

RNA:DNA AS AN INDICATOR OF NUTRITIONAL CONDITION AND GROWTH IN
LARVAL NAKED GOBY, *GOBIOSOMA BOSCH*

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For my family, always my biggest fans

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TABLE OF CONTENTS

DEDICATION.....	ii
ACKNOWLEDGEMENTS.....	iii
LIST OF FIGURES.....	v
ABSTRACT	vii
INTRODUCTION	1
Importance of Wetlands	1
Measuring Habitat Alteration	2
The RNA:DNA Ratio	3
The Naked Goby	5
Study Goals	6
MATERIALS AND METHODS.....	7
Larval Rearing	7
Sampling Procedure	8
Nucleic Acid Analysis	9
Growth and Mortality	10
Statistical Analyses	11
RESULTS	13
Growth and Mortality	13
Nucleic Acid Analyses	17
Wild Larvae	29
DISCUSSION	32
Utility of the RNA:DNA Ratio	32
DNA:DW as an Alternative Index	35
Nucleic Acid Analyses	36
Growth and Mortality.....	39
CONCLUSIONS	42
LITERATURE CITED.....	45
VITA	53

LIST OF FIGURES

Figure 1. <i>Gobiosoma bosc.</i> A) Dry weight ($\mu\text{g DW}$) regressed on standard length (mm SL), and B) \ln dry weight regressed on \ln standard length for larvae from three laboratory food treatments, ranging in age from 1 to 10 DPH (n = 68)	15
Figure 2. <i>Gobiosoma bosc.</i> Growth in SL regressed on age for larvae from three laboratory food treatments: A) 1200 l^{-1} , B) 200 l^{-1} , and C) 20 l^{-1}	16
Figure 3. <i>Gobiosoma bosc.</i> Mean \pm SE of DW regressed on age (DPH) for three laboratory food treatments.....	17
Figure 4. <i>Gobiosoma bosc.</i> Mean \pm SE of daily growth rate estimates regressed on age (DPH) for larvae from three laboratory food treatments: A) length-specific growth ($G_L \text{ d}^{-1}$) for n = 439 individuals, and B) weight-specific growth ($G_W \text{ d}^{-1}$) for n = 68 individuals.....	18
Figure 5. <i>Gobiosoma bosc.</i> Unbiased instantaneous mortality rates ($\hat{Z} \text{ day}^{-1}$) for tanks within each treatment of the experiment.....	19
Figure 6. <i>Gobiosoma bosc.</i> Mean \pm SE of total RNA concentration per whole fish larva from three laboratory food treatments, regressed on age (DPH).....	21
Figure 7. <i>Gobiosoma bosc.</i> Mean \pm SE of total DNA concentration per whole fish larva from three laboratory food treatments, regressed on age (DPH).....	21
Figure 8. <i>Gobiosoma bosc.</i> Regressions of A) \ln total RNA and B) \ln total DNA on \ln SL for fish from three laboratory food treatments.....	22
Figure 9. <i>Gobiosoma bosc.</i> Mean \pm SE of A) RNA and B) DNA concentrations for both laboratory-reared and wild fish in relation to size class (mm SL). Size classes are denoted by midpoints of lengths included in each class.....	23
Figure 10. <i>Gobiosoma bosc.</i> Mean \pm SE of A) total $\mu\text{g RNA } \mu\text{g DW}^{-1}$ and B) total $\mu\text{g DNA } \mu\text{g DW}^{-1}$ in relation to age (DPH) for three laboratory food treatments.....	25
Figure 11. <i>Gobiosoma bosc.</i> A) Line plot and B) regressions with 95% confidence intervals of mean \pm SE of RNA:DNA ratios regressed on age (DPH) for three laboratory food treatments...26	26
Figure 12. <i>Gobiosoma bosc.</i> RNA:DNA ratios of fish from three laboratory food treatments and wild fish in relation to length (mm SL).....	27
Figure 13. <i>Gobiosoma bosc.</i> RNA:DNA ratios of laboratory-reared and wild fish in relation to size class (mm SL). Size classes are denoted by midpoints of lengths included in each class....	28

Figure 14. *Gobiosoma bosc.* RNA:DNA regressed on dry weight ($\mu\text{g DW}$) for three laboratory food treatments.....28

Figure 15. *Gobiosoma bosc.* Mean \pm SE of RNA:DNA ratios in relation to size class ($\mu\text{g DW}$). Only size classes where two or more treatments are represented were included in analysis. Size classes are denoted by midpoints of weights included in each class.....30

Figure 16. *Gobiosoma bosc.* Relationship of mean (\pm SE) RNA:DNA to length-specific growth ($G_L \text{ d}^{-1}$) for three laboratory food treatments.....30

ABSTRACT

Developing organism-based metrics for assessing habitat quality is an important tool in conservation and restoration of aquatic habitats. The use of the RNA:DNA ratio as an early indicator of habitat effects on growth of nekton species has been suggested, but requires species-specific laboratory assessment prior to field application. We used food availability in laboratory treatments to simulate differences in habitat quality. Wild *Gobiosoma bosc* eggs were collected using nest-traps constructed of PVC pipe. Eggs from several different nests collected in the field were hatched in the lab, yielding larvae that were randomly assigned to three feeding treatments in two replicate tanks per treatment. Larvae were fed rotifers, *Brachionus plicatilis*, at densities of 20, 200, or 1200 l⁻¹, in two trials lasting 10 and 8 days. Prey concentrations were measured every 8 hours and adjusted to nominal prey densities. One to five individuals from each tank, totaling 699 larvae, were sampled daily for nucleic acids. Wild larvae were collected using a light trap, and individuals were sampled identically to laboratory-reared fish to allow direct comparisons of growth and nucleic acid concentrations. Experimental fish exhibited significantly higher growth and lower mortality with increasing prey concentration. RNA:DNA ratios declined with age, length, and dry weight (DW) in all treatments. Wild larvae exhibited similar trends in nucleic acid accumulation as laboratory-reared fish. Ratios were lower in fish from higher prey concentrations when related to age, but showed no differences when related to SL, and were higher at higher prey concentration when related to DW. RNA:DNA ratios did not accurately reflect growth rate magnitudes ($G_L d^{-1}$), but trends in growth and RNA:DNA were positively correlated. DNA:DW ratios proved to be a more accurate index of nutritional condition. I hypothesize that inherent growth patterns in larval *Gobiosoma bosc* largely prevent

RNA:DNA from accurately reflecting nutritional condition, which has broad implications for use of this index in the field.

INTRODUCTION

Importance of Wetlands

Coastal wetlands are known to be important areas for biological productivity and perform many functions associated with maintaining healthy ecological systems. Among other things, wetlands serve as buffers to coastal storm systems and potentially destructive wave and surge action, act as natural filters for absorbing pollutants and heavy metals from surrounding waters, and provide food and habitat resources to faunal communities living in and around them. They are important sites of primary and secondary productivity and are also an integral part of nutrient cycles and trophic webs that form the backbone of aquatic ecosystems (Boesch & Turner 1984, Beck et al. 2000). Among the most important of these functions is the role wetlands play in the production of commercially and recreationally important fish and invertebrate species. It is generally recognized that salt marshes and coastal wetlands provide refuge and serve as an ecosystem base for nekton species spending part or all of their life cycles in estuarine systems (Darnell 1958, Darnell 1961, Nixon 1980, Odum & Heald 1975, Naiman & Sibert 1979, Boesch & Turner 1984). The relationship between coastal wetlands and biomass production has rarely been studied on a direct species-specific basis. However, the generally accepted notion is that wetlands and adjacent waters serve as nursery areas for sub-adult life stages of many nekton species, contributing to the likelihood of survival and ultimately year class success (Gunter 1967; Deegan 1993; Minello 1999).

The loss of coastal wetlands has become a pertinent concern worldwide over the past several decades. Anthropogenic influences at the coastal margins are both direct and indirect, and have had significant effects on wetlands and associated habitats (Turner 1997). Nowhere in the United States is this issue more pressing than in the northern Gulf of Mexico, where a total

wetland area of approximately 3,900 km² has been lost since the 1930s (Turner 1990; Boesch et al. 1994), constituting 80% of the nation's total wetland losses (Dahl 1990). Causes for these declines are multi-faceted and include direct losses from construction of canals to service the oil and gas industry (Turner 1997) and lack of riverine sediment deposits due to levee construction along the Mississippi River, as well as more indirect losses from saltwater intrusion, wave-induced erosion of shorelines, and relative sea-level rise (combined effects of subsidence and eustatic sea-level rise) (Boesch et al. 1994, Day et al. 2000).

Measuring Habitat Alteration

It is yet unclear how these dramatic losses of wetland habitat will affect associated nekton populations. Chesney et al. (2000) pointed out the complexity involved in evaluating effects of marsh loss on nekton: 1) marsh edge habitats may be of higher importance to nekton than actual marsh plains; and, 2) the presence of aquatic vegetation could offset losses of marsh area. In addition to actual loss of wetland areas, anthropogenic sources of pollution and other disturbances have increased, putting further pressure on already taxed ecosystems. In the face of these dynamic “stressors” and challenges in evaluating their consequences, management of wetland-associated species necessitates the ability to detect changes to ecosystems and habitat quality at different levels of organization.

At the ecosystem level, changes to community structure and patterns of nekton habitat use have been used extensively to assess the effects of habitat alteration. Minns et al. (1996) evaluated response of fish communities to changes in freshwater habitats. Anthropogenic changes and subsequent restoration of oyster reefs in Chesapeake Bay have been extensively studied using community structure indicators (Harding & Mann 1999, Harding & Mann 2001). Wells et al. (2007) used red snapper assemblages to examine the potential effects of habitat

alteration due to shrimp trawling in the Gulf of Mexico. One downfall of this type of indicator is the latency with which changes are detectable. Negative alteration to environment or loss of habitat can be difficult to detect, must usually occur on a large scale in order to induce changes to community structure, and may take months or years to become measurable.

On a smaller and more individualized level of detection, growth and morphological condition indices have been used to assess effects of environment and habitat type (e.g. Mustafa 1978, Sogard 1992) on nekton populations. Effects of habitat quality have seldom been evaluated using condition indices. Burke et al. (1993) used morphological indices to assess condition in Atlantic croaker *Micropogonias undulatus* along an estuarine pollution gradient. Able et al. (1999) assessed potential impacts of man-made structures on habitat quality via growth of winter flounder *Pseudopleuronectes americanus*. Gossman (2005) used condition factors for multiple species to assess quality of terraced vs. unterraced marsh habitats. Growth-based condition indices have also been used to discriminate habitats of different quality over designated areas (Vila-Gispert et al. 2000, Lloret & Planes 2003, Gilliers et al. 2004). The value of using growth and morphology indices to evaluate habitat quality is that they can undergo detectable changes on a much smaller time scale than indices using measures of community dynamics. There are shortcomings in using these indices to assess habitat effects, however, as changes in growth and morphology can vary widely among individuals and be influenced more by ontogenetic changes due to development than habitat quality (Ferron & Leggett 1994).

The RNA:DNA Ratio

Perhaps a more promising method of evaluating habitat quality is to incorporate physiological metrics as a measure of individual response to habitat. Physiological characteristics can change on the order of hours to days in response to external stimuli, and

therefore have the potential to offer alternate perspectives of habitat effects on nekton populations on a much shorter time scale. One of the more promising and widely-used physiological measures of individual condition is the RNA:DNA ratio. The utility of this ratio as a measure of condition lies in the fact that cellular DNA is relatively constant per cell on a species-specific basis, while RNA content changes in response to the demand for protein synthesis. Thus, the RNA:DNA ratio is an index of protein synthesis occurring on a per-cell basis in an individual at a given time (Raae et al. 1988). Since growth is achieved primarily through protein synthesis, RNA:DNA is generally regarded as an index of growth potential (Bulow 1970). Individuals in good condition are generally characterized by higher RNA:DNA ratios than those in poor condition, and ratios have been shown to respond to changes in feeding conditions rapidly. Latency has been demonstrated to be as short as 1-3 days in some fish species (Martin & Wright 1987, Clemmesen 1996, etc), making the RNA:DNA ratio generally suitable for use as a short-term index of condition.

The use of the RNA:DNA ratio has been studied extensively in relation to nutritional condition and growth in many fish species (see Ferron & Leggett 1994 for a thorough review), but has less frequently been used as an indicator of habitat quality. The potential for its use in this setting lies in the ability of the RNA:DNA ratio to detect changes to the biochemical precursors of growth before somatic changes are manifested. Changes in growth can be caused not only by lack of sufficient food, but also by sub-optimal environmental conditions or other stressors. Biochemical changes are known to be induced by both biotic and abiotic factors including ontogenetic stage (e.g., Westerman & Holt 1994, Rooker and Holt 1996), disease (Steinhart & Eckmann 1992), dissolved oxygen (Peterson & Brown-Peterson 1992, Aday et al. 1999) temperature (Buckley 1982, Buckley 1984, Bulow 1987, Jurss et al. 1987, Mathers et al.

1993), and toxicants (Barron & Adelman 1984). Recently, several studies have focused on utilizing the ratio to measure habitat quality. Dahlhoff & Menge (1996) evaluated ecophysiology of the mussel *Mytilus californianus* in two different rocky intertidal areas using RNA:DNA ratios. Rooker et al. (1997) used RNA:DNA ratios of wild red drum *Sciaenops ocellatus* larvae and juveniles to assess quality of nursery areas. Yamashita et al. (2003) evaluated RNA:DNA ratios in stone flounder *Platichthys bicoloratus* between nearshore and estuarine habitats. Similarly, Gilliers et al. (2004) used RNA:DNA in sole *Solea solea*, dab *Limanda limanda*, and plaice *Pleuronectes platessa* to detect differences in habitat quality from different nursery grounds.

However, before this index can be applied in the field it requires a species-specific laboratory calibration. As with many biochemical measures, the RNA:DNA ratio depends upon temperature, size, species, ration level, ontogenetic stage, and technique, making it highly specific and its comparison across these variables unreliable without calibration (Suthers 1996). Rigorous laboratory trials must be performed to establish baselines for the behavior of RNA:DNA ratios across these variables. It has also been shown that variations in RNA:DNA ratios can depend on the method used for measurement (e.g., Bergeron 1997).

Naked Goby

In light of these considerations, application of the RNA:DNA ratio to the assessment of habitat quality in coastal Louisiana marshes first requires selection of a representative species, followed by thorough laboratory calibration. Species used in studies of habitat quality should exhibit site fidelity for the area to be studied. I chose to use larval naked goby *Gobiosoma bosc* for experimental analysis. Naked goby are ubiquitous to coastal Louisiana and the entire Gulf of Mexico. *Gobiosoma bosc* larvae are pelagic until reaching approximately 18-20 days in age and

8-12 mm in length, at which time they settle to benthic oyster reefs and other hard substrate (Breitburg 1991). Individuals exhibit high site fidelity once settlement has occurred, and have a total life span of approximately 13 months (Conn & Bechler 1996). The fact that this species spends all of its life living in a rather confined area of benthic habitat makes the naked goby a suitable species for use as in assessments of habitat quality. Consequently, past studies have used abundance and occurrence estimates of naked goby to assess habitat quality. Harding & Mann (1999) examined habitat use at a restored oyster reef by measuring patterns of use by naked goby. Hendon et al. (2000) utilized abundance estimates of larval naked goby to assess impacts of shoreline development on recruitment and habitat usage in the Gulf of Mexico.

Study Goals

Because declines in primary and secondary productivity can indicate decreased habitat quality, varying prey concentrations can be used to simulate changes to habitat. We chose to rear *G. bosc* larvae at 3 different prey levels in an effort to induce growth rate-related variation in the RNA:DNA ratio. Upon verification that RNA:DNA responds to changes in condition in a predictable manner, we can utilize it to evaluate condition of larvae *in situ* as well as further explore the effects of other environmental changes on the index. The overall goals of the study were to: 1) determine the effects of food limitation on growth, mortality, and nucleic acid concentrations in larval naked goby; 2) evaluate the applicability of the RNA:DNA ratio as an indicator of condition in individual naked goby exposed to different feeding environments; and 3) use nucleic acid indices formulated for laboratory-reared fish to assess condition of wild naked goby larvae for comparison purposes.

MATERIALS & METHODS

Larval Rearing

Gobiosoma bosc eggs were collected by deploying artificial nest-traps around the perimeter of a brackish marsh pond near Terrebonne Bay, LA. Nest traps were fashioned after Bechler et al. (1990) and were deployed beginning July 2005 during peak *G. bosc* spawning season (Conn 1989) to obtain experimental animals. After approximately 5 days, adult male gobies occupied several traps containing nests with eggs. Traps were checked daily for new egg masses, and those found to be occupied by a male and to contain eggs were carefully removed from the pond, so as not to lose the male occupying the nest, and transferred by bucket to 40 liter (l) aquaria. Eggs laid on a given day were kept separately to keep cohorts together until hatching. Pond conditions averaged 32°C and 10 practical salinity units (psu), and laboratory incubation conditions simulated pond conditions at approximately 27 °C and 10 psu. When 3 to 4 egg masses were ready to hatch at the same time, the nests were exposed to direct light to initiate hatching. Larvae hatched from different nests were mixed and randomly assigned to three feeding treatments with two replicate 40 l tanks per treatment. Feeding treatments were randomly assigned to tanks to avoid pseudoreplication (Hurlbert 1984). Approximately 300 fish were added to each tank, yielding a nominal larval density of 8.82 individuals l⁻¹.

Experimental conditions were maintained for two trials lasting 10 days and 8 days, respectively. Larvae were fed diets of rotifers, *Brachionus plicatilis*, at densities of 20, 200, or 1200 l⁻¹. Rotifer densities were checked every 8 hours using duplicate 2 ml Hensen-Stempel pipette samples, and were adjusted to maintain nominal densities. Rotifers were cultured in 2 l glass Erlenmeyer flasks under direct light and fed *Nannochloropsis* sp. and *Isochrysis galbana* (Tahitian strain). *Nannochloropsis* were added to experimental tanks to maintain rotifers.

Quantities of *Nannochloropsis* were standardized by keeping turbidities in tanks at approximately 2.0 nephelometric turbidity units (ntu) throughout the experiment. Turbidities were read on a Hach 2100N turbidimeter. Tanks were held on a 3.05 m x 1.83 m water table, and were kept at 27°C and 9 ± 1 psu for the duration of the experiment. Temperature and salinity were checked twice per day and adjusted accordingly. Fifty percent water changes with filtered, UV sterilized sea water were carried out daily to maintain water quality.

Sampling Procedures

One to five larvae per tank were sacrificed at 1000 hours daily for nucleic acid analysis. Before the first daily feeding at 0800 hours, larvae were randomly selected from each tank and placed in a beaker of clean sea water for 2 hours to allow evacuation of gut contents. After 2 hours, larvae were individually suctioned onto 13 mm polycarbonate membrane filters using a vacuum-manifold system to remove excess water and to facilitate moving of larvae. The larvae with filters were then placed under a dissecting microscope fitted with a video camera and videotaped for digital length measurement. Live larvae then were placed with their filters into 1.5 ml cryovials, immediately flash frozen in liquid nitrogen, and stored at -196°C until analyzed. Up to four additional larvae per tank were sampled each day for length-weight determination. These larvae were also isolated for 2 hours to allow gut evacuation, but then were placed individually into a watch glass with a seawater solution of clove oil for anesthesia. Larvae were measured to the nearest mm using an ocular micrometer and then preserved in 95% ethanol until weighed.

To allow comparison between condition of laboratory-reared and wild individuals, a light trap designed following Hernandez & Shaw (2003) was deployed overnight in Terrebonne Bay, LA, during the course of the laboratory experiment. The trap was soaked from 2200 h to 0700 h

nightly, retrieved and the catch sorted. Larval *G. bosc* were removed to clean sea water and left for 2 hours to allow gut evacuation. Subsequent sampling for nucleic acid and growth analyses followed the procedure for laboratory-reared individuals.

Nucleic Acid Analysis

RNA and DNA were quantified separately following the methods of Kaplan et al. (2001). Fish were thawed and removed from their filters, and then placed individually into 2 ml centrifuge tubes. Whole individuals were digested at 55°C for 2 hours in 100µl of a solution containing 0.2% sodium dodecylsulfate, 0.1M NaCl, and 10µg ml⁻¹ proteinase K. Samples were then centrifuged at 13,200 RPM for 10 minutes, and supernatants were drawn off and separated into two aliquots for RNA and DNA determinations. Pelleted remains were submerged in distilled water and re-frozen at -20°C to stop digestion processes and preserve otoliths. Supernatants were frozen at -80°C until analysis. When ready for quantification, aliquots for DNA analysis were thawed and duplicate 10µl samples were added to a 96-well plate with 190 µl of a solution containing PicoGreen (Molecular Probes, Oregon). Double-stranded DNA (dsDNA) was quantified fluorometrically via comparison with a bacteriophage lambda dsDNA standard curve (Molecular Probes, Oregon, 0 ng ml⁻¹ -1 µg ml⁻¹). Aliquots for RNA analysis were thawed separately from the DNA aliquots, and a solution containing RNase-free DNase I (bovine pancreas, 2 units, Ambion, Inc., Texas) was added to each sample. Samples were incubated at 37°C for 30 minutes, and then duplicate 5 µl volumes were added to a 96-well plate with 195µl of a solution containing RiboGreen (Molecular Probes, Oregon). Total RNA was determined fluorometrically via comparison with an *Escherichia coli* ribosomal RNA standard curve (Molecular Probes, Oregon, 0 ng ml⁻¹ -1 µg ml⁻¹). Samples for both RNA and DNA measurement were run at 485/538 nm excitation/emission on a SpectraMax M2 microplate

reader (Molecular Devices, California). Post-hoc recovery tests were conducted by subjecting known quantities of both RNA and DNA to extraction and fluorescence procedures identical to those performed on samples.

Growth and Mortality

Larvae stored in ethanol and intended for length-weight analysis extruded fluids that prevented accurate weight estimates. Thus, extra frozen larvae from the nucleic acid samples were used to obtain weights for length-weight regression analysis. Frozen larvae were thawed, removed from their filters, and lengths were measured directly using an ocular micrometer under a dissecting microscope. They were then blotted to remove excess moisture, placed in pre-weighed foil weighing boats and dried overnight at 60°C to a constant weight. Dried fish were weighed twice to the nearest 0.0001 mg using a Sartorius M2P microbalance, and the mean was regressed on standard length (SL). Dried larvae were kept in a desiccator before weighing, and the time out of the desiccator was closely controlled to minimize error due to hydration. Separate regressions were developed for each treatment, and weight estimates for remaining frozen larvae were predicted from digital length measurements.

Growth rates were calculated for larvae from each treatment. Weight-specific growth ($G_W \text{ d}^{-1}$) was calculated for all directly-weighed larvae. Because there were few larvae available for weighing, the starting weight used was a single measurement from the 20 l⁻¹ treatment at age 1 day post hatch (DPH). Fish used in weight-specific growth calculations ranged in age from 2 to 10 DPH. G_W was calculated using the following equation (Ricker 1979):

$$G_W = \frac{(\ln W_2 - \ln W_1)}{t_2 - t_1} \quad (1)$$

where W_1 is the dry weight of sacrificed fish at time t_1 (age 1 DPH), and W_2 is dry weight of sacrificed fish at time t_2 . Similarly, length-specific growth ($G_L \text{ d}^{-1}$) was calculated using the

same equation, but substituting L_2 (length at time t_2) and L_1 (length at time t_1) for W_2 and W_1 , respectively.

Based on the number of larvae stocked per tank (N_0), the number sampled (N_s), and the number remaining alive at the end of the trials (N_t), mean daily instantaneous mortality rates (Z) were calculated using the following formulae (Buckley et al. 1984):

$$Z = \frac{(\ln N_0 - \ln N_t)}{t} \quad (2)$$

where t = number of days in the experimental trial. Calculated Z values were then used to obtain unbiased estimates of mortality (\hat{Z}), accounting for potential survivors removed during sampling:

$$\hat{N}_t = N_t + N_s e^{-Zd} \quad (3)$$

$$\hat{Z} = \frac{(\ln N_0 - \ln \hat{N}_t)}{t} \quad (4)$$

where d = number of days remaining in the trial. \hat{Z} was calculated for each replicate tank in each trial by first obtaining initial Z values, which assume that none of the sampled fish would have survived. Then Equation (2) was solved iteratively to correct \hat{N}_t for use in Equation (3).

Statistical Analyses

All statistical analyses were run using SAS statistical software version 9.1 (SAS Institute 1999). Significance levels for all analyses were set *a priori* to $\alpha = 0.05$. Normality was assessed using the Shapiro-Wilk test for all analyses. The null hypothesis of normality for the experimental error term was not rejected for any analysis (reject at $p \leq 0.05$), and all other assumptions were met unless otherwise noted. Length-weight regressions of laboratory-reared fish were formed using a least-squares approach, and weight-specific growth was compared

between treatments using analysis of covariance (ANCOVA) with SL as a covariate (PROC MIXED). Quadratic trends in growth in SL were tested using PROC MIXED, and PROC NLIN was used to form piecewise regressions of SL on age for treatments with significant quadratic terms. Experiment-wise instantaneous mortality (\hat{Z}) was compared among treatments using a one-way analysis of variance (ANOVA, PROC MIXED). \hat{Z} was calculated for all tanks and all trials with the exception of trial 1, tank 3 (200 l⁻¹) and trial 2, tank 6 (1200 l⁻¹), as final survival counts could not be obtained. Daily G_W and G_L estimates were compared among treatments using ANCOVA with age as a covariate. Differences in nucleic acid content, RNA:DNA and DNA:DW ratios between treatments were analyzed using a series of ANCOVAs with age, SL, and DW as covariates. Fish of ages 0, 9, and 10 DPH were excluded from ANCOVA analyses because age 0-d fish were not assigned a treatment and age 9-d and 10-d fish were not present in the 20 l⁻¹ treatment in trial 1 or any treatments in trial 2. Thus, a total of 400 individuals were included in ANCOVA analyses, covering days one through eight of both trials. RNA:DNA ratios were also compared among treatments within size classes (mm SL, μ g DW) using an ANOVA. Type III sums of squares were utilized for testing in all ANCOVA analyses, while Type I sums of squares were used elsewhere.

RESULTS

Growth and Mortality

A total of 68 individual *G. bosc* larvae, ranging in age from one to 10 DPH, were used to describe length-weight relationships for three treatments (Figure 1). Growth in weight with respect to length was best described by three power functions (Figure 1a). The linear slope of log DW regressed on log SL was significantly lower in the 20 l⁻¹ treatment than in either of the two higher treatments ($t \leq -2.57$, $p < 0.0001$), with no difference in slopes between the two higher treatments ($t = 0.48$, $p = 0.6315$) (Figure 1b). All regressions shared a common intercept. Larvae in the 20 l⁻¹ treatment weighed less (DW per unit SL) than larvae from the other two treatments and fish from the 1200 l⁻¹ treatment were heavier at length than those from the 200 l⁻¹ treatment, indicating treatment effects on growth were proportional to experimental prey concentrations.

Growth in length varied proportionally to prey concentrations, but deceleration of growth in standard length occurred over time in two of three feeding treatments (Figure 2). Piecewise least-squares regression indicated quadratic trends in growth in length in the 200 l⁻¹ treatment ($F = 130.07$, $p < 0.0001$), with growth tapering off around 4.00 mm SL and 8 DPH and in the 1200 l⁻¹ treatment ($F = 210.47$, $p = 0.0002$), an asymptote was predicted near 4.83 mm SL and 11 DPH. In the 20 l⁻¹ treatment, the trend of SL regressed on age remained linear over time with no quadratic trend ($F = 32.88$, $p = 0.5125$) and no deceleration of growth was observed; however, in comparison to sizes from the other treatments, predicted growth in length was only 3.41 mm and 3.65 mm SL at 8 and 11 DPH, respectively. Thus, fish from the lowest prey treatment did not attain sizes comparable to those from the two higher treatments, which may have influenced growth patterns.

Growth in weight exhibited similar patterns, with quadratic trends in the two highest treatments (Figure 3). Individuals in the 200 l⁻¹ treatment (F = 9.99, p = 0.0018) and the 1200 l⁻¹ treatment (F = 10.64, p = 0.0013) experienced tapered growth in weight, predicted to occur near 11 DPH in both treatments. Fish from the 20 l⁻¹ treatment again exhibited no quadratic trend in growth (F = 0.00, p = 0.9498) and grew only marginally during the experiment, reaching final weights of almost 20 µg at 8 DPH while fish in the 1200 l⁻¹ treatment were on average around 6 times heavier at 10 DPH. Fish in the 200 l⁻¹ treatment attained mean weights over twice those in the lowest treatment by 8 DPH. Thus, growth in weight behaved much like growth in length, varying proportionally to experimental prey concentrations.

As expected from quadratic trends in length and weight, daily weight- and length-specific growth rates (G_W d⁻¹ and G_L d⁻¹, respectively) declined with age and demonstrated significant treatment effects (Figure 4). Weight-specific growth rates were highest at 1 DPH and declined with age in the two highest prey treatments. Weight-specific growth did not change with age in the 20 l⁻¹ treatment, having a slope not significantly different than zero (p = 0.508). Slopes were significantly different among all treatments (F = 12.37, p < 0.0001) as were intercepts (F = 47.89, p < 0.0001), with the highest growth rates and most negative slope in the 1200 l⁻¹ treatment and the lowest growth rates and zero slope in the 20 l⁻¹ treatment. Length-specific growth followed similar declining trends from 1 DPH, but demonstrated significant treatment effects (F = 17.86, p < 0.0001) and similar slopes (F = 0.75, p = 0.471) among treatments.

Unbiased instantaneous mortality rates (\hat{Z} d⁻¹) differed among treatments (Figure 5). The mean ± SE of \hat{Z} in the 20 l⁻¹ treatment was 0.209 ± 0.02 d⁻¹, and was significantly higher than in either the 200 l⁻¹ or 1200 l⁻¹ treatments (F = 10.84, p = 0.0072). This indicates that the 20 l⁻¹ treatment produced a feeding environment poor enough to significantly affect both the

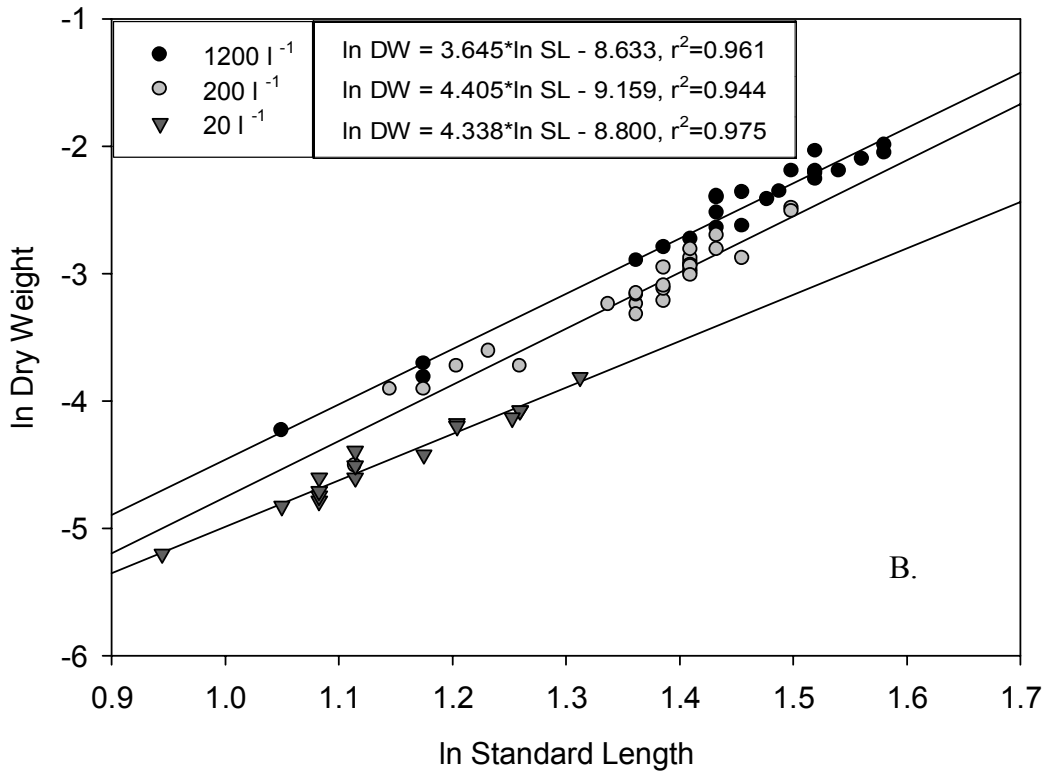
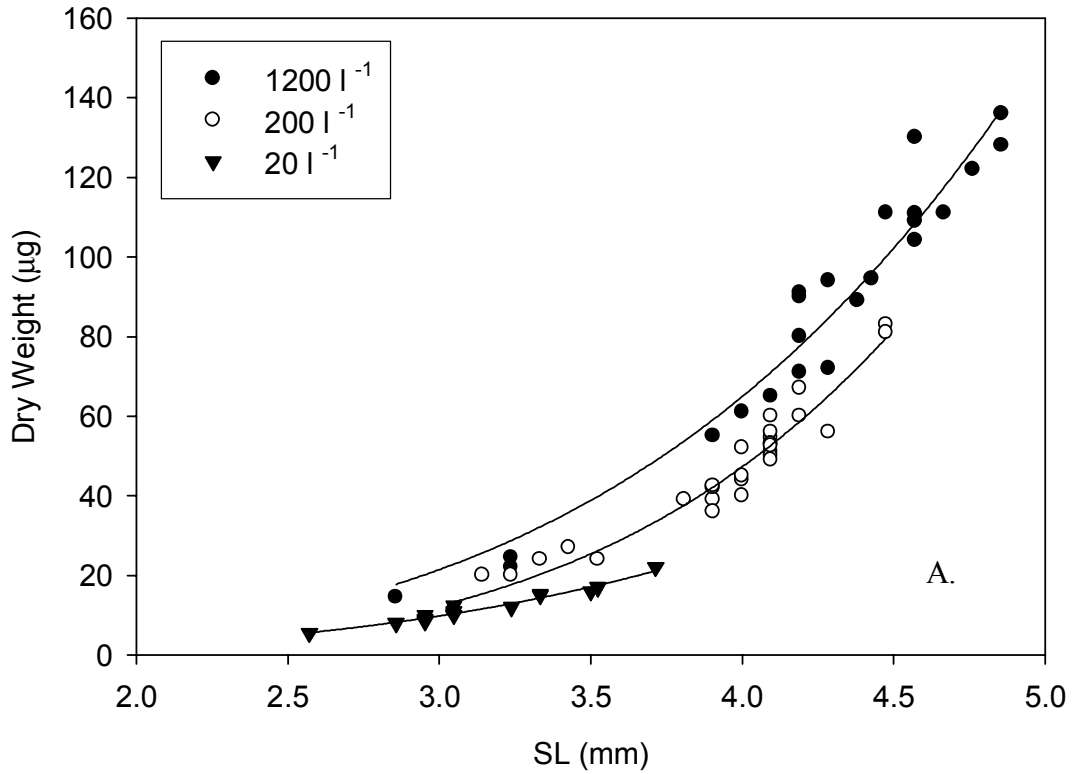


Figure 1. *Gobiosoma bosc.* A) Dry weight ($\mu\text{g DW}$) regressed on standard length (mm SL), and B) ln dry weight regressed on ln standard length for larvae from three food treatments, ranging in age from 1 to 10 DPH ($n = 68$).

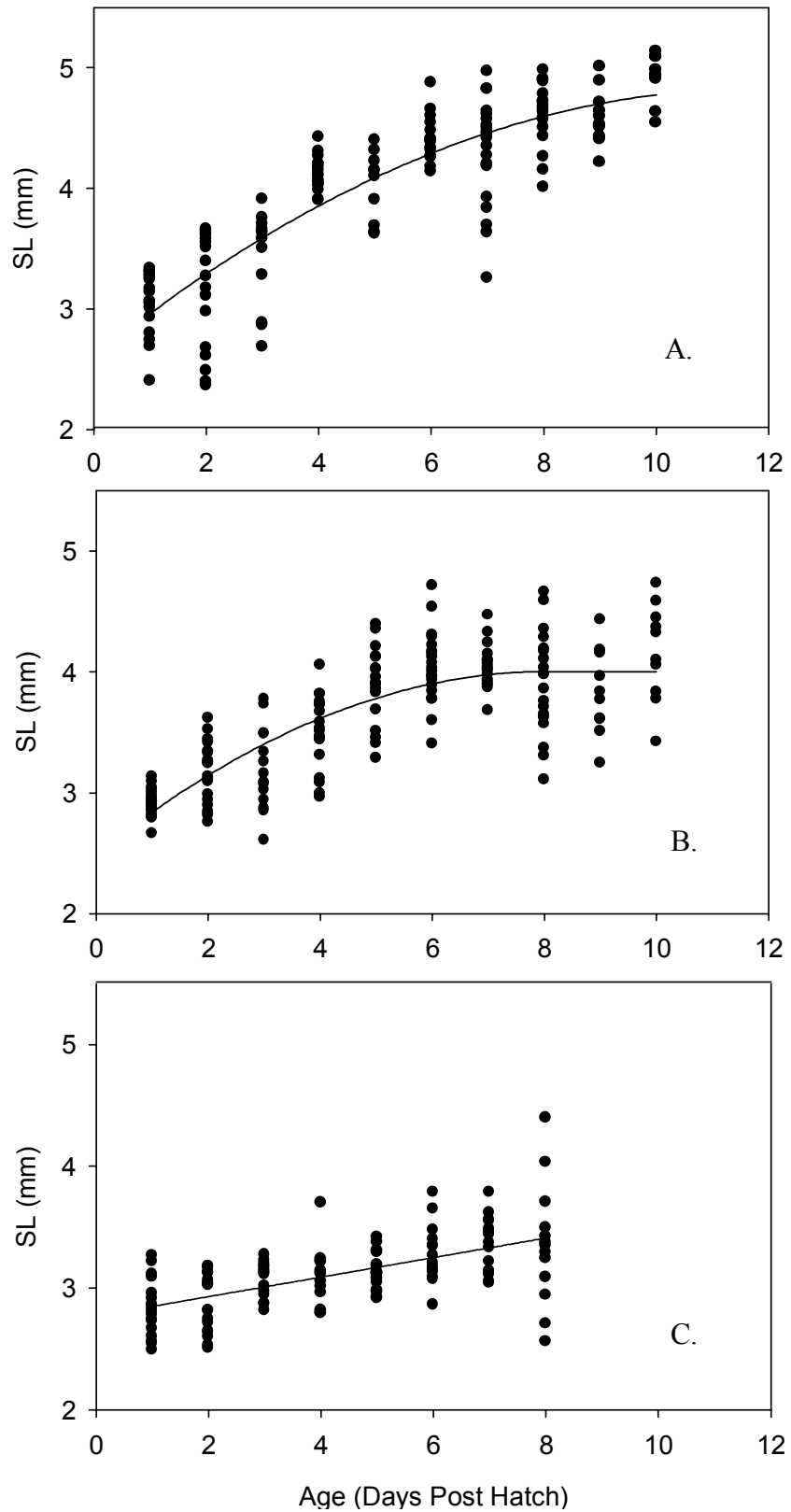


Figure 2. *Gobiosoma bosc*. Growth in SL regressed on age for larvae from three food treatments: A) 1200 l^{-1} , B) 200 l^{-1} , and C) 20 l^{-1} .

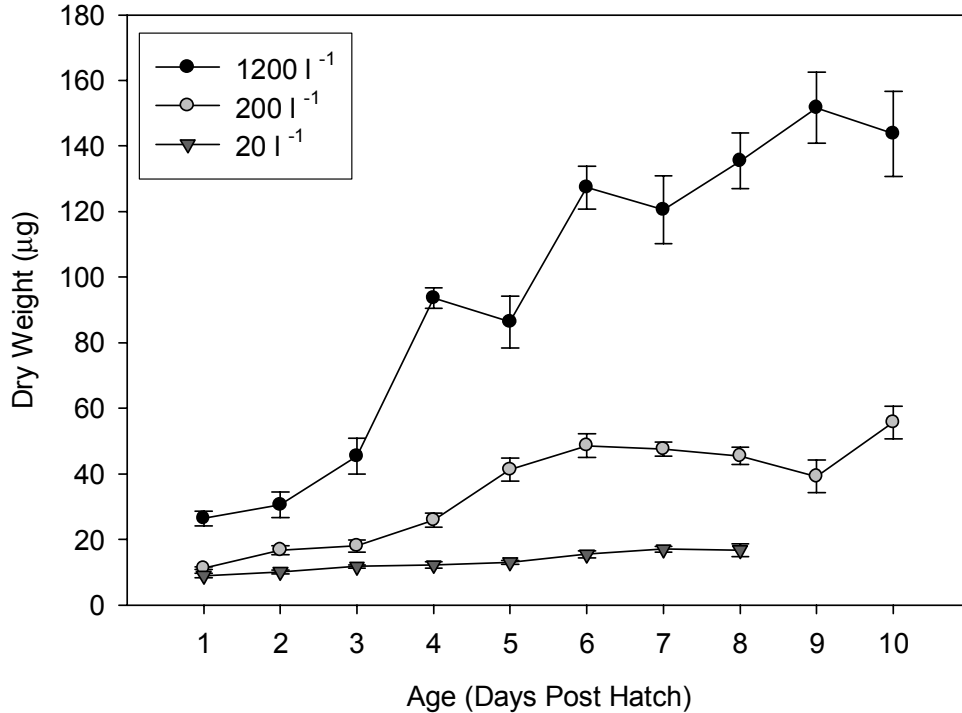


Figure 3. *Gobiosoma bosc*. Mean \pm SE of DW regressed on age (DPH) for three laboratory food treatments.

survival and the condition of *G. bosc* larvae. Mortality was not significantly different between the 200 l⁻¹ (0.079 ± 0.01 d⁻¹) and 1200l⁻¹ (0.073 ± 0.01 d⁻¹) treatments ($t = 0.21$, $p = 0.842$), suggesting that prey concentrations greater than 200 l⁻¹ did not detectably influence survival.

Nucleic Acid Analyses

Total DNA and RNA per fish (mean \pm SE) differed by treatment and age, with age as a covariate. Total DNA concentration was 0.24 ± 0.02 $\mu\text{g fish}^{-1}$ at hatch, decreased on day 1, and then increased with age in all treatments (Figure 6). The overall increase reflected increasing cell numbers associated with growth. Treatment effect was non-significant ($F = 1.43$, $p = 0.240$), but treatment*DPH interaction was significant ($F = 56.27$, $p < 0.0001$). The slope of DNA content regressed on age was lowest in the 20 l⁻¹ treatment and increased significantly with increasing prey concentration (t-tests, all p-values < 0.0001), with all regressions sharing a common intercept (t-tests, all p-values ≥ 0.092). Adjusted mean DNA concentration (ANCOVA,

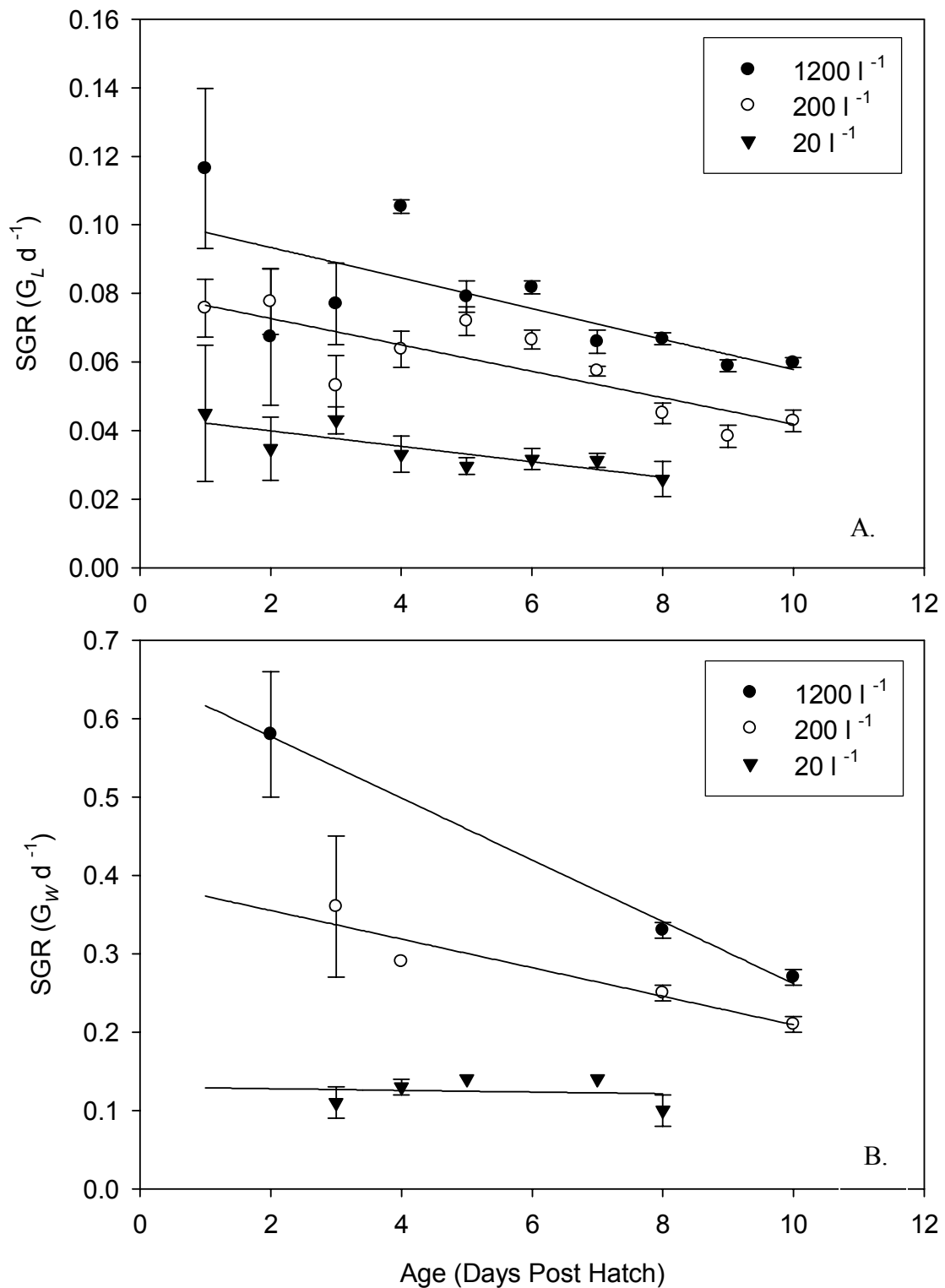


Figure 4. *Gobiosoma bosc*. Mean \pm SE of daily growth rate estimates regressed on age (DPH) for larvae from three laboratory food treatments: A) length-specific growth ($G_L d^{-1}$) for $n = 439$ individuals, and B) weight-specific growth ($G_W d^{-1}$) for $n = 68$ individuals.

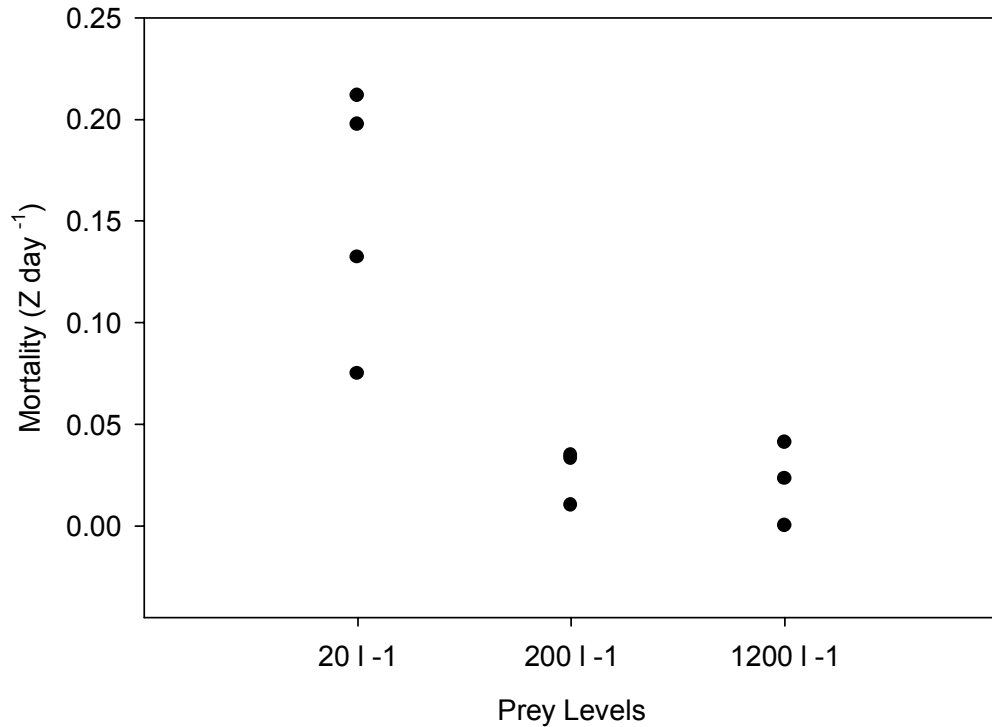


Figure 5. *Gobiosoma bosc.* Unbiased instantaneous mortality rates (\hat{Z} day⁻¹) for tanks within each treatment of the experiment.

covariate = age) became significantly different among all treatments by 3 DPH (all p-values \leq 0.0001), with higher concentrations in higher prey treatments. RNA concentrations followed a similar pattern to DNA concentrations and had a mean of $1.97 \pm 0.15 \mu\text{g fish}^{-1}$ at hatch (Figure 7). Again, treatment effect was not significant ($F = 1.33$, $p = 0.266$), but treatment*DPH interaction was significant ($F = 38.21$, $p < 0.0001$). Slopes of RNA regressed on age were significantly higher at higher prey concentrations (t-tests, all p-values < 0.0001), and shared a common intercept (t-tests, all p-values ≥ 0.324). Mean adjusted RNA concentrations (ANCOVA, covariate = age) also became significantly different among all treatments by 3 DPH (all p-values ≤ 0.0001), with higher concentrations in higher prey treatments. Analytical recovery rates were acceptable at $101.81 \pm 8.1 \%$ and $94.68 \pm 18.0 \%$ for DNA and RNA, respectively.

RNA and DNA concentrations followed different patterns when related to SL (Figure 8). Data in Figure 8 were log-transformed (\ln) to normalize and linearize the data; however, several outliers on the upper tail of the distribution prevented transformations from inducing normality in both cases. Nevertheless, the Shapiro-Wilk statistic was very high, ($W = 0.972$ for DNA, $W = 0.979$ for RNA) and the ANCOVA analysis is robust to such issues; therefore, the analyses were run on the \ln -transformed data. The natural log of both RNA and DNA increased with increasing $\ln(\text{SL})$ for all treatments, but there were no significant differences among slopes (t-tests: RNA, all p-values ≥ 0.282 ; DNA, all p-values ≥ 0.568) or intercepts (t-tests: RNA, all p-values ≥ 0.065 ; DNA, all p-values ≥ 0.386) for either. Treatment effects (RNA: $F = 2.45$, $p = 0.0845$; DNA: $F = 0.94$, $p = 0.395$) and interaction effects (RNA: $F = 1.24$, $p = 0.291$; DNA: $F = 0.44$, $p = 0.642$) were non-significant. Adjusted means (ANCOVA, covariate = $\ln \text{SL}$) were also not significantly different between treatments for either $\ln \text{RNA}$ (all p-values ≥ 0.075) or $\ln \text{DNA}$ (all p-values ≥ 0.099). However, ANOVA analyses of RNA and DNA by size class did reveal some differences between treatments (Figure 9), most notably that mean RNA and DNA concentrations were significantly lower in the 20 l^{-1} treatment than in the other two treatments at the 3.5 and 4.5 mm size classes ($p \leq 0.048$).

Trends were much different when nucleic acids were related to DW (Figure 10). All treatments exhibited declines in both RNA and DNA from days 2 to 4, with concentrations in the 200 l^{-1} and 1200 l^{-1} treatments remaining relatively constant following initial declines. Concentrations in the 20 l^{-1} treatment increased sharply on day 5, and remained higher and more variable for the remainder of the experiment. Concentrations from the 200 l^{-1} treatment were higher and more variable than those from the 1200 l^{-1} treatment on and after day 4. DNA per unit DW (DNA:DW) and RNA per unit DW (RNA:DW) were also \ln -transformed to normalize data

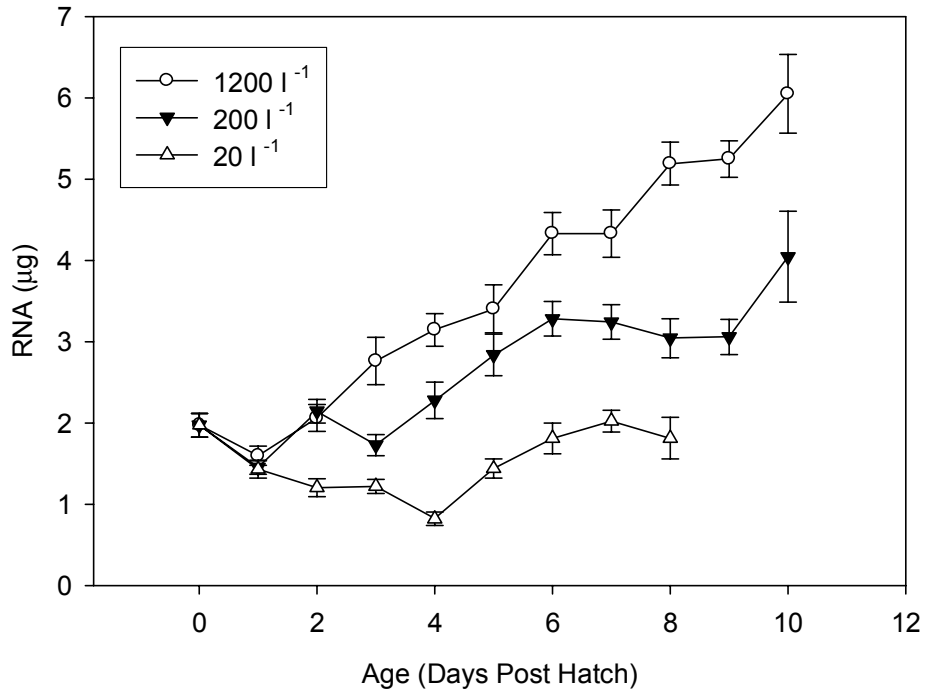


Figure 6. *Gobiosoma bosc*. Mean \pm SE of total RNA concentration per whole fish larva from three laboratory food treatments, regressed on age (DPH).

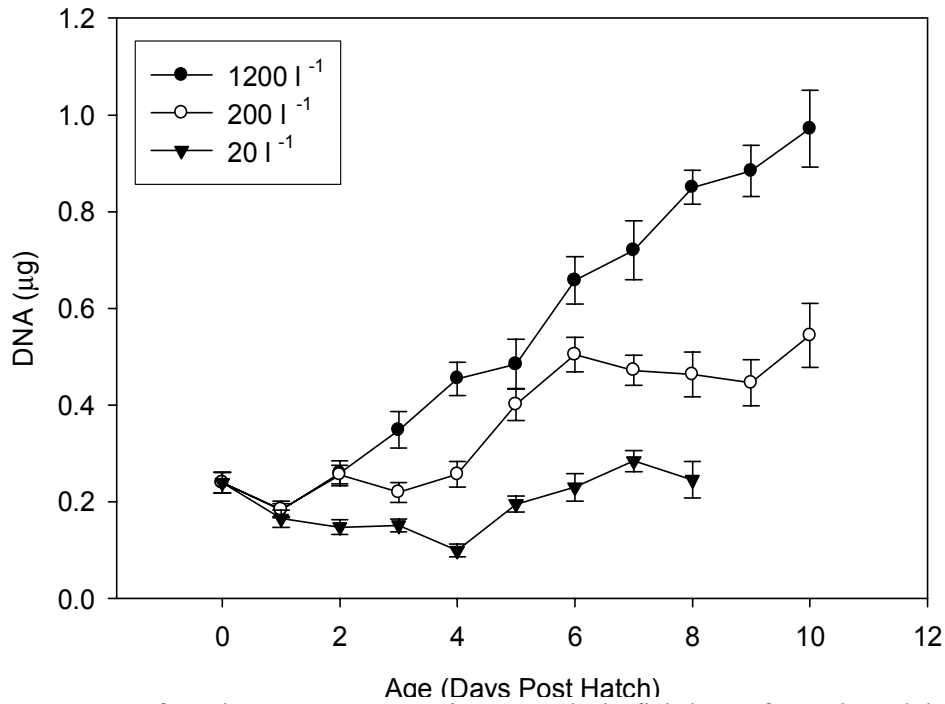


Figure 7. Mean \pm SE of total DNA concentration per whole fish larva from three laboratory food treatments, regressed on age (DPH).

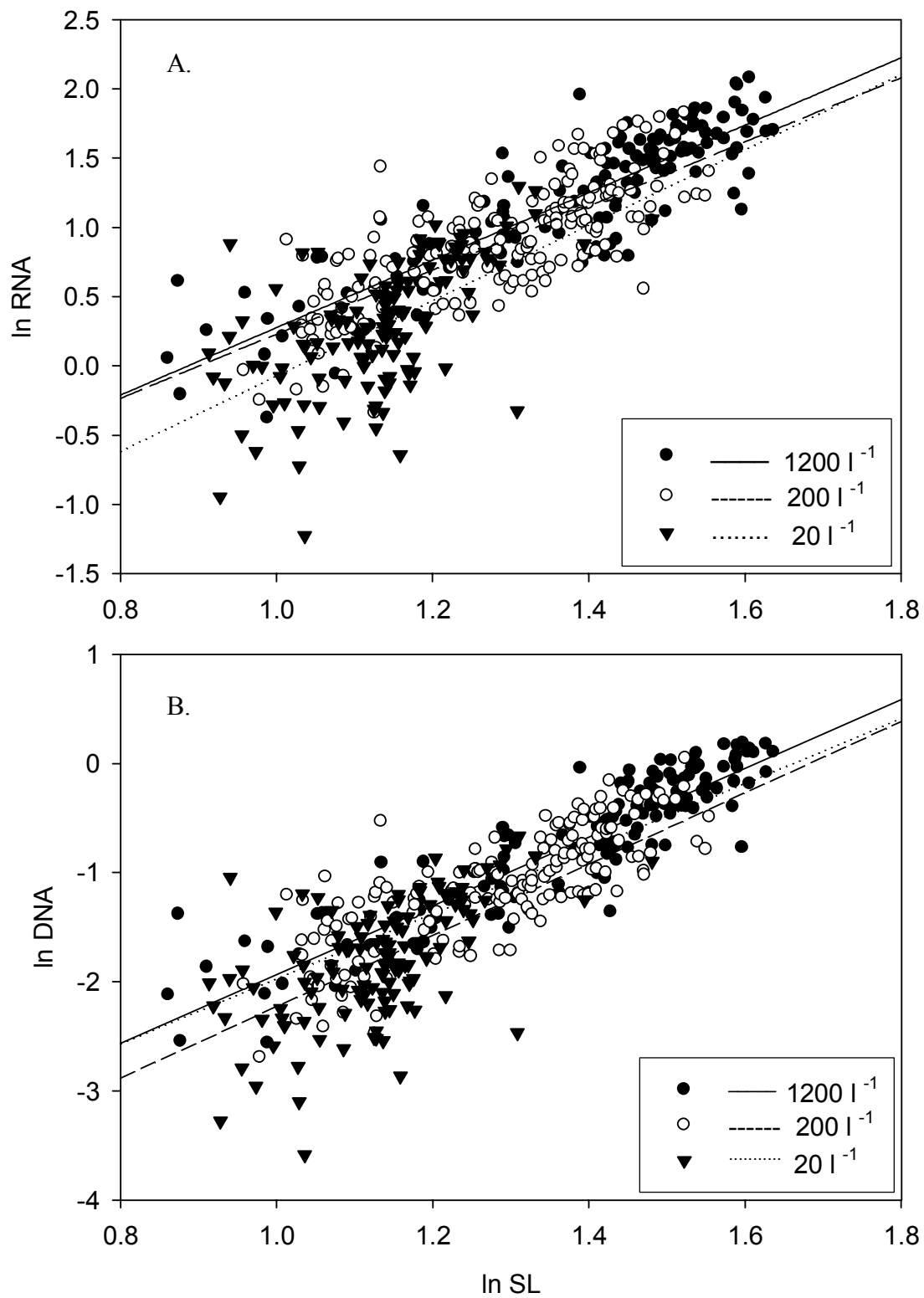


Figure 8. *Gobiosoma bosc.* Regressions of A) ln total RNA and B) ln total DNA on ln SL for fish from three laboratory food treatments.

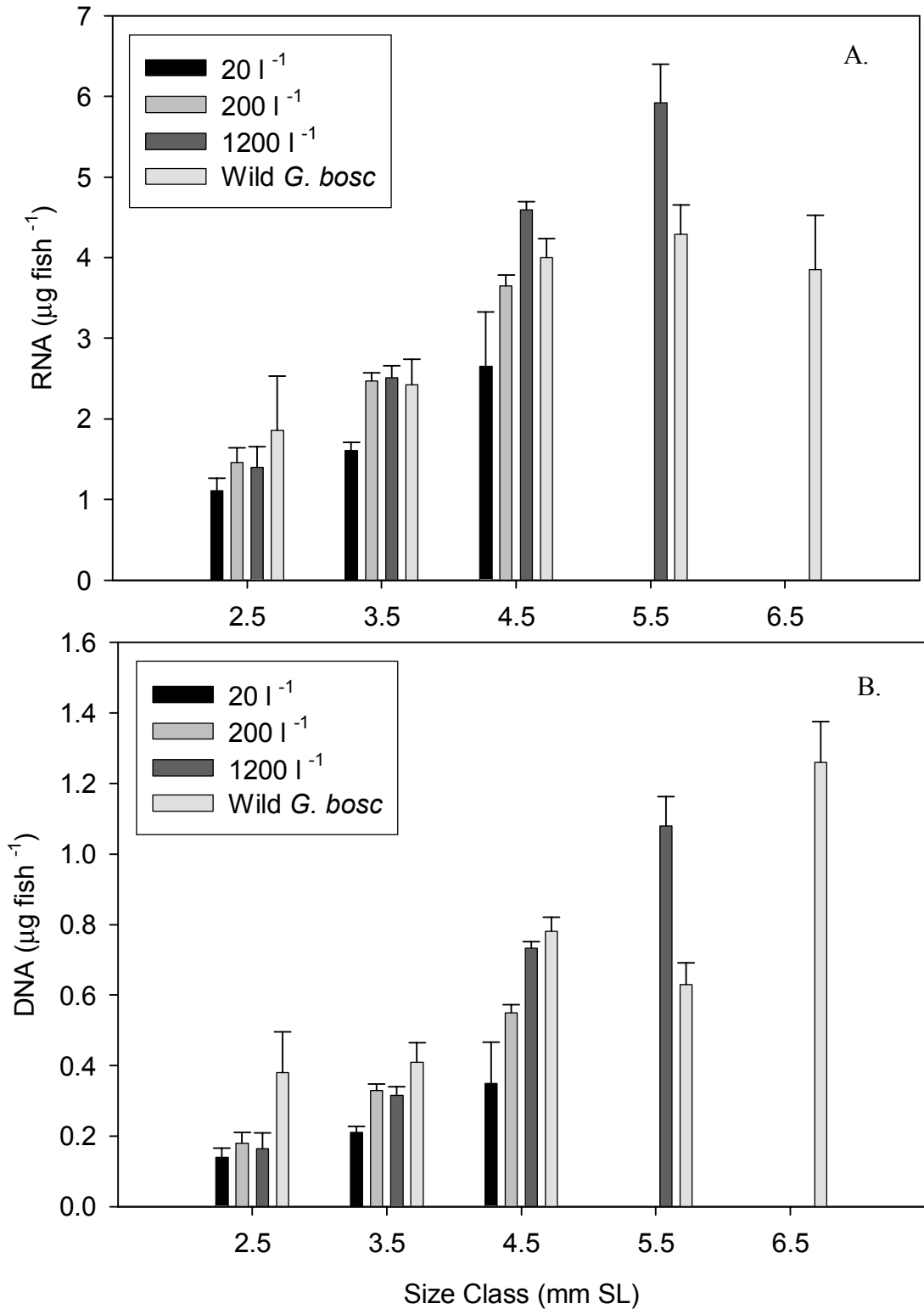


Figure 9. *Gobiosoma bosc*. Mean \pm SE of A) RNA and B) DNA concentrations for both lab-reared and wild fish in relation to size class (mm SL). Size classes are denoted by midpoints of lengths included in each class.

but exhibited the same outlier-induced non-normality as with the previously discussed analysis despite transformation. Again, Shapiro-Wilk values were very high ($W = 0.982$ for \ln DNA:DW, $W = 0.980$ for \ln RNA:DW), thus analyses were performed with \ln -transformed data. With \ln DPH as a covariate, both DNA:DW and RNA:DW exhibited significant treatment (RNA: $F = 25.77$, $p < 0.0001$; DNA: $F = 24.28$, $p < 0.0001$) and interaction (RNA: $F = 7.02$, $p = 0.001$; DNA: $F = 3.28$, $p = 0.039$) effects. Intercepts were different among treatments (t-tests: DNA:DW, all p -values ≤ 0.0001 ; RNA:DW, all p -values ≤ 0.0001). Slope was different between the 20 l^{-1} treatment and the 1200 l^{-1} treatment in both cases (t-tests: DNA:DW, $p = 0.050$; RNA:DW, $p = 0.0009$), but not between the 200 l^{-1} and 1200 l^{-1} treatments (t-tests: DNA:DW, $p = 0.600$; RNA:DW, $p = 0.744$).

The mean (\pm SE) RNA:DNA ratio was 8.41 ± 0.22 at hatch and declined in all treatments from day 1 through the end of the experiment (Figure 11a). Contrary to expected trends, the slope of RNA:DNA regressed on age was significantly less negative in the 20 l^{-1} treatment than in the 1200 l^{-1} treatment ($t = 2.36$, $p < 0.019$). Differences in slope were not significant between the 200 l^{-1} and 1200 l^{-1} treatments ($t = 1.00$, $p < 0.320$), and all treatments shared a common intercept (t-tests, all p -values ≥ 0.783). Using age as a covariate, neither treatment ($F = 0.04$, $p = 0.961$) nor interaction ($F = 2.82$, $p = 0.061$) were significant. However, based on examination of confidence intervals (Figure 11b), differences between the highest and lowest treatments were apparent after approximately 3 DPH.

RNA:DNA ratios exhibited different trends when related to measures of body size. Slopes of RNA:DNA regressed on SL did not vary significantly between treatments (t-tests, all p -values > 0.596), and declined with increasing SL in all treatments (Figure 12). With SL as a covariate, neither treatment ($F = 0.2$, $p = 0.819$) nor interaction ($F = 0.16$, $p = 0.855$) effects were

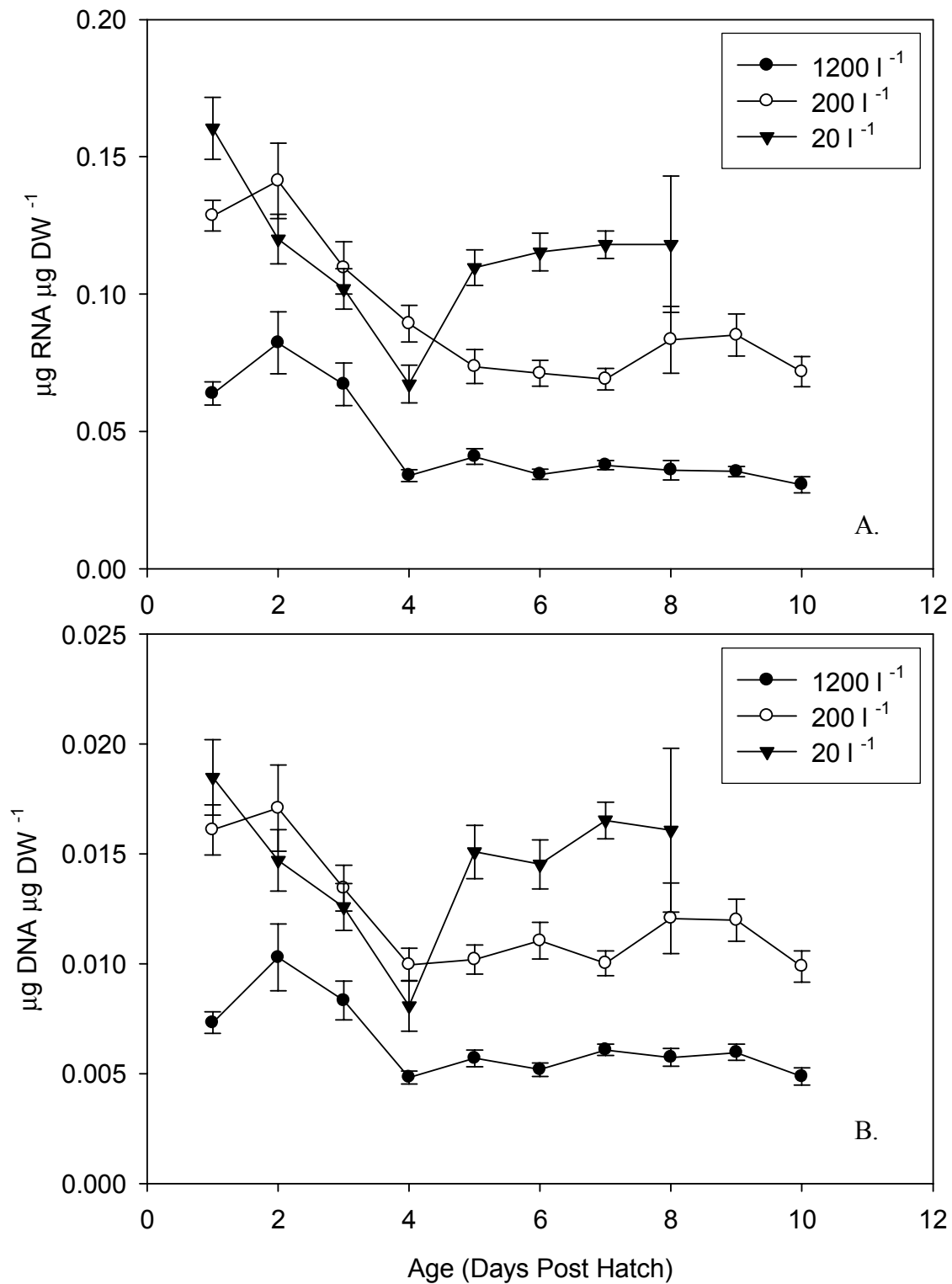


Figure 10. *Gobiosoma bosc.* Mean \pm SE of A) total $\mu\text{g RNA } \mu\text{g DW}^{-1}$ and B) total $\mu\text{g DNA } \mu\text{g DW}^{-1}$ in relation to age (DPH) for three laboratory food treatments.

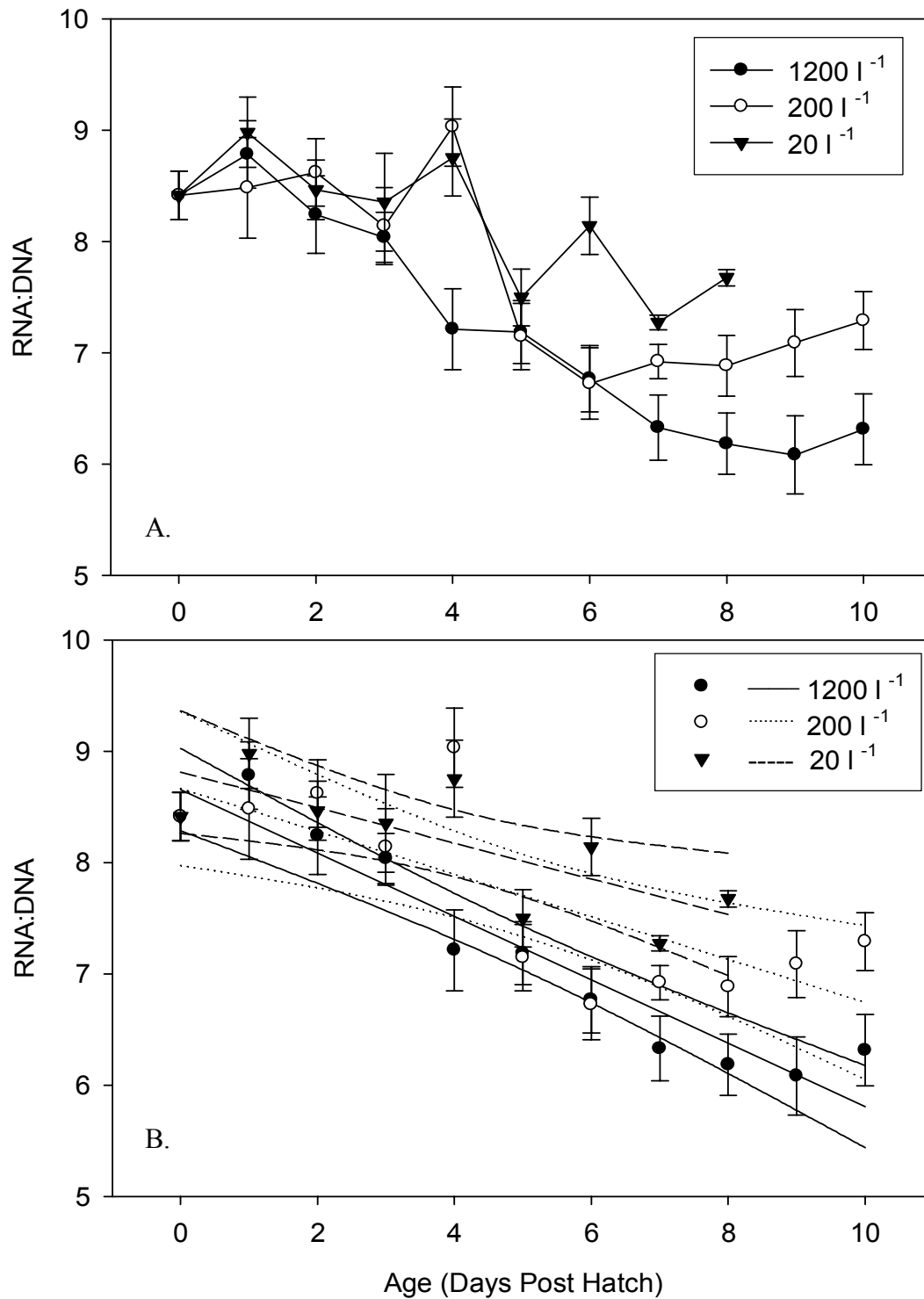


Figure 11. *Gobiosoma bosc.* A) Line plot and B) regressions with 95% confidence intervals of mean \pm SE of RNA:DNA ratios regressed on age (DPH) for three laboratory food treatments.

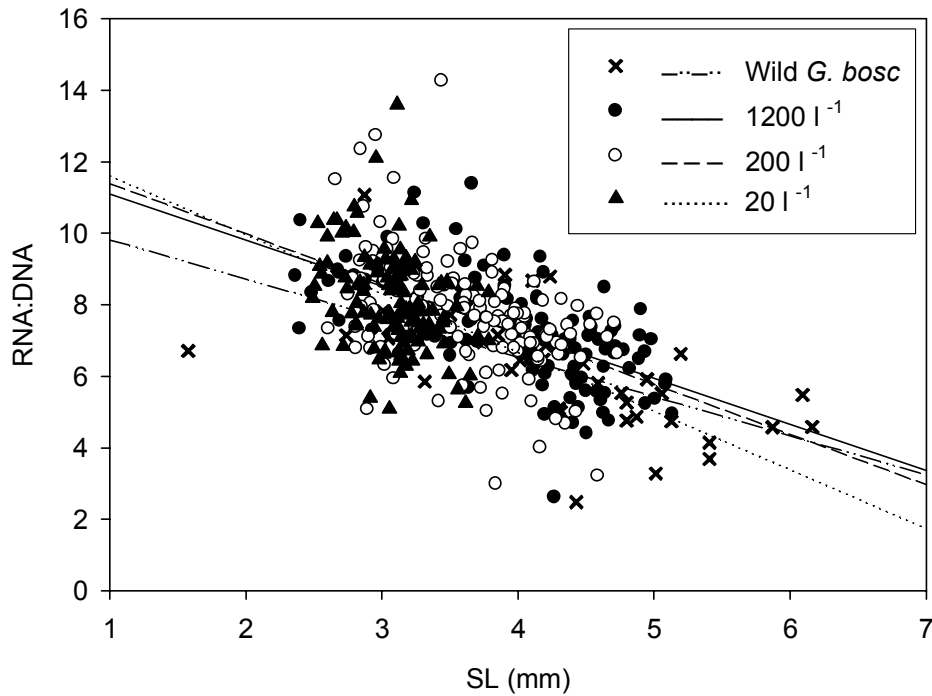


Figure 12. *Gobiosoma bosc*. RNA:DNA ratios of fish from three laboratory food treatments and wild fish in relation to length (mm SL).

significant. Additionally, there were no significant differences between treatments when RNA:DNA was compared between size classes (1mm SL bins) (Figure 13). RNA:DNA was inversely related to DW (Figure 14), and regression slopes were significantly different by treatment (t-tests, $p \leq 0.010$) and shared a common intercept (t-tests, $p \geq 0.507$). The slope was less negative in the 1200 l^{-1} treatment than in either of the other two treatments. Overall treatment effects were not significant ($F = 0.26$, $p = 0.768$) while interaction effect was ($F = 10.43$, $p < 0.0001$).

Additionally, differences in RNA:DNA among treatments were examined for those DW size classes with 2 or more treatments represented (Figure 15). RNA:DNA ratios were not significantly different among treatments in the smallest size class (LSMEANS, $p \geq 0.234$) but

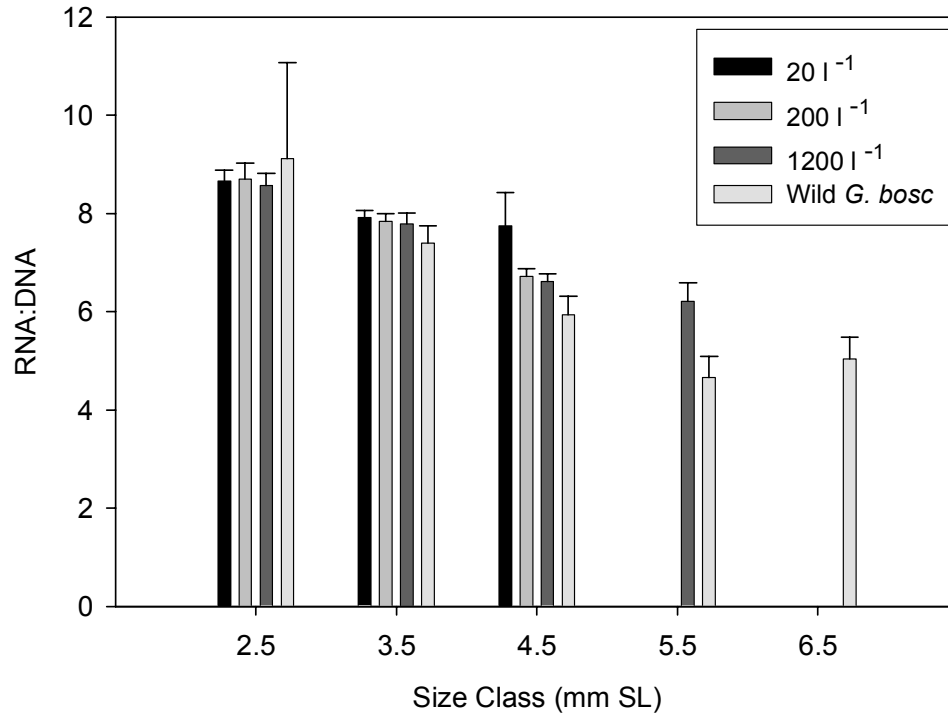


Figure 13. *Gobiosoma bosc.* RNA:DNA ratios of laboratory-reared and wild fish in relation to size class (mm SL). Size classes are denoted by midpoints of lengths included in each class.

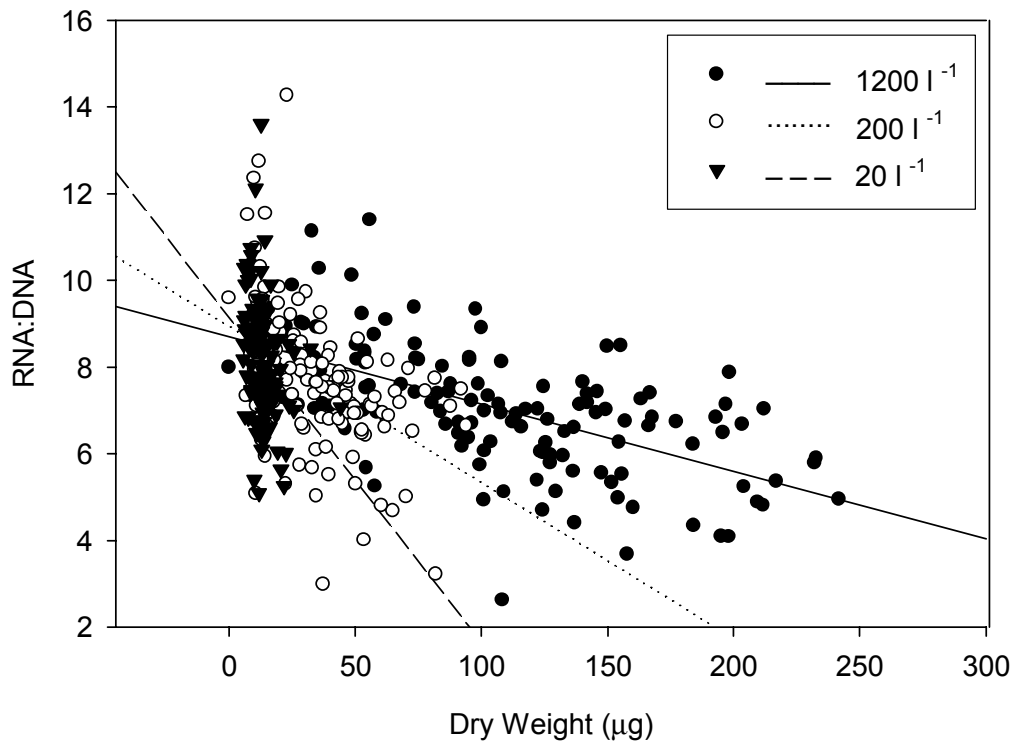


Figure 14. *Gobiosoma bosc.* RNA:DNA regressed on dry weight (µg DW) for three laboratory food treatments.

became different when weights reached the 30 μg size class and remained higher in the 1200 l^{-1} treatment than in the 20 l^{-1} treatment through the 50 μg size class. Ratios were not significantly different between the 200 and 1200 l^{-1} treatments for the two largest size classes presented (LSMEANS, $p \geq 0.151$), nor were they different between the 20 l^{-1} and 200 l^{-1} treatments within any size class (LSMEANS, $p \geq 0.339$). As with previous analyses with size variables, residuals of RNA:DNA regressed on SL and DW were not normally distributed due to outliers, and \ln -transformed data were less normal than untransformed data. Additionally, Shapiro-Wilk values were high on untransformed data ($W = 0.974$), so analyses were performed on untransformed data.

RNA:DNA ratios were directly related to trends in growth rates (Figure 16), as both variables declined with age. However, magnitudes of growth rates were inversely related to RNA:DNA ratios, with higher ratios in individuals with lower growth rates. Regression slopes were not significantly different between treatments, indicating that RNA:DNA increased similarly per unit change in growth rate for all treatments.

Wild Larvae

Wild *G. bosc* larvae were of similar size to those from the laboratory experiment, ranging from 2.74 to 6.16 mm SL with mean length of 4.31 mm ($n = 37$ fish). Wild fish demonstrated trends in RNA and DNA accumulation similar to those from laboratory-reared fish when compared to SL (Figure 8) but seemed to have slightly higher variation. RNA:DNA ratios for wild larvae regressed on SL also followed similar patterns to laboratory-reared fish (Figure 13). RNA:DNA was inversely related to SL, with the regression slope not differing significantly from slopes of laboratory treatments ($t = 0.68$, $p = 0.494$) and declining over the range of sizes collected. Analysis of RNA:DNA by size class detected no significant differences between wild

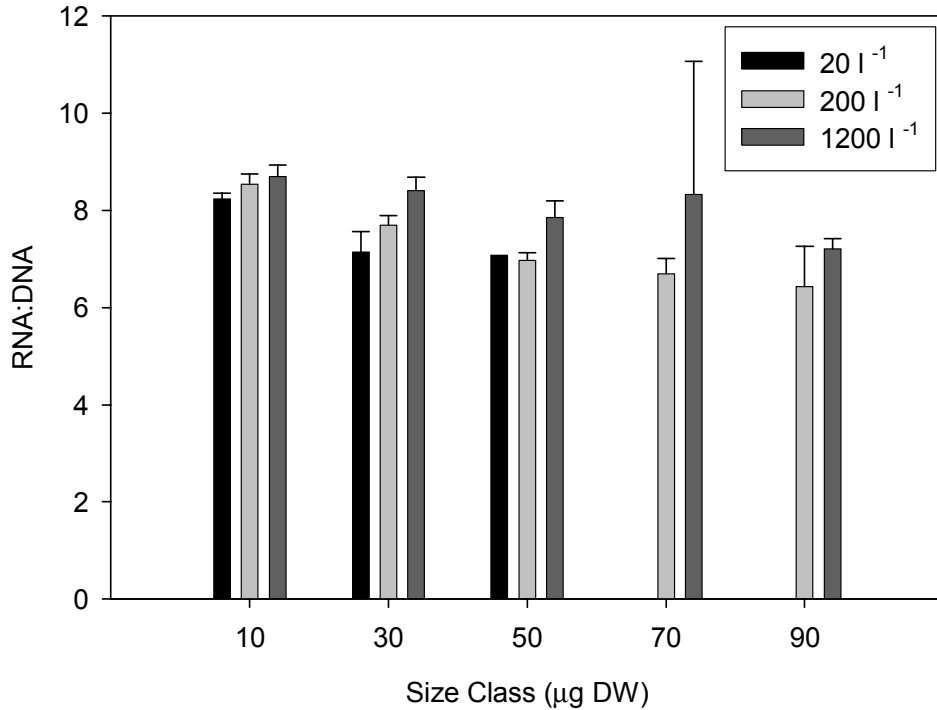


Figure 15. *Gobiosoma bosc.* Mean \pm SE of RNA:DNA ratios in relation to size class ($\mu\text{g DW}$). Only size classes where two or more treatments are represented were included in analysis. Size classes are denoted by midpoints of weights included in each class.

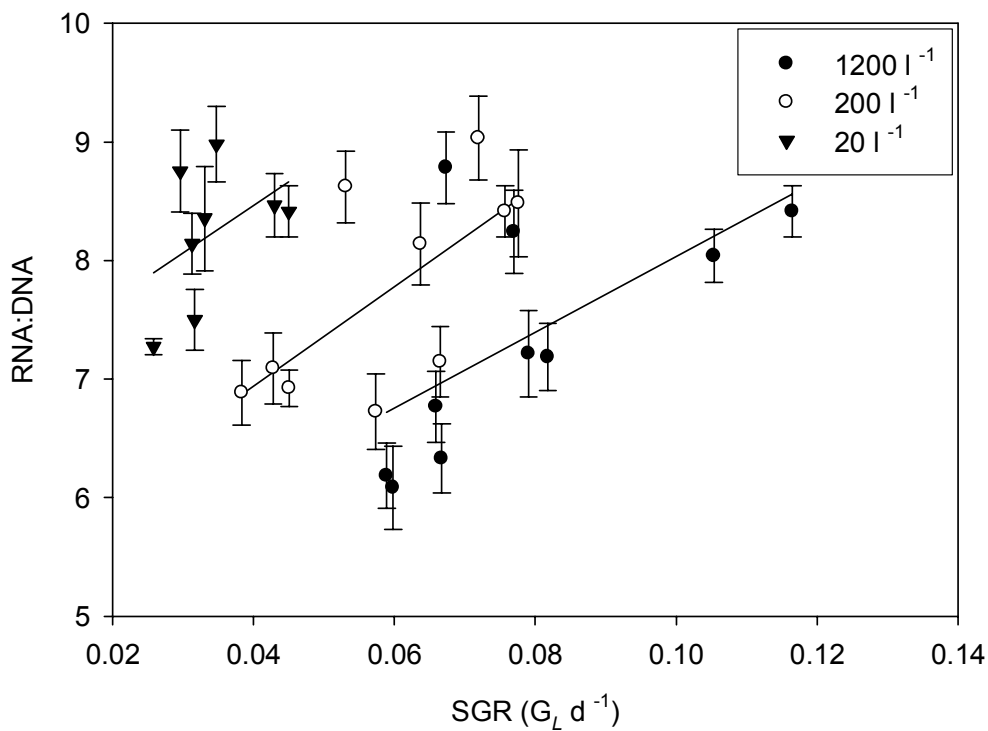


Figure 16. *Gobiosoma bosc.* Relationship of mean (\pm SE) RNA:DNA to length-specific growth ($G_L d^{-1}$) for three laboratory food treatments.

fish and those reared in the laboratory (LSMEANS, $p \geq 0.151$). The fact that RNA:DNA values for wild larvae fell within the range of values obtained from laboratory-reared fish indicated that the range of prey concentrations used in laboratory trials reasonably reflected *in situ* conditions. Environmental data (mean \pm SE, N=3, range) from the field collection site in August 2005 reflected similar means and ranges for temperature (32.5 ± 0.63 , 31.2-34.4°C) and salinity (12.1 ± 0.72 , 9.7-14.0 psu) conditions to those maintained in the laboratory. Otoliths were obtained from wild specimens, but were not able to be aged as yet.

DISCUSSION

This study tested the concept of using RNA:DNA ratios to determine condition of laboratory-reared *Gobiosoma bosc* larvae for future application to fish in the wild. I utilized estimates of instantaneous growth and mortality, measures of body size, known ages, and RNA and DNA measurements in laboratory-reared fish to examine nucleic acid-based indices of condition for individuals from three different food environments. These indices were then compared to measurements from wild larvae to evaluate applicability of the RNA:DNA ratio as an index of individual condition in early life history stages in this species. Overall, the RNA:DNA ratio varied in its ability to distinguish poorly-fed from well-fed larvae depending upon the covariate to which it was related. The relationship of RNA:DNA to prey concentration was dependent upon age and size, and was only a good indicator of nutritional condition in naked goby larvae when compared within the larger size classes found in this experiment. However, trends in RNA:DNA were consistent with trends in growth, while magnitudes of RNA:DNA were higher in poorly-fed individuals. Thus, RNA:DNA ratios were not ideal indicators of growth under these experimental conditions. The ratio of DNA to DW, on the other hand, proved to be successful in delineating poorly-fed from well-fed fish after 4 DPH and seems to be a better index of condition in this situation. All nucleic acid and growth measurements demonstrated ontogenetic effects, reinforcing the need for knowledge of life-history and evaluation of ontogenetic effects on biochemical indices in experimental species.

Utility of the RNA:DNA Ratio

Trends in RNA:DNA with age for all fish in this experiment support the notion of an ontogenetic pattern. Overall declines in RNA:DNA along with periodic increases and decreases denoted changes to cell growth dynamics, which were consistent with the idea of a stressful

developmental period. Declines in RNA:DNA following yolk absorption are in fact a well-documented occurrence in many species of fish. Clemmesen & Doan (1996) reported declines in both fed and starved larval cod *Gadus morhua* in relation to age through 7 DPH. Caldarone et al. (2003) also showed declines in RNA:DNA for larval cod following onset of feeding, and similar trends have been demonstrated in larval herring *Clupea harengus* (Clemmesen 1987), Dover sole (Bergeron & Boulhic 1994), and red drum *Scianops ocellatus* (Westerman & Holt 1994). Falling ratios during early ontogeny are usually attributed to nutritional stress related to learning to feed exogenously or to poor feeding conditions, but can also appear throughout the larval period during stages of rapid development and tissue reorganization (Clemmesen et al. 1987, Raae et al. 1988, Westerman et al. 1988, Bergeron et al. 1991). An interesting pattern is that ratios fell despite overall increases in both RNA and DNA in all treatments, and were lower in fish from higher prey treatments despite higher concentrations of both RNA and DNA in well-fed fish. Thus, the observed trends in RNA:DNA ratios were not caused by stable DNA coupled with declining RNA as has been observed in most studies of this type (i.e., Clemmesen 1994), but by very slight differences in the rate of change for RNA with respect to DNA, both of which exhibited simultaneous patterns of change. Also of interest is that RNA:DNA increased at 1 DPH despite declines in both RNA and DNA on that day in all treatments. This relationship indicates that magnitudes of RNA:DNA ratios were not a result of differences in condition as expected, but were instead closely linked to cellular changes in nucleic acid content occurring during development.

The relationship between RNA:DNA and growth rate further solidifies the argument for ontogeny-based patterns in nucleic acids in larval *G. bosc.* Direct correlation of RNA:DNA and growth rate has been demonstrated in larval cod *Gadus morhua* (Buckley 1979), sand lance

Ammodytes americanus (Buckley et al. 1984), red drum *Sciaenops ocellatus* (Westerman & Holt 1994), and silver sea bream *Sparus sarba* (Deane et al. 2003) among others. RNA:DNA is generally considered a predictor of growth since RNA activity determines protein synthesis on a cellular level. Patterns in the RNA:DNA and growth were directly related in this study, but the magnitude of RNA:DNA was inversely related to growth rate, with higher ratios in fish with lower growth. There are two implications in this relationship: 1) declining RNA:DNA ratios and growth with age, regardless of feeding environment, indicate that ontogeny is likely the main force behind changes to each over the course of the experiment; and 2) inverse relationships among magnitudes of RNA:DNA and growth rate suggest that RNA:DNA is not a good indicator of potential protein synthesis, and thus condition to some extent, in larval naked goby.

There is more than one plausible explanation for the latter. Several authors (Lied et al. 1982, Foster et al. 1991, Foster et al. 1992c) demonstrated that protein synthesis is a result not only of ribosome number (i.e., RNA content) but also specific activity of ribosomes, and that the two variables can vary independently. Additionally, several studies suggest that there may be an uncoupling of RNA concentration and protein synthesis due to the dynamics of cell proliferation (hyperplasia) and cell growth (hypertrophy) in larval fish (Westerman & Holt 1988, Mathers et al. 1993, Pelletier et al. 1995, Gwak 2002). Both of these possibilities may have consequences for this study.

Differences in the activity of ribosomes could have implications here given that RNA:DNA ratios were higher in individuals with lower growth rates. Two pathways for achieving somatic growth in fish have been suggested: increased RNA content, or increased ribosomal activity (Rosenlund et al. 1983, Miglavs & Jobling 1989, Mathers et al. 1993). The dominance of one pathway over another depends on particular species-specific circumstances.

Fish in good condition experiencing high rates of growth may also experience lower cell and protein turnover rates (Mathers et al. 1993), which would lower protein production requirements. It may be metabolically more efficient for a healthy cell to up-regulate ribosomal activity instead of producing new ribosomes. This would lead to fewer ribosomes per cell and thus less RNA per unit DNA in an individual. Fish in poorer condition likely experience higher rates of protein turnover necessitating increased protein synthesis, and may not possess the cellular regulation mechanisms to allow for up-regulation of ribosomal activity. Another possibility is that fish in poor condition employ compensatory mechanisms, where spikes in RNA production may be a last effort to survive, or due to the loss of cellular control mechanisms caused by a lack of nutrition (Raae et al. 1988).

DNA:DW as an Alternative Index

The principle of hyperplasia versus hypertrophy is based upon changes to cell dynamics, in which an organism may experience growth via increasing cell numbers (hyperplasia) or increasing cell size (hypertrophy). It follows that individuals experiencing hyperplasia would demonstrate an increase in genetic material (i.e., DNA) along with associated somatic growth (weight gain, for example) whereas individuals undergoing hypertrophy would experience somatic growth without associated increases in DNA. Measurement of DNA in relation to dry weight has been suggested as a measure of these cellular dynamics (Bergeron et al. 1991, Richard et al. 1991, Bergeron 1997, Malzahn et al. 2003). As both DNA and DW are measures of biomass, it is expected that the DNA:DW ratio would remain relatively constant with age, and would be higher in animals subjected to starvation (Dortch personal communication, Bergeron et al. 1991). Further, it is thought that fish exposed to sub-optimal conditions carry out catabolism in two stages: first, lipid and carbohydrate content in cells is re-absorbed and water content

increased, causing cell sizes to shrink slightly while maintaining genetic integrity of the cell body (Erlich 1974a); and second, catabolism of the cell occurs when starvation progresses to more intense levels. Therefore, nutritional condition in fish undergoing moderate levels of starvation should be measurable by the relation of DNA to DW.

In this study, the DNA:DW ratio showed significant treatment effects when related to age and successfully delineated larvae from different feeding environments. The fact that larvae from the 20 l⁻¹ treatment had higher DNA:DW ratios after 4 DPH indicates that individuals were likely experiencing growth via hyperplasia, and that cells were smaller on average than those in fish from higher prey treatments due to catabolism of lipid and carbohydrate content. Fish from the highest prey treatment had the lowest DNA:DW ratios, indicating that growth via hypertrophy was more prevalent. Declines in DNA:DW for all treatments through 4 DPH suggests ontogenetic growth common to all individuals regardless of feeding history. Bergeron (1997) suggested DNA:DW is a better index of nutritional condition in fish larvae because of the inherent variability in RNA:DNA ratios, and the relative stability of DNA and DW measurements in contrast to highly volatile and sensitive RNA measurements. Richard et al. (1991) and Malzahn et al. (2003) reached similar conclusions. My experiment produced analogous results, and it appears that DNA:DW more accurately reflects nutritional condition of naked goby larvae than RNA:DNA ratios at this stage of development.

Nucleic Acid Indices

Total DNA and RNA levels exhibited highly significant treatment effects and also indicated ontogenetic trends when related to age. Both RNA and DNA declined from hatch to 1 DPH in all treatments, with levels in the 20 l⁻¹ treatment declining through 5 DPH before slowly increasing through 8 DPH. The 200 l⁻¹ treatment increased overall with declines at several

points, while the 1200 l⁻¹ treatment increased continually from 1 DPH. Magnitudes of DNA and RNA differed between treatments, with higher levels of genetic material in the higher prey treatments reflecting higher cell numbers associated with elevated growth. Declines in DNA and RNA in all treatments at 1 DPH were likely due to the switch from endogenous to exogenous feeding, a “critical period” in larval development (May 1974), and experienced by larvae of most fish species upon first feeding. Naked goby experience this shift in energy source at around 24 hours after hatch. Subsequent patterns in RNA and DNA were similar between the 20 and 200 l⁻¹ treatments, but concentrations were lower and changes were delayed by one day in the lowest treatment. This implies that ontogenetic changes underlie the patterns in RNA and DNA and are experienced by all *G. bosc* larvae, though perhaps deferred in the lowest treatment due to poor nutrition. Individuals from the 1200 l⁻¹ treatment may have been in good enough condition that these changes did not cause loss of DNA or RNA.

Fish undergo many physiological changes during the larval period, including pigmentation, flexion of the notochord, development of fins, eyes, and organs, as well as a host of morphological changes. In almost all cases, changes are based upon size and not age and can thus be delayed in slow growing individuals (Zwiefel & Lasker 1976, Fukuhara 1986, Chambers & Leggett 1982, Vollestad 1992). In naked goby, early larval changes are generally estimated to occur along the following size scale (SL): first pigmentation at 3.0 mm; re-positioning of the mouth to a terminal position near 3.3 mm; caudal fin rays partially developed by 4.0 mm; snout becomes pointed and anal and dorsal fins differentiated by 7.5 mm (Scotton et al. 1973). Fish in this study experienced most of these transformations during the 8 and 10 day experiment period and, based on sizes at age, dips in RNA and DNA concentrations coincided with predicted timing of changes. Daily images of individuals (not shown) confirmed that pigmentation

occurred later in fish from the 20 l⁻¹ treatment, and notochord flexion did not occur at all by 8 DPH versus 7-8 DPH in the 1200 l⁻¹ treatment and 9-10 DPH in the 200 l⁻¹ treatment. The fact that length tapered off at around 4 mm in the 200 l⁻¹ treatment indicates that the notochord flexion period initiated around this size may be quite stressful for larvae in sub-optimal condition. Complete mortality in the 20 l⁻¹ treatment also occurred near the time of flexion initiation. Thus it is likely that other morphological changes occurred later and at a higher metabolic cost in the lower prey treatments as well, which would explain more variation in patterns of RNA and DNA accumulation.

RNA and DNA concentrations also showed increasing trends when related to SL, which may be due to accumulation of cells and genetic material with size. Lack of significant differences among treatments in the smallest size class indicate that fish of this size were all experiencing similar condition regardless of feeding environment. By the time fish reached 3 mm SL, those from the 200 and 1200 l⁻¹ treatments had accumulated significantly higher concentrations of both RNA and DNA relative to fish from the lowest treatment, and all treatments became differentiated by 4 mm SL. Because DNA concentration is a measure of cell number and RNA is a measure of protein synthesis potential, these changes in relation to SL denote greater weight at length and higher growth rates in the two highest treatments versus the lowest treatment, which is corroborated by growth data. The fact that wild fish showed similar rates of increase in nucleic acid concentration at length suggests that conditions *in situ* were similar to those induced in laboratory treatments and wild individuals were likely of similar weight to experimental fish.

Growth and Mortality

All three treatments provided adequate prey to induce positive growth through 10 DPH, though

at different rates. Magnitudes of weight-specific growth for the three prey treatments exhibited a direct relationship to prey density as expected, and were higher in the 200 and 1200 Γ^{-1} treatments than published values of $\sim 0.15 \text{ d}^{-1}$ for naked goby (Houde & Zastrow 1993, Campfield 2004). Rates in the 20 Γ^{-1} treatment remained near published estimates for the whole experiment. Differences between rates in higher prey treatments in this study and those from previous studies are likely due to the fact that growth can vary with size, temperature, and life stage (Houde 1997). Published rates are for metamorphosis-stage larvae aged 18-20 DPH from laboratory and wild populations, and temperatures ranging from $\sim 22 - 27^\circ\text{C}$, whereas rates from this study were obtained from larvae up to 10 DPH and held at a constant temperature of 27°C . These differences in larval stage and temperature history could have caused the differences in growth rate. Fish in all treatments experienced declining growth rates throughout the experiment, and it is possible that growth rates in *G. bosc* larvae naturally decline through the early larval period and increase after metamorphosis. Similar declines in growth throughout the larval period have been observed in northern anchovy *Engraulis mordax*, bay anchovy *Anchoa mitchilli*, and walleye pollock *Theragra chalcogramma*, and Houde (1997) hypothesized that many taxa exhibit this pattern during larval stages. It is therefore not likely that the decreased growth in the present study is due to experimental design or error, but more so due to growth patterns inherent in *G. bosc* larvae. Another possibility is the effect of maternal condition on egg quality, which can vary according to a variety of environmental and physiological variables. Multiple batches of progeny were used in an effort to resolve these issues, however.

As expected in light of declining growth rates over the course of the experiment, length at age tapered in the two highest food treatments near the end of the experiment, with fish from the 200 Γ^{-1} treatment attaining smaller size at age and reaching peak length for the experiment at an

earlier age than those from the 1200 l⁻¹ treatment. Faster growth in the 1200 l⁻¹ treatment suggests that those fish would be less hindered by physiological changes than fish offered less prey, thus explaining the tapering in SL at a smaller size and earlier age in fish from the 200 l⁻¹ treatment. A less likely explanation for these trends is the reduced availability of properly sized prey for older and larger larvae. Breitburg (1994) fed rotifers to preflexion *G. bosc* larvae, but fed *Artemia* nauplii to post-flexion larvae. Notochord flexion was just starting around 8 DPH in fish from the 1200 l⁻¹ treatment in this study. In *post hoc* experiments, larvae were offered *Artemia* at 10 DPH and did not consume them until 12 DPH and approximately 7 mm SL, which was larger and older than either experimental trial presented here. Intermediate size fractions of prey were not tested for selection by *G. bosc* larvae, however. If fish in the current study attained sizes requiring larger prey, it is feasible that growth would suffer after that point in the presence of smaller prey items. However, it is also highly possible that growth patterns were a result of ontogenetic development.

Mortality in relation to measures of growth and biochemical changes reveal a more complete picture of larval dynamics associated with ontogeny and condition. Results from this experiment show that the chosen treatments had highly significant effects on mortality rates and thus on larval condition. No data are published on *in situ* mortality rates in naked goby for comparison; however, rates for a relative species *Lepidogobius lepidus* (bay goby) are estimated as ranging from 0.13 to 0.51 (Grossman 1979). Estimates of mortality from this study fall within a reasonably similar range. Mortality was substantially higher in the 20 l⁻¹ treatment than in both other treatments, and total mortality in the 20 l⁻¹ treatment occurred around 7-8 DPH, coinciding with the lowest measured RNA:DNA ratios from the other two treatments (e.g., Caldarone 2005). This indicates that, despite having rather high magnitude ratios overall and having higher

ratios than well-fed fish on several days of the experiment, individuals from the 20 l⁻¹ treatment were in fact in poorer condition than those in other treatments. It also reinforces the point that ontogeny likely plays a role in the declining growth in all treatments and suggests that development occurring near days 7 and 8 may be particularly taxing, so that fish from the 20 l⁻¹ treatment already weakened by poor nutrition experienced high rates of mortality.

CONCLUSIONS

Many studies have successfully utilized nucleic acid indices to describe nutritional condition in marine fish larvae. The most utilized index is the RNA:DNA ratio, which has been correlated with variables including prey availability, growth, temperature, and body size (Ferron & Leggett 1994). Additionally, this index has been demonstrated to accurately reflect changes in condition within as little as 1 to 3 days of food deprivation (Robinson & Ware 1988, Richard et al. 1991, etc.), making it potentially suitable for instantaneous assessment of condition.

There are situations, however, when the RNA:DNA ratio is not a useful index for evaluating condition. High variability in RNA:DNA ratios for healthy fish can prevent detection of differences when compared to fish in poor condition. Clemmesen (1994) found such high variability in fed herring larvae that RNA:DNA ratios actually overlapped with, and at times were lower than, those from larvae starved for 3-4 days. Clemmesen (1996) also found difficulty in differentiating herring larvae from high and low food density treatments using RNA:DNA, as ratios were similar between the two groups and fish from the low food treatment occasionally had higher ratios than fish from the high food treatment. Several other studies have reported highly variable ratios for fed larvae as well (Rae et al. 1988, Westerman & Holt 1988, Bergeron et al. 1991).

As with any biochemical measure, the RNA:DNA index may also vary in its ability to describe condition according to species, age, and environmental variables. It is well documented that comparisons in RNA:DNA between fish of different life stages should not be made, as inherent differences in growth rate, metabolism, and overall physiology confound the assessment (Clemmesen 1994, Ferron & Leggett 1994). Species-specific differences in physiology and life-history characteristics are other factors to consider, as they may influence the overall magnitude

of the ratio and ontogenetic patterns in nucleic acid dynamics. Environment plays a role via effects of temperature, salinity, dissolved oxygen, and potential toxins on nucleic acid concentrations.

This study reinforces the importance of thorough species-specific analyses of physiological dynamics before any biochemical index is utilized for condition estimates. Patterns in growth and nucleic acid accumulation were not solely a function of nutritional condition as expected, but were equally a function of ontogenetic development and life history. The fact that naked goby are a nest-brooding species that are more developed at hatch than most larvae likely influenced growth dynamics and played a role in the magnitude of ratios measured during the experiment. Most fish in poor enough condition to experience increased mortality will have RNA:DNA ratios near 1 or 2 (see Clemessen 1994), while poorly-fed fish in this experiment never exhibited ratios lower than ~6. Growth and mortality data support the notion that poorly-fed fish were indeed less viable than those from higher prey treatments, thus magnitudes of RNA:DNA ratios are not an accurate reflection of condition in larval naked goby. This assertion can also be made considering that RNA:DNA ratios weren't a function of nutritional condition at all in comparison with growth rates.

The fact that the RNA:DNA ratio does not provide insights into the role of ribosomal activity limits its utility as an index of growth and condition (Miglav & Jobling 1989). Indices using RNA measurements are subject to ambiguity regarding the pathways being utilized by a cell to attain protein growth. The DNA:DW ratio, however, is a promising alternative to the RNA:DNA index, and was more appropriate for determining condition in larval naked goby in this experiment. Because the use of the DNA:DW index is not dependent on highly sensitive RNA measurements, variability can be minimized. Further, measuring DNA in relation to DW

gives a better idea of cellular growth dynamics occurring at a given time since DNA content per cell is a constant value within a species. Combining estimates of DNA:DW with other condition factors such as protein and somatic growth rates could prove even more promising in determining overall individual condition.

Biochemical indicators have the potential to be very useful in measuring habitat quality in estuarine marsh environments. Because estuaries are so important to such a large number of nekton species for food, shelter, and nursery grounds, it is extremely imperative to be able to detect negative changes to the ecosystem before patterns of habitat use and community structure are disrupted. Utilizing benthic species with high site fidelity as bio-indicators is a promising line of research, and one that should be pursued. Naked goby *Gobiosoma bosc* are well suited for use as an experimental species, though more work is needed to establish baselines for biochemical and growth dynamics over a broader range of ages and sizes in this species.

Future studies of this kind should strive to combine several condition indices to form comprehensive assessments of habitat quality. One index that is just beginning to be investigated is the relation of otolith increment width to nucleic acid-based condition indices (Clemessen & Doan 1996, Morales-Nin et al. 2002). Coupling otolith research with biochemical indicators of condition has broad implications for use in evaluating habitat quality. The ability to determine a history of condition from an individual occupying an altered habitat would give clues as to the timing of onset of habitat degradation, duration spent in the altered area by the individual, and identification of any trade-offs the individual may be experiencing by residing in the habitat. These important aspects of estuarine ecosystem dynamics are paramount to our understanding of the effects of natural and anthropogenic stressors on nekton assemblages in marsh habitats.

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