (0.37 µg $prey^{-1}$) to obtain this weight-specific ration. These values greatly exceeded most of the prey concentrations recorded. The authors concluded that starvation and food-limited growth were inevitable at all of the sampling sites visited.

Ferron (1991) determined the RNA/DNA ratios of mackerel *(Scomber scombrus)* larvae (6.5-7.5 mm) and related these to *in situ* measures of food concentrations. Larvae and their food (microzooplankton) were sampled hourly in the upper mixed layer, at a fixed station and at discrete depths (0-5, 5-10, and 10-15 m) for five consecutive days. The resulting time series revealed the advection of two distinct water masses and feeding environments past the station. The first which persisted for 3 d, was characterized by food concentrations that increased gradually from 31 to 58 copepod nauplii l^{-1}, followed by a rapid decline (55% decrease over 4h). The second water mass which persisted for the remaining 2 d of sampling, was characterized by food levels ranging from 26-45 nauplii l^{-1}. The RNA/DNA ratios of the larvae sampled from these discrete water masses showed food-limited positive protein growth rates, (based on Buckley's (1984) model Eq. (1)), in 98 % of the cases examined. No significant differences in the frequency histograms of RNA/DNA ratios were detected between the two feeding environments. However, vertical migrations of larvae between the three depth layers sampled became evident shortly after the decline in food concentrations which occurred at the transition between the first and second water masses. Since 7.0 mm mackerel larvae are fully competent foragers (Ware and Lambert, 1985, Buckley *et al.*, 1987), this suggests an increased foraging activity in a food-reduced environment. About 70% of all nauplii sampled were *Oithona similis*, 25% were *Pseudocalanus minutus*, and 5% *Temora longicornis*. Assuming an average dry weight of 0.7 µg $nauplius^{-1}$

(Buckley *et al.*, 1987), the range of prey concentrations (26-58 nauplii l^{-1}) experienced by the larvae was 18.2-40.6 µg l^{-1}.

McGurk *et al.* (1992) measured the RNA/DNA ratios of larvae of Pacific herring *(Clupea pallasi)* and of sand lance *(Ammodytes hexapterus)* collected in two embayments of the Gulf of Alaska. They observed that 11-23% of the first-feeding herring larvae and 45% of the first-feeding sand lance larvae had RNA/DNA ratios typical of starving animals, despite the fact that the two feeding environments were characterized by prey levels (16-84 prey l^{-1} or 11.2-58.8 µg l^{-1}) known to support positive growth rates in the laboratory (Kiørboe and Munk, 1986; Buckley *et al.*, 1987). Moreover, the residuals of regressions of ln (RNA/DNA) on larval length were uncorrelated with prey concentrations or water temperature, whereas positive correlations are expected between these variables based on laboratory results (Buckley, 1982, 1984; Buckley *et al.*, 1984). McGurk *et al.* (1992) proposed that the nutritional condition of first-feeding larvae must be determined by the interaction of prey concentrations, temperature and the ability of the larvae to feed effectively. A further recent example of the lack of correlation between larval condition and food availability is provided by the study of McGurk *et al.* (1993), who collected larvae of Pacific herring from four distinct cohorts in Auke Bay (Alaska). The authors assessed larval condition using multivariate morphometric techniques developed by McGurk (1985a, b). Although the food densities measured (27-30 nauplii l^{-1}, 19-21 µg l^{-1}) were at all times sufficient to support at least 4% larval growth in weight d^{-1}, and were an order of magnitude higher than food rations eliciting starvation in the laboratory (Kiørboe and Munk, 1986), it was estimated that between 8-35% of the herring larvae exhibited condition values characteristic of starving larvae.

There does, however, appear to be a good agreement between the condition of larvae as would be predicted from the maintenance range developed from the review of laboratory experiments (25-35 µg l^{-1}), and the *in situ* prey concentrations reported by Canino *et al.* (1991), Ferron (1991), and McGurk *et*

al. (1992, 1993). Therefore, Canino *et al.* (1991) reported a high proportion of starving larvae at prey concentrations of 8.8-16.2 μg l^{-1} which laboratory studies suggest are below maintenance. Ferron (1991) found little evidence of starvation at prey concentrations of 18.2-40.6 μg l^{-1} which laboratory studies suggest are equal to or above maintenance levels. McGurk *et al.* (1992, 1993) reported 8-45% starving larvae at prey concentrations of 11.2-58.8 μg l^{-1} and 19-21 μg l^{-1}, which range from below to above maintenance levels. This agreement is satisfactory given the numerous sources of error known to be associated with estimates of prey concentrations both in the laboratory and *in situ.* However, the frequent absence of significant relationships between condition and food availability (McGurk *et al.*, 1993) suggests other exogenous or endogenous factors influence feeding success and condition *in situ.* One such exogenous factor, which has not yet been examined in relation to larval condition, is the possible effect of microscale turbulence on feeding success (MacKenzie et al. 1991).

Rosenthal and Alderdice (1976), who examined the effects of a broad variety of environmental stressors (abiotic and biotic) on marine fish eggs and larvae, concluded that sub-lethal effects were often limited to a very similar array of organismal responses, many of which were biochemical in origin. These biochemical changes were often translated, later in time, into histological, morphological, physiological, and ethological responses. Given this background, it is reasonable to expect that biochemical indicators of fish condition will be most sensitive to environmental stress (including food availability), and that sensitivity would then proceed in reduced order to cellular (histological) and organismal (morphometric) level indicators. Although the number of laboratory or field studies which allow evaluation of the sensitivity of condition indices to different food rations is limited, the trend of increased sensitivity as one proceeds from organismal, to tissue, and finally to the cellular levels indices is apparent. Within the array of biochemical measures of condition now available, a trend is also apparent. Short-term energy indicators such as digestive enzymes, glycogen and

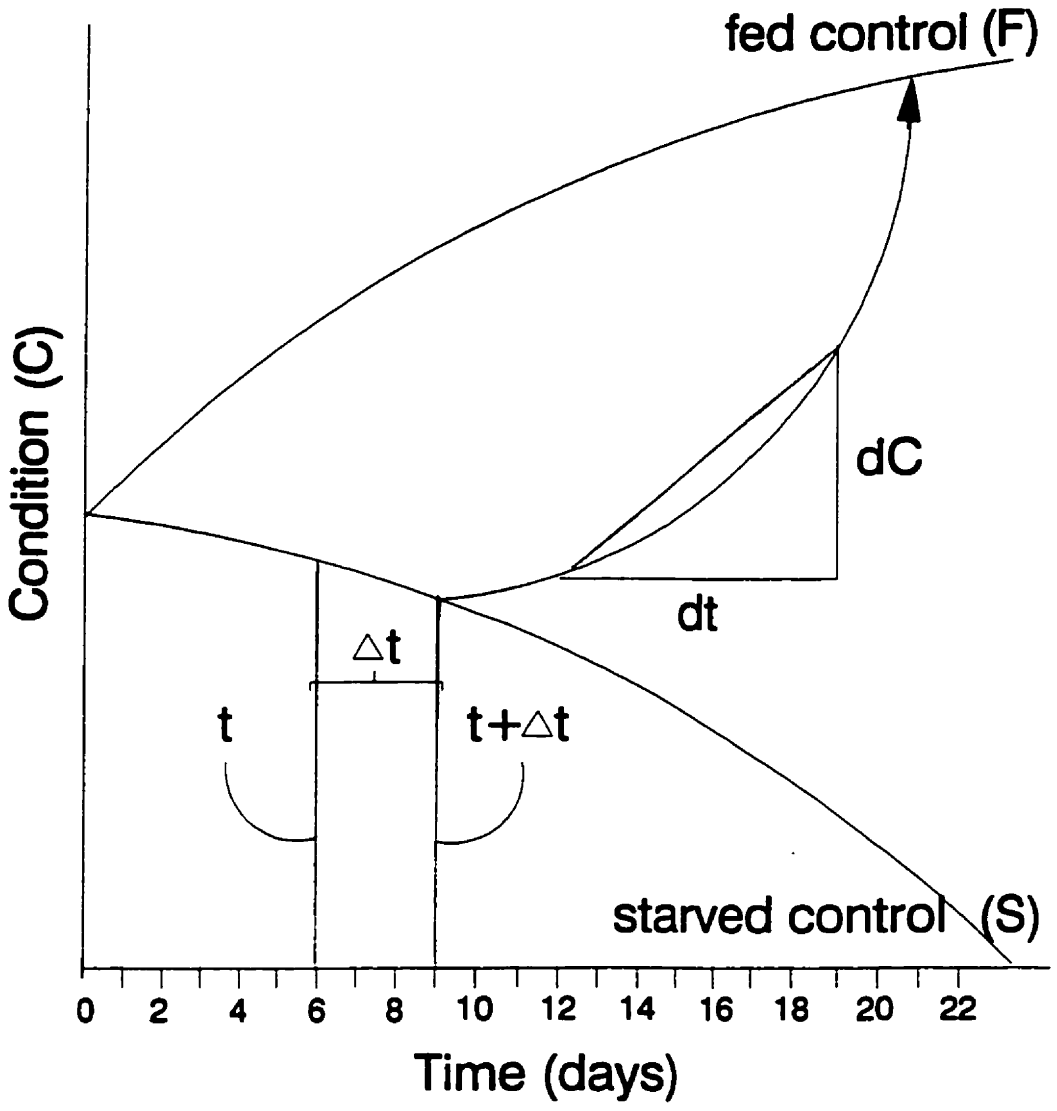
lipids appear to be more sensitive to food ration than are nucleic acids and proteins. Further research is required to confirm these trends and provide reliable measures of sensitivity for the several condition indices currently employed.

4. TIME RESPONSE (LATENCY AND DYNAMICS)

From a consideration of the direct effects of various food rations on condition, we now turn to the measurement of the time response of condition in relation to intermittent food levels. In contrast to predation, which is a near-instantaneous process, the response of larval condition and growth to variation in prey abundance is slower, and the relationship between cause and effect is likely to involve a time lag. Therefore, unless repeated measurements are taken on the same specimens (a logistic impossibility in field studies), the time response of condition to changes in food availability must be known in order to predict the relationship between present condition and feeding history, or to forecast future larval condition from a knowledge of the present feeding status. The time response of condition is also required in order to estimate the duration of different starvation categories, and to determine daily starvation mortality at sea (Theilacker, 1986; Theilacker and Watanabe, 1989).

We define latency as the time required for a given change in food availability to be reflected as a significant change in the particular index of condition used. This lag is a consequence of the time required for the energy balance of the animal to adjust to the new conditions, and for chemical reactions to occur which allow energy to be transformed, and then utilized or stored depending on the physiological needs of the moment. Latency can be viewed as equivalent to sensitivity over time. It is expected that sensitivity and latency will be negatively correlated (higher sensitivity - shorter latency). The dynamics of condition is defined as the rate of change in condition after the response is first detected. These concepts of latency and dynamics are depicted in Figure 2, in which the condition of fed and starved controls is illustrated over time, along with a

FIGURE 2. Time response of condition following feeding *ad libitum* (F) and starvation (S) controls. The arrow refers to the condition trajectory of specimens for which feeding was delayed for 6 d (t=6) before being resumed. Latency, ($L=\Delta t$) is equivalent to the time interval between food introduction (t=6 d) and the time at which a significant increase in condition above the starvation control was recorded ($t+\Delta t$=9 d). The dynamics ($D=dC/dt$), is the rate of increase in condition after the response has been elicited. In the example illustrated an exponential response of condition to food is used. Other shapes are, of course, possible.


fed control (F)
Condition (C)
dC
dt
△t
t
t+△t
starved control (S)
0
2
4
6
8
10
12
14
16
18
20
22
Time (days)

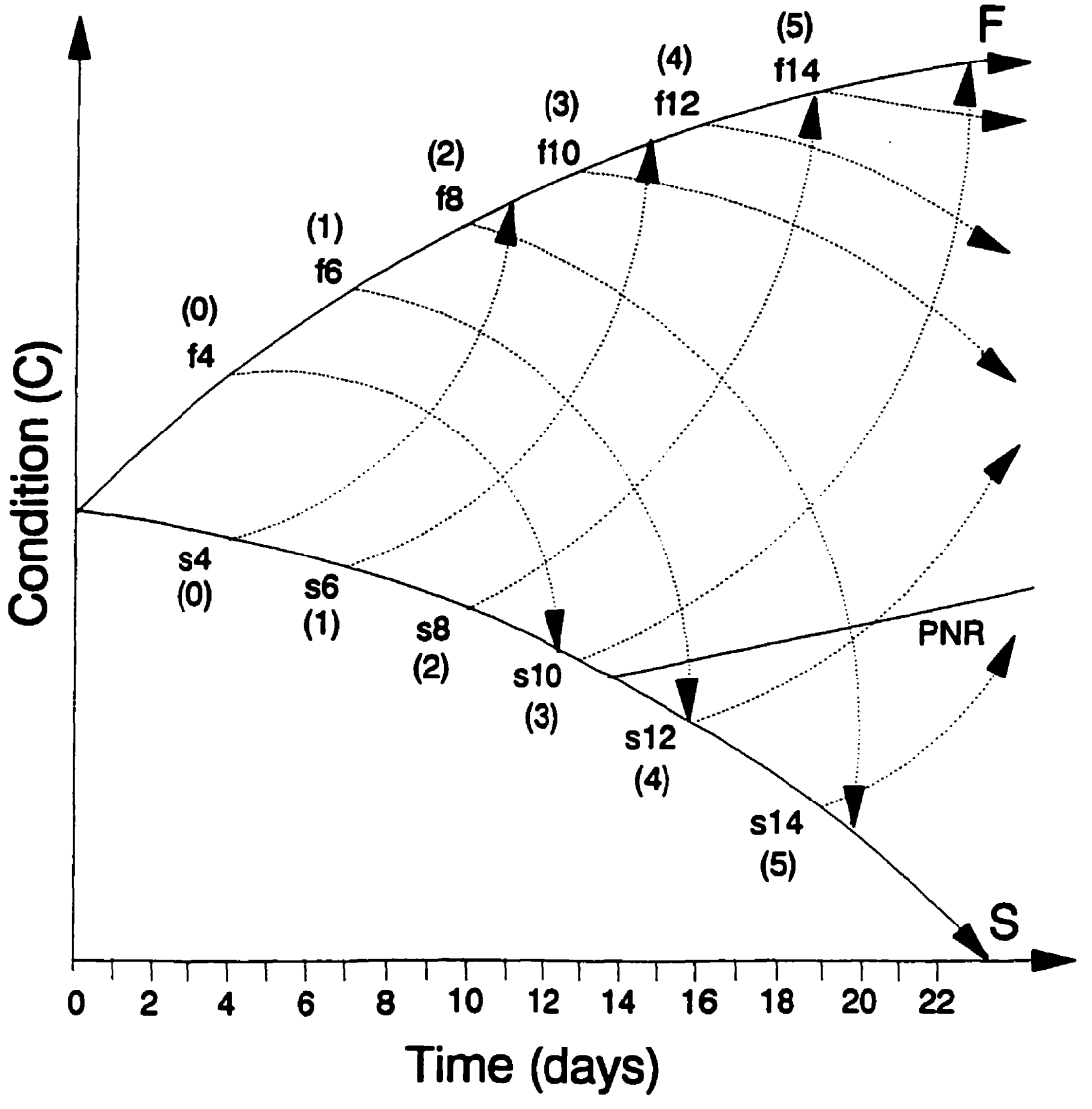
delayed-fed treatment in which the larvae were starved for 6 d before being fed. In the example given, latency ($L=\Delta t$) is equivalent to the lag (3 d) between the time at which food was first given ($t = 6$), and the time a noticeable difference in condition (above the starvation control level) was detected ($t+\Delta t=9$). Dynamics (D) refer to the rate of change dC/dt after the response has been elicited. Below we review laboratory studies which have systematically addressed the questions of latency and dynamics using delayed-fed and delayed-starved experiments. Other indirect measures, which are available from laboratory or field studies, are also used to generate time response predictions for different condition measures.

4.1 Morphometric measurements

Theilacker (1978), who used a stepwise discriminant analysis (SWDA) of 11 morphometric variables (see section 2.1 and Table 1) to relate food level to condition, found that 87% of the fed and 94% of starved jack mackerel larvae could be differentiated after 3d of starvation in the laboratory. However, when the starved group was further divided into moderately starved (starved for 1 or 2 d) and severely starved (starved for 3 d) larvae, the proportion correctly classified declined to 83% for the fed, 96% for the moderately-starved, and only 78% for the severely-starved larvae. Powell and Chester (1985) collected similar data for spot larvae, and found that 84% of the fed and 83% of the starved animals could be distinguished by a SWDA. However, when the moderately and severely starved specimens were separated on the basis of 50% mortality (PNR) values, only 80% of the fed, 50% of the moderately-starved, and 63% of the severely-starved larvae were correctly classified. These results suggest that for the larvae studied, morphological changes have a short latency (<1 day) following starvation. However, after a significant decline over the first two days, the morphometric indices become less affected by prolonged starvation (3 d). Hence, after this interval, moderately and severely-starved animals remain morphologically indistinct.

Wright and Martin (1985) measured standard length, eye diameter, head length, head depth, body height at pectorals and body height at the anus, on larvae of striped bass exposed to various delayed-fed and delayed-starved conditions in the laboratory. Five groups of 8 d post-hatch larvae were exposed to 1000 *Artemia* nauplii/l for 2, 4, 6, 8, and 10 d, and then starved to determine their rate of deterioration. Eight other groups were starved for 1, 2, 3, 4, 5, 7, 9, and 11 d, before being offered food at 1000 nauplii/l to measure their rate of recovery. The experiment which lasted 14 d, included two controls in which the animals were starved or fed for the total duration of the experiment. Wright and Martin (1985) reported the standard length of each group of larvae over time, and fitted parabolic curves to each treatment group. Most of the curves obtained were similar in shape to those illustrated in Figure 3. Dynamics showed a slow response following the application of the treatment (starvation or feeding) followed by a steeper slope when the values approached the control levels. In the early delayed-starved treatment F2 (fed for 2d and starved) and F4 (fed for 4d and starved), Wright and Martin (1985) found that standard length continued to increase for some days after food withdrawal before a shrinkage was recorded. If food was removed later, standard length showed no increase, and began to decrease with an average latency of 1-2 d The dynamics of deterioration was approximately 0.3 mm/d for most of the delayed-starved treatments. Among the delayed-fed treatments, five days of starvation represented the maximum starvation time from which full recovery to fed-control levels was possible (compensatory growth). The dynamics of recovery was in the order of 0.3 mm/d for most delayed-fed treatments applied prior to 5 d, but was much lower (0.1 mm/d) for larvae starved for 7 d or more before being fed. The six morphological variables and all possible ratios were included in a SWDA following Theilacker (1978), and the values for the canonical variables reported in Martin and Wright (1987). The results showed that continuously-fed and starved (control) specimens could be distinguished after only 2 d of feeding delay; overlapping centroids demonstrated, however, that recovering and starving

FIGURE 3. Proposed model for the time response of morphological indices and histological scores following different feeding regimes. The arrows refer to the condition trajectories of larvae exposed to different feeding conditions. F = feeding *ad libitum* control, S = starvation control, PNR = point of no return and defines the condition level below which the larvae will die even if they are fed (also defined as the point of irreversible starvation). Delayed-fed treatments are labeled s*n* (s4-s14), where *n* = number of days feeding was delayed before being resumed. Delayed-starved treatments are labeled f*n* (f4-f14) where *n* = number of days starvation was delayed before being resumed. The numbers in brackets, above and below, refer to latency in days, and increase with the length of time feeding or starvation was initially delayed.


F
(5)
f14
(4)
f12
(3)
f10
(2)
f8
(1)
f6
(0)
f4
Condition (C)
s4
(0)
s6
(1)
s8
(2)
s10
(3)
s12
(4)
s14
(5)
PNR
S
0
2
4
6
8
10
12
14
16
18
20
22
Time (days)

specimens could not be discriminated from the different delayed-starved and delayed-fed treatments when passing through transition phases.

4.2 Histological measurements

Theilacker (1978) showed that 83% of fed, 44% of moderately-starved, and 83% of severely-starved jack mackerel larvae could be correctly classified based on histological scores (Table 1). The fact that only 44% of the larvae in the intermediate class were correctly classified using histological scores, compared with 96% correct classification based on the analysis of morphometric data (see above), suggests an earlier response (shorter latency) of morphological changes to starvation. However, a greater resolution of histological scores within the class of moderately starved larvae was also demonstrated, since 19% were wrongly graded as healthy, 44% were correctly classified as moderately-starved, and 37% were wrongly classified as severely-starved (Table 1). It is expected that the 19% which remained in good condition after 1-2 d of starvation would be able to sustain a longer period of starvation, than the 37% which were in poor condition Theilacker (1978) also obtained good correspondence between morphological and histological assessments of condition (Table 1). The agreement being best (92.9 %) for larvae past the point of irreversible starvation (3 d starvation), and lower for larvae starved for 1 and 2 d (40.7%). This suggest a different dynamic for the two indices.

Kashuba and Matthews (1984) recorded six different histological scores (midgut, pancreas, liver, muscle, cartilage, and notochord) on shad *(Dorosoma* spp.) larvae sampled weekly in a reservoir over a two-month period. They noted a decrease in pancreas, midgut and liver condition following a drop in food availability at the time the larvae were sampled, and a decrease in muscle and notochord scores a week later. The authors concluded that pancreas, midgut and liver scores were indicators of early stages of starvation (latency<1 week), while

muscle and notochord scores were more indicative of later stages of starvation (latency>1 week).

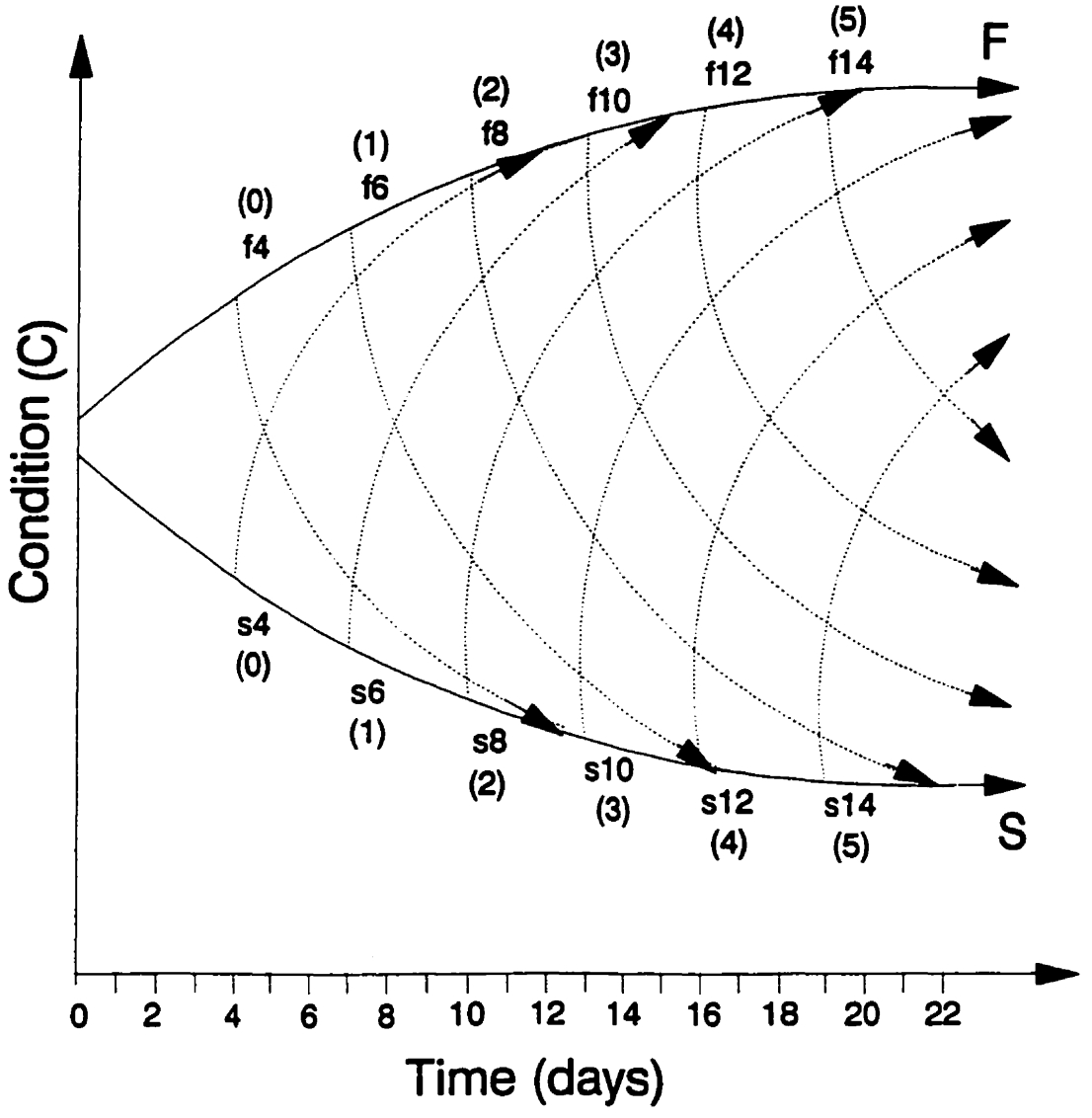
Martin *et al.* (1985) and Martin and Wright (1987), who analyzed the dynamics of laboratory-reared larval striped bass histology, reported significant changes in Theilacker's (1978) histological scores after 2 d of starvation. By defining a boundary histological score to delimit animals in good and poor condition, they were able to determine that more time was required for larvae to recover from starvation than to deteriorate from fed to starvation levels. For both deteriorating or recovering fish, the total time required to go from control to boundary level condition also increased with the duration of the initial starvation or feeding conditions. These linear relationships (computed from data provided in Table 3 of Martin and Wright, 1987) are described by the following equations:

$$\text{Recovery time (days)} = 0.58\ \text{feeding delay (days)} + 2.07 \quad (n=6,\ r^2=0.918) \tag{6}$$

$$\text{Deterioration time (days)} = 0.59\ \text{starvation delay (days)} + 0.53 \quad (n=5,\ r^2=0.927) \tag{7}$$

Although very few data points were reported, the slopes of the two lines is very similar while the intercept is much higher for recovery time than for deterioration time. Based on the shape of the curves of delayed-fed treatments provided by Martin and Wright (1987), the dynamics of histological scores appear to follow the morphological model illustrated in Figure 3. If we picture the histological boundary level as a line running horizontally and equidistant between fed and starved controls, the increasing time required to recover or deteriorate must result from increased latency and divergence of the control lines with increasing feeding or starvation delays. Martin and Wright (1987) found that the first tissue to show signs of deterioration were the last to recover. This makes it difficult, even when assessing the status of several tissues, to determine with

FIGURE 4. Proposed model for the time response of cell height measures and biochemical indices of condition following different feeding regimes. The arrows refer to the condition trajectories of larvae exposed to different feeding conditions. F = feeding *ad libitum* control, S = starvation control, Delayed-fed treatments are labeled s*n* (s4-s14), where *n* = number of days feeding was delayed before being resumed. Delayed-starved treatments are labeled f*n* (f4-f14) where *n* = number of days starvation was delayed before being resumed. The numbers in brackets, above and below, refer to latency in days, and increase with the length of time feeding or starvation was initially delayed. The PNR level was not plotted on this figure because the measures of condition following this type of response (mainly rate indicators) do not provide direct estimates of irreversible starvation.


Condition (C)
F
S
(0)
f4
(1)
f6
(2)
f8
(3)
f10
(4)
f12
(5)
f14
s4
(0)
s6
(1)
s8
(2)
s10
(3)
s12
(4)
s14
(5)
0
2
4
6
8
10
12
14
16
18
20
22
Time (days)

certainty whether the larvae was deteriorating or recovering at the time of capture.

Setzler-Hamilton *et al.* (1987) sampled larval striped bass weekly over several years, from two different rivers. They obtained a good correspondence between the percent of larvae having starved morphometry (computed from SWDA using the same variables as Wright and Martin, 1985) and the average histological score of a sub-sample. The peaks in histological scores corresponded with troughs in the proportion of larvae classified as starving on the basis of their morphometry, with little lag between the two. The one week interval between sampling was, however, too long to allow a precise estimate of the degree of synchrony.

Oozeki *et al.* (1989) detected significant changes in the epithelium cell heights of the anterior, mid, and posterior regions of the gut, and in the liver and pancreatic cells of stone flounder *(Kareius bicoloratus)* larvae, 2-3 d after onset of starvation. These quantitative changes were rapid in the first 2-3 d following the onset of starvation, but stabilized and showed no further change until death. This dynamic was similar for all tissue cells examined, with the exception of the epithelium cell height of the anterior part of the gut, where the initial decrease was more gradual. The curves obtained had the same shape as those illustrated in Figure 4. The apparent difference between the dynamics of traditional histological scores (Figure 3) and cell height measurements (Figure 4) probably reflect the fact that the former integrate the values of a set of qualitative scores, while the latter represents a series of measurements of a single quantitative measure. Therefore, average cell height responds more rapidly to changes in food level than traditional histological scores. Cell height, however, also attains its minimum or maximum value faster, and exhibits more resilience thereafter.

Theilacker and Watanabe (1989) delayed feeding for 0-4 d in post yolk-sac larvae of northern anchovy kept in the laboratory, and measured their standard

length and midgut cell height. Standard length at age and growth rates differed significantly between the five treatments such that the age of a 5 mm larvae could range from 9 to 19 d. The growth curves were more or less linear for the different treatments, and never converged, so differing from the model outlined in Figure 3. However, the decrease in midgut cell height reported for the delayed-fed groups showed a rapid decrease after 1 d of starvation and a lesser change after 2, 3 and 4 d. This conforms to the dynamics reported by Oozeki *et al.* (1989) and the model illustrated in Figure 4. Margulies (1993) reported significant changes in histological scores of black skipjack tuna *(Euthynnus lineatus)* less than 24 h after the onset of starvation in the laboratory, with liver hepatocytes responding in as little as 12 to 15 h. For experiments conducted over 6 d, the histological scores did not change in fed controls, while in starved treatments, a rapid drop was recorded within the first 24 h followed by a more gradual decline thereafter. Larvae fed after 48 h of starvation were able to recover to fed-control levels, but those provided with food after 72 h failed to recover (irreversible starvation). The shape of the recovery curve followed the model outlined in Figure 3, while the shape of the deterioration curve more closely approximated that illustrated in Figure 4.

4.3 Biochemical measurements

Martin *et al.* (1984) measured total fatty acids in larval striped bass exposed to various starvation/feeding regimes in the laboratory. In addition to feeding and starvation controls, they also used four starvation treatments which consisted of delayed feeding for 4, 5, 6, and 7 d. Peaks in fatty acid levels were detected at 6 d after hatching in animals subjected to starvation. This peak was attributed to the catabolism of lipoproteins derived from yolk remains. Changes in growth rates were evident at 7 d but not at 5 d delayed feeding. There was no indication of the accumulation of fatty acids prior to 10 d post-hatching, suggesting that the utilization of dietary sources was complete for at least 4 d after the peak seen at 6 d post-hatch. Commenting on these results, Martin and Wright (1987)

concluded that early feeding influenced fatty acid levels in ways which allowed separation of feeding and non-feeding larvae, but that fatty acid levels could not be used as an indication of present nutritional status or of the capacity of the larvae to withstand starvation because they were, in essence, indicators of the duration of the delay in first-feeding.

Martin *et al.* (1984) provided valuable information on total fatty acid dynamics and on fatty acid composition of striped bass larvae during starvation. It is important to note, however, that striped bass larvae differ from many marine fish larvae in that the egg contains large quantities of neutral fat stores. These are preferentially retained during the embryonic period and are utilized for up to 20 d after hatching, and well beyond the onset of first-feeding (Eldridge *et al.*, 1982, 1983). This contrasts with herring and cod in which most neutral lipid stores are catabolized during early larval development (Fraser *et al.*, 1988). The dynamics of neutral and polar lipids, first proposed as a possible indicator of the nutritional status of marine fish larvae by Fraser *et al.* (1987), remains poorly known. Given this reality, Håkanson (1989a) used data on neutral and polar lipids from his work on copepods (Håkanson, 1984) to predict neutral lipid changes following starvation in larvae of northern anchovy. In copepods, the total triglyceride content of starved specimens was consumed after 3 d, while their wax ester content showed significant change only 1 wk after cessation of feeding. Unfortunately, the fact that fish larvae may differ in their lipid content, composition, diet, and metabolism makes any comparison with copepod values questionable. Nevertheless, Håkanson (1989a) reported a predictable decrease in triglyceride (TAG) levels following 4 d of starvation in larval northern anchovy reared in the laboratory. However, TAG levels were <1.0 µg/larvae for animals up to 18 d post-hatching, and changes in TAG levels after the onset of starvation were small, <1.0 µg. Large changes (1-6 µg) in TAG content were observed only in larvae that had been fed or starved beginning 18 d following hatching. Håkanson (1989a) made no attempt to calculate neutral lipid utilization rates.

The latency of changes in nucleic acid levels of larval fish relative to changes in food availability has been assumed to be on the order of a few days (Buckley 1984, Buckley and Lough, 1987). Buckley (1979, 1980) detected changes in the RNA content (also expressed as changes in the RNA/DNA ratio) of reared larval cod and winter flounder 2 d and 4 d respectively following removal of food. However, in both studies, the frequency of sampling was 2 d, so that the minimum latency period remains undefined. Larval fathead minnows *(Pimephales pomelas)* exposed to sub-lethal levels of hydrogen cyanide exhibited a measurable reduction in their RNA, protein content and RNA/DNA ratios after only 24 h of exposure (Barron and Adelman, 1985). This short latency may result from the fact that toxicants act directly on oxidative processes affecting RNA synthesis, as opposed to changes in the food environment which indirectly influence metabolism through changes in energy substrate pools. Here too, a higher sampling resolution may have revealed an even shorter latency.

Wright and Martin (1985) conducted laboratory experiments involving larval striped bass to investigate the latency and dynamics of changes in the RNA-DNA ratio following various delayed-fed and delayed-starved conditions (see section 4.1 on morphometrics). From the deterioration and recovery curves obtained (which were similar to those illustrated in Figure 4) they determined that the mean latency time for detectable changes in RNA/DNA ratios was 0.66 d (15.8 h) for larvae starved after being fed, and 0.81 d (19.4 h) for larvae fed after being starved. The recovery dynamic, obtained from their plots, was approximately 0.4 RNA/DNA units/d, which is twice as fast as the deterioration dynamic (0.2 units/d). Wright and Martin (1985) concluded that the RNA/DNA ratio was sufficiently sensitive to allow striped bass larvae fed for 8 d and starved for 6 d to be distinguished from those which had been starved for 7 d and fed for 7 d. We disagree with their conclusion since it is very difficult using their curves, to distinguish whether the larvae sampled at any given time were recovering or degenerating without a knowledge of their present and past condition and/or

feeding environment. This restriction also applies to all other indices and to the models proposed in Figures 3 and 4.

Clemmesen (1987) obtained a linear relationship between the RNA/DNA ratio and the starvation interval (measured in degree-days) in groups of laboratory-reared herring and turbot larvae :

$$\text{RNA/DNA} = 2.353 - 0.009 \text{ starvation interval (degree-days)} \quad (r^2=0.39,\ n=21) \qquad (8)$$

The addition of data for fed larvae and for dry weight measures to the relationship, allowed Clemmesen (1987) to explain 62% of the observed variability in the length of the starvation interval (expressed in degree-days), thus allowing evaluation of the prior feeding history :

$$\text{starvation interval} = 66.373 + 27.638 \log \text{dry weight/larvae} - 53.387 \text{ RNA/DNA} \quad (r^2=0.62,\ n=163) \qquad (9)$$

However this model, when applied to sole *(Solea solea)* larvae (Richard *et al.*, 1991), explained only 28% of the variability in the starvation intervals imposed. Moreover, while the relationship described in equation 9 could be used to determine the duration of starvation at a given temperature, it cannot discriminate whether the condition of an individual larvae was improving or deteriorating at the time of sampling. The RNA/DNA ratio deterioration curves provided by Clemmesen (1987) for herring larvae are consistent with the model illustrated in Figure 4, with a sharp decline (0.2 units/d) during the first 4 d of starvation. The subsequent minimum starvation-control values were, however, less clear because very few data were reported in the 1.0-1.5 range.

Ueberschär and Clemmesen (1992) detected a significant ($P<0.05$) decrease (43%) in the RNA/DNA ratio of starved herring larvae after 3 d of starvation. This

was followed by a reduction in the rate of decrease and a stabilization in the ratio at values of approximately 1.0 at 10 and 13 d after the onset of starvation. The rate of decrease (dC/dt) in the RNA/DNA ratio ranged from -0.8 units/d between day 0 and day 3, to 0.2 units/d between day 3 and day 6 and 0.1 units/d thereafter. Although the sampling was conducted at three days intervals, the deterioration curve obtained followed the shape depicted in Figure 4.

In adult fish, the dynamics of the RNA/DNA ratio exhibit different rates of increase and decrease following starvation and re-feeding. Mugiya and Oka (1991) detected significant RNA/DNA ratio differences in juvenile rainbow trout after 2 d of starvation, such that 50% RNA/DNA ratio levels were reached on the third day. However, RNA/DNA ratio recovery was more gradual and levels equal to those observed in control fed fish were attained only after 4d.

Lied *et al.* (1983) measured ribosomal RNA and DNA content, and the rate of incorporation of ^{14}C-phenylalanine from ribosome extracts of epaxial muscle in 2 yr-old cod. No significant changes in the rRNA, DNA and RNA/DNA ratio were detected after 3 d of starvation. However, the amino acid incorporating activity of the muscle was reduced to 55% of the control level observed in fed fish. No significant change in DNA content was recorded (constant number of cells) after 5 and 8 d of starvation, despite the fact that rRNA had declined to 87% and 72%, and the RNA/DNA ratio to 81% and 68% of their original values, respectively, indicating a decrease in the number of ribosomes per cell. Amino acid incorporating activity declined to 39% and 13%, respectively, of the levels of the fed controls during the same time interval. Upon re-feeding of fish starved for 8 d, the rRNA, rRNA/DNA ratio and the amino acid incorporating activity were completely restored within 12 h. These results indicate that, in juvenile cod, recovery of muscle protein synthetic activity after starvation and re-feeding is much faster than is its decrease following starvation. More importantly, they illustrate the fact that protein synthesis is related to both the number of ribosomes present in the muscle (quantity of rRNA) and their specific activity,

and that these two variables can vary independently (Lied *et al.*, 1982). Roselund and Lied (1986) studied the effect of different protein energy levels on the muscle growth of saithe and rainbow trout. Their results indicate that the rate of amino acid incorporation/min/mg RNA was relatively constant at all but the two lowest levels. These findings indicate that the reduced protein synthesis of starving fish was related both to a decrease in rRNA and to a reduced efficiency of amino acid incorporation. In fish fed at higher protein levels, the higher protein synthesis achieved was attributed to higher rRNA levels alone. Loughna and Goldspink (1984) also concluded that reduction in protein synthesis caused by long-term fasting was related to a reduction in the number of white muscle ribosomes and a lowering of their activity.

Miglavs and Jobling (1989) exposed four groups of juvenile Arctic charr *(Salvelinus alpinus)* maintained in the laboratory to periods of food satiation (S) and food restriction (R) for various lengths of time. The first group was satiated for 16 wk, (S16), the second was fed a restricted diet for 8 wk and then sampled, (R8-S0), the third was fed a restricted diet for 8 wk and then satiated for 4, (R8-S4), and the fourth last group was fed restricted for 8 wk and satiated for 8, (R8-S8). The fish maintained on restricted diets and then provided with an excess of food showed compensatory growth and specific growth rates that were significantly higher than those predicted from the RNA/DNA ratio-growth rate relationship established for the satiated group (S16). Two possibilities could explain these results, 1) a differential ribosome protein synthetic activity level or 2) a change in the relationship between anabolic and catabolic processes. Since high somatic growth rate is likely to be associated primarily with higher levels of protein synthesis and very low rates of protein degradation, the first explanation appears more plausible.

If the same decoupling of protein synthesis and RNA content (ribosome number) occurs in larval fish, it is likely that in severely starved animals that have reached a low rRNA level further decreases in protein synthetic capacity will be

reflected only in a reduction in amino acid incorporation rates. In larval fish, the dynamics of changes in protein levels are likely to occur more rapidly since the rate of protein synthesis (and potentially degradation) is reported to be 20 times faster than in the adult fish (Hansen *et al.* 1989). RNA levels measured in starved fish larvae typically decrease to values around 1.0 but never reach zero, suggesting a conservation of at least some RNA (Ueberschär and Clemmesen, 1992). This conservation is reflected in frequency histograms of RNA/DNA ratios which are typically skewed to the right and truncated at values below 1.0 (Buckley, 1984; Buckley and Lough, 1987; Ferron, 1991). Concurrent measurement of protein is needed with nucleic acids in order to determine RNA/DNA threshold values reflecting negative growth (starvation) and positive growth.

A latency between changes in RNA/DNA ratios and protein growth rate is expected, since RNA synthesis precedes any increase in protein growth rate. Haines (1980) reported a 7 d lag between changes in the RNA/DNA ratio and weight increase of adult black crappies *(Pomoxis nigromaculatus)* collected at weekly intervals in the field. Buckley (1984) and Buckley and Lough (1987) inferred from their studies, that the RNA/DNA ratio reflected growth 2-4 d prior to capture. This statement is misleading since RNA/DNA ratio changes should precede and not follow changes in protein growth. Hovenkamp (1990) observed a relationship between growth rates over the last 5 d (as determined from the width of otolith daily increments) and protein growth rates as predicted from Buckley's model (eq.1) for groups of plaice larvae collected at two different sites in the North Sea.

Pedersen and Hjelmeland (1988) provided the first data on the latency and dynamics of proteolytic enzyme activity following food intake. Their sampling of starved herring larvae within 75 min following food ingestion, revealed significant increases in trypsin levels during that short time interval. Further, the measurement of trypsin activity 3 h after food intake, and after a second meal

was offered, showed no further changes suggesting a persistence of high trypsin levels after a single meal and an extensive re-use of the enzyme to facilitate the digestion of several meals. Larvae starved following the ingestion of a single meal exhibited declines in trypsin activity to pre-ingestion levels after 24-48 h, consistent with a 24-48 h protection against autodigestion of the enzyme in the gut. Following more than 6 d of starvation, the trypsin secretory response to a food pulse decreased substantially in spite of the presence of significant quantities of pancreatic trypsinogen, suggesting a defective release of the enzyme proform and a loss of digestive capabilities under extreme food deprivation. Ueberschär and Clemmesen (1992) detected a 90.2% decrease in trypsin activity following 3 d of starvation in herring larvae, but no further changes for the remaining 10 d when the larvae were deprived of food. Proteolytic enzyme dynamics follow the model proposed in Figure 4, but the initial rate of change following onset of feeding or starvation was much higher than is typical for nucleic acids. Latency was also much shorter, with significant responses being recorded on the order of hours.

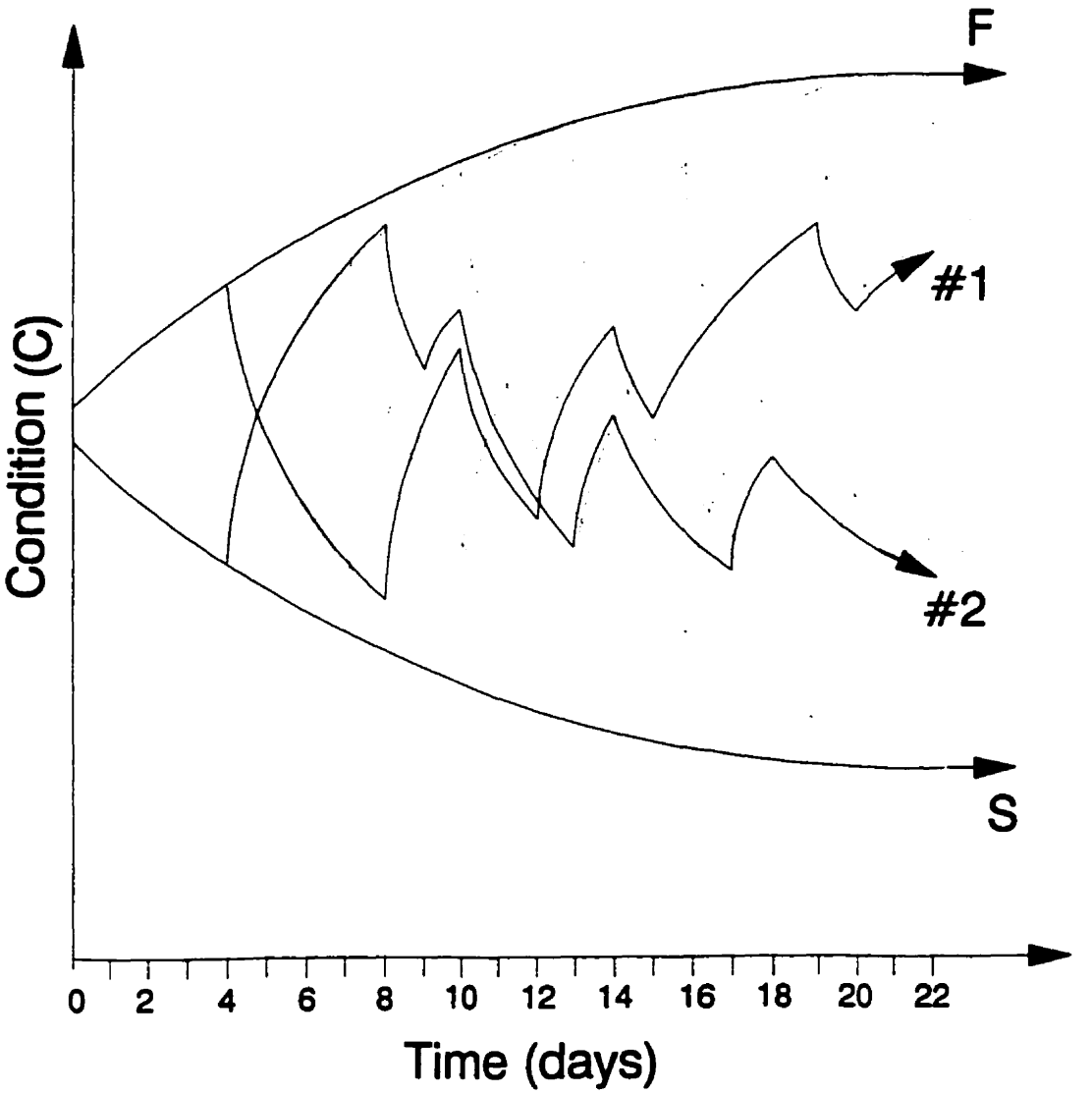
The above review of latency and dynamics of various condition measures, indicates that most indices conform to one of the two models proposed (Figures 3 and 4). Figure 3, characterized by a slow initial response, appears to describe the dynamics of morphometric indices and histological methods which employ qualitative scores. The model illustrated in Figure 4, on the other hand, more appropriately describes the dynamics of histological cell heights, nucleic acids and proteolytic enzymes following intermittent food energy inputs. The indices conforming to the model illustrated in Figure 3 have the disadvantage of responding over long time scales, but have the advantage of accurately assessing long-term effects such as irreversible starvation. Indices which follow the model illustrated in Figure 4 have the advantage of responding over shorter time scales, but their disadvantage lies in their inability to discriminate between individuals situated at the extremes (fed and starved control levels). This dichotomy in the time response of condition indices must be considered when

selecting indices to be used in the field testing of hypotheses. The generalized responses illustrated in Figures. 3 and 4 provide little more than a framework and a first step toward the dynamic modeling of condition and the forecasting of condition trajectories from various feeding and starvation regimes. Figure 5 simulates two condition trajectories, using the framework provided in Figure 4. Similar simulation results were obtained by Sclafani (1992) and Sclafani *et al.* (1993) for individual changes in the vertical distribution of larval cod as a function of their condition. While the models proposed here are heuristically useful, their direct application should be approached with caution, since dynamics and latency are expected to be size and species-dependent. Laboratory calibration is therefore called for before such models are applied to different field situations. We are currently conducting such calibrations.

5. LABORATORY VERSUS FIELD ESTIMATES

There are several examples in which larvae reared in the laboratory were found to vary morphometrically, histologically or biochemically from specimens collected at sea. Since most calibration studies on condition are carried out in laboratory tanks or enclosures, this becomes a major concern when laboratory results are extrapolated to field studies. For morphometric measurements, Hempel and Blaxter (1963) and Blaxter (1971) compared the Fulton-K condition factor (weight/length^3) of herring larvae sampled at sea to those reared in the laboratory. The shrinkage associated with death before fixation was found to be greater in sea-caught larvae (20%) as compared to laboratory-reared larvae (12%). The resulting inflated condition of wild larvae gave the incorrect perception that larvae sampled at sea were in better condition than those maintained in the laboratory. However, at lengths above 12 mm, Blaxter (1971) noted that laboratory-reared herring larvae tended to have a greater body depth for a given length and therefore a higher Fulton-K index than did sea-caught larvae. Many older larvae sampled at sea had a condition factor that was consistently below the experimentally determined starvation level. Three possible

FIGURE 5. Condition trajectories simulated from Figure 4, for two larvae (#1 and #2) exposed for 22 d to different intermittent feeding conditions. The scenarios are for larvae #1: fed 4 d, starved 4 d, fed 2 d, starved 2 d, fed 2 d, starved 1 d, fed 4 d, starved 1 d, and fed 2 d. For larvae #2: starved 4 d, fed 4 d, starved 1 d, fed 1 d, starved 3 d, fed 1 d, starved 3 d, fed 1 d, and starved 4 d. No latency was accounted for between each change in food level.


F
#1
#2
S
Condition (C)
0
2
4
6
8
10
12
14
16
18
20
22
Time (days)

explanations were given by Blaxter (1971) for these differences: 1) different growth characteristics between the two groups, 2) the condition index was sensitive to physical damage suffered by the animal during capture, 3) a selective capture at sea of weak or moribund larvae.

Balbontin *et al.* (1973) reinforced the view that laboratory and field specimens differed morphometrically after finding that laboratory-reared herring larvae ranging in size between 35 and 85 mm, had significantly heavier and deeper-bodies and larger heads than did wild larvae of the same size. This assessment was based on measurements of total, standard, and pre-anal lengths, along with head and sub-orbital head heights. Ehrlich (1975) reported that wild plaice juveniles had a dry weight more than twice that of laboratory animals of the same length Arthur (1976) detected significant differences in relative body depth at the pectorals (body depth/standard length) between ocean-caught and laboratory-reared northern anchovy larvae. He showed a clear divergence between wild and laboratory larvae at lengths over 7.0 mm. While laboratory larvae increased allometrically in relative body depth and weight over their entire larval life, the ocean-caught larvae showed a decrease in the same measure at a mid-larval stage (15-20 mm) followed by an increase at later stages. These differences were attributed to differential food availability in the two environments.

Laurence (1979) suggested that the Fulton-K index, while useful for adult fish, might not be applicable for larval fish because of allometric growth causing the index to become a function of length. He based his assertion on length-weight relationships obtained from seven species of marine fish larvae reared in the laboratory which showed an average length exponent of 4.15 (range 3.76-4.77), far from the 3.0 exponent expected from an isometric index. Recently, Frank and McRuer (1989) obtained a length exponent of 3.03 for field-collected haddock larvae and suggested that the high length exponent (4.48) obtained by Laurence (1979) for the same species could be explained by the fact that these measurements were obtained on laboratory-reared animals which are known to

differ morphologically from field-collected specimens. In support of their findings they noted the exponent values of length obtained by Economou (1987) for five species of field-collected gadoid larvae, which ranged from 2.92 to 3.22. However, Koslow *et al.* (1985) obtained a length exponent of 3.55 for field-collected cod, another gadoid. For herring larvae, Marshall *et al.* (1937) obtained a length exponent of 4.52 and Sameoto (1972) a length exponent of 4.49 for field-collected specimens, which is comparable to the Ehrlich *et al.* (1976) estimate of 4.57 for laboratory-reared animals of the same species. Moreover, Balbontin *et al.* (1973) calculated length-weight relationships for both laboratory-reared and field collected herring larvae, and found that although the intercepts differed significantly, the slopes (3.61 and 3.54) did not. Therefore while isometry could be demonstrated for some field-caught gadoids and not for their laboratory counterparts, herring larvae showed consistent allometry for both wild and laboratory-reared specimens.

The reliability of morphometric indices has often been questioned because of the failure to adequately correct for differential shrinkage occurring in preserved specimens (Theilacker, 1986; Theilacker and Watanabe, 1989). Although shrinkage of laboratory collected larvae is usually constant after preservation, those collected in the field shrink more and show greater variability due to abrasion and damage suffered in the net (Theilacker, 1980a). The average shrinkage measured by Theilacker (1980a) for different body parameters in larval northern anchovy was 3% resulting from net capture alone, and a further 8% resulting from preservation in formalin. This shrinkage has also been measured in terms of length (Hay, 1981, 1982) and dry weight (Hay, 1984) for larval Pacific herring larvae. Theilacker (1980a), Hay (1982) and McGurk (1985a) all found that both the duration of the tow and the time elapsed between capture and preservation were important in determining the shrinkage suffered by collected larvae. Theilacker (1986) found, for instance, that body height could vary by 0 to 23% in jack mackerel larvae after net treatment ranging from 5 to 20 min. Delayed fixation as short as 3 min after capture could lead to a total length

shrinkage of more than 30% in herring larvae (Hay, 1981). Larvae killed prior to fixation also shrank more than larvae killed by fixation (Hay, 1981). Litvak and Leggett (1992) noticed that capelin *(Mallotus villosus)* larvae which were dead prior to preservation curled substantially more than those killed by preservation. They developed a "curl factor" to detect and correct for the presence of animals that were dead at the time of capture. Salinity and the concentration of formalin are also known to have an impact on shrinkage at preservation. In general, high formalin concentration and high salinity tend to maximize length reduction and minimize weight loss, while low formalin concentrations and low salinity tend to minimize length reduction but maximize weight loss (Hay, 1984). Shrinkage due to fixation is an important consideration in the calculation of length-weight relationships. For example, Hay (1984) working with Pacific herring larvae, obtained a length exponent of 2.92 for fixed and 4.12 for unfixed specimens. Caution must also be exercised when using average mean values to correct for shrinkage due to preservation because shrinkage can vary greatly between individuals.

McGurk (1985a), who studied the effect of net collection on differential shrinkage in various morphometric measurements applied on Pacific herring larvae, found that the body dimension most affected by capture was the head, which shrank more in depth and length than other parts. This resulted in an inflation of several morphometric measures and an overestimation of condition. Multivariate analyses neither eliminated the effects of differences in shape between field-collected and laboratory-reared larvae nor corrected for the distortion caused by net capture. The problem of shrinkage due to fixation can be avoided by videotaping live anaesthetized specimens and collecting the required measurements from the digitized images (McClatchie *et al.*, pers. comm.). This technique does not, however, overcome the difficulties associated with damage and distortion of important body parts, or of shrinkage of body dimensions, resulting from mixing and abrasion in the net (McGurk, 1985b).

Turning to histological measurements, O'Connell (1980) observed that some ocean-caught northern anchovy larvae exhibited necrosis of the midgut mucosa, although these characteristics were never observed in laboratory-reared specimens. He explained this difference by the fact that larvae starved in laboratory experiments are never fed and hence do not experience gut function, while wild larvae, in contrast, may experience brief periods of starvation and re-feeding. Theilacker (1986) found that ocean-caught jack mackerel larvae exhibited four tissue conditions not seen in the laboratory. These were related to the presence of lesions in the brain, luminar vacuoles in the midgut, total degeneration of the midgut mucosal cells, and a wavy configuration of the muscle fibers. However, their influence on condition was considered negligible because these characteristics were seen only in a small proportion of the fish examined, and they constituted only a small fraction of the characteristics used to categorize condition. A dark staining of the pancreas, on the other hand, which was very common in wild larvae, was never encountered in laboratory-reared larvae. This prevented the use of that sensitive organ for grading condition in field-collected specimens (Theilacker, 1986). Oozeki *et al.* (1989) noticed that wild-caught stone flounder larvae generally had larger midgut cells than did their fed laboratory counterparts. Liver cells, however, were intermediate in size between fed and starved laboratory specimens.

A further important consideration when applying histological assessments to fish larvae sampled at sea is the autolytic tissue decomposition that can occur within 2 to 3 min after death (Theilacker and Watanabe, 1989; Owen *et al.*, 1989). These changes demand that the time between death and preservation be kept to a minimum if reliable estimates of histological condition are to be obtained. Based on observations of specimens collected at sea, Owen *et al.* (1989) proposed the use of the midgut cell height (Theilacker and Watanabe, 1989) which appears to be unaffected by up to 5-6 min delays between capture and preservation.

A small number of authors have reported differences between the biochemical status of laboratory-reared and field-caught larvae. Balbontin *et al.* (1973) found that the proportions of neutral lipids, polar lipids and protein were significantly lower in field-collected herring larvae than in laboratory-reared larvae of similar size (55-85 mm). This caused the triglyceride/total lipid ratio to be much higher in laboratory animals than in wild specimens. Ehrlich (1975) observed the same phenomenon in wild plaice juveniles which were heavier at a given length, and contained smaller percentages of water, triglycerides and carbon, but higher ash and carbohydrates content than did laboratory-reared animals. Fraser *et al.* (1987) obtained comparable lipid composition and lipid class proportions between herring larvae reared in large enclosures and those collected at sea. However, results from enclosure experiments, when compared with those from experiments conducted in 18 l containers (Tocher *et al.*, 1985a, 1985b), showed that starved larvae from the 18 l tanks had lower triacylglycerol catabolism than did those fed at low levels in enclosures. This suggests that container size can affect larval composition independent of the food provided. Theilacker (1980b) reported similar tank effects on morphological measurements in northern anchovy larvae reared in containers of different size. Håkanson (1989a) found that cholesterol levels were slightly higher, and triglyceride contents much higher, in laboratory-reared northern anchovy larvae as compared with specimens collected at sea. The fact that the larvae were fed only rotifers in the laboratory, which are known to be nutritionally inferior to natural plankton, is one possible reason for these differences.

Wild winter flounder larvae did not appear to differ in their RNA, DNA, and protein content from specimens reared in the laboratory (Buckley, 1980). But when comparing length-chemical constituent relationships, Buckley (1981) found that wild specimens had higher protein, DNA, RNA and total dry weight than did their laboratory counterparts. Sand lance *(Ammodytes* spp.) specimens sampled at-sea also showed higher RNA/DNA ratios than did cultured larvae (Buckley *et al.*, 1984). Buckley (1984) offered three possible explanations: 1) higher food

availability at sea, 2) poor food quality in laboratory rearings, or 3) differential survival favoring faster growing individuals at-sea. Raae *et al.* (1988) observed similar differences in RNA/DNA ratios of cod larvae, with the individuals reared in laboratory tanks having, on average, lower ratios than those reared in outdoor ponds. There is also evidence that the types of food typically used in laboratory rearings may have an influence on larval fish condition. For example, *Artemia* nauplii, which are known to be less nutritional than natural plankton, were suspected of being responsible for the sub-optimal growth conditions in studies conducted by Clemmesen (1987), Richard *et al.* (1991) and Davis and Olla (1992)

Heath (1992) argued that one advantage of biochemical over histological and morphometric condition indices is that these estimates are independent of net damage. However, it has been suggested that rapid enzyme action (lipases, RNases or proteases) shortly after death, can cause deterioration of the sample (Bulow, 1974) and bias its compositional determination. Ferguson and Drahushchak (1989) demonstrated experimentally that this was not the case for nucleic acid content of rainbow trout muscle tissue. Routine methods usually preclude preservation of the specimens in liquid nitrogen immediately after capture and preferably before death. Ferron (1991) found that sorting of live from dead larvae prior to freezing, in order to avoid enzyme action, can lead to an overestimation of the condition of the specimens collected. For these reasons great care must be taken when sorting the samples and interpreting the results from field studies employing biochemical indices.

6. SIZE AND AGE SPECIFICITY

The confounding effect of age and size on the determination of larval fish condition is a major concern for all categories of indices. Hempel and Blaxter (1963), Blaxter (1971) and Vilela and Zijlestra (1971) measured the Fulton-K index (weight/length^3) of herring larvae sampled in different areas of the North

Sea and in different years. The index decreased from hatching to resorption of the yolk, followed by a plateau at lengths between 8 and 15 mm and then a gradual increase which resulted from allometric growth, ossification and deposition of energy reserves in larvae >15 mm. This pattern was consistent for groups of larvae sampled at different sites and in different years, and persistent even when calculated separately for one and two millimeters length intervals. Sameoto (1972) also reported similar changes in the Fulton-K index of field collected herring larvae.

To overcome the allometry problems associated with the use of the Fulton-K index on herring larvae, Chenoweth (1970) computed a relative condition index based on the ratio of the observed weight to the expected weight which was empirically derived from the length-weight relationship of the population from which the samples were collected. Seasonal differences in relative condition could be detected based on this index, but these differences were small and could not be compared with those from other studies for which a different length exponent was demonstrated. Ehrlich *et al.* (1976) provided calculations of both relative and Fulton-K condition indices for herring larvae. However, while the Fulton-K index was found to increase with size, the relative index showed a slow decrease. These authors suggested the use of the Fulton-K index only within very limited size ranges. Body height also increased with size and the Fulton-K index, but changes in body height during starvation could not be used as a starvation index because of the large differences in this measure between individuals of the same size. Von Westernhagen and Rosenthal (1981) demonstrated a decrease in the Fulton-K index of field-collected Pacific herring, from hatching to the complete resorption of the yolk-sac. This decrease was attributed to an increase in total length concurrent with a decrease in yolk-sac volume which lead to a decrease in total weight. The index further increased with size after the onset of first-feeding. This was attributed to allometric growth (von Westernhagen and Rosenthal, 1981). Laurence (1974) reported a similar increasing trend in the Fulton-K index of haddock larvae reared in the laboratory.

In contrast, Neilson *et al.* (1986) reported a decline in this index during the 21 d following initiation of feeding in laboratory-reared larvae of cod. This decline was not correlated with the different prey concentrations offered.

Cone (1989) reviewed the use of three morphological condition indices in adult fish (Fulton-K, relative condition factor, and relative weight). He concluded that these indices all suffered from faulty assumptions that could lead to erroneous conclusions. He recommended, in their place, a contrast between parameters derived from the length-weight regression characterizing the different groups to be compared. This suggestion generated significant controversy over the properties and the wise use of morphological condition indices (see Springer *et al.*, 1990). Cone's reply to these comments (which also appear in Springer *et al.* (1990)) included an important distinction between condition and form, condition being a difference in weight at a given length, and form being the rate of increase in weight as a function of length. Cone also argued that the relative weight should only be used for comparing individuals within the same size-class. Although Cone's comments were applied to adult fishes, his message can be adapted in the context of fish larvae.

Several investigators, who have employed stepwise discriminant analyses of morphological variables (Theilacker, 1978; Powell and Chester, 1985; Martin and Wright, 1987), have suggested that such analysis should be restricted to small size ranges, because most of the ratios used as inputs to the analyses are highly correlated and share size information. Koslow *et al.* (1985), McGurk (1985b) and Powell and Chester (1985) used multivariate approaches to accommodate comparisons of condition between larvae of different sizes. However, these authors, too, cautioned that it was preferable to restrict morphometric analysis to small size ranges in order to avoid allometric problems. It seems that a stepwise discriminant analysis using principal components of shape after extracting principal components of size (McGurk, 1985b) could more effectively overcome problems associated with comparison of animals of different size.

Theilacker (1980a) showed that shrinkage of northern anchovy larvae following net capture is size-dependent. Smaller larvae (4.0 mm) tend to be more sensitive to net damage and, consequently, exhibit greater shrinkage (19%) than larger (18.0 mm) and more robust larvae (8%). Hay (1981, 1984) reported similar length-dependent shrinkage in net-sampled herring larvae.

O'Connell (1976) expressed confidence that the histological grading of larval northern anchovy was independent of size or age effects, the one exception being larvae which retained remnants of yolk or pancreatic zymogen. These specimens were treated as a separate group. O'Connell and Paloma (1981), who compared glycogen levels in the liver with the overall histological grading of small anchovy larvae (<6.4 mm), found high glycogen in robust specimens and low glycogen in emaciated specimens. This relationship did not, however, persist in larger specimens (>6.4 mm). These differences were rationalized by a possible greater tolerance of larger larvae to fluctuations in plankton regimes, as a consequence of the wider range of prey size they can eat. This diversity could make larger larvae less susceptible to a scarcity of some planktonic prey. Both histological and morphometric analyses performed by Theilacker (1986), indicated that starvation was a major source of mortality during the interval between first feeding and 6 d post-hatching in wild jack mackerel larvae, but not for older specimens. Stone flounder larvae, fed *ad libitum* in the laboratory, exhibited a gradual increase in pancreatic, liver, and posterior gut cell height, 30 d after hatching (Oozeki *et al.*, 1989). These changes were explained on the basis that these structures were the sites of active storage following food intake. Midgut cell height, in contrast, followed the same increasing trend with age but only following an abrupt decline 2 d after hatching. A similar decline was noticed in the anterior gut cell height at 20 d after hatching. The midgut cell height, employed by Theilacker and Watanabe (1989) as an index of starvation, could not be used for anchovy larvae >6.0 mm because of the folding of the midgut at that stage. Margulies (1993) used histological grading of the liver and gut to infer

that starvation mortality was very important in field-collected scombridids, but that vulnerability to starvation was highly dependent on development stage. Rice *et al.* (1987) and Sieg *et al.* (1989) both postulated that the ability to detect starvation in field-caught fish larvae depended on the developmental stage of the animal studied. They further suggested that older stages were much less susceptible to starvation than were first-feeding larvae. Recently, Sieg (1992a, 1992b) has suggested that the level of organ development is more important in determining resistance to starvation than is size or age, and that only the condition of specimens belonging to similar developmental stages (or physiological ages) should be compared.

Age and size specificity has been a matter of concern in the application of most biochemical condition indices used to date, (Buckley, 1984; Bulow, 1987; Clemmesen, 1989; Richard *et al.*, 1991; Suthers *et al.*, 1992). Although the order of utilization of major body constituents may remain constant following starvation in individuals of different sizes, the increased stored energy reserves accumulated from the post-yolk sac stage to the juvenile stage inevitably provides a greater "buffer" against changes in macromolecular composition (Love, 1970; Balbontin *et al.*, 1973; Ehrlich, 1974a, 1974b, 1975; Sieg, 1992b). These energy reserves in older fish result from an increase in the surplus energy available for growth as metabolism and activity levels decrease and storage organs become more fully developed with age and size (Blaxter and Ehrlich, 1974; Sieg, 1992a, 1992b). Increased digestive and assimilation efficiencies of the intestine and increased efficiency of energy deposition in the liver are also characteristic of older fish larvae (Sieg, 1992a, 1992b). One consequence of this is the increase in the liver-somatic index (LSI) with size in fish. This LSI becomes a useful indicator of food intake and energy storage in juvenile and adult fish (Heidinger and Crawford, 1977; Bulow *et al.*, 1978; Black and Love, 1986). The concept of physiological age has been recently introduced by Sieg (1992a) to illustrate this development-dependent starvation resistance.

Balbontin *et al.* (1973) demonstrated that neutral fats (triglyceride), carbohydrates, and total lipids did not vary significantly with size in wild herring larvae ranging from 55 to 85 mm in length. Protein and ash content did, however, change with size in laboratory-reared larvae of similar age, and the triglyceride/total lipid ratio was higher in smaller larvae, suggesting that faster growing individuals had used up a greater proportion of their neutral fat stores. This age-dependent starvation was documented by Blaxter and Ehrlich (1974), who reported an increasing time to reach irreversible starvation in plaice and herring larvae as they developed. The sinking rate of herring larvae was also shown to increase as they aged as a consequence of the buildup of muscle tissue, decreases in water content, and ossification of the skeleton. However, when starved, their buoyancy increased regardless of their size.

Gatten *et al.* (1983) reported that Atlantic herring larvae reared in enclosures exhibited, with increasing age, distinct phases in the proportion of triglycerides (TAG) comprising total lipids. A first phase, which occurred between hatching and 20 d, and was characterized by a very low percentage (1-2%) of TAG; a second phase, between 20 and 60 d characterized by TAG comprising about 15-20% of the total lipids, and finally a third phase, which occurred in larvae older than 60 d, and was characterized by the proportion of TAG increasing to more than 60%. During the same developmental period, polar lipids progressively declined as a proportion of total lipids, and decreased from about 30 to 10% between 40 and 90 d post-hatching. In addition, the exponential increase in total lipid levels observed in the following 60 d was mirrored in an exponential increase in growth rate and was mainly attributed to increasing TAG levels. Based on the composition of their fatty acids, Gatten *et al.* (1983) determined that these TAG were primarily obtained from zooplankton rich in wax-esters. Fukuda *et al.* (1986) observed that the high rate of triacylglycerol (TAG) accumulation which occurred throughout Pacific herring larval development, decreased at the onset of metamorphosis. Commenting on these findings, Fraser (1989) attributed the slower rate of TAG accumulation at metamorphosis to the

fact that greater quantities of exogenous lipids were required for the high levels of cell multiplication and differentiation which accompany metamorphosis. This developmental stage was also characterized by lower protein/DNA ratios. The quantities of cholesterol, polar lipids and TAG were reported to increase non-linearly with length in northern anchovy larvae (Håkanson, 1989a, 1989b). Moreover, polar lipids and cholesterol, which are constituents of cell membranes, were found to be linearly related to larval dry weight, confirming their role as useful denominators to account for size differences when using TAG/sterol and TAG/cholesterol ratios.

Although the effects of age and size on the chemical composition of larval fish are typically confounded, the quantity of any major biochemical constituent is commonly more strongly correlated to its size than to its age (Ehrlich, 1974a, 1974b; Buckley, 1981; Clemmesen, 1987; Richard et al., 1991). For nucleic acids and protein, relationships between dry weight (DW) and RNA, DW and DNA, and DW and protein, are often highly significant and are found to be within the same range for different species (Buckley and Lough, 1987; Clemmesen, 1987). However, when the quantity of RNA is divided by the quantity of DNA present in a fish, it is expected that the RNA/DNA ratio resulting will become independent of size, unless one of the two parameters becomes independent of size. Bulow (1987), in his review of RNA/DNA ratios as indicators of growth in fish, recommended that the use of this ratio should be restricted to comparison of discrete size and life history stages. However, Buckley (1980) showed that the RNA/DNA ratio of winter flounder larvae was not significantly affected by size up to the point of metamorphosis, and it is only at that stage that a significant increase in the RNA/DNA ratio was observed. Further, the relationship between protein growth rate, RNA/DNA ratio, and temperature depicted in eq.1, and established by Buckley (1984) using laboratory-reared larvae of eight different species, was not affected by size, except for individuals near or past metamorphosis. Buckley and Lough (1987) showed that in haddock and cod larvae sampled at different sites on Georges Bank, size had no significant effect

on their RNA/DNA ratios. Hovenkamp (1990) found no relationship between RNA/DNA ratios and size or developmental stage in plaice larvae sampled in the North sea.

In contrast, Clemmesen (1992) found that the RNA/DNA ratio of individual Atlantic herring larvae older than 10 d post-hatch increased linearly with size in both starved and fed specimens, and at a rate which depended on the length of the starvation period. She therefore established RNA/DNA ratio-length relationships for different starvation periods (starved 2-3 d, 3-4 d, 4-6 d, 6-9 d), and recommended the use of a starvation range, defined as two standard deviations from each of these regression lines, as a means of determining the extent of starvation for individual larvae sampled at sea. A primary reason for the significant influence of size on RNA/DNA ratios reported by Clemmesen (1992), as contrasted with the absence of such a relationship in earlier studies, was the application of the ratio to individual fish larvae. Chambers (1993), and Pepin and Miller (1993) have recently cautioned against the use of aggregated versus individual based analyses of early life history traits. This caution appears to apply equally to indices of condition.

Richard *et al.* (1991) reported that the RNA/DNA ratio of sole larvae was consistently higher in fed than in starved specimens, but that the differences between the two groups diverged more slowly with increasing age. The rate of decrease (deterioration dynamics) in RNA levels following starvation was also found to be dependent on developmental stage. This caused Richard *et al.* (1991) to suggest the restriction of the RNA/DNA ratio comparisons to narrow size and age classes.

No significant differences were detected in the RNA/DNA ratios of fed and starved yolk-sac larvae analyzed by Buckley (1979, 1980, 1981), Clemmesen (1987), Robinson and Ware (1988) and Richard *et al.* (1991). However, RNA/DNA ratios were observed to decline progressively in both groups as the

larvae aged. Although we do not expect the presence of food to have a significant impact on the growth and rate of yolk utilization of larvae before they feed exogenously, the decreasing RNA/DNA ratio in yolk-sac larvae is contrary to the expectation of positive growth during a stage of intensive tissue build-up. In this connection, McGurk and Kusser (1992) found that RNA/DNA ratios were sensitive to the method of analysis and, in particular, ethidium bromide determinations conducted without appropriate nucleic acid purification yielded lower values than methods using purification steps, especially in yolk-sac larvae. The decreasing RNA/DNA ratio trends observed during yolk-sac resorption could therefore result from procedural artifacts caused by interfering substances present in the yolk. This is supported by studies using purified material. Clemmesen (1989, 1992), who found that starved and fed Atlantic herring larvae had constant RNA/DNA ratios (no trends present) both before yolk exhaustion and up to an age of 9 d post-hatching. The RNA/DNA ratios were also similar between the two groups of fed and starved specimens and could not be used to detect starving animals earlier than 10 d post-hatching. Steinhart and Eckman (1992), too, found no significant differences in RNA/DNA ratios of fed and starved yolk-sac whitefish *(Coregonus* spp.) larvae, even though some of the specimens analyzed showed clear signs of feeding. Mathers *et al.* (1993) found that RNA levels were insensitive to various feeding rations in rainbow trout fry before they reached 30 d post-hatch. The authors associated unchanged RNA levels with the fact that mixed feeding (endogenous and exogenous) supplied the energy during the period preceding total yolk absorption at 40 d post-hatch. The existence of stable RNA/DNA ratios and active protein catabolism (negative growth) during the interval between hatching and yolk absorption is consistent with the absence of cyclic ring deposition on the otolith prior to yolk-sac exhaustion in most marine fish larvae (Campana and Neilson, 1985).

In many marine fish species, the larval mouth opens during or shortly after yolk absorption, and the digestive tract differentiates rapidly to enable the larvae to feed on planktonic prey. At this stage, the larvae possess a functional intestine

with three morphologically distinct parts, the fore-, mid-, and hindgut. However, the larvae typically remain stomachless for some time, often until metamorphosis (Govoni, 1980; Vu, 1983; Lauff and Hofer, 1984; Govoni *et al.*, 1986). For these reasons, the main proteolytic enzymes found in the gut are of the trypsin type, and their activity is concentrated in the mid and posterior part of the intestine (Vu, 1983) where proteins are absorbed via the pinocytotic capacity of the epithelial cells (Govoni *et al.*, 1986; Deplano *et al.*, 1991; Walford and Lam, 1993). The quantity of these proteolytic enzymes increases with age as a consequence of increases in food intake and the size of the secreting organs (pancreas and liver). Pepsin-like enzyme activity only becomes important when the stomach differentiates (Walford and Lam, 1993). In first feeding larvae, exogenous proteolytic enzymes obtained from the diet appear to be essential, since the larvae are unable to produce their own (Lauff and Hofer, 1984). The absence of dietary sources of these enzymes has been identified as one inadequacy of some artificial diets.

Hjelmeland *et al.* (1984) reported a large increase in trypsin activity 4 and 5 d post-hatching, followed by decline shortly after in cod larvae. This peak was independent of feeding status, and appeared to be associated with a period of physiological conditioning prior to first-feeding. This preconditioning precluded the use of tryptic enzyme activity levels as an index of nutritional condition in cod larvae younger than 5 d post-hatch. Pedersen *et al.* (1987) identified three phases of tryptic enzyme activity during the early development of herring larvae. The first yolk-sac phase was characterized by increasing enzyme activity. The second declining phase was observed 7-12 d after hatching. A third phase (13-24 d after hatching) was characterized by increasing trypsin activity and was related to the amount of food offered to the larvae. These results were subsequently confirmed by Pedersen *et al.* (1990). The first and second phases of trypsin activity observed in herring are similar to those described by Hjelmeland *et al.* (1984) for cod larvae. In herring (Pederson *et al.*, 1987, 1990), the largest differences in trypsin activity between fed and starved treatments were observed

during the second phase. Cousin *et al.* (1987) found that proteolytic enzymes activity was equivalent in starved and fed turbot larvae 5 d post-hatching, suggesting that 5 day-old larvae were incapable of controlling the release of these enzymes in quantities proportional to the quantity of food ingested. Clemmesen and Ueberschär (1993) observed a linear increase in tryptic enzymes activity with size in both starved and fed herring larvae. In this case, the enzyme activity-length relationship for starved larvae had a lower slope than for fed larvae, and this slope was related to the length of starvation.

The possibility that the timing of major physiological events may occur at "target sizes" rather than at "target ages" as suggested by Chambers and Leggett (1987) may also mean that major changes in fish body composition are associated with developmental thresholds. This is exemplified in the changes in chemical composition that accompany metamorphosis in bonefish *(Albula* spp.) leptocephalus larvae (Pfeila and Luna, 1984), and in the DNA-dry weight ratio changes seen before and after metamorphosis in sole, (Bergeron *et al.*, 1991). Biochemical changes associated with developmental thresholds are also consistent with the theory of saltatory ontogeny (Balon, 1984), which predicts that development in fishes does not proceed as a continuous series of small histological changes, but rather via short periods of major change in form and function of the organs, separated by longer steady-state intervals. During these intervals, different tissues forming an organ are believed to align their rate of development in order to become simultaneously functional.

7. SPECIES-SPECIFICITY

Biochemical pathways and processes are much more universal than are anatomical characters. As a consequence, a general declining trend in species-specific variability in condition-related responses is seen as one progresses from morphometric, to histological, to biochemical indices. Hence, great care must be exercised in drawing conclusions from between-species comparisons based on

common measures, especially when employing morphometric indices. For exampie, Ehrlich *et al.* (1976) noticed that the top of the head of Atlantic herring larvae changed from convex to concave during starvation. As a consequence, changes in the eye height/head height ratio provided a reliable indication of starvation. However, in plaice no such change occurred. Each species must be considered independently when morphological assessment is made, because shape differences and differential growth of various body parts is characteristic for each species. Laboratory calibration is therefore essential for each species considered.

Histological indices of condition have now been developed for larvae of a considerable number of species: yellowtail flounder *(Seriola quinqueradiata)* (Umeda and Ochiai, 1975), northern anchovy (O'Connell, 1976, 1980; O'Connell and Paloma, 1981; Owen *et al.*, 1989; Theilacker and Watanabe, 1989), jack mackerel (Theilacker, 1978, 1986), stone flounder (Oozeki *et al.*, 1989), (*Vinciguerria* spp.) (Sieg, 1992a, 1992b), black skipjack tuna *(Euthynnus lineatus)*, frigate tuna *(Auxis* spp.), and sierra *(Scomberomorus sierra)* (Margulies, 1993). While some species-specific differences have been noted, the great majority of histological changes in gut epithelium, digestive organs and muscle tissue during starvation were common to all species examined (Kashuba and Matthews, 1984; Theilacker, 1986, Oozeki *et al.*, 1989). It must be emphasized however, that when subjective histological classifications are used, inter-species differences may represent little more than inter-laboratory differences in scoring. Inter-species and inter-laboratory calibrations are, therefore, needed. Quantitative and objective measures such as the midgut cell height (Theilacker and Watanabe, 1989) might provide a solution to the problem of inter-species comparisons, especially if the index is found to show low species-specificity. To date, the number of studies reporting such quantitative measures is too limited to allow a definitive assessment of species-specificity.

The lipid composition of fish eggs exhibits a high degree of species-specificity. Tocher and Sargent (1984) reported that the lipid-rich eggs of capelin and sand lance *(Ammodytes* spp.) had a higher proportion of neutral lipids (mainly triglycerides) relative to total lipids, than did eggs of cod, herring, and saithe, which were characterized by lower overall lipid levels but a higher proportion of polar lipids (mainly phospholipids). The utilization of these lipid classes during the embryonic and larval stages also varies between species. For example, in cod the only lipid class to decline significantly during embryonic development are the phospholipids (Fraser *et al.* 1988). Neutral lipids reserves are utilized only during the larval period. The rate of their utilization increases shortly after the yolk is depleted. Similarly, in striped bass, phospholipids were completely transferred from the yolk to the embryo within 5 d of hatching. Neutral lipids (triglycerides and wax esters), present mainly in the oil globule, were catabolized for up to 20d post-hatching (Eldridge *et al.*, 1982, 1983). In contrast, in species which have relatively large quantities of neutral lipids (in the form of wax esters or triglycerides) in their eggs (red drum, capelin, sand lance or salmonids), these neutral lipid sources are typically catabolized early and throughout their embryonic stage (Vetter *et al.*, 1983; Tocher and Sargent, 1984; Cowey *et al.*, 1985). Vetter *et al.* (1983) calculated, in red drum, that neutral lipids accounted for approximately 98% of the energy requirements of the embryonic stage. If triglycerides are used preferentially for short-term energy needs and polar lipids, which are structural, are not affected by starvation, the species-specific lipid composition of marine fish eggs could seriously bias the reliability of TAG/cholesterol ratios as indicators of condition. Therefore, triglycerides levels in newly-hatch larvae are determined by their levels in the egg content, and will influence the dynamics of their change during starvation and the length of time these changes will be detectable prior to complete exhaustion of the lipid stores.

Because of the central role of nucleic acids in protein synthesis, the RNA-DNA ratio is potentially a reliable index for inter-specific comparisons of growth and condition as evidenced by the wide range of taxa to which it has been

applied (Dagg and Littlepage, 1972; Dortch *et al.*, 1983; Ota and Landry, 1984; Wright and Hetzel, 1985; DeFlaun *et al.*, 1986; Wang and Stickle, 1986; DeBevoise and Taghon, 1988; Clarke *et al.*, 1989; Berdalet and Dortch, 1991; Juinio *et al.*, 1992; see also the review by Frantzis *et al.*, 1993). Clemmesen (1987, 1988) claimed that the rate of decrease (deterioration dynamics) in the RNA-DNA ratio of fish larvae during starvation was species-independent. However, the generality of the relationship between protein growth rate, RNA/DNA ratio and temperature (Buckley, 1984; eq.1) determined for eight marine species was recently questioned by Bergeron and Boulhic ,1994 who found it to be unreliable when applied to laboratory-reared sole larvae. The rate of protein synthesis, as measured by quantifying the amino acid incorporation in the muscle, was also found to differ between and among juveniles of saithe, cod, rainbow trout, and Atlantic herring, and to be positively correlated with their respective growth rate (Roselund *et al.*, 1983).

With respect to proteolytic enzymes, species having a functional stomach at or immediately after hatching (eg. salmonids, Timeyko and Novikov, 1987) routinely produce pepsin-like enzymes, while other species which lack a stomach during all or part of their larval stage produce trypsin-like enzymes (Lauff and Hofer, 1984). The capacity to produce endogenous proteolytic enzymes also seems to differ considerably between species even at comparable stages of digestive tract differentiation (Lauff and Hoser, 1984; Cousin *et al.*, 1987; Munilla-Moran, 1990).

8. PROCESSING TIME, COSTS, AND REQUIREMENTS

The large sample sizes that typically confront biologists who sample larval fish at sea, coupled with the time and cost demands associated with their processing, require that the procedures adopted provide the required data resolution at the minimum cost possible. Setzler-Hamilton *et al.* (1987) reported the time required to process samples in order to obtain indices of condition for larval striped bass

increased as follows: 1) morphometric measurements, 2) RNA/DNA ratio analysis, 3) fatty acid content, and 4) histological scoring. It must be remembered, however, that processing requirements prior to preservation can add considerably to the total cost of developing condition indices. While special care must be taken when preserving samples for morphometric and histological analyses, larvae used for biochemical analyses need only to be sorted from the bulk sample and frozen individually. When pre-preservation costs are included, Setzler-Hamilton *et al.* (1987) determined that the most expensive indices are generally those based on fatty acids determinations, followed by indices based on nucleic acids. Indices based on histological and morphometric measures are generally the least expensive to develop. They therefore recommended the use of morphological and histological indices on the basis of their good correlation with condition, and their low average costs.

Many researchers who have employed morphological indices of condition admit to the potential of biochemical and histological assessments of condition, but note that these methods are too time-consuming and costly to be used routinely in ichthyoplankton surveys (Ehrlich *et al.*, 1976; Theilacker, 1978, 1986; Koslow *et al.*, 1985; Powell and Chester, 1985). One solution is to use less sensitive but less costly indices on all samples. A second is to use the less expensive morphological indices on most samples and to verify the results obtained from these results via histological or biochemical assessments based on a sub-sample (Theilacker, 1986). For the future, given the real limits on improvements in the precision of morphological indices, the most promising developments in this area are likely to be related to methods for reducing the processing time now associated with histological and biochemical indices. For example, the midgut cell height index developed by Theilacker and Watanabe (1989) and similar quantitative histological assessments such as those proposed by Oozeki *et al.* (1989) have proved to be equally sensitive, but less costly, than more traditional histological scores. However, given the rapid development of new molecular approaches, it is likely that the greatest gains in cost reduction will

occur in the analysis of biochemically based indices of condition and growth. Automated continuous-flow systems (Caldarone and Buckley, 1991) have already substantially reduce the time required to process samples, such that a 100 sample carousel can be processed overnight (Sterzel *et al.*, 1985). This is, however, not achieved without concurrent increase in unit costs. The potential for development of fluorescent probes specific to all major biomolecules (Haugland, 1992) could dramatically reduce the costs associated with the quantification of lipids, proteins, carbohydrates, enzymes and nucleic acids, and make possible the development of related indices from very small amounts of tissue and the use of relatively inexpensive equipment. Fluorescence techniques, in addition of being sensitive and specific, can also be automated and may eventually be performed at sea.

Given the recent emphasis on the individual as the unit of study in fish early life history and recruitment studies (Chambers and Leggett, 1987; Chambers *et al.*, 1989; Chambers, 1993; VanWinkle *et al.*, 1993) and the high level of between-individual variation inherent in measures of condition, it is important that new methods be applicable to individual larvae. This has not been a problem with morphological and histological methods. However, the application of new biochemical techniques to individual fish larvae has been hampered by the fact that the quantity of material required for the analyses frequently required the pooling of samples (Buckley, 1984; Hjelmeland *et al.*, 1984; Fraser *et al.*, 1987). Recent advances in methodology have now made it possible to develop indices based on lipids (Håkanson, 1989a), nucleic acids (Clemmesen, 1988, 1989, 1993), and proteolytic enzymes (Ueberschär, 1988; Ueberschär and Clemmesen, 1990; Ueberschär *et al.*, 1992) for individual fish larvae. Several of these measurements can be obtained concurrently on the same specimens, and multivariate biochemical condition indices generated. These individual-based measurements have also highlighted the erroneous conclusions which resulted from the earlier pooling of samples (Clemmesen, 1988; Richard *et al.*, 1991; Ueberschär *et al.*, 1992; Bergeron and Bouhlic, 1994).

9. SUMMARY AND RECOMMENDATIONS

Table 2 provides a summary of key attributes of each category of condition index reviewed. From the table and the discussion above, it is clear that the sources of variability that influence both the nature and the interpretation of index values, remain poorly known for all categories. The sensitivity, the latency and the dynamics of condition have also been very poorly described and the majority of the attributes given in Table 2 are still not well defined. This gap in knowledge is a major limitation to the application of these methods to studies of larval survival and recruitment. All indices exhibited some effect of size, and species-specificity, that must be considered when measuring condition over a range of larval sizes and across species. Processing time, costs and requirements are important considerations in improvement of current condition indices and for the development of new techniques.

Measures of condition integrate feeding success over time, and give an indication of the probability of starvation and survival. During the last two decades, the emphasis of research into the condition of larval fish has changed from a search for correlations with year-class strength (Chenoweth, 1970; Blaxter, 1971; Vilela and Zijlstra, 1971) to much more focused analyses of the impact of food quantity and quality on condition. However, as Neilson *et al.* (1986) have observed, while the number of studies in which one or more measures of condition have been employed continues to grow rapidly, very few have attempted, and even fewer have succeeded, to link directly measures of condition to probability of survival, which is often the stated goal in such studies. Rather the thrust of much of the work recently published have been the development of new and more accurate measures of condition. In such a context, it is perhaps important to restate the reality that the initial, and still center purpose of measuring condition is to predict reliably survival probabilities under

TABLE 2. Summary of key attributes for the seven categories of condition indices reviewed

	morphological indices	*histological (scores)*	*histological (cell heights)*	*nucleic acid and protein*	*lipids*	*digestive enzymes*	*metabolic enzymes*
1. Reliability to detect condition changes	YES, through multivariate analysis	YES, through multivariate analysis	YES	YES	NO, because are likely to be influenced by gut content	NO, because are likely to be influenced by gut content	YES, but with limited data
2. Sources of variability other than nutrition	some identified none studied	some identified none studied	some identified none studied	several identified some studied	some identified few studied	some identified few studied	some identified none studied
3. Sensitivity	believed to be LOW	believed to be LOW	believed to be MODERATE	believed to be MODERATE	believed to be MODERATE	believed to be HIGH	unknown
4. Latency	days	days	hours-day	hours-day	hours-day	hours	unknown
5. Dynamics	SLOW initial changes	SLOW initial changes	MODERATE initial changes	MODERATE initial changes	MODERATE initial changes	RAPID initial changes	unknown
6. Laboratory versus field differences	large	moderate	small	small	large	unknown	unknown
7. Size and age-specificity	large	large	moderate	low	large	large	unknown
8. Species-specificity	large	large	small	small	moderate	large	unknown
9. Relative processing time	short	long	long	long	long	long	long
10. Relative processing costs	low	moderate	moderate	high	high	high	high
11. Processing requirements prior to analysis	high due to differential shrinkage	high due to autolysis	high due to autolysis	low	low	low	low

given food regimes in order that observed larval abundances can be used as early predictors of recruitment (Frank and McRuer, 1989).

The ideal condition index should be capable of providing a direct estimate of starvation mortality, for larvae that have passed the point-of-no-return, and a reliable estimate of susceptibility to starvation or starvation-induced predation for the specimens determined to be at intermediate or high levels of condition. Most of the condition indices reviewed here exhibit a decline shortly after onset of starvation. However, only histological scores and protein measurements continued to change beyond the point of irreversible starvation, a requirement for quantification of the proportion of larvae in a sample clearly destined to die regardless of future feeding conditions. The few estimates of the proportion of starving larvae in sampled populations which do exist suggest that instantaneous starvation mortality may be relatively low (5-35%, O'Connell, 1980; Theilacker, 1986) although it may occasionally account for a high proportion of the mortality experienced by larvae (Theilacker, 1986). It is reasonable to assume, however, from the data available, that most larvae will have been exposed to food-limited growth conditions, and will therefore be characterized as being in moderate or good condition. If this proves true, morphological indices are likely to be only marginally useful when applied to field studies, because they do not differentiate between animals that differ slightly in this degree of starvation. Methods based on analyses of triglycerides and proteolytic enzymes, in contrast, respond quickly to changes in feeding, and their short response time reduces to a very narrow window in time, the period during which moderately-starved animals can be identified. There effective use therefore requires a high resolution sampling which is frequently difficult to achieve at sea. Indices based on histological cell heights and nucleic acid and protein measurements exhibited more gradual changes to both starvation and recovery, therefore providing a longer window of opportunity for detecting moderately-starved animals. Selecting the "ideal index" thus becomes a question of matching the sensitivity, the latency, and dynamics of the index to the needs of the hypothesis to be tested, and to the *a priori* knowledge

of the likely magnitude and duration of food deprivation. In the absence of such insight, indices based on histological, nucleic acid and protein assessments would appear to offer the greatest overall return when applied to larvae that are likely to be in intermediate condition.

Providing that a sufficiently fine-scale sampling of the food environment is achieved, and the advection of the water masses in which larvae occur is well described, the past, present, and future prey field of larval fish can be quantified. Further, through selection of the appropriate index, it should be possible to quantify the proportion of larvae in a given water mass that have died during a defined interval in time, provided the sensitivity and the dynamics of the index are known. These estimates of larval mortality can be validated by monitoring, coincidentally, larval abundances corrected for dispersal losses, which can best be obtained from Lagrangian sampling (Heath, 1992). When dealing with larvae in intermediate condition, relating present condition to previous prey fields or future condition to present prey fields will also require a knowledge of the latency, the dynamics, and the sensitivity of the index. Factors other than food concentration known to influence feeding success (eg. turbulence, larval fish behavior) should also be evaluated in order to improve the predictions Finally it is worth noting that the time and space scales of larval sampling programs must be developed with reference to the characteristics of the condition index to be employed. This has rarely been done, and the result is likely to be a serious bias of the data (Frank and Taggart, 1990).

It will be almost impossible to investigate systematically the relationship between condition and survival in larval fish, unless and until the determination of condition becomes a routine component of larval surveys. Moreover, future research on condition indices is likely to be more productive if it is devoted to the refinement of existing methods, rather than to the search for the ideal condition index. From the review presented here, its should be obvious that there is no "best index" for all circumstances. Rather, a suite of indices chosen with

reference to their particular characteristics, and incorporated into a properly scales sampling program is more likely to yield the answers that are sought.

CHAPTER 2

FEMALE CONTRIBUTIONS TO EGG AND HATCHLING SIZE AND TO THE BIOCHEMICAL CHANGES ASSOCIATED WITH STARVATION IN CAPELIN *(Mallotus villosus)* LARVAE

1.INTRODUCTION

It is widely believed that for many marine fish populations successful recruitment depends on the production of a large quantity of high quality eggs (Kjørsvik *and al.*, 1990). However, while annual egg production is typically correlated with spawning stock biomass, it is weakly, if at all, correlated with the number of young fish surviving their first year (Sissenwine, 1984). For this reason, variable rates of offspring mortality during the first year have repeatedly been hypothesized to be the most important factor controlling population size (Rothschild, 1986; Rice *et al.*, 1987; Sinclair, 1988; Fogarty *et al.*, 1991). At the inter-specific level, marine fish larvae that hatch from larger eggs are believed to have a survival advantage over smaller larvae since they tend to be larger at hatch, can survive longer on their yolk reserves, can search a greater volume of water at first-feeding, can feed on a wider size range of prey at first-feeding, and are better able to escape predators (Hunter, 1981; Blaxter, 1988; Miller *et al.*, 1988). These patterns may also hold intraspecifically, however, they have not received the same attention (Blaxter and Hempel, 1963; Bagenal, 1969; Fowler, 1972; Hulata *et al.*, 1974; Marsh, 1986; Weeks, 1993). In an effort to address these questions, a series of biochemical measures have been developed with which to measure the physiological condition of early larval stages and to study the relationship between these measures and the susceptibility of larvae to starvation and predation mortality (see the review by Ferron and Leggett, 1994). Despite an extensive body of research, it is not yet clear which of these measures are most sensitive to starvation, nor is it known the extent to which they are influenced by the egg provisioning and the nutritional status of the mother.

In this study we sought to a) assess the character and magnitude of associations between egg dry weight and protein content and the quality of larvae they produced, b) determine which of a series of currently used biochemical measures were most responsive to the effects of starvation

subsequent to hatching, and c) determine whether the rate of change (dynamics) of these measures during starvation is influenced by female source.

We focused our research on capelin *(Mallotus villosus:* Osmeridae), a small (<20 cm total length) short-lived (<5 yrs) iteroparous northern circumpolar species, which in Newfoundland, migrate shoreward in spring and summer to spawn in the intertidal and sub-tidal zones of exposed gravel beaches (Leggett and Frank, 1990).

2. MATERIAL AND METHODS

2.1 Experimental design

Pre-spawned adult capelin *(Mallotus villosus)* were collected from fishermen's traps at Bryant's Cove, Newfoundland, on June 29 1989. Males and females were held in separate seawater containers, and were subsequently transferred to separate 300 l tanks provided with ambient running seawater. Sixty females were randomly chosen from the sample on June 30 and videotaped to obtain morphometric measurements. Their total wet weight was recorded to the nearest milligram on a "Mettler P1210N" balance, and a small number of eggs (approximately 100) were stripped from each female to determine their average egg diameter. The fish were then returned to the tanks where each female was isolated in a small (28 x 24 x 15 cm) submerged plastic container to permit their identification in the crosses that were conducted during the following days.

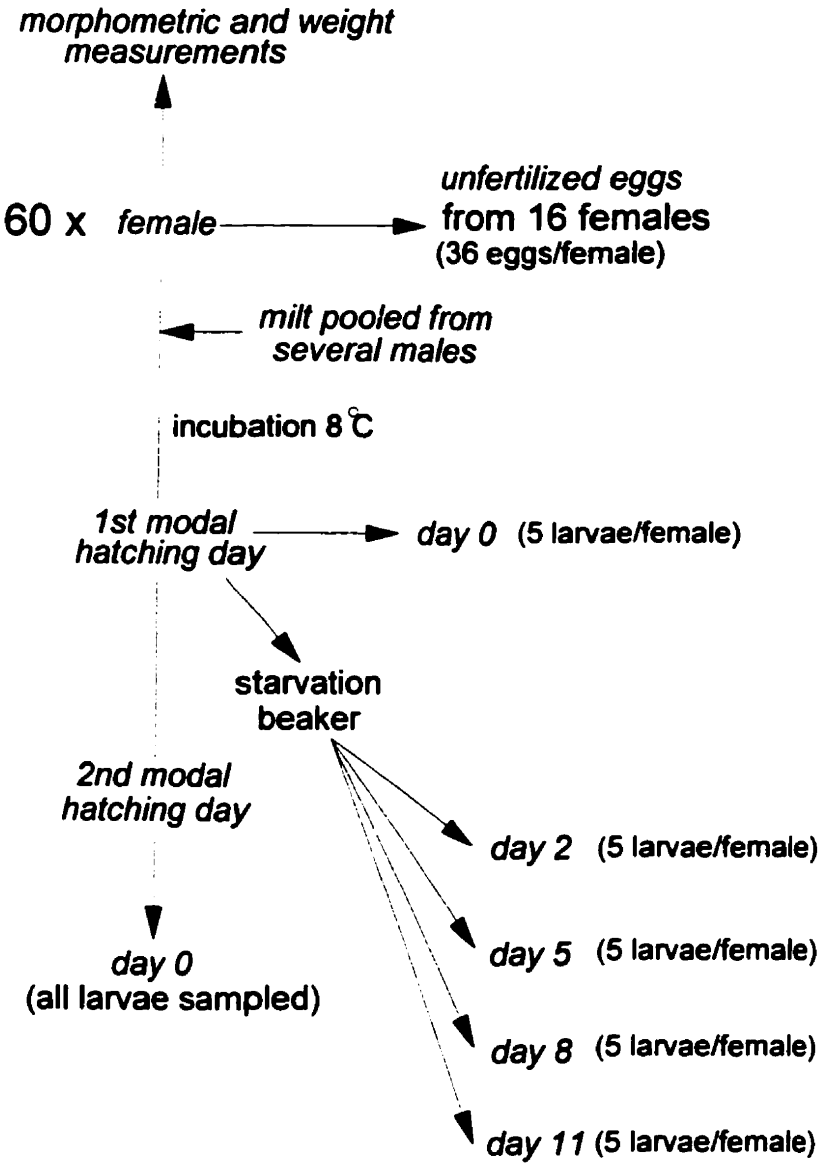
The experimental design and the sampling scheme used in this experiment are summarized in Figure 1. All crosses were performed on July 1 and 2, 1989. Males were stripped to obtain their milt by exerting a gentle pressure along their side from the anterior region of the gonad to the gonopore area. Only fish which yielded a good quantity of opaque white sperm were used as sires. The milt of several males was pooled in plastic containers maintained on ice, and visible

impurities (faeces) were removed with a glass bore pipette. The milt containers were subsequently maintained at 4° C until use.

Females were stripped one by one. Two small batches of eggs (200-400 eggs each) were extruded into separate petri dish maintained on ice from each of the 60 females. Three to four drops of the sperm pool were added to each egg batch and the eggs/sperm were slowly agitated for one minute to ensure thorough mixing of sperm and eggs. A squirt bottle filled with chilled (4°C) 0.45 µm filtered, UV sterilized seawater was then used to spread the eggs over the surface of the dish. The petri dishes were again agitated for approximately five minutes, after which the water was decanted and fresh, chilled (4°C), filtered, sterilized seawater was added to cover the eggs to a depth of approximately 1.0 cm. All fertilized eggs produced by these half-sib crosses were initially incubated in petri dishes at 8°C. After two days of incubation, eggs showing signs of normal embryonic development (transparent with symmetrical blastomeres) were transferred into 100 ml beakers. One single beaker containing between 30 to 300 eggs per family was followed for the experiments described here.

All beakers containing fertilized eggs were maintained on a 12-hr photoperiod at a constant temperature (8° C) in a circulating rectangular water bath. The beakers were assigned a random location to account for possible temperature gradients in the bath. The water in the beakers was changed daily with fresh UV sterilized, filtered seawater. All dead eggs were removed daily with a pipette. The beakers were monitored daily at 8h00-10h00 and hatched larvae were removed. On the first day that more than 30 to 40% of the larvae had hatched in any beaker (identified as the first modal hatching day), five larvae were frozen and the remainder were transferred to a separate "starvation" beaker which was monitored and sampled until all larvae in the beaker had died. Five larvae were sampled from that "starvation beaker" on day 2, 5, 8 and 11 after transfer

FIGURE 1. Layout of the experimental design used for the experiments. The number of eggs or larvae sampled are indicated in brackets.



and were frozen in liquid nitrogen for later analyses (Figure 1). No larvae survived starvation longer than 12 d post-hatch. All larvae hatching on the subsequent day on which more than 30 to 40% of the larvae remaining in the beaker hatched (identified as the second modal hatching day) were collected and frozen in liquid nitrogen (Figure 1). Hatching frequency distributions were uni-modal in most cases and, as a result, the first and second modal hatching days were typically consecutive. However, sampling was separated by more than one day in some instances, either because of bi-modality in hatchling numbers, or because a wide hatching date frequency distribution occurred. All sampled eggs and larvae were maintained in liquid nitrogen. For shipment to our Montreal laboratory, they were transferred on dry ice, and were later held at -80° C at McGill. During the month following transfer to McGill, they were vacuum freeze dried for 24 hours and were then maintained at -20° C on anhydrous calcium sulfate (drierite) in dessicators until biochemical assays were performed.

A small quantity of eggs (approximately 200) was obtained from 16 of the 60 females on the day following the crosses. These unfertilized eggs were placed in 1.5-ml polypropylene microtubes, and were immediately immersed in liquid nitrogen for later biochemical analysis. High mortality was experienced by the spawned fish shortly after the crosses were performed. We sampled unfertilized eggs only from the 16 females that survived because we were concerned about possible *post-mortem* compositional changes in the eggs.

The ovaries of each female used in the experiment were removed after the crosses were completed and the female's somatic and gonad wet weights determined. The carcasses were then frozen at -20° C. Somatic dry mass was obtained by drying the carcasses for 48 hrs at 60° C in a drying oven. The dry carcasses were ground, flooded in diethyl ether and decanted to facilitate lipid extraction. The total lean dry mass was determined after three cycles of lipid extraction (Chambers *et al.* 1989). The lipid content of each female was estimated by subtraction of the lean dry mass from the total dry mass. Total

fecundity estimates were obtained by dividing the ovary dry weight by the mean egg dry weight determined from a sample of 36 unfertilized eggs per female, which were subsequently used for biochemical determination (Figure 1). The unfertilized eggs sampled for biochemical assays and the eggs used for the crosses were included in the total fecundity estimates.

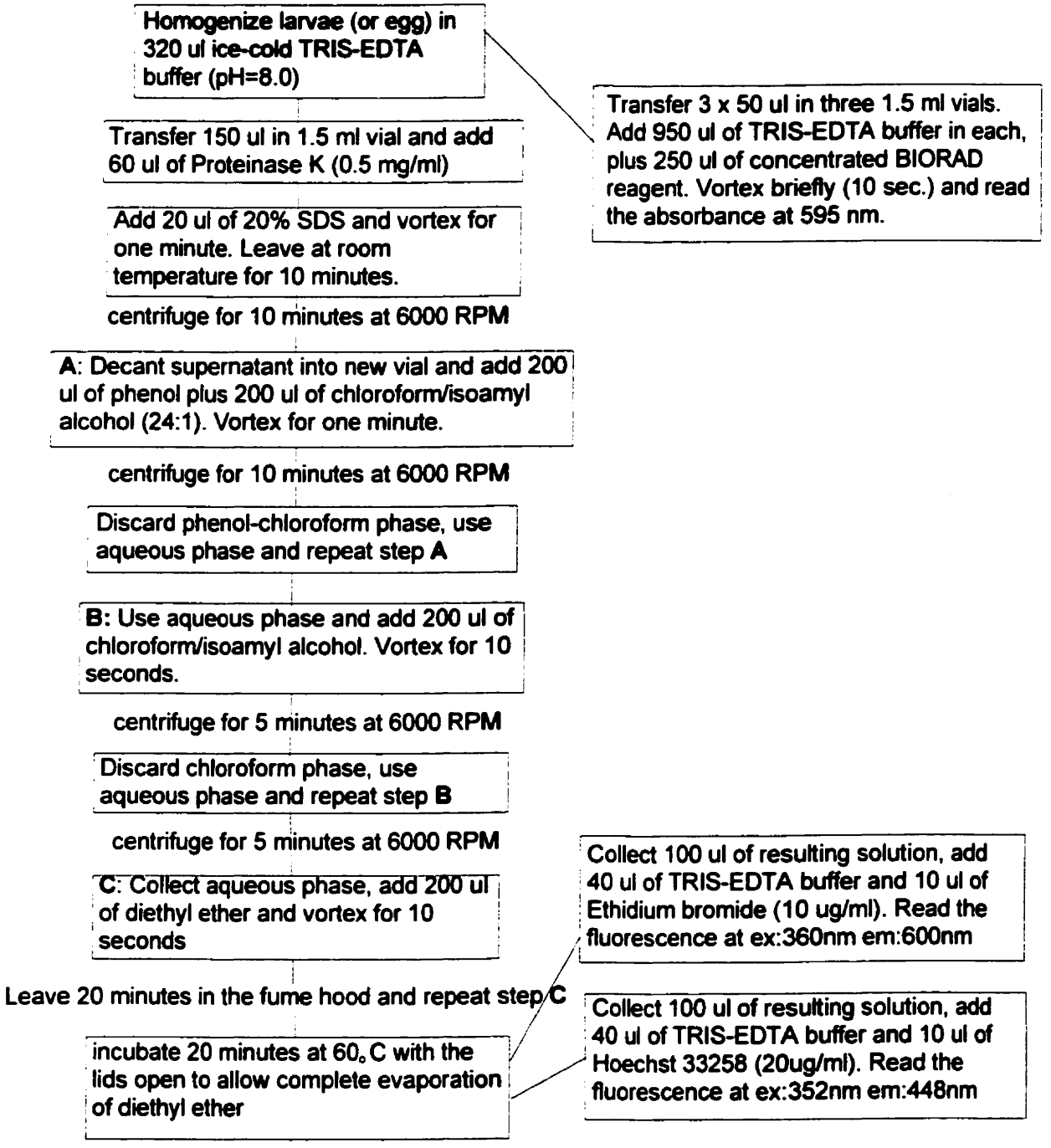
2.2 Biochemical assays

Although fish egg size is most frequently reported as diameter or volume (Elgar, 1990 and Pepin and Myers, 1991), we used dry weight as our measure of egg and larval size. This was done because the quantity of egg nutrients provided by the mother and expressed in dry weight is likely to be a more reliable and direct measure of parental investment (Hutchings, 1991), as it reflects the quantity of energy substrate available for embryonic growth and development. This is important since the assumption that egg diameter or volume is a good predictor of organic and energetic content has recently been questioned and proven to be false for some invertebrates (McEdward and Carson, 1987; McEdward and Coulter, 1987). Kjesbu et al. (1996) found variable water content in cod eggs and used egg dry weight instead of egg diameter or volume as a more reliable measure of egg size. Knutsen and Tilseth (1985) found a significant ($P<0.05$) positive correlation between egg diameter and egg dry weight, and Quattro and Weeks (1991) found a significant positive relationship between egg size and energetic content. However, these relationships have not been tested intra-specifically.

All dried eggs and larvae collected were inspected individually prior to weighing to assure that no parts were missing and to detect the presence of salt crystals which could bias dry weight measurements. Eggs or larvae having parts missing or those covered with unremovable salt deposits were discarded. Intact eggs and larvae were weighed to the nearest 0.1 µg on a Cahn C-31 microbalance.

Larvae sampled from the "starvation beakers" were analyzed individually for their protein and nucleic acid content following the procedures outlined in Figure 2. Unfertilized eggs and the larvae that were sampled on the second modal hatching day were weighed and analyzed for their protein content only. Eggs and larvae were homogenized on ice in 1.5 ml microtubes using disposable polypropylene pellet pestles (Mandel scientific company, Guelph, Ont. Canada). After the sample was homogenized, one aliquot (further subdivided into triplicates) was used for protein content determination using the "Biorad" protein assay following Bradford (1976). The second aliquot was utilized for the extraction and purification of nucleic acids following the methods outlined in Clemmesen (1988). Several modifications to this extraction procedure were required, notably in the volumes used, to allow the quantitation of both protein and nucleic acids on the small aliquots obtained from individual eggs and larvae (Figure 2). Other modifications included the pH of the This-EDTA buffer which was lowered to 8.0 instead of 9.0 in order to yield a higher bound to unbound Hoescht 33258 dye fluorescence ratio (Labarca and Paigen, 1980). This was also employed to prevent instability in the binding of Hoechst 33258, which was observed at higher pH (Cesarone *et al.*, 1979). The gentle mixing between each of the four centrifugations and included in the methods documented (Clemmesen, 1988) was reduced from one and 10 minutes to 10 and 60 seconds mixing on a slow speed vortex. This was done to reduce the total amount of time required to perform the assay. The centrifugations were carried with a Heraeus biofuge 13 microcentrifuge fitted with a HFA 17.1 (7.9 cm radius) polypropylene rotor. In addition to the above modifications to the Clemmesen (1988) protocol, two purification steps using diethyl ether (Maniatis *et al.*, 1992) were included to remove residual traces of phenol or chloroform causing interference with the Hoechst 33258 fluorescence.

FIGURE 2. Flowchart of the detailed procedures used to determine protein and nucleic acid content in the samples.



All chemicals used for the procedures were analytical grade and were obtained from the following sources: Free base 8-hydroxyquinoline (8-hydoxy-1-azanaphtalene), SDS (sodium dodecyl sulfate, sodium salts, 95%), EDTA (ethylenediaminetetraacetic acid disodium dihydrate, disodium salt, 99%), isoamyl alcohol (99%), 2-mercaptoethanol (2-hydroxyethylmercaptan, β-mercaptoethanol), DNA (deoxyribonucleic acid type I, highly polymerized, from calf thymus), and RNA (ribonucleic acid type III from bakers yeast) were all obtained from SIGMA chemical company (St.Louis, MO, USA). Hoechst 332582 - [2 - (4 – hydoxyphenyl) – 6 – benzimidazol] - 6 - (1 – methyl – 4 -piperazyl) benzimidazole, trihydrochloride), ethidiumbromide (2,7 – diamino –10 – ethyl – 9 - phenylphenantridium bromide), and proteinase K (from *tritirachium album*) were obtained from Boehringer Mannheim (Laval, Quebec, Canada). Accugen phenol was bought from Anachemia Science (Lachine, Quebec, Canada) and chloroform, sodium chloride and diethyl ether from BDH chemicals (Toronto, Ontario, Canada). Ultra pure TRIS [TRIS- (hydoxymethyl)-aminomethane] was purchased from Schwarz/Mann Biotech (Cleveland OH, USA). For the protein determination, the "Biorad" protein assay kit was used (BIO-RAD laboratories, Mississauga, Ontario, Canada). The kit includes the dye reagent concentrate (containing Coomassie blue dye, phosphoric acid and methanol) and bovine serum albumin standards.

The Tris-EDTA buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA) was prepared with doubled distilled water, the pH was adjusted to 8.0, and it was maintained at 4° C until use. The ethidium bromide (10µg/ml) and Hoechst (20µg/ml) dyes were diluted in distilled water and kept at 4° C for up to two years in closed flasks wrapped in aluminum foil. SDS was diluted to 20% in distilled water and kept in a closed flask at room temperature for two years. The crystallized phenol was melted on a hot plate and hydroxyquinoline was added to a final concentration of 0.1%. It was then washed 2-3 times with an equivalent volume of 0.5 M Tris buffer (pH = 9.0) until a pH >7.8 was attained (Maniatis et *al.*, 1992). As a final step, 100 ml of 0.1 M Tris was added to the phenol to form a thin layer surface

preventing oxidation. Phenol was stored in a dark glass bottle at 4° C for up to 3-5 weeks, and was discarded if a noticeable color change from yellow to orange (indicative of oxidation) appeared. The chloroform and isoamyl alcohol were mixed in proportions of 24:1 (V/V) and maintained in closed dark bottles at room temperature. Stock solutions of DNA (500 µg/ml), yeast RNA (100 µg/ml), and bovine serum albumine (1 mg/ ml) were made by dilution in distilled water. Proteinase K (500 µg/ml) was diluted in Tris-EDTA buffer. These stock solutions were frozen at -20° C until use. The concentrations of DNA and RNA solutions were determined spectophotometrically using an expected OD_{260} (optical density) of 1.000 for solutions containing 50 µg/ml of double-stranded DNA or 40 µg/ml of single-stranded RNA (Maniatis *et al.*, 1992). The concentrations of bovine serum albumine protein standards were calculated using the following equation from Bashford and Harris (1987):

$$\text{protein (mg/ml)} = 1.55\ OD_{280} - 0.76\ OD_{260}$$

Working standards (100 µg/ml) of RNA, DNA and protein were prepared weekly from frozen stock solutions. These were used for calibrations and internal standards.

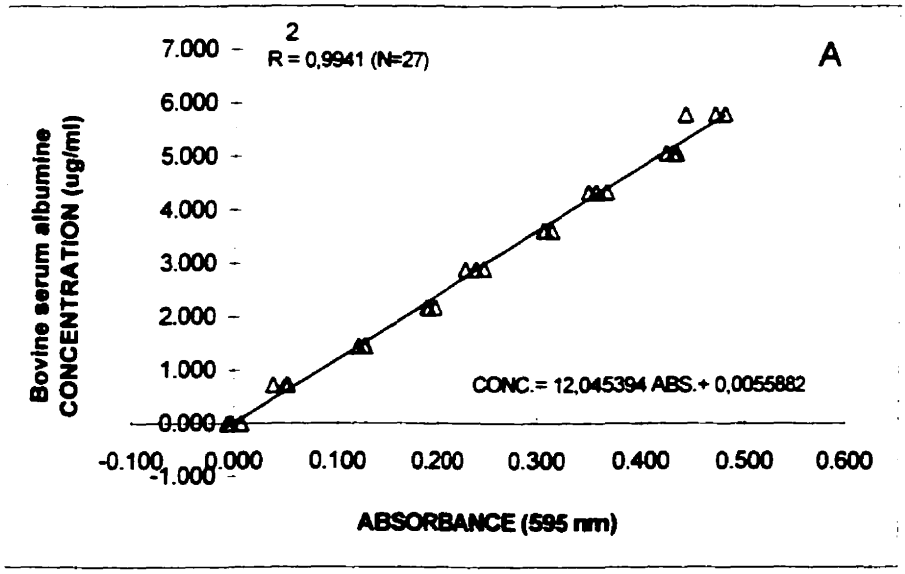
The protein content of the samples was determined by reading the absorbance at 595 nm on a "Pye Unicam PU-8088" spectrophotometer after addition of the Biorad concentrated reagent (microassay procedure) to the sample. The spectrophotometer was set for a bandwidth of 2.0 nm, and an integration and response time of 2.0 sec. Calibrations using bovine serum albumine were conducted weekly and consisted of triplicates of at least 8 different concentrations encompassing the range of all sample values. The calibration was repeated whenever a $R^2<0.99$ was obtained. Triplicates of blanks (Tris-EDTA buffer+dye) and standards were measured for each group of 24 samples processed, and for two concentrations situated within the range of the samples values, to detect and correct for any deviations from calibration values.

The calibration line illustrated in Figure 3a was used for the determination of the protein content of the samples.

The nucleic acid content of the samples was assessed by the fluorescence increase, measured on a "Perkin-Elmer LS-3B" spectrofluorometer, resulting from the binding of Hoechst 33258 (Biz) to DNA and ethidium bromide (EBr) to RNA and DNA. The spectrofluorometer was set to operate on the autorange ordinate scaling (decimal point automatically set in relation to signal intensity), at a slit width of 10 nm, and a scan speed of 60 nm min^{-1}. A 100 µl quartz micro-cuvette (2 x 10 mm light path) was used for these measurements. Wavelength scans showed that the maximum excitation and emission fluorescence for DNA+Biz was obtained at the wavelengths (ex: 352 nm, em: 448 nm) recommended by Clemmesen (1988), but differed slightly in the case of the EBr bounded to DNA and RNA (ex: 360 nm, em: 600 nm as compared with ex: 365 nm, em: 590 nm). The 360 and 600 nm wavelengths were used for all measurements involving EBr. The fluorometer readings for standards and samples in the presence of Biz were unstable. This problem was resolved by maintaining the samples at cold temperature (1° C), and by exposing them to the UV beam for short consecutive exposure times (10-20 seconds), until a stable reading was obtained. We believe that the SDS present in the purified homogenates caused this interference, since washing of the homogenates with diethyl ether (which should have removed any traces of phenol or chloroform) did not alleviate the problem.

Triplicates of blanks (Tris-EDTA buffer+dye) and standards (for two concentrations situated within the range of sample values) were measured for each set of 24 samples processed. The endogenous fluorescence of purified nucleic acid extracts before addition of dyes was negligible (3-6% of the Tris-EDTA+dye values). However, the background (residual) fluorescence increased significantly (+302.5% and +12.5% of Tris-EDTA+dye values for Biz and EBr

FIGURE 3. Calibration lines used to calculate: (A) the protein content of samples from the absorbance measured at 595 nm, and (B) the DNA concentration of the samples from the fluorescence measured at 448 nm



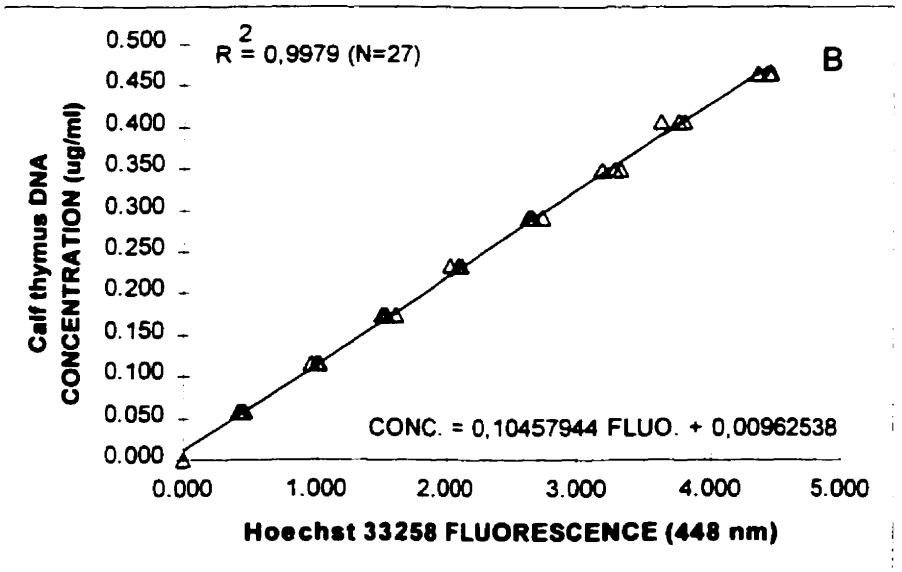
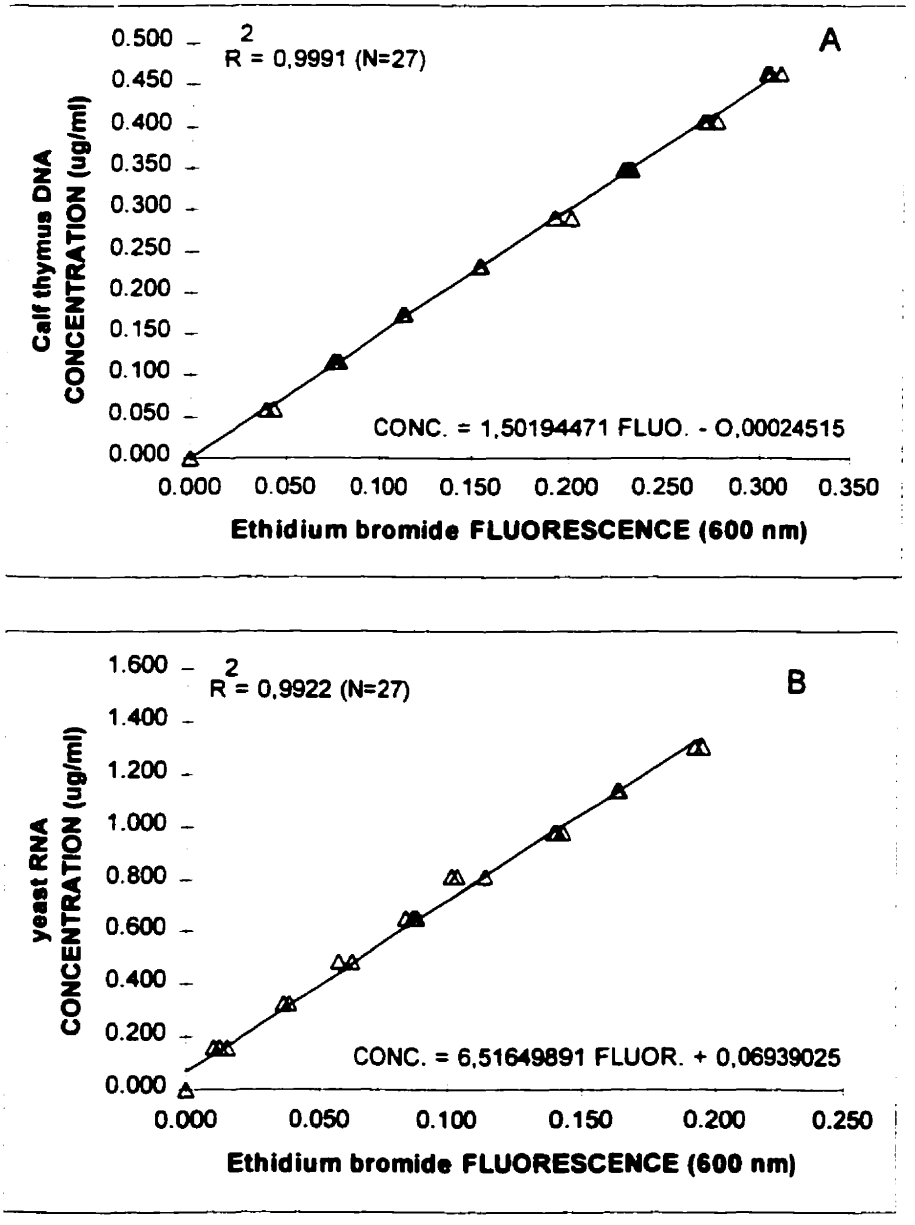


FIGURE 4. Calibration lines used to calculate: (A) the ethidium bromide fluorescence due to DNA from the concentration calculated from the Hoechst fluorescence and (B) the RNA concentration of the samples from the ethidium bromide fluorescence due to RNA.



respectively) upon addition of the dyes to the Tris-EDTA blanks extracted with methods identical to those applied to the samples (Figure 2).This residual fluorescence was obviously created by interference of the fluorochromes (especially Hoechst 33258) with extracting agents (proteinase K, SDS, phenol, chloroform or diethyl ether). Lipman (1989) reported that concentrations of 0.1-0.2% SDS lead to increasing and more variable EBr and Biz fluorescence because of intercalation of these fluorochromes inside the detergent micelles. Our SDS concentrations (1.7%) were well above these values. There is, therefore, the possibility that SDS was the main interfering agent in our protocol. Further testing would be required to confirm this and to determine whether such high concentrations are required for efficient cell disruption or whether they can be advantageously lowered. For each set of samples, we used the residual Tris-EDTA+dye fluorescence values and subtract them from the samples' measurements, rather than using a crude buffer blank value as is usually done. The treated (extracted) buffer blanks were routinely made (one for every set of 12 samples) when conducting the extraction and purification procedure to account for potential day-to-day differences in the effectiveness of the extraction process.

The fluorescence increase resulting when Hoechst 33258 (Biz) is in the presence of DNA derives from the specific binding of the fluorochrome to the A-T base pairs regions of the DNA molecule (Commings, 1975; Müller and Gautier, 1975; Cesarone *et al.*, 1979; Labarca and Paigen, 1980). The fluorescence arising from ethidium bromide in the presence of nucleic acids results from the non-specific intercalation of the fluorochrome inside the helix of RNA and DNA molecules. Therefore, DNA and RNA concentrations can be obtained by measuring the fluorescence yield of Biz and EBr on aliquots of a sample in the following manner. (1) A DNA+Biz calibration line is used to determine the concentration of DNA from the Biz measurement; (2) this concentration is then reported on a DNA+EBr line to obtain the expected EBr fluorescence due to DNA alone; (3) the expected fluorescence is then subtracted from the total EBr

fluorescence (due to DNA and RNA) to provide the EBr fluorescence due to RNA alone; (4) the latter is reported on the RNA+EBr line to yield the concentration of RNA in the sample. The calibration lines for DNA+Biz, DNA+EBr and RNA+Ebr used for this purpose are shown in Figures 3b, 4a and 4b. The calibrations were run weekly and repeated whenever a R^2<0.99 was obtained.

2.3 Data analysis

Correlation patterns between female, egg and larval traits were studied using the protein and dry weight measurements obtained from 576 unfertilized eggs from 16 families and 829 newly-hatched larvae, sampled on the second modal hatching day, obtained from 41 families, together with the weight and morphometric measurements taken on the corresponding mothers. Pearson correlation matrices were used as a first exploration tool, and regression analysis and single-way ANOVA as a means of inspecting the relationships of interest in greater detail (Wilkinson, 1998).

Total dry weight (DW), protein (Prot), DNA and RNA content were obtained for 464 individual larvae ranging in age from 0 to 12 d post-hatch. These were derived from 43 half-sib families for which "starvation beakers" (from first modal hatching day) could be monitored. DNA/DW, RNA/DW, Prot/DW, RNA/DNA, RNA/Prot and Prot/DNA ratios were also computed. Regression analyses were computed using single biochemical measures as the dependent variable and post-hatching age (duration of starvation) as the independent variable to determine which of the above variables were most responsive to starvation. The sensitivity of the various biochemical measures to starvation was assessed by the t-value of their slope when regressed against post-hatching age. Regression of the dependent variables on hatching age was used as a means of testing *a priori* whether any age confounding effect due to variable hatching age was present. Female effects on starvation dynamics were determined using ANCOVA models with female source as the treatment (class) variable and post-hatching

age as the covariate. An interaction term (female*age) was included in the analysis to test *a priori* for the homogeneity of slopes between family regressions. All statistical analyses were performed on SAS version 6.04 (SAS Institute Inc., 1985) or on SYSTAT version 8.0 (Wilkinson, 1998).

3. RESULTS

3.1 Correlation patterns and partitioning of the variance in egg and hatchling size

The mean number of eggs incubated per beaker (116.5 ± 66.4) and the modal hatching age (23.6 ± 4.3 days) differed between families. These confounding effects must be considered when analyzing the biochemical variables measured at the onset of starvation and subsequently. Mean egg dry weight had no significant effect ($P<0.05$) on developmental time (age at hatching). This was true for larvae sampled at both first and second modal hatching days. However, the mean number of eggs incubated per beaker (range 18-270) had a significant (ANOVA, $P<0.001$) impact on the age at which most of the larvae hatched (first modal hatching day). That relationship was positive for 50 monitored beakers, meaning that more densely aggregated eggs tended to hatch later than less densely aggregated ones. This variation in incubation time between-family had no major effect on the size of the larvae, since no significant relationships (ANOVA, $P<0.05$) were found between the dry weight or the protein content of the larvae at hatch and the age at which they hatched.

Unfertilized capelin eggs contained on average 66.2 ± 18.1 µg of protein which represents the bulk (89%) of their average 74.2 ± 7.5 µg total dry weight. Those larvae which hatched on the second modal hatching day (23.6 ± 4.3 days post-fertilization) had an average dry weight of 51.4 ± 7.2 µg of which only 22.6 ± 9.0 µg (43.9%) was composed of protein. The mean dry weight of unfertilized

FIGURE 5. Relationship between egg dry weight and hatchling dry weight. The error bars represent one standard error from the mean family values.

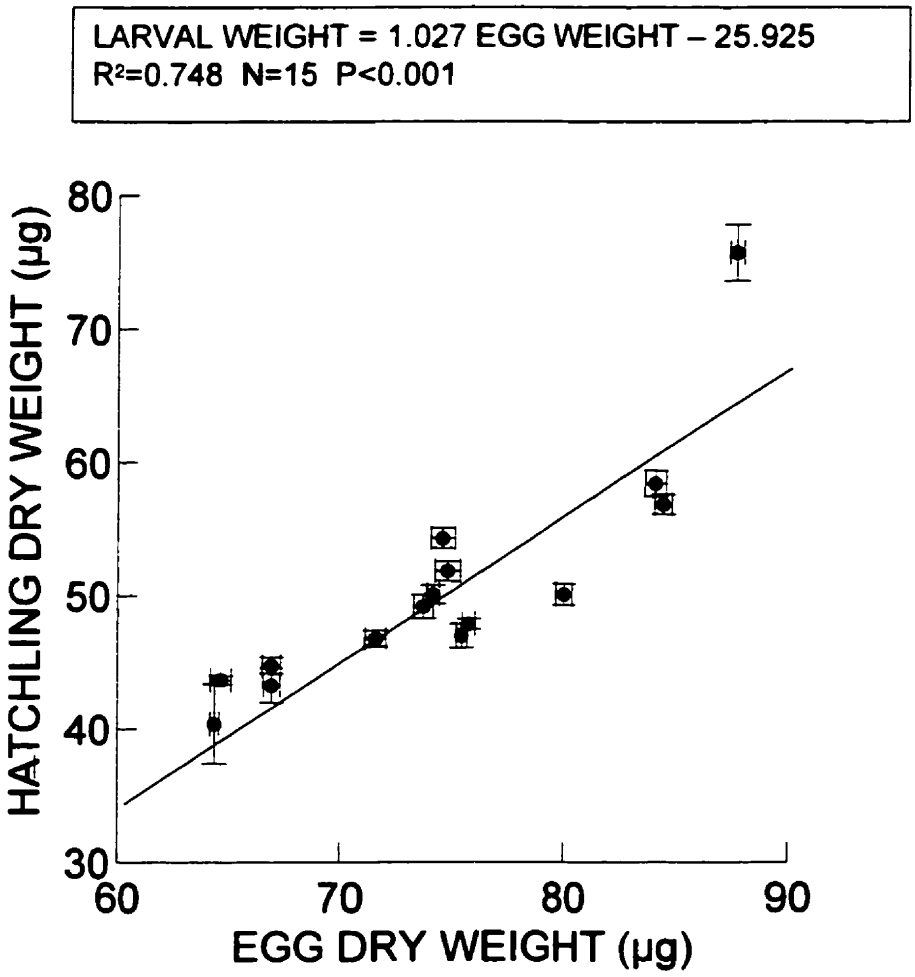
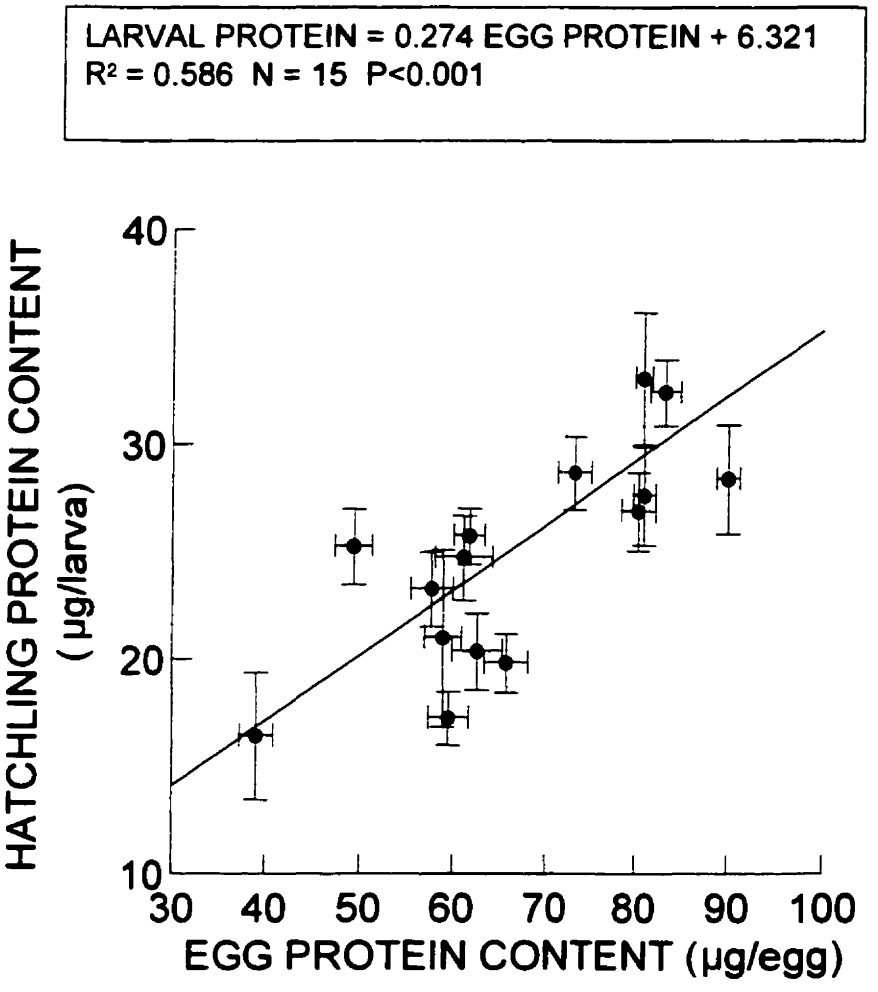


FIGURE 6. Relationship between egg protein content and hatchling protein content. The error bars represent one standard error from the mean family values.



eggs was highly correlated ($P<0.001$) with mean larval dry weight at hatch (Figure 5).There was approximately equal variance (standard error) about the family means for both egg and larval weight, but larval dry weight was more variable in the families exhibiting the two extremes of egg size. The protein content of unfertilized eggs was positively correlated ($P<0.01$) with the protein content (Figure 6) and the dry weight ($P<0.05$, not shown) of newly-hatched larvae. Here too, variance about family means was approximately equal in egg and larval protein content, but the families exhibiting the highest and lowest mean protein content in newly hatched larvae also exhibited the largest variance (Figure 6).

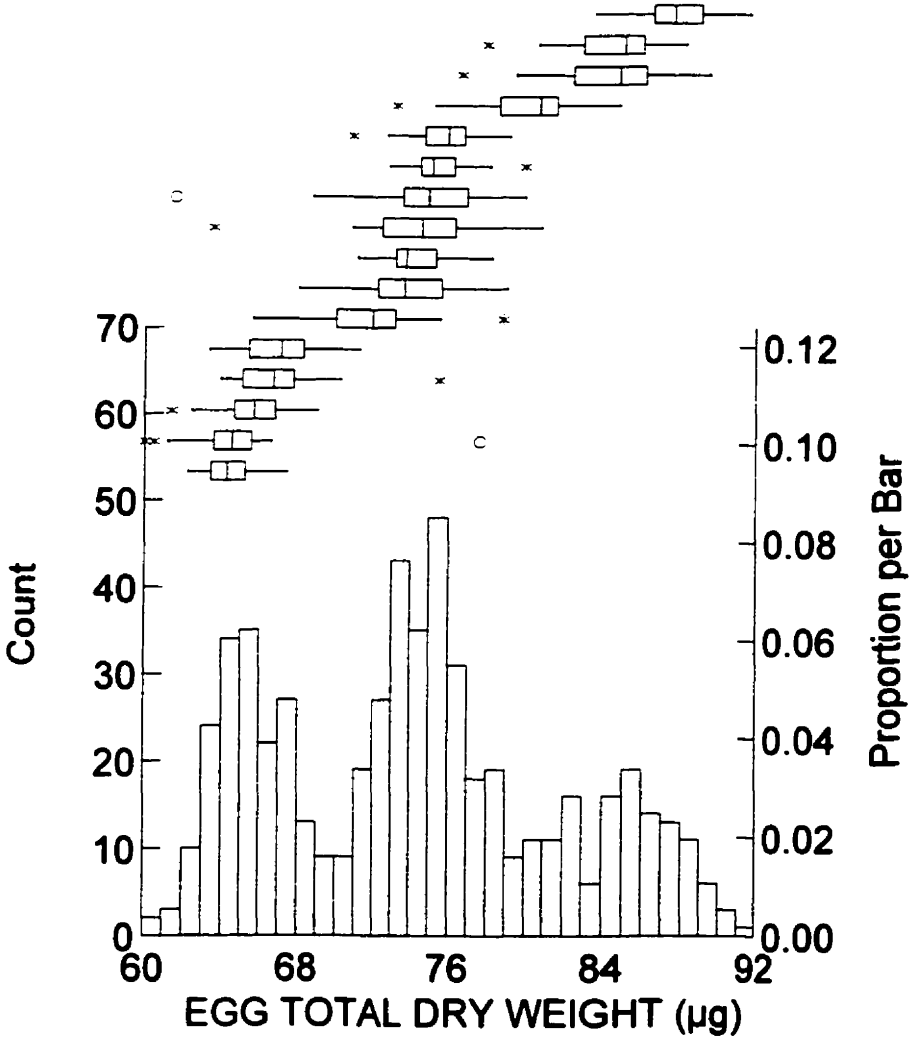
Computation of variance components (SAS, Procedure VARCOMP) revealed that most of the variation in egg and larval dry weight, and in egg and larval protein content, resulted from between-family rather than within-family variability (Table 1). This pattern was most apparent for egg dry weight for which 90.7% of the variability was attributed to between-family variance. Between-family variance was lower for egg protein content (59.4%), and for hatchling dry weight (57.5%) but was still higher than the within family variance. This pattern was reversed for hatchling protein content, for which greater variability (58.0%) occurred within rather than between families (Table 1). Despite the narrow range of egg sizes within females, and large between-family differences, a tri-modal frequency distribution was obtained when unfertilized egg dry weight values were pooled for all the 16 families examined (Figure 7).

Females used in the experiments were on average 140.1 ± 9.7 mm in length and 24.32 ± 5.52 grams in weight. Their average ovary weight (corrected for eggs extruded) was 11.46 ± 2.95 grams for a gonado-somatic index of 0.47 ± 0.04. Their total dry weight was 2.59 ± 0.73 grams and lean dry weight 2.25 ± 0.55 grams which translated into a total lipid content of 0.27 ± 0.11 grams or 10.3%. All of the female traits measured (total length, total wet and dry weight, ovary wet and dry weight, total lean weight, lipid content, gonado-somatic index)

Table 1. Summary results from the computation of between-family variance components for the dependent variables listed.

Dependent variable	df	variance component	estimate	Percent %
EGG dry weight	15	var (female)	53.3181	90.72
	548	var (error)	5.4509	9.28
	563	sum	58.7690	
EGG protein	15	var (female)	193.5505	56.76
	558	var (error)	147.4363	43.24
	573	sum	340.9868	
HATCH dry weight	40	var (female)	30.7980	57.99
	627	var (error)	22.3085	42.01
	667	sum	53.1065	
HATCH protein	40	var (female)	20.0424	24.49
	736	var (error)	61.8009	75.11
	776	sum	81.8433	

FIGURE 7. Frequency distribution of unfertilized egg dry weight for 16 females. Each box plot in the upper part of the figure represent the distribution of egg sizes within a single family. The wiskers on each side of the center box encompass the first and fourth quartiles, while the center box includes the second and third quartiles. The median is represented by the vertical line within the box. Values situated at more than 1.5 interquartile ranges from the edge of the center box are represented by asterisks and those at more than 3.0 interquartile ranges represented by empty circles.



were significantly and positively correlated ($P<0.001$, Pearson correlation matrix) to one another, meaning that longer females were heavier and had larger ovaries. The only exceptions were lipid content and gsi which were uncorrelated with female size.

There was no significant relationship (ANOVA, $P<0.001$) between any of the above female traits and the corresponding family mean egg and larval dry weight or mean egg and larval protein content. Total fecundity and relative fecundity estimates were computed for eight females (Table 2) for which egg dry weight values were available. For those females there was a significant positive relationship (ANOVA, $P<0.001$) between fecundity and ovary dry weight, and between fecundity and total length and weight ($P<0.01$, Figure 8a). Relative fecundity had a negative and significant effect on egg dry weight ($P<0.01$, Figure 8b).

3.2 Effects of starvation on the biochemical composition of the larvae

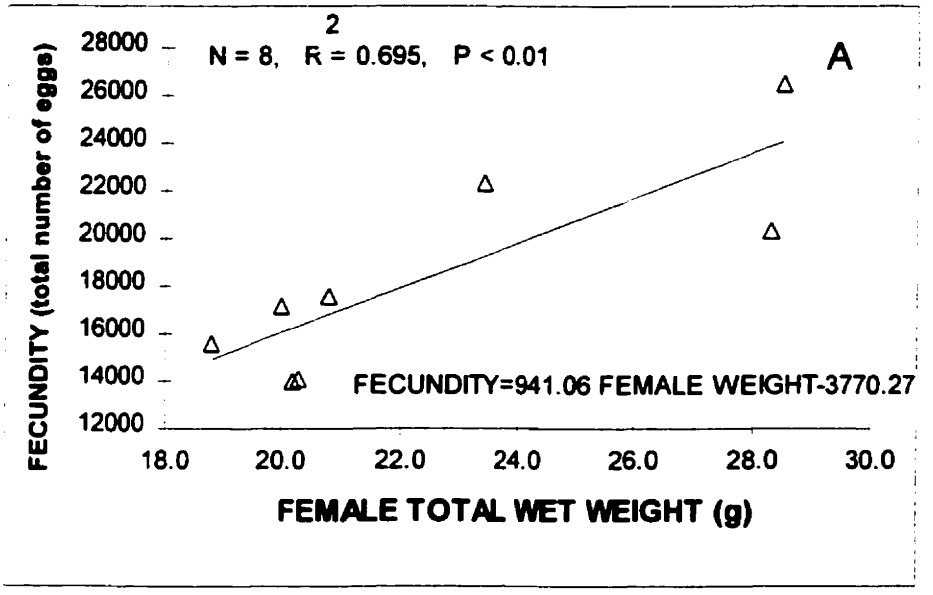
No significant relationship (ANCOVA, $P<0.01$) was found between age at hatch and any of the 10 biochemical variables measured on the larvae that hatched on the first modal hatching day. Thus, as was the case for larvae from the second modal hatching day, we conclude that variation in egg density and hatching age that prevailed between-beakers had no significant influence on the variables measured on starving larvae. These potential sources of variance were therefore ignored in subsequent analyses. There were no significant correlation ($P<0.05$) among any of the variables (total dry weight, DNA, RNA, protein content) measured on starved larvae of the same age.

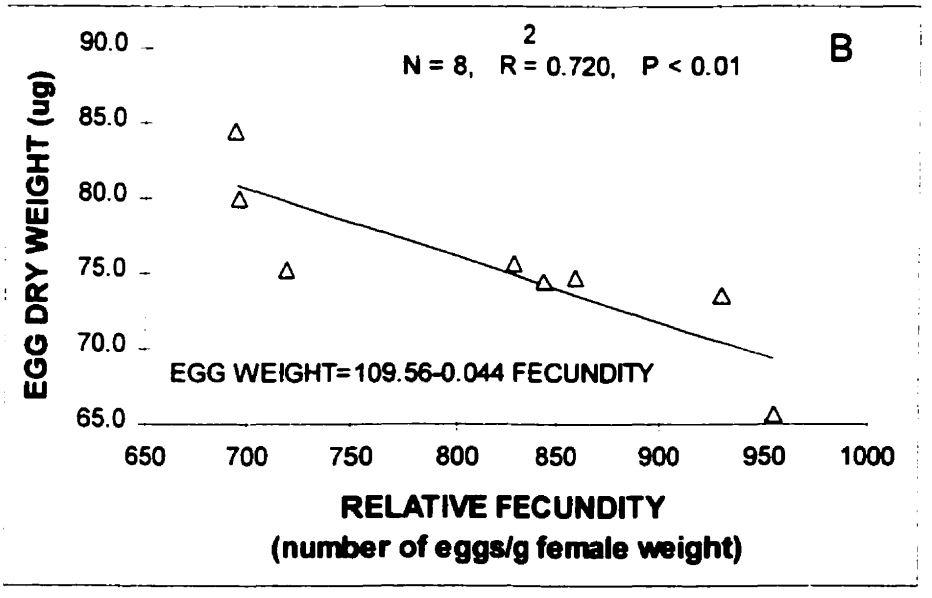
Regression analysis (SYSTAT, GLM), revealed that nine of the ten biochemical measures employed were sensitive to starvation and were significantly ($P<0.01$) affected by post-hatching age. The only variable not affected was DNA content per larva (Table 3). Total larval dry weight was

Table 2. Reproductive parameters for eight of the females used in the experiment and for which the required data were available for computations. The females were ranked by size (length and weight).

Female total length (mm)	female total wet weight (g)	female ovary wet weight (g)	female wet gonado-somatic index	female ovary dry weight (g)	mean egg dry weight (µg)	Fecundity (total number of eggs)	relative fecundity (eggs/g female)
129	18.8	8.40	0.447	1.091	75.7	15 559	828
130	20.0	9.81	0.490	1.206	74.7	17 187	859
133	20.2	8.24	0.408	1.105	84.5	14 013	694
133	20.3	8.56	0.442	1.058	80.0	14 121	696
133	20.8	9.87	0.475	1.236	74.5	17 561	844
138	28.3	15.61	0.552	1.448	75.3	20 354	719
142	23.4	11.46	0.490	1.402	65.6	22 347	955
145	28.5	12.80	0.449	1.876	73.6	26 504	930

FIGURE 8. Relationships between (A) female total wet weight and fecundity and between (B) egg size and female relative fecundity.





most strongly affected by starvation ($P<0.001$). Capelin larvae averaged 50.7 µg at hatching and lost weight at a rate of 2.1 µg d^{-1} reaching an average dry weight of 27.4 µg after 11 days of starvation. This represents a reduction of 46% of their hatching weight. Forty two percent of the variability in larval dry weight during starvation was explained by post-hatching age. DNA/DW (µg DNA/µg DW) was less strongly influenced by starvation, but increased significantly ($P<0.001$) from 0.011 (1.1% of dry weight) at hatch to approximately 0.022 (2.2% of dry weight) at day 11. RNA/DNA ratio decreased ($P<0.001$) during starvation from a value of 5.01 at hatching to 1.38 at day 11. The rate of decrease in the RNA/DNA ratio was 0.33 units d^{-1}. Approximately 22% of the variation in the RNA/DNA ratio was explained by the duration of starvation (Table 3). The quantity of RNA and Protein per larvae, RNA per dry weight and the RNA/Protein ratio decreased significantly ($P<0.001$) during starvation (Table 3). However, the decrease occurred at a slower rate than that for the dry weight and RNA/DNA ratio. In those four cases less than 10% of the variability could be explained by post-hatching age. The percentage of Protein per dry weight increased substantially during starvation ($P<0.01$) while the amount of Protein per DNA decreased ($P<0.01$, Table 3).

3.3 Female effects on the rate of change in the biochemical composition of starving larvae

Analysis of covariance (ANCOVA) tests for differences in intercept between a family of treatment lines. The analysis assumes that the slopes do not differ between treatments. We tested *a priori* for equality of slopes by introducing an interaction term linking the treatment variable and the covariate of interest. Specifically each model relating the dependent variable (Y) to female (class variable) and post-hatching age (covariate) incorporated a female * post-hatch age interaction term. The basic model was thus: Y = constant + female + phage + female * phage. A significant ($P<0.001$) interaction between post-hatching age and female source was detected for dry weight and Protein/dry weight as well as

Table 3. Regression parameters for the relationship between biochemical measures and the duration of starvation (post-hatching age). The dependent variables are ordered by the magnitude of the t-value of their slope.

dependent variable	intercept	slope	Percent change per day	t-value of the slope	R^2
1. Dry weight (DW)	50.745	-2.122	-4.18 %	-18.060***	0.420
2. DNA/DW	0.011	0.001	9.09%	10.559***	0.234
3. RNA/DNA	5.009	-0.330	-6.59%	-10.204***	0.218
4. µg RNA/larva	2.762	-0.187	-6.77%	-9.893***	0.207
5. µg Prot/larva	25.190	-0.761	-3.02%	-6.179***	0.078
6. RNA/DW	0.055	-0.002	-3.64%	-5.153***	0.068
7. RNA/Prot	0.114	-0.005	-4.39%	-4.624***	0.054
8. Prot/DW	0.495	0.009	1.82%	3.057**	0.021
9. Prot/DNA	48.036	-1.047	-2.18%	-2.676**	0.019
10. µg DNA/larva	0.560	-0.002	0.35%	-0.714	0.001

***P<0.001, **P<0.01, *P<0.05

Table 4. F-ratio values from analyses of covariance using the model Y = constant + female + post-hatching age + female * post-hatching age. The dependent variables (Y) are ranked according to the magnitude of their post-hatching age effect (df = degrees of freedom).

dependent variable	df	Female	df	Post-hatching age	df	Post-hatching age * female	R^2
1. Dry weight	40	3.696***	1	7.427**	40	2.857***	0.688
2. DNA/DW	40	0.829	1	2.270	39	0.991	0.453
3. RNA/DNA	39	1.352	1	0.960	40	1.054	0.442
4. RNA/Prot	39	0.997	1	0.876	40	1.261	0.347
5. RNA/DW	40	1.134	1	0.875	39	1.173	0.331
6. µg Prot/larva	40	1.676**	1	0.676	40	1.831**	0.401
7. µg RNA/larva	40	1.180	1	0.186	39	0.906	0.429
8. Prot/DW	40	1.062	1	0.087	40	2.097***	0.337
9. Prot/DNA	40	0.688	1	0.069	39	1.622*	0.383
10. µg DNA/larva	40	0.832	1	0.041	40	0.845	0.256

***P<0.001, **P<0.01, *P<0.05

for the amount of Protein/larva ($P<0.01$) and the Protein/DNA ratio ($P<0.05$, Table 4). This implies that the family lines for those four variables differed significantly between treatments (females) and cannot be assumed to approximate parallel lines. After removing the non-significant interaction term from the six remaining ANCOVA, we found a highly significant ($P<0.001$) post-hatching age effect for five, and a significant ($P<0.05$) treatment effect (difference in family intercepts) for all six (Table 5). The significant differences in the intercepts of these lines underlies significant differences in the biochemical composition of newly hatched larvae from different females.

The magnitude of the F-ratio for the ANCOVA post-hatching term (Table 5) was consistent with the results previously obtained for these variables from the t-value of their regression slope when female effects were not considered (Table 3). The amount of RNA per larva, the RNA/DNA ratio and the DNA/DW were most affected by the duration of starvation, followed by the RNA/DW and the RNA/Protein ratios (Table 5). The amount of DNA per larva was not significantly affected by starvation. The addition of a female and a post-hatch * female interaction term to the simple relationship (Y = post-hatch age), greatly increased the explained variability for all the dependent variables examined (Table 3-4).

4. DISCUSSION

4.1 Egg size, embryonic development rate and larval size

More than 60% of the capelin eggs spawned on Bryant's Cove beach (a typical capelin spawning site) occur at the high tide level where they may be submerged for only one or two hours daily. The remainder of the eggs, which are found at the intermediate and low tide levels, are submerged from two to 24 hrs daily (Frank and Leggett, 1981a). Consequently, incubation temperatures experienced by wild capelin eggs can differ dramatically due to direct exposure to sunlight (>25° C) or flooding of waters whose temperature averages 8-10° C.

Table 5. F-ratio values from analyses of covariance using the model Y = constant + female + post-hatching age. These relationships were tested for the homogeneity of their slopes and the interaction term female*post-hatching age was removed from the analysis. The dependent variables (Y) are ranked according to the magnitude of their post-hatching age effect (df = degrees of freedom).

Dependent variable		female		post-hatching age	R^2
	df		df		
1. µg RNA/larva	40	1.992**	1	109.210***	0.360
2. RNA/DNA	40	1.916**	1	103.313***	0.364
3. DNA/DW	40	1.898**	1	100.729***	0.379
4. RNA/DW	40	1.642*	1	29.681***	0.225
5. RNA/Prot	40	1.960**	1	27.528***	0.235
6. µg DNA/larva	40	1.728**	1	1.753	0.173

***P<0.001, **P<0.01, *P<0.05

Hence, hatching age can vary from 9-11 d at the high tide zone to 22-24 d at the low tide zone (Frank and Leggett, 1981b). The incubation times we observed (mean = 23.6, range 19-35 d) at 8°C are consistent with the range of values reported for larvae experiencing low tide conditions. Frank and Leggett (1981b) determined that as much as 85% of the variability in capelin egg hatching time on Bryant's Cove beach could be explained by temperature differences. However, the substantial between-family variability in median hatching dates we observed at a constant incubation temperature suggests that, independent of temperature, other factors are involved.

The number of eggs incubated per beaker had a significant impact on their rate of development, crowded eggs hatching later than less crowded ones. Since the water was changed only once daily, this suggest that other environmentally based factors possibly oxygen levels may have been involved. Given the range of environmental conditions experienced by capelin eggs on native spawning beaches (egg density, temperature, duration of submersion) these factors are likely to be at play in the wild. While it is possible that the rate of oxygen depletion in the beakers caused the observed variability between family in the rate of embryonic development, we cannot exclude genetic or maternal influences.

For the 16 families for which data were available, we found that between-family differences in egg size did not influence embryonic development rate or the age at which most of the capelin larvae hatched. Araunjo-Lima (1994) have reported similar findings at the inter-population level, as have Blaxter and Hempel (1963), Kazakov (1981), Wallace and Aasjord, 1984; Knutsen and Tilseth (1985), March (1986), and Hutchings (1991) at the intra-population level. The absence of a correlation between egg size and incubation time in capelin contrasts with the results of some interpopulation and interspecies analyses which have shown that at constant temperature, fish embryos from larger eggs typically develop more slowly (Ware, 1975; Hunter, 1981, Duarte and Alcaraz,

1989; Pepin, 1991). Interestingly, studies on amphibians also reveal little or no relationship between hatching age and egg size at the intra-population level, even though a significant relationship between these two variables exists at the inter-population and inter-species level (Kaplan, 1989). This suggests that at constant temperature, most of the variability in the rate of embryonic development relative to egg size occur within populations of single species. It should be noted, however, that the majority of the above conclusions are based on group data (family, population, or species means). Chambers *et al.*, (1989) showed that different results can be obtained when "individual-based" values are used in the analyses. These authors found, for example, that a significant intra-population relationship existed between embryonic development time and egg size in capelin when assessed at the individual level, while no correlation was evident when the analyses were conducted using family means. Our results are consistent with these findings. However, in our analyses variation in age at hatch within family was removed by sampling only on modal hatching dates. Egg size also varied little within family. It is important to note that our experiments were not designed to evaluate the relationship between egg size and incubation time at the individual level. Rather we sought to determine whether variable-hatching age had a significant confounding effect on the condition of emerging and starving larvae. Our results confirm that variable between-family hatching age did not have a significant confounding effect on the condition of starving larvae. The destructive nature of our sampling prevented repeated measurements on individuals. More recently, Benoît and Pepin (1999), using an individual based analysis, concluded that embryonic development in yellowtail flounder was overwhelmingly influenced by temperature and that egg size had a greater impact on the length of the yolk-sac stage than on the embryonic stage. Chambers et al., (1989) reported similar findings for capelin.

Independent of hatching age, capelin larvae hatching from half-sib families having larger eggs (which contained more protein) were larger at hatch. In fish, such correlations have generally been reported in analyses conducted at the

inter-specific level (Hunter, 1981; Duarte and Alcaraz, 1989; Araujo-Lima, 1994; Chambers and Leggett, 1996), at the population levels when measured intra-specifically (Blaxter and Hempel, 1963; Hempel and Blaxter, 1963; Kazakov, 1981; Brown and Taylor, 1992; Thorpe et al., 1984; Wallace and Aasjord, 1984; Knutsen and Tilseth, 1985; Marsh, 1986; Zastrow et al., 1989; Beacham and Murray, 1990; Hinckley, 1990; Hutchings, 1991; McEvoy and McEvoy, 1991); and at the family level (Springate and Bromage, 1985; Rana, 1985; Chambers *et* al., 1989; Brown and Taylor, 1992; Marteinsdottir and Able, 1992; Ojanguren *et* al., 1996; Marteinsdottir and Steinarsson, 1998). Our use of dry weight as a measure of egg and larval size may have improved the likelihood of obtaining this correlation at the family level, since hatchling weight unlike length, is directly affected by the amount of yolk remaining at hatch, and is itself correlated to the amount of egg yolk (Hutchings, 1991).

For capelin, the slope of the relationship we obtained between egg weight and hatchling weight approached 1.0 (1.029), (Figure 5). Assuming a complete transfer of biomass between the egg and the newly-hatched larvae, the intercept (26 µg) represents the sum of metabolic losses during embryonic development plus the weight of the chorion which is lost at hatching. For most families, variance in egg weight was equivalent to the variance in hatchling weight. The exceptions were two clutches which exhibited the lower and upper extremes of egg size. These latter had a much higher variance in hatchling weight relative to egg weight (Figure 5) and hatchling protein relative to egg protein content (Figure 6).

The dry weight and the protein content of the newly hatched capelin larvae was positively related to the quantity of egg protein. This indicates that a constant proportion of the egg yolk protein was utilized for embryonic growth independent of the protein content of the larvae at hatch. Approximately 27% of the unfertilized egg protein was retained in the newly hatched larvae in the form of protein (Figure 6). It has frequently been demonstrated (see review in Ferron and

Leggett, 1994) that yolk proteins are important energy substrates in early larval growth. If, as our results suggest, larvae incorporate a constant proportion of egg protein during embryonic growth, then larvae hatching from larger eggs will incorporate a greater quantity of yolk proteins by hatching, and will achieve a greater weight at hatch.

4.2 Sensitivity and dynamics of biochemical measures of larval condition in relation to starvation.

In our analyses we defined sensitivity as the minimum change in food ration that can be detected by a particular index of condition, and latency as the time required for a given change in food deprivation to be reflected as a significant change in the condition index (Ferron and Leggett, 1994). We hypothesized, following a review of existing literature (Ferron and Leggett, 1994) that sensitivity and latency would be inversely correlated. We defined the dynamics of the time response of condition indices as the rate of change in the condition index following the period of latency and the detection of a significant response (Ferron and Leggett, 1994). Dynamics and latency can be measured only under strictly controlled laboratory conditions in which starvation and feeding controls are monitored concurrently with delayed-starved and delayed-fed treatments.

Daily examination of samples taken from beakers showed that capelin larvae reached complete yolk exhaustion four days after hatching and were expected to initiate feeding shortly after. The duration of post-hatching starvation in capelin larvae was reflected in several biochemical measures. Yolk-sac (0-4 days post-hatch) and post yolk-sac larvae lost significant weight, RNA and protein, but conserved their DNA. The indices which were significantly ($P<0.01$) sensitive to starvation were, in rank order, dry weight (DW), DNA/DW, RNA/DNA ratio, RNA/larva, Prot/larva, RNA/DW, RNA/Prot, Prot/DW and Prot/DNA (Table 3). These findings are consistent with those of earlier studies reviewed in Ferron and Leggett (1994), and with those of more recent work (Table 6).

Table 6. Summary results of the sensitivity of various measures of condition to starvation gathered from laboratory studies conducted since 1994.

Author	Measured variable	Response to starvation	Species	Life stage
Mathers et al., 1994	RNA DNA Protein	Decrease Decrease Decrease	Herring *Clupea harengus*	Larvae
Canino, 1994	RNA DNA RNA/DNA	Decrease Decrease Decrease	Walleye Pollock *Theragra chalcogramma*	Larvae
Malloy and Targett, 1994	RNA/DNA	Decrease	Summer flounder *Paralichthys dentatus*	Juvenile
Clemmesen, 1994	RNA/DNA	Decrease	Herring *Clupea harengus*	Larvae
Bergeron and Boulhic, 1994	RNA/DNA	Decrease	Sole *Solea solea*	Larvae
Bisbal and Bengtson, 1995	Dry weight RNA/DNA Protein	Constant Decrease Decrease	Summer flounder *Paralichthys dentatus*	Larvae and juvenile
Clemmesen and Doan, 1996	RNA/DNA	Decrease	Cod *Gadhus morhua*	Larvae
Rooker and Holt, 1996	RNA/DNA	Decrease	Red drum *Sciaenops ocellatus*	Larvae and juvenile
Suthers et al., 1996	RNA/DNA RNA/Dw	Decrease Decrease	Australian Bass *Macquaria novemaculeata*	larvae
Folkvord et al., 1996	DNA RNA RNA/DNA	Decrease Decrease Decrease	Herring *Clupea harengus*	Larvae
Rooker and Holt, 1997	RNA/DNA	Decrease	Red drum *Sciaenops ocellatus*	Larvae and juvenile
Bergeron and Person-Le Ruyet, 1997	DNA/Dw	Increase	Sea Bass *Dicentrarchus labrax*	Larvae
Kristogu-Baduge et al., 1999	Dry weight DNA RNA RNA/DNA	Decrease Constant Decrease Decrease	Herring *Clupea harengus*	Larvae

The rate of dry weight loss (starvation dynamics) observed in capelin larvae (2.12 μg d^{-1}, 4.2% d^{-1}) was consistent with the loss rate of 5% d^{-1} reported by McGurk, (1984) for starving Pacific herring larvae and by Folkvord et al. (1994) for starving cod larvae. Values reported by Kristogu-Baduge (1999) in starving herring larvae were found to be lower (2.6% at 8°C) and to be temperature-dependent. Bisbal and Bengtson (1995) working with summer flounder larvae and juvenile found no relationship between the rate of loss in body mass and the length of starvation.

DNA content of starving capelin larvae remained essentially unchanged (-0.002 μg $d^{-1,}$ -0,3% $d^{-1)}$. This contributed to the significant RNA/DNA ratio decrease and the significant DNA/DW increase during the same period. Richard et al. (1991) reported DNA content increased in fed sole larvae, but remained stable in larvae starved for up to 15 days. Folkvord et al. (1996) observed a slight decrease (0.01 μg $d^{-1)}$ in the DNA content of starving herring larvae over 11 days, but Kristogu-Baduge (1999) reported no significant decrease (ANOVA, $p<0.05$) in the DNA content of starved herring larvae at temperatures of 5 and 8°C. We conclude, on the basis of the evidence available, that DNA levels remain quite constant during starvation in early stage larvae, and that starving larvae maintain cell number and integrity as their condition deteriorates even during prolonged starvation. This stability which has been largely assumed to date is fundamental to the use of RNA/DNA and DNA/DW ratios as indicators of condition in starved fish larvae. It is important to note however, that Clemmesen (1994), Suthers et al. (1996), Bergeron and Boulhic (1991), Richard et al. (1991) and Bergeron and Person-Le Ruyet (1997) have shown, that distinct DNA trends are associated with growth during the various developmental stages (yolk-sac, pre-flexion and flexion etc.) of fish. These distinctive patterns, which may be related to the saltatory growth model proposed by Balon (1984) should be kept in mind when condition indices based on DNA measures, are considered. Until these stage specific differences are more fully researched, analyses that encompass these growth stages should be approached with caution.

DNA/DW, an index of thinness, can be used to accurately assess the amount of weight loss, since it is expressed in relation to cell number. Its use has been promoted by Bergeron *et al.*, (1991) and Richard *et al.*, (1991) because its sensitivity to starvation approaches that of the RNA/DNA ratio but it has the advantage of exhibiting less inter-individual variability in response to food and temperature. Our experiments with starving capelin larvae, showed that DNA level remained unchanged over 11 days of starvation, while weight loss over the period caused DNA/DW to increase at a rate of 0.001 (9.1%) d^{-1} reaching a value of 0.022 (2.2%) after 11 days. Bergeron et al. (1991) working with sole larvae and juvenile argued that DNA/DW values above 0.030 (3.0%) were indicative of starvation. Richard et al. (1991) reported that the DNA/DW remained between 0.020-0.022 (2.0-2.2%) in fed sole larvae but increased to values well above 0.040-0.050 (4.0 and 5.0%) in starved ones. Kristogu-Baduge et. al. (1999) reported a significant increase in the DNA/DW ratio of starved herring larvae up to 12 days post-hatch at 8°C, with levels ranging from about 0.016 (1.6%) at 5 days post-hatch to 0.023 (2.3%) at 12 days post-hatch for an average increase of 0.1% d^{-1} . They proposed a critical DNA/DW level of 0.022-0.029 (2.2-2.9%) to differentiate between starved and fed individuals of different stage raised at different temperatures. The rate of increase in DNA/DW we observed in starving capelin larvae (0.1% $d^{-1)}$ corresponds precisely with that obtained by Kristogu-Baduge et. al. (1999) for starving herring larvae, and the critical value they suggest 0.022 (2.2%) was consistent with the DNA/DW exhibited by capelin larvae at the point of death (11th d of starvation). However, the variability in DNA/DW reported at starvation 0.03-0.05 in larval and juvenile sole, and 0.02-0.03 for larval capelin and herring suggests that critical DNA/DW levels are probably species-specific and that generalization is dangerous and unjustified.

The latency of the RNA/DNA ratio following starvation has been widely assumed to be on the order of days but has typically not been accurately measured because of the application of inappropriate sampling scale (Ferron and Leggett, 1994). Using daily sampling, Malloy and Targett (1994) found that

RNA/DNA ratio responds to starvation and re-feeding of juvenile summer flounder within 24 hr at 16°C. Rooker and Holt (1996) who sampled at 4 hr intervals, observed diel periodicity in the RNA/DNA of red drum larvae. These findings indicate that latency in the RNA/DNA ratio is likely to be measured on the scale of hours rather than days, and is also likely to be temperature-dependent.

The dynamics (rate of change) of the RNA/DNA ratio after a response has been triggered, has been hypothesized to follow an exponential decay curve, with an abrupt change at the onset of starvation followed by a more gradual decrease towards critical levels (Ferron and Leggett, 1994). From interpolating the data of Wright and Martin (1985) for starving larval striped bass, and those of Clemmesen (1987) for starving larval herring, we estimated the average rate of decrease in the RNA/DNA ratio to be approximately 0.2 unit d^{-1} (Ferron and Leggett, 1994). Although we were unable to distinguish between curvilinear and linear trend in the data presented here, our data for starved capelin larvae indicate a rate of decrease of 0.33 unit (6.6 %) d^{-1} over the 11 d starvation period. Several studies published after our 1994 review (Ferron and Leggett, 1994) have reported curvilinear decays in RNA/DNA ratio during starvation (Table 7). In spite of the wide range of species, rearing temperatures and stages reflected in the data, the overall average rate of decay in the RNA/DNA ratio approximates 0.2-0.3 unit d^{-1} with significantly higher values (1-2 units $d^{-1)}$ recorded during the early stages of starvation.

In capelin, RNA content per larva decreased at a rate approaching 0.2 µg d^{-1} (6.8% d^{-1}) following starvation. This change in RNA with time was responsible for observed changes in the RNA/DNA ratio, since DNA levels remained unchanged and RNA/DNA decreased at similar rate (6.6% $d^{-1)}$. Bergeron and Boulhic (1994) also confirmed the basic assumption of changes in the RNA/DNA ratio associated with changes in RNA levels alone, when comparing DNA, RNA and RNA/DNA ratios in starving sole larvae. Westerman and Holt (1994) measured a

Table 7. Summary results of the dynamics of RNA/DNA ratios following starvation of post yolk-sac larvae and calculated from laboratory studies conducted since 1994.

Author	Type of response	Range and average rates of decay (units d^{-1})	species	Life stage
Clemmesen, 1994	Curvilinear	0.2-0.3	Herring *Clupea harengus*	Larvae
Bergeron and Boulhic, 1994	Curvilinear	0.2	Sole *Solea solea*	Larvae
Bisbal and Bengtson, 1995	Linear	0.58 6 d-old 0.29 16 d-old	Summer flounder *Paralichthys dentatus*	Larvae and juvenile
Clemmesen and Doan, 1996	Curvilinear	0.20-0.30	Cod *Gadhus morhua*	Larvae
Folkvord et al., 1996	Curvilinear	0.55	Herring *Clupea harengus*	Larvae
Kristogu-Baduge et al., 1999	Curvilinear	0.11-0.36	Herring *Clupea harengus*	Larvae

42.6% decrease of the RNA content of starving red drum larvae over the first 4 days following hatching (10.6% $d^{-1)}$, a value slightly higher than the average 6.8% d^{-1} we observed in capelin larvae over 11 days of starvation.

Our results based on experiments with starving capelin larvae indicate that DNA/DW is more responsive to starvation than is the RNA/DNA ratio. This occurs because larvae lose weight more rapidly than they lose RNA. However, it is important to note that the two indices depict simultaneous but quite different processes, weight loss in the case of the DNA/DW ratio and reduced growth potential in the case of the RNA/DNA ratio. Which of weight loss versus reduced growth potential is a more reliable indicator of recovery potential following starvation and of higher survival probability remains unknown. Bergeron (1997) has argued in favor of the DNA/DW over the RNA/DNA ratio on the basis that RNA/DNA ratios are temperature sensitive while DNA/DW are not. RNA/DNA ratios also tend to be more variable between-individuals and harder to replicate than DNA/DW ratios even under strictly controlled feeding conditions.

One unwanted source of variability in the RNA/DNA ratio is believed to result from error magnification due to the probability of analytical errors in RNA and DNA determinations, which are derived from one another. For most techniques based on Karsten and Wollenberger (1977, 1972) and Clemmesen (1993), DNA levels are subtracted from total nucleic acids after RNAse digestion using the ethidium bromide dye. Alternately in other techniques (Clemmesen, 1988) RNA levels could be determined by subtracting DNA measured using Hoechst 33258 dye from the total nucleic acid assesses using the ethidium bromide. However, in either case errors in DNA or RNA will translate into errors in the RNA/DNA ratio which could be multiplicative. The use of DNA/DW ratio instead assures that DNA determination and dry weight are independent estimates.

The rate of Protein loss per larva was significant over starvation (0.76 μg d^{-1}, 3.0% d^{-1}). Richard et al. (1991) reported the protein content of sole larvae

decreased from 50 to about 20 μg larva^{-1} over seven days of starvation (an average rate of 8.5% d^{-1}). The protein content of summer flounder larvae studied by Bisbal and Bengston (1995) decreased at a much higher rate during starvation. Six day-old summer flounder larvae lost on average 0.4 μg (13% d^{-1}) of protein and 16-days-old 15 μg (55% d^{-1}) of protein over the first 24 hours of starvation. Westerman and Holt (1994) reported maximum protein catabolism to be in the order of 20% d^{-1} in red drum larvae just prior to the onset of exogenous feeding. These findings suggest protein dynamics may be more species-specific and temperature-dependent than is the case for the other indices investigated. The use of numerous methods for determining protein content may contribute to the variability reflected in the data reported to date.

4.3 Female effects on the dynamics of condition indices

The rates of change (dynamics) of several of the measures of condition we developed for capelin larvae were significantly affected by female source. Both starvation dynamics and the intercept of measures associated with substrate indices (dry weight, protein/larva, prot/dw) were different for larvae from different females. The dynamics of rate indicators based on RNA and DNA, in contrast were unaffected by female source. There were, however, significant differences in their intercept that are attributable to female source. As a consequence, of the nine measures of condition found to be sensitive to starvation only five were found to have a starvation dynamics that was independent of female source (no significant female*post-hatching age interaction). Of those five, RNA per larvae, RNA/DNA ratio and the DNA/DW were most sensitive in their response to starvation. This study is, to the best of our knowledge, the first to definitely show a maternal contribution to the value of biochemical indices of larval condition during starvation. Future interpretation of the statistical significance of these indices should be approached with this reality in mind.

CHAPTER 3

ONTOGENETIC TRENDS AND PERIODICITES IN THE PROTEIN AND NUCLEIC ACID CONTENT OF CAPELIN *(Mallotus villosus)* EMBRYOS AND LARVAE.

1. INTRODUCTION

The literature review presented in chapter 1 concluded that generalizations draw from the measurement of larval fish condition can be strongly influenced by the time and spatial sampling resolution employed relative to the time and space scales of periodic trends in condition. The existence of diel variations in the nucleic acid content of young fish were first suggested by Ferron (1991) and by Mugiya and Oka (1991). These oscillations, were obscured in previous studies because the daily (or longer) sampling frequencies employed. The masking of oscillations or the aliasing effects that can be created by inappropriate sampling scales can seriously bias the conclusions drawn from larval condition studies. It is known for example, that each dominant cycle (sinusoid) of a periodic phenomenon, must be sampled at a minimum frequency of five $cycle^{-1}$, in order to avoid serious "aliasing" and faulty perceptions of the underlying fluctuations (Frank and Taggart, 1990).

It is believed that circadian (24 hrs) biological clocks are common to all eucaryotes, and that their endogenous periodicity is often entrained or synchronized by abiotic factors (Schwassmann, 1971; Palmer, 1974; Gerkema, 1992; Leatherland et al., 1992). There is also evidence that a single self-oscillating clock is responsible for driving circadian rhythms within each cell of an organism (Palmer, 1974). Circadian and ultradian (<24 hrs) oscillations generated by this clock, which occur at the cellular and subcellular levels, are believed to be at the origin of supracellular rhythms, acting through pacemaker (self-sustained oscillator) activity or coupled cellular oscillators (Peters and Veeneklaas, 1992; Leatherland et al., 1992). In fish, a multioscillator model first proposed by Spieler and Noeske (1984) and later described in greater detail by Meier (1992), illustrates the hierarchy of this process by incorporating primary and secondary pacemakers entrained by exogenous factors. Together these act on the neuroendocrine centers and set neural and hormonal rhythms which, in turn, affect fish behavior. In addition to this physiological regulation of cyclical

behavior, such behaviors can also regulate the physiological status of the animal, thus giving rise to a feedback mechanism (Noakes, 1992).

Numerous studies on fish vertical movement, feeding, predator avoidance, and spawning have revealed diel and ultradian rhythms (Schwassmann, 1971; Gibson, 1992; Noakes, 1992). Further, the concentration of several circulating hormones (prolactine, melatonine, cortisol, thyroxine corticosteroids, gonadotropin) have been shown to exhibit diel periodicity (Hontela, 1984; Meier, 1984, 1992; Spieler and Noeske, 1984), in association with the photoreceptive capacity of the retina and the pineal organ (Falcón et al., 1992; Gern et al., 1992; Tabata, 1992). Circadian and ultradian cellular oscillators have been little studied in fish and remain to be identified (Peters and Veeneklaas, 1992).

Fish larvae often display diel vertical migrations (Neilson and Perry, 1990) and diurnal variation in oxygen consumption following activity rhythms (Rombough, 1988). Given their undifferentiated state of organ development, it is believed that fish embryos lack the capacity to synthesize hormones possessed by older animals, but must rely on maternal sources provided in the egg yolk (Mommsen and Walsh, 1988). Given the general lack of knowledge concerning cellular oscillators in adult fish, it is not surprising that we know even less about the existence of cellular or neurohormonal oscillations in larvae.

Ferron's (1991) studies were designed to investigate the impact of food availability on nutritional condition in marine fish larvae, and revealed circadian oscillations of unknown origin in the protein and nucleic acid content of field-collected mackerel *(Scomber scombrus)* larvae. The amplitude of these oscillations significantly influenced measures of larval condition based on RNA/DNA ratios, and were responsible for an approximately 50% difference between day and night values.

Biochemical estimators of larval condition, and particularly RNA/DNA ratios, are now being widely used to assess the nutritional state and feeding status of larval fishes in the wild. Their use is based on the assumption that feeding and in some instances temperature are the primary factors affecting the estimators (see review chapter 1). Ferron's (1991) findings together with evidence from other studies (Mugiya and Oka, 1991; Rooker and Holt, 1996; Chicharo et al., 1998), suggest that diel variations in RNA/DNA ratios may be common and should be considered when sampling fish to assess their condition. This is particularly important when such condition measures are based on larvae collected in oceanographic ichthyoplankton surveys, where grids of stations are typically visited at different times of the day, and hence aliasing of the data is likely if significant periodicities exist.

In this study we investigated the existence and the ontogeny of circadian and ultadian oscillations in the nucleic acid and protein content of laboratory-reared fish larvae.

2. MATERIAL AND METHODS

2.1 Rearing and sampling protocol

Pre-spawned adult capelin *(Mallotus villosus)* were collected from fishermen's traps in Bryant's Cove (Newfoundland) on June 15-20, 1990. Males and females were maintained in separate net cages attached to a raft moored in the cove. On July 5, 40 males and 40 females were collected from the cages with dip nets, and transferred into separate outdoor 300 L tanks provided with running seawater at ambient temperature and located adjacent to the laboratory facilities. Embryos were produced by artificial crosses performed on July 7 and 11, 1990. Fourteen half-sib families were generated following procedures outlined in Chapter 2. All eggs obtained from each female were fertilized. The eggs were extruded in consecutive batches of 1000-1500 eggs into separate

petri dishes maintained on ice. This was repeated until no additional eggs could be obtained from a single female (usually after 16-18 batches). The eggs were fertilized with the pooled milt obtained from several males. The fertilized eggs were incubated in the petri dishes in which they were fertilized, and all dishes from a single female were maintained separately by floating them in plastic trays supplied with circulating water maintained at 7° C. The water in the petri dishes was changed daily using fresh U.V. sterilized 0.45µm filtered seawater. A 16D:8D photoperiod was used with darkness occurring between 22:00 and 6:00 Newfoundland standard time.

Developing capelin embryos were sampled from a single female (tray). This was done to minimize potential female effects on offspring composition (see Chapter 2). Five to seven embryos were collected every two hours from dishes selected at random from the tray. The eggs were rinsed with distilled water, pipetted into a micro-tube and frozen in liquid nitrogen for later biochemical analysis. The family used was spawned on July 7 and the sampling of embryos was initiated two hours after fertilization, and continued 24 hrs d^{-1} until 19 d post-fertilization, at which time, a large number of larvae started hatching. Because of a shortage of hatchlings from the first female, the larvae from a second female had to be used to provide yolk-sac and post-yolk-sac larvae. Therefore, approximately 1800 larvae were obtained from a second female whose eggs were fertilized on July 11 and transferred as they hatched daily from the petri dishes to a common 38.8 L black tank (40 cm diameter) maintained in a 7°C water bath for 13 consecutive days (hatching age = 17-29 d post-fertilization). These larvae were fed and sampled every two hours for 24 hrs d^{-1} beginning on day 22 post-fertilization (528 hrs) and continuing until 35 d (840 hrs) post-fertilization. Temperature and photoperiod during incubation, hatch, and subsequently was identical to that experienced by the embryos and larvae from the first family. The larvae from female 2 were fed wild plankton daily beginning on the 6th day after the first hatchlings were obtained. The daily ration consisted of 500 ml of cultured algae (*Dunaniella* spp.) together with

natural plankton in quantities required to maintain a density of 1000 copepod nauplii l^{-1} (see Chapter 4 for details of the plankton collections). All embryos and larvae obtained from the two females were sampled every 2 hrs on a 24 hrs cycle at 00h30, 2h30, 4h30, 6h30, 8h30, 10h30, 12h30 14h30, 16h30, 18h30, 20h30 and 22h30 daily. All frozen eggs and larvae were transferred from Newfoundland to Montreal on dry ice, where they were maintained at -80° C. During the month following transfer, they were vacuum freeze dried for 24 hrs and then maintained at -20° C on drierite in dessicators for several months while biochemical assays were performed.

2.2 Biochemical assays

Reagents and procedures used for the preparation of reagents and standards are identical to those detailed in Chapters 2 and 4. We had planned to conduct dry weight, nucleic acid and protein assays on individual embryos and larvae. This proved impossible in many cases because of difficulties encountered in separating the freeze-dried embryos and larvae removed from each microtube without breaking and losing parts. Most homogenates were therefore made of the pooled five embryos or five larvae sampled from a single female at a given time. The protein content of each homogenate was determined on three aliquots of the crude homogenate, while nucleic acids were measured from a single aliquot which was extracted and purified following the procedure outlined in chapter 2. All samples were homogenized and assayed randomly.

Protein content was determined on groups of 12 samples (36 triplicates), along with triplicates of one blank and of four bovine serum albumin standards. The absorbency readings were corrected for a linear temperature effect using the values calculated from chapter 4 data. Most measurements were taken over a four weeks period. A total of 8 calibrations, each comprised of triplicates of 8 different bovine serum albumin concentrations encompassing the range of

samples values were run during the same period. All those calibration lines had $R^2>0.990$, and none differed significantly (ANCOVA, $P<0.001$) from one another, also none of the daily standards differed significantly (ANOVA, $P<0.001$) from the calibration done on corresponding days. The calibration with the intercept closest to zero was chosen to calculate sample protein content and is described by the following relationship

$$[\text{protein ug/ml}] = 14.84019\ O.D_{595nm} + 0.00304 \qquad (N=27,\ R^2=0.997)$$

where O.D. = optical density (absorbency reading).

A total of 566 samples x 3 replicates (1698 values) were measured. Large particles of larval tissue remained in solution in 41/566 (7.2%) of the samples following processing. These appear to have resulted from incomplete homogenization. To avoid bias in the results obtained, these 41 samples were eliminated. The conclusions drawn in this chapter are therefore based on the analysis of 525 samples (including 48 blanks). After calculating the amount of protein in µl/ml and then in µg/homogenate using the appropriate dilution factors, outliers were identified and removed from all sets of triplicates using a Q-value of 0.94 for a 90% confidence level (Smith, 1998). A total of 34/477 (7.1%) of the samples had one triplicate outlier removed this way. The coefficient of variation for the 477 triplicate samples remaining was 12.40 %. The average value from the 48 triplicate blank samples measured was 5.05 ± 6.73 µg of protein per homogenate and 27/48 (56%) of those blanks exhibited values not significantly different from zero (t-test, $P<0.05$).

Nucleic acids were measured by fluorescence using the Hoeschst 33258 (Bizbenzimidazole) and ethidium bromide (Ebr) specific binding dyes (see chapter 2 and 4 for the full details). Two triplicate DNA standards and one triplicate extracted blank were measured along with each set of 24 samples processed. The mean Biz and Ebr fluorescence of all triplicate blanks was used

to subtract the background fluorescence from samples in absence of nucleic acids. All measurements were made within a two week period. Two sets of calibrations were made prior to samples measurements and one set immediately following. Each set of calibrations consisted of 9 triplicates (27) of a range of concentrations of DNA and RNA standards along with the Biz and Ebr dyes. None of the three calibration lines obtained for DNA+Hoechst 33258 (Biz), DNA+Ethidium bromide (EBr), and RNA+EBr differed significantly (ANCOVA, $P>0.001$) from one another. Therefore the calibrations conducted immediately before the samples were assessed were used, and are described by the following relationships:

$$[DNA] = 0.11559\ f_{Biz} + 0.03635 \qquad (N=27,\ R^2=0.988)$$

$$f_{EBr(DNA)} = 0.58254\ [DNA] - 0.01801 \qquad (N=27,\ R^2=0.987)$$

$$[RNA] = 1.56916\ f_{EBr(RNA)} + 0.03816 \qquad (N=27,\ R^2=0.994)$$

Where f = fluorescence reading.

These equations were used to calculate the DNA and RNA concentrations in the samples. The average DNA and RNA content of the 48 blanks measured was 1.51 ± 0.40 and -0.15 ± 1.28 µg/homogenate respectively, and did not differ significantly (t-test, $P>0.001$) from zero.

2.3 Data analysis

Nucleic acid and protein content were determined on pooled eggs or larvae homogenates for a total of 190 embryos and 55 yolk-sac larvae homogenates obtained from female 1; and on 121 post-yolk-sac larvae homogenates from female 2. From this total of 475 homogenates, 30 (6.3%) had a protein content not significantly (t-test, $P>0.05$) different from the blanks and were eliminated. These samples were probably damaged and parts lost during processing. Also

2/475 (0.4%) had a RNA content not significantly (t-test, P>0.05) different from the blanks and had to be eliminated.

Protein, DNA, and RNA values were computed in µg/homogenate. These values were then divided by the number of eggs or larvae homogenized to estimate the quantity per individual (µg/egg or µg/larva). We also expressed the output as µg/dry weight but these data, which were few in numbers are not reported as series. Rather, they are used for comparative purposes only.

RNA/DNA, RNA/Protein and Protein/DNA ratios were computed as sample means and displayed as time series. These time series were constructed for each of the six variables determined (Protein, DNA, RNA content per egg or larva, and RNA/DNA, RNA/Protein, and Protein/DNA). The series were plotted in relation to post-fertilization age. Because the biochemical composition of embryos and of larvae is likely to differ, and may exhibit discontinuities at hatching (Zeitoun et al., 1977; Buckley, 1979, 1980), the embryonic and larval series were analyzed separately.

The newly-hatched larvae sampled from female 1 had identical post-hatching age and post-fertilization age at sampling. Those sampled from female 2 had identical post-fertilization age but varied in post-hatching age by up to 13 days. Only larvae from female 2 were fed. We evaluated these two larval groups separately in the analyses that follow. Time first series included all embryos from female 1 and is thereafter referred to as the embryo series (post-fertilization age = 2-504 hrs, N = 251). The second series included all larvae from female 1 and is hereafter referred to as the newly-hatched series (post-hatching age = 0, post-fertilization age = 448-614 hrs, N = 84). The third series included all larvae from female 2 and is hereafter referenced as the post-yolk-sac series (post-hatching age = 0-13 d, post-fertilization age = 624-944 hrs, N = 161).

Spectral analysis was performed using the SERIES-Fourier procedure of SYSTAT (Wilkinson, 1998). This algorithm applies only to the analysis of series with lengths that are powers of 2. The series were therefore truncated or padded with zeros to fit the nearest power of 2 (Wilkinson, 1998). The embryonic series contained a total of 251 observations. Therefore, five lines of zeros were added at the end of the record to create a record length of 256. The newly-hatched larvae series contained 84 observations, we therefore removed the first 21 lines of data and added one line of zeros at the end to create a record of 64. We chose to remove data from the beginning rather than the end of the series because a greater number of missing values occurred in the early part of the record. At this stage, embryos were still being sampled along with newly-hatch larvae from female 1. For the post-yolk-sac larvae series, the first or last 35 lines of the file were removed, creating two separate records of 128 observations. In this case, because the same number of missing values occurred at the beginning and at the end of the record we applied separate analysis to two segments. Each of the four series described above were detrended prior to analysis to assure stationarity, and to ensure that the mean and variance did not vary across time. Linear trends were removed using two techniques; the SERIES-Trend and the SERIES-Difference procedures of SYSTAT (Wilkinson, 1998). The SERIES-trend utilizes the method of least squares for linear trend fitting. The SERIES-Difference replaces each value by the difference between it and the previous value of the series. This latter technique is considered more efficient in removing trends (Wilkinson, 1998). This was evident in the analyses we conducted. Therefore, in all cases, the periodograms obtained on series detrended using the SERIES-Difference technique showed fewer low-frequency peaks than the periodograms obtained from the same series when detrended using the SERIES-Trend procedure. We report only those results obtained using the SERIES-Difference detrending procedure. Some non-linear trends may remain following this detrending procedure, however, the procedure does reduce the low-frequency variance, and thus renders significant higher frequency periodicities more obvious.

Missing values were interpolated by local quadratic smoothing, a built-in feature of the SERIES algorithm (Wilkinson, 1998). The largest gap interpolated was one of 18 sampling intervals in length. This occurred once in the embryonic time series. This series also contained two gaps of 5 and numerous gaps of one for a total of 61 missing values. The yolk-sac series contained two gaps of 4 and two gaps of two for a total of 22 missing values, while the post-yolk-sac series contained one gap of three sampling intervals and 6 gaps of two for a total of 32 missing values. The effect of interpolating missing values on the overall results of the analysis is judged to have been minimal .

3. RESULTS

Spectral or Fourier analysis decomposes a time series into a finite sum of sine and cosine waves of different frequencies. This facilitates detection of significant inherent non-random cyclic variations. Long-term trends plague spectral analysis because the series, unless detrended, is considered non-stationary and the results of the analysis may become inconclusive or invalid (Wilkinson, 1998). This is usually detected as a high variance at the lowest frequencies on the periodogram. However, the identification of these longer term trends, as they are removed by detrending, can shed insights into the underlying mechanisms involved (Legendre and Dutilleul, 1992). The following section describes the long-term trends that were removed from the series.

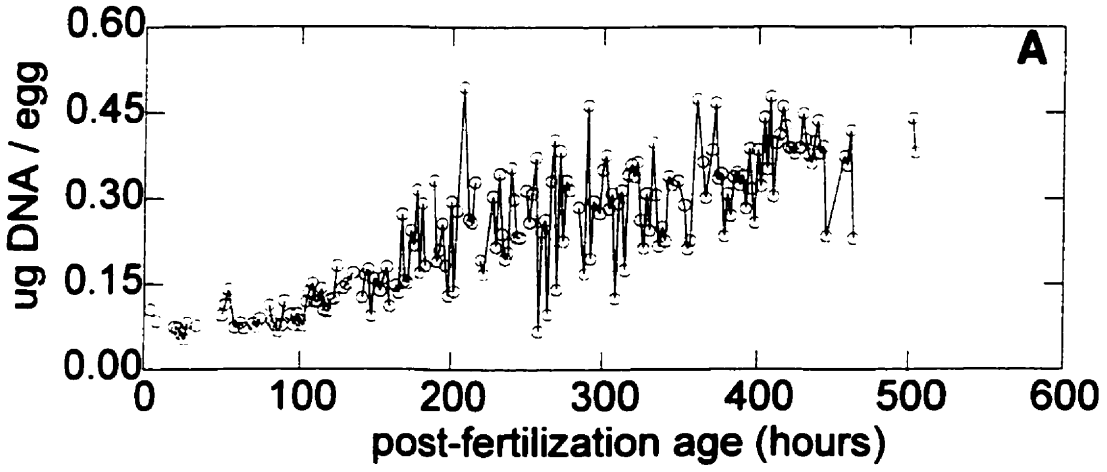
3.1 Ontogenetic trends in biochemical composition

The most obvious long-term trends in the biochemical measurements taken occurred during the period of embryonic development. Both the DNA and the RNA content of the embryos increased constantly during development. Hatching levels of both RNA and DNA (1.25 and 0.40 μg egg $^{-1}$) were approximately five times higher than at fertilization (0.30 and 0.06 μg egg $^{-1}$,

FIGURE 1a. Time series of the mean DNA content per embryo.

FIGURE 1b. Time series of the mean RNA content per embryo.

The embryonic period extends from fertilization until hatching which began at approximately 450 hours post-fertilization



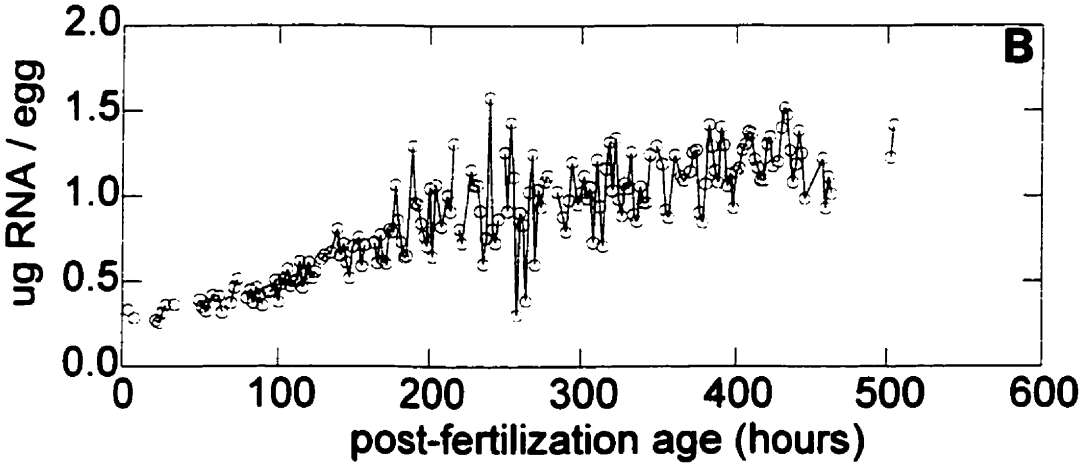
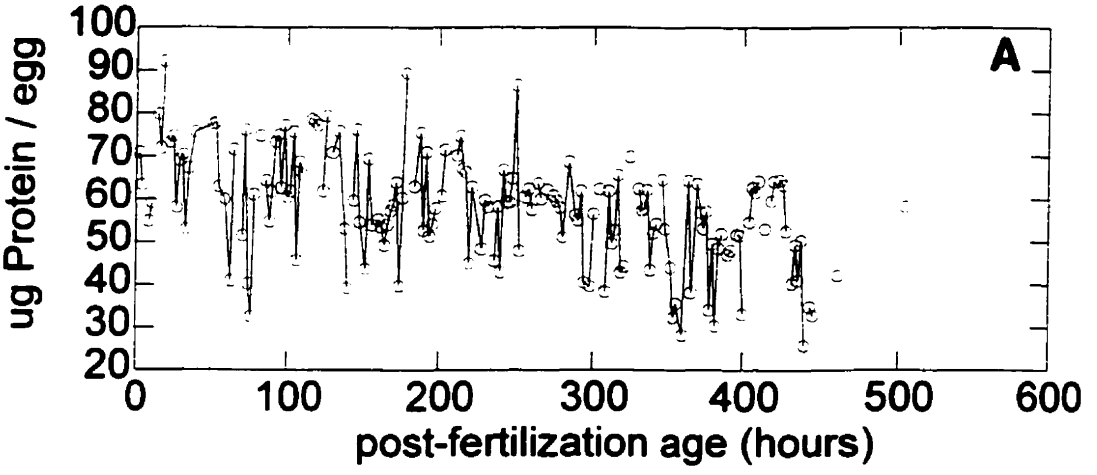


FIGURE 2a. Time series of the mean Protein content per embryo.

FIGURE 2b. Time series of the mean RNA/DNA ratio per embryo.

The embryonic period extends from fertilization until hatching which began at approximately 450 hours post-fertilization



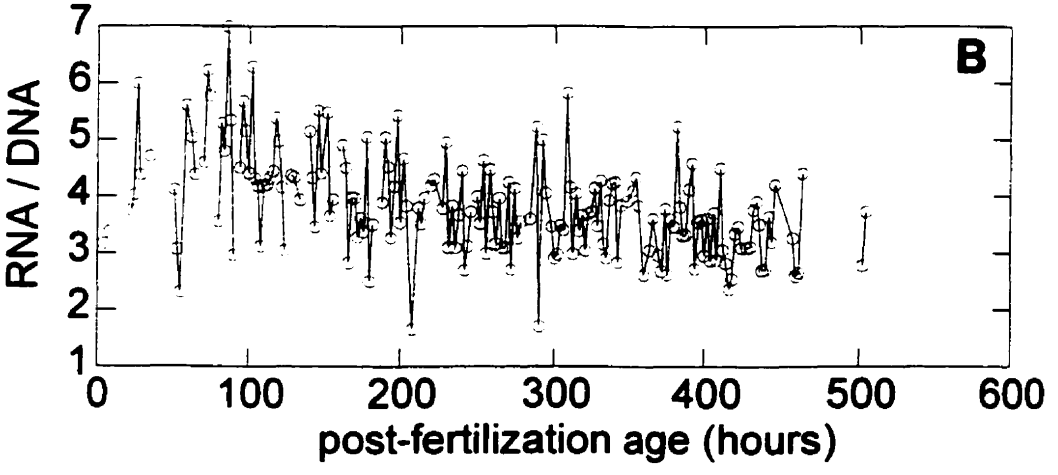
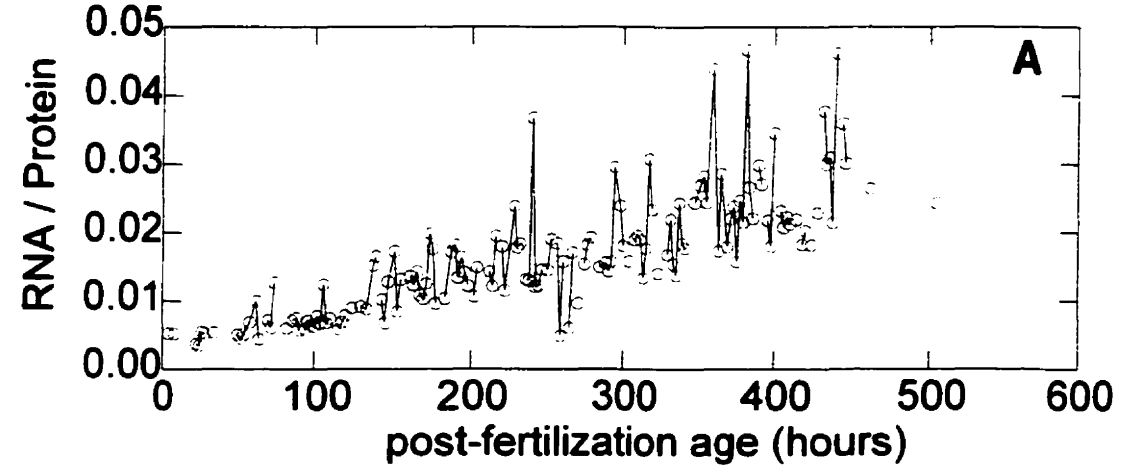
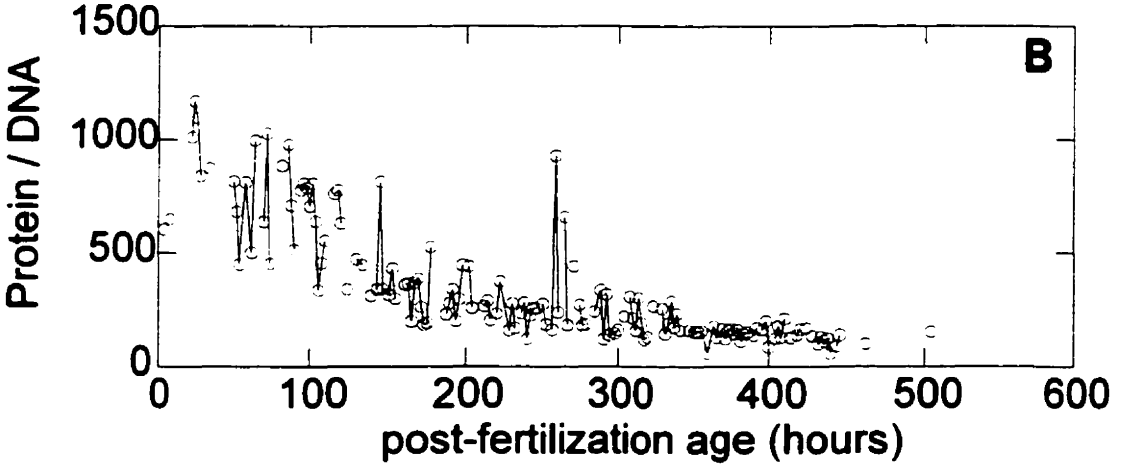


FIGURE 3a. Time series of the mean RNA/Protein ratio per embryo.

FIGURE 3b. Time series of the mean Protein/DNA ratio per embryo.

The embryonic period extends from fertilization until hatching which began at approximately 450 hours post-fertilization





Figures 1b and 1c). No increase in embryonic DNA occurred prior to 100 hrs post-fertilization, while RNA increased slowly during this interval. After 100 hrs post-fertilization, both the RNA and DNA increased significantly until about 300 hrs post-fertilization when a plateau appears to have been reached. A 50% decrease in the protein content of embryos occurred during the period of embryonic development (Figure 2a), with levels declining from approximately 80 $\mu g\ egg^{-1}$ following fertilization, to 40 $\mu g\ egg^{-1}$ prior to hatching. The RNA/DNA ratio of embryos decreased from 5.0 after fertilization to about 3.0 prior to hatching. This occurred because DNA levels increased at a faster rate than RNA levels (Figure 2b). The RNA/Protein and Protein/DNA ratios were affected positively and negatively (respectively); (Figures 3a and 3b), by the decreasing Protein levels. The increasing trend in the RNA/Protein ratio was linear, but the decreasing trend in the Protein/DNA was clearly curvilinear with a rapid drop within the first 200 hours of embryonic development (Figure 3b).

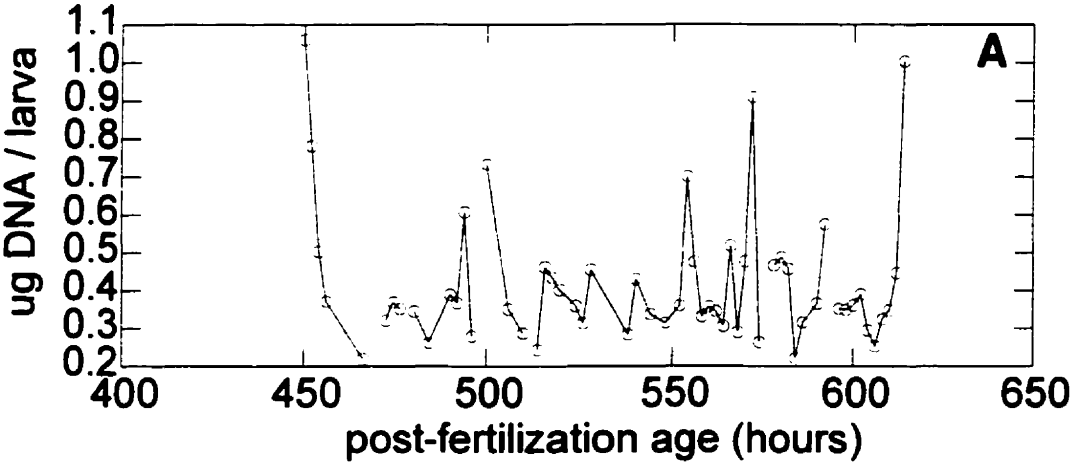
Newly-hatched larvae exhibited no meaningful long-term trends in the biochemical components measured (Figures 4-6). The only exception was the RNA/DNA ratio which decreased linearly with age (Figure 5b). Larvae that hatched later did so at a cost of a lower RNA/DNA ratio. Persistent cyclic trends were evident in Protein (Figure 5a) and in the Protein/DNA ratio (Figure 6b). The highest Protein and Protein/DNA ratio values occurred in the first half of the series prior to 550 hours after post-fertilization. The RNA/Protein and Protein/DNA ratios exhibited higher variance in the second half of the series (Figures 6a and 6b).

Decreasing trends in the Protein and RNA content of post-yolk-sac larvae (Figures 7b and 8a) resulted in a decrease in the RNA/DNA and Protein/DNA ratios with time (Figures 8b and 9b). The Protein, RNA and Protein/DNA series all exhibited a sharp drop at 750 hours followed by a linear decrease thereafter (Figures 7b, 8a and 9b). The RNA/DNA ratio decreased linearly from first sampling to 825 hours post-fertilization (ratio value = 2.0) after which it

FIGURE 4a. Time series of the mean DNA content per larva for newly-hatched larvae derived from female 1.

FIGURE 4b. Time series of the mean RNA content per larva for newly-hatched larvae derived from female 1.

The hatching window extends from 450 to 620 hours post- fertilization.



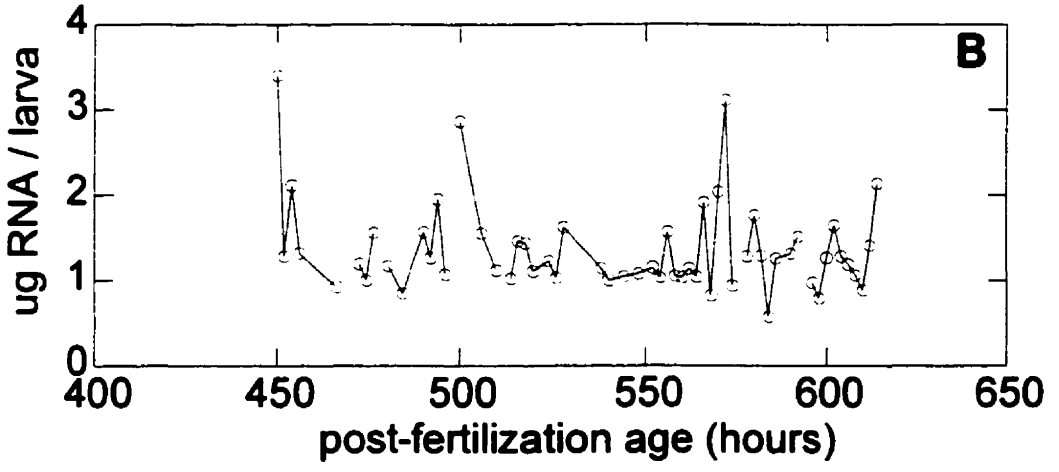
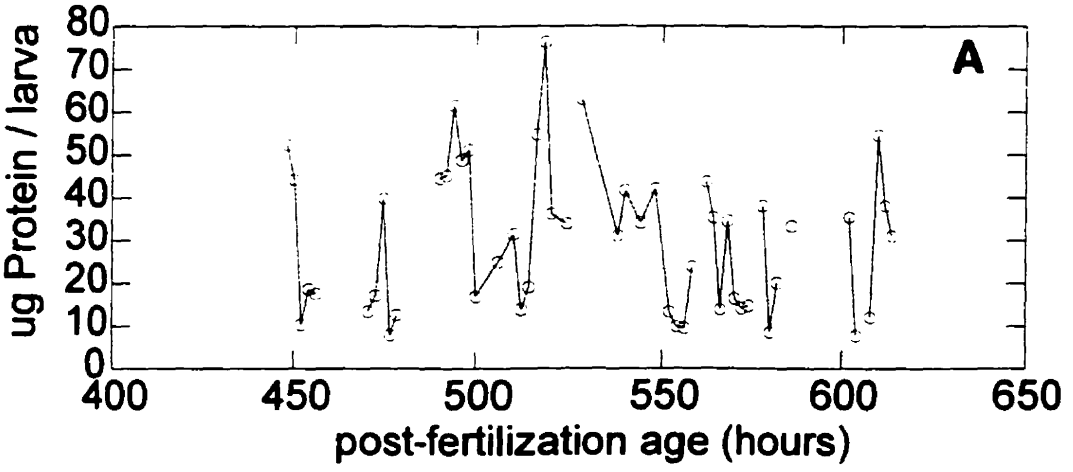


FIGURE 5a. Time series of the mean Protein content per larva for newly-hatched larvae derived from female 1.

FIGURE 5b. Time series of the mean RNA/DNA ratio per larva for newly-hatched larvae derived from female 1.

The hatching window extends from 450 to 620 hours post- fertilization.



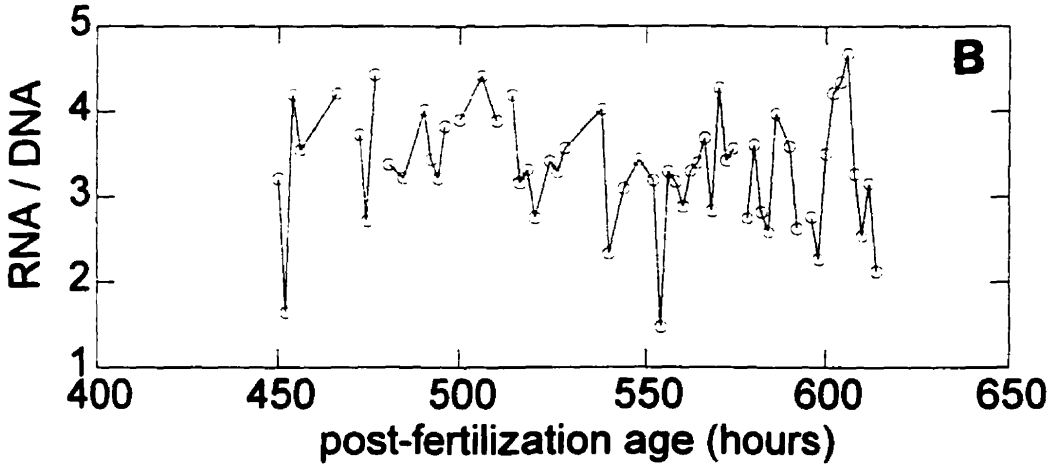
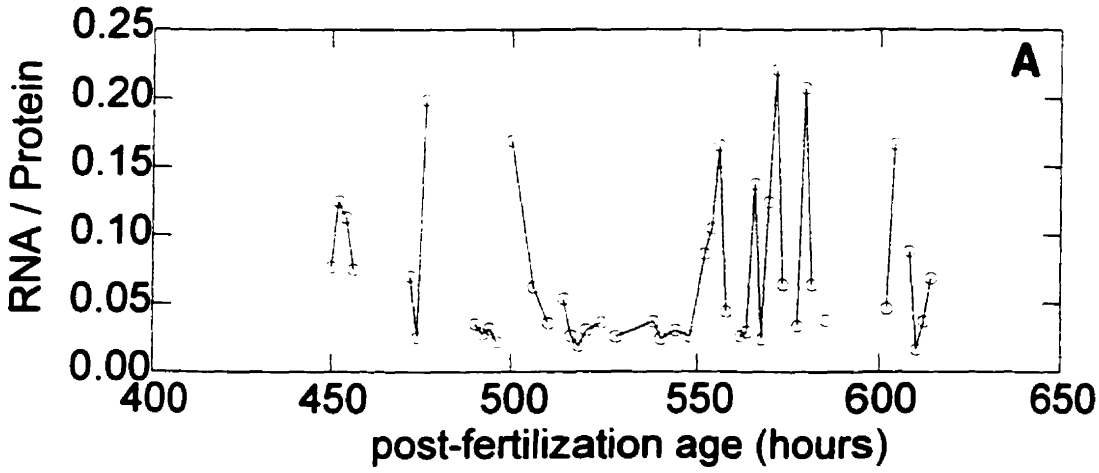
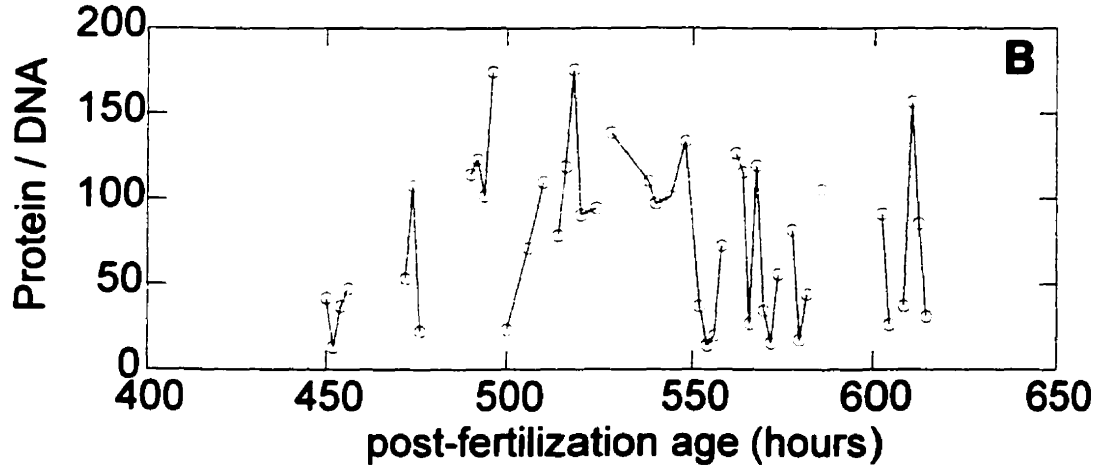


FIGURE 6a. Time series of the mean RNA/Protein ratio per larva for newly-hatched larvae derived from female 1.

FIGURE 6b. Time series of the mean Protein/DNA ratio per larva for newly-hatched larvae derived from female 1.

The hatching window extends from 450 to 620 hours post- fertilization.





stabilized (Figure 8b). The DNA series showed an early increase followed by a slow decline in the later half of the series (Figure 7a).

3.2 Periodicities in biochemical composition

Spectral analysis involves the analysis of the magnitude of the trigonometric components of a series in relation to their frequency. This was performed on the time series to further investigate the existence of inherent periodic components. The magnitude of the peaks revealed on the periodogram are considered to be proportional to the contribution of different waveforms at a given frequency to the overall shape of the series. The results of the spectral analysis for the 18 series described above, showed no significant peaks for 10 of the series. The periodograms of DNA and RNA content and RNA/DNA and RNA/Protein ratios of the embryos, yolk-sac and post-yolk-sac larvae were practically blank. This does not mean that no significant inherent cycles are present in those series. Rather the only conclusion to be safely drawn is that no strong or consistent periodicities could be detected by the analysis performed, and that the variability observed must be interpreted as random. Only the quantity of protein (per egg or larvae) and the Protein/DNA ratio exhibited significant peaks. These periodograms are displayed in Figures 10-13.

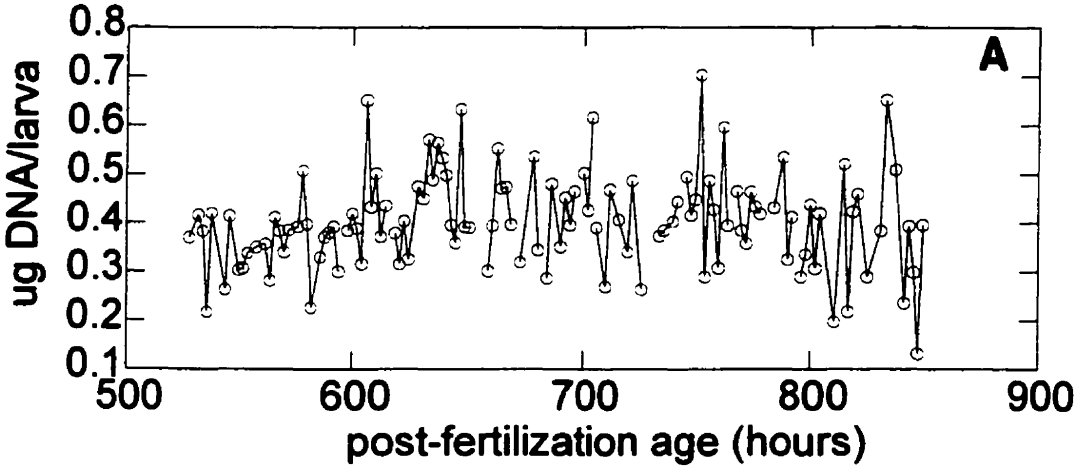
The protein periodogram of developing embryos exhibited a dominant peak at a period equivalent to the length of the series itself (Figure 10a, 256 hrs). This indicates that the series was not fully detrended by the procedures applied. No such low-frequency trends were evident in the Protein/DNA ratio of embryos (Figure 10b) or in the Protein and Protein/DNA ratios of yolk-sac and post-yolk-sac larvae (Figures 11-13), we conclude that these series were efficiently detrended.

The Protein content of embryos displayed peaks at frequencies corresponding to periods of 64.0, 28.4, 10.2, 8.0 and 6.6 hrs (Figure 10a).

FIGURE 7a. Time series of the mean DNA content per larva for yolk-sac and post-yolk-sac larvae derived from female 2.

FIGURE 7b. Time series of the mean RNA content per larva for yolk-sac and post-yolk-sac larvae derived from female 2.

The larvae that hatched on 13 consecutive days were stocked in tanks, sampled starting on the 5th day (528 hrs post-fertilization) and provided with food 8 d after the first hatchlings were stocked (600 hrs post-fertilization).



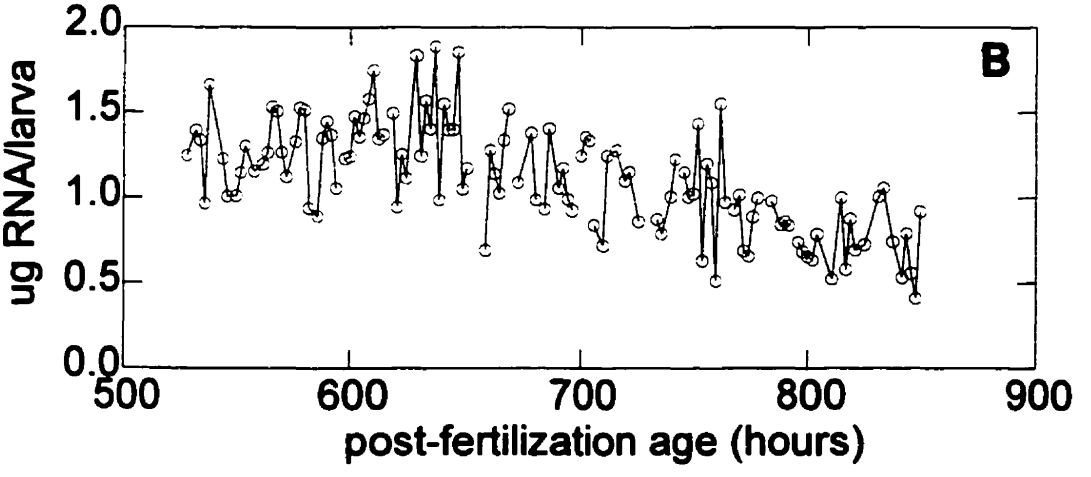
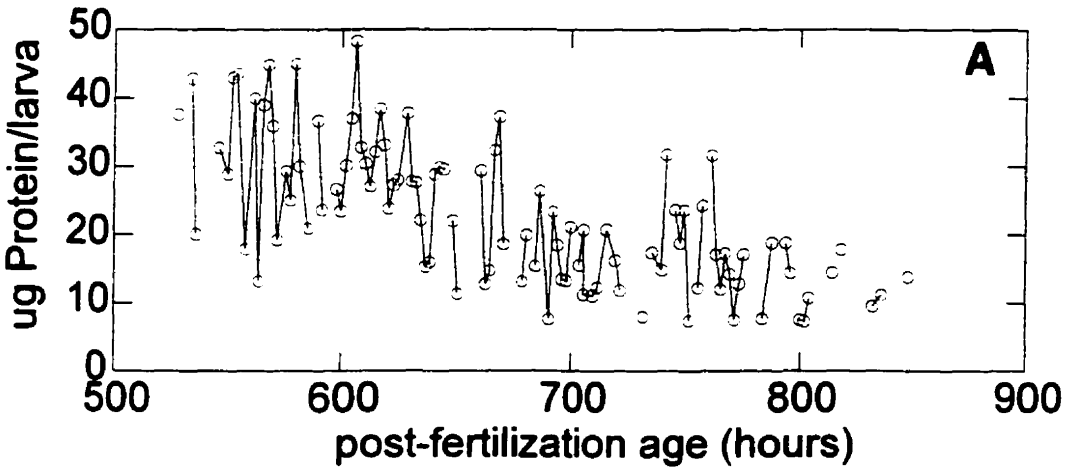


FIGURE 8a. Time series of the mean Protein content per larva for yolk-sac and post-yolk-sac larvae derived from female 2.

FIGURE 8b. Time series of the mean RNA/DNA ratio per larva for yolk-sac and post-yolk-sac larvae derived from female 2.

The larvae that hatched on 13 consecutive days were stocked in tanks, sampled starting on the 5th day (528 hrs post-fertilization) and provided with food 8 d after the first hatchlings were stocked (600 hrs post-fertilization).



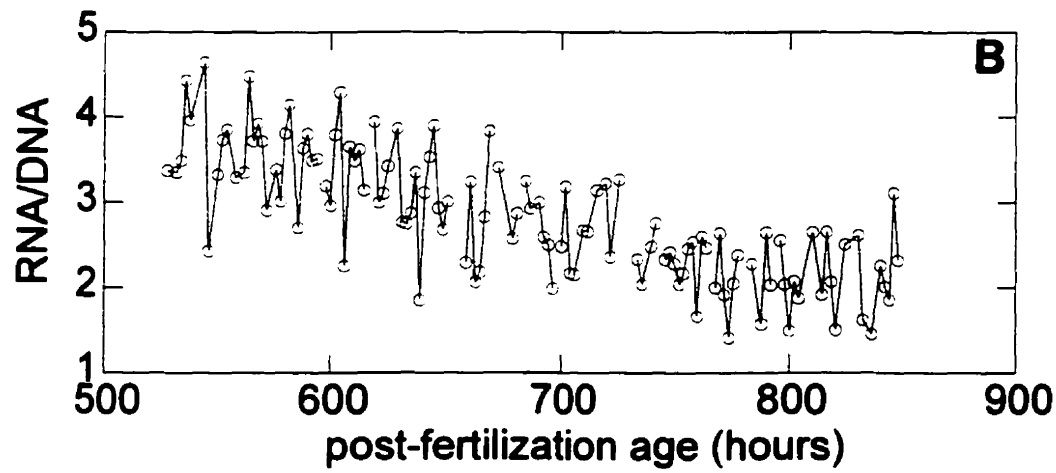
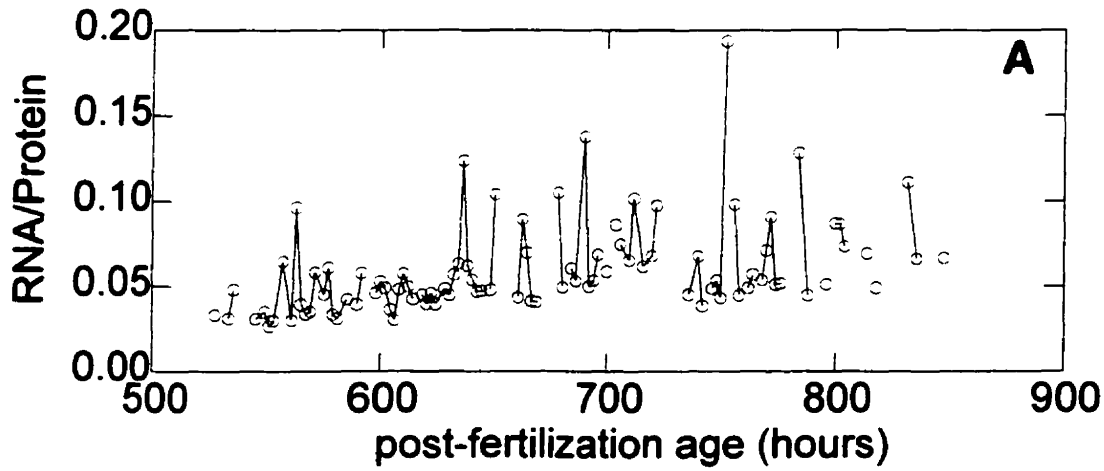


FIGURE 9a. Time series of the mean RNA/Protein ratio per larva for yolk-sac and post-yolk-sac larvae derived from female 2.

FIGURE 9b. Time series of the mean Protein/DNA ratio per larva for yolk-sac and post-yolk-sac larvae derived from female 2.

The larvae that hatched on 13 consecutive days were stocked in tanks, sampled starting on the 5th day (528 hrs post-fertilization) and provided with food 8 d after the first hatchlings were stocked (600 hrs post-fertilization).



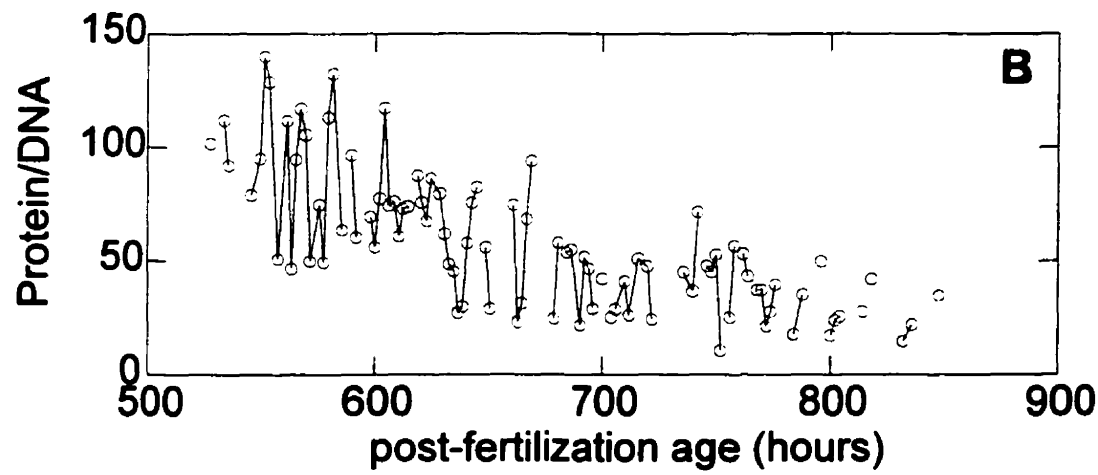


FIGURE 10a. Periodogram obtained from the spectral analysis of the Protein content per embryo time series. The major peaks are identified by the corresponding periods (1/frequency).

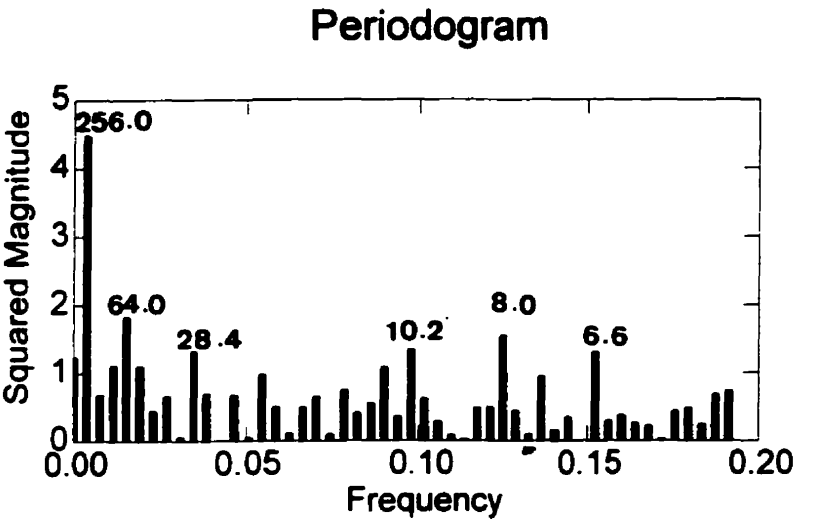


FIGURE 10b. Periodogram obtained from the spectral analysis of the Protein/DNA ratio per embryo time series. The major peaks are identified by the corresponding periods (1/frequency).

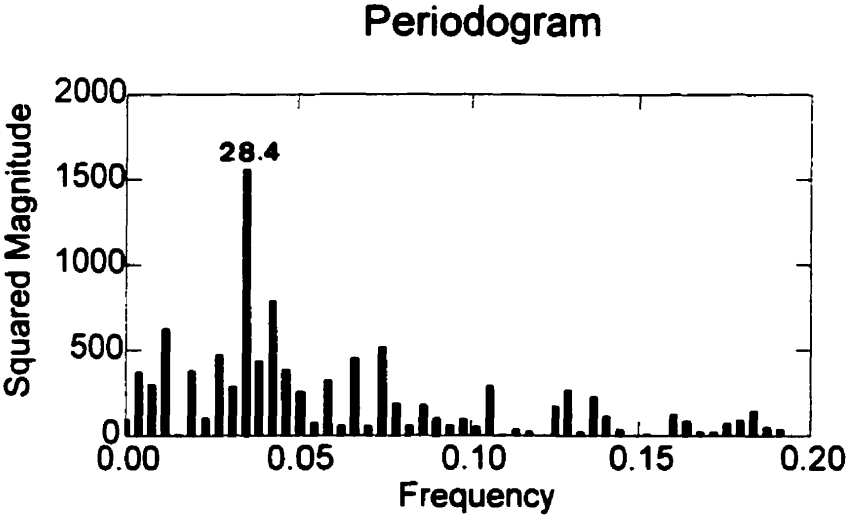


FIGURE 11a. Periodogram obtained from the spectral analysis of the Protein per larva time series for the newly-hatch larvae derived from female 1. The major peaks are identified by the corresponding periods (1/frequency).

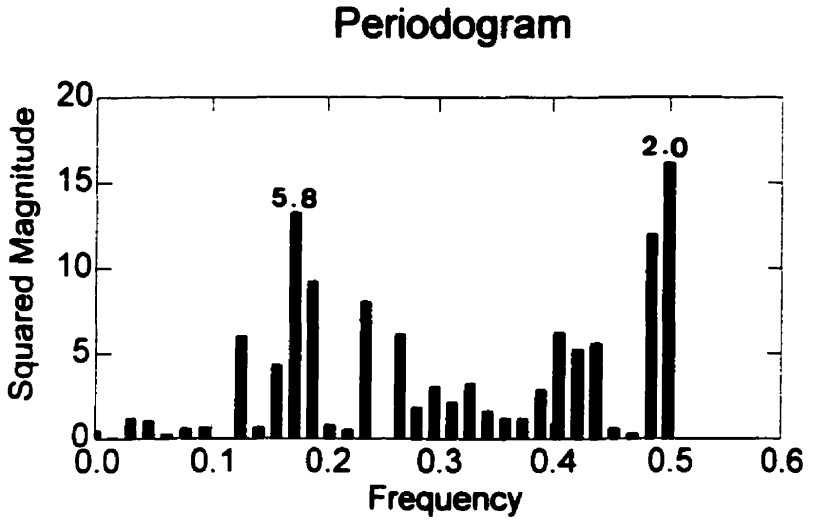


FIGURE 11b. Periodogram obtained from the spectral analysis of the Protein/DNA ratio time series for the newly-hatch larvae derived from female 1. The major peaks are identified by the corresponding periods (1/frequency).

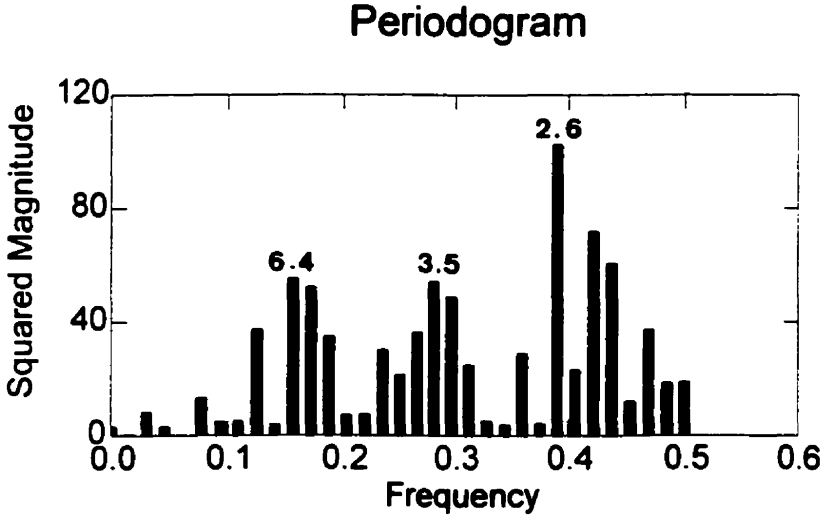


FIGURE 12a. Periodogram obtained from the spectral analysis of the Protein per larva time series for the yolk-sac and post-yolk-sac larvae derived from female 2. The series has been truncated at the beginning of the record prior to analysis. The major peaks are identified by the corresponding periods (1/frequency).

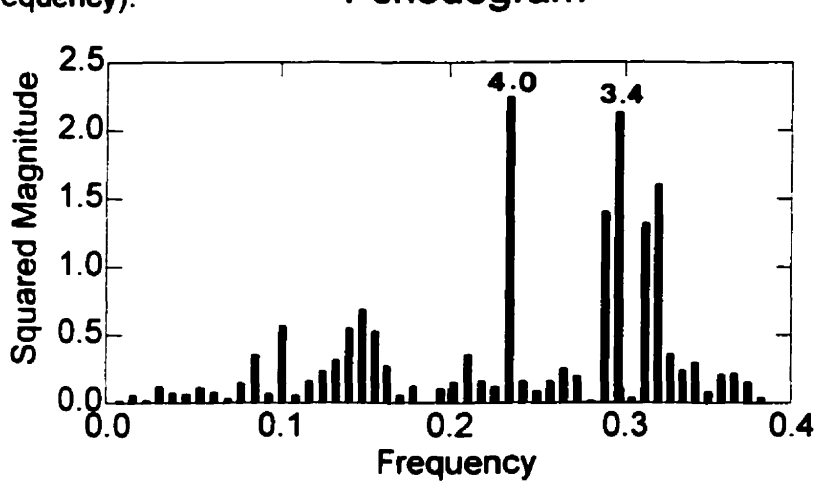


FIGURE 12b. Periodogram obtained from the spectral analysis of the Protein/DNA ratio time series for the yolk-sac and post-yolk-sac larvae derived from female 2. The series has been truncated at the beginning of the record prior to analysis. The major peaks are identified by the corresponding periods (1/frequency).

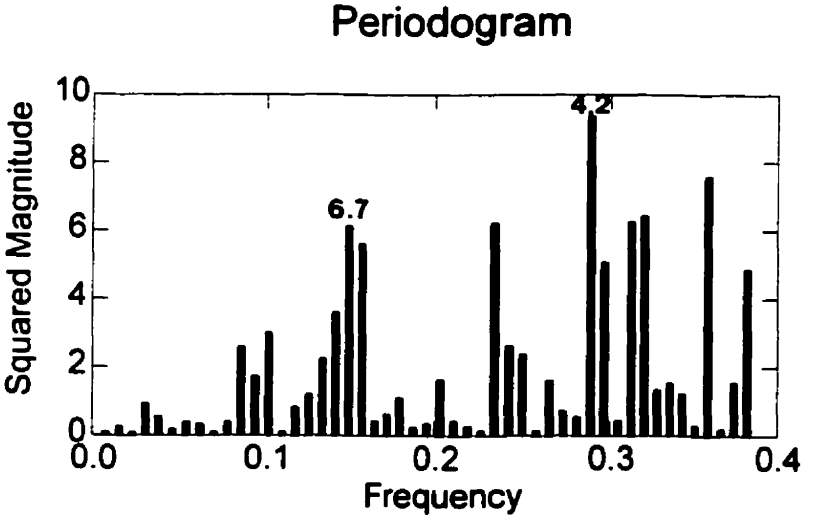


FIGURE 13a. Periodogram obtained from the spectral analysis of the Protein per larva time series for the yolk-sac and post-yolk-sac larvae derived from female 2. The series has been truncated at the end of the record prior to analysis. The major peaks are identified by the corresponding periods (1/frequency).

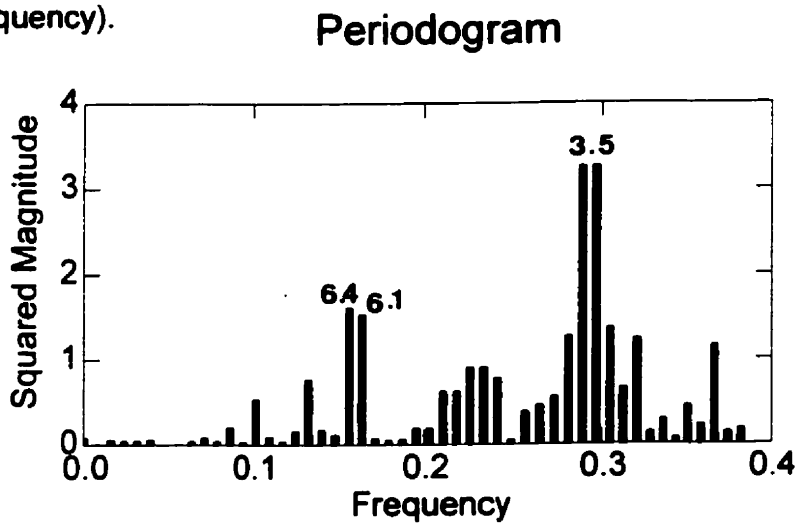
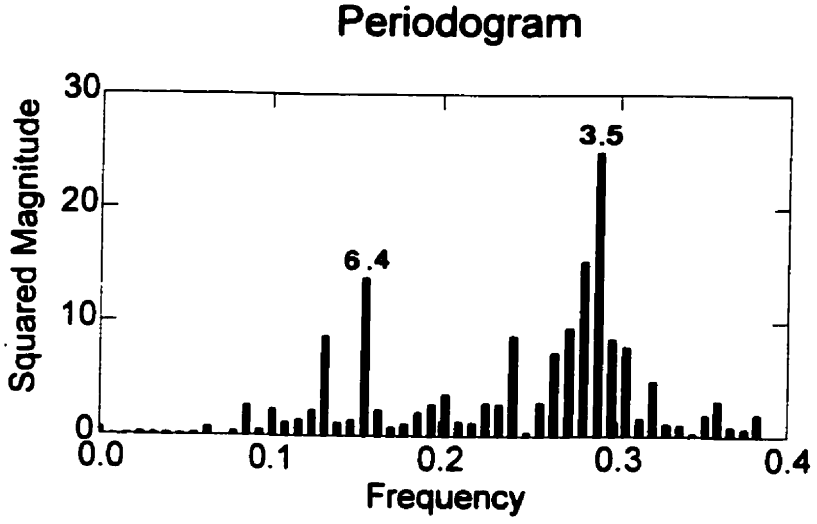


FIGURE 13b. Periodogram obtained from the spectral analysis of the Protein/DNA ratio time series for the yolk-sac and post-yolk-sac larvae derived from female 2. The series has been truncated at the end of the record prior to analysis. The major peaks are identified by the corresponding periods (1/frequency).



These peaks were somewhat masked by the presence of the long-term trend (256 hrs) which was not fully removed by the detrending procedure employed. The periodicity of 64 hrs (2.6 d) is apparent from the plot of the time series itself (Figure 1a) as it repeats itself about six times over the entire embryonic period. The amplitude of the cycles (difference between peaks and troughs) was approximately 20 μg egg^{-1} (Figure 1a). This implies that the protein content of the embryos sampled during the peaks was approximately twice the minimum values of those sampled 32 hrs earlier or later. The Protein/DNA ratio series exhibited one major peak at 28.4 hrs, and very low variance at low frequencies (confirming the effective removal of the non-linear decrease depicted by this ratio; Figure 3b).

The periodograms obtained from the protein content and Protein/DNA ratio of newly-hatched larvae revealed no peaks at low frequencies (Figures 11a and 11b). We conclude that no long-term trends occurred in these series. This was expected from the series plots. Protein values exhibited peaks at 5.8 and 2.0 hrs, while the Protein/DNA ratio exhibited the strongest periodicities at 2.6, 3.5 and 6.4 hrs. The series were characterized by high variability (noise) after 550 hrs post-fertilization. This is likely to have resulted in the occurrence of the periodogram peaks at periods shorter than 6 hours.

The protein content and Protein/DNA ratio of post-yolk-sac larvae decreased with time. These trends were removed effectively as indicated by the absence of peaks at low frequencies (Figures 12a, 12b, 13a and 13b). The spectral analysis of the protein content revealed cycles at 3.5 hrs in both truncated series. At lower frequencies, the timing of the peaks differed. Peaks were obtained at 4.0 hrs in the case of the series truncated at the beginning (Figure 12a), and at 6.4 hrs in the case of the same series truncated at the end (Figure 13a). Protein/DNA periodograms were more consistent between series (Figure 12b and 13b) and exhibited cyclic patterns at 3.5-4.2 and 6.4-6.7 hrs). These were very similar to those obtained for the protein content (Figure 13a).

4. DISCUSSION

4.1 Ontogenetic trends and discontinuities

Amphibian and fish eggs accumulate material in their cytoplasm from the mother's bloodstream during oogenesis, such that when fertilization occurs, they possess all the energy sources, organelles, enzymes, precursors of DNA, RNA and protein synthesis, stored mRNA, structural proteins and morphogenic determinants required to initiate cleavage and embryonic development (Gilbert, 1991). A fish egg is a semi-closed system. Its membrane allows gas exchange but does not allow most solutes to diffuse (Heming and Buddington, 1988). The growing embryos and larvae are, therefore, entirely dependent on endogenous yolk as the energy substrate until the hatchling is able to feed exogenously. The pattern of sequential utilization of the various yolk nutrients during embryonic and larval development are known to be species-specific (see section 7, chapter 1). In general, however, protein and lipids are used preferentially shortly before and after hatching, and carbohydrate (glycogen) use dominates during the embryonic period (Heming and Buddington, 1988). Enzyme activity in developing fish embryos is consistent with these generalizations. This indicates that glycogen is an important embryonic energy (Mommesen and Walsh, 1988). Proteins and lipids tend to be conserved and are translocated from the yolk to the embryo to supply anabolic processes involved in embryogenesis.

The increases in the DNA and RNA content observed in capelin embryos during development are consistent with the rapid cell multiplication (cleavage) that characterizes embryonic growth. This has previously been described by Zeitoun et al. (1977) for rainbow trout *(Oncorhynchus mykiss)*, and Buckley (1980, 1981) for cod *(Gadus morhua)* and winter flounder *(Pleuronectes americanus)* larvae. The rate of increase in the quantity of RNA was lower than that of DNA, with the exception of the first 100 hrs post-fertilization. This resulted in a constantly decreasing RNA/DNA ratio, and suggests that most of

the embryonic growth resulted from cell multiplication rather than cell enlargement. This is confirmed by the non-linear decrease in the Protein/DNA ratio which has been used as a cell size index (Westerman and Holt, 1994; Folkvord et al., 1996). In amphibians, this initial increase in cell number (cleavage) has been associated with cyclins. These are proteins encoded by maternal mRNA present in the yolk of unfertilized egg (Gilbert, 1991). These cyclins are not detectable in unfertilized eggs but rapidly increase shortly after fertilization and decline near the end of the blastula stage. In capelin embryos, it is probable that the quantities of DNA, RNA, and Protein present immediately after fertilization are of maternal origin. The asymptote in DNA and RNA content which occurred at approximately 300 hrs post-fertilization may be coincident with the attainment of the late blastula stage. The mean protein content decreased by half during embryonic development (from approximately 80 to 40 $\mu g\ egg^{-1}$). The most pronounced decline occurred shortly before hatching. This pattern of protein utilization is similar to that described for embryos of rainbow trout *(Oncorhynchus mykiss)* by Zeitoun et al. (1977). However, it varies from the pattern described by Buckley (1981) for cod *(Gadus morhua)* embryos. In this species no changes in protein levels were recorded during embryonic development. The trends we observed in RNA/Protein and Protein/DNA ratios were a direct consequence of the ontogenetic trends in Protein levels.

The DNA and RNA levels of newly-hatched larvae were comparable to levels found in embryos. This implies that nucleic acids were conserved through the hatching process. While variable, the protein content of the newly-hatched capelin larvae was almost as high as the levels found in the late embryo stage (Figures 2a and 5a). This suggests that protein utilization during hatching was small. Buckley (1981) ascribed a significant drop in protein content at hatching to the loss of the egg chorion in cod and winter flounder embryos. In the case of capelin larvae, we observed that the chorion becomes very thin upon hatching. The biochemical composition of eggs and yolk-sac larvae suggest that the chorion contributes little to the protein content measured. The RNA/DNA ratio

exhibited a decreasing trend in larvae that hatched later than 550 hours post-fertilization. This suggests that late hatching carry a cost with respect to post-hatching growth potential.

The DNA, RNA and Protein content of post-yolk-sac larvae was similar to that of newly-hatched larvae. This indicates that no significant increase in cell number (hyperplastic growth) or cell size (hypertrophic growth) occurred between hatching and yolk-sac absorption and that yolk protein rather than being catabolized was reallocated to larval tissue. With time however, the RNA and Protein content of post-yolk-sac larvae decreased continuously (the decline being more important after 7 d post-hatch). This resulted in a decrease in the RNA/DNA and Protein/DNA ratios (Figures 8a, 8b, 9b).

Earlier experiments (see Chapter 2) had revealed that for starved capelin larvae sampled from different females post-hatching age was the main factor affecting condition measures. In those studies, modal hatching age had no significant influence on measures of condition. It was not possible to determine whether the decreasing trends evident in post-yolk-sac larvae in the present study were the result of increasing post-hatching age or of differences in hatching age, since the two are confounded by variable hatching age. However, the differences in biochemical measures we observed between early and late hatching larvae derived from female 1 (Figures 4-6), when contrasted with eggs of modal day hatchlings derived from different females that hatched on different dates (Chapter 2), suggest that there may be a within-family cost (e.g. lower protein) of hatching later when the whole hatching window of offspring from a single female is considered. However, there appears to be little or no cost of hatching at different ages when offspring from different females are considered. The consequences of variation in hatching time on condition (particularly in protein content) may, therefore, be more important within than between-broods.

4.2 Periodicities

The only consistent circadian and longer periodicities we observed in the biochemical components of growing embryos were the 64 hr and 28.4 hr cycles in the protein content and protein/DNA ratios. These oscillations were present throughout the embryonic period, when embryos are believed to be non-photosensitive (Heming and Buddington, 1988). It is unlikely, therefore, that these oscillations were entrained by light. They may result from endogenous self-entrained cellular oscillators or "pacemakers".

However, given that whole eggs were analyzed, and that protein translocations from yolk to embryo would be masked, the question of how the protein content of embryos and their yolk, which are enclosed in a chorion, can fluctuate over a one or 3d period in the absence of exchanges with the surrounding environment must be considered. One possibility is that the spectrophotometric procedure used for the estimation of total protein lacked the necessary specificity. The Coomassie brillant blue G250 dye used in the Biorad protein assay binds to most solubilized polypeptides but does not appear to bind to oligopeptides smaller than 3000 daltons or to free amino acids (Sedmak and Grossberg, 1977). Rønnestad et al., (1992a), who measured the quantity of free amino acid (FAA), protein, and NH_3 excretion during the embryonic and larval development of turbot *(Scophthalmus maximus),* found that approximately 70% of the FAA present in the yolk was used as fuel for aerobic metabolism during embryonic development. The remaining 30% was polymerized and incorporated into embryonic proteins. If yolk FAA are used for metabolism and growth in capelin embryos, and remain undetected by the method we used, the 2.6 d. periodicity in the quantity of polypeptides detected in the egg may have resulted from alternating periods of polymerization (protein synthesis and growth) and depolymerization of yolk proteins to supply FAA as energy for metabolism, or as building blocks for protein synthesis. Because some of the FAA liberated from the depolimerization of yolk proteins are

catabolized and some are incorporated into embryonic growth, the net result would be a slight decrease in the amount of total egg protein during embryonic development. This trend was evident in the data (Figure 2a).

All significant periodogram peaks identified for the protein and protein/DNA ratio of yolk-sac larvae were ultradian (<24 hrs) and occurred in the 5.8-6.4 hrs, and 3.5 hrs periods and at the highest frequencies (2.4-2.0 hrs). DeSilva et al. (1986) reported two peaks in oxygen consumption (VO_2) in newly-hatched larvae of tilapia *(Oreochromis niloticus)*, one soon after sunset and the second shortly before dawn. The six hour periodicity we observed in newly-hatch capelin larvae may be related to similar activity levels. In addition, given the deteriorating nutritional condition of non-feeding larvae with time and the increasing demands imposed by activity, these rhythms are likely to be self-entrained but may be partially synchronized by photoperiod. This possibility could be confirmed only if these variables are found to free-run under constant dark conditions. There are some indications that they may persist in constant darkness. Yamagami (1988) reported that hatching rhythms persisted when larvae of different species were switched from light-dark to constant dark conditions. Photoreceptors present in the eye, or possibly in some form of pineal gland (yet to be identified), could be involved in photoperiod sensing (Yamagami, 1988).

Ultradian (<24 hrs) oscillations of periods approximating 3.5 and 6.5 hrs occurred in the protein content and in the protein/DNA ratio of the post-yolk-sac larvae derived from female 2. Such oscillations have not previously been documented in larval fish perhaps because of the inappropriate sampling frequencies used. Their presence is likely to be related to an endogenous cyclic phenomenon yet to be identified. Circadian oscillations (night highs and day lows) in the RNA content and RNA/DNA ratios were observed in capelin larvae exposed to intermittent feeding (see chapter 4). Such rhythms have also been documented in field-collected mackerel *(Scomber scombrus)* larvae (Ferron,

1991), in laboratory-fed larval and juvenile red drum *(Sciaenops ocellatus)* (Rooker and Holt, 1996), and in field-collected sardine larvae *(Sardina pilchardus)* (Chícharo et al., 1998). However, comparison of our results with those studies is judged unwise because the published data relate exclusively to fish that were feeding exogenously, while our data are derived from capelin larvae which, even at the end of the series, were still at the mixed feeding (endogenous and exogenous) stage (6 d post-hatch) and are likely to have been relying on their oil globule for endogenous energy. Also, the fact that the protein content and RNA/DNA ratio of capelin larvae decreased constantly with no sign of improved condition over time further suggests that these capelin larvae had not yet fully achieved successful exogenous feeding. Other experiments conducted with the same species, and in the same experimental settings and food source, but under intermittent feeding conditions (see Chapter 4) indicate that the capelin larvae experienced difficulty in establishing effective exogenous feeding. Comparison of our results with those from other studies do demonstrate that the development of circadian periodicity in condition may be entrained by circadian periodicity in exogenous feeding and growth, both of which are lacking in fish larvae that rely on endogenous energy reserves.

Leatherland et al. (1992) proposed that endogenous or zeitgeber-entrained rhythmicity is employed by organisms to ensure temporal separation, and efficient integration, of mutually antagonistic physiological events (e.g. lipogenesis and lypolysis in liver cells). Gerkema (1992) termed this the "economic principle" by which physiological coordination and energetic optimization is achieved through alternating periods of rest and energy expenditure. One example of this principle is provided by Farbridge and Leatherland (1987) who found that growth in length and weight were out of phase in coho salmon *(Oncorhynchus kisutch)* parr and smolts. The authors explained this asynchrony by a period of energy assimilation associated with an increase in weight followed by a period of increase in length when the assimilated energy was converted into skeletal and somatic growth. Wieser et

al. (1988), who studied the energy budget of larval cyprinids from Austrian lakes, provides a second example of the same principle. They found no correlation between specific growth rate and the mass specific rate of energy dissipation in larval cyprinids. This contrasts with the strong correlation typically obtained between these two variables in both juvenile and adult fish. Wieser et al. (1988) in explaining this inconsistency, noted that larval stages typically have such a high metabolic rate that energy consuming functions such as activity and growth that may periodically exceed maintenance requirements could not be maximized at the same time under such a tight energy budget because they compete for a limited energy supply. They therefore speculated that larval cyprinids employed a "switching strategy" to achieve both high rates of growth and high levels of swimming activity within these energetic constraints.

A similar switching strategy could underly the circadian periodicities displayed by mackerel larvae which are known to search for prey and feed actively during the day and appear to grow (synthesize protein) during the night by using the energy assimilated during the preceding daylight interval (Ferron, 1991). In the case of capelin larvae, the absence of such diel patterns would suggest that exogenous feeding is a necessary precondition to the development of these cycles.

The confirmation of these patterns of energy partitioning during embryogenesis and larval development awaits the results of further studies. However, the results of the present study do clearly highlight the importance of evaluating the potential existence of circadian and ultradian oscillations in metabolic components when employing biochemical measurements as indicators of larval fish condition. The presence of such rhythms in measures widely used as indicators of larval condition (Protein, RNA, RNA/DNA or Protein/DNA ratios) also implies that sampling must be conducted at times and or at a frequency sufficient to detect or avoid the existence of these

periodicities. Failure to sample at a consistent time in the cycle is likely to lead to aliasing of the data and to the drawing of false conclusions.

It should also be emphasized that where such periodicities exists, changes in condition resulting from food supply or other factors are likely to be reflected not in the absolute value of the unit measured but rather in the magnitude of the variation in the amplitude of one or several of these components relative to a baseline oscillation (Clemmesen, 1996). The evidence of ontogenetic phase shifts in the periodically fluctuating measures of condition examined in this study also carries with it the warning that different developmental stages should be analyzed separately.

CHAPTER 4

THE TIME RESPONSE OF CHANGES IN THE PROTEIN AND NUCLEIC ACID COMPOSITION OF LABORATORY-REARED CAPELIN (*Mallotus villosus*) LARVAE UNDER INTERMITTENT FEEDING REGIMES

1. INTRODUCTION

The lack of a direct relationship between recruitment and spawning biomass in marine fish populations has led researchers to look at the effects of factors responsible for the mortality of young stages. The large variation in growth and mortality during the first year of life typically displayed by most marine fish is still widely believed to be a major driving force behind recruitment variability (Ferron and Leggett, 1994). In that context, a series of condition indices have been developed and tested in the laboratory to provide a way of measuring starvation mortality and susceptibility to predation in the field (reviewed in Ferron and Leggett, 1994). While laboratory calibrations have shown a link between condition and food availability, estimates of the condition of marine fish larvae obtained from animals collected at sea have revealed a large inter-individual variability and overall poor correlations with *in situ* food concentrations (Ferron and Leggett, 1994; Blom et al., 1997, Chicharo, 1997, 1998; Chicharo et al., 1998).

This discrepancy between larval condition and *in situ* food concentrations is believed to be due in part to our inability to accurately reproduce, in the laboratory, the naturally patchy feeding environment of marine fish larvae. Another reason may be that food concentrations encountered by fish larvae at the time of sampling may have little to do with their current condition, since a time lag is to be expected between feeding or lack thereof and detectable changes in condition (Ferron and Legett, 1994). Since variable feeding environments and time-lags are involved, we defined and proposed to use different terms related to time response of changes in relation to the food environment (Ferron and Leggett, 1994). We coined the term sensitivity to describe the minimum variation in food levels required to trigger a measurable change in condition, latency to describe the time required before a response could be detected, and dynamics to describe the rate of change in condition after a latency interval is passed and a response has been elicited. Sensitivity and latency are inversely related, the

more sensitive an index, the shorter its latency. These definitions are now part of the lexicon of studies of larval fish condition (Kristogu-Baduge et al. 1999).

While most researchers now acknowledge the possible existence of a time-lag between condition and food availability, and a temperature effect, the time response correction used or inferred is typically hypothesized without empirical data to support it. Further, the existing data on larval condition have typically been collected at time scales much longer than the scale at which inferences from the data are made. Ueberschär and Clemmesen (1992) for example, reported that the RNA/DNA ratio of laboratory-reared herring *(Clupea harengus)* larvae did not reflect short-term changes in food availability, and that sudden changes in food concentrations lead to an increase in the activity of the ribosomes but not in their numbers. Their measurements were, however, taken at 2 d intervals, notwithstanding the fact that ribosome activity can change within hours (Lied *et al.*, 1983). Many recent studies using the RNA/DNA ratio as a condition index assume a 2-3 d lag between changes in food availability and condition even though significant differences in the RNA/DNA ratio in response to starvation and re-feeding have been recorded within one day or even within hours (Mugiya and Oka, 1991; Malloy and Targett, 1994; Bisbal and Bengston, 1995; Rooker and Holt, 1996, 1997). Several authors including Westerman and Holt (1994) have since called for fine-scale laboratory studies of selected species to aid in the interpretation of field-collected data.

Wright and Martin (1985) investigated the response of notochord length and the RNA/DNA ratio to changes in the feeding status of striped bass *(Morone saxatilis)* larvae raised in the laboratory. While their data provide the first evidence that deterioration and recovery responses may be non-linear, their curves were extrapolated beyond the data, and the data used to generate the curves did not appear in their paper. Therefore, the variability about the lines they reported and the goodness-of-fit cannot be evaluated, nor can the curves be used to predict condition responses.

In this study we sought to determine (1) which of a series of biochemical measures derived from nucleic acid and protein measurements were significantly affected by intermittent (delayed-fed and delayed-starved) feeding conditions in post-yolk-sac larvae of capelin *(Mallotus villosus)*, (2) the dynamics (rate of change) and the shape of the time responses of these measures to various starvation and feeding delays, and (3) which of the empirical data obtained were consistent with the time response models originally proposed (Ferron and Leggett, 1994).

2. MATERIAL AND METHODS

2.1-Experimental design

All larvae used in our experiments reported here were obtained from eggs collected at the low tide level on Bryant's Cove beach (Newfoundland) between July 8 and 12, 1990. The larvae used for the starvation and delayed-fed treatments were collected over a 36 hrs period between July 8 and 10, while the larvae used for the fed controls and the starvation-delayed treatments were collected over a 36 hours period extending from July 10 and July 12. The larvae were initially counted and placed in 28 x 24 x 15 cm containers filled with 0.45µm filtered and U.V. sterilized seawater. A total of 3000 ± 100 larvae were added to each container. These larvae were immediately transferred to 38.8 liters (dimensions: 40 cm diameter and height) black cylindrical tanks to yield stocking densities of 77.3 larvae liter^{-1}. The tanks were filled with 0.45µm filtered and U.V. sterilized seawater.

The tanks were placed in two 2.4 x 1.2 m water baths maintained at an average temperature of 8 ± 1° C. Replicated treatment tanks were assigned a random location within each half of the baths (Figure 1) consistent with a randomized two block design (Hurlbert, 1984). This was done to account for any temperature gradient that may have occurred between the baths' inlet and outlet.

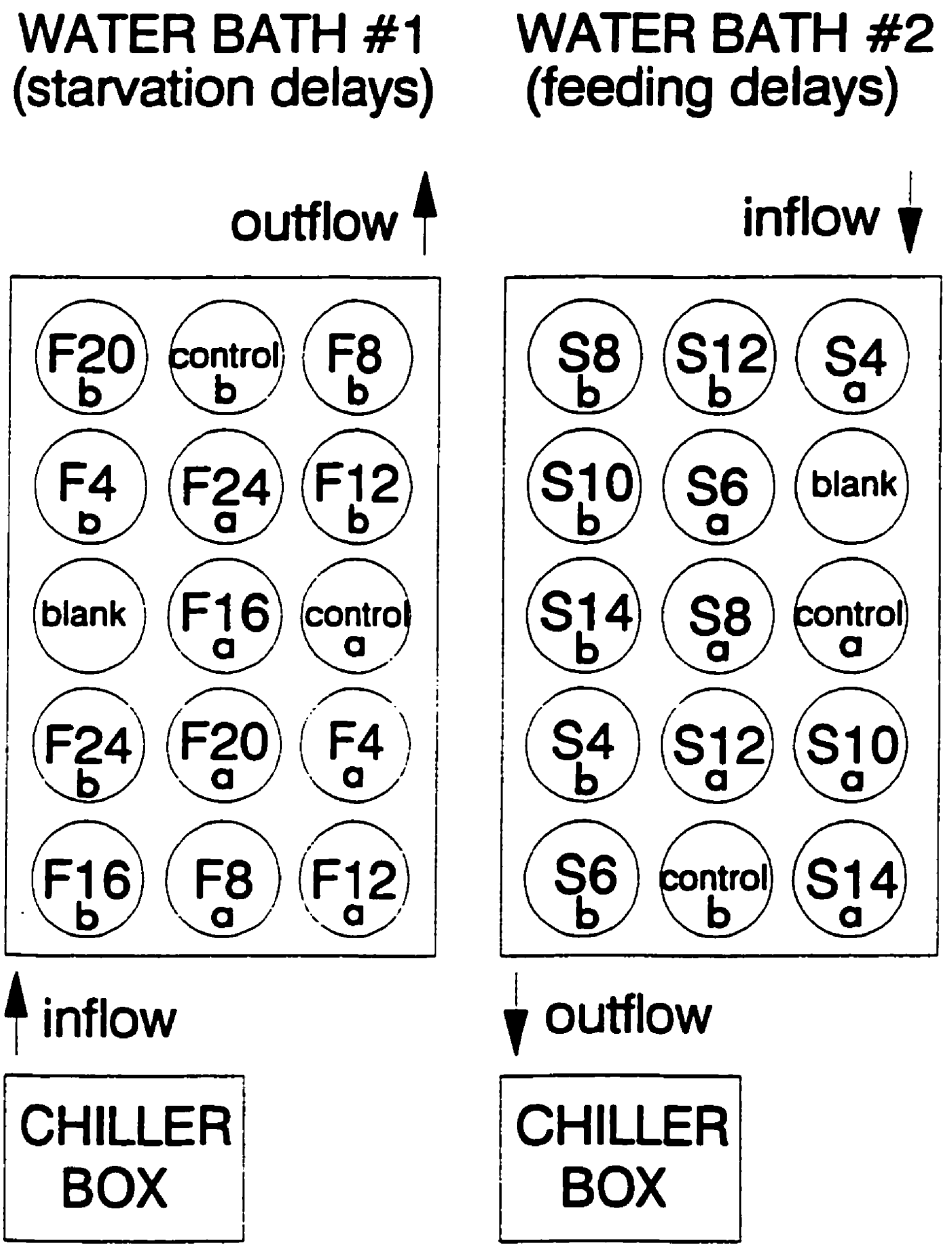
The water temperature of each tank was recorded three times daily (6h45, 14h45 and 22h45) and was adjusted as required to maintain the 8°C target temperature. Fluorescent lights suspended 80 cm above each of the baths were regulated to simulate natural summer light regimes (8D: 16L), with the dark period occurring between 22h00 and 6h00.

Water level in the tanks was maintained via adjustable inflow and outflows. The larvae were maintained in static conditions for the duration of the experiments. Approximately 1/6 of the tank volume was flushed and replaced with fresh filtered and sterilized seawater daily. A small volume (500 ml) of concentrated algae culture *(Dunaniella spp.)* was introduced into each tank daily starting on the first day to improve the condition, growth and survival of the larvae (Blaxter, 1988; Reitan *et al.*, 1993).

The experimental design employed six replicated starvation delay and six replicated feeding delay treatments along with two replicated controls (Figure 1). In the starvation-delay treatments, the larvae were fed for 4, 8, 12, 16, 20 and 24 d after being stocked in the tanks and then transferred to containers free of food and supplied with sterilized-filtered seawater. These treatments were labeled as f4, f8, f12, f16, f20 and f24 (a and b for the two replicates). In the feeding-delay treatments, feeding was delayed for 4, 6, 8, 10, 12, and 14 d after stocking and then food was provided. These treatments are labeled as s4, s6, s8, s10, s12 and s14 (a and b for the two replicates). Replicated tanks provided with *ad libitum* food and labeled (ad) served as controls for the starvation delay trials and tanks not provided with food and labeled (st) were used as controls for the feeding delay trials.

Wild zooplankton was used as food. Plankton was collected daily using a 0.6 m diameter plankton net (80 µm mesh size) fished near the mouth of Bryant's Cove Bay (Newfoundland). The collected plankton was passed through 500 and 120 µm sieves. The fraction below 120 µm was used as food, and three 1.0 ml

FIGURE 1. Location of the tanks within water baths. Tanks from the delayed-starvation bath are labeled f(n) where n = number of days the larvae were fed before being starved. Tanks from the delayed-feeding bath are labeled s(n) where n = number of days the larvae were starved before being fed.



aliquots were counted daily to assess the number of copepod nauplii present in that food sample. The quantity of food provided was regulated in relation to the nauplii concentration measured daily. A sub-sample of 25 ml was also preserved in formalin for further analysis of prey types. Three 1.0 ml aliquots were collected from each of the rearing tanks daily to determine the actual concentrations of nauplii present. Food was added to the tanks between 9h00 and 10h00 each morning, starting 24 hrs after the larvae were stocked in the tanks, and in quantities required to maintain a food concentration of 1000 nauplii l^{-1} within each tank throughout the experiments.

Sampling of the larvae from the control tanks began 4 d (96 hrs) after the beach-collected eggs were hatched and the larvae were stocked and continued for the duration of the experiment (17 d). This period of 4 d allowed complete yolk exhaustion and corresponds with the start of the exogenous feeding in capelin larvae (see chapter 2). Sampling of larvae from the delayed-feeding treatments began on day 4 post-stocking in the s4 tanks, on day 6 in the s6 tanks etc. A similar regime was followed for the f4, f8 etc. groups in the delayed-starvation treatments. Five larvae were sampled at random every four hours from each control tanks for the duration of the experiment, and five larvae were sampled from replicated treatment tanks every four hours for six days (36 samples over 144 hrs.) once the treatment was applied (food introduction or withdrawal). These larvae were immediately rinsed with distilled water on a sieve to prevent the formation of salt crystals and were then frozen in liquid nitrogen. Following their transfer to Montreal on dry ice, the larvae were vacuum freeze dried and then stored at -20° C on drierite in a dessicator until biochemical assays were performed.

Prior to the assays, the larvae were separated and inspected individually to ensure that no body parts were missing and that no salt crystals a source of bias in dry weight measurements were attached to the larvae. In many instances, the five larvae preserved together in a single microtube could not be separated

without damage to the larvae. In these cases, a single measure of dry weight was obtained for the group of larvae which could not be separated, and individual weights were obtained for the remaining larvae. Individual larvae damaged or found to have missing parts were discarded. Dry weights were recorded to the nearest 0.1 µg on a Cahn C-31 micro-balance.

The number of individual larvae successfully recovered from each microtube ranged from 2 to 5 depending on the integrity of the larvae found. For most samples, at least two larvae could not be separated. Therefore, rather than restricting the biochemical analyses to the available individual larvae, we treated the intact larvae present in each microtube as a single sample and conducted the assays on pooled 5 larvae homogenates. For some of the samples containing only intact and separated individual larvae, homogenates were made using single individuals. Samples of pooled and individual larvae from the various treatment/controls were processed at random.

2.2- Biochemical assays

All chemicals used in the procedures were analytical grade. They were obtained from the sources cited in Chapter 2. The Tris-EDTA buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA), the ethidium bromide (10µg/ml) and Hoechst (20µg/ml) dyes, the SDS (20%), the phenol, and the chloroform and isoamyl mix (24:1) were all prepared and stored as outlined in Chapter 2. Stock solutions of DNA (500 µg/ml), yeast RNA (100 µg/ml), and bovine serum albumine (1 mg/ml) were made and frozen at -20°C until use. The concentrations of DNA and RNA standards were determined using an expected optical density OD_{260} of 1.000 for solutions containing 50 µg/ml of double-stranded DNA or 40 µg/ml of single-stranded RNA (Maniatis *et al.*, 1992). The concentrations of bovine serum albumine protein standards were calculated using OD_{260} and OD_{280} with the following equation from Bashford and Harris (1987):

$$\text{protein (mg/ml)} = 1.55\ OD_{280} - 0.76\ OD_{260}$$

Working standards (100 µg/ml) of RNA, DNA and protein were prepared weekly from frozen stock solutions and used for calibrations and internal standards.

Protein measurements were made on three aliquots of the crude larval homogenate, while nucleic acids were measured on a single extracted and purified aliquot following the procedure illustrated in Chapter 2. The protein content of the samples was determined by reading the absorbance at 595 nm on a "Pye Unicam PU-8088" spectrophotometer after adding the Biorad concentrated reagent (microassay procedure) to the homogenate. Triplicates of blanks (Tris-EDTA buffer+dye) and of two bovine serum albumine standards were measured for each group of 12 samples (36 triplicates) processed. The two standard concentrations were chosen from within the range of the sample values to detect and correct for any deviations from calibration values. After comparing blanks recorded before and after the sample measurements, we detected a decreasing trend in absorbance values. This was probably caused by temperature changes that resulted from samples held at room temperature for up to one hour while performing the measurements. This linear temperature effect was subtracted from the samples absorbance using the blank and standard values.

Calibrations using bovine serum albumine were conducted at least weekly over the course of sample measurements and consisted of triplicates of 8 different concentrations encompassing the range of all sample values. The calibration was repeated whenever a $R^2<0.99$ was obtained. None of the 12 calibration lines obtained differed significantly (ANCOVA, $P<0.001$) from one another, and none of the daily standards differed significantly (ANOVA, $P<0.001$) from the corresponding calibration lines. Therefore, the calibration line with the

best fit and lowest intercept (described by the following equation) was used for the determination of the protein content of the samples.

$$[protein]=14.8040\ O.D_{595nm}\ -0.1591,\quad N=27,\quad R^2=0.9967$$

where O.D.=optical density (absorbance reading).

Following determination of the amount in micrograms of protein per homogenate in each of the triplicates (using the appropriate dilutions factors), the outliers were detected and removed using the Q-test (Smith, 1998). We employed a Q-value > 0.94 to ensure a 90% confidence level in the evaluation. A total of 18/759 (2.9%) triplicates had one outlier detected and removed. The overall coefficient of variation for the remaining 736 triplicates was 13.27%. From the 69 triplicate blanks measured, 54 (78%) had a protein content not significantly different than 0 (t-test, $P>0.05$) for an average of 2.08 ± 3.84 µg of protein per homogenate.

The nucleic acid content of the samples was assessed on a "Perkin-Elmer LS-3B" spectrofluorometer, by measuring the fluorescence increase resulting from the binding of Hoechst 33258 (Biz) to DNA and ethidium bromide (EBr) to RNA and DNA. The fluorometer was operated at a slit width of 10 nm, and a scan speed of 60 nm min^{-1}. The excitation and emission wavelengths used for all measurements were 360 and 600 nm for Ebr, and 352 nm and 448 nm for Biz.

Triplicates of blanks (Tris-EDTA buffer+dye) and standards (for two concentrations situated within the range of sample values) were measured for each set of 24 samples processed. Samples were not replicated since the amount of homogenate remaining after the extraction procedure (210 µl) was sufficient for one Ebr and one Biz fluorescence measurement only (see flowchart in chapter 2). The endogenous fluorescence of purified nucleic acid extracts before the addition of dyes was negligible (3.0% for the Tris+EBr values and

6.4% for the TRIS+Biz). However, the background (residual) fluorescence increased significantly upon addition of dyes to the Tris-EDTA blanks extracted with methods identical to those applied to the samples. This fluorescence increase was 313 % for Tris-Biz and 6 % for Tris-EBr respectively. For each set of samples, we used this mean extracted buffer+dye fluorescence value and subtract it from the samples' measurements to obtain a corrected fluorescence. One extracted buffer blank was routinely made (one for every sets of 12 samples) when conducting the extraction and purification procedure. This was done to account for potential day-to-day differences in the effectiveness of the extraction process. This blank value was subtracted from both the Ebr and Biz fluorescence measurements of corresponding samples before calculating the amount of RNA and DNA. Extraction and purification recovery tests performed by adding spike DNA and RNA to buffer and homogenates yielded a mean percentage recovery of 92.8% for DNA and 102.2% for RNA.

RNA and DNA calibrations were run weekly and repeated whenever a $R^2<0.99$ was obtained. Since all fluorescence measurements were conducted within two weeks, two sets of calibration lines were made, one before and the second one after the samples were read. These two sets of calibration lines did not differ significantly one from another (ANOVA, $P<0.001$). We used the calibration lines obtained immediately before sample measurements because the fit of the data to the line was tighter and the intercept lower. The DNA+Biz calibration line used to determine the concentration of DNA from the Biz fluorescence measurement was described by $[DNA]=0.1916\ f_{Biz}+0.0374$ ($N=27$, $R^2=0.9864$). This concentration was reported on a DNA+EBr line described by $[DNA]=2.2180\ f_{Ebr(DNA)}+0.0024$ ($N=27$, $R^2=0.9989$) to obtain the expected EBr fluorescence due to DNA alone. The expected fluorescence so derived was then subtracted from the total EBr fluorescence (due to DNA and RNA) measured, to provide the EBr fluorescence due to RNA alone. As a last step, that fluorescence was reported on the RNA+EBr line described by $[RNA]=2.7474\ f_{Ebr(RNA)}+0.0482$ ($N=27$, $R^2=0.9915$) to yield the concentration of RNA in the sample. DNA, RNA,

and Protein content were either expressed in µg larva^{-1} or in percent dry weight since individual larval dry weight measurements were available in most cases.

2.3- Data analysis

Because of the destructive nature of the sampling and the mortality that occurred within the tanks, the number of larvae available was insufficient in some treatment tanks to provide data over the total duration of the experiment. The two *ad libitum* replicate control tanks were sampled for 280 and 308 hrs, respectively (70-77 samples taken over 12-13 d for a survival rate of 11.6% and 12.8% respectively for 17 d post-hatch). The s4, s6, and s8 delayed-feeding replicated treatment tanks provided sufficient larvae for the entire 144 hr (6 d) sampling period following the 4, 6, and 8 days of starvation. The replicated s10 tanks provided samples for 132 and 120 hrs, the replicated s12 tanks for 72 and 84 hrs, and the replicated s14 tanks for 24 hrs only. The number of samples collected from the s14 treatment tanks was inadequate for most of the statistical analyses that follow. We include the s14 values for a comparison with treatments, but do not attempt statistical interpretation. The replicated starvation control tanks were sampled for a total of 248 and 272 hrs respectively (over 10-11 d, survival rate 10.3% and 11.3% respectively). Of the starvation-delayed treatments, only the f4 and f8 tanks provided sufficient larvae to facilitate sampling over the full 6 days (144 hrs) after feeding was resumed. The f12 tanks yielded sufficient larvae to provide samples for 8 hrs following 12 days of feeding and were therefore excluded from the data analysis. The number of larvae surviving until the beginning of sampling was also too low to allow meaningful sample sizes to be collected from the f16, f20 and f24 replicated tanks.

Nucleic acid and protein content were determined on a total of 593 pooled larvae homogenates and 167 individual larvae homogenates obtained from two replicated controls (*ad libitum* and starvation) and seven replicated treatment tanks (s4, s6, s8, s10, s12, f4 and f8). The average quantity of DNA, RNA, and

protein per homogenate in both pooled and single larvae homogenates was significantly greater than the average recorded for the blanks (t-test, $P<0.001$) that were measured concurrently. When expressed as µg DNA larva^{-1}, µg RNA larva^{-1}, and µg protein larva^{-1}, the average values were again significantly greater than the average recorded for blanks (t-test, $P<0.001$).

The average quantity of DNA larva^{-1} derived from larvae analyzed individually (0.881 ± 0.191 µg) was significantly greater (t-test, $P<0.001$) and less variable (CV=21.6) than the average calculated from pooled larvae homogenates (0.573 ± 0.143 µg, CV=25.0). Similarly the quantity of RNA larva^{-1} derived from single larva homogenates (2.464 ± 0.553 µg) was significantly greater (t-test, $P<0.001$) and less variable (CV=22.4) than the quantity derived from homogenates of pooled larvae (1.833 ± 0.452 µg, CV=24.7). The quantity of protein larva^{-1} in the single larva homogenates (15.064 ± 6.624 µg, CV=44.0) was by contrast significantly lower and as variable as that in pooled larvae homogenates (17.923 ± 7.870 µg, CV=43.9). For this reason, the data from single larva and pooled larvae homogenates were analyzed separately. The quantity of DNA, RNA, and Protein per unit dry weight in the single and pooled samples were computed using the individual or mean dry weights as appropriate. RNA/DNA, RNA/protein and protein/DNA ratios were independently calculated for each replicated treatment/sampling time.

Published models describing the probable dynamics of biochemical measures of condition predict that the shape of the condition response curves following intermittent feeding regimes will be non-linear (Ferron and Leggett, 1994). We therefore fitted quadratic regression curves of the type $Y = a + bX + cX^2$ (where Y = dependent variable and X = post-stocking age in hours) separately to each replicated treatment/control or replicated tank using SYSTAT-SCATTERPLOT (Wilkinson, 1998). These lines were fitted only within the range of existing data. The significance of the polynomial regressions were assessed using the F-ratio statistic. The significance of the constant (a), linear (b) and the non-linear

coefficients (c) was evaluated by a t-test of their deviation from a zero slope or curvature. A polynomial regression was preferred over a nonlinear estimation because we had no *a priori* theoretical model to test. Rather, our objectives were to determine the shape of the response curves using an empirical fitting procedure not restricted by linearity, and to explore its dynamics (rate of change) as defined in Ferron and Leggett (1994), in order to provide a modeling framework. Data gathered from several studies have repeatedly shown non-linearity of condition measures with age (Wright and Martin, 1985; Canino, 1994). This further justified the use of non-linear models.

We used SYSTAT-GLM ANCOVA procedures (Wilkinson, 1998) to test for significant differences in the slope and intercept between replicated tanks of each control/treatment and for differences between treatment and control lines. The basic model used was $Y = a + TREAT + bX + cX^2 + TREAT*X + TREAT*X^2$ where Y = dependent biochemical variable, X = post-stocking age (covariate), TREAT = replicated tank or treatment class variable, a = intercept, b = linear coefficient, and c = non-linear coefficient. As a first step, the interaction terms ($TREAT*X$ or $TREAT*X^2$) were tested and, if significant ($P<0.05$), the original model was retained and the hypothesis of homogeneity of slopes rejected. Significant treatment effects could then be assigned to significant differences in slopes. When the interactions $TREAT*X$ or $TREAT*X^2$ were found to be non-significant ($P<0.05$), they were removed from the model, the hypothesis of homogeneity of slopes was accepted, and the simple model $Y = a + TREAT + bX + cX^2$ was re-run without interactions. In such cases, significant treatment effects were then attributed to significant differences in the intercepts. The level of significance for each term of the model was assessed using the F-ratio. Since sampling was conducted every 4 hrs for approximately 12-13 d in control tanks and for 6 d in each treatment tank, the potential for circadian (diel) patterns effects (Ferron and Leggett, 1994, Rooker and Holt, 1997) on the shape and on the goodness-of-fit of the responses measured was real. We tested for this effect

using SYSTAT-ANOVA (Wilkinson, 1998) on each biochemical variable measured. We employed sampling time as the categorical variable.

3. RESULTS

3.1 Tank (replicate) effects

The purpose of replication is to account for the effects of unexplained influences on measured values (noise, random variation or error), and thus to increase precision (Hurlbert, 1984). In our experiments, the experimental units (tanks) were sampled sequentially in time in order to determine the time response of various condition measures to intermittent feeding. Hurlbert (1984) cautioned that when sampling time is considered as the treatment, sequential measurements cannot be taken as being independent one from another. We overcome this potential error by using Analysis of Covariance (ANCOVA) to test for significant treatment or tank effects in the biochemical measures after adjusting for treatment/control differences in the covariate (post-stocking age). We tested *a priori* for homogeneity of slopes by including an interaction between the treatment/tank effect and the covariate. The presence of a significant interaction term indicates that the slopes differ significantly between replicates and that their intercept could not be compared. In the absence of significant interactions, the comparison of the lines obtained for each replicated tank provides a means of testing whether the time responses occurred more than by chance alone, and whether treatment/control lines fitted for pooled-replicates reliably represented responses within each replicate.

3.1.1 Pooled larvae homogenates

Six of 90 (9 treatments/controls × 10 variables) polynomial regressions computed from pooled larvae homogenates, exhibited a significant interaction (ANCOVA, $P<0.01$) between the tank effect and the covariate (age). These

included Protein larva^{-1} in s6, RNA and Protein per dry weight in f8, and the RNA/DNA, RNA/Protein and Prot/DNA ratios in the starvation control tanks (labeled as TANK in Table 1a). Only two of the 84 remaining treatment/control x variable combinations exhibited significant (ANCOVA, $P<0.01$) between-replicate differences in intercept. The lines fitted to the quantity of RNA larva^{-1} and to the RNA/DNA ratio for the larvae sampled from the *ad libitum* control tanks and those fitted for the relative protein content (protein/dw) for larvae sampled from the two replicate f8 tanks differed significantly (ANCOVA, $P<0.01$) between-replicates in their intercept (Table 1a). Our analysis were therefore based on pooled replicates from 82 of the 90 treatment/control x variable data sets for which no interactions or intercept differences (tank effect) were detected.

3.1.2 Individual larvae homogenates

Only the feeding and starvation controls and the f4, s6 and s8 treatments could be tested for tank effects because of sample size limitation in the f8 ($n = 10$) and s4 ($n = 13$) treatments. In addition, sufficient larvae were obtained from only one of the two replicated tanks in the s10, s12 and s14 treatments. Significant tank effects (ANCOVA, $P<0.01$) were detected in 5 out of 60 (6 treatment/control x 10 variables) regressions. These involved the dry weight of larvae sampled from the *ad libitum* feeding control, the DNA per dry weight of larvae sampled from the feeding control and f4 starvation-delay treatment (labeled as TANK in Table 1b), and the dry weight and RNA content of larvae sampled from the s8 treatment tanks. Our analysis of individual larva homogenates was therefore based on pooled-replicates for 55 of the 60 remaining variable/treatment responses for which no tank effects were detected.

3.2 Differences between treatments and controls and the effect of age

3.2.1 Feeding vs starvation controls

Analysis of pooled larvae homogenates revealed that larvae sampled from the starvation control tanks lost significant RNA (0.00813 hr^{-1} = 0.20 µg d^{-1}) and Protein (0.13796 hr^{-1} = 3.31 µg d^{-1}, $P<0.01$, Table 1a) over the course of the 10-11 days of starvation. Larvae fed *ad libitum* for 12-13 days also lost Protein and showed a reduced Protein/DNA ratios (3.13 d^{-1} , $P<0.05$, Table 1a).

The greatest difference between the capelin larvae sampled from the starvation (st) and the feeding (ad) controls was reflected in the percentage of protein per dry weight (ANCOVA, $P<0.001$, Table 2a). Protein content of starved larvae declined from an average of 42% at the onset of sampling to approximately 25% over 10-11 days. Larvae fed *ad libitum* gained protein over the first 5 days measuring from 43% to 45% and subsequently declined to an average of 30% at 13 days (Figure 2a). This protein loss was also reflected in the absolute levels of protein (µg protein $larva^{-1}$). Larvae from the starvation controls lost protein at a significantly higher rate than those sampled from the feeding controls (ANCOVA, $P<0.01$, Table 2a). Hence, while starved and fed larvae exhibited similar protein content (29 and 25 µg $larva^{-1}$ respectively) at the start of the experiment, starved larvae lost protein at a higher rate and levels at termination of the 11-12 day experiment averaged 10 µg $larva^{-1}$ relative to 15 µg $larva^{-1}$ for fed larvae (Figure 2b).

At the outset, the mean dry weight of larvae from the starvation control tanks (58 µg $larva^{-1}$) was greater than that of larvae from the fed control tanks (47 µg $larva^{-1}$, Figure 3a). Subsequently, starved larvae lost weight while fed larvae gained weight. The amount of DNA $larva^{-1}$ in the starvation control tanks was higher at the onset of the experiment but constantly decreased. DNA levels in larvae sampled from the fed control tanks were relatively lower but increased to approximately 0.6 µg $larva^{-1}$ after 10 d and then reached a plateau (Figure 3b). The amount of RNA per dry weight also varied between fed and starvation controls (ANCOVA, $P<0.01$, Table 2a).

TABLE 1 Summary of results for the polynomial regressions describing the fitted response line for each control/treatment. The regressions are described by the model $Y = a + bX + cX^2$, where Y = dependent variable, X = post-stocking age in hours (independent variable), a = intercept, b = linear coefficient and c = non-linear coefficient. The level of significance for the regressions was tested using the F-ratio and is displayed with the R^2 value. The constant and age term significance were tested using a t-test. Only significant ($P<0.01$) regressions are shown. Ad = ad libitum feeding control, St = starvation control; F4, F8 (delayed-starvation treatments, fed for 4 and 8 d before being starved); S4, S6, S8, S10, S12 (delayed-feeding treatments, starved for 4, 6, 8, 10 and 12 d before being fed). ***$P<0.001$, **$P<0.01$, *$P<0.05$, TANK = significant tank effect ($P<0.01$).

Table 1a-Pooled larvae homogenates

Dependent variable	Independent variable	Controls/Treatment						
		Ad	F4	F8	St	S4	S6	S8
		N=106	N=52	N=51	N=90	N=57	N=57	N=54
Dry weight			R^2=0.223**		R^2=0.154**			
	constant		0.06070***		0.05843***			
	Age		-0.00058		-0.00014			
	Age^2		0.00000		0.00000			
µgRNA		TANK	R^2=0.425***	R^2=0.213**	R^2=0.454***			
	constant		2.45080***	2.03882***	2.52576***			
	Age		-0.00849*	-0.00836*	-0.00813***			
	Age^2		0.00002	0.00004	0.00002			
µgProtein		R^2=0.113**	R^2=0.317***		R^2=0.457***		TANK	
		24.58679***	24.48118***		29.32060***			
	Age	-0.03611	0.02795		-0.13796***			
	Age^2	0.00001	-0.00079		0.00026*			
Prot/dw				TANK	R^2=0.281***			
	constant				0.49289***			
	Age				-0.00134			
	Age^2				0.00000			
RNA/DNA		TANK	R^2=0.249**	R^2=0.248***	TANK	R^2=0.338***	R^2=0.178**	
	constant		4.19301***	3.49868***		4.55168***	3.89779***	
	Age		-0.01115	-0.01454**		-0.01565*	-0.00707	
	Age^2		0.00002	0.00007*		0.00004	0.00001	
RNA/Prot			R^2=0.262***	R^2=0.181**	TANK	R^2=0.182**		
	constant		0.12358***	0.11279***		0.08778***		
	Age		-0.00128	-0.00076		0.00018		
	Age^2		0.00001	0.00001		0.00001		
Prot/DNA		R^2=0.235***	R^2=0.241**		TANK		R^2=0.222**	R^2=0.260***
	constant	45.13418***	41.35114***				38.15972***	44.24328***
	Age	-0.13038*	0.11479				0.08473	-0.12665
	Age^2	0.00020	-0.00167				-0.00139	-0.00044

Table 1b-Individual larvae homogenates

Dependent variable	Independent variable	Controls/Treatments				
		Ad	F4	F8	St	S4
		N=36	N=36	N=10	N=20	N=14
Dry weight		TANK		R^2=0.857**		
	Constant			0.00638*		
	Age			0.00179**		
	Age^2			-0.00001*		
Dna/Dw		TANK	TANK		R^2=0.489**	
	Constant				0.01399***	
	Age				0.00002	
	Age^2				0.00000	
RNA/Dw			R^2=0.280**	R^2=0.845**		
	Constant		0.05467***	0.10621***		
	Age		0.00107**	-0.00227**		
	Age^2		-0.00002	0.00002*		
Prot/dw		R^2=0.282**		R^2=0.745**		
	Constant	0.26143***		0.47320***		
	Age	0.00242*		-0.00931		
	Age^2	-0.00001*		0.00005		
RNA/Prot		R^2=0.478***				R^2=0.661**
	Constant	0.22888***				0.27178***
	Age	-0.00154**				-0.00244*
	Age^2	0.00001***				0.00001

TABLE 2 Summary results for Analyses of Covariance (ANCOVA) used to test for the significance of differences in slopes and intercepts between treatments and controls. The model used was: $Y = a + TREAT + bX + cX^2 + TREAT * X + TREAT * X^2$, where Y = dependent variable, X = post- stocking age (covariate), TREAT = treatment class variable, a = intercept, b = linear coefficient and c = non-linear coefficient. The interaction term $TREAT * AGE^2$ was tested first and its probability level displayed when significant. When found to be non-significant, the term was eliminated and the ANCOVA re-run to test for the interaction term TREAT * AGE alone. Again if deemed non-significant the ANCOVA was re-run without interaction terms. Only significant ANCOVA $P<0.01$ are shown together with their respective F-ratio for the treatment effect and the interaction effect (***$P<0.001$, **$P<0.01$, *$P<0.05$). TANK = significant tank effect ($P<0.05$). Ad = *ad libitum* feeding control, St = starvation control; F4, F8 (delayed-starvation treatments, fed for 4 and 8 d before being starved); S4, S6, S8, S10, S12 (delayed-feeding treatments, starved for 4, 6, 8, 10 and 12 d before being fed).

Dependent variable	Effect	Control-treatment couplings							
		Ad-st	Ad-f4	Ad-f8	St-s4	St-s6	St-s8	St-s10	St-s14
Dry weight	Treatment	8.774**		13.656***		10.553**	9.960**		39.860***
	Age*treat	5.038*		12.390**		4.912*	7.537**		
DNA	Treatment	8.361**	37.087***			9.252**	7.611**	8.433**	12.105**
	Age*treat	9.710**	31.379***						12.627**
RNA	Treatment	TANK	TANK	TANK		6.957**			7.103**
	Age*treat								7.586**
Protein	Treatment	9.397**		10.203**	24.886***	TANK			
	Age*treat				24.678***				
DNA/dw	Treatment		10.519**			11.316**	21.204***	7.135**	8.199**
	Age*treat		9.991**			5.377*		6.333*	8.080**
RNA/dw	Treatment	7.959**		TANK		23.637***	11.507**		
	Age*treat								
Prot/dw	Treatment	14.138***		TANK	10.903**				
	Age*treat				12.567**				
RNA/DNA	Treatment	TANK	TANK	TANK	TANK	TANK	TANK	TANK	TANK
	age*treat								
RNA/Prot	Treatment				TANK	TANK	TANK	TANK	TANK
	age*treat								
Prot/DNA	Treatment		14.454***		TANK	TANK	TANK	TANK	TANK
	age*treat		10.347**						

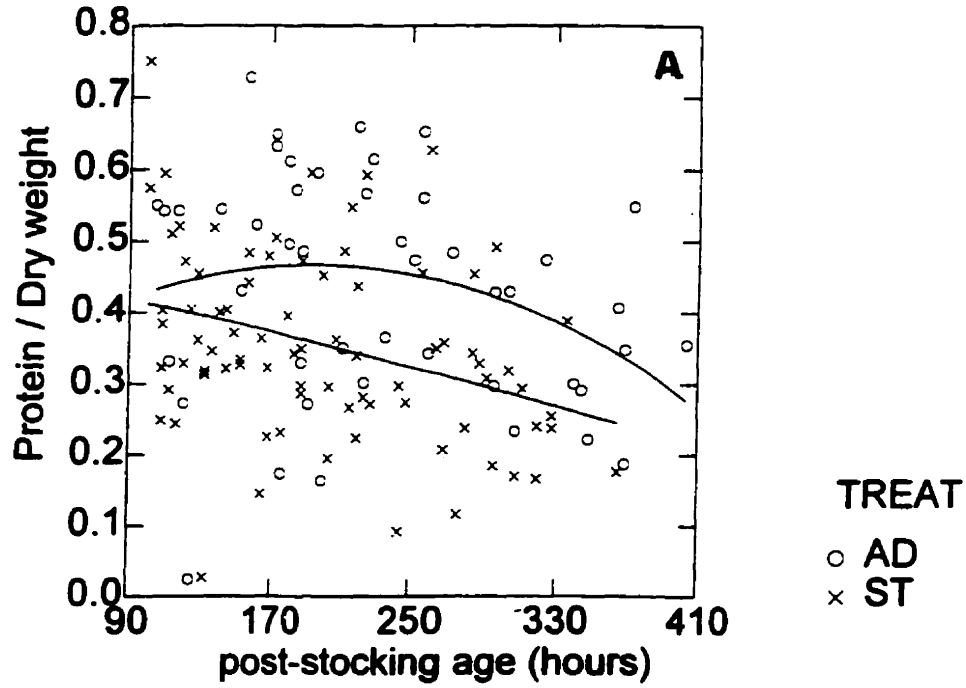
TABLE 2a Pooled larvae homogenates

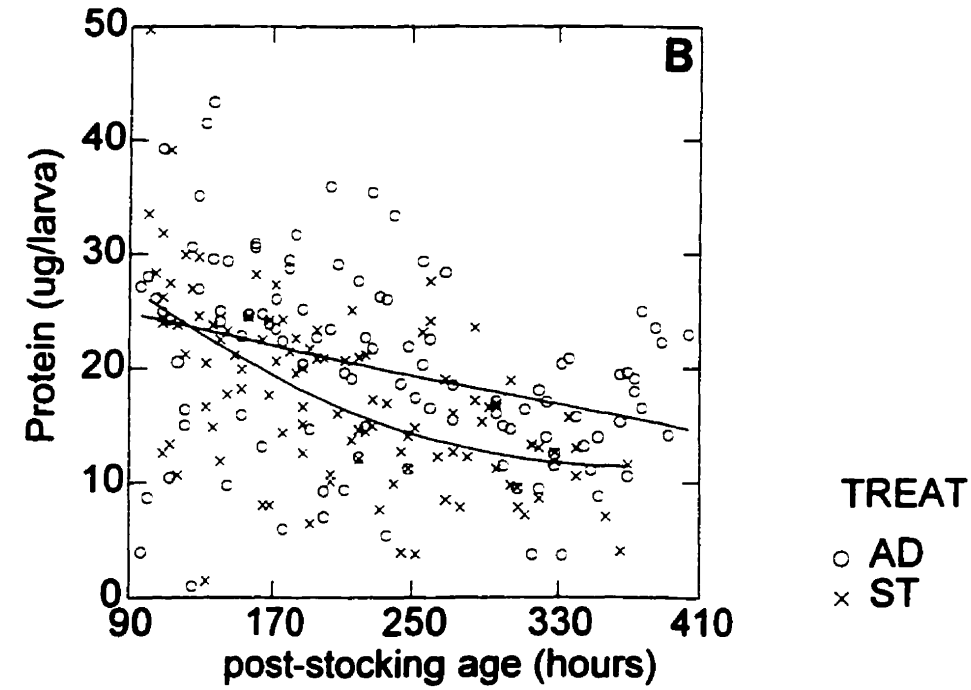
Dependent variable	Effect	Control-treatment couplings								
		Ad-st	Ad-f4	Ad-f8	St-s4	St-s6	St-s8	St-s10	St-s12	St-s14
Dry weight	Treatment	TANK	TANK	TANK	7.326**	17.083***	TANK	12.334**	7.923**	
	age*treat				5.391*	11.199**		13.101***	10.171**	
RNA	Treatment		13.402***							
	age*treat		27.474***							
DNA/dw	Treatment	TANK	TANK	TANK		9.187**	7.311**	13.980***		
	age*treat					12.204***	8.478**	23.240***		
RNA/dw	Treatment					11.418**	8.008**	11.145**		
	age*treat					10.906**	8.2211**	15.796***		
Prot/dw	Treatment			12.097***		9.343**				
	age*treat			11.297**		6.032*				
RNA/DNA	Treatment				7.971**					
	age*treat				3.357					
RNA/Prot	Treatment									8.245**
	age*treat									8.032**
Prot/DNA	Treatment	12.193***								
	age*treat	7.437**								

TABLE 2b Individual larvae homogenates

FIGURE 2a. Time response for the quantity of Protein per dry weight for the larvae sampled from the a*d libitum* feeding control and the starvation control tanks. The data points and fitted lines are plotted separately for each of the two control tanks. Data are from pooled larvae homogenates. Ad = *ad libitum* feeding control, st = starvation control.

FIGURE 2b. Time response for the quantity of Protein per larva for the larvae sampled from the a*d libitum* feeding control and the starvation control tanks. The data points and fitted lines are plotted separately for each of the two control tanks. Data are from pooled larvae homogenates. Ad = *ad libitum* feeding control, st = starvation control.


A
Protein / Dry weight
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
ST


B
Protein (ug/larva)
0
10
20
30
40
50
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
ST

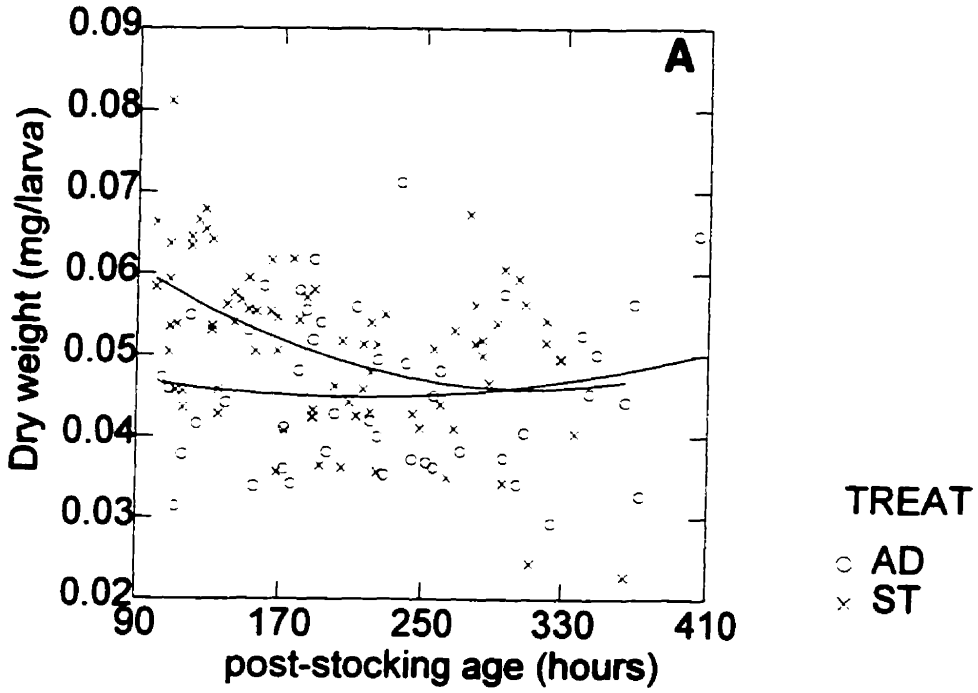
Results based on homogenates of individual larvae varied only slightly from those derived from pooled larvae homogenates. In this case the only significant polynomial regressions obtained were for the DNA/Dry weight response of larvae sampled from the starvation control tanks and the Protein/Dw and RNA/Protein ratio of the larvae sampled from the fed control tanks (Table 1b). Only the latter two variables were significantly ($P<0.01$) affected by post-stocking age. Fed larvae increased their protein content at a rate of 0.06% d^{-1} and RNA/Protein decreased by 0.04 d^{-1} (Table 1b). The only significant differences identified between fed and starved controls was in the slopes of the Protein/DNA ratio. Between-replicate differences in the size (Dry weight and DNA/Dw) of the larvae from the fed control tanks precluded further comparisons with the starvation controls.

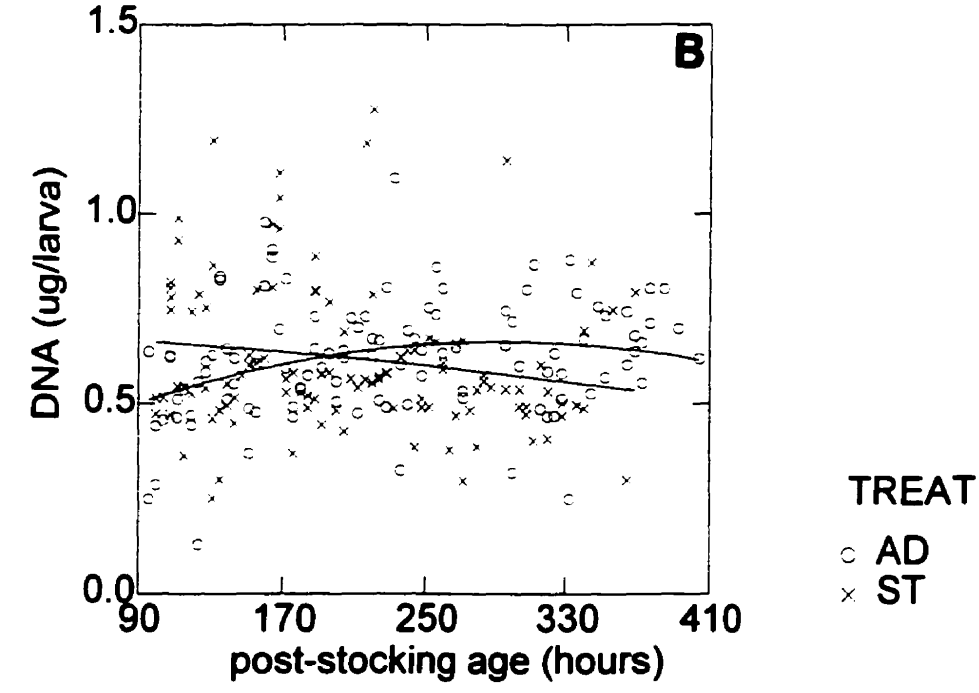
3.2.2 Feeding controls vs delayed-starvation treatments

Most models relating the biochemical components of pooled larvae homogenates to age were significant ($P<0.01$) for the f4 treatment, but only the RNA content changed significantly ($P<0.05$) with age when the larvae were starved after being fed for 4 days (Table 1a). Those larvae lost significant RNA (0.20 µg d^{-1}) after being deprived of food. Despite non-significant differences over six days of starvation, the larvae sampled from the f4 treatment tanks had the slope of the mean DNA content, the DNA/Dry weight and the Prot/DNA ratio against time which was significantly different (ANCOVA, $P<0.01$, Table 2a) than that of fed controls. The larvae from the f4 treatment tanks possessed higher DNA levels (0.8 µg $larva^{-1}$) than did the fed controls (0.5 µg $larva^{-1}$) at the onset of starvation, but lost DNA during starvation at rates that caused DNA to be lower than that of the controls after 6 days of starvation (Figure 4a). Prot/DNA ratios in the f4 treatments (25) were lower than those in fed controls (45) and increased to 35 to reach equal levels by the end of the 6 days of starvation (Figure 4b).

FIGURE 3a. Time response for the Dry weight of the larvae sampled from the *ad libitum* feeding control and the starvation control tanks. The data points and fitted lines are plotted separately for each of the two control tanks. Data are from pooled larvae homogenates. Ad = *ad libitum* feeding control, st = starvation control.

FIGURE 3b. Time response for the quantity of DNA per larva for the larvae sampled from the *ad libitum* feeding control and the starvation control tanks. The data points and fitted lines are plotted separately for each of the two control tanks. Data are from pooled larvae homogenates. Ad = *ad libitum* feeding control, st = starvation control.


A
Dry weight (mg/larva)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
ST


B
DNA (ug/larva)
1.5
1.0
0.5
0.0
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
ST

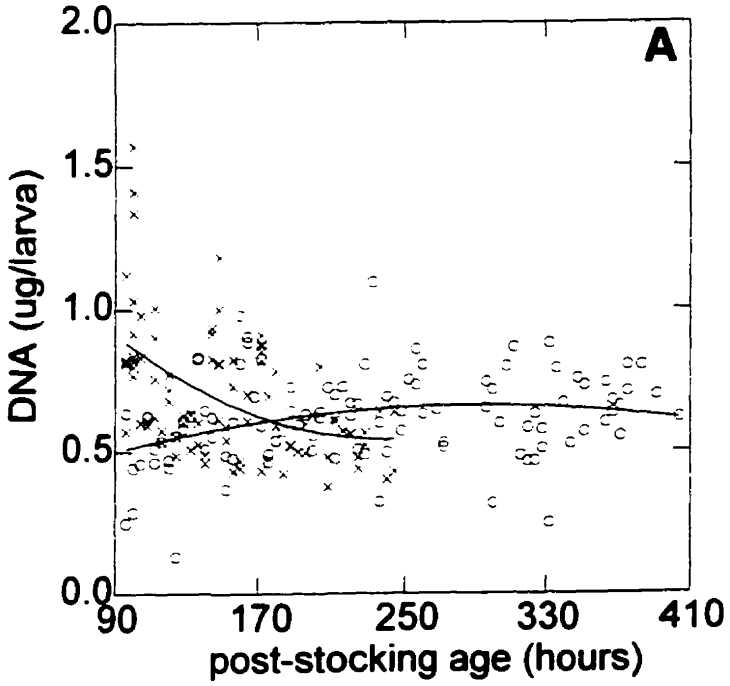
Larvae sampled from the f4 treatment tanks and analyzed individually revealed no significant effect of post-hatching age on any of the variables, except for the quantity of RNA per dry weight. This increased significantly ($P<0.01$) from initial levels of 5.47% at a rate of 0.11% d^{-1} over the six days of starvation (Table 1b). The quantity of RNA $larva^{-1}$ in the f4 treatment tanks diverged significantly ($P<0.001$, ANCOVA, Table 2b) from that of larvae sampled from the feeding treatment tanks. RNA $larva^{-1}$ in the f4 treatment tanks decreased from initial levels of 2.8 µg $larva^{-1}$ to approximately 1.5 µg $larva^{-1}$ at day 6. Over the same interval fed larvae from the control tanks maintained RNA levels above 2.5 µg $larva^{-1}$ (Figure 5a).

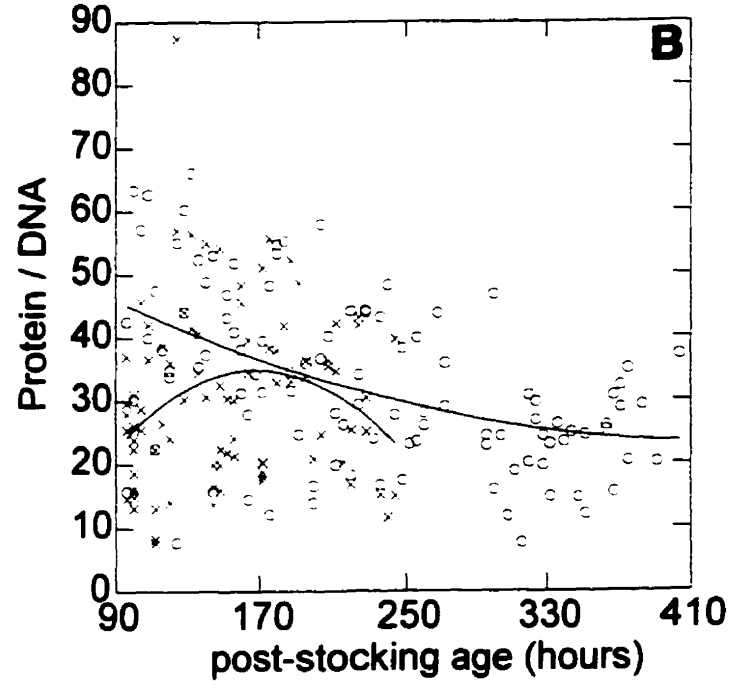
Larvae in the f8 treatment tanks (fed for 8 days and starved for 6 days) exhibited significant ($P<0.05$, Table 1a) reductions in RNA levels and RNA/DNA ratios. The quantity of RNA lost was similar to that recorded for the f4 treatment (0.00836 hr^{-1} = 0.20 µg d^{-1}). The RNA/DNA ratio decreased at a rate of 0.015 d^{-1} from initial levels of 3.5 at the onset of starvation (Table 1a). The principal differences between larvae sampled from the f8 treatment and from the fed controls were in dry weight and in protein $larva^{-1}$ (ANCOVA, $P<0.001$, Table 2a). Larval dry weights averaged approximately 45 µg $larva^{-1}$ in the fed controls over the entire experiment. Corresponding initial values in the f8 treatment tanks were approximately 30-40 µg at the onset of starvation, but increased to 60-70 µg $larva^{-1}$ after 6 days of starvation (Figure 5b). Protein quantity declined from initial values of approximately 20 µg $larva^{-1}$ (lower than fed control levels) to approximately 13 µg $larva^{-1}$ after 6 days of starvation (Figure 6a).

Individual larvae from the f8 treatment tanks exhibited significant weight gain and significant reductions in the RNA/Dw during the six days of starvation ($P<0.05$, Table 1b). However, when compared to the fed controls, only the relative protein content (Prot/dw) was found to diverge significantly ($P<0.01$, ANCOVA, Table 2b). Protein content decreased from 40-45% in both the f8 and

FIGURE 4a. Time response for the quantity of DNA per larva for the larvae sampled from the a*d libitum* feeding control and the f4 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. Ad = *ad libitum* feeding control, f4 = delayed-starved for 4 days.

FIGURE 4b. Time response for the Protein/DNA ratio for the larvae sampled from the a*d libitum* feeding control and the f4 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. Ad = *ad libitum* feeding control, f4 = delayed-starved for 4 days.


A
DNA (ug/larva)
2.0
1.5
1.0
0.5
0.0
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
F4


B
Protein / DNA
90
80
70
60
50
40
30
20
10
0
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
F4

fed controls, but the rate of decrease was higher from the onset of starvation in the f8 treatment tanks (Figure 6b).

3.2.3 Starvation controls vs delayed-feeding treatments

Several polynomial regressions fitted to the six delayed-feeding treatments (s4, s6, s8, s10, s12 and s14) were significant ($P<0.01$), but only one of the biochemical components measured was significantly affected by post-stocking age ($P<0.05$, Table 1a). The RNA/DNA ratio of the larvae sampled from the s4 treatment decreased significantly ($P<0.05$) with age (0.01565 hr^{-1} = 0.38 d^{-1}). Despite this relative lack of variation in condition, the overall response of these variables to delayed-feeding was quite distinct from the response observed in the starvation control tanks. For example, we found significant ($P<0.01$, ANCOVA, Table 2) differences the slopes of the absolute and relative Protein content responses in larvae sampled from the s4 tanks and the starvation control tanks. Larvae fed after having been starved for 4 days had lower protein content (17 µg $larva^{-1}$, 38% dry weight) than those ones from the starvation control tanks (28 µg $larva^{-1}$, 42%) at the onset of feeding (Figures 7a and 7b), but subsequently gained protein and attained levels of 23 µg $larva^{-1}$ (50%) 6 days after the commencement of feeding. Over the same interval the larvae from starvation controls continued to loose protein achieving terminal values of 15 µg $larva^{-1}$ (35% dry weight), (Figures 7a and 7b).

Only the RNA/Protein ratio for individual larvae in the s4 treatment was influenced by post-stocking age ($P<0.05$, Table 1b). This ratio decreased at a rate of 0.06 d^{-1} following feeding. The slopes of the dry weight and RNA/DNA ratio differed between the larvae sampled from the s4 treatment and starvation control tanks ($P<0.01$, ANCOVA, Table 2b). The mean size of the larvae in the s4 treatment was lower but remained stable while that of larvae in the starvation control tanks decreased. This produced a significant ($P<0.01$, Table 2a) divergence (Figure 8a). The RNA/DNA ratio of larvae in the s4 treatment was

FIGURE 5a. Time response for the amount of RNA per larva for the larvae sampled from the *ad libitum* feeding control and the f4 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. Ad = *ad libitum* feeding control, f4 = delayed-starved for 4 days.

FIGURE 5b. Time response for the Dry weight of the larvae sampled from the *ad libitum* feeding control and the f8 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. Ad = *ad libitum* feeding control, f8 = delayed-starved for 8 days.

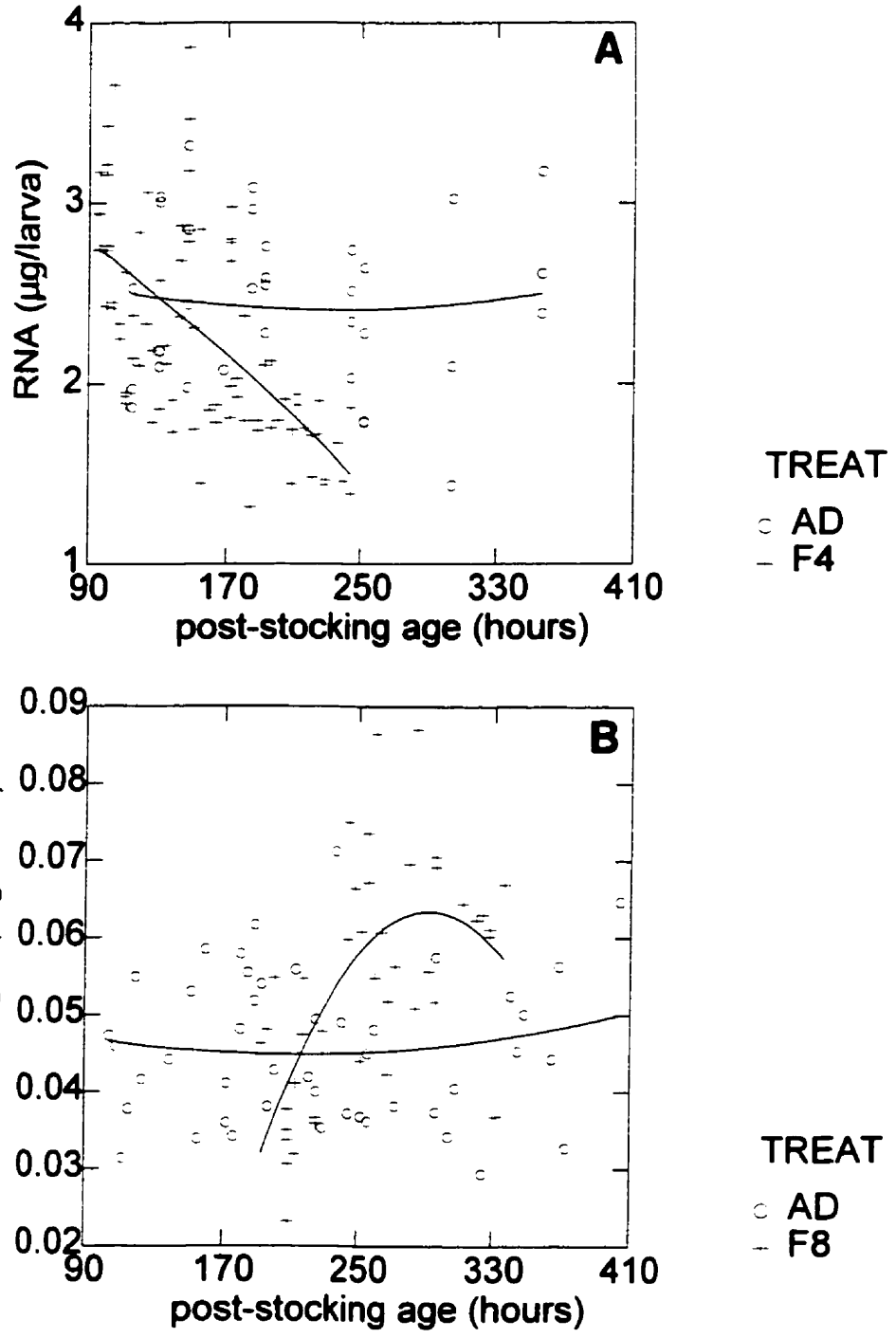
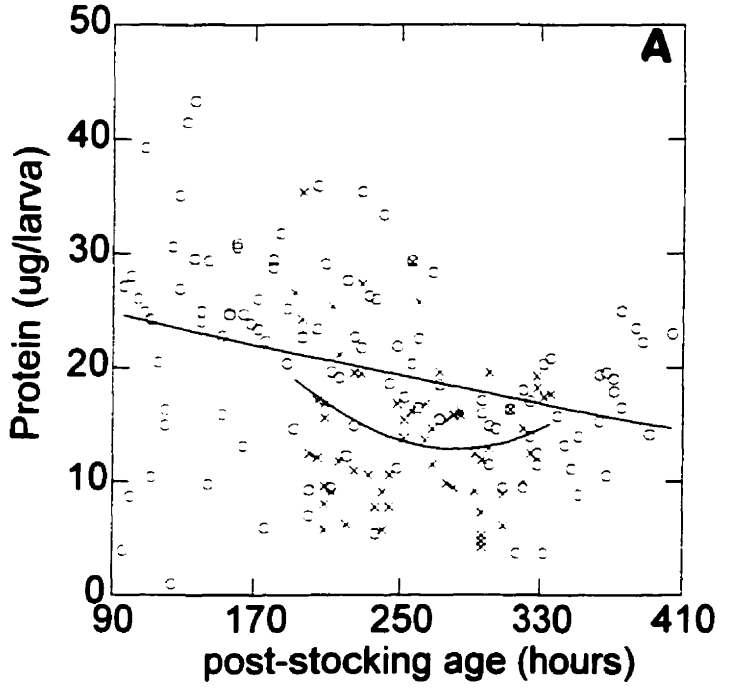
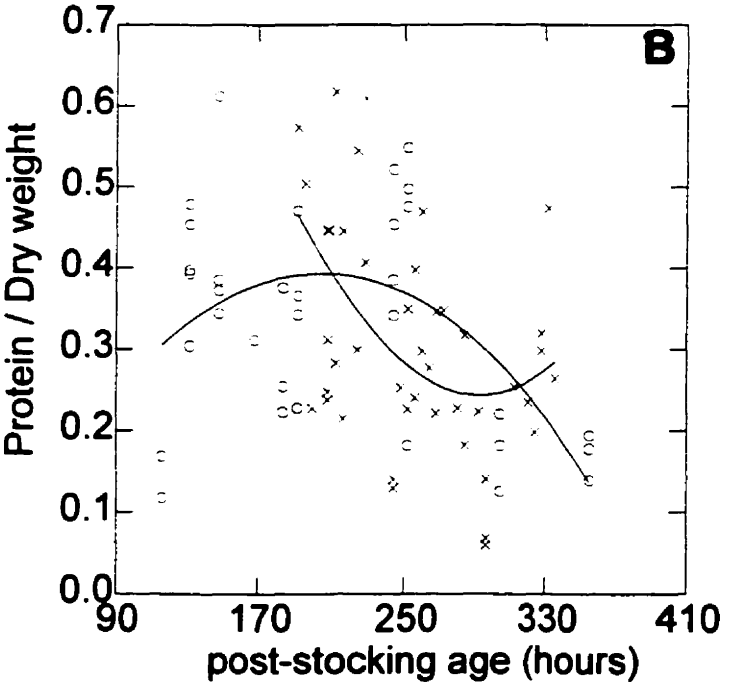

A
RNA (µg/larva)
4
3
2
1
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
F4
B
Dry weight (mg/larva)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
F8

FIGURE 6a Time response for the amount of Protein per larva for the larvae sampled from the a*d libitum* feeding control and the f8 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. Ad = *ad libitum* feeding control, f8 = delayed-starved for 8 days.

FIGURE 6b. Time response for the amount of Protein per dry weight for the larvae sampled from the a*d libitum* feeding control and the f8 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. Ad = *ad libitum* feeding control, f8 = delayed-starved for 8 days.


A
Protein (ug/larva)
50
40
30
20
10
0
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
F8


B
Protein / Dry weight
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
90
170
250
330
410
post-stocking age (hours)
TREAT
AD
F8

also lower (3.8) but stable and differed significantly ($P<0.01$, ANCOVA, Table 2b) from that of the controls (4.2). (Figure 8b).

Some variables measured on pooled larvae homogenates sampled from the s6, s8, s10 and s14 treatment tanks yielded significant regressions (RNA/DNA and Protein/DNA ratios) but none varied significantly with age ($P<0.01$, Table 1a). However, the pooled data indicate that larvae in these treatment groups differed significantly ($P<0.01$, ANCOVA, Table 2a) from the starvation controls in dry weight and in absolute and relative DNA and RNA responses (Figures 9a, 9b, 10a, 10b, 11a and 11b).

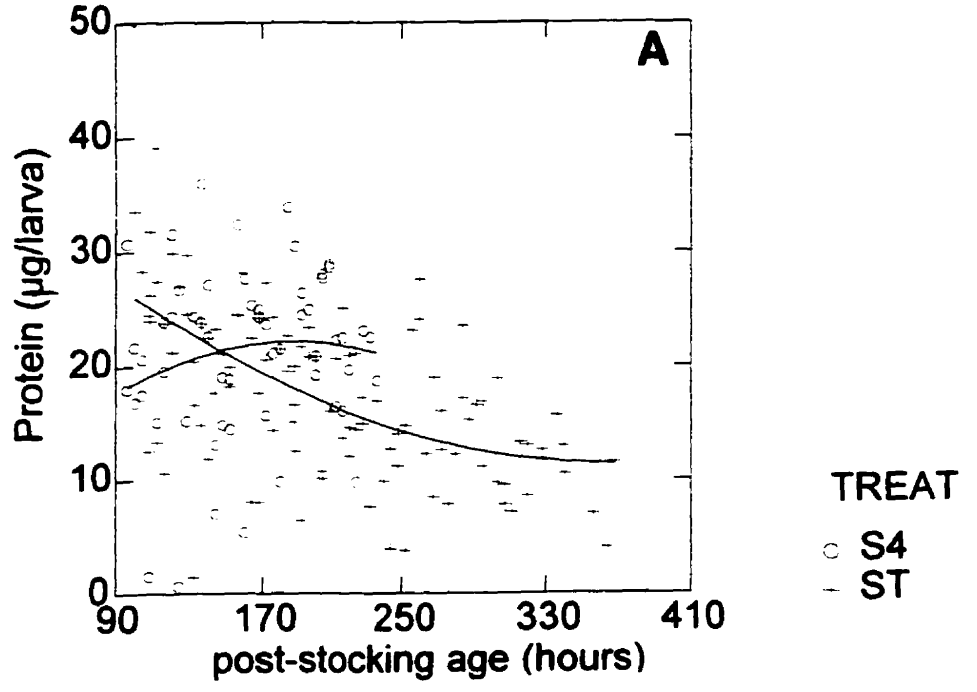
The dry weight of delayed-fed treatments and starvation controls diverged significantly ($P<0.01$, ANCOVA, Table 2a) in the s6 and s8 treatments. Intercepts differed significantly between the s14 treatment and the starvation controls. In these cases, the average size of larvae at the onset of feeding (40 μg $larva^{-1}$) was close to, or below that of starved larvae but quickly increased to weights above those of controls (55 μg $larva^{-1}$) soon after feeding (Figure 9a).

The DNA responses were less dramatic but the larvae in treatments s6, s8, s10 and s14 differed significantly ($P<0.01$, ANCOVA, Table 2b) from starvation controls. With the single exception of the s14 treatment which differed from the control in its slope, these differences were all in the intercept of the fitted lines. Larvae in the s14 treatment had DNA levels equal to those of the control at the onset of feeding but DNA content rapidly increased well above control levels (Figure 9b). The RNA content of the s14 treatments also increased and diverged significantly from the starvation controls (Figure 10a).

The slope of the DNA/Dw responses differed significantly from the controls in the s6, s8, s10 and s14 treatments ($P<0.01$, ANCOVA, Table 2a). DNA/Dry weight was higher by approximately 1% in the s8 and s10 treatments after 6, 8 and 10 days of starvation, but subsequently declined to near control levels

FIGURE 7a. Time response for the amount of Protein per larva for the larvae sampled from the starvation control and the s4 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. St = starvation control, S4 = delayed-fed for 4 days.

FIGURE 7b. Time response for the amount of Protein per dry weight for the larvae sampled from the starvation control and the s4 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. St = starvation control, S4 = delayed-fed for 4 days.


A
Protein (µg/larva)
50
40
30
20
10
0
90
170
250
330
410
post-stocking age (hours)
TREAT
S4
ST

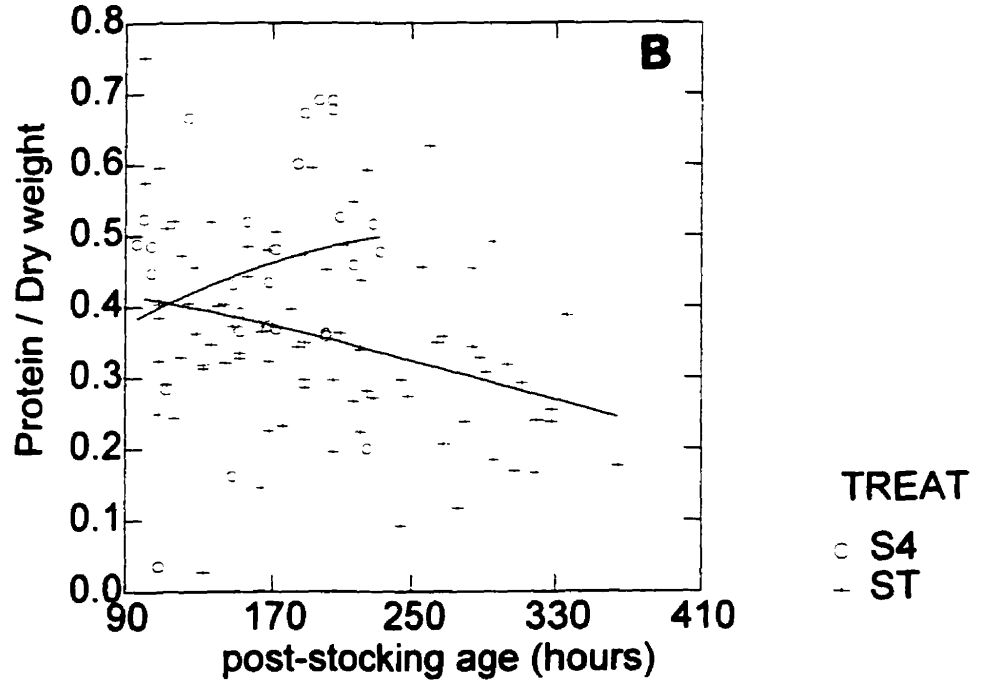
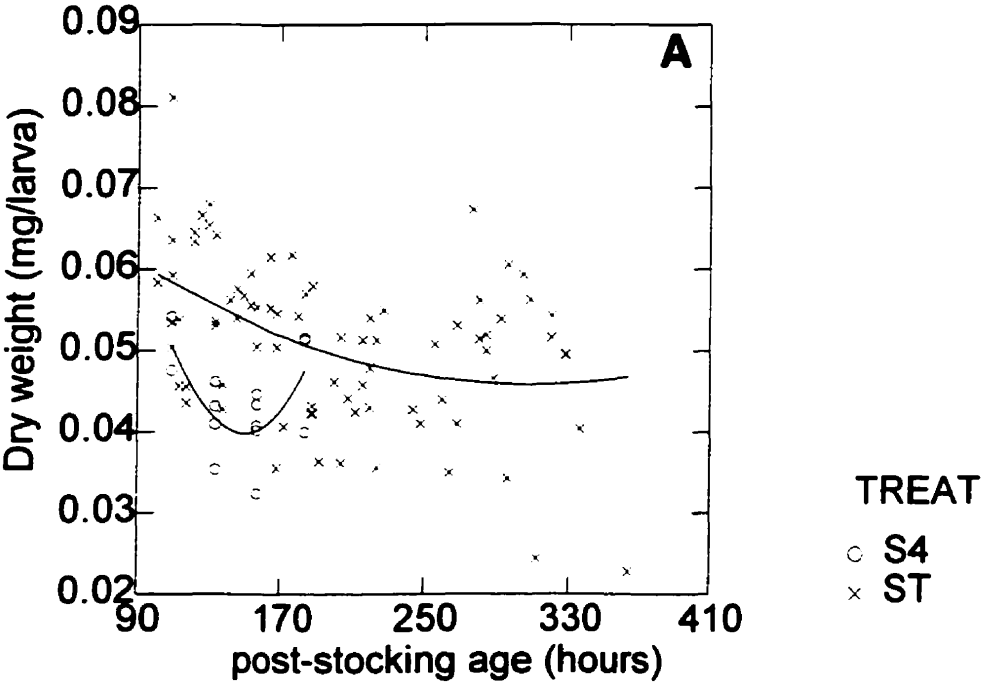
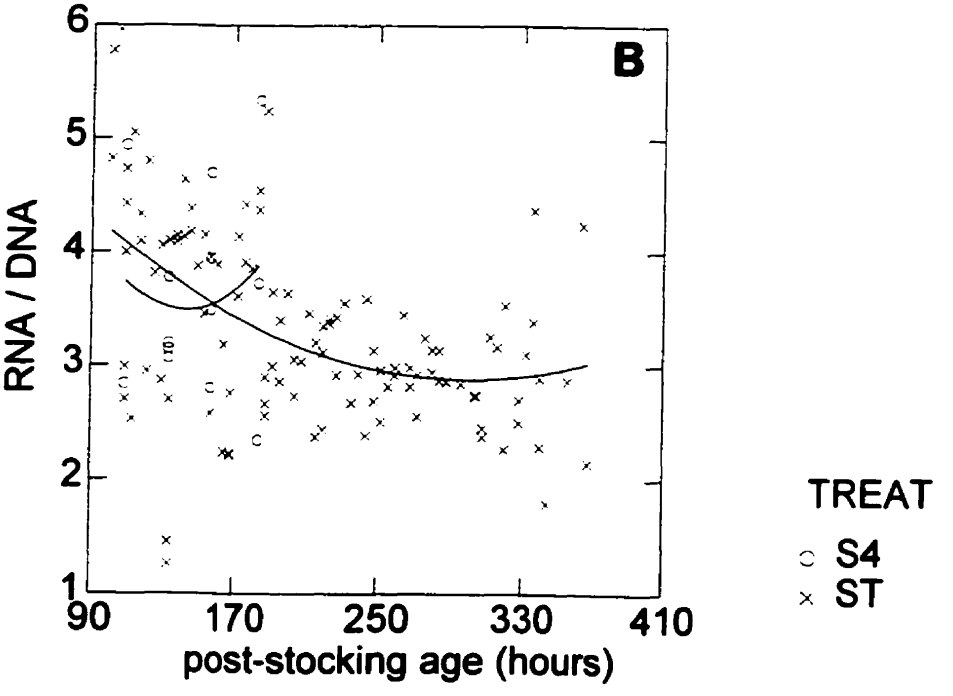

B
Protein / Dry weight
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
90
170
250
330
410
post-stocking age (hours)
TREAT
S4
ST

FIGURE 8a. Time response for the Dry weight of the larvae sampled from the starvation control and the s4 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. St = starvation control, S4 = delayed-fed for 4 days.

FIGURE 8b. Time response for the RNA/DNA ratio of the larvae sampled from the starvation control and the s4 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. St = starvation control, S4 = delayed-fed for 4 days.


A
Dry weight (mg/larva)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
90
170
250
330
410
post-stocking age (hours)
TREAT
S4
ST


B
RNA / DNA
6
5
4
3
2
1
90
170
250
330
410
post-stocking age (hours)
TREAT
S4
ST

(1.0%) following 6 days of feeding (Figure 10b, 11a). The larvae sampled from the s14 treatment tanks exhibited the opposite pattern being below control levels at the onset of feeding and increasing thereafter.

The RNA/Dw of larvae in the s6 and s8 treatments differed significantly ($P<0.01$, ANCOVA, Table 2) in intercept from control levels. Levels were higher (5.0%) than the starvation controls after 6 and 8 days of starvation, but converged to control levels (close to 5.0%) after 6 days of feeding (Figure 11b). Significant ($P<0.01$) differences in the RNA/DNA, RNA/Protein and Protein/DNA ratios in larvae sampled from the replicated starvation control tanks prevented comparisons with the delayed-fed treatments (Table 2a).

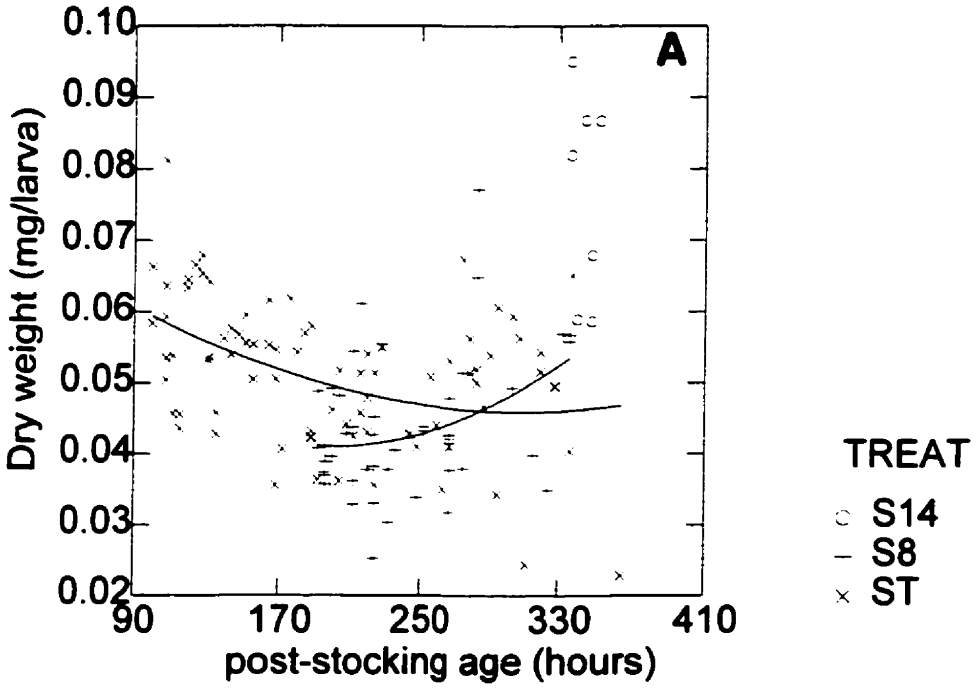
None of the index values calculated for individual larvae sampled from the delayed-fed treatments were significantly affected by age ($P<0.01$, Table 1b). However, the average dry weight of the larvae sampled from the s6, s8, s10 and s12 treatment tanks was similar or lower than starvation controls when feeding resumed (40 to 45 $\mu g\ larva^{-1}$), but increased rapidly and was significantly greater than that of controls after six days of feeding (Figure 12a and 12b). It is worth noting that this weight gain was greatest in larvae that were starved for the longest interval (s12 and s10) relative to shorter periods (s6 and s8). This contributed to the decrease and to the significant ($P<0.01$, ANCOVA, Table 2b) divergence in DNA/Dw and RNA/Dw in the treatment versus control larvae (Figures 13a, 13b, 14a and 14b).

3.3 Time of the day effects

Sampling of the feeding controls and starvation-delayed treatment tanks was conducted every day at 3h00, 7h00, 11h00, 15h00, 19h00 and 23h00. The starvation controls and delayed-fed treatment tanks were sampled daily at 5h00, 9h00, 13h00, 17h00, 21h00, and 1h00.

FIGURE 9a. Time response for the Dry weight of the larvae sampled from the starvation control and the s4 and s14 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. St = starvation control, S4 = delayed-fed for 4 days, s14 = delayed-fed for 14 days.

FIGURE 9b. Time response for the amount of DNA per larva for the larvae sampled from the starvation control and the s10 and s14 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. St = starvation control, S10 = delayed-fed for 10 days, s14 = delayed-fed for 14 days.


A
Dry weight (mg/larva)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
90
170
250
330
410
post-stocking age (hours)
TREAT
S14
S8
ST

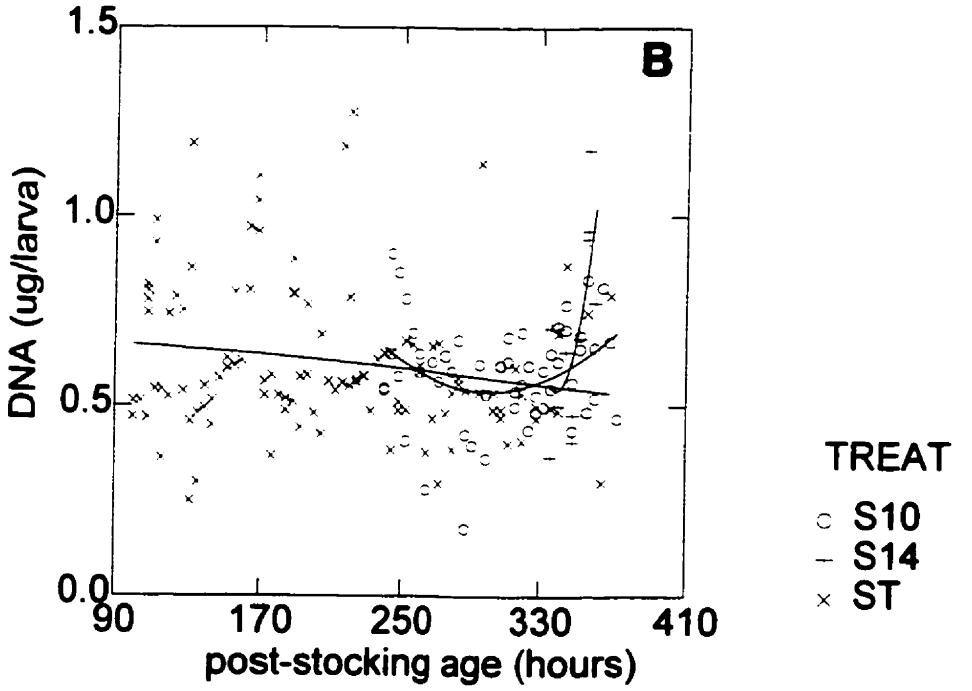
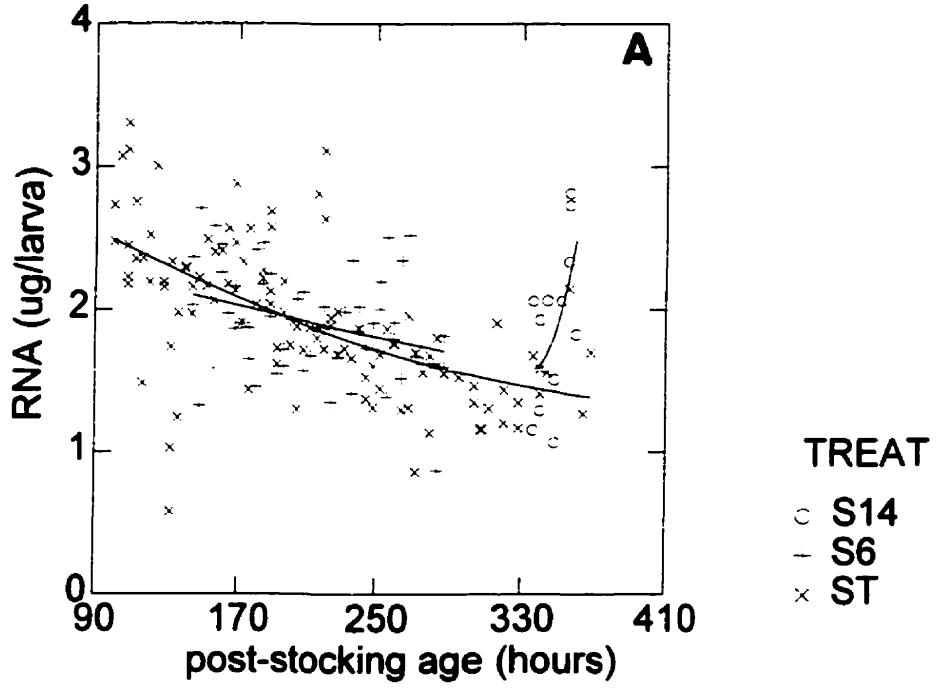
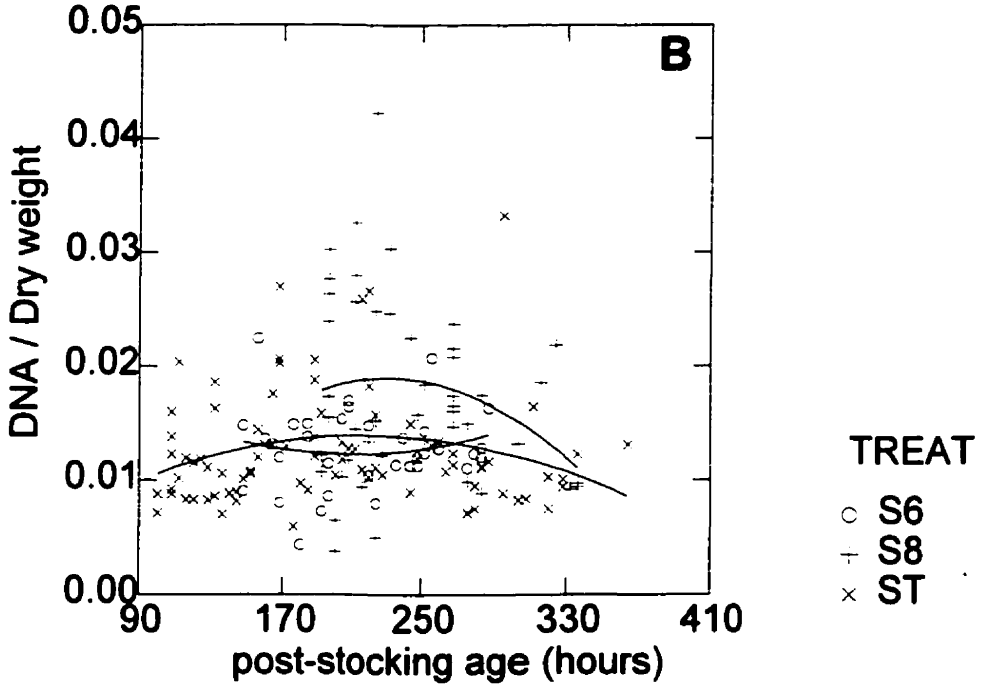

B
DNA (ug/larva)
1.5
1.0
0.5
0.0
90
170
250
330
410
post-stocking age (hours)
TREAT
S10
S14
ST

FIGURE 10a. Time response for the amount of RNA per larva for the larvae sampled from the starvation control and the s6 and s14 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. St = starvation control, S6 = delayed-fed for 6 days, s14 = delayed-fed for 14 days.

FIGURE 10b. Time response for the amount of DNA per dry weight of the larvae sampled from the starvation control and the s6 and s8 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. St = starvation control, S6 = delayed-fed for 6 days, s8 = delayed-fed for 8 days.


A
RNA (ug/larva)
0
1
2
3
4
90
170
250
330
410
post-stocking age (hours)
TREAT
S14
S6
ST


B
DNA / Dry weight
0.00
0.01
0.02
0.03
0.04
0.05
90
170
250
330
410
post-stocking age (hours)
TREAT
S6
S8
ST

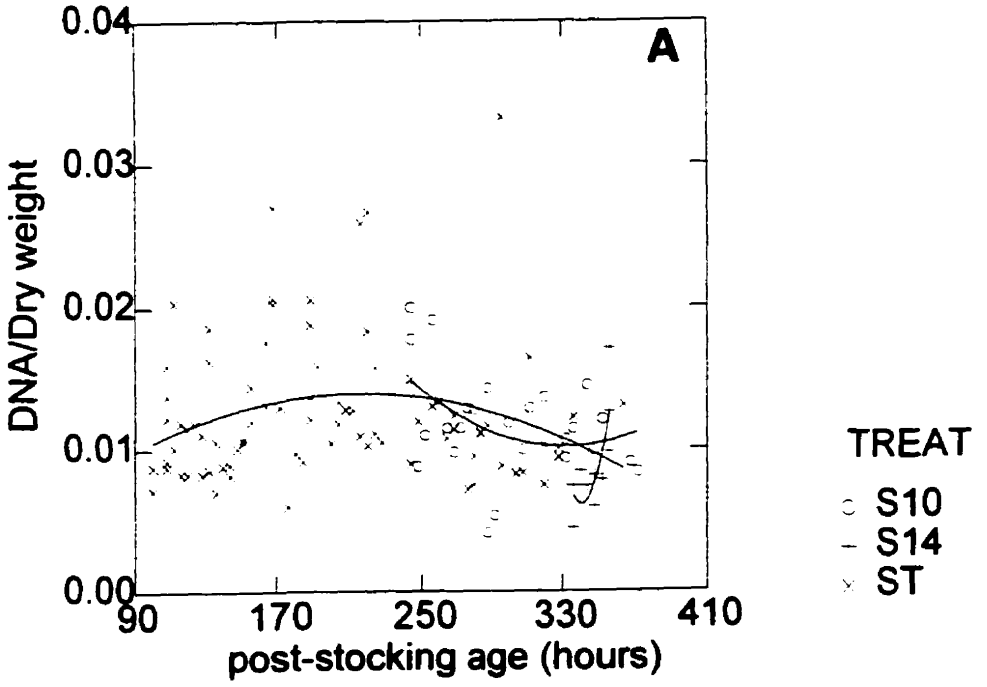
Larval DNA and RNA content (both absolute and relative to dry weight) was significantly related to the time of the day (ANOVA, $P<0.01$). Most larvae sampled from the feeding control and early delayed-fed treatments (s4 and s6) tanks exhibited two peaks and two lows troughs daily (Figures 15, 16 and 17), RNA/Dw and DNA/Dry weight being highest at dusk and late afternoon (7h00 and 17h00) and lowest at night and at mid-day (1h00-3h00 and 11h00, Figures 15a and 16b). This pattern was also evident in the larvae sampled from the longer delayed-fed (s8, s10, s12, and s14) treatments but the peaks here were lagged 2 hrs because of different sampling time, and occurred at 9h00 and 21h00 while the lows occurred at 5h00 and 13h00 (Figures 15b and 17a). Only one peak and one low was noticeable In the starvation controls and delayed-starved f4 and f8 treatments, higher DNA/Dw and RNA/Dw occurring at approximately 5h00, and lower levels in the afternoon (13h00 to 17h00, Figures16a, 17b). These diel patterns, while real, should not affect the shape of the time responses fitted for the various variables/treatments because the least-square fit of the sinusoid would approximate the fitted line. However, their presence undoubtedly contributed to the variability about the fitted lines and decreased the explained variability accounted for by post-stocking age in the polynomial regression models (Tables 1a & 1b).

4. DISCUSSION

Prior to Clemmesen (1987), the methods used to quantify nucleic acid and protein levels in fish larvae were constrained by the amount of tissue required, and this required that larvae be pooled (Buckley, 1979). Recent improvements in techniques (Clemmesen, 1993; Canino and Caldarone, 1995) have obviated this problem. However, to date the data on nucleic acid and protein levels in individual larvae have revealed high variability (Richard et al., 1991; Bergeron and Boulhic, 1994; Clemmesen, 1994; Clemmesen, 1996; Suthers et al. 1996; Folkvord et al., 1996; Kristogu-Baduge et al. 1999). This variability is all too often

FIGURE 11a. Time response for the amount of DNA per dry weight of the larvae sampled from the starvation control and the s10 and s14 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. St = starvation control, S10 = delayed-fed for 10 days, s14 = delayed-fed for 14 days.

FIGURE 11b. Time response for the amount of RNA per dry weight of the larvae sampled from the starvation control and the s6 and s8 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from pooled larvae homogenates. St = starvation control, S6 = delayed-fed for 6 days, s8 = delayed-fed for 8 days.


A
DNA/Dry weight
0.04
0.03
0.02
0.01
0.00
90
170
250
330
410
post-stocking age (hours)
TREAT
S10
S14
ST

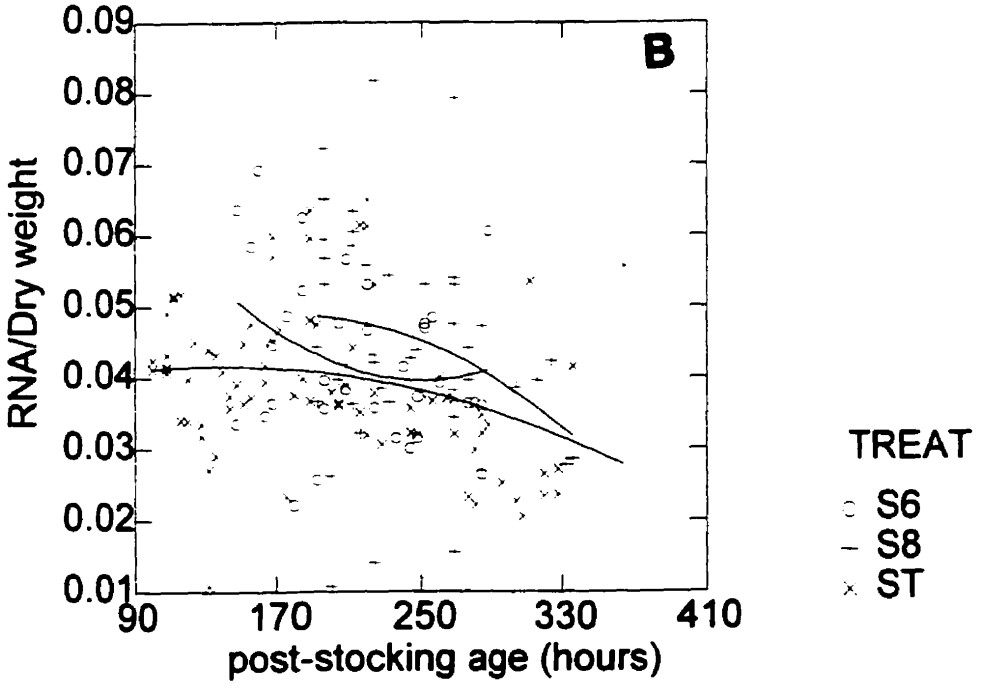
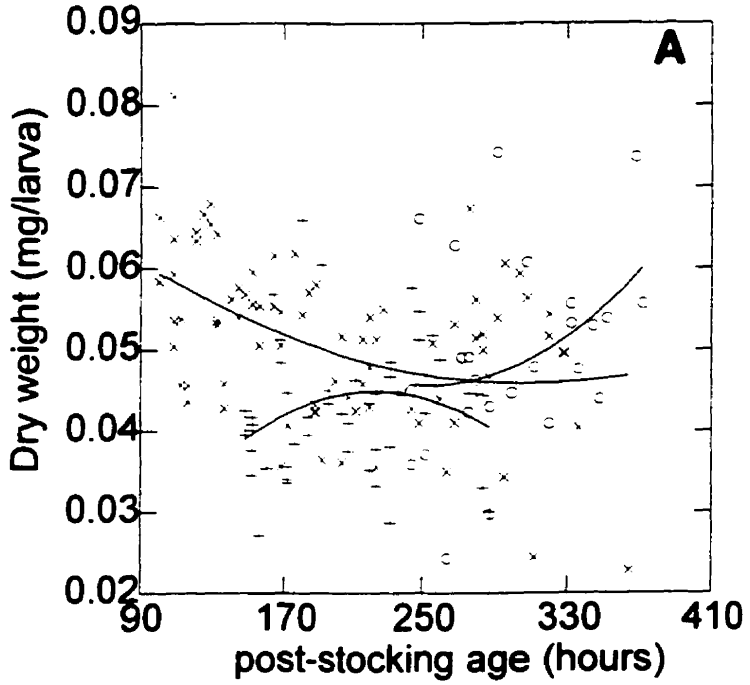

B
RNA/Dry weight
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
90
170
250
330
410
post-stocking age (hours)
TREAT
S6
S8
ST

FIGURE 12a. Time response for the Dry weight of the larvae sampled from the starvation control and the s6 and s10 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. St = starvation control, S6 = delayed-fed for 6 days, s10 = delayed-fed for 10 days.

FIGURE 12b. Time response for the Dry weight of the larvae sampled from the starvation control and the s10 and s12 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. St = starvation control, S10 = delayed-fed for 10 days, s12 = delayed-fed for 12 days.


A
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
Dry weight (mg/larva)
90
170
250
330
410
post-stocking age (hours)
TREAT
S10
S6
ST

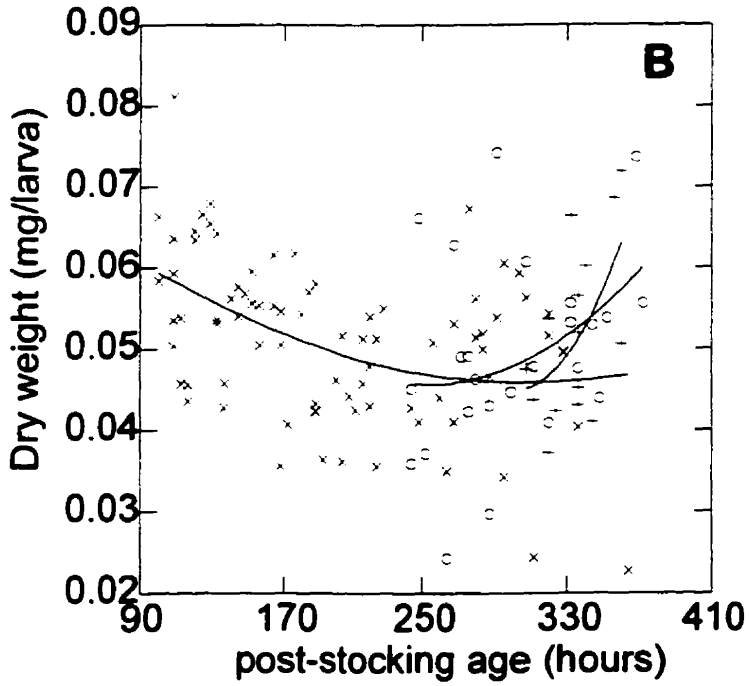
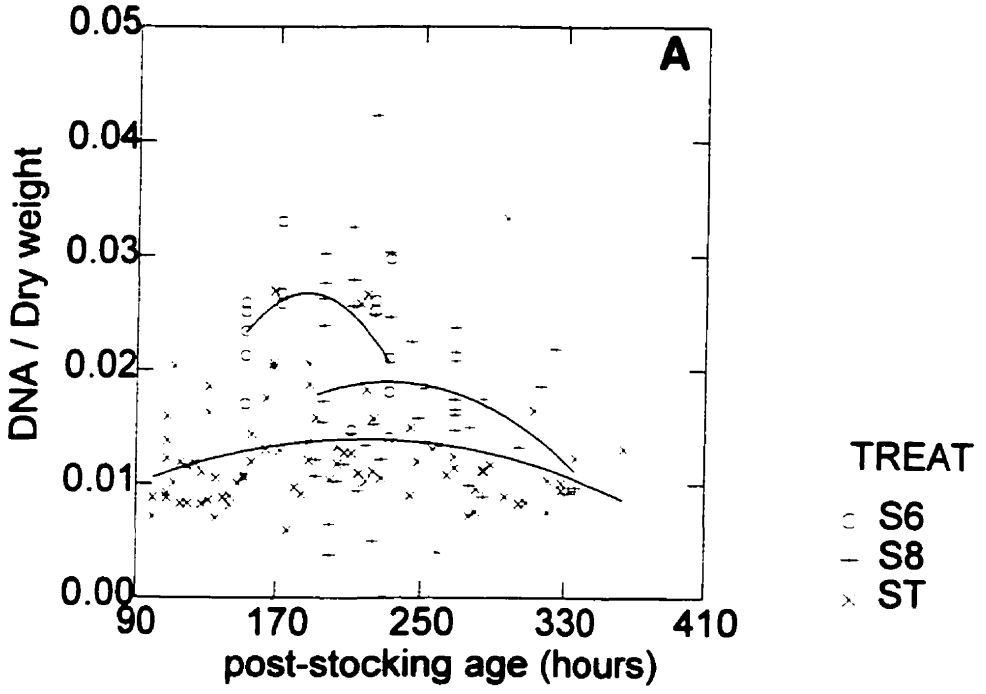

B
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
Dry weight (mg/larva)
90
170
250
330
410
post-stocking age (hours)
TREAT
S10
S12
ST

FIGURE 13a. Time response for the amount of DNA per dry weight of the larvae sampled from the starvation control and the s6 and s8 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. St = starvation control, S6 = delayed-fed for 6 days, s8 = delayed-fed for 8 days.

FIGURE 13b. Time response for the amount of DNA per dry weight of the larvae sampled from the starvation control and the s8 and s10 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. St = starvation control, S8 = delayed-fed for 8 days, s10 = delayed-fed for 10 days.


A
0.05
0.04
0.03
0.02
0.01
0.00
DNA / Dry weight
90
170
250
330
410
post-stocking age (hours)
TREAT
S6
S8
ST

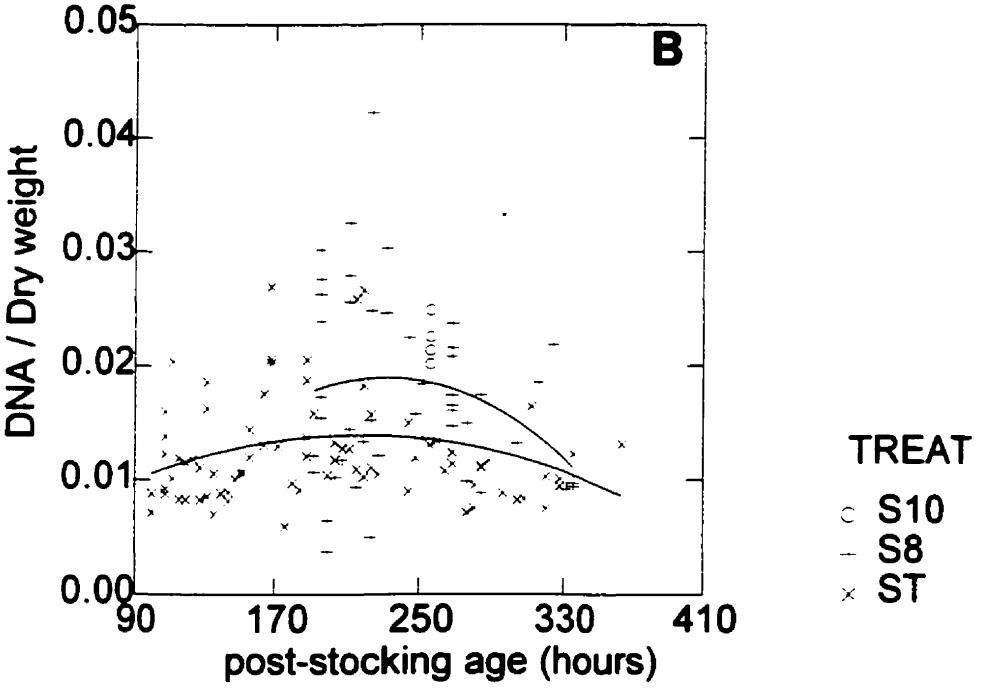
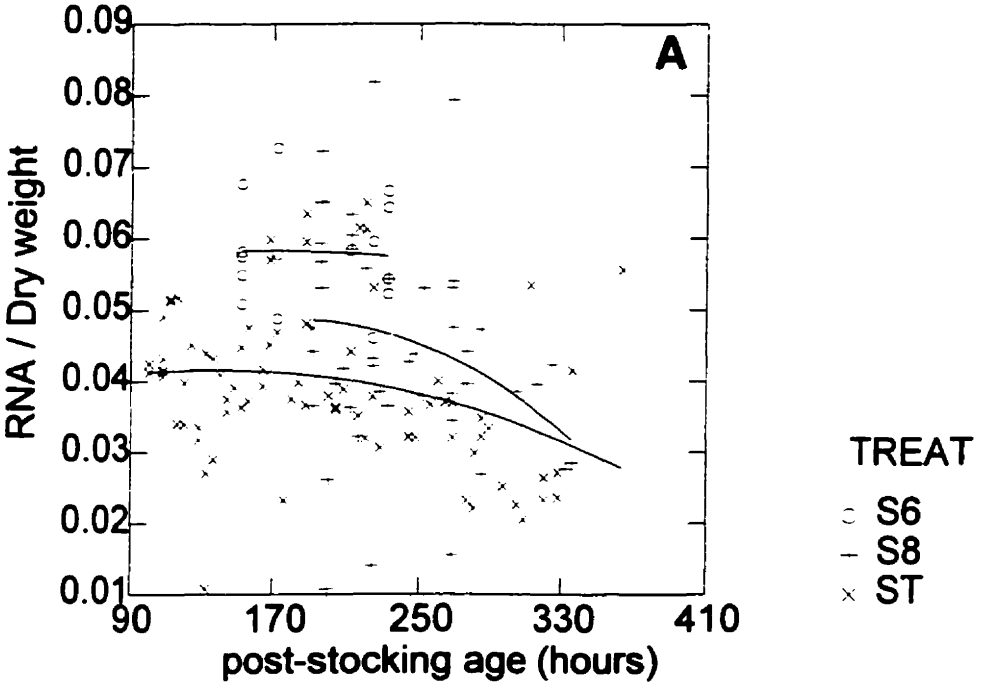
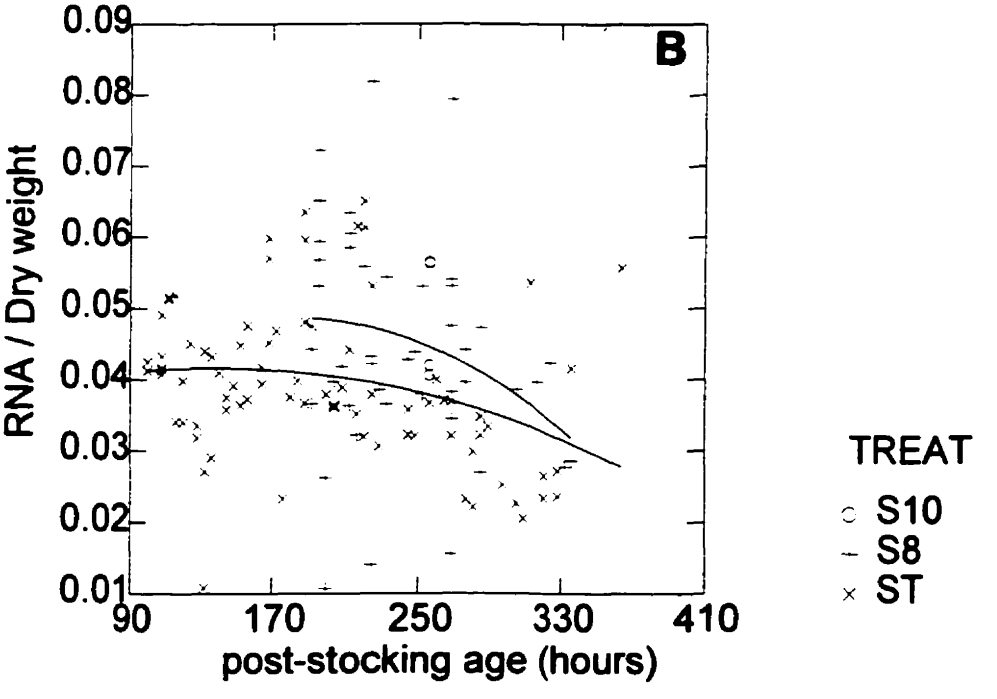

B
0.05
0.04
0.03
0.02
0.01
0.00
DNA / Dry weight
90
170
250
330
410
post-stocking age (hours)
TREAT
S10
S8
ST

FIGURE 14a. Time response for the amount of RNA per dry weight of the larvae sampled from the starvation control and the s6 and s8 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. St = starvation control, S6 = delayed-fed for 6 days, s8 = delayed-fed for 8 days.

FIGURE 14b. Time response for the amount of RNA per dry weight of the larvae sampled from the starvation control and the s8 and s10 treatment tanks. The data points and fitted lines are plotted separately for each of the two treatment and control tanks. Data are from individual larvae homogenates. St = starvation control, S8 = delayed-fed for 8 days, s10 = delayed-fed for 10 days.


A
RNA / Dry weight
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
90
170
250
330
410
post-stocking age (hours)
TREAT
S6
S8
ST


B
RNA / Dry weight
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
90
170
250
330
410
post-stocking age (hours)
TREAT
S10
S8
ST

marked by the reporting of group means and data smoothing (Mathers et al. 1994; Bisbal and Bengsten, 1995; Clemmesen and Doan, 1996; Rooker and Holt, 1996; Canino, 1997).

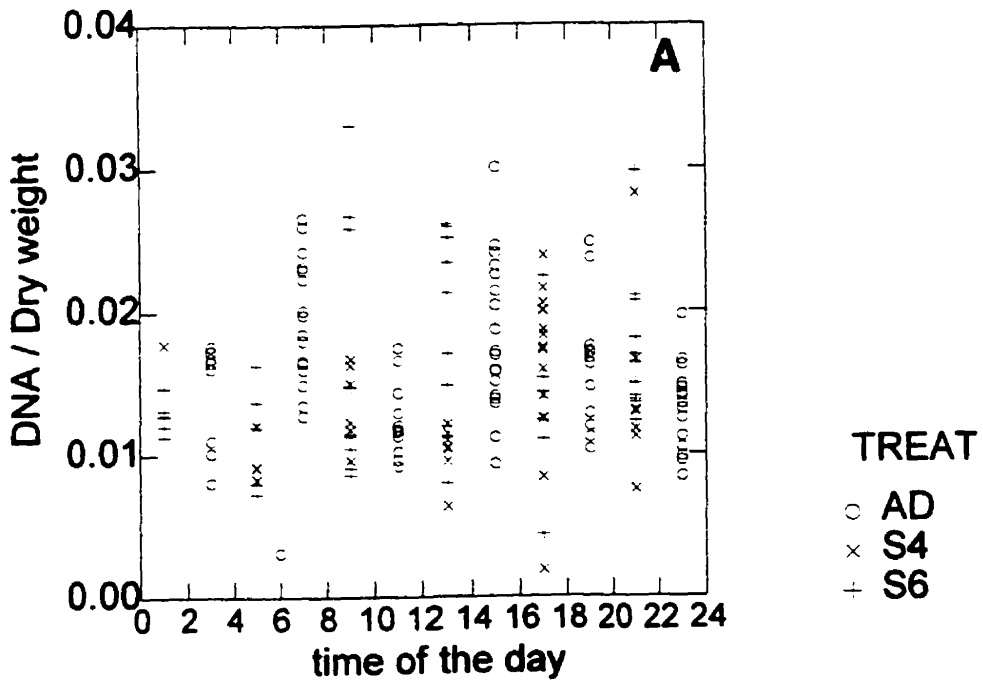
This work provides one of the first comparisons of data obtained from individual larvae and from pooled larvae homogenates. Our data confirm that the quantity of DNA, RNA and Protein per larva can and does vary significantly depending on the analytical approach (individual vs homogenized in groups). However, the overall variability (e.g. coefficient of variation) in these same measures is not affected by the way the larvae are homogenized and analyzed. The time response of condition measures to intermittent feeding was also affected differentially by the way the larvae are analyzed. While unequal sample sizes may have contributed to some degree to this difference, larvae analyzed individually exhibited lower variability about the fitted time responses, and more consistent patterns of divergence between treatment and controls. For these reasons, we recommend that individual-based analyses in which the full range of variability is retained and reported should become the standard in future experiments of this sort. This is also relevant to questions of the effects of condition on larval fishes since it is at the individual level that selection for growth and survival operates. In the discussion that follows we make no further distinction between data collected from individual versus pooled larvae

4.1 Variables most affected by age and most sensitive to intermittent feeding

Dry weight exhibited the most consistent response to intermittent feeding differing significantly (ANCOVA, $P<0.01$, Table 2a and 2b) in 6 out of 9 control-treatment comparisons. Larvae in the starvation controls were larger than those in the fed controls (60 versus 50 μg larva^{-1}) at the beginning of the experiments, but this pattern was reversed by the end of the experiments, larvae from the

FIGURE 15a. Scatterplot showing the amount of DNA per dry weight in relation to the time of the day sampling for the *ad libitum* control and the delayed-fed treatments. The data points are plotted separately for each treatment and control tanks supplied with food. Ad = ad libitum feeding, s4 = delayed-fed for 4 days, s6 = delayed-fed for 6 days.

FIGURE 15b. Scatterplot showing the amount of DNA per dry weight in relation to the time of the day sampling for the delayed-fed treatments. The data points are plotted separately for each treatment and control tanks supplied with food. S8, s10, s12 and s14 = delayed-fed for 8, 10, 12 and 14 days.


A
DNA / Dry weight
0.04
0.03
0.02
0.01
0.00
0 2 4 6 8 10 12 14 16 18 20 22 24
time of the day
TREAT
AD
S4
S6

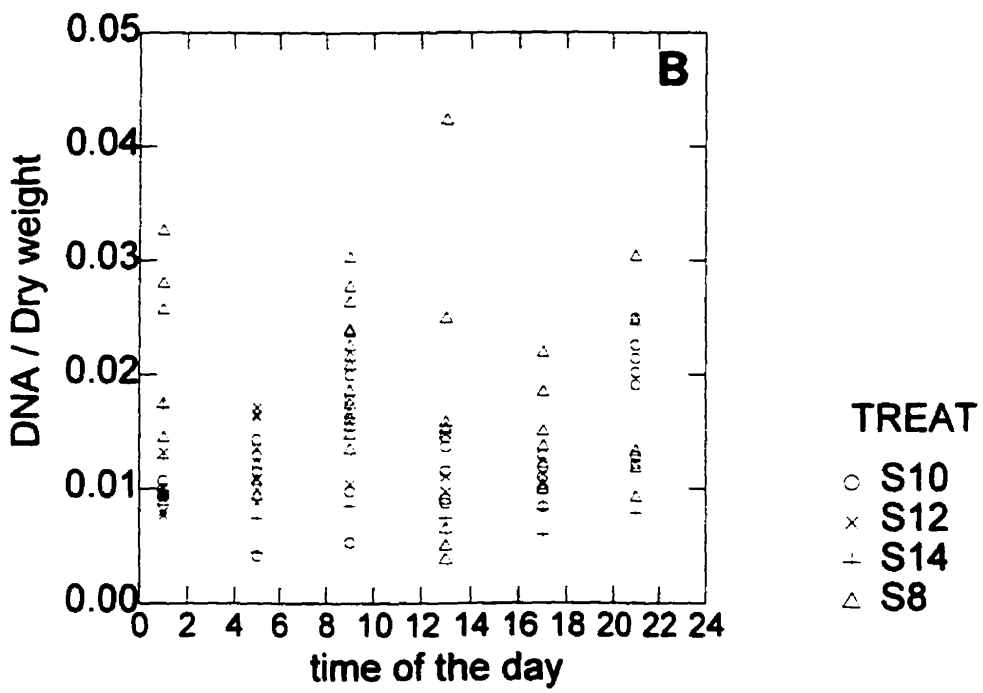
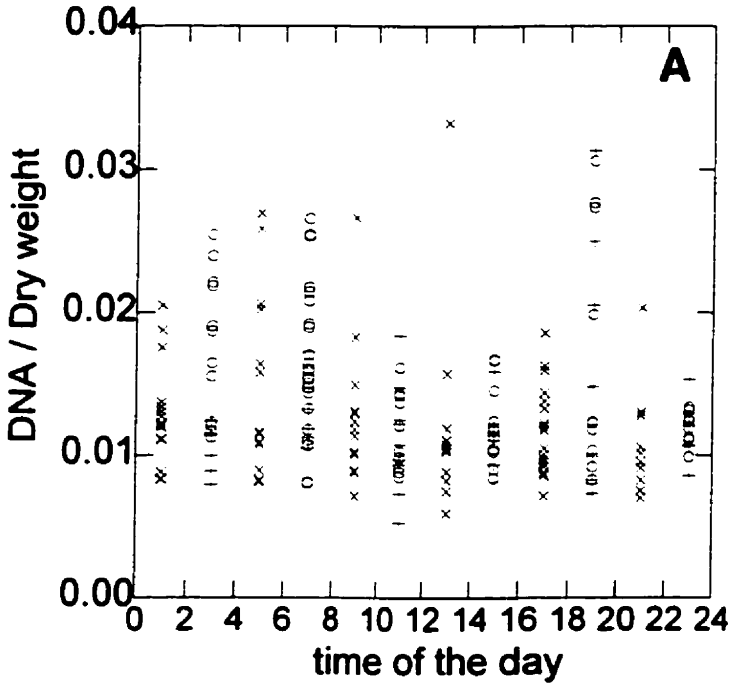

B
DNA / Dry weight
0.05
0.04
0.03
0.02
0.01
0.00
0 2 4 6 8 10 12 14 16 18 20 22 24
time of the day
TREAT
S10
S12
S14
S8

FIGURE 16a. Scatterplot showing the amount of DNA per dry weight in relation to the time of the day sampling for the starvation control and the delayed-starved treatments. The data points are plotted separately for each treatment and control tanks not supplied with food. F4 and f8 = delayed-starved for 4 and 8 days.

FIGURE 16b. Scatterplot showing the amount of RNA per dry weight in relation to the time of the day sampling for the *ad libitum* control and the delayed-fed treatments. The data points are plotted separately for each treatment and control tanks supplied with food. Ad = ad libitum feeding, S4 and s6 = delayed-fed for 4 and 6 days.


A
DNA / Dry weight
0.04
0.03
0.02
0.01
0.00
0 2 4 6 8 10 12 14 16 18 20 22 24
time of the day
TREAT
F4
F8
ST

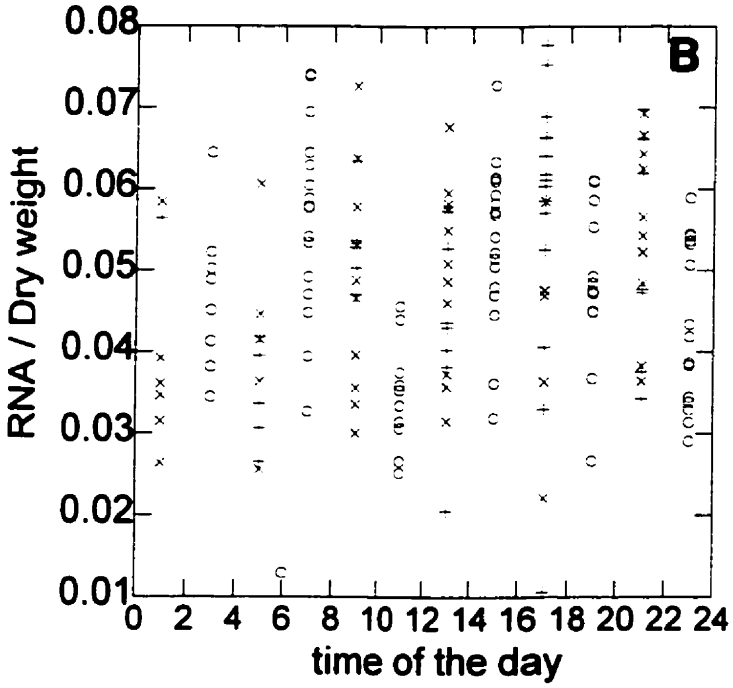
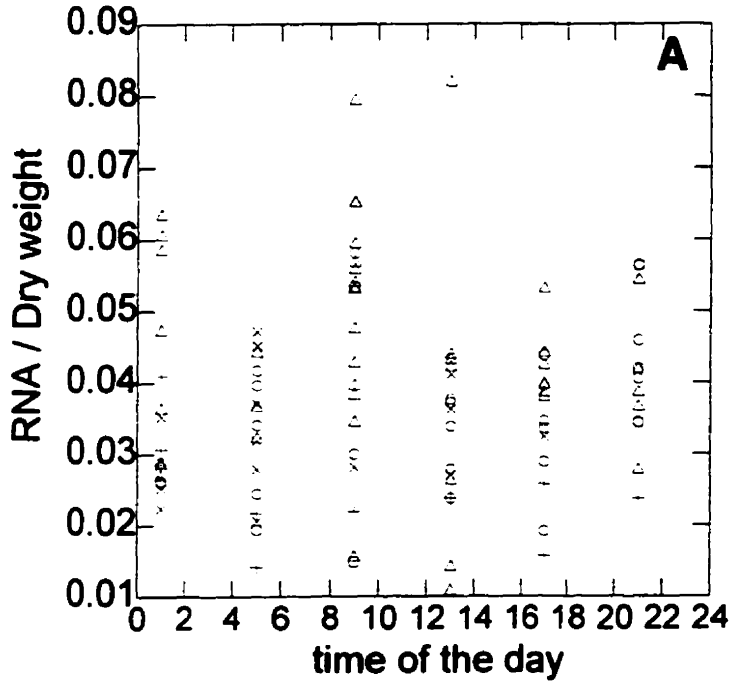
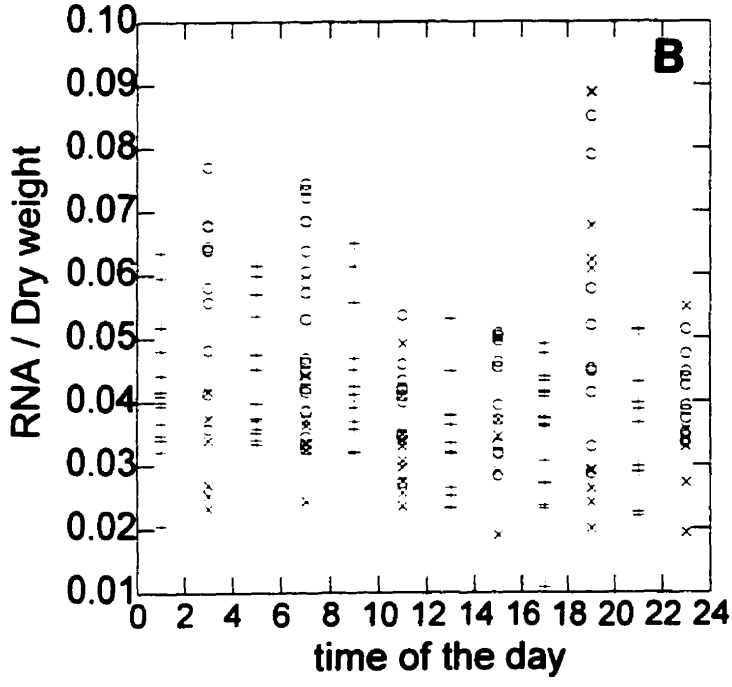

B
RNA / Dry weight
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0 2 4 6 8 10 12 14 16 18 20 22 24
time of the day
TREAT
AD
S4
S6

FIGURE 17a. Scatterplot showing the amount of RNA per dry weight in relation to the time of the day sampling for the delayed-fed treatments. The data points are plotted separately for each treatment and control tanks supplied with food. S8, s10, s12 and s14 = delayed-fed for 8, 10, 12 and 14 days.

FIGURE 17b. Scatterplot showing the amount of RNA per dry weight in relation to the time of the day sampling for the starvation control and the delayed-starved treatments.. The data points are plotted separately for each treatment and control tanks not supplied with food. St = starvation, f4 and f8 = delayed-starved for 4 and 8 days.


A
RNA / Dry weight
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0 2 4 6 8 10 12 14 16 18 20 22 24
time of the day
TREAT
S10
S12
S14
S8


B
RNA / Dry weight
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0 2 4 6 8 10 12 14 16 18 20 22 24
time of the day
TREAT
F4
F8
ST

starvation controls being smaller (50 µg larva^{-1}) than those from feeding controls. (60 µg larva^{-1}). Larvae sampled from the starvation controls at the termination of the experiments were also shorter than those that fed *ad libitum* (mean notochord length of 9.9 and 11.5 mm respectively). This finding is consistent with the results reported in chapter 2 which showed that dry weight was more sensitive to starvation than any of the other condition indices tested (Tables 3 and 4, chapter 2).

The rate of decrease in dry weight of larvae in the starvation control tanks ranged from 1-3 µg d^{-1} (1.6-5.0% d^{-1}) for pooled and individual larvae (Figures 3a and 8a). The mean of those rates (2 µg d^{-1}, 3.3% d^{-1}) was very similar to the 2.1 µg d^{-1} (4.2 % d^{-1}) weight loss recorded for starving capelin larvae from the experiments described in chapter 2, and to the 2.6 % d^{-1} weight loss reported by Kristogu-Baduge et al. (1999) for starving herring *(Clupea harengus)* larvae maintained at 8°C. Bisbal and Bengtson (1995) reported a weight loss of 10.4% d^{-1} in 16 d old feeding summer flounder *(Paralichthys dentatus)* larvae starved for 3 d, but no significant weight loss in 6 d old first-feeding larvae after 2.5 d of starvation in the same experiments.

Surprisingly, the mean dry weight of the capelin larvae fed *ad libitum* for 13 days in our experiments did not increase significantly. This suggest that feeding conditions may have been sub-optimal. Nevertheless, the positive trends evident in the majority of the delayed-fed (s4, s8, s10, s12 and s14) treatments (Figures 8a, 9a, 12a, 12b) are consistent with the non-linear improved condition predicted in Ferron and Leggett (1994). This non-linear recovery was particularly impressive in the s14 treatment group (Figure 9a), whose dry weights ultimately reached values twice those of larvae in the starvation controls.

Folkvord et al. (1996) reported that dry weight responds very quickly to starvation and or renewed feeding in herring larvae, relative to changes in protein content and length, the latter being uncoupled from weight changes and

exhibiting a significant time lag. We too found dry weight to be highly sensitive to intermittent feeding. Our data also point to the close relationship between dry weight and age. This requires that dry weight as an index of feeding success be used with care always in association with size-at-age data. Buckley et al. (1991a) found that dry weight was more highly correlated with survival over the first month of life than any of nucleic acid, protein, lipid measurements when these analyses were applied to winter flounder *(Pseudopleuronectes americanus)* larvae. While our experiments with capelin were of shorter duration, (maximum of 17 days), the results of the delayed-fed treatment s14 also indicate that larger survive longer starvation intervals and show more rapid response in both size and condition once feeding resumes.

DNA content per larva was not significantly affected by starvation over the course of our trials (Table 1a, 1b, Figures 3b, 9b). However, we did find that larvae in the *ad libitum* trials exhibited significantly higher DNA levels than did starved larvae (Table 2a, Figure 3b). This is consistent with the findings reported in chapter 2, and reinforces and further validate the use of DNA to standardize for size differences between animals subjected to condition measures are desired (Ferron and Leggett, 1994; Bergeron, 1997; Bergeron et al., 1997; Bergeron and Person-Le Ruyet, 1997). Folkvord et al. (1996) and Kristogu-Baduge et al. (1999) have also reported that starved larvae conserve DNA but that fed larvae usually grow by increasing their DNA content.

The responses curves of DNA/dw were, as a consequence of DNA stability, primarily influenced by variation in dry weight (see Figures 9a, 10b, 12a, 12b, 13a and 13b). This index was unaffected ($P<0.01$) by post-stocking age (Tables 1a and 1b) with the exception of the larvae in the starvation controls. There were, however, significant and consistent differences in the DNA/dw between controls and treatments (5 out of 9 comparisons, Tables 2a and 2b). DNA/Dw was thus the second most consistent index of intermittent feeding after dry weight. Its advantage over dry weight alone is more independence from age.

DNA per dry weight index was first suggested by Richard et al. (1991) and Bergeron et al. (1991), who worked with sole *(Solea solea)* larvae. They found DNA/Dw to be more responsive to starvation and less variable and more reproducible than proteins, nucleic acids and dry weight. They concluded that DNA/Dw indices >3% were indicative of starvation. Kristogu-Baduge et al. (1999) found DNA/Dw to be consistently higher in starved than fed larvae of herring *(Clupea harengus)*, and concluded that levels >2.2% were indicative of starvation. In our experiments with capelin larvae, levels above 3% were rare. However, a significant percentage of the larvae that had been starved for 6, 8 and 10 days exhibited DNA/Dw indices between 2 and 3% at the onset of feeding (Figures 10b, 11a, 13a, 13b). The index value then rapidly declined below 2% following exposure of the larvae to food. In all of our experiments, the origin of our response curves for the index was approximately 1.8-1.9%. This result suggests a value of 2% as the transition between fed and starved larvae.

The RNA/dw and Protein/dw indices both decreased significantly ($P<0.01$) in relation to post-stocking age (Tables 1a and 1b). This occurred in the f4 and f8 treatments and in the starvation controls. The treatments also diverged significantly ($P<0.01$) from the controls 4 of 9 cases (Tables 2a and 2b). RNA/Dw and Protein/Dw thus ranked third in their effectiveness as condition indices sensitive to intermittent feeding. The most evident of these responses occurred in the Protein/Dw, and facilitated the separation of fed and starved controls (Figure 2a). The deterioration and subsequent recovery of Protein/dw in the s4 treatment (Figure 7b) was consistent with predictions (Ferron and Leggett, 1994). However, RNA/Dw responses exhibited by larvae in the delayed-fed treatments (s6, s8, s10) were inconsistent with expectations (Figures 11b, 14a, 14b). Reference to Figures 13a, 13b, 14a, and 14b, reveals that the variations in RNA/Dw as with DNA/Dw, were primarily driven by variations in dry weight and not to variations in the RNA content of the larvae. The Protein per dry weight (Protein/Dw), index however, differed significantly between fed and starvation controls, and

responded as (Figure 6b, 7b) and was even less sensitive to changes in dry weight alone. Based on the responses reflected in the s4 and f8 treatments, we conclude that Protein/Dw indices <30% are likely to be indicative of starvation (Figures 6b, 7b).

All larvae sampled from the starvation controls and from the f4 and f8 treatments experienced significant reductions in RNA ($P>0.05$) over the time course of the experiments (Table 1a, Figures 6a, 10a). The dynamics (rate of change) of these responses approximated 0.20 $\mu g\ d^{-1}$. As a result, the RNA/DNA ratio index also decreased significantly in the starvation control and the s4 treatment and at a rate approximating 0.35-0.37 d^{-1}. Notwithstanding some tank effects, our findings indicate that the RNA/DNA, RNA/Protein and Protein/DNA ratios were most strongly affected by post-stocking age (Table 1a). However, we observed only 3 of 9 significant treatment-control differences for RNA and Protein content per larva, only 2 of 9 for the Protein/DNA ratio and only one of 9 for the RNA/DNA and RNA/Protein ratios (Table 2a and 2b) making them the least sensitive measures of intermittent feeding among the series of variables compiled. The most significant divergences we observed occurred in the RNA content of larvae from the f4 treatment relative to fed controls (Table 1b, Figure 5a) and in the Protein and Protein/Dw of larvae in the s4 treatment relative to the starvation controls (Figures 7a and 7b). RNA/DNA ratios obtained from the starvation and fed controls tanks, and the RNA/Protein and Protein/DNA ratios of larvae in the starvation control tanks could not be accurately replicated (tank effects), and this prevented their comparison with corresponding delayed-fed and delayed-starved treatments (Table 2a).

The RNA/DNA ratio has been widely used as an index of the time response of condition to intermittent feeding in fish larvae (reviewed in Ferron and Leggett, 1994), but some ontogenetic changes have to be considered. Clemmesen (1994), for example, found the ratio in laboratory-reared herring *(Clupea harengus)* larvae to lie between 4.0 and 5.0, 2-3 days after hatching, after which

it declined exponentially to values between 2.0 and 3.0, 4 to 8 days post-hatch. This decline occurred in both starved and fed larvae. Changes in the RNA/DNA ratio attributable to starvation were evident only after 3 days of starvation and only in larvae which were fed continuously for 16 days or more. Bergeron and Boulhic (1994) working with sole *(Solea solea)* larvae and Bisbal and Bengston (1995) working with summer flounder *(Paralichthys dentatus)* larvae reported similar responses. Both reported a significant decrease in the RNA/DNA ratio between hatching and 4-7 days post-hatch, and found differences between fed and starved larvae only when starvation was imposed at least 16 days post-hatch.

The RNA/DNA ratio we measured in capelin larvae at the beginning of the experiments (4.0-5.0, 4 days post-hatch) were similar to those reported by Clemmesen (1994) for herring larvae, by Bisbal and Bengston (1995) for summer flounder larvae and by Canino (1997) for walleye pollock larvae. Clemmesen and Doan (1996) reported slightly higher values (7.0) for cod larvae. Several authors, have reported an exponential decline in RNA/DNA ratio immediately following hatching. This includes Clemmesen (1994) for herring larvae between 3 and 10 days post-hatch, Bergeron and Boulhic (1994) for sole larvae between 0 and 5 days post-hatch, and Clemmesen and Doan (1996) for cod larvae between 4 and 12 days post-hatch. We observed a similar exponential decline in capelin larvae between 4 and 16 days post-hatch (96-404 hours post-stocking). The minimum RNA/DNA ratio we observed ranged from 2.0-3.0. A minimal (critical) RNA/DNA ratio of 1.0 has often been reported for starving larvae of different species in several studies (Clemmesen, 1994; Bergeron and Boulhic, 1994; Canino, 1994). However, Bisbal and Bengsten (1994) who measured RNA/DNA ratio of fed and starved summer flounder larvae reported minimum critical ratios of 2.0 after 60 to 216 hours (2.5 to 9 days) of starvation in 6 to 60 d old larvae. Canino (1997) observed minimum ratios of 2.0 for walleye pollock larvae raised at different prey densities up to 10 d post-feeding. And Kristogu-Baduge et al. (1999) reported that RNA/DNA ratios remained above 2.0 for starved herring larvae of different

size classes. These findings including ours, contrast strongly with the critical RNA/DNA ratio of 1.0 proposed by several authors as the definite index value for starvation in larvae, and this generalization should be abandoned.

We were unable to evaluate the patterns of changes in RNA/DNA ratios from the f12, f16, f20 and f24 delayed-starved treatments because larvae numbers at the time of sampling were inadequate. We also experienced difficulties obtaining consistent RNA/DNA responses in replicates of the feeding and starvation controls (tank effects). This precluded the use of those data to compare delayed-fed and delayed-starved treatments and fed and starved controls. Bergeron and Boulhic (1994) working with sole larvae also experienced similar difficulties. They attributed those difficulties to differences in egg source and minor variations in rearing conditions. It is important to note that in the majority of the studies reported above, ours being an exception, no attempt to replicate was made in the experimental design used. Based on our findings and those of Bergeron and Boulhic (1994) we suggest that great care should taken interpreting the significance of RNA/DNA ratio changes observed under experimental conditions.

Mathers et al. (1994) measured the dry weight, DNA, RNA, and Protein content of fed and starved herring *(Clupea harengus)* larvae, and computed indices of DNA/dw, RNA/dw, Protein/dw, RNA/DNA, RNA/Protein, and Protein/DNA. In their study, DNA, RNA and protein content were most sensitive to starvation over 7 days of starvation in 14, 53 and 66 d post-hatch larvae. RNA/DNA, RNA/Protein and Protein/DNA were less sensitive due presumably to concurrent changes in their numerator and denominator. While elements of this study have been questioned by Folkvord and Moksness (1995), it does reveal the evolution of the raw variables measured in combination with various computed ratios, and thus gives a better overall picture of the underlying processes. Suthers et al. (1996) reported the RNA/DNA ratio to be less sensitive to starvation than RNA alone in Australian bass *(Macquaria novemaculata)* and used relative indices developed from the residuals of the RNA to dry weight

relationships to assess starvation. This approach has the advantage of being independent of size effects. Kristogu-Baduge et al. (1999) demonstrated that fed and starved herring larvae could be distinguished from the residuals of the relationship between RNA and DNA. However, this distinction was more effective when applied to older pre-metamorphosis larvae. Residuals developed for first-feeding larvae showed great overlap.

The Protein/DNA ratio, an index of cell size growth, has seldom been reported from laboratory studies, probably because few authors have measured protein concurrently with nucleic acids. Westerman and Holt (1994), noted an increase in the Protein/DNA ratio of red drum *(Sciaenops ocellatus)* larvae immediately after the onset of first-feeding. Rooker and Holt (1996) reported an exponential decrease in the DNA/Protein ratio which represents an exponential increase in Protein/DNA ratio, in 7 mm larvae of the same species during active feeding. Folkvord et al. (1996) combined measures of DNA derived for herring larvae raised in mesocosms with protein data derived from (Buckley, 1984) model to compare the two. They observed that protein growth rates were higher than DNA growth rates when larval growth rates (measured as increments in length) were high. The inverse was true when the growth rates were low and the larvae were starving. These findings support the hypothesis that high Protein/DNA ratio reflect high growth and low ratios reflect poor condition in starving larvae. Our results also tend to confirm that.

In our experiments, the RNA/Protein ratio was less affected than other variables by intermittent feeding in capelin larvae. Only one of 9 treatment responses significantly diverged from the controls (Table 2a and 2b). A parallel decrease in the two components (RNA and Protein) of the ratio produced a constant ratio throughout the experiments. Berdalet *et al.*, (1994) reported identical results in experiments involving the dinoflagellate *Heterocapsa* sp. when exposed to nitrogen and phosphorus starvation. Ribosome efficiency (RNA/Protein), therefore appears to be insensitive to starvation and sub-optimal

feeding. Rooker and Holt (1996) demonstrated an exponential decrease in the RNA/Protein and DNA/Protein ratio of developing red drum larvae. This was associated with rapid cell multiplication followed by cell enlargement. The capelin larvae we studied had probably not yet reached this stage of exponential growth to detect significant changes in the RNA/Protein ratio.

4.2 Shape of the responses to various feeding conditions

To have utility in the *in situ* study of larval condition, biochemical measures must exhibit significant differences in their time response under extreme feeding conditions (e.g. starvation and *ad libitum* feeding). In our experiments, dry weight, quantity of DNA and Protein per larva, RNA/dw and Protein/dw all differed significantly between controls. In addition, the condition of the larvae exposed to intermittent feeding should change over time and be significantly affected by age in both the controls and the treatments, and diverge from one another (Ferron and Leggett, 1994). In our experiments, several treatments and controls showed significant divergence relative to the controls that was independent of post-stocking age. This is misleading and is related to the observation that in cases where this occurred recovery lines suggesting sub-optimal feeding. The best illustration of this effect is the quantity of protein larva^{-1} which decreased significantly in the starvation control but remained unchanged in the s4 treatment (Figure 7a). In this case the stability of the protein content in the larvae from the s4 treatment was not a product of its insensitivity to food levels, but rather to the fact that fed larvae experienced sub-optimal feeding levels that were at the best slightly higher than those experienced by the larvae in the starvation control.

In all our measures, the shape of the starvation controls were consistent with an exponentially deteriorating condition, however, the responses observed in the *ad libitum* feeding control were also consistent with deteriorating condition. This prevented, in most cases, a clear divergence in the response line of the

starvation and *ad libitum* feeding controls. This suggest that either (1) the condition measures used were unreliable as indices of starvation/feeding, or (2) that the condition of the larvae sampled in these experiments was not affected differentially by starvation and *ad libitum* feeding. We judge the second to be more likely, because survival rates were similar between feeding and starvation controls (see below), and most of the biochemical measures we evaluated have been shown to be acceptable indices of starvation for laboratory-reared fish larvae (Ferron and Leggett, 1994, and chapter 2).

Folkvord and Moksness (1995) have strongly recommended that survival data be reported along with growth rates in experimental studies of larval fish growth and condition as a general indicator of the adequacy of the rearing conditions experienced. In our experiments the overall survival rate to 17 d post-hatch was 12.2% for *ad libitum* feeding control tanks and 10.8% for larvae in the starvation control tanks. While very few authors report survival rates from laboratory experiments, Folkvord et al. (1996) observed that in those that have reported those rates, the larvae experienced poor growth and high mortality in spite of the provision of excess in food. They obtained a 27% survival rate for herring larvae raised in enclosures for approximately 60 days. Westerman and Holt, (1994) computed survival rates for red drum raised in tanks for the first 18 days post-hatching, and reported that survival was in the range of 28 to 63%. They considered those to be "normal" based on values calculated from other studies. Kristogu-Baduge (1999) obtained a mortality rate of 2.1% d^{-1} for herring larvae raised up to 27 d post-hatch in tanks. This translates to an overall cumulative survival rate of about 42.4%, which is almost double that reported by Folkvord et al. (1996), and four times the rate we obtained for capelin larvae at 17 days post-hatch. The survival rates we obtained for capelin (12%) were low relative to the above studies. This fact plus the observation that survival rates were comparable between fed and starved control lead us to conclude that the *ad libitum* feeding conditions we offered provided no survival advantage relative to starved larvae.

Frank and Leggett (1986) determined that wild caught zooplankton in the size range of 40-51 µm produced maximum growth rates in capelin larvae reared in large volume enclosures, and that the survival of first-feeding capelin larvae was most closely related to the abundance of prey within the 40-51 µm size range. Frank and Leggett (1986) also reported the occurrence of large numbers of tintinnids, diatoms and copepod eggs and nauplii in waters typically occupied by larval capelin at Bryant's Cove (Newfoundland). Our plankton collections were made at the same site and consistently yielded a wide variety of prey types including large numbers of copepod eggs and nauplii of the genus *Calanus*, *Pseudocalanus* and *Oithona* which are believed to be appropriate for first-feeding capelin larvae. It is possible that the 80 µm mesh size plankton net employed inadequately retained in the 40-51 µm size prey, thereby reducing their overall abundance to the profit of larger prey size in our tanks.

However, over the course of experiments, we made three replicate daily counts of copepod nauplii densities in our tanks, and densities were always above 1000 L^{-1}. These densities are at least 10 times higher than those typically encountered by larvae at sea (Frank and Leggett, 1986), and were not inconsistent with densities used in many studies (Buckley et al., 1991a; Canino, 1994; Clemmesen, 1994; Westerman and Holt, 1994; Clemmesen and Doan, 1996; Rooker and Holt, 1996; Rooker and Holt, 1997; Suthers et al., 1996). In fact they were double the densities found by Grønkjaer et al. (1997) and Canino (1997) to be required to produce good survival and growth of fish larvae in rearing tanks.

Folkvord et al., (1996) pointed out that most studies using rearing tanks to cultivate marine fish larvae have yielded poor growth and high mortality in spite of excess feeding. And that the most promising rearing success came from studies using enclosures, where high growth could be achieved at plankton densities comparable to those found in the field. Although the use of sub-optimal preys like *Brachionus* and *Artemia* can explain some of the poor feeding

conditions seen in some laboratory calibrations, it is still unclear why poor growth and survival result from good feeding in high natural plankton densities in many studies including ours.

One possible explanation may come from the larval stomach contents. Examination of the gut contents of capelin larvae sampled at the end of the s4, s6, and s8 treatments, which had all been feeding for six days, indicated that most had fed predominantly on small bivalve larvae. These mollusk larvae are difficult to digest because of the slow penetration of digestive enzymes in the interior of the shells (Govoni *et al.*, 1986) and this may have lead to a zero or negative net energy balance, with more energy being expended in capture and processing than was yielded by digestion and assimilation. The deterioration of the condition of the larvae fed *ad libitum* and the absence of a sustained recovery from starvation levels in the delayed-fed treatments, may be partially explained by the preference of capelin larvae for these low mobility high contrast bivalve larvae which occurred in great numbers on the bottom of the treatment/control tanks, over the more abundant but more active copepod nauplii.

These facts condition the conclusions to be drawn from the experiments described here. The sub-optimal feeding conditions require that we compare the condition of larvae which have starved to those which have fed poorly, rather than comparing the condition of starved larvae to those which have fed optimally. The consequence is reduced divergence between fed and starved controls and between controls and delayed-fed and delayed-starved treatments, and less conformity to predicted response curves which were developed based on optimal feeding experiments.

A further important confounding factor influencing the comparison of the condition of beach-collected larvae is the potential variability in their post-hatching age. We have already shown that post-hatching age had a significant impact on the condition of capelin larvae raised in the laboratory (chapter 2). In

addition to a variability induced by a 36 hrs beach collection time for larvae used in the experiments, it is known that larval emergence can also vary from beach sediments collected at one point in time. For example, Frank and Leggett (1981a, 1982b, 1983) have determined that hatching capelin larvae can accumulate in the interstices of gravel beaches and emerge synchronously during infrequent near-shore warming events. However, we are able to estimate the probable post-hatching age of the beach-collected larvae used in the intermittent feeding experiments, by comparing their condition to those larvae hatched and starved in the laboratory (chapter 2), since capelin larvae are not expected to feed during their beach residence time.

We know, for instance, that the size at hatch of capelin larvae from different half-sib families (Chapter 2) was 50.74 µg larva^{-1} at hatch and decreased at a rate of 2.12 µg d^{-1} during starvation, that their RNA content was 2.76 µg larva^{-1} (5.5% dry weight) at hatch and decreased at a rate of 0.19 µg d^{-1} (0.2% d^{-1}) during starvation, and that their Protein content was 25.19 µg larva^{-1} (49.5% dry weight) at hatch and decreased at a rate of 0.76 µg d^{-1} (0.9% d^{-1}) during starvation (data derived from Table 3, of chapter 2). In the experiments outlined here, the larvae collected from the beach and sampled from the starvation controls tanks at the beginning of the experiments (4 d after stocking) had a mean size of about 58.3 µg larva^{-1}, a RNA content of 2.52 µg larva^{-1} (4.2% dry weight), and a protein content of 29.3 µg larva^{-1} (49 % dry weight) (data derived from Table 2a). Since most values are comparable with values reported for newly-hatched larvae, it can therefore be assumed that differences in post-hatching age were small and had little impact on the biochemical condition of the larvae sampled later from the tanks. In other words, post-hatching age plus four days closely approximates the post-stocking age in the experiments described here. This is further supported by the following observation. While disturbing the beach-collected sediment to collect larvae, most larvae would swim up immediately after disturbance. However, many larvae kept emerging in containers, even after the sediment was disturbed repeatedly up to one hour

after being collected from the beach, suggesting that larvae continued to hatch during that time interval.

Another confounding factor when looking at the time response of nucleic acids to starvation of early emerging larvae, is the fact that RNA content and RNA/DNA ratios usually decrease after hatching and for several days until yolk absorption is completed and the larvae have initiated feeding (Clemmesen, 1996; Bergeron, 1997). This decrease which Westerman and Holt (1994) described as a "biochemical critical period" in the Hjort's hypothesis sense, and which Canino (1997) describe as an "energetic bottleneck", has been observed and well documented in the early larval stages of sole *(Solea solea)* (Bergeron and Boulhic, 1994), herring *(Clupea harengus)* (Clemmesen, 1994), walleye pollock *(Theragra chalcogramma)* (Canino, 1994; Canino 1997), cod *(Gadus morhua)* (Clemmesen and Doan, 1996) and red drum *(Sciaenops ocellatus)* (Westerman and Holt, 1994). This decrease seems to occur in both fed and starved larvae, and prevents any reliable comparison between the two groups at these important early stages. Mathers et al. (1993) measured RNA, DNA and RNA/DNA ratios in rainbow trout *(Onchorhynchus mykiss)* larvae fed commercial diets and starved, and concluded that larval RNA concentrations only become nutritionally dependant at 27 days post-hatch, after the larvae have fully resorbed their yolk and are relying on exogenous feeding. This shows that the ability to distinguish between fed and starved individuals in the earlier stages using nucleic acids, may be more related to the presence of endogenous feeding than to the ability of the larvae to feed.

4.3 Application of findings to field studies

The prediction of larval fish condition in the field is related to (1) the ability to quantify the food energy present in the environment (prey types, sizes, nutritional quality, and energy gain) (2) to contrast this with the sensitivity of reliable measures of larval condition, and (3) to translate these measures into condition

responses based on their known dynamics to intermittent feeding. Further, providing that a high space resolution is achieved while sampling the fish larvae and their prey, it may be possible from the measurement of larval condition at the time of sampling, to forecast the condition trajectories based on past and present prey fields. If food availability is tightly coupled with condition, which still remains to be confirmed (Ferron and Leggett, 1994; Chicharo, 1998), then the abundance estimates obtained using condition measurements and those obtained from concurrent sampling should correspond.

The condition of larvae sampled *in situ* is typically highly variable (Ferron and Leggett, 1994). Recent studies have also shown that very few of the larvae sampled at sea (<5%) are starving based on their RNA/DNA ratio (Blom et al., 1997, Chicharo, 1997, 1998; Chicharo et al., 1998). If the measures of condition investigated in this study are to be useful in field studies, the dynamics of the measures in response to intermittent feeding conditions must be defined. Given the results of the laboratory experiments reported here, we suggest that forecasting the broad trends in condition may be possible if energy availability is accurately determined. However, such forecasts will have a low predictive power due to large differences in individual responses to given feeding conditions and to the difficulty to replicate *in situ* feeding environments in the laboratory.

We conclude that field identification of potential survivors within cohorts, based on analysis of condition indices, is not achievable given the current knowledge of the dynamics, latency and sensitivity of commonly used condition measures. Greater effort is required to define and investigate those parameters that must closely mimic those encountered by larvae in the field. Our experience also indicates that very great care will be required in the design, execution and analysis of the experiments performed if this is to be meaningful.

We conclude, based on the totality of our analysis of the problem, that the best that is likely to be achieved, given the current state of knowledge, is an

analysis of the mean condition of larval cohorts at-sea. Finally, our data indicate that this limited objective is most likely to be achieved through the use of dry weight, and DNA/dw, RNA/dw and Protein/dw. Our data also suggest that the interpretation of condition in first-feeding larvae exposed to intermittent and sub-optimal feeding based on RNA/DNA, RNA/Protein and Protein/DNA indices should be approached with extreme caution.

GENERAL CONCLUSION

A comprehensive review on the use of various condition indices to assess larval fish nutritional status (chapter 1), revealed that the number of field-based studies was small relative to those conducted in the laboratory. This may explain the strong focus of this research which has been on the improvement of methods for studying the association between food quantity and larval condition, rather than the search for correlations between larval condition and year-class strength. Although laboratory calibrations are essential to field studies, they have often become and end in themselves. Greater effort towards refinement of existing methods followed by testing in the field would be more valuable. Seven main methods to measure larval fish condition were identified, namely: morphological indices, histological scores, histological cell heights, nucleic acids and proteins, lipids, digestive and metabolic enzymes. Most of these methods reliably detect condition changes related to variable food availability. The exceptions are lipids and digestive enzymes, both of which require further refinement to eliminate the confounding effects of gut content.

The sensitivity, latency and dynamics of the seven index categories used are not clearly defined. These characteristics must be known if the indices are to be effectively employed. The limited data available on their time response (dynamics), reveal a wide range of time and space scales. We distinguished three major groups on this basis. The first group, morphological and histological scores, have low sensitivity, long latency and slow dynamics (Figure 3, chapter 1). They should only be used to detect condition changes integrated over several days, and consequently over broader areas of the sea (tens to hundreds of kilometres). A coarse estimate of food availability at those scales is sufficient, since condition will likely reflect the feeding environment experienced by the larvae several days prior to sampling. A reliable estimate of the proportion of larvae that are past the nutritional point-of-no-return is achievable using these methods. The second group, histological cell heights, nucleic acids, proteins and

lipids, exhibited moderate sensitivity, latency, and dynamics (Figure 4, chapter 1), and appear to respond on a scale of hours to days. They are more applicable to field surveys conducted at scales of one to tens of kilometers. Most of these indices are substrate indicators. Therefore, they allow the detection of starving larvae in response to sub-daily changes in feeding success, and should be indicative of sub-optimal growth and condition. For indices in this class to be effective, the sampling of the larvae and their food should be conducted several times daily. The third group, digestive and metabolic enzymes are, in essence, rate indicators. They are believed to have high sensitivity, short latency and rapid dynamics. Their potential is likely to be maximized in intensive small-scale studies covering hundreds of meters to a few kilometres, where feeding success is being contrasted directly and almost simultaneously with food availability.

Our review of the studies to date led us to hypothesize on the time response (dynamics) of larval condition. We tested dynamics models (Figures 3 & 4, chapter 1) by measuring the time course of nucleic acids and proteins in larvae of capelin submitted to intermittent feeding trials. Although several substrate indicators such as dry weight, and relative DNA, RNA and Protein content did vary between controls and treatments, the results did not conform to the recovery dynamics proposed, apparently due to sub-optimal feeding conditions in the *ad libitum* feeding controls and in the delayed-fed treatments. However, the non-linear deterioration dynamics conformed to the predictions in the starvation controls and several of the delayed-starvation treatments. These findings clearly show that optimal feeding conditions are required in order for larvae that have experienced starvation to demonstrate recovery dynamics of the type hypothesized. Only under ideal feeding conditions can the dynamics (rates of deterioration and recovery) be calculated accurately and used for prediction curves. Our experiments also revealed two major limitations to the development of reliable prediction of nutritional dynamics. First, large inter-individual variability in larval condition is typical within-tanks and second, *ad libitum* feeding conditions are very difficult to replicate. While replication of feeding conditions

may be improved by using single prey items rather than mixed prey assemblages (at the expense of increasing the differences between laboratory and field conditions), inter-individual variability in feeding intensity, feeding success, growth, and condition is probably unavoidable and thus, inherent in any laboratory setting.

Our studies revealed that *a priori* knowledge of the sensitivity, the latency and the dynamics of condition indices is essential to any attempt to link food availability to larval condition. In addition, several other practical considerations are also important for the successful use of these indicators in field studies. Most critically, the larvae and their food must be sampled concurrently and at the time and space scales appropriate to the condition measures chosen, and the movement of the water masses described in enough details to allow the reconstruction of the larger scale picture.

While variability in larval fish condition has always been associated with changes in food availability, our review of the literature revealed several other sources of variability which may confound or mask the results. Among the biotic factors identified, diel periodicity and genetic factors are central. Diel periodicity appears to be problematic principally with histological indices, nucleic acids, proteins, lipids and digestive enzymes. Our experiments on diel variations in nucleic acids and proteins (chapter 3), revealed clear ultradian (6 hr) patterns of protein catabolism and anabolism in post-yolk-sac larvae. These cycles which can be represented by two daily peaks and lows, were also present in the DNA and RNA content of larvae exposed to intermittent feeding (Figures 15-17, Chapter 4). The presence of such rhythms can create severe aliaising of data and faulty interpretation if they are not accommodated in the sampling design used. The reported diel periodicity in RNA content and RNA/DNA ratios was restricted to studies where exogenously-feeding larvae were sampled in the laboratory or at sea. If, as we hypothesize, these circadian patterns are caused by alternating periods of rest and activity in larval fish, there is a high probability

that similar patterns are present in other condition measures. It is quite possible that their presence has been masked to date by the use of sampling intervals which are typically longer than the rhythms identified (daily or longer). For all biochemical indicators, and for some histological scores known to follow diel patterns, sampling should be conducted at least five times daily (every 5 hrs, Taggart and Frank, 1990) in order to avoid potentially serious aliaising of the data.

To assess the possible influence of genetic factors on larval fish condition (suggested in the literature) we investigated female contribution to egg and larval size, and to the nucleic acid and protein deterioration dynamics of starving larvae, through a half-sib experimental design (Chapter 2). When capelin larvae derived from different females were starved, dry weight and protein content (substrate indicators) decreased substantially after hatching. The rate of decrease varied between families. The RNA content and RNA/DNA ratio (rate indicators) were not influenced by female source, but were maximally influenced by post-hatching age (length of starvation). These findings indicate that laboratory studies should employ half or full-sib design to avoid any undesirable maternal contribution to larval condition. This caution is not achievable in field studies since the larvae collected will display the full potential of genetic diversity. In this case, rate indicators such as nucleic acids (RNA and DNA) should be used in place of substrate indicators such as proteins and dry weight whose dynamics are affected by female source.

The rate of decline in the quantity of substrate indicators following hatching differed between-clutches and were positively related to larval size at hatch. This suggests a pre-determined maximal time to irreversible starvation that is independent of egg size or larval size at hatch. The results also suggest that condition will be related to larval size at hatch, only if the larvae are fed within that starvation window. This important finding is contrary to the popular belief that larger larvae at hatch can withstand longer periods of starvation (Blaxter and

Hempel, 1963; Bagenal, 1969; Fowler, 1972; Hulata *et al.*, 1974; Hunter, 1981; Marsh, 1986; Blaxter, 1988; Miller *et al.*, 1988; Weeks, 1993) and deserves further investigation.

Our experiments designed to assess female contribution to larval condition also demonstrated that, for capelin, between-clutch variability in egg size was much higher than among-clutch variability. This partitioning of variance in egg size was not related to the nutritional status of the female but rather to an energy investment strategy centred around variable size-specific fecundity. These results while consistent with a small number of similar findings for other species, (Marsh, 1984, 1986; Marteinsdottir and Able, 1988; Zastrow *et al.*, 1989; and Hinckley, 1990) do provide one of the few examples of an optimal parental investment strategy (McGinley *et al.*, 1987) which consists of providing near-equal investment in each offspring in the absence of density-dependent offspring fitness, and in environments characterized by spatial heterogeneity.

These experiments also revealed that egg size and larval size at hatch (as measured by weight and protein content) were correlated at the family level, despite the absence of a family relationship between egg size and age at hatch or embryonic developmental rate. This finding is contrary to most interspecies and interpopulation analyses conducted to date (see Hunter, 1981 for a review). However, the absence of a relationship between egg size and embryonic developmental rate coupled, with the positive correlation between egg size and larval size, provide good evidence that, independent of egg size, a constant proportion of the egg yolk volume may be used for embryonic development in fishes. The fact that hatching time varied substantially between-clutches reared at constant temperatures is also consistent with the concept of a maternally-driven embryonic development rate. We should, however, point out that the larvae used in these experiments had similar within-clutch hatching ages. Sampling of larvae of different hatching ages over 24 hrs. cycles (chapter 3) revealed that within-clutch variation in hatching age could also contribute to

variability in the condition of starving larvae, reflected primarily in an energy cost (lower protein content per larva) associated with late hatching within a clutch.

Where discrepancies exist between our findings and those of others, the difference is likely to result from the fact that the analyses were conducted at different levels (species, population, family, or individuals), all of which are known to affect the correlation patterns obtained (Chambers *et al.*, 1989). This fact underlines the danger of drawing conclusions from data obtained at different levels and the need to assess early life history traits at several levels in order to fully understand the key variables involved in any given process.

Among the suite of indices computed from the nucleic acid and protein measurements generated in the three sets of experiments conducted (chapters 2, 3 and 4), DNA/dry weight produced some of the most consistent response to predictions in the intermittent feeding trials (chapter 4), was one of the most affected by starvation independent of female source (chapter 2), but was affected by ultradian periodicity in the experiments described in chapter 4. RNA content per larva ranked first before the RNA/DNA ratio, in the list of indices most affected by starvation which were independent of female contribution (Table 5, chapter 2), but was ranked low in the intermittent feeding trial (chapter 4). While RNA/dry weight was affected by starvation independent of female source (chapter 2), was ranked third in the intermittent feeding trials, but was affected by ultradian variability (chapter 4). Since female contribution cannot be evaluated in field situations and diel variability can be controlled by the choice of appropriate sampling intervals, the quantity of DNA and RNA per dry weight should be least affected by confounding effects and have the greatest potential for accurately assessing larval condition of all the measures we evaluated. The results also show that under certain circumstances (e.g. sub-optimal feeding conditions) the quantity of DNA per dry weight would exhibit greater sensitivity and would therefore be superior to the RNA/DNA ratio for detecting differences in larval condition. In any case, since both the quantity of RNA and DNA per larva are

obtained from the same procedures, we recommend that RNA/DNA ratio be computed and inspected separately. We also strongly recommend the computation of DNA and RNA/dry weight indices, and that all samples analyzed be weighted (dry weights) at a 0.1 µg precision on a microbalance

Collectively the results of our laboratory experiments reveal 1) the importance of considering female parent contributions when assessing larval fish condition by means of biochemical indices in the laboratory, 2) the need to consider the organization level employed when early-life history correlations are evaluated 3) the possibility of determining experimentally the deterioration and recovery dynamics of biochemical condition measures, 4) the difficulty of reliably reproducing and replicating *ad libitum* feeding conditions in the laboratory, 5) the biasing induced by inherent circadian and ultradian rhythms, mainly in the RNA and Protein content of fish larvae, and 6) the superiority of the quantity of RNA per larva over all other protein/nucleic acid based indices for accurately assessing larval fish condition under various feeding regimes.

On a more general level, this work has shown that methods for the evaluation of condition in larval fish should be selected on the basis of their potential to reliably assess condition on the time and space scales imposed by field sampling. In order to better achieve this, a combination of condition indices is likely to be preferable to the use of one index. Processing time, costs and requirements associated with their use must also be considered since they too vary greatly and could compromise the analyses. The refinement of existing methods should concentrate on reducing sample processing time, costs and requirements.

REFERENCES

Araujo-Lima,C.A.R.M.(1994). Egg size and larval development in Central Amazonian fish. *Journal of Fish Biology*, **44**, 371-389.

Arthur, D.K. (1976). Food and feeding of larvae of three fishes occurring in the California current, *Sardinops sagax*, *Engraulis mordax*, and *Trachurus symmetricus*. *Fishery Bulletin U.S.*, **74(3)**, 517-530.

Bagenal, T.B. (1969). Relationship between egg size and fry survival in Brown trout *Salmo trutta* L. *Journal of Fish Biology*, **1**, 349-353.

Bailey, K.M. and Houde, E.D. (1989). Predation on eggs and larvae of marine fishes and the recruitment problem. *Advances in Marine Biology*, **25**,1-83.

Bailey, K.M. and Spring, S.M. (1992). Comparison of larval, age-0 juvenile and age-2 recruit abundance indices of walleye pollock, *Theragra chalcogramma* , in the western Gulf of Alaska. *ICES Journal of Marine Science,* **49**, 297-304.

Balbontin, F., De Silva, S.S. and Ehrlich, K.F. (1973). A comparative study of anatomical and chemical characteristics of reared and wild herring. *Aquaculture*, **2**, 217-240.

Balon, E.G. (1984). Reflections on some decisive events in the early life of fishes. *Transactions of the American Fisheries Society,* **113**, 178-185.

Barron, M.G. and Adelman, I.R. (1984). Nucleic acid, protein content, and growth of larval fish sublethally exposed to various toxicants. *Canadian Journal of Fisheries and Aquatic Sciences*, **41**, 141-150.

Barron, M.G. and Adelman, I.R. (1985). Temporal characterization of growth of fathead minnow, *Pimephales promelas*, larvae during sublethal hydrogen cyanide exposure. *Comparative Biochemistry and Physiology* , **81C (2)**, 341-344.

Bashford, C.L. and Harris, D.A. (1987). Spectrophotometry and spectrofluorimetry. a practical approach. IRL Press, Oxford & Washington D.C. 176 pages.

Beacham, T.D. and Murray, C.B. (1985). Effect of female size, egg size, and water temperature on developmental biology of chum salmon *Oncorhynchus keta* from the Nitinat river, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 1755-1765.

Beacham, T.D. and Murray, C.B. (1990). Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: A comparative analysis. *Journal of the Fisheries Research Board of Canada,* **119**, 927-945.

Bengtson, D.A., Barkman, R.C. and Berry, W.J. (1987). Relationship between maternal size, egg diameter, time of spawning season, temperature and length at hatch of Atlantic silverside (*Menidia menidia). Journal of Fish Biology*, **31**, 697-704.

Benoît, H.P. and Pepin, P. (1999). Interaction of rearing temperature and maternal influence on egg development rates and larval size at hatch in yellowtail flounder *(Pleuronectes ferrugineus). Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 785-794.

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

Berdalet, E., Latasa, M. and Estrada, M. (1994). Effects of nitrogen and phosphorus starvation on nucleic acid and protein content of *Heterocapsa* sp. *Journal of Plankton Research*, **16 (4)**, 303-316.

Bergeron, J.P.(1997). Nucleic acids in ichthyoplankton ecology : a review, with emphasis on recent advances for new perspectives. *Journal of Fish Biology* , **51 (Suppl. A)**, 284-302.

Bergeron, J.P.et Boulhic M..(1994). Rapport ARN/ADN et évaluation de l'état nutritionnel et de la croissance des larves de poissons marins : un essai de mise au point expérimentale chez la sole (*Solea solea L.). ICES Journal of Marine Science*, **51**, 181-190.

Bergeron, J.P. and Person-Le Ruyet.,J. (1997). Teneur en ADN de la larve de *Dicentrarchus labrax* : évolution onogénétique et effet de la privation de la nourriture. *Aquatic Living Ressources*, **10**, 247-250.

Bergeron, J.-P., Bouhlic, M. and Galois, R. (1991). Effet de la privation de nourriture sur la teneur en ADN de la larve de sole *Solea solea. ICES Journal of Marine Science*, **48**, 127-134.

Bisbal ,G.A. and Bengtson D.A. (1995). Description of the starving condition in summer flounder, *Paralichthys dentatus*, early life history stages. *Fishery Bulletin U.S.*, **93,** 217-230.

Black, D. and Love, R.M. (1986). The sequential mobilization and restoration of energy reserves in tissues of Atlantic cod during starvation and re-feeding. *Journal of Comparative Physiology B*, **156**, 469-479.

Blaxter, J.H.S. (1971). Feeding and condition of Clyde herring larvae. *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer,* **160**, 128-136.

Blaxter, J.H.S. (1988). Patterns and variety in development. *In* Fish Physiology vol. XI: the physiology of developing fish. *Edited by* Hoar, W.S. and Randall, D.J., Academic Press, London, pp.1-58.

Blaxter, J.H.S. and Ehrlich, K.F. (1974). Changes in behaviour during starvation of herring and plaice larvae. *In-* The early life history of fish (Blaxter J.H.S., ed.), pp. 575-588. Sringer-Verlag, Berlin.

Blaxter, J.H.S. and Hempel, G. (1963). The influence of egg size on herring larvae *Clupea harengus. Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer,* **28**, 211-240.

Blom G.,.Folkvord ,A. ,Johannesen, A. and Fossum P. (1997). Interannual variations in condition indices of larval Norwegian spring-spawning herring. *Journal of Fish Biology*, **51 (Suppl. A)**, 370-384.

Boujard, T. and Leatherland, J.F. (1992). Circadian rhythms and feeding time in fishes. *Environmental Biology of Fishes,* **35**, 109-131.

Bradford, M.J. (1992). Precision of recruitment predictions from early life stages of marine fishes. *Fishery Bulletin U.S.*, **90**, 439-453.

Bradford, M.M.(1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248-254.

Brown, R.W. and Taylor, W.W. (1992). Effects of egg composition and prey density on the larval growth and survival of lake whitefish *(Coregonus clupeaformis* Mitchill). *Journal of Fish Biology*, **40**, 381-394.

Buckley, L.J. (1979). Relationship between RNA-DNA ratio, prey density, and growth rate in Atlantic cod *Gadus morhua*. *Journal of the Fisheries Research Board of Canada*, **36**, 1497-1502.

Buckley, L.J. (1980). Changes in ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder *Pseudopleuronectes americanus*, and effect of starvation. *Fishery Bulletin U.S.*, **77(3)**, 703-708.

Buckley, L.J. (1981). Biochemical changes during ontogenesis of cod *Gadus morhua* and winter flounder *Pseudopleuronectes americanus* larvae. *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer*, **178**, 547-552.

Buckley, L.J. (1982). Effects of temperature on growth and biochemical composition of larval winter flounder *Pseudopleuronectes americanus*. *Marine Ecology Progress Series*, **8**, 181-186.

Buckley, L.J. (1984). RNA-DNA ratio: An index of larval fish growth in the sea. *Marine Biology*, **80**, 291-298.

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

Buckley, L.J. and McNamara, P. (1993). Estimation of zooplankton and ichthyoplankton growth and condition using nucleic acid probe techniques. *U.S. Globec news*, **4**, 12.

Buckley, L.J., Turner, S.I., Halavik, T.A., Smigielski, A.S., Drew, S.M. and Laurence, G.C. (1984). Effects of temperature and food availability on growth, survival, and RNA-DNA ratio of larval sand lance *Ammodytes americanus*. *Marine Ecology Progress Series*, **15**, 91-97.

Buckley, L.J., Halavik, T.A., Smigielski, A.S., and Laurence, G.C. (1987). Growth and survival of the larvae of three species of temperate marine fishes reared at discrete prey densities. *American Fisheries Society Symposium*, **2**, 82-92.

Buckley, L.J., Smigielski, A.S., Halavik, T.A. and Laurence, G.C. (1990). Effects of water temperature on size and biochemical composition of winter flounder *Pseudopleuronectes americanus* at hatching and feeding initiation. *Fishery Bulletin U.S.*, **88**, 419-428.

Buckley, L.J., Smigielski, A.S., Halavik, T.A., Caldarone, E.M., Burns, B.R. and Laurence, G.C. (1991a). Winter flounder *Pseudopleuronectes americanus* reproductive success. I. Among-location variability in size and survival of larvae reared in the laboratory. *Marine Ecology Progress Series*, **74**, 117-124.

Buckley, L.J., Smigielski, A.S., Halavik, T.A., Caldarone, E.M., Burns, B.R. and Laurence, G.C. (1991b). Winter flounder *Pseudopleuronectes americanus* reproductive success. II. Effects of spawning time and female size on size, composition and viability of eggs and larvae. *Marine Ecology Progress Series*, **74**, 125-135.

Bulow, F.J. (1970). RNA-DNA ratios as indicators of recent growth rates of a fish. *Journal of the Fisheries Research Board of Canada*, **27**, 2343-2349.

Bulow, F. J. (1974). A review of the literature concerning the relationship between nucleic acids and the growth rates of fish. *The Tennessee Technical Journal*, **9**, 17-23.

Bulow, F.J. (1987). RNA-DNA ratios as indicators of growth in fish: A review. *In*- "The age and growth of fish" (Summerfelt R.C., and Hall G.E., eds.), pp.45-64. The Iowa State University Press, Ames, Iowa.

Bulow, F.J., Coburn, C.B. and Cobb, C.S. (1978). Comparisons of two bluegill populations by means of the RNA-DNA ratio and liver-somatic index. *Transactions of the American Fisheries Society*, **107(6)**, 799-803.

Caldarone, E. and Buckley, L.J. (1991). Quantitation of DNA and RNA in crude tissue extracts by flow injection analysis. *Analytical Biochemistry*, **199**, 137-141.

Campana, S. and Neilson, J. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 1014-1032.

Canino,M.F. (1994). Effect of temperature and food availability on growth and RNA/DNA ratios of walleye pollock *Theragra chalcogramma* (Pallas) eggs and larvae. *Journal of Experimental Marine Biology and Ecology*, **175**, 1-16.

Canino M.F. (1997). Nucleic acid contents and growth of first-feeding walleye pollock larvae in response to prey densities typical of sub-Arctic ecosystems. *Journal of Fish Biology*, **51**, 41-52.

Canino, M.F., and Caldarone,E.M. (1995). Modification and comparaison of two fluorometric techniques for determining nucleic acid content of larval fish. *U.S. Fishery Bulletin* , **93**, 158-165.

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

Cesarone, C.F., Bolognesi, C., and Santi, L. (1979). Improved microfluorometric DNA determination in biological material using 33258 Hoechst. *Analytical Biochemistry*, **100**, 188-197.

Chambers, R.C. (1993). Phenotypic variability in fish populations and its representation in individual-based models. *Transactions of the American Fisheries Society*, **122(3)**, 404-414.

Chambers, R.C. and Leggett, W.C. (1987). Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder *Pseudopleuronectes americanus* with a review of variation in other species. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**, 1936-1947.

Chambers, R.C. and Leggett, W.C. (1996). Maternal influences on variation in egg sizes in temperate marine fishes. *American Zoologists*, **36**, 180-196.

Chambers, R.C., Leggett, W.C. and Brown, J.A. (1989). Egg size, female effects, and the correlations between early life history traits of capelin, *Mallotus villosus*: An appraisal at the individual level. *Fishery Bulletin U.S.*, **87**, 515-523.

Checkley, D.M. Jr. (1984). The relation of growth to ingestion for larvae of Atlantic herring *Clupea harengus* and other fish. *Marine Ecology Progress Series*, **18**, 215-224.

Chenoweth, S.B. (1970). Seasonal variations in condition of larval herring in Boothbay area of the Maine coast. *Journal of the Fisheries Research Board of Canada*, **27**, 1875-1879.

Chicharo, M.A. (1997). Starvation percentages in field caught Sardina pilchardus larvae off southern Portugal . *Scientia Marina*, **61 (4)**, 507-516.

Chicharo M..A. (1998). Nutritional condition and starvation in *Sardina pilchardus* (L .) larvae off southern Portugal compared with some environmental factors. *Journal of Experimental Marine Biology and Ecology*, **225**, 123-137.

Chicharo,M..A.,Chicharo,L.,Valdés,L.,López-Jamar,E.andRé,P. (1998). Estimation of starvation and diel variation of the RNA/DNA ratios in field-caught Sardina pilchardus larvae off the north of Spain. *Marine Ecology Progress Series*, **164**, 273-283.

Clarke, A., Rodhouse, P.G., Holmes, L.J. and Pascoe, P.L. (1989). Growth rate and nucleic acid ratio in cultured cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda). *Journal of Experimental Marine Biology and Ecology*, **133**, 229-240.

Clarke, M.E., Calvi, C., Domeier, M., Edmonds, M., and Walsh, P.J. (1992). Effect of nutrition and temperature on metabolic enzymes activities in larval and juvenile red drum, *Sciaenops ocellatus*, and lane snapper, *Lutjanus synagris*. *Marine Biology*, **112**, 31-36.

Clemmesen, C. (1987). Laboratory studies on RNA-DNA ratios of starved and fed herring *Clupea harengus* and turbot *Scophthalmus maximus* larvae. *Journal du Conseil international pour l'Exploration de la Mer*, **43**, 122-128.

Clemmesen, C. (1988). A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. *Meeresforsch*, **32**, 134-143.

Clemmesen, C. (1989). RNA-DNA ratios of laboratory-reared and wild herring larvae determined with a highly sensitive fluorescence method. *Journal of Fish Biology*, **35(Suppl. A)**, 331-333.

Clemmesen, C. (1992). The effect of food availability, age or size on the RNA/DNA ratio of laboratory reared individually measured herring larvae. *International Council for the Exploration of the Sea, Council Meeting*, 1992/L:33.

Clemmesen, C. (1993). Improvements in the fluorometric determination of the RNA and DNA content of individual marine fish larvae. *Marine Ecology Progress Series*, **100**, 177-183.

Clemmesen, C . (1994). The effect of food availability, age or size on the RNA / DNA ratio of individually mesured herring larvae : laboratory calibration. *Marine Biology*, **118**, 377-382.

Clemmesen, C. (1996). Survival Strategies in Early life stages of Marine Ressources : Importance and limits of RNA / DNA ratios as a mesure of nutritional condition in fish larvae. *Proceeding of an International workshop/Yokohama /Japan,* 11-14 october.

Clemmesen, C. and Ueberschär, B. (1993). Application of RNA/DNA ratio and tryptic enzyme activity on laboratory-reared and wild-caught herring larvae-short communication. *In-* Fish Ecotoxicology and Ecophysiology (Braunbeck, T.,

Hanke, W. and Segner, H., eds.), pp. 227-232. VCH Verlagsgesellschaft mbH, D-6940 Weinheim.

Clemmesen, C. and 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. *Marine Ecology Progress Series*, **138**, 33-39.

Comings, D.E. (1975). Mechanisms of chromosome binding. VIII. Hoechst 33258-DNA interaction. *Chromosoma*, **52**, 229-243.

Cone, R.S. (1989). The need to reconsider the use of condition indices in fishery science. *Transactions of the American Fisheries Society*, **118**, 510-514.

Cousin, J.C.B., Balouet, G. and Baudin-Laurencin, F. (1986). Altérations histologiques observées chez des larves de turbot *Scophthalmus maximus* en élevage intensif. *Aquaculture*, **52**, 173-189.

Cousin, J.B.C., Baudin-Laurencin, F. and Gabaudan, J. (1987). Ontogeny of enzymatic activities in fed and fasting turbot, (*Scophthalmus maximus*). *Journal of Fish Biology*, **30**, 15-33.

Cowey, C.B., Bell, J.G., Knox, D., Fraser, A. and Youngson, A. (1985). Lipids and lipid antioxidant systems in developing eggs of salmon (*Salmo salar*). *Lipids*, **20**, 567-572.

Cushing, D.H. (1972). The production cycle and the numbers of marine fish. *Symposium of the Zoological Society of London*, **29**, 213-232.

Dabrowski, K. (1982). Proteolytic enzyme activity decline in starving fish alevins and larvae. *Environmental Biology of Fishes*, **7(1)**, 73-76.

Dabrowski, K. and Glogowski, J. (1977). Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. *Hydrobiologia*, **54**, 129-134.

Dagg, M.J. and Littlepage, J.L. (1972). Relationships between growth rate and RNA, DNA, protein and dry weight in *Artemia salina* and *Euchaeta elongata*. *Marine Biology*, **17**, 162-170.

Davis, M.W. and Olla, B.L. (1992). Comparison of growth, behavior and lipid concentrations of walleye pollock *Theragra chalcogramma* larvae fed lipid-enriched, lipid-deficient and field-collected prey. *Marine Ecology Progress Series*, **90**, 23-30.

DeBevoise, A.E. and Taghon, G.L. (1988). RNA/DNA ratios of the hydrothermal-vent vestimentiferans *Ridgeia piscesae* and *R. phaeophiale* indicate variations in growth rates over small spatial scales. *Marine Biology*, **97**, 421-426.

DeFlaun, M.F., Paul, J.H. and Davis, D. (1986). Simplified method for dissolved DNA determinations in aquatic environments. *Applied Environmental Microbiology*, **52(4)**, 654-659.

Delauney, F., Marty, Y., Moal, J. and Samain, J.-F. (1992). Growth and lipid class composition of *Pecten maximus* larvae grown under hatchery conditions. *Journal of Experimental Marine Biology and Ecology*, **163**, 209-219.

De March, B.G.E. (1991). Genetic, maternal, and tank determinants of growth in hatchery-reared juvenile Arctic charr *Salvelinus alpinus*. *Canadian Journal of Zoology*, **69**, 655-660.

Denman, K.L. and Powell, T.M. (1984). Effects of physical processes on planktonic ecosystems in the coastal ocean. *Oceanography and Marine Biology Annual Review*, **22**, 125-168.

Deplano, M., Connes, R., Diaz, J.P. and Barnabé, G. (1991). Variation in the absorption of macromolecular proteins in larvae of the sea bass *Dicentrarchus labrax*, during transition to the exotrophic phase. *Marine Biology*, **110**, 29-36.

DeSilva, C.D., Premawansa, S., and Keemiyahetty, C.N. (1986). Oxygen consumption in *Oreochromis niloticus* in relation to development, salinity, temperature and time of day. *Journal of Fish Biology*, **29,** 267-277.

DeVlaming, V.L., Sage, M. and Tiegs, R. (1975). A diurnal rhythm of pituitary prolactin activity with diurnal effects of mammalian and teleostean prolactin on total body lipid deposition and liver lipid metabolism in teleost fishes. *Journal of Fish Biology*, **7**, 717-726.

Dortch, Q., Roberts, T.L., Clayton, J.R. and Ahmed, S.I. (1983). RNA/DNA ratios and DNA concentration as indicators of growth rate and biomass in planktonic marine organisms. *Marine Ecology Progress Series*, **13**, 61-71.

Duarte, C.M. and Alcaraz, M. (1989). To produce many small of few large eggs: a size-independent reproductive tactic of fish. *Oecologia*, **80**, 401-404.

Economou, A. (1987). Ecology of survival in some gadoid larvae of the northern North Sea. *Environmental Biology of Fishes*, **19(4)**, 241-260.

Ehrlich, K.F. (1974a). Chemical changes during growth and starvation of larval *Pleuronectes platessa*. *Marine Biology*, **24**, 39-48.

Ehrlich, K.F. (1974b). Chemical changes during growth and starvation of herring larvae. *In*-"The early life history of fish" (Blaxter J.H.S., ed.), pp.301-323. Springer-Verlag, Berlin.

Ehrlich, K.F. (1975). A preliminary study of the chemical composition of sea-caught larval herring and plaice. *Comparative Biochemistry and Physiology*, **51B**, 25-28.

Ehrlich, K.F., Blaxter, J.H.S. and Pemberton, R. (1976). Morphological and histological changes during the growth and starvation of herring and plaice larvae. *Marine Biology*, **35**, 105-118.

Eldridge, M.B., Whipple, J.A. and Bowers, M.J. (1982). Bioenergetics and growth of striped bass, *Morone saxatilis*, embryos and larvae. *Fishery Bulletin U.S.*, **80(3)**, 461-474.

Eldridge, M.B., Joseph, J.D., Taberski, K.M. and Seaborn, G.T. (1983). Lipid and fatty acid composition of the endogenous energy sources of striped bass *Morone saxatilis* eggs. *Lipids*, **18(8)**, 510-513.

Elgar, M.A. (1990). Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. Oikos, **59**, 283-287.

Falcón, J., Thibault, C., Begay, V., Zachmann, A., and Collin, J.-P. (1992). Regulation of the rhythmic melatonin secretion by fish pineal photoreceptor cells. *In*-"Rhythms in Fishes", (Ali, M.I. Ed.), pp. 167-198. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

Farber-Lorda, J. (1991). Multivariate approach to the morphological and biochemical differentiation of Antarctic krill *Euphausia superba* and *Thysanoessa macrura*. *Deep Sea Research*, **38**, 771-779.

Farbridge, K.J. and Leatherland, J.F. (1987). Lunar cycles of coho salmon *Oncorhynchus kisutch*. II. Scale amino acid uptake, nucleic acids, metabolic reserves and plasma thyroid hormones. *Journal of Experimental Biology*, **129**, 179-189.

Ferguson, M.M. and Danzmann, R.G. (1990). RNA/DNA ratios in white muscle as estimates of growth in rainbow trout held at different temperatures. *Canadian Journal of Zoology*, **68**, 1494-1498.

Ferguson, M.M. and Drahushchak, L.R. (1989). Effects of tissue collection and storage methods on nucleic acid determinations in white muscle of fishes. *Transactions of the American Fisheries Society*, **118**, 709-713.

Ferron, A. (1991). État nutritionnel des larves de maquereau *Scomber scombrus* en rapport avec la disponibilité de nourriture et la structure physique côtière dans le sud-ouest du Golfe Saint-Laurent. **MSc Thesis**, *Departement d'Océanographie, Université du Québec à Rimouski, Rimouski, Québec, 195 pages.*

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

Fleming, I.A. and Gross, M.R. (1990). Latitudinal clines: A trade-off between egg number and size in Pacific salmon. *Ecology,* **71(1)**, 1-11.

Fogarty, M.J. (1993). Recruitment in randomly varying environments. *ICES Journal of Marine Science*, **50**, 247-260.

Fogarty, M.J., Sissenwine, M.P. and Cohen, E.B. (1991). Recruitment variability and the dynamics of exploited marine populations. *Trends in Ecology and Evolution*, **6(8)**, 241-246.

Folkvord ,A., and Moksness, E.. (1995). RNA /DNA ratios and growth of herring larvae. *Marine Ecology Progress Series*, **121**, 311-312.

Folkvord, A., Øiestad, V. and Kvenseth, P.G. (1994). Growth patterns of three cohorts fo Atlantic cod larvae (Gadus morhua L.) studies in a macrocosm. *ICES. Journal of Marine Science*, **51**, 325-336.

Folkvord, A., Ystanes, L., Johannessen, A. and Moksness, E. (1996). RNA:DNA ratios and growth of herring *(Clupea harengus)* larvae reared in mesocosms. *Marine Biology*, **126**, 591-602.

Foster, A.R., Houlihan, D.F. and Hall, S. J. (1993). Effects of nutritional regime on correlates of growth rate in juvenile Atlantic cod, *Gadus morhua*. Comparison of morphological and biochemical measurements. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 505-512.

Foster, A.R., Houlihan, D.F., Hall, S.J. and Burren, L.J. (1992). The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod *Gadus morhua*. *Canadian Journal of Zoology*, **70**, 2095-2102.

Fowler, L.G. (1972). Growth and mortality of fingerling chinook salmon as affected by egg size. *Progressive Fish Culturist*, **34**, 66-69.

Frank, K.T. and Leggett, W.C. (1981a). Wind regulation of emergence times and early larval survival in capelin *Mallotus villosus*. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 215-223.

Frank, K.T. and Leggett, W.C. (1981b). Prediction of egg development and mortality rates in capelin (*Mallotus villosus)* from meteorological, hydrographic,

and biological factors. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1327-1338.

Frank, K.T. and Leggett, W.C. (1982b). Coastal water mass replacement: its effect on zooplankton dynamics and the predator-prey complex associated with larval capelin (*Mallotus villosus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **39**, 991-1003.

Frank, K.T. and Leggett, W.C. (1983). Survival value of an opportunistic life-stage transition in capelin (*Mallotus villosus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **40**, 1442-1448.

Frank, K.T. and Leggett, W.C. (1986). Effect of prey abundance and size on the growth and survival of larval fish: an experimental study employing large volume enclosures. *Marine Ecology Progress Series*, **34,** 11-22.

Frank, K.T. and Leggett, W.C. (1994). Fisheries ecology in the context of ecological and evolutionnary theory. *Annual Reviews in Ecology and Systematics*, **25**, 401-422.

Frank, K.T., and 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. *Canadian Journal of Fisheries and Aquatic Sciences*, **46 (Suppl.1)**, 125-133.

Frantzis, A., Grémare, A. and Vétion, G. (1993). Taux de croissance et rapports ARN/ADN chez le bivalve dépositivore *Abra ovata* nourri à partir de différents détritus. *Oceanologica acta*, **16(3)**, 303-313.

Fraser, A.J. (1989). Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. *Canadian Journal of Fisheries and Aquatic Sciences*, **46**, 1868-1873.

Fraser, A.J., Tocher, D.R. and Sargent, J.R. (1985). Thin-layer chromatography-flame ionization detection and the quantitation of marine neutral lipids and phospholipids. *Journal of Experimental Marine Biology and Ecology*, **88**, 91-99.

Fraser, A.J., Sargent, J.R., Gamble, J.C. and MacLachlan, P. (1987). Lipid class and fatty acid composition as indicators of the nutritional condition of larval Atlantic herring. *American Fisheries Society Symposium*, **2**, 129-143.

Fraser, A.J., Gamble, J.C. and Sargent, J.R. (1988). Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod *Gadus morhua*. *Marine Biology*, **99**, 307-313.

Frolov, A.V. and Pankov, S.L. (1992). The effect of starvation on the biochemical composition of the rotifer *Brachionus plicatilis*. *Journal of the marine biological association of the United Kingdom*. **72**, 343-356.

Fukuda, M., Nakano, H. and Yamamoto, K. (1986). Biochemical changes in Pacific herring during early development stages. *Hokkaido Daigaku Faculty of Fisheries Bulletin*, **37**, 30-37.

Fyhn, H.J. (1989). First feeding of marine fish larvae: Are free amino acids the source of energy? *Aquaculture*, **80**, 111-120.

Fyhn, H.J. and Serigstad, B. (1987). Free amino acids as energy substrate in developing eggs and larvae of the cod *Gadus morhua*. *Marine Biology*, **96**, 335-341.

Gallager, S.M., Mann, R. and Sakaki, G.C. (1986). Lipid as an index of growth and viability in three species of bivalve larvae. *Aquaculture*, **56**, 81-103.

Gatten, R.R., Sargent, J.R. and Gamble, J.C. (1983). Diet-induced changes in fatty acid composition of herring larvae reared in enclosed ecosystems. *Journal of the marine biological association of the United Kingdom*, **63**, 575-584.

Gerkema, M.P. (1992). Biological rhythms: Mechanisms and adaptive values. *In*-"Rhythms in Fishes", (Ali, M.I. Ed.), pp. 27-37. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

Gern, W.A., Greenhouse, S.S., Nervina, J.M., and Gasser, P.J. (1992). The rainbow trout pineal organ: An endocrine photometer. *In*- Rhythms in Fishes, (Ali, M.I. Ed.), pp. 199-218. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

Gibson, R.N. (1992). Tidally-synchronized behaviour in marine fishes. *In*- Rhythms in Fishes, (Ali, M.I. Ed.), pp. 63-81. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

Gilbert, S.F. (1991). Developmental biology 3rd Edition. Sinauer Associates Inc. Publishers, Sunderland MA, USA.

Gleeson, M. and Maughan, R.J. (1986). A simple enzymatic fluorometric method for the determination of triglycerides in 10 µl of serum. *Clinica Chimica Acta*, **156**, 97-103.

Goolish, E.M. and Adelman, I.R. (1987). Tissue-specific cytochrome oxidase activity in largemouth bass: The metabolic costs of feeding and growth. *Physiological Zoology*, **69**, 454-464.

Goolish, E.M. and Adelman, I.R. (1988). Tissue-specific allometry of an aerobic respiratory enzyme in large and small species of cyprinid (Teleostei). *Canadian Journal of Zoology*, **66**, 2199-2208.

Goolish, E.M., Barron, M.C. and Adelman, I.R. (1984). Thermoacclimatory response of nucleic acid and protein content of carp muscle tissue: influence of growth rate and relationship to glycine uptake by scales. *Canadian Journal of Zoology*, **62**, 2164-2170.

Govoni, J.J. (1980). Morphological, histological, and functional aspects of alimentary canal and associated organ development in larval *Leiostomus xanthurus*. *Revue Canadienne de Biologie*, **39(2)**, 69-80.

Govoni, J.J., Boehlert, G.W. and Watanabe, Y. (1986). The physiology of digestion in fish larvae. *Environmental Biology of Fishes*, **16(1-3)**, 59-77.

Grønkjaer,P., Clemmesen, C.and St.John, M. (1997). Nutritional condition and vertical distribution of Baltic cod larvae. *Journal of Fish Biology*, **51(Suppl. A)**, 352-369.

Haines, T.A. (1973). An evaluation of RNA-DNA ratio as a measure of long-term growth in fish populations. *Journal of the Fisheries Research Board of Canada*, **30**, 195-199.

Haines, T.A. (1980). Seasonal patterns of muscle RNA-DNA ratio and growth in black crappie, *Pomoxis nigromaculatus*. *Environmental Biology of Fishes*, **5(1)**, 67-70.

Håkanson, J.L. (1984). The long and short term feeding condition in field-caught *Calanus Pacificus*, as determined from the lipid content. *Limnology and Oceanography*, **29(4)**, 794-804.

Håkanson, J.L. (1989a). Analysis of lipid components for determining the condition of anchovy larvae, *Engraulis mordax*. *Marine Biology*, **102**, 143-151.

Håkanson, J.L. (1989b). Condition of larval anchovy *Engraulis mordax* in the southern California Bight, as measured through lipid analysis. *Marine Biology*, **102**, 153-159.

Hansen, P.E., Lied, E. and Børresen, T. (1989). Estimation of protein synthesis in fish larvae using an *in vitro* polyribosome assay. *Aquaculture*, **79**, 85-89.

Harris, G.P. (1980). Temporal and spatial scales in phytoplankton ecology. Mechanisms, methods, models and management. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 877-900.

Harris, R.K., Nishiyama, T. and Paul, J. (1986). Carbon, nitrogen and caloric content of eggs, larvae, and juveniles of the walleye pollock, *Theragra chalcogramma*. *Journal of Fish Biology*, **29**, 87-98.

Haugland, R.P. (1992). Handbook of fluorescent probes and research chemicals 5th edition, Molecular Probes Inc. Eugene, OR 97402-0414, 421 pages.

Hay, D.E. (1981). Effects of capture and fixation on gut contents and body size of Pacific herring larvae. *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer*, **178**, 395-400.

Hay, D.E. (1982). Fixation shrinkage of herring larvae: Effects of salinity, formalin concentration, and other factors. *Canadian Journal of Fisheries and Aquatic Sciences*, **39**, 1138-1143.

Hay, D.E. (1984). Weight loss and change of condition factor during fixation of Pacific herring, *Clupea harengus pallasi*, eggs and larvae. *Journal of Fish Biology*, **25**, 421-433.

Heath, M.R. (1992). Field investigations of the early life stages of marine fish. *Advances in Marine Biology*, **28**, 1-153.

Heidinger, R.C. and Crawford, S.D. (1977). Effect of temperature and feeding rate on the liver-somatic index of the largemouth bass, *Micropterus salmoides*. *Journal of the Fisheries Research Board of Canada*, **34**, 633-638.

Heming, T.A. and Buddington, R.K. (1988). Yolk absorption in embryonic and larval fishes. *In*- Fish physiology vol.XI: The physiology of developing fish, part A, (Hoar, W.S. and Randall, D.J. Eds.), pp. 408-446, Academic Press, New York.

Hempel, G. and Blaxter, J.H.S. (1963). On the condition of herring larvae. *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer*, **154**, 35-40.

Hewitt, R.P., Theilacker, G.H. and Lo, N.C.H. (1985). Causes of mortality in young jack mackerel. *Marine Ecology Progress Series*, **26**, 1-10.

Hislop, J.R.G. (1988). The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus*, in British waters. *Journal of Fish Biology*, **32**, 923-930.

Hjelmeland, K., and Jørgensen, T. (1985). Evaluation of radioimmunoassay as a method to quantify trypsin and trypsinogen in fish. *Transactions of the American Fisheries Society*, **114**, 619-621.

Hjelmeland, K., Pedersen, B.H. and Nilssen, E.M. (1988). Trypsin content in intestines of herring larvae, *Clupea harengus*, ingesting inert polystyrene spheres or live crustacea prey. *Marine Biology*, **98**, 331-335.

Hjelmeland, K., Huse, I., Jorgensen, T., Molovik, G. and Raae, J. (1984). Trypsin and trypsinogen as indices of growth and survival potential of cod *Gadus morhua*, larvae. *In*-The propagation of cod *Gadus morhua* Part 1, (Dahl E., Danielssen, D.S., Moksness, E. and Solemdal, P., eds.), pp.189-202. Flodevigen biological station, Arendal, Norway.

Hjort, J. (1914). Fluctuations in the great fisheries of northern Europe viewed in the light of the biological research. *Rapports et Procès-verbaux des Réunions. Conseil permanent international pour l'Exploration de la Mer,* **20**, 1-228.

Hinckley, S. (1990).Variation of egg size of walleye pollock *Theragra chalcogramma* with a preliminary examination of the effect of egg size on larval size. *Fishery Bulletin U.S.*, **88**, 471-483.

Hislop, J.R.G. 1988. The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus,* in British waters.*Journal of Fish Biology.* **32**: 923-930.

Hontela, A. (1984). Daily cycles of serum gonadotropin hormone in fish. *Transactions of the American Fisheries Society,* **113,** 458-466.

Horne, E.P.W. and Platt, T. (1984). The dominant space and time scales of variability in the physical and biological fields on continental shelves. *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer,* **183**, 8-19.

Houde, E.D. (1982). Micro and fine-scale biology. *In*- Fish Ecology III-A foundation for REX a recruitment experiment (Rothschild, B.J. and Rooth, C., eds.), pp.96-122. University of Miami, Miami FL, 389 pages.

Houde, E.D. (1987). Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium*, **2**, 17-29.

Houde, E.D. (1989). Comparative growth, mortality, and energetics of marine fish larvae: Temperature and implied latitudinal effects. *Fishery Bulletin U.S*, **87**, 471-485.

Houde, E.D. and Schekter, R.C. (1983). Oxygen uptake and comparativeenergetics among eggs and larvae of three subtropical marine fishes. *Marine Biology*, **72**, 283-293.

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. *Marine Ecology Progress Series*, **58**, 205-215.

Hovenkamp, F. and Witte, J. IJ. (1991). Growth, otolith growth and RNA-DNA ratios of larval plaice *Pleuronectes platessa* in the North Sea 1987 to 1989. *Marine Ecology Progress Series*, **70**, 105-116.

Hulata, G., Moav, R. and Wohlfarth, G. (1974). The relationship of gonad and egg size to weight and age in the European and chinese races of the common carp *Cyprinus carpio* L. *Journal of Fish Biology*, **6**, 745-758.

Hunter, J.R. (1976). Report of a colloquium on larval fish mortality studies and their relation to fishery research, January 1975. NOAA Technical Report, National Marine Fisheries Service, CIRC-395, 5 p.

Hunter, J.R. (1981). Feeding ecology and predation of marine fish larvae. *In-* Marine fish larvae (Lasker, R., ed.), pp. 34-77. University of Washington Press.

Hunter, J.R. (1984). Inferences regarding predation on the early life stages of cod and other fishes. *In-* The propagation of cod *Gadus morhua*, Part 2 (Dahl E., Danielssen D.S., Moksness E., and Solemdal P., eds.), pp. 533-552. Flodevigen biological station, Arendal, Norway.

Hurlbert, S.H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecological monographs*, **54 (2),** 187-211.

Hutchings, J.A. (1991). Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution*, **45 (5)**, 1162-1168.

Juinio, M.A.R., Cobb, J.S., Bengtson, D. and Johnson, M. (1992). Changes in nucleic acids over the molt cycle in relation to food availability and temperature in *Homarus americanus* postlarvae. *Marine Biology*, **114**, 1-10.

Jürss, K., Bittorf, Th., and Vökler, Th. (1986). Influence of salinity and food deprivation on growth, RNA/DNA ratio and certain enzyme activities in rainbow trout *Salmo gairdneri*. *Comparative Biochemistry and Physiology*, **83B(2)**, 425-433.

Jürss, K., Bittorf, Th., Vökler, Th. and Wacke, R. (1987). Effects of temperature, food deprivation and certain enzyme activities in rainbow trout *Salmo gairdneri*. *Comparative Biochemistry and Physiology*, **87B**, 241-253.

Kamler, E. (1992). Early life history of fish. An energetics approach. Fish and fisheries series 4, Chapman & Hall, New York. 267 pages.

Kaplan, R.H. (1989). Ovum size plasticity and maternal effects on the early development of the frog, *(Bombina orientalis,* Boulenger) in a field population in Korea. *Functional Ecology*, **3**, 597-604.

Karsten, U. and Wollenberger, A. (1972). Determination of DNA and RNA in homogenized cells and tissues by surface fluorometry. *Analytical Biochemistry,* **46**, 135-148.

Karsten, U. and Wollenberger, A. (1977). Improvements in the ethidium bromide method for direct fluorometric estimation of DNA and RNA in cell and tissue homogenates. *Analytical Biochemistry,* **77**, 464-470.

Kashuba, S.A. and Matthews, W.J. (1984). Physical condition of larval shad during spring-summer in a southwestern reservoir. *Transactions of the American Fisheries Society*, **113**, 199-204.

Kayes, T. (1978). Effects of hypophysectomy and beef growth hormone replacement therapy on morphometric and biochemical indicators of growth in the fed versus starved black bullhead *Ictalurus melas. General Comparative Endocrinology*, **35**, 419-431.

Kazakov, R.V. (1981). The effect of the size of Atlantic salmon, *Salmo salar* L., eggs on embryos and alevins. *Journal of Fish Biology*, **19**, 353-360.

Keast, A. and Eadie, J.M. (1985). Growth depensation in year-0 largemouth bass: The influence of diet. *Transactions of the American Fisheries Society*, **114**, 204-213.

Kiørboe, T. (1989). Growth in fish larvae. Are they particularly efficient? *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer*, **191**, 383-389.

Kiørboe, T. and Munk, P. (1986). Feeding and growth of larval herring, *Clupea harengus,* in relation to density of copepod nauplii. *Environmental Biology of Fishes*, **17(2)**, 133-139.

Kiørboe, T., Munk P., and Richardson, K. (1987). Respiration and growth of larval herring *Clupea harengus:* Relation between specific dynamic action and growth efficiency. *Marine Ecology Progress Series*, **40**, 1-10.

Kjesbu, O.S., Solemdal, P., Bratland, P. and Fonn, M. (1996). Variation in annual production in individual captive Atlantic cod *(Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 610-620.

Kjørsvik, E., Mangor-Jensen, A. and Holmefjord, I. (1990). Egg quality in fishes. *Advances in Marine Biology*, **26**, 71-113.

Klungsøyr, J., Tilseth, S., Wihelmsen, S., Falk-Petersen, S. and Sargent, J.R. (1989). Fatty acid composition as an indicator of food intake in cod larvae *Gadus morhua* from Lofoten, northern Norway. *Marine Biology*, **102**, 183-188.

Knutsen, G.M. and Tilseth, S. (1985). Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. *Transactions of the American Fisheries Society*, **114**, 507-511.

Koslow, J.A. (1992). Fecundity and the stock-recruitment relationship. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 210-217.

Koslow, J.A., Brault, S., Dugas, J., Fournier, R.O., and Hughes, P. (1985). Condition of larval cod *Gadus morhua* off southwest Nova Scotia in 1983 in relation to plankton abundance and temperature. *Marine Biology*, **86**, 113-121.

Kristogu-Baduge, S., Folkvord, A., and Johannessen, A. (1999). Responsiveness of selected condition measures of herring, *Clupea harengus*, larvae to starvation in relation to ontogeny and temperature. *Environmental Biology of Fishes*, **54**, 191-204.

Labarca,C. and Paigen, K. (1980). A simple, rapid and sensitive DNA assay procedure. *Analytical Biochemistry*, **102**, 344-352.

Lasker, R. (1962). Efficiency and rate of yolk utilization by developing embryos and larvae of the Pacific sardine, *Sardinops caerulea*. *Journal of the Fisheries Research Board of Canada*, **19(5)**, 867-875.

Lasker, R. (1981). The role of a stable ocean in larval fish survival and subsequent recruitment. *In-* Marine fish larvae (Lasker, R., ed.), pp. 80-87. University of Washington Press.

Lasker, R. and Zweifel J.R. (1978). Growth and survival of first-feeding northern anchovy larvae *Engraulis mordax* in patches containing different proportions of large and small prey. *In-* Spatial patterns in plankton communities (Steele, J.H., ed.), pp. 329-354. Plenum Publ. Co. New York.

Lauff, M. and Hofer, R. (1984). Proteolytic enzymes in fish development and the importance of dietary enzymes. *Aquaculture*, **37**, 335-346.

Laurence, G.C. (1974). Growth and survival of haddock *Melanogrammus aeglefinus* larvae in relation to planktonic prey concentration. *Journal of the Fisheries Research Board of Canada*, **31**, 1415-1419.

Laurence, G. C. (1977). A bioenergetic model for the analysis of feeding and survival potential of winter flounder *Pleuronectes americanus* larvae during the period from hatching to metamorphosis. *Fishery Bulletin U.S.*, **75 (3)**, 529-546.

Laurence, G.C. (1979). Larval length-weight relations for seven species of northwest Atlantic fishes reared in the laboratory. *Fishery Bulletin U.S.*, **76(4)**, 890-895.

Leatherland, J.F., Farbridge, K.J. and Boujard, T. (1992). Lunar and semi-lunar rhythms in fishes. *In*- Rhythms in Fishes, (Ali, M.I. Ed.), pp. 83-107. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

LeCren, E.D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch *Perca flavescens*. *Journal of Animal Ecology*, **20**, 201-219.

Legendre, P. and Dutilleul, P. (1992). Introduction to the analysis of periodic phenomena. *In*- Rhythms in Fishes, (Ali, M.I. Ed.), pp. 11-25. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

Leggett, W.C. (1986). The dependence of fish larval survival on food and predator densities. *In*-The role of freshwater outflow in coastal marine ecosystems (Skreslet, S., ed.), pp. 117-137. NATO ASI series, Vol. G7.

Leggett, W.C. and Frank, K.T. (1990). The spawning of the capelin. *Scientific American*, 102-107.

Lied, E., and Rosenlund, G. (1984). The influence of the ratio of protein energy to total energy in the feed on the activity of protein synthesis *in vitro*, the level of ribosomal RNA and the RNA-DNA ratio in white trunk muscle of Atlantic cod *Gadus morhua*. *Comparative Biochemistry and Physiology*, **77A**, 489-494.

Lied, E., Lund, B. and Von Der Decken, A. (1982). Protein synthesis *in vitro* by epaxial muscle polyribosomes from cod, *Gadus morhua*. *Comparative Biochemistry and Physiology*, **72B**, 187-193.

Lied, E., Rosenlund, G., Lund, B. and Von Der Decken, A. (1983). Effect of starvation and re-feeding on *in vitro* protein synthesis in white trunk muscle of Atlantic cod *Gadus morhua*. *Comparative Biochemistry and Physiology*, **76B**, 777-781.

Litvak, M. and Leggett, W.C. (1992). Age and size-selective predation on larval fishes: the bigger- is- better hypothesis revisited. *Marine Ecology Progress Series*, **81**, 13-24.

Lone, K.P. and Ince, B.W. (1983). Cellular growth responses of rainbow trout *Salmo gairdneri* fed different levels of dietary protein, and an anabolic steroid ethylestrenol. *General Comparative Endocrinology*, **49**, 32-49.

Loughna, P.T. and Goldspink, G. (1984). The effects of starvation upon protein turnover in red and white myotomal muscle of rainbow trout, *Salmo gairdneri*. *Journal of Fish Biology*, **25**, 223-230.

Love, R.M. (1970). The Chemical Biology of Fishes, Vol.1. Academic Press, London and New York, 547 pages.

Love, R.M. (1980). The Chemical Biology of Fishes, Vol.2: Advances 1968-1977. Academic Press, London and New York, 943 pages.

Lowery, M.S. and Somero, G.N. (1990). Starvation effects on protein synthesis in red and white muscle of barred sand bass *Paralabrax nebulifer*.*Physiological Zoology*, **63**, 630-648.

Lowery, M.S., Roberts, S.J. and Somero, G.N. (1987). Effects of starvation on the activities and localization of glycolytic enzymes in the white muscle of the barred sand bass *Paralabrax nebulifer*. *Physiological Zoology*, **60**, 538-549.

MacKas, D.L., Denman, K.L., and Abbott, M.R. (1985). Plankton patchiness: Biology in the physical vernacular. *Bulletin of Marine Sciences*, **37(2)**, 652-674.

MacKenzie, B.R. and Leggett, W.C. (1991). Quantifying the contribution of small-scale turbulence to the encounter rates between larval fish and their zooplankton prey: Effects of wind and tide. *Marine Ecology Progress Series*, **73**, 149-160.

MacKenzie, B. R., Leggett, W.C. and Peters, R.H. (1990). Estimating larval fish ingestion rates: Can laboratory derived values be reliably extrapolated to the wild? *Marine Ecology Progress Series*, **67**, 209-225.

Malloy ,K.D. and T.E. Targett. (1994). The use of RNA :DNA ratios to predict growth limitation of juvenile summer flounder (*Paralichthys dentatus*) from Delaware and North Carolina estuaries. *Marine Biology*, **118**, 367-375.

Maniatis, T. , Fritsch, E.F. and Sambrook, J. (1992). Molecular cloning. A laboratory manual. Cold Sping Harbour laboratory publications, New York.

Marsh, E. (1984). Egg size variation in central Texas populations of *Etheostoma spectabile* (Pisces: Percidae). *Copeia*, **1984(2)**, 291-301.

March, E. (1986). Effects of egg size on offspring fitness and maternal fecundity in the orangethroat darter *Etheostoma spectabile* (Pisces: Percidae). *Copeia*, **1**, 18-30.

Margulies, D. (1993). Assessment of the nutritional condition of larval and early juvenile tuna and spanish mackerel (Pices:Scombridae) in the Panama Bight. *Marine Biology*, **115**, 317-330.

Marshall, S.M., Nicholls, A.G. and Orr, A.P. (1937). On the growth and feeding of the larval and post-larval stages of the Clyde herring. *Journal of the marine biological association of the United Kingdom*, **23**, 245-267.

Marteinsdottir, G. and Able, K.W. (1988). Geographic variation in egg size among populations of the mummichog, *Fundulus heteroclitus* (Pisces: Fundulidae), *Copeia*, **2**, 471-478.

Marteinsdottir, G. and Able, K.W. (1992). Influence of egg size on embryos and larvae of *Fundulus heteroclitus* (L.). *Journal of Fish Biology*, **41**, 883-896.

Marteinsdottir, G. and Steinarsson, A. (1998). Maternal influence on the size and viability of Iceland cod *Gadus morhua* egg and larvae. *Journal of Fish Biology*, **52**, 1241-1258.

Martin, F.D. and Wright, D.A. (1987). Nutritional state analysis and its use in predicting striped bass recruitment: Laboratory calibration. *American Fisheries Society Symposium*, **2**, 109-114.

Martin, F.D., Wright, D.A. and Means, J.C. (1984). Fatty acids and starvation in larval striped bass *Morone saxatilis*. *Comparative Biochemistry and Physiology*, **77B (4)**, 785-790.

Martin, F.D., Wright, D.A, Means, J.C. and Setzler-Hamilton, E.M. (1985). Importance of food supply to nutritional state of larval striped bass in the Potomac river estuary. *Transactions of the American Fisheries Society*, **114**, 137-145.

Mathers, E.M., Houlihan, D.F. and Cunningham, M.J. (1992). Nucleic acid concentrations and enzyme activities as correlates of growth rate of the saithe *Pollachius virens* : Growth-rate estimates of open-sea fish. *Marine Biology*, **112**, 363-369.

Mathers, E.M., Houlihan, D.F., McCarthy, I.D. and Burren, L.J. (1993). Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Oncorhynchus mykiss:* effects of age and temperature. *Journal of Fish Biology*, **43**, 245-263.

Mathers, E .M ., Houlihan, D.F. and Burren, L.J. (1994). RNA,DNA and protein concentration in fed and starved herring *Clupea harengus* larvae. *Marine Ecology Progress Series*, **107**, 223-231.

May, R.C. (1971). Effects of delayed feeding on larvae of the grunion, *Leuresthes tenuis*. *Fishery Bulletin U.S.*, **69(2)**, 411-425.

May, R.C. (1974). Larval mortality in marine fishes and the critical period concept. *In*-The early life history of fish (Blaxter, J.H.S., ed.), pp. 3-19. Springer-Verlag, Berlin.

McEdward, L.R. and Coulter, L.K. (1987). Egg volume and energetic content are not correlated among sibling offspring of starfish: implications for life-history theory. *Evolution*. **41(4)**, 914-917.

McEdward, L.R. and Carson, S.F. (1987). Variation in egg organic content and its relationship with egg size in the starfish *Solaster stimpsoni*. *Marine Ecology Progress Series*, **37**, 159-169.

McEvoy, L. A. and McEvoy J. (1991). Size fluctuation in the eggs and newly hatched larvae of captive turbot *Scophthalmus maximus*. *Journal of the marine biological association of the United Kingdom*, **71**, 679-690.

McGinley, M.A., Temme, D.H., and Geber, M.A. (1987). Parental investment in offspring in variable environments: theoretical and empirical considerations. *The American Naturalist* **130**, 370-398.

McGurk, M.D. (1984). Effects of delayed feeding and temperature on the age of irreversible starvation and on the rates of growth and mortality of Pacific herring larvae. *Marine Biology*, **84**, 13-26.

McGurk, M.D. (1985a). Effect of net capture on the postpreservation morphometry, dry weight, and condition factor of Pacific herring larvae. *Transactions of the American Fisheries Society*, **114**, 348-355.

McGurk, M.D. (1985b). Multivariate analysis of morphometry and dry weight of Pacific herring larvae. *Marine Biology*, **86**, 1-11.

McGurk, M.D. and Kusser, W.C. (1992). Comparison of three methods of measuring RNA and DNA concentrations of individual Pacific herring, *Clupea harengus pallasi*, larvae. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 967-974.

McGurk, M.D., Warburton, H.D., Galbraith, M. and Kusser, W.C. (1992). RNA-DNA ratio of herring and sand lance larvae from Port Moller, Alaska: Comparison with prey concentration and temperature. *Fisheries Oceanography*, **1(3)**, 193-207.

McGurk, M.D., Paul, A.J., Coyle, K.O., Ziemann, D.A. and Haldorson, L.J. (1993). Relationships between prey concentration and growth, condition and

mortality of Pacific herring, *Clupea pallasi,* larvae in an Alaska subarctic embayment. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 163-180.

Meffe, G.K. (1987). Embryo size variation in mosquitofish: Optimality vs plasticity in propagule size. *Copeia*, **1987 (3)**, 762-768.

Meffe, G.K. (1990). Offspring size variation in eastern mosquitofish *(Gambusia holbrooki*: Poeciliidae) from contrasting thermal environments. *Copeia*, **1990 (1)**, 10-18.

Meier, A.H. (1984). Temporal synergism of circadian neuroendocrine oscillations regulates seasonal conditions in the Gulf killifish. *Transactions of the American Fisheries Society,* **113,** 422-431.

Meier, A.H. (1992). Circadian basis for neuroendocrine regulation. *In*-Rhythms in Fishes, (Ali, M.I. Ed.), pp. 109-126. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

Miglavs, I. and Jobling, M. (1989). Effects of feeding regime on food consumption, growth rates and tissue nucleic acids in juvenile Arctic charr, *Salvelinus alpinus*, with particular respect to compensatory growth. *Journal of Fish Biology*, **34**, 947-957.

Miller, T.J., Crowder, L.B., Rice, J.A. and Marschall, E.A. (1988). Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 1657-1670.

Moon, T.W. (1983). Metabolic reserves and enzyme activities with food deprivation in immature American eels, *Anguilla rostrata. Canadian Journal of Zoology*, **61**, 802-811.

Moon, T.W. and Johnson, I.A. (1980). Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscles and liver of the plaice, *Pleuronectes platessa*. *Journal of Comparative Physiology*, **136**, 31-38.

Mommsen, T.P. and Walsh, P.J. (1988). Vitellogenesis and oocyte assembly. *In*- Fish physiology vol.XI: The physiology of developing fish, part A, (Hoar, W.S. and Randall, D.J. Eds.), pp. 347-406, Academic Press, New York.

Mugiya, Y. and Oka, H. (1991). Biochemical relationship between otolith and somatic growth in the rainbow trout *Oncorhynchus mykiss*: Consequence of starvation, resumed feeding, and diel variations. *Fishery Bulletin U.S.*, **89**, 239-245.

Müller, W. and Gautier, F. (1975). Interactions of heteroaromatic compounds with nucleic acids. A T-specific non-intercalating DNA ligands. *European Journal of Biochemistry* **54**, 385-394.

Munilla-Moran, R., Stark, J.R. and Barbour, A. (1990). The role of exogenous enzymes in digestion in cultured turbot larvae *Scophthalmus maximus*. *Aquaculture*, **88**, 337-350.

Navarro, J.C. and Sargent, J.R. (1992). Behavioural differences in starving herring *Clupea harengus* larvae correlate with body levels of essential fatty acids. *Journal of Fish Biology*, **41**, 509-513.

Neilson, J.D. and Perry, R.I. (1990). Diel vertical migrations of marine fishes: an obligate or facultative process? *Advances in Marine Biology,* **26,** 115-168.

Neilson, J.D., Perry, R.I., Valerio, P. and Waiwood, K.G. (1986). Condition of Atlantic cod *Gadus morhua* larvae after the transition to exogenous feeding:

morphometrics, buoyancy and predator avoidance. *Marine Ecology Progress Series*, **32**, 229-235.

Nemeth, P.M., Hitchins, O.E., Solanki, L. and Cole, T.G. (1986). Fluorometric procedures for measuring triglyceride concentrations in small amounts of tissue and plasma. *Journal of Lipid Research*, **27**, 447-452.

Noakes, D.L.G. (1992). Behaviour and rhythms in fishes. *In*-Rhythms in Fishes, (Ali, M.I. Ed.), pp. 39-50. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

O'Connell, C.P. (1976). Histological criteria for diagnosing the starving condition in early post yolk sac larvae of the northern anchovy *Englaulis mordax*. *Journal of Experimental Marine Biology and Ecology*, **25**, 285-312.

O'Connell, C.P. (1980). Percentage of starving northern anchovy *Engraulis mordax*, larvae in the sea as estimated by histological methods. *Fishery Bulletin U.S.*, **78(2)**, 475-489.

O'Connell, C.P. (1981). Estimation by histological methods of the percent of starving larvae of the northern anchovy *Engraulis mordax* in the sea. *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer*, **178**, 357-360.

O'Connell, C.P., and Paloma, P.A. (1981). Histochemical indications of liver glycogen in samples of emaciated and robust larvae of the northern anchovy *Engraulis mordax*. *Fishery Bulletin U.S.*, **79(4)**, 806-812.

Ojanguren, A.F., Reyes-Gavilan, F.G. and Braña, F. (1996). Effects of egg size on offspring development and fitness in brown trout, *Salmo trutta* L. *Aquaculture*,**147**, 9-20.

Olivier, J.D., Holeton, G.F. and Chua, K.E. (1979). Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores, and environmental temperature. *Transactions of the American Fisheries Society*, **108**, 130-136.

Oozeki, Y, Ishii, T. and Hirano, R. (1989). Histological study of the effects of starvation on reared and wild-caught larval stone flounder, *Kareius bicoloratus*. *Marine Biology*, **100**, 269-275.

Ota, A.Y. and Landry, M.R. (1984). Nucleic acids as growth rate indicators for early developmental stages of *Calanus Pacificus*. *Journal of Experimental Marine Biology and Ecology*, **80**, 147-160.

Owen, R.W., Lo, N.C.H., Butler, J.L., Theilacker, G.H., Alvarino, A., Hunter, J.R. and Watanabe, Y. (1989). Spawning and survival patterns of larval northern anchovy, *Engraulis mordax*, in contrasting environments-A site intensive study. *Fishery Bulletin U.S.*, **87**, 673-688.

Palmer, J.D. (1974). Biological clocks in marine organisms. John Wiley and Sons Inc., New York, 173 pages.

Panagiotaki, P. and Geffen, A.J. (1992). Parental effects on size variation in fish larvae. *Journal of Fish Biology*, **41 (Suppl. B)**, 37-42.

Pedersen, B.H. and Hjelmeland, K. (1988). Fate of trypsin and assimilation efficiency in larval herring *Clupea harengus* following digestion of copepods. *Marine Biology*, **97**, 467-476.

Pedersen, B.H., Nilssen, E.M. and Hjelmeland, K. (1987). Variations in the content of trypsin and trypsinogen in larval herring *Clupea harengus* digesting copepod nauplii. *Marine Biology*, **94**, 171-181.

Pedersen, B.H., Ugelstad, I. and Hjelmeland, K. (1990). Effects of a transitory, low food supply in the early life of larval herring *Clupea harengus* on mortality, growth and digestive capacity. *Marine Biology*, **107**, 61-66.

Pepin, P. (1991). Effects of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 503-518.

Pepin, P. and R. A. Myers. (1991). Significance of egg and larval size to recruitment variability of temperate marine fish. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 1820-1828.

Pepin, P. and Miller, T. J. (1993). Potential use and abuse of general empirical models of early life history processes in fish. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 1343-1345.

Pepin, P., Shears, T.H. and DeLafontaine, Y. (1992). Significance of body size to the interaction between a larval fish *Mallotus villosus* and a vertebrate predator *Gasterosteus aculeatus*. *Marine Ecology Progress Series*, **81**, 1-12.

Peterman, R.M., Bradford, M.J., Lo, N.C.H., and Methot, R.D. (1988). Contribution of early life stages to interannual variability in recruitment of northern anchovy *Engraulis mordax*. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 8-16.

Peters, R.C. and Veeneklaas, R.J. (1992). Ultradian rhythms in fishes. *In*- Rhythms in Fishes, (Ali, M.I. Ed.), pp. 51-61. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

Peterson, M.S. and Brown-Peterson, N. (1992). Growth under stressed conditions in juvenile channel catfish *Ictalurus punctatus* as measured by nucleic acids. *Comparative Biochemistry and Physiology*, **103A (2)**, 323-327.

Pfeila, E. and Luna, A. (1984). Changes in biochemical composition and energy utilization during metamorphosis of leptocephalus larvae of the bonefish *Albula*. *Environmental Biology of Fishes*, **10(4)**, 243-251.

Platt, T. and Denman, K.L. (1975). Spectral analysis in ecology. *Annual Review of Ecology and Systematics*, **6**, 189-210.

Powell, A.B. and Chester, A.J. (1985). Morphometric indices of nutritional condition and sensitivity to starvation of spot larvae. *Transactions of the American Fisheries Society*, **114**, 338-347.

Powell, A.B., Chester, A.J., Govoni, J.J. and Warlen, S.M. (1990). Nutritional condition of spot larvae associated with the Mississippi river plume. *Transactions of the American Fisheries Society*, **119**, 957-965.

Purcell, J.E., and Grover, J.J. (1990). Predation and food limitation as causes of mortality in larval herring at a spawning ground in British Columbia. *Marine Ecology Progress Series*, **59**, 55-61.

Quattro, J.M. and S.C. Weeks. (1991). Correlations between egg size and energetic content within and among biotypes of the genus *Poeciliopsis*. *Journal of Fish Biology*, **38**, 331-334.

Raae, A.J., Opstad, I., Kvenseth, P. and Th. Walther, B. (1988). RNA, DNA and protein during early development in feeding and starved cod *Gadus morhua*. *Aquaculture*, **73**, 247-259.

Rana, K.J. (1985). Influence of egg size on the growth, onset of feeding, point-of-no-return, and survival of unfed *Oreochromis mossambicus* fry. *Aquaculture*, **46**, 119-131.

Reitan, K.I., Rainuzzo, J.R., Øie, G., and Olsen, Y. (1993). Nutritional effects of algal addition in first-feeding of turbot *(Scophtalmus maximus)* larvae. *Aquaculture*, **118,** 257-275.

Rice, J.A., Crowder, L.B. and Binkowski, F.P. (1987). Evaluating potential sources of mortality for larval bloater *Coregonus hoyi*: Starvation and vulnerability to predation. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**, 467-472.

Rice, J.A., Miller, T. J., Rose, K., Crowder, L. B., Marschall, E. A., Trebitz, A. S. and DeAngelis, D. L. (1993). Growth rate variation and larval survival: Inferences from an individual-based size-dependent predation model. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 133-142.

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

Robinson, S.M.C. and Ware, D.M. (1988). Ontogenetic development of growth rates in larval Pacific herring, *Clupea harengus pallasi*, measured with RNA-DNA ratios in the strait of Georgia, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 1422-1429.

Rombough, P.J. (1988). Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. *In*-Fish physiology vol.XI: The physiology of developing fish, part A, (Hoar, W.S. and Randall, D.J. Eds.), pp. 59-161, Academic Press, New York.

Rønnestad, I., Fyhn, H.J. and Gravningen, K. (1992a). The importance of free amino acids to the energy metabolism of eggs and larvae of turbot *Scophthalmus maximus*. *Marine Biology*, **114**, 517-525.

Rønnestad, I., Finn, R.N., Groot, E.P. and Fyhn, H.J. (1992b). Utilization of free amino acids related to energy metabolism of developing eggs and larvae of lemon sole *Microstomus kitt* reared in the laboratory. *Marine Ecology Progress Series*, **88**, 195-205.

Rooker, J. R. and Holt, G. J. (1996). Application of RNA :DNA ratios to evaluate the condition and growth of larval and juvenile Red Drum (*Sciaenops ocellatus*). *Marine Freshwater Ressources*, **47**, 283-290.

Rooker, J.R., Holt, G.J. and Holt, S.A..(1997). Condition of larval and juvenile red drum (*Sciaenops ocellatus*) from estuarine nursery habitats. *Marine Biology*, **127**, 387-394.

Roselund, G. and Lied, E. (1986). Growth and muscle protein synthesis *in vitro* of saithe *Pollachius virens* and rainbow trout *Salmo gairdneri* in response to protein-energy intake. *Acta Agriculturae Scandinavica*, **36**, 195-204.

Roselund, G., Lund, B., Lied, E. and Von Der Decken, A. (1983). Properties of white trunk muscle from saithe *Pollachius virens*, rainbow trout *Salmo gairdneri*, and herring *Clupea harengus*: Protein synthesis *in vitro*, electrophoretic study of proteins. *Comparative Biochemistry and Physiology*, **74B(3)**, 389-397.

Rosenthal, H. and Alderdice, D.F. (1976). Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. *Journal of the Fisheries Research Board of Canada*, **33**, 2047-2065.

Rothschild, B.J. (1986). Dynamics of marine fish populations. Harvard University Press, Cambridge, Mass. 277 pages.

Rothschild, B.J. and Rooth, C. (1982). Fish Ecology III, a foundation for REX a recruitment experiment, University of Miami, Miami FL, 389 pages.

Sale, P.F. (1990). Recruitment of marine species: Is the bandwagon rolling in the right direction? *Trends in Ecology and Evolution*, **5(1)**, 25-27.

Sameoto, D.D. (1972). Distribution of herring *Clupea harengus* larvae along the southern coast of Nova Scotia with observations on their growth and condition factor. *Journal of the Fisheries Research Board of Canada*, **29**, 507-515.

Schwassmann, H.O. (1971). Biological rhythms. *In-* Fish physiology vol.VI: Environmental relations and behaviour, (Hoar, W.S. and Randall, D.J. Eds.), pp. 371-428, Academic Press, New York.

Sclafani, M. (1992). Vertical migration of marine larval fish: Patterns, models and application to recruitment research. **MSc Thesis,** *Department of Oceanography, Dalhousie University, Halifax, N.S., 144 pages.*

Sclafani, M., Taggart, C.T., and Thompson, K.R. (1993). Condition, buoyancy and the distribution of larval fish: implications for vertical migration and retention. *Journal of Plankton Research*, **15(4)**, 413-435.

Sedmak, J.J. and Grossberg, S.E. (1977). A rapid, sensitive, and versatile assay for protein using Coomassie Brilliant Blue G250. *Analytical Biochemistry*, **79**, 544-552.

Segner, H. and Möller, H. (1984). Electron microscopical investigations on starvation-induced liver pathology in flounders *Platichthys flesus*. *Marine Ecology Progress Series*, **19**, 193-196.

Setzler-Hamilton, E.M., Wright, D.A., Martin, F.D., Millsaps, C.V. and Whitlow, S.I. (1987). Analysis of nutritional condition and its use in predicting striped bass recruitment: Field studies. *American Fisheries Society Symposium*, **2**, 115-128.

Shelbourne, J.E. (1957). The feeding and condition of plaice larvae in good and bad plankton patches. *Journal of the marine biological association of the United Kingdom*, **36**, 539-558.

Shultz, D.L. (1991). Parental investment in temporally varying environments. *Evolutionary Ecology*, **5**, 415-427.

Sieg, A. (1992a). A histological study on the nutritional condition of larval and metamorphosing fishes of the genus *Vinciguerria* (Photichthyidae, Pices) sampled in two contrasting environments. *Journal of Applied Ichthyology*, **8**, 154-163.

Sieg, A. (1992b). Histological study of organogenesis in the young stages of the mesopelagic fish *Vinciguerria* (Photichthyidae, Pices). *Bulletin of Marine Sciences*, **50(1)**, 97-107.

Sieg, A. (1993). Histological study on larval nutritional condition of the southwest Atlantic anchovy *Engraulis anchoita*, caught in three hydrographically differing

frontal systems of the southwest-Atlantic. International Council for the Exploration of the Sea, Council Meeting, 1993/L: 57.

Sieg, A., Clemmessen, C. and Ueberschär, B. (1989). Comparison of biochemical and histological methods for the evaluation of the *in situ* nutritional condition of marine fish larvae. International Council for the Exploration of the Sea, Council Meeting,1989/L:4

Sinclair, M. (1988). Marine populations: An essay on population regulation and speciation. University of Washington Press, Seattle, 252 pages.

Sissenwine, M.P. (1984). Why do fish populations vary? *In-* Exploitation of marine communities (May, R.M., ed.), pp.59-94. Springer-Verlag, New York.

Smith, P. E. (1985). Year-class strength and survival of 0-group clupeoids. *Canadian Journal of Fisheries and Aquatic Sciences*, **42 (Suppl. 1)**, 69-82.

Smith, S. (1957). Early development and hatching *In-* The physiology of fishes, (Brown, M., ed.), pp.323-359. New York Academic Press.

Smith, S.S. (1998). Evaluation of analytical data. In Food analysis, (Second Edition) (S. Suzanne Nielson, ed.) pp. 55-69. Aspen Publishers Inc, Maryland.

Soivio, A., Niemistö, M. and Bäckström, M. (1989). Fatty acid composition of *Coregonus muskun* Pallas: Changes during incubation, hatching, feeding and starvation. *Aquaculture*, **79**, 163-168.

Spieler, R.E. and Noeske, T.A. (1984). Effects of photoperiod and feeding schedule on diel variations of locomotor activity, cortisol, and thyroxine in goldfish. *Transactions of the American Fisheries Society,* **113,** 528-539.

Springate, J.R.C. and Bromage, N.R. (1985). Effects of egg size on early growth and survival in rainbow trout *(Salmo gairdneri* Richardson). *Aquaculture,* **47**, 163-172.

Springer, T.A. and Murphy, B.R.; Gutreuter, S.; Anderson, R.O.; Miranda, L.E. and Jackson, D.C.; Cone, R.S. (1990). Properties of relative weight and other condition indices. *Transactions of the American Fisheries Society*, **119**, 1048-1058.

Steele, J. H. (1978). Spatial pattern in plankton communities. Plenum press, New-York.

Steinhart, M. and Eckmann, R. (1992). Evaluating the nutritional condition of individual whitefish *Coregonus* spp. larvae by the RNA/DNA ratio. *Journal of Fish Biology*, **40**, 791-799.

Sterzel, W., Bedford, P. and Eisenbrand, G. (1985). Automated determination of DNA using the fluorochrome Hoechst 33258. *Analytical Biochemistry,* **147**, 462-467.

Stryer, L. (1981). Biochemistry, second edition, W.H. Freeman and Company, San Francisco. 949 pages.

Sullivan, K.M. and Somero, G.. (1980). Enzyme activities of fish skeletal muscle and brain as influenced by depth of occurrence and habits of feeding and locomotion. *Marine Biology*, **60**, 91-99.

Sundby, S., Bjørke, H., Soldal, A. V. and Olsen, S. (1989). Mortality rates during the early life stages and year-class strength of northeast Arctic cod *Gadus morhua. Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer*, **191**, 351-358.

Suthers, I.M., Fraser, A. and Frank, K.T. (1992). Comparison of lipid, otolith and morphometric condition indices of pelagic juvenile cod *Gadus morhua* from the Canadian Atlantic. *Marine Ecology Progress Series*, **84**, 31-40.

Suthers, I. M. ,Cleary, J. J. ,Battaglene S. C. and Evans R. (1996). Relative RNA content as a mesure of condition in larval and juvenile Fish. *Marine Freshwater Ressources*, **47**, 301-307.

Tabata, M. (1992). Photoreceptor organs and circadian locomotor activity in fishes. *In-* Rhythms in Fishes, (Ali, M.I. Ed.), pp. 223-234. NATO ASI series, series A: Life Sciences Vol. 236, Plenum Press, New York.

Taggart, C. T. and Frank, K. T. (1990). Perspectives on larval fish ecology and recruitment processes. Probing the scales of relationships. *In-* Large marine ecosystems, patterns, processes and yields, (Sherman, K., Alexander, L.M. and Gold, B. J., eds.), pp.151-164. American Association for the Advancement of Science, Washington D.C.

Theilacker, G.H. (1978). Effect of starvation on the histological and morphological characteristics of jack mackerel *Trachurus symmetricus*, larvae. *Fishery Bulletin U.S.*, **76(2)**, 403-414.

Theilacker, G.H. (1980a). Changes in body measurements of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservation. *Fishery Bulletin U.S.*, **78(3)**, 685-692.

Theilacker, G.H. (1980b). Rearing container size affects morphology and nutritional condition of larval jack mackerel, *Trachurus symmetricus*. *Fishery Bulletin U.S.*, **78(3)**, 789-791.

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

Theilacker, G.H., and Watanabe, Y. (1989). Midgut cell height defines nutritional status of laboratory raised larval northern anchovy *Engraulis mordax*. *Fishery Bulletin U.S.*, **87**, 457-469.

Thorpe, J.E., Miles, M.S., and Keay, D.S. (1984). Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. *Aquaculture*, **43**, 289-305.

Timeyko, V.N. and Novikov, G.G. (1987). Proteolytic activity in the digestive tract of Atlantic salmon, *Salmo salar* during larval development. *Journal of Ichthyology*, **27(4)**, 27-33.

Tocher, D.R. and Sargent, J.R. (1984). Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. *Lipids*, **19(7)**, 492-499.

Tocher, D.R., Fraser, A.J., Sargent, J.R. and Gamble, J.C. (1985a). Fatty acid composition of phospholipids and neutral lipids during embryonic and early larval development in Atlantic herring, *Clupea harengus*. *Lipids*, **20(2)**, 69-74.

Tocher, D.R., Fraser, A.J., Sargent, J.R. and Gamble, J.C. (1985b). Lipid class composition during embryonic and early larval development in Atlantic herring, *Clupea harengus*. *Lipids*, **20(2)**, 84-89.

Ueberschär, B. (1988). Determination of the nutritional condition of individual marine fish larvae by analyzing their proteolytic enzyme activities with a highly sensitive fluorescence technique. *Meeresforsch*, **32**, 144-154.

Ueberschär, B. and Clemmesen, C. (1992). A comparison of the nutritional condition of herring larvae as determined by two biochemical methods-tryptic enzyme activity and RNA/DNA ratio measurements. *ICES Journal of Marine Science*, **49**, 245-249.

Ueberschär, B., Pedersen, B.H. and Hjelmeland, K. (1992). Quantification of trypsin with a radioimmunoassay in herring larvae *Clupea harengus*, compared with a highly sensitive fluorescence technique to determine tryptic enzyme activity. *Marine Biology*, **113**, 469-473.

Umeda, S. and Ochiai, A. (1975). On the histological structure and function of digestive organs of the fed and starved larvae of the yellowtail, *Seriola quinqueradiata*. *Japanese Journal of Ichthyology*, **21(4)**, 213-219.

Underwood, A.J. and Fairweather, P.G. (1989). Supply-side ecology and benthic marine assemblages. *Trends in Ecology and Evolution*, **4(1)**, 16-19.

Van Winkle, W., Rose, K. A. and Chambers, R. C. (1993). Individual-based approach to fish population dynamics: An overview. *Transactions of the American Fisheries Society*, **122 (3)**, 397-403.

Vetter, R.D., Hodson, R.E. and Arnold, C. (1983). Energy metabolism in a rapidly developing marine fish egg, the red drum *Sciaenops ocellata*. *Canadian Journal of Fisheries and Aquatic Sciences*, **40**, 627-634.

Vilela, M.H. and Zijlstra, J.J. (1971). On the condition of herring larvae in the central and southern North Sea. *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer*, **160**, 137-141.

Vlymen, W. J. (1977). A mathematical model of the relationship between larval anchovy *Engraulis mordax*, growth, prey microdistribution and larval behaviour. *Environmental Biology of Fishes*, **2**, 211-233.

Von Westernhagen, H. and Rosenthal, H. (1981). On condition factor measurements in Pacific herring larvae. *Helgoländer Meeressunters*, **34**, 257-262.

Vu, T.T. (1983). Étude histoenzymologique des activités protéasiques dans le tube digestif des larves et des adultes de bar, *Dicentrarchus labrax*. *Aquaculture*, **32**, 57-69.

Walford, J. and Lam, T.J. (1993). Development of digestive tract and proteolytic enzyme activity in seabass *Lates calcarifer* larvae and juveniles. *Aquaculture*, **109**, 187-205.

Wallace, J.C. and Aasjord, D. (1984). An investigation of the consequences of egg size for the culture of Arctic charr, *Salvelinus alpinus* (L.). . *Journal of Fish Biology*, **24**, 427-435.

Wang, S.Y. and Stickle, W.B. (1986). Changes in nucleic acid concentration with starvation in the blue crab *Callinectes sapidus*. *Journal of Crustacean Biology*, **6**, 49-56.

Ware, D.M. (1975). Relation between egg size, growth, and natural mortality of larval fish. *Journal of the Fisheries Research Board of Canada*, **32**, 2503-2512.

Ware, D.M. and Lambert, T. C. (1985). Early life history of Atlantic mackerel *Scomber scombrus* in the southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 577-592.

Weeks, S.C. (1993). Phenotypic plasticity of life-history traits in clonal and sexual fish (*Poeciliopsis)* at high and low densities. *Oecologia*, **93**, 307-314.

Westerman, M. E. and Holt, G. J. (1988). The RNA-DNA ratio: Measurement of nucleic acids in larval *Sciaenops ocellatus*. *Contributions to Marine Sciences*, **30 (Suppl.)**, 117-124.

Westerman, M. , Holt,G.J..(1994). RNA:DNA ratio during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. *Marine Biology*, **121**, 1-9.

Wicker, A.M. and Johnson, W.E. (1987). Relationship among fat content, condition factor, and first-year survival of Florida largemouth bass. *Transactions of the American Fisheries Society,* **116**, 264-271.

Wieser, W., Forstner, H., Medgyesy, N., and Hinterleitner, S. (1988). To switch or not to switch: partitioning of energy between growth and activity in larval cyprinids (Cyprinidae: Teleostei). *Functional Ecology,* **2,** 499-507.

Wilkinson, L. (1998). SYSTAT 8.0 statistics. SPSS Inc., Chicago IL.

Wilson, D. C. and Millermann, R. E. (1969). Relationships of female age and size and size to embryo number and size in the shiner perch, *Cymatogaster aggregata*. *Journal of the Fisheries Research Board of Canada*, **26**, 2339-2344.

Wright, D.A. and Hetzel, E.W. (1985). Use of RNA:DNA ratios as an indicator of nutritional stress in the American oyster *Crassostrea virginica*. *Marine Ecology Progress Series*, **25,** 199-206.

Wright, D.A. and Martin, F.D. (1985). The effect of starvation on RNA-DNA ratios and growth of larval striped bass, *Morone saxatilis*. *Journal of Fish Biology*, **27**, 479-485.

Wyatt, T. (1972). Some effects of food density on the growth and behaviour of plaice *Pleuronectes platessa* larvae. *Marine Biology*, **14**, 210-216.

Yamagami, K. (1988). Mechanisms of hatching in fish. *In-* Fish physiology vol.XI: The physiology of developing fish, part A, (Hoar, W.S. and Randall, D.J. Eds.), pp. 447-499, Academic Press, New York.

Yamashita, Y. and Bailey, K. M. (1989). A laboratory study of the bioenergetics of larval walleye pollock, *Theragra chalcogramma*. *Fishery Bulletin U.S.*, **87**, 525-536.

Yin, M.C. and Blaxter, J.H.S. (1986). Morphological changes during growth and starvation of larval cod *Gadus morhua* and flounder *Platichthys flesus*. *Journal of Experimental Marine Biology and Ecology*, **104**, 215-228.

Yin, M. C. and Blaxter, J. H. S. (1987). Temperature, salinity tolerance, and buoyancy during early development and starvation of Clyde and North Sea herring, cod and flounder larvae. *Journal of Experimental Marine Biology and Ecology*, **107**, 279-290.

Zastrow, C.E., Houde, E.D. and Saunders, E.H. (1989). Quality of striped bass *Morone saxatilis* eggs in relation to river source and female weight. *Rapports et Procès-verbaux des Réunions. Conseil international pour l'Exploration de la Mer*, **191**, 34-42.

Zeitoun, I.H., Ullrey, D.E., Bergen, W.G. and Magee, W.T. (1977). DNA, RNA, protein and free amino acids during ontogenesis of rainbow trout *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada*, **34**, 83-88.