ABSTRACT

DEL TORO SILVA, FELIX M. Evaluation of Nursery Habitat: an Ecophysiological Approach. (Under the direction of John M. Miller.)

Abiotic conditions can determine biomass production within habitats and this can influence the relative contribution of nursery habitats to adult populations, yet few studies have addressed how the interactions of abiotic parameters can influence production. Through a combination of laboratory experiments, simulation modeling, and field experiments the effects of abiotic dynamics on fish growth were examined. The objectives of this study were to: (1) assess the effects of oxygen and temperature dynamics on growth; (2) test three indices of metabolic capacity (MMS, RMR, LOC) as indicators of fish performance relative to abiotic dynamics; (3) develop a simulation model to predict growth based on abiotic conditions; (4) validate the model in four nursery areas within the Pamlico River Estuary; and (5) compare habitat classification based on model results with juvenile abundance classification. *Paralichthys lethostigma* (southern flounder) was used as model organism in this study. Results showed significant main and interaction effects of oxygen and temperature on growth. Growth rates were best described with an optimum curve as function of temperature, while low oxygen levels negatively affected growth rate. The interaction effects were detected near the optimum temperature for growth indicating oxygen limitation at levels above the conventional hypoxia threshold of 2.00 mg/L. No significant abiotic effects on RMR were detected, but significant effects were detected for the other two indices (MMS and LOC). MMS indices showed a pattern similar to the observed growth rates in response to temperature and oxygen treatments. The model was successfully parameterized

with laboratory data and validated through simulations of field trials using environmental data as input. The model successfully reproduced growth rates in the field using environmental data (oxygen, temperature and salinity) as input variables and more importantly, it reproduced in a fine temporal scale dynamics of growth rate within the simulations. Comparisons of habitat classification between juvenile abundance and model simulations were not possible because environmental conditions during field experiments were so sever that most of the experiments resulted in negative growth rates. Overall, the study demonstrated the importance of abiotic dynamics on individual performance and resulting biomass production within a habitat and that the ecophysiological framework is an adequate model to develop and test hypothesis of mechanism affecting production within nursery habitats.

Evaluation of Nursery Habitat: an Ecophysiological Approach

by
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BIOGRAPHY

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Introduction

1.1 Introduction

Population growth and demand of marine resources threaten most marine ecosystems (Rosen et al., 2000). Increasing numbers of fisheries are overexploited with the resulting depletion of many wild populations. Among the most notable examples in North America is the collapse of the cod fishery (Roughgarden and Smith, 1996; Myers et al., 1997; Overholtz, 2002). The inability to prevent the reduction of many wild stocks, due to poor understanding of stock dynamics or to overwhelming political interests, has been a major component of the problem (Walters and Collie, 1988). Recently a publication by Worm et al., (2006) has predicted that if current trends continue fisheries production would collapse by the year 2048. The combined effects of economics, politics, and the limitations of current scientific understanding of stock dynamics have contributed to the decline of fisheries world wide.

Fisheries scientists have expanded their focus beyond catch regulations into linkages between stocks and habitats to improve understanding of stock dynamics. Research efforts have shifted onto the role of habitat in fisheries, due in part to the enactment of the Sustainable Fisheries Act (Pub.L. no. 104-297) through the reauthorization of the Magnuson-Stevens Act. Under the Act, the US government must identify, designate and protect "essential fish habitat". Essential fish habitat (EFH) is defined as: "those waters and substrate necessary to fish for spawning, breeding, feeding or growth to maturity" (Pub. L. no. 94-265). In compliance with the Act, scientists and managers must develop methods to evaluate habitat for purposes of management and conservation. Understanding habitat components and how these influence its quality should lead to better management of fisheries.

Over the years, ecologists have developed various criteria in the evaluation of habitat quality. But, is habitat quality correctly evaluated with presence/absence data, indicator species, abundance, or diversity of organisms? Fisheries ecologists have attempted to evaluate habitats based on characteristics assumed to be biologically meaningful (e.g., substrate, structure) and measurements of abundance, but not all habitat used by the organism represents essential habitat (Minello, 1999). The presence of an organism within a given habitat does not provide information on its performance or the contribution by the habitat to its population. A shortcoming of correlative approaches is that they fail to provide mechanisms by which environmental factors influence habitat quality for an organism at various spatial and temporal scales.

The National Marine Fisheries Service has identified 4 levels of information for the identification of EFH. Level 1, presence-absence data, describes the geographical distribution of the species relative to habitat. Level 2 uses habitat specific densities and correlations to habitat characteristics, under the assumption that density estimates are proportional to habitat quality (Minello and Zimmerman, 1992; Minello and Webb Jr., 1997; Able, 1999; Minello, 1999). This approach fails to provide mechanisms explaining the functional relationships between species and habitat characteristics (Minello, 1999). For example, although densities of *Palaemonetes pugio* were not significantly different among natural and created marshes, shrimp mean size was significantly larger in natural marshes (Minello and Webb Jr., 1997). Level 3 information uses measurements of growth, reproduction or survival for habitat evaluation. The integration of all types of Level 3 information is used to produce Level 4 criteria, biomass production within the habitat.

Ultimately, habitat evaluation will depend on measures of growth, survival, reproduction and how these relate to biomass production (Able, 1999). To decipher the links between habitat production and habitat characteristics requires an understanding of the mechanisms operating on the affecting the organisms within the habitat.

One approach to habitat quality evaluation has been through the development of habitat suitability indices (HSI). HSI incorporate Geographic Information Systems (GIS) into models combining spatial distribution and species abundance or density (Levels 1 and 2) to predict habitat quality for single and multiple species (Brown et al., 2000; Kupschus, 2003; Store and Jokimäki, 2003). These methods attempt to integrate biotic and abiotic elements into a spatial framework to evaluate habitat. Although valuable management tools, such models result in correlative analyses, lacking mechanistic explanations that could lead to models with greater predictive power. Without understanding the processes responsible for biomass production, habitat evaluation can be biased.

For example, when factors such as colonization processes are not considered, habitat evaluation based on abundance indices is inadequate (Guindon and Miller, 1995). In marine environments, circulation patterns are a driving mechanism in colonization processes and variations in these patterns could account for variation of yearly recruitment (Higgins et al., 1997; Taylor et al., *in preparation*). Habitat classification relying on presence-absence data (Level 1), in a year of low recruitment a habitat suitability index could determine poor habitat quality without identifying the underlying mechanisms for such classification. Because this type of habitat assessment does not account for mechanisms influencing settlement processes

management decisions may be taken, potentially compromising the conservation of valuable resources.

In their review, Beck et al. (2001) summarized the status of nursery habitat research and emphasized that correlative analysis (Level 1 and 2 criteria) cannot provide strong inference on nursery habitat quality. The alternative is to follow a more rigorous experimental approach focused on hypothesis testing and mechanism determination. A mechanistic approach emphasizing ecosystem dynamics and their relationship to growth and survival (Level 3 information criteria) will provide greater predictive power in habitat assessment and ultimately yield production estimates (Level 4). In summary, effective habitat evaluation requires the development of mechanistic explanations of how habitat characteristics affect Level 3 criteria (survival and growth) and Level 4 (production of organisms).

In fishes, growth is assumed to be an integrator of the environmental conditions. Faster growth rates imply ample food resources and favorable abiotic conditions (Able 1999). It has been shown for various species that growth rates are indicative of habitat quality and recruitment success to the adult population (Hare and Cowen, 1997; Meng et al., 2000; Bergenius et al., 2002; Ross, 2003). Ultimately, nursery habitat evaluation aims to quantify the net biomass output of these habitats into adult populations. Understanding the mechanisms behind fish growth is the first step in evaluating and comparing nursery habitats.

One approach to the evaluation of nursery habitat is the assessment of potential production as a measurement of habitat quality as suggested by Guindon and Miller (1995). Production can be defined through the equation $P=B^{(G-Z)}$. Where biomass (B) is a function of

colonization and immigration processes, growth (G) is a function of available resources and abiotic factors, and mortality (Z) is the loss due to natural mortality, predation, and emigration. Potential production, biomass production within a habitat without mortality and colonization effects, provides the advantage of identifying and empirically testing the mechanisms affecting fish growth within a habitat.

Estuaries are highly productive areas with abundant resources, able to support rich communities of fishes and invertebrates and serving as nurseries for many species (Teal, 1962; Beck et al., 2001). When utilizing potential production as a measure of habitat quality of these nursery areas it is necessary to consider available food resources and the effects of abiotic factors. If food resources are limiting, growth will be affected and potentially survival as well. Although a relationship between estuaries and fishery production is evident, the means by which this occurs remain to be elucidated (Kneib, 1997; Kneib, 2000; Minello et al., 2003).

In North Carolina estuaries food resources do not appear to be limiting. Previous studies have determined that nursery areas within the Pamlico-Albemarle estuarine system have abundant food resources and are likely below carrying capacity, suggesting that other factors regulate fish production (Kamermans et al., 1995; Ross, 2003). Based on those conclusions, the present study focused on the effects of abiotic parameters on fish growth within nursery habitats; because these parameters regulate the physiological performance of individuals (Fry, 1947; Fry, 1971; Brett and Groves, 1979; Peters, 1971; Iwata et al., 1994; Seikai et al., 1997; Madon, 2002). In the absence of food limitation, the effects of abiotic

parameters on individual growth must be understood in order to assess habitat quality through potential production.

Dissolved oxygen is an abiotic factor of concern in relation to habitat quality in nursery areas (Gibson, 1994). Oxygen limited waters have become more prominent in coastal zones throughout the nation (Paerl, 1998; Rabalais et al., 2002; Hagy et al., 2004). Hypoxic events are most common during the summer months when stratification of the water column occurs and bottom waters become anoxic. In North Carolina, storms or periods of strong winds can produce upwelling of deeper anoxic waters onto the upper layers of the water column. Hypoxic events can also be driven by nocturnal respiration of the aquatic community within shallow waters of the esturary. Among the most dramatic effects of hypoxia are the fish kills observed during the summer season in some of North America's estuaries (Paerl, 1998). These events coincide with the residence period of many juvenile stages in the estuaries. Oxygen limited waters reduce habitat quality and restrict quantity of habitat for estuarine fishes, which can translate into reduced fishery production (Gibson, 1994).

Oxygen dynamics are particularly relevant to benthic species such as *Paralichthys lethostigma*. Benthic fishes are likely to be exposed to limited DO waters and the first to be affected by upwelling events. *P. lethostigma* is a very tolerant species, able to cope with variations in salinity from 35 ‰ to salinities near fresh. It is able to survive DO concentrations as low as 2 mg/L and temperatures of 38 °C (Taylor and Miller, 2001; van Maaren et al., 1999). *Paralichthys lethostigma* is an extremely valuable species both for commercial and recreational anglers. This species had estimated average commercial

landings ranging of 2,297,531 lbs., valued at, $4,870,780 during 2006. Based on the NC DMF 2004 stock assessment, the stock is over fished and has been for at least 10 years (NCDMF, 2005). The current conditions of NC stocks increase the urgency of studies that can improve the understanding of juvenile ecology.

During the juvenile stages, *P. lethostigma* inhabits the shallow waters in the upper reaches of the estuary. These are dynamic areas affected by temperature changes, inland discharges, wind driven tides and upwelling events, which makes their habitat ideal study sites. The eutrophic shallow waters in estuaries can be saturated with oxygen during the day and become hypoxic-anoxic at night, while water temperature can vary as much 5 C within 24hrs in North Carolina estuaries. Under such conditions, an integrating framework that can capture system dynamics is needed to assess the performance of organism within the estuary. The synoptic properties of metabolism provide the means to evaluate habitat quality from the organism's perspective (Miller at al., 2000; Neill et al., 2004). The ecophysiological approach exposed in this document provides such framework by modeling the effects of abiotic factors on fish physiological responses to these dynamics and how these in turn affect their performance within that habitat.

1.2 Rationale

The ecophysiological model is based on the relationship between the metabolic capacity of the organism and its biological performance. Metabolism, defined as the sum of reactions yielding energy for the activities of the organism, supports all regulatory processes, locomotion, reproduction, growth, etc (Fry, 1971). Conditions under which energetic costs

of maintenance are minimized and maximum metabolism is not limited will yield maximum energy for other biological activities. In juvenile fishes most of the remaining energy is allocated towards growth (Yamashita et al., 2001). Establishing the relationship between fish metabolic capacity and its environment will yield a functional relationship for the evaluation of habitat quality.

A mechanistic framework, such as the ecophysiological model, allows the integration of environmental effects into the evaluation of habitat quality (Miller et al., 1997, Miller et al., 2000; Neill et al., 2004). The ecophysiological model predicts fish growth through the application of physiological responses of metabolism to varying environmental conditions and its effects on individual bioenergetics. The effect of interactions among environmental variables on metabolism is used to estimate the energetic gains and expenditures of the organism within a habitat. Through this approach, metabolism provides the means to evaluate the effects of abiotic factors on the individual and how these factors influence habitat quality relative to the organism.

Under the ecophysiological framework, environmental factors operate on metabolism under six categories (Fry, 1947). (1) Controlling factors are identities governing metabolic rate through their operating on the internal medium where metabolism takes place (e.g. temperature). (2) Limiting factors are identities governing metabolism by their operation on the metabolic chain (e.g. oxygen, substrate). (3) Accessory (loading) factors are identities imposing additional metabolic demands raising the maintenance rate (e.g. salinity). (4) Lethal factors ultimately cause the death of the organism (e.g. toxins). (5) Masking factors are identities that prevent a second factor from acting naturally and can be the product of

complex interactions between factors. (6) Finally, directive factors can produce a response from an organism which is directed in relation to a gradient or away from a deterrent. Response to directive factors can be extrinsic (e.g. movement) or intrinsic (e.g. acclimation). The metabolic capacity of the fish is the product of all six categories acting upon fish metabolism. The metabolic capacity (i.e. metabolic scope) of the organism provides the means to link individual bioenergetics with abiotic conditions. In theory, environmental conditions where metabolic scope is maximized represent the best habitat for the organism.

Considering the dynamic nature of oxygen and temperature in North Carolina's estuaries and the anthropogenic influence on those dynamics, it is imperative to understand how these factors affect fish metabolism in order to establish any relationship to habitat quality. Dissolved oxygen and temperature have been shown to be the dominant factors influencing metabolism and growth in fishes (Peters, 1971; Claireaux and Lagardère, 1999; Madon, 2002; Wuenschel et al., 2004). Under low oxygen levels, maximum metabolic capacity of an individual is restricted and growth is reduced. Under Fry's model, even when food resources are plentiful, if oxygen levels are not adequate aerobic metabolism is curtailed resulting in reduced growth. This phenomenon was demonstrated under laboratory conditions with *Paralichthys lethostigma* (southern flounder) (Taylor and Miller, 2001). Under field conditions DO levels in combination with high summer temperatures will have a significant effect on the performance of fishes.

1.3 Research Goals

The goal of this study was to evaluate the role of spatiotemporal dynamics of abiotic factors and their interacting effects on fish's metabolic capacity and the relationship to *P. lethostigma* nursery habitat quality. I employed a combination of laboratory, field experiments and simulation modeling to accomplish study objectives. Fish performance (i.e. growth) was evaluated as a function of abiotic factors, mainly oxygen and temperature, in the laboratory and in the field. The specific objectives were: (1) to develop an ecophysiological model of southern flounder that predicts growth under time varying environmental regimes, (2) evaluate model predictions with field trials on selected sites in Pamlico Estuary throughout the summer season, thus providing information of spatial and temporal patterns within the estuary, (3) test two field estimates of fish metabolic performance: marginal metabolic scope (MMS) and limiting oxygen concentration (LOC), and (4) compare these measures with functional criteria of habitat quality (i.e., growth).

The objectives of the study can be summarized in the following hypotheses:

Ha1: Estimates of MMS, RMR, and LOC will reflect environmental conditions and reflect relative habitat quality.

DO effects

$Ha_{1.1}$: LOC will be positively correlated to DO conditions.

$Ha_{1.2}$: Growth rate will be negatively correlated to DO conditions.

Temperature effects

$Ha_{1.3:}$ RMR will be positively correlated to temperature experienced by the organism.

$Ha_{1.4}$: Growth rate will be positively correlated to temperature.

Interaction effects

$Ha_{1.5:}$ Under low DO conditions MMS will be relatively higher under low temperature than under higher temperatures.

$Ha_{1.6}$: Reduction in growth rates under low DO conditions will be greater under high temperatures.

Ha2: Temporal dynamics of abiotic factors can be used to simulate fish growth.

$Ha_{2.1}$: High frequency of hypoxic events will be negatively correlated to growth rate and metabolic scope.

$Ha_{2.2}$: The extent of hypoxic periods will be negatively correlated to growth rate and metabolic scope.

$Ha_{2.3}$: Effects of hypoxic events on growth and metabolic scope will have a greater magnitude under higher temperature.

$Ha_{2.4:}$ Habitats with high temperature and low frequency of hypoxic events will produce higher growth rates and greater metabolic scope.

1.4 References

Able, K.W., 1999. Measure of juvenile fish habitat quality: examples from a national estuarine research reserve. In: Beneka, L.R. (Ed.), Fish habitat: essential fish habitat and rehabilitation. American Fisheries Society. American Fisheries Society, Bethesda, Maryland, pp. 134-147.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.R., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51, 633-641.

Bergenius, M.A.J., Meekan, M.G., Robertson, D.R., McCormick, M.I., 2002. Larval growth predicts the recruitment success of a coral reef fish. Oecologia 131, 521-525.

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. In: Hoar, W.S., D.J. Randall and J.R. Brett (Ed.), Fish physiology: bioenergetics and growth. Academic Press, New York, pp. 279-353.

Brown, S.K., K.R. Buja, S.H. Jury, M.E. Monaco, and A. Banner. 2000. Habitat suitability indices models for eight fish and invertebrate species in Casco and Sheepscot Bays, Maine. North American Journal of Fisheries Management 20, 408-435.

Claireaux, G., Lagardère, J.P., 1999. Influence of temperature, oxygen, and salinity on the metabolism of the European sea bass. Journal of Sea Research 42, 157-168.

Fry, F.E.J., 1947. Effects of the environment on animal activity. University of Toronto Studies Biological Series 55.

Fry, F.E.J., 1971. The efffect of environmental factors on the physiology of fish. In: Hoar, W.S.a.D.J.R.D.J. (Ed.), Fish Physiology. Academic Press, NY, pp. 1-98.

Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. Netherlands Journal of Sea Research 32, 191-206.

Guindon, K.Y.a.J.M.M., 1995. Growth potential of juvenile southern flounder, Paralichthys lethostigma, in low salinity nursery areas of Pamlico Sound, North Carolina, USA. Netherlands Journal of Sea Research 34, 89-100.

Hagy, J.D., W. R. Boyton, C. W. Keefer, and K. V. Wood, 2004. Hypoxia in Chesapeake Bay, 1950-2001: Long-term change in relation to nutrient loading and river flow. Estuaries 27, 634-658.

Hare, J.A., Cowen, R.K., 1997. Size, growth, development, and survival of the planktonic larvae of Pomatomus saltatrix (Pisces: Pomatomidae). Ecology 78, 2415-2431.

Higgins, K., Hastings, A., Sarvela, J.N., Botsford, L.W., 1997. Stochastic Dynamics and Deterministic Skeletons: Population Behavior of Dungeness Crab. Science 276, 1431-1435.

Iwata, N., Kikuchi, K., Honda, H., Kiyono, M., Kurokura, H., 1994. Effects of temperature on growth of Japanese flounder. Fisheries Science 60, 527-531.

Kamermans, P., Guindon, K.Y., Miller, J.M., 1995. Importance of food availability for growth of juvenile southern flounder (Paralichthys lethostigma) in the Pamlico River estuary, North Carolina, USA. Netherlands Journal Of Sea Research 34, 101-109.

Kneib, R.T., 1997. The role of tidal marshes in the ecology of estuarine nekton. Oceanography and Marine Biology an Annual Review 35, 163-220.

Kneib, R.T., 2000. Salt marsh ecoscapes and production transfers by estuarine nekton in the southeastern United States. Kluwer Academic Publishers, Dordrecht, Boston & London.

Kupschus, S., 2003. Development and evaluation of statistical habitat suitability models: an example based on juvenile spotted seatrout Cynoscion nebulosus. Marine Ecology-Progress Series 265, 197-212.

Madon, S.P., 2002. Ecophysiology of juvenile California halibut Paralichthys californicus in relation to body size, water temperature and salinity. Marine Ecology Progress Series 243, 235-249.

Meng, L., Gray, C., Taplin, B., Kupcha, E., 2000. Using winter flounder growth rates to assess habitat quality in Rhode Island's coastal lagoons. Marine Ecology Progress Series 201, 287-299.

Miller, J.M., Neill, W.H., Duchon, K.A., Ross, S.W., 2000. Ecophysiological determinants of secondary production in salt marshes: a simulation study. In: Weinstein, M.P.D.A.K. (Ed.), Concepts and controversies in tidal marsh ecology. Kluwer Academic Publishers, Dordrecht, Boston & London., pp. 315-332.

Miller, J.W., W.H. Neill, and K.A. Duchon., 1997. An ecophysiological model for predicting performance of released fish. Bulletin of National Research Institute of Aquaculture (Japan) 3, 87-91.

Minello, T.J., 1999. Nekton densities in shallow estuarine habitats of Texas and Louisiana and the identification of essential fish habitat. In: Beneka, L.R. (Ed.), Fish habitat:

essential fish habitat and rehabilitation. American Fisheries Society Symposium, Bethestda, Maryland, pp. 43-75.

Minello, T.J., Zimmerman, R.J., 1992. Utilization Of Natural And Transplanted Texas Salt Marshes By Fish And Decapod Crustaceans. Marine Ecology-Progress Series 90, 273-285.

Minello, T.J., Webb, J.W., 1997. Use of natural and created Spartina alterniflora salt marshes by fishery species and other aquatic fauna in Galveston Bay, Texas, USA. Marine Ecology-Progress Series 151, 165-179.

Minello, T.J., Able, K.W., Weinstein, M.P., Hays, C.G., 2003. Salt marshes as nurseries for nekton: Testing hypotheses on density, growth and survival through meta-analysis. Marine Ecology Progress Series 246, 39-59.

Myers, R.A., Mertz, G., Fowlow, P.S., 1997. Maximum population growth rates and recovery times for Atlantic cod, Gadus morhua. Fishery Bulletin 95, 762-772.

NCDMF, 2005. Southern Flounder Fisheries Management Plan. In: DENR (Ed.). State of North Carolina, pp. 335.

Neill, W.H., Brandes, T.S., Burke, B.J., Craig, S.R., Dimichele, L.V., Duchon, K., Edwards, R.E., Fontaine, L.P., Gatlin, D.M., Hutchins, C., Miller, J.M., Ponwith, B.J., Stahl, C.J., Tomasso, J.R., Vega, R.R., 2004. Ecophys.Fish: A simulation model of fish growth in time-varying environmental regimes. Reviews In Fisheries Science 12, 233-288.

Overholtz, W.J., 2002. The Gulf of Maine-Georges Bank Atlantic herring (Clupea harengus): spatial pattern analysis of the collapse and recovery of a large marine fish complex. Fisheries Research 57, 237-254.

Paerl, H.W., Pinckney, J.W., Fear, J.M., Peierls, B.L., 1998. Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia and the eutrophying Neuse River Estuary, NC, USA. Marine Ecology Progress Series 166, 17-25.

Peters, D.S., 1971. Growth and energy utilization of juvenile flounder, Paralichthys dentatus and Paralichthys lethostigma, as affected by temperature, salinity, and food availability., Zoology. North Carolina State, Raleigh, pp. 68.

Rabalais, N.N., Turner, R.E., Wiseman, W.J., 2002. Gulf of Mexico hypoxia, aka "The dead zone". Annual Review Of Ecology And Systematics 33, 235-263.

Rosen, C., L. Roberts, G. Mock, W. Vannaselt, J. Overton, 2000. World Resources 2000-2001, people and ecosystems, the fraying web of life. In: Program, U.N.D. (Ed.). World Resources Institute, Washington D.C., pp. 389.

Ross, S.W., 2003. The relative value of different estuarine nursery areas in North Carolina for transient juvenile marine fishes. Fishery Bulletin 101, 384-404.

Roughgarden, J., Smith, F., 1996. Why fisheries collapse and what to do about it. Proceedings Of The National Academy Of Sciences Of The United States Of America 93, 5078-5083.

Seikai, T., Takeuchi, T., Gwang Sic, P., 1997. Comparison of growth, feed efficiency, and chemical composition of juvenile flounder fed live mysids and formula feed under laboratory conditions. Fisheries Science 63, 520-526.

Store, R., Jokimaki, J., 2003. A GIS-based multi-scale approach to habitat suitability modeling. Ecological Modelling 169, 1-15.

Taylor, J.C., Miller, J.M., 2001. Physiological performance of juvenile southern flounder, Paralichthys lethostigma (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. Journal Of Experimental Marine Biology And Ecology 258, 195-214.

Taylor, J.C., Miller, J.M., Pietrafesa, L.J., Dickey, D., Ross, S., *In preparation*. Winter winds and river discharge determine juvenile southernflounder abundance and distribution in North Carolina estuaries. Fisheries Oceanography.

Teal, J.M., 1962. Energy flow in the saltmarsh ecosystem in Georgia. Ecology 43, 614-624.

Walters, C.J., Collie, J.S., 1988. Is Research On Environmental-Factors Useful To Fisheries Management. Canadian Journal Of Fisheries And Aquatic Sciences 45, 1848-1854.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., Watson, R., 2006. Impacts of biodiversity loss on ocean ecosystem services. Science 314, 787-790.

Yamashita, Y., Tanaka, M., Miller, J.M., 2001. Ecophysiology of juvenile flatfish in nursery grounds. Journal of Sea Research 45, 205-218.

Chapter 2

Influence of Oxygen and Temperature on Growth and Metabolic Performance of *Paralichthys lethostigma* (Pleuronectiformes: Paralichthyidae)

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Abstract

This study applies Fry's (1947) classification of environmental factors to demonstrate the limiting effects of oxygen and its interaction with temperature on the growth of juvenile *P. lethostigma*. We also evaluated the properties of two metabolic indices, marginal metabolic scope (MMS) and limiting oxygen concentration (LOC), as indicators of metabolic scope. We found that oxygen limitation has its greatest impact near the optimum temperature for growth of the species. At 29 C, a reduction from 6.00 mg/L to 4.00mg/L in dissolved oxygen (DO) caused a 50% reduction in growth rate, while at 27 C the reduction in DO had no significant effect on growth rate. These results are particularly relevant because these temperatures and oxygen concentrations are commonly observed in estuarine fish nursery areas during summer months. At all temperatures, fish from the lowest oxygen treatment (1.75mg/L DO) had negative growth rates. Comparisons between daily oscillating oxygen treatments and constant DO treatments failed to demonstrate significant effects on growth. At temperatures past the optimum, growth rates between the 6.00mg/L and 4.00mg/L DO treatments were not statistically different. LOC was significantly affected by temperature, oxygen, and their interaction. Estimates of LOC were positively correlated with oxygen treatment ($R^2 > 0.71$) and negatively correlated with temperature at moderate and low oxygen concentrations ($R^2 > -0.84$). MMS was significantly affected by temperature and oxygen, and was negatively correlated with oxygen concentration ($R^2 > -0.91$), but correlations between MMS and temperature were not as strong. In conclusion, oxygen and temperature interactions have significant effects on metabolic scope and growth rates of fish, well above

the accepted hypoxia threshold of 2.00mg/L and MMS has proved a useful estimator of the metabolic scope of the organism within an environment.

2.1 Introduction

Life in the aquatic medium presents water-breathing organisms with different problems compared to respiration in air. In water, oxygen concentrations are relatively low and dependent on solubility. Because oxygen solubility is inversely proportional to temperature, it will decrease with increasing temperature (Libes, 1992). Given that most aquatic organisms are ectotherms, changes in temperature pose additional difficulties because, while reducing oxygen solubility, temperature also increases metabolic rate and the potential for oxygen limitation (Fry, 1947). The interaction of these two physico-chemical parameters is expected to affect the capacity of an organism to perform its biological activities. Given that dissolved oxygen and temperature patterns are affected by eutrophication (Flemer, 1972; Reyes and Merino, 1991; Turner and Rabalais, 1991) and global warming respectively, (Linton et al., 1998) human activity can affect habitat quality for many aquatic organisms. This is particularly relevant to estuarine dependent fishes whose habitat is characterized by naturally fluctuating environmental conditions. For these reasons, it is important to understand how these parameters influence the biology of aquatic organisms in order to predict how anthropogenic effects can alter habitat quality and the biological processes within.

Growth in fishes is assumed to integrate the effects of environmental conditions within a habitat; including food resources and physico-chemical (or abiotic) conditions such as temperature, oxygen, and salinity (Fry, 1947; Brett and Groves, 1979; Peters, 1971; Iwata et al., 1994; Gibson, 1994; Seikai et al., 1997; Madon, 2002) and has been used as an

indicator of habitat quality (Able, 1999; Meng et al, 2002). Although the interacting effects of temperature and dissolved oxygen (DO) on growth have long been recognized (Fry, 1947), few publications have investigated the interacting effects of these factors on growth rate (Mallekh el al., 1998; Claireaux and Lagardère, 1999; Claireaux et al., 2000; Mallekh and Lagardère, 2002; McNatt and Rice 2004; Cerezo and García, 2005; Stierhoff et al., 2006).

Fry (1947) recognized that metabolism is the means by which organisms perform all activities (e.g., growth) and defined six categories or factors by which environmental variables affect metabolism. Central to Fry's (1947) approach is the concept of metabolic scope, which he defined as the difference between the minimum or standard (SMR) and maximum or active (MMR) metabolic rates. The difference represents the net metabolic capacity for the organism to perform all biological activities including both reproductive and somatic growth (Fry, 1947, Fry 1971). Under Fry's classification, temperature operates as a controlling factor, determining the minimum metabolic rate of the organism, while oxygen operates as a limiting factor determining the maximum metabolic rate. Changes in either of these factors can increase or reduce the amount of scope available to an organism in a given habitat. According to this theory, the scope of the organism is tightly correlated with its growth and can be used as a predictor of individual performance within a given environment.

For example, according to traditional bioenergetic principles with unlimited food supply the growth rate of a fish is expected to increase exponentially with temperature as it approaches an optimum after which it declines sharply. But applying Fry's principles, the optimum temperature is dependent on the interaction of temperature with other environmental factors such as oxygen. A decline in DO (limiting factor) can reduce growth

at higher temperatures (i.e., reduction in scope; Fig. 2.1a) by effectively reducing the capacity for cellular respiration. Such reduction in scope at higher temperatures should produce a displacement of the optimal temperature for growth given DO restricted conditions (Fig. 2.1b; Fry, 1947, 1971; Neill and Bryan, 1991). Accordingly, conditions for optimal growth are not only dependent on temperature and food consumption, but will be dependent on adequate oxygen availability to maximize metabolic scope.

An empirical approximation of metabolic scope can be estimated through closed respirometry assays (Neill and Bryan, 1991). A fish within a respirometry chamber is assumed to maintain its routine metabolic rate (RMR) through compensatory processes (e.g., increased gill ventilation rate) as DO decreases within the chamber. However, at some oxygen concentration compensatory processes fail to support RMR and metabolic rate declines, becoming oxygen dependent. This DO concentration is defined as the limiting oxygen concentration (LOC; Neill and Bryan, 1991). Accordingly, at LOC RMR is equivalent to MMR. The slope at LOC, defined as marginal metabolic scope (MMS), is proportional to metabolic scope and can be used as a surrogate (Fig. 2.2). These principles have been adapted into an ecophysiological framework for the evaluation of habitat quality based on relationships between metabolic scope and fish bioenergetics within a given habitat (Miller et al., 2000; Neill et al., 2004).

The main objective of the present study was to evaluate the limiting effects of oxygen and its interaction with temperature on the growth and metabolic scope of juvenile *Paralichthys lethostigma*. Temperate juvenile fishes seem to allocate most of their metabolic scope towards growth (Yamashita et al., 2001; Munch and Conover, 2003). Therefore, we

expected to observe reduction in growth (i.e., scope) with a moderate reduction in oxygen at temperatures near the reported optimum for the species, because oxygen should become limiting to the metabolic demands at this temperature. We also expected that interactions between temperature and oxygen would result in a reduction in optimum temperature for growth with decreasing DO. A second objective was to evaluate the properties of MMS and LOC as indices of metabolic capacity of the organism. The responses of MMS and LOC to the interaction of temperature and oxygen, the two dominant abiotic factors in estuaries, have not been specifically addressed in laboratory studies and their use as a surrogate of metabolic scope has received limited attention (Claireaux and Lagardère, 1999; Claireaux et al., 2000). MMS estimates were compared to the observed growth rates and experimental treatments to assess their utility as an indicator of metabolic capacity and habitat quality.

2.2 Material and methods

2.2.1 Experimental design

The effects of oxygen and temperature were tested under controlled conditions in the laboratory. Light periods were set to twelve hours of light and twelve hours of darkness. A split plot design was employed with six temperatures and three dissolved oxygen (DO) concentrations. Oxygen treatment levels were achieved using nitrogen-stripping columns (Taylor, 2001) and included a low level treatment (1.75 mg/L), a moderate treatment (4.0 mg/L), and a high oxygen treatment (6.00mg/L). All DO treatments were conducted at 33, 31, 29, 27, 25 and 23 C for a two-week period. These temperatures were selected to represent a range of temperatures possibly encountered during the summer season in nursery

areas. In addition to constant DO, an experiment with oscillating DO conditions at 25 and 27 C were conducted to simulate natural diel cycles in the field. Oscillating DO conditions ranged from 1.75- 6.00 mg/L (low treatment), 4.00- 6.00 mg/L (moderate) and 6.00 mg/L (high or control). Each treatment had three replicate tanks per experiment with three fish per tank. Experiments were conducted with hatchery-reared fish from NC State University. Fish were individually tagged with acrylic paint injected subcutaneously. Environmental conditions were monitored daily to insure desired conditions throughout the experiment. All experimental manipulations were conducted under NC State University approved animal use protocols.

2.2.2 Respirometer design

Four water-tight circular acrylic respirometers were constructed. Each unit consisted of a removable flat top with two concentric o-rings and a bottom sealed permanently to two concentric cylinders (15.24 cm and 20.32 cm in diameter), both 4 cm tall. The lid was secured with six stainless steel bolts (Fig. 2.3). Water was circulated through the chamber with a submersible pump to insure proper mixing and avoid oxygen depletion near the DO probe. Food colorant was used to test circulation within the chamber and to locate any potential leaks. The outermost chamber connected to the pump intake and was under negative pressure. The water exited the pump into the PVC T passing by the DO probe. Oxygen and temperature were measured with a Yellow Spring Instrument DO meter (YSI 52). The innermost cylinder contained the fish and received the water after it had passed the DO probe. Once the respirometer was sealed, water could only pass between chambers through

small holes of the inner chamber located 1 cm from the bottom and spaced 1 cm from each other. In addition, two ball valves were connected to the lid leading to each chamber. The valves were used to purge any air bubbles and to convert the respirometer into a flow-through chamber when open. The estimated total volume of the chamber was 1L. The system was meant to be portable and applicable in laboratory and field experiments.

2.2.3 Respirometry trial

A respirometry trial is essentially a challenge test. The fish was placed within the chamber in flow-through mode under normal oxygen conditions (6.00 mg/L) and left undisturbed for an hour to allow normalization of respiration rates after handling. After that period, the valves were closed and oxygen concentration was recorded until LOC was reached. All trials were conducted within a temperature regulated water bath at the temperature from which the fish originated to prevent temperature fluctuations. An external water heater was employed to maintain the desired temperature in the tanks containing the respirometry chambers.

Oxygen measurements were recorded using a laptop taking approximately 54 readings per minute. Recordings were averaged per minute and oxygen uptake rate was estimated from the difference in DO concentrations per minute (DO_{t+1}-DO_t). An empty chamber was used during the trials to calculate background oxygen demand (BOD) of the respirometer, which was subtracted from the respiration rates of the chamber and fish combined. Uptake rates were divided by the weight of the fish to correct for differences in fish size between individuals. Data were plotted as uptake versus oxygen concentration of

the chamber. A non-linear model (Proc NLIN in SAS®) was employed to determine LOC and MMS from this relationship. The model fits two linear regressions to the data using least-squares and finds the break-point between the two lines. The break-point parameter is defined as the LOC and the slope below this point (with intercept of zero) is defined as the MMS (Neill and Bryan, 1991).

2.2.4 Statistical analysis

After two weeks of exposure to experimental conditions, response variables (metabolic indices and growth) of individual fish were measured. Growth rate was calculated following Ricker's (1975) formula for instantaneous growth (g/day). Growth analyses were conducted with the average growth rate per tank, while metabolic analyses were conducted on individual fish. We employed mixed-effect models (PROC MIXED in SAS®) to evaluate treatment and interaction effects among dissolved oxygen and temperature treatment levels. Because significant interactions between factors were expected, we conducted multiple comparisons tests. We used Tukey's correction factor to test for significant difference between least-squared means. All statistical analyses were conducted using SAS 9.1® (SAS, 2004), and considered significant at $\alpha < 0.05$.

2.3 Results

2.3.1 Growth

Growth rate of *P. lethostigma* was affected by temperature and dissolved oxygen concentration after two weeks of exposure. Growth rate increased with temperature and reached a maximum at 29 C for the high oxygen treatment (Table 2.1). Likewise, relative

growth rate for the moderate oxygen treatment increased with temperature, but reached a maximum at 27 C. The low oxygen treatment group had negative growth rates at all temperatures. Growth rates of the high DO treatment groups over the temperature range studied is best described as an optimum function, increasing exponentially with temperature until it reaches a maximum at 29 C and declining quickly afterwards (Fig. 2.4). The moderate DO treatment group (4.00 mg/L) can be similarly described with an optimum curve reaching its maximum at 27 C and declining at higher temperatures with negative growth at 33 C (Fig. 2.4). At both high and moderate oxygen concentrations, growth rate approached zero at 31 C and became negative at 33 C (Fig. 2.4). The low oxygen concentration group (1.75 mg/L) is best described as a linear function with negative growth at all temperatures and no survivors at 33 C (Fig. 2.4).

A two-factor analysis of variance showed significant effects of both treatments ($p < 0.01$) on relative growth rate (Table 2.2). In addition, a significant interaction effect between the two factors was detected ($p < 0.01$). The interaction effects are evident when a decrease in oxygen concentration of 2 mg/L caused a 50% reduction of growth rate at 29 C. The reduction in growth rate was accompanied by a displacement of the optimal temperature for growth of *P. lethostigma* from 29 C to 27 C (Fig. 2.4). Multiple comparisons indicate that at 27 C, the 4.00 and 6.00 mg/L treatments groups were not significantly different from the 6.00 mg/L at 29 C, but at 29 C the 6.00 mg/L and 4.00 mg/L were significantly different (Fig. 2.5). The growth rates at 23 C and 25 C of the low and moderate DO treatments were not significantly different thus grouped with all oxygen levels at 31 C and 33 C (Fig 2.5).

Analyses of DO oscillation effects on growth indicate that oxygen oscillation did not have a statistically significant effect on growth rate (Table 2.3).

2.3.2 Metabolic indices

The limiting oxygen concentration (LOC) of *P. lethostigma* was affected by both treatments and the estimates are reported in Table 2.1. LOC estimates tended to decline in value with both temperature and oxygen treatment. With the exception of the 25 C treatment group, mean adjusted LOC values were correlated with DO (R^2 >0.71). Similarly, mean adjusted LOC values were strongly correlated with temperature at moderate and low DO concentrations (-0.84 > R^2 > -0.95). Limiting oxygen concentration was positively affected by DO concentration and effects were greatest at high temperatures and low oxygen combinations (Fig. 2.6). A two-factor analysis of variance indicates a significant effect of temperature and oxygen on LOC, but a significant interaction term was also detected (Table 2.2). Both factors were significantly related to LOC estimates, but this effect was enhanced at 29 C and low DO concentration. No statistically significant difference in LOC was detected between constant and oscillating treatments (Table 2.3).

The analysis of variance on MMS estimates showed a significant temperature effect (Table 2.2). With the exception of the 25 C treatment group, marginal metabolic scope estimates within temperature were strongly correlated to dissolved oxygen treatment (R^2< -0.91). Beyond 29 C the optimum temperature for growth was exceeded, hence growth rate and MMS declined regardless of oxygen concentration. If the 31 and 33 C treatment groups are excluded, multiple comparison analyses grouped the 23 C and 25 C treatments together

and the 27 C and 29 C treatments separately. In a similar manner the 1.75 mg/L treatment tended to group separately from either the 4.00mg/L or the 6.00 mg/L at 29 C and 27 C (Fig. 2.7). Analysis of variance between oscillating and constant DO conditions showed a significant DO regime effect (Table 2.3). At both temperatures the constant treatments tended to have higher MMS values than the oscillating groups.

2.4 Discussion

2.4.1 Growth

Results indicate that at 6.00mg/L growth rate increased exponentially with temperature, reaching an optimum at 29 C. These results are in close agreement with Peters' (1971), which reported similar growth rates and optimal temperature at 30 C for *P. lethostigma*. Growth rates at this DO concentration follow bioenergetics prediction of exponential increase to an optimum temperature and a rapid decline past this point (Fry, 1947; Fry, 1971; Kitchell et al., 1977; Brett and Groves, 1979). The optimal temperature for growth presumably coincides with the optimal temperature for metabolic scope. At low temperatures, growth rate is affected by slower rates of catabolic and anabolic processes within cells, while a reduction in growth rate at temperatures past the optimum can be interpreted under Fry's (1947) framework as a reduction in scope because SMR approaches MMR (Fig. 2.1a). Most likely the reduction in scope past the optimum is due to a breakdown of cellular processes caused by excessive temperature (Smith and Jones, 1982; de Ory et al., 1998). Therefore, based on our results, growth rate below 29 C at 6.00 mg/L was controlled

by temperature, while past this temperature enzymatic breakdown was likely responsible for the reduced growth rate.

At 4.00 mg/L, like the 6.00 mg/L treatment, growth exhibited an optimal curve with similar growth rates between 23 C and 27 C and 31 C and 33 C. However, unlike the 6.00mg/L, the growth rate at 29 C was significantly different between the two DO treatments. A moderate oxygen reduction of 2.00 mg/L caused a 50% reduction in growth rate at 29 C (Fig. 2.4) resulting in a displacement of the optimum temperature for growth to 27 C at 4.00mg/L oxygen concentration. As predicted by Fry (1941), a reduction in oxygen had a limiting effect on metabolic scope and hence growth rate. The predicted reduction in metabolic scope corresponded with observations of reduced food consumption at 4.00 mg/L, which suggest consumption regulation by the organisms given its available scope. These observations are supported by studies correlating reduced food consumption with decreasing oxygen concentrations and presumably reduced metabolic scope (Mallekh el al., 1998; Mallekh and Lagardère, 2002; Stierhoff et al., 2006). The 27 C group, unlike the 29 C treatment, did not reflect oxygen limitation effects on growth rate between 6.00 and 4.00 mg/L, suggesting that oxygen limitation occurs at concentrations below 4.00 mg/L at 27 C.

Under conditions of severe hypoxia (1.75 mg/L) growth rate was affected at all temperatures with no survivors at 33 C, suggesting that this concentration significantly affected metabolic scope e even at the lowest temperature treatment (Fig. 2.4). Similar to the 29 C and 4.00 mg/L treatment, a reduction in food consumption was observed at all temperatures under hypoxic conditions. Interestingly, the negative growth rates observed

were similar across all temperatures, suggesting regulatory mechanisms prevented further weight loss with increasing temperature (Fig. 2.5).

According to traditional fish bioenergetics models, respiration rate is temperature dependent and consequently consumption should increase with temperature for weight maintenance. On the other hand, Hochachka and Lutz (2001) proposed a suppression of ATP turnover as a mechanism of defense against hypoxia. Such mechanism suggests a survival response in which the organism reduces metabolic demands, and would explain in part the apparent lack of temperature effect on growth rate under severe hypoxia. Further research is needed to elucidate possible mechanisms responsible for the observed results if we are to improve our predictions of fish bioenergetics in response to these environmental factors.

2.4.2 Metabolic indices

The response of MMS to our treatments is a function of LOC. We expected to observe a decrease in LOC as an adaptation to the limiting effects of oxygen. With the exception of the moderate and high oxygen groups at 25 C, LOC behaved as expected (Fig. 2.6). A reduction in LOC is a shift of the MMR curve closer to the origin. A shift to the left increases the area under the curve at lower DO concentrations relative to individuals having MMR curves farther to the right as long as both organisms have similar SMR. A greater area under the MMR curve corresponds to a higher metabolic scope and hence higher MMS at a given oxygen concentration. For this reason, organisms adapted to the low oxygen treatment exhibited a relatively higher MMS compared to the control individuals once the oxygen limitation was removed.

The adaptive response of LOC can be explained by several physiological adaptations reported in the literature. Two blood-oxygen binding properties are: (1) affinity, the resistance to binding between hemoglobin (Hb) and O_2 and (2) capacity, the amount of oxygen being carried by the blood. Both of these properties can be affected differently by environmental factors (Nikinma et al., 1980). Increasing temperature reduces oxygen solubility in the water and blood plasma and reduces hemoglobin's oxygen affinity ($Hb\text{-}O_2$) (Cameron, 1989). Although a reduction in $Hb\text{-}O_2$ increases the difficulty of uptake at the gills, it can increase the rate of oxygen unloading at the tissues (Nikinmaa and Salama, 1998); which suggests that under high DO concentrations the reduced uptake at the gills is compensated by higher ventilation frequency and oxygen unloading rate at the tissues. Such compensatory response at high DO concentrations could account for the lack of statistical difference in MMS among the 6.00 mg/L groups as temperature increased. The reduction of $Hb\text{-}O_2$ to temperature is advantageous as long as the oxygen gradient at the gill-water interface is large enough to compensate the reduced $Hb\text{-}O_2$ and guarantee oxygen loading to meet metabolic demands (Nikinmaa and Salama, 1998).

In contrast, adaptive responses to low DO are to increase oxygen capacity and affinity either by elevating haematocrit levels (Weber et al., 1976; Soivio et al., 1980) and/or reducing nucleoside triphosphate (NTP) in erythrocytes (Nikinmaa et al., 1980; Soivio et al., 1980), respectively. An increase in concentration and affinity will increases oxygen loading at the gill interface relative to the low environmental concentrations and will maintain the delivery of oxygen to the tissues (Wood et al., 1975; Weber and Lykkeboe, 1978; Nikinmaa et al., 1980).

An increased HB-O_2 affinity will increase oxygen uptake, but once the oxygen limitation is removed these physiological adaptations could provide an advantage relative to organisms that have occupied constant normoxic conditions. This hypothesis is supported by studies where after exposure to oxygen limitation, fish exhibited compensatory growth (Bejda, 1992; Person-Le Ruyet et al., 2003). It is possible that organisms adapted to low DO conditions will outperform organisms lacking acclimation to low DO once the limitation is removed due to the increase rate of oxygen delivery. In our study the high MMS estimates in response to DO treatments seems to support this assessment, but the mechanism responsible has yet to be determined.

2.4.3 Conclusions and implications for habitat assessment

Although Diaz and Rosenberg (1995) defined hypoxia as oxygen concentrations below 2.00 mg/L, our study clearly demonstrates interacting effects of oxygen and temperature on growth rate above this concentration and rejects the generalized view of a threshold response to oxygen. If we were to limit our evaluation of temperature and oxygen interactions to the highest and lowest temperatures in our data we would conclude that oxygen effects are not significant until levels fall below 1.75 mg/L. This is because at these extremes no statistical difference was detected between the high and moderate oxygen treatments (Fig. 2.5). This situation has caused some researchers to conclude that effects of hypoxia on growth (McNatt and Rice, 2004), movement (Wannamaker and Rice, 2000; Bell et al., 2003; Bell and Eggleston, 2005) and habitat quality (Rabelais et al., 2002; Eby and Crowder, 2002) are a threshold response to oxygen concentrations below 2.00 mg/L. In light

of Hochachka and Lutz's (2001) discussion the proposed threshold response seems more likely a function of minimum oxygen requirements, which would account for avoidance responses, but does not preclude negative effects to be appreciable above this concentration as demonstrated in this study and by Stierhoff et al. (2006).

A generalized threshold response effect on growth proves overly simplistic because it ignores the interaction effects of temperature and oxygen concentration on metabolic reaction rates. Aerobic metabolism is a second order kinetic process dependent on oxygen concentration and substrate in addition to temperature. Our results clearly demonstrate that the interaction between oxygen and temperature have an impact on the metabolic demands and the capacity to meet these demands in a given oxy-thermal environment. It also demonstrates that hypoxia, rather than a fixed value, should be considered a continuum along the temperature gradient, with minimum DO requirements increasing with temperature (Fig. 2.4).

We conclude that oxygen should not be evaluated as a threshold value independent of temperature. A reduction from 6.00 to 4.00mg/L at 29 C produced a 50% reduction in growth rate over two weeks. This has important implications for the ecological assessments of nursery habitat quality in estuaries. Normal temperatures during spring to summer period range from 22 to 32 C in many of the North Carolina nursery areas (Guindon and Miller, 1995; Del Toro-Silva *unpublished data*). The observed temperatures coincide with DO concentrations that on average are below 4.00 mg/L (Del Toro-Silva, *unpublished data*). According to our results, we expect reduced metabolic scope and suboptimal growth within these habitats as temperature increases and DO declines during the ensuing summer and fall.

An interesting finding in our study was the poor correlation between oxygen saturation and growth rates. Although oxygen saturation units account for the physico-chemical interactions between solubility and temperature, they fail to capture the effects of these interactions relative to the requirements of the biota. For example, a 6.00mg/L DO concentration is approximately 90.54% saturation at 29 C and 87.60% saturation at 27 C, while a 4.00mg/L concentration is approximately 60.36% saturation and 58.40% saturation, respectively (Table 2.4). The resulting difference in oxygen saturation between the two DO concentrations was 30.18% saturation at 29 C and 29.20% saturation at 27 C. When we examined the difference between the two temperatures at 4.00mg/L the resulting difference was only 1.96%. The percent difference between the two temperatures does not correlate with a 50% reduction in growth rate at 29 C (Fig. 2.4). Our results demonstrate that although the difference in saturation between the two temperatures at 4.00mg/L was only 1.96%, growth rate was significantly limited at 29 C. Hence, reports of oxygen saturation, while capturing some of the physico-chemical interactions between solubility and temperature, do not accurately portray the ecological requirements of an organism as demonstrated in our study.

The significant response of MMS to a limiting factor supports its utility as a predictor of habitat quality. The observed increase in MMS values in flounder exposed to low DO demonstrate its utility as an indicator of environmental quality. Although the response of MMS in this experiment seems counter intuitive since organisms under the most adverse conditions and lowest growth had the highest MMS values, we remind the reader that the index represents the metabolic capacity of the organism immediately after the oxygen

limitation had been removed. Under normal oxygen conditions the physiological adaptations to low DO should reflect a greater metabolic capacity relative to control fish under equal oxygen and temperature conditions. The application of this metabolic index requires a mechanistic framework to properly identify its results. In field tests, the index should provide an instantaneous estimate of relative habitat quality because metabolic capacity estimates represent conditions currently experienced by the organism. Hence, we predict that habitats in which metabolic scope is restricted by environmental factors (e.g. contaminants, parasites, or disease) will have lower MMS indices.

A mechanistic framework (e.g. Fry's ecophysiological approach) under which environmental effects can be interpreted will lead to the development of testable hypotheses on habitat assessment. Because physiology governs biological processes in response to external stimuli, the ecophysiological approach can be used to predict the energetic response of an organism. For example, under the ecophysiological model we can hypothesize that emigration from nursery areas is regulated by metabolic scope. As conditions deteriorate (reduction in scope) with the progression of summer, specifically conditions of increased temperature and reduced dissolved oxygen, organisms should begin to emigrate in search of more favourable habitat. Although we don't argue that the approach used here is the best alternative, we do emphasize that mechanistic approaches are needed to understand the relationships between habitat characteristics and the ecological requirements of organisms.

2.5 References

Able, K.W., 1999. Measure of juvenile fish habitat quality: examples from a national estuarine research reserve. In: Beneka, L.R. (Ed.), Fish habitat: essential fish habitat and rehabilitation. American Fisheries Society. American Fisheries Society, Bethesda, Maryland, pp. 134-147.

Bejda, A.J., Phelan, B.A., Studholme, A.L., 1992. The effect of dissolved oxygen on the growth of young of the year winter flounder, *Pseudopleuronectes americanus*. Environmental Biology of Fishes 34, 321-327.

Bell, G.W., Eggleston, D.B., 2005. Species-specific avoidance responses by blue crabs and fish to chronic and episodic hypoxia. Marine Biology 146, 761.

Bell, G.W., Eggleston, D.B., Wolcott, T.G., 2003. Behavioral responses of free-ranging blue crabs to episodic hypoxia. 1. Movement. Marine Ecology Progress Series, Vol. 259, p.

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. In: Hoar, W.S., D.J. Randall and J.R. Brett (Ed.), Fish physiology: bioenergetics and growth. Academic Press, New York, pp. 279-353.

Cameron, J.N., 1989. The Respiratory Physiology of Animals. Oxford University Press. New York.

Cerezo, J., Garcia, B.G., 2004. The effects of oxygen levels on oxygen consumption, survival and ventilatory frequency of sharpsnout sea bream (Diplodus puntazzo Gmelin, 1789)

at different conditions of temperature and fish weight. Journal of Applied Ichthyology 20, 488-492.

Claireaux, G., Lagardère, J.P., 1999. Influence of temperature, oxygen, and salinity on the metabolism of the European sea bass. Journal of Sea Research 42, 157-168.

Claireaux, G., Webber, D.M., Lagardère, J.P., Kerr, S.R., 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). Journal of Sea Research 44, 257-265.

Diaz, R.J., Rosenberg, R. 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and Marine Biology Annual Review 33: 245-303.

de-Ory, I., Romero, L.E., Cantero, D., 1998. Modelling the kinetics of growth of *Acetobacter aceti* in discontinuous culture: influence of the temperature of operation. Applied Microbiology and Biotechnology 49, 189-193.

Eby, L.A., Crowder, L.B., 2002. Hypoxia-based habitat compression in the Neuse River Estuary: Context-dependent shifts in behavioral avoidance thresholds. Canadian Journal of Fisheries and Aquatic Sciences 59, 952.

Flemer, D.A., 1972. Current status of knowledge concerning the cause and biological effects of eutrophication in Chesapeake Bay. Chesapeake Science 13(SUPPLEMENT), S144-S149.

Fry, F.E.J., 1947. Effects of the environment on animal activity. University of Toronto Studies Biological Series 55.

Fry, F.E.J., 1971. The efffect of environmental factors on the physiology of fish. In: Hoar, W.S.a.D.J.R.D.J. (Ed.), Fish Physiology. Academic Press, NY, pp. 1-98.

Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. Netherlands Journal of Sea Research 32, 191-206.

Guindon, K.Y., Miller, J.M., 1995. Growth potential of juvenile southern flounder, *Paralichthys lethostigma*, in low salinity nursery areas of Pamlico Sound, North Carolina, USA. Netherlands Journal of Sea Research 34, 89-100.

Hochachka, P.W., Lutz, P.L., 2001. Mechanism, origin, and evolution of anoxia tolerance in animals. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 130, 435-459.

Iwata, N., Kikuchi, K., Honda, H., Kiyono, M., Kurokura, H., 1994. Effects of temperature on growth of Japanese flounder. Fisheries Science 60, 527-531.

Kitchell, J.F., Stewert, D.J., Weigner, D., 1977. Application of bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). Journal of Fish Research Board Canada 34, 1922-1935.

Libes, S.M., 1992. An introduction to marine biogeochemestry. John Wiley & Sons, Inc., New York.

Linton, T.K., I.J.Morgan, Walsh, P.J., Wood, C.M., 1998. Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth, and metabolism. Canadian Journal of Fisheries and Aquatic Sciences 55, 576-586.

Madon, S.P., 2002. Ecophysiology of juvenile California halibut *Paralichthys californicus* in relation to body size, water temperature and salinity. Marine Ecology Progress Series 243, 235-249.

Mallekh, R. and Lagardère, J.P., 2002. Effect of temperature and dissolved oxygen concentration onthe metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. Journal of Fish Biology. 60:1105-1115.

Mallekh, R. and Lagardère, J.P., Be´gout Anras, M.L., and Lafaye, J.Y. 1998. Variability in appetite of turbot, Scophthalmus maximus under intensive rearing conditions: the role of environmental factors. Aquaculture 160: 123-138.

McNatt, R.A. and Rice, J.A., 2004. Hypoxia induced growth rate reduction in two juvenile estuarine dependent species. Journal of Experimental Marine Biology and Ecology. 311, 147-156.

Meng, L., Gray, C., Taplin, B., Kupcha, E., 2000. Using winter flounder growth rates to assess habitat quality in Rhode Island's coastal lagoons. Marine Ecology Progress Series 201, 287-299.

Miller, J.M., Neill, W.H., Duchon, K.A., Ross, S.W., 2000. Ecophysiological determinants of secondary production in salt marshes: a simulation study. Kluwer Academic Publishers, Dordrecht, Boston & London.

Munch, S.B., Conover, D.O., 2003. Rapid growth results in increased susceptibility to predation in *Menidia menidia*. Evolution 57, 2119-2127.

Neill W.H., Bryan, J.D., 1991. Responses of fish to temperature and oxygen, and response integration through metabolic scope. In: Tomasso, D.E.B.a.J.R. (Ed.), Aquaculture and water quality: advances in world aquaculture, pp. pp. 30-57.

Neill, W.H., Brandes, T.S., Burke, B.J., Craig, S.R., Dimichele, L.V., Duchon, K., Edwards, R.E., Fontaine, L.P., Gatlin, D.M., Hutchins, C., Miller, J.M., Ponwith, B.J., Stahl, C.J., Tomasso, J.R., Vega, R.R., 2004. Ecophys.Fish: A simulation model of fish growth in time-varying environmental regimes. Reviews In Fisheries Science 12, 233-288.

Nikinmaa, M., Salama, A., 1998. Oxygen transport in fish. In: Perry, S.F., Tufts, B. (Eds.), Fish physiology: fish respiration. Academic Press, London, pp. 141-184.

Nikinmaa, M., Tuurala, H., Soivio, A., 1980. Thermoacclimation changes in blood oxygen binding properties and gill secondary lamellar structure of *Salmo gairdneri*. Journal of Comparative Physiology 140, 255-260.

Paerl, H.W.J.W.P., J.M. Fear, B.L. Peierls., 1998. Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia and the eutrophying Neuse River Estuary, NC, USA. Marine Ecology Progress Series 166, 17-25.

Person-Le Ruyet, J., Lacut, A., Le Bayon, N., Le Roux, A., Pichavant, K., Quéméner, L., 2003. Effects of repeated hypoxic shoks on growth and metabolism of turbot juveniles. Aquatic Living Resources 16, 25-34.

Peters, D.S., 1971. Growth and energy utilization of juvenile flounder, *Paralichthys dentatus* and *Paralichthys lethostigma*, as affected by temperature, salinity, and food availability. Zoology. North Carolina State, Raleigh, pp. 68.

Rabalais, N.N., Turner, R.E., Wiseman, W.J., 2002. Gulf of Mexico hypoxia, aka "The dead zone". Annual Review of Ecology and Systematics 33, 235-263.

Reyes, E., Merino, M., 1991. Diel dissolved oxygen dynamics and eutrophication in a shallow, well-mixed tropical lagoon (Cancun, Mexico). Estuaries 14, 372-381.

Ricker, W.E., 1975. Computational interpretation of biological statistics of fish populations. Bulletin Fisheries Research Board of Canada 191, 382.

Seikai, T., Takeuchi, T., Gwang Sic, P., 1997. Comparison of growth, feed efficiency, and chemical composition of juvenile flounder fed live mysids and formula feed under laboratory conditions. Fisheries Science 63, 520-526.

Smith, F.M., Jones, D.R., 1982. The effect of changes in blood oxygen-carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). Journal Experimental Biology 97, 325-334.

Soivio, A., Nikinmaa, M., Westman, K., 1980. The blodd binding properties of hypoxic *Salmo gairdneri*. Journal of Comparative Physiology 136, 83-87.

Stierhoff, K.L., Targett, T.E., Grecay, P.A., 2003. Hypoxia tolerance of the mummichog: the role of access to the water surface. Journal of Fish Biology 63, 580-592.

Taylor, J.C., Miller, J.M., 2001. Physiological performance of juvenile southern flounder, *Paralichthys lethostigma* (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. Journal Of Experimental Marine Biology And Ecology 258, 195-214.

Turner, R.E., Rabalais, N.N., 1991. Changes in Mississippi River Water Quality This Century. BioScience 41, 140-147.

Wannamaker, C.M., Rice, J.A., 2000. Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. Journal of Experimental Marine Biology And Ecology 249, 145-163.

Weber, R.E., De Wilde, J.A.M., 1976. Multiple hemoglobins in plaice and flounder and their functional properties. Comparative Biochemistry and Physiology B. 54, 433-437.

Weber, R.E., Lykkeboe, G., 1978. Respiratory adaptations in carp blood: influences of hypoxia, red cell organic phosphates, divalent cations and CO_2 on hemoglobin-oxygen affinity. Journal of Comparative Physiology 128, 127-137.

Weber, R.E., Wood, S.C., Lomholt, J.P., 1976. Temperature acclimation and oxygen-binding properties of blood and multiple hemoglobins of rainbow trout. Journal of Experimental Biology 65, 333-345.

Wood, S.C., Johansen, K., Weber, R.E., 1975. Effects of ambient PO_2 on hemoglobin-oxygen affinity and red cell ATP concentrations in a benthic fish, *Pleuronectes platessa*. Respiration Physiology 25, 259-267.

Yamashita, Y., Tanaka, M., Miller, J.M., 2001. Ecophysiology of juvenile flatfish in nursery grounds. Journal of Sea Research 45, 205-218.

Table 2.1 Least squares means of treatment responses of juvenile southern flounder *P. lethostigma* *oscillating dissolved oxygen treatment.

Temperature C	Oxygen mg/L	G rate g/day	SE	MMS	SE	LOC mg/L	SE	RMR $mgO_{2*}min^{-1}*g^{-1}$	SE
	1.75	0.000	0.0043	0.0021	0.00031	2.67	0.263	0.0054	0.00090
23	**4.00**	0.011	0.0039	0.0020	0.00049	3.97	0.416	0.0078	0.00142
	6.00	0.018	0.0039	0.0016	0.00049	3.59	0.416	0.0058	0.00142
	1.75	-0.003	0.0039	0.0015	0.00035	2.29	0.294	0.0035	0.00101
25	**4.00**	0.006	0.0041	0.0013	0.00049	3.40	0.416	0.0043	0.00142
	6.00	0.009	0.0045	0.0012	0.00070	2.00	0.588	0.0024	0.00201
	1.75	-0.001	0.0030	0.0015	0.00022	2.65	0.416	0.0049	0.00088
25*	**4.00**	0.012	0.0040	0.0017	0.00027	3.67	0.416	0.0058	0.00088
	6.00	0.018	0.0030	0.0032	0.00022	2.14	0.509	0.0038	0.00108
	1.75	-0.014	0.0056	0.0032	0.00040	2.44	0.340	0.0067	0.00116
27	**4.00**	0.031	0.0039	0.0026	0.00035	2.46	0.294	0.0057	0.00101
	6.00	0.029	0.0048	0.0023	0.00049	2.76	0.416	0.0059	0.00142
	1.75	-0.007	0.0035	0.0018	0.00038	2.66	0.720	0.0049	0.00153
27*	**4.00**	0.017	0.0028	0.0017	0.00019	2.86	0.360	0.0052	0.00076
	6.00	0.017	0.0032	0.0016	0.00019	2.85	0.360	0.0044	0.00076
	1.75	-0.008	0.0047	0.0027	0.00040	1.18	0.340	0.0027	0.00116
29	**4.00**	0.021	0.0039	0.0023	0.00031	2.66	0.263	0.0061	0.00090
	6.00	0.042	0.0041	0.0020	0.00040	3.57	0.340	0.0068	0.00116
	1.75	-0.005	0.0054	0.0029	0.00035	1.51	0.294	0.0042	0.00101
31	**4.00**	0.006	0.0048	0.0030	0.00040	1.67	0.340	0.0045	0.00116
	6.00	0.008	0.0045	0.0034	0.00035	2.39	0.294	0.0077	0.00101
	1.75	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
33	**4.00**	-0.022	0.0069	0.0026	0.00049	2.00	0.416	0.0026	0.00142
	6.00	-0.033	0.0056	0.0023	0.00040	2.74	0.340	0.0063	0.00116

Table 2.2 Type III test of fixed effects on response variables for constant oxygen treatments

Variable	Effect	df	F-Value	prob > F
Relative growth rate (g/day)	**Temperature**	66	21.14	<0.0001
	DO	66	44.76	<0.0001
	Temperature x DO	66	5.21	0.0003
MMS	**Temperature**	35	5.68	0.0006
	DO	35	1.09	0.3462
	Temperature x DO	35	0.57	0.8160
LOC	**Temperature**	35	6.54	0.0002
	DO	35	10.14	0.0003
	Temperature x DO	35	2.29	0.0387
RMR	**Temperature**	35	1.95	0.1107
	DO	35	2.05	0.1441
	Temperature x DO	35	1.6	0.1525

Table 2.3 Type III test of fixed effects on response variables of constant vs. oscillating treatments

Variable	Effect	df	F-Value	prob > F
Growth rate (g/day)	**DO**	69	68.37	<.0001
	Temperature	69	9.76	0.0026
	Osc. Vs. Const	69	0.09	0.7612
	Temperature x DO	69	13.19	<.0001
MMS	**DO**	23.3	3.11	0.0636
	Temperature	23.3	7.9	0.0099
	Osc. Vs. Const	23.3	27.18	<.0001
	Temperature x DO	23.3	1.82	0.184
LOC	**DO**	23.3	3.54	0.0454
	Temperature	23.3	0.75	0.3952
	Osc. Vs. Const	23.3	0.02	0.8771
	Temperature x DO	23.3	3.99	0.0324
RMR	**DO**	23.3	1.3	0.2904
	Temperature	23.3	1.08	0.3097
	Osc. Vs. Const	23.3	1.44	0.2427
	Temperature x DO	23.3	0.87	0.4317

Table 2.4 Percent saturation for each treatment combination

Temperature	6.00 mg/L	4.00 mg/L	1.75 mg/L
33.00	96.71	64.48	28.21
31.00	93.46	62.31	27.26
29.00	90.54	60.36	26.41
27.00	87.60	58.40	25.55
25.00	84.73	56.49	24.71
23.00	81.76	54.50	23.85

Percent saturation estimates derived from interpolation of values provided in YSI Model 52 Operations Manual (YSI, 2000).

Figure Legend

Fig. 2.1a Limiting effects of reduced oxygen on maximum metabolism (Fry, 1941). A reduction in dissolved oxygen (dotted arrow) will cause a depression in the maximum metabolic rate curve (dotted line). The depression of the maximum metabolic rate will reduce the area under the curve and the standard metabolic rate curve.

Fig. 2.1b Limiting effects of oxygen on metabolic scope (Fry 1941) Metabolic scope under oxygen saturation (solid line) and under oxygen limitation (dotted line). The reduction in oxygen causes a reduction of metabolic scope and a displacement of the optimum temperature for scope. Arrows point the optimum temperature for scope.

Fig. 2.2 Derivation of marginal metabolic rate (adapted from Neill and Bryan, 1991). Maximum metabolic rate is represented by the exponential curve (MMS). The routine metabolic rate is represented by the horizontal line (RMR). The area between the MMS and RMR represents metabolic scope. The point at which both rates intercept is defined as the limiting oxygen concentration (LOC). The slope along LOC is defined as marginal metabolic scope (MMS) and it is proportional to metabolic scope.

Fig. 2.3 Schematic diagram of respirometer. Arrows point to the direction of water flow through the apparatus once it is sealed.

Fig. 2.4 Growth rates of *Paralichthys lethostigma* after 14 days of constant oxygen and temperature treatment. Solid arrow indicates abnormally low growth rates at 4.00 and 6.00 mg/L.

Fig. 2.5 Multiple comparisons of *Paralichthys lethostigma* growth rates after 14 days of constant oxygen and temperature treatment. Dotted ellipses contain groups that were found to be NS (non-significant).

Fig. 2.6 Limiting oxygen concentration (LOC) of *Paralichthys lethostigma* after 14 days of constant oxygen and temperature treatment.

Fig 2.7 Marginal metabolic scope (MMS) of *Paralichthys lethostigma* after 14 days of constant oxygen and temperature treatment.

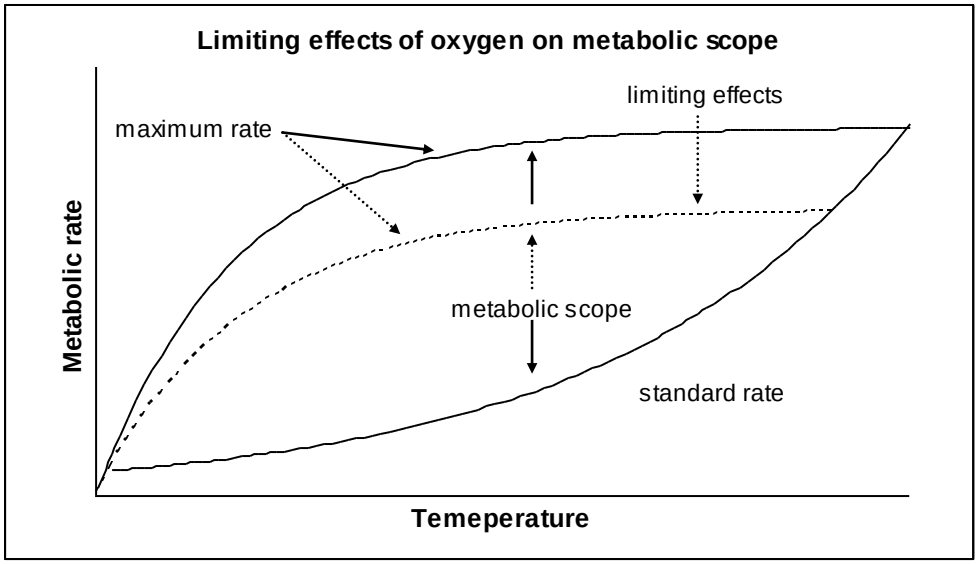


Fig. 2.1a Limiting effects of reduced oxygen on maximum metabolism (Fry, 1941). A reduction in dissolved oxygen (dotted arrow) will cause a depression in the maximum metabolic rate curve (dotted line). The depression of the maximum metabolic rate will reduce the area under the curve and the standard metabolic rate curve.

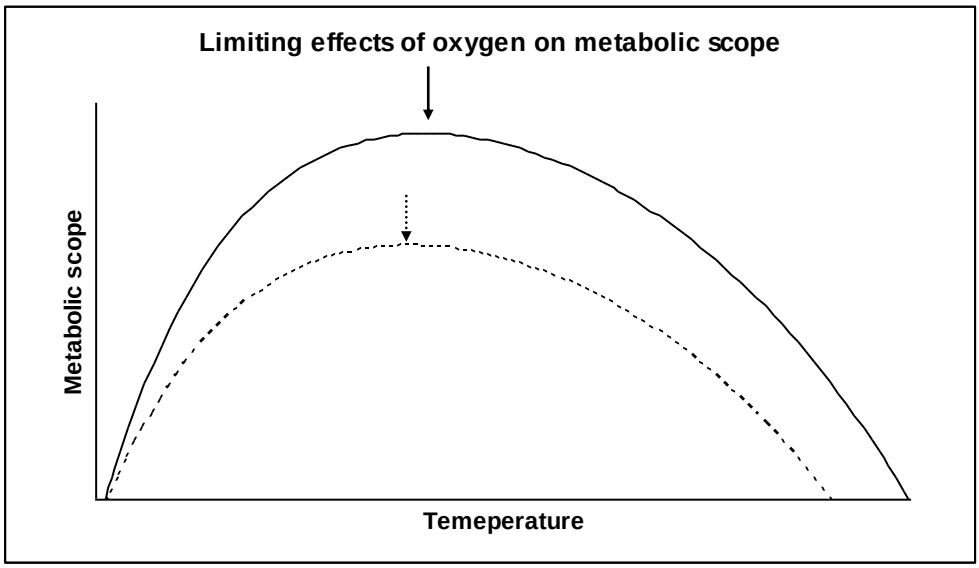


Fig. 2.1b Limiting effects of oxygen on metabolic scope (Fry 1941) Metabolic scope under oxygen saturation (solid line) and under oxygen limitation (dotted line). The reduction in oxygen causes a reduction of metabolic scope and a displacement of the optimum temperature for scope. Arrows point the optimum temperature for scope.

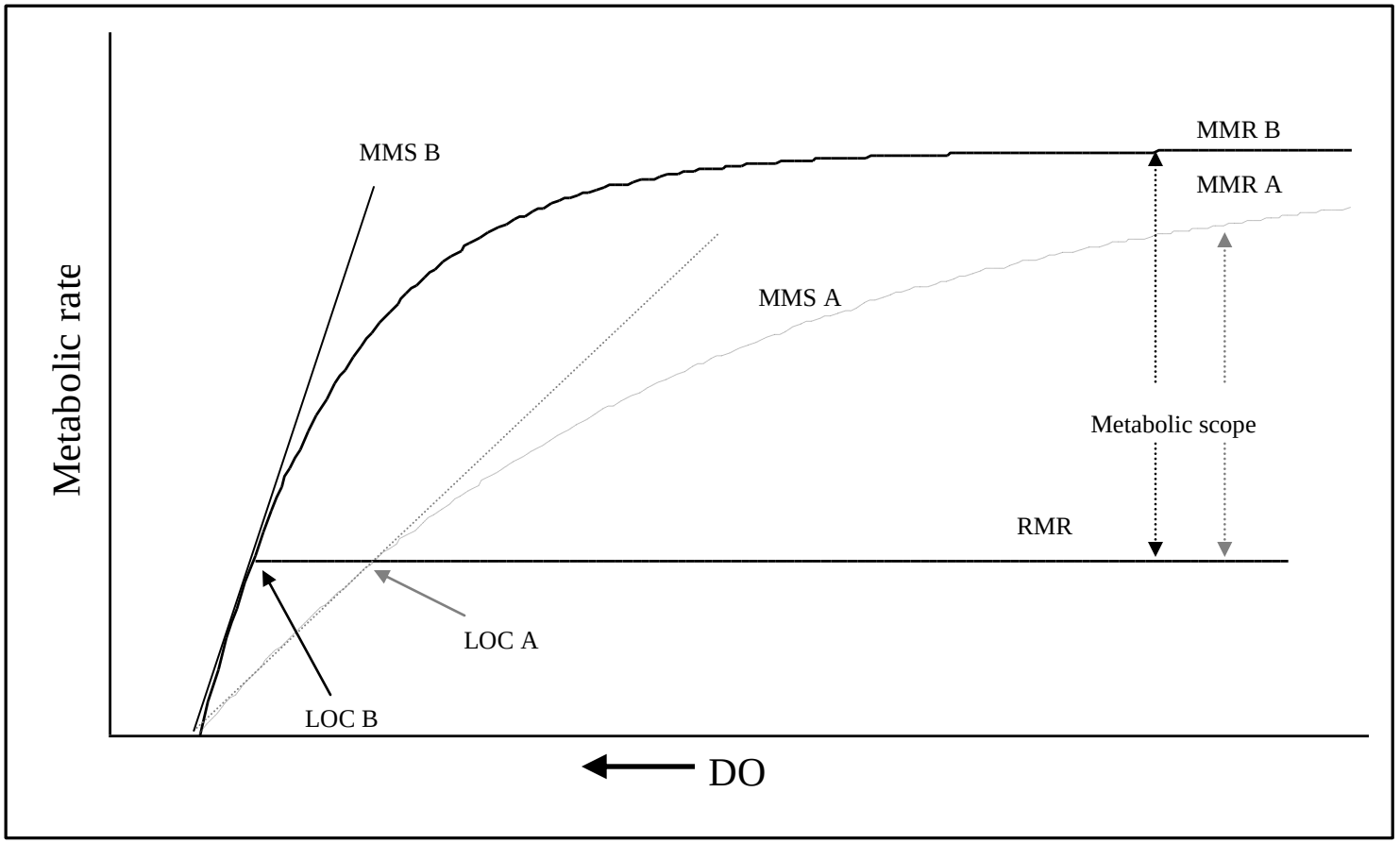


Fig. 2.2 Derivation of marginal metabolic rate (adapted from Neill and Bryan, 1991). Maximum metabolic rate is represented by the exponential curve (MMS). The routine metabolic rate is represented by the horizontal line (RMR). The area between the MMS and RMR represents metabolic scope. The point at which both rates intercept is defined as the limiting oxygen concentration (LOC). The slope along LOC is defined as marginal metabolic scope (MMS) and it is proportional to metabolic scope.

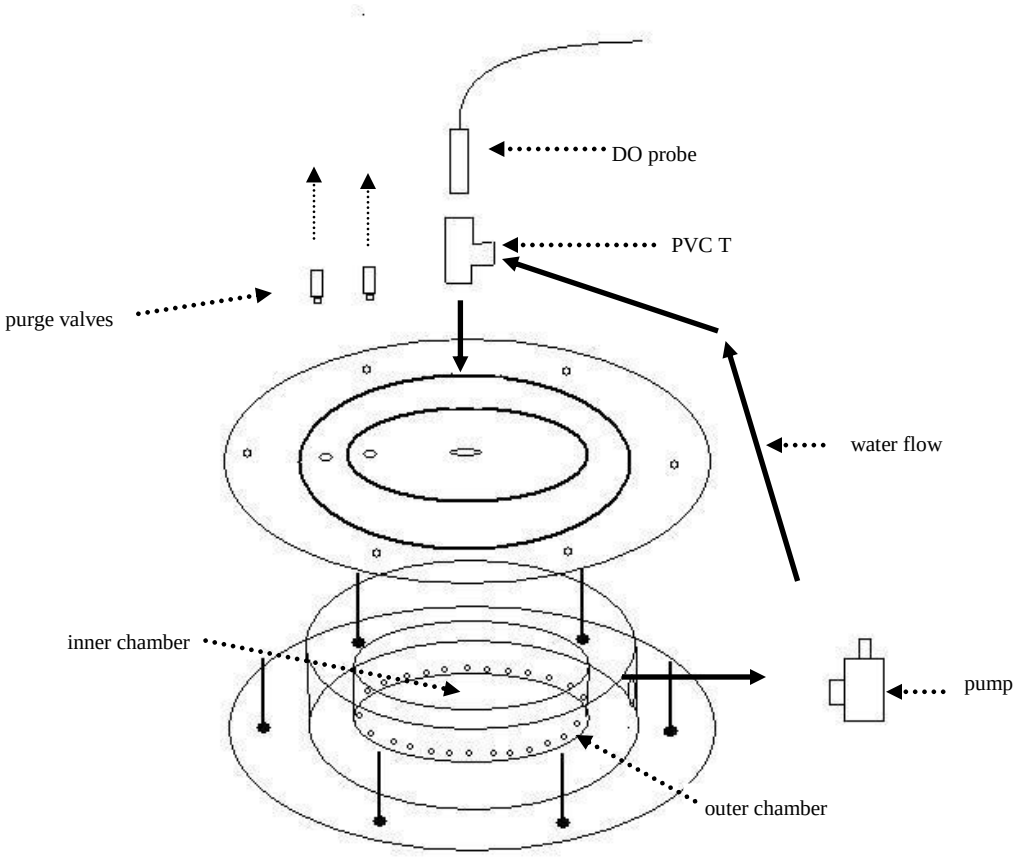


Fig. 2.3 Schematic diagram of respirometer. Arrows point to the direction of water flow through the apparatus once it is sealed.

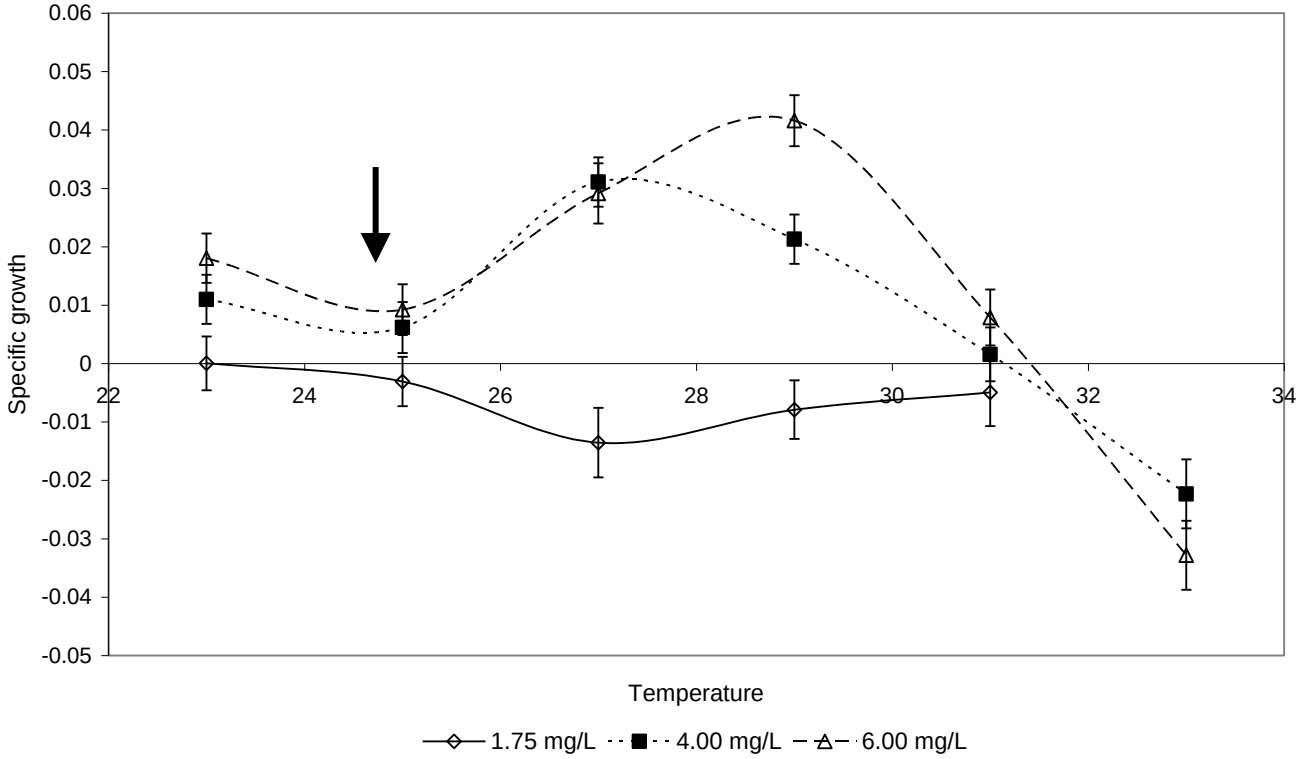


Fig. 2.4 Growth rates of *Paralichthys lethostigma* after 14 days of constant oxygen and temperature treatment. Solid arrow indicates abnormally low growth rates at 4.00 and 6.00 mg/L.

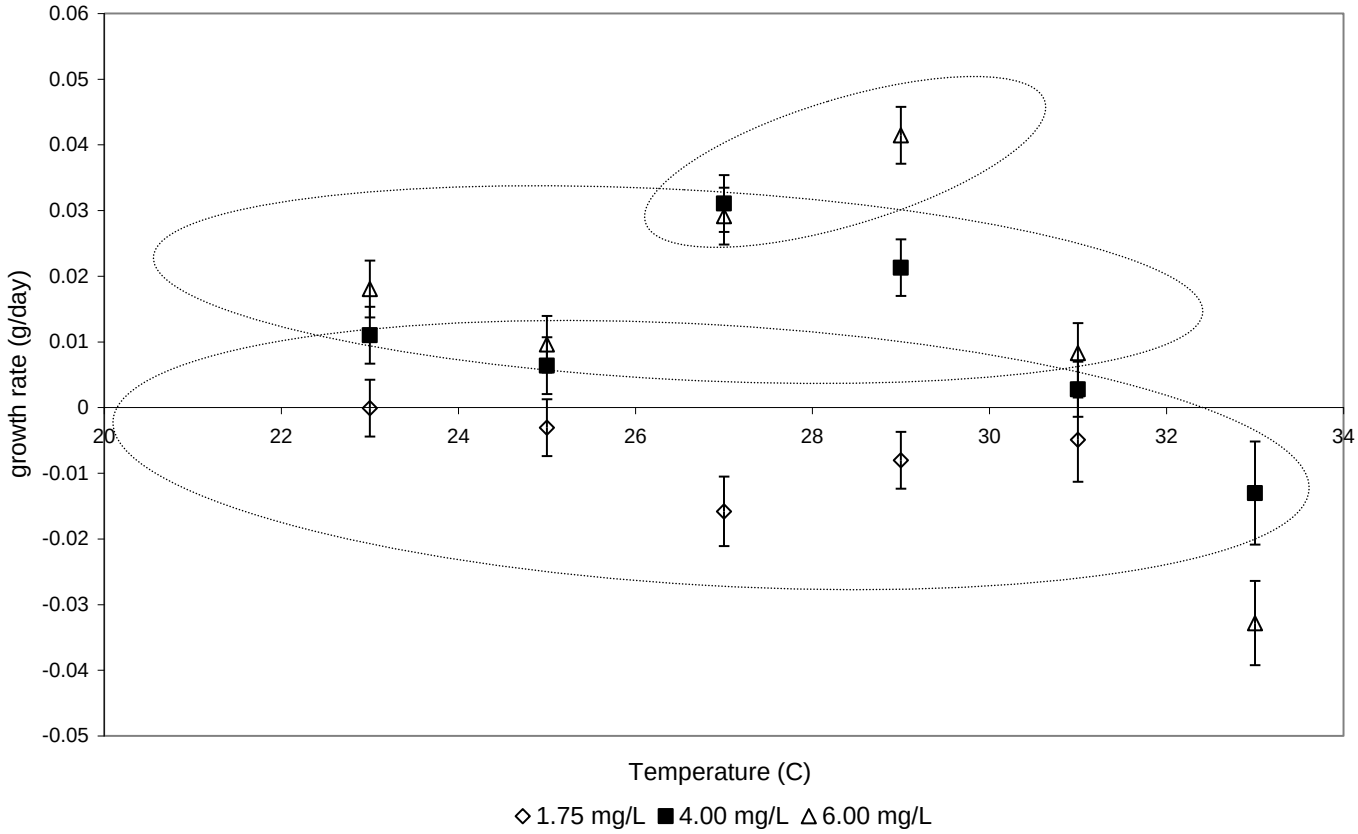


Fig. 2.5 Multiple comparisons of *Paralichthys lethostigma* growth rates after 14 days of constant oxygen and temperature treatment. Dotted ellipses contain groups that were found to be NS (non-significant).

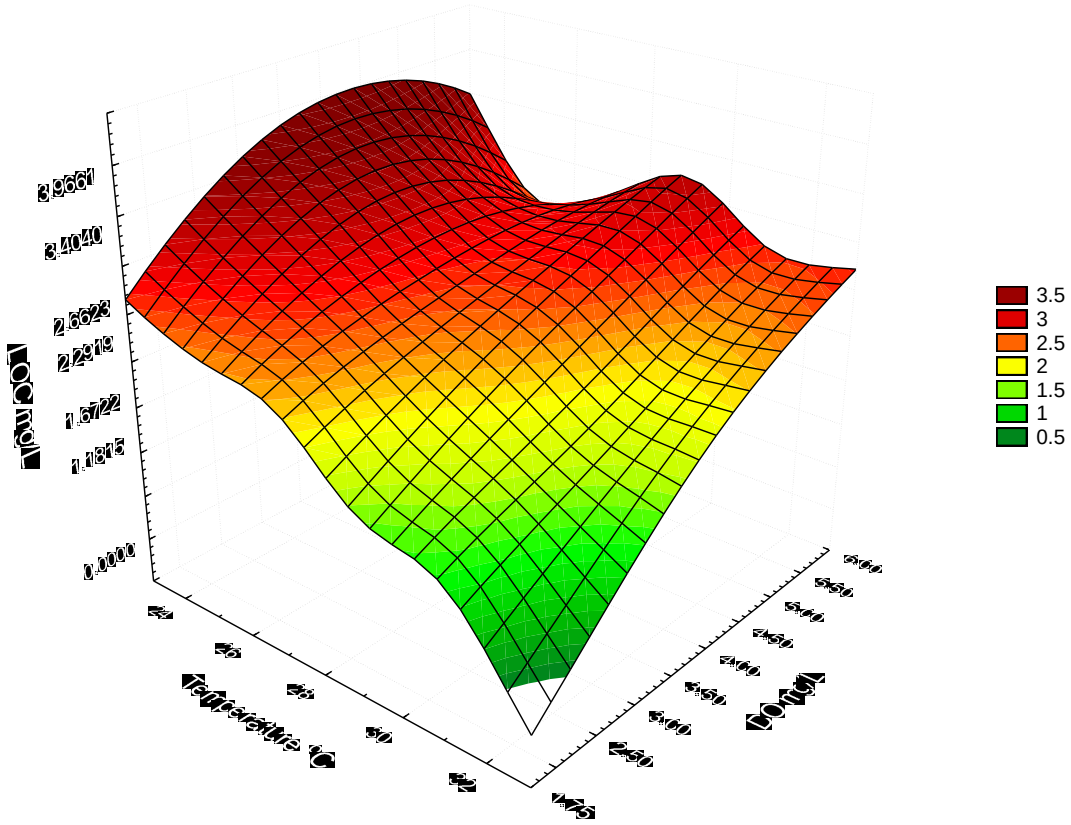


Fig. 2.6 Limiting oxygen concentration (LOC) of *Paralichthys lethostigma* after 14 days of constant oxygen and temperature treatment.

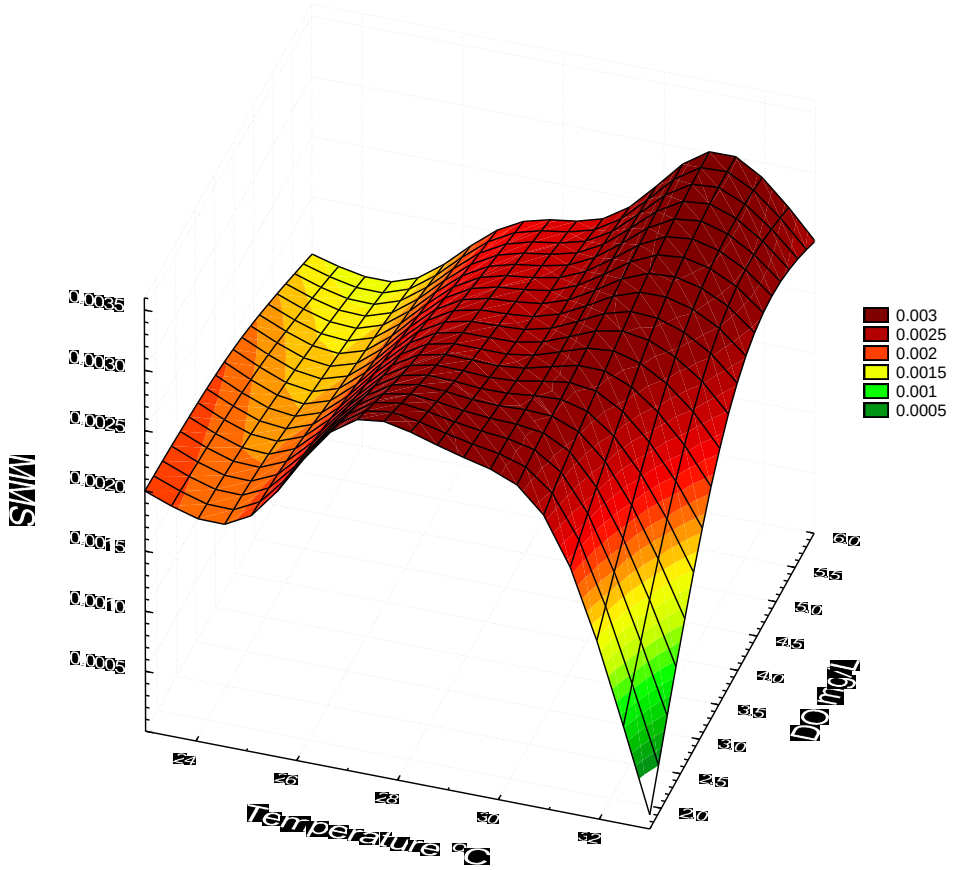


Fig 2.7 Marginal metabolic scope (MMS) of *Paralichthys lethostigma* after 14 days of constant oxygen and temperature treatment.

Chapter 3

Individual Growth Simulation of *Paralichthys lethostigma* (Pleuronectiformes: Paralichthyidae)

Abstract

Eutrophication of coastal ecosystems and the resulting hypoxia of bottom waters have severe negative impacts such as direct mortality of the fauna, restriction of available habitat and a reduction in habitat quality. Although sub-lethal effects of hypoxia on estuarine fauna have previously been addressed, most approaches have considered mean oxygen levels and ignored interactions with temperature. The focus of this chapter was the development of a simulation model based on ecophysiological principles developed by Fry (1947). Fry's (1947) model provides a mechanistic framework to quantitatively assess the effects of abiotic dynamics on fish growth. The model inputs are environmental data (oxygen, temperature, and salinity) and predicts individual growth. The model was parameterized for southern flounder (*Paralichthys lethostigma*) using published laboratory data and goodness of fit tests were used to assess model performance. The goodness of fit test failed to reject the null hypothesis of no difference between observed final weight and model predictions. It was concluded that the model accurately described the growth of *P. lethostigma* under various environmental conditions.

3.1 Introduction

Habitat quality, particularly in nursery areas, plays an important role in fisheries production (Able, 1999; Beck et al., 2001; Yamashita, 2001; Dahlgren et al., 2006). Nursery areas are thought to contribute disproportionately to the recruitment of new individuals to the adult population and are critical to the maintenance of fisheries stocks (Thorrold et al., 1998; Yamashita et al., 2000; Beck et al., 2001; Thorrold et al., 2001; Gillanders, 2002; Gillanders et al., 2003). Although a strong linkage between recruitment and nursery habitat has been established (Thorrold et al., 1998; Yamashita et al., 2000; Gillanders, 2002), the relative importance of the mechanisms behind these linkage are not well understood (Kneib, 1997; Able, 1999; Kneib, 2000; Beck et al., 2001; Adams et al., 2006). Determining which characteristics and mechanisms influence habitat quality requires further research.

Biomass production is presumably the best measurement of habitat quality (Able, 1999; Minello, 1999; Beck et al., 2001; Dahlgren et al., 2006). According the National Marine Fisheries Service's information criteria of habitat evaluation, production estimates is the preferred measurement of habitat quality (Minello, 1999), but direct estimates of production are hardly ever possible to achieve. In lieu of production estimates, potential production has been proposed as a surrogate measurement (Guindon and Miller, 1995). This approach uses growth rate estimates to evaluate habitats. Growth rates have been used with free ranging and caged fishes to evaluate coastal habitats and recruitment throughout North America (Guindon and Miller, 1995; Hare and Cowen, 1997; Meng et al., 2000; Bergenius et al., 2002; Ross, 2003).

Growth rate is dependent on the environmental conditions experienced by the organism and is commonly accepted as an indicator to habitat quality. Previous studies linking juvenile growth to survival suggest that faster growth increases survival (Houde, 1987; Hare and Cowen, 1997; Houde, 1997). Although, the "bigger is better" hypothesis has come under scrutiny (Taylor, 2003; Munch and Conover, 2003; Takasuka et al., 2004), it is generally accepted that growth rate is inversely related to predator vulnerability (Houde, 1987; Hare and Cowen, 1997; Houde, 1997; Sogard, 1997; Takasuka et al., 2003). The roles of abiotic factors, temperature in particular, on the growth rate of fish has long been recognized by biologists (Fry, 1947; Fry, 1971; Brett et al., 1969; Brett and Groves, 1979;). The relationship between temperature and growth was exploited by Kitchell et al., (1977) in the development of a mass balance model that predicts individual fish growth based on temperature and consumption.

The modeling framework developed by Kitchell et al. (1977) uses the relationship between temperature and growth rate to (1) estimate consumption or (2) to predict individual growth, based on consumption estimates and known temperatures. Their bioenergetics model was based on a mass balance equation which stipulates that growth depends on the amount of energy ingested minus losses due to expenditures such as: excretion, egestion, and transformation of energy (Kitchell et al., 1977). The rates at which these processes occur are determined by the controlling effects of temperature, whereas consumption is the only limiting step in the accumulation of biomass. Controlling identities, such as temperature, govern metabolic rate by operating on the internal medium where metabolism takes place,

while limiting identities govern metabolism by their operation on the metabolic chain (Fry, 1947).

The modeling approach developed by Kitchell et al., (1977) has been extensively applied in fisheries science at the population and individual levels (DeAngelis et al., 1993; LaBar, 1993; Mason and Patrick, 1993; Burke and Rice, 2002) to evaluate predator prey interactions (Goyke and Brandt, 1993; Breck, 1993) and habitat suitability (Brandt and Kirsch, 1993; Luo et al., 2001). Two of the biggest difficulties in the application of this modeling approach are the estimation of activity and consumption (Ney, 1993). Regardless of these difficulties, the modeling approach has been applied to evaluate predator prey dynamics, habitat suitability, and potential production in estuarine systems throughout the world (Luo et al., 2001; Sharf et al.., 2006; Stevens et al., 2006).

The bioenergetics model by Kitchell et al., (1977) was developed in northern oligotrophic lakes, where prey consumption acts as the main limiting agent to the growth rate of fish and the effects of other environmental parameters are not implicitly built into the model. In these oligotrophic lake systems growth is restricted to a narrow time window in the summer; hence the model focuses mostly on the effects of temperature and prey availability. Although growth rates are dependent on the amount of energy available and the rate at which it can be processed, it does not consider other environmental factors, in addition to temperature, that can influence growth. The model only considers a single limiting step in the growth process (i.e. consumption) and does not account for other environmental factors such as dissolved oxygen (DO) and salinity, which can fluctuate greatly in space and time in estuaries. These mechanistic omissions can affect its performance in scenarios where other

limiting steps may influence growth. Besides these omissions, bioenergetic models generally use average values as input, which are not adequate in highly dynamic environments like estuaries.

The Pamlico-Albemarle estuarine system is the largest lagoonal and second largest estuarine system in the United States (Giese et al., 1979). The system is separated from the Atlantic by a series of barrier islands. The estuary has an average depth of 4.5m, but can reach 7-8m in the deepest basin (Pietrafesa et al., 1986). Access to the ocean is limited to a few relatively narrow inlets which restricts the flow into and from the estuarine system. The geomorphology of the system makes the dynamics of the estuary mostly wind driven rather than by lunar tides (Pietrafesa et al., 1986). The wind driven currents have direct effect on the transport of larvae across the sound and can ultimately determine larval settlement (Xie and Eggleston, 1999; Taylor et al., *in preparation*). Therefore, the settlement fate of larvae will depend on weather patterns during the ingress period into the estuary (Etherington and Eggleston 2003; Reyns et al., 2007; Taylor et al., *in preparation*). Consequently, habitat quality assessment will require an understanding of how environmental dynamics within settlement areas impact growth and potential juvenile production by these nursery areas.

For example, the impact of wind driven currents is most dramatic in the upper reaches of the Neuse River Estuary, where changes in wind patterns can cause upwelling of hypoxic bottom water. The phenomenon has been linked to mass mortality events of fishes (Pearl et al., 1998). Hypoxic events are most common during the summer months when temperature and salinity cause stratification of the water column and high levels of respiration by the aquatic community reduce DO levels in the water column. These events coincide with the

residence period of many juvenile stages in the estuaries. Oxygen limited waters reduce habitat quality and restrict quantity of habitat for estuarine fishes, which can translate into reduced fishery production (Gibson, 1994).

In contrast to the bioenergetic model by Kitchell et al. (1977), which does not account for environmental effects other than temperature, the ecophysiological approach (Neill et al., 2004) incorporates the effects of other environmental variables besides temperature. The ecophysiological model is based on the relationship between the metabolic capacity of the organism and its environment. The framework allows the simulation of interacting effects of dynamic environmental conditions on metabolism in a fine temporal scale. Metabolism, defined as the sum of reactions yielding energy for the activities of the organism, supports all biological processes (e.g. locomotion, reproduction, growth) (Fry, 1971). In juvenile fishes, most of the remaining energy is allocated towards growth (Yamashita, 2001). Establishing the relationship between fish metabolic capacity and its environment will yield a functional relationship for the evaluation of habitat quality.

The objectives of this study were to: (1) the parameterize an ecophysiological model of *Paralichthys lethostigma,* and (2) the simulate of the effects of temporal dynamics of abiotic factors and their interactions on metabolic capacity and resulting fish growth. The model was coded utilizing the Stella® software and was constructed to simulate fish metabolism on an hourly time step. The model was parameterized from laboratory data published by Del Toro-Silva et al., (2008). Model simulations were compared to results from laboratory experiments to assess goodness of fit. Finally, a sensitivity analysis was performed on the model to determine relative parameter impact on model predictions.

3.2 Methods

3.2.1 Metabolic Module

The model has two major modules with subunits within the first module. First, the metabolic module is where the environmental data enters the model and reproduces the effects of abiotic factors on metabolism according to Fry's theories in the different subunits. According to Fry's paradigm, environmental factors are classified in six categories: 1) controlling, 2) limiting, 3) loading, 4) masking, 5) directive, and 6) lethal, according to their effects on metabolic scope. Controlling factors are identities that govern metabolic rate by operating on the internal medium where metabolism takes place (e.g. temperature). That is, processes occurring at the cellular level which rates are determined by these factors. Limiting factors are those governing metabolism by their operation on the metabolic chain (e.g. oxygen, substrate). Limiting factors can curtail the rate of metabolic processes when they become scarce within the medium. For example, a reduction in the concentration of O_2 within the cell would reduce the rate of cellular respiration. Loading factors impose additional metabolic demands, raising the maintenance rate (e.g. salinity), whereas masking factors prevent a second factor from acting naturally (Fry, 1947). Masking factors can be complex interactions between factors (Fry, 1947). Directive factors elicit a response from an organism which is directed in relation to a gradient or away from a deterrent. The response by the organism to directive factors can be extrinsic (e.g. movement) or intrinsic (e.g. acclimation). The last category, lethal factors, ultimately causes the death of the organism

(e.g. toxins). The metabolic capacity of a fish is a complex function of all six categories acting upon fish metabolism. The model presented here focuses on the effects of factors in the first three categories: temperature, dissolved oxygen (DO), and salinity, respectively on metabolic scope (Fig. 3.1). The main output of the metabolic module is metabolic scope, which is defined as the difference between the standard and maximum metabolic rates of the organism (Fry, 1947).

3.2.2 Controlling Factors subunit

According to Fry (1947; 1971), controlling factors affect metabolic scope by determining the rates at which metabolic processes take place. Temperature is the controlling factor in this model (Fig. 3.2). The effects of temperature were modeled as the exponential function:

Eq. 1 $M_{std} = S * EXP(q1 * T_{accl}) * EXP(q2 * T_{stress}) * W_{effect}$

The estimation of M_{std} (Eq.1) was adapted from Neill et al.'s (2004) ecophysiological model for red drum (*Sciaenops ocellatus*). The equation defines standard metabolism (M_{std}) as a function of a salinity dependent intercept (S) elevated to the exponential of the acclimation temperature (T_{accl}) and temperature stress (T_{stress}) (Neill et al., 2004). The variable (S) is estimated in the Loading Effects module and its derivation is explained in the following section (3.2.3). The W_{effect} is a variable accounting for allometric effects on respiration rate due to differences in size.

Eq. 2 $W_{effect} = W_{fish}^{Wexp}$

Where W_{fish} is the weight of the fish and W_{exp} is a constant with value of -0.01 was derived by trial an error to fit growth rate data obtained in the laboratory (Del Toro-Silva et al., 2008). The constants q1 was set to 0.07 (Burke and Rice 2002) and q2 was set arbitrarily to 0.03.

The component T_{accl} represents the steady state component of temperature to which the organism is acclimated (Neill et al., 2004) and it is affected by $T_{acclChg}$:

Eq. 3 $T_{accli} = T_{accl0} + T_{acclChgi}$

and

Eq. 4 $T_{acclChgi} = T_{accl0} * EXP(-kaccl_0 * (T_{accl0} - T_{ai})/T_{accl0}) - T_{accl0}$

The acclimation temperature at time *i* is estimated as the sum of T_{accl0} (T_{accl} at time 0) and $T_{acclChgi}$ (acclamation change). The estimation of $T_{acclChgi}$, is through an exponential function (Eq. 4) in which as T_{accli} draws near T_a (ambient temperature), $T_{acclChgi}$ (rate of change) approaches 0 because the organism becomes acclimated to the environmental temperature. The coefficient $kaccl_0$ is equal to M_{std0} making the rate of acclimation faster for warmer acclimated fish vs. cold acclimated fish. The second and final temperature component in the estimation of M_{std} (T_{stress}) is defined as:

Eq. 5 $T_{stress} = T_{accl} - T_a$,

which accounts for stress effects on M_{std} due to quick temperature changes in the environment relative to T_{accl} (Neill et al., 2004).

3.2.3 Loading Factors subunit

Salinity acts as a loading factor directly altering standard metabolism (Fig. 3.3). Adapting the equation from Neill et al. (2004), the effects of salinity were modeled as the intercept S:

Eq. 6 $S = S_{int} + (S_{var} * S_{mult})$,

where S_{int} is an intercept with a values of 0.0172 and S_{mult} is a factor equal to 0.1042. The third variable S_{var} is defined:

Eq. 7 $S_{var} = ((S_a - S_{aOpt}) / (S_{aL} - S_{aOpt}))^2$

Where S_a represents the observed salinity, S_{aOpt} is the optimal salinity of the species in this case set to 10ppt. The variable S_{aL} is the same as defined by Neill et al., (2004). According to Eq. 6 a minimum value of S occurs when ambient salinity (S_a) approaches the salinity optimum for the species (S_{aOpt}). In this manner salinity changes away from the optimum act as a loading factor by increasing M_{std} through its intercept S.

3.2.4 Limiting Factors subunit

Environmental dissolved oxygen concentration was treated as a limiting factor to fish metabolic scope through its effects on active metabolism (M_{act}).

Eq. 8 $M_{act} = MMSO * (A^{\wedge Mactexp}) * A_{factor} * MMS_{effect} * W_{effect}$

The effects of ambient dissolved oxygen (DO_a) on Mact were modeled as a logistic function. These effects were mainly implemented through the temperature dependent parameter DO_{lim}, the DO concentration below which active metabolism becomes oxygen dependent (Fig. 3.4). The function used to compute DO_{lim} was derived from the equation used by Neill et al. (2004):

Eq. 9 $DO_{lim} = v_2 + ((T_{ratio}\text{^}^{Hill}/(v_3 + T_{ratio}\text{^}^{Hill})) * v_1)$.

The equation incorporates the effects of temperature through the parameter T_{ratio}, which is the ratio of the ambient temperature (T_a) to inflection temperature (T_{infl}). Therefore, as temperature approaches the optimal temperature for growth (T_{opt}), DO_{lim} and metabolic scope increases, provided that enough oxygen is available in the environment. For a temperature acclimated *P. lethostigma*, DO_{lim} was estimated at 4.00 mg/L at 27°C from laboratory growth rates (Del Toro-Silva et al., 2008).

Estimates of DO_{lim} are used to calculate A, the adjusted DO used in the computation of active metabolism (Neill et al., 2004):

Eq. 10 $A = Min (DO_a, DO_{lim} * T_{damg})$.

The variable T_{damg} is a variable used to predict A past T_{opt} and it is explained in more detail in the section Critical Temperature Effects. The variable A is modified in Eq.8 by the exponent M_{actexp} and A_{factor} parameters. Both of these parameters were used to fit M_{act} to the observed growth rates from laboratory experiments. M_{actexp} is a constant with a value of 1.8 and was arbitrarily assigned to fit the data. A_{factor} was derived from a modification of the K_A (Θ) algorithm (Thornton and Lessem, 1978). Where the derivation of A_{factor}:

Eq. 11 $A_{factor} = A_{int} + (A_{factor1} * \exp (^{I1*(DOa\ DOlim)})) / (1 + (A_{factor1} * \exp (^{I1*(DOa\ DOlim)}) - 1)$

and

Eq. 12 $I_1 = 1 / (A_{factor2} - DO_{lim}) * \ln (A_{factor3} * (1 - A_{factor1}) / (A_{factor1} *(1 - A_{factor3})$.

The A_{factor} function is a logistic equation that approaches 1 as DO_a nears DO_{lim}. This function was included to account for the non linear effects of oxygen limitation on M_{act}. The

variable MMSO acts as an intercept in the equation and is adjusted to fit the observed data and is assumed to represent the inherent metabolic efficiency of fish/environment system (Neill et al., 2004).

The variable MMS_{effect} in Eq. 8 is estimated in the $DO_{accl\ effect}$ subunit of the model and is explained in the section titled $DO_{accl\ effect}$ subunit. MMS_{effect} is dependent on DO_{accl}, the oxygen concentration to which the organism is acclimated, which is estimated in the Limiting Factors subunit. The estimation of DO_{accl} is analogous to the derivation of T_{accl}:

Eq. 13 $DO_{accl} = DO_{accl0} + DO_{acclChgi}$

where

Eq. 14 $DO_{acclChgi} = DO_{accl0} * EXP(-raccl_0 * (DO_{accl0} - DO_{ai}) / DO_{accl0}) - DO_{accl0}$

As DO_{accl0} draws near DO_a (ambient DO), $DO_{acclChg}$ approaches 0 when the organism becomes acclimated to the environmental DO. The coefficient $raccl_0$ is proportional to M_{std0} making the rate of acclimation dependent on the metabolic rate of the organism.

3.2.5 Critical temperature effects

The effects of temperatures above the optimum are usually reflected as a reduction in growth performance. Critical temperature effects were modeled in a manner similar to A_{factor} using a portion of the algorithm developed by Thornton and Lessem (1978). The variable T_{damg}:

Eq. 15 $T_{damg}: (K_4 * \exp^{(I_2 * (T_4 - T_a))}) / (1 + ((K_4 * \exp^{(I_2 * (T_4 - T_a))}) - 1)$

IF ($T_a > T_{opt}$) else T_{damg}: (1).

where

Eq. 16 $I_2 = 1 / (T_4 - T_3) * \ln (K_3 * (1 – K_4) / (K_4 *(1 - K_3)))$.

The variable acts on metabolic scope through its effects on A when $T_a > T_{opt}$. The variable T_{damg} approaches 0 as T_a increases past T_{opt}, effectively reducing the metabolic scope available. The reported lethal temperature for *P. lethostigma* is 40°C (Peters, 1971), but according to the ecophysiological framework the interaction with other environmental variables will affect this value. For example van Mareen, (1999) demonstrated that increments in salinity decreased the critical temperature of *P. lethostigma*. The inclusion of T_{damg} allows the effects of extreme temperature to directly reduce M_{scope}.

3.2.6 $DO_{accl\ effect}$ subunit

Changes in DO_a elicit physiological changes (e.g. blood chemistry) and behavioral changes in the organism (e.g. increased ventilation rates) (Weber et al., 1976; Wood et al., 1975; Weber and Lykkeboe, 1978; Nikinmaa et al., 1980; Soivio et al., 1980). After exposure to low DO_a, organisms can exhibit compensatory growth responses (Bejda, 1992; Person-Le Ruyet et al., 2003). Such responses have brought forth the hypothesis that low DO_a acclimation can result in enhanced performance once DO_a levels are no longer limiting and quality food is available (Neill et al., 2004). Empirical evidence also supports enhanced metabolic capacity of fish acclimated to low DO_a once oxygen levels rise (Del Toro-Silva et al., 2008). The acclimation response to oxygen was modeled within the $DO_{accl\ factor}$ subunit using results from laboratory data to produce a fit in MMS (Fig. 3.5). The derivation of MMS_{effect} is:

Eq. 17 $MMS_{effect} = MMS_{accl} / MMS_a$

where MMS_{accl} is the estimated marginal metabolic scope for DO_{accl} and MMS_a is the expected marginal metabolic scope for DO_a. The proportion gives an index of the relative oxygen efficiency of a fish, which is used as a multiplier in the estimation of M_{act}. In practice, the lower the DO_{accl} is relative to DO_a, the higher the coefficient. The function is analogous to the equations used to estimate A_{factor} in the Limiting Factors module.

Eq. 18 $MMS_{accl} = MMS_{int} + (MMS_{factor2} * \exp (^{MMSfactor1 *(DOlim - DOaccl)})) / (1 + (MMS_{factor2} * \exp (^{MMSfactor1*(DOlim - DOaccl)}) - 1)$

and

Eq. 19 $MMS_{factor1} = 1 / (DO_{lim} – MMS_{factor3}) * \ln(MMS_{factor4} * (1 - MMS_{factor2}) / MMS_{factor2} *(1 - MMS_{factor4})$.

whereas

Eq. 20 $MMS_a = MMS_{int} + (MMS_{factor2} * \exp (^{MMSfactor1 *(DOlim - DOa)})) / (1 + (MMS_{factor2} * \exp (^{MMSfactor1*(DOlim - DOa)}) - 1)$

The parameters used in these equations were derived by fitting laboratory data on MMS published by Del Toro-Silva et al., (2008)

3.2.7 Metabolic Scope

The central component of the ecophysiological modeling approach is the estimation of metabolic scope. Metabolic scope (M_{scope}) is defined in the model as the difference between M_{act} and M_{std}:

Eq. 21 $M_{scope} = M_{act} – M_{std}$.

The quantity represents the maximum rate of oxygen utilization by the organism to perform its biological activities once the minimum metabolic costs to sustain life are met. According to theory, energy consumption is determined by the M_{scope} available (Neill et al., 2004). Empirical studies with turbot support this idea; the species regulated their consumption in response to its metabolic capacity, given environmental conditions (Mallekh et al., 1998). To reflect this phenomenon in the model, a new variable ($M_{scope\ max}$) was created in this version of the ecophysiological model. The variable, analogous to $M_{scope,}$ represents the maximum possible scope for the organism if DO_a was not limiting. That is, in the calculation of $M_{scope\ max}$, A always equals DO_{lim}, while normally A varies between the minimum of DO_a and DO_{lim}. The proportion between ambient scope (M_{scope}) and potential scope ($M_{scope\ max}$) gives the variable $M_{scope\ proportion}$. This proportion is used in the bioenergetics module to estimate consumption.

3.2.8 Bioenergetics Module

The second module or bioenergetics module receives the predicted metabolic capacity and converts estimates of oxygen consumption and metabolic capacity into caloric equivalents and biomass accumulation. In essence, the module is similar to traditional mass balance models like the Wisconsin fish bioenergetics model (Kitchell et al., 1977). The current model differs from the original ecophysiological model in its bioenergetics module. The current module has two equivalent sections, one tracks the movement of mass consumed (grams) and the second tracks the same material in calories (Fig. 3.6). The modification was performed to simulate conditions in which environmental constraints may affect the

assimilation of previously consumed material. The modification allows for material to move through the digestive track without being completely assimilated by the organism. The model assumes that assimilation of consumed mass is determined by metabolic scope and stomach contents may pass through the digestive tract without being completely assimilated into tissue growth.

The mass component of the bioenergetics module simulates the consumption and evacuation of material. The contents of the reserve, labeled Stomach, are the product of the balance between the input of food or Feeding and Evacuation (Fig 3.6). The feeding rate:

Eq. 22 $Feeding = M_{scope\ proportion} * Feeding_{Prob} * C_{max}$

is defined by the proportion of scope available ($M_{scope\ proportion}$), the physiological maximum of consumption (C_{max}), and a feeding probability variable. The physiological maximum was defined arbitrarily as a proportion of fish weight (g).

Eq. 23 $C_{max} = W_{fish} * W_{proportion}$

The variable $Feeding_{Prob}$ is the product of food encounter and stomach vacuity:

Eq. 24 $Feeding_{Prob}: = p_{encounter} * S_{vacuity}$

where,

Eq. 25 $S_{vacuity} = k_1 * \exp\left(^{-((Sprop\ /\ k2)\wedge k0)}\right)$

and

Eq. 26 $p_{encounter}$ = If(random(0,1) < p) then (1) else (0).

A Weibull function was adapted to describe $S_{vacuity}$ (Eq. 25), where S_{prop} is the ratio between the amount of material in the stomach and C_{max}. Accordingly, as S_{prop} approaches 1 $S_{vacuity}$ approaches 0, hence $Feeding_{Prob}$ approaches 0 making the probability of feeding partially dependent on the capacity of the stomach to hold more food. The other component of Eq. 24, $p_{encounter}$ was defined by a uniformly random function and a probability constant (p) (Eq. 26). The random function generates values between 0 and 1 in each time step. When the random function generates a number less than p the fish encounters food (1) else food is not present (0). Hence, feeding probability ($Feeding_{Prob}$) is the product of stomach fullness and a random function representing encounter probability, while Feeding is the product of the $Feeding_{Prob}$ and C_{max} modified by the allowable scope given environmental conditions.

The advantage of the definition of consumption within the model is that it makes consumption a function of metabolic scope. Accordingly, under conditions that allow maximum M_{scope} feeding rate should approach C_{max}, while environmental conditions limiting scope should curtail the amount of food consumed even when S_{prop} is 0. This definition is supported by empirical work from Mallekh et al., (1998), who demonstrated that fish feeding activity was correlated to environmental parameters. In particular, they found that when O_2 saturation dropped below 95% fish appetite was significantly reduced. The $p_{encounter}$ function introduces randomness into the model simulations, which so far is mostly deterministic. In summary, the definition of Feeding employed in the model allowed for the simulation of a mechanism regulating consumption based upon environmental parameters and the ability of

the organism to assimilate energy given environmental and physical constraints while including variability in resource availability within a habitat.

A second rate affecting the mass component of the bioenergetics module is the evacuation rate of the stomach. Evacuation was modeled using the exponential equation:

Eq. 27 $x * \exp(b * Mstd)$,

where x acts as an intercept and b is a constant. The equation is analogous to the temperature dependent gut evacuation rate of He and Wurtsbaugh (1993), but scaled to Mstd. The equation used allows evacuation to be proportional to standard metabolism and is independent of metabolic scope limitation on energy assimilation.

The equivalent component to the mass component of the bioenergetics module tracks the flux of material in its caloric equivalent. The calories ingested are equal to Feeding multiplied by the caloric density of the feed (GE_{feed} in cal/g).

Eq. 28 Caloric input = Feeding * GE_{feed}

The ingested calories are available in a reservoir (Available Calories), which are affected by egestion, excretion, and assimilation processes (Fig. 3.6). The egestion rate is proportional to the evacuation rate (Neill et al., 2004):

Eq. 29 Egestion = Evacuation * GE_{feed},

while the excretion rate is a function of food digestibility and the rate of assimilation (Neill et al., 2004):

Eq. 30 Excretion = (1-$Food_{digestibility}$) * Assimilation + 0.05 Assimilation.

The rate of assimilation, energy available to the fish is:

Eq. 31 Assimilation = M_{act} * Oxycal

where Oxycal is the conversion factor of oxygen to calories (3.4 Cal/mg O_2) (Neill et al., 2004). Accordingly, the Available Calories for Assimilation are the product of Caloric input minus egestion and excretion processes.

The assimilated calories go into the Fish Energy reservoir, where maintenance, activity, and processing cost are subtracted. The balance of calories after these costs are met is used to calculate the biomass of the fish. The cost of maintenance is estimated as:

Eq. 32 Maintenance = M_{std} * Oxycal,

and the processing as:

Eq. 33 $Processing_{cost}$ = sda * Assimilation.

The specific dynamic action (sda) is a constant accounting for the energy lost in the conversion of calories ingested into a form usable by the organism. It is traditionally some proportion of the energy assimilated and is this model was assumed to have a value of 0.12.

Eq. 34 $Activity_{Cost}$ = Activity * Oxycal,

where,

Eq. 35 Activity = If (M_{act} > (Winberg*M_{std}) then [(Winberg * M_{std}) – M_{std}]

else (M_{act}-M_{std}).

The $Activity_{Cost}$ is defined as energetic cost incurred by a routinely active fish and is the product of Activity and Oxycal. Because in nature the cost of activity can approach M_{act} it was necessary to define Activity in a way that it could vary between M_{act} and M_{std}. Winberg (1960) deduced that the cost of a routinely active fish is approximately twice M_{std}. This idea has been accepted by convention and applied in previous individual growth models (Kitchell et al., 1977; Neill et al., 2004). The definition used in Eq. 35 allows for Activity to have a maximum value of Winberg*M_{std}, when metabolic scope allows for it or the difference between M_{act} and M_{std}, when M_{act} is restricted by limiting fators and is less than Winberg*M_{std} . The balance between Assimilation and the expenditures of Maintenance, Activity, and $Processing_{costs}$ are converted into fish biomass estimate by the equation:

Eq. 36 $W_{fish} = Fish_{energy} / GE_{fish}$,

where GE_{fish} is the caloric density of the fish which was assumed to be about 1000 cal/g (Neill et al., 2004). Finally, the estimated W_{fish} is used in to calculate the G_{rate}:

Eq. 37 $G_{rate} = \exp(^{\ln(Wfish) - \ln(Wfish0)} / _{(time+1) / 24}) - 1$,

where W_{fish0} is the initial weight of the fish and time is the total length of the simulation. The estimates G_{rate} and W_{fish} are the final outputs of the model once the effects of the initial inputs, temperature, oxygen, salinity are accounted for.

3.2.9 Model Fit

The model was parameterized to fit results from laboratory experiments. The data used were growth rates of *P. lethostigma* after two weeks of being exposed to various dissolved oxygen and temperature combinations (Del Toro-Silva et al., 2008). Simulations were conducted at two degree intervals ranging from 23 C to 33 C and three constant DO concentrations. The oxygen concentrations used were 1.75mg/L, 4.00mg/L, and 6.00mg/L. In addition, two simulations were conducted at 25 C and 27 C under cyclical DO conditions to determine model accuracy under dynamic conditions. Dissolved oxygen conditions were varied every 12 hours from normoxia (6.00 mg/L) to either treatment of 1.75mg/L or 4.00 mg/L. Results of model simulations were compared to observed growth rates to determine the accuracy of model predictions. Model outputs were analyzed with a X^2 test to detect differences between observed and predicted growth rates. The test requires values to be greater than 5, because growth rates do not conform to this requirement, final weight for a 10.00g fish were estimated from the observed growth rates (Del Toro-Silva et al., 2008). The projected weights based on the observed laboratory growth rates of a standardized 10.00 g fish were compared to those predicted by the model and tested for goodness of fit.

3.2.10 Sensitivity Analysis

Individual Parameter Perturbation (IPP) analysis was used to determine the relative sensitivity of model output to each parameter. In the analysis, each parameter was independently varied by ±5% of its initial value while keeping all other parameters constant. Relative sensitivity was determined by the percent change in model output at the end of the simulation and each parameter was ranked according to its impact on simulation results.

This method allows for the detection of the parameters with the highest impact on model predictions, hence identifying those needing better accuracy in order to improve model performance.

3.3 Results

The model was parameterized to fit data from laboratory studies. The list of parameters used and their respective values are reported in Table 3.1. The X^2 test did not reveal significant difference between model predictions and laboratory observations (α = 0.05; df = 21). Results for two week long simulations along with the respective laboratory results are summarized on Table 3.2 and 3.3. Under constant DO conditions, the model successfully predicted the final weights of *P. lethostigma* between 23 C and 29 C (Fig. 3.7). The X^2 failed to detect significant difference between model predictions and the observed growth rates and final weights, hence we cannot reject the null hypothesis of different distributions between model and laboratory results ($p > 0.05$). Within this range of temperatures the largest errors observed corresponded to simulations at 1.75 mg/L (Table 3.2). At 27 C and 29 C, although observed and simulated fish lost weight, the model over predicted final fish weight by as much as 17.4% and 10.11% respectively (Fig 3.7). The model also over predicted the final fish weight at 25 C at 4.00mg/L and 6.00mg/L. Past the assumed optimal temperature of 29 C, the accuracy of model predictions to the observed data declines, particularly at 33 C where the model over predicted growth by 31.75%. No comparisons were made at 1.75mg/L for 31 C or 33 C because model simulations predicted negative M_{scope} which produced non senssical results. According to theory, negative scope

eventually results in fish fatality, which was observed at 33 C and with few survivors at 31 C. Finally, simulations of cyclical DO patterns over predicted growth predominantly for the 1.75 mg/L treatments by as much as 27.83%.

Sensitivity analysis indicates that the four parameters with the greatest impact on model simulations were T_{opt}, M_{actexp}, T_{infl}, and Hill (Fig. 3.8). T_{opt} had the greatest impact with a 27.57% change on model output, followed by M_{actexp} with a 17.26%. The other two parameters, T_{infl} and Hill had a -16.19% and -8.19% changes on model output, respectively. None of the other parameters used in the model had a sensitivity index in excess of ±5 % on simulation output. A summary of the sensitivity analysis is provided in Table 3.4.

3.4 Discussion

In general the model performed well predicting growth rates observed in the laboratory. Although the percent error in some of the cases seems large, these fall within the confidence interval of the observed data (Fig 3.9). Because of the small weight change being simulated a small discrepancy among simulated and observed growth results in a large percent error. In addition the model is able to simulate with accuracy the final weights of *P. lethostigma* under 22 different conditions including 4 sets of changing dissolved oxygen concentrations. In conclusion, the proximity in valued among observed and predicted values and the ability of the model to perform under 22 different conditions was concluded as evidence of successful model accuracy.

Results from sensitivity analysis were encouraging, considering that only four parameters had a sensitivity index greater than ±5 %. The four parameters with the highest

sensitivity were expected considering the role they play in the estimation of M_{act}. T_{opt} is defined as the temperature at which maximum scope will be observed. The parameter operates by restricting $T_{acclChg}$, (if $T_a \geq T_{opt}$ $T_{acclChg}$ equals 0) capping T_{accl} to a predetermined T_{opt}. Restricting maximum T_{accl} to T_{opt} in turn insures that maximum M_{scope} is at T_{opt}. Reducing T_{opt} causes a displacement on the optimum temperature for growth in addition to a reduction in $M_{scope.}$ An increase of T_{opt} causes the opposite, resulting in an over prediction of growth rate at higher temperatures. Changes in value of Tinfl had the opposite effect of T_{opt} due to its role in the estimation of T_{ratio} and the resulting DO_{lim}. A third parameter, Hill, acts as an exponent in the DO_{lim} estimate. Changes of the Hill parameter affect the steepness of the DO_{lim} curve as temperature increases. The previous three parameters have an indirect effect on M_{act} through their effects on the DO_{lim} equation. The M_{actexp} parameter directly affects the magnitude of M_{act} through its effects on A. Although error in the estimation of any parameter can change the results of a simulation, the results of the sensitivity analysis reaffirm the mechanics and assumptions in the model. The greatest effect observed was limited to the optimum temperature and parameters related to the estimation of M_{act}. These results are expected since temperature controls the rate at which metabolic processes take place. Although errors in the estimation of T_{opt} can significantly influence simulation results, the estimates used in this model are in close agreement with those published for *P. lethostigma* (Peters, 1971; Del Toro-Silva et al. 2008) which supports the accuracy of model predictions.

The ecophysiological model for *P. lethostigma* was successfully parameterized to reproduce the observed growth rates in the laboratory (Fig. 3.9). Although some

discrepancies were observed, the behavior of the model performed according to theory. Standard metabolism increased in proportion with temperature. Salinity acted as a loading factor increasing standard metabolism, while dissolved oxygen concentration (DO) acted as limiting factor to metabolic scope. Under constant conditions, the model accurately predicted final fish weights with the exception of low oxygen conditions. Although final weights were over predicted, the model described weight loss on all simulations at low DO. Over prediction at 25 C and 4.00mg/L and 6.00mg/L are confounded with lower than expected growth rates under these conditions (Del Toro-Silva et al., 2008). The major discrepancies were observed at the lowest oxygen conditions when negative growth rates were recorded. Although the model predicts biomass changes within the individual, it is intended to simulate positive changes through aerobic metabolism and does not incorporate physiological mechanism for weight loss or possible anaerobic processes. This can affect the accuracy of the model predicting weight loss under prolonged hypoxic conditions. Fish condition can vary under adverse environmental conditions; energy content was demonstrated to vary in response to abiotic conditions (Ellis, 2007). Nevertheless, the metabolic module of the model successfully described the expected dynamics of metabolic scope given the environmental conditions utilized in the model. At temperatures past the optimum model over predictions fell within the confidence interval of the observed data, hence were considered valid. In conclusion, the model's fit and behavior under all of these conditions was interpreted as confirmation of model assumptions, particularly since it is intended to predict fish growth and not weight loss.

Examination of temporal dynamics was conducted using cyclical oxygen conditions. Even though these simulations over predicted growth, mechanistically they behaved correctly. The model assumes that adaptation to lower oxygen conditions increases oxygen acquisition efficiency. The function MMS_{effect} was fit to the estimated MMS values of *P. lethostigma* after two weeks exposure to experimental conditions (Del Toro-Silva et al., 2008). These MMS values were positively correlated with temperature and inversely related to DO. Accordingly, simulations under cyclical DO conditions predicted higher M_{effect} when DO levels increased after exposure to low oxygen resulting in a compensatory response of M_{scope} for these individuals. The reduction in model performance of the dynamic DO simulations can be attributed in part to lower observed growth rates at 6.00 mg/L and 4.00mg/L at 27 C under cyclical DO conditions relative to growth rates under constant conditions. Under the cyclical DO regime, growth rates at both concentrations were approximately 50% less than under constant conditions (Table 3.2). Despite the discrepancy in the predicted growth rates, simulations fell within the confidence interval of the observed growth rate. This was not the case under cycling low DO conditions, where the model over predicted growth rate by 43%. One possible explanation could be the unnatural way in which oxygen levels were manipulated in the experiment. The oxygen regime used in the laboratory changed abruptly from low oxygen levels during night hours to high oxygen levels during day hours, unlike natural systems where diel changes are generally gradual.

As an initial response to oxygen limitation, organisms tend to increase their ventilation rate. When this strategy does not suffice, they increase both blood oxygen carrying capacity and oxygen affinity (Weber et al., 1976; Wood et al., 1975; Weber and

Lykkeboe, 1978; Nikinmaa et al., 1980; Soivio et al., 1980). Finally, when these strategies are not adequate, modifications at the molecular level result in the suppression of ATP turnover (Hochachka and Lutz, 2001; Wu, 2002). These molecular modifications result in the suppression of metabolic rate and down regulation of protein synthesis. Although a reduction of ATP turnover protects tissues from hypoxia, it is possible that a lag response to hypoxia could exist. A lag response would result in an over prediction of metabolic scope and in the decoupling between the predicted and the observed growth rates.

Alternatively, recent studies have demonstrated that rapid re-oxygenation can result in superoxide radical (O_2^-) production resulting in oxidative stress and potential tissue damage (Zenteno-Savin, 2005). It is possible that the rapid re-oxygenation regime used in the laboratory increased the propensity of oxidative stress in the low oxygen treatments, which would account for lower than predicted growth rates. Unfortunately, the mechanics of the model do not account for oxidative stress effects on growth. Further research on the acclimation response to changes in oxygen concentrations are required to improve predictions of growth rate. Meanwhile the model is considered to perform well under a variety of conditions based on goodness of fit and sensitivity analyses.

3.5 References

Able, K.W., 1999. Measure of juvenile fish habitat quality: examples from a national estuarine research reserve. In: Beneka, L.R. (Ed.), Fish habitat: essential fish habitat and rehabilitation. American Fisheries Society. American Fisheries Society, Bethesda, Maryland, pp. 134-147.

Adams, A.J., Dahlgren, C.P., Kellison, G.T., Kendall, M.S., Layman, C.A., Ley, J.A., Nagelkerken, I., Serafy, J.E., 2006. Nursery function of tropical back-reef systems. Marine Ecology Progress Series 318, 287-301.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.R., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51, 633-641.

Bejda, A.J., Phelan, B.A., Studholme, A.L., 1992. The effect of dissolved oxygen on the growth of young of the year winter flounder, *Pseudopleuronectes americanus*. Environmental Biology of Fishes 34, 321-327.

Bergenius, M.A.J., Meekan, M.G., Robertson, D.R., McCormick, M.I., 2002. Larval growth predicts the recruitment success of a coral reef fish. Oecologia 131, 521-525.

Brandt, S.B., Kirsch, J., 1993. Spatially explicit models of striped bass growth potential in Chesapeake Bay. Transactions of the American Fisheries Society 122, 845-869.

Breck, J.E., 1993. Foraging theory and piscivorous fish: are forage fish just big zooplankton. Transactions of the American Fisheries Society 122, 902-911.

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. In: Hoar, W.S., D.J. Randall and J.R. Brett (Ed.), Fish physiology: bioenergetics and growth. Academic Press, New York, pp. 279-353.

Brett, J.R., Shelbour, J.E., Shoop, C.T., 1969. Growth rate and body composition of fingeling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. Journal of the Fisheries Research Board of Canada 26, 2363-2394.

Burke, B.J., Rice, J.A. 2002. A linked foraging and bioenergetics model for southern flounder. Transactions of the American Fisheries Society 131, 120-131.

Dahlgren, C.P., Kellison, G.T., Adams, A.J., Gillanders, B.M., Kendall, M.S., Layman, C.A., Ley, J.A., Nagelkerken, I., Serafy, J.E., 2006. Marine nurseries and effective juvenile habitats: concepts and applications. Marine Ecology Progress Series 312, 291-295.

Deangelis, D.L., Shuter, B.J., Ridgway, M.S., Scheffer, M., 1993. Modeling growth and survival in an age-0 fish cohort. Transactions of the American Fisheries Society 122, 927-941.

Del Toro-Silva, F.M., Miller, J.M., Taylor, J.C., Ellis, T.A., 2008. Influence of oxygen and temperature on growth and metabolic performance of *Paralichthys lethostigma* (Pleuronectiformes: Paralichthyidae). Journal of Experimental Marine Biology and Ecology doi: 10.1016/j.jembe.2008.01.019.

Ellis, T.A., 2007. Assessing nursery quality for southern flounder, *Paralichthys lethostigma*, through fish energy content and habitat abiotic conditions, Zoology. NC State, Raleigh.

Fry, F.E.J., 1947. Effects of the environment on animal activity. University of Toronto Studies Biological Series 55.

Fry, F.E.J., 1971. The efffect of environmental factors on the physiology of fish. In: Hoar, W.S.a.D.J.R.D.J. (Ed.), Fish Physiology. Academic Press, NY, pp. 1-98.

Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. Netherlands Journal of Sea Research 32, 191-206.

Giese, G.L., Wilder, H.B., Parker-Jr., G.G., 1979. Hydrology of major estuaries and sounds of North Carolina, U.S. Geological Survey Water Resources Investigations 79-46. U.S. Geological Survey, pp. 175.

Gillanders, B.M., 2002. Connectivity between juvenile and adult fish populations: do adults remain near their recruitment estuaries? Marine Ecology Progress Series 240, 215-223.

Gillanders, B.M., Able, K.W., Brown, J.A., Eggleston, D.B., Sheridan, P.F., 2003. Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. Marine Ecology Progress Series 247, 281-295.

Goyke, A.P., Brandt, S.B., 1993. Spatial models of salmonine growth-rates in Lake Ontario. Transactions of the American Fisheries Society 122, 870-883.

Guindon, K.Y.a.J.M.M., 1995. Growth potential of juvenile southern flounder, *Paralichthys lethostigma*, in low salinity nursery areas of Pamlico Sound, North Carolina, USA. Netherlands Journal of Sea Research 34, 89-100.

Hare, J.A., Cowen, R.K., 1997. Size, growth, development, and survival of the planktonic larvae of *Pomatomus saltatrix* (Pisces: Pomatomidae). Ecology 78, 2415-2431.

He, E.Q., Wurtsbaugh, W.A., 1993. An empirical model of gastric evacuation rates for fish and analysis of digestion in piscivorous brown trout. Transactions of the American Fisheries Society 122, 717-730.

Hochachka, P.W., Lutz, P.L., 2001. Mechanism, origin, and evolution of anoxia tolerance in animals. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 130, 435-459.

Houde, E.D., 1987. Fish early life dynamics and recruitment variability. American Fisheries Society Symposium Series. 2, 17-29.

Houde, E.D., 1997. Patterns and trends in larval-stage growth and mortality of teleost fish. Journal of Fish Biology 51(A), 52-83.

Kitchell, J.F., Stewert, D.J., Weigner, D., 1977. Application of bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). Journal of Fish Research Board Canada 34, 1922-1935.

Kneib, R.T., 1997. The role of tidal marshes in the ecology of estuarine nekton. Oceanography and Marine Biology an Annual Review 35, 163-220.

Kneib, R.T., 2000. Salt marsh ecoscapes and production transfers by estuarine nekton in the southeastern United States. Kluwer Academic Publishers, Dordrecht, Boston & London.

Labar, G.W., 1993. Use of bioenergetics models to predict the effect of increased lake trout predation on rainbow smelt following sea lamprey control. Transactions of the American Fisheries Society 122, 942-950.

Luo, J.G., Hartman, K.J., Brandt, S.B., Cerco, C.F., Rippetoe, T.H., 2001. A spatially-explicit approach for estimating carrying capacity: an application for the atlantic menhaden *Brevoortia tyrannus*) in Chesapeake Bay. Estuaries 24, 545-556.

Mallekh, R., Lagardere, J.P., Anras, M.L.B., Lafaye, J.Y., 1998. Variability in appetite of turbot, *Scophthalmus maximus* under intensive rearing conditions: the role of environmental factors. Aquaculture 165, 123-138.

Mason, D.M., Patrick, E.V., 1993. A model for the space-time dependence of feeding for pelagic fish populations. Transactions of the American Fisheries Society 122, 884-901.

Meng, L., Gray, C., Taplin, B., Kupcha, E., 2000. Using winter flounder growth rates to assess habitat quality in Rhode Island's coastal lagoons. Marine Ecology Progress Series 201, 287-299.

Minello, T.J., 1999. Nekton densities in shallow estuarine habitats of Texas and Louisiana and the identification of essential fish habitat. In: Beneka, L.R. (Ed.), Fish habitat: essential fish habitat and rehabilitation. American Fisheries Society Symposium, Bethestda, Maryland, pp. 43-75.

Munch, S.B., Conover, D.O., 2003. Rapid growth results in increased susceptibility to predation in *Menidia menidia*. Evolution 57, 2119-2127.

Neill, W.H., Brandes, T.S., Burke, B.J., Craig, S.R., Dimichele, L.V., Duchon, K., Edwards, R.E., Fontaine, L.P., Gatlin, D.M., Hutchins, C., Miller, J.M., Ponwith, B.J., Stahl, C.J., Tomasso, J.R., Vega, R.R., 2004. Ecophys.Fish: A simulation model of fish

growth in time-varying environmental regimes. Reviews in Fisheries Science 12, 233-288.

Ney, J.J., 1993. Bioenergetics modeling today: growing pains on the cutting edge. Transactions of The American Fisheries Society 122, 736-748.

Nikinmaa, M., Tuurala, H., Soivio, A., 1980. Thermoacclimation changes in blood oxygen binding properties and gill secondary lamellar structure of *Salmo gairdneri*. Journal of Comparative Physiology 140, 255-260.

Paerl, H.W.J.W.P., J.M. Fear, B.L. Peierls., 1998. Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia and the eutrophying Neuse River Estuary, NC, USA. Marine Ecology Progress Series 166, 17-25.

Person-Le Ruyet, J., Lacut, A., Le Bayon, N., Le Roux, A., Pichavant, K., Quéméner, L., 2003. Effects of repeated hypoxic shocks on growth and metabolism of turbot juveniles. Aquatic Living Resources 16, 25-34.

Peters, D.S., 1971. Growth and energy utilization of juvenile flounder, *Paralichthys dentatus* and *Paralichthys lethostigma*, as affected by temperature, salinity, and food availability., Zoology. North Carolina State, Raleigh, pp. 68.

Pietrafesa, L.J., Janowitz, G.S., Chao, T.Y., Weisberg, R.H., Askari, F., Noble, E., 1986. The Physical Oceanography of Pamlico Sound. UNC Sea Grant Publication, UNC-SG-WP-86-5, 126 pp.

Ross, S.W., 2003. The relative value of different estuarine nursery areas in North Carolina for transient juvenile marine fishes. Fishery Bulletin 101, 384-404.

Scharf, F.S., Buckel, J.A., K.A., R., Juanes, F., Cowan, J.H., 2006. Effects of variable prey and cohort dynamics on growth of young-of-the-year estuarine bluefish: evidence for interactions between spring and summer spawned cohorts. Transactions of the American Fisheries Society 135, 1266–1289.

Sogard, S.M., 1997. Size-selective mortality in the juvenile stage of teleost fishes: A review. Bulletin of Marine Science 60, 1129-1157.

Soivio, A., Nikinmaa, M., Westman, K., 1980. The blodd binding properties of hypoxic *Salmo gairdneri*. Journal of Comparative Physiology 136, 83-87.

Stevens, M., Maes, J., Ollevier, F., 2006. A bioenergetics model for juvenile flounder *Platichthys flesus* Journal of Applied Ichthyology 22, 79-84.

Takasuka, A., Aoki, I., Mitani, I., 2003. Evidence of growth selective predation on larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. Marine Ecology Progress Series 252, 223-238.

Takasuka, A., Aoki, I., Mitani, I., 2004. Three synergistic growth-related mechanisms in the short term survival of larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. Marine Ecology Progress Series 270, 217-228.

Taylor, D.L., 2003. Size-dependent predation on post-settlement winter flounder *Pseudopleuronectes americanus* by sand shrimp *Crangon septemspinosa*. Marine Ecology Progress Series 263, 197–215.

Taylor, J.C., Miller, J.M., Pietrafesa, L.J., Dickey, D., Ross, S., In prep. Winter winds and river discharge determine juvenile southernflounder abundance and distribution in North Carolina estuaries. Fisheries Oceanography.

Thornton, K.W., Lessem, A.S., 1978. A temperature algorithm for modifying biological rates. Transactions of the American Fisheries Society 102, 284-287.

Thorrold, S.R., Jones, C.M., Swart, P.K., Targett, T.E., 1998. Accurate classification of juvenile weakfish *Cynoscion regalis* estuarine nursery areas based on chemical signatures in otoliths. Marine Ecology Progress Series 173, 253-265.

Thorrold, S.R., Latkoczy, C., Swart, P.K., Jones, C.M., 2001. Natal homing in marine fish metapopulation. Science 291, 297-299.

van Maaren C.C., J. K., Daniels, H.V., 1999. Temperature tolerance and oxygen consumption rates for juvenile southern flounder *Paralichthys lethostigma* acclimated to five different temperatures. Kihei, Hawai'i, University of Hawai'i Sea Grant College Program.

Weber, R.E., De Wilde, J.A.M., 1976. Multiple hemoglobin in plaice and flounder and their functional properties. Comparative Biochemistry and Physiology B 54, 433.

Weber, R.E., Lykkeboe, G., 1978. Respiratory adaptations in carp blood: influences of hypoxia, red cell organic phosphates, divalent cations and CO2 on hemoglobin-oxygen affinity. Journal of Comparative Physiology 128, 127-137.

Winberg, G.G., 1960. Rate of Metabolism and Food Requirement of Fishes. Fisheries Research Board of Canada, Translation Series 194.

Wood, S.C., Johansen, K., Weber, R.E., 1975. Effects of ambient Po_2 on hemoglobin-oxygen affinity and red cell ATP concentrations in a benthic fish, *Pleuronectes platessa*. Respiration Physiology 25, 259-267.

Wu, R.S.S., 2002. Hypoxia: from molecular responses to ecosystem responses. Marine Pollution Bulletin 45(SI), 35-45.

Xie, L., Eggleston, D.B., 1999. Computer simulations of wind-induced estuarine circulation patterns and estuary-shelf exchange processes: the potential role of wind forcing on larval transport. Estuarine Coastal and Shelf Science 49, 221-234.

Yamashita, Y., Otake, T., Yamada, H., 2000. Relative contributions from exposed inshore and estuarine nursery grounds to the recruitment of stone flounder, *Platichthys bicoloratus*, estimated using otolith Sr:Ca ratios. Fishery Oceanography 9, 316-327.

Yamashita, Y., Tanaka, M., Miller, J.M., 2001. Ecophysiology of juvenile flatfish in nursery grounds. Journal of Sea Research 45, 205-218.

Zenteno-Savin, T., 2005. Oxidative stress in response to environmental hypoxia/reoxygenation in two crustacean species, Pacific white shrimp (*Litopenaeus vannamei*) and red claw crayfish (*Cherax quadricarinatus*). Free Radical Biology and Medice 39(S1), S177-S177.

Table 3.1 Parameter values for the ecophysiological model of *Paralichthys lethostigma*.

Parameter	value	Parameter	value
Food digestibility	0.95	$MMS_{Factor3}$	0
GEFeed	4000	$MMS_{Factor4}$	0
M_{actexp}	1.8	MMS_{int}	0.112
MMSO	0.5	k0	0.336
OxyCal	3.4	k1	0.168
sda	0.12	k2	0.169
W_{exp}	-0.01	p	0.112
Weigberg	2	A_{fact1}	2.916
x	0.101	A_{fact2}	-0.225
b	3.42	A_{fact3}	-0.112
$W_{proportion}$	0.15	Hill	-8.188
K3	0.8	int	1.514
K4	0.71	v1	3.978
q1	0.07	v2	4.655
q2	0.06	v3	-0.729
T3	29.5	Sa_{LL}	0.112
T4	30.5	Sa_{Opt}	-0.112
T_{infl}	47.2	Sa_{UL}	0.616
T_{opt}	29	Sa_{int}	0.112
$MMS_{Factor2}$	0.507	Sa_{Mult}	-0.504

Table 3.2 Summary of simulated and observed *Paralichthys lethostigma* growth rates.

	1.75mg/L			4.00 mg/L			6.00mg/L		
Temperature C	Observed	SE	Model	Observed	SE	Model	Observed	SE	Model
23	0.000	0.0043	$-1.23\ e^{-4}$	0.011	0.0039	$1.49\ e^{-2}$	0.018	0.0039	$1.49\ e^{-2}$
25	-0.003	0.0039	$-5.20\ e^{-4}$	0.006	0.0041	$1.81\ e^{-2}$	0.009	0.0045	$1.81\ e^{-2}$
25*	-0.001	0.0030	$9.84\ e^{-3}$	0.012	0.0040	$1.81\ e^{-2}$	0.018	0.0030	$1.81\ e^{-2}$
27	-0.014	0.0056	$-4.45\ e^{-4}$	0.031	0.0039	$2.99\ e^{-2}$	0.029	0.0048	$2.99\ e^{-2}$
27*	-0.007	0.0035	$1.64\ e^{-2}$	0.017	0.0028	$2.97\ e^{-2}$	0.017	0.0032	$2.99\ e^{-2}$
29	-0.008	0.0047	$-4.18\ e^{-4}$	0.021	0.0039	$2.16\ e^{-2}$	0.042	0.0041	$4.24\ e^{-2}$
31	-0.005	0.0054	N/A	0.006	0.0048	$7.81\ e^{-3}$	0.008	0.0045	$7.81\ e^{-3}$
33	N/A	N/A	N/A	-0.022	0.0069	$-5.98\ e^{-4}$	-0.033	0.0056	$-5.98\ e^{-4}$

Table 3.3 Simulated and estimated final weights of a 10.00g *Paralichthys lethostigma.*

	Dissolved Oxygen Concentration					
	1.75mg/L		4.00 mg/L		6.00mg/L	
Temperature C	Observed	Model	Observed	Model	Observed	Model
23	9.93	9.98	12.23	12.30	12.23	12.30
25	9.59	9.93	11.10	12.86	11.10	12.86
25*	9.86	11.47	12.32	12.86	12.32	12.86
27	8.21	9.94	15.13	15.10	15.13	15.10
27*	9.06	12.62	12.66	15.10	12.66	15.10
29	8.94	9.94	13.38	13.49	17.79	17.88
31	9.32	N/A	11.03	11.15	11.03	11.15
33	N/A	N/A	6.77	9.92	6.77	9.92

Table 3.4 Sensitivity analysis percent change in Paralichthys lethostigma model output. The parameters with the greatest sensitivity where: T_{opt}, Hill, M_{actexp}, and $T_{infl.}$

Food digestibility	0.056	$MMS_{Factor3}$	50
GEFeed	-0.168	$MMS_{Factor4}$	0.245
M_{actexp}	17.26	MMS_{int}	-0.359
MMSO	4.916	k0	4.49
OxyCal	4.302	k1	1
sda	-0.952	k2	0.54
W_{exp}	-0.225	p	0.9
Weigberg	-0.336	A_{fact1}	0.749
x	0	A_{fact2}	0.999
b	-0.448	A_{fact3}	0.0305
$W_{proportion}$	-0.169	Hill	23.2
K3	-0.056	int	0.25
K4	0.168	v1	2.85
q1	-1.232	v2	2.63
q2	0.112	v3	0.00000263
T3	-0.279	Sa_{LL}	5
T4	0.279	Sa_{Opt}	10
T_{infl}	-16.191	Sa_{UL}	40
T_{opt}	27.573	Sa_{int}	0.0172
$MMS_{Factor2}$	0.280	Sa_{Mult}	0.104

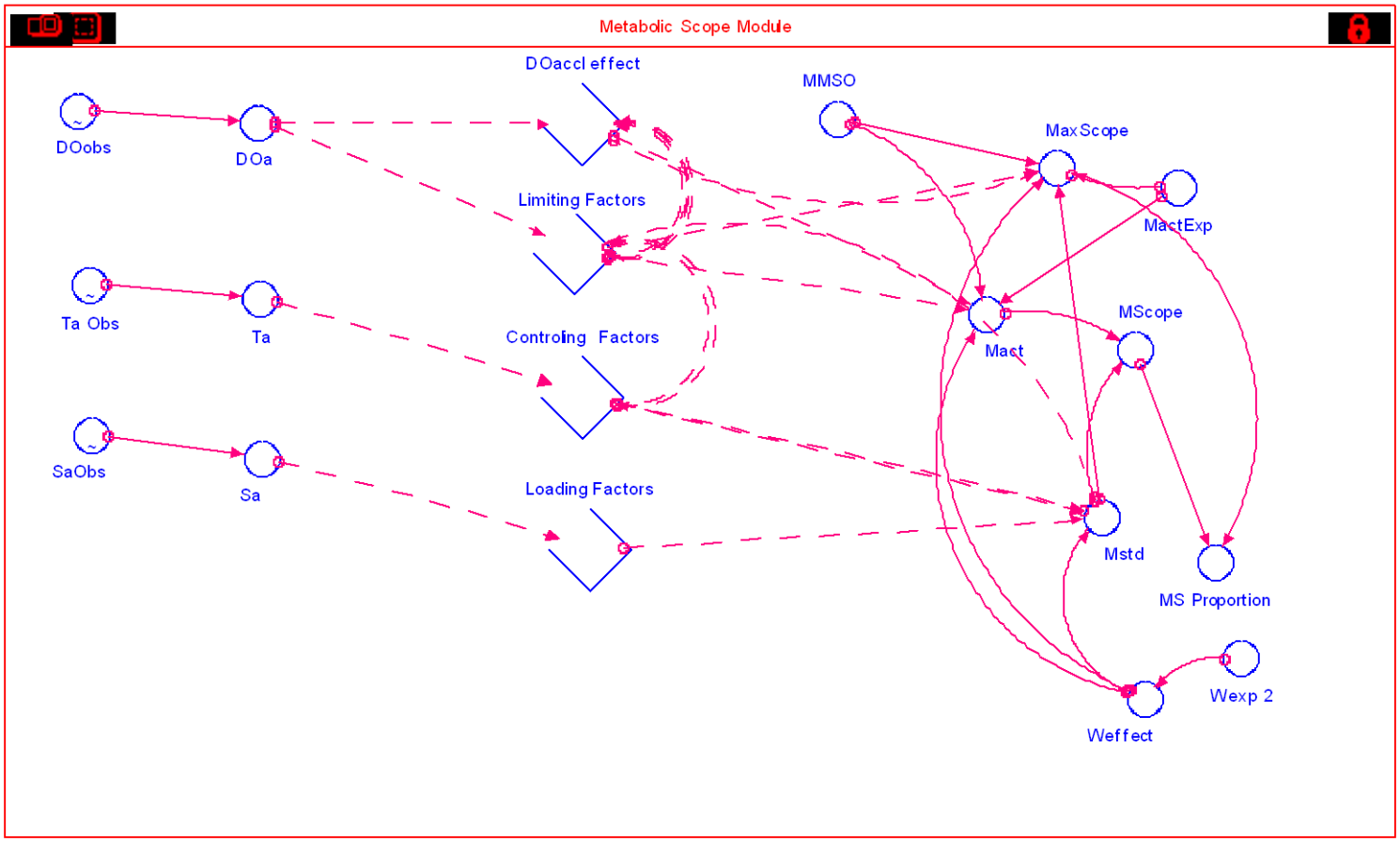


Fig. 3.1 Metabolic Module

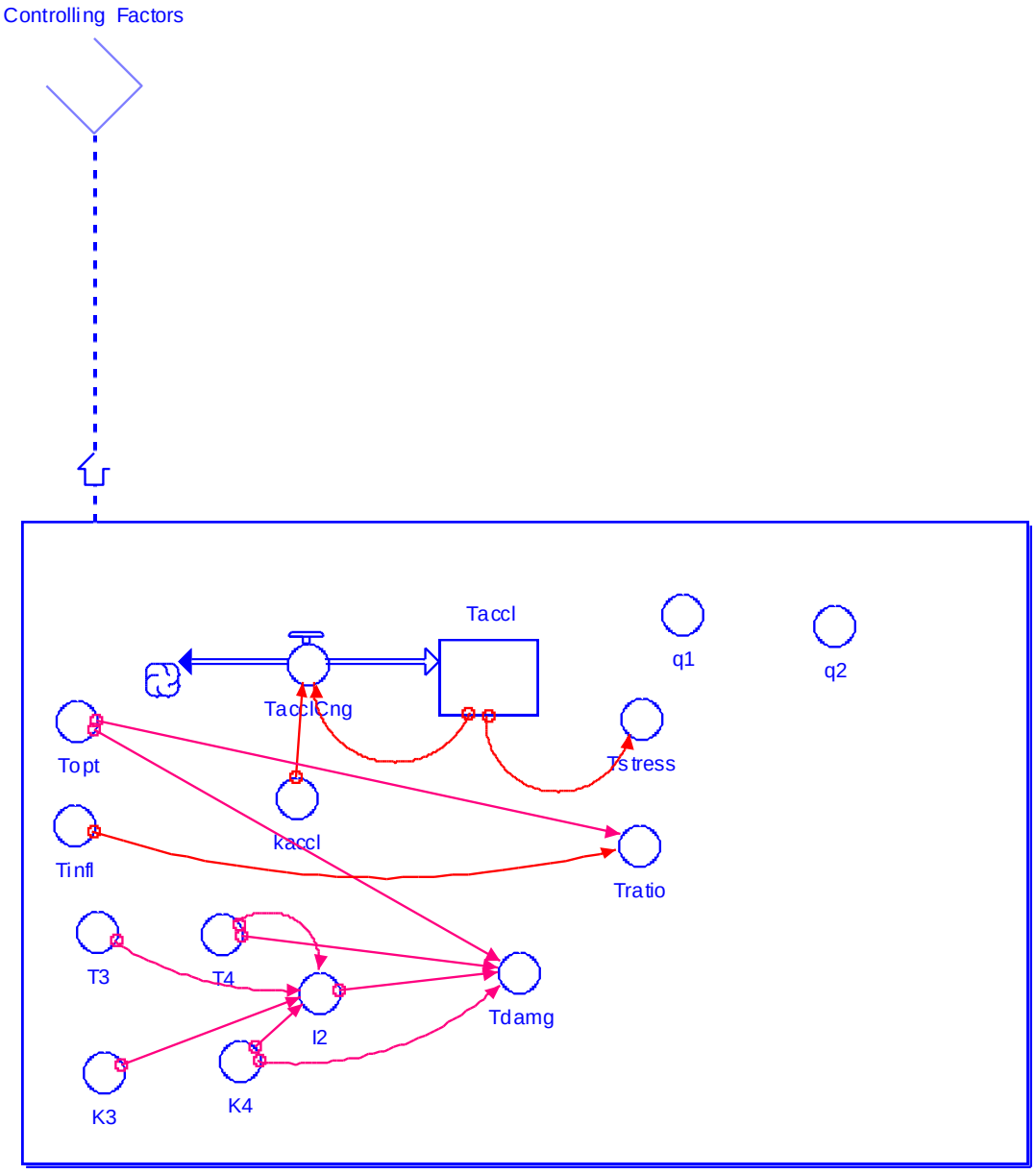


Fig. 3.2 Controlling Factors subunit

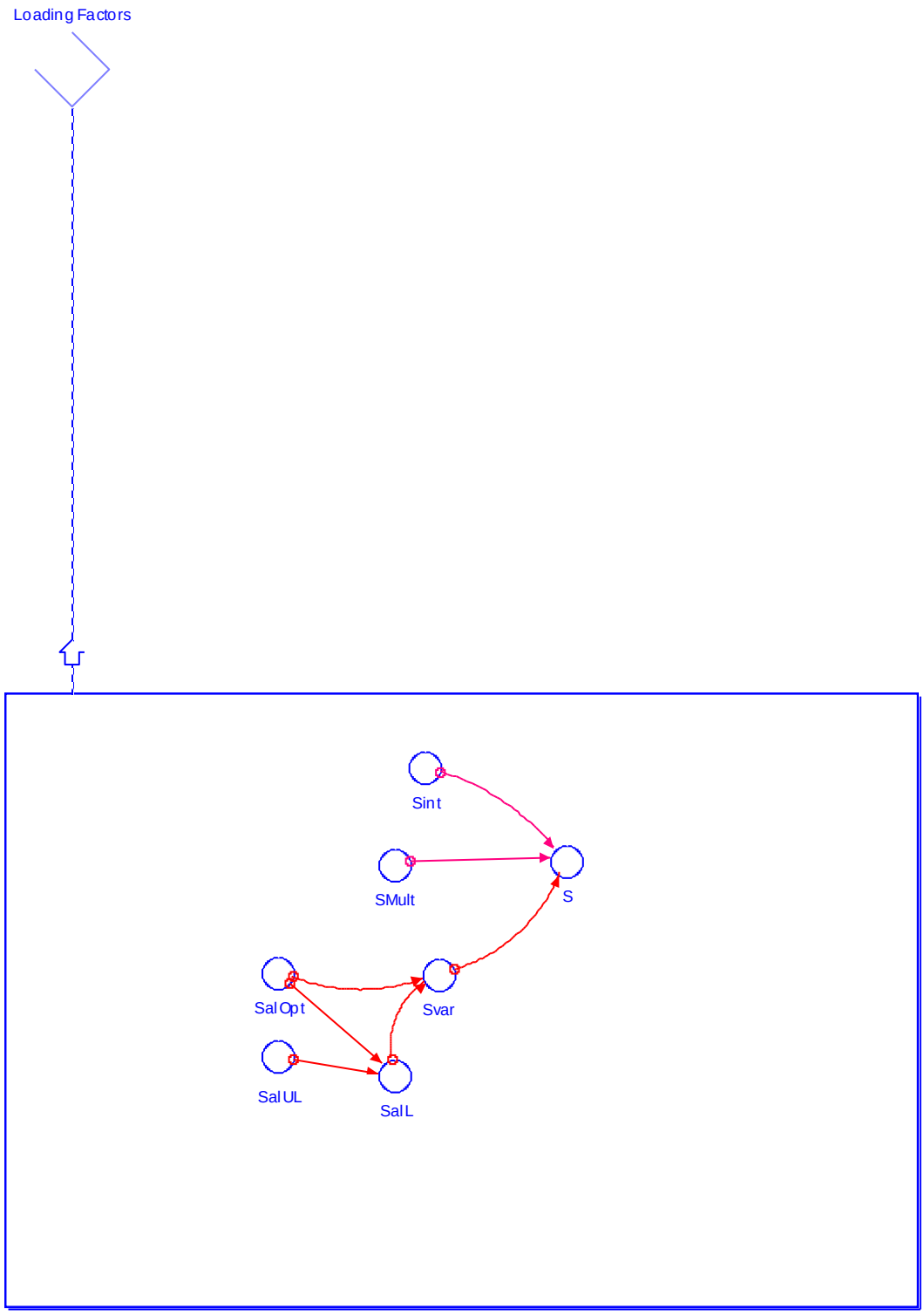


Fig.3.3 Loading Factors subunit

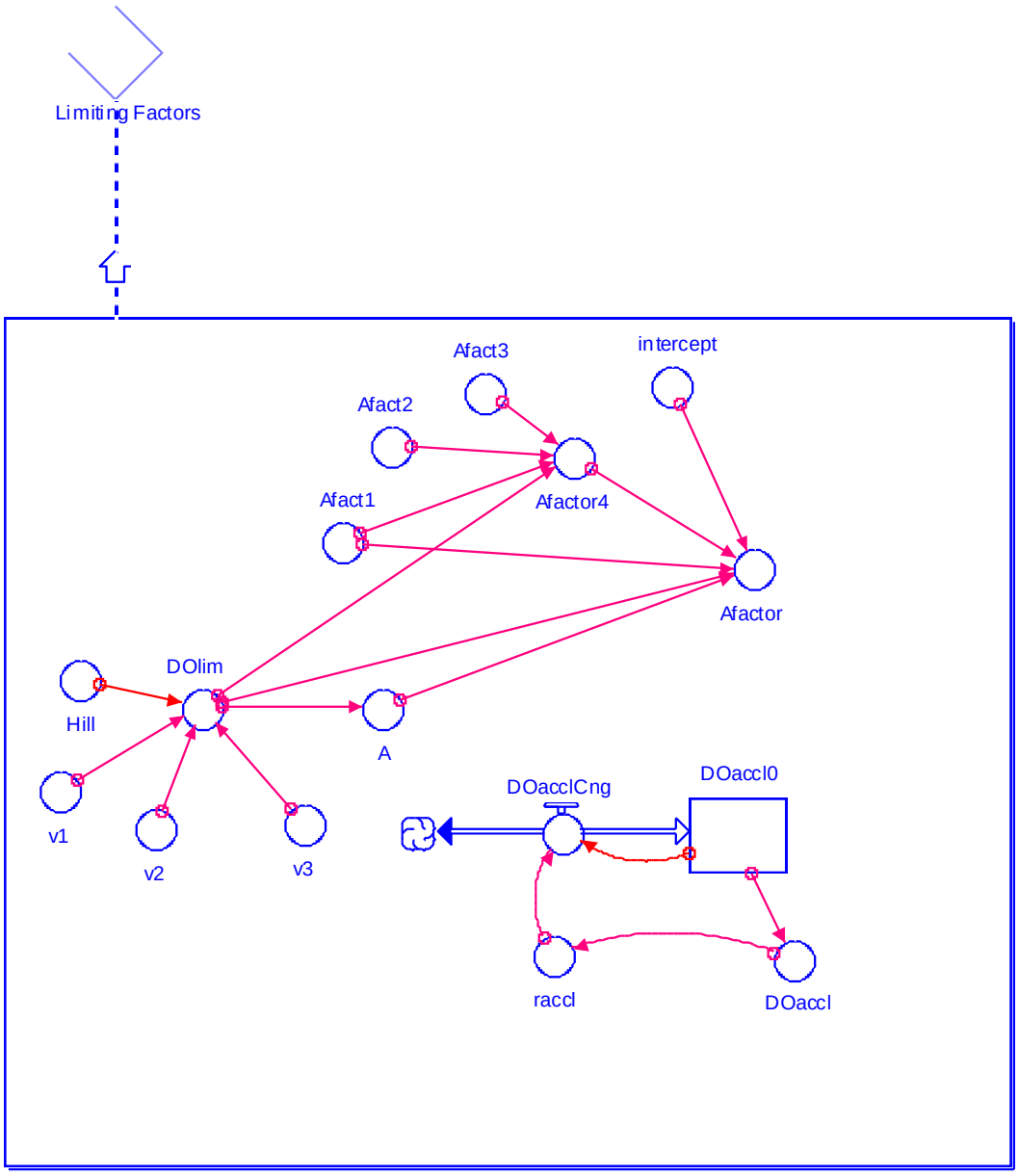


Fig. 3.4 Limiting Factors subunit

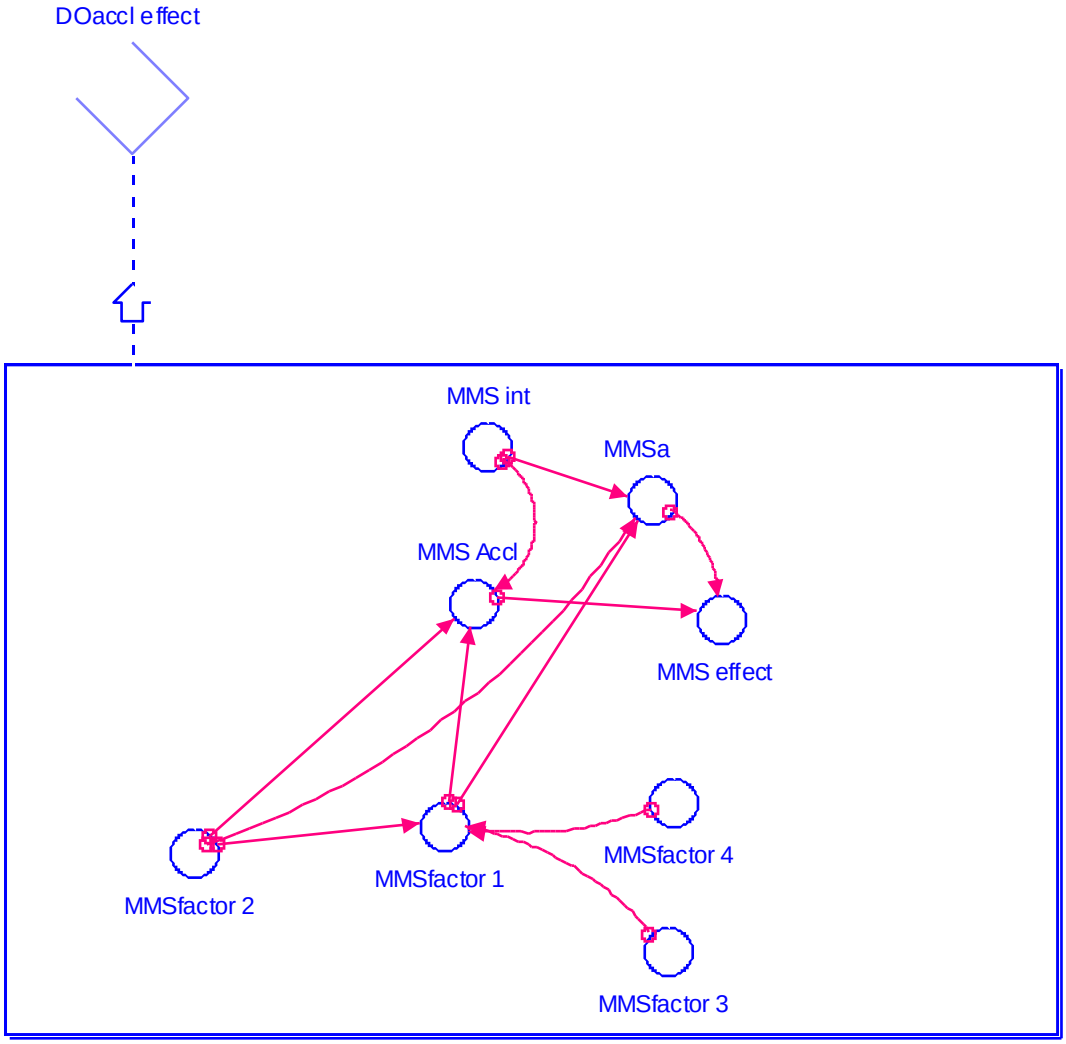


Fig. 3.5 DOaccl factor subunit

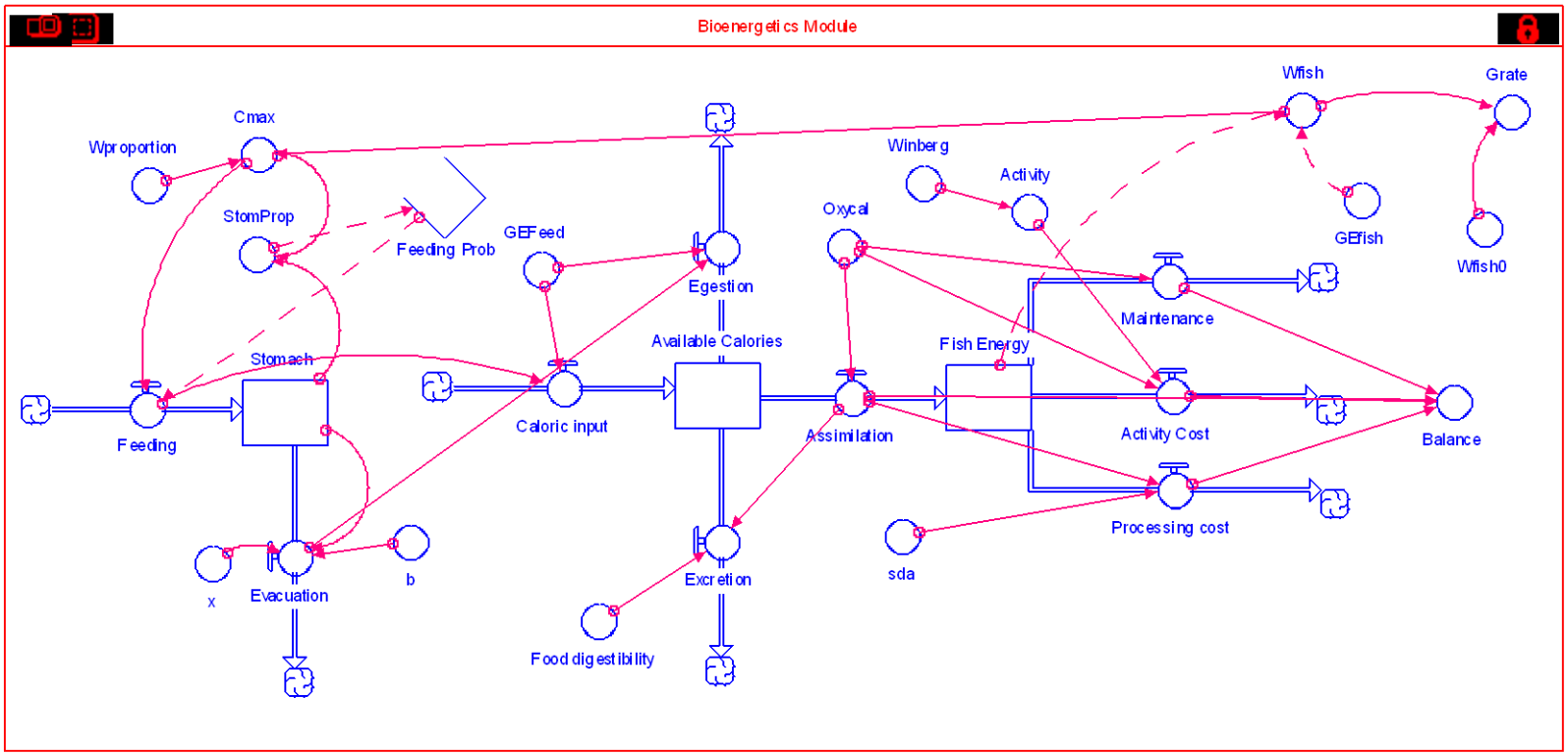


Fig. 3.6 Bioenergetic Module

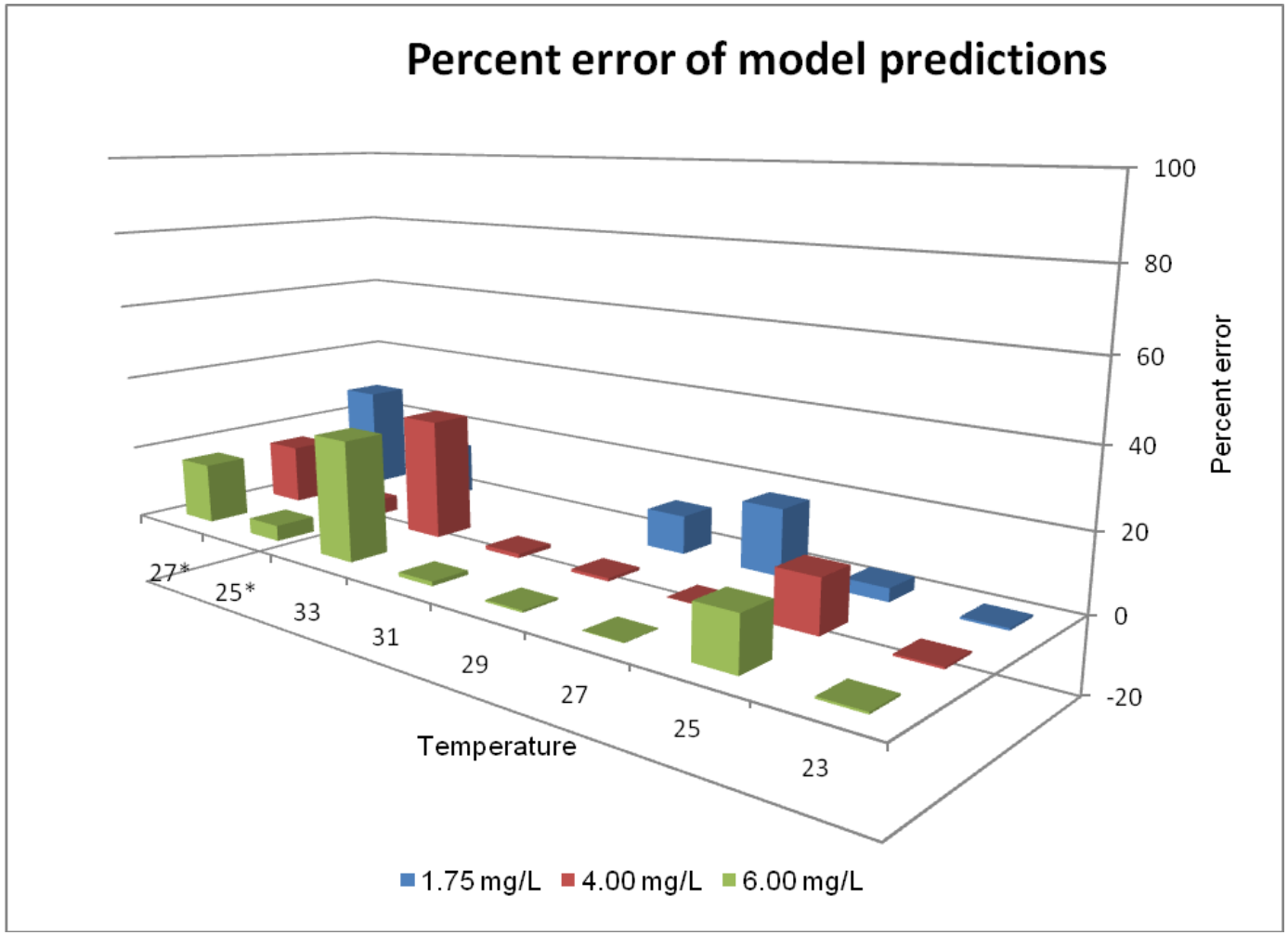


Fig. 3.7 Percent error of model predictions from observations

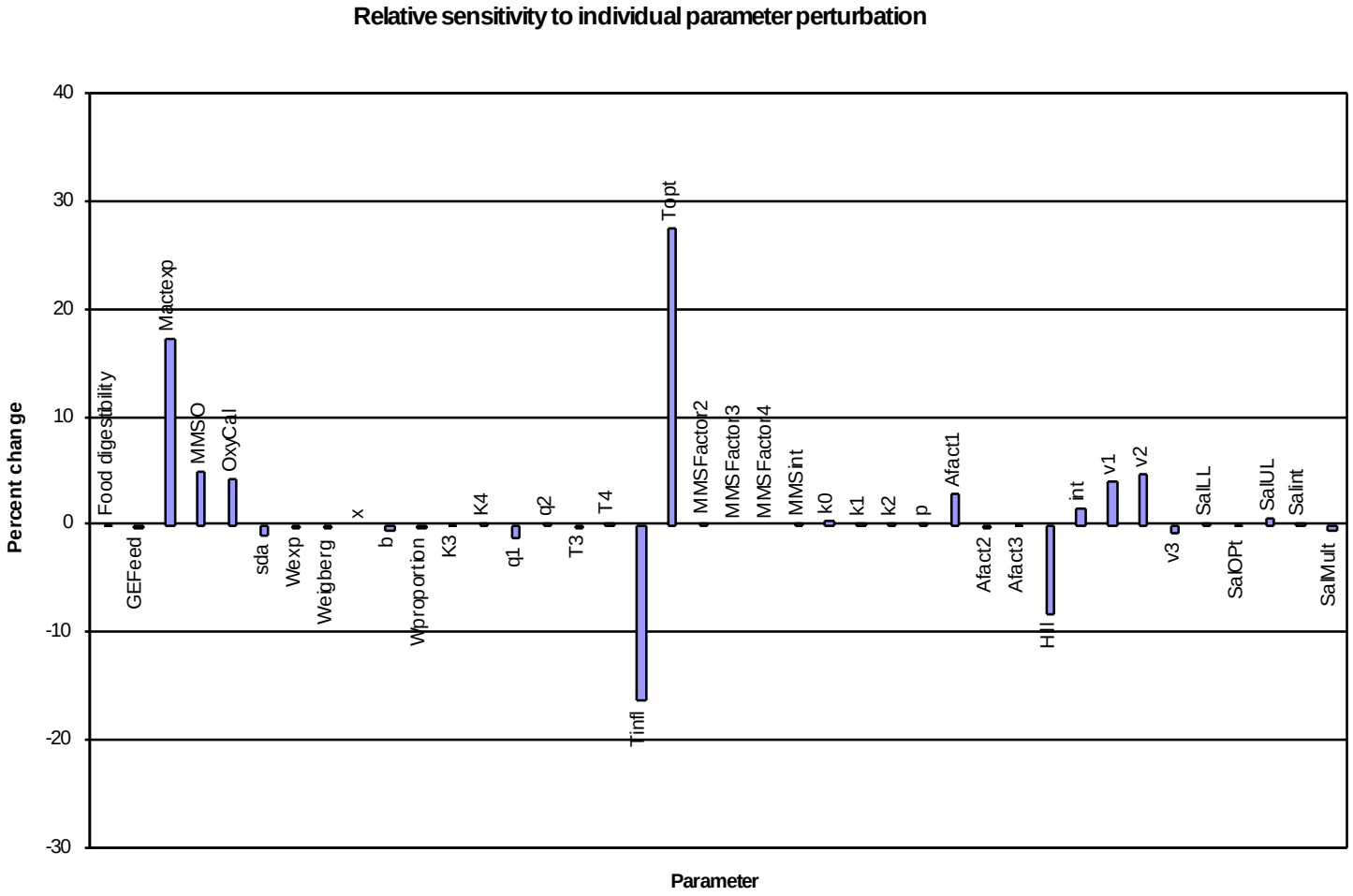
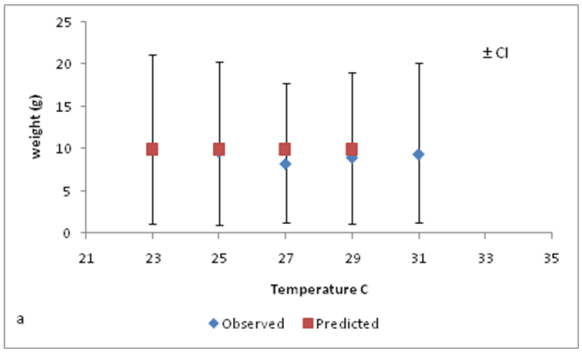
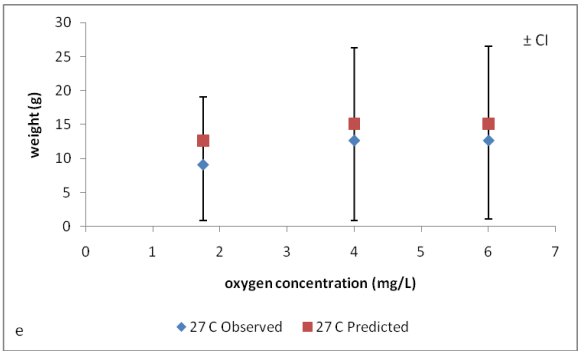


Fig. 3.8 Percent change per individual parameter perturbation





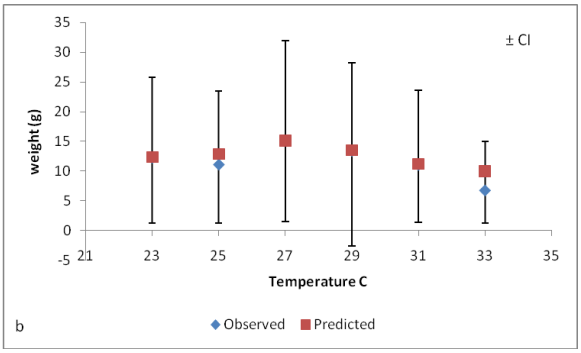
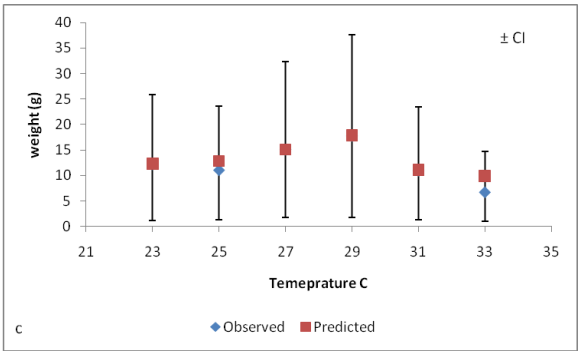
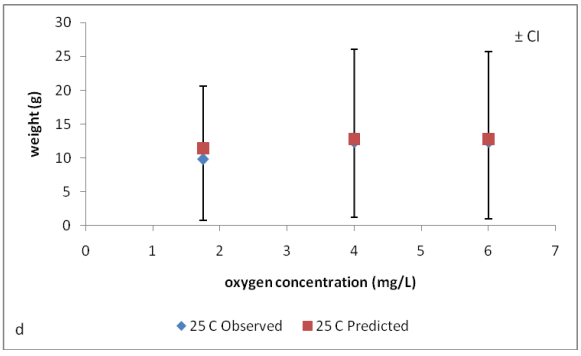


Fig. 3.9 Comparison of observed laboratory weights and model simulations after two weeks under various dissolved oxygen and temperature conditions; a) final weights after two weeks under 1.75 mg/L DO, b) final weights after two weeks under 4.00 mg/L DO; c) final weights after two weeks under 6.00 mg/L DO; d) final weights after two weeks under cycling 1.75-6.00 mg/L DO at 25 C; e) final weights after two weeks under cycling 4.00-6.00 mg/L DO at 27 C.





Chapter 4

Assessing *Paralichthys lethostigma* (Pleuronectiformes: Paralichthyidae) Nursery Habitat Quality in North Carolina Estuaries: Application of an Ecophysiological Model

Abstract

This chapter presents results from the application of *P. lethostigma* ecophysiological model to four nursery habitats of the Pamlico River estuary in North Carolina. The sites were selected based on NC Division of Marine Fisheries juvenile abundance data where "good" habitats represent areas of high juvenile abundance and "bad" sites represent the lower range of abundance in the system. Two sites from each classification were selected in replicate experiments in the summer of 2005. The study employed a combination of caging growth studies, *in situ* metabolic measurements, and computer simulations. The caging experiment resulted in negative growth rates at almost all sites with the exception of one location in the early summer. These results precluded conclusions on habitat quality comparing classifications based on juvenile abundance and growth rates. Although the caging experiments resulted in negative growth rates, the results are explained by the observed abiotic conditions within the system. The simulation model successfully predicted bioenergetic changes of organisms within the cages, which also serves as an independent validation of the model developed in Chapter 3. Estimates of MMS and LOC failed as general indicators of habitat quality in the field, probably due to the ephemeral nature of these physiological adaptations to changes in environmental conditions. The study demonstrated that spatial and temporal heterogeneity of environmental conditions are important determinants of growth rate and resulting assessments of habitat quality. In conclusion, the model successfully predicts changes in metabolic scope and growth in response to abiotic dynamics. In addition, environmental fluctuations and their effects on

metabolic scope are more meaningful biologically than averages and future habitat quality assessments should take this into consideration.

4.1 Introduction

The global decline of fisheries (Roughgarden and Smith, 1996; Myers et al., 1997; Overholtz, 2002) has prompted fisheries scientists to expand their focus from traditional stock assessments to linkages between habitat and stock production (Beck et al., 2001; Dahlgren et al., 2006). The decline of fisheries seems so severe that a publication by Worm et al., (2006) stated that if current trends in fisheries decline continued, world fisheries production will collapse by the year 2048. In the US research efforts have shifted to habitat studies, in part due to the enactment of the Sustainable Fisheries Act through the reauthorization of the Magnuson-Stevens Act by the U.S. government (Pub.L. no. 104-297). Scientists and managers must develop methods to evaluate habitat for management and conservation purposes. An improved understanding of the linkages between habitat components and their relationship to habitat quality should lead to better fisheries management.

Ecologists have attempted to evaluate habitats based on characteristics assumed biologically meaningful (e.g. substrate, structure) and measurements of abundance. But, is habitat quality correctly evaluated with presence/absence data, indicator species, abundance, or diversity of organisms? Not all habitat used by an organism represent essential habitat (Minello, 1999) and presence does not provide information on the individual's performance within a given habitat or the habitat's contribution to the population. These types of studies are based mostly on correlations, which in general fail to provide mechanisms influencing a habitat's quality. Ultimately, habitat evaluation will depend on the ability of ecologists to

decipher the mechanisms influencing performance, linking habitat production and habitat characteristics.

In a fisheries framework, biomass production is probably the best measurement of habitat quality (Able, 1999; Minello, 1999; Beck et al., 2001; Dahlgren et al., 2006). Although production estimates is the preferred measurement of habitat quality (Minello, 1999), direct estimates of production are hardly ever possible to achieve. In lieu of production estimates, potential production has been proposed as a surrogate measurement for habitat evaluation (Guindon and Miller, 1995). Estimates of potential production can serve as a management and policy making tool for habitat protection and enhancement.

Understanding the links between habitat characteristics and potential production is particularly critical in estuarine environments. Estuaries are dynamic environments with highly variable water quality conditions. In North Carolina, the Pamlico-Albemarle estuarine system is the largest lagoonal and second largest estuarine system in the United States (Giese et al., 1979). The geomorphology of the system makes the dynamics within mostly wind driven (Pietrafesa et al., 1986), which have direct effects on larval transport across the sound and can ultimately determine larval settlement (Xie and Eggleston, 1999; Taylor et al., in preparation). Therefore, dynamics within settlement areas can have significant impacts on the growth and potential recruitment contribution by nursery areas. Consequently, it is essential to understand how environmental dynamics affect individual performance and biomass production within these nursery areas to assess habitat quality.

This chapter addresses the question of how environmental dynamics affect individual performance within nursery areas to assess habitat quality in terms of biomass production. It

addresses the question thorough the application of an ecophysiological model of *Paralichthys lethostigma* in four nursery habitats, in addition to caging studies and *in situ* metabolic measurements within the estuarine system. The specific objectives were to: (1) validate the ecophysiological model of *P. lethogstigma* with independent field data and (2) test the effectiveness of MMS and LOC as indices of habitat quality.

4.2. Methods

4.2.1 Study Sites and Habitat Characterization

The Pamlico River Estuary provides nursery habitats critical to the growth, survival and recruitment of various estuarine dependent species in North Carolina. The upper reaches of this estuary provide areas of low-salinity and soft bottoms preferred by southern flounder juveniles as nurseries (Powell and Schwartz, 1977; Burke et al., 1991). Site selection was based on abundance data from NC Department of Marine Fisheries (NCDMF) yearly juvenile catch survey program and work conducted by Guindon and Miller (1995) in the area. Sites classified as "good" represent areas of high juvenile abundance and "bad" sites represent the lower range of abundance in the system. Two "good" sites, East Fork Creek (EC) and Porter Creek (PC), and two "bad" sites, Back Creek (BC) and Long Creek (LC), were selected. All of the sites are tributaries to the Pamlico River with LC and PC on the southern bank and EC and BC on the northern bank of the Pamlico River (Fig. 4.1). Two experiments (early and late summer) were conducted, whereas early summer experiments occurred between May 24 and June 7, while late summer experiments occurred between June

21 and July 14 of 2005. The arbitrary division intended to establish spatial and temporal differences among locations during the summer of 2005.

Abiotic conditions were monitored throughout the duration of the experiments with a Yellow Springs Instruments 6000 Sonde. The instrument recorded dissolved oxygen concentration (DO), temperature, and salinity every half hour. Field experiments were of two and three weeks in duration in the early summer and late summer respectively. The YSI 6000 Sondes were deployed at depths of 1-1.5m, anchored at approximately 20cm from the bottom. *Paralichthys lethostigma* is a benthic predator exhibiting relative little vertical displacement in the water column, for this reason measuring abiotic conditions as close as possible to the bottom was deemed appropriate. All YSI 6000 Sondes were serviced weekly to insure the most accurate measurements possible. The oxygen sensors in particular were sensitive to biological fouling and it was necessary to clean and calibrate the sensors weekly.

In addition to abiotic data, potential prey was sampled at each study site. Although previous studies have concluded that food is not a limiting in North Carolina nursery areas (Kamermans et al., 1995; Ross, 2003), prey samples were collected to estimate potential differences in abundance among sites. Prey samples were collected with an epibenthic sled with a 0.19 m^2 opening and fitted with a plankton net (100 µm mesh) with a cod end. The sled is designed to capture prey present on the surface of the sediment and in the water column above it (see Kamermans et al., 1995). Five tows, approximately 1 minute duration and covering approximately 20 meters, were made at each site. Each transect was performed within 10 meters of the general area of the experiment. All samples were immediately preserved in 95% ethanol for later analysis in the laboratory.

Samples were strained from the ethanol using a 100 µm sieve and rinsed with water. Prey items were identified in the laboratory and counted under a dissection microscope to obtain estimates of prey abundance within the nursery area. With a few exceptions samples were counted and identified in their entirety; however the high number of mysids in some cases made sub-sampling necessary. Estimates of mysid abundance were considered particularly important because young of the year southern flounder mainly consume mysids during the early residence in nursery habitats (Fitzhugh, 1993). In those instances were sub-sampling was necessary, all large prey items were identified and counted first. The remainder of the sample was placed in 500 ml of water and stirred to uniformly distribute the organisms. Three 50 mL aliquot were taken and prey items were identified and counted as before. The average of the three subsamples was taken and used to estimate the total number of prey in the entire sample and number/m^3.

4.2.2 Caging studies and metabolic indices

Caging experiments were conducted at each site during both periods to estimate growth within these nursery areas and used as independent data to validate the ecophysiological model of *P. lethostigma* (Chapter 3). Fish where caged to insure retrieval of the individuals at the end of the experiment and to insure exposure to the recorded abiotic conditions. The cage material also excluded potential predators while allowing prey items to enter. All planned experiments were conducted with hatchery reared fish from NC State University's Lake Wheeler Hatchery Facility. The use of hatchery reared fish presumably reduces sources of variation due to fish origin and increased the potential to detect

differences among habitats. Five replicate cages were used at each location with five fish per cage in the first experiment. Each fish was individually tagged subcutaneously with acrylic paint. Individual weight change was estimated and used to determine the average per cage at the end of the experiment. The average of the five cages was used to determine the average growth rate per site. During the second experiment the number of cages was reduced to three with four fish per cage. Weight was measured only at the end of the two week period of the early summer experiment, while in the late summer run weight was measured weekly.

In addition to the planned caging experiments, two experiments utilizing wild caught *P. lethostigma* juveniles were conducted within PC and BC. The experiments followed the same protocol as the hatchery reared fish experiments. These fishes were collected with a beam trawl within the site (Ellis, 2007). The experiment lasted two weeks and weight change was recorded every week.

Finally, one mesocosm experiment was conducted in the summer of 2006 with hatchery reared fish. The experiment was conducted in a concrete pond at the Institute of Marine Sciences of UNC with circulating water from Bogue Sound. In these mesocosm experiments six cages with three fish per cage were fed daily pellet food. The hatchery fish were fed their normal pellet diet during the experiment. The pellets had a caloric density of approximately 4000 Cal/g while mysids and shrimp have a reported mean caloric density of approximately 1000 Cal/g (Thayer et al., 1973). The concern was that the lower caloric density of wild feed was affecting the growth rates of the hatchery fish in the field, by limiting the total amount of calories available to the fish per meal. As a proxy, two feeding regimes where implemented to determine whether a reduced caloric ration equivalent to ¼ of

their normal diet would result in a significantly different growth rate relative to the high calorie hatchery feeding regime.

The fish were fed daily at 9:00 am with a ration adjusted to 15% of the total cage biomass, assuming that 15% is the physiological maximum of consumption, and weights were monitored weekly. To reflect the caloric difference, the ration for the cages representing fish on a 1000 Cal/g diet had a ration ¼ that of the 4000 Cal/g treatments, because pellet feed had four times the caloric density of natural feed. According to the ecophysiological model consumption and assimilation are regulated by the available metabolic scope. Hence, excess calories should not represent an advantage in growth if conditions restrict maximum scope. Also, energy consumption should vary accordingly to the available scope. If no difference in growth was detected between the treatments, it was inferred that scope regulated consumption is an appropriate model assumption and excess calories are not assimilated into biomass. In addition, it was inferred that excess calories consumed are not an advantage if metabolic scope is limiting.

In addition to the collection of abiotic data and field cage growth studies, *in situ* metabolic measurements were conducted on fish sub-sampled from the cages in all four nursery habitats. Individual fish were extracted from the cages and placed inside respirometry chambers following the protocol in Chapter 2. Respirometry trials were conducted from the boat at the end of the experimental period using water from the location within the chamber. A maximum of eight samples per location were collected. Response variables, MMS and LOC were estimated at the end of the experiment utilizing the statistical routines described in Chapter 2. Estimates of MMS and LOC were analyzed with a two

factor (i.e., creek and period) Proc Mixed routine in SAS 9.1® (SAS, 2004) to determine differences among the groups of fish.

4.2.3 Model Simulations

The environmental data collected during the field experiments were used to simulate *P. lethostigma* growth with the ecophysiological model. The data were summarized into hourly means due to model requirements (Chapter 3). The hourly data were inputted to the model and MMSO values were adjusted to fit observed weight changes. Due to loss of weight in all field experiments an *a posteriori* feeding experiment was conducted to determine whether weight loss was due to a lag in feeding transition from a pellet diet to a wild prey diet by the hatchery reared fish, since food was readily observed within the cages during fish extraction.

The feeding transition experiment consisted of 14 tanks each holding a hatchery reared *P. lethostigma*. Three treatments were applied randomly to the tanks, with four tanks under the no food present treatment (NF), five tanks were exposed to live shrimp (WF), and five tanks were a control treatment (C) fed the regular pellet diet. Both WF and C groups were fed *ad lib* to satiation throughout the duration of the experiment. The experiment lasted a total of 23 days and weights were recorded every two days. A time dependent feeding probability function was derived (p) from this experiment and included in the model to account for the delay in diet switch of the hatchery fish. Because feeding is dependent on whether the probability is greater than the output of a uniformly random function (Chapter 3), 500 simulations were used to calculate the mean growth trajectory of the virtual fish. The

average of these simulations was taken to represent the predicted growth of *P. lethostigma* within the studied nursery habitats. During the simulations with environmental data from the wild fish experiments the time lag function was eliminated and feeding probability was set to 0.9 because these fish were assumed to be accustomed to natural feed. The high probability value was assumed to represent no food limitation while allowing room for a small level for randomness.

4.2.4 Data Analyses

Temporal and spatial comparisons of abiotic factors among the nursery habitats were conducted with a two factor ANOVA comparing effects of location and period on environmental conditions. Since the data is autocorrelated, it violates the ANOVA assumption of independence an autoregressive correction is needed. A repeated measure ANOVA with a first-order autoregressive process was employed for this purpose (Ellis, 2007). The test models the correlation between measurements and allows for the error term at a given time (t) to be partially predicted by the preceding error at time (t-1). Because of the frequency of data points (every 30 minutes) it was necessary to bin data into 4 hour bins. Grouping of the data provided a reduction of noise and increased the ability of the test to account for daily oscillations in temperature and oxygen. The ANOVA model used the Proc Mixed routine from SAS 9.1® (SAS, 2004). The two class variables used were location and period. The rankings of the spatio-temporal habitat characteristics were compared to rankings of metabolic indices and model predictions to assess the model accuracy. Similarly, prey data was analyzed with a two factor ANOVA with the Proc Mix routine to detect

differences between habitats or periods. Statistical analyses were considered significant at $\alpha = 0.05$.

4.3. Results

4.3.1 Study Sites and Habitat Characterization

The descriptive statistics of abiotic conditions during experimental periods 1 and 2 are summarized in Tables 4.1 and 4.2 respectively. Mean temperatures during the first experiment were highest on BC at 24.82 C, followed by PC (24.17 C), EC (23.16 C), and LC (22.71 C) successively. The coefficient of variation during this period ranked BC highest (7.87%), followed by EC (6.23%), PC (5.97%), and LC last (5.70%). During the second experimental period, mean temperatures were highest at EC with 29.54 C, followed by BC (28.47 C), PC (28.25 C), and LC having the lowest temperature (27.96 C). The respective coefficient of variation during this period ranked in descending order EC (6.34%), LC (5.31%), PC (5.21%), and BC last (4.57%). On average, mean temperatures were 4.76 C higher during the second experimental period.

Mean DO concentrations during the first period was highest at LC, with 6.01 mg/L, followed by EC (4.75 mg/L), PC (4.43 mg/L), and BC having the lowest concentration at 3.81 mg/L. The coefficient of variation during this period was highest at BC (63.84%) followed in descending order by EC (45.18%), PC (28.08%), and LC (27.11%) in last place. During the second experimental period mean DO levels were highest at EC (5.09 mg/L) followed by PC (4.60 mg/L), LC (4.10 mg/L), and BC (3.50 mg/L) successively. The coefficient of variation ranked BC (57.91%) highest followed y LC (54.55%), EC (36.42%),

and PC (34.08%) last. With the exception of EC, mean DO levels were lower during the second experimental period.

Salinity estimates during both periods correlated with proximity to the mouth of the Pamlico River (Fig. 4.1). During the first period, mean salinity estimates ranked EC highest (6.35ppt), followed by LC (6.27ppt), PC (3.76ppt), and finally BC (3.67ppt). Mean salinity during the second period ranked EC (7.01ppt), LC (6.17ppt), BC (4.69ppt), and PC last (4.54ppt). In general, mean salinities were higher during the second experimental period of the study. Coefficients of variations during the first period ranked PC (8.98%), EC (8.88%), BC (8.67%), and LC (3.84%) last. Meanwhile, rankings during the second period were BC (15.13%), EC (8.29%), LC (8.18%), and PC (7.76%) last. All of the sites exhibited salinities in the oligohaline range preferred by *P. lethostigma* (Burke, et al., 1991).

Results from the repeated measures ANOVA are reported in Table 4.3. The analyses failed to detect significant differences in temperature due to creek, period or their interaction. This was not the case with salinity and DO, where analyses of variance detected significant differences among creeks and period respectively. Salinity was significantly different among sites while DO was significantly different between periods. No significant interaction effects were detected for any of the three abiotic parameters.

A two factor ANOVA was employed to analyze the prey data collected from the sites. The analysis was limited to the density of mysids within the area, because in a concurrent study 50% of the full stomach of wild caught fish contained mysids only and 70-95% of the stomach samples contained mysids and some other food category (Ellis, 2007). The analysis did not include data from BC during the second experimental period because the experiment

was interrupted due to complete fish mortality. The ANOVA yielded significant site, period and interaction effects on mysid density (Table 4.4). The density of mysids was greater in the second experimental period at all sites, but only at PC were the values significantly higher than the rest of the sites with an estimated density of 751 mysids/m^3 (Fig. 4.2).

4.3.2 Caging studies and metabolic indices

Average initial fish weights per cage during the early summer experiment ranged from 15.51 to 20.05. During this period, with the exception of LC, caged fish in the remaining nursery areas lost weight. The greatest weight loss was observed at BC, followed by EC and PC. The observed negative growth rates at all sites were significantly different from 0 during this period and only LC had positive growth rate (Table 4.5). The late summer experiment resulted in overall negative growth rate, but weekly growth rates showed weight gain between the second and third weeks at PC ($3.93e^{-3}$and $7.27e^{-3}$ respectively) (Table 4.6). A two factor ANOVA on growth rates detected significant differences among sites and period, but also indicated significant interaction effects (Table 4.7).

During the pond experiment overall growth rate was negative for both treatments. A two factor ANOVA failed to detect statistically significant differences between the two treatments. Therefore, it was concluded that consumption and assimilation are subjugated to the available metabolic scope and excess calories were not advantageous to growth rate when metabolic scope was limited. The result also supports the assumption that resources in the field were not limiting and that restrictions to metabolic scope were responsible for poor growth rates.

Analyses of metabolic indices, LOC and MMS, are reported on Table 4.8. The statistical test indicated no significant effects for either creek or period on LOC and only a significant effect on MMS from creek. Multiple comparisons of MMS among creeks yielded a significant difference between BC (0.0119) and the rest of the creeks (Table 4.9). The rest of the creeks were similar in their mean MMS values, ranging from 0.0021 to 0.0025 (Table 4.9).

4.3.3 Model simulations

The results of the diet switch experiments indicate that initially fish submitted to the wild feed treatment lost weight, while the control fish demonstrated positive growth (0.009 g/day) from the onset of the experiment. The wild feed treatment exhibited an initial weight loss similar to the no feed treatment, -0.018 g/day and -0.017 g/day respectively, but after day 10 the wild group reduced its trend in growth rate by an order of magnitude (Table 4.10). Although the trend was reduced, the fish did not recovered the lost mean weight until after day 19 (Fig. 4.3). Based on these results an *ad hoc* function was created, 1-EXP(-0.0005*time), in which the feeding probability approached 1 over time reaching 0.5 at after day 10. The function was incorporated into the model to account for the assumption of delayed prey switch by the hatchery fish in the field trials.

The results from the simulations and observed field weights for the early summer experiment are summarized in Table 4.11. Except for BC all predicted final weights fell within the standard error of the observed weights (Fig. 4.4). A goodness of fit test failed to reject the hypothesis of no difference between predicted and observed weights for all sites.

The late summer experiment is summarized in Table 4.12 for each week of its duration and the corresponding predicted weights are also presented. After the first week most predicted weights fell within the standard error margin of the observed weights (Fig. 4.5). By the end of the second week all fish from BC had died and simulation results are not reported. For all other sites, although the fit was not as good as in the first week a goodness of fit test failed to detect significant differences between simulated and observed weights (Fig. 4.6). This was also the case at the end the third week, were goodness of fit test failed to detect differences between observed and predicted weights (Fig. 4.7).

Finally, the two field trials conducted with wild fish are summarized in Table 4.13. The simulation means were in accordance with the observed weight changes and fell within 1 SE of the expected means. According to the goodness of fit test, the predicted weights were not statistically different from the observed weights (Fig. 4.8).

4.4 Discussion

4.4.1 Habitat Characterization

In general, the patterns of the abiotic parameters examined throughout the study behaved as expected. The observed mean water temperatures for all four study sites were higher during the second experimental period. The difference can be explained partially by the high heat capacity of water which allows it to accumulate heat from the increasingly longer daylight hours and lose little heat to the atmosphere during night time (Libes, 1992). This property can also explain the reduction in temperature variation, because as summer temperatures approach their peak, the heat capacity of water acts as a buffer allowing little variation late in the summer. In the context of metabolic scope, higher temperatures will

produce a higher standard metabolic rate in an organism. Higher temperatures will promote higher growth as long as temperature does not exceed the physiological optimum of the organism and no limiting factors are acting upon its metabolism. Although mean temperatures during the first experimental period did not exceed the physiological optimum of the species (29 C), temperatures approaching the optimum were recorded (Table 1). In the second period mean temperatures reached the assumed optimum of 29 C with recorded temperatures as high as 34.32 C (Table 2). These extreme temperatures can have deleterious effects on the organisms and result in poor growth (Chapter 2).

While higher temperatures promote growth, reduced oxygen concentrations can have limiting effects upon growth. As expected, mean DO levels were lower during the second experimental period of the study. The reduction of DO is partially explained by the reduced O_2 solubility with increasing water temperature (Libes, 1992). In addition to this physical phenomenon, enhanced primary productivity with the advancement of summer causes greater oxygen biological demand at nights, increasing the magnitude of diel DO fluctuations (Flemer, 1972; Reyes and Merino, 1991). The increased primary productivity and reduced oxygen solubility in the later part of the summer can account for greater diel oxygen fluctuations in shallow nursery habitats observed during this study.

Salinity patterns unlike DO and temperature are not as clear. The only discernible pattern relates the higher salinity values on LC and EC over both periods. The pattern is not really surprising considering that both of these tributaries of the Pamlico River are closer in proximity to the mouth of the river, hence receiving a greater salt water influx than the other two sites. All of the study sites can be classified as oligohaline, environments well within the

range preferred by juvenile *P. lethostigma* during their settlement and early development (Powell and Schwartz, 1977; Burke et al., 1991).

4.4.2 Caging studies and metabolic indices

4.4.2.1 Field growth rates

Initially, the study design aimed to explain growth rates with the abiotic patterns and test the hypothesis that early in the season conditions would be favorable to growth and with the advancement of summer conditions would deteriorate resulting in reduced growth. Unfortunately, with the exception of LC, all field trials resulted in overall negative growth rates. In the context of this study, the observed results are attributed to the already deteriorating conditions during the first experimental period. During the first experiment, LC was the only site without the occurrence of hypoxic periods while having the lowest mean temperature. The interactions between oxygen and temperature restricted metabolic scope and affected growth rates at all sites. During the second experimental period, mean temperatures near the optimum of the species in combination with mean DO concentrations below the limiting oxygen concentrations for growth were recorded at all study sites (Chapter 2). In addition, the frequency and duration of hypoxic events increased during the second experimental period. The combination of hypoxic periods and high temperatures produced conditions less favorable to growth over the late summer season.

The combination of high temperatures and low oxygen conditions will result in a restricted scope for growth (Fry, 1947). The interaction of these factors significantly affects the ability of juvenile *P. lethostigma* to assimilate and accumulate biomass. Ellis (2007) found that the energetic content of *P. lethostigma* varies significantly at fine temporal scales

and that in addition to mean temperature and oxygen, the frequency of hypoxic events and the duration of these events are significant factors in this variation. Although other environmental factors such as feed availability can affect growth, data from the literature suggest that in North Carolina estuaries food is not restricted (Kammermans et al., 1995; Ross, 2003). The results from field experiments and model simulations support that energy assimilation is regulated by the available metabolic scope and that the interaction of the abiotic parameters measured can be used to predict the resulting growth. These results are supported in a published study by Malleck et al., (1998) which concluded that DO concentrations below 95% saturation affect feed consumption. Hence, growth rates were significantly affected by the abiotic parameters monitored within this study through their effect on metabolic scope.

4.4.2.2 Metabolic indices

The metabolic indices estimates from the field study correlated to the abiotic parameters measured throughout the experiment, but these relationships were not as strong as those observed in the laboratory (Chapter 2). Although higher average temperatures along with lower DO concentrations were observed in the second period, statistical analyses failed to detect significant temporal effects on either metabolic index. In contrast, differences in MMS values were detected among creeks. Multiple comparisons showed that BC was different from all other having the highest MMS estimate in the first period ($1.19e^{-2}$) (Table 4.9). This site had the lowest mean DO concentrations (3.81 mg/L), consistently lower DO levels, and highest temperature for the period (24.82 C) (Table 4.1), while exhibiting the greatest weight loss from all four creeks (Table 4.5). The combination of low DO and high

temperatures in the field was analogous to the experimental conditions in the laboratory which produced the highest MMS estimates at 29 C and 1.75 mg/L DO concentration (Chapter 2).

Alternatively, LC, the only positive growth rate throughout the study (1.06 e^{-3}) (Table 4.5), had the lowest average temperature (22.71 C), highest mean DO concentration (6.01 mg/L) (Table 4.1), and the lowest MMS. Unfortunately the relationship between MMS and growth rate did not hold; although EC and PC had mean DO concentration above the limiting oxygen concentration (LOC) for the respective mean temperatures, they still exhibited negative growth yet their respective MMS estimates were similar to LC. These sites, EC and PC, had a higher frequency and longer periods of hypoxia than LC, which accounts for the negative growth rate, but the events were of less magnitude and duration than BC, which might have not sufficed to elicit a response in MMS and could account for the lack of statistical significance among MMS estimates. Alternatively, it is possible that the adaptive responses responsible for higher MMS estimates are short lived once oxygen levels increase. Unfortunately, the laboratory study did not address the time required for the effects to DO levels to produce a response on MMS or the duration of these physiological adaptations. In summary, environmental conditions were severe enough to produce an effect on MMS at BC, but their effects on these estimates at the other sites did not correlate to their cumulative effects on growth rates.

During the second period episodes of hypoxia/anoxia were recorded at all sites, but BC in particular had events lasting up to 23 hours. Estimates of MMS from BC were not available for the second experimental period because no survivors were available by the end

of the second week. In the remainder sites, conditions had deteriorated further with higher mean temperatures, lower mean DO concentrations, and higher frequency of hypoxic events. Even though mean DO concentrations were lower relative to the early summer period, MMS estimates were not statistically different from the first period. This might be due to the cyclical nature of conditions in the field. Unlike the laboratory study which demonstrated that constant temperatures and DO affect MMS estimates, the variable conditions in the field, particularly during the late summer, did not produce a clear effect on the metabolic index. In general, MMS estimates tended to be variable and did not correlate strongly with environmental conditions in the field.

4.4.3 Model simulations

Overall simulation results for both experimental periods and all sites were in agreement with the observed weights (Table 4.11 - 4.13). Although the simulations are within the standard error of most estimates, it must be noted that the model is intended to predict biomass gain and not weight loss. Initially, the study design considered that initial and final weights would be adequate to test the simulations, but the unexpected weight loss and lack of temporal resolution of weight changes during the first experimental period made obvious the necessity of weekly weight estimations. The measurements at weekly intervals in the following experiments provided a greater temporal resolution, which allowed the test of model behavior throughout the simulations in relation to the input variables. Even though the model accuracy can be questioned since weight changes were small and mostly negative, the model was able to track the behaviour of weight changes of organisms.

As discussed in section 4.2.1, most of the experiments resulted in overall weight loss, but in PC and the BC experiments with hatchery and wild fish respectively, positive growth rates were observed within the experimental period. When examined closely, after an initial weight loss at the end of week one, fish from PC showed positive growth rate in weeks two and three. The model successfully captured the changes in growth rate observed in the field during this period (Fig. 4.9). Even more reassuring is the ability of the model to predict with high temporal resolution biomass changes of an organism within the habitat, given the variable environmental conditions (Fig 4.10). The individual simulation tracks the initial drop in weight and growth rate of *P. lethostigma* within the first few days of the simulation and shows the recovery in growth rate by 125 hours or approximately 5 days.

Additional validation to the model was provided by the simulation of wild *P. lethostigma* within BC during the late summer. The loss of weight by hatchery fish in the field added another variable to the simulation and model validation. The unexpected weight loss required the incorporation of the feeding probability function in order to address the lag in prey switch from pellet to wild feed which added to the model complexity. Fortunately, the experiment conducted with wild fish afforded the opportunity to simplify the model by removing the time delay function of feeding in the bioenergetics module. During this simulation, the feeding probability function was replaced by a fixed probability 0.9. This value was arbitrarily selected to represent no food limitation while allowing a small probability of no encounter. Although overall growth rate was negative in this experiment, after an initial biomass loss at the end of week 1 the average response was an increase in growth rate by week 2 (Fig. 4.11). The simulated growth rate was interpreted as evidence of

the model's effectiveness to reproduce, in a fine temporal scale, the effects of abiotic factors on metabolic scope and on individual bioenergetics even when weight loss occurred.

In addition, the wild fish simulation also supported the assumption that food is not limited within these nursery areas and that consumption is regulated by the available metabolic scope. The high fixed probability assigned during this simulation assumes that food is not limiting within the habitat and biomass gain is mainly restricted by the ability of the organism to assimilate the available energy into biomass. Hence, the resulting changes in biomass are a product of environmental conditions and their effect on metabolic scope. The model accurately tracks the behavior of the observed growth rates, suggesting that the mechanisms incorporated within are sufficient to explain the variability in growth rate as predicted by the input environmental variables.

Additional evidence for metabolic scope regulated consumption is provided by the caging experiments conducted in the IMS ponds. During this time period temperatures as high as 34 C were recorded while low oxygen conditions (<2.00mg/L) were experienced daily. As predicted, the combination of low DO and high temperatures produced conditions restricting metabolic scope and hence growth. The lack of statistical difference between the food ration treatments was interpreted as confirmation that consumption was regulated by metabolic scope and that abiotic conditions, not calories, were restricting growth. Simulation results predicted growth rates in close agreement with the observed changes from the experiments (Fig. 4.12). The simulation of growth rates during this period are in agreement with results presented in Chapter 2, in which fish under extreme abiotic conditions had negative growth rates. The model predicted a restriction in consumption under such

conditions which is also supported by empirical results from Malleck et al., (1998) and Mallekh and Lagardère (2002). Although, it was not possible to conclude there were any effects of caloric density on growth rate from this experiment, empirical and modeling results supports the hypothesis that energy assimilation is regulated by metabolic scope

The MMSO parameter (Chapter 3) represents the remaining variability not explained by the input variables and was defined by Neill et al., (2004) as the inherent metabolic efficiency of the fish/environment system. This parameter remained fairly constant over time with the exception of differences between early summer and late summer simulations at LC and PC (Tables 4.11 and 4.12). The two instances were MMSO changed coincided with two independent events. At LC a large barnacle colonization event occurred, which dramatically increased the fouling of the cages. This in turn could have altered the conditions of the system relative to the first experiment. At PC due to conflicts with commercial fishers the location of the cages was moved closer to the mouth of the creek resulting in a higher MMSO value for the second period. Although MMSO varied between early and late summer, the simulations conducted with wild fish during the late summer at PC did not require a different MMSO from simulations with hatchery reared fish, suggesting that differences between early summer and late summer simulations with hatchery fish were not an artifact of the model.

4.5 Conclusions and implications to habitat evaluation

The environmental parameters recorded throughout this study reaffirm the high levels of variability within estuarine nursery habitats. The agreement between the observed growth

rates and model simulations serve as validation of model assumptions and performance. Results from model simulations reaffirm the importance of interactions among environmental parameters, through its effects on metabolic scope, on individual bioenergetics. Unfortunately, field estimates of MMS had poor sensitivity as an indicator of overall habitat quality. The poor performance of MMS can be attributed to the ephemeral nature of environmental conditions and the corresponding acclimation by the organism. Enzymatic changes within a system can occur in the span of hours in which case, MMS would reflect the most recent conditions experienced by the organism and not necessarily the cumulative effects of environmental history. In conclusion, the model accurately captures the behavior of metabolic scope in response to the environment and its effects on growth, while MMS and LOC proved to be poor indicators of habitat quality in rapidly changing systems.

The study also demonstrates that spatial and temporal heterogeneity of environmental conditions are important determinants of overall habitat quality. Although LC was classified as a "bad" habitat, it was the only site with positive growth rates during the first experimental period. Concurrently, EC and PC both which were classified as "good" habitats had negative growth rates. Interestingly, this was not the case during the second experimental period. In this instance, PC was the only site with positive growth rate while LC like the rest of the sites had negative growth rate. Although this study only covers one summer season which precludes long term predictions, the results suggest that in all habitats events of adverse conditions occur, but it is rather the frequency of these events and the extent of these events within these areas which will determine the nursery quality of a given habitat. Finally, the study also demonstrates that environmental fluctuations and their effects on scope are more

meaningful biologically than averages; therefore future assessments of habitat quality should take this into consideration.

4.6 References

Able, K.W., 1999. Measure of juvenile fish habitat quality: examples from a national estuarine research reserve. In: Beneka, L.R. (Ed.), Fish habitat: essential fish habitat and rehabilitation. American Fisheries Society. American Fisheries Society, Bethesda, Maryland, pp. 134-147.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.R., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51, 633-641.

Burke, J.S., Miller, J.M., Hoss, D.E., 1991. Immigration and settlemtent pattern of *Paralichthys dentatus* and *P. lethostigma* in an estuarine nursery ground, North Carolina, USA. Netherlands Journal of Sea Research 27, 393-405.

Dahlgren, C.P., Kellison, G.T., Adams, A.J., Gillanders, B.M., Kendall, M.S., Layman, C.A., Ley, J.A., Nagelkerken, I., Serafy, J.E., 2006. Marine nurseries and effective juvenile habitats: concepts and applications. Marine Ecology Progress Series 312, 291-295.

Ellis, T.A., 2007. Assessing nursery quality for southern flounder, *Paralichthys lethostigma*, through fish energy content and habitat abiotic conditions, Zoology. NC State, Raleigh.

Fitzhugh, G.R., 1993. An individual-based approach to understanding patterns of differential growth and population size structure in juvenile southern flounder (*Paralichthys lethostigma*), Zoology. NC State, Raleigh.

Flemer, D.A., 1972. Current status of knowledge concerning the cause and biological effects of eutrophication in Chesapeake Bay. Chesapeake Science 13(SUPPLEMENT), S144-S149.

Fry, F.E.J., 1947. Effects of the environment on animal activity. University of Toronto Studies Biological Series 55.

Giese, G.L., Wilder, H.B., Parker-Jr., G.G., 1979. Hydrology of major estuaries and sounds of North Carolina, U.S. Geological Survey Water Resources Investigations 79-46. U.S. Geological Survey, pp. 175.

Guindon, K.Y.a.J.M.M., 1995. Growth potential of juvenile southern flounder, *Paralichthys lethostigma*, in low salinity nursery areas of Pamlico Sound, North Carolina, USA. Netherlands Journal of Sea Research 34, 89-100.

Kamermans, P., Guindon, K. Y., Miller, J. M., 1995. Importance of food availability for growth of juvenile southern flounder (Paralichthys lethostigma) in the Pamlico River estuary, North Carolina, USA. Netherlands Journal of Sea Research 34, 101-109.

Libes, S.M., 1992. An introduction to marine biogeochemestry. John Wiley & Sons, Inc., New York.

Mallekh, R. and Lagardère, J.P., 2002. Effect of temperature and dissolved oxygen concentration onthe metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. Journal of Fish Biology 60, 1105-1115.

Mallekh, R. and Lagardère, J.P., Be´gout Anras, M.L., and Lafaye, J.Y. 1998. Variability in appetite of turbot, Scophthalmus maximus under intensive rearing conditions: the role of environmental factors. Aquaculture 160, 123-138.

Minello, T.J., 1999. Nekton densities in shallow estuarine habitats of Texas and Louisiana and the identification of essential fish habitat. In: Beneka, L.R. (Ed.), Fish habitat: essential fish habitat and rehabilitation. American Fisheries Society Symposium, Bethestda, Maryland, pp. 43-75.

Myers, R. A., G. Mertz and P. S. Fowlow 1997. "Maximum population growth rates and recovery times for Atlantic cod, Gadus morhua." Fishery Bulletin 95, 762-772.

Neill, W.H., Brandes, T.S., Burke, B.J., Craig, S.R., Dimichele, L.V., Duchon, K., Edwards, R.E., Fontaine, L.P., Gatlin, D.M., Hutchins, C., Miller, J.M., Ponwith, B.J., Stahl, C.J., Tomasso, J.R., Vega, R.R., 2004. Ecophys.Fish: A simulation model of fish growth in time-varying environmental regimes. Reviews in Fisheries Science 12, 233-288.

Overholtz, W. J. 2002. "The Gulf of Maine-Georges Bank Atlantic herring (Clupea harengus): spatial pattern analysis of the collapse and recovery of a large marine fish complex." Fisheries Research 57(3): 237-254.

Pietrafesa, L.J., Janowitz, G.S., Chao, T.Y., Weisberg, R.H., Askari, F., Noble, E., 1986. The Physical Oceanography of Pamlico Sound. UNC Sea Grant Publication, UNC-SG-WP-86-5, 126 pp.

Powell, A.B., Schwartz, F.J., 1977. Distribution of paralichthid flounders (Bothidae: Paralichthys) in North Carolina estuaries. Chesapeake Science 18, 334-339.

Reyes, E., Merino, M., 1991. Diel dissolved oxygen dynamics and eutrophication in a shallow, well-mixed tropical lagoon (Cancun, Mexico). Estuaries 14, 372-381.

Ross, S. W. 2003. The relative value of different estuarine nursery areas in North Carolina for transient juvenile marine fishes. Fishery Bulletin 101, 384-404.

Roughgarden, J. and F. Smith 1996. Why fisheries collapse and what to do about it. Proceedings of the National Academy of Sciences of the United States Of America 93, 5078-5083.

SAS, 2004. SAS/STAT 9.1 User's Guide. SAS Institute Inc. Cary, NC.

Taylor, J.C., Miller, J.M., Pietrafesa, L.J., Dickey, D., Ross, S., In prep. Winter winds and river discharge determine juvenile southernflounder abundance and distribution in North Carolina estuaries. Fisheries Oceanography.

Thayer, G.W., Shaaf, W.E., Angelovic, J.W., LaCroix M.W. 1973. Caloric measurements of some estuarine organisms. Fishery Bulletin 71, 289-296.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., Watson, R. 2006. Impacts of biodiversity loss on ocean ecosystem services. Science 314, 787-790.

Xie, L., Eggleston, D.B., 1999. Computer simulations of wind-induced estuarine circulation patterns and estuary-shelf exchange processes: the potential role of wind forcing on larval transport. Estuarine Coastal and Shelf Science 49, 221-234.

Table 4.1 Summary statistics for nursery habitats in the Pamlico River during early summer (May 24-June 7) 2005. (*Temp = temperature in C , DO = dissolved oxygen concentration in mg/L, and Sal = salinity in ppt.*)

	BC			EC			LC			PC		
	Temp	DO	Sal	Temp	DO	Sal	Temp	DO	Sal	Temp	DO	Sal
Mean	24.82	3.81	3.67	23.16	4.75	6.35	22.71	6.01	6.27	24.17	4.43	3.76
Standard Error	0.07	0.09	0.01	0.06	0.08	0.02	0.05	0.06	0.01	0.06	0.05	0.01
Median	24.58	3.72	3.55	22.95	4.52	6.19	22.76	6.12	6.22	24.05	4.47	3.69
Mode	21.98	0.00	3.50	21.71	2.48	6.15	23.15	5.47	6.01	21.68	4.87	3.39
Standard Deviation	1.95	2.43	0.32	1.44	2.15	0.56	1.30	1.63	0.24	1.44	1.24	0.34
Sample Variance	3.81	5.91	0.10	2.08	4.61	0.32	1.68	2.65	0.06	2.08	1.54	0.11
Range	7.84	9.89	1.68	6.36	6.14	3.85	8.06	8.45	1.24	6.46	7.92	1.91
Minimum	21.47	0.00	2.91	20.31	2.30	4.79	18.73	1.54	5.60	21.62	1.09	3.25
Maximum	29.31	9.89	4.59	26.67	8.44	8.64	26.79	9.99	6.84	28.08	9.01	5.16
Count	730.00	730.00	730.00	675.00	675.00	675.00	680.00	680.00	680.00	683.00	683.00	683.00
Confidence Level(95%)	0.14	0.18	0.02	0.11	0.16	0.04	0.10	0.12	0.02	0.11	0.09	0.03
Coefficient of Variation	7.87	63.84	8.67	6.23	45.18	8.88	5.70	27.11	3.84	5.97	28.08	8.98

Table 4.2 Summary statistics for nursery habitats in the Pamlico River during late summer (June 21-July-14) of 2005. (*Temp = temperature in C , DO = dissolved oxygen concentration in mg/L, and Sal = salinity in ppt.*)

	BC			EC			LC			PC		
	Temp	DO	Sal	Temp	DO	Sal	Temp	DO	Sal	Temp	DO	Sal
Mean	28.47	3.50	4.69	29.54	5.09	7.01	27.96	4.10	6.17	28.25	4.60	4.54
Standard Error	0.05	0.08	0.03	0.06	0.05	0.02	0.05	0.07	0.02	0.05	0.05	0.01
Median	28.20	3.37	4.81	29.59	5.09	6.91	27.78	3.74	6.31	27.89	4.65	4.48
Mode	26.97	0.14	5.26	27.12	6.41	7.67	26.82	3.49	6.54	26.77	5.68	4.24
Standard Deviation	1.30	2.03	0.71	1.87	1.76	0.58	1.48	2.24	0.50	1.47	1.57	0.35
Sample Variance	1.70	4.12	0.50	3.51	3.11	0.34	2.21	5.00	0.25	2.16	2.46	0.12
Range	5.95	9.13	4.46	8.46	9.61	2.35	7.55	18.58	2.48	7.04	7.20	1.59
Minimum	26.07	0.14	1.10	25.86	0.04	5.57	24.29	0.00	4.37	24.82	0.78	4.00
Maximum	32.02	9.27	5.56	34.32	9.65	7.92	31.84	18.58	6.85	31.86	7.98	5.59
Count	683	683	683	1049	1039	1049	968	905	968.	999	951	999
Confidence Level(95.0%)	0.10	0.15	0.05	0.11	0.11	0.04	0.09	0.15	0.03	0.09	0.10	0.02
Coefficient of Variance	4.57	57.91	15.13	6.34	34.62	8.29	5.31	54.55	8.18	5.21	34.08	7.76

Table 4.3 Type III test of fixed effects on field variables for nursery habitats in the Pamlico River during the summer 2005

Variable	Effect	df	F-Value	prob > F
Temperature	Creek	3	0.44	0.73
	Period	1	0.12	0.73
	Creek x Period	3	0.88	0.48
DO	Creek	3	1.73	0.18
	Period	1	20.42	0.0001
	Creek x Period	3	1.90	0.15
Salinity	Creek	3	Inf.	<0.0001
	Period	1	0.12	0.74
	Creek x Period	3	0.69	0.56

Table 4.4 Type III test of fixed effects on site and period on the proportion of mysid at four nursery habitats in the Pamlico River during the summer of 2005

Variable	Effect	df	F-Value	prob > F
Mysid density ($3/m^3$)	**Creek**	3	13.44	<0.0001
	Period	1	20.53	0.0001
	Creek x Period	2	13.00	0.0001

Table 4.5 Results of early summer (May 24-June 7) of 2005 caging experiments in nursery areas of the Pamlico River Estuary, NC. *(*Reported variables are: IW= initial weights, FW= final weight, and Grate=exp((LN(IW)- LN(FW))/(days))-1. BC = Back Creek, EC= Eastfork Creek, LC = Long Creek, and PC = Porter Creek*)

	Site Average.			Standard Deviation			Standard error		
	IW (g)	FW (g)	Grate (g/day)	IW (g)	FW (g)	Grate (g/day)	IW (g)	FW (g)	Grate (g/day)
BC	20.05	18.77	-5.60E-03	3.85	3.58	2.16E-03	1.93	1.79	1.08E-03
EC	17.12	15.98	-4.98E-03	2.84	2.53	9.39E-04	1.27	1.13	4.20E-04
LC	15.51	15.67	1.06E-03	3.42	3.31	9.55E-04	1.53	1.48	4.27E-04
PC	19.28	18.15	-3.50E-03	5.13	4.38	1.73E-03	2.30	1.96	7.76E-04

Table 4.6 Results of late summer (June 21-July-14)caging experiments in nursery areas of the Pamlico River Estuary, NC. *(*Reported variables are: IW= initial weights, FW= final weight, Grate=exp((LN(IW)-LN(FW))/(days))-1, and* N/A = *not available. Sites are: BC = Back Creek, EC= Eastfork Creek, LC = Long Creek, and PC = Porter Creek.*)

		Site Average.			Standard Deviation			Standard error		
	Site	IW (g)	FW (g)	Grate (g/day)	IW (g)	FW (g)	Grate (g/day)	IW (g)	FW (g)	Grate (g/day)
Week1	**BC**	14.24	13.58	-6.63E-03	0.64	0.55	3.39E-03	0.37	0.32	1.96E-03
	EC	14.20	13.89	-2.95E-03	1.15	1.17	6.97E-04	0.66	0.68	4.02E-04
	LC	14.09	13.04	-1.03E-02	1.93	1.69	1.55E-03	1.12	0.97	8.94E-04
	PC	12.01	11.87	-1.56E-03	0.89	0.90	2.08E-03	0.51	0.52	1.20E-03
Week2	**BC**	13.58	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	EC	14.75	14.20	-5.09E-03	1.45	1.15	3.08E-03	0.83	0.66	1.78E-03
	LC	13.04	12.90	-1.02E-02	1.69	1.47	3.42E-03	1.53	0.85	1.98E-03
	PC	11.87	12.37083	0.003932	0.90	0.90	3.76E-03	0.52	0.52	2.17E-03
Week3	**BC**	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	EC	13.89	13.38	-5.83E-03	1.17	1.23	1.59E-03	0.68	0.71	9.17E-04
	LC	12.90	11.97	-1.61E-02	1.47	2.96	7.46E-03	1.53	2.09	5.28E-03
	PC	12.37	12.54	7.27E-0.3	0.90	0.78	4.88E-03	0.52	0.45	2.82E-03

Table 4.7 Type III test of fixed effects on field variables for nursery habitats in the Pamlico River during the summer of 2005

Variable	Effect	df	F-Value	prob > F
Growth rate (g/day)	**Creek**	3	7.56	0.0011
	Period	1	8.37	0.0082
	Creek x Period	3	12.53	<0.0001

Table 4.8 Type III test of fixed effects on metabolic indices from nursery habitats in the Pamlico River during the summer of 2005

Variable	Effect	df	F-Value	prob > F
LOC	Creek	3	1.01	0.4015
	Period	1	0.26	0.6145
	Creek x Period	2	0.38	0.6845
MMS	Creek	3	5.00	0.0054
	Period	1	0.01	0.9403
	Creek x Period	2	0.03	0.9697

Table 4.9 Mean of MMS and LOC estimates from nursery habitats in the Pamlico River during the summer of 2005

Site	LOC Early summer	SE	MMS Early summer	SE	LOC Late summer	SE	MMS Late summer	SE
BC	1.74	0.266	0.0119	0.0483	N/A	N/A	N/A	N/A
EC	2.36	0.363	0.0024	0.0130	2.26	0.407	0.0017	0.0124
LC	2.25	0.244	0.0020	0.0068	2.34	0.340	0.0025	0.0155
PC	2.02	0.242	0.0027	0.0110	2.35	0.327	0.0024	0.0072

Table 4.10 Mean growth rate of hatchery raised *P. lethostigma* under three different diets. Treatments were: Wild (live shrimp), Control (pellets), and No (no food).

	Wild		Control		No	
day	**Mean (g/day)**	**standard error**	**Mean (g/day)**	**standard error**	**Mean (g/day)**	**standard error**
2	-0.018	0.014	0.024	0.017	-0.017	0.012
4	-0.009	0.009	0.018	0.011	-0.015	0.005
6	-0.005	0.005	0.010	0.006	-0.013	0.004
8	-0.005	0.004	0.006	0.004	-0.010	0.003
10	-0.004	0.003	0.011	0.003	-0.010	0.003
15	-0.001	0.002	0.006	0.002	-0.008	0.003
19	-0.0002	0.002	0.006	0.002	-0.009	0.002
21	0.0004	0.002	0.006	0.001	-0.009	0.002

Table 4.11. Observed and predicted weights of *P. lethostigma* for early (May 24-June 7) summer of 2005 experiment.

Site	Initial weight (g)	Final weight (g)	Predicted weight (g)	MMSO
BC	19.62	17.52	19.89	.07
EC	17.12	15.98	17.03	.1
LC	15.51	15.67	15.66	.18
PC	19.28	18.15	19.10	.1

Table 4.12. Observed and predicted weights of *P. lethostigma* for late (June 21-July-14) summer experiment in 2005.

Site	Initial (g)	Week 1 Observed (g)	Week 1 Predicted (g)	Week 2 Observed (g)	Week 2 Predicted (g)	Week 3 Observed (g)	Week 3 Predicted (g)	MMSO
BC	14.24	13.58	12.94	N/A		N/A		0.07
EC	14.75	14.20	14.68	13.89	14.61	13.38	14.55	0.1
LC	14.09	13.04	13.97	12.90	14.00	12.67	13.91	0.08
PC	12.01	11.87	11.92	12.37	12.28	12.54	12.53	0.5

Table 4.13. Observed and predicted weights of wild *P. lethostigma* for late (June 21-July-14) summer experiment in 2005.

Site	Initial (g)	Week 1 Observed (g)	Week 1 Predicted (g)	Week 2 Observed (g)	Week 2 Predicted (g)	MMSO
BC	8.76	8.58	8.67	8.83	8.71	0.07
PC	8.56	8.13	8.55	7.82	8.47	0.5

Figures Legend

Fig. 4.1 Map coastal North Carolina. The field study were conducted during the summer of 2005 in four tributaries of the Pamlico River. The sites were: Back Creek (1), Eastfork Creek (2), Long Creek (3), and Porter Creek (4).

Fig. 4.2 Mysid densities at four nursery areas of the Pamlico River Estuary during the summer of 2005. The sites were: Back Creek (1), Eastfork Creek (2), Long Creek (3), and Porter Creek (4).

Fig. 4.3 Growth rate trajectories of hatchery reared fish under three types of diet. The treatments were: shrimp (Wild), pellet feed (Control), and no food (No).

Fig. 4.4 Comparison of simulated and observed weights at the end of early summer (May 24-June 7) experiment of 2005. Solid diamonds represent observed final weights and open squares represent simulated final weights. The sites were: Back Creek (BC), Eastfork Creek (EC), Long Creek (LC), and Porter Creek (PC).

Fig. 4.5 Comparison of simulated and observed weights at the end of week 1 in late summer (June 21-July-14) experiment of 2005. Solid diamonds represent observed final weights and open squares represent simulated final weights. The sites were: Back Creek (BC), Eastfork Creek (EC), Long Creek (LC), and Porter Creek (PC).

Fig. 4.6 Comparison of simulated and observed weights at the end of week 2 in late summer (June 21-July-14) experiment of 2005. Solid diamonds represent observed final weights and open squares represent simulated final weights. The sites were: Back Creek (BC), Eastfork Creek (EC), Long Creek (LC), and Porter Creek (PC). No observations or simulations were made for BC during this period.

Fig. 4.7 Comparison of simulated and observed weights at the end of week 3 in late summer (June 21-July-14) experiment of 2005. Solid diamonds represent observed final weights and open squares represent simulated final weights. The sites were: Back Creek (BC), Eastfork Creek (EC), Long Creek (LC), and Porter Creek (PC). No observations or simulations were made for BC during this period.

Fig. 4.8 Comparison of simulated and observed weights of wild caught fish from the late (June 21-July-14) summer experiment of 2005. Solid diamonds represent observed final weights and open squares represent simulated final weights. The sites were: Back Creek (BC and Porter Creek (PC).

Fig 4.9. Comparison of average growth rates (g/day) between simulated and field observations of hatchery reared *P. lethostigma* in Porter Creek during a late (June 21-July-14) summer experiment in 2005. Solid diamonds represent observed growth rates and open squares represent simulated growth rates.

Fig. 4.10 Three week long simulation of an indivdiual *P. lethostigma* from PC in late (June 21-July-14) summer 2005. After an initial weight loss concomitant with a drop in dissolved oxygen the simulated fish ends with a net biomass gain. The variables in the graph are fish weight (Wfish), growth rate (Grate) in g/day, dissolved oxygen (DO), and temperature (Ta) in C.

Fig. 4.11 Comparison of growth rates (g/day) of simulated and field observations of wild *P. lethostigma* in Back Creek from a late (June 21-July-14) summer experiment in 2005. Solid diamonds represent observed growth rates and open squares represent simulated growth rates.

Fig. 4.12 Comparison of simulated and observed growth rates (g/day) of hatchery reared *P. lethostigma* in July 2006. Solid diamonds represent observed final weights and open squares represent simulated growth rates.

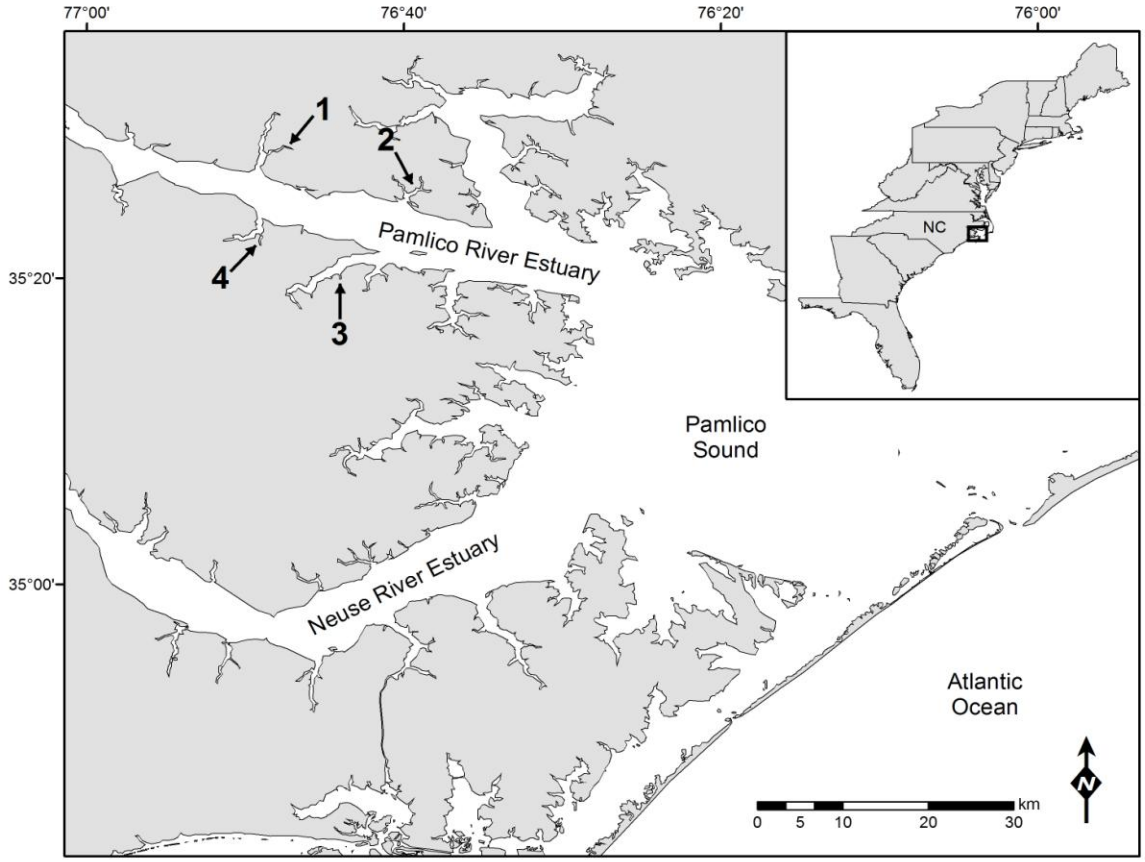


Fig. 4.1 Map coastal North Carolina. The field study were conducted during the summer of 2005 in four tributaries of the Pamlico River. The sites were: Back Creek (1), Eastfork Creek (2), Long Creek (3), and Porter Creek (4).

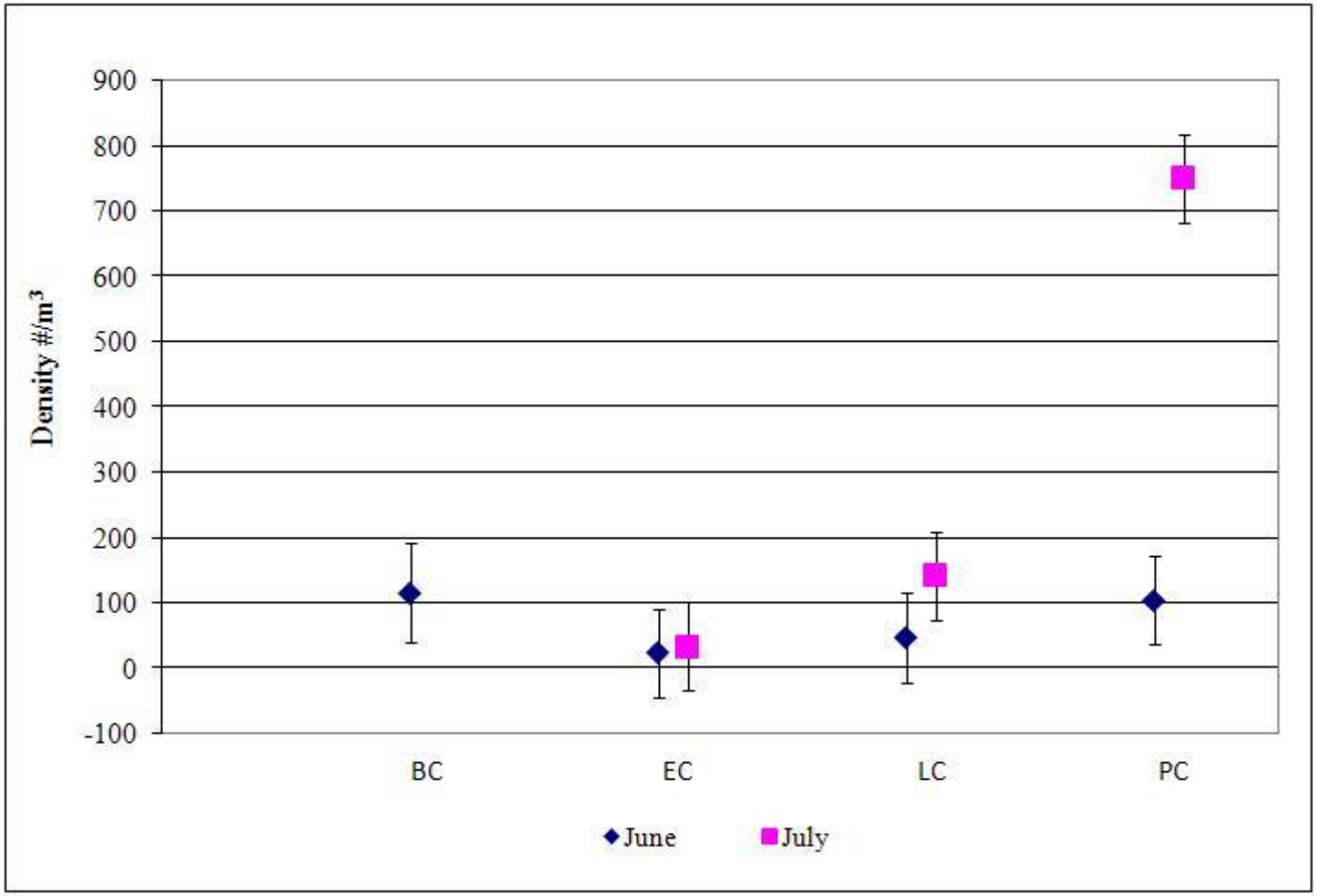


Fig. 4.2 Mysid densities at four nursery areas of the Pamlico River Estuary during the summer of 2005. The sites were: Back Creek (1), Eastfork Creek (2), Long Creek (3), and Porter Creek (4).

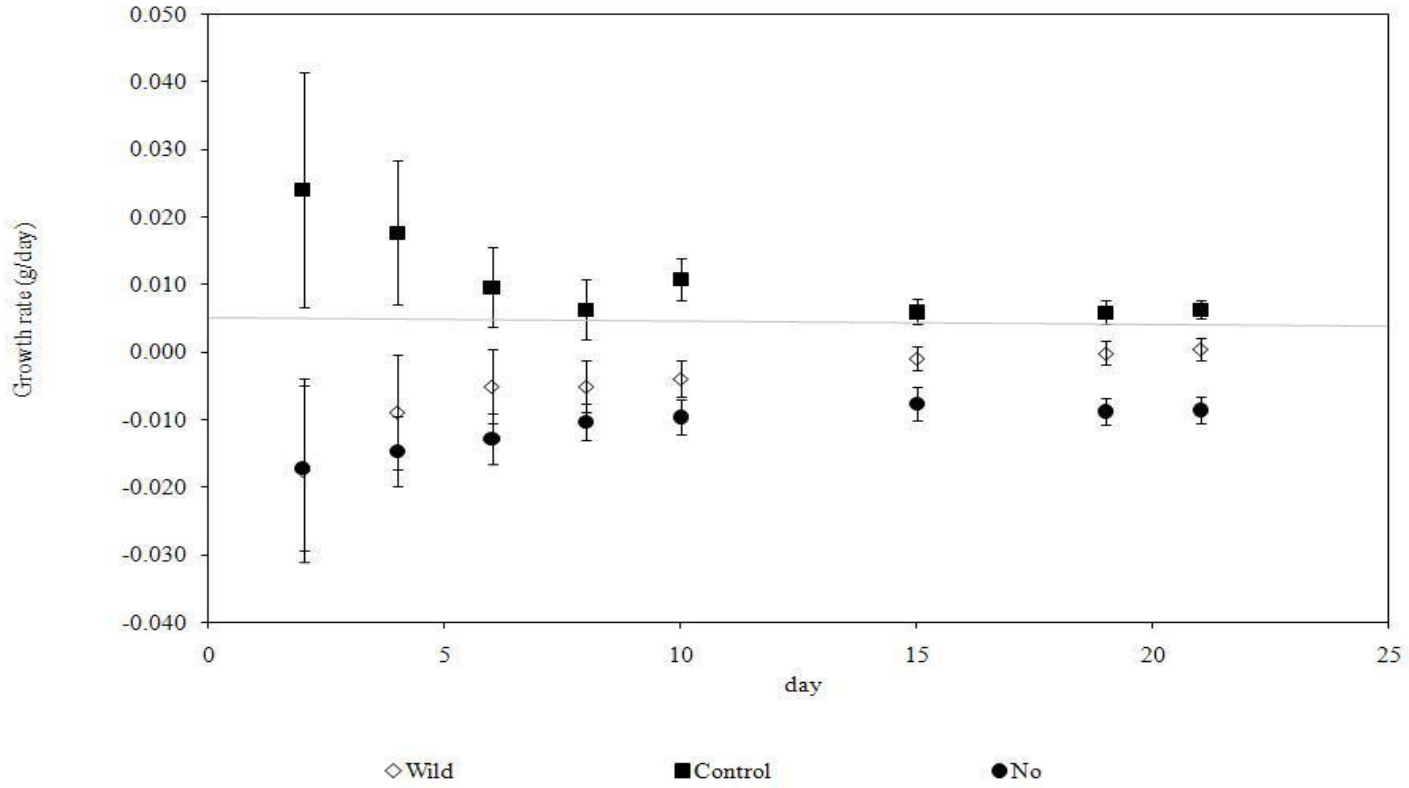


Fig. 4.3 Growth rate trajectories of hatchery reared fish under three types of diet. The treatments were: shrimp (Wild), pellet feed (Control), and no food (No).

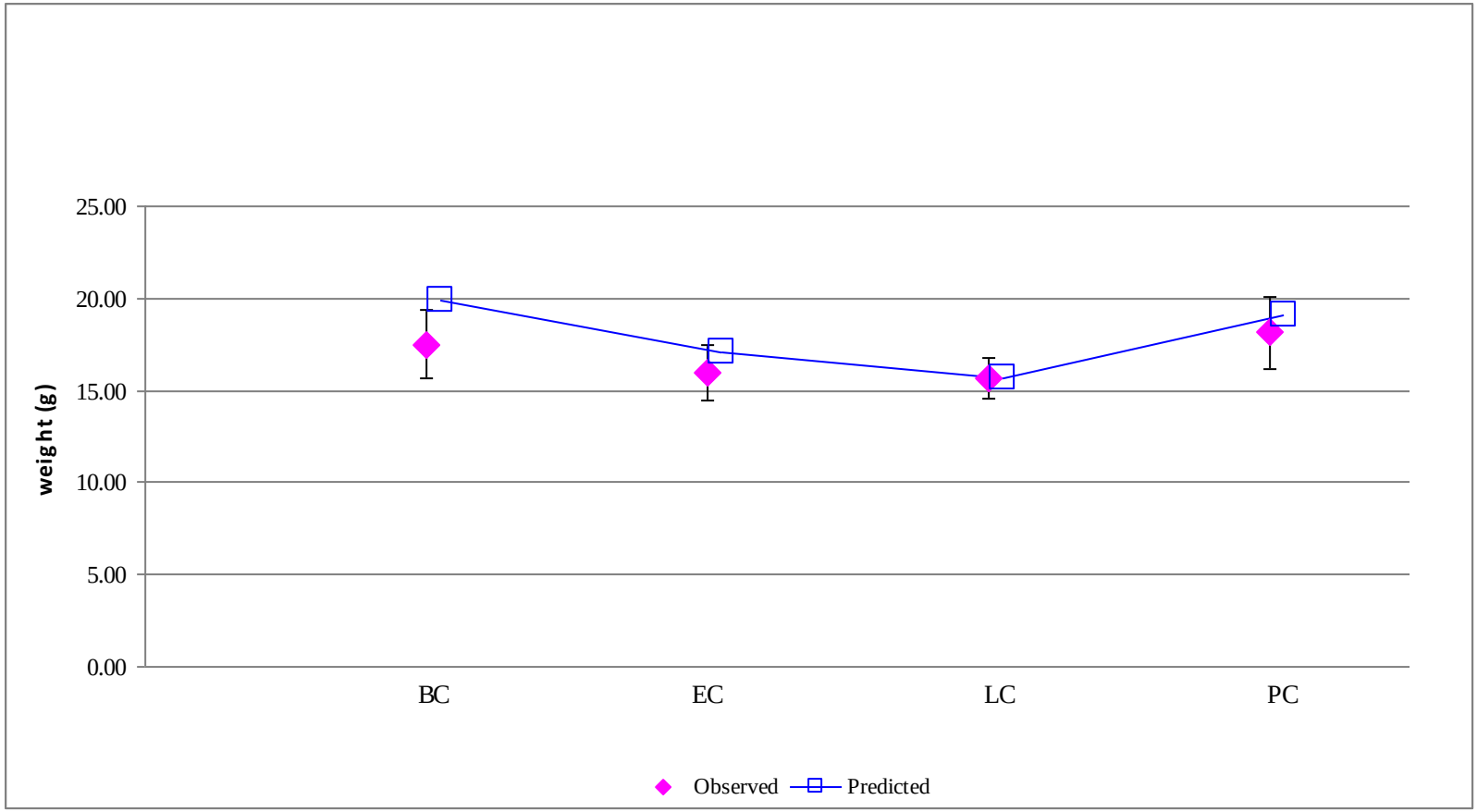


Fig. 4.4 Comparison of simulated and observed weights at the end of early summer (May 24-June 7) experiment of 2005

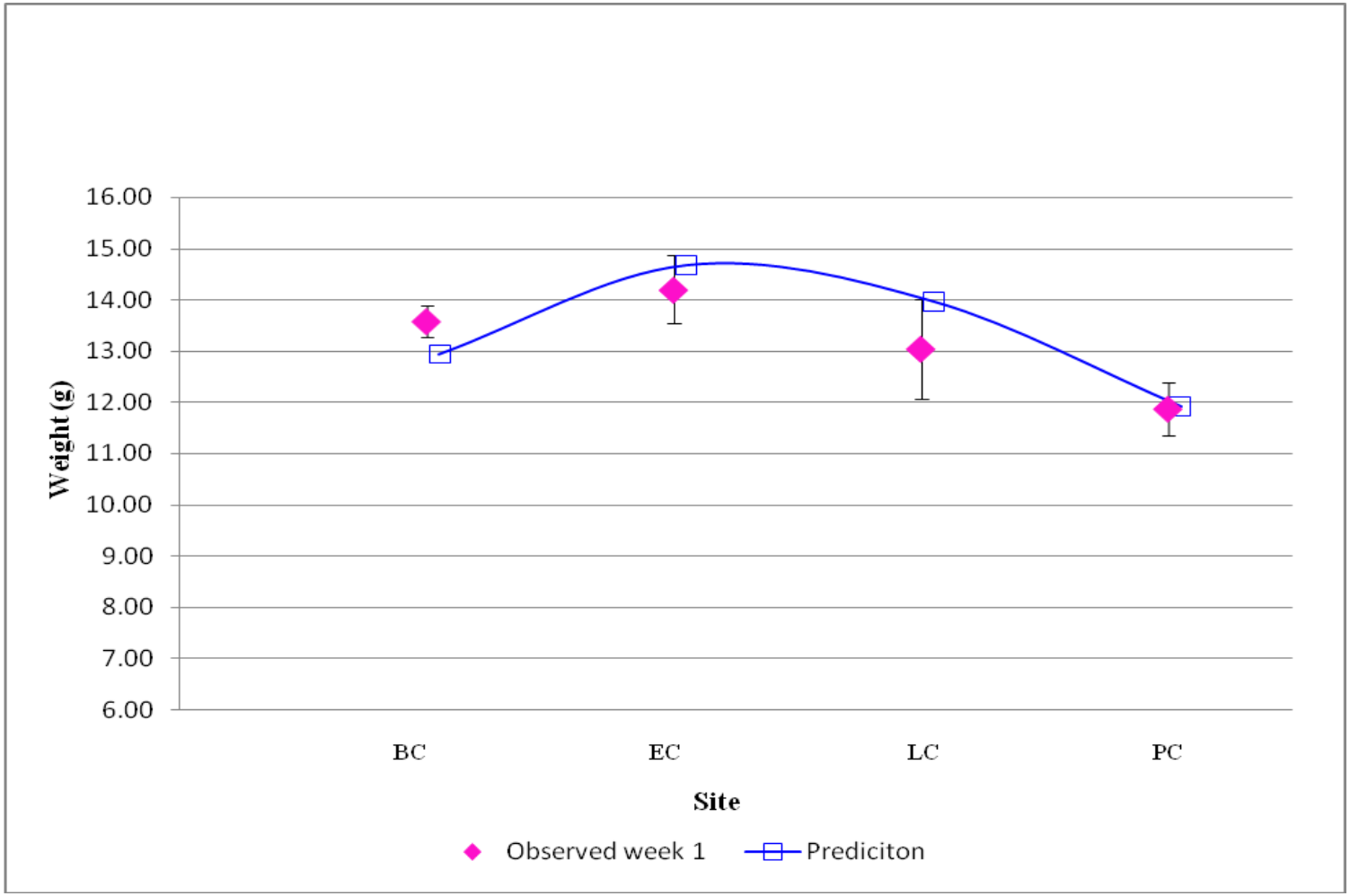


Fig. 4.5 Comparison of simulated and observed weights at the end of week 1 in latesummer (June 21-July 14) experiment of 2005

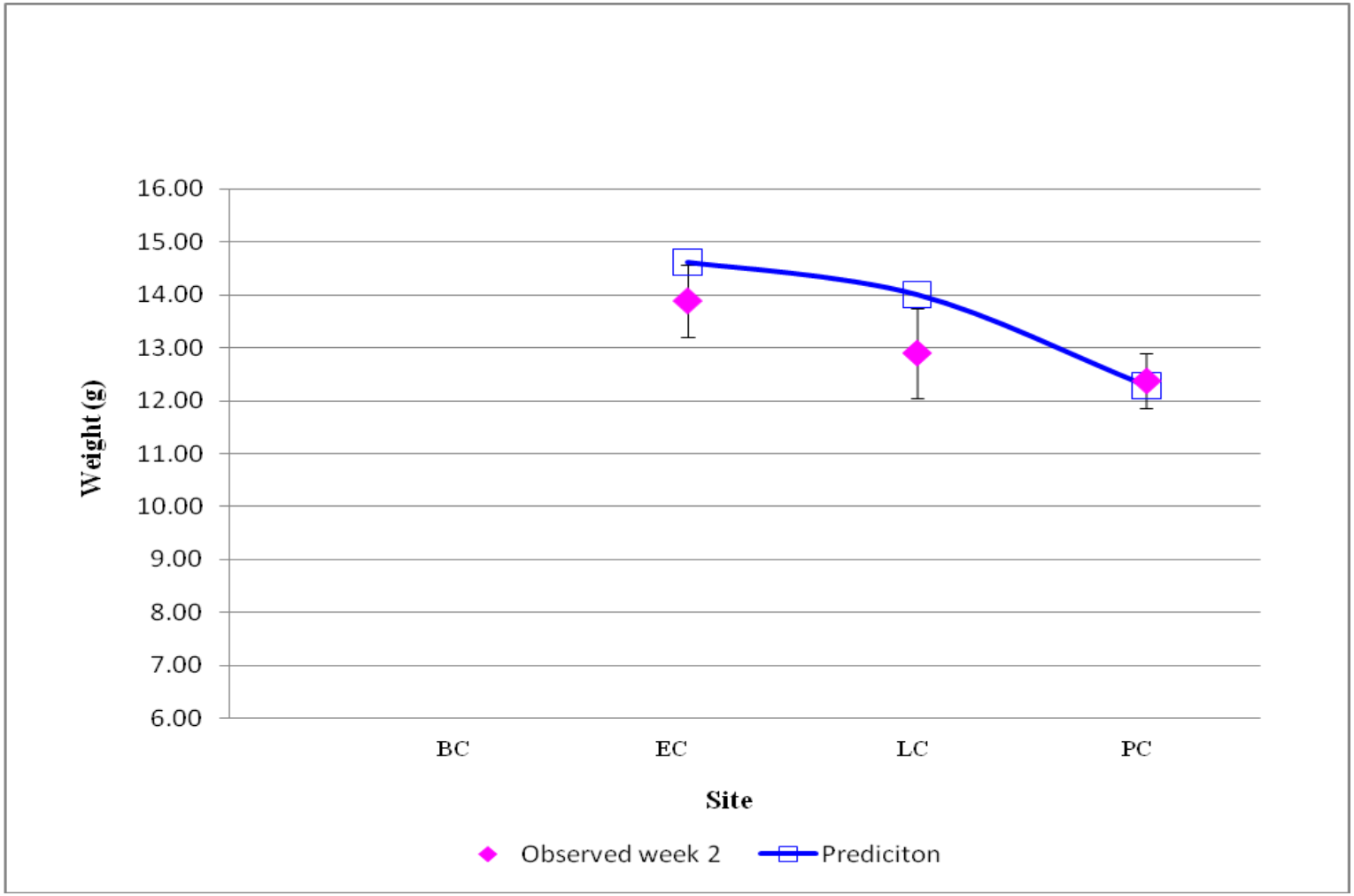


Fig. 4.6 Comparison of simulated and observed weights at the end of week 2 in late summer (June 21-July 14) experiment of 2005

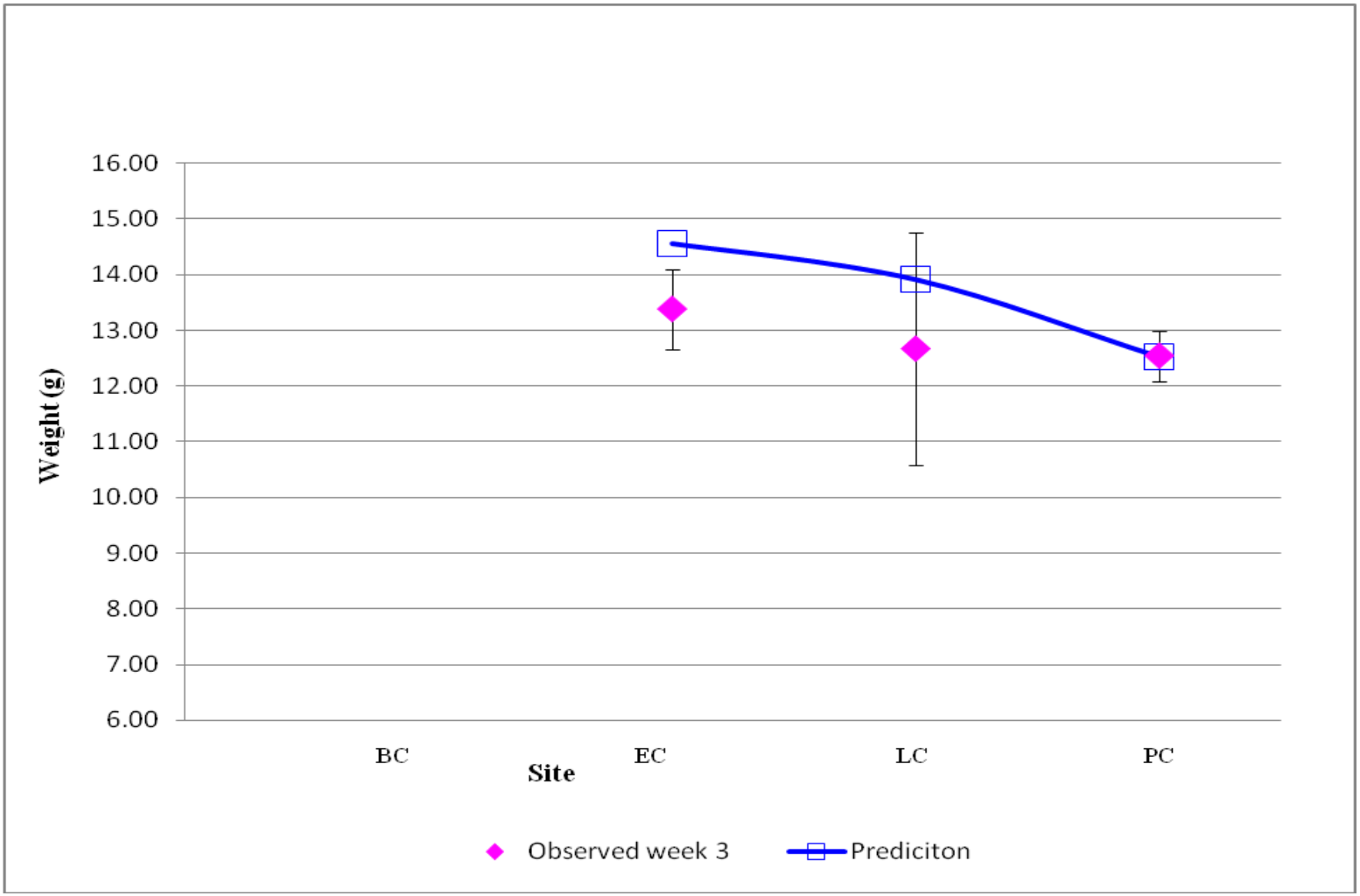


Fig. 4.7 Comparison of simulated and observed weights at the end of week 3 in late summer (June 21-July 14) experiment of 2005

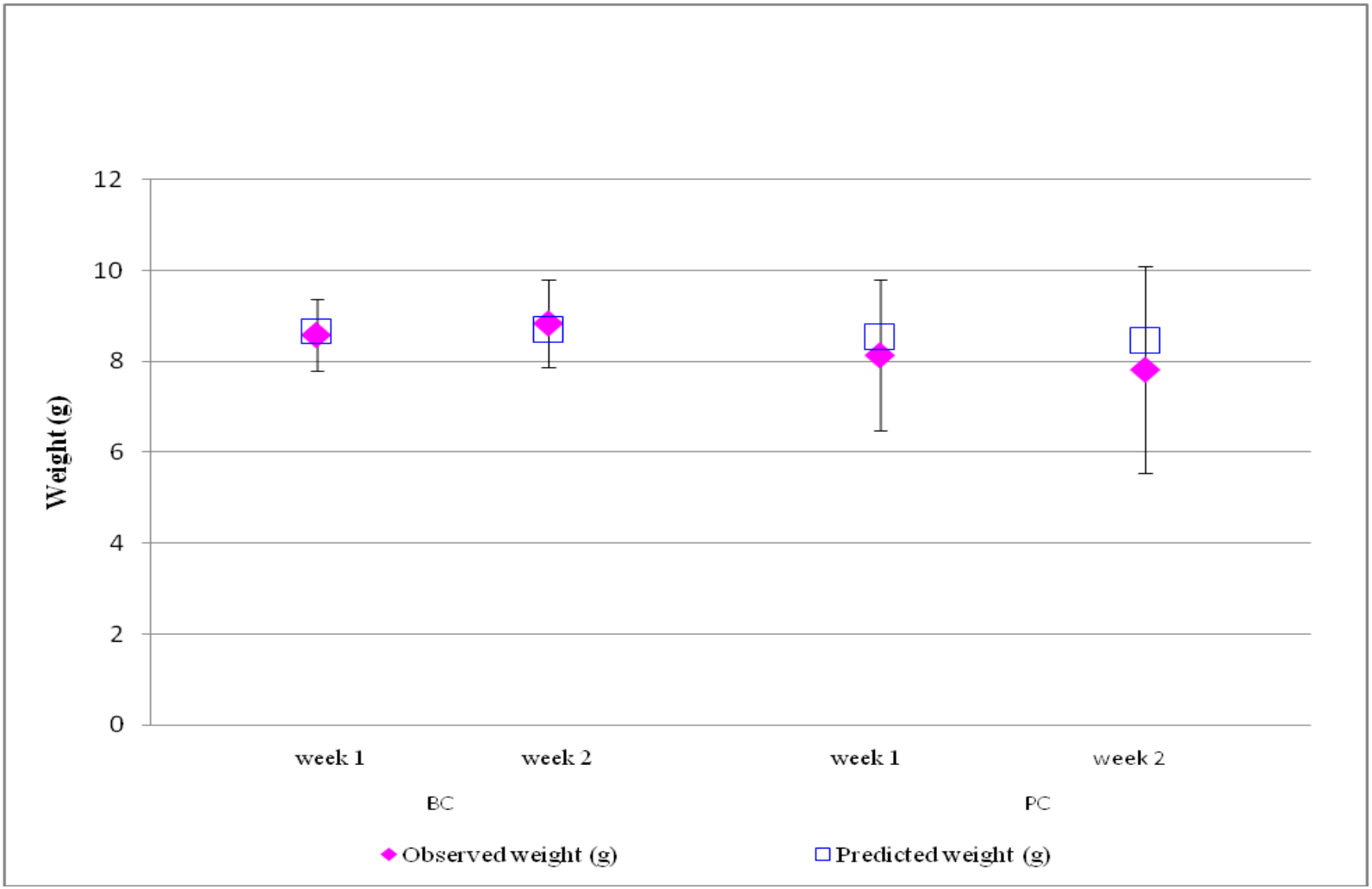


Fig. 4.8 Comparison of simulated and observed weights of wild caught fish from the late summer (June 21-July 14) experiment of 2005.

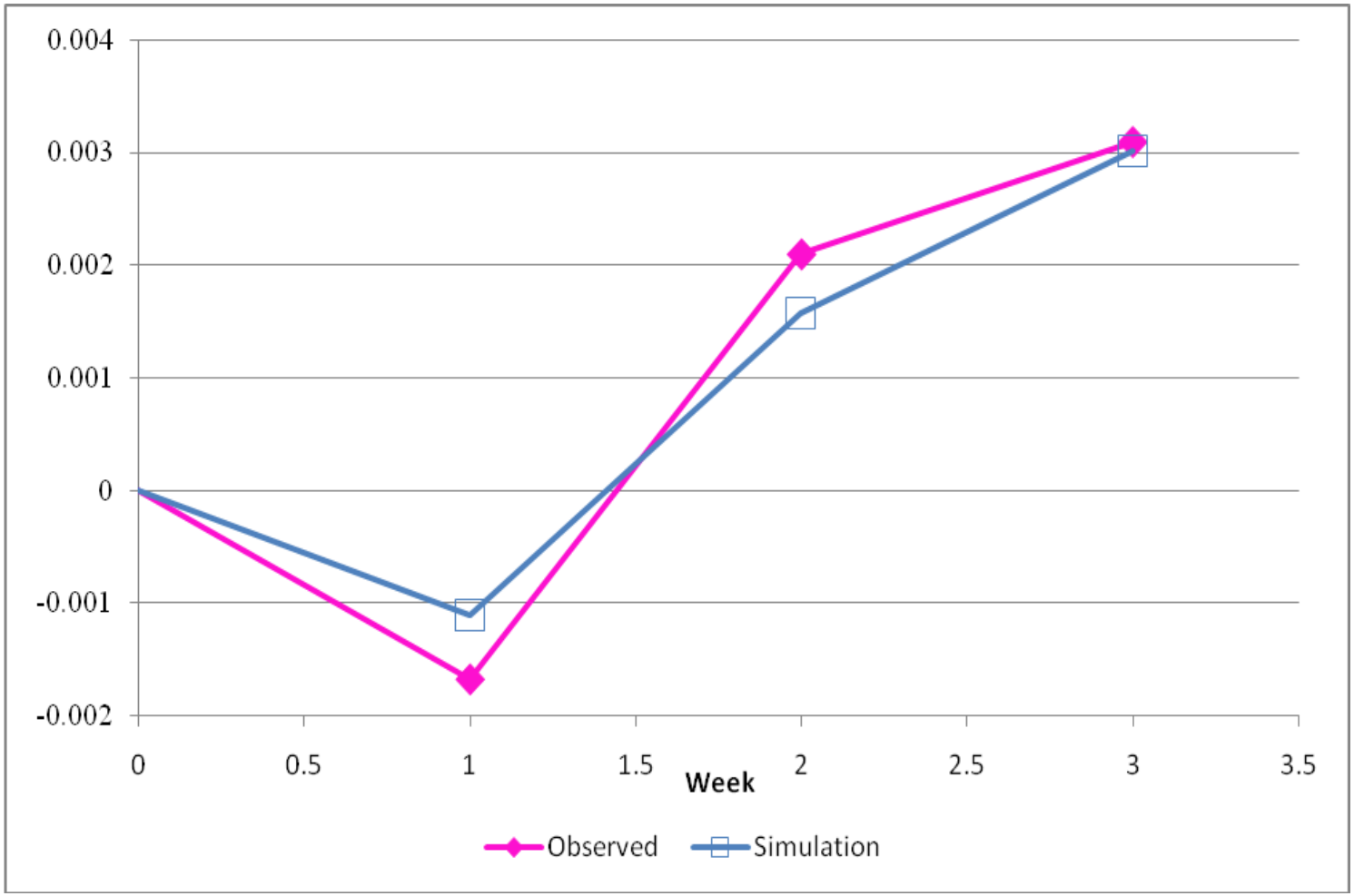


Fig 4.9 Comparison of average growth rates (g/day) between simulated and field observations of hatchery reared *P. lethostigma* in Porter Creek during a late summer (June 21-July 14) experiment in 2005

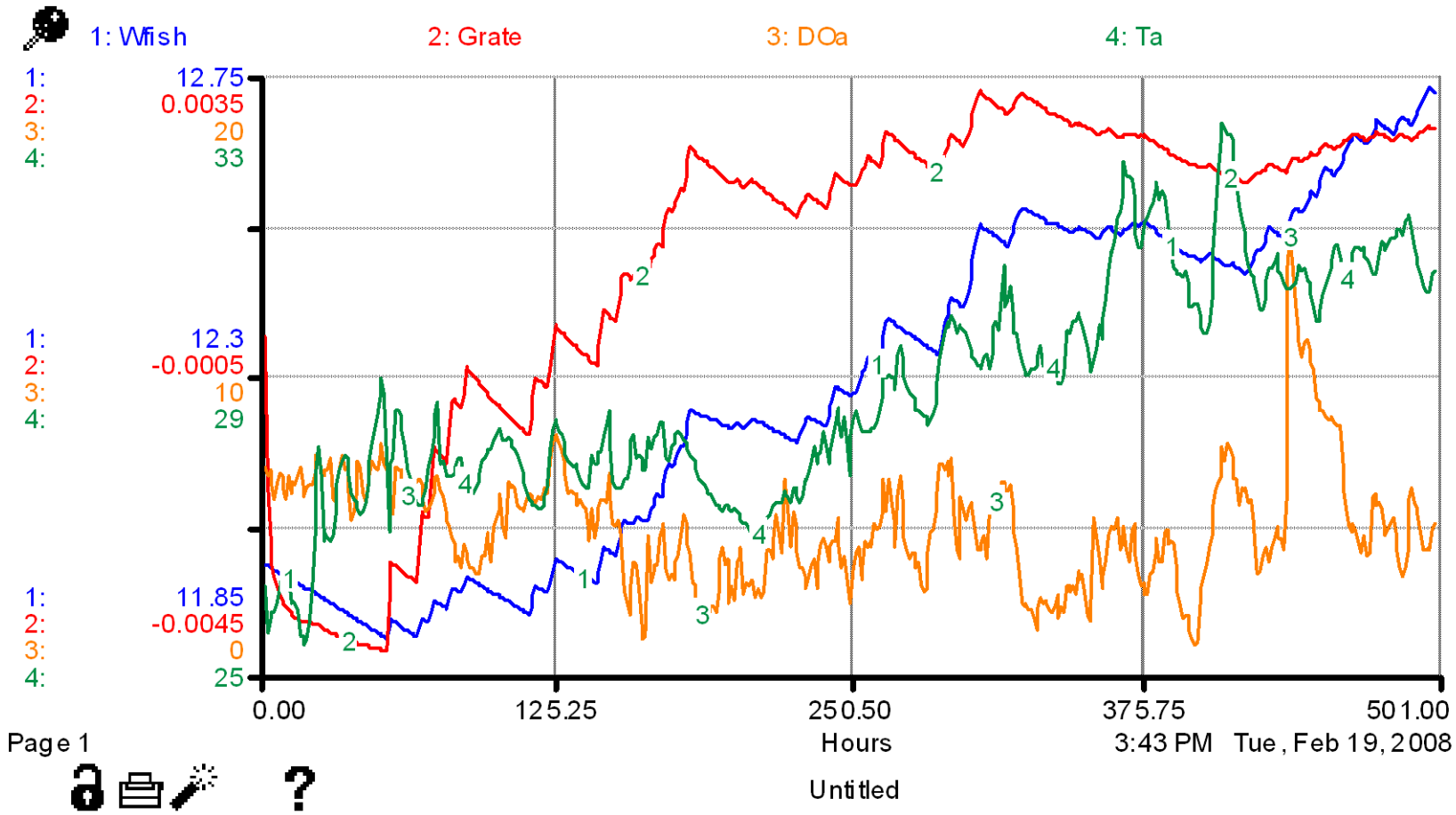


Fig. 4.10 Three week long simulation of an indivdiual *P. lethostigma* from PC in late summer 2005. After an initial weight loss concomitant with a drop in dissolved oxygen the simulated fish ends with a net biomass gain

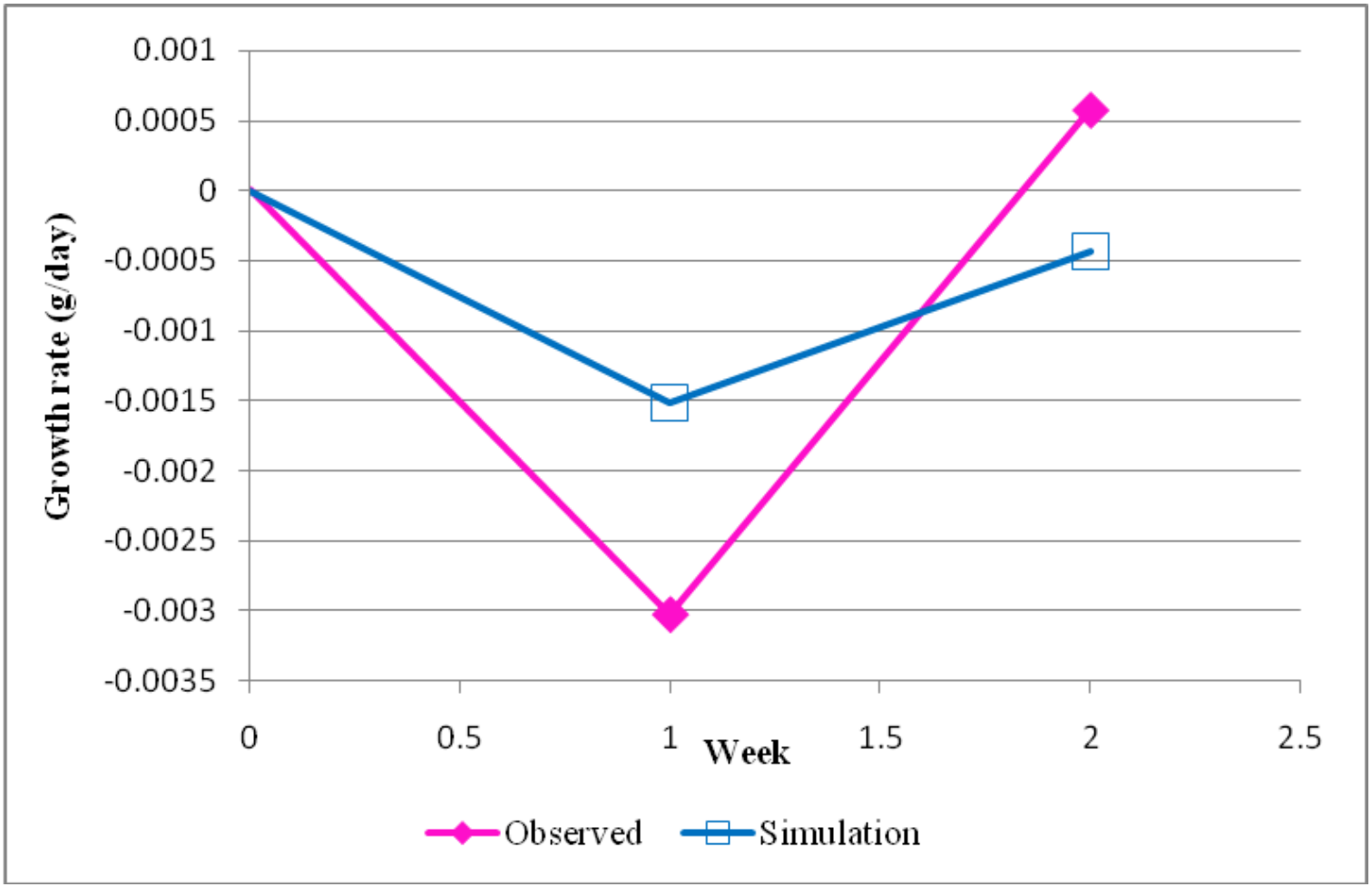


Fig. 4.11 Comparison of growth rates (g/day) of simulated and field observations of wild *P. lethostigma* in Back Creek from a late summer experiment in 2005

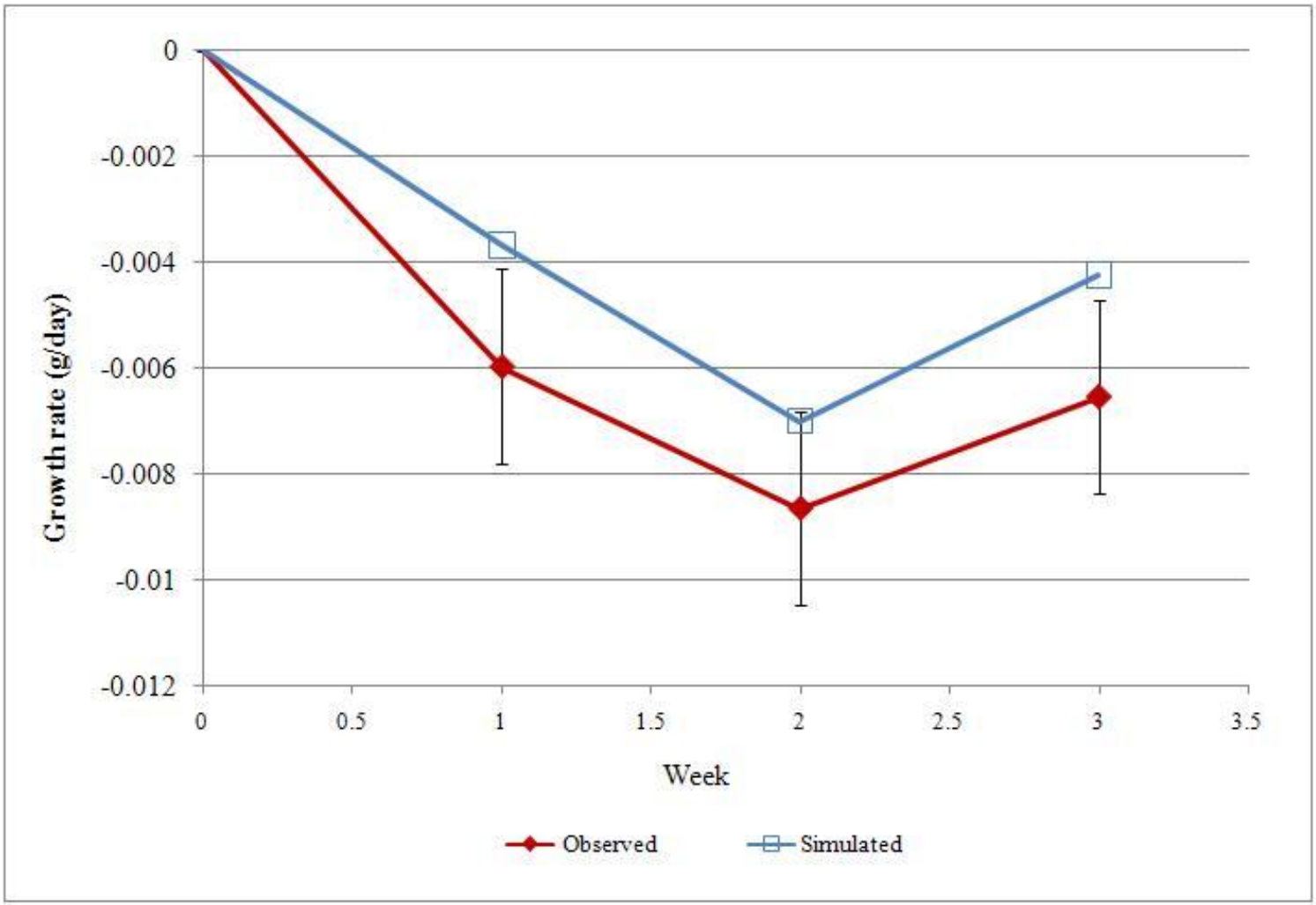


Fig. 4.12 Comparison of simulated and observed growth rates (g/day) of hatchery reared *P. lethostigma* in July 2006. Solid diamonds represent observed final weights and open squares represent simulated growth rates

Chapter 5

Summary

Overall, the study successfully demonstrated the role that abiotic factors have as determinants metabolic scope and growth rate. The laboratory portion of the study (Chapter 2) tested the hypotheses relating estimates of MMS, RMR, and LOC to environmental conditions and hence relative habitat quality (Chapter 1). Results indicated that LOC was positively correlated to DO (R^2>0.71) while within temperature treatment growth was negatively correlated to DO at 29 C, but effects were only detected at the lowest DO level (1.75mg/L) at all other temperatures bellow 29 C. While DO effects on LOC were significant, temperature effects on RMR were not statistically significant as proposed in (Chapter 1). This was not the case for growth rates which at high DO concentrations followed an optimum curve reaching a maximum at 29 C and declining shortly after this temperature (Fig. 2.4).

Interaction effects between DO and temperature growth rate proved significant. At the optimum temperature (29 C), growth rate was reduced by 50% with a 2.00mg/L reduction in DO, yet a similar reduction at 27 C did not affect growth rate demonstrating the significant interaction effects between the two treatments (Fig. 2.4). At the extremes of the temperature range used in the experiments, DO concentrations seemed to act differently on growth. At temperatures below the optimum DO was not limiting to growth except at 1.75mg/L. While at temperatures past the optimum DO effects were not significant, presumably due to breakdown of cellular processes caused by excessive temperature. The dynamics of DO and temperature interactions on growth demonstrated that the threshold model (i.e. hypoxia DO< 2.00mg/L) is not an appropriate model. Hypoxia should be evaluated as a continuum along the temperature gradient, with minimum DO requirements increasing towards the optimum

(Fig. 2.4). Although, it was predicted that at the lowest DO treatments weight loss would increase with temperature (i.e. increased respiration with increased temperature), no significant effects were detected. It is possible that a suppression of ATP turnover in response to severe hypoxia reduced activity at higher temperatures, reducing respiration rates at those temperatures and net weight loss.

Laboratory results indicated significant interaction effects on MMS mainly due to a reduction in LOC in response to acclimation to lower DO concentrations. The acclimation resulted in increased metabolic capacity at low oxygen conditions relative to non-acclimated fish when compared at normoxic conditions. MMS estimates correlated positively with temperature until the optimum was reached after which MMS tended to decline with increasing temperature. Alternatively, MMS was negatively correlated to DO which when evaluated in conjunction with temperature caused a shift in MMS that mirrored growth rate.

Although, lab results concluded that metabolic scope correlates to growth rate, MMS proved to be an ineffective field indicator of habitat quality. Because, growth rates are the product of the cumulative effects of the environment on metabolic scope, while MMS estimates only reflect the most recent conditions to which the organism has acclimated. This was evident in the poor correspondence between MMS estimates and estimated growth rates during field studies. For example, MMS estimates were inversely related to growth rate during the first experiment when only BC and LC are considered. This relationship falls apart once the other two sites are included because although EC and PC had negative growth rates, their respective MMS estimates were not statistically different than LC. In conclusion, MMS estimates are useful indicators of metabolic scope in response to recent environmental

conditions, but under variable environmental conditions it failed to capture the cumulative effects on the organism.

Alternatively, the simulation model effectively captured the cumulative effects of environmental variability on metabolic scope and accurately predicted its effects on growth. Simulation results support the hypothesis that the extent and frequency of hypoxic events will result in reduced metabolic scope, hence reduce growth rate within the habitat. Although the magnitude in scope reduction associated with hypoxia increased with temperature, the corresponding reduction in growth did not increase significantly with temperature. Presumably this was due to a reduction in activity by the organism. Although negative growth rates were recorded in most of the cage trials, growth dynamics within simulations of these trials accurately replicated observed growth patterns and the resulting weight predictions validated the accuracy of the ecophysiological model.

Further research is needed to refine model accuracy. In particular, further validation with long term growth experiments to assess the accuracy of the model over prolonged simulations. Although the model accurately predicted weight changes within the simulated periods, it was not clear from the results if cumulative error will significantly affect growth rate predictions over longer periods of time. Experiments monitoring growth rate from ingress into the nursery area to late in the summer would provide the data needed to conduct this type of model assessment. Alternatively, long term pond experiments could be used to this effect. Through this type of simulations, the ecophysiological model can provide a framework to develop hypothesis and test mechanism regulating emigration from nursery areas. According to results, the advancement of summer conditions within nursery areas

restricted metabolic scope, which adversely affected growth and survival within these habitats. The model can be used to test if environmental conditions are determinants of emigration and develop predictions relating environmental conditions and time of emigration.

The study demonstrated that within habitats environmental conditions can be unfavourable even in locations of traditionally high juvenile abundance. Which raises the question: do all habitats have environmental conditions restricting metabolic scope and if so why are these organisms found within these areas? The ecophysiological model could be coupled with a spatially explicit model of environmental conditions to predict on a per area basis growth potential within a nursery area and test if habitats differ significantly in production on a fine spatial scale and to what extent of unfavourable conditions are experienced within an area.

A spatially explicit model could also be used to test hypotheses predicting habitat use and movement within nurseries. Spatially explicit models could also be used to evaluate how predator prey interactions are affected by conditions within a habitat. The scale at which organisms face environmental variability can influence these relationships. For example, if bottom water hypoxia causes migration of benthic species it can increase predation rates by increasing their availability to pelagic predators. Alternatively, if hypoxic events are large enough to affect the whole water column the model can be used to test whether predation rates will increase due to increase encounter probability or if environmental conditions will affect predator and prey alike resulting in reduce predation due to depressed metabolic scope.

Although the model is a simplification of fish responses to the environment, this study has demonstrated that it is a valid representation of the processes affecting the organism within nursery habitats. More importantly, it provides an integrating framework in which new hypothesis can be developed and tested not only in regards to the physiology of the organism but its ecology. Even though further research is needed, the model and the approach used in this study is a new tool in the study of linkages between fisheries production and habitat quality.