Nutritional Condition of Striped Bass (Morone Saxatilis) Larvae as Determined by Constituent Lipid Analysis

William Frank Patterson III
Old Dominion University

Follow this and additional works at: https://digitalcommons.odu.edu/biology_etds

Part of the Marine Biology Commons, and the Population Biology Commons

Recommended Citation
Patterson, William F.. "Nutritional Condition of Striped Bass (Morone Saxatilis) Larvae as Determined by Constituent Lipid Analysis" (1995). Master of Science (MS), Thesis, Biological Sciences, Old Dominion University, DOI: 10.25777/a99m-yh34
https://digitalcommons.odu.edu/biology_etds/241

This Thesis is brought to you for free and open access by the Biological Sciences at ODU Digital Commons. It has been accepted for inclusion in Biological Sciences Theses & Dissertations by an authorized administrator of ODU Digital Commons. For more information, please contact digitalcommons@odu.edu.
NUTRITIONAL CONDITION OF STRIPED BASS (*MORONE SAXATILIS*)
LARVAE AS DETERMINED BY CONSTITUENT LIPID ANALYSIS

by
William Frank Patterson III
B.A. May 1991, University of Virginia

A Thesis submitted to the Faculty of Old Dominion University in Partial Fulfillment of the Requirement for the Degree of

MASTER OF SCIENCE
BIOLOGY

Old Dominion University
September, 1995

Approved by

James H. Cowan Jr. (Co-director)

Cynthia Jones (Co-director)

Harold G. Marshall
ABSTRACT

NUTRITIONAL CONDITION OF STRIPED BASS (MORONE SAXATILIS) LARVAE AS DETERMINED BY CONSTITUENT LIPID ANALYSIS

William Frank Patterson III
Old Dominion University, 1995
Co-Director: Dr. James H. Cowan, Jr.
Co-Director: Dr. Cynthia M. Jones

Larval striped bass were reared in the laboratory from 6 days post-hatch (dph) to 21 dph under four different feeding regimes to assess the effect of food concentration on triacylglycerol to sterol ratios (TG:ST) of larvae. Larvae were fed Peru strain Artemia salina nauplii in concentrations of 0, 10, 100, and 1000 l⁻¹, with three replicate tanks within each feeding treatment. Larvae were sampled on 14 and 21 dph and frozen in liquid nitrogen. Lipids were extracted from larvae in a 90:10:1 solution of dichloromethane:hexane:formic acid and analyzed with thin-layer chromatography/ flame ionization detection. Analysis of larval constituent lipids, and calculation of vital rates of instantaneous daily mortality (Z) and weight-specific growth (G) from different treatments, suggested that a TG:ST ratio of 0.75 may be a conservative estimate of the boundary between larvae in good and poor nutritional condition.

This laboratory-calibrated standard was employed to determine the nutritional condition of wild-caught striped bass larvae from the Pamunkey River, Virginia. On five cruises during spring 1994, larval striped bass and zooplankton were collected from the area of peak spawning activity in the Pamunkey. Analysis of constituent lipids of wild-caught larvae showed later-spawned cohorts of larval striped bass were in worse
nutritional condition than early-spawned fish. Nutritional condition declined as food levels fell, especially larger cladocerans, and was positively correlated to temperature (p = 0.03). This method for determining relative health of larval fish was successful in determining nutritional condition of larval striped bass and easily could be incorporated into existing larval surveys.
DEDICATION

This work is dedicated to the memory of
Dr. Ray S. Birdsong.

Thank you for the opportunity.
ACKNOWLEDGEMENTS

I would like to thank my committee members, Dr. Ray S. Birdsong, Dr. James H. Cowan, Jr., Dr. Cynthia Jones, and Dr. Harold G. Marshall, for their guidance and criticisms. In particular, I would like to thank Dr. Ray S. Birdsong for his academic, moral, and financial support of me and this project. Dr. Birdsong’s untimely passing was a great loss to Old Dominion University, and he is sorely missed by his graduate students, colleagues, and friends.

I am also indebted to many of my fellow graduate students for generously giving of their time and energy. I thank Michael Normandt, Gyung-Soo Park, and John Rose for helping with the design and construction of the rearing system, and with running the feeding experiment. I thank George Mateja, Tim Rach, Richard Fulford, John Rose, and Richard Ramsdell for their help in field collections. I thank Dr. George McManus and Alan Foster of the Dauphin Island Sea Lab, Dauphin Island, Alabama, for their help in lipid extraction, analysis, and interpretation of results. I thank Dr. Simon Thorrold and Michael Lane for advice concerning statistical analysis. And I thank Tom Dooley for help in figure presentation, and for making us laugh.

I thank the Virginia Department of Game and Inland Fisheries personnel and staff for providing striped bass larvae, especially the personnel and staff of the Brookneal Striped Bass Hatchery. I thank the Department of Biological Sciences of Old Dominion University for use of laboratory space, the R/V Beagle Too, and all equipment used in field collections.
Above all, I would like to thank my Mother and Father for their love and support along the way.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF TABLES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Striped Bass Early Life History</td>
<td>1</td>
</tr>
<tr>
<td>Research Setting and Rationale.</td>
<td>4</td>
</tr>
<tr>
<td>Hypotheses Tested and Objectives of the Study</td>
<td>6</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>9</td>
</tr>
<tr>
<td>I. Feeding Experiment</td>
<td>9</td>
</tr>
<tr>
<td>Rearing System</td>
<td>9</td>
</tr>
<tr>
<td>Feeding Experiment</td>
<td>12</td>
</tr>
<tr>
<td>Lipid Analysis</td>
<td>16</td>
</tr>
<tr>
<td>II. Field Collection</td>
<td>18</td>
</tr>
<tr>
<td>Zooplankton Collection and Enumeration</td>
<td>18</td>
</tr>
<tr>
<td>Striped Bass Larvae Collection and Lipid Analysis</td>
<td>20</td>
</tr>
<tr>
<td>RESULTS</td>
<td>22</td>
</tr>
<tr>
<td>I. Feeding Experiment</td>
<td>22</td>
</tr>
<tr>
<td>II. Field Results</td>
<td>27</td>
</tr>
<tr>
<td>Water Quality Parameters</td>
<td>27</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>32</td>
</tr>
<tr>
<td>Larval Striped Bass Condition.</td>
<td>36</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>41</td>
</tr>
<tr>
<td>I. Feeding Experiment</td>
<td>41</td>
</tr>
<tr>
<td>Six Day Post-hatch Larvae</td>
<td>41</td>
</tr>
<tr>
<td>Growth and Mortality in the Feeding Experiment</td>
<td>42</td>
</tr>
<tr>
<td>TG:ST Ratios in Striped Bass Larvae</td>
<td>44</td>
</tr>
<tr>
<td>II. Field Collections</td>
<td>47</td>
</tr>
<tr>
<td>Water Quality Parameters</td>
<td>47</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>48</td>
</tr>
<tr>
<td>Nutritional Condition in Wild-caught Striped Bass Larvae</td>
<td>49</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>53</td>
</tr>
<tr>
<td>TABLE</td>
<td>PAGE</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>1. Ranges of water quality parameters for the feeding experiment. Ranges are for parameters measured in all tanks combined throughout the feeding experiment.</td>
<td>14</td>
</tr>
<tr>
<td>2. Lab results from weeks one and two of the feeding experiment. Standard errors are given along with means of each variable for each treatment in weeks one and two of the feeding experiment.</td>
<td>25</td>
</tr>
<tr>
<td>3. Biotic and abiotic parameters measured in the Pamunkey River on cruises one through five. Standard errors are given along with mean values for each biotic and abiotic parameter measured in the field.</td>
<td>33</td>
</tr>
<tr>
<td>4. Results from correlations between TG:ST of field caught larvae and temperature, and mean <em>Bosmina</em> abundance.</td>
<td>40</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>DESCRIPTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Maps of the Chesapeake Bay and York River System, Virginia. Sampling stations within the Pamunkey River are illustrated.</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>Schematic diagram of recirculating rearing system used in the feeding experiment.</td>
<td>10</td>
</tr>
<tr>
<td>3.</td>
<td>Regression of Dwt on St for all 6, 14, and 21 dph larvae.</td>
<td>23</td>
</tr>
<tr>
<td>4.</td>
<td>Frequency histogram of the TG:ST ratios from 28 samples of 6 dph larvae pooled in groups of three.</td>
<td>24</td>
</tr>
<tr>
<td>5.</td>
<td>Nonlinear regressions of Dwt and G on TG:ST ratios from 14 dph larvae.</td>
<td>28</td>
</tr>
<tr>
<td>6.</td>
<td>Nonlinear regressions of Dwt and ASL on TG:ST ratios from 21 dph larvae. Regression equations, p-values and coefficients are given.</td>
<td>29</td>
</tr>
<tr>
<td>7.</td>
<td>Nonlinear regressions of G and Z on TG:ST ratios from 21 dph larvae.</td>
<td>30</td>
</tr>
<tr>
<td>8.</td>
<td>Linear regression of G on Z and nonlinear regression of G/Z on TG:ST ratios from 21 dph larvae.</td>
<td>31</td>
</tr>
<tr>
<td>9.</td>
<td>Estimates of the abundances of the dominant taxa of zooplankton prey available to striped bass larvae in the Pamunkey River in spring 1994.</td>
<td>34</td>
</tr>
<tr>
<td>10.</td>
<td>Estimates of the abundances of total copepod adults and copepodites and total cladocerans available as prey for striped bass larvae in the Pamunkey River in spring 1994.</td>
<td>35</td>
</tr>
<tr>
<td>11.</td>
<td>Boxplots of mean TG:ST ratios of sampled striped bass larvae from the Pamunkey River in spring 1994, with weekly sample size given within each box.</td>
<td>38</td>
</tr>
<tr>
<td>12.</td>
<td>The SL of field sampled striped bass larvae plotted against their TG:ST ratios for each of the five cruises.</td>
<td>39</td>
</tr>
</tbody>
</table>
INTRODUCTION

Striped Bass Early Life History

The wide range in observed recruitment success of striped bass in Chesapeake Bay has been reported to result primarily from density-independent environmental factors which act while striped bass are young (Ulanowicz and Polgar 1980; Houde and Lubbers 1986; Uphoff 1989; Tsai 1991; Chesney 1993; Cowan et al. 1993). The environmental factors cited as affecting recruitment include reduction of suitable habitat for young striped bass (Coutant 1985; Coutant and Benson 1990), organic and inorganic pollution, including acid rain (Hall et al. 1985, 1991; Goodyear 1985; Palawski et al. 1985; Uphoff 1989; Hall 1991; Tsai et al. 1991), water temperatures during egg and larval stages (Rogers and Westin 1981; Morgan et al. 1981; Boreman 1983; Chesney 1993; Cowan et al. 1993, Rutherford and Houde 1995), and larval mortality due to starvation or predation (Martin et al. 1985; Setzler-Hamilton et al. 1987; McGovern and Olney 1988; Tsai 1991).

Laboratory and model simulation studies have shown striped bass larvae are resistant to starvation (Eldridge et al. 1981; Houde 1987; Tsai 1991; Cowan et al. 1993), even though the vital rates of weight specific growth (G) and instantaneous daily mortality (Z) are driven by variations in prey densities and temperature (Rogers and Westin 1981; Eldridge et al. 1981, 1982; Houde and Lubbers 1986; Tsai 1991; Chesney 1993; Cowan et al. 1993). Although cohorts of wild striped bass larvae are susceptible to episodic losses due to temperature fluctuations, they are unlikely to suffer catastrophic losses due to
starvation (Cowan et al. 1993, Rutherford and Houde 1995). However, subtle variations in daily growth and mortality rates can have a great effect on recruitment (Houde 1987; Houde 1989). Poor nutritional condition may slow growth and extend the larval period, thus exposing larval striped bass to potential predators for a protracted period of time (Cushing 1975; McGurk 1986; Miller et al. 1988; Pepin 1989, 1991; Cowan and Houde 1992).

Several researchers have examined nutritional condition of striped bass larvae and attempted to differentiate "healthy" larvae from those in poor nutritional condition (Martin et al. 1984, 1985; Martin and Wright 1987; Setzler-Hamilton et al. 1987; Setzler-Hamilton and Cowan 1993). Martin and Wright (1987) reared larval striped bass under different feeding regimes designed to yield larvae in variable nutritional conditions, after which they assessed four different methods for ability to diagnose larvae in good and poor condition. The four methods tested were histologic analyses, morphometric measures, RNA/DNA ratios, and measurements of fatty acid composition (Martin and Wright 1987). Martin and Wright (1987) reported that the histologic method did not distinguish between starving larvae and those recovering from starvation, and that morphometric measures should be restricted to comparisons of larvae within a limited size range (5.5-12 mm). They also reported that differences in RNA/DNA ratios between fed and starved larvae were clear two days after first-feeding, but suggested RNA/DNA ratios respond quickly to individual "bouts of feeding," and thus their usefulness may be limited (Martin and Wright 1987; Setzler-Hamilton et al. 1987). The fourth method calibrated by Martin and Wright (1987) involved quantifying total fatty acids, which proved to be a poor measure of nutritional
condition (Setzler-Hamilton et al. 1987).

In addition, Martin et al. (1985) and Setzler-Hamilton et al. (1987) conducted field studies in the Potomac and Choptank Rivers, Maryland, using the methods described by Martin and Wright (1987) for assessing nutritional condition of striped bass larvae. Martin et al. (1985) found the nutritional condition of striped bass larvae in the Potomac River in 1981 to be correlated to zooplankton concentration (copepod and cladoceran concentrations in particular), and rising water temperatures. Setzler-Hamilton et al. (1987) reported that field studies analyzing nutritional condition of wild-caught striped bass larvae could be used in conjunction with water quality data and zooplankton abundance to predict recruitment success of Potomac River striped bass. Moreover, Setzler-Hamilton and Cowan (1993) analyzed Potomac River striped bass larvae based on morphometric and length-weight regression parameters and observed a relationship between high numbers of larvae in poor nutritional condition and a low index of recruitment for Potomac River striped bass.

More recently, Fraser (1989) proposed a "novel biochemical index" for larval fish based on constituent lipid composition. The storage lipid triacylglycerol (TG) is a good indicator of physiologic state of larvae and the ratio of TG to highly conservative lipids such as sterol (ST) or polar lipid (PL) is an indicator of nutritional condition (Fraser 1989). Hakanson (1989 a,b) successfully employed this method in determining the nutritional condition of larval anchovy, *Engraulis mordax*, in the Southern California Bight and correlated larval anchovy condition to energy stores in the copepod *Calanus pacificus*. Suthers et al. (1992) examined TG content in pelagic juvenile cod, *Gadus*
morhua, and indicated that the utility of a lipid-based condition index may be species- and life-stage specific.

Research Setting and Rationale

In late winter, coastal migratory striped bass enter Chesapeake Bay, and begin to move up rivers in early spring to spawn. In spring, the Pamunkey River, Virginia, is generally characterized by high flow and is well-mixed (Marshall and Alden 1990; Birdsong 1992). High spring river flow has been demonstrated to be important to successful striped bass spawns in several river systems (Setzler-Hamilton et al. 1981; Stevens 1980; Stevens et al. 1985), but spring river flow is inversely related to zooplankton concentration in the Pamunkey River (Birdsong 1992). Peak striped bass spawning takes place in the Pamunkey River in late April (Grant and Olney 1991) when zooplankton concentration may not be high enough to support good larval growth.

The Virginia Chesapeake Bay Water Quality and Living Resources Monitoring Program (CBMP) monitors the zooplankton community monthly at one tidally-fresh station in the Pamunkey River (Figure 1). The average concentration of zooplankton at this station from 1986 to 1989 was 6.8 plankters l⁻¹. In the spring months (April, May, June) during 1986 to 1989 the average zooplankton concentration was $7.6 \pm 2.36$ plankters l⁻¹, with a peak concentration of 27.4 plankters l⁻¹ in June 1989, and a low concentration of 0.2 plankters l⁻¹ in May 1989 (Birdsong 1992). Although caution should
Figure 1. Maps of the Chesapeake Bay and York River system, Virginia. Sampling stations within the Pamunkey River are illustrated.
Chesapeake Bay

York River System

Mattaponi

Pamunkey

York
be used when applying results from larval feeding studies conducted in the laboratory to wild larvae, the literature on prey density requirements for striped bass indicate that spring zooplankton densities in the Pamunkey River may not be adequate to support good larval growth (Rogers and Westin 1981; Eldridge et al. 1981, 1982; Martin et al. 1984; Houde and Lubbers 1986; Martin and Wright 1987; Tsai 1991; Cowan et al. 1993).

Martin et al. (1985) found temporal variation in zooplankton concentration in the Potomac River did not explain poor nutritional condition of Potomac River striped bass larvae, even though ambient river zooplankton concentrations were not high enough to support good growth in the laboratory. Rutherford and Houde (1995) also reported that differences in growth and survival of cohorts of larval striped bass in the Potomac River were not correlated to zooplankton concentration. However, the combined concentrations of copepods and cladocerans in the Potomac River approached 100 l⁻¹ throughout Martin et al.'s (1985) study and approached 1000 l⁻¹ during Rutherford and Houde's (1995) study, which is much higher than observed prey concentrations in the Pamunkey River in spring (Birdsong 1992). Therefore, the potential for prey concentrations to restrict larval striped bass growth and survival may be greater in the Pamunkey River than in the Potomac River.

Hypotheses to be Tested and Objectives of the Study

Ho₁: There is no difference in TG:ST ratios in starved versus fed striped bass larvae.

Corollary: Differences in the vital rates of weight-specific growth or instantaneous daily
mortality of striped bass larvae can be attributed to amount of prey offered.

The first objective was to determine the efficacy of an index of nutritional condition of striped bass larvae based on the constituent lipid of fed and starved larvae reared in the laboratory. The ratio of TG to ST has been shown to be an effective measure of condition in other species (Fraser 1989; Hakanson 1989 a, b), and its utility in determining condition in striped bass larvae was tested.

Ho 2: There is no difference in zooplankton concentrations between sampled stations in the Pamunkey River, Virginia.

Corollary: Zooplankton concentrations at each station is sufficient to support growth in striped bass larvae.

The second objective of this work was to examine the zooplankton community in the area of peak striped bass spawning activity in the Pamunkey River. The Virginia CBMP estimates meso- and micro-zooplankton concentrations at only one station (TF 4.2, Figure 1), once monthly in the tidally-fresh portion of the Pamunkey River (Birdsong 1992). This current study estimated zooplankton concentrations at four different stations within the area of peak striped bass spawning activity (Figure 1) to more accurately estimate zooplankton prey available to larval striped bass (Grant and Olney 1991).

Ho 3: The nutritional condition of striped bass larvae is not related to zooplankton concentrations or water mass characteristics in the Pamunkey River.

The third objective of this work was to analyze nutritional condition of larval striped bass in the Pamunkey River, Virginia. Larval striped bass were collected throughout the 1994 spawning season in the Pamunkey and their nutritional condition co-related to water mass characteristics and zooplankton concentrations. The goal was to determine physical
factors which limit striped bass larval growth in the Pamunkey River, and to determine if potential prey for striped bass larvae was adequate to support larval growth.
MATERIALS AND METHODS

I. Feeding Experiment

Rearing System

The closed rearing system for the feeding experiment consisted of twelve 38 l glass aquaria and a 40 l sump tank (Figure 2). The aquaria were arranged side by side in two banks of six aquaria on rubberized metal racks 125 cm off the floor. A 1.91 cm hole was drilled in the bottom each aquarium. The drilled holes were located in the same place in each aquarium (12 cm from the front edge and 6 cm from the side edge of each) and fitted with a PVC bushing which was sealed with clear silicone chalk. The bushing had an inner diameter (ID) of 2.54 cm on the inside of the aquarium and an outer diameter (OD) of 3.81 cm on the underside of each aquarium.

A 23 cm tall 2.54 cm OD PVC standpipe was fitted into the inside bushing of each aquarium to maintain a volume of 30 l of water when full. A second, 6.35 cm OD PVC standpipe surrounded the 2.54 cm OD standpipe and was "glued" to the floor of each aquarium with clear silicone chalk. Nine 5.08 cm holes were drilled and filed in each of the larger standpipes to allow water to flow freely through the system. The larger standpipes were fitted with 153 µm Nitex® mesh which was glued to the standpipe with clear silicone chalk. The Nitex® mesh allowed free flow of water, but did not allow mixing of larvae and prey organisms throughout the system.

Below each aquarium, a piece of stretched 5.72 cm ID soft plastic tubing was fixed to
Figure 2. Schematic drawing of recirculating rearing system used in the feeding experiment.
Treatments:

1. 38 l aquarium
2. internal standpipe
3. external standpipe
4. drum valve
5. PVC pipe to aquaria
6. PVC pipe returning to sump
7. sump tank and chiller unit
8. 1/15 h.p. centrifugal pump
9. support rack
10. rubber tubing from pump to delivery pipe
11. PVC bushing exiting aquarium
the outer bushing. The plastic tubing from each aquarium was directed into a length of 6.35 cm OD PVC, which sloped down to the sump tank. The sump tank contained copper coils, which were wrapped with PVC tubing and sealed (no copper leached into the system). Coolant was pumped into the coils by a 1,000 BTU compressor unit located under the sump. The chilling potential of the compressor was easily adjusted and allowed for the water temperature of the rearing system to be precisely controlled. The sump tank was also heavily aerated with air forced through air stones.

Water passed through the bottom of the sump tank and was pumped back to the top of the system with a 1/15 horsepower, 2.3 amp electric centrifugal pump. The pump had a back pressure valve which allowed for regulation of water flow (approximately 0.67 l·min per aquarium). Water was pumped through 3.81 ID cm soft plastic tubing back to the top of the system. At the top, the rubber tubing was attached to 3.81 cm OD PVC tubing which was branched to each bank of tanks. At each branch, the 3.81 cm OD PVC pipe feed into 3.81 cm OD to 2.54 cm ID PVC reducer T's at each tank, and then back to 3.81 cm OD PVC pipe until the last tank where the line was capped with a PVC cap. The 2.54 cm arm of the reducer T was fitted with a 2.54 cm OD, threaded 1.91 cm ID PVC bushing. A threaded 1.91 cm OD PVC drum valve was wrapped counter-thread with teflon tape and screwed into each threaded bushing. A 25 cm long piece of 1.27 cm OD rubber tubing was placed inside each drum valve and run to the near side of each aquarium to lessen the "shock" of falling water. Throughout the system, all PVC to PVC connections were made with PVC cement which was cured for 48 hours outside of the rearing room whenever possible.
Design of Feeding Experiment

Twenty thousand 5 day post-hatch (dph) striped bass larvae for the rearing experiment were obtained from the Virginia Department of Game and Inland Fisheries' Brookneal Striped Bass Hatchery in May 1994. The larvae were the progeny of two small striped bass females; one female was 5.6 kg and the other was 6.3 kg. The larvae were acclimated to conditions in the rearing system for 24 hours and then stocked into the rearing system aquaria. Just prior to stocking, a representative sample of 20, 6 dph striped bass larvae were measured to 0.1 mm standard length with an ocular micrometer, then dried in an oven at 60°C for 24 hours and weighed to the nearest µg. Also, a representative sample of 75, 6 dph larvae, in groups of three, were placed in 2 ml Nunc® cryovials, frozen in liquid nitrogen, and stored at -80°C for later lipid analysis.

The 15 day feeding experiment had four feeding treatments, each with three replicate tanks. Striped bass larvae were stocked at a density of 15 l⁻¹ in each aquarium for a total of 450 per tank; dead larvae from each tank were removed after 24 hours and replaced before the feeding experiment was begun. The larvae were fed Peru strain Artemia salina nauplii in nominal treatment concentrations of 0, 10, 100, and 1,000 nauplii l⁻¹ (treatments B, A, D, and C respectively). Artemia eggs were hatched daily in inverted 2 liter soda bottles. Eggs were hatched at a temperature of 23°C, a salinity of 22‰, and under moderately heavy aeration. The nauplii were harvested each morning, washed with distilled water, and placed in 1% sea water. Ambient Artemia nauplii concentration in each tank was monitored at least 4 times a day. The water in each aquarium was stirred...
by scooping 200 ml of aquarium water in each of two beakers and pouring the water back against opposite sides of the aquarium (Tsai 1991). Fifty ml aliquots of water were sampled from each aquarium and the nauplii density enumerated using a petri dish and a dissecting microscope. Treatment nauplii concentrations were then adjusted from a stock of known concentration.

The water quality of each aquarium was monitored every other day with a Hydrolab® Scout II, and the physical parameters of the water were maintained within the optimal range for striped bass larvae (Table 1). Ammonia concentration and water hardness ([Ca++]i) were determined using test kits. The salinity of the rearing system was maintained at 1 psu and 33% of the system's water was changed every other day. All salt water was artificially made using Instant Ocean® in distilled water. Each aquarium was moderately aerated with an air stone to promote mixing of Artemia nauplii and maintain high oxygen levels.

The feeding experiment ran until larvae were 21 dph. On day 7 of the experiment, three subsamples of three 14 dph larvae were randomly taken from each tank, placed in cryovials, frozen in liquid nitrogen, and stored at -80°C for lipid analysis. This subsampling was repeated on the last day of the experiment (larvae were 21 dph). At the end of the experiment, all larvae were harvested and counted from each tank. Ten fish from each tank were measured to SL and instantaneous daily mortality (Z) was calculated for larvae in each aquarium following Ricker (1975):
Table 1. Ranges of Water Quality Parameters for Feeding Study. Ranges are for Parameters Measured in All Tanks Combined Throughout the Feeding Experiment.

<table>
<thead>
<tr>
<th>Water Quality Parameter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>8.50 ± 0.30</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>19.00 ± 0.70</td>
</tr>
<tr>
<td>pH</td>
<td>7.85 ± 0.20</td>
</tr>
<tr>
<td>Salinity (psu)</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Ammonia concentration (ppm)</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Hardness (Ca++ ppm)</td>
<td>200 ± 5</td>
</tr>
</tbody>
</table>
\[ Z_1 = \frac{\ln(N_0/N_t)}{t} \]  
(Equation 1)

\[ Z_1 = \text{instantaneous daily mortality} \]
\[ N_t = \text{number of larvae alive at time } t \text{ (21 dph)} \]
\[ N_0 = \text{number of larvae alive at time } 0 \text{ (450 larvae)} \]
\[ t = \text{time in days (14)} \]

Because it is unlikely all sampled 14 dph larvae would have died by the end of the experiment, \( Z_1 \) was corrected by substituting into equation 2 the number of larvae sampled (\( N_0 = 9 \)) and \( Z_1 \) to solve for a conservative estimate of the number of sampled larvae (\( N'_t \)) expected to be alive at the end of the experiment. Following Ricker (1975):
\[ N'_t = N_0 e^{Z_1 t} \]  
(Equation 2)
\[ t = 7 \text{ days} \]

A corrected \( Z (Z_2) \) for each aquarium then was calculated by summing \( N_t \) and \( N'_t \) and substituting this value into equation one.
\[ Z_2 = \frac{\ln(N_0/(N_t + N'_t))}{t} \]  
(Equation 3)
\[ N_0 = 450 \text{ larvae} \]
\[ t = 14 \text{ days} \]

Larval samples were weighed to the nearest µg as part of the lipid extraction procedure. The mean weights of 6 and 21 dph larvae were used to calculate weight-specific growth (G) for the larvae in each aquarium. G was calculated following Ricker (1975):
\[ G = \frac{\ln(W_t/W_0)}{t} \]  
(Equation 4)
\[ G = \text{instantaneous daily growth} \]
\[ W_t = \text{weight at time } t \text{ (21 days post-hatch)} \]
\[ W_0 = \text{weight at time } 0 \text{ (6 days post-hatch)} \]
Lipid Analysis

For lipid analysis, samples of larvae were fast-frozen in liquid nitrogen and stored at 80°C for later analysis. The frozen larvae were transferred to Dauphin Island Sea Lab, Dauphin Island, Alabama, in a thick-walled styrofoam cooler packed with dry ice, and were stored at -80°C until samples were processed. Sample processing protocol is described below.

First, small pieces of aluminum foil were weighed to the nearest µg. Individual samples of larvae were placed on pieces of foil and freeze dried for 8 to 12 hours. After the samples were dried, the foil plus dried larvae were re-weighed (µg) and larval weight estimated by difference. The lipids from each sample then were extracted.

To begin extraction, larval samples first placed in a small glass mortar and pestle which had been rinsed twice with dichloromethane to remove any organic residue. Larval samples were ground in a small aliquot of dichloromethane and transferred to 40 ml test tubes containing 10 ml of dichloromethane mixed with 10 ml of methanol. Ten ml of distilled water then were added to samples to separate extracted lipids from larval carcasses. Extracted lipids were drawn from samples with a glass pasteur pipette and placed in 20 ml test tubes which had been rinsed twice with dichloromethane. Polar solvents were evaporated from test tubes under a stream of N₂ for approximately 30 minutes. Once dry, test tube sides were rinsed with dichloromethane and samples were re-
evaporated. After the solvents totally evaporated the second time, more dichloromethane was added and the solutions were transferred to small glass vials and evaporated under N₂ once again. After this final evaporation, 50 µl of dichloromethane were added to the samples, and they were transferred to small glass tubes which were placed inside the small glass vials.

Lipids were analyzed using an Iatroscan® TH-10 thin layer chromatography/flame ionization detection system, which has a sensitivity of 50 ng per lipid class at a signal to noise ratio >5. Between 2 and 5 µl of each sample were spotted on three SII chromarods, which had been blanked by the Iatroscan and humidified over nitric acid. Each chromarod rack contained 10 rods, so three samples were spotted per rack, along with a known standard (Matreya® mix). After spotting, the rack was placed back in a humidity chamber for ten minutes and then developed. Chromarods were developed in a 90:10:0.1 mixture of hexane:diethyl ether:formic acid for between 56 and 58 minutes. Rods then were scanned by the Iatroscan and lipid peaks recorded using Iatroscan software on a Dell 486 personal computer. Development separated triacylglycerol, free fatty acid, sterol ester, sterol, and phospholipid lipid classes. Standard curves and calibration relationships between peak area and lipid concentrations were used to calculate µg of constituent lipids present in samples.

After the lipids from laboratory-reared fish was separated and quantified, the mean ratio of TG to ST ± SE was calculated. Striped bass larvae switch to exogenous energy sources at ~7 dph, thus the ratio of TG to ST calculated for samples of 6 dph larvae yields a measure of nutritional condition at the time when most of their endogenous energy
reserves have been absorbed. Also, to determine if ST was conservative during starvation, a regression of sterol on dry weight for all 6, 14, and 21 dph larvae was run (SAS Inc. 1986).

Results of the feeding experiment were designed to provide a means of distinguishing between healthy and starved larvae caught in the field based on the ratio of TG to ST. Analyses of variance (ANOVAs) were run on results from week 1 of the study, with TG:ST, DWt, and G of larvae as the dependent variables and Artemia nauplii concentration as the independent variable, to determine if there were significant differences among feeding treatments (SAS Inc. 1986). Tukey's studentized range test was employed to group means of treatment groups (SAS Inc. 1988). Also, non-linear regressions were run with TG:ST as the dependent variable and DWt and G as the independent variables.

ANOVAs and Tukey's studentized range test were run on results from week 2 of the experiment, with the addition of Z and ΔSL (mm·d⁻¹) as dependent variables (SAS Inc. 1986). Non-linear regressions were run on results from week 2, with the addition of Z, ΔSL, and G/Z as independent variables. Also, a linear regression was run with G as the independent variable and Z as the dependent variable (SAS Inc. 1988)

II. Field Collections

Zooplankton Collection and Enumeration
Zooplankton was collected in the first 15 km of the tidally-fresh portion of the Pamunkey River in spring 1994. The sampling dates were April 20, April 27, May 6, May 13, and May 20, 1994. Zooplankton was collected at four stations, one each at river km 31, 36, 41, and 45, and labeled PR1, PR2, PR3, and PR4 respectively (Figure 1). Also, salinity, temperature, pH, conductivity, and dissolved oxygen were measured with a Hydrolab® Scout II on each cruise at stations PR1 and PR4.

Zooplankton collections were made while anchored on station. A 40 l·min⁻¹ diaphragm pump was used to pump 100 l of river water through a 73 µm Nitex® net to sample zooplankton. Plankton was pumped at 2m from the bottom, 1m below the surface, and half-way between the two. Four replicate samples were taken at each station and preserved in 5% formalin in Pamunkey River water. Also on each cruise, two replicate zooplankton samples were collected at PR2, placed in cryovials, and frozen for later lipid analysis.

Zooplankton samples were enumerated by first splitting samples to approximately 50 plankters using a Folsom plankton splitter. Individual splits then were enumerated using a petri dish and dissecting microscope. The highest number split was read first, and then each subsequent split, until a dominant species was present at 20 plankters/split. All other species were recorded and counted. Lower splits were read until a subdominant species was found, also at 20 plankters/split (Alden et al. 1982). Differences in the zooplankton concentrations between sampling stations and sampling dates were tested using multivariate analysis of variance (MANOVA) (SAS Inc. 1988).
Striped Bass Larvae Collection

Samples of striped bass larvae were collected on the same cruises as were zooplankton samples, and along the same reach of the Pamunkey River. On each cruise, after zooplankton samples were collected at station PR4, striped bass larvae were collected on the return trip down river. Larvae were collected using a paired bongo net with 202 µm Nitex® mesh towed at 2 knots for 5-10 minutes. Nets were retrieved and washed down, and larvae removed from the cod-ends. Larvae were collected for analysis by placing small amounts of water and detritus from the cod-ends in a plastic bowl, diluting the mixture with water, and picking out individual larvae with a pasteur pipette. Larvae were placed in cryovials and frozen in liquid nitrogen for later lipid analysis at the Dauphin Island Sea Lab. To prevent lipid metabolism in sampled larvae, all unpicked samples were discarded 30 min after collection in the net-tows.

In the laboratory, individual cryovials containing 40-50 larvae each were thawed and their contents placed in a glass petri dish which was resting on crushed ice. Individual larvae were identified to species using a dissecting microscope. All white perch larvae, *Morone americana*, were counted and discarded, while individual striped bass larvae were measured (SL to the 0.1 mm) and placed on pre-weighed pieces of aluminum foil. The foil pieces were frozen until all cryovials for the respective sampling date had been processed. The larvae then were freeze dried, weighed, and lipids extracted as before.

Mean TG to ST ratios ± SE along with mean length ± SE of collected larvae were calculated for each sampling date. Differences in TG:ST and SL between sampling dates
were tested with ANOVA (SAS Inc. 1986). The mean TG:ST for each sampling date was correlated with zooplankton concentrations, *Eurytemora affinis* condition, and water quality parameters (e.g., temperature, pH, and dissolved oxygen) (SAS Inc. 1988).
RESULTS

I. Rearing Experiment

To determine whether ST was conservative in striped bass larvae, a regression of DWt on ST from all the 6, 14, and 21 day post-hatch larvae was run. The regression was significant (p < 0.01) with an $r^2 = 0.92$ and equation $y = 49.9x - 261.2$ (Figure 3).

Thirty samples of 6 dph larvae (n = 3) had a mean SL of $5.9 \pm 0.12$ mm and a mean DWt of $136 \pm 2.58 \mu$g. Two of these samples later were discarded due to contamination. The 28 samples from which lipids were extracted and analyzed had a mean TG:ST of $1.01 \pm 0.11$ (Figure 4). The plotted frequency histogram of TG:ST ratios of 6 dph larvae clearly shows a bimodal distribution (Figure 4), which may have been caused by intrassexual differences in egg quality (Zastrow et al. 1992; Monteleone and Houde 1992; Secor 1992).

Mean TG:ST of 14 dph larvae for treatments A, B, C, and D was 0.74, 0.74, 1.22, and 0.78 respectively (Table 2), and there was a significant difference between treatments (ANOVA, p < 0.01). Tukey's studentized range test (p < 0.05) for TG:ST grouped the treatments into 2 groups; A, B, and D were similar and different than C. Mean TG:ST of 21 dph larvae for treatments A, B, C, and D was 0.75, 0.75, 1.26, and 0.78 respectively (Table 2); these means differed significantly between treatments (ANOVA, p < 0.01). As in week one, Tukey's studentized range test (p < 0.05) grouped treatments A, B, and D together, while C was dissimilar to the other three.
Figure 3. Regression of Dry Weight on Sterol content for all 6, 14, and 21 day post-hatch larvae sampled in the lab. The regression equation is \( y = 49.86x - 261.6 \) \((n = 98; p = 0.01)\), with \( r^2 = 0.91 \).
Figure 4. Frequency histogram of the TG:ST ratios from 28 samples of 6 dph striped bass larvae pooled in groups of 3. Mean TG:ST ratio for the 28 samples $1.01 \pm 0.11$. 
Table 2. Lab Results From Weeks One and Two of the Feeding Experiment. Standard Errors are Given Along With Means of Each Variable for Each Treatment in Weeks One and Two of the Feeding Experiment.

### Lab Results Week 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TG:ST</th>
<th>SE</th>
<th>Dwt (µg)</th>
<th>SE</th>
<th>G</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.74</td>
<td>0.16</td>
<td>122.7</td>
<td>9.16</td>
<td>-0.015</td>
<td>0.006</td>
</tr>
<tr>
<td>B</td>
<td>0.74</td>
<td>0.19</td>
<td>114.3</td>
<td>7.40</td>
<td>-0.025</td>
<td>0.005</td>
</tr>
<tr>
<td>C</td>
<td>1.20</td>
<td>0.11</td>
<td>352.9</td>
<td>22.92</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>D</td>
<td>0.78</td>
<td>0.14</td>
<td>261.8</td>
<td>27.03</td>
<td>0.093</td>
<td>0.02</td>
</tr>
</tbody>
</table>

### Lab Results Week 2

<table>
<thead>
<tr>
<th>Trt</th>
<th>TG:ST</th>
<th>SE</th>
<th>Mean ΔSL (mm/d)</th>
<th>SE</th>
<th>Dwt(µg)</th>
<th>SE</th>
<th>G</th>
<th>SE</th>
<th>Z</th>
<th>SE</th>
<th>G/Z</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.75</td>
<td>0.056</td>
<td>0.094 0.007</td>
<td>450.5</td>
<td>42.95</td>
<td>0.085</td>
<td>0.007</td>
<td>0.130</td>
<td>0.022</td>
<td>0.664</td>
<td>0.073</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.75</td>
<td>0.042</td>
<td>-0.006 0.004</td>
<td>92.3</td>
<td>10.79</td>
<td>-0.028</td>
<td>0.008</td>
<td>0.162</td>
<td>0.006</td>
<td>-0.169</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.26</td>
<td>0.046</td>
<td>0.317 0.010</td>
<td>1545.8</td>
<td>183.7</td>
<td>0.173</td>
<td>0.009</td>
<td>0.053</td>
<td>0.004</td>
<td>3.09</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.78</td>
<td>0.039</td>
<td>0.185 0.007</td>
<td>670.8</td>
<td>27.87</td>
<td>0.117</td>
<td>0.003</td>
<td>0.077</td>
<td>0.003</td>
<td>1.41</td>
<td>0.055</td>
<td></td>
</tr>
</tbody>
</table>
Mean DWt of the 14 dph larvae for treatments A, B, C, and D was 123, 114, 353, and 262 µg respectively (Table 2). There was a significant difference between treatments (ANOVA, p < 0.01). Tukey's studentized range test (p < 0.05) for DWt formed three groups; treatments A and B grouped together, and C and D were dissimilar to A and B and to each other. Mean DWt of 21 dph larvae for treatments A, B, C, and D were 450.5, 92.3, 1545.8, and 670.8 µg respectively (Table 2). There was a significant difference in DWt between treatments (p < 0.01). Tukey's studentized range test (p < 0.05) grouped the treatments into three groups, with A and D grouping together, and B and C dissimilar to B and D and to each other.

Mean G of 14 dph larvae for treatments A, B, C, and D was -0.015, -0.025, 0.14, and 0.093 respectively (Table 2), and there was a significant difference among treatments (ANOVA, p < 0.01). Again, Tukey's studentized range test (p < 0.05) grouped treatments A and B together, while C and D remained ungrouped. Mean G of 21 dph larvae for treatments A, B, C, and D was 0.085, -0.028, 0.173, and 0.117 respectively (Table 2). There was a significant difference between treatments (ANOVA, p < 0.01); all treatments sorted into independent groupings with Tukey's studentized range test (p < 0.05).

Mean ΔSL (mm·d⁻¹) of 21 day post-hatch larvae for treatments A, B, C, and D were 0.094, -0.006, 0.317, and 0.185 respectively (Table 2). There was a significant difference in ΔSL between treatments (p < 0.01). All treatments sorted into independent groups with Tukey's studentized range test (p < 0.05).

Mean Z of 21 day post-hatch larvae for treatments A, B, C, and D were 0.130, 0.162, 0.053, and 0.077 respectively (Table 2). There was a significant difference in Z between
treatments (ANOVA, p < 0.01). Tukey's studentized range test (p < 0.05) grouped the
treatments into three groups, with C and D together, and A and B dissimilar to C and D
and each other.

Nonlinear regressions run between TG:ST and DWt and G of 14 dph larvae were
significant (p < 0.01), with $r^2 = 0.95$ and 0.99, and equations of $y = 1976645.5 +$
$1977013.1(1-e^{-12.21x})$ and $y = 0.1473 + -3004122.7(e^{-0.04426})$ (Figure 5). Nonlinear
regressions run between mean TG:ST and DWt, ΔSL, G, Z, and G/Z of 21 dph larvae
were significant (p < 0.01) (Figures 6 and 7). Nonlinear regressions of DWt and ΔSL on
TG:ST of 21 dph larvae had $r^2 = 0.94$ and 0.87, and equations of $y = -1169183.6 +$
$1170756.1(1-e^{-0.98x})$ and $y = -2749.1 + 2749.5(1-e^{-12.31x})$. Nonlinear regressions of G, Z,
and G/Z on TG:ST of 21 dph larvae had $r^2 = 0.64$, 0.92, and 0.91, and equations of $y = -$
$2962.32 + 2962.51(1-e^{-13.29x}), y = 0.055 + (1.86 \cdot 10^5)(x^{-29.57}),$ and
$y = -8719.5 + 8722.6(1-e^{-10.72x})$ respectively (Figures 6 and 7). The linear regression of G
on Z for the 21 dph was significant (p < 0.01) with $r^2 = 0.91$ and equation $y = -1.67 x +$
0.268 (Figure 8).

II. Field Results

Water Quality Parameters

Temperature, pH, and dissolved oxygen were measured on each cruise at stations
Figure 5. Nonlinear regressions of DWt and G on TG:ST ratios from 14 dph larvae. The regression equations (p < 0.01) are $y = 1976645.5 + 1977013.1(1-e^{-0.03x})$, $r^2 = 0.95$, and $y = 0.1473 + -3004122.7(e^{0.000326x})$, $r^2 = 0.99$, respectively.
Figure 6. Nonlinear regressions of DWt and ΔSL on TG:ST ratios of 21 dph larvae. The regression equations (p < 0.01) are

\[ y = -2749.1 + 2749.5(1-e^{\cdot.098}), \quad r^2 = 0.87, \text{ and} \quad x \]

\[ y = -1169183.6 + 1170756.1(1-e^{\cdot9.098}), \quad r^2 = 0.94, \text{ respectively.} \]
TG:ST

Delta SL mm

DWT µg
Figure 7. Nonlinear regressions of $G$ and $Z$ on TG:ST ratios of 21 dph larvae. The regression equations ($p < 0.01$) are
\[
y = -2962.3 + 2962.5 \left(1-e^{-\frac{x}{y}}\right), \quad r^2 = 0.64, \quad \text{and} \\
y = 0.055 + \left(1.86\times10^5\right)x^{29.57}, \quad r^2 = 0.92, \quad \text{respectively.}
\]
Figure 8. Linear regression of G on Z and nonlinear regression of G/Z on TG:ST ratios of 21 dph larvae. The regression equations (p < 0.01) are $y = -1.67x + 0.268$, $r^2 = 0.91$, and $y = -8719.5 + 8722.6(1-e^{-x})$, $r^2 = 0.92$, respectively.
PR1 and PR4. Readings from these two stations were averaged and means reported for each cruise (Table 3). Mean temperature increased in week 2, but then decreased through week 5. Both pH and dissolved oxygen did not exhibit any clear temporal patterns (Table 3).

Zooplankton

The major zooplankton taxa available as prey for larval striped bass in the Pamunkey River in the spring of 1994, were *Eurytemora affinis* copepods and copepodities, copepod nauplii, *Bosmina longirostris*, and rotifers. Statistical analyses of plankton concentrations were performed only on these taxa because they always were present and usually in high numbers (except for rotifers). Other taxa sometimes present in samples included cyclioid, harpacticoid and calanoid copepods, cladocerans other than *Bosmina*, barnacle nauplii, and trochophore larvae. The total copepods and copepodites and total cladocerans also were estimated for each week.

Mean concentrations $1^{-1}$ of zooplankton for cruises 1, 2, 3, 4, and 5 respectively were 20.8, 18.9, 24.9, 18.7, and 9.0 *Eurytemora affinis* copepods and copepodities; 13.9, 43.0, 50.9, 23.8, and 21.4 copepod nauplii; 8.9, 7.1, 7.2, 2.8, and 2.2 *Bosmina longirostris*; and 1.3, 1.5, 1.5, 0.8, and 1.7 rotifers (Table 3, Figure 9). Mean concentrations $1^{-1}$ of total copepods and copepodites and for total cladocerans for cruises 1 through 5 were 23.3, 31.5, 31.0, 27.5, and 19.0; and 10.2, 7.5, 11.8, 4.6, and 3.4 respectively (Figure 10).

The zooplankton concentration data for *Eurytemora affinis* copepods and
Table 3. Biotic and Abiotic Parameters Measured in the Pamunkey River on Cruises 1 through 5. Standard Errors are Given Along With Mean Values for Each Biotic and Abiotic Parameter Measured in the Field.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4-20-94</th>
<th>4-27-94</th>
<th>5-6-94</th>
<th>5-13-94</th>
<th>5-20-94</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eurytemora affinis</em> adults and copepodites l(^{-1})</td>
<td>20.8 (3.16)</td>
<td>18.9 (4.9)</td>
<td>24.9 (14.82)</td>
<td>18.7 (6.89)</td>
<td>9.0 (3.45)</td>
</tr>
<tr>
<td>Copepod nauplii l(^{-1})</td>
<td>13.9 (7.34)</td>
<td>43.0 (13.00)</td>
<td>50.9 (13.58)</td>
<td>23.8 (5.72)</td>
<td>21.4 (6.44)</td>
</tr>
<tr>
<td><em>Bosmina longirostris</em> l(^{-1})</td>
<td>8.9 (4.93)</td>
<td>7.1 (3.72)</td>
<td>7.2 (2.96)</td>
<td>2.8 (0.59)</td>
<td>2.2 (0.51)</td>
</tr>
<tr>
<td>Rotifers l(^{-1})</td>
<td>1.3 (0.40)</td>
<td>1.5 (0.79)</td>
<td>1.5 (0.58)</td>
<td>0.8 (0.28)</td>
<td>1.7 (0.42)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>18.73 (0.11)</td>
<td>19.71 (0.13)</td>
<td>18.80 (0.12)</td>
<td>18.49 (0.017)</td>
<td>17.77 (0.13)</td>
</tr>
<tr>
<td>pH</td>
<td>7.07 (0.049)</td>
<td>7.13 (0.17)</td>
<td>6.83 (0.056)</td>
<td>7.02 (0.023)</td>
<td>7.24 (0.079)</td>
</tr>
<tr>
<td>Dissolved oxygen (mg l(^{-1}))</td>
<td>8.05 (0.13)</td>
<td>6.62 (0.21)</td>
<td>7.04 (0.17)</td>
<td>7.44 (0.089)</td>
<td>7.48 (0.079)</td>
</tr>
<tr>
<td>TG:ST <em>Eurytemora affinis</em></td>
<td>4.53 (0.95)</td>
<td>1.79 (0.12)</td>
<td>2.24 (0.23)</td>
<td>5.66 (1.57)</td>
<td>4.93 (0.37)</td>
</tr>
<tr>
<td>TG:ST <em>Morone saxatilis</em> larvae</td>
<td>2.09 (0.36)</td>
<td>3.71 (0.44)</td>
<td>1.56 (0.25)</td>
<td>0.38 (0.11)</td>
<td>0.34 (0.08)</td>
</tr>
<tr>
<td>Percent <em>Morone saxatilis</em> in poor condition</td>
<td>9.09</td>
<td>18.18</td>
<td>38.89</td>
<td>80.77</td>
<td>88.00</td>
</tr>
</tbody>
</table>
Figure 9. Estimates of the abundances of the dominant taxa of zooplankton prey available to striped bass larvae in the Pamunkey River in spring 1994. Abundances are given for *Eurytemora affinis* copepods and copepodites, copepod nauplii, *Bosmina longirostris* and rotifers.
Figure 10. Estimates of the abundances of the total copepod adults and copepodites and total cladocerans available as prey for striped bass larvae in the Pamunkey River in spring 1994.
Copepod adults and copepodites
Cladocera

Abundance per liter

Date

April 20
April 27
May 6
May 13
May 20

0
5
10
15
20
25
30
35
copepodites, copepod nauplii, *Bosmina longirostris*, and rotifers was sorted by cruise and station and a MANOVA was run on each. There was a significant difference in zooplankton concentrations within station across cruises (MANOVA, p < 0.01). PR2 had the most consistent zooplankton concentrations across the five cruises, followed by PR4 and PR1, with PR3 the most inconsistent. There was also a significant difference in zooplankton concentration within cruise between stations (MANOVA, p < 0.01). The general trend across stations was a decrease in zooplankton concentration from PR1 to PR4. This was particularly true for cruises 2, 3, and 4, but less so for 1 and 5.

Lipid analysis performed on *Eurytemora affinis* showed no temporal trend over the five cruises. The mean TG:ST for the adult *Eurytemora affinis* was 4.53, 1.79, 2.24, 5.66, and 4.93 for cruises 1 through 5 respectively (Table 3).

**Larval Striped Bass Condition**

There was a higher catch per unit effort (CPUE) of *Morone sp.* larvae on the first two cruises than on the last two. This indicated striped bass larvae were more abundant during the weeks of April 20th and April 27th, than in subsequent weeks. This is consistent with historical data (Grant and Olney, 1991). Before lipid analyses could be performed on field caught striped bass larvae, they were separated from the congener *Morone americana* larvae. *M. americana* outnumbered striped bass larvae 7.8, 7.9, 9.4, 6.6, and 10.3 to 1 on cruises 1 through 5 respectively.

The field caught striped bass larvae had mean DWts of 197, 282, 365, 1026, and 548
µg for cruises 1 through 5 respectively. The larvae had mean TG:ST of 2.09, 3.71, 1.56, 0.38, and 0.34 for cruises 1 through 5, which were significantly different (ANOVA, p < 0.01) (Table 3; Figure 11). The larvae had mean SLs of 7.5, 7.2, 7.9, 10.9, and 9.6 mm for cruises 1 through 5 (Figure 12), which also were significantly different (ANOVA, p < 0.01).

Correlation analyses were run between mean TG:ST of striped bass larvae collected on the 5 cruises, and the water quality parameters of temperature, dissolved oxygen, and pH. Correlations were also run between larval striped bass TG:ST and abundances of total copepods and copepodites, total cladocerans, combined total copepods and copepodites and total cladocerans, *Eurytemora affinis* copepods and copepodites, copepod nauplii, *Bosmina longirostris*, rotifers; and mean TG:ST of *Eurytemora affinis* copepods. Only the correlation between larval striped bass condition and temperature was significant (p = 0.03, r = 0.92) (Table 4). The correlation between mean TG:ST of the striped bass larvae and abundance of *Bosmina longirostris* was nearly significant (p = 0.14, r = 0.75) (Table 4).
Figure 11. Boxplots of mean TG:ST ratio of sampled striped bass larvae in the Pamunkey River in spring 1994, with weekly sample size given within each box. Upper and lower sides of the boxes represent the 75th and 25th percentiles of the mean, while upper and lower extended bars represent the 90th and 10th percentiles.
Figure 12. SL of field sampled striped bass larvae plotted against their TG:ST ratios for each of 5 cruises. Vertical lines indicate a TG:ST ratio of 0.75.
Table 4. Results from Correlations Between mean TG:ST of field-caught larvae and Temperature and *Bosmina* Abundance

<table>
<thead>
<tr>
<th></th>
<th>Mean TG:ST</th>
<th>Temperature °C</th>
<th><em>Bosmina</em> abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean TG:ST</strong></td>
<td>1.00</td>
<td>0.92</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>Temperature °C</strong></td>
<td>0.00</td>
<td>0.03</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Bosmina abundance</strong></td>
<td>0.92</td>
<td>1.00</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.00</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>0.55</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.33</td>
<td>0.00</td>
</tr>
</tbody>
</table>
DISCUSSION

I. Feeding Experiment

It has been shown in several copepod and larval fish species that triacylglycerol content is highly labile and a good indicator of physiologic state (Ehrlich 1974a,b, 1975; Hakanson 1984, 1989a, b, Fraser et al. 1987, 1988; Ohman 1988; Fraser 1989). Absolute TG content, however, is not an indicator of nutritional condition. TG content must be expressed in a ratio that "accounts for the size-dependency of TG content" (Fraser 1989). Sterol content has been shown in several species to be conservative and highly correlated with dry weight (Hakanson 1984, 1989a, b; Fraser et al. 1987, 1988; Fraser 1989). In order for the TG:ST ratio to be used as a measure of condition, it first must be shown that ST is conservative (Fraser 1989). The regression of DWt on ST for all 6, 14, and 21 dph striped bass larvae was significant (p = 0.01) and ST content was shown to be highly correlated with the DWt of the larvae (r^2 = 0.92) (Figure 3). Therefore, the ratio of TG:ST is a valid indicator of nutritional condition in striped bass larvae.

6 Day Post-hatch Larvae

The striped bass larvae cultured in the feeding experiment had a mean TG:ST of 1.01 at 6 dph, and their TG:ST ratios showed a high degree of variation (Figure 4). The
bimodal distribution of the TG:ST ratios, and the spread within each mode, of the 6 dph larvae in this study probably was caused by both inter- and intrafemale differences in egg quality. There is evidence that maternal contribution to size of eggs and relative lipid stores can vary significantly within and between females (Rogers and Westin 1981; Zastrow et al. 1989; Monteleone and Houde 1990; Secor 1992). Zastrow et al. (1989) reported a high degree of variability in total lipid per egg within brood from twenty-one female striped bass in the upper Chesapeake Bay. Eldridge et al. (1981) also found endogenous energy reserves of eggs and larvae of seven west coast striped bass females to vary greatly between females.

Growth and Mortality in the Feeding Experiment

In week 1 of the feeding experiment DWt and G were significantly different between feeding treatments. Larvae fed at a density of 1000 Artemia naplii 1·1 (treatment C) showed the greatest growth, while starved larvae (treatment B) and those fed at 10 Artemia naplii 1·1 (treatment A) lost weight. Weight loss in starved larvae was not unexpected; however, previous studies have not reported weight loss by minimally fed larvae (Eldridge et al. 1982; Houde and Lubbers 1986; Chesney 1989; Tsai 1991; Meng 1993). The weight loss in week 1 of larvae in treatment A was slight, only 0.015 d·1, and by week 2 the larvae in this treatment were growing by 0.085 d·1 (Table 2).

The calculated weight loss of treatment A larvae in week 1 may have been the result of variability in initial size of larvae, but larvae were pooled in groups of three, so any
variability in initial weight should have been masked. Another possible explanation for the weight loss may have been currents in the aquaria caused by the flow through system. Some larvae may not have been able to meet the increased metabolic demands of swimming in the current established by the flow through system.

In week 2 of the feeding experiment growth (mm·d⁻¹), DWt, G, and Z all were significantly different among treatments. Treatment A larvae had the greatest growth in length of 0.317 mm·d⁻¹ while starved larvae had negative growth of 0.006 mm·d⁻¹. Treatment A larvae also had the greatest G in week 2 with a G of 0.173 d⁻¹, while starved larvae had a G of -0.028 d⁻¹. As expected, treatment A also had the lowest Z on 21 dph (Z of 0.053 d⁻¹). Starved larvae had the highest Z at 0.162 d⁻¹ (Table 2).

Lengths and weights of the 21 dph larvae were similar to values from other experiments found in the literature in which striped bass larvae were fed Artemia nauplii (Eldridge et al. 1981; Houde and Lubbers 1986; Chesney 1989; Lemm and Lemarie 1991; Tsai 1991). Instantaneous daily mortality (Z) values, however, were much higher in this study, averaging 0.162, 0.130, 0.077, and 0.053 d⁻¹ for Artemia concentrations of 0, 10, 100, 1000 l⁻¹ (Table 2). Eldridge et al. (1981), using 81 containers with a stocking density of 3 larvae l⁻¹, reared larvae to 31 dph and estimated Z values of 0.12, 0.051, 0.027, and 0.008 d⁻¹ for treatments of 0, 10, 100, and 1000 Artemia nauplii l⁻¹. Houde and Lubbers (1986) cultured striped bass larvae in 36 l aquaria at larval densities of 4 l⁻¹ until 30 dph, and had estimated Z values of 0.0069 d⁻¹ for nauplii densities of 100 l⁻¹ and 0.0066 d⁻¹ for nauplii densities of 500 l⁻¹. Tsai (1991), using 15 l test tanks stocked at a density of 4 larvae l⁻¹ and growing larvae to 19 dph, reported daily mortality rates of 0.08, 0.11, 0.04,
and 0.01 d\(^{-1}\) for Artemia nauplii densities of 0, 10, 100, and 500 l\(^{-1}\).

There are several possible reasons for the high Z values in this study; Artemia prey may have had a lower nutritive value than those used in other studies, stocking densities of striped bass larvae may have been too high in this study (15 l\(^{-1}\)), or the flow through system may caused higher larval mortality. It is unlikely that prey quality or larval stocking density are responsible for higher mortality rates because larvae grew at similar rates and reached similar weights as those of other studies (Eldridge et al. 1981; Houde and Lubbers 1986; Chesney 1989; Lemm and Lemarie 1991; Tsai 1991), but these studies used static systems to culture larvae. While the recirculating system in this study maintained environmental parameters within narrow ranges (Table 1), currents that were established around the standpipes as water exited each aquaria tended to trap smaller larvae on the Nitex\textsuperscript{®} mesh surrounding the outer standpipes. This became less of a problem in week 2 of the experiment as smaller larvae in treatments A and B were less dense due to mortality, and larvae in treatments C and D were too large to be effected by the standpipes.

TG:ST Ratios in Striped Bass Larvae

The feeding experiment was designed to yield larvae in both healthy and poor condition, but Ehrlich (1975) found laboratory-reared feeding larvae of plaice (Plueronectes platessa) and herring (Clupea harengus) had much higher TG contents than similar sized wild-caught larvae. Fraser et al. (1987) also showed laboratory-reared larval
herring had lower rates of TG catabolism than larvae in 300 m³ enclosures. Fraser (1989) cautioned about the applicability of using nutritional condition indices derived from laboratory-reared larvae to wild-caught larvae. He suggested that it may be possible to determine "threshold" TG:ST ratios from young wild-caught larvae or from first feeding larvae when there exogenous food sources begin to effect TG reserves.

TG:ST ratios of the 6 dph larvae in this study yield a "threshold" TG:ST ratio (1.01) by which to evaluate healthy larvae and those in poor condition (Figure 4). Results from weeks 1 and week 2 of the feeding experiment also offer a threshold value of TG:ST. Larval treatments fed at Artemia nauplii densities of 0 and 101·· (B and A) both had mean TG:ST ratios of 0.74 and 0.75, respectively, on 14 and 21 dph (Table 2). Larvae in these treatments experienced weight loss in week 1, but larvae in treatment A had a mean G of 0.085 d⁻¹ by 21 dph. Treatment D, fed 100 nauplii 1⁻¹, had TG:ST ratios of only 0.78 for both weeks of the experiment, but had significantly greater growth in length and weight than did treatment A.

Tukey’s studentized range test grouped mean TG:ST values of treatments A, B and D together for both weeks 1 and 2 of the feeding experiment (p<0.05). A priori it was expected that TG:ST ratio from all treatment groups would separate into distinct groups. There are two probable reasons why this did not happen. The starved larvae had a high mean TG:ST ratio compared to fed treatments A and D, and the starved larva's TG:ST ratio did not decrease from week 1 to week 2. Doroshev (1970) and Eldridge et al. (1981) showed larval striped bass were initially conserved uptake of their oil globule, which contains endogenous lipid stores. Eldridge et al. (1981) cultured striped bass larvae
to 31 dph with no well-defined point-of-no-return for larvae. They hypothesized that when food is not available to striped bass larvae, larvae may conserve their oil globule as an energy store: this hypothesis was echoed by Tsai (1991). Eldridge et al. (1981) also found the rate of absorption of the oil globule in larval striped bass in culture tanks was directly related to amount of food offered.

It also is possible that mean TG:ST ratios of treatments A, B and D grouped together because TG:ST ratios of treatments A and D failed to increase and therefore separate themselves from B, and each other. Dergaleva and Shatunovskiy (1978) noted at the switch to exogenous feeding, striped bass larvae begin to accumulate TG, but accumulation of TG can only occur once basal metabolic needs of the larvae are met (Fraser 1989). Meng (1993) estimated striped bass larva need 57 *Artemia* nauplii d⁻¹ to support metabolism and growth. Although daily food rations were not estimated in this study, Eldridge et al. (1982) estimated that individual striped bass larvae consumed 0.2, 10.3, and 53.7 *Artemia* nauplii d⁻¹ in treatments of 10, 100, and 1000 nauplii l⁻¹ on 13 dph, and 16.6, 51.6, and 102.6 *Artemia* nauplii d⁻¹ for the same treatments on 23 dph.

Therefore, larvae in treatments A and D may have been able to support basal metabolism and growth, but were not consuming enough nauplii to store excess lipid in the form of TG.

The regressions of G and DWt on TG:ST for the 14 dph larvae and G, Z, ΔSL, and G/Z on TG:ST for the 21dph larvae illustrate the relationships of TG:ST to larval size-at-age and vital rates (Figures 5, 6, 7 & 8). Larvae that have higher TG:ST ratios grow faster and have lower mortality than larvae with low TG:ST. The regression of G/Z on
TG:ST shows that G/Z approaches 1 (point when larval cohorts begin to accumulate biomass (Houde 1989)) as TG:ST approaches 0.75 (Figure 8). This relationship suggests TG:ST ratios are good indicators of health in striped bass larvae and can be predictive of vital rates.

Based on lipid analyses of the 6, 14 and 21 dph larvae in this study, I have two estimates of a "threshold" TG:ST ratio for healthy striped bass larvae. The 6 dph larvae had a mean TG:ST ratio of 1.01, but they had not yet switched to exogenous feeding and their TG:ST ratio could be expected to fall some by the time they had fully switched (Dergaleva and Shatunovskiy 1978; Fraser 1989). The lipid results from week 1 and 2 of the feeding experiment indicate starved and poorly fed larvae had a TG:ST of about 0.75. Striped bass larvae are resistant to starvation (Eldridge et al. 1981; Houde 1987; Tsai 1991; Cowan et al. 1993) and this may have affected the results from the feeding experiment, but a TG:ST of 0.75 appears to be a conservative estimate for larvae in good condition.

II Field Collections

Water Quality Parameters

The water quality parameters measured showed no clear temporal patterns over the course of the field collections. Salinity was never more than 1 psu except at PR1 where it was never greater than 3 psu. Dissolved oxygen and pH showed no pattern, but were
within the optimal range for striped bass larvae (Table 3) (Rogers et al. 1981). Temperature did increase on cruise 2, but then decreased through cruise 5. Temperature was only measured on the dates of the sampling cruises and was never below 17° C (Table 2).

Zooplankton

In the spring mesozooplankton in the tidally-fresh portion of the Pamunkey River are typically sparse (Birdsong 1992). It has been hypothesized that the concentration of mesozooplankton in the Pamunkey in the spring is too low to support good striped bass larval survival and growth (Jacobs 1993). Although the sampling methods in this study differ from those used to sample mesozooplankton by the CBMP, densities reported here are much higher than those reported by the CBMP (Birdsong 1992; Birdsong unpublished data) (Figures 9 & 10). The stations sampled in this study (PR1, PR2, PR3 and PR4) were located half way between the tidally-fresh station in the Pamunkey of the CBMP (TF4.2) and the mouth of the Pamunkey river (Figure 1). This is significant because stations sampled in this study overlap the area of peak striped bass spawning activity reported by Grant and Olney (1991), and the concentration of mesozooplankton generally decreased up river. Although CBMP’s station TF4.2 was not sampled concurrently with the stations in this study, or with the same methodology, it appears that historic zooplankton concentrations at TF4.2 may underestimate zooplankton prey available to striped bass larvae.
In studies of striped bass larvae in the Potomac River, Maryland, in 1981, 1987, 1988 and 1989, combined densities of copepod adults and copepodites, and cladocerans rarely were lower than 100 \( l^{-1} \) (Martin et al. 1985; Rutherford and Houde 1995). In fact, the combined densities never were less than 100 \( l^{-1} \) in 1988 and approached 1000 \( l^{-1} \) during spring 1987 (Rutherford and Houde 1995). In spring 1994 in the Pamunkey River, combined densities of copepod adults and copepodites and cladocerans were greater than 40 \( l^{-1} \) only once (cruise 3), and averaged 33.2 plankters \( l^{-1} \) (Figure 10). Therefore, it appears prey for larval striped bass has a greater potential to be limiting in the Pamunkey River than in the Potomac.

Nutritional Condition in Wild-caught Striped Bass Larvae

The previously described standards for the TG:ST ratio of striped bass larvae in poor condition were applied to striped bass larvae sampled from the Pamunkey River. The mean TG:ST for larvae increased on cruise two, but then decreased through cruise five (Table 3). The percent of sampled striped bass which sorted into poor condition, however, increased from cruise one through cruise five. Martin et al. (1985) and Setzler-Hamilton et al. (1987) found that nutritional condition of striped bass larvae in the Potomac River (judged by a morphometric index, a histologic index, fatty acid analysis, and RNA:DNA ratios) generally increased over the spawning season. They reported that after May most larvae sorted into healthy categories (Martin et al. 1985; Setzler-Hamilton et al. 1987); \textit{a priori} the same phenomenon was expected to occur in the Pamunkey
Larval condition in the Pamunkey did not correlate to concentration of mesozooplankton prey available to larvae. The correlation between larval condition and *Bosmina* abundance was nearly significant (*p* = 0.14), but the only significant correlation was between mean larval condition and temperature (Table 4). Larval striped bass select for *Bosmina* in the Potomac River (Beaven and Mihursky 1979; Setzler-Hamilton et al. 1981; Martin et al. 1985), and Martin et al. (1985) suggested larger plankters such as cladocerans become increasingly important to larger larvae. But, Martin et al. (1985) found nutritional condition in striped bass larvae had a negative correlation to combined densities of copepods and cladocerans, which they labeled as "counter-intuitive." They also found a negative correlation between striped bass larval condition and temperature in the Potomac River, which again was not expected.

Rutherford and Houde (1995) were unable to correlate growth and survival of cohorts of striped bass larvae in the Potomac to abundance of zooplankton prey, but did see a pattern of high G/Z ratios and high recruitment in years when the planktonic prey of the larvae was highest. They found that high G/Z ratios were correlated to temperature and suggested temperature was the dominant factor driving striped bass recruitment, and it may have overshadowed all other factors (Rutherford and Houde 1995). The results from the feeding experiment in this study show G/Z is highly correlated to nutritional condition in striped bass larvae (Figure 8), and the field data shows TG:ST ratios of the larvae are highly correlated to water temperature (Table 4). Therefore, I deduce that later spawned cohorts of striped bass in the Pamunkey in 1994 had lower G/Z ratios as water
temperature dropped (Table 4, Figures 8, 11 & 12).

It was expected that once larvae reached a certain "critical" size they would be able to forage effectively and would be beyond the initial threat of starvation, but the ratio of TG:ST was not correlated to SL. Cowan et al. (1993) and Rutherford and Houde (1995) suggested year class strength in Potomac River larvae is essentially set by the time larvae reach 8 mm in length, but in this study only ten larvae over 8 mm SL sorted into the healthy category (Figure 12). Regardless of size, striped bass larvae sampled later during the spring 1994, spawn in the Pamunkey River were not as healthy and probably not growing as rapidly as earlier spawned cohorts (Figure 12).

Fraser (1989) warned that when applying TG:ST ratios as condition measures to larval animals, one has to be careful not to confuse catabolism of lipid stores at times of meta-morphosis as an indicator of poor condition. But the striped bass larvae sampled in the Pamunkey all were under 13.5 mm SL, which is well shy of the 20 mm SL when striped bass metamorphose to juveniles. Also, larvae in laboratory treatments C and D were grown threw the finfold stage (8-10 mm) and their TG levels were not effected. It is possible that larvae sampled in the first weeks of the field study had not yet burned up their oil globules, but this does not explain the very high TG:ST of some of the larvae (Figures 11 & 12).

The third and most likely hypothesis for the decrease in mean larval TG:ST ratios over the five cruises is that the later spawned cohorts encountered unfavorable conditions and were not doing as well as the early spawned larvae (Figures 11 & 12). Although planktonic prey did not correlate well with mean larval TG:ST, zooplankton abundance
did decrease with temperature (Table 3, Figures 8 & 9). Temperature was correlated to mean TG:ST in the larvae and decreased over the five cruises, and may have overshadowed some of the effects of decreased zooplankton abundance (Rutherford and Houde 1995).

*Bosmina* concentration in particular decreased over the five cruises, and other cladocerans mostly were absent from the zooplankton samples. Beavan and Mihursky (1979) reported that larger striped bass larvae select for *Bosmina* in the Potomac River, and Martin et al. (1985) hypothesized that larval striped bass depend on larger plankters, such as *Bosmina* for their nutritional well being. Therefore, I hypothesize that the absence of larger plankters, the decrease in *Bosmina* abundance, and the decrease in water temperature were the causes of the decrease in the numbers of healthy versus starved striped bass larvae during the 1994 spawning season in the Pamunkey River.

The use of TG:ST ratios to determine nutritional condition in larval fish is a relatively recent concept, and papers on this subject are relatively few. This study suggests that using TG:ST ratios as an indicator of nutritional condition is also applicable to striped bass larvae, but more work is needed to "fine-tune" the index. For example, larvae from many striped bass females of many different sizes need to be sampled to obtain a better estimate of "baseline" TG:ST by which to judge the health of field-caught larvae. Pulse feeding studies should also be employed to better understand what effect offering food to first feeding larvae and then starving them has on their TG:ST ratios. Also, the ability of TG:ST ratios in determining the nutritional condition of older larvae needs to be tested further.
LITERATURE CITED


Dergaleva, Z.T. and M.I. Shatunovskiy. 1978. Data on the lipid metabolism of the larvae


Hall, L.W., Jr. 1991. A synthesis of water quality and contaminants data on the early life


Suthers, I.M., A. Fraser, and K.T. Frank. 1992. Comparison of lipid, otolith and morphometric condition indices of pelagic juvenile cod *Gadus morhua* from the


