ECOPHYSIOLOGY OF GROWTH IN THE PACIFIC WHITE SHRIMP

(*LITOPENAEUS VANNAMEI*)

A Dissertation

by

SCOTT JEFFERY WALKER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2009

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

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ABSTRACT

Ecophysiology of Growth in the Pacific White Shrimp

(*Litopenaeus vannamei*). (May 2009)

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Co-Chairs of Advisory Committee: Dr. Addison L. Lawrence
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Ecophysiological responses of *Litopenaeus vannamei* were evaluated as functions of 1) salinity and animal size, 2) temperature and the animal's nutritive state, and 3) dissolved-oxygen concentration and animal size. Growth rate, routine metabolic rate, limiting oxygen concentration for routine metabolism, and marginal metabolic scope were determined for *L. vannamei* maintained and tested at salinities of 2, 10, and 28 ppt, all at 28 ^{o}C. Routine metabolic rate (RMR) was not demonstrably dependent on salinity but decreased with increasing shrimp weight. Limiting oxygen concentration for routine metabolism (LOCr) was independent of shrimp weight up to 9 g; but, for larger shrimp, decreased with increasing weight. Marginal metabolic scope (MMS = RMR/LOCr) also decreased with increasing shrimp weight and was independent of salinity for shrimp weighing up to 9 g; but, like LOCr, MMS was dependent on salinity for larger shrimp. Growth rate was significantly less at 2 ppt than at 10 or 28 ppt, which gave similar growth rates. The effects of four temperatures (20, 24, 28, and 32 ºC) on growth, RMR, LOCr, and MMS were examined for fed and starved *L. vannamei*.

Routine metabolic rate increased with increased temperature both for fed and starved shrimp. Marginal metabolic scope and growth appeared to be positively related and, at 20 ºC, seemed to induce a state of metabolic torpor. Data from the study of chronic effects of hypoxia (~2 mg $O_2 \cdot L^{-1}$) vs. normoxia (> 5 mg $O_2 \cdot L^{-1}$) on ecophysiological responses indicated that although low-DO environments can depress RMR and growth in *L. vannamei*, animals grown under hypoxic and normoxic conditions did not differ in their metabolic responses upon acute exposure to hypoxia, providing no evidence of acclimation to hypoxia in *L. vannamei*.

Data from the above experiments were used to parameterize Ecophys.Shrimp, a computer simulation model of shrimp growth in time-varying environmental regimes. One unified model was able to simulate all my experiments; and, with only minimal adjustment of the model parameter MMSO, it also adequately simulated studies taken from the literature. Thus, Ecophys.Shrimp seems capable of realistically representing the ecophysiological dynamics of shrimp metabolism and growth in various culture systems.

DEDICATION

To my friends and family,

without whom this would not

have been possible

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TABLE OF CONTENTS

Page

ABSTRACT iii

DEDICATION v

ACKNOWLEDGEMENTS vi

TABLE OF CONTENTS vii

LIST OF TABLES x

LIST OF FIGURES xii

CHAPTER

I INTRODUCTION 1

Objectives 7

II EFFECT OF SALINITY AND BODY WEIGHT ON ECOPHYSIOLOGICAL PERFORMANCE OF THE PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*) 8

Introduction 8
Materials and Methods 9
Shrimp and Experimental Culture System 9
Salinity Acclimation of Postlarvae 10
Experimental Respirometry System 11
Respirometry and Data Acquisition 12
Statistical Analysis 15
Results 16
Water Quality 16
Growth and Respirometry 16
Discussion 24

III EFFECTS OF TEMPERATURE, STARVATION AND DIETARY PROTEIN ON ECOPHYSIOLOGICAL PERFORMANCE OF THE PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*) 27

CHAPTER Page

Introduction 27
Materials and Methods 28
Shrimp and Experimental Culture System 28
Preparation of Experimental Diets 29
Effects of Temperature on Fed and Starved Shrimp 30
Effects of Temperature and Dietary Protein Level 31
Respirometry 31
Statistical Analysis 32
Results 33
Water Quality 33
Effects of Temperature on Growth and Survival 33
Effects of Temperature on Respirometric Responses 36
Discussion 47

IV EFFECT OF DISSOLVED OXYGEN CONCENTRATION ON ECOPHYSIOLOGICAL PERFORMANCE OF THE PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*) 51

Introduction 51
Materials and Methods 53
Shrimp and Experimental Culture System 53
Experimental Respirometry System 57
Statistical Analysis 57
Results 58
Water Quality 58
Effects of DO on Growth and Survival 58
Effects of DO on Respirometric Responses 58
Discussion 62

V PARAMETERIZATION OF ECOPHYS.SHRIMP: A DETERMINISTIC SIMULATION MODEL OF SHRIMP GROWTH IN TIME-VARYING ENVIRONMENTAL REGIMES FOR THE PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*) 64

Introduction 64
Adaptation of Ecophys.Shrimp from Ecophys.Fish 64
Materials and Methods 68
Parameterization of Ecophys.Shrimp 68
Statistical Analysis 69
Results 70
Salinity 70

CHAPTER Page

Temperature: Commercial Feed 73
Temperature: 25 and 35% Protein Diets 75
Temperature: Starvation 79
Dissolved Oxygen 82
Discussion 89
Metabolic Rates 89
Growth 98

VI SUMMARY AND CONCLUSIONS 102

Salinity 102
Temperature, Starvation, and Feed Protein 102
Dissolved Oxygen 103
Ecophys.Shrimp 103

REFERENCES 106

APPENDIX A 115

APPENDIX B 118

VITA 125

LIST OF TABLES

TABLE		Page
1	Mean ± SD for water-quality parameters in the culture system, once salinity had attained its nominal treatment levels	16
2	Composition of 25 and 35% protein diets	29
3	Mean ± SD for culture tank water quality parameters in experiment 1 (fed and starved) and experiment 2 (25 and 35% crude protein diet)	34
4	Mean growth rate (g/d) and survival up to and through respirometry for fed and starved shrimp at four different temperatures	35
5	Mean growth rate (g/d) and survival up to and through respirometry for shrimp offered 25 and 35% crude protein diets at 24 and 28 ºC	36
6	Mean RMR (mg $O_2 \cdot g^{-1} \cdot h^{-1}$), LOCr (mg $O_2 \cdot L^{-1}$), and MMS (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) for fed and starved *L. vannamei* subjected to respirometric measurement at four different temperatures	37
7	Analysis of covariance in ecophysiological responses with temperature and feed-protein level	47
8	Mean ± SD for water-quality parameters in the culture system	59
9	Mean growth rate (g/d) and survival (up to 30-d)	59
10	Mean RMR (mg $O_2 \cdot g^{-1} \cdot h^{-1}$), LOCr (mg $O_2 \cdot L^{-1}$), and MMS (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) for L. vannamei redistributed between and within high-DO (>5 mg$\cdot L^{-1}$) and low-DO (2 mg$\cdot L^{-1}$) treatments and subjected to respirometric measurement	60
11	Analyses of covariance for comparison of observed	

TABLE Page

responses versus responses simulated with Ecophys.Shrimp at three salinities: 2, 10, and 28 ppt 70

12 Analyses of covariance for comparison of observed results (shrimp fed commercial production feed, Rangen 45-10) versus Ecophys.Shrimp results at four temperatures: 20, 24, 28, and 32 ºC 73

13 Analyses of covariance for comparison of observed results (shrimp fed either 25 or 35% protein feed) versus Ecophys.Shrimp results at two temperatures: 24 and 28 ºC 76

14 Analyses of covariance for comparison of ecophysiological responses for empirical results versus computer simulated results for shrimp starved at four temperatures: 20, 24, 28, and 32 ºC 80

15 Analyses of covariance for comparison of ecophysiological responses for empirical results versus computer simulated results for four DO regimes 83

16 Comparison of oxygen consumption rates (VO_2) obtained by Rosas et al. (2001a) to those obtained by Ecophys.Shrimp where Mstd is standard metabolic rate 91

17 Routine metabolic rates (RMR) of *L. vannamei* obtained by Villarreal et al. (1994) and simulated RMR and standard metabolic rates (Mstd) obtained from Ecophys.Shrimp 97

LIST OF FIGURES

FIGURE		Page
1	Schematic representation of automated routine respirometer	13
2	Routine metabolic rate (RMR) versus shrimp wet-weight (W) for 2, 10, and 28 ppt salinity treatments	17
3	Marginal metabolic scope (MMS) versus shrimp wet-weight (W) for 2, 10, and 28 ppt salinity treatments	18
4	Limiting oxygen concentration (LOCr) versus shrimp wet-weight (W) for 2, 10, and 28 ppt salinity treatments	19
5	Routine metabolic rate (RMR) versus shrimp wet-weight (W) at 2, 10, and 28 ppt, with power-models at each salinity fitted separately for shrimp weighing ≤ 9 g and shrimp weighing >9 g	20
6	Limiting oxygen concentration (LOCr) versus shrimp wet-weight (W) at 2, 10, and 28 ppt, with power-models at each salinity fitted separately for shrimp weighing ≤ 9 g and shrimp weighing >9 g	21
7	Marginal metabolic scope (MMS) versus shrimp wet-weight (W) at 2, 10, and 28 ppt, with power-models at each salinity fitted separately for shrimp weighing ≤ 9 g and shrimp weighing >9 g	22
8	Linear growth rate from day 50 vs. MMS adjusted to a common weight of 15 g	23
9	Routine metabolic rate (RMR) over time for shrimp within the four temperature treatments: A, 20 ºC (fed: $y = 0.285 x^{-0.105}$; starved: $y = 0.378 x^{-0.275}$); B, 24 ºC (fed: $y = 0.260 x^{0.105}$; starved: $y = 0.719 x^{-0.392}$); C, 28 ºC (fed: $y = 0.397 x^{0.042}$;	

FIGURE Page

starved: y = 1.854 x $^{-0.759}$); and, D, 32 ºC (fed: y = 0.347 x $^{0.143}$; starved: y = 0.1.614 x $^{-0.639}$ 38

10 Limiting oxygen concentration for routine metabolism (LOCr) over time for shrimp within the four temperature treatments: A, 20 ºC (fed: y = 2.204 x $^{-0.060}$; starved: y = 1.842 x $^{0.017}$); B, 24 ºC (fed: y = 0.921 x $^{0.220}$; starved: y = 2.088 x $^{-0.023}$); C, 28 ºC (fed: y = 1.771 x $^{0.022}$; starved: y = 2.462 x $^{-0.047}$); and, D, 32 ºC (fed: y = 1.644 x $^{0.045}$; starved: y = 1.802 x $^{0.076}$)..... 40

11 Marginal metabolic scope (MMS) over time for shrimp within the four temperature treatments: A, 20 ºC (fed: y = 0.095 x $^{0.041}$; starved: y = 0.147 x $^{-0.182}$); B, 24 ºC (fed: y = 0.260 x $^{-0.080}$; starved: y = 0.358 x $^{-0.379}$); C, 28 ºC (fed: y = 0.222 x $^{0.022}$; starved: y = 0.636 x $^{-0.661}$); and, D, 32 ºC (fed: y = 0.216 x $^{0.105}$; starved: y = 0.895 x $^{-0.708}$)..... 43

12 Linear growth rate of fed shrimp vs. MMS adjusted to a common weight of 6 g..... 45

13 Linear rate of weight change of starved shrimp vs. MMS adjusted to a common weight of 6 g 46

14 Schematic representation of oxygen-stripping columns 55

15 Close-up view of component J, N2 gas connection manifold, from Figure 14 56

16 Routine metabolic rate (RMR) versus shrimp wet-weight (W) for *L. vannamei* redistributed between and within high-DO (>5 mg·L^{-1}) and low-DO (2 mg·L^{-1}) treatments..... 60

17 Limiting oxygen concentration (LOCr) versus shrimp wet-weight (W) for *L. vannamei* redistributed between and within high-DO (>5 mg·L^{-1}) and low-DO (2 mg·L^{-1}) treatments 61

FIGURE Page

18 Marginal metabolic scope (MMS) versus shrimp wet-weight (W) for *L. vannamei* redistributed between and within high-DO (>5 $mg \cdot L^{-1}$) and low-DO (2 $mg \cdot L^{-1}$) treatments 62

19 Ecophys.Shrimp: STELLA® model of shrimp growth in response to ecophysiological factors 66

20 Graphical output of shrimp growth under the model Ecophys.Shrimp where y-axis is shrimp weight (g) and x-axis is time (h) 68

21 Routine metabolic rate (RMR) versus shrimp wet-weight (W) at 2, 10, and 28 ppt for observed (obs) and simulated (sim) shrimp 71

22 Marginal metabolic scope (MMS) versus shrimp wet-weight (W) at 2, 10, and 28 ppt for observed (obs) and simulated (sim) shrimp 72

23 Shrimp wet-weight (W) versus time (months) at 2, 10, and 28 ppt for observed (obs) and simulated (sim) shrimp 72

24 Routine metabolic rate (RMR) versus time (d) at 20, 24, 28, and 32 ºC for observed (obs) and simulated (sim) shrimp fed a commercial production feed (Rangen 45-10) 74

25 Marginal metabolic scope (MMS) versus time (d) at 20, 24, 28, and 32 ºC for observed (obs) and simulated (sim) shrimp fed a commercial production feed (Rangen 45-10) 74

26 Weight change (ΔW) versus time (d) at 20, 24, 28, and 32 ºC for observed (obs) and simulated (sim) shrimp fed a commercial production feed (Rangen 45-10) 75

27 Routine metabolic rate (RMR) versus shrimp wet-weight (W) at 24 and 28 ºC for observed and simulated shrimp fed a 25% protein diet 76

FIGURE Page

28 Marginal metabolic scope (MMS) versus shrimp wet-weight (W) at 24 and 28 ºC for observed and simulated shrimp fed a 25% protein diet 77

29 Weight change (ΔW) versus time (d) at 24 and 28 ºC for observed and simulated shrimp fed a 25% protein diet 77

30 Routine metabolic rate (RMR) versus shrimp wet-weight (W) at 24 and 28 ºC for observed and simulated shrimp fed a 35% protein diet 78

31 Marginal metabolic scope (MMS) versus shrimp wet-weight (W) at 24 and 28 ºC for observed and simulated shrimp fed a 35% protein diet 78

32 Weight change (ΔW) versus time (d) at 24 and 28 ºC for observed and simulated shrimp fed a 35% protein diet 79

33 Routine metabolic rate (RMR) versus time (d) for observed (obs) and simulated (sim) shrimp starved at 20, 24, 28, and 32 ºC 81

34 Marginal metabolic scope (MMS) versus time (d) for observed (obs) and simulated (sim) shrimp starved at 20, 24, 28, and 32 ºC 81

35 Weight change (ΔW; g/d) versus time (d) for observed (lab) and simulated (sim) shrimp starved at 20, 24, 28, and 32 ºC 82

36 Routine metabolic rate (RMR) versus shrimp wet weight (W) for observed (obs) and simulated (sim) shrimp maintained for 30-d at near-air-saturation DO and then transferred to near-air-saturation DO 84

37 Marginal metabolic scope (MMS) versus shrimp wet-weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at near-air-saturation DO and then transferred to near-air-saturation DO 84

FIGURE Page

38 Routine metabolic rate (RMR) versus shrimp wet-weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at near-air-saturation DO and then transferred to 2 mg·L^{-1} DO 85

39 Marginal metabolic scope (MMS) versus shrimp wet weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at near-air-saturation DO and then transferred to 2 mg·L^{-1} DO 85

40 Routine metabolic rate (RMR) versus shrimp wet-weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at 2 mg·L^{-1} DO and then transferred to near-air-saturation DO. 86

41 Marginal metabolic scope (MMS) versus shrimp wet-weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at 2 mg·L^{-1} DO and then transferred to near-air-saturation DO 86

42 Routine metabolic rate (RMR) versus shrimp wet-weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at 2 mg·L^{-1} DO and then transferred to 2 mg·L^{-1} DO 87

43 Marginal metabolic scope (MMS) versus shrimp wet-weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at 2 mg·L^{-1} DO and then transferred to 2 mg·L^{-1} DO 87

44 Weight change (ΔW) versus time (d) for observed and simulated shrimp maintained for 30-d at near air saturation DO 88

45 Weight change (ΔW) versus time (d) for observed and simulated shrimp maintained for 30-d at 2 mg·L^{-1} DO 88

46 Comparison of respiratory rates at 30 ºC reported in Palacios et al. (1996) to simulated results obtained from Ecophys.Shrimp 93

FIGURE Page

47 Comparison of respiratory rates at 25 ºC reported in Palacios et al. (1996) to simulated results obtained from Ecophys.Shrimp 94

48 Comparison of respiratory rates at 20 ºC reported in Palacios et al. (1996) to simulated results obtained from Ecophys.Shrimp 94

CHAPTER I

INTRODUCTION

The Pacific white shrimp *Litopenaeus vannamei* (Boone 1931) is one of the most widely cultured shrimp species in the world, and it dominates shrimp mariculture in Texas and the U.S. This decapod crustacean occurs naturally in the eastern Pacific Ocean, from northern Mexico to northern Peru (Holthius, 1980). As with all members of family *Penaeidae*, adult *L. vannamei* live in the open ocean and spawn in coastal waters (Edwards and Bowers 1974). After hatching from pelagic eggs, young shrimp develop through a series of planktonic larval stages into postlarvae (PLs) and migrate into lower-salinity bays, lagoons, and estuaries before returning to the open ocean as young adults (Edwards and Bowers 1974). Penaeid shrimp experience rapid growth during their first six to nine months at which point their growth rate slows (Rothlisberg 1998). It is the period of rapid growth that is exploited in aquaculture (Rothlisberg 1998). The very extensive use of *L. vannamei* in aquaculture makes this shrimp species an appropriate model and target for investigation of the interacting effects of environment on shrimp growth.

Considerable research has been conducted to determine the effects of single-factor environment on growth and performance of penaeid shrimp. As a euryhaline species, *L. vannamei* can tolerate a wide range of salinities, from slightly brackish (1-2 ppt) to hypersaline (40 ppt), according to Stern et al. (1990). However, as noted by Bray et al. (1994), tolerance may define geographic range but it does not necessarily imply an

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This dissertation follows the style and format of the Journal of Crustacean Biology.

optimal level for growth. Previous experiments conducted to evaluate the effect of salinity on *L. vannamei* growth vary both in terms of experimental design and results. In an outdoor tank study designed to mimic pond conditions at various salinities (5, 15, 25, 35, and 49 ppt), Bray et al. (1994) reported maximal growth between 5-15 ppt and least growth at 49 ppt. Huang (1983) measured the growth of postlarval *L. vannamei* over 30-d exposure to 5, 15, 25, 35, and 45 ppt; highest growth was obtained at 25 ppt and lowest growth at 45 ppt. These results are consistent with the observation by Castile and Lawrence (1981), that *L. vannamei* hemolymph is iso-osmotic with seawater diluted to 24.7 ppt. However, the finding of Castile and Lawrence (1981) is somewhat at variance with that of Rodriguez (1981), who reported the iso-osmotic point for *L. vannamei* to be 18-20 ppt.

Wyban et al. (1995) conducted an experiment designed to determine the effects of temperature both in pond culture and in nature on growth of *L. vannamei*. Results reported by Wyban et al. (1995) indicated that reduced growth can be expected for all sizes of shrimp at water temperatures below 23°C and above 30°C for shrimp larger than 16.0 g. Research conducted by Palacios et al. (1996) to determine the effects of temperature and body weight on oxygen consumption showed that the log respiratory rates of *L. vannamei* were linearly related to the log of body weight.

Seidman and Lawrence (1985) conducted experiments with *L. vannamei* and *Penaeus monodon* (Fabricius 1798) postlarvae, with mean ± SD weights of 60.2 ± 0.27 and 55.5 ± 0.23 mg, respectively, to determine the effect of dissolved-oxygen concentration (DO) on growth and feed digestibility at 27.7 ± 0.5 ^{o}C and 30.5 ± 0.7 ppt

salinity. Although the researchers reported no difference in feed digestibility at levels of DO between 1 and 4 mg $O_2 \cdot L^{-1}$, growth was reduced in shrimp held at the lowest DO level. The results of this study suggest that the limiting DO level for growth occurs between 1.17 and 1.91 mg/L for *L. vannamei* and between 1.21 and 2.22 mg $O_2 \cdot L^{-1}$ for *P. monodon*, under the experimental temperature and salinity regime. The researchers opined that growth reduction was possibly caused by reduced feed consumption at low DO; however, they offered no empirical data to support this. Furthermore, the researchers noted that the limiting DO levels obtained were only applicable to shrimp of similar size.

Fry (1947) developed a "physiological classification of environment" whereby components of environment are categorized according to the way in which they influence metabolism of the individual and, consequently, activities such as growth. The six factor classes proposed by Fry (1947) were lethal, masking, directive, controlling, limiting, and accessory. Lethal factors are those factors which kill the organism by interdicting metabolism. A masking factor is a factor that prevents another factor from operating as it normally would if it were not in the presence of the masking factor. Directive factors "allow or require a response on the part of the organism directed in some relation to a gradient of the factor". Controlling factors govern the metabolic rate at the site of metabolism (internal medium). Limiting factors restrict metabolic rate "by virtue of their operation within the metabolic chain". Lastly, accessory lethal factors are combinations/interactions of the previously mentioned factors which result in the death of the organism. Fry (1947) also introduced what he called "metabolic scope for

activity", which he defined as the difference between minimum and maximum rates of aerobic metabolism.

The aforementioned tenets proposed by Fry (1947, 1971) were later elaborated upon and modified by Neill and Bryan (1991) and Neill et al. (2004). Neill et al. (2004) combined those concepts with conventional bioenergetics to form the theoretical basis of Ecophys.Fish, a computer simulation model of fish growth parameterized for the red drum *Sciaenops ocellatus* and bluegill *Lepomis macrochirus*. Ecophys.Fish uses time-varying environmental variables (dissolved oxygen concentration, temperature, pH, salinity, and feed quantity and quality) as inputs, with growth as the primary output. Dissolved oxygen (DO; mg $O_2 \cdot L^{-1}$) is modeled as a limiting factor. The model also accommodates mortality (lethal factor) due to low levels of DO; however, this is the only variable for which lethal effects are modeled within Ecophys.Fish. Salinity is modeled as a loading (masking) factor. Combined with fish weight, salinity is used to establish standard metabolism. The effects of temperature and pH are both modeled as controlling factors. In Ecophys.Fish, directive factors are not addressed and accessory factors are treated as interactions of the other four factors.

According to Brown et al. (2004) "...metabolic rate is the fundamental biological rate because it is the rate of energy uptake, transformation, and allocation." Oxygen consumption rate, via respirometry, has long been used as an indirect measure of metabolic rate, especially in aquatic animals (Keys 1930; Clausen 1936; Fry 1947, 1971). The terms and relationships relevant to the metabolism studies in this dissertation are consistent with those used by Fry (1947, 1971), Springer and Neill (1988), Neill and

Bryan (1991), and Fontaine et al. (2007). The standard and active metabolic rates are those corresponding with minimal and maximum levels of sustained aerobic metabolism, respectively. Routine metabolic rate (RMR) is the rate at which oxygen is consumed by a fasted animal, engaged only in voluntary activity. In aquatic systems, RMR tends to be independent of ambient concentration of dissolved oxygen (DO) over a broad range of values (zone of respiratory independence; Fry 1971). However, for situations in which DO declines to values well below those corresponding with air saturation, an animal that is an oxygen regulator in the zone of respiratory independence may enter the zone of respiratory dependence, where it becomes an oxygen conformer. This transition DO is called the limiting oxygen concentration for routine metabolism (LOCr, which is analogous to the critical oxygen concentration for standard metabolism). Below the LOCr, the animal's respiratory and circulatory systems no longer can meet oxygen demands for standard metabolism and routine activity; consequently, routine activity is constrained and there is a decline in oxygen consumption. Thus, LOCr is a measure of the minimum DO required for biological maintenance and routine activity under a particular set of environmental conditions.

Fry (1947) defined metabolic scope (MS) as the difference between active and standard metabolic rates; thus, MS estimates an animal's capacity for aerobic metabolism beyond that required for biological maintenance. Given the experimental difficulties associated with measuring both active and standard metabolic rates in aquatic poikilotherms, Neill and Bryan (1991) proposed the concept of marginal metabolic scope (MMS), which involves the use of RMR and LOCr data to estimate the marginal

rate of increase in MS available to support animal growth and other activities beyond biological maintenance. Marginal metabolic scope (MMS) is defined as the ratio of RMR to the limiting oxygen concentration for that rate (LOCr), multiplied by 1 mg $O_2 \cdot L^{-1}$ (to make the dimensions of MMS the same as those of MS); thus, MMS is the rate at which MS increases per unit increase in DO, when DO is near LOCr (thus, "marginal"). Neill and Bryan (1991) proposed that MMS reflects the capacity of the animal-environment system to support growth and could therefore be regarded as an integrative measure of environmental quality for animal performance and production. The MMS concept subsequently was incorporated into Ecophys.Fish by Neill et al. (2004), with the parameter MMSO representing the residual intercept of MMS, given the joint effects of temperature, pH, DO, salinity, and animal size. The parameter MMSO can be estimated by iterative simulation of growth trials that terminate with respirometric estimates of MMS (Neill et al. 2004).

Although previous models of shrimp growth have been reported in the literature (Jackson and Wang, 1998; George and Grant, 1983), none has approached the issue from the standpoint of multivariate environmental effects on metabolism and consequent responses as growth. A growth-simulation model based on the effects of ecophysiological variables (dissolved oxygen, pH, temperature, salinity, and feed management) on metabolism could utilize data from existing research to provide users with a basis for aquaculture management and future experimentation as well as providing a teaching tool for educators. To date, no ecophysiology-based simulation

model has been developed and formally presented for *L. vannamei* or any other penaeid shrimp.

Objectives

The specific objectives of this study were 1) to conduct the growth and respirometry experiments necessary to 2) refine and parameterize a deterministic STELLA® simulation model of "growth" (both positive and negative biomass changes) in juvenile *L. vannamei* under time-varying conditions of environmental temperature, salinity, and dissolved oxygen, with accommodation for variation in animal size between 0.02 and 30.0 g, and for extreme nutritive states (satiety vs. starvation).

CHAPTER II

EFFECT OF SALINITY AND BODY WEIGHT ON ECOPHYSIOLOGICAL PERFORMANCE OF THE PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*)

Introduction

The natural life cycle of the Pacific white shrimp *Litopenaeus vannamei* (Boone 1931) involves both marine and estuarine phases. Adults depend on the stable salinity of offshore marine waters for reproduction; whereas, postlarvae and juveniles are adapted to the more euryhaline waters of estuaries (Bishop et al. 1980; Castille and Lawrence 1981; Re et al. 2004). Menz and Bowers (1980) reported that out-migration from estuarine to offshore waters occurs when *L. vannamei* reach a total length of 100 – 200 mm; according to Wyban et al. (1995), this corresponds to a body weight of 8 – 10 g.

As a euryhaline species, *L. vannamei* can tolerate a wide range of salinities, from slightly brackish (1-2 ppt) to hypersaline (40 ppt), according to Stern et al. (1990). However, as noted by Bray et al. (1994), tolerance does not necessarily define the optimum for ecophysiological performance even though tolerance limits may bound geographic distribution. At the core of ecophysiological performance are metabolic responses to variation in environmental factors (Fry 1947, 1971).

Due in part to extensive use of penaeid shrimp in aquaculture, numerous studies have been conducted to determine the effects of short-term variation in salinity on metabolism as reflected in respiration rates (Villarreal et al. 1994; Rosas et al. 2001(b); Pillai and Diwan 2002; Setiarto et al. 2004; Re et al. 2004). However, relatively few studies have approached the issue from the perspective of chronic effects of salinity

(Roy et al. 2007) and body weight (Palacios et al. 1996) on metabolic rate in salinity-acclimated shrimp. One objective of the present study was to measure the effects of salinity at three levels—2, 10, and 28 ppt—on MMS and its components, for *L. vannamei* growing through a range of sizes (1.9 to 30.7g). A second objective was to assess the relationship between MMS and growth rate.

Materials and Methods

Shrimp and Experimental Culture System

Specific-pathogen-free postlarvae (PLs) of *Litopenaeus vannamei* (~ 0.02 g, "Kona" strain; Hennig et al. 2004) for this study were obtained from The Oceanic Institute, Makapu Point, HI, and reared in a semi-closed recirculating seawater system at the Texas AgriLife Mariculture Research Lab, a Texas A&M System research facility in Port Aransas, Texas. Seawater was obtained from the Corpus Christi (Texas) Ship Channel and pumped through a series of high-volume, pressurized sand filters, and then into outdoor storage tanks. The experimental culture system consisted of three indoor recirculating subsystems, each containing three 3.7-m-diameter circular tanks, connected in parallel with a biofilter, intake sump, return sump, and foam fractionator. Flow rate of re-circulated water through each tank was set at 30 $L \cdot min^{-1}$ using adjustable inflow meters. Total volume of each subsystem was approximately 19,000 L. Water temperature of each subsystem was maintained near 28 ºC using central heating and air-conditioning. Dissolved oxygen concentration (DO) for each culture tank was maintained near air saturation using two fused-silica airstones per tank connected to a low-pressure blower. A 12h:12h photoperiod was implemented with an automated light-

control system. Temperature, salinity and DO were monitored at least daily, using a YSI 85® meter (YSI Inc., Yellow Springs, OH). Tests for ammonia, nitrate, and nitrite were conducted weekly, using methods adapted from those of Spotte (1979a,b) and Solarzano (1969) for ammonia; Spotte (1979a,b) and Mullen and Riley (1955) for nitrate; and, Spotte (1979a,b) and Strickland and Parsons (1972) for nitrite. A YSI pH 100® meter was used to measure pH once weekly.

Salinity Acclimation of Postlarvae

Prior to the introduction of shrimp, two of the three previously described culture subsystems were filled with seawater diluted to 10 ppt with municipal water and de-chlorinated using sodium thiosulfate. The third subsystem was filled with seawater from the facilities storage tanks (35 ppt). Approximately 6000 *L. vannamei* PLs were equally distributed among the three culture tanks of the third subsystem. Over a 30-d period, the salinity of the third subsystem was lowered from 35 ppt to 10 ppt, by dilution with de-chlorinated municipal water. Shrimp PLs then were harvested from the third subsystem and restocked into all three subsystems at a stocking density of 50/m^2 (525 shrimp per tank). One subsystem remained at 10 ppt while the salinities of the other two were raised to 28 ppt and lowered to 2 ppt, respectively, over a 15-d time span. The three subsystems then were maintained at salinities of 28, 10, and 2 ppt, respectively, for the duration of the experiment. Batch water exchanges (20% every 3 days) were conducted using seawater from outdoor storage tanks diluted to nominal salinities with reverse-osmosis (RO) water. Shrimp in each culture tank were offered a commercial feed

(Rangen 45-10; Rangen Feeds, Buhl, ID) in excess, using automatic feeders, for the duration of the study. Uneaten feed, feces, and exuviae were removed daily.

Experimental Respirometry System

Automated routine respirometry (Springer and Neill 1988) was conducted in three water baths, each consisting of a fiberglass tank (1.52 x 1.52 x 0.44 m deep) containing 690 L of water. Each water bath was set up as a closed recirculating system, with temperature maintained near 28 ºC via an in-line heat exchanger, and salinity at 2, 10, or 28 ppt, via addition of reverse-osmosis (RO) water or seawater. DO in each water bath was maintained at near air-saturation using two fused-silica airstones connected to a low-pressure blower. Because experimental shrimp were maintained in nine culture tanks, but my respirometry system had only eight units, an incomplete randomized-block sampling regime was employed. Twenty-four hours prior to respirometry, one shrimp from eight of the nine culture tanks was netted and transferred to a square polyethylene bucket (0.23 x 0.23 x 0.37 m) placed inside the respirometry water-bath with the same salinity. This was done to prevent ingestion of feed during the 24 h prior to respirometry. Each bucket had a plastic-mesh-covered, 0.04-m-diameter hole on each of the four sides below the water line to allow for water exchange.

Automated routine respirometry was performed on 72 shrimp—8 from each treatment tank—over a 9-day period each month for six months, for a total of 432 shrimp. At the end of each 9-day period, the respirometry water-bath tanks were drained and cleaned. Seventy-two hours prior to the next 9-day respirometry episode, the tanks were filled with the appropriate mix of seawater and RO water to yield the desired level

of salinity (2, 10, or 28 ppt) and to give time for the water to reach 28 ºC. Including the period of time for salinity acclimation, total length of this experiment was 211 days (January - August, 2006).

Respirometry and Data Acquisition

Following methodology adapted from Springer and Neill (1988), Neill and Bryan (1991), Clark (2003), and Fontaine et al. (2007), automated routine respirometry was performed to determine the effects of salinity and shrimp weight on RMR, LOCr, and MMS. The eight shrimp for a given day's trial were weighed individually, then placed into the respirometry chambers (Figure 1). Because respirometric analysis was performed on shrimp ranging in size from 1.9 to 30.7g, three different sizes of respirometry chamber (total respirometer volumes of 0.77, 1.25, and 1.75 L, respectively) were used, depending on the size of the shrimp to be accommodated. Respirometry was conducted with the respirometers submerged in the previously described respirometry water-baths. Each of the eight respirometers consisted of glass and plastic chambers into which a YSI model 600R multi-environmental probe had been inserted. The YSI probes monitored environmental data every 15 sec and transmitted that information to a microcomputer; then, for each of the eight respirometers, the four values of a given environmental variable obtained each minute were averaged and written to the computer's hard-drive. Two powerhead-pumps (Micro-Jet MC320, Aquarium Systems Inc.) were attached to each respirometer and controlled by the computer. One pump was used to recirculate water within the respirometry chamber;

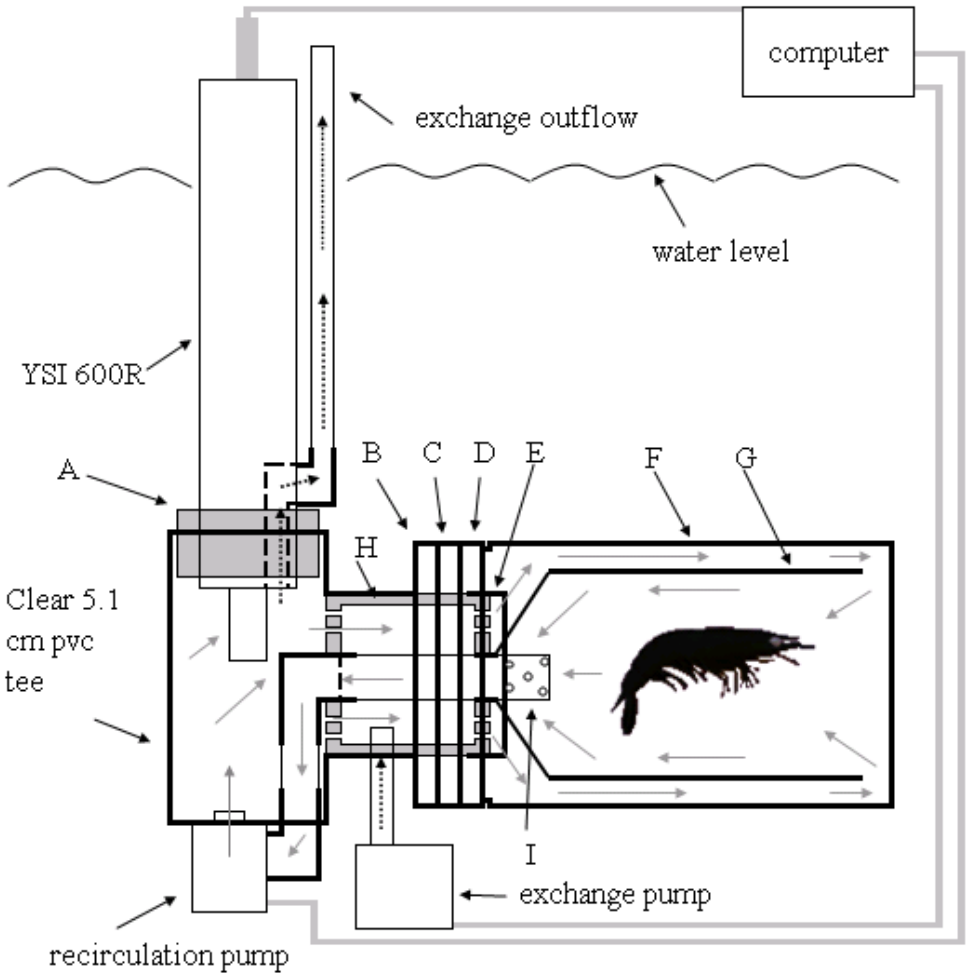


Figure 1. Schematic representation of automated routine respirometer. A = 5.1 x 3.2 cm polyvinyl chloride (pvc) reducer bushing; B = 1.0-cm-long segment of schedule 80 pvc pipe glued to a pvc tee with outside diameter (O.D.) of 9.4 cm, one side (facing YSI) routed to a depth of 0.5 cm with an inside diameter (I.D.) of 7.8 cm and the other side (facing jar) having an inside diameter of 7.0 cm; C = 1.0-cm-thick, ring-shaped, closed-cell neoprene gasket, with I.D. 7.0 cm and O.D. 9.4 cm; D = Qorpak® jar lid and 40-mm pvc gasket, both with a 7.0-cm-I.D. center hole; E = 5.1-cm threaded pvc coupling cut to a length of 1.5 cm; F = Qorpak® straight-side round glass jar (480 ml, 960 ml, or 1460 ml); G = clear plastic insert sleeve; H = 5.1 x 7.2 cm pvc nipple threaded on the jar side of the respirometer, held in place by the exchange pump connection, with a perforated manifold glued to one side (facing YSI) ; and, I = support for G, with a perforated manifold fitting flush with the I.D. of H. Components B – E and H form a compression fitting that seals the jar to the respirometer housing. Arrows within the respirometer show direction of water flow.

whereas, the other was used for intermittent exchange of oxygen-depleted water with aerated water from the surrounding water-bath. Pumps were controlled by an automated respirometry program written in LabVIEW (National Instruments, Inc.) and functionally equivalent to that described by Springer and Neill (1988). The LabVIEW program accepted user inputs of shrimp weight and chamber volume, monitored DO, and computed rate of oxygen consumption (VO_2, mg $O_2 \cdot g^{-1} \cdot h^{-1}$) in each respirometer every minute, using the following formula:

$$VO_2 = ((DO_i - DO_{i-1}) \cdot vol)/((t_i - t_{i-1}) \cdot W)$$

where DO is the per-minute average of dissolved oxygen concentration, in mg $O_2 \cdot L^{-1}$; vol is respirometer chamber volume, less shrimp volume, in L; W is shrimp weight, in g; and, t is time, in h. The program then applied the Springer and Neill (1988) algorithm for determining LOCr, and restarted the water-exchange pump on a given respirometer as soon as the LOCr had been declared. The LOCr algorithm detected statistically significant deviation in VO_2 from the cumulative linear trend established by the series of preceding values in that "run," a run being the series of VO_2 values recorded as DO in the closed respirometer declined from 5.25 mg/L. The value of regressed VO_2 at the LOCr was declared the RMR for that run.

Clark (2003) and Fontaine et al. (2007) advocated not using data collected in the first four hours of respirometry because those data may be compromised by disturbance from human activity and/or increased metabolic activity due to handling stress. In this study, shrimp were held in the respirometry chambers in a state of continuous water exchange with the surrounding bath for 4 h before data acquisition began. At the end of

this habituation period, shrimp were subjected to 18-19 h of respirometry, removed from the respirometry chambers, and a "blank run" then was conducted with each respirometer for ~30 min in order to estimate biological and chemical oxygen demand (BCOD; mg $O_2 \cdot g^{-1} \cdot h^{-1}$), expressed per gram of weight for the shrimp just removed. Immediately following BCOD determination, new shrimp were loaded into the respirometry chambers and each YSI probe was equipped with a new DO membrane and calibrated according to the manufacturers' instructions. For each shrimp subjected to respirometric analysis, mean values of RMR (adjusted for BCOD; mg $O_2 \cdot g^{-1} \cdot h^{-1}$); LOCr (mg $O_2 \cdot L^{-1}$); and, their ratio, MMS (RMR/LOCr), were calculated. Typically, 10-15 values of RMR, LOCr, and MMS—one set for each of 10-15 runs—were obtained for each shrimp subjected to respirometry. Observed values of MMS that were greater or less than one standard deviation from the mean were rejected (as well as their component RMR and LOCr values), and adjusted mean values then were calculated.

Statistical Analysis

Statistical analyses were performed using SPSS (version 13.0, SPSS Inc., Chicago, Illinois). Final-weight data (n = 25 shrimp/tank) from culture-tank shrimp were analyzed using one-way analysis of variance to determine if significant differences for growth existed among treatments. Bonferroni multiple comparison tests (Ott and Longnecker 2001) were used to resolve differences among treatment means. Respirometry data then were subjected to analysis of covariance to test for effects of salinity and body weight (W) on RMR, LOCr, and MMS, where the dependent variable

was either lnRMR, lnLOCr, or lnMMS; the factor was salinity; and, the covariate was lnW. Differences were considered significant at $P < 0.05$.

Results

Water Quality

Means (± SD) for DO, temperature, salinity, total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3) and pH are shown in Table 1.

Table 1. Mean ± SD for water-quality parameters in the culture system, once salinity had attained its nominal treatment levels. Temperature, salinity, and dissolved oxygen (DO) were measured daily. Total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), and pH were measured weekly.

	Salinity		
Parameter	2 ppt	10 ppt	28 ppt
Temperature (ºC)	28.0 ± 0.61	28.0 ± 0.66	28.2 ± 0.77
Salinity (ppt)	2.1 ± 0.13	10.1 ± 0.20	28.3 ± 0.72
DO ($mg \cdot L^{-1}$)	6.5 ± 0.31	6.2 ± 0.27	5.5 ± 0.32
TAN ($mg \cdot L^{-1}$)	1.3 ± 0.81	0.2 ± 0.10	0.1 ± 0.05
NO_2 ($mg \cdot L^{-1}$)	0.1 ± 0.09	0.1 ± 0.11	0.1 ± 0.10
NO_3 ($mg \cdot L^{-1}$)	10.0 ± 9.29	8.2 ± 7.47	10.4 ± 9.66
pH	7.2 ± 0.81	7.3 ± 0.71	7.7 ± 0.41

These physiochemical factors remained within acceptable ranges for penaeid shrimp, based on recommendations of Allan et al. (1990),Chen and Lei (1990), and Chen and Lin (1991).

Growth and Respirometry

Of the 432 shrimp randomly selected for respirometry, no data were collected from four animals, either because of death during respirometry or equipment failure. In

addition, data from three other animals were rejected on the basis of a test for outliers (standardized residuals of lnMMS). The final analysis was based on a total sample of 425 shrimp—140 at 2 ppt, 143 at 10 ppt, and 142 at 28 ppt.

Final mean (± SD) values for individual body weight (W) of culture-tank shrimp from the 2, 10, and 28 ppt treatments were 20.2 (±3.97), 24.7 (± 2.74), and 25.7 (± 3.22) g, respectively. One-way ANOVA indicated a significant difference ($P < 0.001$) among treatments. Bonferroni multiple comparison tests declared the 2 ppt treatment significantly different from the 10 and 28 ppt treatments ($P < 0.001$, for both comparisons), but the 10 and 28 ppt treatments not significantly different from each other ($P = 0.212$).

Routine metabolic rate (RMR, mg $O_2 \cdot g^{-1} \cdot h^{-1}$) decreased with increasing shrimp weight for all three salinities tested (Figure 2).

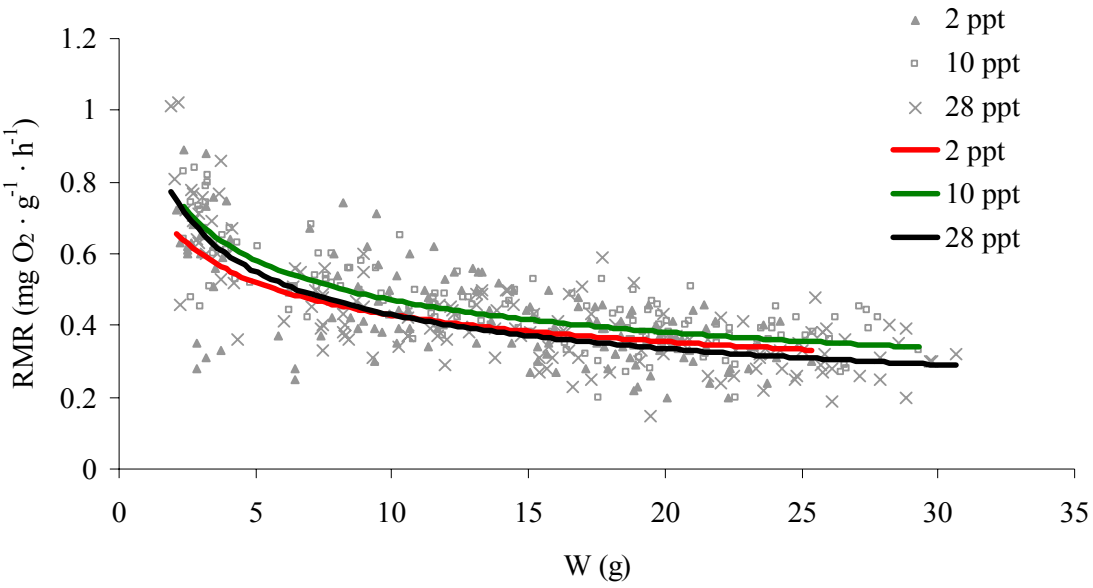


Figure 2. Routine metabolic rate (RMR) versus shrimp wet-weight (W) for 2, 10, and 28 ppt salinity treatments.

Regression of lnRMR (y) on lnW (x) yielded the following power-model equations: $y = -0.210 x^{-0.276}$, $y = -0.053 x^{-0.304}$, and $y = -0.024 x^{-0.357}$ for the 2, 10, and 28 ppt treatments, respectively. Analysis of covariance showed no significant difference in lnRMR among salinity treatments (P = 0.067). Marginal metabolic scope (MMS, mg $O_2 \cdot g^{-1} \cdot h^{-1}$) decreased with increasing shrimp weight for all three salinities tested (Figure 3). Regression of lnMMS on lnW yielded the following power-model equations: $y = -0.998 x^{-0..281}$, $y = -1.005 x^{-0.233}$, and $y = -0.970 x^{-0.260}$ for the 2, 10, and 28 ppt treatments, respectively. Analysis of covariance showed no significant difference among salinity treatments (P = 0.293). Limiting oxygen concentration (LOCr, mg $O_2 \cdot L^{-1}$) also decreased with increasing shrimp weight for the 10 and 28 ppt treatments, but not for the 2 ppt treatment; however, that relationship presented greater variability than

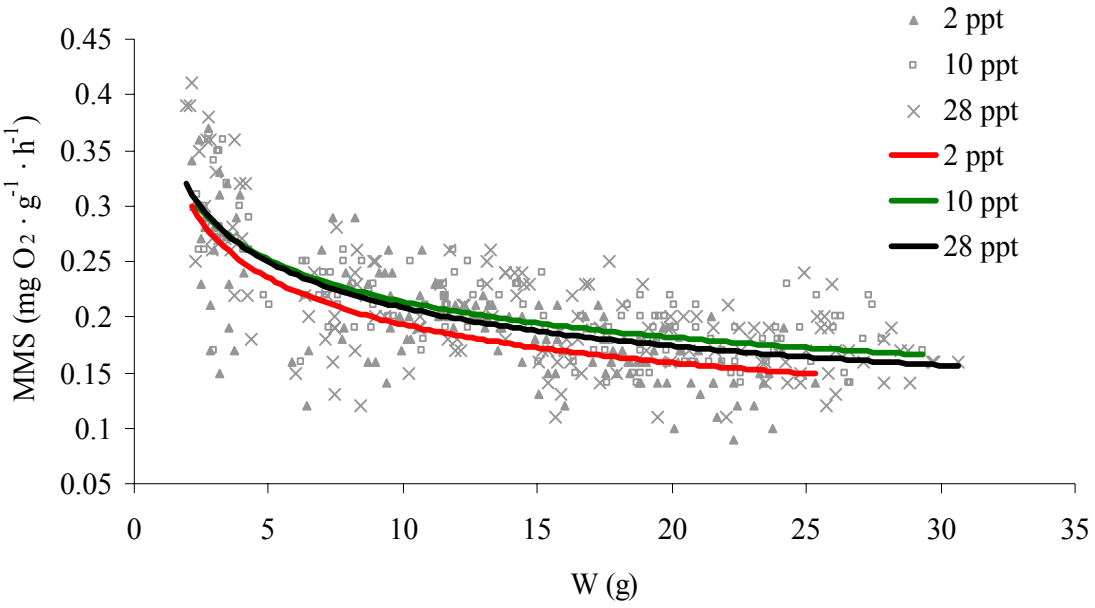


Figure 3. Marginal metabolic scope (MMS) versus shrimp wet-weight (W) for 2, 10, and 28 ppt salinity treatments.

the one with RMR (Figure 4). Regression of lnLOCr on lnW yielded the following power-model equations: $y = 0.788 x^{0.005}$, $y = 0.951 x^{-0.071}$, and $y = 0.946 x^{-0.097}$ for the 2, 10, and 28 ppt treatments, respectively. Analysis of covariance showed a significant difference between the intercept for the 2-ppt treatment and those for the 10- and 28-ppt treatments ($P = 0.013$ and $P < 0.014$, respectively) and between the slope for the 2-ppt treatment and those for the 10- and 28-ppt treatments ($P = 0.004$ and $P < 0.001$, respectively). However, there was no significant difference between the intercepts or slopes for the 10- and 28-ppt treatments ($P = 0.928$ and $P = 0.287$, respectively).

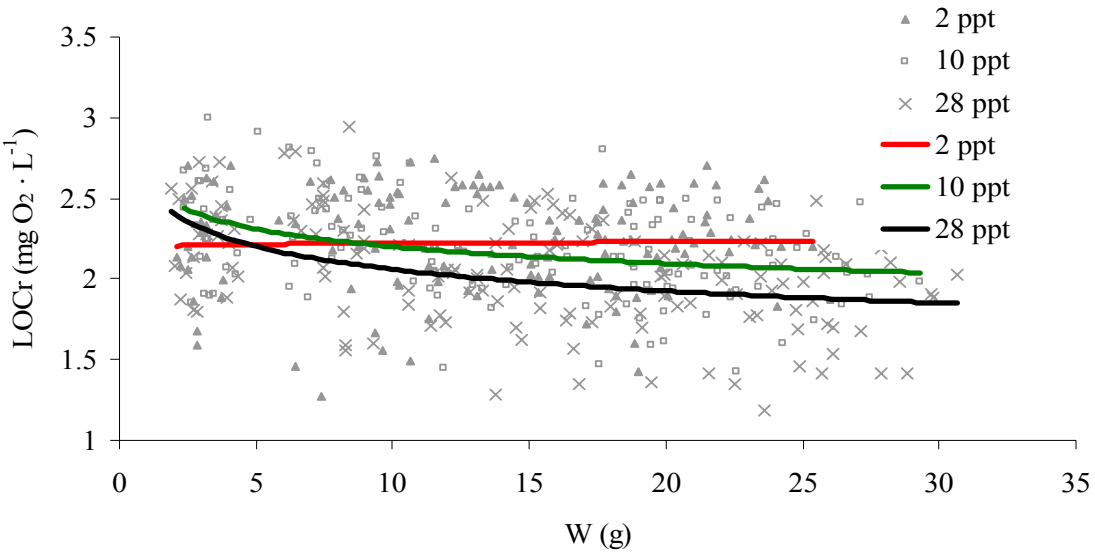


Figure 4. Limiting oxygen concentration (LOCr) versus shrimp wet-weight (W) for 2, 10, and 28 ppt salinity treatments.

Reconciliation of final growth data with ecophysiological responses obtained in this study prompted consideration of the natural history of *L. vannamei*, specifically the

point of out-migration from estuarine to offshore waters. Therefore, the full dataset was partitioned into two discrete subsets (shrimp ≤ 9 g and > 9 g) and analyzed in the same manner as before. For shrimp ≤ 9 g, RMR decreased with increased W for all three salinities (Figure 5). Regression of lnRMR on lnW yielded the following power-model equations: $y = -0.266 x^{-0.262}$, $y = -0.121 x^{-0.259}$, and $y = 0.092 x^{-0.437}$ for the 2, 10, and 28 ppt treatments, respectively. Analysis of covariance showed no significant difference

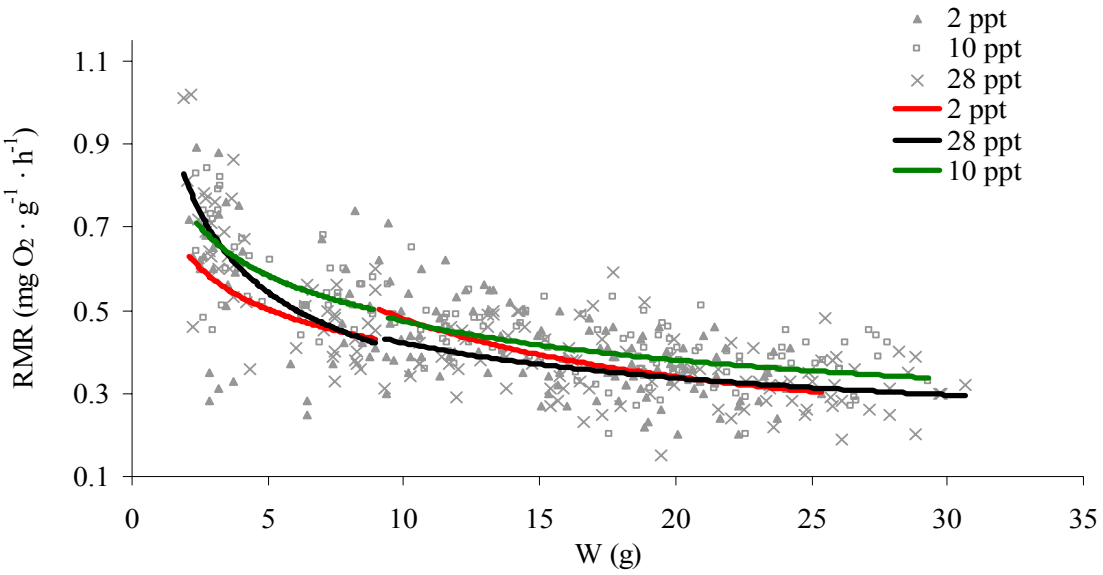


Figure 5. Routine metabolic rate (RMR) versus shrimp wet-weight (W) at 2, 10, and 28 ppt, with power-models at each salinity fitted separately for shrimp weighing ≤ 9 g and shrimp weighing >9 g.

in lnRMR among salinity treatments (P = 0.126). LOCr increased slightly with increased W for the 2 and 10 ppt treatments, and decreased slightly for the 28 ppt treatment (Figure 6). Regression of lnLOCr on lnW yielded the following power- model equations: $y = 0.777 x^{0.011}$, $y = 0.812 x^{0.025}$, and $y = 0.817 x^{-0.002}$ for the 2, 10, and 28 either slopes or intercepts for the salinities tested (P = 0.921). MMS decreased with

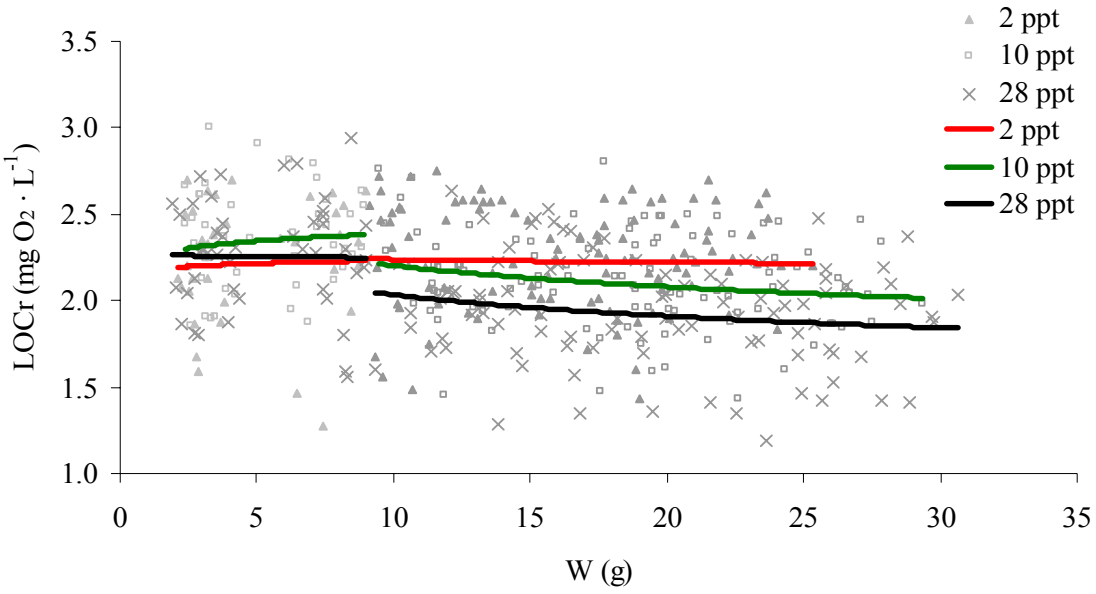


Figure 6. Limiting oxygen concentration (LOCr) versus shrimp wet-weight (W) at 2, 10, and 28 ppt, with power-models at each salinity fitted separately for shrimp weighing ≤ 9 g and shrimp weighing >9 g.

increasing shrimp weight for all three salinities tested (Figure 7). Regression of lnMMS on lnW yielded the following power-model equations: $y = -1.043x^{-0.274}$, $y = -0.933 x^{-0.284}$, and $y = -0.725 x^{-0.435}$ for the 2, 10, and 28 ppt treatments, respectively. Analysis of covariance showed no significant difference among salinity treatments (P = 0.142).

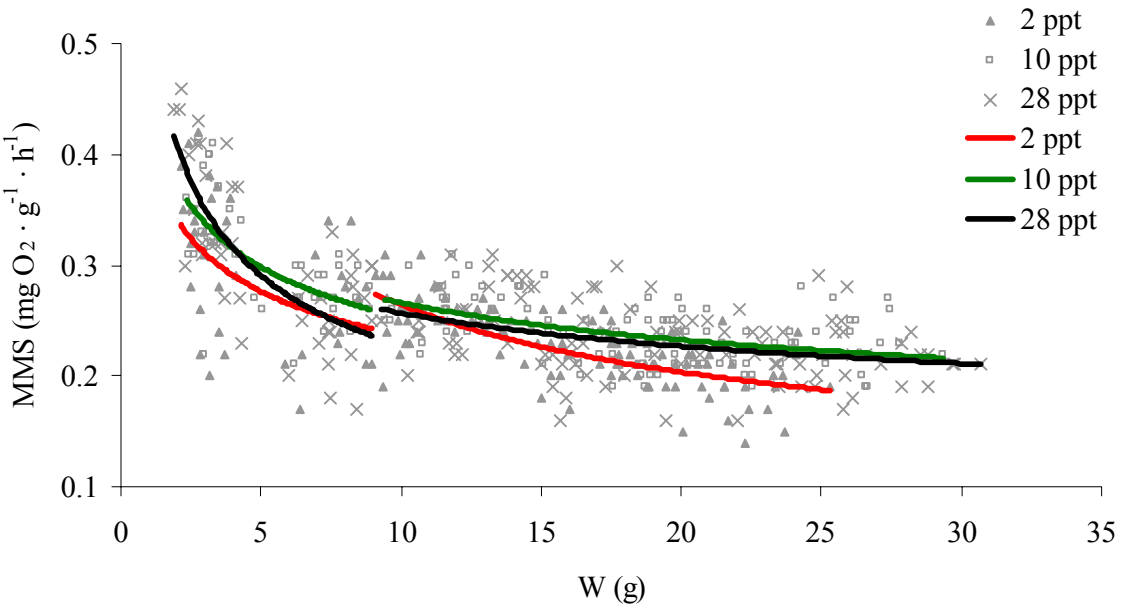


Figure 7. Marginal metabolic scope (MMS) versus shrimp wet-weight (W) at 2, 10, and 28 ppt, with power-models at each salinity fitted separately for shrimp weighing ≤ 9 g and shrimp weighing >9 g.

For shrimp > 9 g, RMR decreased with increased W for all three salinities. Regression of lnRMR on lnW yielded the following power-model equations: $y = 0.410 x^{-0.496}$, $y = -0.004 x^{-0.322}$, and $y = 0.131 x^{-0.319}$ for the 2, 10, and 28 ppt treatments, respectively. Analysis of covariance showed no significant difference in lnRMR among salinity treatments (P = 0.116). LOCr decreased slightly with increased W for all salinities tested. Regression of lnLOCr on lnW yielded the following power-model equations: $y = 0.830 x^{-0.010}$, $y = 0.979 x^{-0.082}$, and $y = 0.915 x^{-0.089}$ for the 2, 10, and 28 ppt treatments, respectively. Analysis of covariance showed no significant difference in either slopes or intercepts for the salinities tested (P = 0.469). MMS decreased with increasing shrimp weight for all three salinities tested (cf. Figure 5). Regression of lnMMS on lnW yielded the following power-model equations: $y = -0.420x^{-0.486}$, y = -

0.983 x $^{-0.240}$, and y = -1.046 x $^{-0.230}$ for the 2, 10, and 28 ppt treatments, respectively. Analysis of covariance showed a significant difference between the intercept for the 2 ppt treatment and those for the 10 and 28 ppt treatments (P = 0.008 and P = 0.003, respectively), as well as between the slope for the 2 ppt treatment and those for the 10 and 28 ppt treatments (P = 0.001 and P = 0.001, respectively). There were no significant differences between slopes or intercepts for the 10 and 28 ppt treatments (P = 0.769 and P = 0.891, respectively).

Regression of linear growth rate (y, g/d) on MMS (x, obtained after 50 days from initiation of respirometry, when the majority of shrimp had surpassed 9 g wet weight) adjusted to a common shrimp weight (15 g) showed a positive relationship for all treatments (Figure 8), and generated the following equations: y = 0.176x + 0.091 (R^2 = 0.058), y = 0.158x + 0.106 (R^2 = 0.030), and y = 0.117x + 0.127 (R^2 = 0.036) for the 2, 10, and 28 ppt treatments, respectively.

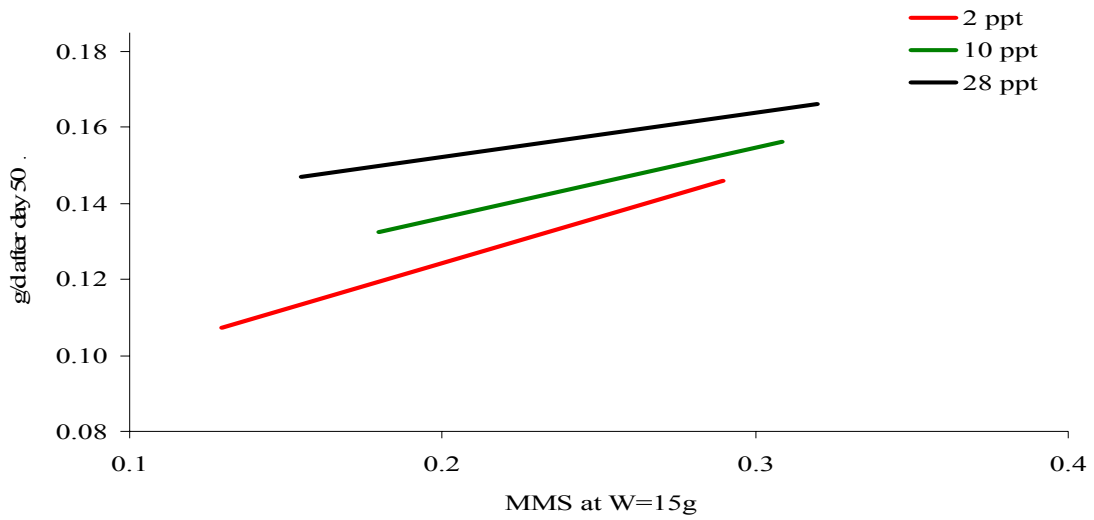


Figure 8. Linear growth rate from day 50 vs. MMS adjusted to a common weight of 15 g.

Discussion

Ion-osmoregulation in shrimp depends in part on the shrimp's ability to absorb or excrete ions against a concentration gradient (hypo- or hyper-osmoregulation), and a corresponding metabolic load. Several studies have sought to evaluate the increase in metabolic cost as reflected by increased oxygen consumption; however, as previously stated, most of these studies focused on acute salinity changes rather than on long-term effects of chronic exposure. Furthermore, most studies have been limited to a single size-class of shrimp.

It is well known that oxygen consumption increases with increased body weight but decreases on a per-unit-weight basis. Palacios et al. (1996) measured the effects of temperature and body weight on respiration rate for *L. vannamei* ranging in weight from 1 to 50 g (for their 30 ºC treatment). Transformation of the results obtained by Palacios et al. (1996) to dimensions of measurement used in the present study gives values that are very similar to those presented here. Although the values reported by Palacios et al. (1996) for oxygen consumption rate are slightly higher than those found in this study, the general trend is the same and the difference in reported values could be explained by the slightly higher temperature used by Palacios et al. (1996).

In a study to determine the chronic effects of various ionic compositions in low-salinity (4 ppt) environments on the growth of *L. vannamei* (~ 4-5 g), Roy et al. (2007) reported that differences in growth were not reflected by differences in routine rate of oxygen consumption. The same can be said for the present study.

Roy et al. (2007) did not measure the LOCr response. Seidman and Lawrence (1985) estimated LOCr to be between 1.17 and 1.91 mg $O_2 \cdot L^{-1}$ for *L. vannamei* and 1.21 to 2.22 mg $O_2 \cdot L^{-1}$ for *Penaeus monodon* (Fabricius 1798) post-larvae weighing ~ 0.060 and 0.056 g, respectively. Villarreal et al. (1994) reported a value of 1.3 mg $O_2 \cdot L^{-1}$ for *L. vannamei* weighing ~ 0.15 g. Rosas et al. (1997) reported exceptionally high LOCr values for *Litopenaeus schmitti* and *Litopenaeus setiferus* post-larvae exposed to various salinities: 4.5 to 5.0 mg $O_2 \cdot L^{-1}$ for *L. schmitti* (~ 0.0023 g) exposed to salinities from 15 to 38 ppt, and for *L. setiferus* (~ 0.0024 g) at salinities from 1 to 37 ppt. In all cases, respirometry was conducted with unfed shrimp that had been fasted for 12h. To my knowledge, no other studies have obtained LOCr values from shrimp over a size range as extensive as that in the present study, nor with as much replication.

I partitioned the full dataset at W = 9 g. This is the approximate size at which *L. vannamei* migrate from estuaries to offshore waters (Menz and Bowers 1980; Wyban et al. 1995). Trial division of my dataset at 1-g increments from 7 – 11 g, gave best overall explanation of MMS variance with division at 9 g ($\leq$ 9 g: $R^2 = 0.39$; > 9 g: $R^2 = 0.31$). This study evaluated the chronic effects of salinity and body weight on the metabolic responses of *L. vannamei*. Those responses were significantly different for shrimp at 2 ppt as compared with those at 10 ppt and 28 ppt salinities (W > 9 g), and decreased with increased body weight. This indicates higher metabolic cost for the shrimp maintained at the lowest salinity evaluated, and, considering the natural history of *L. vannamei*, suggests that migration from estuaries to offshore waters is coincident with a size-related change in the pattern of underlying metabolic response. Comparison of linear growth

rate and MMS adjusted to a common weight supports the idea that MMS and growth are positively related. This study reports for the first time MMS values for *L. vannamei* at three different salinities (2, 10, 28 ppt) and documents the informative value of MMS in discerning differences among salinity treatments where RMR alone does not.

CHAPTER III

EFFECTS OF TEMPERATURE, STARVATION AND DIETARY PROTEIN ON ECOPHYSIOLOGICAL PERFORMANCE OF THE PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*)

Introduction

Like all penaeids, *Litopenaeus vannamei* (Boone 1931) has a life history characterized by the use of both marine (adults) and estuarine (postlarval to juvenile) habitats (Edwards, 1977; Bishop et al. 1980). Both types of habitat are subject to fluctuations in abiotic factors, of which temperature is the most prominent in controlling metabolic rate.

Respirometric measurement of oxygen-uptake rate in penaeid shrimp is well established as a means for indirectly estimating metabolic rate (Kurmaly et al. 1989; Rosas et al. 1992, 1997, 1999a, 1999b, 2001a, 2001b; Villarreal et al. 1994; Palacios et al. 1996; Dai et al. 1999; Racotta et al. 2000; Salvato et al. 2001; Re et al. 2004; Tian et al. 2004; Roy et al. 2007). The relevant terms and relationships that describe the respirometry and metabolism studies in this chapter are consistent with those in the previous chapter of this dissertation. This study evaluated the effects of four temperatures—20, 24, 28, and 32 ºC—on survival, growth, and metabolic performance for *L. vannamei* under fed and starved conditions. Furthermore, at two of the four temperatures —24 and 28 ºC—this study evaluated the effects of dietary crude protein level (25 and 35%) on ecophysiological performance of *L. vannamei*.

Materials and Methods

Shrimp and Experimental Culture System

Specific-pathogen-free postlarvae (PLs) of *Litopenaeus vannamei* ("Kona" strain, Hennig et al. 2004) for this study were obtained from The Oceanic Institute, Makapu Point, HI, and reared in a semi-closed, recirculating seawater system at the Texas AgriLife Mariculture Research Lab, a Texas A&M System research facility in Port Aransas, Texas. Animals were maintained at 26 ºC until application of treatment temperatures. During this period shrimp were offered a commercial feed (Rangen 45-10; Rangen Feeds, Buhl, ID) at a rate of 0.5 g of dry feed/(shrimp·day). System seawater was obtained from the Corpus Christi (Texas) Ship Channel and pumped through a series of high-volume pressurized sand filters prior to storage in outdoor tanks. The effect of temperature on positive and negative "growth" was evaluated indoors, in two temperature-controlled rooms (Experiment 1). The effect of temperature and dietary protein level was evaluated in a third, adjoining room (Experiment 2). Each room contained two semi-closed, recirculating seawater systems, each comprised of 36 culture tanks (28 x 28 x 61 cm), intake sump, biofilter, return sump, foam fractionator and pressurized sand filter. Seawater from the outdoor storage tanks was added to each recirculating system at a rate of 3.8 $L \cdot min^{-1}$. Flow rate of incoming treated seawater into each tank was set at 45.4 $L \cdot h^{-1}$ using drip-irrigation flow restrictors. Water depth in each tank was 46 cm, with 15 cm freeboard (36.1 L).

Preparation of Experimental Diets

Two semi-purified diets (25 and 35% crude protein) were manufactured via cold extrusion with a Hobart mixer at the Texas AgriLife Research Mariculture Lab (Port Aransas, TX) following the methodology of Siccardi (2006). Ingredient compositions of

Table 2. Composition of 25 and 35% protein diets.

	Inclusion (%)	
Ingredient	25% protein	35% protein
alginate[5]	2.00	2.00
calcium carbonate[2]	1.46	1.46
α-cellulose[4]	1.38	5.37
cholesterol[2]	0.20	0.20
diatomaceous earth[4]	-	3.38
methionine[2]	0.04	-
fish meal[2]	13.61	13.61
potassium chloride[3]	1.85	1.85
krill meal[1]	9.07	9.07
magnesium oxide[3]	1.73	1.73
phospholipid[1]	4.20	4.20
dicalcium phosphate[2]	6.56	6.56
sodium hexametaphosphate[3]	1.00	1.00
mineral mix[1]	0.27	0.27
vitamin mix[1]	0.23	0.23
isolated soy protein[1]	-	11.11
soy bean oil[1]	1.43	-
squid meal[1]	13.61	13.61
vitamin C[1]	0.05	0.05
wheat starch[2]	41.30	24.29

[1]Zeigler Brothers, Gardners, PA, USA.
[2]MP Biomedicals, Cleveland, OH, USA.
[3]Fisher Scientific, Fair Lawn, NJ, USA.
[4]Sigma, St. Louis, MO, USA.
[5]Keltone HV Alginate, NutraSweet-Kelco Company, Chicago, IL.
[6]Omega Protein Corporation Inc., Houston, TX, USA.

the 25 and 35% crude protein semi-purified diets are shown in Table 2. Prepared diets were stored at 4°C until use.

Effects of Temperature on Fed and Starved Shrimp

Shrimp performance at four experimental temperatures (20, 24, 28, and 32 °C) was evaluated in four different semi-closed, recirculating systems over a 38-d trial, to determine the controlling effects of temperature on metabolism and weight change in fed and starved *L. vannamei*. Temperature was managed using in-line heat exchangers, and salinity was maintained at 28 ppt for all systems by adding seawater or reverse-osmosis (RO) purified water as necessary. Shrimp with an initial mean weight of 6.1 g (± 0.48 g) were stocked one per tank. Each system contained two experimental treatments, each replicated in 18 tanks. The assignment of tanks to treatment was done randomly. Shrimp in the first treatment were offered a commercial production feed (Rangen 45-10; Rangen Feeds, Buhl, ID) in excess using automatic feeders; while shrimp in the second treatment were not fed. Uneaten feed, feces, and exuvia were removed from each "fed" tank daily. A 12:12 photoperiod was implemented with an automated light-control system. Temperature, salinity and dissolved oxygen (DO) were monitored daily using a YSI 85® meter (YSI Inc., Yellow Springs, OH). Tests for ammonia, nitrate, nitrite and pH were conducted weekly using methods adapted from those of Spotte (1979a,b) and Solarzano (1969), Spotte (1979a,b) and Mullen and Riley (1955), Spotte (1979a,b) and Strickland and Parsons (1972), and a YSI pH 100® meter, respectively. After 4 days of treatment imposition, one shrimp from each treatment was removed every other day, weighed, and subjected to automated routine respirometry. Total trial length was 38 d.

Effects of Temperature and Dietary Protein Level

Two experimental temperatures, 24 and 28 °C, were imposed in two different semi-closed, recirculating systems over the 38-d trial. Temperature was managed using in-line heat exchangers, and salinity was maintained at 28 ppt for all systems. Each system—one at 24 and the other at 28 °C—received two experimental feed-protein treatments, each replicated in 18 tanks. The assignment of tanks to treatment was done randomly. Shrimp with an initial mean weight of 6.1 g (± 0.50 g) were stocked one per tank. Shrimp were offered either the 25% or the 35% protein diet fed in excess using automatic feeders. Uneaten feed, feces, and exuvia were removed from each tank daily. A 12:12 photoperiod was implemented with an automated light-control system. Temperature, salinity and dissolved oxygen (DO) were monitored daily using a YSI 85® meter (YSI Inc., Yellow Springs, OH). Tests for ammonia, nitrate, nitrite and pH were conducted weekly using methods adapted from those of Spotte (1979a,b) and Solarzano (1969), Spotte (1979a,b) and Mullen and Riley (1955), Spotte (1979a,b) and Strickland and Parsons (1972), and a YSI pH 100® meter, respectively. At the end of the 38-d growth trial, two shrimp from each treatment were removed daily, weighed, and subjected to automated routine respirometry. Total trial length was 55 d.

Respirometry

In both experiments, automated routine respirometry (Springer and Neill 1988) was performed following procedures consistent with those described in Chapter II of this dissertation. Experiment 1 required the use of four fiberglass tanks (1.52 x 1.52 x 0.44 m deep, volume 690 L) as water baths. Each tank was set up as a closed recirculating

system, with water temperature maintained at 20, 24, 28, or 32 ºC by use of in-line heat exchangers. Dissolved oxygen levels were maintained near air saturation using two 5.08 cm fused-silica airstones connected to a low pressure blower. Salinity in each tank was maintained at 28 ppt by adding seawater diluted with RO water. Twenty-four hours prior respirometry, one shrimp from each treatment was netted and transferred to its own square polyethylene bucket (0.23 x 0.23 x 0.37 m) placed inside the appropriate respirometry water-bath. This was done to prevent ingestion of feed (for the fed treatments) during the 24 h prior to respirometry. Each of the 8 buckets (two per tank) had a plastic-mesh-covered, 0.04-m-diameter hole on each of the four sides below the water line to allow for water exchange. Experiment 2 involved the same protocols, except only two water baths were used—one at 24 ºC and the other at 28 ºC.

Statistical Analysis

Statistical analyses for both experiments were performed using SPSS (version 13.0, SPSS Inc., Chicago, Illinois). For Experiment 1, respirometry data (RMR, LOCr, and MMS) as well as data on rate of weight change (g/d) from culture-tank shrimp were analyzed using one-way analysis of variance, to determine if significant differences ($P \leq 0.05$) existed among treatment means. Student-Newman-Keuls multiple comparison test (Ott and Longnecker, 2001) was used to resolve differences among treatment means. Respirometry data were further subjected to analysis of covariance to test for effects of temperature through time on RMR, LOCr, and MMS, where the dependent variable was RMR, LOCr, or MMS; the factors were temperature (ºC) and treatment within

temperature (fed or starved); and, the covariate was time (d). Differences were considered significant at $P < 0.05$.

Ecophysiological data obtained from experiment 2 were analyzed using one-way analysis of variance to determine if differences existed among treatment means. Respirometry data then were subjected to analysis of covariance to test for effects of temperature, dietary protein level and body weight (W) on RMR, LOCr, and MMS, where the dependent variable was either lnRMR, lnLOCr, or lnMMS; the factor was either temperature or dietary protein level; and, the covariate was lnW. Differences were considered significant at $P < 0.05$.

Results

Water Quality

Means (± SD) of dissolved oxygen (DO), temperature, salinity, total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3) and pH are shown in Table 3. Physiochemical factors remained within acceptable values for penaeid shrimp, as judged by the recommendations by Allan et al. (1990), Chen and Lei (1990), and Chen and Lin (1991).

Effects of Temperature on Growth and Survival

Experiment 1.—Significant differences in daily growth rate (g/d) were observed among temperatures for the fed shrimp, but not for the starved shrimp. The various responses and statistics are presented in Table 4 to document the differences in growth rate (g/d), % survival prior to respirometry, and % survival through respirometry, among temperature treatments and between feed treatments.

Table 3. Mean ± SD for culture tank water quality parameters in experiment 1 (fed and starved) and experiment 2 (25 and 35% crude protein diet). Total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), and pH were measured weekly. Temperature, salinity, and dissolved oxygen (DO) were measured daily

	Temperature Treatment					
	Experiment 1				Experiment 2	
Parameter	20 ºC	24 ºC	28 ºC	32 ºC	24 ºC	28 ºC
Temperature (ºC)	20.4 ± 1.03	23.9 ± 0.45	27.8 ± 0.53	31.9 ± 0.22	23.9 ± 0.50	28.0 ± 0.20
Salinity (ppt)	27.0 ± 1.54	27.5 ± 1.53	27.8 ± 1.47	28.3 ± 1.55	27.2 ± 1.49	28.1 ± 1.46
DO ($mg \cdot L^{-1}$)	7.5 ± 0.26	6.9 ± 0.16	6.2 ± 0.15	5.7 ± 0.13	6.8 ± 0.17	6.2 ± 0.14
TAN ($mg \cdot L^{-1}$)	0.2 ± 0.12	0.2 ± 0.16	0.2 ± 0.16	0.2 ± 0.14	0.3 ± 0.23	0.2 ± 0.20
NO_2 ($mg \cdot L^{-1}$)	0.0 ± 0.01	0.1 ± 0.11	0.1 ± 0.12	0.1 ± 0.15	0.1 ± 0.14	0.1 ± 0.12
NO_3 ($mg \cdot L^{-1}$)	0.1 ± 0.03	0.1 ± 0.04	0.2 ± 0.12	0.3 ± 0.21	0.1 ± 0.08	0.2 ± 0.19
pH	8.3 ± 0.02	8.3 ± 0.02	8.3 ± 0.03	8.4 ± 0.02	8.3 ± 0.03	8.3 ± 0.02

Table 4. Mean growth rate (g/d) and survival up to and through respirometry for fed and starved shrimp at four different temperatures.

Treatment	Mean growth rate (g/d)	Survival to respirometry (%)	Survival through respirometry (%)
Fed			
20 ºC	0.02[a]	94.4	88.9
24 ºC	0.07[b]	94.4	88.9
28 ºC	0.16[c]	100	83.3
32 ºC	0.17[c]	100	83.3
Mean Square Error	0.004	-	-
P value	< 0.001	-	-
Starved			
20 ºC	0.00[a]	89.9	83.3
24 ºC	0.00[a]	83.3	66.7
28 ºC	0.00[a]	66.7 (0 after 30 d)	55.6
32 ºC	-0.02[a]	61.1 (0 after 26 d)	27.8
Mean Square Error	0.001	-	-
P value	0.510	-	-

Values with like-superscripts within treatments indicate statistically similar groups ($P<0.05$). Survival rates are calculated as percentage of shrimp alive on day they had been scheduled for respirometry, and percentage of those that survived respirometry.

Experiment 2.—Significant differences in daily growth rate (g/d) were observed between temperatures within diets, but not between diets at the same temperature (24 ºC: $P = 0.814$; 28 ºC: $P = 0.872$). Results are presented in Table 5 to document the differences in growth rate (g/d), % survival to respirometry, and % survival through respirometry between temperatures within diets.

Table 5. Mean growth rate (g/d) and survival up to and through respirometry for shrimp offered 25 and 35% crude protein diets at 24 and 28 ºC.

Treatment	Mean growth rate (g/d)	Survival to respirometry (%)	Survival through respirometry (%)
25 % protein			
24 ºC	0.12	77.8	72.2
28 ºC	0.16	100.0	94.4
P value	0.024	-	-
35 % protein			
24 ºC	0.12	94.4	94.4
28 ºC	0.17	88.9	83.3
P value	<0.001	-	-

Effects of Temperature on Respirometric Responses

Experiment 1.—Mean values of RMR, LOCr, and MMS are shown in Table 6. RMR and MMS tended to increase with temperature, both within fed and starved treatments; whereas, LOCr was statistically invariate across temperatures, both within fed and starved treatments. For fed shrimp, RMR at each temperature differed significantly from that at every other temperature; for starved shrimp, for which respirometry sample sizes were smaller, differences in RMR could be resolved only between the highest-temperature treatment (32 ºC) and the lower three. Both for fed and starved shrimp, patterns of statistical difference in MMS vs. temperature were similar to those in RMR.

Fed shrimp had consistently higher values of RMR and MMS, and lower values of LOCr, than their starved counterparts at each temperature (Table 6). Comparisons of fed and starved treatments through time within temperatures (ANCOVA) indicated significant differences in RMR for shrimp maintained at 24 ºC ($p < 0.000$), at 28 ºC ($p = 0.022$), and at 32 ºC ($p = 0.003$), but not for those at 20 ºC ($p = 0.347$) (Figure 9).

Table 6. Mean RMR (mg $O_2 \cdot g^{-1} \cdot h^{-1}$), LOCr (mg $O_2 \cdot L^{-1}$), and MMS (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) for fed and starved *L. vannamei* subjected to respirometric measurement at four different temperatures.

Treatment	*n*	RMR (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)	LOCr (mg $O_2 \cdot L^{-1}$)	MMS (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)
Fed				
20 ºC	16	0.21^{a}	1.93^{a}	0.12^{a}
24 ºC	16	0.35^{b}	1.79^{a}	0.21^{b}
28 ºC	15	0.45^{c}	1.90^{a}	0.24^{b}
32 ºC	15	0.54^{d}	1.91^{a}	0.30^{c}
Mean Square Error		0.007	0.203	0.002
P value		< 0.001	0.828	<0.001
Starved				
20 ºC	15	0.18^{a}	2.02^{a}	0.10^{a}
24 ºC	12	0.26^{a}	2.04^{a}	0.14^{a}
28 ºC	8	0.27^{a}	2.24^{a}	0.12^{a}
32 ºC	5	0.40^{b}	2.22^{a}	0.19^{b}
Mean Square Error		0.008	0.312	0.003
P value		0.001	0.751	0.005

Values with like-superscripts within treatments indicate statistically similar groups ($P<0.05$).

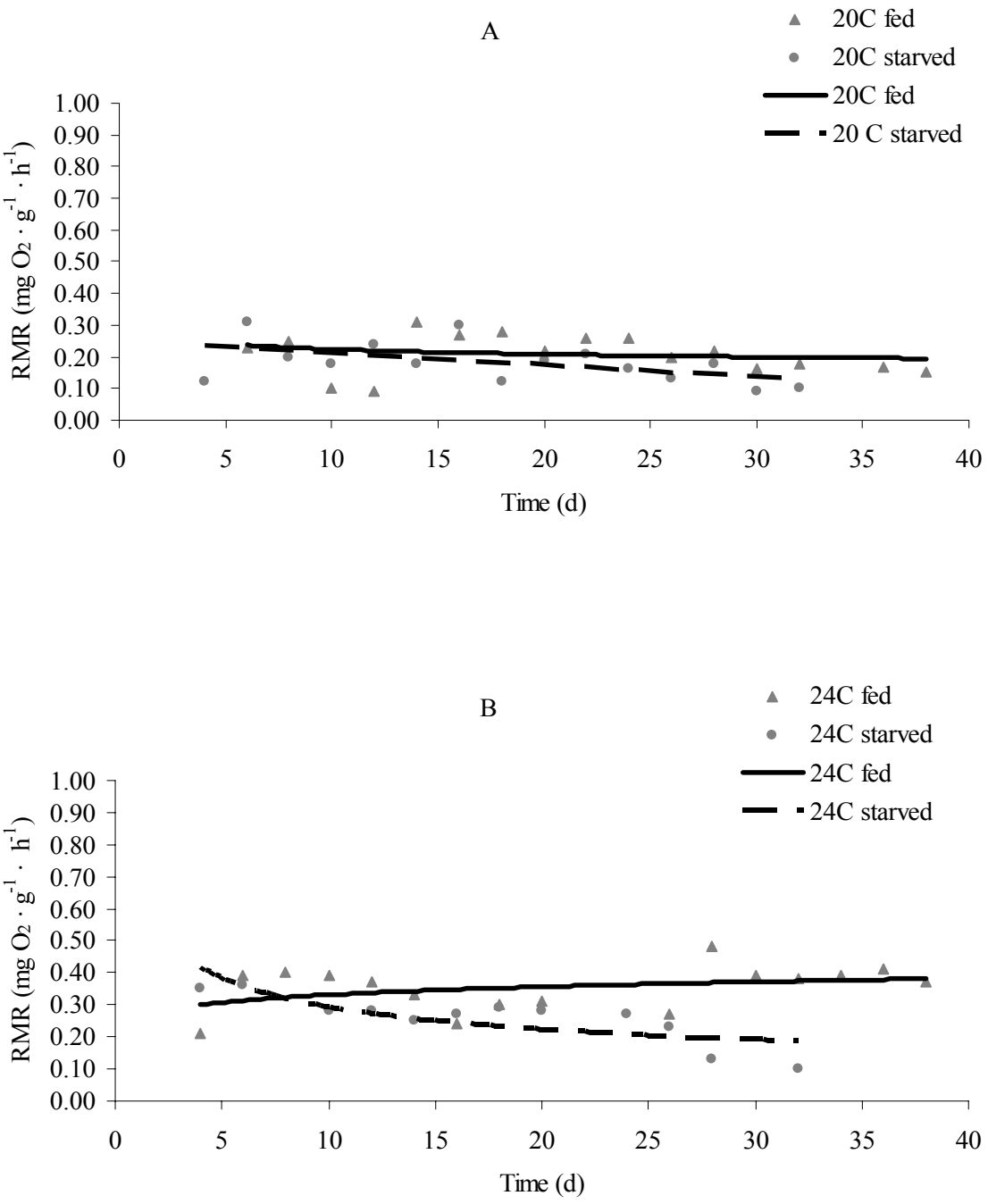
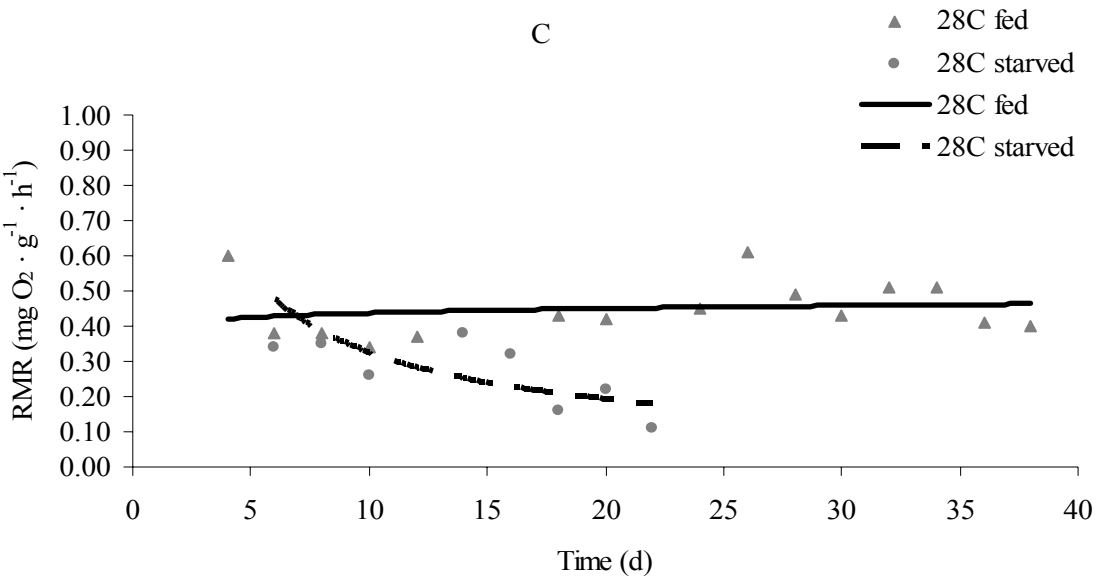


Figure 9. Routine metabolic rate (RMR) over time for shrimp within the four temperature treatments: A, 20 ºC (fed: $y = 0.285 x^{-0.105}$; starved: $y = 0.378 x^{-0.275}$); B, 24 ºC (fed: $y = 0.260 x^{0.105}$; starved: $y = 0.719 x^{-0.392}$); C, 28 ºC (fed: $y = 0.397 x^{0.042}$; starved: $y = 1.854 x^{-0.759}$); and, D, 32 ºC (fed: $y = 0.347 x^{0.143}$; starved: $y = 0.1.614 x^{-0.639}$



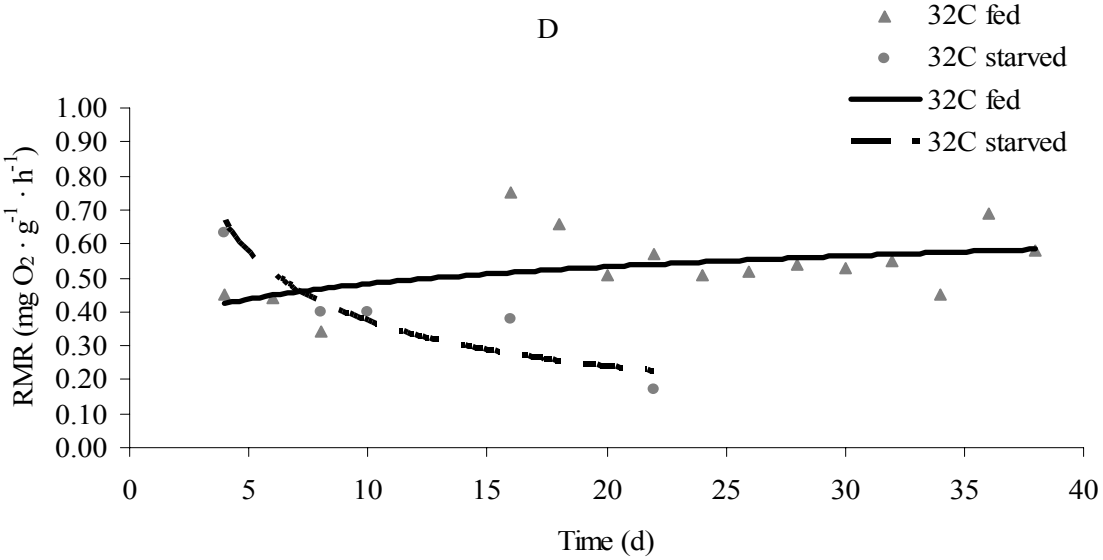


Figure 9. Continued.

Analysis of covariance detected no significant differences in LOCr responses through time among temperatures within fed/starved treatments (20 ºC; p = 0.555, 24 ºC; p = 0.351, 28 ºC; p = 0.553, and 32 ºC; p = 0.679) (Figure 10).

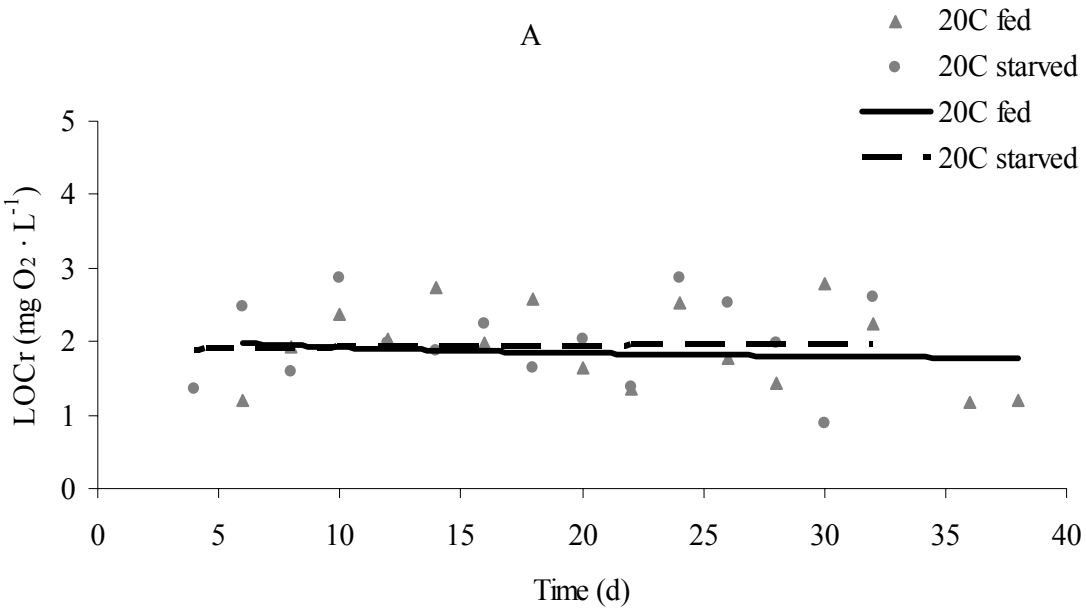
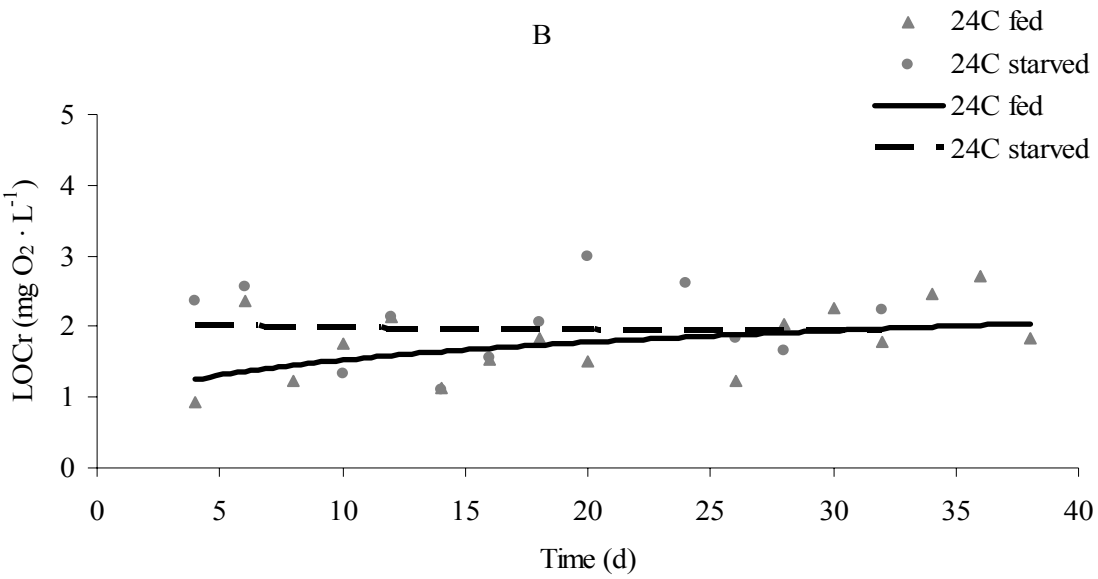


Figure 10. Limiting oxygen concentration for routine metabolism (LOCr) over time for shrimp within the four temperature treatments: A, 20 ºC (fed: $y = 2.204 x^{-0.060}$; starved: $y = 1.842 x^{0.017}$); B, 24 ºC (fed: $y = 0.921 x^{0.220}$; starved: $y = 2.088 x^{-0.023}$); C, 28 ºC (fed: $y = 1.771 x^{0.022}$; starved: $y = 2.462 x^{-0.047}$); and, D, 32 ºC (fed: $y = 1.644 x^{0.045}$; starved: $y = 1.802 x^{0.076}$).



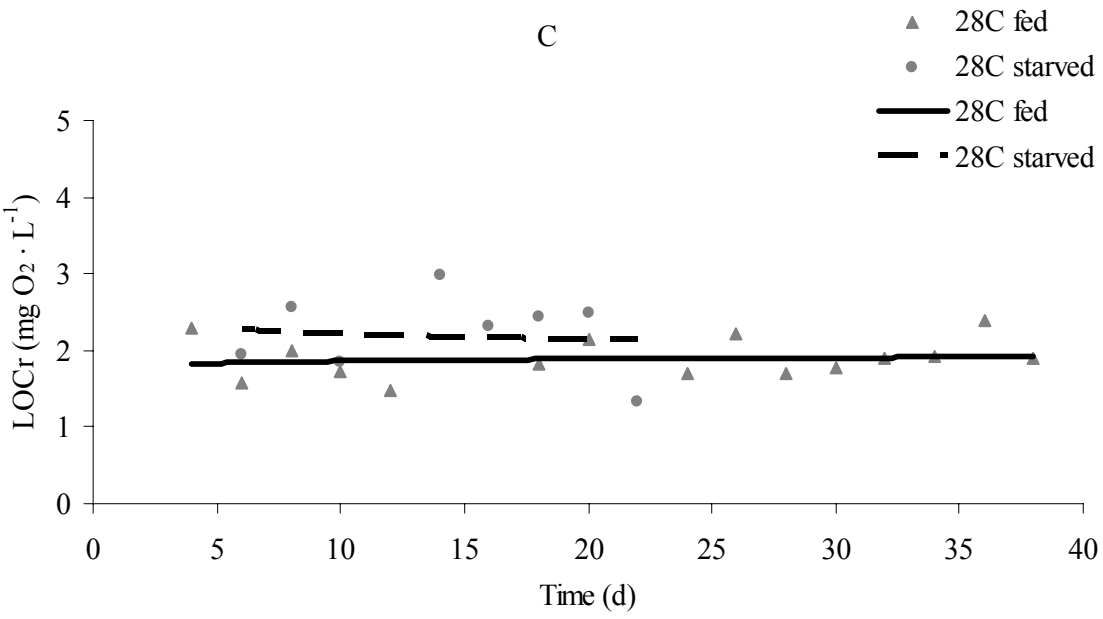


Figure 10. Continued.

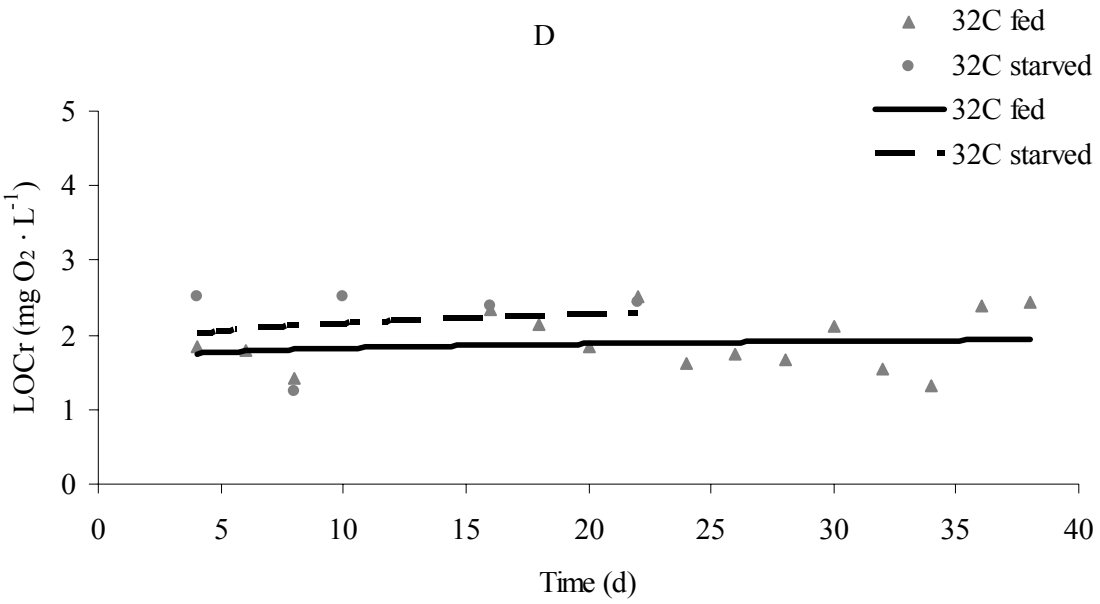


Figure 10. Continued.

Comparisons of fed and starved treatments through time within temperatures (ANCOVA) did show significant differences in MMS for shrimp maintained at 28 ºC (p = 0.009), and at 32 ºC (p = 0.001), but not at 20 ºC (p = 0.488) or 24 ºC (p = 0.176) (Figure 11).

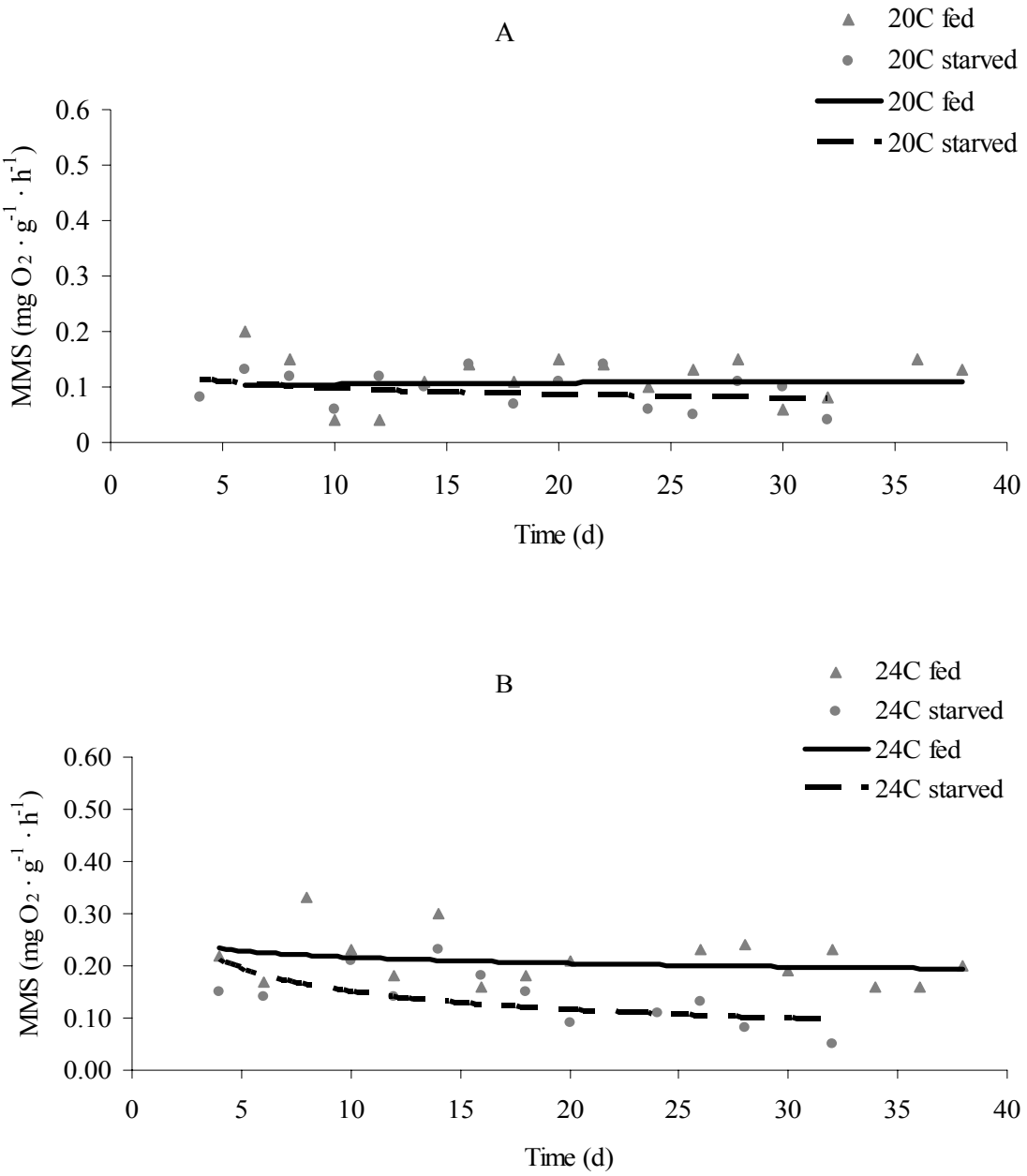
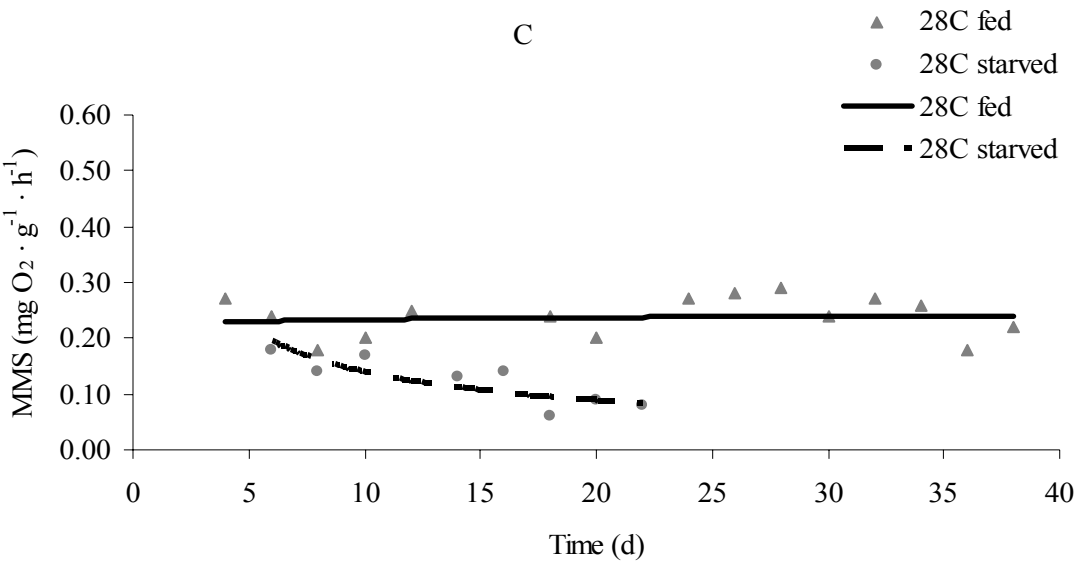


Figure 11. Marginal metabolic scope (MMS) over time for shrimp within the four temperature treatments: A, 20 ºC (fed: $y = 0.095 x^{0.041}$; starved: $y = 0.147 x^{-0.182}$); B, 24 ºC (fed: $y = 0.260 x^{-0.080}$; starved: $y = 0.358 x^{-0.379}$); C, 28 ºC (fed: $y = 0.222 x^{0.022}$; starved: $y = 0.636 x^{-0.661}$); and, D, 32 ºC (fed: $y = 0.216 x^{0.105}$; starved: $y = 0.895 x^{-0.708}$).



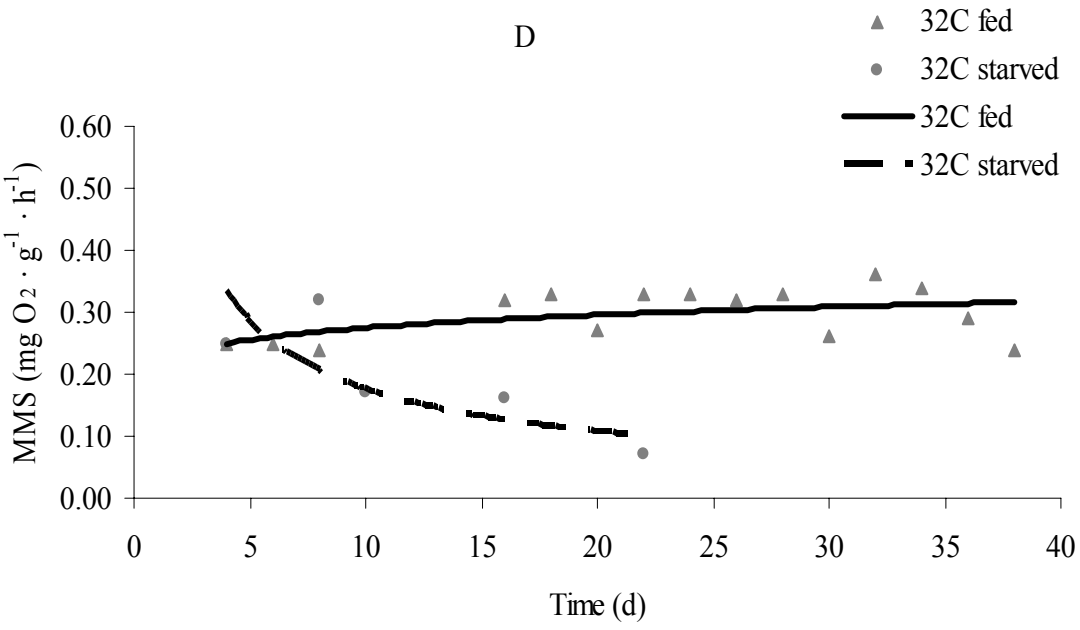


Figure 11. Continued.

Regression of linear growth rate (g/d) on MMS adjusted to a common shrimp weight (6g) showed a positive relationship for all temperatures (Figure 12), and generated the following equations: $y = 0.2343x - 0.0234$ ($R^2 = 0.35$); $y = 0.1175x + 0.0126$ ($R^2 = 0.01$); $y = 0.6042x + 0.0256$ ($R^2 = 0.12$); $y = 0.3156x + 0.0329$ ($R^2 = 0.04$) for the 20, 24, 28, and 32 ºC fed treatments, respectively. Note that, at a common value of MMS (e.g., 0.25 mgO2/(g*h)), growth rate increases from 20 to 28 ºC, then declines dramatically at 32 ºC. But, note also that 32 ºC seems to promote highest values of MMS, thus off-setting the effect of lowered growth rate per unit MMS. This pair of relationships implies, for 6-g *L. vannamei* at 28-ppt salinity, greater metabolic capacity at 32 ºC, but greater metabolic efficiency at 28 ºC.

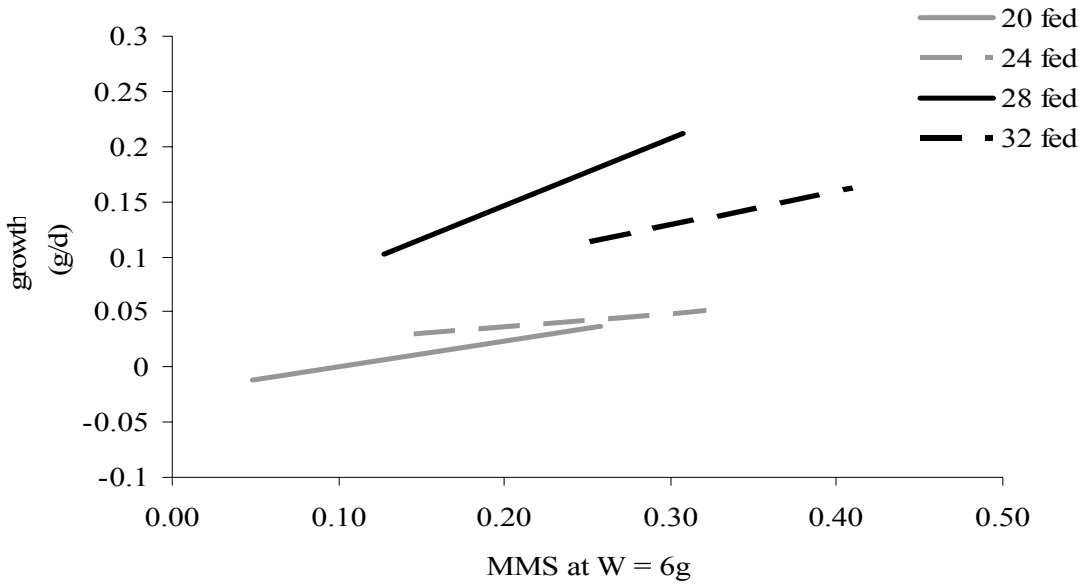


Figure 12. Linear growth rate of fed shrimp vs. MMS adjusted to a common weight of 6 g.

Similar treatment of the data for starved shrimp again showed a positive relationship between rate of weight change and MMS for all temperatures (Figure 13), generating the following equations: $y = 0.3451x – 0.02$ ($R^2 = 0.03$); $y = 0.3531x - 0.0569$ ($R^2 = 0.10$); $y = 0.7579x - 0.0979$ ($R^2 = 0.76$); $y = 0.2809x - 0.1395$ ($R^2 = 0.13$) for the 20-, 24-, 28-, and 32-ºC starved treatments, respectively. Here, the 20-ºC and 32-ºC treatments emerged as most and least favorable, respectively. Essentially, the higher the temperature, the faster the shrimp starved.

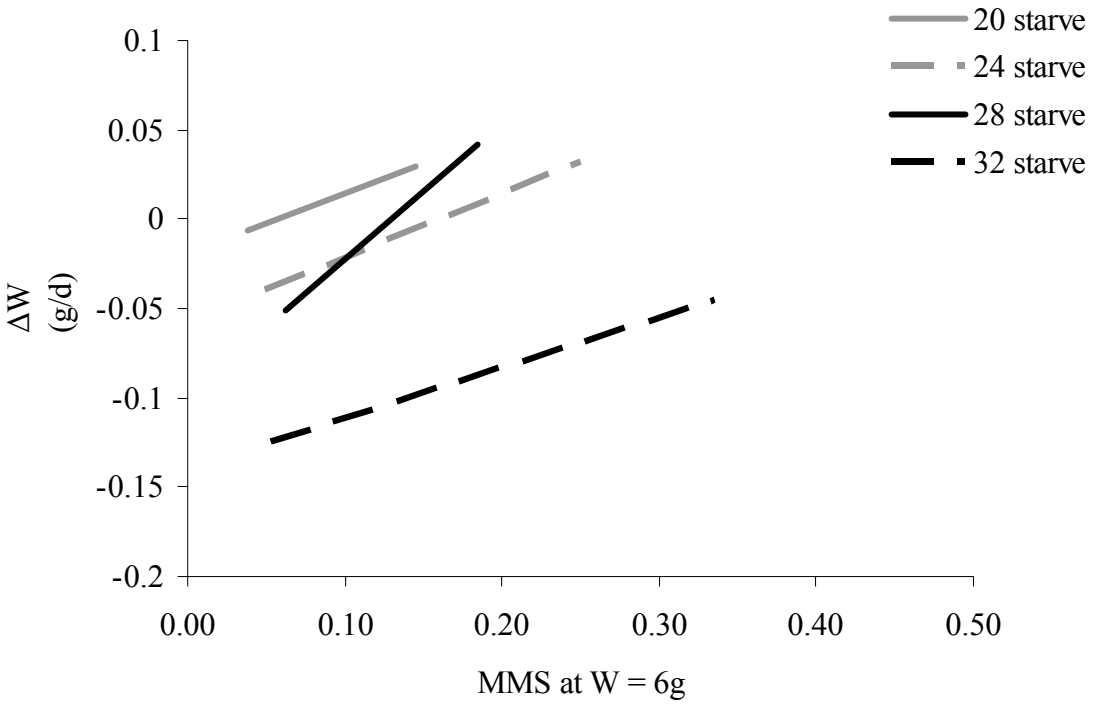


Figure 13. Linear rate of weight change of starved shrimp vs. MMS adjusted to a common weight of 6 g.

Experiment 2.—Analysis of covariance detected no significant variation in any ecophysiological response, either with temperature or with feed-protein level (Table 7).

Values of RMR, LOCr, and MMS were similar to those observed for fed shrimp in Experiment 1 at the equivalent temperature.

Table 7. Analysis of covariance in ecophysiological responses with temperature and feed-protein level.

Treatment	n	*P* value		
		lnRMR ($mg\ O_2 \cdot g^{-1} \cdot h^{-1}$)	lnLOCr ($mg\ O_2 \cdot L^{-1}$)	lnMMS ($mg\ O_2 \cdot g^{-1} \cdot h^{-1}$)
25% protein				
24 ºC	13			
28 ºC	17	0.869	0.089	0.232
35% protein				
24 ºC	16			
28 ºC	15	0.800	0.696	0.823
24 ºC				
25% protein	13			
35% protein	16	0.780	0.134	0.144
28 ºC				
25% protein	17			
35% protein	15	0.886	0.859	0.879

Discussion

As noted by Wyban et al. (1995), the thermal regime of the eastern tropical Pacific's upper mixed layer is relatively stable and rarely departs from 25 – 27 ºC in offshore areas where adult *L. vannamei* occur naturally; however, estuarine environments in nearshore areas are subject to dramatic diurnal and seasonal temperature fluctuations depending on weather conditions. Because of this, *L. vannamei* PLs and juveniles (1 to 10 g) are adapted to highly variable thermal conditions and may be considered eurythermal (Wyban et al. 1995).

Previous studies have investigated the effect of temperature on *L. vannamei* from the standpoints both of growth (Wyban et al. 1995) and oxygen consumption (Palacios et al. 1996; Villarreal et al. 1994). The effects of temperature on growth in *L. vannamei* was quantified by Wyban et al. (1995) in a series of four experiments (three utilizing aquaculture pond water and one using well water) intended to measure growth for three size classes of shrimp (3.9, 10.8, and 16.0g) at three constant temperatures (23, 27, and 30 ºC). Wyban et al. (1995) reported that for shrimp similar in size to those used in this study, reduced growth and feeding can be expected when water temperature is below 23 ºC. The same can be said of the present study in terms of positive growth and although not quantified in the present study, daily visual inspection indicated that feed consumption in the 20 and 24 ºC treatments was less than that in the 28 and 32 ºC treatments.

As with the present study, previous studies investigating the effect of temperature on oxygen consumption in *L. vannamei* have reported decreased respiration rates with decreased temperatures (Villarreal et al. 1994; Palacios et al. 1996). Villarreal et al. (1994) measured the effect of various salinity and temperature combinations on RMR and LOCr in *L. vannamei* postlarvae ($W = 0.15 \pm 0.05$g) and found both responses to be correlated with temperature. In the present study, LOCr did not vary with temperature, which may be reflective of the different sizes of shrimp used compared with those used by Villarreal et al. (1994). Palacios et al. (1996) measured the effect of temperature (20, 25, and 30 ºC) and body weight (< 1 to > 50g) on oxygen consumption and obtained RMR values of 0.382 and 0.314 mg $O_2 \cdot g^{-1} \cdot h^{-1}$ at 25 ºC for 5 and 10 g shrimp,

respectively; these values compare favorably with the mean value obtained in the present study for fed shrimp at 24 ºC (cf. Table 3). Interpretation of graphical results presented by Palacios et al. (1996) also indicates consistency with results of the present study in terms of RMR for similar temperatures and shrimp weights.

Previous studies investigating the effects of starvation on penaeid shrimp have primarily focused on compensatory growth (Wu and Dong 2002), changes in body composition (Palacios et al. 2004; Siccardi et al. 2006; Stuck et al. 1996) and utilization of energy reserves (Schafer 1968; Cuzon et al. 1980; Barclay et al. 1983; and Chandumpai et al. 1991). Measurement of oxygen consumption in starved crustaceans has mostly been limited to studies done with krill. Meyer and Oettl (2005) measured RMR in starved *Euphausia superba* and found respiration rate decreased significantly after only 3-d of starvation. Salomon et al. (2000) reported significant reduction in oxygen consumption for starved versus fed *Meganyctiphanes norvegica* after 6d. These results are consistent with the results of the present study with regards to RMR for the 24-, 28- and 32-ºC fed-shrimp treatments, but not for 20 ºC which may be indicative of temperature-induced metabolic torpor.

The use of MMS as an integrative measure of environmental quality for animal performance and production appears to have been justified, in that the results presented a consistent tendency for positive relationship between rate of weight change and MMS, both for fed and starved shrimp. Moreover, for starved shrimp, MMS deteriorated over time, in keeping with the pace of mortality.

It is important to recognize that MMS and its respirometric components are point-measures of the metabolic potential for performance. Growth is an integrative measure of realized performance over a relatively long interval of time. Thus, there can be no expectation of correlation between MMS and growth of a feed-deprived shrimp—until that deprivation has persisted long enough to have caused deterioration of the metabolic machinery.

CHAPTER IV

EFFECT OF DISSOLVED OXYGEN CONCENTRATION ON ECOPHYSIOLOGICAL PERFORMANCE OF THE PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*)

Introduction

The Pacific white shrimp, *Litopenaeus vannamei* (Boone 1931), occurs naturally along the eastern Pacific coast from the Gulf of California to northern Peru (Perez-Farfante and Kensley 1997), and is exploited globally as an aquaculture species. Productivity of *L. vannamei* can be influenced by several abiotic factors, one of which is the dissolved oxygen (DO) concentration of the water in which they live. Dissolved oxygen concentration can be a limiting factor with regards to the metabolism of aerobic aquatic animals because, at reduced levels (under hypoxic conditions), oxygen supply to tissues is restricted, and this may result in reduced capacity of the organism to perform metabolically powered activities (Fry 1947, 1971). Hypoxic conditions—< 2.8 mg $O_2 \cdot L^{-1}$ at 20 ppt, 25 ºC, and 1 atmospheric pressure (Diaz and Rosenberg 1995)—can occur both in aquaculture and in natural habitats. Hypoxic conditions in aquaculture are often seen in culture ponds with dense algal blooms at night and whenever photosynthesis is greatly exceeded by respiration. In intensive shrimp ponds, biological and chemical oxygen demand (BCOD) usually exceeds oxygen production by primary producers, thus requiring the use of mechanical aeration to sustain the cultured shrimp (McGraw et al. 2001). Occurrence of hypoxia in estuaries is primarily attributed to eutrophication, often the consequence of anthropogenic loading of nutrients containing nitrogen and

phosphorus into marine coastal ecosystems (Diaz and Rosenberg 1995, Wannamaker and Rice 2000, Wu et al. 2002).

Previous studies of penaeid shrimp in hypoxic conditions have sought to quantify lethal DO levels and evaluate sub-lethal performance. Reported lethal values of DO vary by species, methodology, and duration of exposure. Allan and Maguire (1991) estimated the 96-h LC_{50} of DO for *Penaeus monodon* to be 0.9 mg $O_2 \cdot L^{-1}$. The 48-h LC_{50} of DO for postlarval *Litopenaeus setiferus* was reported by Martinez et al. (1998) to be 1.27 mg $O_2 \cdot L^{-1}$ and, for juveniles, the 72-h LC_{50} of DO was reported to be 1.16 mg $O_2 \cdot L^{-1}$. Chen and Nan (1992) reported an average lethal DO of 0.74 mg $O_2 \cdot L^{-1}$ for *Fenneropenaeus chinensis* weighing between 0.31 and 10.54 g. Wu et al. (2002) reported an 8-h LC_{50} of DO for *Metapenaeus ensis* to be 0.77 mg $O_2 \cdot L^{-1}$. The lethal DO for *L. vannamei* under observed conditions was reported to be 0.2 mg $O_2 \cdot L^{-1}$ after 1-h of exposure (Perez-Rostro et al. 2004), and about 1.0 mg $O_2 \cdot L^{-1}$ in aquaculture pond conditions (Hopkins et al. 1991).

The effects of tolerable hypoxia on growth, survival, feeding, molting, behavior, osmoregulatory capacity, and immune response of penaeid shrimp have also been reported (Clark 1986; Renaud 1986; Allan and Maguire 1991; Charmantier et al. 1994; Moullac et al. 1998; Wannamaker and Rice 2000; McGraw et al. 2001; Wu et al. 2002; Perez-Rostro et al. 2004; Mugnier and Soyez 2005). There seem to be no published studies detailing the effect of tolerable hypoxia on the metabolic rate of *L. vannamei*.

Oxygen uptake via respirometry is well established as a means for estimating metabolic rate in penaeid shrimp (Kurmaly et al. 1989; Rosas et al. 1992, 1997, 1999a,

1999b, 2001a, 2001b;Villarreal et al. 1994; Palacios et al. 1996; Dai et al. 1999; Racotta et al. 2000; Salvato et al. 2001; Re et al. 2004; Tian et al. 2004; Roy et al. 2007). The relevant terms and relationships that describe the metabolism studies in this chapter are consistent with those in Chapter II of this dissertation. This study evaluated the effects of two levels of DO—2 and >5 mg $O_2 \cdot L^{-1}$—on MMS and its components for *L. vannamei*. The selection of 2 mg $O_2 \cdot L^{-1}$ as the lower DO level used in this study was based on the mean LOCr responses of 2.1 and 1.9 mg $O_2 \cdot L^{-1}$ determined in Chapters II and III of this dissertation, respectively, for shrimp maintained at 28 ºC.

Materials and Methods

Shrimp and Experimental Culture System

Specific-pathogen-free postlarvae (PLs) of *L. vannamei* ("Kona" strain, Hennig et al. 2004) were obtained from The Oceanic Institute, Makapu Point, HI, and reared at 28 ºC in a semi-closed, recirculating seawater system at the Texas AgriLife Mariculture Research Lab, a Texas A&M System research facility in Port Aransas, Texas. During this period shrimp were offered a commercial feed (Rangen 45-10; Rangen Feeds, Buhl, ID) at a rate of 0.5 g of dry feed/(day · $shrimp^{-1}$). System seawater was obtained from the Corpus Christi (Texas) Ship Channel and pumped through a series of high-volume, pressurized sand filters prior to storage in outdoor tanks. The effects of ambient DO on growth and other ecophysiological responses were evaluated indoors, in a temperature-controlled room. The room contained four semi-closed, recirculating seawater systems, each comprised of a fiberglass culture tank (1.52 x 1.52 x 0.44 m deep; volume, 690 L), recirculation pump, and in-line heat exchanger. Within each culture tank was a plastic-

mesh cage (1.21 x 1.21 x 0.42 m deep) partitioned into 16 equal-sized compartments (0.30 x 0.30 x 0.42 m deep). Just below the water line of each compartment, a 5-cm-wide "lip" constructed of plastic mesh was attached. For two of the four culture tanks, seawater from the outdoor storage tanks passed first through O_2-stripping columns at a rate of 1.89 $L \cdot min^{-1}$ before flowing into each of the two culture tanks at a rate of 0.95 $L \cdot min^{-1} \cdot tank^{-1}$ (Figures 14 and 15). The remaining two culture tanks were supplied with fresh seawater from outdoor storage tanks at a rate of 0.95 $L \cdot min^{-1} \cdot tank^{-1}$ and maintained at near air-saturation using two fused-silica airstones connected to a low pressure blower. Culture tank temperatures were maintained at 28 ºC using in-line heat exchangers, and salinity was allowed to fluctuate with the ambient salinity of the Corpus Christi Ship Channel, although fluctuations were damped by mixing in the facility's storage tanks.

Two experimental DO levels (2.0 and >5.0 $mg \cdot L^{-1}$) were tested in four different semi-closed recirculating systems over a 30-d trial to determine effects of hypoxia on growth and survival. Shrimp with an initial mean weight of 14.5 g (± 1.97 g) were stocked one/cage-compartment. At stocking, DO in each tank was at or near air saturation. Immediately after shrimp were stocked, N_2 gas was passed through the oxygen-stripping columns and for two of the culture tanks, DO level was reduced over a 3-d period to 2.0 $mg \cdot L^{-1}$ and maintained there. Coincident with completion of stocking, one sheet of plastic "bubble wrap" (0.30 x 0.30 m) was placed under the "lip" of each compartment opening. This was done to prevent shrimp escape, and, in the case of the shrimp in the 2.0 $mg \cdot L^{-1}$ DO treatment, to prevent shrimp from coming into contact with

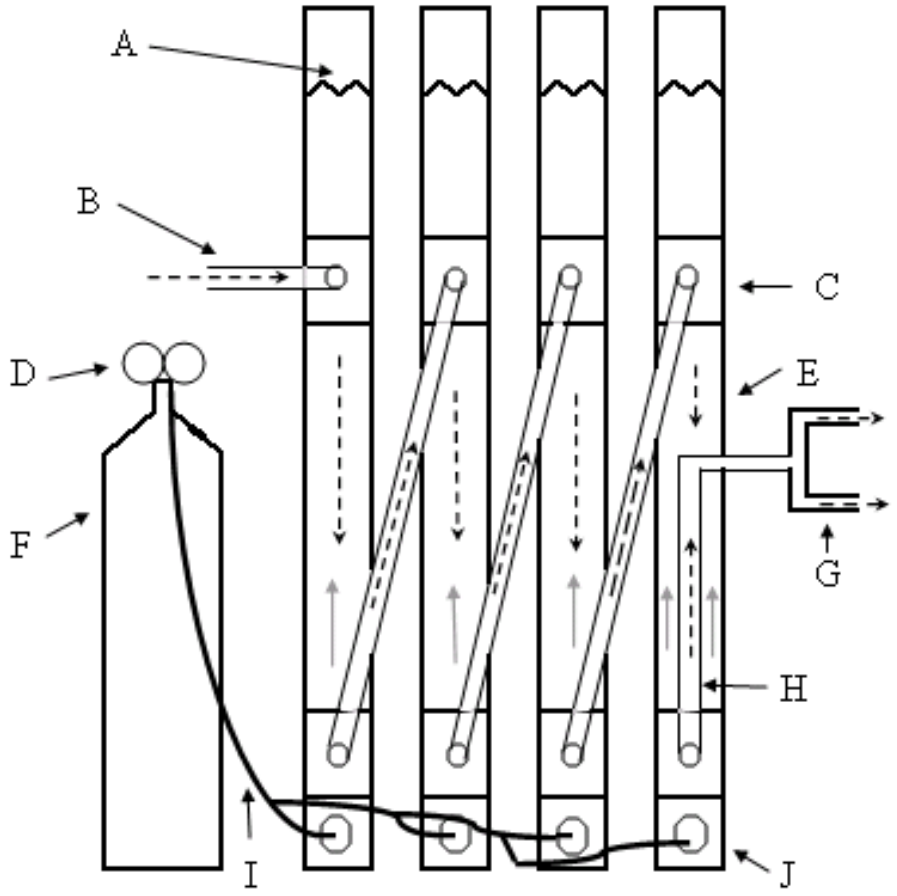


Figure 14. Schematic representation of oxygen-stripping columns. A = water level; B = seawater inflow; C = 5.1 cm slip/1.3 cm threaded pvc tee; D = gas regulator; E = 5.1 cm clear acrylic pipe; F = compressed N_2 gas cylinder; G = seawater outflow; H = 1.3 cm I.D. pvc pipe; I = 0.6 cm inside-diameter braided polyvinyl chloride (pvc) tubing; and J = 5.1 cm slip/5.1 cm threaded pvc tee and N_2 gas connection manifold. Gray arrows represent direction of N_2 gas flow. Dashed arrows represent direction of seawater flow. Total height of each stripping column is 243.8 cm.

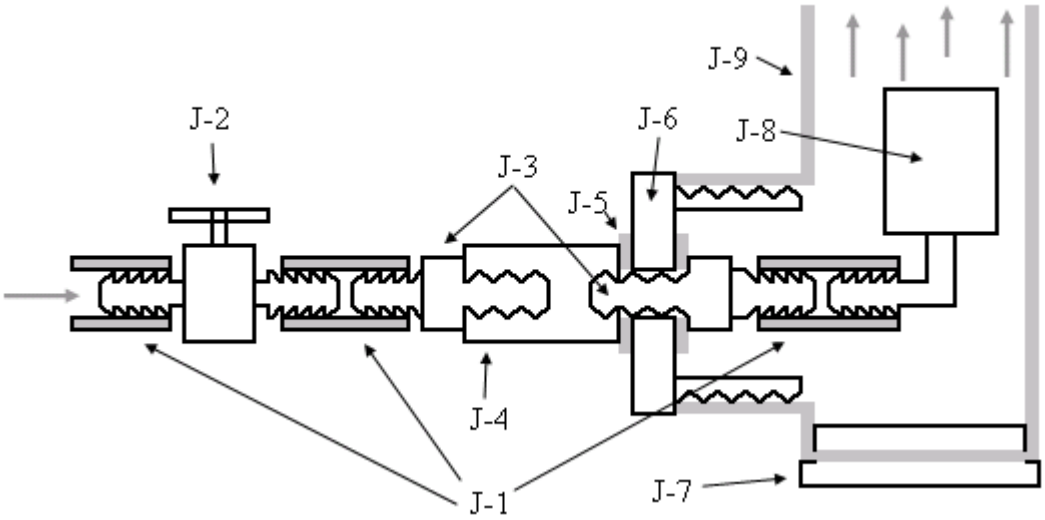


Figure 15. Close-up view of component J, N_2 gas connection manifold, from Figure 14. J-1 = 0.6 cm inside-diameter braided polyvinyl chloride (pvc) tubing; J-2 = gas needle valve; J-3 = 0.6 cm barbed/threaded polyethylene connector ; J-4 = 1.3 cm threaded pvc coupling; J-5 = 0.5 cm thick, ring-shaped closed cell neoprene gasket; J-6 = 5.1 threaded pvc plug with 1.9 cm diameter hole drilled in center; J-7 = 5.1 slip pvc plug; J-8 = Coral Life® lime wood "Stubby Stone"; J-9 = 5.1 cm slip/5.1 cm threaded pvc tee. Gray arrows represent direction of N_2 gas flow.

the air-water interface as well as to reduce the opportunity for partial reoxygenation of water. Shrimp were offered a commercial production feed (Rangen 45-10; Rangen Feeds, Buhl, ID) twice daily at a rate of 0.25 g of dry feed · shrimp^{-1}. Uneaten feed, feces, and exuvia were removed from each tank daily. A 12:12 photoperiod was implemented with an automated light-control system. Temperature, salinity, and DO were monitored twice daily using a YSI 85® meter (YSI Inc., Yellow Springs, OH). Tests for ammonia, nitrate, nitrite and pH were conducted weekly using methods adapted from those of Spotte (1979a,b) and Solarzano (1969), Spotte (1979a,b) and Mullen and Riley (1955), Spotte (1979a,b) and Strickland and Parsons (1972), and a YSI pH 100® meter, respectively. At the end of the 30-d growth and survival trial, shrimp

were weighed and redistributed in the following manner, in an effort to assess the extent of oxygen acclimation: 16 shrimp from the near-air-saturation DO treatment were transferred to the 2.0 $mg \cdot L^{-1}$ DO treatment (high to low), 16 shrimp from the near-air-saturation DO treatment were transferred to the same treatment, but different culture tank (high to high), 16 shrimp from the 2.0 $mg \cdot L^{-1}$ DO treatment were transferred to the near-air-saturation DO treatment (low to high), and 16 shrimp from the 2.0 $mg \cdot L^{-1}$ DO treatment were transferred to the same treatment, but different culture tank (low to low). Redistribution of shrimp was conducted in the above manner to ensure equal handling of individuals.

Experimental Respirometry System

Automated routine respirometry (Springer and Neill 1988) was conducted, using the two near- air-saturation DO culture tanks as water baths. Two shrimp from each of the four treatments were randomly selected and subjected to respirometric measurements of respirometric responses (RMR, LOCr, and MMS) daily for 8-d. Feeding of shrimp chosen for respirometry ceased 24-h prior to conducting respirometry in order to prevent feed ingestion.

Statistical Analysis

Statistical analyses were performed using SPSS (version 13.0, SPSS Inc., Chicago, Illinois). Growth rate (g/d) data from the 30-d growth and survival trial were analyzed using one-way analysis of variance to determine if significant differences ($P \leq 0.05$) existed among treatment means. Respirometric data (RMR, LOCr, and MMS) were analyzed using one-way analysis of variance to determine if differences existed

among treatment means. Respirometric data then were subjected to analysis of covariance to test for effects of DO and body weight (W) on RMR, LOCr, and MMS, where the dependent variable was either lnRMR, lnLOCr, or lnMMS; the factor was DO, and the covariate was lnW. Differences were considered significant at $P < 0.05$.

Results

Water Quality

Means (± SD) of dissolved oxygen (DO), temperature, salinity, total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3) and pH are shown in Table 8. Physiochemical factors remained within acceptable values for penaeid shrimp based on recommendations by Allan et al. (1990), Chen and Lei (1990), and Chen and Lin (1991).

Effects of DO on Growth and Survival

Significant differences in daily growth rate (g/d) were observed between shrimp maintained in the low- and high-DO treatments; low-DO shrimp tended to grow more slowly than high-DO shrimp (Table 9). DO treatment had little apparent effect on survival, which was over 90% for both treatments.

Effects of DO on Respirometric Responses

Mean values of RMR, LOCr, and MMS are shown in Table 10. Analysis of variance declared none of the respirometric responses to be significantly different ($P > 0.05$) among treatments. Routine metabolic rate (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) decreased with increasing shrimp weight for all four treatments tested (Figure 16). Regression of lnRMR (y) on lnW yielded the following power-model equations: $y = 0.998 \, x^{-0.733}$, $y = -0.629 \, x^{-0.212}$, $y = 0.356 \, x^{-0.546}$, and $y = -0.405 \, x^{-0.239}$ for the high to high, high to low,

low to high, and low to low treatments, respectively. lnRMR (y) on lnW yielded the following power-model equations: $y = 0.998 \times^{-0.733}$, $y = -0.629 \times^{-0.212}$, $y = 0.356 \times^{-0.546}$, and $y = -0.405 \times^{-0.239}$ for the high to high, high to low, low to high, and low to low treatments, respectively.

Table 8. Mean ± SD for water-quality parameters in the culture system. Observations of total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), and pH were taken weekly. Observations of temperature, salinity, and dissolved oxygen (DO) were taken twice daily (after attainment of 2 mg $O_2 \cdot L^{-1}$ for the low DO treatment).

	DO	
Parameter	2.0 ($mg \cdot L^{-1}$)	near air saturation ($mg \cdot L^{-1}$)
Temperature (ºC)	28.0 ± 0.15	27.9 ± 0.05
Salinity (ppt)	31.6 ± 4.16	31.6 ± 4.16
DO ($mg \cdot L^{-1}$)	2.2 ± 0.36	5.5 ± 0.25
TAN ($mg \cdot L^{-1}$)	0.1 ± 0.04	0.1 ± 0.03
NO_2 ($mg \cdot L^{-1}$)	0.0 ± 0.01	0.0 ± 0.01
NO_3 ($mg \cdot L^{-1}$)	0.2 ± 0.07	0.2 ± 0.05
pH	8.0 ± 0.06	8.1 ± 0.05

Table 9. Mean growth rate (g/d) and survival (up to 30-d).

treatment	mean growth rate (g/d)	survival to respirometry (%)
low DO	0.09	93.8
high DO	0.11	90.6
P value	0.010	-

Table 10. Mean RMR (mg $O_2 \cdot g^{-1} \cdot h^{-1}$), LOCr (mg $O_2 \cdot L^{-1}$), and MMS (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) for *L. vannamei* redistributed between and within high-DO (>5 mg$\cdot L^{-1}$) and low-DO (2 mg$\cdot L^{-1}$) treatments and subjected to respirometric measurement.

treatment	n	RMR (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)	LOCr (mg $O_2 \cdot L^{-1}$)	MMS (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)
high to high	14	0.33	1.83	0.19
high to low	15	0.30	1.87	0.16
low to high	15	0.31	1.71	0.19
low to low	14	0.35	1.88	0.19
P value	-	0.062	0.451	0.110

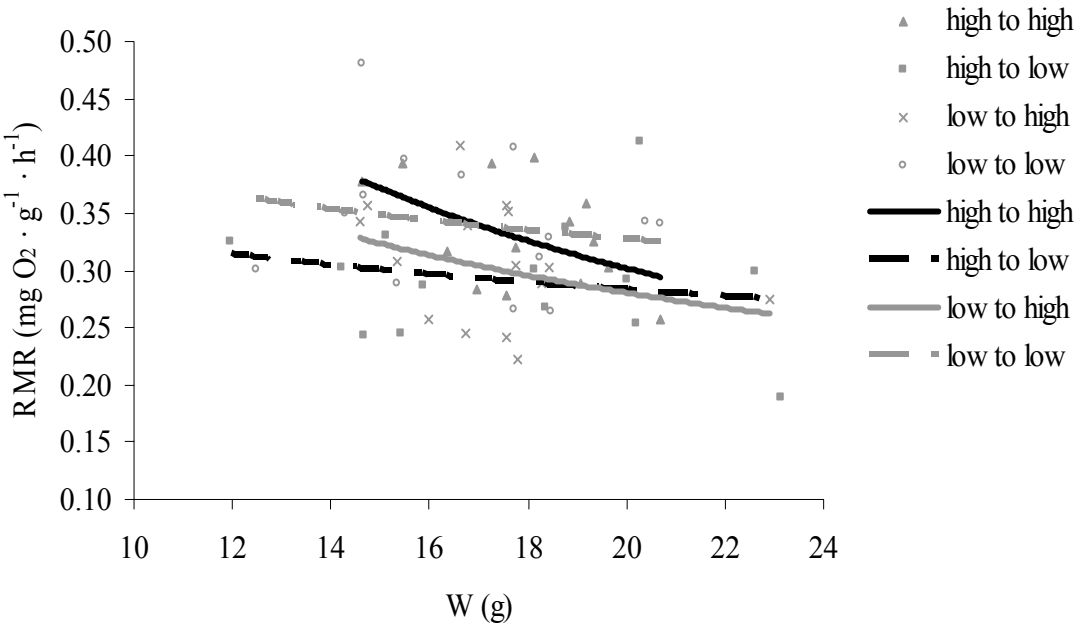


Figure 16. Routine metabolic rate (RMR) versus shrimp wet-weight (W) for *L. vannamei* redistributed between and within high-DO (>5 mg$\cdot L^{-1}$) and low-DO (2 mg$\cdot L^{-1}$) treatments.

Analysis of covariance showed no significant difference in lnRMR among treatments (P = 0.728). Limiting oxygen concentration (mg $O_2 \cdot L^{-1}$) also decreased with increasing

shrimp weight for all four treatments (Figure 17). Regression of lnLOCr (y) on lnW yielded the following power-model equations: $y = 1.470 x^{-0.305}$, $y = 1.482 x^{-0.305}$, $y = 2.688x^{-0.762}$, and $y = 1.188 x^{-0.204}$ for the high to high, high to low, low to high, and low to low treatments, respectively. Analysis of covariance showed no significant difference in lnLOCr among treatments (P = 0.476). Marginal metabolic scope (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) decreased with increasing shrimp weight for the high to high and low to low treatments,

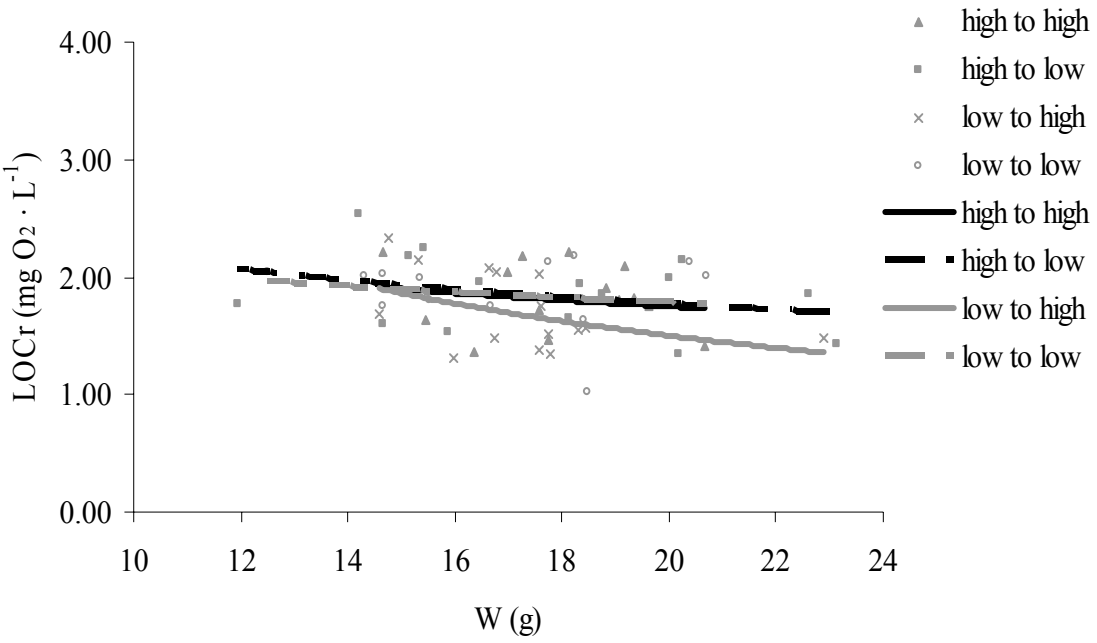


Figure 17. Limiting oxygen concentration (LOCr) versus shrimp wet-weight (W) for *L. vannamei* redistributed between and within high-DO (>5 mg·L^{-1}) and low-DO (2 mg·L^{-1}) treatments.

but not for low to high and high to low treatments (Figure 18). Regression of lnMMS (y) on lnW yielded the following power-model equations: $y = -0.598 x^{-0.380}$, $y = -2.086 x^{0.093}$, $y = -2.388x^{0.246}$, and $y = -1.393 x^{-0.099}$ for the high to high, high to low, low to

high, and low to low treatments, respectively. Analysis of covariance showed no significant difference in lnMMS among treatments (P = 0.894).

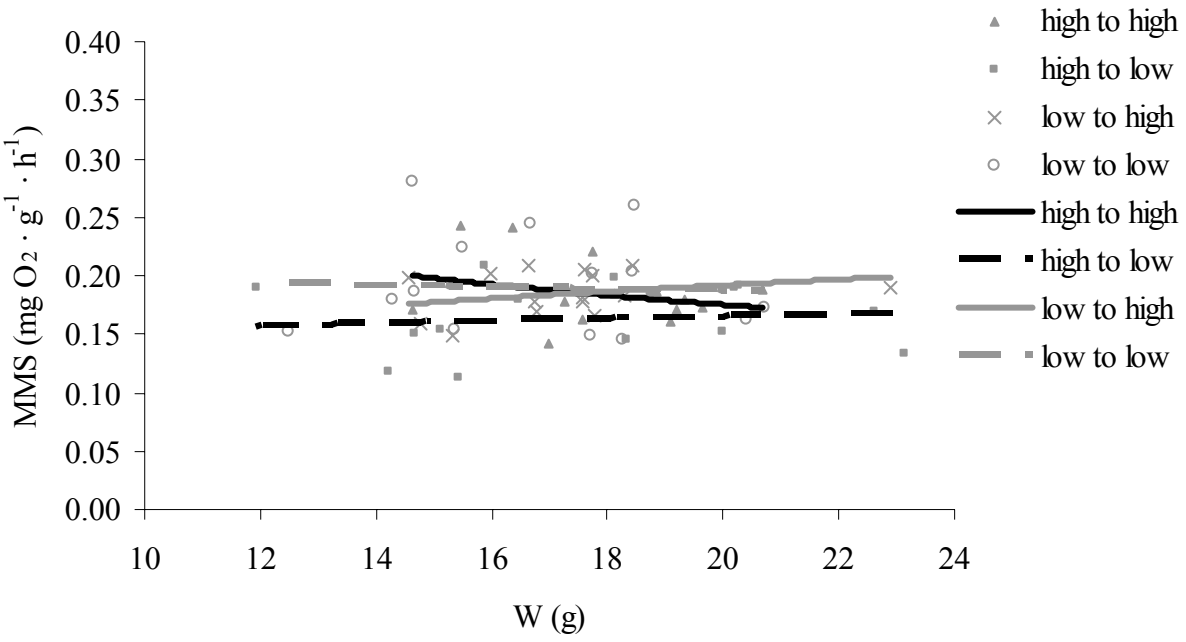


Figure 18. Marginal metabolic scope (MMS) versus shrimp wet-weight (W) for *L. vannamei* redistributed between and within high-DO (>5 mg·L^{-1}) and low-DO (2 mg·L^{-1}) treatments.

Discussion

In the present study, the daily growth rate of *L. vannamei* maintained under tolerable hypoxic conditions was significantly less than the growth rate of shrimp maintained under normoxic conditions. These results concur with results reported by Seidman and Lawrence (1985), who observed reduced growth of *L. vannamei* at constant DO values under 2.0 mg O_2·L^{-1}. Seidman and Lawrence (1985) attributed reduced growth in their study to reduction in feed consumption by shrimp under low-DO

conditions, but reduction in feed consumption was not quantified. Rosas et al. (1999b) reported decreased oxygen consumption for *Litopenaeus setiferus* maintained at 2 and 3 mg $O_2 \cdot L^{-1}$ relative to oxygen consumption of shrimp maintained between 4 and 5.8 mg $O_2 \cdot L^{-1}$, both at 15 and 35 ppt salinity. Clark (1986) noted that molting of *Penaeus semisulcatus* was reduced under hypoxic conditions, and that molting was coincident with return to normoxic conditions. As shrimp must molt in order to grow, this could be a possible factor explaining reduced growth under hypoxic conditions. However, molt frequency was not assessed in the present study.

A possible explanation for the disagreement between the ecophysiological response data (weight · treatment interaction) and the growth data may be that hypoxic conditions have little or no lasting effect on the metabolic capacity of *L. vannamei* once the common oxygen regime of respirometry is established. This, of course, is not to say that developmental hypoxia will not affect adult respiratory responses in *L. vannamei*. Previous studies with crustaceans have shown that hypoxic exposure during embryonic or larval periods has resulted in increases in oxygen uptake and metabolic capacity (Kobayashi et al. 1988, Hervant et al. 1995, Astall et al. 1997, Wiggins and Frappell 2000). However, results of this experiment indicate *L. vannamei*, in the size class evaluated, does not exhibit any substantial acclimatory response to changes in dissolved oxygen concentrations over the interval of 2.0 to >5.0 $mg \cdot L^{-1}$.

CHAPTER V

PARAMETERIZATION OF ECOPHYS.SHRIMP: A DETERMINISTIC SIMULATION MODEL OF SHRIMP GROWTH IN TIME-VARYING ENVIRONMENTAL REGIMES FOR THE PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*)

Introduction

Adaptation of Ecophys.Shrimp from Ecophys.Fish

Neill et al. (2004) developed the STELLA® model "Ecophys.Fish" for simulating growth of finfish in time-varying environment. They parameterized and tested the model for the euryhaline red drum *Sciaenops ocellatus* and the freshwater bluegill *Lepomis macrochirus*. Their model built upon the framework of F.E.J. Fry's "physiological classification of environment" (Fry 1947, 1971) and conventional fish bioenergetics theory (Warren 1967). Ecophys.Shrimp adopts the structure and rationale of Ecophys.Fish (Neill et al. 2004) in all regards with the exception of the invocation of processes contained in a "Molt Function" of the model (Figure 19).

From its inception, the goal of Ecophys.Shrimp was a unified model that could best describe multiple observed experiments with *L. vannamei*. For any simulation model, a near 1:1 relationship between observed and simulated results can be achieved for a given data set. But that does not mean the model will work for a different data set. That being said, Ecophys.Shrimp was programmed to best describe the experiments in Chapters II – IV with a minimal number of conditional statements. For that reason, the model does not contain environment-specific constraints (i.e. IF – THEN statements

regarding salinity, temperature, or DO) outside of those found in Ecophys.Fish. The constraints placed on the model are those I believe to be consistent with the natural life history of *L. vannamei*, or consistent with either growth or starvation experiments that I performed. Of the model parameters that were changed, I believe two in particular deserve mention: Winberg and Wexp. In Ecophys.Fish, the value for Winberg was set to a constant throughout simulation; however, for Ecophys.Shrimp the value for Winberg was set at 2 while the simulated shrimp were kept under culture conditions at which point Winberg degraded with body weight during the period of time the simulated shrimp (> 3.5 g) underwent respirometry. This was necessary in order to get acceptable fit of simulated shrimp to observed shrimp, and I believe it to be an artifact of the physical constraint placed on the shrimp while inside the respirometry chambers. The 3.5g "cut-off" is also used in the Wexp function of the model. Shrimp weighing < 3.5g are assigned a weight exponent of -0.42, whereas shrimp > 3.5g are assigned a weight exponent of -0.30. The division at 3.5g is coincident with the natural shift from near-exponential to near-linear growth in *L. vannamei*. For quick reference, differences between the Ecophys.Fish and Ecophys.Shrimp models' equations are presented in APPENDIX A. Definitions for all terms in the Ecophys.Shrimp model can be found in APPENDIX B.

Development of a deterministic model for positive and negative shrimp growth presented a problem unique to crustaceans. In order for shrimp to experience "growth" (either positive or negative), they must molt. Molting is the process by which shrimp exchange an old exoskeleton for a new exoskeleton. During growth, a shrimp's tissues

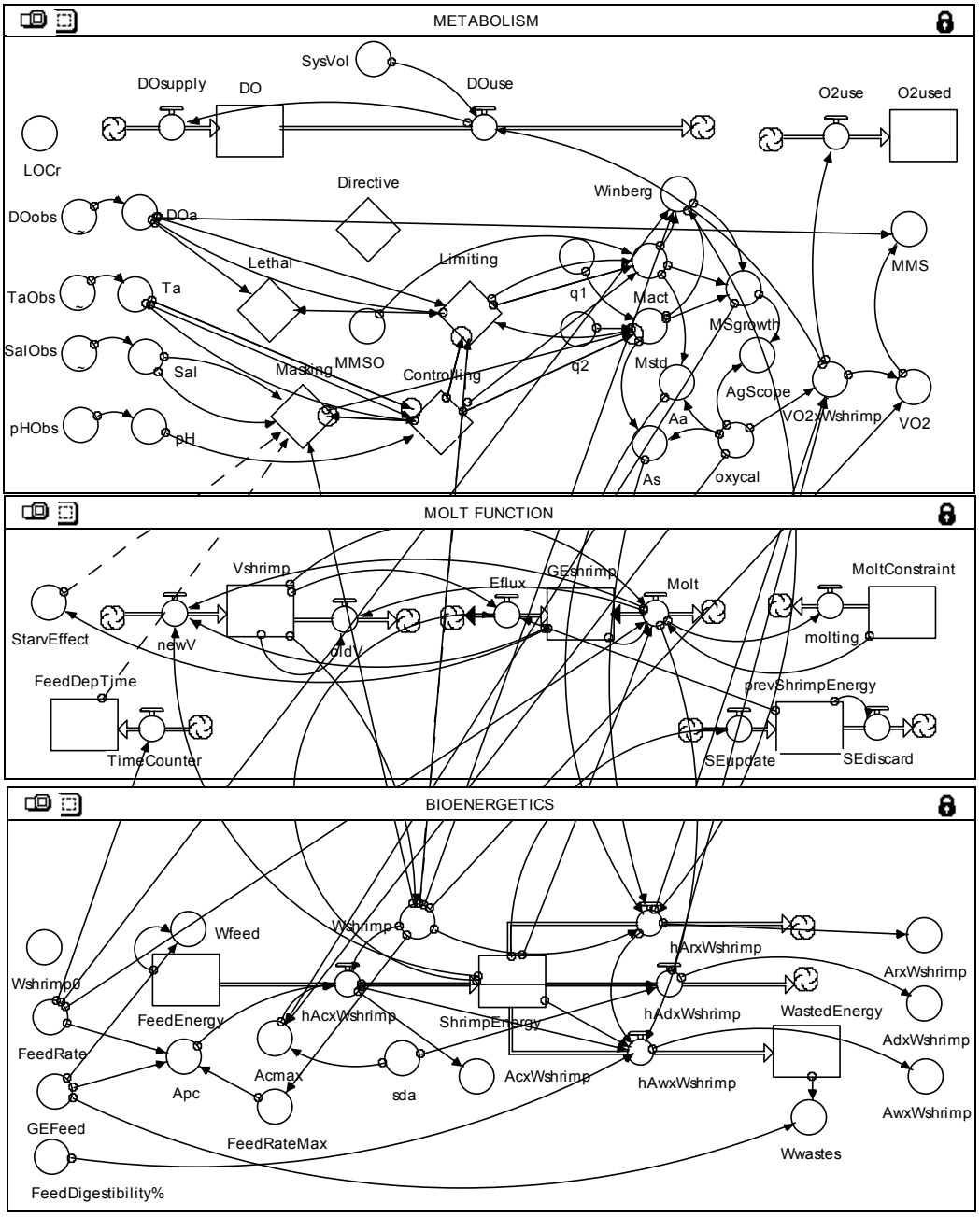


Figure 19. Ecophys.Shrimp: STELLA® model of shrimp growth in response to ecophysiological factors.

will fill the confines of its hardened exoskeleton, thereby decreasing interstitial space. In order to make room for continued growth, the shrimp will simultaneously stop eating

and dehydrate itself. The shrimp then will molt and exchange its old hardened exoskeleton for a new soft exoskeleton. Because the new exoskeleton is soft (not yet mineralized), it can be expanded when the shrimp hydrates its tissues, thus increasing volume of the new exoskeleton and allowing for continued growth. However, this process may be reversed for starving shrimp, allowing the new exoskeleton to be smaller in volume than the old exoskeleton (Siccardi et al. 2006).

To account for molting, the simulation model linked the molting phenomenon with the simulated gross energy of the shrimp. In the language of STELLA®, this relationship is represented in the "molt" biflow by the statement:

IF FeedRate > 0 AND GEshrimp > 1200 AND (ShrimpEnergy/GEshrimp > Vshrimp) THEN (1200 - 1100)/DT ELSE IF FeedRate = 0 AND GEshrimp < MoltConstraint · 950 THEN -(MoltConstraint · (1000 - 950)) /DT ELSE 0

This statement can be explained as follows: For growing shrimp, as energy density reaches a maximum value of 1200 cal·g^{-1} (live weight), the animal molts and its energy density is reset by hydration to a value of 1100 cal·g^{-1}. For starving shrimp (where feed rate = 0), the model incorporates the state variable "MoltConstraint" and unidirectional-flow variable "molting" (c.f. Figure 19). These two variables act together to constrain the molting phenomenon so that final simulated shrimp weight approaches that of final shrimp weight observed in laboratory trials. It must be noted that for real shrimp, change in size occurs immediately after molting, but increase in tissue density through protein synthesis occurs though the intermolt period (Zanotto and Wheatly 2003). However, for Ecophys.Shrimp, no distinction is made between change in size and

change in weight, as a 1:1 relationship between tissue density and water density is assumed. Thus, for the simulated shrimp, all growth (positive or negative) occurs at the point of molting and biomass remains static until the next molt (Figure 20).

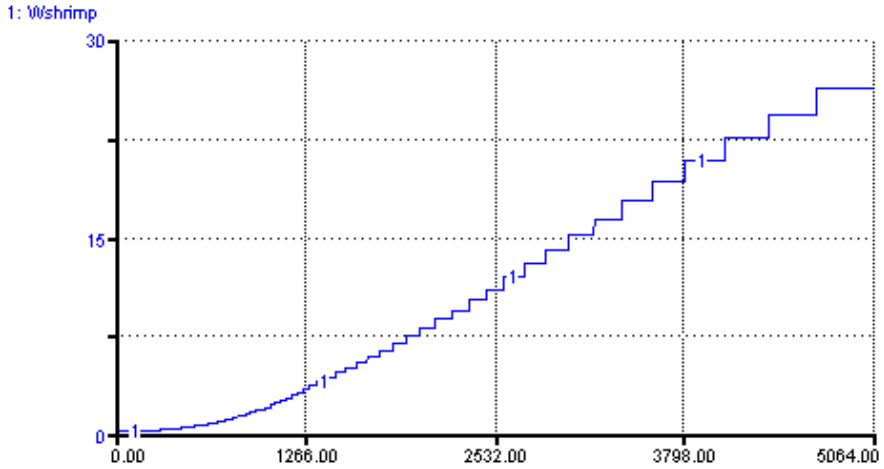


Figure 20. Graphical output of shrimp growth under the model Ecophys.Shrimp where y-axis is shrimp weight (g) and x-axis is time (h).

Materials and Methods

Parameterization of Ecophys.Shrimp

For each shrimp subjected to respirometric assay in Chapters II-IV, a simulated shrimp was created in the Ecophys.Shrimp Stella® computer program. Each simulated shrimp was given the same environmental history (salinity, temperature, and DO) as the real shrimp. Furthermore, each simulated shrimp was "run" for the same amount of time as that for each real shrimp, between stocking and respirometry. Model inputs for each simulated shrimp were the initial gross energy of the shrimp (in all cases, this was set at 1100 cal·g^{-1}, the assumed value for a newly molted shrimp), gross energy of the feed

($cal \cdot g^{-1}$), feed digestibility (assumed to be 80%), feed availability (assumed not to be limiting), starting weight of the shrimp (or, in the case of the salinity experiment in Chapter II, the mean starting weight), and mean LOCr value from respirometry which was incorporated into the DO history of each shrimp as the final value of DO. Model outputs used for comparison of the observed shrimp to the simulated shrimp were MMS, RMR, and final shrimp weight.

Statistical Analysis

Laboratory results from Chapter II were compared to simulation results using ANCOVA. Comparison of respirometric responses was done with lnRMR or lnMMS as the dependent variable, lnW as the covariate, and the fixed factor was observed or simulated. Final weights (at respirometry), simulated and observed, from the salinity experiment were also analyzed with ANCOVA. Weight was the dependent variable, time (month of simulation) was the covariate, and observation or simulation was the fixed factor. Observed results from Chapter III were compared to simulation results using ANCOVA. Comparison of respirometric responses was done with RMR or MMS as the dependent variable, time as the covariate, and the fixed factor either observation or simulation. Weight change (from stocking W to respirometry W), simulated and observed, from the temperature experiment (shrimp fed commercial feed and starved) were also analyzed with ANCOVA. Weight change was the dependent variable, time (d) was the covariate, and observation or simulation was the fixed factor. Comparisons of simulated and observed results were done for each salinity tested in Chapter II; each

temperature, treatment (fed or starved), and diet in Chapter III; and, each DO regime in Chapter IV. Differences were considered significant at $P < 0.05$.

Results

Salinity

Results from analysis of covariance comparing observed results to simulated results are presented in Table 11. Graphical comparisons of RMR, MMS and W versus

Table 11. Analyses of covariance for comparison of observed responses versus responses simulated with Ecophys.Shrimp at three salinities: 2, 10, and 28 ppt.

	Salinity		
Parameter	2 ppt	10 ppt	28 ppt
RMR			
P-value	0.151	0.702	0.374
R^2	0.612	0.804	0.746
MMS			
P-value	0.132	0.722	0.689
R^2	0.650	0.701	0.567
W			
P-value	0.177	0.858	0.788
R^2	0.952	0.960	0.971

time are shown in Figures 21 – 23. In every case, there was no significant difference between empirical values and simulated values. In Chapter II, it was hypothesized that an underlying metabolic change occurs in *L. vannamei* when they reach 9 g in body weight. Accounting for this in Ecophys.Shrimp was accomplished by making the parameter "smin" conditional, so that:

$$\text{IF Wshrimp} < 9 \text{ THEN } 0.065 \cdot \text{Wshrimp}^{-0.2} \text{ ELSE } 0.064 \cdot \text{Wshrimp}^{-0.2}$$

Although the equation above may seem to have minimal differential effects at Wshrimp = 9, the effect over time is rather dramatic with regards to final W.

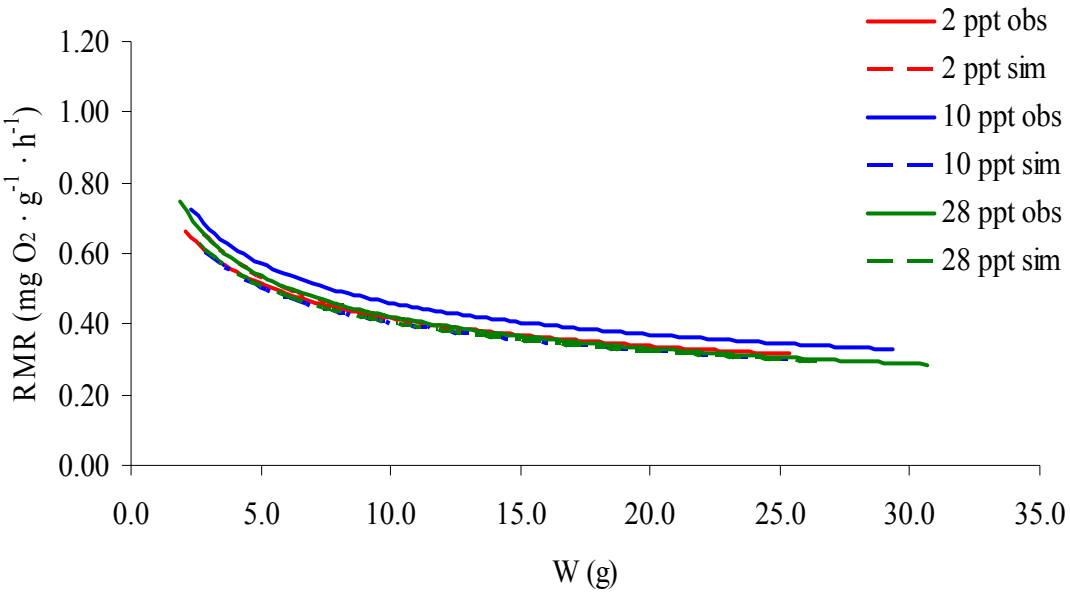


Figure 21. Routine metabolic rate (RMR) versus shrimp wet-weight (W) at 2, 10, and 28 ppt for observed (obs) and simulated (sim) shrimp.

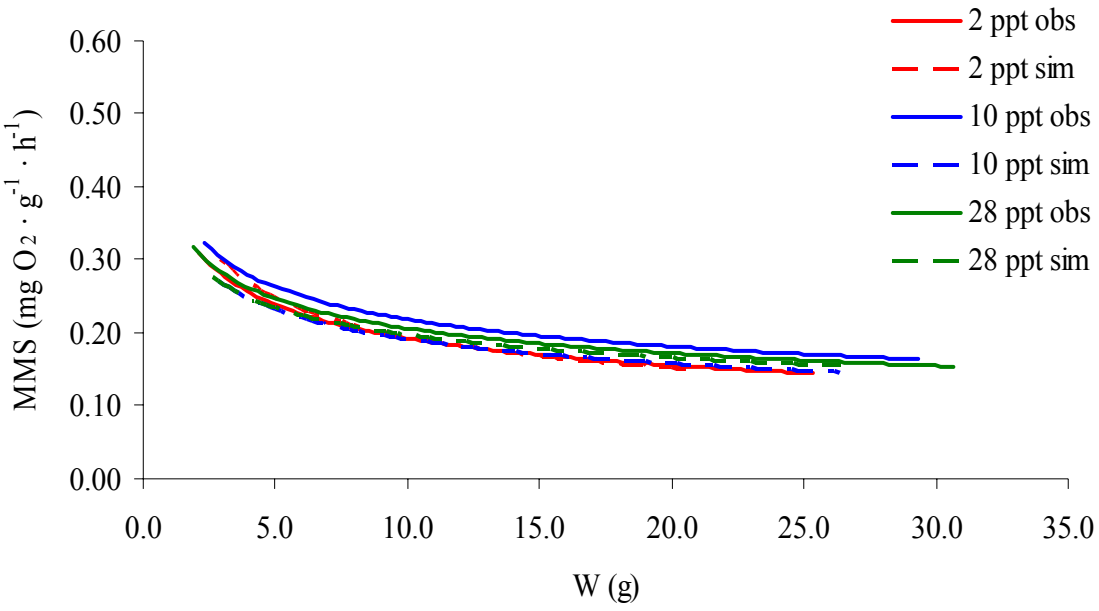


Figure 22. Marginal metabolic scope (MMS) versus shrimp wet-weight (W) at 2, 10, and 28 ppt for observed (obs) and simulated (sim) shrimp.

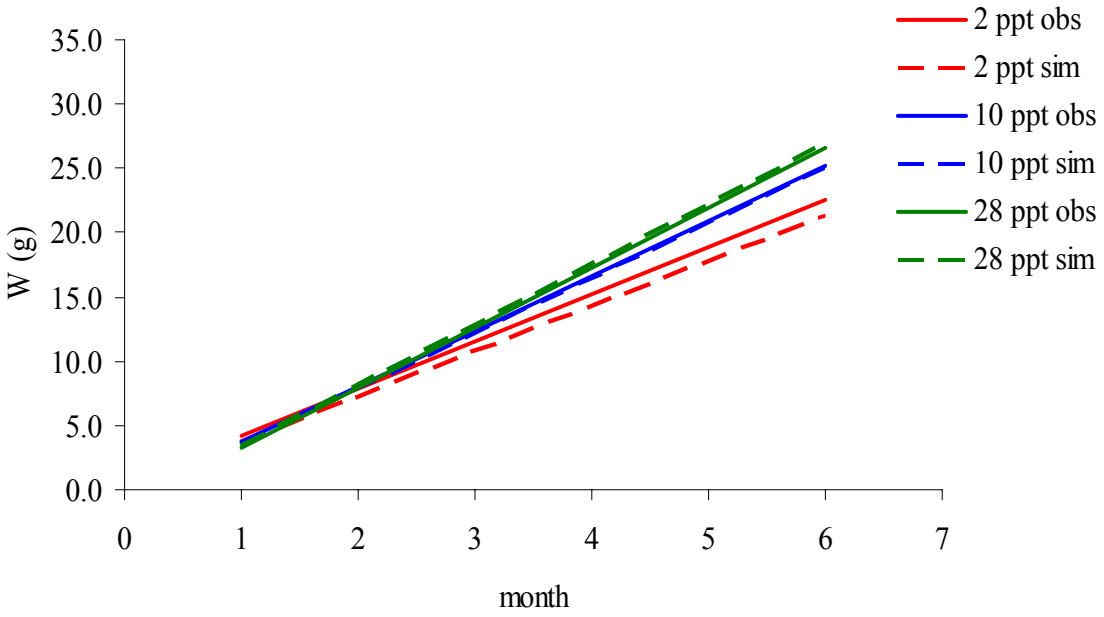


Figure 23. Shrimp wet-weight (W) versus time (months) at 2, 10, and 28 ppt for observed (obs) and simulated (sim) shrimp.

Temperature: Commercial Feed

Results from analysis of covariance comparing observed results to simulated results are depicted in Table 12. Graphical comparisons of RMR and MMS versus time are shown in Figures 24 – 26. In every case, there was no significant difference between empirical and modeled responses—but, in several cases, differences approached significance.

Table 12. Analyses of covariance for comparison of observed results (shrimp fed commercial production feed, Rangen 45-10) versus Ecophys.Shrimp results at four temperatures: 20, 24, 28, and 32 ºC.

	Temperature			
Parameter	20 ºC	24 ºC	28 ºC	32 ºC
RMR				
P-value	0.958	0.097	0.078	0.070
R_2	0.310	0.192	0.072	0.030
MMS				
P-value	0.823	0.705	0.130	0.062
R_2	0.177	0.006	0.026	0.036
ΔW				
P-value	0.691	0.319	0.402	0.574
R_2	0.160	0.628	0.385	0.175

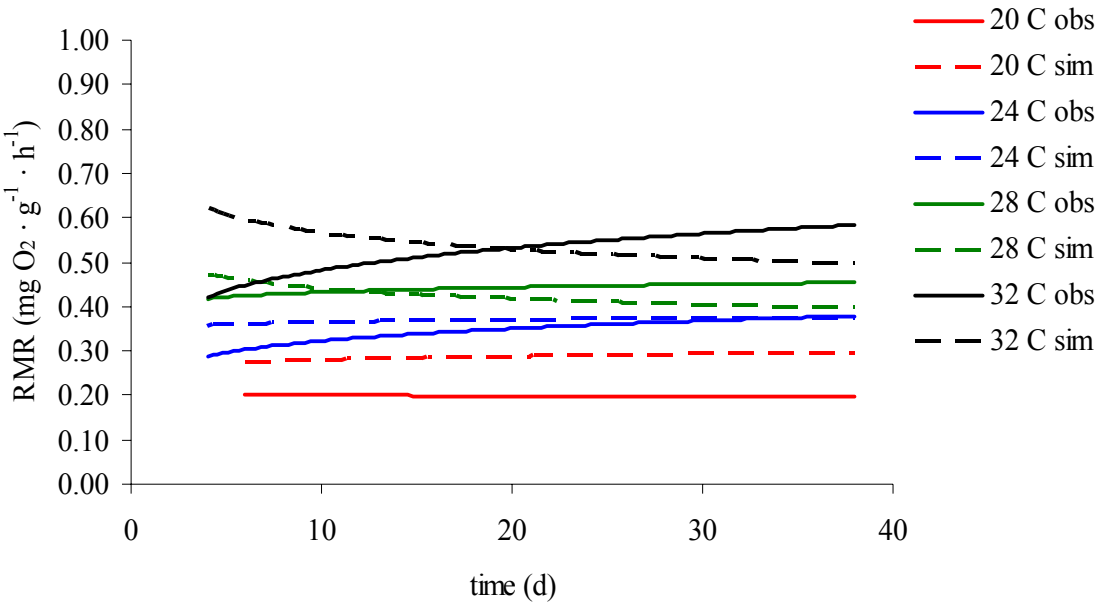


Figure 24. Routine metabolic rate (RMR) versus time (d) at 20, 24, 28, and 32 ºC for observed (obs) and simulated (sim) shrimp fed a commercial production feed (Rangen 45-10).

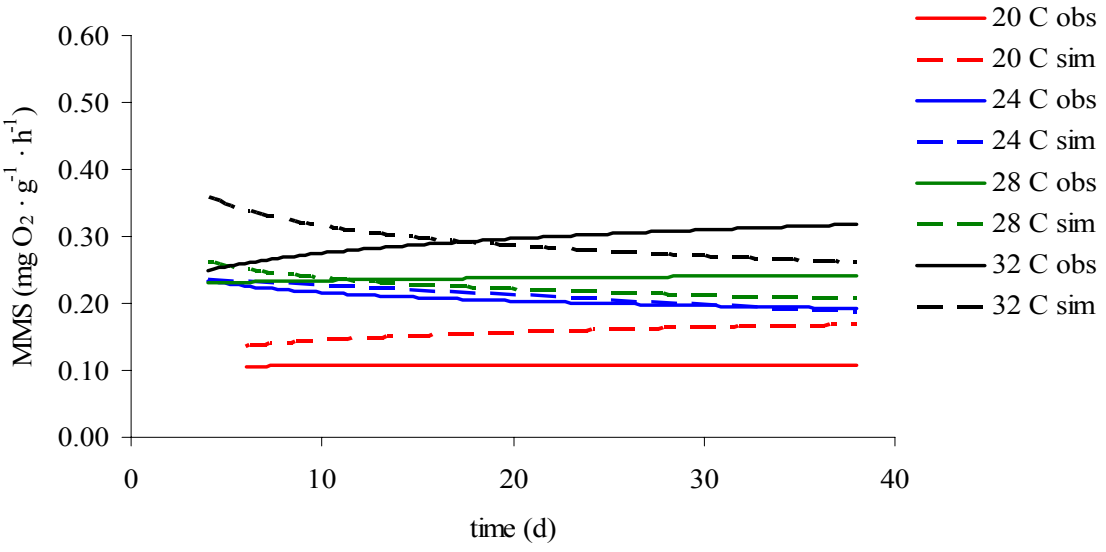


Figure 25. Marginal metabolic scope (MMS) versus time (d) at 20, 24, 28, and 32 ºC for observed (obs) and simulated (sim) shrimp fed a commercial production feed (Rangen 45-10).

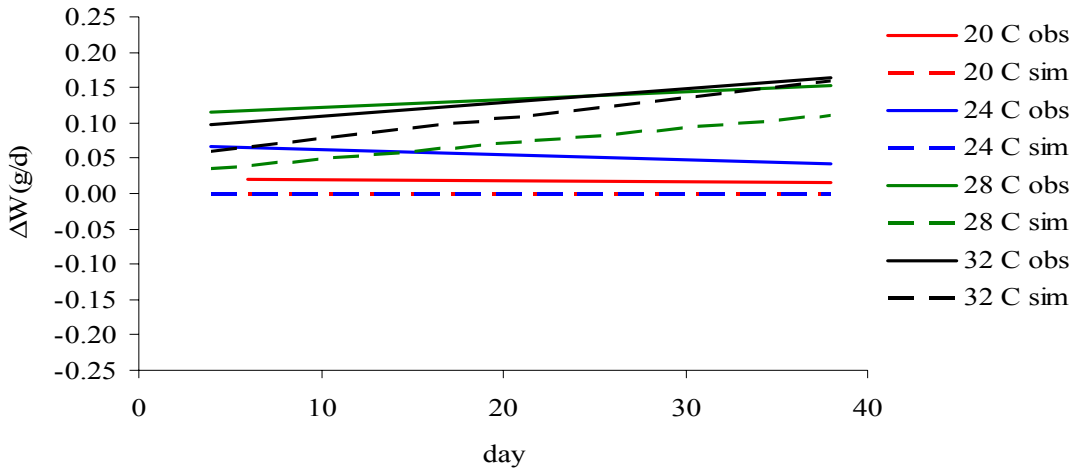


Figure 26. Weight change (ΔW) versus time (d) at 20, 24, 28, and 32 ºC for observed (obs) and simulated (sim) shrimp fed a commercial production feed (Rangen 45-10).

Temperature: 25 and 35% Protein Diets

Results from analysis of covariance comparing observed results to simulated results are depicted in Table 13. Graphical comparisons of observed and simulated results for RMR and MMS versus shrimp wet-weight are shown in Figures 27 – 32. In every case, there was no significant difference between empirical values and modeled values (P = 0.05).

Table 13. Analyses of covariance for comparison of observed results (shrimp fed either 25 or 35% protein feed) versus Ecophys.Shrimp results at two temperatures: 24 and 28 ºC.

	Treatment			
	25% protein feed		35% protein feed	
Parameter	24 ºC	28 ºC	24 ºC	28 ºC
RMR				
P-value	0.484	0.531	0.626	0.979
R_2	0.040	0.059	0.229	0.040
MMS				
P-value	0.284	0.862	0.366	0.154
R_2	0.098	0.053	0.196	0.184
ΔW				
P-value	0.098	0.199	0.207	0.357
R_2	0.529	0.085	0.530	0.114

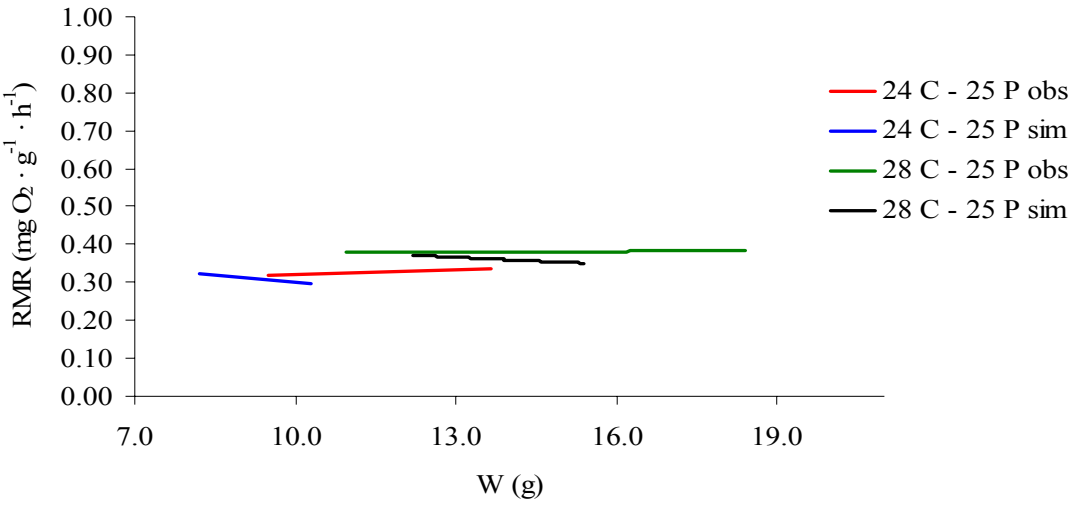


Figure 27. Routine metabolic rate (RMR) versus shrimp wet-weight (W) at 24 and 28 ºC for observed and simulated shrimp fed a 25% protein diet.

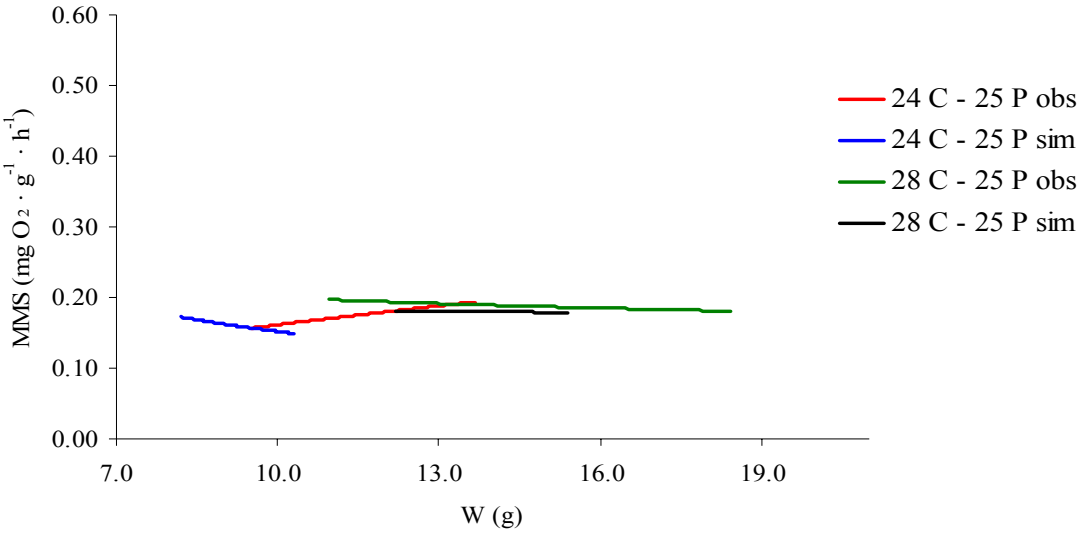


Figure 28. Marginal metabolic scope (MMS) versus shrimp wet-weight (W) at 24 and 28 ºC for observed and simulated shrimp fed a 25% protein diet.

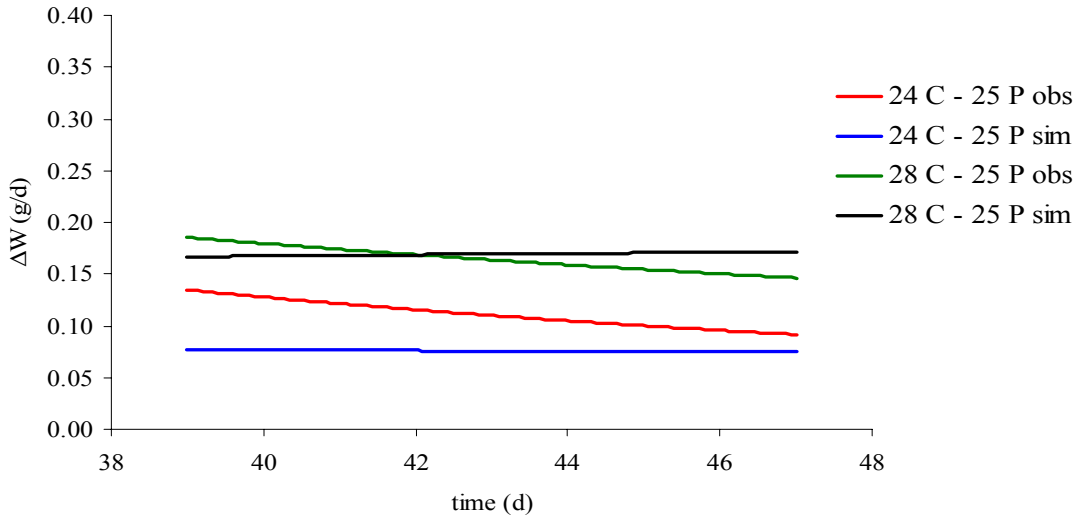


Figure 29. Weight change (ΔW) versus time (d) at 24 and 28 ºC for observed and simulated shrimp fed a 25% protein diet.

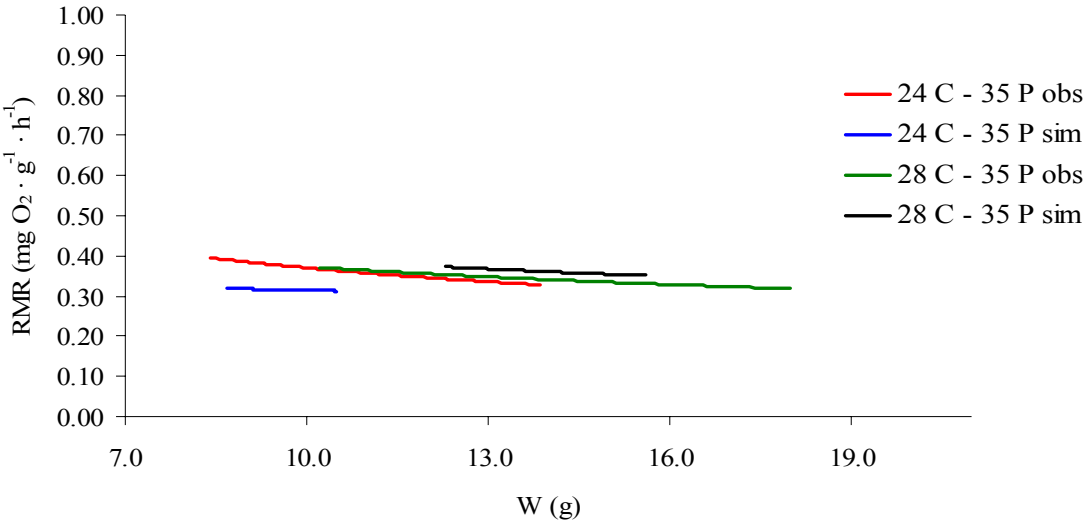


Figure 30. Routine metabolic rate (RMR) versus shrimp wet-weight (W) at 24 and 28 ºC for observed and simulated shrimp fed a 35% protein diet.

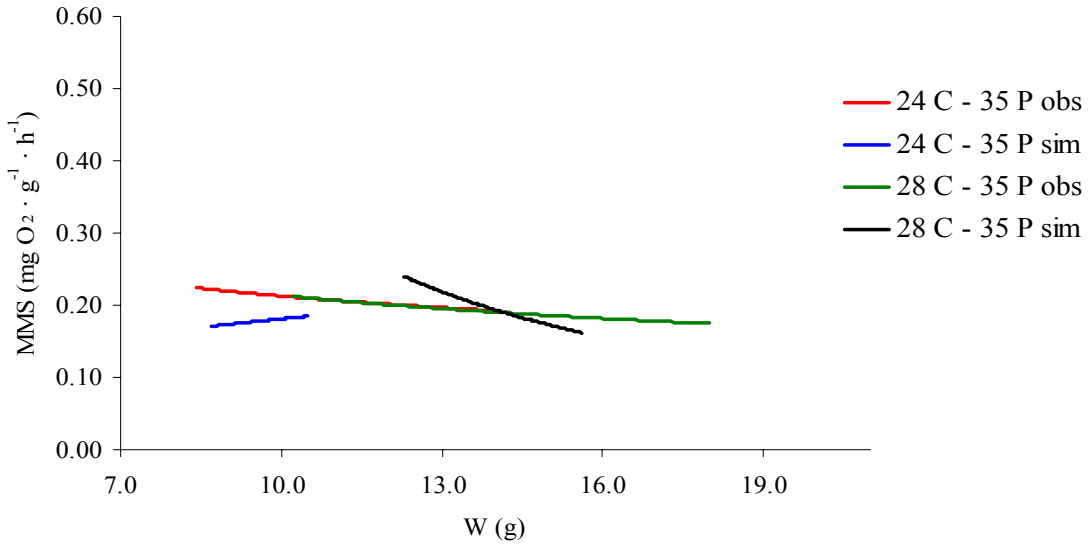


Figure 31. Marginal metabolic scope (MMS) versus shrimp wet-weight (W) at 24 and 28 ºC for observed and simulated shrimp fed a 35% protein diet.

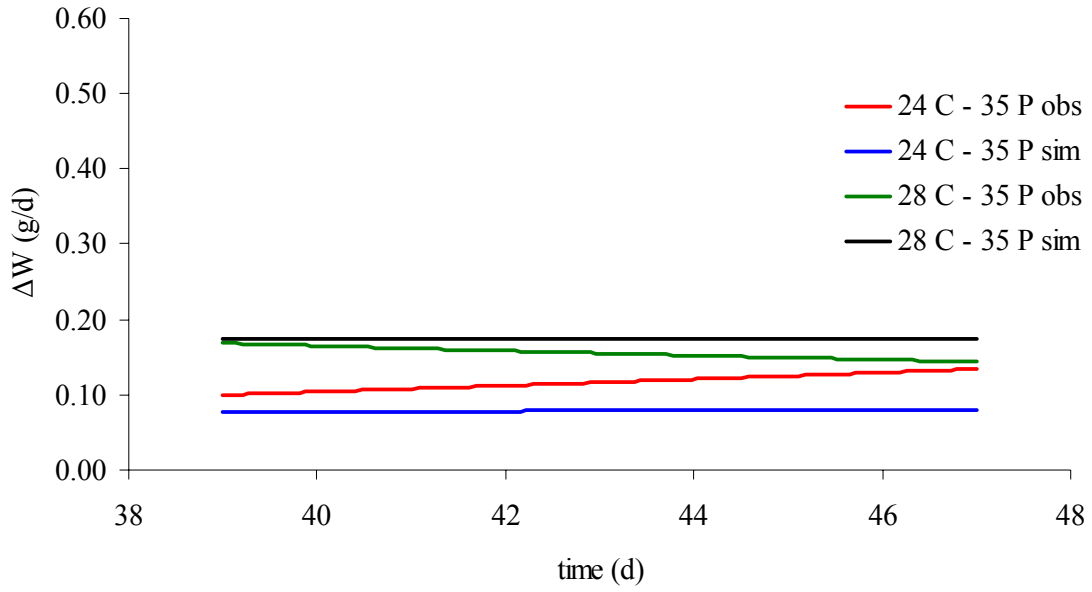


Figure 32. Weight change (ΔW) versus time (d) at 24 and 28 ºC for observed and simulated shrimp fed a 35% protein diet.

Temperature: Starvation

Results from analysis of covariance comparing observed results to simulated results are depicted in Table 14. Graphical comparisons of observed and simulated results for RMR, MMS, and daily change in weight (ΔW; g/d) versus time (d) are shown in Figures 33 – 35. There was no significant difference between empirical values and modeled values ($P < 0.05$).

Table 14. Analyses of covariance for comparison of ecophysiological responses for empirical results versus computer simulated results for shrimp starved at four temperatures: 20, 24, 28, and 32 ºC.

	Temperature			
Parameter	20 ºC	24 ºC	28 ºC	32 ºC
RMR				
P-value	0.138	0.893	0.410	0.648
R_2	0.123	0.199	0.514	0.539
MMS				
P-value	0.709	0.940	0.283	0.698
R_2	0.022	0.115	0.654	0.289
ΔW				
P-value	0.105	0.059	0.149	0.307
R_2	0.179	0.394	0.284	0.422

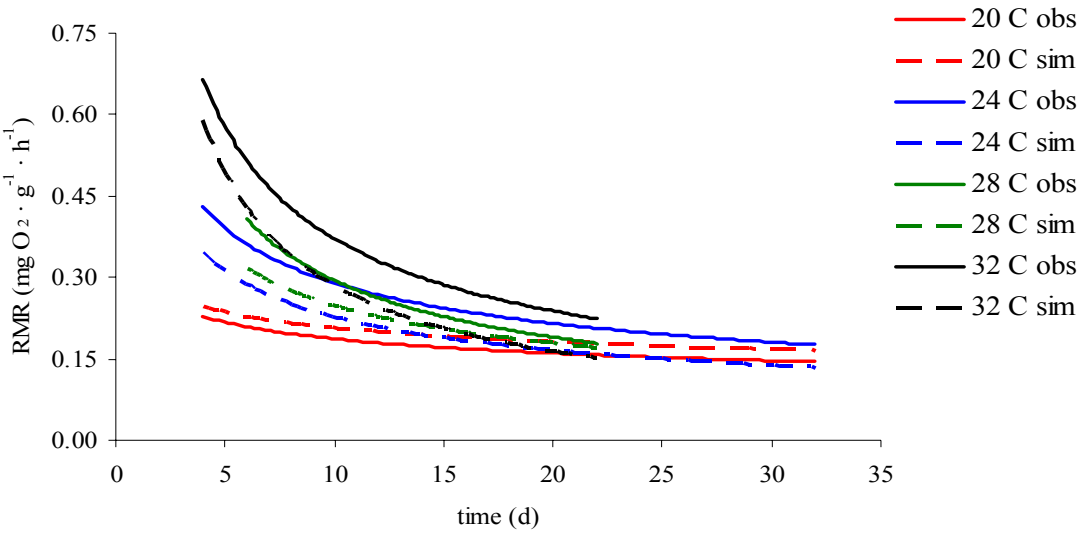


Figure 33. Routine metabolic rate (RMR) versus time (d) for observed (obs) and simulated (sim) shrimp starved at 20, 24, 28, and 32 ºC.

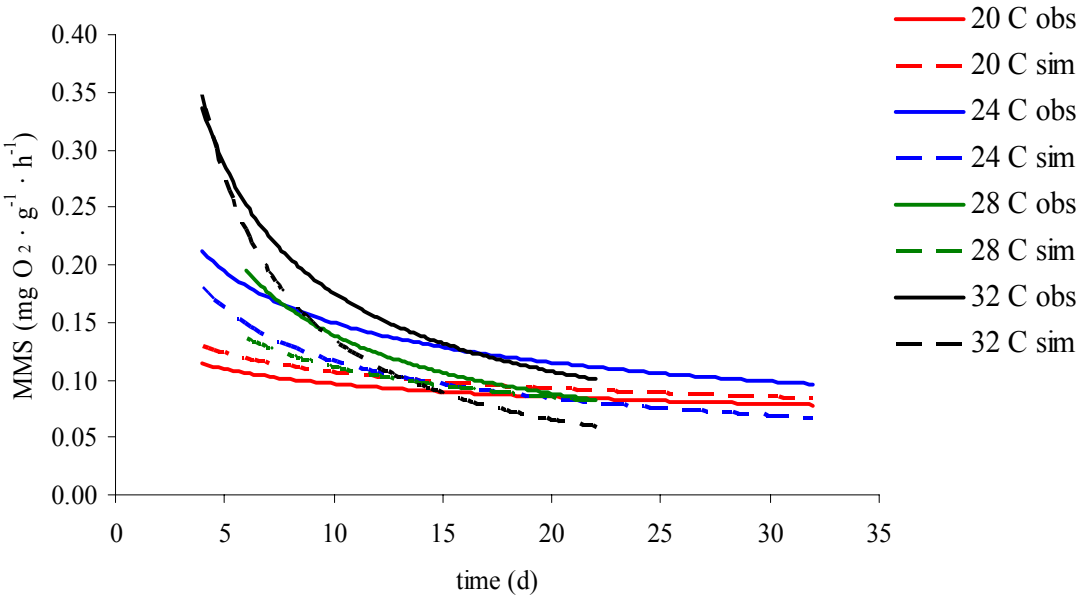


Figure 34. Marginal metabolic scope (MMS) versus time (d) for observed (obs) and simulated (sim) shrimp starved at 20, 24, 28, and 32 ºC.

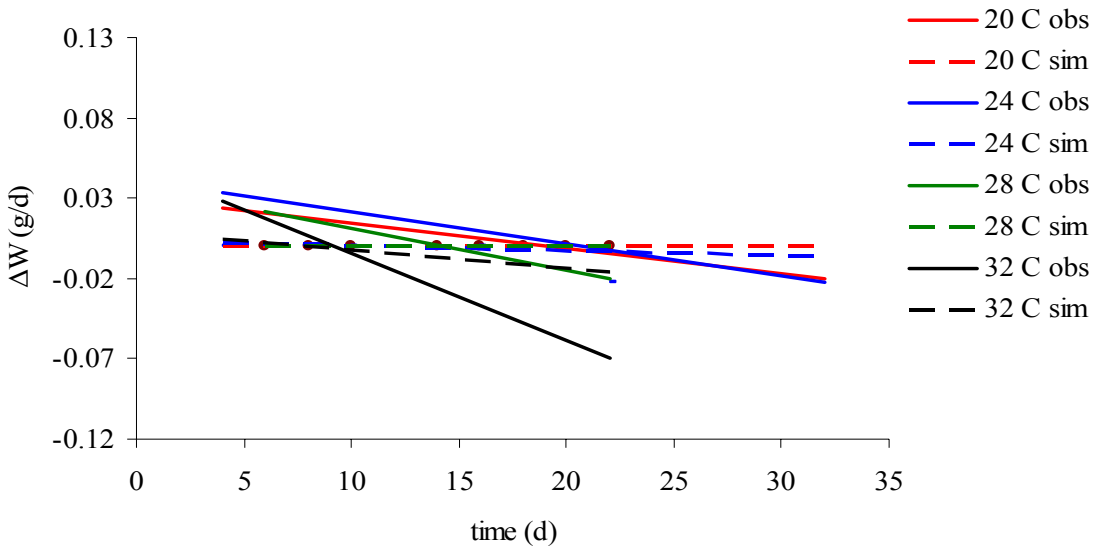


Figure 35. Weight change (ΔW; g/d) versus time (d) for observed (lab) and simulated (sim) shrimp starved at 20, 24, 28, and 32 ºC.

Dissolved Oxygen

Results from analysis of covariance comparing observed results to simulated results are depicted in Table 15. Graphical comparisons of observed and simulated results for RMR and MMS versus time are shown in Figures 36 – 45. In every case, there was no significant difference between empirical values and modeled values.

Table 15. Analyses of covariance for comparison of ecophysiological responses for empirical results versus computer simulated results for four DO regimes. Shrimp transferred from near air saturation to near air saturation (high to high), shrimp transferred from near air saturation to 2 $mg \cdot L^{-1}$ (high to low), shrimp transferred from 2 $mg \cdot L^{-1}$ to near air saturation (low to high), and shrimp transferred from 2 $mg \cdot L^{-1}$ to 2 $mg \cdot L^{-1}$ (low to low) after 30 d of growth at either 2 $mg \cdot L^{-1}$ or near air saturation DO. Shrimp growth (g/d) comparisons are between shrimp maintained for 30 d at either 2 $mg \cdot L^{-1}$ or near air saturation DO.

	DO regime			
	30-d growth high DO		30-d growth low DO	
Parameter	high to high	high to low	low to high	low to low
RMR				
P-value	0.175	0.915	0.630	0.854
R_2	0.326	0.200	0.183	0.077
MMS				
P-value	0.758	0.642	0.314	0.781
R_2	0.028	0.068	0.193	0.043
ΔW				
P-value	0.294		0.233	
R_2	0.475		0.615	

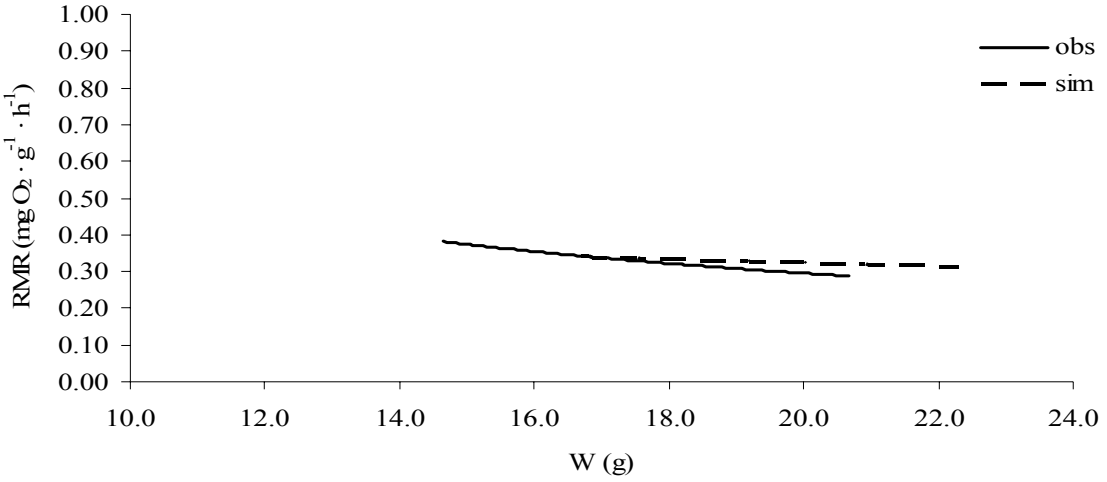


Figure 36. Routine metabolic rate (RMR) versus shrimp wet weight (W) for observed (obs) and simulated (sim) shrimp maintained for 30-d at near-air-saturation DO and then transferred to near-air-saturation DO.

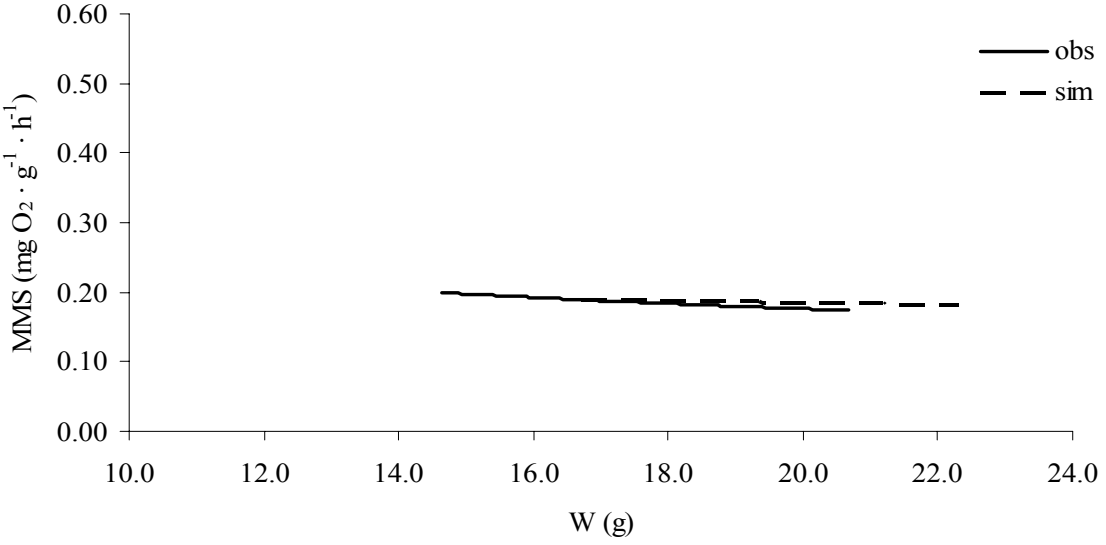


Figure 37. Marginal metabolic scope (MMS) versus shrimp wet weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at near-air-saturation DO and then transferred to near-air-saturation DO.

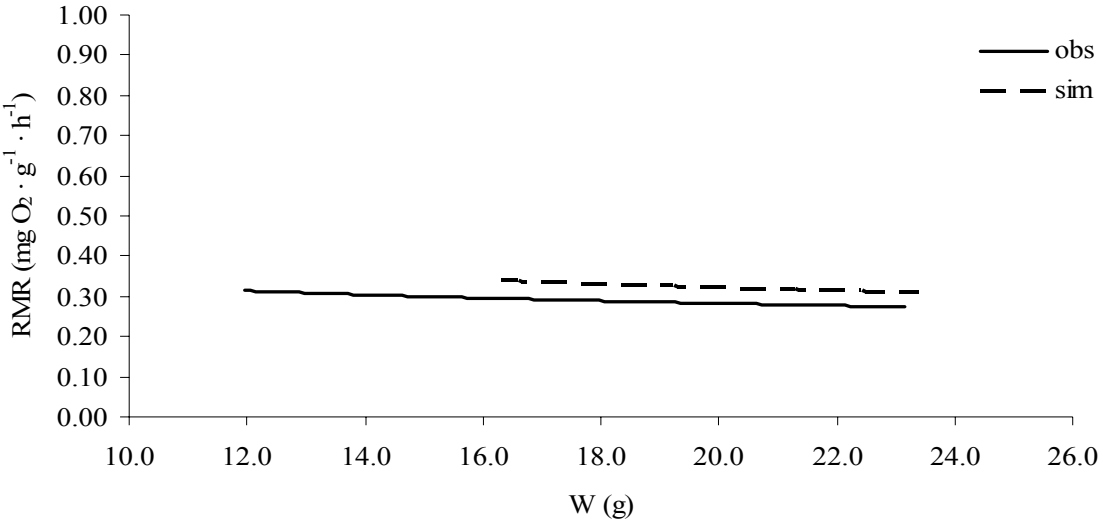


Figure 38. Routine metabolic rate (RMR) versus shrimp wet weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at near-air-saturation DO and then transferred to 2 mg·L^{-1} DO.

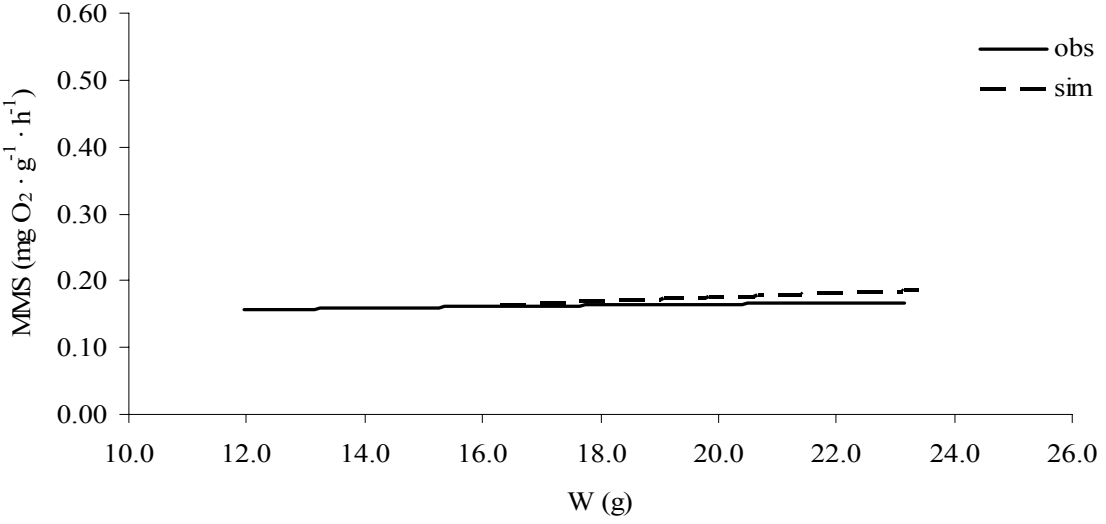


Figure 39. Marginal metabolic scope (MMS) versus shrimp wet weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at near-air-saturation DO and then transferred to 2 mg·L^{-1} DO.

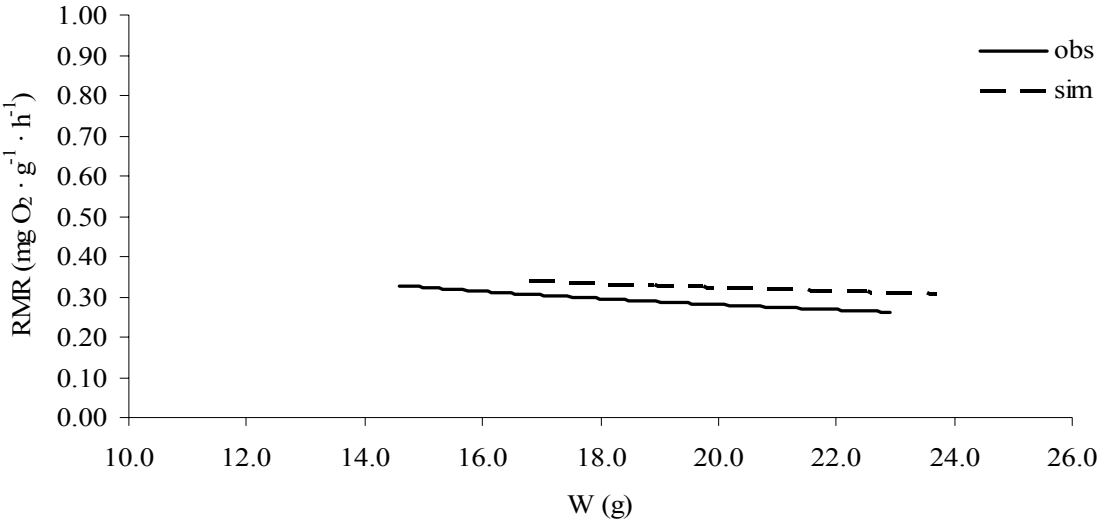


Figure 40. Routine metabolic rate (RMR) versus shrimp wet weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at 2 mg·L^{-1} DO and then transferred to near-air-saturation DO.

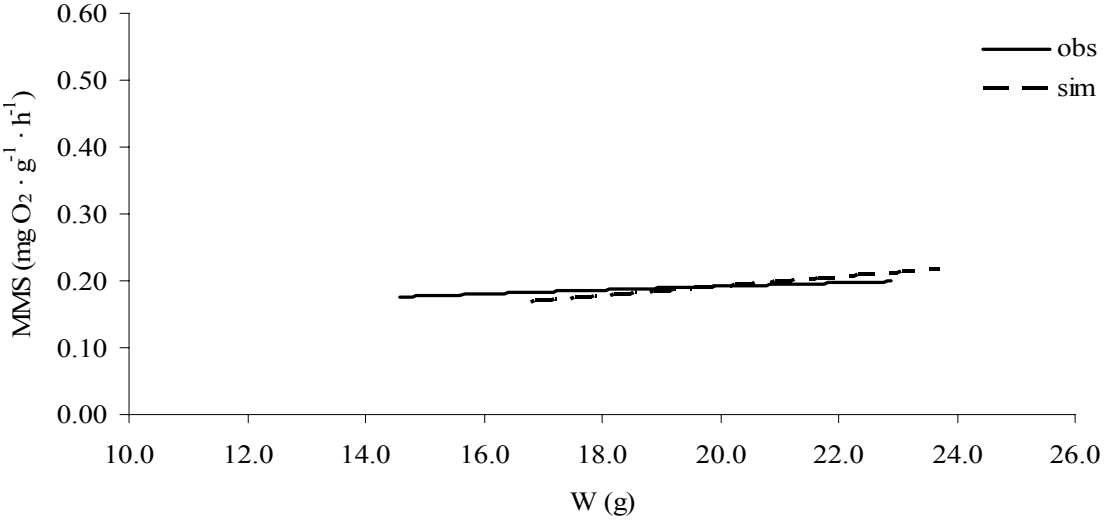


Figure 41. Marginal metabolic scope (MMS) versus shrimp wet weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at 2 mg·L^{-1} DO and then transferred to near-air-saturation DO.

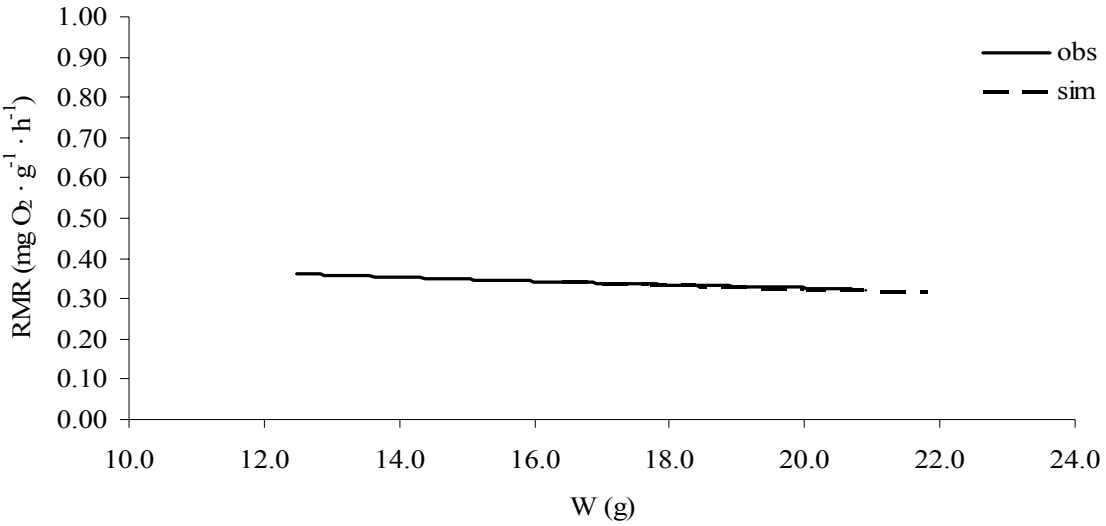


Figure 42. Routine metabolic rate (RMR) versus shrimp wet weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at 2 mg·L^{-1} DO and then transferred to 2 mg·L-1 DO.

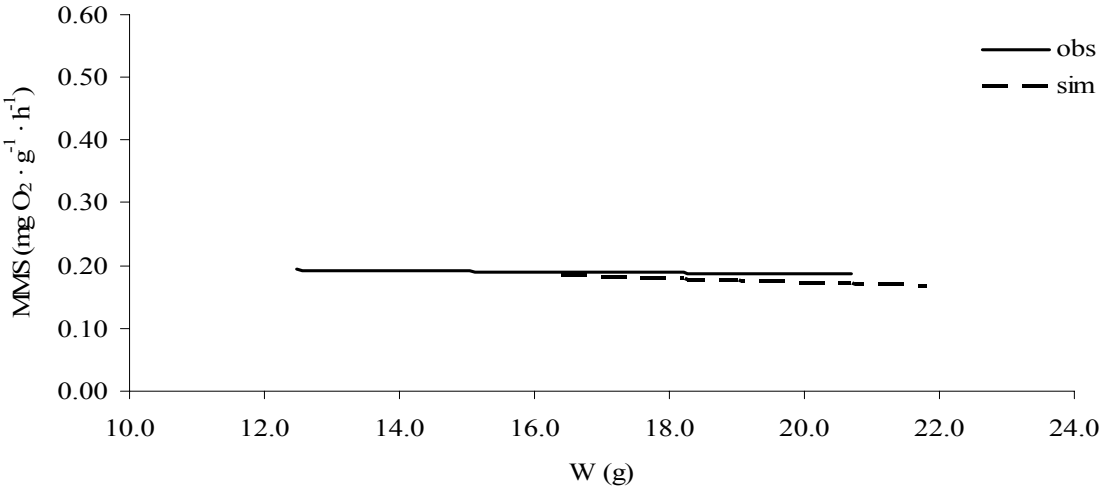


Figure 43. Marginal metabolic scope (MMS) versus shrimp wet weight (W) for observed (lab) and simulated (sim) shrimp maintained for 30-d at 2 mg·L^{-1} DO and then transferred to 2 mg·L^{-1} DO.

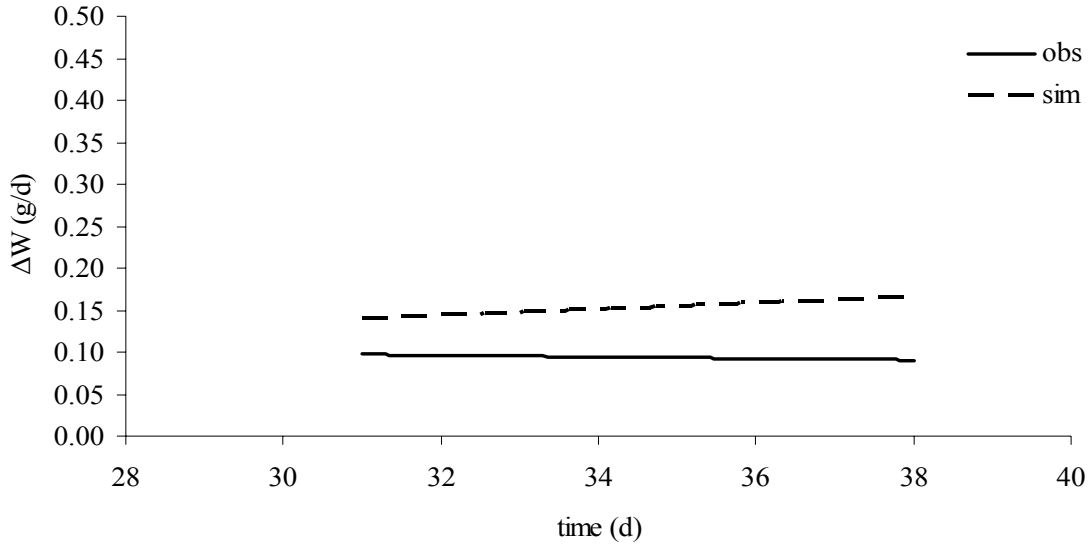


Figure 44. Weight change (ΔW) versus time (d) for observed and simulated shrimp maintained for 30-d at near air saturation DO.

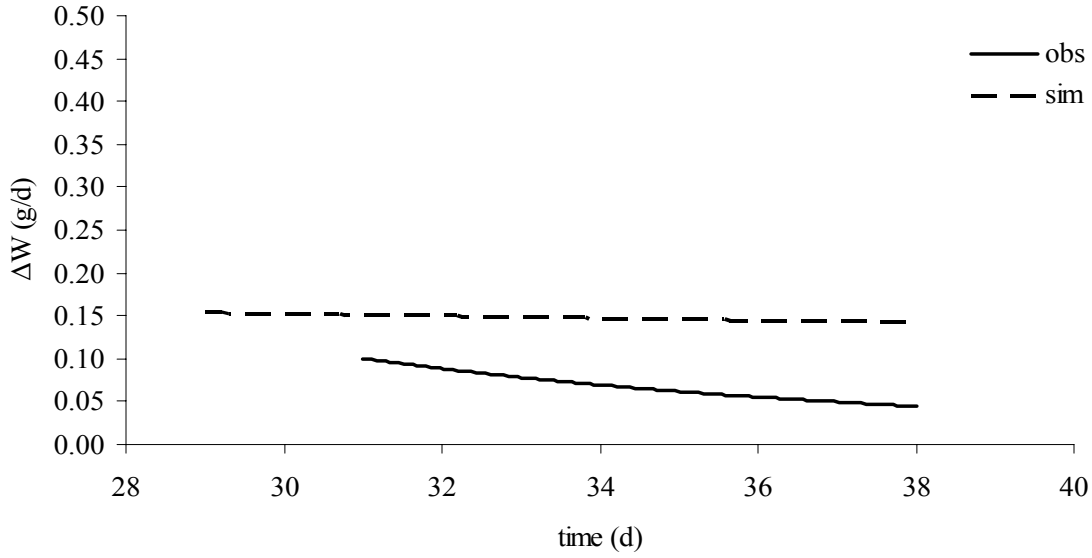


Figure 45. Weight change (ΔW) versus time (d) for observed and simulated shrimp maintained for 30-d at 2 $mg \cdot L^{-1}$ DO.

Discussion

Metabolic Rates

As evident in the results section of this chapter, one unified model was reasonably able to simulate the results of observed trials presented in Chapters II-IV. In many cases, the fraction of variability accounted for under the model was small. I believe this had two main causes: low rates of replication and high inherent metabolic variability among individuals.

In any case, the efficacy of Ecophys.Shrimp is not to be judged entirely in its ability to replicate the results of the experiments presented here, but in its ability to simulate the results of other, independent experiments. Extension of model evaluation beyond the present body of experiments is necessary, because, after all, even though the experiments presented in Chapter II-IV were conducted in different experimental systems, using different batches of shrimp, the respirometry equipment and approach were the same, and, perhaps more importantly, so was the principal investigator. In an attempt to evaluate Ecophys.Shrimp in a more encyclopedic way, I used the model to simulate a selection of published studies which reported respiration rates of *L. vannamei* under environmental conditions which were independent from (but sometimes similar to) those presented in Chapters II-IV.

The inherent difficulty of simulating the work of others is often magnified by a lack of key information. When this occurs, one is forced to make assumptions in the model that may or may not be correct. Such is the case with Rosas et al. (2001a). Although seemingly innocuous, the authors reported oxygen consumption rates of *L.*

vannamei in terms of mg $O_2 \cdot h^{-1} \cdot gdw^{-1}$ where dw is dry weight. While there is nothing wrong with this, and it is probably more accurate than reporting VO_2 on a wet-weight basis, the authors failed to provide even a mean value of percent body-moisture for the shrimp used in their study. Therefore, in order to convert their VO_2 results to dimensions used by the model, an assumption of 75% body moisture was used (Siccardi 2006). Rosas et al. (2001a) attempted to determine the effect of salinity acclimation on oxygen consumption for juvenile *L. vannamei*. The portion of their results that I simulated are for their "4-d acclimated" shrimp. The initial starting weight of their shrimp was 4 ± 1 g wet weight; the animals then were held for 2-d at 28 ºC, in 30 ppt "natural sea water," with dissolved oxygen concentration of 5.0 mg/L and pH of 8.3 ± 1. During acclimation, these shrimp were fed fish flesh twice daily (5,020 joules/g dw). Rosas et al. (2001a) reported that their shrimp were held in the above conditions for 2-d and then acclimated to 30, 15, and 5 ppt over the course of 4-d with no change in the other environmental parameters or feeding schedule. Shrimp then were placed in the respirometry chambers 12-h (in the absence of feed) before any measurements were made. Three variants of Ecophys.Shrimp were programmed to run for a total of 168-h and replicated the salinity changes reported by Rosas et al. (2001a). The starting weight in each model was 4 g and the pH was 8.3. Salinity and temperature were set to the values reported by the authors. Feed rate was set to zero after 156-h of run-time. A comparison of the results obtained by Rosas et al. (2001a) and those produced by Ecophys.Shrimp are shown in Table 16. For the shrimp acclimated to 5 ppt, the result obtained from Ecophys.Shrimp matched the result obtained by Rosas et al. (2001a).

However, for the 30 and 15 ppt 4-d acclimated shrimp, the routine metabolic rates (VO_2) are much higher under the model than the rates reported by Rosas et al. (2001a). Interestingly, the standard metabolic rates obtained under the model correspond well with those obtained by Rosas et al. (2001a). Review of their work reveals that although they used a "continuous flow respirometer in closed circuit" (from Rosas et al. 1998), which appears functionally equivalent to the system used to parameterize Ecophys.Shrimp, their reported recirculating flow rate was 1.2 $L \cdot h^{-1}$; whereas the flow rate inside the respirometer described in Chapter II was 4.6 $L \cdot h^{-1}$. This may have

Table 16. Comparison of oxygen consumption rates (VO_2) obtained by Rosas et al. (2001a) to those obtained by Ecophys.Shrimp where Mstd is standard metabolic rate.

Treatment/Parameter	Rosas et al. (2001a)	Ecophys.Shrimp
30 ppt		
VO_2 (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)	0.33 ± 0.05	0.53
Mstd (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)		0.31
15 ppt		
VO_2 (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)	0.33 ± 0.05	0.53
Mstd (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)		0.30
5 ppt		
VO_2 (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)	0.54 ± 0.08	0.54
Mstd (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)		0.31

caused the shrimp used by Rosas et al. (2001a) to have a metabolic rate closer to standard than routine. In the case of the lowest salinity used by Rosas et al. (2001a), the increased metabolic rate may have been due to an increase in shrimp activity in response to the low salinity treatment. However, this is merely supposition on my part. Regardless, contemplation of the results suggests that the metabolic rates observed in my

respirometry (and thus the rates used to parameterize the model) are similar to those obtained by Rosas et al. (2001a), at least in terms of the range between standard and routine metabolism (which Ecophys.Shrimp sets to twice standard).

Palacios et al. (1996) reported the effects of temperature and body weight on the oxygen consumption of *L. vannamei*. Those authors used what they termed a "temporarily-closed-flow design" respirometer to determine "resting respiratory rate" of shrimp in a range of body weights from 1 to 23g at temperatures of 20 and 25 ºC and a body weight range of 1 to 50g at 30 ºC. The authors used shrimp held at 28 ºC and 35 ppt until 24-h prior to respirometric measurements, after which the animals were maintained at their experimental temperatures. Each temperature treatment was simulated with Ecophys.Shrimp using the environmental values reported by Palacios et al. (1996). Because the authors did not report the starting weight of their shrimp (only the final weights, as it was not a growth trial), each simulation was run only for 72-h. The first 24 h replicated the culture conditions reported by the authors with an assumed pH of 8.0 and DO of 5.5 mg/L. The second 24 h period replicated the different temperature treatments and the simulated shrimp were deprived of feed. Although the authors did not report depriving the animals of feed 24 h prior to respirometry, they did not report feeding them, either. Because the general consensus in the literature is that "resting rates" do not include specific dynamic action, I believe the decision to deprive the simulated shrimp of feed 24-h prior to and during respirometry was appropriate. The final 24 h period corresponds with the time the shrimp were subjected to respirometric measurements. The authors presented their results graphically (log respiratory rate,

mgO2·kg^{-1}·h^{-1}, versus log body weight, g), which, for the purposes of this comparison, were translated to units of measurement used by Ecophys.Shrimp. Furthermore, the authors provided data points for individual animals clustered around trend lines. Due to the nature of their graph, only the clearly labeled delineations on the x-axis were used in conjunction with the trend lines as best estimates of values on the y-axis. For this reason, the simulated results do not go to the same weight extremes as those reported in the paper. Graphical comparisons of results obtained by Palacios et al. (1996) and simulated results from Ecophys.Shrimp are shown in Figures 46-48.

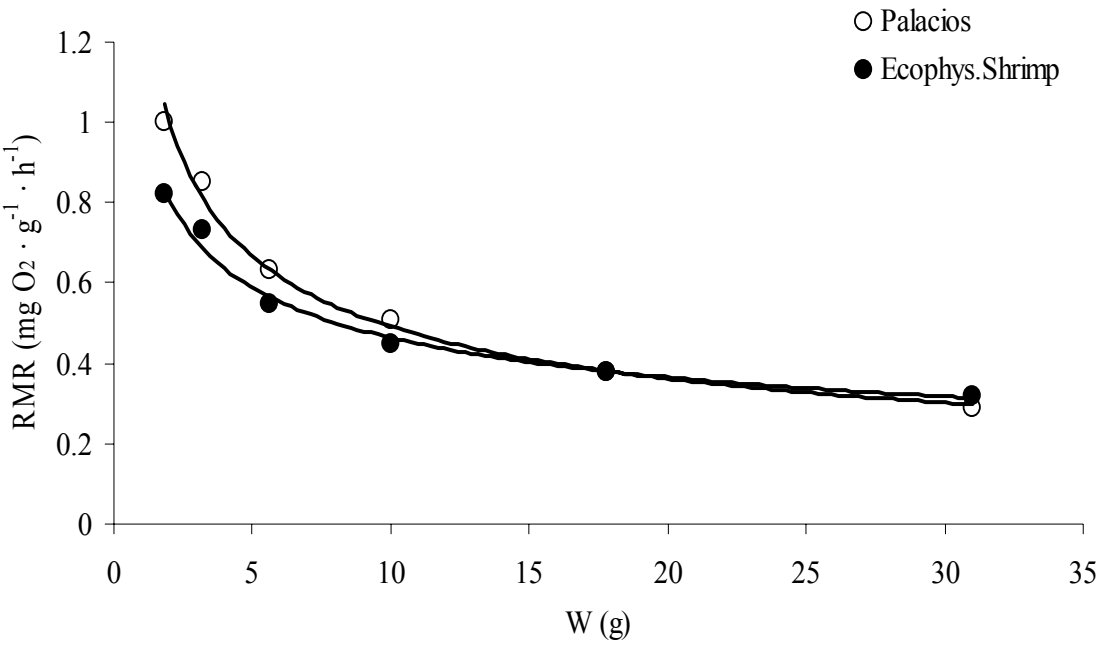


Figure 46. Comparison of respiratory rates at 30 ºC reported in Palacios et al. (1996) to simulated results obtained from Ecophys.Shrimp.

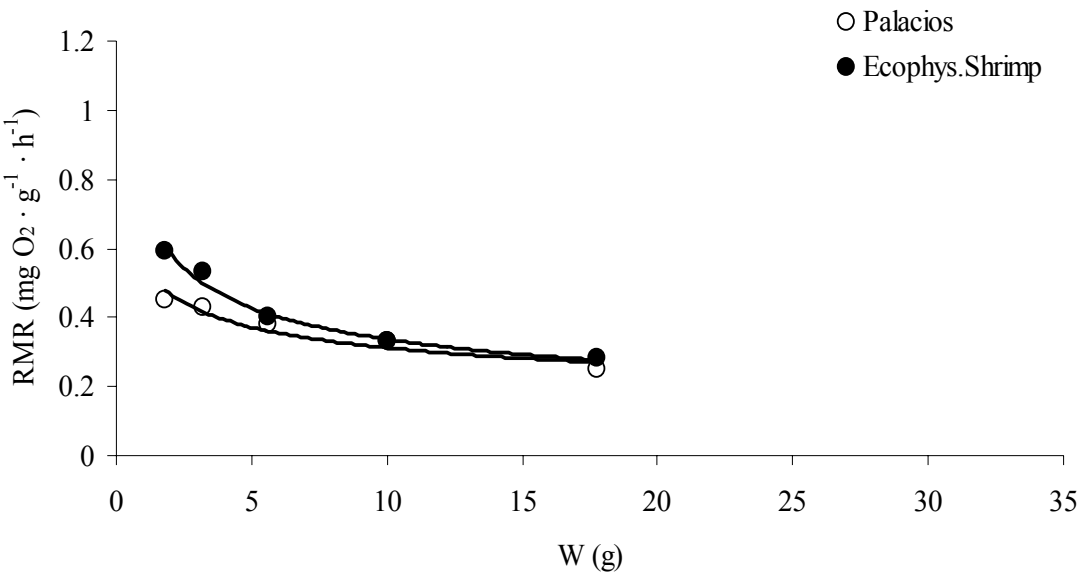


Figure 47. Comparison of respiratory rates at 25 ºC reported in Palacios et al. (1996) to simulated results obtained from Ecophys.Shrimp.

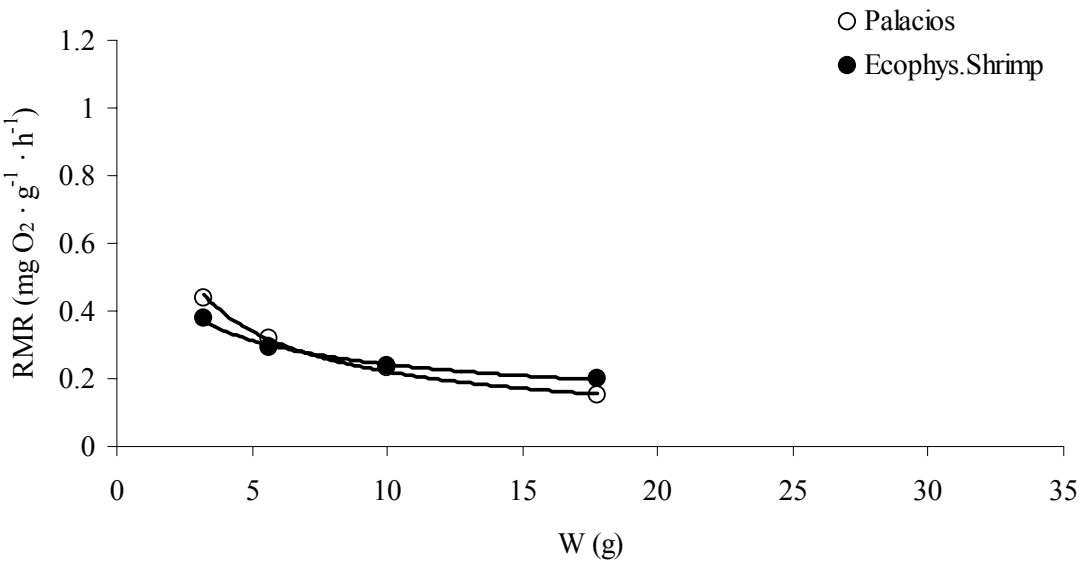


Figure 48. Comparison of respiratory rates at 20 ºC reported in Palacios et al. (1996) to simulated results obtained from Ecophys.Shrimp.

Due to the number of assumptions that had to be made with regards to the interpretation of Palacios et al.'s (1996) results, it is difficult to make claims about the accuracy of model performance, especially when one considers that it was not possible to determine deviations from their trend lines with any real confidence. However, the values and trends observed in the comparisons go further to support the model than detract from it.

Also of note, comparison of the values obtained by Palacios et al. (1996) to those of Rosas et al. (2001a) under similar conditions (28 ºC and 30 ºC, respectively) show rather pronounced differences in oxygen consumption for shrimp of similar weight. In general, the values reported by Palacios et al. (1996) are in much better agreement with those simulated by Ecophys.Shrimp than are those reported by Rosas et al. (2001a).

Racotta and Herrera (2000) reported changes in oxygen consumption of *L. vannamei* in response to increasing levels of ambient ammonia. The starting weight of their shrimp was 10.2 ± 1.1 g. Their shrimp were acclimated for 2 weeks in 39 ppt sea water, at a pH of 7.8 and temperature of 28 ± 1 ºC. The authors fed their shrimp a 25% protein commercial shrimp feed with a daily ration of 5% biomass. Prior to respirometry, shrimp were not fed for 24 h. After 24 h in the absence of feed, shrimp were exposed to four levels of ambient ammonia-N. For comparison to Ecophys.Shrimp, the range of respiratory responses in treatments which Racotta and Herrera (2000) reported as not significantly different were 0.007 (control), 0.36, and 1.07 mmol/L ambient ammonia treatments. Ecophys.Shrimp was programmed with a run time of 384-h and a feed rate set to zero after 336-h. Salinity and pH were set to the values reported by Racotta and Herrera (2000), and initial weight and temperature were

set at 10.2 g and 28 ºC, respectively. Racotta and Herrera (2000) reported oxygen consumption values (interpreted from their graphs) of 4.7 to 5.9 $\mu mol \cdot g^{-1} \cdot h^{-1}$ (0.15 - 0.19 mg $O_2 \cdot g^{-1} \cdot h^{-1}$) for the control, 0.36, and 1.07 mmol/L ambient ammonia treatments. The value of VO_2 obtained from Ecophys.Shrimp was 0.39 mg $O_2 \cdot g^{-1} \cdot h^{-1}$ with a standard metabolic rate of 0.25 mg $O_2 \cdot g^{-1} \cdot h^{-1}$. Even though the respirometry system described by Racotta and Herrera (2000) can be considered "static," it produced VO2 values so much below those simulated by Ecophys.Shrimp—and those observed in my parameterization experiments—that the discrepancy must be regarded as irreconcilable.

A more favorable comparison was obtained for another study, which also used static respirometry, versus Ecophys.Shrimp. Villarreal et al. (1994) measured the effect of temperature and salinity on the oxygen consumption of *L. vannamei* postlarvae. The initial weight of their shrimp was 0.15 ± 0.05 g; the animals were held for 24-h at 24 ± 1 ºC with salinities of 25, 30, 35, 40, or 45 ppt. The postlarval shrimp then were transferred to different temperature treatments of 20, 24, 28, or 32 ºC, and maintained in the absence of feed for an additional 24 h. Respirometric measurements then were taken for 60 min in static chambers covered with aluminum foil and with "minimum disturbance" of the shrimp. Ecophys.Shrimp was programmed with a run time of 49 h with deprivation of food after 24 h. Programmed values of temperature and salinity mirrored those reported by Villarreal et al. (1994) and initial weight was set to 0.15 g. Initial dissolved oxygen was set at 5.5 mg $O_2 \cdot L^{-1}$ and pHwas set to 8.0 (both values assumed). Comparisons of results obtained by Villarreal et al. (1994) with those from Ecophys.Shrimp are shown in Table 17.

Table 17. Routine metabolic rates (RMR) of *L. vannamei* obtained by Villarreal et al. (1994) and simulated RMR and standard metabolic rates (Mstd) obtained from Ecophys.Shrimp.

		Villarreal et al.(1994)	Ecophys.Shrimp	
Temp. (ºC)	Salinity (ppt)	RMR (± SD) (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)	RMR (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)	Mstd (mg $O_2 \cdot g^{-1} \cdot h^{-1}$)
20	25	0.36 ± 0.09	0.69	0.34
	30	0.40 ± 0.04	0.69	0.34
	35	0.36 ± 0.04	0.69	0.35
	40	0.41 ± 0.07	0.70	0.35
	45	0.38 ± 0.06	0.70	0.35
24	25	0.56 ± 0.10	0.9	0.45
	30	0.55 ± 0.12	0.9	0.45
	35	0.52 ± 0.15	0.91	0.45
	40	0.63 ± 0.08	0.91	0.45
	45	0.56 ± 0.11	0.91	0.46
28	25	0.61 ± 0.16	1.18	0.59
	30	0.70 ± 0.08	1.18	0.59
	35	0.79 ± 0.06	1.18	0.59
	40	0.70 ± 0.17	1.18	0.59
	45	0.77 ± 0.03	1.18	0.59
32	25	0.73 ± 0.10	1.52	0.76
	30	0.65 ± 0.09	1.52	0.76
	35	1.10 ± 0.15	1.53	0.76
	40	0.88 ± 0.11	1.53	0.77
	45	0.85 ± 0.11	1.54	0.77

Note that in most cases, the values reported by Villarreal et al. (1994) for "routine rate of oxygen consumption" correspond with those values obtained under the model for standard metabolic rate. In fact, aggregating the entire dataset (20 pairs of values), the linear regression of observed VO_2 (Y) on simulated standard VO_2 (X) is Y = 0.05 +

1.08*X, with $r^2 = 0.77$. Given the respirometric methodology used by Villarreal et al. (1994) to obtain their results, relative to the methodology used to parameterize Ecophys.Shrimp, it is not surprising that they observed routine rates which were close to the standard rates obtained under Ecophys.Shrimp.

Growth

It seems relatively clear from the comparisons of respiratory rates in the previous section that protocol can have a great deal of impact on experimental results. Such appears to be the case when attempting to simulate growth of *L. vannamei* as described in various published literature. Experimental conditions (culture conditions, length of study, quality of feed, quality of animals, etc...) are by no means uniform for the many studies in the literature which have reported growth of *L. vannamei* under various environmental conditions. One way to account for this is the previously described MMSO function in Ecophys.Shrimp. For example, Patnaik et al. (2008) conducted a study to determine the effects of stocking density and feed rate on the growth of *L. vannamei* in a recirculating indoor system. The authors reported using the same commercial feed (Rangen 45-10, Rangen Inc., Buhl Idaho, USA) as that in the studies used to parameterize Ecophys.Shrimp. The initial size of their shrimp was 4.7 g, and mean environmental parameters (± SD) were 5.1 ± 0.4 $mg \cdot L^{-1}$, 29.8 ± 0.9 ºC, and 33.8 ± 1.0 ppt, for DO, temperature, and salinity, respectively. The value reported for pH was 7.96. The length of their study was 43 days. Patnaik et al. (2008) reported final weights ranging from 10.2 to 13.6 g. Simulation of their study using Ecophys.Shrimp yielded a final weight of 11.3 g. Final weight obtained by Patnaik et al. (2008) under density and

feed rate conditions similar to those used in the salinity study from Chapter II was 13.3 g. This result was obtained under the model only when MMSO was raised from 0.71 to 0.74.

Conversely, attempts to simulate the results of Gomez-Jimenez et al. (2005) required a reduction in MMSO. The authors conducted a 25-d experiment to determine the effects of dietary protein level on growth, survival and ammonia efflux rate of *L. vannamei* raised in zero-water-exchange culture systems. Water quality parameters for the study were (mean ± SD) 28.5 ± 0.3 ºC, 36.7 ± 0.4 ppt, 5.8 ± 0.2 $mg \cdot L^{-1}$, and 8.1 ± 0.2 for temperature, salinity, DO, and pH, respectively. The authors reported no significant difference among the dietary treatments (25, 30, 35, and 40% protein diet) for final weight. Although not significantly different from the other treatments, the 40% protein feed produced largest numeric weight gain. The final weight obtained in this treatment by the researchers was 4.27 ± 0.07 g. Ecophys.Shrimp was programmed with the mean values of the water quality parameters and run for a simulated 25-d. Initial starting weight for Ecophys.Shrimp was 1.94 g, which was the mean starting weight reported by Gomez-Jimenez et al. (2005) for the 40% protein feed treatment. Using an MMSO value of 0.71, the final weight under the model was 5.07 g. Replication of the final weight reported by Gomez-Jimenez et al. (2005) required an MMSO value of 0.68 (4.32 g final weight).

Finally, significantly different final weights were obtained by Samocha et al. (2008) in a 92-d super-intensive grow-out study conducted in greenhouse-enclosed raceways to evaluate the effect of foam fractionators and settling tanks on shrimp

performance. The researchers reported initial *L. vannamei* stocking weights of 1.25 ± 0.17 g into four raceways, two with settling tanks and two with foam fractionators. Stocking density was 530 shrimp/m^3. Samocha et al. (2008) reported no significant differences in water quality between treatments. The actual water quality parameters (DO, temperature, salinity, and pH) for their study (2 measurements/day) were obtained from Susmita Patnaik (via personal communication) and programmed into Ecophys.Shrimp. The final weights reported by Samocha et al. (2008) for the raceways operated with settling tanks were 18.4 and 18.5 g. Using an MMSO of 0.73, a final weight of 18.9 g was obtained under the model. For the raceways operated with foam fractionators, the empirical values of 17.3 and 17.4 g were reported by the researchers. Using an MMSO value of 0.72 gave a simulated result of 17.5 g.

Neill et al. (2004) made similar adjustments in MMSO to account for among-experiment differences in growth rate. As with Ecophys.Fish (Neill et al. 2004), the results of Ecophys.Shrimp also suggest that requisite variation in MMSO is a measure of differences in environment-animal-performance systems that go beyond the effects of variation in the abiotic components of environment (i.e., temperature, DO, salinity, and pH).

What were the true values of MMSO for the published experiments I have simulated here? That, we can not know, in the absence of accompanying respirometry data. I can say only that the requisite values of MMSO to achieve good fit of simulated to observed growth rates did not require much deviation from the nominal value, 0.71—

which was the one used in simulation of all the parameterization experiments reported here.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Salinity

Metabolic responses of *L. vannamei* were significantly different for shrimp at 2 ppt as compared with those at 10 ppt and 28 ppt salinities. Over all three salinities, routine metabolic rate (RMR) and marginal metabolic scope (MMS) decreased with increasing body weight. Limiting oxygen concentration for routine mebabolism (LOCr) also tended to decrease with shrimp size, in the experiments at 10 and 28 ppt, but not at 2 ppt. Metabolic-variable fits were improved by partitioning the data at W = 9 g. These results indicate higher metabolic cost for the shrimp maintained at the lowest salinity evaluated, and, considering the natural history of *L. vannamei*, suggest that migration from estuaries to offshore waters is coincident with a size-related change in the pattern of underlying metabolic response. Comparison of linear growth rate and MMS adjusted to a common weight supports the idea that MMS and growth are positively related.

Temperature, Starvation, and Feed Protein

For fed shrimp, reduced growth and respiration of *L. vannamei* was coincident with reduction in temperature. Results of oxygen consumption measurement in starved *L. vannamei* PLs are consistent with those obtained with other crustaceans (Salomon et al. 2000; Meyer and Oettl 2005;) with regards to RMR for the 24-, 28- and 32-ºC fed-shrimp treatments, but not for 20 ºC which may be indicative of temperature-induced metabolic torpor. The use of MMS as an integrative measure of environmental quality for animal performance and production appears to have been justified, in that the results

presented a consistent tendency for positive relationship between rate of weight change and MMS, both for fed and starved shrimp. Moreover, for starved shrimp, MMS deteriorated over time, in keeping with the pace of mortality. Feed protein level appeared to have no effect on respiratory responses or growth, suggesting that the lowest level tested (25%) was not limiting for *L. vannamei*.

Dissolved Oxygen

The daily growth rate of *L. vannamei* maintained under tolerable hypoxic conditions was significantly less than the growth rate of shrimp maintained under normoxic conditions. However, there were no significant differences between treatments in terms of respirometric responses. This suggests that hypoxic conditions have little or no lasting effect on the metabolic capacity of *L. vannamei* once the common oxygen regime of respirometry is established. Thus, in the size class evaluated, *L. vannamei* does not exhibit any substantial acclimatory response to changes in dissolved oxygen concentrations over the interval of 2.0 to >5.0 $mg \cdot L^{-1}$.

Ecophys.Shrimp

At various points in this dissertation, I have made reference to the nature of shrimp growth; specifically, the molting process. As stated in Chapter V, Ecophys.Shrimp is essentially the same model, functionally speaking, as that proposed by Neill et al. (2004) with the addition of the "Molt Function". That shrimp molt in order to grow is, of course, not a revelation. However, when one thinks in terms of comparing Ecophys.Fish to Ecophys.Shrimp, it becomes readily apparent that slight deviations in model parameters, specifically MMSO, can have rather dramatic impact

with regard to the models output of final weight. Repeated use of the model, initially in the process of parameterization and finally in the attempt to reproduce observed results, has shown that differences in growth over time between treatments are actually a reflection of the number of molts that take place in a given amount of time. As intuitive as this may seem, I find it very promising that: 1) Ecophys.Shrimp can predict growth as well as it can, in light of the fact that, as an hourly time-step model, it is only one time-step away from either molting or not; and 2) by only manipulating the input value of MMSO one can effectively "fine-tune" the number of times a simulated shrimp molts in a particular shrimp-environment system.

Under Ecophys.Shrimp, the simulated responses of *L. vannamei* to salinity, temperature, and DO, versus its own size and nutritive status have been shown to be biologically reasonable and consistent with the parameterization experiments conducted in Chapters II-IV as well as other published studies. The goal of converting Ecophys.Fish to Ecophys.Shrimp was achieved, in that only minor adjustments to model structure and re-parameterization were required to accommodate molting and provide adequate fit to both my own experiments and those taken from the literature. Although some lack of fit was evident in the parameterization experiments, in no case was observed performance significantly different from simulated performance at $P = 0.05$.

The simulation model Ecophys.Shrimp appears to be capable of simulating the effects of time-varying environmental conditions on the growth and respiratory responses of *L. vannamei*. Its ability to do so, however, is subject in large part to the nature of the culture systems it simulates. For future researchers to use the model to

make predictions about the growth of *L. vannamei*, it may be necessary to simulate previous studies conducted in the same experimental system to obtain the proper value of MMSO.

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APPENDIX A

Table A-1. STELLA® model equation differences between Ecophys.Shrimp and Ecophys.Fish.

	Model Equation	
Parameter	Ecophys.Shrimp	Ecophys.Fish
DOlim	0.10+ (Tratio^Hill/(1+Tratio^Hill)) *(1-0.005*Tstress^2)*4.5	0.35+(Tratio^Hill/(1+Tratio^Hill))*(1-0.005*Tstress^2)*8.15
Eflux	(ShrimpEnergy-prevShrimpEnergy)/Vshrimp	IF (hAdxWfish < 0.2* hArxWfish) AND (GEfish > 800) THEN (-(0.003/24)*GEfish) ELSE (IF (hAdxWfish > 0.2*hArxWfish) AND (GEfish <1400) THEN (+(0.003/24)*GEfish) ELSE 0)
FedDepTime	0	N/A
FeedRateMax	0.15*Wshrimp^(0.0*LOGN (Wshrimp)-0.60)	0.18*Wfish^(0.0105*LOGN(Wfish)-0.25)
hAwxWshrimp	((100-FeedDigestibility%)/100)*hAcxWshrimp + 0.05*MAX(hAcxWshrimp, hArxWshrimp) + (IF Molt > 0 then 0.05*ShrimpEnergy/dt else 0)	((100-FeedDigestibility%)/100)*hAcxWfish + 0.05*MAX(hAcxWfish, hArxWfish)
Hill	3	2
INIT GEshrimp	1100	1000
INIT MoltConstraint	1	N/A
INIT prevShrimpEnergy	ShrimpEnergy	N/A
INIT Vshrimp	Wshrimp0	N/A

MMSO	0.71	variable
Molt	IF FeedRate > 0 AND GEshrimp > 1200 AND (ShrimpEnergy/GEshrimp > Vshrimp) THEN (1200-1100)/DT ELSE IF FeedRate = 0 AND GEshrimp < MoltConstraint*950 THEN -(MoltConstraint*(1000 - 950)) /DT ELSE 0	N/A
MoltConstraint(t)	MoltConstraint(t - dt) + (-molting) * dt	N/A
molting	IF Molt <> 0 THEN 0.29 ELSE 0	N/A
newV	IF NOT(Molt = 0) Then (ShrimpEnergy/GEshrimp)/DT Else 0	N/A
oldV	IF NOT(Molt = 0) Then Vshrimp/DT Else 0	N/A
prevShrimpEnergy(t)	prevShrimpEnergy(t - dt) + (SEupdate - SEdiscard) * dt	N/A
q1	0.065	0.05
SalLL	5	0.1
SalOpt	20	10
SalUL	45	50
SEdiscard	prevShrimpEnergy/DT	N/A
SEupdate	ShrimpEnergy/DT	N/A
Sgain	IF (Taccl>18) THEN .001 ELSE .001+(0.0001*((18-Taccl)^2)*-Tstress)	IF (Taccl>18) THEN .003 ELSE .003+(0.0001*((18-Taccl)^2)*-Tstress)
ShrimpEnergy(	ShrimpEnergy(t - dt) +	FishEnergy(t - dt) + (hAcxWfish -

t)	(hAcxWshrimp - hArxWshrimp - hAwxWshrimp - hAdxWshrimp) * dt	hArxWfish - hAwxWfish - hAdxWfish) * dt
Smin	(IF Wshrimp < 9 THEN 0.065*Wshrimp^-0.2 ELSE 0.064*Wshrimp^-0.2) *(IF FeedDepTime >120 THEN StarvEffect ELSE 1)	0.1*Wfish^-0.2
StarvEffect	(0.0012*GEshrimp) - 0.3783	N/A
TimeCounter	IF FeedRate = 0 THEN 1 ELSE 0	N/A
Vshrimp(t)	Vshrimp(t - dt) + (newV - oldV) * dt	N/A
Wexp	if Wshrimp < 3.5 then -.42 else -.3	-0.22
Winberg	IF FeedRate > 0 THEN (1+0.5*(Mact-Mstd)/Mstd)*0 + 2 ELSE IF FeedRate = 0 AND Wshrimp < 3.5 THEN 2 ELSE 2 * Wshrimp^-0.1	(1+0.5*(Mact-Mstd)/Mstd)*0+2
Wshrimp	Vshrimp	FishEnergy/GEFish

APPENDIX B

Parameters, Equations, and Definitions for Ecophys.Shrimp

A: min(DOa, DOlim/phfactor*Weffect – The adjusted DO (mg $O_2 \cdot L^{-1}$) for computing active metabolic rate. An animal-weight-adjusted minimum of DOa and DOlim, with the later diminished by the Bohr effect when ambient pH < 7.

Aa: 24*Mact*oxycal – The daily active metabolic rate multiplied by the oxycaloric equivalent.

Acmax: max(0,248(MSgrowth/sda)*oxycal) – The metabolism available to support feed processing and assimilation.

AcxWshrimp: hAcxWshrimp*24 – The daily, per-shrimp rate of feed-energy consumption.

AdxWshrimp: hAdxWshrimp*24 – The daily, per-shrimp energy loss for feed processing and metabolism.

AgScope: MSgrowth/oxycal – Metabolic scope for growth divided by the oxycaloric equivalent.

Apc: MIN(FeedRate, FeedRateMax)*GEfeed – Hourly rate at which feed energy is presented (to the metabolic subsystem) for processing.

ArxWshrimp: hArxWshrimp*24 – The daily, per-shrimp rate of energy loss for routine metabolism.

As: 24*Mstd*oxycal – Daily standard metabolism multiplied by the oxycaloric equivalent.

AwxWshrimp: hAwxWshrimp*24 – The daily, per-shrimp rate of energy loss as waste.

DO(t): DO(t-dt) + (DOsupply – DOuse) * dt – DO level at any given time in the simulation.

DOa: DOobs – Ambient DO (mg $O_2 \cdot L^{-1}$).

DOaccl: max (min(DOaccl0, DOlim), minDOaccl) – DO acclimation state (mg $O_2 \cdot L^{-1}$).

DOaccl0(t): DOaccl0(t - dt) + (DOacclCng)*dt – Initial DO acclimation state at onset of simulation.

DOacclCng: IF (DOaccl0 < minDOaccl) THEN (minDOaccl - DOaccl0) ELSE IF (DOaccl0 < DOlim) THEN (DOlim - DOaccl0) ELSE (DOa - (DOa - DOaccl0) * EXP (-raccl/24) - DOaccl0) – Change in DO acclimation state.

DOlim: 0.1 + (Tratio^Hill/(1+Tratio^Hill))*(1-0.005*Tstress^2)*4.5 – The temperature dependent DO below which active metabolic rate becomes DO-dependent.

DOobs: user input – Dissolved oxygen level (mg $O_2 \cdot L^{-1}$).

DOStress: DOa-DOaccl – Difference between ambient DO and DO acclimation state.

DOsupply: DOuse – Amount of oxygen consumed by the shrimp. This parameter is redundant with VO2xWshrimp/SysVol to maintain programming continuity in STELLA®.

DOuse: VO2xWshrimp/SysVol – Amount of oxygen consumed by the shrimp.

Eflux: (ShrimpEnergy-prevShrimpEnergy)/Vshrimp – Change in shrimp energy relative to shrimp volume.

FeedDigestibility%: user input – Percent feed digestibility.

FeedDepTime(t): FeedDepTime(t - dt) + (TimeCounter) * dt – Feed deprivation time used to count the number of hours the simulated shrimp has been without feed.

FeedEnergy(t): FeedEnergy(t-dt) + (-hAcxWshrimp) * dt – Energy content of feed available for presentation per individual.

FeedRate: user input – Rate at which feed is offered to an animal as a percent of the animals body weight.

FeedRateMax: 0.15*Wshrimp^(0*LOGN(Wshrimp)-0.60) – Maximum daily rate of feed consumption under metabolism- and feed- unlimited conditions.

GEFeed: user input – Gross energy content (cal/g) of the feed as consumed.

GEshrimp(t): GEshrimp(t-dt) + (Eflux-Molt) * dt – Gross energy content (cal/g) of the shrimp tissue.

hAcxWshrimp: (min(Apc, Acmax) * Wshrimp)24 – The hourly, per-shrimp rate of feed-energy consumption.

hAdxWshrimp: sda * hAcxWshrimp – The hourly, per-shrimp energy loss for feed processing metabolism.

hArxWshrimp: (min (Winberg * As, Aa)/24 * Wshrimp – The hourly, per-shrimp rate of energy loss for routine metabolism.

hAwxWshrimp: ((100-FeedDigestibility%)/100) * hAcxWshrimp + 0.05 * max(hAcxWshrimp, hArxWshrimp) + (IF Molt > 0 THEN 0.05 * ShrimpEnergy/dt ELSE 0) – The hourly, per-shrimp rate of energy loss as wastes.

Hill: 3 – Used as the functional basis for computing DOlim.

INIT DO: user input – The initial value of DO (mg $O_2 \cdot L^{-1}$) at the onset of simulation.

INIT DOaccl0: max(min(DOa, DOlim), minDOaccl) – Initial DO (mg $O_2 \cdot L^{-1}$) acclimation at the onset of the simulation.

INIT FeedEnergy: user input – Initial energy (cal/g) of feed.

INIT GEshrimp: 1100 – Starting gross energy value of the animal at the beginning of simulation.

INIT MoltConstraint: 1 – Starting value for MoltConstraint. This variable works in conjunction with "molting" to restrain the molt phenomenon during periods of starvation.

INIT O2used: 0 – Sets the initial value of O_2 consumed by the shrimp to zero at the beginning of the simulation.

INIT prevShrimpEnergy: ShrimpEnergy – Initial energy (cal/g) level of the shrimp tissue.

INIT PrMORT: 0 – Initial probability of mortality.

INIT ShrimpEnergy: Wshrimp0*GEshrimp – Initial energy (cal/g) level of the shrimp tissue.

INIT Taccl: min(Ta, 34) – Initial acclimation temperature of the shrimp/environment system.

INIT Vshrimp: Wshrimp0 – Initial shrimp weight at the onset of simulation.

INIT WastedEnergy: 0 – Initial amount of wasted energy at the onset of simulation.

kaccl: IF ((Ta > 34) AND (Taccl > 34)) THEN 0 ELSE (24*EXP (-2.15 + (Taccl-Ta)/Topt -((Topt-Ta)/8.5)^2))) – The function for thermal acclimation rate coefficient.

Mact: MMSO*pHfactor*A*DOlim*DOaccl^-0.9 – Active metabolic rate.

minDOaccl: 0.45*DOlim – Minimum acclimation DO.

MMS: VO2/DOa – Marginal metabolic scope. Routine metabolic rate divided by the limiting oxygen concentration for the routine rate (mg $O_2 \cdot g^{-1} \cdot h^{-1}$).

MMSO: 0.71 – Residual intercept of MMS. Inherent metabolic efficiency of the shrimp/environment system after the effects of temperature, pH, DO, salinity, and animal size have been taken into account.

Molt: IF FeedRate > 0 AND GEshrimp > 1200 AND (ShrimpEnergy/GEshrimp > Vshrimp) THEN (1200-1100)/dt ELSE IF FeedRate = 0 and GEshrimp < MoltConstraint*950 THEN-(MoltConstraint*(1000-950))/dt ELSE 0 – The function which enables the simulated shrimp to grow. When the gross energy of the simulated shrimp reaches 1200 cal/g the shrimp molts and its energy density is reset to 1100 cal/g. For starving shrimp, the function allows for the molting process when the shrimps energy density falls below 950 cal/g.

MoltConstraint(t): MoltConstraint(t-dt) + (-molting) * dt – Regulates the "molt down" phenomenon in starving shrimp.

molting: IF Molt <> 0 THEN 0.4 ELSE 0 – The function which regulates the amount by which a simulated shrimps weight will decrease during starved conditions.

MORTdec: 0*PrMORT – Decreasing probability of mortality.

MORTinc: IF((DOStress) < -2.45) THEN -(DOStress)*0.023 ELSE 0 – Increasing probability of mortality.

MSgrowth: Mact-Winberg*Mstd – Metabolic scope for growth.

Mstd: S*EXP(q1*Taccl)*EXP(q2*Tstress) – Standard metabolism.

newV: IF NOT(Molt = 0) THEN (ShrimpEnergy/GEshrimp)/dt ELSE 0 – New shrimp volume after molting.

O2use: VO2xWshrimp – Oxygen consumption.

O2used(t): O2used(t-dt) + (O2use) * dt – Amount of oxygen consumed by the shrimp over time.

oldV: IF NOT(Molt = 0) THEN Vshrimp/dt ELSE 0 – Shrimp volume prior to molting.

oxycal: 3.4 – Oxycaloric equivalent (cal/mg O2).

pH: pHObs – Observed pH.

pHfactor: 1-EXP((-pHgain/max(0.01,(max(pH, 7)))) – Dimensionless transform of pH designed to enable implementation of a crude Bohr effect.

pHgain: 0.56 – Rate constant used to decay pHfactor.

pHObs: user input – Observed pH.

preShrimpEnergy(t): preShrimpEnergy(t-dt) + (SEupdate - SEdiscard) * dt – Energy (cal/g) level of the shrimp tissue as it changes through time.

PrMORT(t): PrMORT(t-dt) + ((MORTinc - MORTdec) * dt – Probability of mortality as it changes through time.

q1: 0.065 – Steady state rate constant.

q2: 0.09 – Transient state rate constant.

raccl: IF DOaccl0 >= minDOaccl AND DOaccl0 <= DOlim THEN 1*Mstd ELSE 0 – The rate coefficient by which DOaccl decays toward DOa or the appropriate interval boundary.

S: Smin + Sgain * Svar – The intercept value combined with shrimp weight which establishes standard metabolism.

Sal: SalObs – The observed salinity (mg/L).

SalL: IF (Sal < SalOpt) THEN SalLL ELSE SalUL – The relevant unit loading value of salinity.

SalLL: 5 – The lower unit loading value of Sal for the shrimp.

SalObs: user input – Observed value of salinity.

SalOpt: 20 – Optimum value of salinity.

SalUL: 45 – The upper unit loading value of Sal for the animal.

sda: 0.15 – Specific dynamic action. Proportion of the feed's energy used in processing feed.

SEdiscard: prevShrimpEnergy/dt – Previous shrimp energy divided by change in time.

SEupdate: ShrimpEnergy/dt – Shrimp energy divided by change in time.

Sgain: IF (Taccl > 18) THEN 0.001 ELSE 0.001 + (0.0001 * ((18-Taccl)^2) * -Tstress) – A scaling parameter presumed to be temperature sensitive, increasing with cold stress and decreasing with relief from cold stress.

ShrimpEnergy0(t): ShrimpEnergy(t - dt) + (hAcxWshrimp - hArxWshrimp - hAwxWshrimp - hAdxWshrimp) * dt – Energy (cal/g) level of the shrimp tissue through time.

Smin: (IF Wshrimp < 9 THEN 0.065*Wshrimp^-0.2 ELSE 0.064*Wshrimp^-0.2) * (IF FeedDepTime > 120 THEN StarvEffect ELSE 1) – The minimum value of S. In the context of this model, Smin is weight dependent at 9 g shrimp weight. This variable can also be degraded by starve effect when the simulated shrimp has been deprived of feed for greater than 120 hrs.

StarvEffect: (0.0012*GEshrimp)-0.3783 – Sets the standard metabolism for a starving shrimp as a function of shrimp gross energy.

Svar: ((Sal-SalOpt)/(SalL-SalOpt))^2 – The difference between SalOpt and the relevant unit-loading value of salinity, SalL.

SysVol: 100 – Water volume of system in liters, per shrimp.

Ta: TaObs – Ambient temperature.

Taccl(t): Taccl(t-dt) + (TacclCng) * dt – Acclimation temperature.

TacclCng: Ta-(Ta-Taccl) * EXP(-kaccl/24)-Taccl – Change in temperature acclimation.

TaObs: user input – Observed temperature.

Topt: 28 – Optimum temperature.

TimeCounter: IF FeedRate = 0 THEN 1 ELSE 0 – Flow variable that begins counting elapsed time once the simulated shrimp is not being fed.

Tinfl: 32 – Inflection temperature.

Tratio: Ta/Tinfl – Ambient temperature divided by the inflection temperature.

Tstress: Ta-Taccl – The deviation of acclimation temperature from ambient temperature.

VO2: VO2xWshrimp/Wshrimp – Rate of oxygen consumption (mg $O_2 \cdot g^{-1} \cdot h^{-1}$).

VO2xWshrimp: (hArxWshrimp + hAdxWshrimp)/oxycal – Routine rate of oxygen uptake.

Vshrimp(t): Vshrimp(t-dt) +(newV – oldV) * dt – Shrimp volume through time.

Wasted Energy(t): WastedEnergy(t-dt) +)hAwxWshrimp) * dt. Amount of wasted energy through time.

Weffect: Wshrimp^Wexp – The proportion of A achieved by a shrimp weighing W shrimp g, relative to that achieved by a 1-g shrimp.

Wexp: IF Wshrimp < 3.5 then -0.42 ELSE -0.3 – The exponent used to calculate Weffect.

Wfeed: FeedEnergy/GEFeed – This variable is intended to indicate W of feed remaining, but result is spurious subsequent to any change in gross energy of feed being fed.

Winberg: IF FeedRate>0 THEN (1+0.5*(Mact-Mst)/Mst)*0 + 2 ELSE IF FeedRate = 0 AND Wshrimp < 3.5 THEN 2 ELSE 2 * Wshrimp^-0.1 – This function allows Winberg parm to be set to 1.0 plus a fraction (here, 0.5) of metabolic scope divided by Mstd, or a constant value of 2.0. For the shrimp simulation model, Winberg is set to 2.0 while the shrimp are living in culture conditions and are less than 3.5 g. Shrimp above 3.5 g have Winberg degraded with shrimp weight.

Wshrimp: Vshrimp – The gram weight of the shrimp. Assumed ratio of 1:1 with shrimp volume.

VITA

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