MIGRATION DYNAMICS, WITHIN-ESTUARY BEHAVIORS AND

CARDIORESPIRATORY RESPONSES OF SUMMER FLOUNDER TO SELECTED

ESTUARINE CONDITIONS

‗‗‗‗‗‗‗‗‗‗‗‗‗‗‗‗

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Science

‗‗‗‗‗‗‗‗‗‗‗‗‗‗‗‗

by

Karen M. Capossela

2010

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Science

_____

Karen M. Capossela

Approved, by the Committee, April 2010

_____

Mary C. Fabrizio, Ph.D.
Committee Chairman/Co-Advisor

_____

Richard W. Brill, Ph.D.
Co-Advisor

_____

John M. Brubaker, Ph.D.

_____

Timothy E. Targett, Ph.D.
University of Delaware
Lewes, DE

TABLE OF CONTENTS

ACKNOWLEDGMENTS

This thesis could not have been conducted or completed without the assistance of my co-advisors, Dr. Mary Fabrizio and Dr. Richard Brill. I am grateful for the opportunity to explore my interests in fish physiology and behavior in an environment that fostered my development and skills as a scientist. I also recognize my committee members, Dr. John Brubaker and Dr. Timothy Targett, for the time, support and guidance that contributed to the completion of this project.

Two people deserve special mention for their generous support of this work. First, I am indebted to Dr. Peter Bushnell for his enthusiastic assistance in both the field and the laboratory. Thank you for answering a multitude of questions about fish physiology and for the sarcasm and wit that made summers on the Eastern Shore so memorable. Second, my gratitude goes to Mark Henderson for his help in the acoustic telemetry portion of this project. You have been an amazing sounding board for me, and I appreciate your insight and willingness to 'go the extra mile.'

To Hank Brooks, Jennifer Greaney, Aimee Halvorson, Wendy Lowery, Ashleigh Rhea and Troy Tuckey from the VIMS Juvenile Fish and Blue Crab Trawl Survey, thank you for your assistance with this project and for patiently teaching me the ropes of trawling with the best. My sincere appreciation goes to the people who created two unforgettable summers of work and fun at the VIMS Eastern Shore laboratory, especially to Dr. Mark Luckenbach, Sean Fate, Reade Bonniwell, Josh Smith, Edward Smith, and Linda Ward for providing the best support a graduate student could ask for. To my labmate and classmate, Branson Williams, thank you for sharing every step of this graduate experience…keep hope alive! I also thank the other members of my lab whom I have been privileged to work with: Justine Woodward, Leonard Machut and Ryan Schloesser.

Some things in life cannot be learned in the classroom. Thank you to my comrades, Valerie Arthur, Alvana Cundey, Jessica Currey, Courtney Ford, Christina Huie, Katie McBroom, Brittany Naylor and Jenn Stokes. You make me smile both inside and out! I also thank past and present VIMS friends: Todd Clardy, Carolina Funkey, Stefanie Gera, Andrij Horodysky, Chris Magel, Leonard Pace, Erin Reilly, Amy Then, Stephanie Wilson and Heather Wiseman. And I would be remiss if I did not extend a special thank you to my officemate, Pat McGrath, for putting up with quirks and questions and for the many laughs.

Finally, I wish to thank my family for their constant support and love. I especially thank my parents, Merritt and Pamala Capossela, who first taught me the value of hard work and commitment and encouraged my curiosity of the world. Also, many thanks to my siblings, Diane, Julie and David, for the loyalty and friendship that always see me through.

LIST OF TABLES

LIST OF FIGURES

CHAPTER 2. Metabolic and cardiorespiratory responses of summer flounder to hypoxia at two temperatures

ABSTRACT

A comprehensive understanding of migration dynamics, within-estuary behaviors and physiological tolerances of fish is needed to assess and predict future responses of fish to environmental disturbance and to protect habitat necessary for growth and survival. This study adds to current knowledge of summer flounder (*Paralichthys dentatus*) responses to environmental conditions by (1) examining migration and within-estuarine behaviors of adult summer flounder and (2) establishing summer flounder metabolic requirements and cardiorespiratory responses to variations in hypoxia and temperature.

To elucidate use of a southern mid-Atlantic Bight estuary by adult summer flounder, the movements of 45 fish were monitored using acoustic telemetry within a seaside lagoon along Virginia's Eastern Shore. Mean residence time in this region was 1.5 times greater than previously reported for this species. The majority of fish remained within the estuary until mid-October, although some fish dispersed from the system earlier, and some temporarily exited and re-entered the system before final dispersal. Migration into or out of the system most closely followed seasonal changes in mean water temperature, but photoperiod and other factors may also play a role. Fish resided primarily in the deeper regions of the lagoon system, where strong currents preclude the development of suboptimal conditions such as hypoxia. Summer flounder also generally exhibited low levels of large-scale activity (e.g., 100s of meters). Variations in the migratory and within-estuary behaviors indicate that individual fish use different strategies to maximize food and habitat resources. Additional research is necessary to identify preferences and behaviors of summer flounder within estuaries and along the inner continental shelf and to relate these to prey distribution.

To provide a metabolic framework for understanding how summer flounder respond to hypoxia, stopflow respirometry was used to measure changes in resting metabolic rate, oxygen extraction, gill ventilation and heart rate during progressive hypoxia at an acclimation temperature (22°C) and after an acute increase to 30°C. Summer flounder experience a 6-fold increase in gill ventilation while maintaining oxygen extraction above 50%. However, the critical oxygen saturation increased significantly from 27% at 22°C to 39% at 30°C, suggesting a lower tolerance to hypoxia after exposure to an acute increase in temperature. In addition, fish subjected to 30°C were unable to increase gill ventilation to as great an extent as at 22°C. At both temperatures, significant bradycardia was only observed when oxygen levels decreased to levels below critical oxygen saturation. Due to their ability to maintain aerobic metabolism in low oxygen conditions, summer flounder are not likely to avoid hypoxic conditions to maintain aerobic metabolism in the wild. However, exposure to hypoxia can decrease aerobic scope and consequently affect somatic and gonadal growth rates. Additional studies specifically examining the effects of hypoxia and temperature on aerobic scope are necessary to fully understand the effects of variable environmental conditions on growth and reproduction in this species.

MIGRATION DYNAMICS, WITHIN-ESTUARY BEHAVIORS AND

CARDIORESPIRATORY RESPONSES OF SUMMER FLOUNDER TO

SELECTED ESTUARINE CONDITIONS

INTRODUCTION

Estuaries are among the most productive aquatic ecosystems in the world, supporting valuable fisheries and providing seasonal nursery habitat and feeding grounds for many coastal species. Both juvenile and adult migratory fishes take advantage of the abundant food resources, warm summer temperatures and protection from predation found in estuaries to grow rapidly or prepare for spawning (Peters and Angelovic 1971, Miller et al. 1985, Gibson 1994, Scharf et al. 2006). Estuaries can also be challenging environments, due to the potential for significant environmental variability. Many estuarine organisms live near their tolerance limits, and exposure to suboptimal conditions may affect growth, recruitment success and survival.

Anthropogenic development and consequences of climate change threaten to increase the extent and duration of environmental perturbations in estuaries (Kuo and Nelson 1987, Kennedy 1990, Diaz and Rosenberg 1995). Increase in the rate of organic matter input from land associated with coastal development has been the primary cause of declining estuarine quality observed over the past three decades and is a major contributor to the development of hypoxic conditions in estuaries (dissolved oxygen less than 5 mg $O_2 \, l^{-1}$; Pinckney et al. 2001, Cooper and Brush 1991, Boesch 2002). Global warming, caused by the trapping of radiant energy by excessive greenhouse gases emitted into the atmosphere, may also play a part in altering estuarine systems (Kennedy 1990, Diaz and Rosenberg 1995, Diaz and Rosenberg 2008). On a global scale, water temperatures rise as the atmosphere warms. Temperature is the primary driver of the

abundance and distribution of aquatic organisms, and temperature-linked shifts in organismal distributions have already been observed in response to a changing climate (Perry et al. 2005, Parmesan 2006). Warmer temperatures will lead to decreased solubility of oxygen in water (Kennedy 1990), which, along with the predicted rise in sea level and increased precipitation (affecting nutrient input and stratification), may exacerbate hypoxic events (Kennedy 1990, Roessig et al. 2004). Together, these processes result in alteration of the extent of suitable habitat for estuarine species.

When exposed to suboptimal environmental conditions, estuarine fishes maintain homeostasis through avoidance or adaptive mechanisms. These responses are important for survival, but may compromise fish performance in other ways. For example, alterations in the distribution of fish allow individuals to avoid suboptimal conditions but may in turn increase energy expenditure, the risk of predation, or an individual's access to food resources (Kramer 1987). Conversely, fish that remain in suboptimal conditions may be limited in aerobic scope (the metabolic confines within which all aerobic activities must be undertaken; Claireaux and Lefrançois 2007), which in turn affects physiological processes such as growth, disease resistance, metamorphosis, or fecundity (Noga 1993, Yamashita et al. 2001, Pörtner and Knust 2006, Stierhoff et al. 2006, Landry et al. 2007, Stierhoff et al. 2009). Individual organisms therefore employ a combination of behavioral and physiological strategies in response to environmental changes. A clear understanding of migration behaviors, estuarine habitat requirements and environmental tolerances is needed to assess and predict future responses of fish to environmental disturbances and to protect habitats necessary for growth and survival.

Summer flounder

Summer flounder (*Paralichthys dentatus*) depend seasonally on estuaries for growth and survival and, as such, may be affected by increasing environmental variability. Summer flounder range from Nova Scotia to Florida, but are primarily concentrated between Cape Cod, Massachusetts and Cape Hatteras, North Carolina. Both juveniles and adults use mid-Atlantic estuaries during the spring and summer months as nursery and feeding grounds before adults move offshore to spawn in the fall. Summer flounder appear to exhibit site fidelity, returning to the same estuary after undertaking spawning migrations to the edge of the continental shelf (Desfosse 1995, Sackett et al. 2008).

Because seasonal estuarine use is important to the recruitment success and productivity of summer flounder stocks, studies are needed to clearly define estuarine habitat use and understand the affects of suboptimal conditions. Previous work primarily investigated juvenile use of estuaries as nursery habitats and physiological responses to environmental conditions (e.g., Malloy and Targett 1991, Roundtree and Able 1992, Szedlmayer and Able 1993, Tyler 2004, Necaise et al. 2005, Stierhoff et al. 2006, Stierhoff et al. 2009). Only recently have studies using acoustic telemetry investigated the ways larger juvenile and adult fish use estuaries and the precise timing of migration (Fabrizio et al. 2007, Sackett et al. 2007, Sackett et al. 2008). Similarly, despite the importance of understanding responses to environmental conditions in a metabolic framework, limited information exists on the metabolic requirements of summer flounder (Klein-MacPhee 1979). Moreover, information on the ability of summer flounder to

maintain aerobic metabolism through cardiorespiratory adjustments in the face of environmental stressors such as hypoxia or temperature changes is lacking.

Thesis Objectives

Given the limited understanding of adult summer flounder use of estuaries and their metabolic tolerances to fluctuating estuarine conditions, the objectives of this thesis research were to (1) examine migratory and within-estuary behaviors of adult summer flounder (Chapter 1) and (2) establish a metabolic framework for understanding summer flounder metabolic requirements and cardiorespiratory responses to hypoxia and temperature changes (Chapter 2). This research will add to current understanding of summer flounder behaviors, with which to assess future impacts of anthropogenic and climatic changes.

LITERATURE CITED

Boesch, D.F. 2002. Challenges and opportunities for science in reducing nutrient over enrichment of coastal ecosystems. Estuaries 25(4b):886-900.

Claireaux, G. and C. Lefrançois. 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Philosophical Transactions of the Royal Society 362:2031-2041.

Cooper, S.R. and G.S. Brush. 1991. Long-term history of Chesapeake Bay anoxia. Science 254:992-996.

Desfosse, J.C. 1995. Movements and ecology of summer flounder, *Paralichthys dentatus*, tagged in the southern mid-Atlantic Bight. Ph.D. Dissertation. College of William and Mary, Gloucester Point, VA.

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

Diaz, R.J, and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321:926-929.

Fabrizio, M.C., Henderson, M.J. and J.A. Lucy. 2007. Understanding localized movements and habitat associations of summer flounder in Chesapeake Bay using passive acoustic arrays: final report, 1 December 2007, submitted to Virginia Marine Resources Commission, Marine Recreational Fishing Advisory Board, grant #V771030.

Gibson, R.N. 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. Netherlands Journal of Sea Research 32(2):191-206.

Kennedy, V.S. 1990. Anticipated effects of climate change on estuarine and coastal fisheries. Fisheries 15:16-25.

Klein-MacPhee, G. 1979. Growth, activity and metabolism studies of summer flounder *Paralichthys dentatus* (L.) under laboratory conditions. PhD Dissertation. University of Rhode Island, Kingston, RI.

Kramer, D.L. 1987. Dissolved oxygen and fish behavior. Environmental Biology of Fish 18(2):81-92.

Kuo, A.Y. and B.J. Nelson. 1987. Hypoxia and salinity in Virginia's estuaries. Estuaries 10(4):277-283.

Landry, C.A., Steele, S.L., Manning, S. and A.O. Cheek. Long term hypoxia suppresses reproductive capacity in the estuarine fish, *Fundulus grandis*. Comparative Biochemistry and Physiology, Part A 148:317-323.

Malloy, K.D. and T.E. Targett. 1991. Feeding, growth and survival of juvenile summer flounder *Paralichthys dentatus*: experimental analysis of the effects of temperature and salinity. Marine Ecological Progress Series 72:213-223.

Miller, J.M., Crowder, L.B. and M.L. Moser. Migration and utilization of estuarine nurseries by juvenile fishes: an evolutionary perspective. In: *Migration: mechanisms and adaptive significance*. Ed. M.A. Rankin. Port Aransas, TX, Contributions in Marine Science Vol. 27, 1985.

Necaise, A.M.D, Ross, S.W. and J.M. Miller. 2005. Estuarine habitat evaluation measured by growth of juvenile summer flounder *Paralichthys dentatus* in a North Carolina estuary. Marine Ecology Progress Series 285:157-168.

Noga, E.J. Fungal diseases of marine and estuarine fishes. In: *Pathobiology of Marine and Estuarine Organisms*. Eds. J.A. Couch and J.W. Fournie. Boca Raton, CRC Press, 1993.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution and Systematics 37:637-669.

Perry, A.L., Low, P.J., Ellis, J.R. and J.D. Reynolds. 2005. Climate change and distribution shifts in marine fishes. Science 308:1912-1915.

Peters, D.S. and J.W. Angelovic. 1971. Effect of temperature, salinity, and food availability on growth and energy utilization of juvenile summer flounder, *Paralichthys dentatus*. Ed. D.J. Nelson. Proc. 3rd Natl. Symp. Radioecology USAEC Conf.,-710501-PI. p. 545-554. National Technical Information Service, Springfield, VA.

Pinckney, J.L., Paerl, H.W., Tester, P. and T.L. Richardson. 2001. The role of nutrient loading and eutrophication in estuarine ecology. Environmental Health Perspectives 109(5):699-706.

Pörtner, H.O. and R. Knust. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95-97.

Roessig, J.M., Woodley, C.M., Cech, J.J. and L.J. Hansen. 2004. Effects of global climate change on marine and estuarine fishes and fisheries. Reviews in Fish Biology and Fisheries 14:251-275.

Roundtree, R.A. and K.W. Able. 1992. Foraging habits, growth, and temporal patterns of salt-marsh creek habitat use by young-of-the-year summer flounder in New Jersey. Transactions of the American Fisheries Society 121:765-776.

Sackett, D.K., Able, K.W. and T.M. Grothues. 2007. Dynamics of summer flounder *Paralichthys dentatus*, seasonal migrations based on ultrasonic telemetry. Estuarine, Coastal and Shelf Science 74:119-130.

Sackett, D.K., Able, K.W. and T.M. Grothues. 2008. Habitat dynamics of summer flounder *Paralichthys dentatus* within a shallow USA estuary based on multiple approaches using acoustic telemetry. Marine Ecology Progress Series 364:199-212.

Scharf, F.S., Manderson, J.P. and M.C. Fabrizio. 2006. The effects of seafloor habitat complexity on survival of juvenile fishes: species-specific interactions with structural refuge. Journal of Experimental Marine Biology and Ecology 335:167-176.

Stierhoff, K.L., Targett, T.E. and K. Miller. 2006. Ecophysiological responses of juvenile summer and winter flounder to hypoxia: experimental and modeling analyses of effects on estuarine nursery quality. Marine Ecological Progress Series 325:255-266.

Stierhoff, K.L., Targett, T.E. and J.H. Power. 2009. Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA:DNA. Canadian Journal of Fisheries and Aquatic Sciences 66:1033-1047.

Szedlmayer, S.T. and K.W. Able. 1993. Ultrasonic telemetry of age-0 summer flounder, *Paralichthys dentatus*, movements in a southern New Jersey estuary. Copeia 3:728-736.

Tyler, R.M. 2004. Distribution and avoidance patterns of juvenile summer flounder (*Paralichthys dentatus*) and weakfish (*Cynoscion regalis*) in relation to hypoxia: field studies in a temperate coastal lagoon tributary and laboratory choice-trial experiments. PhD thesis. University of Delaware, Newark, DE.

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

CHAPTER 1

MIGRATION AND WITHIN-ESTUARY BEHAVIORS OF ADULT

SUMMER FLOUNDER IN A VIRGINIA EASTERN SHORE SEASIDE

LAGOON USING PASSIVE ACOUSTIC TELEMETRY

ABSTRACT

To elucidate use of a southern mid-Atlantic Bight estuary by adult summer flounder, the movements of 45 tagged fish were monitored using acoustic telemetry within a seaside lagoon along Virginia's Eastern Shore. The mean residence time of fish monitored from 8 June 2007 to 17 January 2008 (when the last fish was detected leaving the system) was conservatively estimated to be 130 days (range: 18-223 days), which is 1.5 times longer than previously reported. The majority of fish remained within the lagoon system until mid-October, although some fish dispersed out of the system earlier, and some temporarily exited and re-entered the system before final dispersal. Larger fish were more likely to disperse before mid-October and may have moved to other estuaries or the inner continental shelf during the summer and early fall. Fish resided primarily in the deeper regions of the system (upper channels) and generally exhibited low levels of large-scale (100s of meters) activity. Only 27% of fish released in the upper channels were detected in the lower channels, and they dispersed later in the fall than individuals that remained solely in the upper channels. Fish were detected returning to the system from 7 February to 7 April 2008. Fish that dispersed after mid-October were most likely to return to the system the following spring. Migration into or out of the system most closely followed seasonal changes in mean water temperature, but photoperiod and other factors may also play a role. Different migration and within-estuary behaviors indicate that individual summer flounder use different strategies to maximize food and habitat resources. Additional research is necessary to identify preferences and behaviors of summer flounder within estuaries and along the inner continental shelf and to relate these to prey distribution.

INTRODUCTION

Seasonal estuarine residence plays a vital role for juvenile and adult summer flounder (*Paralichthys dentatus*) by providing the optimal temperatures, food resources and protection from predation necessary for growth and survival during the spring and summer months. Summer flounder migrate offshore to spawn over the edge of the continental shelf in the fall and winter before migrating back inshore the following spring, often returning to the same estuary in subsequent years (Sackett et al. 2007). As a result, stock abundance is likely be influenced by local estuarine conditions (Ray 2005). A clear understanding of how summer flounder use these estuarine systems is necessary, especially in light of the continued anthropogenic alteration and degradation of estuarine environments resulting from eutrophication, shoreline development, and global climate change. The use of estuaries as nursery habitat and responses of juvenile summer flounder to estuarine conditions has been extensively examined (e.g., Malloy and Targett 1991, Roundtree and Able 1992b, Szedlmayer and Able 1993, Malloy and Targett 1994, Able and Kaiser 1994, Tyler 2004, Necaise et al. 2005, Stierhoff et al. 2006, Stierhoff et al. 2009) but significant gaps exist in our understanding of residency times and within-estuary behaviors of adult summer flounder.

The timing of adult summer flounder spawning migration varies with latitude, proceeding from north to south along the range of distribution (Nova Scotia to Florida; Smith 1973, Morse 1981, Able and Kaiser 1994, Kraus and Musick 2001). Migration timing has traditionally been determined by assessing the abundance of fish in an estuary

over time using standard fisheries methods, such as bottom trawl surveys. However,

population-level monitoring is insufficient to understand the dynamics of emigration.

Recently, the precise timing of migration was investigated for individual summer

flounder using acoustically monitored adults in the Mullica River-Great Bay estuary (NJ).

Here, a large number of fish departed the estuary in July (well before the fall), but the

precise timing varied between years (Sackett et al. 2007). Therefore, variation in

emigration may exist not only by latitude, but also among individual fish within a system,

and by year. Similarly, adult summer flounder are known to return to the same estuary in

subsequent years (Poole 1962, Murawski 1970, Sackett et al. 2007, Fabrizio et al. 2007b),

but not all summer flounder exhibit this site fidelity and factors influencing site fidelity in

summer flounder are not well understood.

Adult summer flounder are found in a variety of habitats within estuaries,

including tidal creeks and seagrass beds (Bigelow and Schroeder 1953, Dahlberg 1972,

Orth and Heck 1980, Roundtree and Able 1992a), but there is a high degree of individual

variability regarding within-estuary behaviors. Adult fish in the Mullica River-Great Bay

estuary were primarily distributed in the deeper areas of the bay near the inlet during the

summer, while more mobile fish resided in other areas of this estuary (Sackett et al.

2008). Likewise, most fish remained in the Mullica River-Great Bay system until

emigration to the outer shelf, but several adults also exited and re-entered the system

multiple times (i.e., 'temporary emigrants'). Similar patterns were observed in the

Chesapeake Bay, where sedentary adults resided at structured sites, but more active fish

traveled longer distances (Fabrizio et al. 2007b). Such variations have been noted in other

species, and may be strategies used by individual fish to maximize food and habitat resources (Kobler et al. 2009, Pautzke et al. 2010).

This study examined the use of a seaside lagoon of Virginia's Eastern Shore by individual adult summer flounder. Located in the southern portion of the mid-Atlantic Bight (MAB), these lagoons are recognized as one of the primary nursery grounds for juvenile summer flounder (Schwartz 1961, Norcross and Wyanski 1994, Kraus and Musick 2001, Desfosse 1995), but also support a large number of adults and an active recreational fishery (Richards 1970, Desfosse 1990). Previous descriptions of summer flounder use of these systems are limited to juvenile habitat preferences (Wyanski 1990, Norcross and Wyanski 1994) and adult migration patterns determined by traditional mark-recapture methods (Kraus and Musick 2001, Desfosse 1995). The timing of emigration and length of estuarine residency have not been examined on an individual basis in this region of the MAB and likely vary from those fish in systems further north. In addition, these lagoon systems experience the large temperature range typical of most MAB systems (0-30°C), but are different from larger estuaries in that they are shallow (mean depth < 3m), well-mixed, and polyhaline (>28‰) with restricted access to the ocean, minimal freshwater input, and a moderate tidal range (1.2-1.4 m, NOAA Tidal Current Prediction Center 2007). Therefore, within-system distribution and behaviors may differ between fish that use the Virginia Eastern Shore lagoons and those that primarily inhabit larger estuaries and systems exhibiting a salinity gradient.

My objectives were to describe patterns of seasonal immigration and emigration and within-estuary behaviors of summer flounder from an estuary in the southern portion of the MAB. To meet this objective, the movements and residency time of acoustically

tagged adult summer flounder were monitored in an Eastern Shore lagoon. These data were used to determine: (1) immigration/emigration rates and duration of residency and (2) spatio-temporal distribution and activity of fish within the system. It was not within the scope of this study to examine small-scale abiotic influences on migratory movements or distributions (i.e., temperature, dissolved oxygen, barometric pressure; see Sackett et al. 2007, 2008). However, the effects of seasonal temperature and photoperiod, as well as fish size, on immigration, emigration, and residency were considered (Smith 1973, Able and Kaiser 1994, Rogers and Van Den Avyle 1983, Packer et al. 1999). Similarly, because tidal stage, time of day and temperature have been associated with flatfish activity (Casterlin and Reynolds 1982, Wirjoatmodjo and Pitcher 1984, Olla et al. 1972, Malloy and Targett 1991, Szedlmayer and Able 1993, Fabrizio et al. 2007b), these factors were also considered when examining within-estuary activity.

METHODS

Study Site

The Eastern Shore lagoon system behind the barrier islands near Wachapreague, Virginia (Fig. 1) is primarily connected to the Atlantic Ocean via Wachapreague Inlet, although oceanic water from Metompkin Inlet to the north and Quinby Inlet to the south also penetrate the barrier islands. The two main channels leading from Wachapreague Inlet further divide into channels that cut through the marsh before opening into large, shallow tidal flats. The entire system is characterized by strong currents due to constrictions at the inlet and in the channels (Conrath 2005), although channel currents generally dissipate with distance from the inlet. Channels were identified as areas approximately 3-12 m deep inside the inlet constricted by *Spartina alterniflora* dominated salt marsh. Sediment type follows the energy gradient, with coarse sand within and near the inlet, and progressively finer (muddy) sediments at increasing distance from the inlet (Wyanski 1990).

I divided the Wachapreague lagoon system into four regions (Fig 1):

1. Wachapreague Inlet (approximately 625 m wide) – the primary point of ingress and egress of fish characterized by depths of approximately 6-15 m and strong currents;

2. Upper channels – the channel leading north away from Wachapreague Inlet and its divergent channels;

3. Lower channels – the channel leading south away from Wachapreague Inlet and its

    divergent channels;

4. Tidal flats (also known locally as 'bays') – the shallowest bodies of water included in

    the study (approximately 1-2 m). Although several tidal flats are present in this

    area, only Swash Bay was included due to its popularity with summer flounder

    anglers for recreational fishing.

        Environmental conditions in the inlet, channels and tidal flat were monitored from

8 June 2007 to 29 July 2008 with three YSI multi-parameter water quality data loggers

(Model 6920-O, Yellow Springs Instruments) and six temperature loggers (U22-001,

HOBO; Fig.1). The latter recorded water temperature once per hour. The YSI data

loggers recorded temperature, salinity and dissolved oxygen concentration ([DO]) once

per hour. YSI units were replaced with calibrated units every 1-2 weeks in the summer

and, as fouling diminished, every 2-4 weeks thereafter. Despite such precautions,

erroneous recordings due to membrane fouling, battery failure and calibration drift did

occur; these recordings were easily detected and removed upon examination of the data.

Photoperiod for each day in the study was acquired from tide prediction software (Jtides,

www.arachnoid.com/JTides).

Telemetry and Tagging

        On 22 May 2007, 50 adult summer flounder were captured by hook and line and

internally tagged with individually coded transmitters using established procedures

(Fabrizio and Pessutti 2007). Tagged fish (261-558 mm TL) were considered adults

because summer flounder can reach maturity at 240-300 mm TL (Morse 1981). All fish

were tagged and released in the upper channels with the exception of one fish that was captured, tagged and released in the tidal flat. Transmitters (V9-2L-R64K, 69 kHz; VEMCO) were 30 mm long and 9 mm in diameter with a delay time between 60 and 180 seconds and had a projected 14-month battery life. Immediately after capture, summer flounder were anesthetized with 60 mg l^{-1} AQUI-S before surgical implantation and were allowed to fully recover in an onboard aquarium before release at the capture site. Before release, fish were measured (TL) and externally tagged with an individually numbered T-bar anchor tag inserted near the caudal peduncle to alert anglers to report recaptures.

Fish locations were passively monitored with 27 receivers (VR2, VR2W; VEMCO) deployed throughout the study site between 21 May and 8 June 2007 (Fig. 1, 1-27). Receivers were attached to an anchored line fitted with a buoy and positioned near the bottom of the water column (≤ 1 m) with the hydrophone oriented downward to better detect bottom-dwelling summer flounder. Range tests in this system were performed in a similar manner to those conducted by Fabrizio et al. (2007b). Approximately 50% of emitted pings from a test transmitter (VEMCO) were detected by a VR2 receiver (VEMCO) 350 m away, and receivers were therefore placed ≥ 700 m apart to monitor fish movements on the scale of 100s of meters.

Most receivers were retrieved on 31 January 2008; eight receivers remained in the vicinity of the inlet to permit detection of fish that returned to the Wachapreague system the following year (16-18, 22, 24-27). Eleven receivers from a separate study on rays (Josh Smith and John A. Musick; Fig 1, receivers marked "J") were deployed in the system on 26 June 2007 (J01-J03) and 26 July 2007 (J05-J09, J11-J12, J14), and retrieved on 17 November 2007, with the exception J14 which remained in the system

until 12 December 2007. The data from four of those receivers were included as upper

channel (J01, J02, J07) and inlet (J14) region data in our analyses of distribution and

activity due to their proximity to our own receivers. The remaining seven receivers

provided supplementary coverage of the Wachapreague system. Several receivers were

lost before the retrieval dates, most likely due to strong currents, but gaps in receiver

coverage did not inhibit assessment of immigration and emigration or regional use due to

the presence of other nearby receivers.

Summer flounder migratory and within-estuary behaviors were examined from 8

June 2007 (last date of receiver deployment) until the last fish departed on 17 January

2008. The start date (8 June 2007) was approximately two weeks after the release of

tagged fish. This limited possible atypical activity patterns due to recovery from capture

and surgery (Knights and Lasse 1996, Rogers and White 2007). The number and timing

of summer flounder returns to the Wachapreague system were also monitored from 18

January through 29 July 2008, after which all instruments were retrieved.

Migratory Behaviors

A fish was considered to have emigrated on the last day it was detected in the

Wachapreague system. Likewise, fish were considered to have immigrated (i.e., to have

returned) to the Wachapreague system when they were first redetected at the inlet or

within the system after dispersing. Because Wachapreague Inlet is the primary location

for fish emigration, dispersals were only considered for statistical analysis when fish

were last detected at the inlet. The fate of fish after last being detected elsewhere within

the system could not be conclusively determined, and as such these were treated as

censored observations for statistical analysis. Fish detected at or near the inlet (17-22, J14), but undetected for two consecutive weeks, potentially exited and re-entered the system before final emigration (Sackett et al. 2007) and were considered temporary emigrants.

Data were examined over weekly intervals to determine patterns of seasonal migration and to estimate mean residence times. Dispersal and return rates were calculated with the Kaplan-Meier (KM) estimator, a nonparametric approach requiring no assumptions about the underlying hazard function and accommodating censored observations (Pollock et al. 1989, Bennetts et al. 2001). Temporary migrants were considered residents until the final dispersal out of the inlet. The residence time of uncensored fish was used to calculate a mean residence time for summer flounder in the Wachapreague system.

A piecewise linear regression was used to identify the abrupt change in dispersal rate (the change-point); non-linear least-square estimation was used to fit this model to the data (NLIN procedure in SAS, SAS Institute Inc., Cary, NC). A piecewise linear regression allows for different relationships between the response and the explanatory variable depending on the ranges of values for the explanatory variable (e.g., Ryan et al. 2002). The time before the change-point was considered to be the 'residency period,' a time during which most fish were found within the system. The time after the change-point was considered the 'emigration period,' during which most fish were observed emigrating from the Wachapreague system. An odds ratio was used to test whether the timing of emigration (i.e., during the residency or emigration period) affected the likelihood of a fish returning to the Wachapreague system the following year.

The effects of mean monthly temperature and mean monthly photoperiod on monthly emigration (log transformed to improve homogeneity of variance) were examined with a multiple linear regression (GLM procedure in SAS, SAS Institute Inc., Cary, NC). The statistical model fit to the data was:

$$Y_i = \beta_0 + \beta_1\, X_{1i} + \beta_2\, X_{2i} + \varepsilon_i \tag{1}$$

where Y_i is the percent of fish that dispersed for month i, β_0 is the overall mean, β_1 measures the change in Y_i per unit change in X_{1i}, X_{1i} is the mean temperature for month i, β_2 measures the change in Y_i per unit change in X_{2i}, X_{2i} is the mean photoperiod for month i, and ε_i is the random error for the ith observation.

In addition to these environmental variables, I examined the effect of fish size on the probability of emigration before or after the change-point (i.e., residency or emigration period) using logistic regression (LOGISTIC procedure in SAS, SAS Institute Inc., Cary, NC). The statistical model fit to the data was:

$$g(x) = \beta_0 + \beta_1\, X_1 \tag{2}$$

where $g(x)$ is the natural log of the odds of a fish dispersing before or after the change-point, β_0 is the intercept, β_1 measures the change in $g(x)$ per unit change in X_1, and X_1 is fish size. Goodness of fit statistics were calculated to assess the fit of the model.

Within-Estuary Behaviors

Temporal and spatial distribution of summer flounder in the Wachapreague system were described by examining summer flounder use of the defined regions (inlet, upper channels, lower channels, tidal flat) on a monthly basis until all fish dispersed. The proportion of fish in each region, and the proportion of time fish spent in each region were calculated by month. In a given month, the proportion of time fish spent in all regions summed to one, but because a single fish could occupy more that one region in any given month, the sum of the proportions of fish in each region for a given month could exceed one. This same methodology was used to calculate proportions of time and fish in each region by residency and emigration periods (excluding October, the month in both periods), and the z statistic was used to test for differences in the proportions for the two periods (Fleiss 1981). For fish that moved between regions, the length of time between the last region-specific detection to the next region-specific detection was not included in the calculations because fish location during that interval could not be objectively assigned to a region. All proportions were expressed as percentages.

Movement between receivers was used to develop an activity index, taken as the total number of times a fish moved between receivers during a given period of time. A fish was assigned an activity index value of one for each arrival at a new receiver. An activity index of zero indicated that a fish was detected at a receiver, but did not move between receivers. Consequently, the activity index was a discrete count. Activity index was summarized weekly for individual fish by tidal stage (ebb, slack before ebb, flood, slack before flood) within each time of day interval (day or night). Day (10:00-16:00) and night (22:00-4:00) were restricted to non-consecutive six hour periods to minimize

autocorrelations associated with successive observations on the same fish during day and night periods (Rogers and White 2007). Mean temperature was computed for each period (tidal stage, time of day and week combination) from temperature recordings from the two YSI receivers in the upper channels.

To determine how activity changed, the relationship between activity indices and week, time of day, tidal stage and temperature was examined with a repeated measures generalized linear model (GENMOD procedure in SAS, SAS Institute Inc., Cary, NC). The statistical model fit to the data was:

$$\log(\lambda_{ijk}) = \mu + \alpha_i + \delta_j + \tau_k + \gamma \qquad (3)$$

where $\log(\lambda_{ijk})$ describes the link function, λ_{ijk} is the mean activity for week i, time of day j and tidal stage k, μ is the overall mean activity, α is the week effect ($i = 1\text{-}32$), δ is the time of day effect (j=day, night), τ is the tidal stage effect (k=ebb, flood, slack before ebb, slack before flood), and γ is the effect of mean temperature. All plausible interactions (temperature*tidal stage, temperature*time of day, temperature*week) were investigated, but were removed from the final model if non-significant at an alpha level of 0.05. All effects in this model were considered fixed effects. Because activity index data are count data, the fit of the negative binomial and the Poisson distribution were examined using the FitDistr script in R (R Foundation for Statistical Computing, Vienna, Austria). The negative binomial distribution was selected because the fit had the lowest Akaike's Information Criterion (AIC) value (Burnham and Anderson 2002).

Activity index data represented observations on the same fish over time (repeated measures), observations which are likely to be correlated. In a repeated measures generalized linear model, generalized estimating equations (GEEs) are used to calculate the working correlation matrix that describes the nature of the correlation among repeated measurements within subjects (Littell et al. 2006). Several correlation structures were tested, and the Quasi-likelihood Information Criterion (QIC; a modification of AIC applied to models fit by GEE) was used to identify the correlation matrix that best described the relationship among activity indices for a given fish (Littell et al. 2006). Several plausible correlation structures (independent, exchangeable, first-order autoregressive and unstructured; Littell et al. 2006) were examined, and the first-order autoregressive structure best fit the observations. This structure assumes correlation of observations is a function of their distance in time (Littell et al. 2006), which is appropriate for acoustic data because fish movements closer together in time may be more correlated than those farther apart in time.

RESULTS

Migratory Behaviors

Fish included in all subsequent analyses were those that were alive and detected at receivers as of 8 June 2007. As a result, 45 out of 50 tagged fish were included in analyses (278-558 mm TL). Of the 5 fish eliminated from analyses, one was assumed dead (all detections were at a single receiver), another departed through the inlet before 8 June 2007 (and was detected in Delaware Bay on 9 June 2007, Dewayne Fox, *pers. comm.*), and the remaining 3 were not detected by moored receivers in the Wachapreague system (these fish may have departed undetected or may have been harvested by recreational anglers).

Three types of behaviors were observed among summer flounder in the Wachapreague system: those that emigrated early (during the residency period), those that temporarily emigrated (i.e., exited and re-entered the system multiple times during the study period), and those that emigrated late (during the emigration period). Fish emigrated from the Wachapreague system throughout the study but dispersal rates changed significantly in mid-October [change-point=week 18 (11 October 2007); F=212.2, P<0.05; Fig 2]. Based on the KM model, only 27% of tagged summer flounder were estimated to have dispersed by 11 October 2007. Accordingly, the period from 8 June 2007 to 11 October 2007 was identified as the residency period (Fig. 2), and the majority of fish that dispersed during this period emigrated shortly after tagging in June (Fig. 3). June was also the month with the highest number of censored fish (i.e., fish of

unknown fate; Fig. 3). The emigration period was identified from 11 October 2007 until the last fish departed on 17 January 2008 (Fig. 2). During this period, emigration rates increased such that 50% of fish had departed by 11 November 2007, but most fish (31%) dispersed in December. Seven fish were temporary migrants, remaining undetected for 14-154 days after detection at or near the inlet before redetection and final emigration for the season. The mean (± SE) residency time for summer flounder in the Wachapreague system was 130 ± 13 days, or about 4.3 months (range: 18-223 days).

A total of 17 summer flounder (36%) returned to the Wachapreague system between 7 February and 7 April 2008 (Fig. 4). Four of these fish were fish that had been censored in 2007, and thus their dispersal dates were unknown. Of the returning fish with known dispersal dates, 58% (n=11) had dispersed during the emigration period and 29% (n=2) had dispersed during the residency period. Consequently, the odds of returning to the Wachapreague system were 3.5 times greater if a fish departed during the emigration period than if a fish departed during the residency period (odds ratio=1.4/0.4). Additional fish may have returned to the Wachapreague system but were not detected due to the limited number of receivers in the system from February to July 2008.

The emigration period was characterized by large seasonal variation in water temperature as compared with the residency period ($CV_{residency}$=9.5%, $CV_{emigration}$=46%). Photoperiod, however, was characterized by a smooth and almost constant change over time ($CV_{residency}$=7.7%, $CV_{emigration}$=5.8%). Emigration from the Wachapreague system appeared to follow more closely the steep decline in temperature rather than the gradual shift in day length (Figs. 5, 6). The multiple linear regression that included both temperature and photoperiod as predictors was significant (F=20.3, P<0.05), and

explained 89% of the variance in monthly dispersals. Mean monthly temperature was significant (Type III SS, F=6.39, P<0.05), although the addition of photoperiod did not contribute to the significance of the model (Type III SS, F=0.94, P>0.05). The period of time over which fish returned (3 months, see results below) was inadequate to statistically examine the effect of mean monthly temperature or mean monthly photoperiod on fish returns. Data from the YSI data loggers confirmed that the system was polyhaline (>28‰) and that [DO] remained above the critical oxygen level (27.2%, 1.97 mgO$_2$l^{-1}) for adult summer flounder experiencing typical summer temperatures (22°C; Capossela, Chapter 2).

The logistic regression relating dispersal probabilities and fish size showed that smaller fish were more likely to emigrate during the emigration period, and this probability is inversely related to fish size (χ^2=8.45, P<0.05). In other words, larger fish are more likely to leave the system in summer or early fall than are smaller fish. The measure of fit indicated that predicted and observed frequencies were not significantly different (χ^2=3.86, P>0.05), thereby making the logistic regression an adequate model. The mean (± SE) sizes of fish that emigrated during the residency and dispersal periods were 437±21 mm TL and 367±13 mm TL, respectively. Again, insufficient return data precluded statistical analysis of fish size and returns.

Within-Estuary Behaviors

Summer flounder primarily used the upper channels during the residency period, although fish were detected in all habitats over the course of the study (Fig. 7, 8). Fish occupied the upper and lower channels for 78% and 19% (respectively) of the total time

that fish were detected in the Wachapreague system (Table 1). With the exception of the single fish released in the tidal flat, all fish were detected in the upper channels, but only 27% ($n=12$) of fish that were detected in the upper channels were also detected in the lower channels. This suggests that the majority of fish released in the upper channels (73%, $n=32$) remained in the upper channels near the release site or the inlet until emigration.

The proportion of time and the proportion of fish in the upper channels was significantly greater during the residency period than during the emigration period, (Table 3; z_{time}=17.0 $P<0.05$; z_{fish}=4.22, $P<0.05$). Use of the lower channels was greatest during the dispersal period, both in terms of proportions of time spent in these habitats and the number of fish detected (Table 1; z_{time}=14.6, $P<0.05$; z_{fish}=2.62, $P<0.05$). Most fish (85%) detected in the lower channels occupied the upper channels for a mean ($\pm$ SE) of 132 ($\pm$14) days before they were detected in the lower channels. Fish detected in both the upper and lower channels had a later mean emigration date (15 November 2007) than fish that did not use the lower channels (24 August 2007).

Summer flounder did not appear to regularly occupy the additional portions of the Wachapreague system monitored by the ray study's receivers. Only 7% ($n=3$) of summer flounder were detected by these supplementary receivers, although fish presence may have been underestimated due to the limited coverage and the shorter period of deployment. The mean ($\pm$ SE) time fish occupied these areas was 6 ± 4 days (range: 0.2-13 days). Similarly, only 4% ($n=2$) of summer flounder briefly occupied the tidal flat between October and December 2007 (6 ± 5 days, range: 1.0-11 days).

Although the inlet region was frequented by summer flounder over the course of the study (Figs. 7, 8), fish spent a smaller proportion of time at the inlet (2%) than in the upper and lower channels (97%; Table 1). The mean ($\pm$ SE) time at the inlet was 2 ± 0.6 days. Not surprisingly, the proportions of time and fish at the inlet were greatest during the emigration period (Table 1; z_{time}=4.85, P<0.05; z_{fish}=2.98, P<0.05; Fig. 5, 6).

Examination of activity was not possible without a sufficient number of fish present. For this reason, examination of activity was limited to fish occupying the upper channel region (receivers: 23-27, J01-J02), where a majority of the fish resided, during the residency period (8 June – 11 October 2007). Summer flounder in the upper channels were inactive during the residency period, and only five fish moved between receivers more than 10 times (activity index >10; Fig. 11). According to the repeated measures generalized linear model, the observed activity did not change significantly by week (χ^2=20.52, P>0.05), but did change significantly with time of day. The mean activity index was significantly greater during night than day (χ^2=8.52, P<0.05) and twice as many fish were active during night (n=12) than during day (n=6). Although most activity occurred on flood tide or during slack before ebb, mean activity was only marginally significantly different among tidal stages (χ^2=7.50, P=0.06). This marginal significance was not associated with differences in mean temperature for a given tidal stage in the upper channels because fish activity did not vary with temperature (χ^2=0.02, P>0.05).

DISCUSSION

Migratory Behaviors

The observed timing of summer flounder emigration from the Wachapreague system (primarily emigrated from October through January, with the largest number of fish emigrating in December) is consistent with the established seasonal progression of spawning migration from north to south (Smith 1973, Morse 1981, Kraus and Musick 2001, Sackett et al. 2007). Further north in New Jersey, acoustically tagged fish generally emigrate between August and December (Able et al. 1990, Roundtree and Able 1992b, Szedlmayer and Able 1992, Szedlmayer and Able 1993). Fabrizio et al. (2005) reported that 75% of tagged summer flounder had dispersed from a study site on the inner shelf (near NJ) by mid-September. In contrast, 75% of tagged fish in the Wachapreague system had not dispersed until early December. Because fish dispersed later from the Wachapreague system, the mean residency time was 1.5 times longer (130 days, June – January) than those previously reported in New Jersey (86 days, May – December; Sackett et al. 2008). The timing of summer flounder emigration from Wachapreague system most closely matched that reported for nearby Chesapeake Bay (fish primarily emigrated from October through December, with some fish detected in the Bay as late as February; Desfosse 1995, Fabrizio et al. 2007b). However, dispersal rates and residency time in the Bay have not yet been studied.

Previous research has not addressed the fate of summer flounder that emigrated early in the summer (residency period) or that temporarily emigrate. These early or

temporary emigrations are not likely related to offshore spawning. Rather, fish that

departed early (including censored fish, which may have dispersed undetected), or those

that temporarily emigrated (a behavior also noted by Sackett et al. 2007), may have

occupied habitats on the inner continental shelf. However, based on the observation of a

single fish that was subsequently detected in Delaware Bay, summer flounder may also

move between coastal estuarine systems before establishing residency.

Based on what is known about summer flounder life history, fish that departed

during the emigration period were most likely moving offshore to spawn. Furthermore,

we also speculate that after spawning fish are more likely to return to the estuary last

occupied. Fish that dispersed from the Wachapreague system during the emigration

period had significantly greater odds of returning to Wachapreague the following year

than fish that had dispersed during the residency period. In addition, it was the larger

adults that were more likely to disperse early or temporarily emigrate from the system.

Larger fish have been reported to commence spawning migrations earlier than smaller

fish (Smith 1973), but my results suggest that emigration behaviors not associated with

spawning may be related to the size of the fish.

The percentage of fish returning to the Wachapreague system (36%) was similar

to that reported for northern estuaries (25-35% and 39% in New York and New Jersey,

respectively; Poole 1962, Sackett et al. 2007). Unlike a previous mark-recapture study

(Desfosse 1995), returns to the Wachapreague system after April were not detected

despite a tag battery life that would have permitted detection through July 2008.

However, summer flounder did return to the Wachapreague system as early as February,

suggesting that the calculated residency time of 130 days (4.3 months) is conservative

and that some fish may actually reside in this system upward of 10 months (February-December).

Seasonal changes in temperature strongly influenced the length of residency in the Wachapreague system due to the close association observed between the seasonal decline in temperature and the increase in emigration rates. Photoperiod, in contrast, did not appear to influence dispersal rates as much as temperature. However, spawning migration begins near the peak of summer flounder gonadal development (Smith 1973), and both temperature and photoperiod are known to cue seasonal gonad maturation in teleosts (Crim 1982). The exact role of photoperiod and temperature (or the interaction between these factors) in cuing summer flounder spawning migrations is unknown, but other factors such as prey availability may be important as well.

Within-Estuary Behaviors

The distribution of summer flounder in the Wachapreague system was comparable to that observed in Great Bay, NJ (Sackett et al. 2008); in both studies, adult summer flounder were primarily detected in the deeper areas near the inlet. Fish rarely occupied regions farther from the inlet that are characterized by finer sediment, lower current velocities, and shallower depths. Even though supplementary coverage of areas monitored by the ray study was conducted with a small number of receivers over a shorter time, the strong presence of fish in the upper channels negates the possibility that many fish used these areas undetected.

Substrate, current, prey availability and depth all potentially influenced the observed within-lagoon distribution of individual fish. Adult summer flounder prefer

hard, sandy substrate in which they can easily bury (Bigelow and Schroeder 1953, Schwartz 1964, Powell and Schwartz 1977). In small lagoon systems, the sandier substrate is found in high velocity waters, such as those in channels nearest the inlet, whereas tidal flats are generally characterized by finer grained (i.e., mud) substrate. Also, fishes and crustaceans comprise a large portion of the adult summer flounder diet (Packer et al. 1999, Latour et al. 2008), and higher current velocities may deliver more potential prey species into an area per unit time. Summer flounder may also prefer deeper areas, like those in the channel and inlet regions, which are more likely to exhibit stable environmental conditions (Sackett et al. 2008). The Wachapreague system is considered to be relatively well-mixed, but rapid fluctuations in environmental conditions (i.e., hypoxia, high temperatures) possibly occurred undetected during the summer in shallower areas, like the tidal flat (Shen et al. 2008, Tyler et al. 2009). Summer flounder occupied the tidal flat only from October to December, suggesting that the shallow waters of the tidal flat may have been too warm for fish to frequent them in the summer months. All monitored habitats were polyhaline (salinity >28‰), therefore salinity preferences of summer flounder could not be examined as was previously done (Able and Kaiser 1994, Sackett et al. 2008).

Once summer flounder assume residency in an estuary, they have been described as becoming sedentary and making only minor movements until the fall emigration (Desfosse 1995). This behavior characterized fish in the Wachapreague system that resided primarily in the upper channels. Larger-scale movements (100s of meters) between receivers were only rarely observed. However, fish could have been active at smaller scales (< 100s of meters). Active tracking of summer flounder in a NJ estuary

revealed that fish remained within small areas (0.18 km^2) but were in motion for most of the observed time within these areas (Sackett et al. 2008).

I did not observe significant effects of temperature or tidal stage on large-scale fish activity in the upper channels during the residency period. Unlike age-0 summer flounder that use tidal currents to reduce energy costs while feeding in tidal creeks (Szedlmayer and Able 1993), and other flatfish that use high tide to access prey in shallow tidal flats (Wirjoatmodjo and Pitcher 1984), the fish in the Wachapreague system primarily resided in deeper areas. In these high current regions fish may have an ample supply of prey and may not need to modify activity or use energetically beneficial tidal conditions. Sackett et al. (2008) also found that small-scale movement of summer flounder in deeper waters was not related to tidal currents or temperature. Rather, they attributed small-scale adult summer flounder activity to feeding, competition or territorial behaviors. Fish activity was significantly greater at night than during the day in the upper channels during the residency period. A feeding behavior study conducted in the laboratory suggested that summer flounder were more active during the day (Olla et al. 1972), but activity was considered on a much smaller scale (in 10.6 x 4.5 x 3.0 m seawater tank). Similar large-scale (200-400 m) activity of summer flounder in the Chesapeake Bay was also greatest at night (Fabrizio et al. 2007b). However, a recent study of the visual system of flounder (Horodysky et al. *In press*) suggest that their visual system is not particularly effective at very low light levels and thereby may limit prey capture at night.

In contrast to the majority of inactive fish that primarily resided in the upper channels, a distinct group of transient fish (*n*=12) used the lower channels. This use of

the lower channels increased as the study period progressed and these transient fish had a later mean dispersal date than fish that avoided the lower channels. Fabrizio et al. (2007b) observed some summer flounder in the lower Chesapeake Bay remained in the same area for long periods of time but others traveled greater distances, possibly in search of optimal habitats for foraging and refuge. The transient fish in the Wachapreague system may have moved into other potentially less favored habitats (e.g., lower channels, tidal flat) to avoid fitness reductions in response to resource limitation in the upper channels. This coexistence of behavioral types (transients and residents) has been noted in other species and is postulated to result in approximately equal fitness among individuals (Bolnick et al. 2003, Kobler et al. 2009)

Identifying differences in behaviors within a population are one of the benefits of using acoustic telemetry to monitor individual fish movements. This study confirms that even though summer flounder life history and migration dynamics are well-described, individual fish are not uniform in their use of estuaries during summer residencies throughout the MAB region. For a majority of summer flounder, it appears to be bioenergetically advantageous for fish to reside in deeper, stable waters within an estuary until undertaking their spawning migration. However, some fish are more active (100s of meters) within a system, and larger adults potentially make use of multiple coastal systems before migrating offshore to spawn, making summer flounder differentially vulnerable to estuarine conditions, predation and human impacts. Further establishment of a network of strategic acoustic monitoring stations along MAB estuaries and the continental shelf would enable monitoring of fish in these habitats and could help clarify the fate of early dispersals or temporary migrants (Grothues 2005, Able and Grothues

2007). Similarly, studies combining prey distribution and availability with acoustic monitoring may help explain observed distributions and why some fish appear to be more active than others.

Finally, summer flounder exhibited longer residence times in this southern MAB lagoon than those previously reported for summer flounder in the northern MAB, which appears to be related to seasonal temperature variation, but may also be associated with favorable conditions within this well-mixed system. The long residence times and high percentage of returning adults to this Eastern Shore lagoon, along with the already confirmed use of these areas as nursery grounds, make local conditions important to population success. These lagoons are currently only moderately impacted by anthropogenic sources (Giordano 2009), but in general coastal lagoons are more susceptible to changes in land use and nutrient enrichment than river-dominated estuaries (Boynton et al. 1996, McGlathery et al. 2001). A continued understanding of summer flounder preferences and behaviors in estuaries along their range of distribution is essential for protecting habitat necessary for promoting year-class strength and spawning success.

LITERATURE CITED

Able, K.W., Matheson, R.E., Morse, W.W. and G. Shepherd. 1990. Patterns of summer flounder *Paralichthys dentatus* early life history in the mid-Atlantic Bight and New Jersey estuaries. Fishery Bulletin 88(1):1-12.

Able, K.W. and S.C. Kaiser. 1994. Synthesis of summer flounder habitat parameters. NOAA Coastal Ocean Program, Decision Analysis Series No.1, Silver Spring, MD.

Able, K.W. and T.M. Grouthes. 2007. An approach to understanding habitat dynamics of flatfishes: advantages of biotelemetry. Journal of Sea Research 58:1-7.

Bennetts, R.E., Nichols, J.D., Lebreton, J., Pradel, R., Hines, J.E. and W.M. Kitchens. 2001. Methods for estimating dispersal probabilities and related parameters using marked animals. Pages 2-17 in J. Clobert, J.D. Nichols, E. Danchin and A. Dhondt, eds. Dispersal. Oxford University Press, Oxford, UK.

Berghahn, R., Bullock, A.M. and Karakiri M. 1993. Effects of solar radiation on the population dynamics of juvenile flatfish in the shallows of the Wadden Sea. Journal of Fish Biology 42:329-345.

Bigelow H.B. and W.C. Schroeder. 1953. Fishes of the Gulf of Maine. U.S. Fish and Wildlife Service. Fishery Bulletin 53(74):85-118.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Husley, C.D. and M.L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. American Naturalist 161:1-28.

Boynton, W.R., Hagy, J.D., Murray, L. and C. Stokes. 1996. A comparative analysis of eutrophication patterns in a temperate coastal lagoon. Estuaries 19:408-421.

Burnham, K.P. and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd edition. Springer Science and Business Media, LLC, New York, NY.

Capossela, K.M. 2010. Chapter 2: Metabolic and cardiorespiratory responses of summer flounder to hypoxia at two temperatures. In: Migration dynamics, within-estuary behaviors and cardiorespiratory responses of summer flounder to selected estuarine conditions. M.S. Thesis. College of William and Mary, Gloucester Point, VA.

Castorlin, M.E. and W.W. Reynolds. 1982. Thermoregulatory behavior and diel activity of yearling winter flounder, *Pseudopleuronectes americanus* (Walbaum). Environmental Biology of Fishes 7:177-180.

Conrath, C.L. 2005. Nursery delineation, movement patterns, and migration of the sandbar shark, *Carcharhinus plumbeus*, in the eastern shore of Virginia. PhD Dissertation. College of William and Mary, Gloucester Point, VA.

Crim, L.W. 1982. Environmental modulation of annual and daily rhythms associated with reproduction in teleost fishes. Canadian Journal of Fisheries and Aquatic Sciences 39:17-21.

Dahlberg, R.M. 1972. An ecological study of Georgia coastal fishes. Fishery Bulletin (U.S.) 70:323-353.

Desfosse, J.C., Musick, J.A., Estes, A.D. and P. Lyons. 1990. Stock identification of summer flounder (*Paralichthys dentatus*) in the southern mid-Atlantic Bight. Final Report submitted to Virginia Marine Resources Commission, WB-86-01-04; Federal Aid for Sports Fisheries Restoration, F-61-R. College of William and Mary, Virginia Institute of Marine Science, Gloucester Point.

Desfosse, J.C. 1995. Movements and ecology of summer flounder, *Paralichthys dentatus*, tagged in the southern mid-Atlantic Bight. Ph.D. Dissertation. College of William and Mary, Gloucester Point, VA.

Fabrizio, M.C., Pessutti, J.P., Manderson, J.P., Drohan, A.F. and B.A. Phelan. 2005. Use of the historic area remediation site by black sea bass and summer flounder. NOAA, NEFSC Center Reference Document 05-06 [www.nefsc.noaa.gov/nefsc/publications/crd/crd0506/].

Fabrizio, M.C. and J.P. Pessutti. 2007a. Long-term effects and recovery from surgical implantation of dummy transmitters in two marine fishes. Journal of Experimental Marine Biology and Ecology 351:243-254.

Fabrizio, M.C., Henderson, M.J. and J.A. Lucy. 2007b. Understanding localized movements and habitat associations of summer flounder in Chesapeake Bay using passive acoustic arrays: final report, 1 December 2007, submitted to Virginia Marine Resources Commission, Marine Recreational Fishing Advisory Board, grant #V771030

Fleiss, J.L. 1981. Statistical methods for rates and proportions, 3rd Edition. Wiley, Hoboken, NJ.

Giordano, J.C.P. 2009. Nutrient loading and system responses in the coastal lagoons of the Delmarva Peninsula. Master's Thesis. College of William and Mary, Gloucester Point, VA.

Grothues, T.M., Able, K.W., McDonnell, J. and M.M. Sisak. 2005. An estuarine observatory for real-time telemetry of migrant macrofauna: design, performance and constraints. Limnology and Oceanography: Methods 3:275-289.

Heupel, M.R., Semmens, J.M. and A.J. Hobday. 2006. Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. Marine and Freshwater Research 57:1-13.

Horodysky, A.Z., Brill. R.W., Warrant, E.J., Musick, J.A. and R.J. Latour. *In press*. Comparative visual function in four piscivorous fishes inhabiting Chesapeake Bay. Journal of Experimental Biology.

Kjerfve, B. 1986. Comparative oceanography of coastal lagoons, p. 63-81. *In* V.S. Kennedy (ed.), Estuarine Variability. Academic Press, New York.

Knights, B.C. and B.A. Lasse. 1996. Effects of implanted transmitters on adult bluegills at two temperatures. Transactions of the American Fisheries Society 125:440-449.

Kobler, A., Klefoth, T., Mehner, T. and R. Arlinghaus. 2009. Coexistence of behavioral types in an aquatic top predator: a response to resource limitation? Oecologia 161:837-847.

Kraus, R.T. and J.A. Musick. 2001. A brief interpretation of summer flounder, *Paralichthys dentatus*, movements and stock structure with new tagging data on juveniles. Marine Fisheries Review 63(3):1-6.

Latour, R.J., Gartland, J., Bonzek, C.F. and R.A. Johnson. 2008. The trophic dynamics of summer flounder (*Paralichthys dentatus*) in Chesapeake Bay. Fishery Bulletin 106:47-57.

Littell, R.C., Milliken, G., Stroup, W.W., Wolfinger, R. and O. Schabenberger. 2006. SAS for mixed models, 2nd edition. SAS Institute, Cary, North Carolina.

Malloy, K.D. and T.E. Targett. 1991. Feeding, growth and survival of juvenile summer flounder *Paralichthys dentatus*: experimental analysis of the effects of temperature and salinity. Marine Ecological Progress Series 72:213-223.

Malloy, K.D. and T.E. Targett. The use of RNA:DNA ratios to predict growth limitation of juvenile summer flounder (*Paralichthys dentatus*) from Delaware and North Carolina estuaries. Marine Biology 118:367-375.

McGlathery, K.J., Anderson, I.C. and A.C. Tyler. 2001. Magnitude and variability of benthic and pelagic metabolism in a temperate coastal lagoon. Marine Ecology Progress Series 216:1-15.

Morse, W.W. 1981. Reproduction of the summer flounder, *Paralichthys dentatus* (L.).
    Journal of Fish Biology 19:189-203.

Murawski, W.S. 1970. Results of tagging experiments of summer flounder,
    Paralichthys dentatus, conducted in New Jersey waters from 1960 to 1967.
    NJDEP Division of Fish Game and Wildlife, 73 pp.

Necaise, A.M.D, Ross, S.W. and J.M. Miller. 2005. Estuarine habitat evaluation
    measured by growth of juvenile summer flounder *Paralichthys dentatus* in a
    North Carolina estuary. Marine Ecology Progress Series 285:157-168.

NOAA Tidal Currents Prediction Center. 2007. Internet access (accessed 2 March
    2010). http://tidesandcurrents.noaa.gov/tides07/tab2ec2b.html#44.

Norcross, B.L. and D.M. Wyanski. 1994. Interannual variation in the recruitment pattern
    and abundance of age-0 summer flounder, *Paralichthys dentatus*, in Virginia
    estuaries. Fishery Bulletin 92:591-598.

Olla, B.L., Samet, C.E. and A.L. Studholme. 1972. Activity and feeding behavior of the
    summer flounder (*Paralichthys dentatus*) under controlled laboratory conditions.
    Fish. Bull. (U.S.) 70:1127-1136.

Orth, R.J. and K.L. Heck, Jr. 1980. Structural components of eelgrass (*Zostera marina*)
    meadows in the lower Chesapeake Bay – fishes. Estuaries 3:278-288.

Packer, D.B., Griesbach, S.J., Berrien, P.L., Zetlin, C.A., Johnson, D.L. and W.W.
    Morse. 1999. Summer Flounder, *Paralichthys dentatus*, life history and habitat
    characteristics. NOAA Technical Memorandum NMFS-NE-151, Woods Hole,
    MA.

Pautzke, S.M., Mather, M.E., Finn, J.T., Deegan, L.A. and R.M. Muth. 2010. Seasonal
    use of a New England estuary by foraging contingents of migratory striped bass.
    Transactions of the American Fisheries Society 139:257-269.

Pollock, K.H., Winterstein, S.R., Bunck, C.M. and P.D. Curtis. 1989. Survival analysis
    in telemetry studies: the staggered entry design. J. Wildl. Manage. 53(1):7-15.

Poole, J.C. 1962. The fluke population of Great South Bay and their significance to the
    sport fishery. N.Y. Fish and Game Journal 9:93-117.

Powell, A.B. and F.J. Schwartz. 1977. Distribution of paralichthid flounders (Bothidae:
    Paralichthys) in North Carolina estuaries. Chesapeake Science. 18(4):334-339.

Rantin, F.T., Kalinin, A.L. and M.L. Glass. 2007. The effects of temperature on
    respiratory and cardiac function of teleost fish. In *Fish Respiration and
    Environment* (Fernandes, M.N., Rantin, F.T., Glass, M.L., Kapoor, B.G., eds.),
    pp.55-82. New Hampshire: Science Publishers.

Ray, G.C. Connectivities of estuarine fishes to the coastal realm. 2005. Estuarine, Coastal and Shelf Science 64:18-32.

Richards, C.E. and M. Castagna. 1970. Marine fishes of Virginia's Eastern Shore (inlet and marsh, seaside waters). Chesapeake Science 11(4):235-248.

Rogers, S.G. and M.J. Van Den Avyle. 1983. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic) – summer flounder. U.S. Fish and Wildlife Service FWS/OBS-82/11.15. 14 pp.

Rogers, K.B. and G.C. White. 2007. Analysis of movement and habitat use from telemetry data. In: Brown, M.L. and C.S. Guy (Eds.) Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda, MD.

Roundtree, R.A. and K.W. Able. 1992a. Fauna of polyhaline marsh creeks in southern New Jersey: composition, abundance and biomass. Estuaries 15:171-186.

Roundtree, R.A. and K.W. Able. 1992b. Foraging habits, growth, and temporal patterns of salt-marsh creek habitat use by young-of-the-year summer flounder in New Jersey. Transactions of the American Fisheries Society 121:765-776.

Ryan, S.E., Porth, L.S. and C.A. Troendle 2002. Defining phases of bedload transport using piecewise regression. Earth Surface Processes and Landforms 27(9): 971 990.

Sackett, D.K., Able, K.W. and T.M. Grothues. 2007. Dynamics of summer flounder *Paralichthys dentatus*, seasonal migrations based on ultrasonic telemetry. Estuarine, Coastal and Shelf Science 74:119-130.

Sackett, D.K., Able, K.W. and T.M. Grothues. 2008. Habitat dynamics of summer flounder *Paralichthys dentatus* within a shallow USA estuary based on multiple approaches using acoustic telemetry. Marine Ecology Progress Series 364:199-212.

Schwartz, F.J. 1964. Fishes of Isle of Wight and Assawoman bays near Ocean City, Maryland. Chesapeake Sci. 5:172-193.

Shen, J., Wang, T., Herman, J., Mason, P. and G.L. Arnold. 2008. Hypoxia in a coastal embayment of the Chesapeake Bay: a model diagnostic study of oxygen dynamics. Estuaries and Coasts 31:652-663.

Smith, W.G. 1973. The distribution of summer flounder, *Paralichthys dentatus*, eggs and larvae on the continental shelf between Cape Cod and Cape Lookout, 1965-66. Fish. Bull. (U.S.) 71:527-548.

Stierhoff, K.L., Targett, T.E. and K. Miller. 2006. Ecophysiological responses of juvenile summer and winter flounder to hypoxia: experimental and modeling analyses of effects on estuarine nursery quality. Marine Ecological Progress Series 325:255-266.

Stierhoff, K.L., Targett, T.E. and J.H. Power. 2009. Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA:DNA. Canadian Journal of Fisheries and Aquatic Sciences 66:1033-1047.

Szedlmayer, S.T. and K.W. Able. 1993. Ultrasonic telemetry of age-0 summer flounder, *Paralichthys dentatus*, movements in a southern New Jersey estuary. Copeia 3:728-736.

Tyler, R.M. 2004. Distribution and avoidance patterns of juvenile summer flounder (*Paralichthys dentatus*) and weakfish (*Cynoscion regalis*) in relation to hypoxia: field studies in a temperate coastal lagoon tributary and laboratory choice-trial experiments. PhD thesis. University of Delaware, Newark, DE.

Tyler, R.M., Damian, B.C. and T.E. Targett. 2009. Temporal and spatial dynamics of diel-cycling hypoxia in estuarine tributaries. Estuaries and Coasts 32:123-145.

Watters, K.W. and L.S. Smith. 1973. Respiratory dynamics of the starry flounder *Platichthys stellatus* in response to low oxygen and high temperature. Marine Biology 19:133-148.

Wirjoatmodjo, S. and T.J. Pitcher. 1984. Flounders follow the tides to feed: evidence from ultrasonic tracking in an estuary. Estuarine, coastal and shelf science 19:231-241.

Wyanski, D.M. 1990. Patterns of habitat utilization in 0-age summer flounder. Masters Thesis. College of William and Mary, Gloucester Point, VA.

Table 1. Summer flounder use of the Wachapreague system, VA. The proportion of time

fish spent in each region (upper channels, lower channels and inlet) by month, period

(residency and emigration) and overall (from 8 June 2007 – 17 January 2008). Also

presented is the proportion of fish found in each region by month, period, and overall.

The residency period was from 8 June to 10 October 2007 and the emigration period was

from 11 October 2007 to 17 January 2008. Tidal flat and supplementary channel use was

not included due to low fish presence in these areas,

		Proportion of Time			Proportion of Fish		
		Upper Channels	Lower Channels	Inlet	Upper Channels	Lower Channels	Inlet
Month							
2007	June	97%	2%	0.6%	98%	2.%	18%
	July	99%	1%	0.1%	96%	8%	8%
	August	84%	15%	1%	88%	19%	38%
	September	61%	34%	5%	55%	35%	15%
	October	54%	44%	2%	59%	41%	29%
	November	49%	46%	2%	47%	53%	53%
	December	49%	29%	18%	30%	60%	90%
2008	January	33%	43%	25%	50%	50%	50%
Period							
	Residency	86%	11%	1%	98%	18%	40%
	Emigration	49%	15%	6%	50%	56%	87%
Overall							
	Total	78%	19%	2%	98%	29%	68%

Figure 1. Eastern Shore lagoon system near Wachapreague, VA in the southern mid-Atlantic Bight, indicating locations of acoustic receivers, and YSI loggers. HOBO temperature loggers were attached to receiver moorings at receiver 2, 4, 9, 13, 17, 20 and 22. Regions specified in the text are as follows: Wachapreague Inlet = receivers 21-22, J14; Upper Channels = 19-20, 23-27, J01-J02, J07; Lower Channels = 1-18; Tidal Flat = 11-15. Receivers J03, J05-J09, J11 and J12 from the independent study provided supplementary data about use of the upper channels.
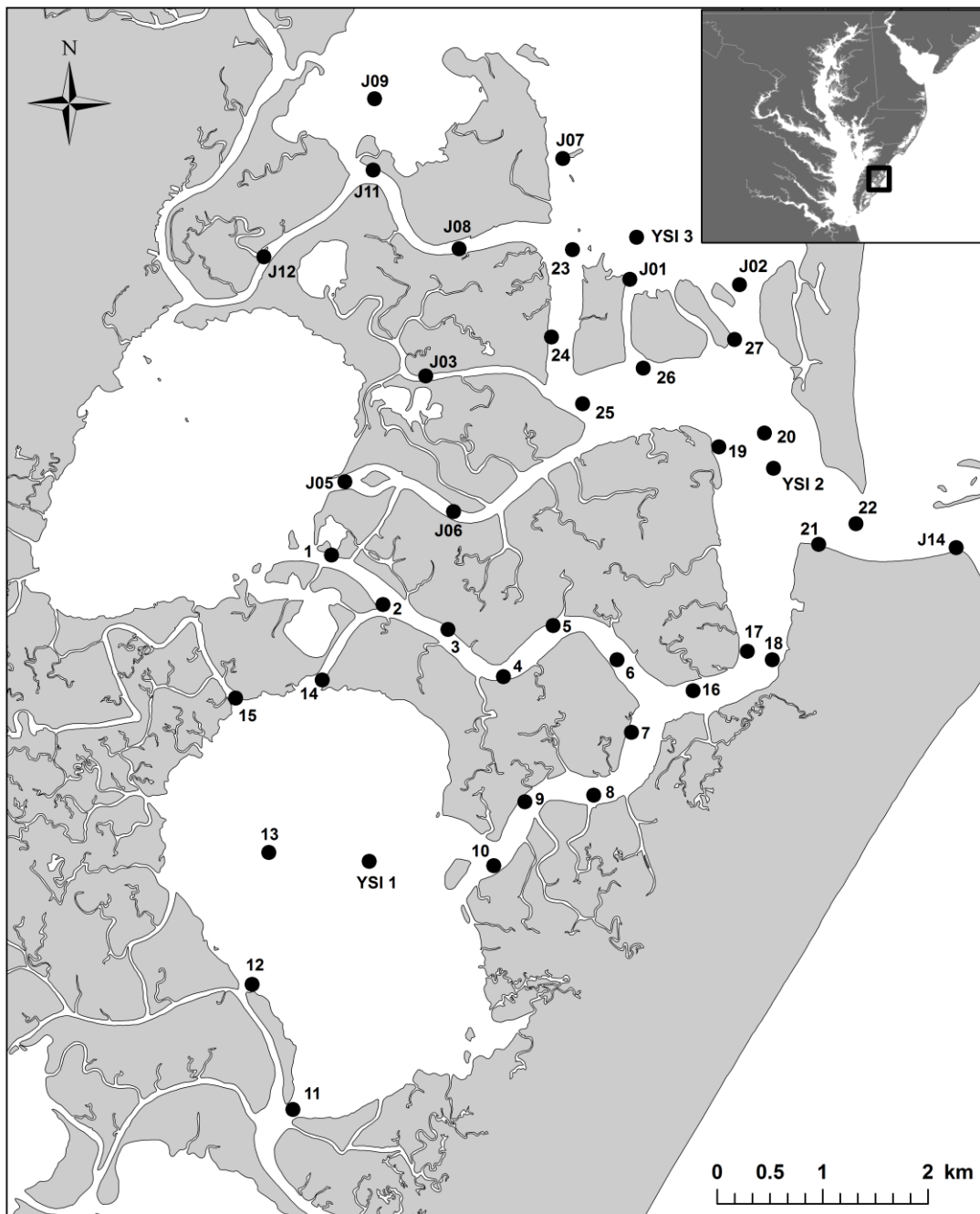
Figure 2. Dispersal rates of summer flounder from the Wachapreague system, VA, based

on the Kaplan-Meier estimator (solid line) with 95% CI intervals (dotted line). Dispersal

rates are expressed as the probability of residency (i.e., probability of fish not dispersing).

Fish were tagged on 22 May 2007 and emigration was monitored from 8 June 2007

(week 1) until the last fish emigrated on 17 January 2008 (week 32). Dispersal rates

changed significantly at week 18 (11 October 2007). The time before the change-point

was considered the 'residency period,' a time during which most fish were found within

the system. The time after the change-point was considered the 'emigration period,'

during which most fish were observed departing from the Wachapreague system.
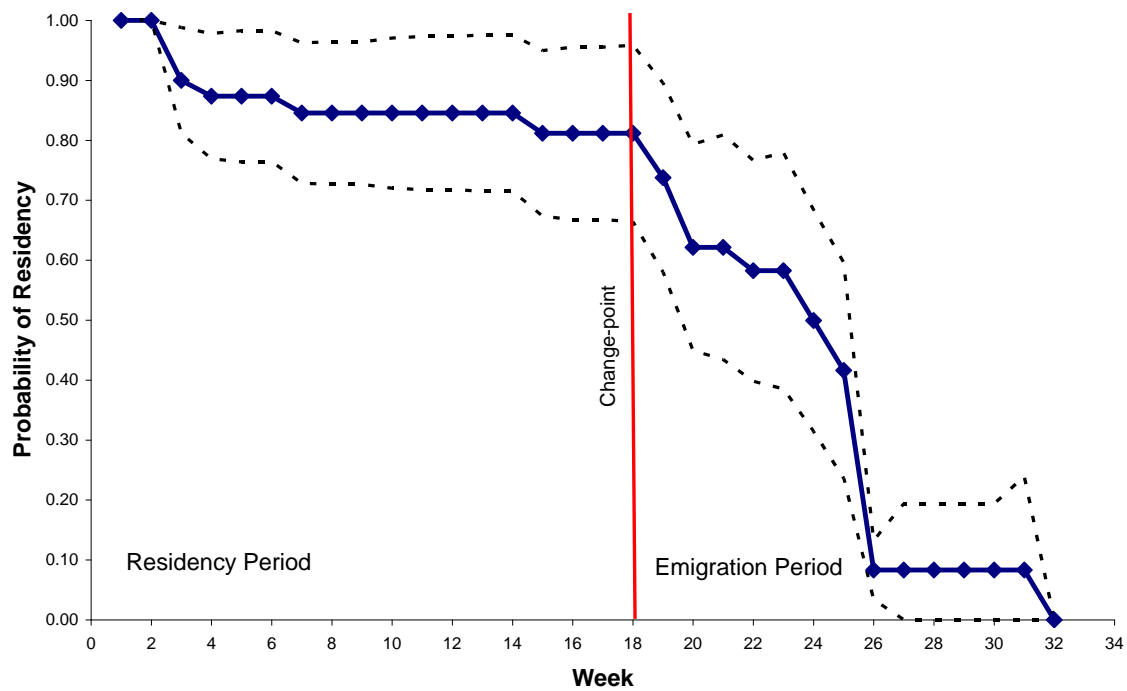
Figure 3. Proportion of summer flounder that dispersed (black) or were censored (gray) each month (n=45 fish). Fish that did not disperse at the inlet had unknown fates and were excluded (censored) from the number of fish present in the system after the week during which they were last detected. Note that sample sizes for each month were 45 (Jun), 31 (Jul), 30 (Aug), 25 (Sep), 23 (Oct), 16 (Nov), 10 Dec, and 2 (Jan).
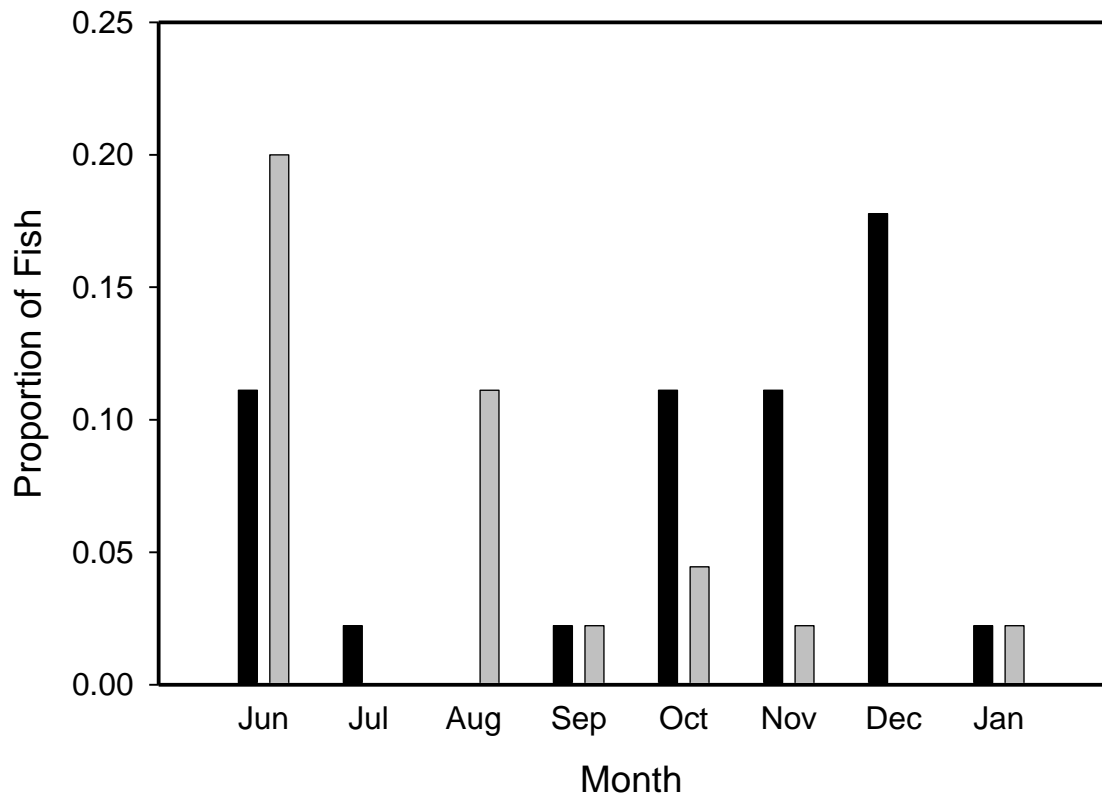
Figure 4. Return rates of summer flounder to the Wachapreague system based on the

Kaplan-Meier estimator (solid line) with 95% CI intervals (dotted line). Return rates are

expressed as the probability of residency. Immigration of acoustically tagged fish was

monitored from 18 January 2008 (week 33) until the last detected immigration on 7 April
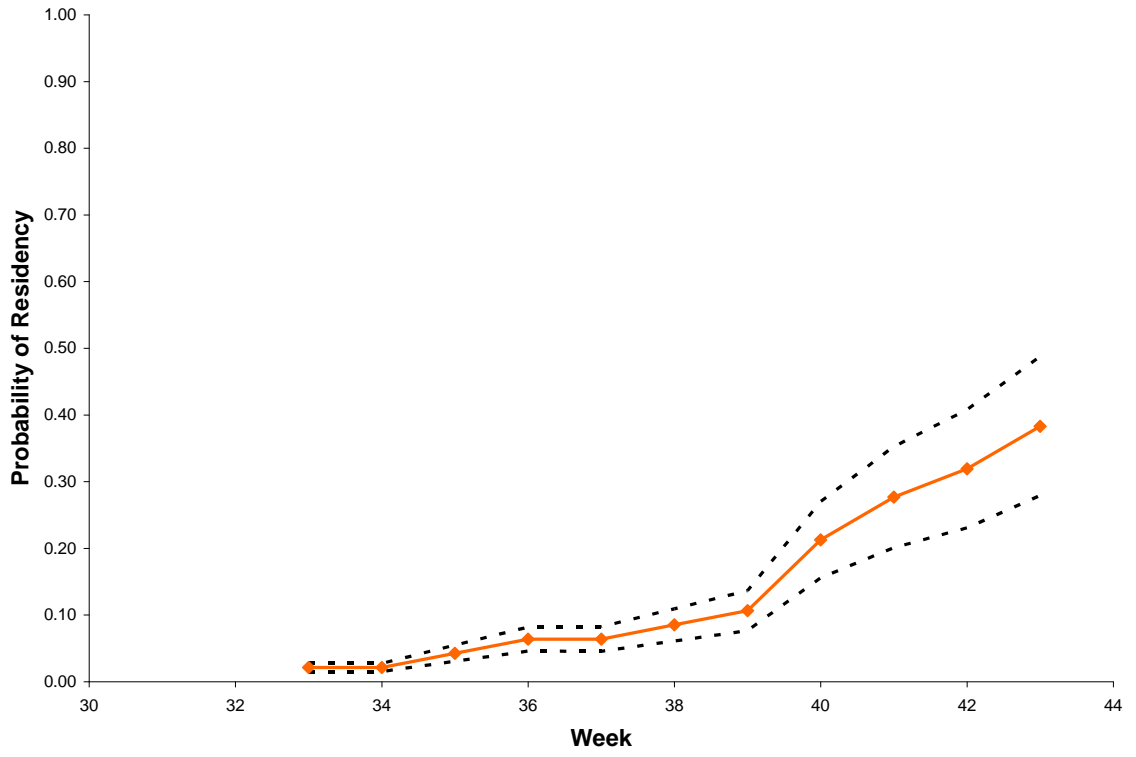
2008 (week 43).

Figure 5. Dispersal (blue line) and return (orange line) rates of summer flounder in the Wachapreague system, VA based on the Kaplan-Meier estimate. Mean daily temperature (°C; gray line) is also plotted. Mean daily temperature was calculated from temperature logger data. Confidence intervals were omitted for clarity.
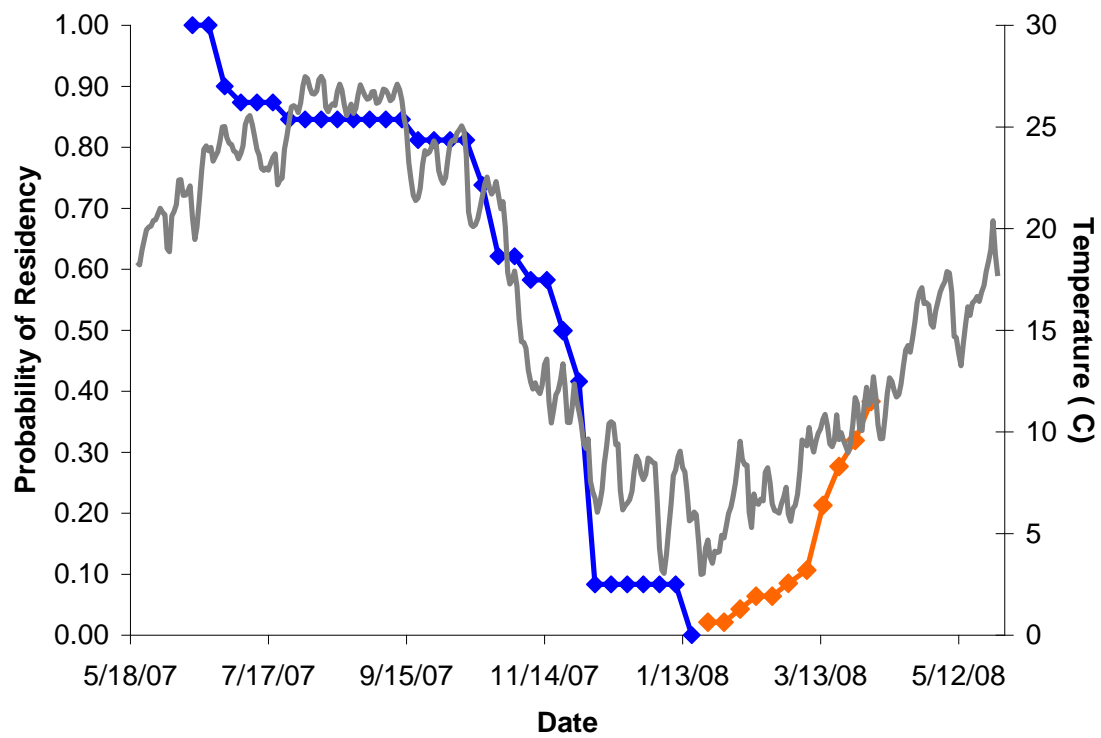
Figure 6. Dispersal (blue line) and return (orange line) rates of summer flounder in the

Wachapreague system, VA based on the Kaplan-Meier estimate. Photoperiod (day

length in hours; gray line) is also plotted. Photoperiod for each day in the study was

acquired from tide prediction software (Jtides, www.arachnoid.com/JTides). Confidence
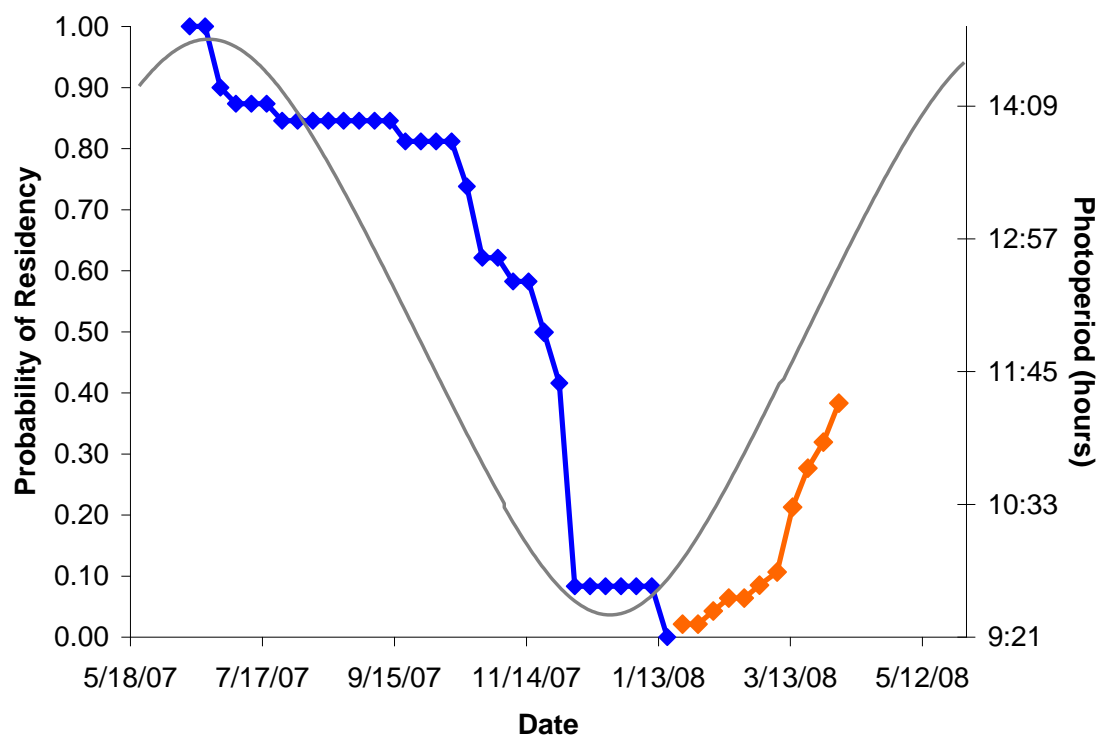
intervals were omitted for clarity.

Figure 7. Monthly proportion of known summer fish in the upper channels (black), lower channels (gray) and inlet (white). The proportion of fish occupying a region was determined as the ratio of the number of individual fish identified in that region to the number of fish detected in the system that month. The sum of the proportions of fish in each region for a given month could exceed one because a single fish could occupy more than one region in any given month.
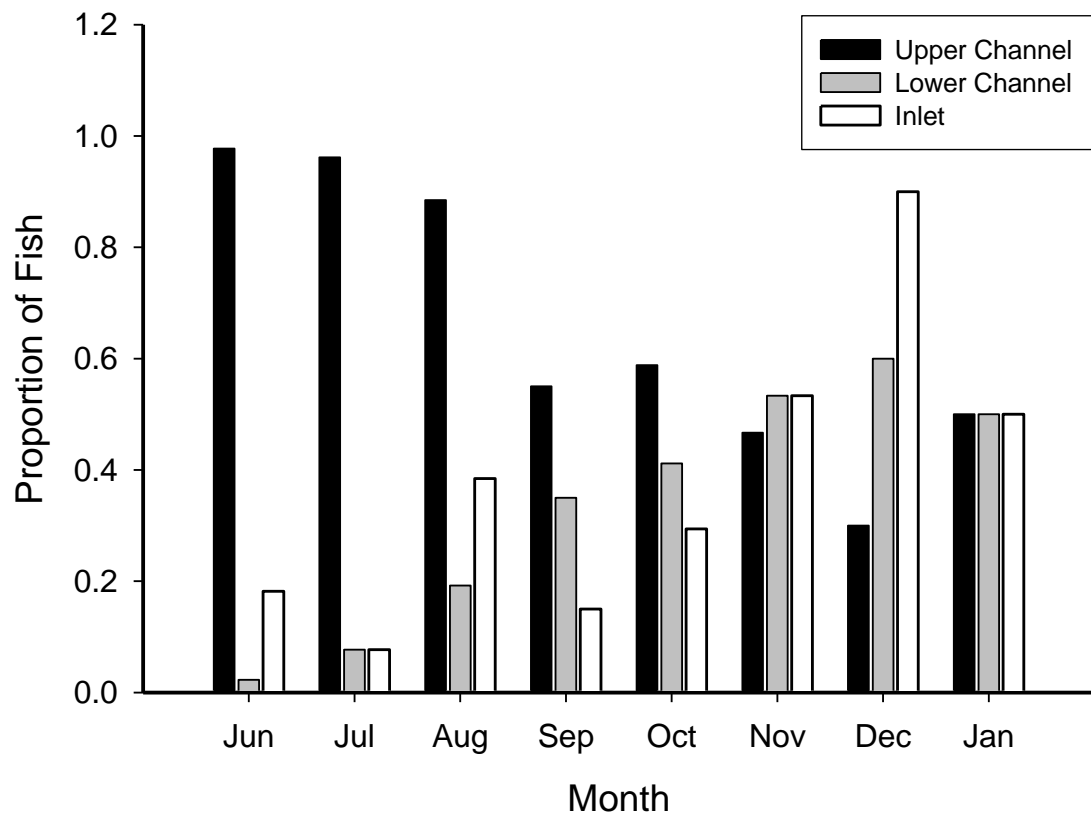
Figure 8. Monthly proportion of time summer fish occupied the upper channels (black),

lower channels (gray) and inlet (white). For a given month, the proportion of time

individual fish occupied each region was calculated as the ratio of the amount of time a

fish resided in a region to the total time it was detected that month, with proportions for a

month adding to one.
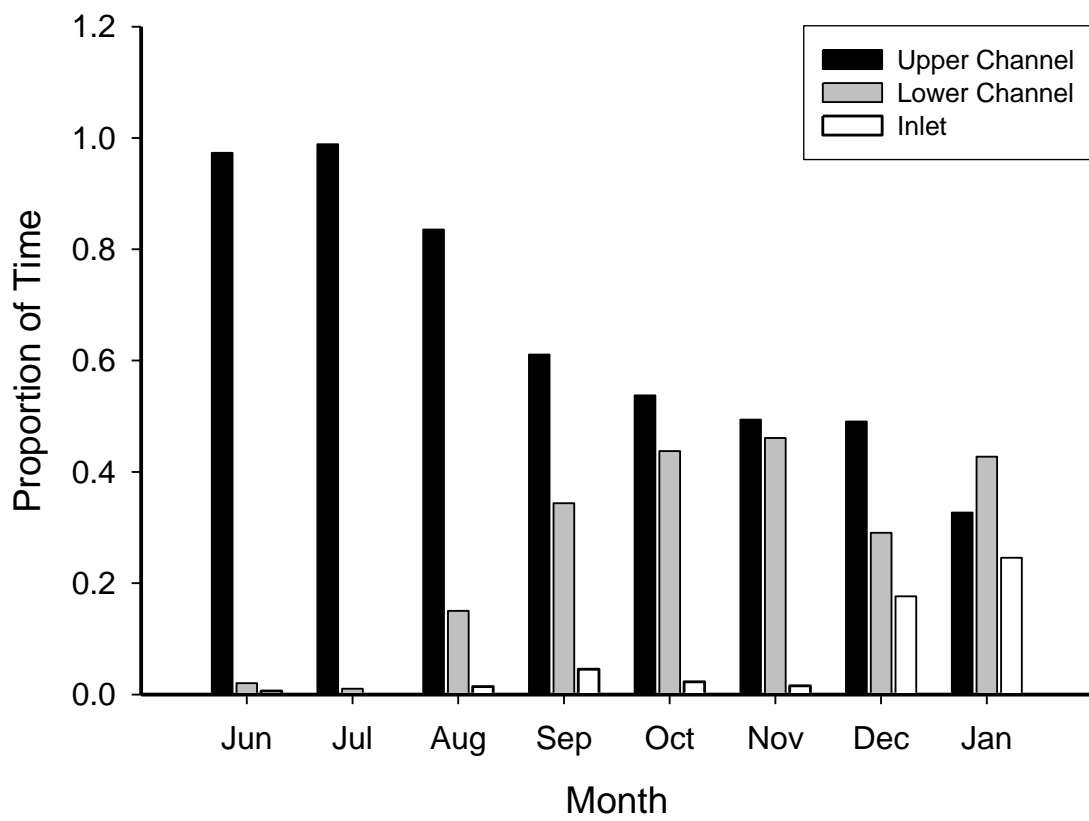
Figure 9. Activity of individual summer flounder (Fish ID = 4184-4233) in the

Wachapreague system, VA, during the residency period (8 June to 10 October 2007) in

the upper channel region. The activity index represents the total number of times a fish

moved between receivers during the residency period (8 June to 10 October 2007). A

fish was assigned an activity index value of 1 for each arrival at a new receiver. An

activity index of 0 indicated that a fish was detected at a receiver, but did not move
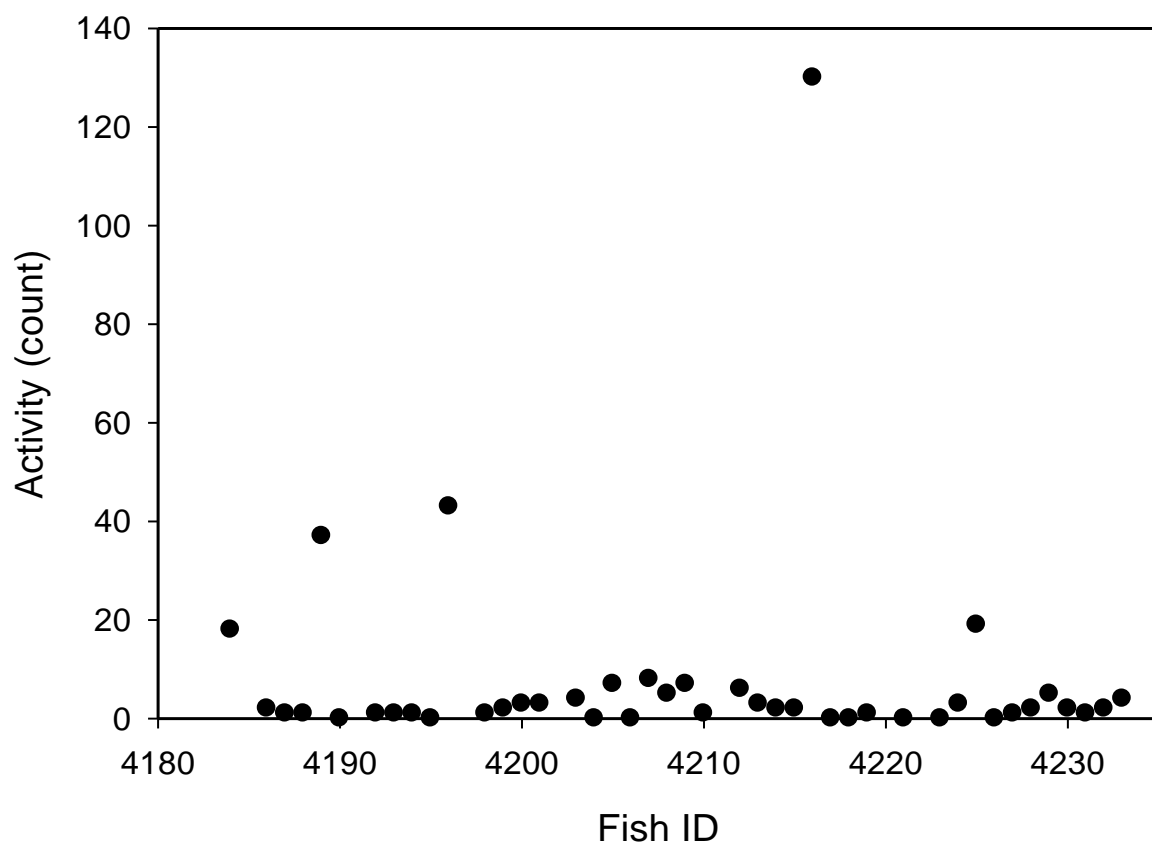
between receivers.

CHAPTER 2

METABOLIC AND CARDIORESPIRATORY RESPONSES OF

SUMMER FLOUNDER TO HYPOXIA AT TWO TEMPERATURES

ABSTRACT

This study provides a metabolic framework for understanding how summer flounder (*Paralichthys dentatus*) are affected by increasing anthropogenic alterations to coastal waters, specifically hypoxia. Stopflow respirometry was used to measure changes in resting metabolic rate, gill ventilation, oxygen extraction and heart rate during progressive hypoxia at an acclimation temperature (22°C) and after an acute temperature increase (to 30°C). The mean (± SE) critical oxygen saturation (i.e., the oxygen saturation after which fish could no longer maintain aerobic metabolism) increased significantly from 27% ± 0.02 at 22°C to 39% ± 0.02 at 30°C, suggesting a lower tolerance to hypoxia after exposure to an acute increase in temperature. Gill ventilation in normoxia was 0.13 ± 0.01 and 0.37 ± 0.02 $l\,kg^{-1}\,min^{-1}$ at 22°C and 30°C (acute) and increased immediately with onset of hypoxia, reaching 0.81 ± 0.05 and 1.00 ± 0.08 $l\,kg^{-1}\,min^{-1}$ (at 22 and 30°C) at the lowest oxygen levels tested. Oxygen extraction in normoxia was 86.7 ± 0.02 and 80.2 ± 0.02 % (at 22 and 30°C) and likewise decreased immediately with onset of hypoxia, reaching 50.1 ± 0.05 and 54.8 ± 0.02 % (at 22 and 30°C) at the lowest oxygen levels tested. Extraction was linearly related to gill ventilation at both temperatures, implying that the increase in gill ventilation, rather than the decrease in ambient oxygen concentrations, was primarily responsible for the decrease in oxygen extraction. At normoxia, mean heart rates were 46.4 ± 5.26 and 79.7 ± 1.65 beats min^{-1} (at 22 and 30°C), but significant bradycardia was not observed until oxygen levels decreased to levels below critical oxygen saturation. Due to their ability to effectively increase gill ventilation while maintaining oxygen extraction above 50%, summer flounder are among the more hypoxia-tolerant flatfish species studied to date.

INTRODUCTION

Estuaries and lagoons of the U.S. east coast serve as important nursery and feeding grounds for numerous fish species, including summer flounder, *Paralichthys dentatus* (L.). This ecologically and economically valuable flatfish resides primarily in mid-Atlantic Bight (MAB) estuaries in the spring and summer before migrating offshore to spawn over the continental shelf in the fall and winter (Bigelow and Schroeder 1953, Smith and Daiber 1977). It is during their estuarine residence that fish are most likely to encounter hypoxic events, and these events are increasing in frequency and duration worldwide due to increasing coastal eutrophication and directional climate change (Diaz 2001, Diaz and Brietburg 2009). For example, Chesapeake Bay, commonly used by summer flounder during the summer, has experienced more widespread and severe hypoxic events since the 1960s than previously recorded (Hagy et al. 2004). As benthic fish, summer flounder may experience hypoxia in deeper estuaries like Chesapeake Bay, when water column stratification and a high supply of organic matter coincide. In addition, fish may experience diel-cycling hypoxia caused by algal respiration in shallow estuaries or lagoons (Shen et al. 2008, Tyler et al. 2009, Stierhoff et al. 2009).

Fish generally depend on aerobic respiration to meet metabolic requirements. As a result, oxygen acts as a limiting factor constraining activity due to a reduction in aerobic scope (the metabolic confines within which all aerobic activities must be undertaken; Claireaux and Lefrançois 2007). Fish may avoid hypoxic areas by altering their movement or distribution, but such movements may increase energy expenditure and the

risk of predation, or decrease food availability (Kramer 1987). If fish remain in hypoxic areas, they must make physiological adjustments to fulfill oxygen requirements. Most teleosts act as oxygen-regulators, maintaining oxygen consumption (i.e., metabolic rate) during hypoxia by regulating respiratory parameters and cardiac function (Perry et al. 2009, Gamperl and Driedzic 2009). However, at critical oxygen saturation, the cardio-respiratory system can no longer maintain sufficient oxygen delivery and fish must resort to anaerobic metabolism. Obviously, with few exceptions, fish cannot survive for extended periods at oxygen levels below the critical oxygen saturation.

Water temperature also affects the metabolic requirements of fish and may significantly influence fish regulatory responses to hypoxia and critical oxygen saturation. Temperature acts as a controlling factor, governing reactions involved in metabolism. In addition, higher temperatures elevate oxygen requirements while decreasing oxygen solubility and thus place further constraints on aerobic scope (Pörtner and Knust 2007). This is especially true if an acute rise in temperature accompanies a hypoxic event. Fish exposed to acute increases in temperature have higher metabolic requirements than those that are acclimated (Duthie and Houlihan 1982), and these higher metabolic requirements may limit regulatory capabilities during hypoxia. Conversely, fish in hypoxic conditions often succumb to high temperatures that are not lethal in well-oxygenated water (Brett 1956).

Because environmental influences (i.e., hypoxia, temperature) on fish activity are mediated primarily through aerobic metabolism, understanding the metabolic responses of fish to such influences is useful (Claireaux and Lefrançois 2007, Pörtner and Knust 2007, Chabot and Claireaux 2008). Therefore, assessing basic metabolic requirements,

critical oxygen saturation, and the concomitant cardiorespiratory adjustments occurring as critical oxygen saturation is approached, is necessary for understanding hypoxia tolerance and thus, the effects of hypoxia on fish movement and distribution. Metabolic and regulatory responses to hypoxia have been examined in other flatfish species and are known to vary according to species-specific physiological characteristics (e.g., gill surface area, cardiac anatomy, blood oxygen affinity). These responses also vary according to the extent or frequency of hypoxic episodes experienced in the wild, with species that are more hypoxia-tolerant able to inhabit areas prone to hypoxia (Steffensen et al. 1982).

Previous laboratory studies primarily described the effects of hypoxia and temperature on juvenile summer flounder growth, feeding rates, avoidance and survival (e.g., Tyler 2004, Necaise et al. 2005, Stierhoff et al. 2006), but little to no information is available regarding summer flounder metabolic requirements (Klein-MacPhee 1979) or cardiorespiratory responses to hypoxia. In general, flatfishes have low metabolic rates, reflective of a sedentary lifestyle. Most flatfishes act as oxygen regulators, but some act as oxygen-conformers, (metabolic rate declining with progressive hypoxia; Steffensen et al. 1982). To understand how summer flounder metabolism is affected by hypoxia, I investigated the metabolic requirements, critical oxygen saturation and regulatory responses to hypoxia and elevated temperature. I specifically measured the effects of hypoxia on resting metabolic rate ($\dot{V}_{O_2}$), gill ventilation ($\dot{V}_g$), oxygen extraction (E_{O_2}) and heart rate (f_H) of summer flounder at a normal acclimation temperature and after an acute rise in temperature.

METHODS

Experimental Protocol

All experiments were conducted at the Virginia Institute of Marine Science

Eastern Shore Laboratory (Wachapreague, VA) from June through August 2008. Summer

flounder (214-924 g) were captured using hook and line from the tidal lagoons near

Wachapreague Inlet and maintained in a closed system tank at an acclimation

temperature of 22°C ($\pm$ 2) and 30-34‰ salinity. Fish were held in captivity for at least

two weeks and fasted for at least 36 hours prior to use in an experiment.

Individuals were anesthetized in seawater containing 6 mg l^{-1} benzocaine (ethyl-p-

aminobenzoate) until they did not respond when gently lifted by the caudal peduncle.

Anesthetized fish were placed on a surgery table and the gills continuously irrigated with

seawater containing anesthetic. A polyethylene cannula (I.D. 0.86 mm, O.D. 1.27 mm)

was inserted through the cleithrum near the gills and used to sample exhalent water

(Saunders 1961, Saunders 1962, Randall et al. 1967), and electrocardiogram (EKG) wire

leads were placed subcutaneously near the heart. Fish were then moved to a 22.5 l

respirometer (constructed of 9.5 mm thick acrylic) and allowed to recover overnight at

normoxia and 22°C. A layer of sand on the bottom of the respirometer allowed the fish to

bury. The respirometer was also covered with black plastic to reduce visual disturbance.

Hypoxia trials were conducted at the fishes' acclimation temperature (22°C, *n*=9)

and after an acute increase in temperature to 30°C (*n*=9). In the latter case, fish were

allowed to recover in the respirometer overnight at 22°C before the temperature was

increased to 30°C (over approximately 2 hours). Hypoxia trials commenced once metabolic rate had stabilized after the temperature change (over approximately 3 hours). Data were collected during normoxia (oxygen levels > 85% oxygen saturation), and after stepwise reductions in oxygen to 75, 50, 30 and 20% saturation. Approximately 30 minutes was needed to achieve each stepwise decline in oxygen saturation. Experiments at 30°C were terminated at 30% saturation to avoid overly stressing the fish. Once the experiment was complete, oxygen levels were returned to normoxia and fish were allowed to recuperate in the respirometer overnight. Instrumentation was subsequently removed, and fish were held for a minimum of 22 days before being returned to the tidal lagoons from which they were captured.

Stopflow respirometry (Steffensen 1989) was used to determine the resting metabolic rate ($\dot{V}o_2$ in mgO$_2$ kg^{-1} h^{-1}), which is the oxygen consumption of quiescent, post-absorptive (fasted) fish exhibiting low levels of spontaneous activity (Jobling 1993). In this method, the respirometer is submerged in an outer water bath, and the intermittent flushing of the respirometer with outer bath water eliminated the washout effect common to flow-through respirometry and the effects of the accumulation of waste products common to static-system respirometry (Steffensen 1989). Oxygen levels in the outer water bath were controlled via an oxygen stripping column bubbled with air (during normoxia) or nitrogen (during hypoxia). These oxygen levels were monitored, and the flow of nitrogen to the stripping column controlled, with an oxygen meter (Oxy-Reg OX10000, Loligo Systems, Tjele, Denmark), and water temperature was controlled with a chiller and immersion heaters. Oxygen levels in the respirometer were monitored with an oxygen meter (WTW Oxi 3150, Global Water Instrumentation, Gold River, CA, USA)

and controlled by the computer-driven flushing of the respirometer with outer bath water at set intervals using a small pump submerged in the outer bath. When flushing ceased, the respirometer was closed to the outer bath water. Water within the respirometer was continually mixed with a submerged recirculating pump.

The partial pressure of oxygen (PO_2) and water temperature were continuously measured in the respirometer and recorded with an automated data acquisition system and custom-designed software developed in Dasylab 7.0 (National Instruments Inc., Austin, TX, USA) which interfaced with a series of macros executed in QuattroPro 11.0 (Corel Inc., Mountain View, CA, USA). PO_2 values were converted to oxygen content (mgO_2 l^{-1}) and oxygen saturation (%) using standard equations (following Richards 1965) within the Dasylab software routines. Resting metabolic rate, $\dot{V}o_2$ (mgO_2 kg^{-1} h^{-1}), was calculated over approximately10-minutes intervals (when the flush pump was turned off) using the following equation:

$$\dot{V}o_2 = \Delta C_w O_2 \times \Delta t^{-1} \times V_{resp} \times M^{-1} \qquad (1)$$

where $\Delta C_w O_2$ is the rate of change in water oxygen content, Δt is the length of the time interval over which $\dot{V}o_2$ was measured (h), V_{resp} is the volume (l) of the respirometer adjusted for fish volume, and M is the mass of the fish (kg).

Oxygen extraction (Eo_2 in %) was calculated during closed-respirometer intervals as:

$$Eo_2 = (S_I - S_E)/(S_I) \qquad (2)$$

where S_I is the oxygen content of the water in the respirometer (i.e., inhalant water) and S_E is the oxygen content of the exhalent water. Eo_2 was measured every 2-3 seconds and

a mean Eo_2 was calculated at each oxygen level from exhalent water drawn over a fiber-optic oxygen electrode (Microx TX3, Presens, Regensburg, Germany); exhalent water was pumped back into the respirometer using a roller pump. Eo_2 values for a given oxygen saturation level were averaged, resulting in one mean Eo_2 value per oxygen saturation level (including normoxia).

Gill ventilation ($\dot{V}_g$ in $l\ kg^{-1}\ min^{-1}$) was calculated from the calculated resting metabolic rate and oxygen extraction data using:

$$\dot{V}_g = \dot{V}o_2 / Eo_2 \qquad (3)$$

As before, a mean $\dot{V}_g$ value was calculated for each stepwise oxygen saturation level.

Calculations for $\dot{V}_g$ were made under the assumption that extrabranchial uptake of oxygen (cutaneous respiration) was insignificant.

Heart rate, (f_H in beats min^{-1}) was calculated from the EKG signal, which was amplified using a DAM50 amplifier (World Precision Instruments, Sarasota, FL, USA). Heart rates were recorded continuously during closed-respirometer intervals, and were highly variable. Therefore, instead of calculating a mean heart rate for each interval as before, individual heart rates for a given oxygen saturation were plotted as a frequency histogram and a Gaussian four-parameter model was fit to the heart rate data to estimate the most frequent heart rate at each oxygen level using Sigma Plot (9.0, SYSTAT Software, Inc., San Jose, CA, USA).

For each response, the differences in measured and calculated values of the response between 22°C and 30°C (acute) at normoxia were quantified as Q_{10} values to

permit comparison among similar studies. Q_{10} is the factor by which a response changes

for a rise in 10°C:

$$Q_{10} = (X_{T2} / X_{T1})^{10/(T2-T1)} \tag{4}$$

where X_{T1} and X_{T2} are the values at temperature T1 (22°C) and T2 (30°C).

Statistical Analysis

I tested the effect of oxygen saturation and temperature on the mean of each

response using a two-way repeated measures analysis of variance (ANOVA; MIXED

procedure in SAS, SAS Institute Inc., Cary, NC). Multiple measurements on individual

fish over time (i.e., stepwise oxygen levels) can violate the assumption of independence

in an ANOVA, and a repeated measures model allows the selection of the proper

covariance matrix to describe the relationship between consecutive measurements from

the same subject (Littell et al. 2006). The statistical model fit to the data was:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha * \beta)_{ij} + \varepsilon_{ij} \tag{5}$$

where Y_{ij} is the response ($\dot{V}o_2$, $\dot{V}_g$, Eo_2 or f_H), α_i is the effect of the ith oxygen

saturation level, β_j is the effect of the jth temperature level, $\alpha*\beta$ is the interaction between

the ith oxygen saturation level and the jth temperature, and ε is the random error associated

with each observation. The Akaike's Information Criterion value for small sample sizes

(AICc) was used to identify the correlation matrix that best described the covariance

structure for a given response. The first-order autoregressive model best described the

covariance structure of the $\dot{V}o_2$, Eo_2 and f_H responses, and the heterogeneous compound

symmetry model best described the covariance structure of the $\dot{V}_g$ response. Oxygen

saturation level (normoxia, 70, 50, 30 and 20%) and temperature (22 and 30°C) were considered fixed factors in the model, as was the interaction between these two factors. Resting metabolic rate data were log-transformed to improve homogeneity of variance, a necessary assumption of ANOVA.

Because responses were not measured at 20% saturation for experiments at 30°C, the experimental design was unbalanced. As a result, two-way ANOVA could not be used to estimate the oxygen saturation level at which the mean response differed from the mean response at normoxia. I therefore used one-way ANOVA to examine the mean effects associated with oxygen saturation levels at each temperature separately. *A priori* contrast statements were constructed to test for differences in mean responses at normoxia versus varying levels of oxygen saturation.

The critical oxygen saturation (S_{crit}) is defined as the oxygen saturation after which fish can no longer maintain aerobic metabolism. My study used a more objective approach to calculate S_{crit} than has been used previously (e.g., Fernandes et al. 1995). For each experiment, a piecewise linear regression was fit to the data using non-linear least-square estimation (NLIN procedure in SAS; e.g., Fig. 1). A piecewise linear regression fits multiple linear models to data that exhibit different linear relationships between the response and the explanatory variable over the range of values observed for the explanatory variable; these models objectively determine the point where the relationship changes by minimizing the overall residual error (e.g., Ryan et al. 2002). For 22°C experiments, the slope of the line for $\dot{V}o_2$ values greater than S_{crit} was set to 0 because the corresponding slope parameter estimates did not differ from 0 (based on the 95% CI for the slope parameter). For all experiments, if the model did not converge, model

parameters could not be estimated and that particular experiment was excluded from the S_{crit} analysis.

A *t*-test was used to test for a difference in mean S_{crit} at 22 and 30°C. Similarly, a *t*-test was used to determine if the change in each response at normoxia after experiencing an acute rise in temperature from 22 to 30°C was significant. For all statistics, significance was determined at α=0.05. All response values are reported as mean ± one standard error (SE).

RESULTS

Responses to Acute Temperature Change during Normoxia

Resting metabolic rate ($\dot{V}o_2$), gill ventilation ($\dot{V}_g$) and heart rate (f_H) increased significantly, and oxygen extraction (Eo_2) decreased significantly from mean values observed during normoxia when temperature was increased to 30°C (Table 1). The Q_{10} effect of acute temperature increase from 22 to 30°C on $\dot{V}o_2$, Eo_2, $\dot{V}_g$, f_H at normoxia was 2.5, 0.91, 3.7 and 2.0, respectively.

Responses to Hypoxia at Two Temperatures

Summer flounder acted as oxygen regulators (i.e., maintained oxygen consumption until S_{crit}) at 22°C and after an acute increase in temperature to 30°C. Overall oxygen saturation and temperature effects could not be assessed for mean $\dot{V}o_2$ because the interaction between oxygen saturation and temperature was significant (two-way ANOVA, F=18.8, $P<0.05$). However, examination of responses for each temperature level separately revealed that mean $\dot{V}o_2$ remained unchanged until 20% saturation at 22°C and 30% saturation at 30°C (Fig. 2). The calculated mean S_{crit} for fish at 22 and 30°C was 27.2% $\pm$ 0.02 (1.97 mg $O_2 l^{-1}$) and 39.0% $\pm$ 0.02 (2.45 mg $O_2 l^{-1}$). This increase in mean S_{crit} with increasing temperature was significant (Table 1).

Similar to $\dot{V}o_2$, overall oxygen saturation and temperature effects could not be

assessed for mean $\dot{V}_g$ because of a significant interaction between oxygen saturation and

temperature (two-way ANOVA, $F=12.4$, $P<0.05$). However, at both 22 and 30°C, a

significant increase in mean $\dot{V}_g$ was apparent immediately (70% oxygen saturation; Fig.

3). At the lowest hypoxic levels tested, mean $\dot{V}_g$ increased 6.3-fold from normoxia at

22°C, and 2.7-fold from normoxia at 30°C.

Both oxygen saturation and temperature had significant effects on the mean Eo_2

(two-way ANOVA, oxygen saturation: $F=41.5$, $P<0.05$; temperature: $F=4.54$, $P<0.05$;

interaction effect was not significant). Eo_2 was highest during normoxia (>80%

saturation at both temperatures). At both 22 and 30°C, a significant decrease in mean

Eo_2 was apparent at 70% oxygen saturation (Fig. 4). At the lowest oxygen saturation

levels tested, mean Eo_2 decreased 1.7-fold from normoxia at 22°C, and 1.5-fold from

normoxia at 30°C; however, fish were able to extract greater than 50% of available

oxygen at the lowest oxygen saturation tested. Eo_2 was a linear function of $\dot{V}_g$ (Fig. 5),

implying that hypoxia *per se* does not affect extraction, but rather Eo_2 decreases due to

the increase in $\dot{V}_g$ in response to hypoxia.

As observed with Eo_2, both oxygen saturation and temperature significantly

affected mean f_H (2-way ANOVA, oxygen saturation: $F=5.68$ $P<0.05$, temperature:

$F=33.5$, $P<0.05$; interaction effect was not significant). However, mean f_H was not

significantly different from normoxic values until 20% saturation at 22°C and 30%

saturation at 30°C (Fig. 6).

DISCUSSION

Normoxic Responses at Two Temperatures

The mean resting metabolic rate of summer flounder was similar to that of other flatfishes at normoxia (Table 1) and may reflect the relatively sedentary lifestyle of this group of fish. Metabolic requirements of benthic flatfishes are often much lower than the requirements of species with more active lifestyles (e.g., rainbow trout; Table 1). Mean gill ventilation and heart rate for summer flounder were also similar to those reported for other flatfishes, but oxygen extraction was on the higher end of the known flatfish range, resembling that of carp (Table 1).

An acute increase in temperature resulted in changes in mean resting metabolic rate, gill ventilation, oxygen extraction and heart rate during normoxia that are typical of responses observed in teleosts (Rantin et al. 2007). The largest compensatory response was a 2.8-fold increase in mean gill ventilation (Q_{10}=3.7) similar to the 3-fold increase exhibited by starry flounder (*Platichthys stellatus*) exposed to a temperature change from 15 to 20°C (Watters and Smith 1973). The increase in mean gill ventilation is presumably required to meet the temperature-induced increase in resting metabolic rate (Q_{10}=2.5) in an environment with lower oxygen solubility. The observed increase in mean resting metabolic rate can be attributed to increased metabolic requirements at higher temperatures or a stress ('excitement') response to the acute change in temperature to which fish were exposed in the lab. Duthie and Houlihan (1982) observed a large increase in mean metabolic rate (Q_{10}=3.9) in European flounder (*Platichthys flesus*)

exposed to an acute rise in temperature from 5 to 15°C. Over 20 hours, however, mean metabolic rate declined to that observed in European flounder acclimated at 15°C, implying either metabolic compensation or recovery from 'excitement metabolism.' Continued exposure of summer flounder to 30°C may have resulted in a similar response.

Summer flounder showed a 1.7-fold increase in heart rate (Q_{10}=2.0) which, along with an increase in cardiac output, is known to assist with blood oxygen transport during acute exposure to higher temperatures (Stevens et al. 1972, Heath and Hughes 1973). Cardiac output was not measured in my study, but in another flatfish (winter flounder, *Pseudopleuronectes americanus*) heart rate increased (Q_{10}=2.4) with an acute rise in temperature (15-20°C; Cech et al. 1976). Oxygen extraction in teleosts is generally not largely affected by changes in temperature during normoxia (Rantin et al. 2007), and results from my study support this general trend. Even though summer flounder exhibited a significant decrease in mean oxygen extraction after an acute temperature change, this decrease was small (Q_{10}=0.91) and may not have been biologically significant. An accentuated reduction in Eo_2 at higher temperatures during hypoxia was also not observed in summer flounder, as has been reported for tilapia (*Oreochrornis niloticus*; Fernandes and Rantin 1989). In tilapia, oxygen extraction in hypoxia decreased from 53% at 20°C to 35% at 35°C (Fernandes and Rantin 1989).

Responses to Hypoxia at Two Temperatures

The predominant and most immediate response of summer flounder to hypoxia was an increase in gill ventilation, a common strategy shared by other water-breathing fishes (Randall 1982). In general, fish appear to maintain as high a partial pressure of

oxygen in the arterial blood as possible, and increasing gill ventilation promotes branchial oxygen transfer by bringing more oxygen in contact with the gills and by minimizing the mean difference in oxygen partial pressure between inspired water and the blood in the gills. The 6-fold increase in mean gill ventilation during progressive hypoxia at a normal acclimation temperature (22°C) observed in summer flounder is much higher than the 1.6-fold increase reported for plaice, *Pleuronectes platessa* (10°C, Steffensen et al. 1982), the 2-fold increase reported for European flounder (8-10°C, Kerstens et al. 1979, Steffensen et al. 1982) and the 3-fold increase for starry flounder (9-12°C, Watters and Smith 1973). However, total ventilation volume may have been

underestimated in all of these studies because only gill ventilation ($\dot{V}_g$) was considered. Cutaneous respiration has been reported to account for a significant portion of oxygen uptake in flatfishes: 33% in European flounder (Nonnotte and Kirsch 1978) and 27% in plaice (Steffensen et al. 1981). The extent of cutaneous respiration in summer flounder is unknown.

As hypoxia progressed, the mean resting metabolic rate of summer flounder did not increase despite increases in branchial muscle work related to higher gill ventilation (as seen in Beamish 1964). This lack of increase in mean resting metabolic rate is consistent with other benthic fishes (Watters and Smith 1973, Kerstens et al. 1979, Jorgensen and Mustafa 1980, Steffensen et al. 1982, Van den Thillart et al. 1994, Maxime et al. 2000, Taylor and Miller 2001). The energy costs associated with increasing gill ventilation may be met through anaerobic metabolism, as evidenced by increasing blood and muscle lactate concentrations in flatfish experiencing hypoxia (turbot, *Scophthalmus maximus*; Maxime et al. 2000). Other theories explaining this phenomenon

include a low cost of ventilation and declining oxygen uptake in specific tissues due to blood flow rearrangement (Steffensen et al. 1982, Maxime et al. 2000).

The degree of hypoxia tolerance in fishes depends in part on the ability to maintain oxygen extraction in the face of increasing gill ventilation (Steffensen et al. 1982). As gill ventilation increases, water moves more quickly by the gills and functional dead space increases; this generally results in a decrease in oxygen extraction (Saunders 1962, Randall1982). Hypoxia-tolerant carp maintained oxygen extraction at about 82% in the face of a 5 to 6-fold increase in gill ventilation during hypoxia (Lomholt and Johansen 1979). In contrast, oxygen extraction of rainbow trout (considered a hypoxia-intolerant species) decreased from 55 to 20% following a 13-fold increase in gill ventilation during hypoxia (Holeton and Randall 1967). Summer flounder in my study experienced a decrease in mean oxygen extraction due to an increase in mean gill ventilation (as exhibited by the linear relationship between these two responses; Fig. 5). However, the ability of summer flounder to extract oxygen is comparable to that of the European flounder during progressive hypoxia [oxygen extraction declined from 78-65% and 76-52%; Steffensen et al. (1982) and Kerstens et al. (1979)]. European flounder are considered more hypoxia tolerant than oxygen-conforming plaice due to their ability to maintain the same oxygen extraction as plaice, but at a higher gill ventilation (Steffensen et al. 1982). In the same way, summer flounder may be more hypoxia tolerant than either of these flatfishes due to the extent to which they can increase gill ventilation, which rivals that of carp.

Bradycardia is a typical teleost response to hypoxia (Gamperl and Driedzic 2009), but the role of bradycardia in maintaining arterial oxygen levels remains unclear. In most

teleosts, bradycardia is accompanied by an increase in stroke volume, which maintains cardiac output while increasing blood pressure (Randall 1982). Previously, this increase in pressure was thought to promote gill diffusion capacity by increasing lamellar recruitment (i.e., number of secondary lamellae perfused with blood) and evenly distributing blood within the lamellae (Farrell et al. 1979, Farrell et al. 1980, Booth 1979, Randall 1982). However, arterial blood gases in rainbow trout are unaffected by the presence or absence of bradycardia during hypoxia (Perry and Desforges 2006). Regardless, bradycardia does appear to have a cardio-protective effect in the face of declining venous oxygen (Farrell 2007), which flatfishes depend on for supplying oxygenated blood to the cardiac muscle (Farrell and Jones 1992, Farrell 2007).

Bradycardia was observed in summer flounder only at low oxygen levels, shortly after fish reached S_{crit}. In contrast, bradycardia in winter flounder was observed at oxygen levels as high as ~60% saturation when acclimated to 8°C and exposed to progressive hypoxia (Mendonça 2009). However, these results for winter flounder are unusual because winter flounder did not experience any bradycardia during progressive hypoxia at higher acclimation temperatures (10°C, 15°C; Cech et al. 1977, Mendonça 2009). After an acute increase in temperature, S_{crit} and bradycardia in summer flounder occurred at a higher oxygen saturation level than at the acclimation temperature, suggesting decreased tolerance to hypoxia. However, both the absence of bradycardia or the occurrence of bradycardia only after S_{crit} is reached imply a very high tolerance for hypoxia in flatfish cardiac muscle and possibly the ability for substantial anaerobic energy production (Jørgensen and Mustafa 1980, Mendonça et al. 2007). Further

investigations into summer flounder cardiac function may provide a more complete understanding of how this species responds to hypoxia.

The mean S_{crit} for summer flounder at 22°C expressed as percent oxygen saturation (27%) fell within the range of S_{crit} values reported for other flatfishes experiencing progressive hypoxia at their acclimation temperatures: turbot (13-20% saturation, Maxime et al. 2000), sole, *Solea solea* (12-20% saturation, Van den Thillart et al. 1994), starry flounder (30% saturation, Watters and Smith 1973) and European flounder (40-50% saturation, Steffensen et al .1982). S_{crit} has been reported to increase and decrease in response to increasing temperature (Fry and Hart 1948, Ultsch et al. 1978, Fernandes and Rantin 1989, Schurmann and Steffensen 1997). S_{crit} can also remain independent of an increase in temperature (Ott et al. 1980). For summer flounder, mean S_{crit} increased in response to an acute rise in temperature (from 22 to 30°C). This increase in S_{crit} may be due to a decreased capacity for increasing gill ventilation at higher temperatures (Hughes and Shelton 1962), as seen in the relatively small 3-fold increase in mean gill ventilation during hypoxia at 30°C, as compared with the 6-fold increase at 22°C. Gill ventilation is limited by the branchial pump capacity (Farrell and Steffensen 1987), and lower temperatures usually permit greater scope for increase during hypoxia (Fernandes 1995). Because mean gill ventilation was higher during normoxia at 30 versus 22°C, summer flounder had a limited ability to increase gill ventilation at 30°C as hypoxia progressed.

Implications

The large capacity to which summer flounder are able to increase gill ventilation while maintaining oxygen extraction above 50% suggests that they are well-adapted to tolerating the hypoxic conditions they are likely to encounter in mid-Atlantic estuaries. An acute increase in temperature limits the ability of summer flounder to increase gill ventilation, thereby increasing S_{crit} and reducing hypoxia tolerance. However, S_{crit} values at both 22 and 30°C indicate that summer flounder are able to maintain metabolic requirements down to low levels of oxygen saturation (27% saturation or 2.0 mg O_2 l^{-1} at 22°C, 39% saturation or 2.5 mg O_2 l^{-1} at 30°C). The length of time summer flounder can survive at oxygen levels below S_{crit} is unknown, although fish may exhibit behavioral avoidance to hypoxia before reaching S_{crit}. However, only when oxygen concentrations declined below 1 mg O_2 l^{-1} did summer flounder exhibit avoidance in the laboratory (Tyler 2004), and it seems unlikely that the growing duration and extent of hypoxia in areas like Chesapeake Bay will significantly alter summer flounder movement or distribution.

Despite the ability to remain in hypoxic situations, the cost of maintaining resting metabolic rate during low oxygen saturation levels higher than S_{crit} may deflect energy away from other important processes. For example, exposure to hypoxia affects feeding and growth rates in juvenile summer flounder even at moderate hypoxic levels (50-70% oxygen saturation), and especially at higher temperatures (30°C; Stierhoff et al. 2006). Hypoxia has also been shown to negatively affect feeding, growth, predation efficiency, predator escape responses, disease resistance and reproduction in fishes (Noga 1993, Tallqvist et al. 1999, Yamashita et al. 2001, Wu et al. 2003, Domenici et al. 2007, Landry

et al. 2007, Chabot and Claireaux 2008). These responses are related energetically to aerobic scope, the decrease of which can limit important activities (Claireaux and Lefrançois 2007). For this reason, further work establishing the aerobic scope of summer flounder and examining how aerobic scope is affected by hypoxia and temperature is necessary. The metabolic requirements and cardiorespiratory responses of summer flounder established in this study provide the requisite baseline data necessary to proceed with such future metabolic studies on summer flounder.

LITERATURE CITED

Beamish, F.W.H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. III. Influence of oxygen. Canadian Journal of Zoology 42(3):355-366.

Bigelow H.B. and W.C. Schroeder. 1953. Fishes of the Gulf of Maine. U.S. Fish and Wildlife Service. Fish. Bull. 53(74):85-118.

Booth, J.H. 1979. The effects of oxygen supply, epinephrine, and acetylcholine on the distribution of blood flow in trout gills. Journal of Experimental Biology 83:31-39.

Brett, J. R. 1956. Some principles in the thermal requirements of fishes. Quarterly Review of Biology 31:5–87.

Cech, J.J., Bridges, D.W., Rowell, D.M. and P.J. Balzer. 1976. Cardiovascular responses of winter flounder, *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. Canadian Journal of Zoology 54:1383-1388.

Cech, J.J., Rowell, D.W. and J.S. Glasgow. 1977. Cardiovascular responses of the winter flounder *Pseudopleuronectes americanus* to hypoxia. Comparative Biochemistry and Physiology 57A:123-125.

Chabot, D. and G. Claireaux. 2008. Environmental hypoxia as a metabolic constraint on fish: the case of Atlantic cod, *Gadus morhua*. Marine Pollution Bulletin 57:287-294.

Claireaux, G. and C. Lefrançois. 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Philosophical Transactions of the Royal Society 362:2031-2041.

Diaz, R.J. 2001. Overview of hypoxia around the world. Journal of Environmental Quality 30:275-281.

Diaz, R. J. and D. L. Breitburg. 2009. The hypoxic environment. Chapter 1 in: Hypoxia. Fish Physiology Vol. 27, C. G. Richards, A. P. Farrell, and C. J. Brauner (eds.), Academic Press, San Diego, CA.

Domenici, P., Lefrançois, C. and A. Shingles. 2007. Hypoxia and the antipredator behaviors of fishes. Philosophical Transactions of the Royal Society 362:2105-2121.

Duthie, G.G. and D.F. Houlihan. 1982. The effect of single step and fluctuating temperature changes on the oxygen consumption of flounders, *Platichthys flesus* (L.): Lack of temperature adaptation. Journal of Fish Biology 21:215-226.

Farrell, A.P., Daxboeck, C. and D.J. Randall. 1979. The effect of input pressure and flow on the pattern and resistance to flow in the isolated perfused gill of a teleost fish. Journal of Comparative Physiology 133B:233-240.

Farrell, A.P., Sobin, S.S., Randall, D.J. and S. Crosby. 1980. Intralamellar blood flow patterns in fish gills. American Journal of Physiology 239:R428-R436.

Farrell, A.P. and J.F. Steffensen. 1987. An analysis of the energetic cost of the branchial and cardiac pumps during sustained swimming in trout. Fish Physiology and Biochemistry 4:73-79.

Farrell, A.P., and Jones, D. R. 1992. The heart, p. 1-88. In: Fish Physiology, edited by W. Hoar, D. Randall, and A. Farrell. Vol. XII, Part A. Academic Press, Inc., San Diego.

Farrell, A. P. 2007. Tribute to P. L. Lutz: a message from the heart – why hypoxic bradycardia in fishes. Journal of Experimental Biology 210: 1715-1725.

Fernandes, M.N. and F.T. Rantin. 1989. Respiratory responses of *Oreochromis niloticus* (Pisces, Cichlidae) to environmental hypoxia under different thermal conditions. Journal of Fish Biology 35:509-519.

Fernandes, M.N., Barrionuevo, W.R. and F.T. Rantin. 1995. Effects of thermal stress on respiratory responses to hypoxia of a South American Prochilodontid fish, *Prochilodus scrofa*. Journal of Fish Biology 46:123-133.

Fry, F.E.J. and J.S. Hart. 1948. The relation of temperature to oxygen consumption in the goldfish. Biological Bulletin 94:66-77.

Gamperl, A.K. and W.R. Driedzic. 2009. Cardiovascular function and cardiac metabolism. Chapter 7 in: Hypoxia. Fish Physiology Vol. 27, C. G. Richards, A. P. Farrell, and C. J. Brauner (eds.), Academic Press, San Diego, CA.

Hagy, J.D., Boynton, W.R., Keefe, C.W. 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.

Heath, A.G. and G.M. Hughes. 1973. Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). Journal of Experimental Biology 59:323-338.

Holeton, G.F. and D.J. Randall. 1967. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of the rainbow trout. Journal of Experimental Biology 46:317-327.

Hughes, G.M. 1960. A comparative study of gill ventilation in marine teleosts. Journal of Experimental Biology 37:28-45.

Hughes, G.M. and G. Shelton. 1962. Respiratory mechanisms and their nervous control in fish. In *Comparative Physiology and Biochemistry*. Ed. O. Lowenstein. Vol. I, pp.275-364. London: Academic Press.

Hughes, G.M. 1973. Respiratory responses to hypoxia in fish. American Zoologist 13:475-489.

Jobling, M. Bioenergetics: feed intake and energy partitioning. In: *Fish Ecophysiology*. Eds. J.C. Rankin, F.B. Jensen. London, Chapman & Hall, 1993.

Jørgensen, J.B. and T. Mustafa. 1980. The effect of hypoxia on carbohydrate metabolism in flounder (*Platichthys flesus* L.) – I. Utilization of glycogen and accumulation of glycolytic end products in various tissues. Comparative Biochemistry and Physiology 67B:243-248.

Kerstens, A., Lomholt, J.P. and K. Johansen. 1979. The ventilation, extraction and uptake of oxygen in undisturbed flounders, *Platichthys flesus*: responses to hypoxia acclimation. Journal of Experimental Biology 83:169-179.

Klein-MacPhee, G. 1979. Growth, activity and metabolism studies of summer flounder *Paralichthys dentatus* (L.) under laboratory conditions. PhD Dissertation. University of Rhode Island, Kingston, RI.

Kramer, D.L. 1987. Dissolved oxygen and fish behavior. Environmental Biology of Fish 18(2):81-92.

Landry, C.A., Steele, S.L., Manning, S. and A.O. Cheek. Long term hypoxia suppresses reproductive capacity in the estuarine fish, *Fundulus grandis*. Comparative Biochemistry and Physiology, Part A 148:317-323.

Lewis, J.M., Costa, I., Val, A.L., Almeida-Val, V.M.F., Gamperl, A.K. and W.R. Driedzic. 2007. Responses to hypoxia and recovery: repayment of oxygen debt is not associated with compensatory protein synthesis in the Amazonian cichlid, *Astronotus ocellatus*. Journal of Experimental Biology 210:1935-1943.

Littell, R.C., Milliken, G., Stroup, W.W., Wolfinger, R. and O. Schabenberger. 2006. SAS for mixed models, 2nd edition. SAS Institute, Cary, North Carolina.

Lomholt, J.P. and K. Johansen. 1979. Hypoxia acclimation in carp – how it affects O_2 uptake, ventilation and O_2 extraction from water. Physiological Zoology 52:38-49.

Maxime, V., Pichavant, K., Boeuf, G. and G. Nonnotte. 2000. Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. Fish Physiology and Biochemistry 22:51-59.

Mendonça, P.C., Genge, A.G., Deitch, E.J. and A.K. Gamperl. 2007. Mechanisms responsible for the enhanced pumping capacity of the in situ winter flounder heart (*Pseudopleuronectes americanus*). American Journal of Physiology 293:R2112-R2119.

Mendonça, P.C. 2009. The cardiovascular physiology of winter flounder (*Pleuronectes americanus*): Control Mechanisms and Environmental Influences. PhD. Dissertation. Memorial University of Newfoundland, St. John's, Newfoundland.

Noga, E.J. Fungal diseases of marine and estuarine fishes. In: *Pathobiology of Marine and Estuarine Organisms*. Eds. J.A. Couch and J.W. Fournie. Boca Raton, CRC Press, 1993.

Nonnotte, G. and R. Kirsch. 1978. Cutaneous respiration in seven seawater teleosts. Respiration Physiology 35:111-118.

Ott, M.E., Heisler, N. and G.R. Ultsch. 1980. A re-evaluation of the relationship between temperature and the critical oxygen tension in freshwater fishes. Comparative Biochemistry and Physiology 67A:337-340.

Perry, S.F. and P.R. Desforges. 2006. Does bradycardia or hypertension enhance gas transfer in rainbow trout (*Oncorhynchus mykiss*)? Comparative Biochemistry and Physiology 144A:163-172.

Perry, S.F., Jonz, M.G. and K.M. Gilmour. 2009. Oxygen sensing and the hypoxic ventilatory response. Chapter 5 in: Hypoxia. Fish Physiology Vol. 27, C. G. Richards, A. P. Farrell, and C. J. Brauner (eds.), Academic Press, San Diego, CA.

Pörtner, H.O. and R. Knust. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95-97.

Randall, D.J., Holeton, G.F. and E.D. Stevens. 1967. The exchange of oxygen and carbon dioxide across the gills of rainbow trout. Journal of Experimental Biology 46:339-348.

Randall, D. 1982. The control of respiration and circulation in fish during exercise and hypoxia. Journal of Experimental Biology 100:275-288.

Rantin, F.T., Kalinin, A.L. and M.L. Glass. 2007. The effects of temperature on respiratory and cardiac function of teleost fish. In *Fish Respiration and Environment* (Fernandes, M.N., Rantin, F.T., Glass, M.L., Kapoor, B.G., eds.), pp.55-82. New Hampshire: Science Publishers.

Richards, F.A. 1965. Dissolved gases other than carbon dioxide. In: *Chemical oceanography*. (Riley, J.P. and Skirrow, G., eds.), Academic Press, New York, NY.

Roundtree, R.A. and K.W. Able. 1992. Foraging habits, growth, and temporal patterns of salt-marsh creek habitat use by young-of-the-year summer flounder in New Jersey. Transactions of the American Fisheries Society 121:765-776.

Ryan, S.E., Porth, L.S. and C.A. Troendle 2002. Defining phases of bedload transport using piecewise regression. Earth Surface Processes and Landforms 27(9): 971 990.

Sackett, D.K., Able, K.W. and T.M. Grothues. 2008. Habitat dynamics of summer flounder *Paralichthys dentatus* within a shallow USA estuary based on multiple approaches using acoustic telemetry. Marine Ecology Progress Series 364:199-212.

Saunders, R.L. 1961. The irrigation of the gills in fishes I: Studies of the mechanism of branchial irrigation. Canadian Journal of Zoology 39:637-653.

Saunders, R.L, 1962. The irrigation of the gills in fishes II: Efficiency of oxygen uptake in relation to respiratory flow, activity and concentrations of oxygen and carbon dioxide. Canadian Journal of Zoology 40:817-62

Schurmann, H. and J.F. Steffensen. 1997. Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. Journal of Fish Biology 50:1166-1180.

Shen, J., Wang, T., Herman, J., Mason, P. and G.L. Arnold. 2008. Hypoxia in a coastal embayment of the Chesapeake Bay: a model diagnostic study of oxygen dynamics. Estuaries and Coasts 31:652-663.

Smith, R.W. and F.C. Daiber. 1977. Biology of the summer flounder, *Paralichthys dentatus*, in Delaware Bay. Fisheries Bulletin 75(4):823-830.

Steffensen, J.F., Lomholt, J.P. and K. Johansen. 1981. The relative importance of skin oxygen uptake in the naturally buried plaice, *Pleuronectes platessa*, exposed to graded hypoxia. Respiration Physiology 44:269-275.

Steffensen, J.F., Lomholt, J.P. and K. Johansen. 1982. Gill ventilation and O_2 extraction during graded hypoxia in two ecologically distinct species of flatfish, the flounder (*Platichthys flesus*) and the plaice (*Pleuronectes platessa*). Environmental Biology of Fishes 7(2):157-163.

Steffensen, J.F. 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiology and Biochemistry 6(1):49-59.

Stevens, E.D., Bennion, G.R., Randall, D.J. and G. Shelton. 1972. Factors affecting arterial pressures and blood flow from the heart in intact, unrestrained lingcod, *Ophiodon elongates*. Comparative Biochemistry and Physiology 43A:681-695.

Stierhoff, K.L., Targett, T.E. and K. Miller. 2006. Ecophysiological responses of juvenile summer and winter flounder to hypoxia: experimental and modeling analyses of effects on estuarine nursery quality. Marine Ecological Progress Series 325:255-266.

Stierhoff, K.L., Targett, T.E. and J.H. Power. 2009. Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA:DNA. Canadian Journal of Fisheries and Aquatic Sciences 66:1033-1047.

Taylor, C.J. and J.M. Miller. 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.

Tallqvist, M., Sandberg-Kilpi, E. and E. Bonsdorff. 1999. Juvenile flounder, *Platichthys flesus* (L.), under hypoxia: effects on tolerance, ventilation rate and predation efficiency. Journal of Experimental Marine Biology and Ecology 242:75-93.

Tyler, R.M. 2004. Distribution and avoidance patterns of juvenile summer flounder (*Paralichthys dentatus*) and weakfish (*Cynoscion regalis*) in relation to hypoxia: field studies in a temperate coastal lagoon tributary and laboratory choice-trial experiments. PhD thesis. University of Delaware, Newark, DE.

Tyler, R.M., Damian, B.C. and T.E. Targett. 2009. Temporal and spatial dynamics of diel-cycling hypoxia in estuarine tributaries. Estuaries and Coasts 32:123-145.

Ultsch, G.R., Boschung, H. and M.J. Ross. 1978. Metabolism, critical oxygen tension, and habitat selection in darters (Etheostoma). Ecology 59:99-107.

Van den Thillart, G., Dalla Via, J., Vitali, G. and P. Cortesi. 1994. Influence of long term hypoxia exposure on the energy metabolism of *Solea solea*. I. Critical O2 levels for aerobic and anaerobic metabolism. Marine Ecology Progress Series 104:109-117.

Watters, K.W. and L.S. Smith. 1973. Respiratory dynamics of the starry flounder *Platichthys stellatus* in response to low oxygen and high temperature. Marine Biology 19:133-148.

Wu, R.S.S., Zhou, B.S., Randall, D.J., Woo, N.Y.S. and P.K.S. Lam. 2003. Aquatic hypoxia is an endocrine disrupter and impairs fish reproduction. Environmental Science and Technology. 37:1137-1141.

Wyanski, D.M. 1990. Patterns of habitat utilization in 0-age summer flounder. Masters Thesis. College of William and Mary, Gloucester Point, VA.

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

Table 1. Summary of cardiorespiratory parameters in summer flounder and other fishes at normoxia. Resting metabolic rate ($\dot{V}o_2$), critical saturation (S_{crit}), oxygen extraction (Eo_2), ventilation volume ($\dot{V}_g$) and heart rate (f_H) of summer flounder acclimated to 22°C and following an increase to 30°C. Values are means ± standard error. Cardiorespiratory parameters of representative flatfishes, rainbow trout (hypoxia-intolerant) and carp (hypoxia-tolerant) are included for comparison. Values are for normal temperatures or after an acute increase in temperature ([a]). Asterisks denote significant difference at α=0.05 relative to results observed at 22°C.

Species		Temp (°C)	VO_2 (mgO$_2$ kg^{-1} h^{-1})	S_{crit} (%)	EO_2 (%)	V_g (l kg^{-1} min^{-1})	f_H (beats min^{-1})	References
Paralichthys dentatus	Summer flounder	22	45.3 ± 2.29	27.2 ± 0.02	86.7 ± 0.02	0.130 ± 0.01	46.4 ± 5.26	This study
		30[a]	92.8 ± 5.46*	39.0 ± 0.02*	80.2 ± 0.02*	0.369 ± 0.03*	79.7 ± 1.65*	This study
Platichthys flesus	European flounder	10	42.8	40-50	78.1	0.106		Steffensen et al. 1982
Pleuronectes platessa	Plaice	10	31.0		69.0	0.089		Steffensen et al. 1982
Pseudo-pleuronectes americanus	Winter flounder	15	43.1				46	Cech et al. 1976
		20[a]	77.5				71	Cech et al. 1976
Platichthys stellatus	Starry flounder	9-11	37.1	31.8	55.0	0.142		Watters & Smith 1973
		19[a]	68.2			0.438		Watters & Smith 1973
Oncorhynchus mykiss	Rainbow trout	15	113.7		55.0	0.274		Holeton & Randall 1967
Cyprinus carpio	Carp	20	64.3		82.0	0.195		Lomholt & Johansen 1979

Figure 1. The determination of S_{crit} from the resting metabolic rate of two fish from this study experiencing progressive hypoxia, one fish acclimated to 22°C (●) and the other fish measured acutely at 30°C (○). Arrows indicate the S_{crit} values estimated from piecewise linear regressions (22°C, S_{crit}=26%; 30°C, S_{crit}=40%). The S_{crit} values for individual experiments were used to calculate mean S_{crit} values for each temperature.
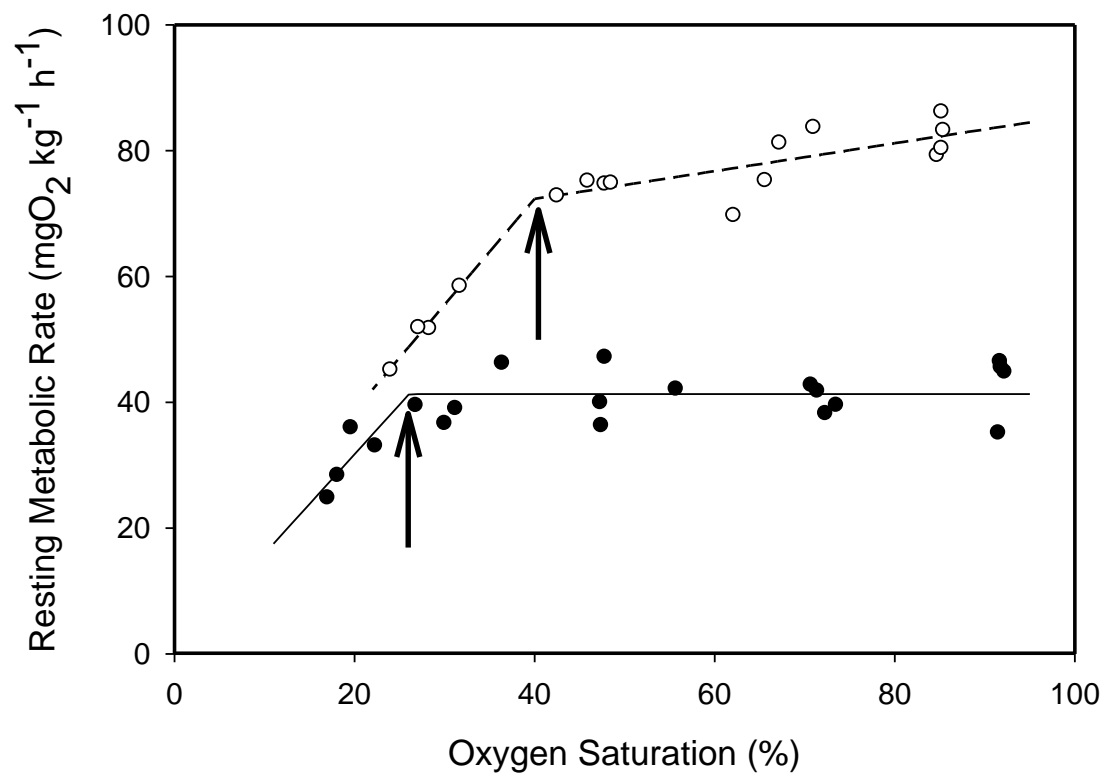
Figure 2. Mean resting metabolic rate ($\dot{V}o_2$) of summer flounder (*Paralichthys dentatus*) acclimated to 22°C (●) and acutely exposed to 30°C (○) in relation to changing oxygen saturation levels. Asterisks denote significant difference at α=0.05 relative to normoxia within the same temperature.
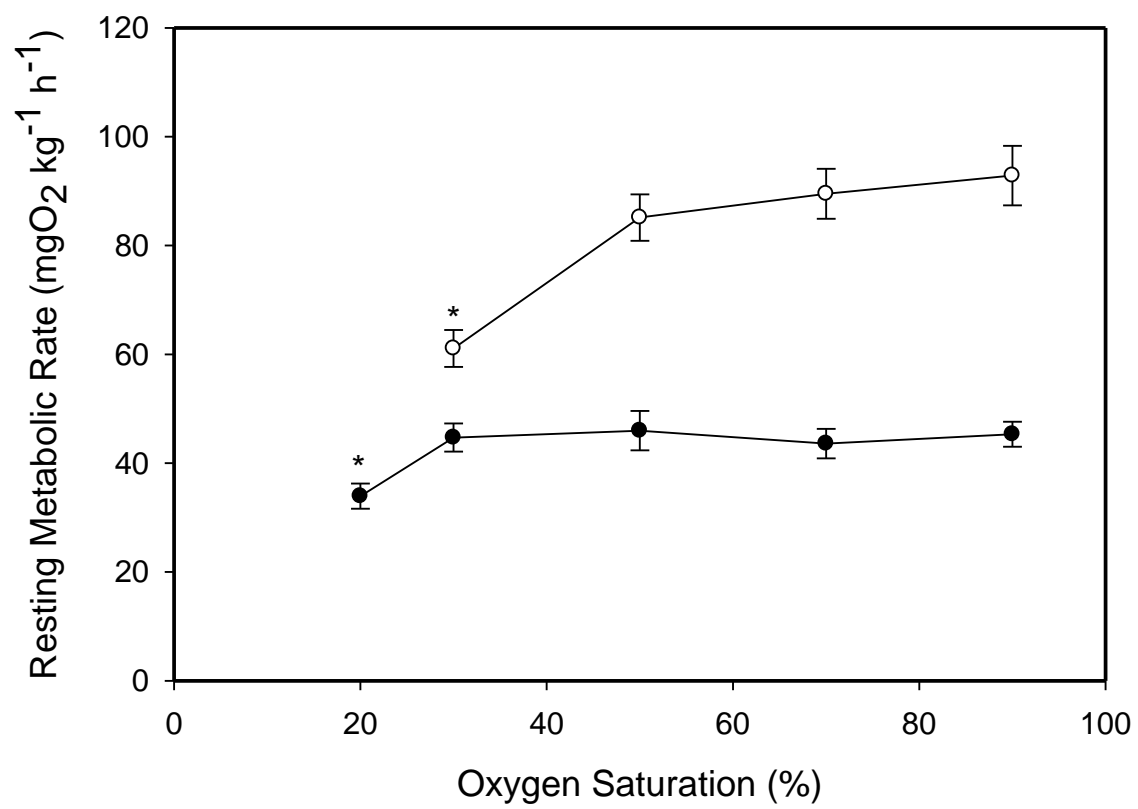
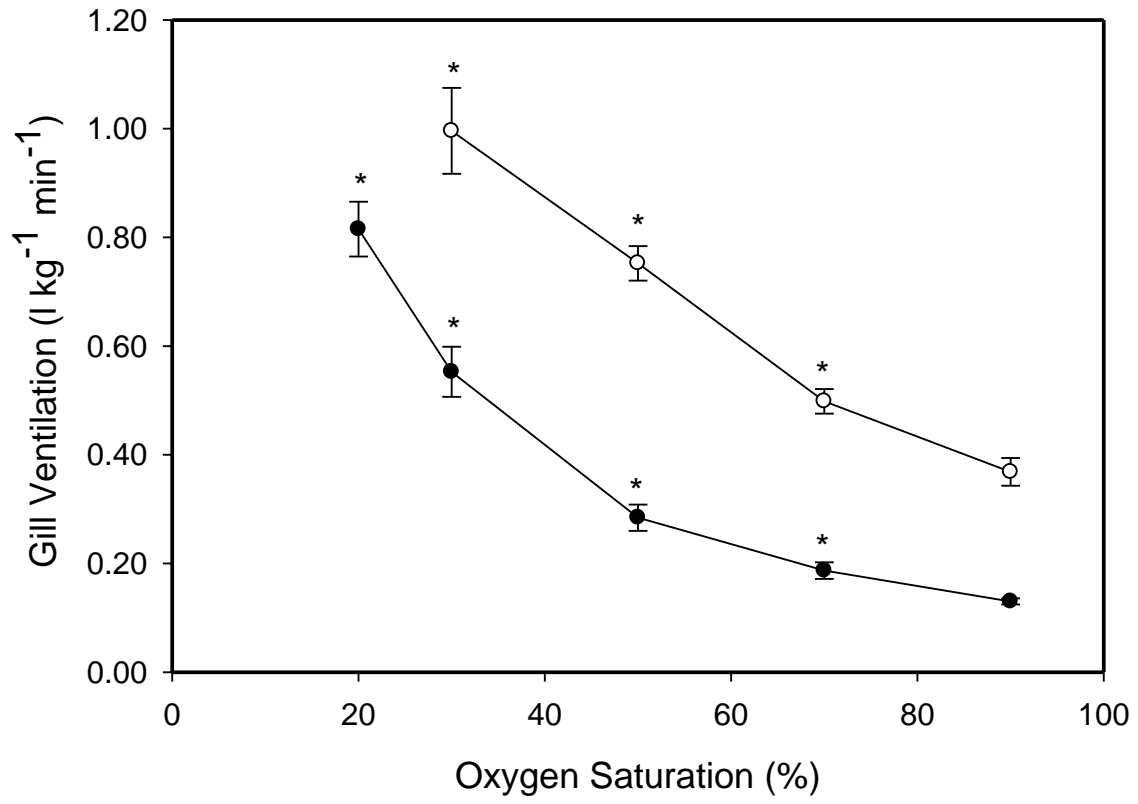Figure 3. Mean gill ventilation ($\dot{V}_g$) of summer flounder (*Paralichthys dentatus*) acclimated to 22°C (●) and acutely exposed to 30°C (○) in relation to changing oxygen saturation levels. Asterisks denote significant difference at α=0.05 relative to normoxia within the same temperature.

Figure 4. Mean oxygen extraction (Eo_2) of summer flounder (*Paralichthys dentatus*) acclimated to 22°C (●) and acutely exposed to 30°C (○) in relation to changing oxygen saturation levels. Asterisks denote significant difference at α=0.05 relative to normoxia within the same temperature.
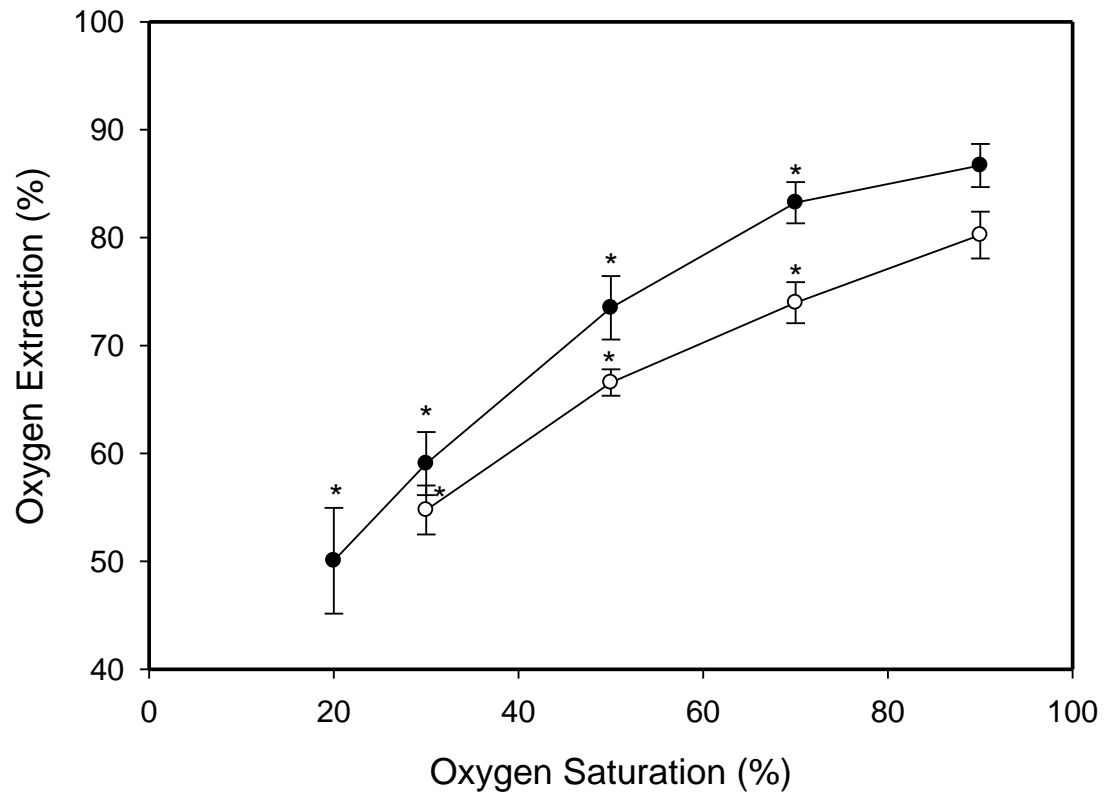
Figure 5. Oxygen extraction (Eo_2) expressed as a linear function of gill ventilation ($\dot{V}_g$) of summer flounder (*Paralichthys dentatus*) acclimated to 22°C ($\bullet$) and acutely exposed to 30°C ($\circ$)The symbols represent mean ± one standard error for $\dot{V}_g$ (horizontal) and Eo_2 (vertical).
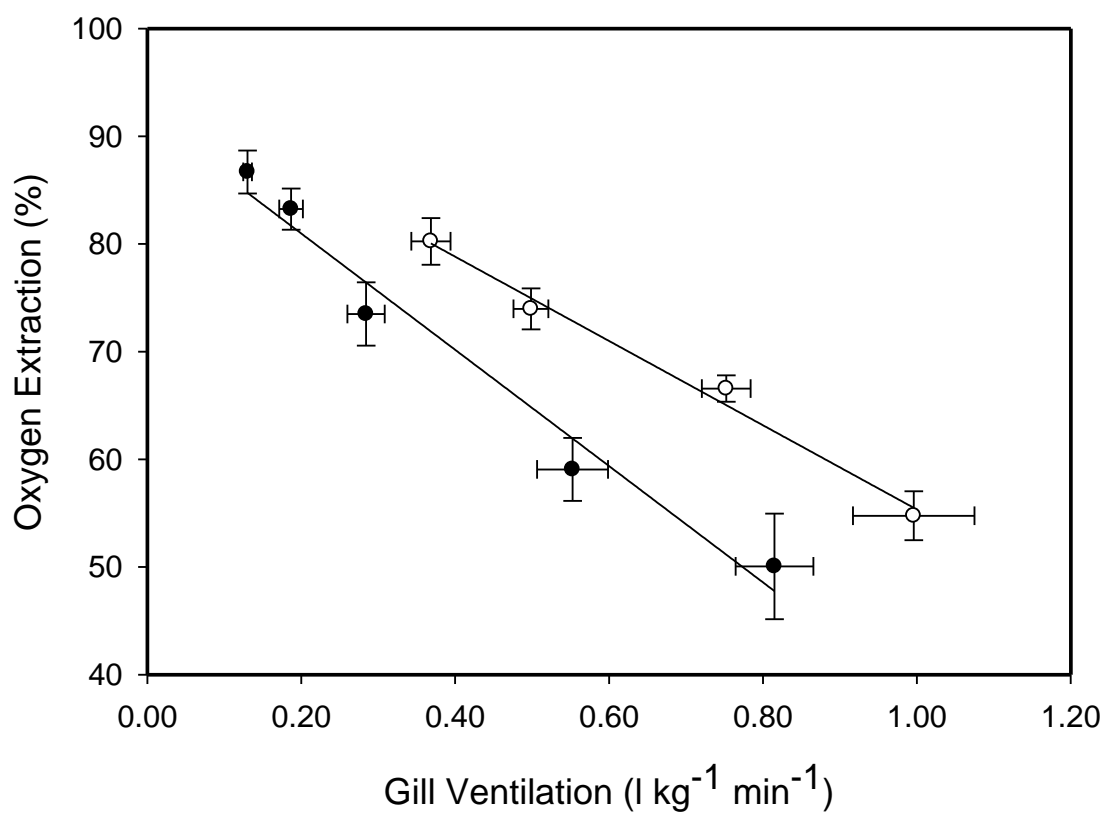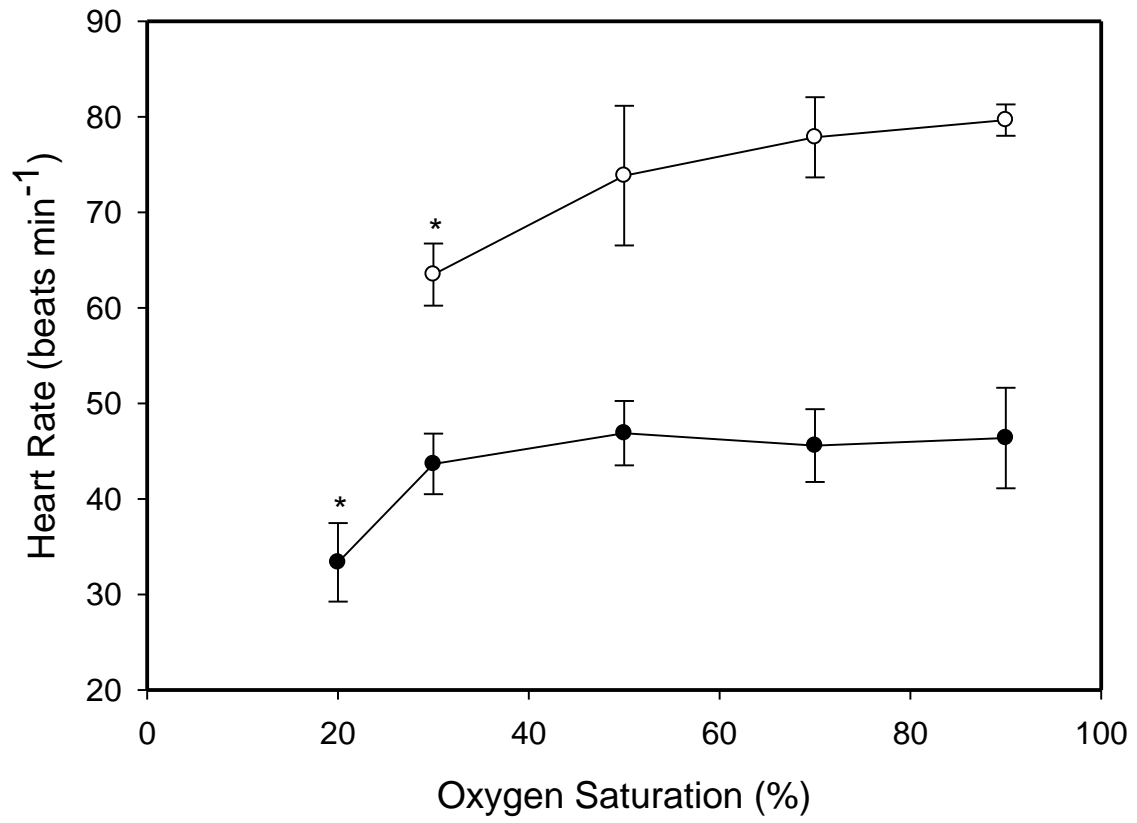
Figure 6. Mean heart rate (f_H) of summer flounder (*Paralichthys dentatus*) acclimated

to 22°C (●) and acutely exposed to 30°C (○) in relation to changing oxygen saturation

levels. Asterisks denote significant difference at α=0.05 relative to normoxia within the

same temperature.

VITA

Karen Marie Capossela

Born in Castro Valley, California on 14 April 1981. Graduated from Piedmont Hills High School in San Jose, California in 1999. Graduated *summa cum laude* from The College of William and Mary in Virginia with a Bachelor of Science in Biology in May 2004. Entered the Master of Science program at the School of Marine Science, Virginia Institute of Marine Science, at The College of William and Mary in 2006.