Recruitment dynamics and otolith chemical signatures of juvenile gray snapper, *Lutjanus griseus,* among West Florida estuarine and coastal marine ecosystems

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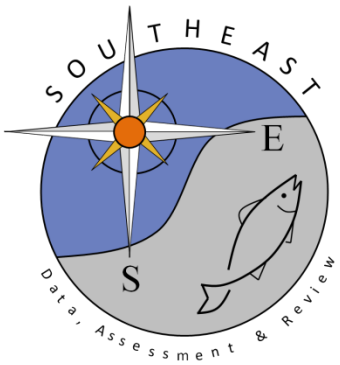

RECRUITMENT DYNAMICS AND OTOLITH CHEMICAL SIGNATURES OF JUVENILE GRAY SNAPPER, *LUTJANUS GRISEUS*, AMONG WEST FLORIDA ESTUARINE AND COASTAL MARINE ECOSYSTEMS

by

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ABSTRACT

RECRUITMENT DYNAMICS AND OTOLITH CHEMICAL SIGNATURES OF JUVENILE GRAY SNAPPER, *LUTJANUS GRISEUS*, AMONG WESTFLORIDA ESTUARINE AND COASTAL MARINE ECOSYSTEMS

Cecelia Lounder

A hierarchical approach to examine nursery function of coastal systems was tested for gray snapper, *Lutjanus griseus*. Juveniles were sampled with trawls and seines in four regions along west Florida. The first two tiers of habitat evaluation showed that seagrass was a significant variable for presence/absence and density of gray snapper (Binomial GLM; $p < 0.001$, Delta Lognormal GLM; $p = 0.002$). Significant differences in growth rates were observed in 2007 (ANCOVA test for equal slopes; $p < 0.001$) but not 2006, driven by fastest growth in the Southwest region and slowest in the Big Bend region. Region-specific natural tags from otolith chemistry were derived from element:Ca ratios (Ba:Ca, Li:Ca, Mg:Ca, Mn:Ca, and Sr:Ca) and carbonate stable isotopes values ($\delta^{13}C$ and $\delta^{18}O$). Significant differences were found among study regions and between sampling years (MANOVA Pilia's trace; $p < 0.001$ for both effects). Cross validated classification accuracies using four regions were 77.9% in 2006, 85.0% in 2007, and 76.3% when years were modeled jointly. Using three regions raised classification success to 82.6% in 2006, 92.3% in 2007, and 83.3% when years were modeled jointly. Subsequent studies may employ tags based on otolith chemical signatures to estimate nursery sources of adults.

INTRODUCTION

Estuaries and coastal waters are among the more productive marine ecosystems and serve as prime nursery habitat for a vast number of fish species during their juvenile life stage. Beck et al. (2001) reported the origin of the nursery-role concept dates to the beginning of the 20th century when ecologists first examined densities and growth rates of fishes that used estuaries during early life stages before moving offshore, and occupying distinct juvenile and adult habitats is a well-documented aspect of ontogeny in many marine species. Therefore, as concern over marine habitat degradation escalates, defining nursery areas for ecologically and economically important species has become crucial.

Beck et al. (2001) proposed that the underlying criterion for defining nursery habitat is whether the contribution per unit area of a particular species to production of individuals recruiting to adult populations is, on average, greater than from other habitats where juveniles occur. Subsequent studies on nursery function have explored this benchmark definition in greater depth to gain insight and improve management of essential habitat for fish populations (Gillanders et al. 2003; Heck et al. 2003). One caveat to Beck et al.'s (2001) nursery definition is the possibility that a larger habitat may contribute more juvenile recruits to the adult population but have a relatively lower production per unit area than less expansive habitats (Dahlgren et al. 2006). Therefore, the ultimate test of significant nursery contribution to adult populations may be to

directly measure movement of individuals from juvenile habitats to the adult population (Gillanders et al. 2003; Patterson et al. 2005; Dahlgren et al. 2006).

The nursery-role hypothesis focuses on life strategies where a separation exists between juvenile and adult habitats (Beck et al. 2001). Increasing age and size of fish with depth is usually related to ontogentic habitat shifts but can vary between rapid, directional migrations, to indirect and overlapping patterns where nursery habitat may occur as a series of different juvenile habitats (Beck et al. 2001; Gillanders et al. 2003). Early life-stage is a crucial period of growth and development and access to food and nutrients are essential to ensure survival and recruitment to adult populations. High primary and secondary productivity in coastal habitats provides essential food sources for sub-adult fish that may be resource-limited at early stages (Houde 1987). As larvae settle among bays and estuaries, the availability of food and complex habitat structure have significant impacts on year-class strength. In a review of seagrass nursery habitats, Heck et al. (2003) state that simply the effect of structure enhances survival of juveniles by serving as a refuge from predators. Exponential growth rate is also fundamental because developing juveniles are more vulnerable to predation at smaller sizes (Houde 1987). Abundant prey resources promote rapid growth and habitat complexity offers refugia, both of which reduce predation-mediated mortality and enhance the likelihood of survival (Bailey and Houde 1989).

The early studies of nursery habitat tended to look at the function of estuaries as a whole, but research has since focused in on smaller habitats within estuaries as being particularly productive. High densities of juvenile fishes within vegetated habitats has led to studies focusing on the role marshes, mangroves and seagrass beds as potential

nurseries (Weinstein and Heck 1979; Nixon 1980; Beck et al. 2001). Other recent studies have examined densities and growth and survival rates as evidence of nursery function among microhabitat such as seagrass, marsh, mangrove and reef habitat (Nagelkerken et al. 2002; Gillanders et al. 2003; Heck et al. 2003). Estuarine marshes provide juvenile habitat for fishes and invertebrate species (Boesch and Turner 1984), while mangroves are well documented as habitat for fish early life stages, particularly in the southern ranges of Florida and in the Caribbean (Nagelkerken et al. 2000; Chittaro et al. 2005).

While vegetated habitats clearly are important as nurseries, recent evidence has expanded the nursery concept to include other structured habitats such as oyster reefs, corals, and hard bottom substrates (Lindeman 1997; Beck et al. 2001). Structural complexity of these biotopes provides necessary shelter and foraging opportunities similar to vegetated habitats. Recent work has also investigated important juvenile habitats which play a similar role to nurseries by sustaining adult populations and often encompass similar characteristics as nursery habitat (Dahlgren et al. 2006). Shallow water, high food abundance and low occurrence of predators are key characteristics noted among valuable juvenile habitats (Dahlgren et al. 2006).

Survival and production of estuarine-dependent reef fishes is critical for commercial and recreational fisheries. The majority of estuarine fish populations are targeted by these industries, utilizing nurseries in early life then undergoing an ontogenetic shift with increasing size and age to adult habitats (Weinstein and Heck 1979; Laegdsgaard and Johnson 1995; Nagelkerken et al. 2002). In fisheries, the high potential for export of biomass, nutrients, and energy from estuaries to offshore foodwebs underscores the role of estuarine nurseries in fish recruitment dynamics (Nagelkerken et

al. 2000; Beck et al. 2001; Gillanders et al. 2003; Eggleston et al. 2004). With the strong evidence for estuarine microhabitats as nurseries for commercial and recreational species, the widespread losses of these habitats have prompted studies focused on understanding the effect that anthropogenic disturbances have on fish populations.

Awareness of the significant role structured habitats play in supporting diversity and high productivity of coastal ecosystems highlights the severity of threats to their existence. Excess nutrients, mainly phosphorous and nitrogen, inundate wetlands and estuaries through runoff from urban areas and agriculture, causing increased algal and phytoplankton biomass and depletion of dissolved oxygen (Howarth et al. 2000; Livingston 2007). The incidence of eutrophication is a global concern in marine ecosystems and in the Gulf of Mexico the drainage from the Mississippi and Atchafalaya Rivers contributes to the largest zone of oxygen-depleted waters in the western Atlantic (Rabalais et al. 2002). The effects of eutrophication include decreasing species diversity, altering ecosystem structure and function, and destroying fish habitat. Certain fishing practices, such as trawling, dredging, and aquaculture, also readily impact invaluable habitat either through pollution or removal of structure (Coleman and Williams 2002). The incidence of harmful algal blooms of a neurotoxic dinoflagellate, *Karenia brevis*, off the coast of Texas and Florida causes widespread fish kills and chokes habitat by decreasing sunlight in the water column (Walsh et al. 2002). Ecosystem fragmentation also is a serious concern for species among estuarine and marine ecosystems, where fitness and viability of organisms suffer long-term affects from disruptions in hydrologic connectivity (Rypel and Layman 2008). The scale of habitat destruction occurs at both the microhabitat level, in cases such as dredging or trawling, and also on larger scales

where entire estuaries are altered due to nutrient overloads and changes in salinity regimes. The degradation of these habitats is widespread, and fish populations are simultaneously threatened by habitat loss and overexploitation (Fourqurean et al. 2003).

Regional declines in fish stocks and water quality throughout the state of Florida have resulted in increased awareness of the extent and impacts of seagrass mortality. Florida Bay represents one of the most extensive seagrass beds in North America, with another extensive seagrass area occurring along north Florida's Gulf coast known as the Big Bend. Between 1984 and 1994, the estimated biomass of turtle grass declined by 28%, manatee grass by 88%, and shoal grass by 92% in Florida Bay (Dawes et al. 2004). Small-scale destruction of seagrass beds occurs in many cases from improper boat operation, where boat propellers scar seagrass beds in shallow waters. More widespread losses have occurred in South Florida as well, with a mass seagrass die-off in 1987 attributed to a combination of factors, including hypoxia, sediment sulfide toxicity, hypersalinity, eutrophication, and pathogens (LaPointe et al. 1990; Carlson et al.1994; Durako and Kuss 1994; Fourqurean and Robblee 1999).

Essential Fish Habitat

The Magnuson-Stevens Fishery Conservation and Management Act (MSA), originally enacted in 1976, directs marine fisheries management in United States federal waters. The 1996 reauthorization of the act, deemed the Sustainable Fisheries Act (SFA), focused on rebuilding overfished fisheries, protecting essential fish habitat (EFH), and reducing bycatch (Public Law 104-297). In particular, the recognition of habitat conservation as a part of sustainable fisheries was a groundbreaking aspect of the legislation. The most recent reauthorization of the MSA, the Fishery Conservation and

Management Reauthorization Act (MSRA) of 2006, was signed into law in January 2007. The MSRA includes a regional plan to address habitat impacts of 2005 hurricanes on Gulf Coast states, in particular the loss of fisheries habitat, and recommendations were made for more precise and comprehensive baseline habitat assessments.

Essential fish habitat was defined in the SFA as "waters and substrate necessary for fish spawning, breeding, feeding or growth to maturity," (National Marine Fisheries Service (NMFS) 1997, p. 66531) but this definition has proven to be vague directives for managers tasked with protecting EFH. Fisheries management plans (FMPs) are required to explain the physical, biological, and chemical characteristics of EFH and how these characteristics influence the use of EFH by individual species or life stages. Fishery management plans must include a fish's geographic range and habitat requirements by life stage and should cover the life history information necessary to understand each species' relationship to or dependence on its various habitats. Therefore, a tiered framework was developed by National Oceanic and Atmospheric Adminstration (NOAA) scientists to assess habitat quality and delineate EFH (NOAA 2004).

At the first level, or tier, the evaluation criterion is whether a species or life stage is present or absent among different habitats. EFH includes those habitats that support the different life stages of each managed species, but a single species may use many different habitats throughout its life to support breeding, spawning, nursery, feeding, and protection functions. Results of several studies have revealed the importance of nursery habitat as EFH (Beck et al. 2001; Nagelkerken et al. 2002; Gillanders et al. 2003; Heck et al. 2003; Mangel et al. 2006; Faunce and Serafy 2008; Patterson et al. 2008) because critical impacts on year-class strength occur in early life when growth is exponential and

small fluctuations in growth and mortality can cause changes in biomass on orders of magnitude (Houde 1987). Nursery habitat has become a focus of fisheries management and research in recent years due to growing awareness of the role estuarine habitats play in supporting coastal resources. However, reviews of nursery habitat research have shown that, historically, the majority of studies have lacked comparative data from different structured habitat where individuals of a given species or life stage may occur (Nagelkerken et al. 2000; Beck et al. 2001). It is also suggested that considerations for the surrounding habitats need to be included in order to understand spatial connectivity among different habitat types (Adams et al. 2006).

Beyond estimating whether a given species or life stage is present in a particular habitat, it is important to know the scale to which individuals are present there. Therefore, the second level of NOAA's (2004) EFH analysis involves examining habitat-specific fish density. Densities and abundances are also included in the second tier of nursery evaluation by Adams et al. (2006). Estimates of density provide a more robust measure of the role of a given habitat as a nursery by providing an estimate of the relative numbers of individuals present there.

Studies that have examined densities of fish among several habitat types also have revealed ontogentic shifts where biotope utilization is correlated to fish size and, theoretically, age (e.g. Nagelkerken et al. 2000; Eggleston et al. 2004). Age then, is an important parameter in distinguishing which habitats function as nurseries or EFH, and is important for conducting a third-tier EFH analysis, which concentrates on population dynamics rates. For example, growth and mortality rates provide an index of habitat quality as proxies for the availability of resources and the ability to avoid predation.

These vital rates are influenced by density-dependent or unpredictable density-independent factors. Examples of density-independent factors could be catastrophic weather events or unusual algal blooms, whereas predation mortality and growth are more likely to be affected by density-dependent processes. Biomass increase (i.e., net production) is achieved when growth (G) exceeds mortality (Z). Density-dependent factors affecting production are fundamentally related to habitat quality and availability, and if lower population-density enhances overall growth then the potential for recruits increases. Consequently, habitat that provides adequate prey resources and functions as a refuge from predation may act as a compensatory mechanism.

The fourth, and highest level of NOAA's (2004) guidelines for EFH analysis, is to compare estimates of habitat-specific production. This highest tier of analysis is the most data intensive, as fish abundance, growth, and mortality are all required to estimate production. Therefore, few habitat quality studies have produced estimates of habitat-specific production versus fish presence/absence or density estimates for species of concern (Mangel et al. 2006).

Beck et al. (2001) concluded from their review of nursery habitat research that proof of the movement of individuals from nursery to adult habitat should perhaps be viewed as the ultimate measure of nursery function. Other authors also have reported that evidence of connectivity between juvenile habitat and adult populations is the conclusive evidence of the nursery role of different habitats (Beck et al. 2001; Chittaro et al. 2005; Secor and Rooker 2005; Dahlgren et al. 2006). However, the assumption that habitats with higher densities or production of juveniles contribute more recruits to adult populations is not frequently tested (Beck et al. 2001; Gillanders et al. 2003). Estimation

of long-term movement patterns and mixing rates are vital information of fish population dynamics and again require another layer of analysis through tagging studies. A variety of methods for tracking fish movement are currently used to collect this kind of data but are often costly or fail to yield a high rate of return. But the improved technology of artificial tags (e.g. external, internal, or telemetry) and the introduction of natural tags, such as otolith elemental or stable isotope signatures, provide powerful tools to conclusively test the nursery hypothesis and, therefore, evaluate EFH (Gillanders et al. 2003).

Gray Snapper as a Model Species

Gray snapper, *Lutjanus griseus*, exhibit a marked ontogenetic shift from inshore nurseries to offshore habitats (Eggleston et al. 2004; Faunce and Serafy 2007); thus, they are a model species in which to examine components of the nursery hypothesis. They are found throughout the western Atlantic and Gulf of Mexico, from North Carolina to Brazil, with rare occurrences of individuals documented as far north as New England (Hoese and Moore 1977). Although they would be unlikely to survive cold winter temperatures in the northern reaches of their range for extended periods, gray snapper's tolerance to salinity and temperature is broad and their geographic distribution is expansive (Wuenschel et al. 2004).

Adults off the west coast of Florida spawn from June through September, with peak spawning occurring in mid-July. However, back-calculated fertilization dates have demonstrated early spawning in south Florida (Allman and Grimes 2002). Oceanic currents transport pelagic eggs and larvae that are spawned offshore, and larvae remain in the plankton for approximately 25 days until they settle in estuaries or coastal marine

habitats (Starck and Schroeder 1971; Allman and Grimes 2002). Throughout their range, juvenile gray snapper utilize estuarine nurseries to provide protection from predation and grow rapidly to less vulnerable sizes. They can be found among seagrass beds, mangrove thickets, and other shallow, structured habitats (Nagelkerken et al. 2000; Burton 2001; Allman and Grimes 2002; Nagelkerken et al. 2002). The variety of habitats in which gray snapper juveniles can be found suggest relative comparisons across habitats may provide useful data for nursery habitat delineation.

Mangroves, channels, reefs, low-relief hard bottom, and seagrass beds are habitats shown to be occupied by later-stage juveniles and adult gray snapper (Burton 2001; Allman and Grimes 2002; Eggleston et al. 2004). Gray snapper reach sexual maturity at 175-180 mm, or 3 years of age and undergo an ontogenetic shift to offshore adult populations between ages 3-5 years (Starck and Schroeder 1971; Burton 2001). Estimates of juvenile abundances in nurseries have been correlated with recruitment to adult populations in reef fish species with similar emigration patterns and estuarine-dependence (Koenig and Coleman 1998; Allman and Grimes 2002). For an economically important species such as gray snapper, this fishery-independent data can be vital for projecting year-class strength for adults recruiting to the fishery.

Gray snapper are targeted by commercial and recreational fisheries throughout their range and are among the more popular gamefish in south Florida (Harper et al. 2000; Figure 1). Along the Atlantic coast of the US, the majority of gray snapper landings come from Florida, but south Florida landings for both recreational and commercial fisheries exceed those from north Florida (Burton 2001). In the Gulf of

Mexico, recreational landings surpass commercial landings, which declined significantly over a fifteen-year period into the late 1990s (Allman and Grimes 2002).

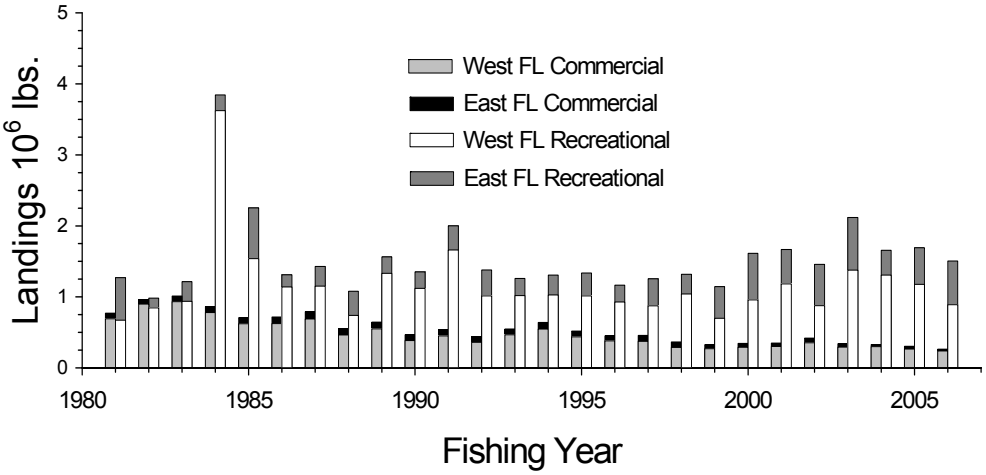

Figure 1. Total annual landings of gray snapper on the Atlantic and Gulf coasts of Florida, 1982-2006. Data sources: NOAA's Marine Recreational Fisheries Statistics Survey (MRFSS) and NOAA's Fisheries Statistics and Economics Division.

A recent assessment of gray snapper in the Florida Keys suggests juveniles are moderately abundant in south Florida but mean landed fish size has been close to the minimum size limit for more than two decades (Ault et al. 2002). Along the east coast of Florida adult gray snapper reach a smaller maximum size at age and younger maximum age in the south than in the north (Burton 2001). These differences in size are likely driven by exploitation rates in south Florida, where faster growing fish are removed from the population first. Long-term changes can then occur such as genetic shift with more slower growing individuals in the population, and can alter the reproductive characteristics of the population where fecundity is a function of size and age. In southeastern Florida, size-distribution patterns observed between a no-take reserve and adjacent fished areas show significantly larger fish in the sanctuary, suggesting even

small-scale variation in population structure exist which is potentially driven by fishing pressure (Faunce et al. 2002).

Similar to trends along the Atlantic coast of Florida, adult gray snapper along the west coast of Florida reach smaller maximum size at age than their southern counterparts (Allman and Goetz in review). Given the differences in fishing pressure over gray snapper's distribution in Florida waters, and estimates of population status that indicate overfishing is likely occurring in south Florida (Burton 2001), region-specific population dynamics and recruitment patterns might provide more comprehensive data for determining essential fish habitat. Little is known about recruitment and movement of gray snapper along the west Florida shelf, but latitudinal variation in growth and reproduction indicate that subpopulations exist which should be managed as separate units (Burton 2001; Allman and Goetz in review). More information needs to be obtained on the relative productivity of various estuarine systems as nursery grounds and the connectivity between nurseries and adult populations, however, to examine population structure in gray snapper.

Examining Nursery Value for Gray Snapper

Production is a function of a population's abundance and population-specific growth and mortality rates over time. Estimating both growth and mortality is dependent on accurate estimation of fish age, which is typically accomplished via analysis of otolith microstructure due to the chronometric properties of otoliths (Campana and Neilson 1985). Alternating opaque and translucent zones, corresponding to daily or annual growth, can be enumerated to reveal fish age (Panella 1971), and several studies have

examined daily growth of juvenile gray snapper through analysis of otolith microstructure (Allman and Grimes 2002; Tzeng et al. 2003; Denit and Sponaugle 2004).

Allman and Grimes (2002) compared juvenile gray snapper sagittae and lapilli for the reliability of estimating fish age from counts of daily opaque zones in otoliths. Both otolith types formed alternate opaque and translucent growth zones, verified as daily increments by alizarine markings in tank experiments, but lapilli were easier to process and increments more distinguishable (Allman and Grimes 2002). Using age and size data, estimates of instantaneous daily growth rates showed similar trends among sampled regions along a latitudinal gradient of west Florida (Allman and Grimes 2002), but Denit et al. (2004) observed different growth rates along the southeast coast of Florida with faster growth in juveniles from southern sites. While growth can be influenced by various factors, Wuenschel et al. (2004) reported a direct correlation between growth and temperature in juvenile gray snapper.

Otolith analysis may also provide information on hatch dates, thus fish spawning cycles (Allman and Grimes 2002). Previous estimates of gray snapper pelagic larval duration and hatch dates indicated the majority of adults spawn between May and September, with a peak from June to August (Domeier et al.1996; Allman and Grimes 2002; Tzeng et al. 2003; Denit and Sponaugle 2004). The lunar cycle has been associated with peaks in spawning, inferred from macroscopic examination of gonads, occurring on the new and full moons (Starck and Schroeder 1971; Domeier et al. 1996). However, analysis of otolith microstructure revealed less conclusive lunar periodicity, with spawning occuring throughout the lunar cycle (Allman and Grimes 2002; Tzeng et al. 2003; Denit and Sponaugle 2004). Settlement marks in otoliths also may reveal rates and

timing of larval transport to a given system and provide an estimate of pelagic larval duration prior to settlement in nursery habitat (Allman and Grimes 2002; Denit and Sponaugle 2004).

Net juvenile production from nursery habitat is valuable as an estimate of the number of individuals potentially available to recruit to adult populations (Beck et al. 2001). However, the estimation of juvenile production alone does not suffice as an indication of recruitment to adult populations if juveniles cannot be linked to adult habitats. Scales of movement vary from meters to kilometers and days to years, and migration is difficult to estimate when juvenile and adult habitat overlaps (Gillanders et al. 2003). Direct estimation of successful movement from juvenile to adult habitat is frequently overlooked or assumed to exist, but the conclusive test rests on the ability to estimate connectivity (Beck et al. 2001; Gillanders 2003; Heck et al. 2003).

Little information exists on gray snapper post-settlement movement patterns and population connectivity in the Gulf of Mexico, especially where genetics fails to discriminate stocks or reveal metapopulation structure (Thorrold et al. 2001; Allman and Goetz in review). Latitudinal differences in growth have been observed in both juvenile and adult populations (Denit and Sponaugle 2004; Allman and Goetz in review) and landings data show gray snapper from north Florida achieve greater maximum size and age than their southern counterparts along both the Gulf and Atlantic (Burton 2001; Allman and Goetz in review). If size-selective mortality from fishing pressure truncates size at age in the south, then knowledge of regional scales of movement could reveal degrees of isolation between northern and southern populations, which in turn may affect

management strategies for gray snapper (Manooch and Matheson 1981; Johnson et al. 1994; Burton 2001; Allman and Goetz in review).

By using tags to identify sources of recruits to adult populations offshore, estimates of relative proportions from different regions could be estimated. Comprehensive tagging studies have been proposed as one means to estimate movement patterns and reveal population connectivity (Burton 2001). The use of artificial tags is one conventional method, but a suite of difficulties reduce their effectiveness: tags are frequently too large relative to the size of juvenile organisms; juvenile natural mortality rates are high; recapture rates of tagged fish is low; and large numbers of individuals need to be tagged to produce even low numbers of recaptures (Gillanders et al. 2003). Instead, the innovation of using biological tags as indicators of estuarine origin has become an increasingly widespread method for tracking movement (e.g., Patterson et al. 1998; Campana and Thorrold 2001; Hanson et al. 2004; Patterson et al. 2004). Otoliths retain chemical components of bodies of water inhabited by the fish, in metabolically inert state, which can be analyzed to generate a biological tag from distinct habitats. Combined with evidence from latitudinal variability in size-at-age of adult gray snapper showing that populations are isolated from north to south, recruitment patterns from nursery to adult habitats would illustrate where subpopulations occur along the Florida coast. With estimates of contributions and sources of contributions from different regions, EFH analysis would be accomplished.

Natural tags derived from otolith chemistry have been shown to be effective for examining source nurseries and population connectivity. Stable isotopes and elements that are incorporated into otoliths occur naturally and differ among water masses where

fish reside (Simkiss 1974; Martin et al. 2004). Otoliths are composed chiefly of a biogenic form of calcium carbonate, aragonite, which precipitates out of endolymphatic fluid in a highly regulated environment compared with other biomineralized aragonite (Campana and Thorrold 2001). Otoliths are formed under membrane regulation while still exposed to surrounding physiological processes, theoretically allowing reconstruction of environmental conditions based on measures of isotopes and elements from their concentrations in otoliths (Mugiya et al. 1981; Payan et al. 1997; Campana and Thorrold 2001). Contamination by trace metals is relatively low in otoliths but element:Ca ratios can be measured as environmental proxies with considerable accuracy (Campana and Thorrold 2001; Patterson et al. 2004). Analytical techniques include solution-based inductively coupled plasma-mass spectrometry (ICP-MS) and isotope ratio mass spectrometry (IR-MS), which measure elemental ratios to calcium carbonate and stable isotope ($\delta^{13}C$ and $\delta^{18}O$) values, respectively. Signatures are derived through multivariate statistical analysis. Analysis of the core of an adult otolith provides information on the elemental and stable isotope values incorporated while the fish was a juvenile, thus can provide information pertaining to nursery of origin if nursery-specific otolith chemical signatures of juveniles from that year class exist (Gillanders 2005; Hamer et al. 2005; Patterson et al. 2008).

The fact that otolith chemistry is a function of the environmental conditions experienced by a fish allows the application of otolith chemistry as an indication of geographic distribution (Campana and Thorrold 2001), with the caveat being that few trace elements found in otoliths are incorporated in direct proportion to the environment (Campana 1999). Differences in environmental tolerances are a reflection of energetic

expenditures which may affect the chemical composition of otoliths. For euryhaline gray snapper, the passive and active transport of ions during osmoregulation may obscure a direct relationship between element concentrations in the environment and otoliths (Martin and Wuenschel 2006). Metabolically related discrepancies also are evident in cases where somatic growth rates affect otolith elemental concentrations (Hoie et al. 2003; Martin and Thorrold 2005; Martin and Wuenschel 2006), especially among larval and juvenile fishes that exhibit higher and more variable growth rates (Martin and Wuenschel 2006).

The complexity of biotic mechanisms and environmental sources of variability for otolith chemistry does not preclude the use of otolith chemical signatures to derive nursery signatures if measurable differences in otolith chemical signatures can be detected on the spatial scale of interest (Edmonds et al. 1999; Campana 1999). In studies to date, Sr and Ba typically have been employed as proxies for ambient salinity and temperature, and occasionally Mg concentration has been applied similarly (Campana and Thorrold 2001; Hanson et al. 2004; Hamer et al. 2006; Martin and Wuenschel 2006). Manganese, Fe, Pb (and perhaps Li, Cu and Ni) are other measurable elements that may elucidate sources of environmental variability in otoliths (Campana 1999; Gillanders 2005). Overall, otolith elemental signatures have been applied in several studies to examine the source of recruits from nursery sources, as well as population connectivity among regions. In the Gulf of Mexico, authors have reported successful direct classification of reef fishes to nursery regions (> 90% accurate) over successive year classes using a suite of elements and applying discriminate function analysis to create otolith chemical signatures (Patterson et al. 1998, 2008; Hanson et al. 2004).

Analysis of stable isotope chemistry of fish otolith carbonate is not as well-established as otolith elemental chemistry applications but has been applied to reconstruct temperature histories, metabolic history, and differentiate between groups of fish (Kalish 1991ab, Thorrold et al. 1998). Moreover, combining stable isotope values with trace elements has been demonstrated to provide more powerful discrimination of fish groups than the application of elemental signatures alone (Thorrold et al. 1998). Precipitation of stable isotopes to otoliths occurs under membrane control, either in equilibrium or disequilibrium (Kalish 1991b). It appears that oxygen isotopes in otoliths are deposited very near to equilibrium with the surrounding water (Kalish 1991a; Thorrold et al. 1997). In equilibrium, the determining factors for isotopic fractionation are based on temperature and solution concentration, thus otolith $\delta^{18}O$ can be used as an indicator of ambient temperature (Kalish 1991b; Thorrold et al. 1997). However, interpreting $\delta^{13}C$ values in otoliths is more complex. Trophic fractionation of carbon isotopes, as well as the isotopic composition of dissolved inorganic carbon (DIC) in seawater, have been shown to affect otolith $\delta^{13}C$ values (Kalish et al. 1991b; Thorrold et al. 1997; 1998). Kinetic effects and metabolic activity influence carbon isotopes, with depleted $\delta^{13}C$ found in otoliths of fish experiencing heightened metabolic rates (Kalish 1991b; Høie et al. 2003; Martin and Thorrold 2005). Aside from metabolic activity and dietary sources of $\delta^{13}C$, correlations between temperature and $\delta^{13}C$ can be confounding and the interactions are more complex than can often be predicted (Kalish 1991b; Campana 1999).

Despite the complex relationships between abiotic factors, physiological influences, and otolith precipitation rates, the underlying assumption of elemental and isotope analysis used to detect movement patterns or determine sources of recruits only

requires statistically significant and distinguishable differences among regions or populations at the scale of interest (Gillanders et al. 2003). Estimates of population connectivity or nursery sourcing revealed from otolith analysis have had significant implications in population models (Thorrold et al. 2001; Hamer et al. 2005). Therefore, otolith chemistry may provide an ideal natural tag to link gray snapper juvenile production in estuaries and coastal ecosystems to adult populations offshore. In the current study, a tiered approach to juvenile gray snapper habitat assessment was attempted, beginning with estimating habitat-specific presence/absence, densities, and growth. The final component of my thesis research was deriving natural tags of nursery region from analysis of otolith chemistry, with the ultimate goal being to employ the tags to inshore juvenile production with recruitment to the adult population.

Objectives

The overall goal of my thesis research was to examine the nursery function of west Florida estuarine and coastal habitats and ecosystems for juvenile gray snapper. Over a two-year period, sampling of juvenile gray snapper was conducted in nine estuarine systems from Pensacola Bay to Florida Bay. A tiered approach to habitat evaluation was attempted based on NOAA's (2004) EFH guidelines, with habitat-specific production rates as the highest tier of assessment. Natural region-specific tags derived from otolith chemical signatures also were tested for applicability in subsequent studies to estimate the nursery origin of adults and to examine gray snapper population connectivity along west Florida.

Null Hypotheses Tested:

$H_{0\text{-}1}$: There was no difference in juvenile gray snapper presence among samples collected within a range of west Florida estuarine and coastal ecosystems.

$H_{0\text{-}2}$: Densities of juvenile gray snapper was not significantly different among west Florida sampling regions or years.

$H_{0\text{-}3}$: Growth of juvenile gray snapper was not significantly different among west Florida sampling regions.

$H_{0\text{-}4}$:Juvenile gray snapper otolith chemical signatures were not significantly different among west Florida sampling regions or years.

METHODS

Sampling

Gray snapper were collected from a total of nine estuarine and coastal ecosystems extending along the West Florida shelf that represented four study regions: the Florida Panhandle, Big Bend, Southwest and South Florida (Figure 2). Gray snapper juveniles were sampled in collaboration with the Florida Fish and Wildlife Research Institute's (FWRI) Fisheries-Independent Monitoring (FIM) program. University of West Florida personnel sampled Pensacola Bay, St. Andrews Bay, Keaton Beach, and Ten Thousand Islands. FWRI personnel sampled Apalachicola Bay, Cedar Key, Tampa Bay, Charlotte Harbor, and Florida Bay. All sampling was conducted based on the stratified random sampling protocol established by the FWRI-FIM program. Each depth and habitat type was divided into 1-minute by 1-minute areas and again into 100 smaller grids. Grid selection was random but stratified relative to habitat type and depth in each system.

Sampling by FWRI was conducted with a 21.3-m seine and 6.1-m otter trawls, while UWF personnel sampled with a 6.1-m trawl. The FWRI-FIM 21.3-m seine protocol prescribes that seines cover a fixed distance of 9.1 m of bottom in no more than 1.8 m of water depth, while trawls are deployed in depths between 1.8 and 7.6 m and towed over a distance of 0.2 nm. Depending on conditions in the field, these protocols were followed

somewhat loosely, but distances and areal coverage of gears were recorded with accuracy. Trawling was conducted by UWF personnel over the course of two years on a monthly basis between August and December, corresponding to peak spawning and presence of juveniles in estuaries. FWRI-caught samples were sent to UWF from this period as well.

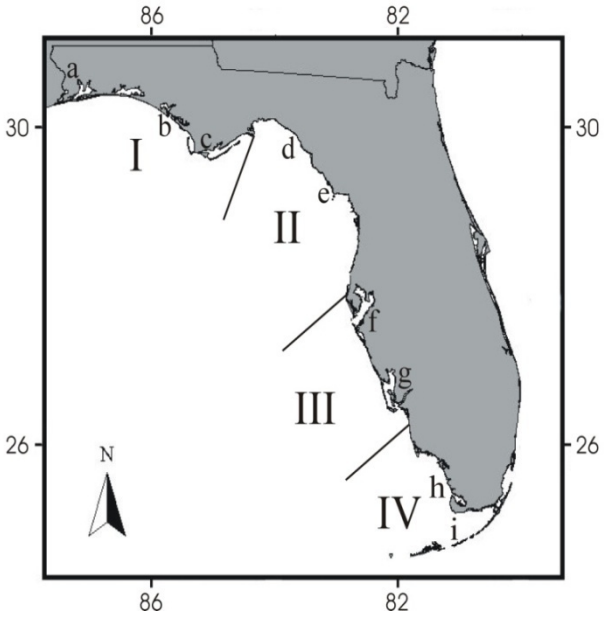

Figure 2. Map of four zones along the west coast of Florida in which gray snapper were sampled in 2006 and 2007: zone I = Panhandle, sone II = Big Bedn. Zone III = Southwest, zone IV = South Florida. The following locations also are labeled on the map: a) University of West Florida and Pensacola Bay, b) NOAA Fisheries Panama City Lab and St. Andrews Bay, c) Apalachicola Bay FIM Lab, d) Keaton Beach, e) Cedar Key FIM Lab, f) Tampa Bay FIM Lab, g) Charlotte Harbor FIM Lab, h) Ten Thousand Islands, and i) Marathon FIM Lab and Florida Bay.

In the field, hydrographic parameters (e.g., water depth, salinity, dissolved oxygen, and water temperature) were measured at a given sampling station with either a Yellow Springs Instrument (YSI) Handheld Multiparameter Instrument or a Seabird 19plus Conductivity-Temperature-Depth (CTD) sensor. Other physical conditions, such as latitude/longitude, wind direction, sea state, cloud cover, bottom vegetation type,

percent cover of vegetation, distance to nearest shoreline and shore type (e.g. mangrove, marsh, oysters, etc), also were recorded. All of the hydrographic parameters recorded by FWRI personnel were sent in to the University of West Florida directly from the FIM database.

The 21.3-m seine deployed by FWRI personnel was a small mesh net with PVC poles attached to the ends of the net. The seine was made of 3 mm #35 knotless nylon mesh with the top supported at the surface by floats and the bottom held on the substrate by lead weights. The net was 1.8 m high with a 1.8 m x 1.8 m x 1.8 m bag placed in the center. Trawls were conical in shape with a wide elliptical opening that gradually tapered backwards toward a narrow bag. The main body of the net was constructed of #9 twine 38 mm stretch mesh and was 4.7 m long. The bag (cod end) was constructed of #18 twine (38 mm stretch mesh) and was 3.2 m long. A Delta #35 3 mm knotless nylon mesh liner was sewn into the bag 46 cm from the bag seam. Each side of the trawl mouth had lines attached to rectangular doors made of 12.7 mm plywood. A tow line pulled the net through the water and a tickler chain dragged along the bottom to startle benthic organisms into the water column where they were swept up by the trawl. All species captured in a given sample were sorted, identified, and recorded in the field; gray snapper were removed and placed in plastic bags on ice. Approval was obtained from the Animal Care and Use Committee at UWF for this collection method (Appendix A). Gray snapper kept by FWRI personnel were frozen at the respective field laboratory and shipped with ice packs to UWF.

Presence/Absence and Density

Density was estimated as the number of individuals captured in a given sample divided by the area sampled in hectares (ha). Density estimates were calculated based on fish captured in the 6.1-m trawl and the 21.3-meter seine. The areal coverage of the trawl was calculated as the distance towed multiplied by 4.0-m. FWRI uses this width as the distance between doors while the trawl is being pulled through the water in order to factor in the arc created by resistance in the water. Area covered by each seine pull was $140m^2$ (FWRI correspondence). Mean densities were calculated for each month, each system, each gear and in seagrass and non-seagrass habitat.

Habitat-specific percent occurrence and mean density were calculated among all samples from 2007 and 2008. To compute habitat-specific mean density, data first were sorted by seagrass presence. Then, samples where seagrass was not present were sorted by habitat type: marsh, oyster reef, mangrove, manmade structure, and open. Habitat-specific density estimates then were truncated to only include sampling events where distance to a given habitat was between 0-10 m. Unbiased habitat-specific mean densities then were computed with the delta method proposed by Pennington (1983) to adjust for the zero inflation caused by a high percentage (80-97%) of zeroes in the habitat-specific data. Where gray snapper only occupy part of the total sampled area, the zero data can then represent unused or unsuitable habitat. The non-zero data are lognormally distributed in this case, with high frequencies of low densities and low frequencies of higher densities. This distribution is called a delta (Δ) distribution (Aitchinson 1955). Unbiased estimates of mean density were obtained with the delta method using the following formula (Pennington, 1983):

$$c = m/n(\exp(y)*G(s^2/2)) \quad \text{equation 1}$$

where:

c = unbiased estimate of the mean

m = number of nonzero values

n = sample size

y = mean of ln of the nonzero values

s^2 = variance of the mean of the ln of the nonzero values

and

$$G(t) = 1 + \frac{n-1}{n}t + \left\{\sum_{j=2}^{\infty} \frac{(n-1)^{[j]-j}}{n^j (n+1)(n+2)\ldots(n+2j-2)}\right\}\left(\frac{t^j}{j!}\right) \quad \text{equation 2}$$

A convergence criterion of 0.0001 was set for calculating this series. Unbiased variance estimates were obtained using the following formula (Pennington, 1983):

$$d = m/n(\exp(2y)\{G(2s^2) - (m-1/n-1)*G[(m-2*s2)/m-1)] \quad \text{equation 3}$$

where:

d = unbiased estimate of the variance of the mean

and the rest of the notation follows as above.

Juvenile gray snapper catch data from the months of August to December 2006 and 2007 were analyzed to test various factors on the presence of fish and on the density of fish. The effect of, sampling region, year, month, depth, temperature, salinity, presence of seagrass, and gear (as a covariate) on juvenile presence and density was tested with a generalized linear modeling approach using a delta-lognormal model computed in SAS (Lo et al. 1992; Ingram et al. 2007). In this approach, the effect of the independent variables on the occurrence of an event (e.g., presence of juvenile gray snapper) first was

tested with a binomial submodel with Proc GLIMMIX in SAS, then the variables' effect on fish density was tested with a lognormal generalized linear model with Proc MIXED in SAS (SAS version 11.3, SAS, Inc. 2004). This modeling approach was necessitated due to the large incidence of zeroes in the catch data; hence, data could not be normalized by conventional transformations. Instead, the delta lognormal model reduced the weight of zeros for data where the distribution of individuals is over-dispersed (Ingram et al. 2007).

Juvenile Age and Growth

Estimates of growth were dependent on accurate estimates of fish age. Otolith based estimates of juvenile gray snapper daily age were previously validated by Allman and Grimes (2002), and their methods for determining daily age of juveniles were utilized in this study. Gray snapper collected by FWRI personnel and shipped frozen to UWF were thawed prior to measurement and analysis. Standard and total lengths (SL and TL, respectively) were measured to the nearest mm and whole weights to the nearest 0.01 g. Fish less than 120 mm were considered small enough for ageing using the lapilli (Allman and Grimes 2002). The lapilli in fish greater than 120 mm tend to grow with decreasing regularity and increments become increasingly indistinguishable and estimated ages lack agreement among readers (Allman and Grimes 2002).

Lapilli were removed from the cranium of each fish and cleaned. After extraction, one lapillus was placed on the index finger and manually sanded on a 3200 grit sand paper then polished on both lateral surfaces against a 4000 grit sand paper. Once a thin transverse section was obtained it was mounted on a glass slide using Cytoseal®

mounting medium. Under a compound microscope, counts of daily opaque zones were made from the primordium to the edge of the otolith and a correction factor of three days was added based on the assumption that the first increment is not formed until the first feeding (Lindeman 1997). For a given sample, three independent counts of increments were averaged if the difference was no greater than 5%. If counts varied by greater than 5% a fourth count was made and rejected if 95% consistency was not achieved (Allman and Grimes 2002). Between-reader precision was tested by having a second reader (Robert Allman of NOAA Fisheries' Panama City Laboratory) age randomly selected samples (17% of 2006 and 16 % of 2007 samples). Average percent error between reader age estimates was computed following Beamish and Fournier (1981) as

$$\frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right] \qquad \text{equation 4.}$$

where R is the number of readers, N is the sample size, i and j correspond to two difference readers and $|X_{ij} - X_j|$ is the absolute value of the difference between reader counts for a given sample. An APE of <5% is generally viewed as high precision between readers (Beamish and Fournier 1981).

Fish hatch date was determined by subtracting estimated age from date of capture. Juveniles were binned in weekly cohorts based on hatch dates and hatch-date frequencies generated to infer differences in spawning periods among each region. Growth functions then were computed by linear regression of total length (TL) on age (SAS, Inc. 2004). Growth estimates were confined to fish estimated to be less than 110 days old, as beyond that age interpreting daily opaque zones becomes unreliable (Allman and Grimes 2002). Linear growth functions were computed for each region where sample sizes permitted.

Difference in growth rate among systems was tested with an analysis of covariance (ANCOVA) test for equal slopes (SAS, Inc. 2004).

Estimating mortality was not possible, hence production was not computed, due to low sample sizes. Mortality rate estimation requires adequate sample sizes for specified cohorts, and I did not obtain this volume of samples to estimate either weekly or monthly cohorts. Therefore, these computations could not be made nor could regional comparisons of production be conducted. Instead, indices of abundance and density were used as proxies of habitat quality and growth rates were compared as measures of region-specific recruitment patterns.

Element and Stable Isotope Analysis

Fish between 50-150mm were randomly selected among regions to examine otolith chemistry. Both sagittae were extracted from each sample and stored dry in plastic vials. The goal was to select at least 50 samples from each of the four sampling regions in each year of the study. Prior to chemical analysis, otoliths were cleaned in 1% ultra-pure nitric acid for 20 seconds under a class-10 clean hood, then repeatedly flooded with double deinonized (18 MΩ cm^{-1}) ultra-pure water (DDH_20). Samples were left to air dry under the clean hood, placed in acid-leached cell wells, and weighed to nearest 10 μg. Samples were kept in acid-leached vials until chemical analysis. All solutions that came in contact with otoliths were made with (DDH_20) and all plasticware and glassware that came in contact with samples were acid leached and quintuple rinsed in (DDH_20).

Clean, dry otoliths were dissolved in 1% ultra-pure nitric acid, with a dilution factor of 1,000x, prior to elemental analysis. Samples were transferred to the laboratory

of Dr. Alan Shiller at the University of Southern Mississippi and analyzed with a Finnigan Element2 high resolution inductively coupled plasma mass spectrometer (HR)-ICP-MS. Samples were spiked with In (2.5 ppb) as an internal standard and analyzed for Ba, Li, Mg, Mn, and Sr, and expressed as molar concentrations to Ca molar concentrations. Element-specific detection limits were estimated as mean concentration +3 standard deviations of blank solutions that were interspersed with otolith solutions for analysis. Matrix effects and instrument drift were estimated and corrected for by running solutions of a certified reference material interspersed with samples.

The second sagitta of each fish was prepared for IR-MS analysis. Clean, dry otoliths were pulverized with acid-leached glass mortar and pestles. Samples were weighed and C and O stable isotopes were analyzed on a Finnigan MAT 251 IR-MS maintained in the laboratory of Dr. Howard Spero at the Department of Geology at the University of California at Davis; sample analysis were performed by Mr. David Winter. Oxygen and carbon isotopic composition of otoliths was reported as delta values in the standard parts per mil (‰) notation:

$$\delta X = [(R_{sample}/R_{standard})\text{-}1] \times 10^3 \qquad \text{equation 5.}$$

Where X is ^{13}C or ^{18}O and R is the corresponding ratio $^{13}C/^{12}C$ or $^{18}O/^{16}O$ (Peterson and Fry 1987). The carbon isotope standard was Pee Dee Belemnite and the oxygen isotope standard was mean ocean water. A carbonate reference standard that had been rigorously tested against the International Atomic Energy Agency's carbonate standard, NBS-19, was run concurrently with samples to control for instrument drift.

Statistical techniques were used to derive region- and year-specific otolith chemical signatures juveniles. Correlation analysis was conducted in SAS to test whether fish size (TL) had a significant linear relationship with element:Ca ratios or stable isotope values. Where a correlation was significant, the slope of the least squares linear relationship between the variables was multiplied by TL and that product was then subtracted from the element:Ca or stable isotope value to remove the effect of TL. Shapiro-Wilk's and Kolmogorov univariate tests for normality and heterogeneity of variance were then performed, and appropriate transformations conducted as needed.

A multivariate analysis of variance (MANOVA) was computed in SAS to test for significant differences among regions and year classes in otolith chemical signatures, with element:Ca ratios and stable isotope delta values as dependent variables and nursery region and year as the independent, or classification variables. Two-way analysis of variance (ANOVA) was performed for each element:Ca ratio and stable isotope delta value individually to determine annual and regional sources of variability. All element:Ca ratios and isotope delta values then were entered into a quadratic discriminant function analysis (QDFA), with computed functions serving as region-specific natural tags (Patterson et al. 2005; SAS, Inc. 2004). Years were modeled separately and jointly. Applicability of natural tags was tested by computing jackknifed cross-validated classification accuracies of individual fish to their nursery region (SAS, Inc. 2004).

RESULTS

Gray snapper were sampled from nine systems across the two years of this study. Seagrass and nonseagrass samples were enumerated across regions and months of the study, with some variability in sampling between seagrass and nonseagrass samples among system (Tables 1-3). Forty-four gray snapper were collected with hook and line along the fringes of mangroves in Ten Thousand Islands by UWF. In Florida Bay, 50 gray snapper were caught with hook and line among bridges and small boat channels by UWF. In both systems, fish were caught on small bait hooks baited with fresh shrimp. The fish from Florida Bay were found primarily among non-seagrass habitat, but habitat was structured with rock outcroppings or relief from manmade structure. Young of the year fish captured in trawls and 21.3-m seines were depicted in standard length size distributions (Figure 3).

Table 1. Stratified Random Sampling Conducted Through the FIM Program By FWRI Personnel in 5 Systems Along the West Florida Shelf: Apalachicola Bay, Cedar Key, Charlotte Harbor, Tampa Bay and Florida Bay. Sampling Efforts Summarized Here Describe the Number of 21.3-m Seine Hauls and 6.1-m Trawl Tows Across Either Seagrass Habitat or Non-Seagrass Habitat in Each System in Each Month From Aug-Dec of 2006.

System		Seine Samples		Trawl Samples	
	Month	Seagrass	Non-seagrass	Seagrass	Non-seagrass
Apalachicola Bay	Aug	10	32	0	30
	Sep	11	32	0	27
	Oct	9	33	0	33
	Nov	8	34	0	25
	Dec	8	33	0	29
Cedar Key	Aug	7	35	0	22
	Sep	6	30	1	14
	Oct	7	30	1	13
	Nov	5	30	2	13
	Dec	1	40	0	17
Charlotte Harbor	Aug	42	40	2	43
	Sep	35	44	4	42
	Oct	34	47	0	43
	Nov	35	44	1	46
	Dec	36	50	0	47
Tampa Bay	Aug	22	108	1	65
	Sep	14	105	2	51
	Oct	20	111	2	56
	Nov	17	90	0	51
	Dec	13	91	0	49
Florida Bay	Aug	88	5	28	3
	Sep	0	0	0	0
	Oct	46	13	18	4
	Nov	0	0	0	0
	Dec	0	0	0	0

Table 2. Stratified Random Sampling Conducted Through the FIM Program by FWRI Personnel in 5 Systems Along the West Florida Shelf: Apalachicola Bay, Cedar Key, Charlotte Harbor, Tampa Bay and Florida Bay. Sampling Efforts Summarized Here Describe the Number of 21.3-m Seine Hauls and 6.1-m Trawl Tows Across Either Seagrass Habitat or Non-Seagrass Habitat in Each System in Each Month From Aug-Dec of 2007.

		Seine Samples		Trawl Samples	
System	Month	Seagrass	Non-seagrass	Seagrass	Non-seagrass
Apalachicola Bay	Aug	14	20	0	35
	Sep	11	37	0	41
	Oct	9	36	0	37
	Nov	12	25	1	23
	Dec	10	23	2	24
Cedar Key	Aug	5	40	1	15
	Sep	3	33	0	17
	Oct	5	30	0	15
	Nov	4	30	1	14
	Dec	4	31	0	17
Charlotte Harbor	Aug	30	55	3	43
	Sep	35	49	1	43
	Oct	34	51	0	45
	Nov	36	56	0	45
	Dec	39	51	0	43
Tampa Bay	Aug	20	87	1	50
	Sep	15	93	0	54
	Oct	20	88	2	48
	Nov	20	86	3	50
	Dec	20	86	1	50
Florida Bay	Aug	65	1	19	3
	Sep	0	0	0	0
	Oct	65	2	8	12
	Nov	0	0	0	0
	Dec	0	0	0	0

Table 3. Stratified Random Sampling Conducted by UWF Personnel in 3 Systems Along the West Florida Shelf: St. Andrews Bay, Keaton Beach and Pensacola Bay. Sampling Efforts Summarized Here Describe the Number of 6.1-m Trawl Tows Across Either Seagrass Habitat or Non-Seagrass Habitat in Each System in Each Month From Aug-Dec of 2006 and 2007.

System	Date	Seagrass	Non-seagrass
St. Andrews Bay	7/24/2006	6	6
	9/5/2006	11	3
	10/23/2006	11	4
	12/11/2006	8	2
Keaton Beach	7/26/2006	6	4
	9/7/2006	8	2
	10/25/2006	13	2
	12/12/2006	12	1
Pensacola Bay	10/15/2006	6	0
	10/29/2007	5	3
St. Andrews Bay	8/13/2007	11	1
	9/17/2007	10	3
	10/23/2007	13	2
	12/12/2007	14	1
Keaton Beach	8/14/2007	10	2
	9/18/2007	10	3
	10/25/2007	6	0
	12/13/2007	9	0

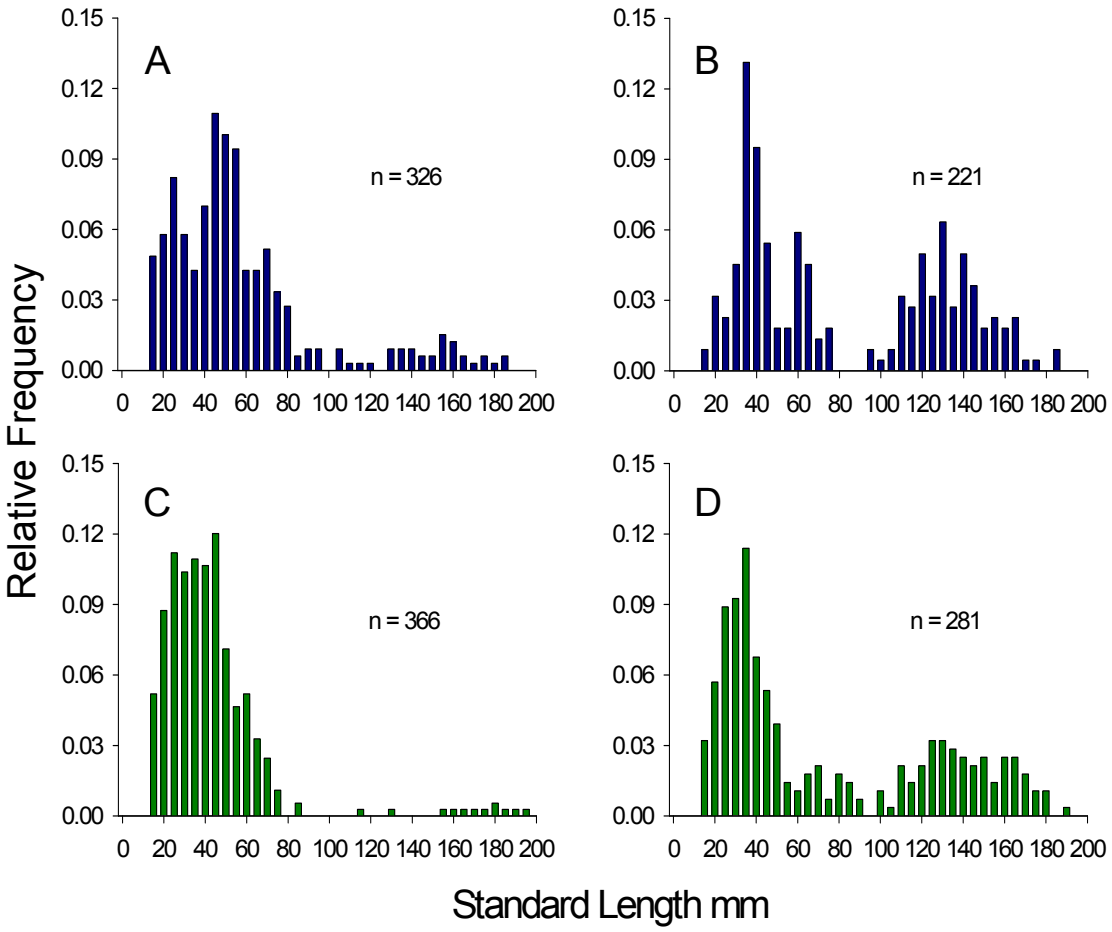

Figure 3. Gear-specific size distributions of juvenile gray snapper sampled along the west coast of Florida with a 6.1-m trawl in A) 2006 and B) 2007 and with a 21.3-m seine in C) 2006 and D) 2007. Sample sizes are given on panels.

Salinity, temperature, and depth data were recorded from study regions during sampling in August through December in both years of the study (Figure 4). However, data were not available for Ten Thousand Islands and Pensacola Bay but likely reflected other systems within their respective regions. Mean water temperature ranging between 14 and 30 °C (Figure 4). Mean temperatures decreased from August to December in all regions. Salinities were as low as 6.6 psu in region I (November 2007) but mean salinities generally fell between approximately 20 and 30 psu. A slight decrease in salinity appeared to occur across months in regions I and II, but remained relatively constant across months in region III. Average depths generally were between 0 and 3 m. The exception to that was a mean depth of 3.4 m in region II during August 2007.

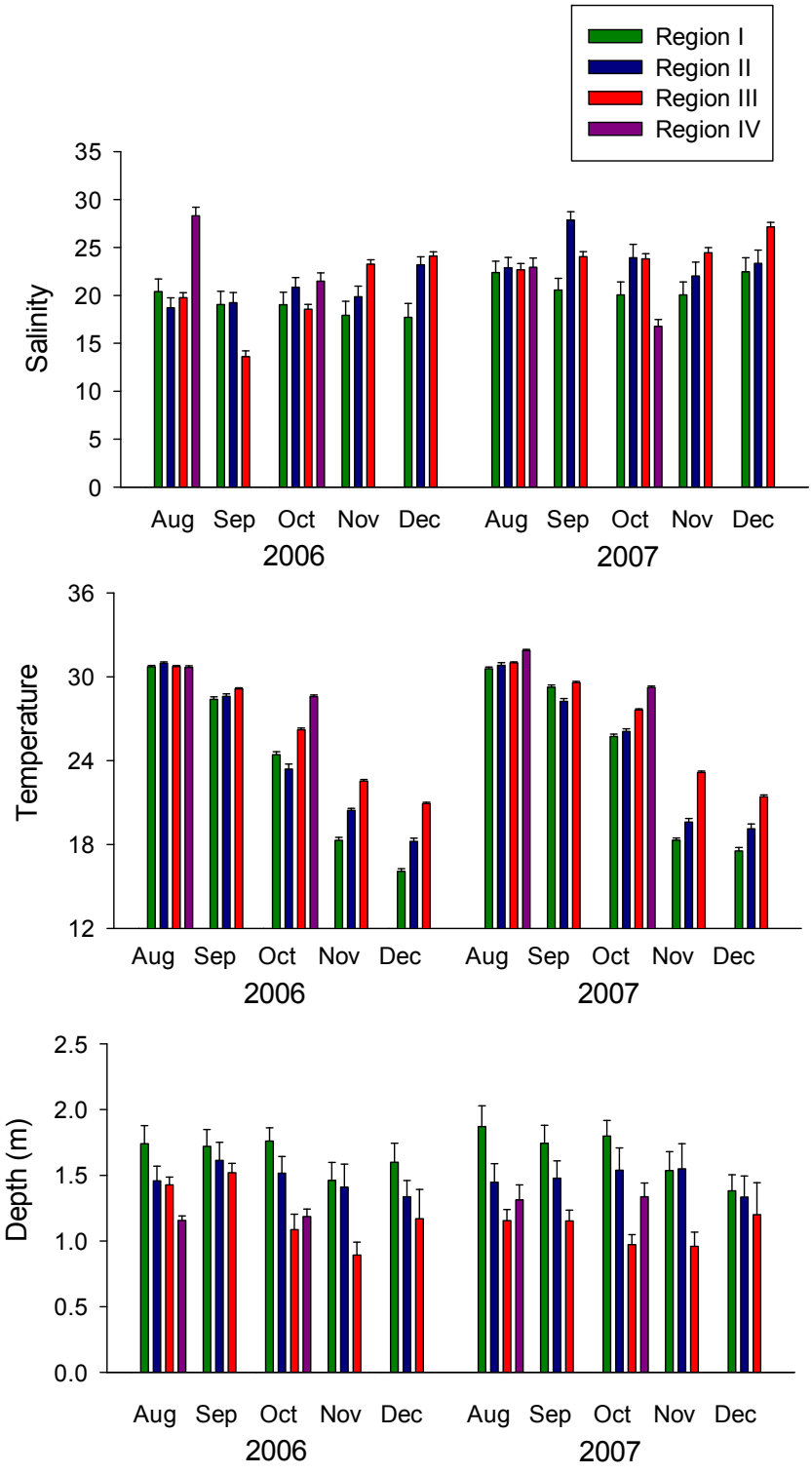

Figure 4. Monthly mean values for salinity concentrations, water temperature and water depth calculated from data collected at sampling stations in each system, grouped by the corresponding region (I-IV) between the months of Aug-Dec in 2006 and 2007. Standard error bars are given for each mean value.

Presence/Absence

Among all structured habitats, juvenile gray snapper occurred most frequently when seagrass beds were present (Figure 5). They were present in 19.89% of samples where seagrass was recorded, compared to only 4.7% of non-seagrass samples. The percent occurrence of gray snapper juveniles in seagrass beds compared to when captured near other structured habitat showed the highest occurrences in seagrass beds, followed by marsh, oyster, mangrove, manmade, and finally open habitat (Figure 5).

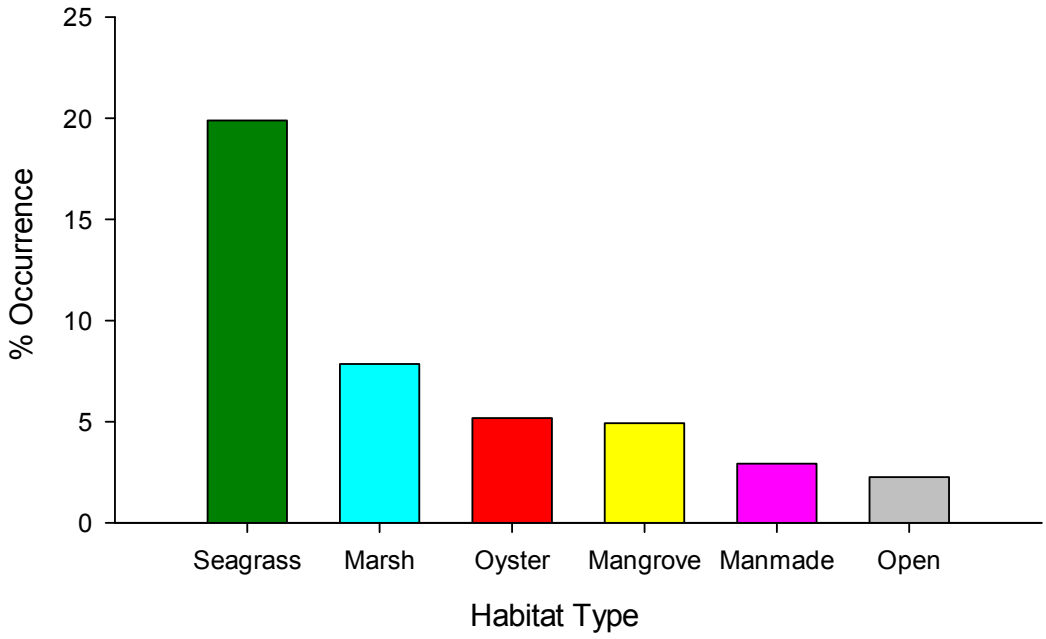

Figure 5. Percent occurrence of gray snapper juveniles captured among various habitats in estuaries along the west Florida shelf in 2006 and 2007.

The binomial portion of the delta lognormal model also showed that seagrass had a significant effect on the presence and absence of gray snapper juveniles (Table 4). Sampling region, month, temperature and depth also significantly affected the presence

of juveniles, and the covariate, gear, also was significant (Table 4). Model results did not indicate that salinity significantly affected the presence of gray snapper.

Table 4. Delta Lognormal Model Results for Binomial Generalized Linear Model Test of Fixed Effects (Gear, Year, Seagrass, Region, Month, Temperature, Salinity, and Depth) On the Presence or Absence of Gray Snapper Juveniles Among West Florida Estuaries.

Type 3 Tests of Fixed Effects				
Effect	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	Pr > F
Gear	1	4311	5.80	0.016
Year	1	4311	0.25	0.619
Veg	1	4311	138.90	<0.001
Region	3	4311	23.29	<0.001
Month	5	4311	7.84	<0.001
Temp	1	4311	13.03	<0.001
Salinity	1	4311	0.07	0.794
Depth	1	4311	8.03	0.005

Monthly differences were related to seasonal temperature shifts, with fewer fish captured later in the year (November and December). Lower temperatures were indicative of lower occurrences of gray snapper, likely due to emigration from juvenile habitat in the north toward the onset of colder winter temperatures. Regional effects were driven by lower abundances in the Big Bend (region II) and South Florida (region IV). Greater depths were also found to have a lower occurrence of gray snapper. Gray snapper did occur more frequently in seine (9.6%) than trawl (4.4%) samples, which was likely due to the fact that FWRI trawl samples typically are taken at greater depths than seine

samples. By including gear as a covariate in the model, however, I was able to account for the variance due to gear and effectively remove it from the error term.

Density Estimates

Juvenile gray snapper mean density was much higher in seagrass versus non-seagrass habitats, which was consistent among sampling gears (Figures 6-9). Densities computed from seine collections tended to be higher overall than densities from trawl collections. Differences between seagrass and non-seagrass densities for fish captured in seines also tended to be several fold greater in seagrass habitat, compared to less marked differences in trawl collections. In 2006, densities in region I tended to be higher than other regions, for both gear types. This typically was not the case in 2007 though, where trends were more variable among regions. Monthly differences in densities showed a decrease in densities across months, and somewhat higher densities in region III in late fall (November and December) than in other regions during this time.

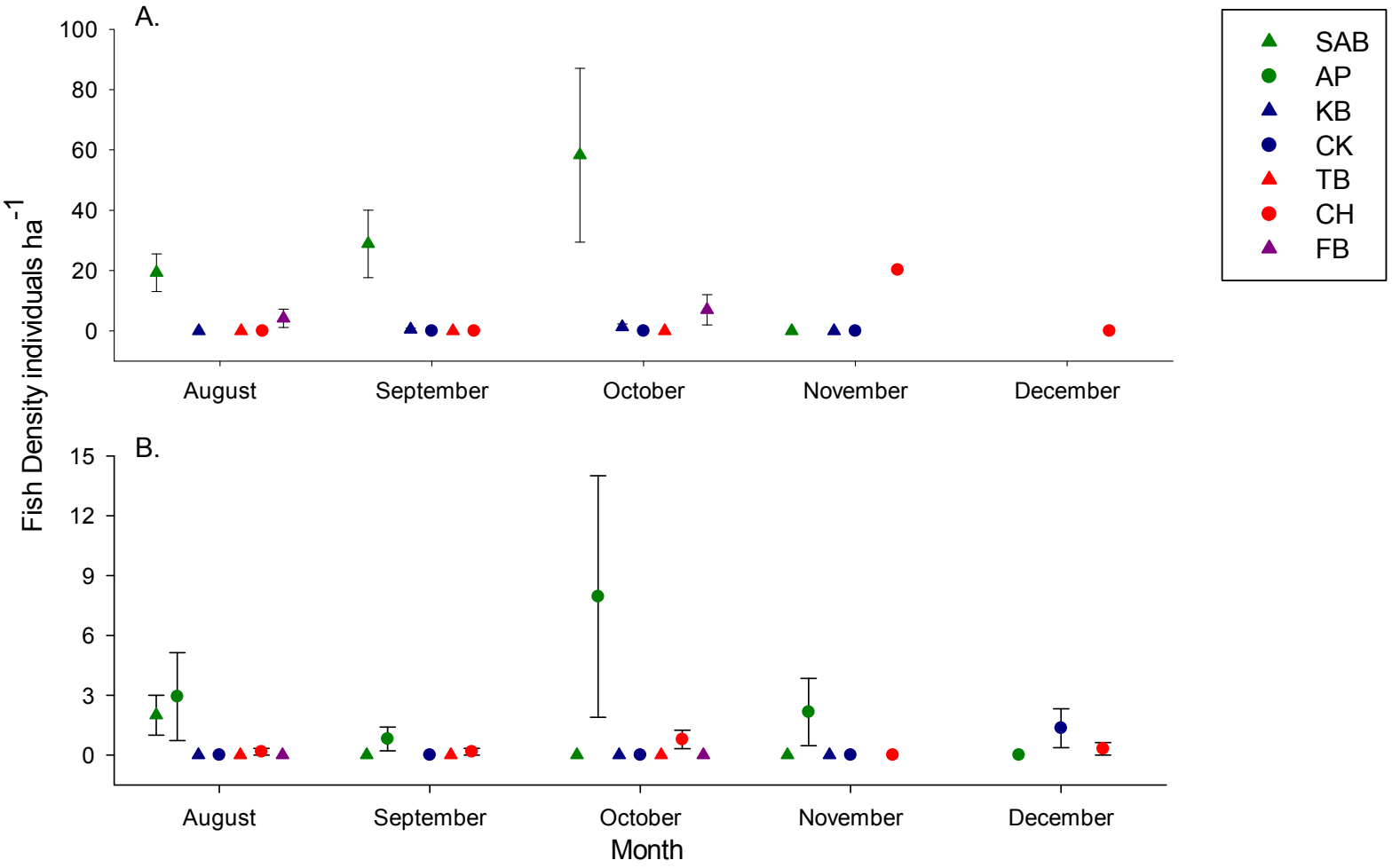

Figure 6. Mean densities (± SE) of juvenile gray snapper captured in 6.1-m trawl samples among A) seagrass habitat and B) non-seagrass habitat in 2006.

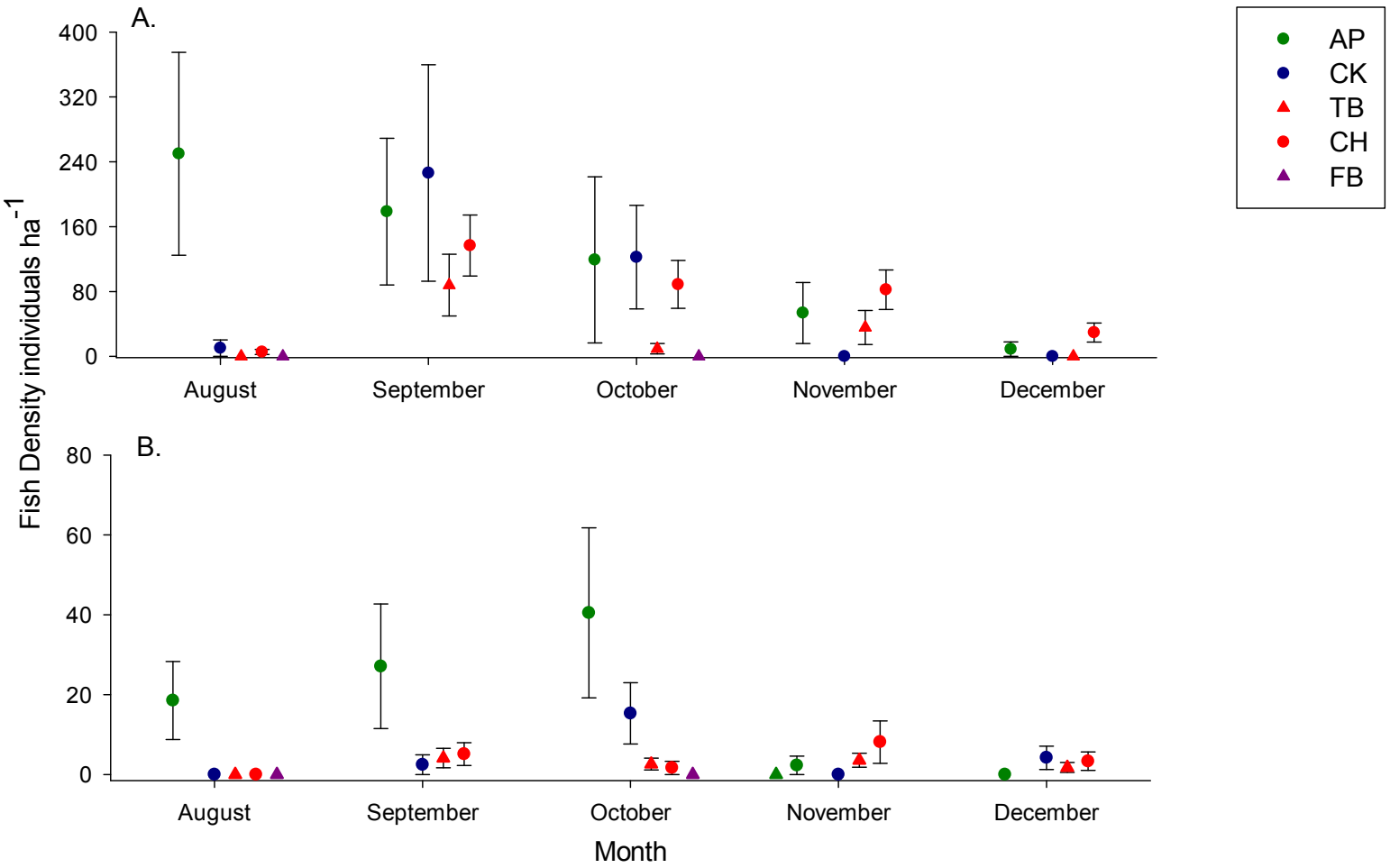

Figure 7. Mean densities (± SE) of juvenile gray snapper captured in 21.3-m seine samples among A) seagrass habitat and B) non-seagrass habitat in 2006.

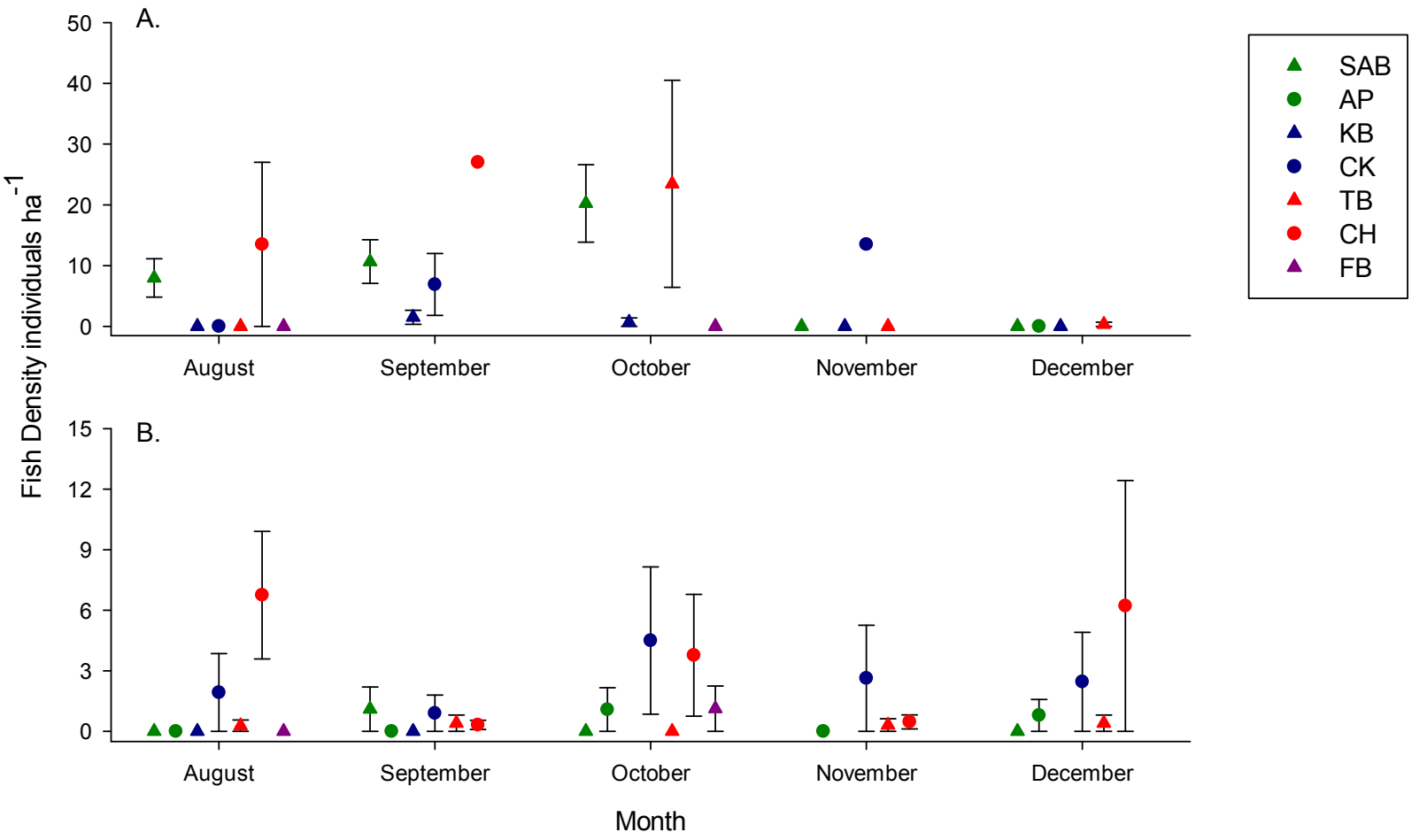

Figure 8. Mean densities (± SE) of juvenile gray snapper captured in 6.1-m trawl samples among A) seagrass habitat and B) non-seagrass habitat in 2007.

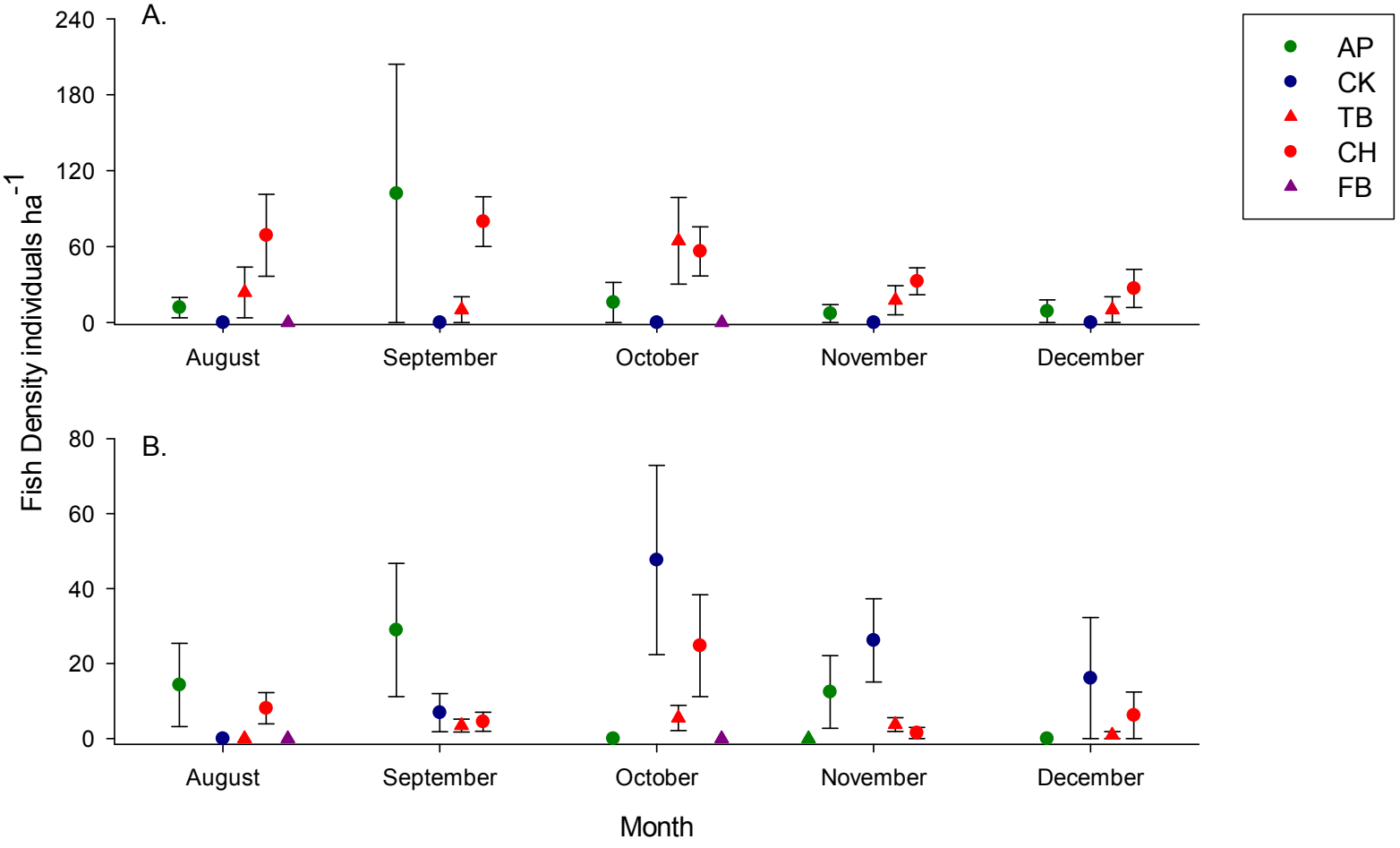

Figure 9. Mean densities (± SE) of juvenile gray snapper captured in 21.3-m seine samples among A) seagrass habitat and B) non-seagrass habitat in 2007

Density estimates computed with the delta method also showed juveniles were several fold more dense, on average, in seagrass versus other structured habitats where seagrass was not present (Figure 10). Density patterns among various habitats were identical to occurrence patterns, from most to least dense, among seagrass, marsh, oyster, mangrove, manmade, and open habitats (Figure 10).

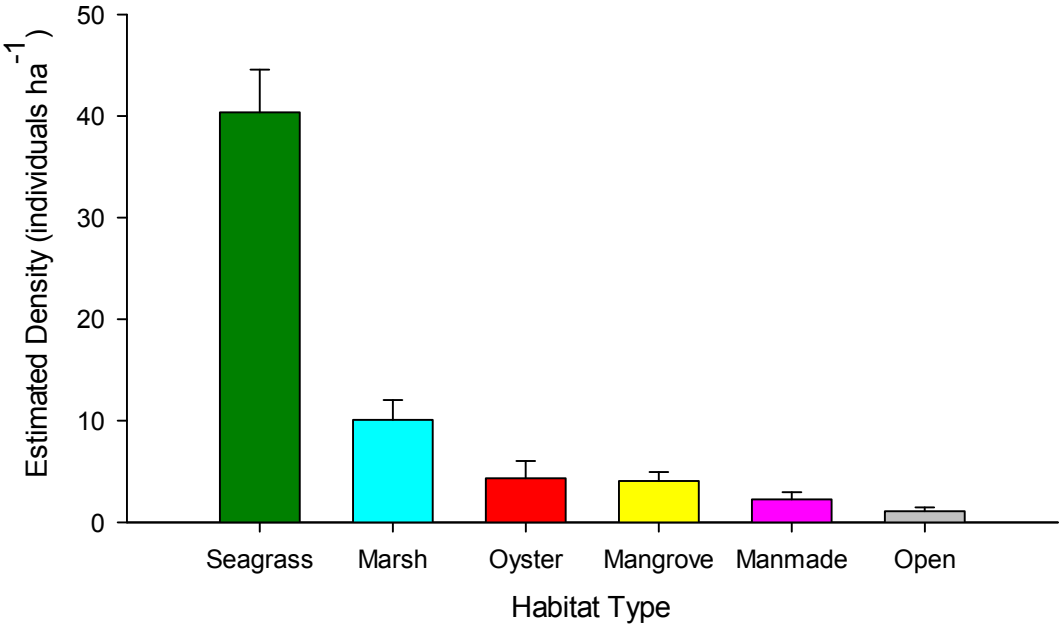

Figure 10. Mean densities (±SD) of gray snapper juveniles captured among various habitats in estuaries along the west Florida shelf in 2006 and 2007. Mean density in non-seagrass habitats was computed from samples collected within 10 m of habitat.

The presence of seagrass had a significant effect on juvenile gray snapper density (Table 5), as might be expected from examination of habitat-specific monthly density estimate plots (Figures 6-9). Other significant variables computed from the delta lognormal model testing effects on densities were: gear, region, month and depth. Compared to effects on presence/absence, the only difference is the lack of temperature effect on densities.

Table 5. Delta Lognormal Model Results Testing Fixed Effects (Year, Seagrass, Region, Month, Temperature, Salinity, and Depth, With Gear as a Covariate) On the Density of Gray Snapper Juveniles Among West Florida Estuaries.

Type 3 Tests of Fixed Effects				
Effect	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	Pr > F
Gear	1	350	259.51	<0.001
Year	1	350	0.89	0.346
Veg	1	350	9.70	0.002
Region	3	350	3.08	0.028
Month	5	350	2.48	0.031
Temp	1	350	3.11	0.079
Salinity	1	350	0.00	0.949
Depth	1	350	11.22	0.001

Sampling region significantly affected juvenile density with higher densities in regions I and III than II and IV. However, it should be stressed that low sampling effort in region IV may indicate those samples are do not represent that region well. The covariate, gear, was also significant in the density model with higher densities in seines than trawls. Higher densities were found in shallower depths and this again is likely due to the differences in shallower depth range for seines and higher densities in seines than in trawls. And lastly, monthly effects were again significant, with the same pattern of lower densities in the later months of the year.

Age and Growth

Sample sizes for fish aged varied among systems, with region I typically generating the highest proportion of samples that could be reliably aged. Region II fish were fewer, but tended to fall more within the size range of fish viable to age, thus a greater percentage of samples collected in this region versus the others were able to be aged (Table 6). In 2006 and 2007, 209 and 312 were received from region III respectively, but these fish tended to be larger than could be reliably aged. In Florida Bay, growth rates could not be determined based on large size of juveniles collected there. Only 3 fish could be aged from 2007 in Florida Bay, and few 2007 fish from Tampa Bay were small enough to age reliably.

Table 6. Sample Sizes of Juvenile Gray Snapper Collected in 2006 and 2007 From West Florida Systems, the Number of Fish <120 mm TL for Which Aging Was Attempted, and the Percent of Samples Which Were Successfully Aged.

	2006			2007		
System	n	attempted aged	% aged	n	attempted aged	% aged
Pensacola Bay	0	0	--	15	15	100
St. Andrew's Bay	256	180	70	118	99	84
Apalachicola Bay	156	61	39	40	39	100
Keaton Beach	4	4	100	3	0	0
Cedar Key	45	45	100	38	27	76
Tampa Bay	57	26	46	111	5	5
Charlotte Harbor	172	92	53	201	16	8

Table 6. Continued.

System	2006 n	2006 attempted aged	2006 % aged	2007 n	2007 attempted aged	2007 % aged
Ten Thousand Ils.	0	0	--	44	0	0
Florida Bay	29	0	0	65	3	5
Total	720	408		635	204	

A percentage of the lapilli prepared for aging in each year were rejected due to indistinct or overlapping opaque zones which made difficult to assign age accurately. From 2006 samples, 13% (n =51/408) of samples were discarded either due to overlapping of outer increments or an indistinguishable core. Of fish collected in 2007, 12% (n = 24/204) of samples were discarded for the same reasons. The second reader made at least one count for 17% (n = 68) of samples from 2006 and 16% (n = 38) of samples in 2007. There was close agreement in age estimates between readers, with combined average percent error calculated as 5.0% (Figure 11). The close relationship between reader, as well as the low APE between counts, indicates high precision between age estimates and instills confidence in the aging conducted by the primary reader.

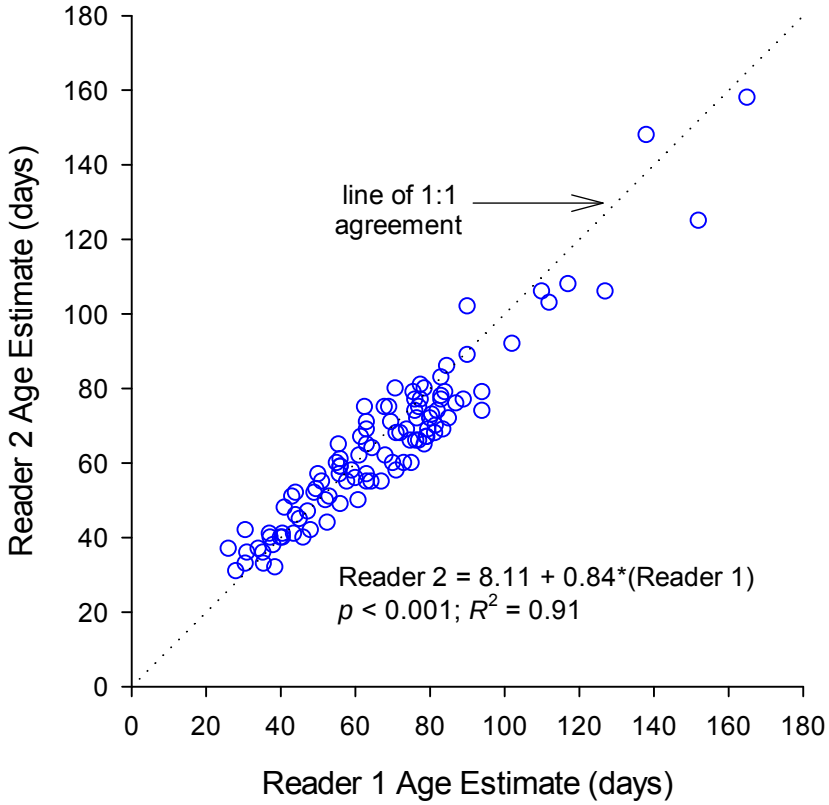

Figure 11. Reader agreement of age validation from a percentage (17%) of total gray snapper juveniles aged from west Florida estuaries in 2006 and 2007 compared as a linear regression equation and the computed R^2 value as an estimate of the relationship to 1:1 agreement.

Hatch date distributions among aged samples showed the earliest spawning occurred in May and the latest in October (Figures 12 and 13). The majority of fish aged in this study were spawned between July and September. That trend was consistent between years, although 2007 samples from region I indicated some early spawning occurred, while region II fish from 2007 had the narrowest range of spawning dates.

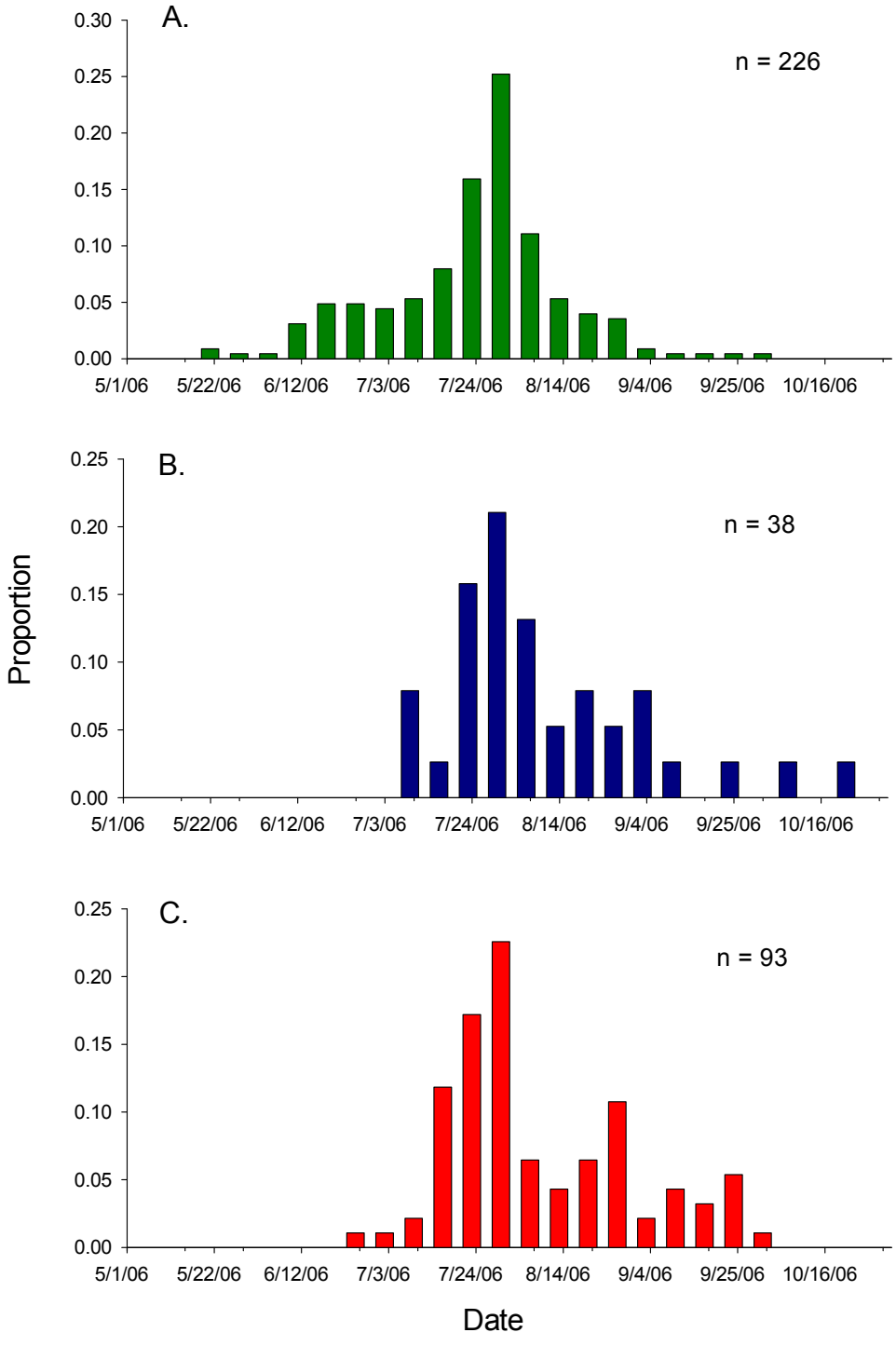

Figure 12. Juvenile gray snapper hatch date frequencies of weekly cohorts sampled for A) region I, B) region II, and C) region III during 2006.

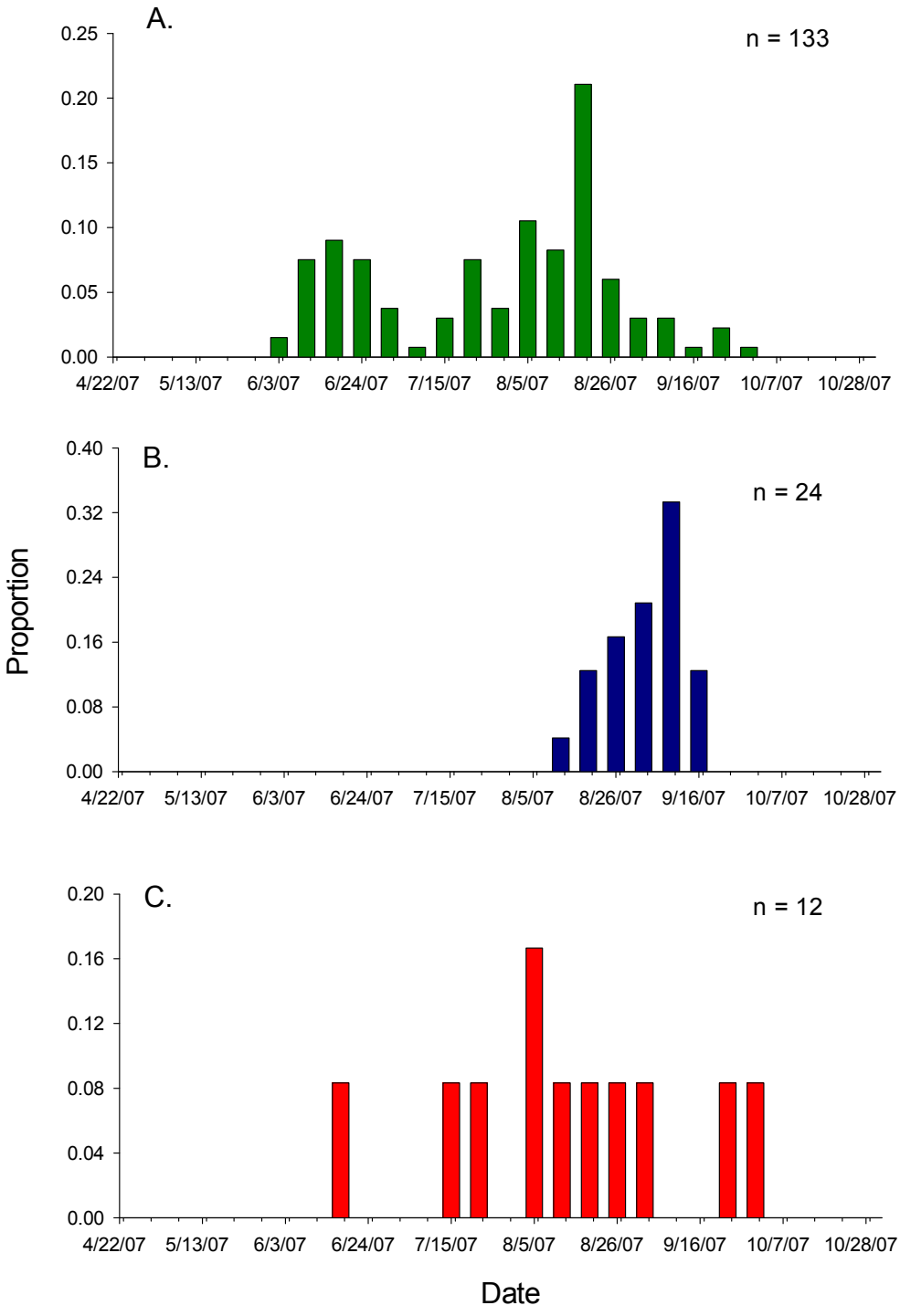

Figure 13. Juvenile gray snapper hatch date frequencies of weekly cohorts sampled for A) region I, B) region II, and C) region III during 2007. Sample sizes indicated on the panel.

There were sufficient samples from regions I-III in both study years to compute juvenile growth rates (Table 7). Region did not significantly affect juvenile gray snapper growth rate in 2006 (Fig. 14; ANCOVA test of equal slopes: $F_{5,348} = 144.63$, $p = 0.5247$). Growth was estimated to be slightly higher, overall, in region III, but substantial variability in size ate age among systems precluded a statistically significant difference in slopes. Growth rates were found to be significantly different among regions in 2007 (Figure. 14; ANCOVA test of equal slopes: $F_{5,163} = 130.47$, $p = <.0001$). Slow growth estimated in region II during 2007 drove this difference, but it is important to note that very few samples within size limits were available from either regions II or III for aging in 2007.

Table 7. Growth Rates and Sample Sizes of Juvenile Gray Snapper Data in 2006 and 2007 for Regions I, II, and III Along the West Florida Shelf.

Year	Region	N	Growth rate (mm/day)
2006	I	226	0.66
	II	38	0.62
	III	90	0.73
2007	I	133	0.79
	II	24	0.46
	III	12	1.12

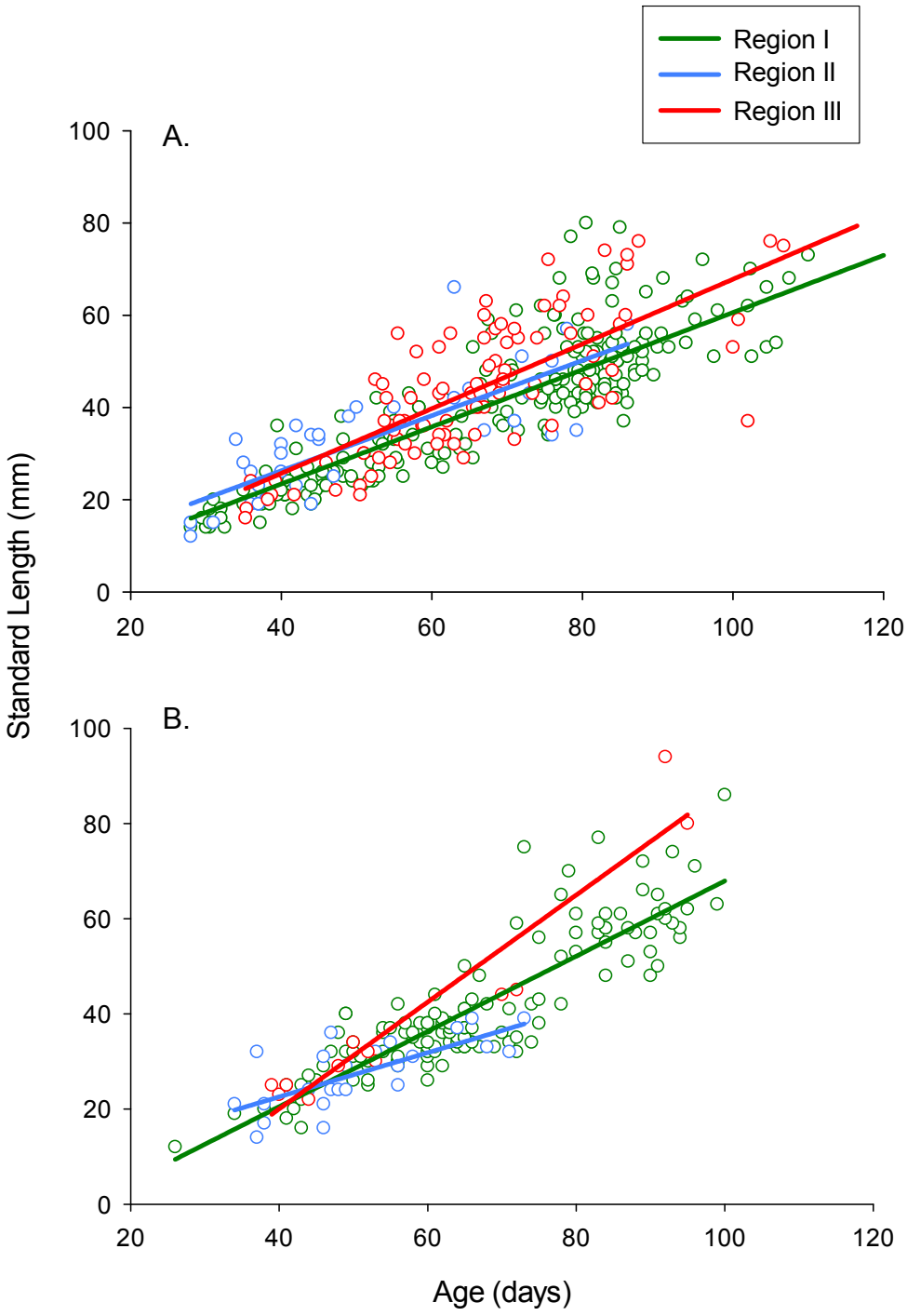

Figure 14. Growth rates of juvenile gray snapper from regions I, II, III along the west Florida shelf in A) 2006 and B) 2007. Sample sizes in 2006 for region I, n= 226, region II, n=38; region III, n=90. Sample sizes in 2007 for region I, n=133; region II, n=24; region III, n=12.

Otolith Chemistry

A total of 387 samples were analyzed for elemental:Ca ratios and stable isotope delta values (Table 8). The size range of fish used in analysis ranged between approximately 30 mm standard length and 160 mm standard length (Figure 15). In most regions fish were of similar size range; however, fish from region IV were larger than those from other regions.

Table 8. Sample Sizes of Gray Snapper Juveniles for Otolith Elemental and Stable Isotope Analysis From Four Regions Along the West Florida Shelf in 2006 and 2007.

System	2006	2007	Both years	Region	2006	2007	Both Years
Pensacola Bay	2	5	7				
St. Andrews Bay	57	42	99	I	97	77	174
Apalachicola Bay	38	30	68				
Keaton Beach	2	0	2	II	17	15	32
Cedar Key	15	15	30				
Tampa Bay	27	15	42	III	75	51	126
Charlotte Harbor	48	36	84				
Ten Thousand Islands	0	23	23	IV	5	50	55
Florida Bay	5	27	32				

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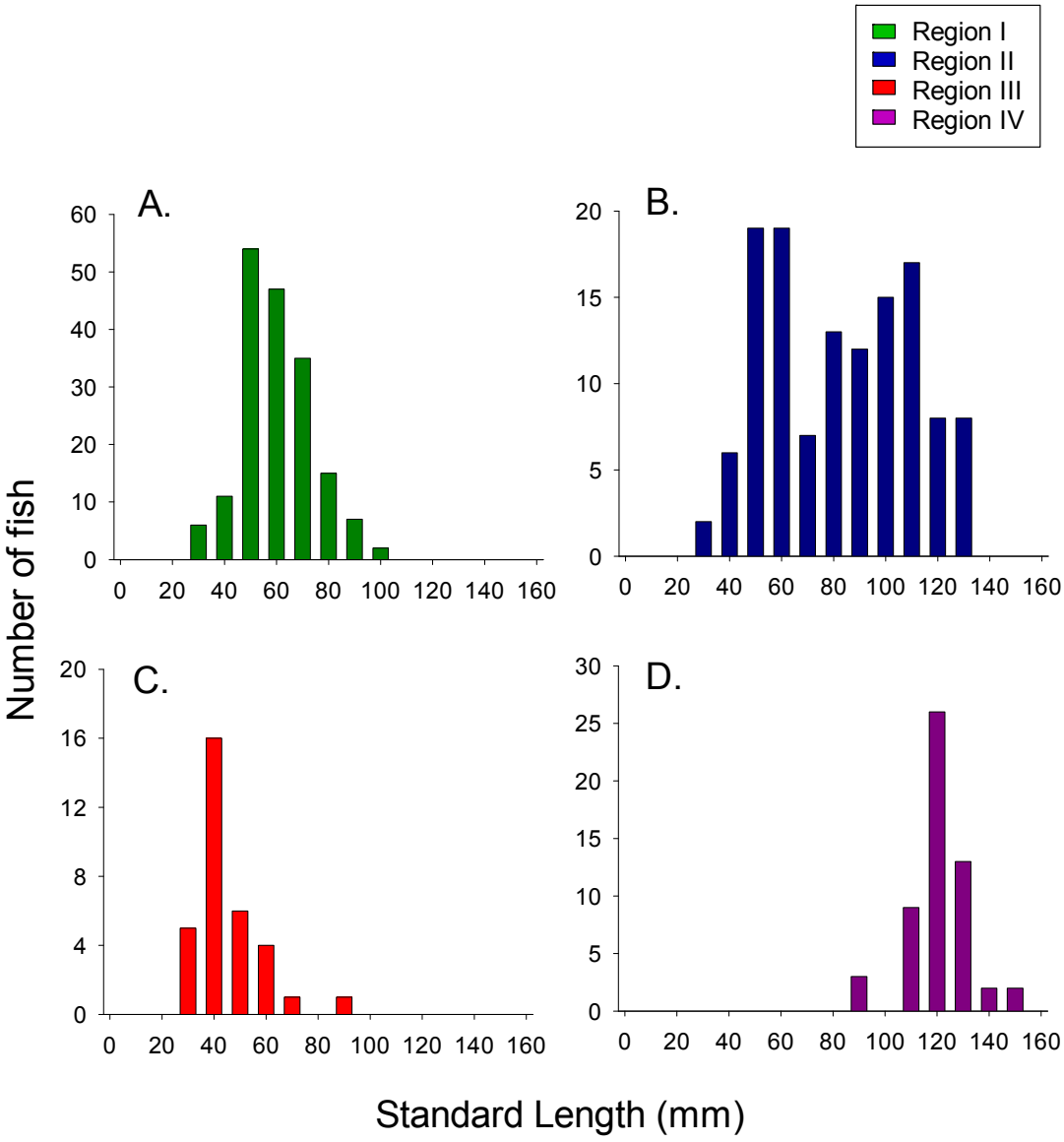

Figure 15. Standard length distributions of juvenile gray snapper collected from west Florida regions in 2006 and 2007 used for otolith elemental and stable isotope analysis. Figure legend is provided above panel B.

Plots of mean element:Ca ratios and stable isotope delta values demonstrate that regional trends were similar between years (Figures 16 and 17). All element:Ca ratios and both stable isotope values were non-normal and heteroscedastic; however, Li:Ca, Ba:Ca, Mn:Ca, and Sr:Ca had the most substantial deviations from parametric assumptions. The

Box-Cox power transformation proved effective at reducing variance for Mn:Ca, Mg:Ca and Sr:Ca, and improved normality for Ba:Ca. Li:Ca and $\delta^{13}C$ tended to be bimodal and transformations were unsuccessful. Transformations for $\delta^{18}O$ data were also not wholly successful due to its considerably leptokurtic distribution.

The MANOVA computed on chemistry data indicated that year was a significant effect in the model (MANOVA Pilia's trace; $F_{7,347} = 8.87$; $p < 0.001$). The significant multivariate year affect was driven by significant year effects for Mn:Ca, Mg:Ca, and $\delta^{13}C$ (ANOVAs; $F_{1,380} > 10.2$; $p < 0.01$) (Table 9). However, it should be reiterated the regional trends in element:Ca ratios and stable isotope delta values were similar between years even for variables that had a significant year effect.

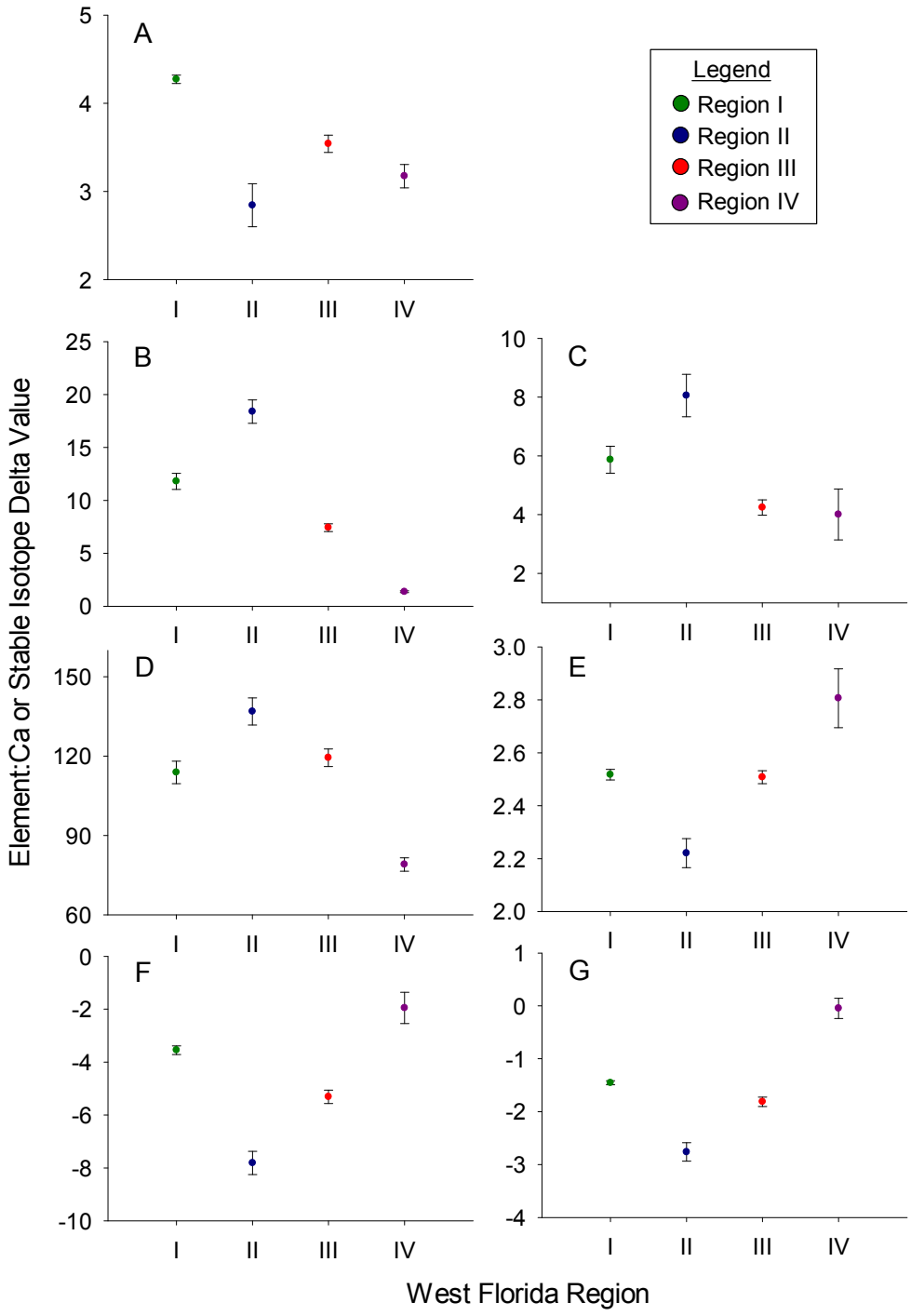

Figure 16. Otolith chemistry plots of 2006 mean (±SE) element:Ca ratios and stable isotope delta values for A) Li:Ca, B) Mn:Ca, C) Ba:Ca, D) Mg:Ca, E) Sr:Ca, F) $\delta^{13}C$, and G) $\delta^{18}O$. Units for all element:Ca ratios are μmol mol^{-1} except Sr:Ca, which is mmol mol^{-1}.

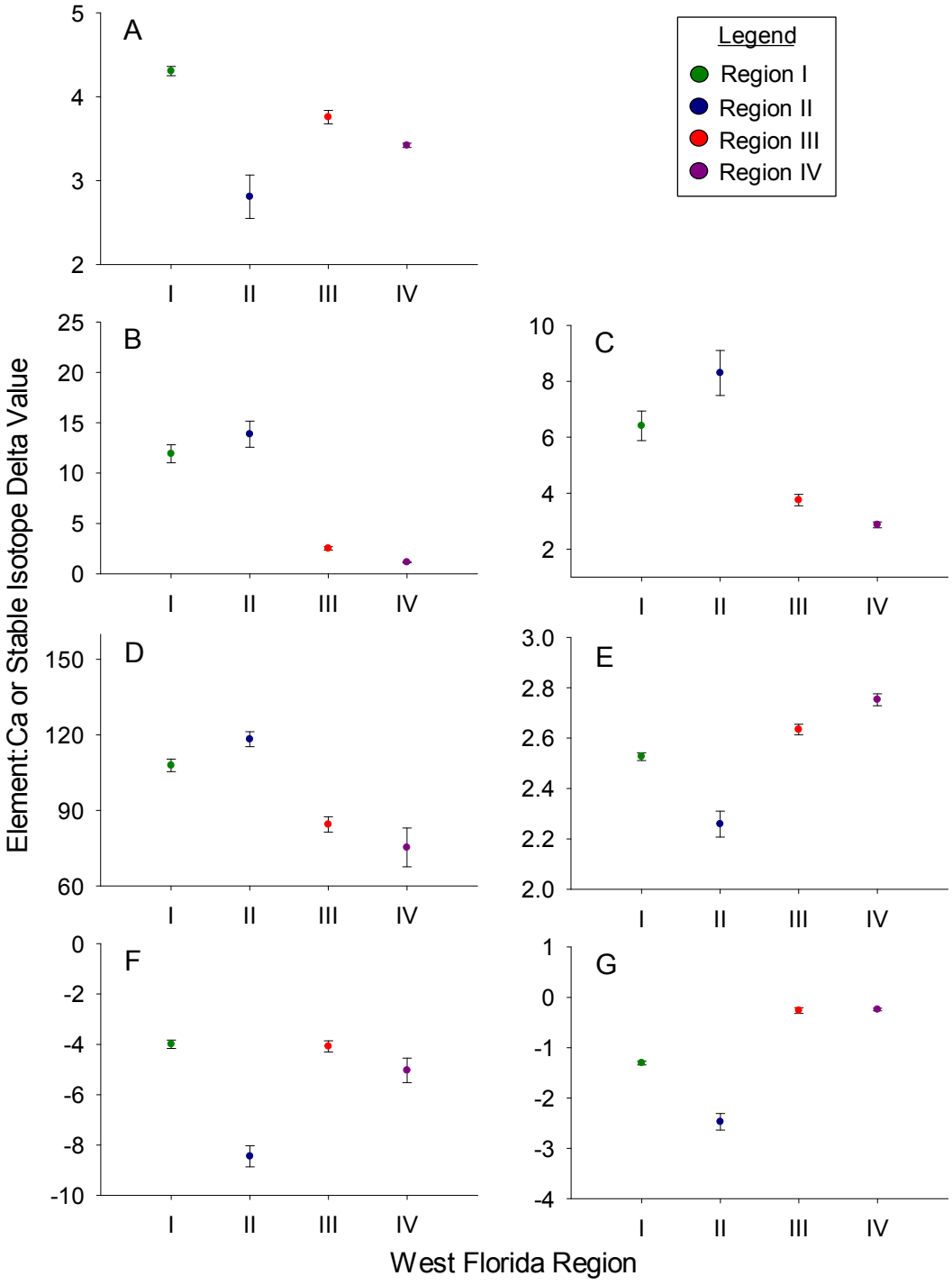

Figure 17. Otolith chemistry plots of 2007 mean (±SE) element:Ca ratios and stable isotope delta values for A) Li:Ca, B) Mn:Ca, C) Ba:Ca, D) Mg:Ca, E) Sr:Ca, F) $\delta^{13}C$, and G) $\delta^{18}O$. Units for all element:Ca ratios are μmol mol^{-1} except Sr:Ca, which is mmol mol^{-1}.

Table 9. Individual ANOVAs for Each Element:Ca and Stable Isotope Concentrations From Juvenile Gray Snapper Otolith Chemistry Data Testing Differences Among Years and Regions. Li:Ca data Were Not Transformed, $\delta^{13}C$ and $\delta^{18}O$ Data Were Detrended for Size, and Ba:Ca, Mn:Ca, Mg:Ca, and Sr:Ca Data Were Detrended for Size and Transformed With Box-Cox Power Transformations.

Model	Source	Degrees of Freedom	Mean Square Error	F Value	Pr > F
Li:Ca	Model	7	6.01	4.93	<0.001
	Year	1	2.38	1.95	0.163
	Region	3	5.86	4.80	0.003
	Error	380	1.22		
Ba:Ca	Model	7	1.95×10^{-3}	22.88	<0.001
	Year	1	3.10×10^{-4}	3.63	0.058
	Region	3	5.74×10^{-4}	6.71	<0.001
	Error	380	8.54×10^{-5}		
Mn:Ca	Model	7	1.36	17.94	<0.001
	Year	1	1.69	22.17	<0.001
	Region	3	1.18	15.55	<0.001
	Error	380	0.08		
Mg:Ca	Model	7	1.84	6.28	<0.001
	Year	1	6.25	21.28	<0.001
	Region	3	1.28	4.35	0.005
	Error	380	2.94		
Sr:Ca	Model	7	17.55	10.40	<0.001
	Year	1	3.43	2.03	0.156
	Region	3	2.20	1.31	0.272
	Error	380	1.68		
$\delta^{13}C$	Model	7	49.36	6.83	<0.001
	Year	1	73.94	10.23	0.002
	Region	3	12.37	1.71	0.164
	Error	380	7.23		
$\delta^{18}O$	Model	7	6.20	7.68	<0.001
	Year	1	0.52	0.64	0.423
	Region	3	3.57	4.43	0.005
	Error	380	0.81		

Quadratic discriminant function results indicate that otolith chemical signatures effectively distinguished gray snapper nursery regions whether year classes were modeled separately or jointly (Figure 18). The mean classification success among samples was 77.9% in 2006, 85.0% in 2007, and 76.3% when years were modeled jointly (Figure 18). The most significant misclassification occurred in 2006 when 0% of the region IV fish were correctly classified. However, only five fish were available for analysis from region IV in 2006. When both years were modeled jointly, region IV fish were distinguished with much greater accuracy. The lack of classification success for region IV fish in 2006 was further explored by pooling samples from regions III and IV and re-running year-specific and joint year models (Figure 19). Results indicate even greater success was achieved in this approach as overall classification success was 82.6% in 2006, 92.3% in 2007, and 83.3% when both years modeled jointly.

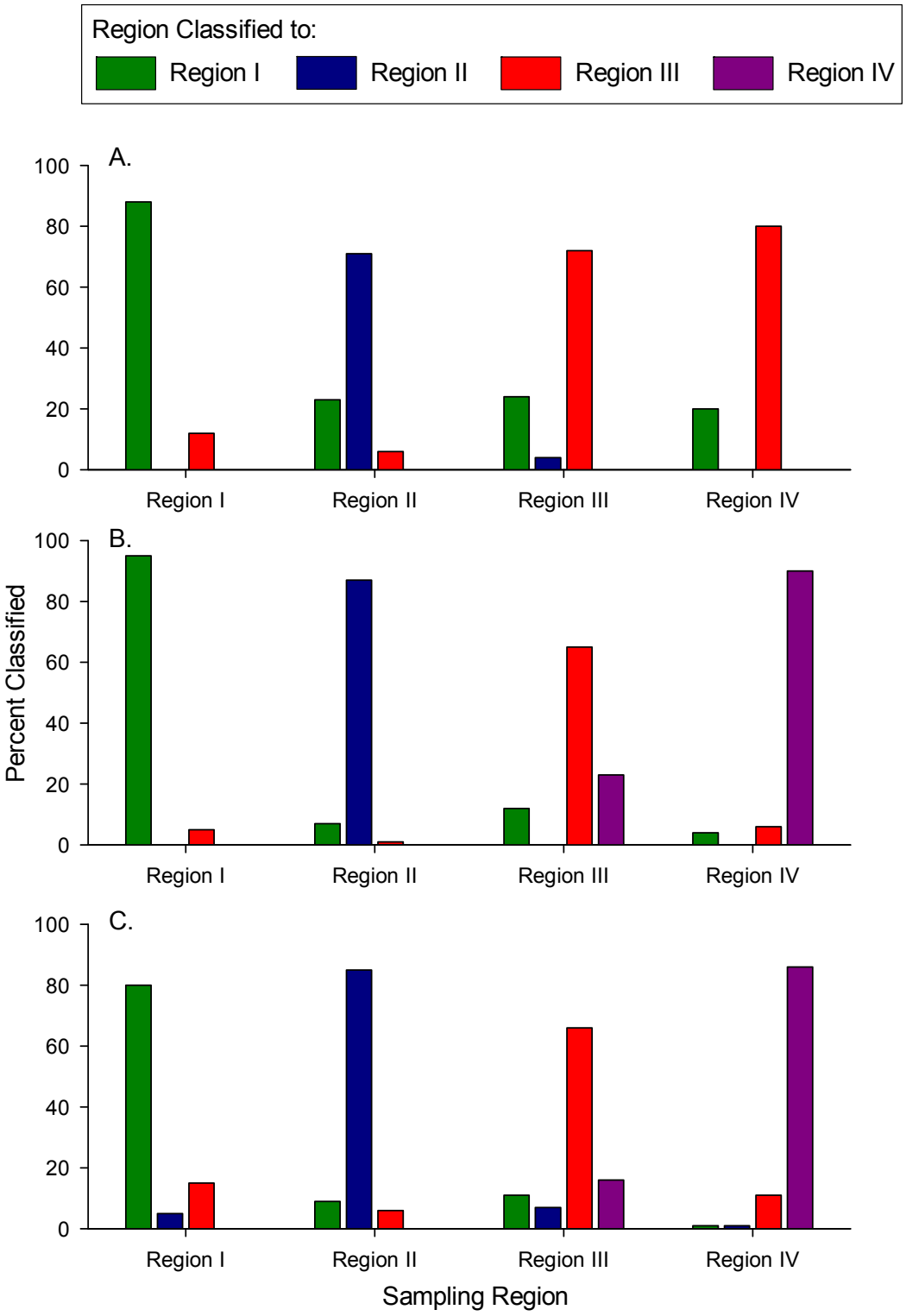

Figure 18. Crossvalidated quadratic discriminant function results for region-specific juvenile gray snapper otolith chemical signatures for fish sampled along the west Florida shelf in A) 2006, B) 2007, C) 2006 and 2007.

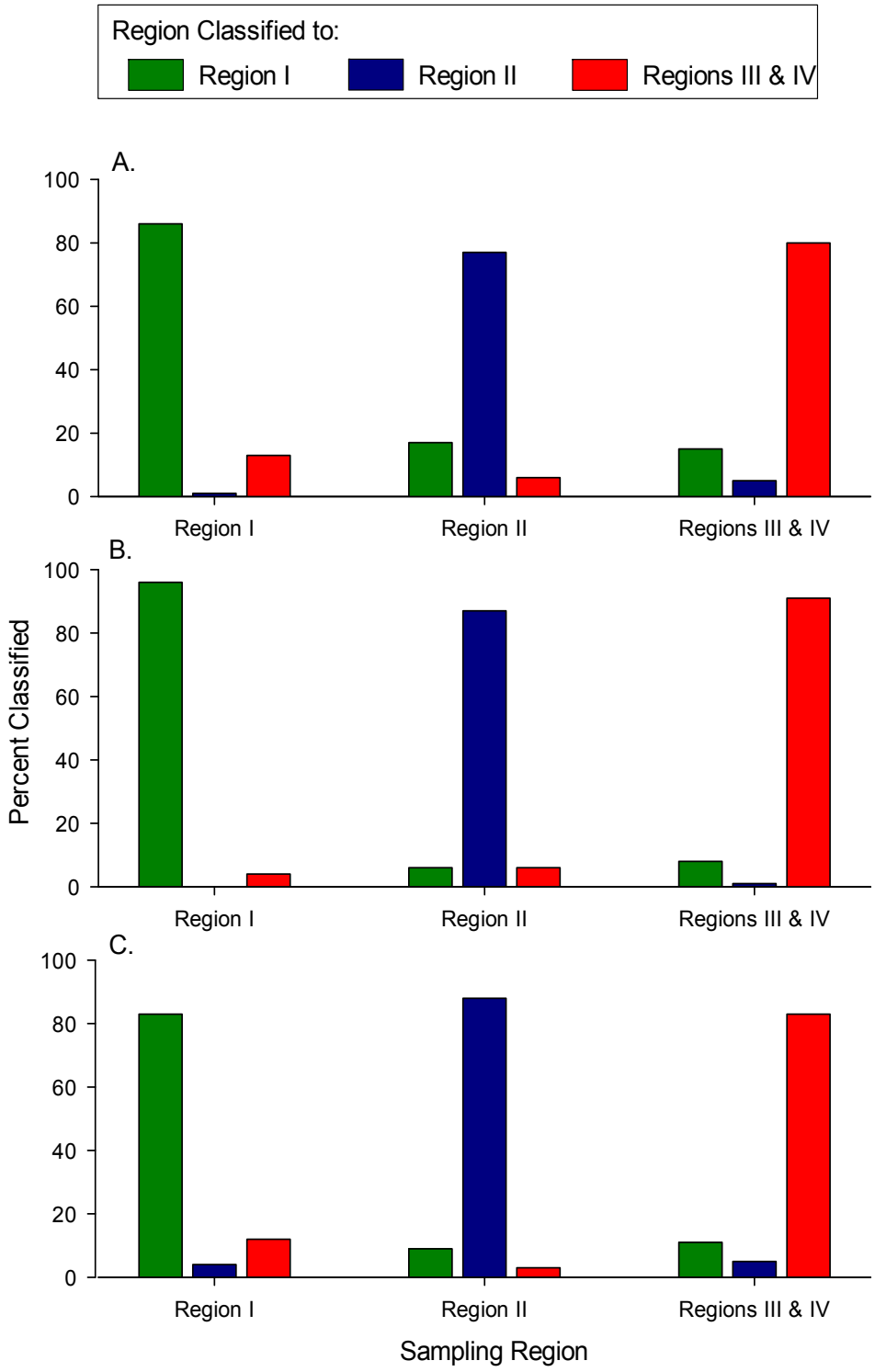

Figure 19. Crossvalidated quadratic discriminant function results for region-specific juvenile gray snapper otolith chemical signatures for fish sampled along the west Florida shelf in A) 2006, B) 2007, C) 2006 and 2007. Fish sampled in regions III and IV were pooled in this model.

DISCUSSION

Evaluating Habitat

Results of this study clearly demonstrate that seagrass beds are the most significant nursery habitat for post-settlement juvenile gray snapper along west Florida. The prevalence of gray snapper in the coastal systems of Florida is well-documented and seagrass beds have been described in numerous studies as likely nursery habitat for juvenile of gray snapper (Starck and Schroeder 1971; Allman and Grimes 2002; Faunce et al. 2002; Denit and Sponaugle 2004; Eggleston et al. 2004; Faunce and Serafy 2007, 2008). However, gray snapper also have been previously reported as occurring in several other types of structured habitat and the relative importance of various habitats had not been tested. The tiered approach to evaluating juvenile gray snapper EFH in this study produced unequivocal results from multiple levels of analysis that seagrass is the most important habitat for juvenile gray snapper.

Juvenile gray snapper were rarely observed in open, non-seagrass habitat and densities were low when they were encountered. Seagrass beds are known to serve as important nursery habitat for early life stages of many nektonic organisms by offering refugia from predators and an abundant food supply (Heck et al. 2003). The proximity to other structured habitats did have some effect on densities, indicating that gray snapper juveniles may utilize these habitats to some degree as well. Marsh, oyster,

mangrove and manmade habitat were each associated with juvenile gray snapper presence and higher densities than open or unstructured habitat. For example, densities in non-seagrass habitat around Cedar Key were low but consistently higher than densities in non-seagrass habitat in other systems. It is important to note that Cedar Key has extensive oyster reefs adjacent to substantial marsh habitat, which may explain the higher densities of juveniles in non-seagrass habitats there.

Gray snapper juveniles have been observed among various habitat-types in South Florida and the Caribbean as well but mangroves, marshes and oyster beds may actually be more important for later juvenile development than the first few months immediately post settlement (Nagelkerken et al. 2000; Eggleston et al. 2004; Faunce and Serafy 2008). For example, south Florida habitat-specific density and size-distributions indicated seagrass was important post-settlement habitat and mangroves became more important later in the juvenile stage (Faunce and Serafy 2007). A similar pattern also was observed in a multi-habitat comparison of fish assemblages in the Florida Keys, where the smallest juveniles were found exclusively among seagrass beds (Eggleston et al. 2004). Continuous seagrass beds in Charlotte Harbor also have been described as habitat where gray snapper occur most frequently from results of spatial modeling of fish distributions (Whaley et al. 2007). The prevailing trend is the function of seagrass beds in estuaries as essential nursery habitat for juvenile gray snapper.

From the two tiers of analysis employed here to test EFH for gray snapper, the significant effect of depth and the significant covariate, gear, also provide further evidence for the importance of shallow seagrass habitat. Separate estimates were derived for seines and trawls, and differences observed in abundances and densities between

gears display the overall significance of seagrass as nursery habitat. However, it is unclear if differences in the scale of density estimates between gear types is due to differences in catchability, selectivity, or gear avoidance. It could have been that prop wash disturbed seagrass habitat, thus juveniles moved to adjacent habitat as the sampling boat approached. Size distributions in samples collected with trawls versus seines were similar, therefore it appears unlikely that selectivity differences affected gear-specific density estimates. Perhaps the most likely cause of differences, however, was the difference in the depth distributions of samples. By sampling in shallow waters, seines sampled more continuous seagrass habitat, thus explaining the difference in frequency of occurrence in seine versus trawl samples. If shallower seagrass habitat is more valuable as gray snapper nursery habitat than deeper seagrass or other structured habitats, then the issue of prop scarring of seagrass beds would be acutely important for gray snapper population ecology.

Depth was both found to have a significant effect on presence/absence and densities of gray snapper. Individuals tended to be more concentrated among shallow zones, which can typically be linked to the photic zone or the depth at which photosynthesis can occur. Depth affects also relate to the relationship between high densities among vegetation, where seagrass beds require penetration of sunlight for growth. Seagrass coverage and seasonalilty are linked as well, where seagrass beds die back or undergo a dormancy in the cooler (<15° C) temperatures winter temperatures of Panhandle estuaries (Zimmerman and Livingston 1976).

Monthly effects on gray snapper densities showed a significant decrease in presence/absence and densities in the late fall. August through November demonstrated

highest concentrations of gray snapper, with December densities being consistently low. Temperature drops and seasonal spawning patterns offshore are likely correlated to monthly differences in densities. Peak spawning occurs offshore between May and September, thus peak abundances in newly settled recruits would be expected to occur among nursery habitats immediately following these events and persist for the first several weeks of growth and development (Allman and Grimes 2002). In southeast Florida the use of mangroves as habitat by gray snapper varies seasonally as well, with a preference for offshore waters in the summer and inshore waters or embayments in the winter (Faunce and Serafy 2008). The movement of gray snapper indicates that habitat selection may occur seasonally and occupation of nursery habitat is confined to warmer months.

Other characteristics of gray snapper habitat were not as influential on their presence there or densities. Salinity did not have a significant effect on the presence or densities of juvenile gray snapper. The lack of evidence for a salinity effect is not surprising given gray snapper's range of tolerance for salinity and the findings that gray snapper often inhabit oligohaline waters (Wuenschel et al. 2004). Systems in the Big Bend tend not to have the same bar-built estuaries that typify other systems along the west-Florida shelf and instead the coastal regions are marked with freshwater tributaries that flow directly into the Gulf of Mexico. It might be that juveniles utilize structured habitats in small tributaries in this region. If true, then capturing individuals in those areas would have increased the overall range of salinity and reduce its effect on the presence of gray snapper. Temperature also did not significantly affect juvenile density, corroborated by other studies that state gray snapper have a wide physiological elasticity and tolerance

to temperature and salinity (Peterson, 2003; Wuenschel et al. 2004). In some cases though, the highest occurrence of gray snapper in Charlotte Harbor has been correlated with high salinities (Whaley et al. 2007). The effect of temperature on presence of gray snapper is likely related to seasonal or monthly effects, especially in the northern systems where juveniles are virtually absent in the bays during the colder winter months. The inference is that they either move to deeper waters where daily temperature fluctuations are more moderate or that they simply do not survive these colder temperatures (Allman and Grimes 2002).

Regional effects on the presence and density of gray snapper were evident. Although fish were present among all systems, there were clear differences in densities of fish captured on a regional scale. The Big Bend (region II) and South Florida (region IV) had the lowest densities of juvenile gray snapper. It should be noted that region IV was clearly undersampled in this study and results from previous research has demonstrated that Florida Bay contains significant nursery habitat for juvenile gray snapper (Denit and Sponaugle 2004; Egglseton et al. 2004; Fauce and Serafy 2008), but density estimates were contingent on FWRI sampling in Florida Bay and their efforts were restricted to a small area in the northern part of the Bay. It may be that in both these regions densities are lower, dispersed over greater areas, but given the vast expanses of seagrass beds it is likely that significant contributions are coming from these habitats which were not evident at the level of density estimates.

There was no significant difference on juvenile presence or densities between study years, suggesting that data are temporally consistent between years. Predictions of future patterns of presence/absence and density could then potentially be made. Applying

a tiered approach to evaluate nursery habitat, it was evident from the first two tiers of analysis that juvenile gray snapper utilized seagrass beds disproportionately over other habitats sampled. Therefore, seagrass habitat appears to be the most nursery habitat based on level-1 and level-2 EFH analyses (NOAA 2004). There is also evidence that gray snapper juveniles occur at greater frequencies and higher densities in other structured habitats, such as marshes, oyster reefs, mangroves and manmade structures, versus open habitat, thus suggesting that those other structured habitats are important to juvenile gray snapper as well, but just not as important or essential as seagrass habitat appears to be.

Age and Growth

Growth functions were computed as part of a third-level habitat analysis. Sample sizes were insufficient to compare growth rates among habitats within systems; hence, only regional comparisons were made. Differences in growth existed between the Panhandle, the Big Bend and Southwest Florida. Growth in the Panhandle was intermediate compared to growth in the other two regions. Seagrass beds there are not as expansive as southern regions of Florida, but they persist in patches in St. Andrews Bay, Apalachicola Bay and Pensacola Bay. Densities in this region were also relatively high. The slowest growth among regions was observed in the Big Bend where gray snapper densities were lowest among all sampled seagrass beds, but where seagrass beds on the coast are more expansive. Growth tended to be highest in southwest Florida, where densities also were high and seagrass bed distributions were similar to what exists in the Panhandle. The trends tended to show faster growth in regions with higher densities and more patchy seagrass habitat.

The use of growth as a parameter to assess habitat implies that faster growth rates are reflective of optimal habitat conditions. Certain factors that can affect growth rates include environmental conditions, availability of food resources, selective mortality, and density dependence within a system, and these factors usually act in concert with one another. Studies show that few significant differences in growth and survival of juvenile fishes in seagrass beds compared to other structured habitats exist, thus implying that the presence of structure is more important as a refuge from predation than for enhancing growth rates (Heck et al. 2003).

Selective mortality and density dependence may also be significant sources of variability in growth among different regions if availability of optimal habitat differs. Resources may be more accessible, and physiochemical characters more optimal in a given habitat, but if fish are more subject to predation or negative density-dependence in this zone, the overall production could be significantly diminished (Peterson 2003). In this study, regions where growth was faster actually had the relatively high densities of juveniles, thus suggesting density-dependence did not have a significant effect on growth. Additional biotic components such as competition and predation can have significant impacts on the use of habitat, and thus, observed growth rates (Peterson 2003).

It has been documented that bias of growth functions is generated if previously occupied habitat altered growth rates or if fast growing fish recruit to other habitats prior to sampling (Searcy et al. 2007). If growth rates are examined after a period where either faster growers or slower growers were selectively removed from the population, growth analysis may be biased towards interpreting the habitat as either lower or higher quality, respectively, than would have been calculated earlier (Searcy et al. 2007). In south

Florida gray snapper move from nursery habitat in seagrass beds to adjacent mangrove habitat while still in their juvenile stages (Faunce and Serafy 2008). It is likely that recruitment to these habitats occurs first among fast-growers, thus growth rates in these habitats may not necessarily be reflective of habitat quality. Without sufficient sample sizes of early stage juveniles in region IV, it was not possible to include fish from that region in growth comparisons. However, future applications of a tiered approach to examining juvenile gray snapper EFH in south Florida should include a concerted effort to sample Florida Bay seagrass beds and other structured habitats more comprehensively than I was able to with assistance from FWRI personnel.

The nursery role of various habitats and regions were not tested via production estimates in my study due to data limitations, but that level is likely to provide the most thorough assessment of habitat value. Future work may consider alternate methods of producing mortality estimates in difficult to sample habitats, such as the visual survey techniques employed by Faunce and Serafy (2008) to estimate growth and production of juvenile gray snapper in southeast Florida. These estimates combined with results from presence/absence, densities and growth trends, would cover the full gambit of the hierarchical framework of investigation and provide the inclusive index of nursery habitat for gray snapper in the Gulf of Mexico.

Authors of other studies examining nursery function have warned that assessing habitat value based on per unit area production rates alone carries the risk of overlooking important, expansive habitats that have lower juvenile production on per unit area basis (Patterson et al. 2005; Dahlgren et al. 2006). The links between nursery, juvenile and adult habitat can be complex and the fact that not all juvenile habitat is considered a

nursery elicits considerations for evaluating juvenile habitat as well. Effective Juvenile Habitat, EJH, may occur where a greater proportion of individuals are contributed to adult populations, regardless of habitat dimension (Dahlgren et al. 2006). The Big Bend area of Florida may be an area in which this concept of EJH versus Beck et al.'s (2001) per unit area production-based paradigm is important for evaluating gray snapper nursery habitat. The Big Bend areas that I sampled had low juvenile densities, but seagrass in the Big Bend is more extensive (2.5×10^5 ha) than in the Panhandle (1.7×10^4 ha) or Southwest Florida (4.3×10^4ha). Hence, the Big Bend may produce just as many or more recruits to the adult population despite low densities observed in this study.

Hatch date frequencies demonstrated that fish aged in this study were hatched during the same time frame across all regions, typically June through late September. This is consistent with a May-September spawning season reported in previous studies (Starck and Schroeder 1971; Domeier et al. 1996; Allman and Grimes 2002). The pattern was more scattered in 2007 in Southwest Florida and truncated in the Big Bend. The Panhandle displayed an identical pattern to previous data. Big Bend samples were only spawned in late August and early September. Southwest Florida had one fish hatched in April, and the remainder between July and September. With future analysis of settlement marks indicating pelagic larval duration, inferences about sources of spawners could be made (Allman and Grimes 2002; Denit and Sponaugle 2004). For example, if a fish from Tampa Bay hatched on the same day as a fish from St. Andrews Bay with an identical pelagic larval duration, it would be unlikely that those individuals were derived from spawning events in close geographic proximity to one another. Longer pelagic durations

in one region versus another, combined with oceanographic data or modeling, might suggest a common source of eggs, hence spawners, was possible.

Estimating Population Connectivity

Natural tags were successfully derived from the region-specific gray snapper otolith chemical signatures along west Florida. Despite the significant effect of year on signatures, temporal consistency in interregional trends between years, and the fact that overall classification success from discriminant functions remained strong when years were combined, suggests that multiyear signatures might be possible. This is contrary to the trend typically reported (e.g., Patterson et al. 2008), however, and should be explored with sampling of subsequent year classes.

Sources of variability between years were attributed to Mn:C, Mg:Ca, and $\delta^{13}C$. A significant relationship between otolith Mg concentrations and somatic growth among other fishes has been documented previously (Elsdon and Gillanders 2002; Martin and Thorrold 2005), thus interannual differences in growth have driven differences in Mg:Ca between years. Temperature and salinity may be somewhat influential in the incorporation of Mn (Martin and Thorrold 2005). Effects of diet, kinetic and metabolic effects, and amounts DIC have all been shown to influence quantities of otolith $\delta^{13}C$ (Kalish 1991b; Thorrold et al. 1997; Martin and Thorrold 2005). These variables may indicate some degree of interannual variability in water composition which are reflected in otolith composition, but the stability of other elements and stable isotopes demonstrate a strong degree of consistency across years. The concentrations of Li:Ca, Ba:Ca, Sr:Ca and $\delta^{18}O$ were found not to be statistically significant across years, as proxies of ambient salinity and temperature (Elsdon and Gillanders 2005; Martin and Wuenschel 2006).

Ratios of Mn:Ca, Sr:Ca, and Ba:Ca, as well as $\delta^{13}C$ values, appeared to be the most influential factors in distinguishing fish from different regions. The relationship between Mn-enriched sediment and otoliths have been traced along the western Florida shelf in a previous study of gag, *Mycteroperca microlepsis*, in which a strong correlation between otolith and sediment [Mn] followed a latitudinal gradient (Hanson et al. 2004). Significant differences in gag otolith Mn values were observed between northern and southern systems along west Florida, which was similar to the pattern of manganese-rich soils in northern regions that lead increased levels of dissolved manganese in the water column (Hanson et al. 2004). Importantly, a similar latitudinal gradient in gray snapper otolith Mn:Ca was observed in the current study. Furthermore, Mn, as well as Sr and Ba, have also been shown to be significant sources of regional variability in otolith chemical signatures in red snapper, *Lutjanus campechanus*, among other regions of the Gulf of Mexico (Patterson et al. 2008). All three variables have potential for strong classification success along west Florida given the variability in freshwater inputs (Hanson et al. 2004).

The correlation between salinity and otolith [Sr] has been well documented in several fishes (reviewed in Campana and Thorrold 2001). The relationship between temperature and Sr is weaker but is a possible factor to consider in explaining Sr:Ca values in gray snapper otoliths (Martin and Wuenschel 2006). Ratios of Sr:Ca were higher in region I, III, and IV that region II where mean salinity was much higher than the other regions. The wide salinity tolerance of gray snapper allows fish to inhabit more saline environments in region II. Salinity ranges can contribute to a strong signal for juveniles derived from this area.

Barium concentrations in otoliths also have been strongly correlated to ambient salinity (Campana and Thorrold 2001; Hamer et al. 2006; Elsdon and Gillanders 2005). Ratios of Ba:Ca in gray snapper otoliths were highest consistently in region I, lowest in region II, and region III levels were intermediate. Freshwater inputs of Ba may be a highly significant source of Ba in otoliths, particularly from Apalachicola Bay and Pensacola Bay where mean Ba:Ca values higher than among other regions. The Apalachicola Bay watershed in northwest Florida drains waters from Georgia, Alabama, and Florida, and has the distinctive trait of its headwaters beginning in the southern Appalachian Mountains (Livingston et al. 1974). In all other systems the means ranged between 6 and 8, suggesting that Ba inputs in these systems contribute significantly to a temporally stable region-specific signature. Evidence is strong that Ba:Ca may serve as a reliable proxy for ambient Ba and that its incorporation into otoliths is similar across life stages (Elsdon and Gillanders 2005; Hamer et al. 2006).

The myriad factors influencing amounts of $\delta^{13}C$ in otoliths make tracing the sources of carbon more complex, but the trend appears similar to other element:Ca examined here, where region II stands out clearly from regions I, III, or IV. The kinetic and metabolic effects on carbon isotopes tend to be inversely related in previous studies, but they do not act alone on the fractionation rates of otolith $\delta^{13}C$; diet and water composition are other sources of $\delta^{13}C$ variability (Kalish 1991b; Martin and Thorrold 2005). There appears to be a more depleted carbon supply in region II otoliths than either far north or southern reaches of juvenile habitat. More densely vegetated areas may indeed support greater species richness, or diverse trophic interactions which could contribute to higher $\delta^{13}C$ values in these regions. The contribution from dissolved

inorganic carbon is a significant source in most cases as well, and a likely contributor to the variability found across these regions (Kalish 1991b; Thorrold et al. 1997; 1998).

With strong classification success and temporal stability of regional signals in this study, the applicability of otolith chemistry was clearly demonstrated. Lara et al. (2008) attempted smaller-scale classification of gray snapper juveniles within Florida Bay. Their discrimination success when looking at regions within Florida Bay was not as strong as we were able to obtain across a more broad geographic scale, and they employed the use of rare earth elements which were below detection limits in some cases (Lara et al. 2008). Comparatively, the classification success rates computed here were up to 20 points higher, and by sampling the Panhandle to Florida Bay, a larger geographic scale was addressed. This is likely a more suitable application of otolith chemistry where the hydrogeochemical conditions and thus regional signals are more distinctive over a broad range.

The data presented here demonstrate that there are particular elements and isotopes in otoliths that can distinguish juvenile gray snapper nursery regions and could potentially be a stable signal over time. These findings are promising not only as markers for the 2006 and 2007 cohorts, but also prove the utility of otolith chemistry for future studies of gray snapper population connectivity. With knowledge of region-specific signatures along the west Florida shelf, analysis of otolith cores from adults caught offshore can potentially be linked to the chemical markers derived from juveniles in this study. The study of otolith chemistry on fine-scales to distinguish among different habitats occupied through a fish's life is accomplished by a few different techniques. Laser ablation ICP-MS, electron microbe and micromilling techniques have all been

shown to successfully remove small portions of the adult otolith corresponding to particular life-stages for analysis of trace elements or stable isotopes (Campana et al. 1997; Elsdon and Gillanders 2003). Cross-sections of adult otoliths are mapped and a predetermined area is outlined corresponding to the age or time-period of interest, and either a small section several μm in diameter is removed for analysis or transects are run and analyzed (Elsdon and Gillanders 2003). The mechanical removal and analysis of adult red snapper (*Lutjanus campechanus*) sagittal cores as material representative of nursery stage was shown to be successful (Barnett and Patterson 2008). Red snapper adult cores also showed no significant difference between right and left sagittae when element:Ca concentrations of Ba, Li, Mg, Mn, Sr, and Pb were compared as well as $\delta^{13}C$ and $\delta^{18}O$ values (Barnett and Patterson 2008). Other studies have also successful sourced adults to natal habitats by analyzing the core portion of adult otoliths using the various techniques (eg. Gillanders and Kingsford 1996; Thorrold et al. 1998; Patterson et al. 2004).

Information on the interregional population connectivity of gray snapper is particularly important for effective fisheries management given differences in fishing pressure and population demographics among regions. Gray snapper in south Florida are a heavily targeted recreational species and observed differences in size at age are correlated with exploitation rates (Allman and Goetz in review). Allman and Goetz (in review) also suggest that the nearshore recreational fishery in south Florida is more susceptible to overfishing than the offshore commercial fisheries, indicating further reason for regional management of fisheries and their respective habitats. Knowledge of

population connectivity between inshore and offshore would provide the information necessary to make population-specific management decisions.

Population genetics analysis indicates there are three genetically distinct populations of gray snapper in U.S. waters: east Florida, north central and northeastern Gulf, and northwestern Gulf (Gold et al. in review). However, there was no difference detected between fish sampled off south Florida, in the Big Bend, and in the Panhandle. Differences in fishing pressure are evident along the eastern Gulf and the Atlantic coast of Florida, with lower average size-at-age occurs in south Florida and differences in fishing pressure between recreational and commercial fisheries (Burton 2001; Allman and Goetz in review). The degree of mixing in adult populations is unknown but latitudinal variations in growth are indicative of geographically isolated sub-populations of gray snapper that are likely supported by recruitment from specific nurseries (Burton 2001; Allman and Grimes 2002). Where genetics fall short in discerning population mixing on ecological time scales, natural tags based on otolith chemistry can be applied to estimate possible nursery sources of adult recruits and examine interregional mixing.

A thorough evaluation of the nursery function of juvenile habitat should include a multi-level approach to examine spatial patterns of abundances and densities, growth and mortality, and at the most robust level, production rates. Applying this approach I was able to assess nursery habitat for juvenile gray snapper on a broad geographic scale, clearly establishing the nursery value of seagrass beds based on abundances and densities. In addition to these estimates, the comparison of growth provides another layer of region-specific population dynamics within nursery habitats. Further studies may explore parameter estimates farther by calculating juvenile gray snapper production

within systems or regions as well. Finally, I was able to derive natural tags from otolith chemical signatures which were highly successful for classifying region-specific recruits. This tool can be used in future work in order to address the critical recruitment question of connectivity from nursery to adult habitat, and subsequently offer a framework from which to incorporate a region-specific management approach to the gray snapper fishery in the eastern Gulf of Mexico.

REFERENCES

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

Aitchison, J. 1955. On the distribution of a positive random variable having a discrete probability mass at the origin. Journal of the American Statistical Association 50:901-908.

Allman, R. J., and L. A. Goetz. *In press*. Latitudinal variation in the population structure of gray snapper, *Lutjanus griseus*, along the west Florida shelf.

Allman, R. J., and C. B. Grimes. 2002. Temporal and spatial dynamics of spawning, settlement, and growth of gray snapper (*Lutjanus griseus*) from the West Florida shelf as determined from otolith microstructures. Fisheries Bulletin 100:391-403.

Ault, J. S., S. G. Smith, G. A. Meester, J. Luo, J. A. Bohnsack, and S. L. Miller. 2002. Baseline Multispecies Coral Reef Fish Stock Assessment for Dry Tortugas. NOAA Technical Memorandum NMFS-SEFSC-487 117 pp.

Bailey, K. M., and E. D. Houde. 1989. Predation on egg and larvae of marine fishes and the recruitment problem. Advanced Marine Biology 25:1-83.

Barnett, B. K., and W. F. Patterson III. 2008. Development of coring methods to extract nursery signatures from red snapper otoliths. Poster presentation: American Fisheries Society 138th Annual National Meeting, Ottawa, Canada.

Beamish, R. J., and D. A. Fournier. 1981. A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences. 38:982-983.

Beck, M. W., K. L. Heck Jr., K. W. Able, D. L. Childers, D. B. Eggleston, and B. M. Gillanders. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51(8):633-641.

Boesch, D. F., and Turner, R. E. 1984. Dependence of fishery species on salt marshes: the role of food and refuge. Estuaries 7:460-468.

Burton, M. L. 2001. Age, growth, and mortality of gray snapper, *Lutjanus griseus,* from the east coast of Florida. Fisheries Bulletin 99:254-265.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188:263-297.

Campana, S. E., and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014-1032.

Campana, S. E., and S. R. Thorrold. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations. Canadian Journal of Fisheries and Aquatic Science 58:30-38.

Campana, S. E., S. R. Thorrold, C. M. Jones, D. Gunther, M. Turbrett, H. Longerich, S. Jackson, N. M. Halden, J. M. Kalish, P. Piccoli, H. de Pontual, H. Troadec, J. Panfili, D. H. Secor, K. P. Severin, S. H. Sie, R. Thresher, W. J. Teesdale, and J. L. Campbell. 1997. Comparison of accuracy, precision and sensitivity in elemental assays of fish otoliths using the election microscope, proton-induced X-

ray emission, and laser ablation inductively coupled plasma mass spectrometry. Canadian Journal of Fisheries and Aquatic Science 54:2068-2079.

Carlson, P. R., L. A. Yarbro, and T. R. Barber. 1994. Relationship of sediment sulfide to mortality of *Thallasia tesudinum* in Florida Bay. Bulletin of Marine Science. 54:733-746.

Chittaro, P. M., P. Usseglio, and P. F. Sale. 2005. Variation in fish density, assemblage composition and relative rates of predation among mangrove, seagrass and coral reef habitats. Environmental Biology of Fishes 72:175-187.

Coleman, F. C., and S. L. Williams. 2002. Overexploiting marine ecosystem engineers: potential consequences for biodiversity. TRENDS in Ecology and Evolution. 17:40-44.

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

Dawes, C. J., R. C. Phillips, and G. Morrison. 2004. Seagrass communities of the Gulf Coast of Florida: status and ecology. Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute and the Tampa Bay Estuary Program. St. Petersburg, FL iv + 74 pp.

Denit, K., and S. Sponaugle. 2004. Growth, variation, settlement, and spawning of gray snapper across a latitudinal gradient. Transactions of the American Fisheries Society 133:1339-1355.

Domeier, M. L., C. Koenig, and F. Coleman. 1996. Reproductive biology of the gray snapper (*Lutjanus griseus*), with notes on spawning for other western Atlantic snappers (Lutjanidae). In Biology and culture of tropical snappers and groupers (F.Arreguin-Sanchez, J.L. Munro, M.C. Blagos, and D. Pauly, eds.) p.189-201 ICLARM Conference Proceedings 48.

Durako, M. D., and K. M. Kuss. 1994. Effects of *Labyrinthula* infection on the photosynthetic capacity of *Thallasia testudinum*. Bulletin of Marine Science. 54:727-732.

Edmonds, J. S., R. A. Steckis, M. J. Moran, N. Caputi, and M. Morita. 1999. Stock delineation of pink snapper and tailor from Western Australia by analysis of stable isotope and strontium/calcium ratios in otolith carbonate. Journal of Fish Biology 55:243-259.

Eggleston, D. B., C. P. Dahlgren, and E. G. Johnson. 2004. Fish density, diversity and size-structure within multiple back reef habitats of Key West National Wildlife Refuge. Bulletin of Marine Science 75:175-204.

Elsdon, T. S, and B. M. Gillanders. 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. Canadian Journal of Fisheries and Aquatic Sciences. 59: 1796-1808.

Elsdon , T. S., and B. M. Gillanders. 2003. Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. Reviews in Fish Biology and Fisheries 13:219-235.

Elsdon, T. S., and B. M. Gillanders. 2005. Alternative life history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. Canadian Journal of Fisheries and Aquatic Sciences 62:1143-1152.

Faunce, C. H., J. J. Lorenz, J. A. Ley, and J. E. Serafy. 2002. Size structure of gray snapper (*Lutajnus griseus*) within a mangrove 'no-take' sanctuary. Bulletin of Marine Science 70:211-216.

Faunce, C. H., and J. E. Serafy. 2007. Nearshore habitat use by gray snapper (*Lutjanus griseus*) and bluestriped grunt (*Haemulon sciurus*): environmental gradients and ontogentic shifts. Bulletin of Marine Science 80:473-495.

Faunce, C. H., and J. E. Serafy. 2008. Selective use of mangrove shorelines by snappers, grunts, and great barracuda. Marine Ecology Progress Series 356:153-162.

Fourqurean, J. W., and M. B. Robblee. 1999. Florida Bay: a history of recent ecological changes. Estuaries 22:345-357.

Fourqurean, J. W., L. M. Rutten, J. C. Durako, and T. A. Frankovich. 2003. Assessing change in seagrass ecosystems on a regional scale: the relative merits of fixed stations and systematic random sampling. Gulf of Mexico Science 21:133

Gillanders, B. M. 2005. Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. Estuarine, Coastal and Shelf Science 64: 47-57.

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

Gillanders, B. M., and M. J. Kingsford. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to coastal reef populations of a temperate reef fish. Marine Ecology Progress Series 141:13-20.

Gold, J. R., E. Saillant, N. D. Ebelt, and S. Lem. *In review*. Conservation genetics of gray snapper (*Lutjanus griseus*) in U.S. waters of the northern Gulf of Mexico and western Atlantic ocean. In press.

Hamer, P. A., G. P. Jenkins, and P. Coutin. 2006. Barium variation in *Pagrus auratus* (Sparidae) otoliths: A potential indicator of migration between an embayment and ocean waters in south-eastern Australia. Estuarine, Coastal and Shelf Science 68:686-702.

Hamer, P. A., G. P. Jenkins, and B. M. Gillanders. 2005. Chemical tags in otoliths indicate the importance of local and distant settlement areas to populations of a temperate sparid, *Pagrus auratus*. Canadian Journal of Fisheries and Aquatic Science 62:623-630.

Hanson, P. J., C. C. Koenig, and V. S. Zdanowicz. 2004. Elemental composition of otoliths used to trace estuarine habitats of juvenile gag *Mycteroperca microlepis* along the west coast of Florida. Marine Ecology Progress Series 267:253-265.

Harper, D. E., J. A. Bohnsack, and B. R. Lockwood. 2000. Recreational fisheries in Biscayne National, Florida, 1976-1991. Marine Fisheries Review. 26 p.

Heck Jr., K. L., G. Hays, and R. J. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Prgoress Series 253:123-136.

Hoese, H. D., and R. H. Moore. 1977. Fishes of the Gulf of Mexico: Texas, Louisiana, and adjacent waters, 2nd Edition. Texas A&M University Press, College Station 327 p.

Høie, H., A. Folkward, and E. Otterlei. 2003. Effect of somatic and otolith growth rate on stable isotope composition of early juvenile cod (*Gadus morhua* L.) otoliths. Journal of Experimental Marine Biology and Ecology 289:41-58.

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

Howarth, R., D. Anderson, J. Cloern, C. Eflring, C. Hopkinson, B. Lapointe, T. Malone, N. Marcus, K. McGlathery, A. Sharpley, and D. Walker. 2000. Nutrient pollution of coastal rivers, bays, and seas. Issues in Ecology 7 [np].

Ingram, G. W. (Jr), W. J. Richards, G. P. Scott, and S. C. Turner. 2007. Development of indices of bluefin tuna (*Thunnus thynnus*) spawning biomass in the Gulf of Mexico developed using delta-lognormal models. ICCAT Collective Volume of Scientific Papers 60:1057-1069.

Johnson, A. G., L. A. Collins, and C. P. Keim. 1994. Age-size structure of gray snapper from the southeastern United States: A comparison of two methods of back-calculating size at age from otolith data. Proceedings of the forty-eighth annual conference of the southeastern association of fish and wildlife agencies 592-600.

Kalish, J. M. 1991a. Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). Marine Biology. 110:37-47.

Kalish, J. M. 1991b. ^{13}C and ^{18}O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. Marine Ecology Progress Series 75:191-203.

Koenig, C. C., and F. C. Coleman. 1998. Absolute abundance and survival of juvenile gags in sea grass beds of the northeastern Gulf of Mexico. Transactions of American Fisheries Society 127:44-55.

Laegdsgaard, P., and C. R. Johnson. 1995. Mangrove habitats as nurseries: unique assemblages of juvenile fish in subtropical mangroves in eastern Australia. Marine Ecology Progress Series 126:67-81.

LaPointe, B. E., J. D. O'Connell, and G. S. Garrett. 1990. Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. Biogeochemistry 10:289-307.

Lara, M.R., D. L. Jones, Z. Chen, J. T. Lamkin, and C. M. Jones. 2008. Spatial variation of otolith elemental signatures among juvenile gray snapper (*Lutjanus griseus*) inhabiting southern Florida waters. Marine Biology 158:235-248.

Lindeman, K. C. 1997. Development of grunts and snappers of southeast Florida: cross-shelf distributions and effects of beach management alternatives. Doctoral dissertation, University of Miami, Coral Gables, Florida.

Livingston, R. J. 2007. Phytoplankton bloom effects on a *gulf estuary*: water quality changes and biological response. Ecological Applications 17:110-128.

Livingston, R. J., R. L. Iverson, R. H. Estabrook, V. E. Keys, and J. Taylor Jr. 1974. Major features of the Apalachicola Bay system: physiography, biota, and resource management. Q. J. Florida Academy Science 37:245-271.

Lo, N. C. H., L. D. Jacobson, J. L. Squire. 1992. Indices of relative abundance from fish spotter data based on delta-lognormal models. Canadian Journal of Fisheries and Aquatic Sciences. 49:1515-1526.

Mangel, M., P. Levin, and A. Patil. 2006. Using life history and persistence criteria to prioritize habitat for management and conservation. Ecological Applications 16:797-806.

Manooch, C.S. III, and R. H. Matheson III. 1981. Age, growth, and mortality of gray snapper collected from Florida waters. Proceedings of the thirty-fifth annual conference of the southeastern association of fish and wildlife agencies 35:331-344.

Martin, G. B., S. R. Thorrold, and C. M. Jones. 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xantharus*). Canadian Journal of Fisheries and Aquatic Sciences 61:34-42.

Martin, G. B., and S. R. Thorrold. 2005. Temperature and salinity effects on magnesium, manganese and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xantharus*. Marine Ecology Progress Series 293:223-232.

Martin, G. B., and M. J. Wuenschel. 2006. Effect of temperature and salinity on otolith element incorporation in juvenile gray snapper *Lutjanus griseus*. Marine Ecology Progress Series 324:229-239.

Mugiya, Y.N. Watabe, J. Yamada, J. M. Dean, D. G. Dunkelberger, and M. Schimuzu. 1981. Diurnal rhythm in otolith formation in the goldfish, *Carassius auratus*. Comparative Biochemistry and Physiology 68A:659-662.

Nagelkerken, I., G. van der Velde, M. W. Gorissen, G. J. Meijer, T. van't Hof, and C. den Hartog. 2000. Importance of mangroves, seagrass beds and the shallow coral reef as a nursery for important coral reef fishes, using a visual census technique. Estaurine, Coastal and Shelf Science 51:31-44.

Nagelkerken, I., C. M. Roberts, G. van der Velde, M. Dorenbosch, M.C. van Riel, E. Cocheret de la Moriniere, and P.H. Nienhuis. 2002. How important are mangroves and seagrass beds for coral-reef fish? The nursery hypothesis tested on an island scale. Marine Ecology Progress Series 244:299-305.

National Marine Fisheries Service (NMFS). 1997. Interim final rules on essential fish habitat. 50 CFR Part 600. Federal Register 62 (December 19, 1997): 66531-66559.

Nixon, S. W. 1980. Between coastal marshes and coastal waters: a review of twenty years of speculation and research on the role of salt marshes in estuarine productivity and water chemistry. Pares 437-525 *in* Hamilton P, and K.B. MacDonald, editors. Estuarine and wetland processes. Plenum Press, New York.

NOAA. 2004. Essential Fish Habitat Guidance. NOAA-NMFS Office of Habitat Conservation. Silver Spring, MD. 80 p.

Panella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173:1124-27.

Patterson, H. M., R. S. McBride, and N. Julien. 2004. Population structure of red drum (*Sciaenops ocellatus*) as determined by otolith chemistry. Marine Biology 144:855-862.

Patterson, H. M., R. G. Taylor, and R. S. McBride. 2005. Coastal origin of common snook, *Centropomus undecimalis*, in FL Bay. Gulf and Caribbean Research 17:25-30.

Patterson III, W. F., J. H. Cowan Jr., E. Graham, and W. B. Lyons. 1998. Otolith microchemical fingerprints of age-0 red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico. Gulf of Marine Science 16:83-91.

Patterson III, W. F., J. H. Cowan Jr., C. A. Wilson, and Z. Chen. 2008. Temporal and spatial variability in juvenile red snapper otolith elemental signatures in the northern Gulf of Mexico. Transactions of the American Fisheries Society 137:521-532.

Payan, P., H. Kossman, A. Watrin, N. Mayer-Gostan, and G. Boeuf. 1997. Ionic compotition of endolymph in teleosts: origin and importance of endolymph alkalinity. Journal of Experimental Biology 200:1905-1912.

Pennington, M. 1983. Efficient estimators of abundance, for fish and plankton surveys. Biometrics 39: 281-286.

Peterson, B. J., and B. Fry. 1987. Stable istopes in ecosystem studies. Annual Review of Ecological Systems 18:293-320.

Peterson, M. S. 2003. A conceptual view of environment-habitat-production linkages in tidal river estuaries. Reviews in Fisheries Science 11:291-313.

Rabalais, N., R. E. Tunrer, and D. Scavia. 2002. Beyond science into policy: Gulf of Mexico hypoxia and the Mississippi River. Bioscience 52:129-142.

Rypel, A. L., and C. A. Layman. 2008. Degree of aquatic ecosystem fragmentation predicts population characteristics of gray snapper (*Lutjanus griseus*) in Caribbean tidal creeks. Canadian Journal of Fisheries and Aquatic Sciences 65:335-339.

SAS Institute, Inc. 2004. Statistics Version 11.3. SAS Institute Inc., Box 8000, Cary, North Carolina 27511-8000.

Searcy, S. P., D. B. Eggleston, and J. A. Hare. 2007. Is growth a reliable indicator of habitat quality and essential fish habitat for a juvenile estuarine fish? Canadian Journal of Fisheries and Aquatic Sciences 64:681-691.

Secor, D. H., and J. R. Rooker. 2005. Connectivity in the life history of fishes that use estuaries. Editorial / Estuarine, Coastal and Shelf Science 64:1-3

Simkiss, K. 1974. Calcium metabolism in relation to ageing. In T. Bagenal (Ed.), *Ageing of Fish* (pp. 1-2). London: Unwin Bros.

Starck, W. A., II, and R. E. Schroeder (eds.). 1971. Investigations on the gray snapper, *Lutjanus griseus*. Stud. Trop. Oceanogr. No. 10, Univ. Miami Press, Coral Gables, FL, 224 p.

Sustainable Fisheries Act, Public Law Number 104-297. 110 Statute, 3559 (1996).

Thorrold, S. R., S. E. Campana, C. M. Jones, and P. K. Stewart. 1997. Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. Geochemica and Cosmochimica Acta 61: 2909-2919.

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

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

Tzeng, M. W., J. A. Hare, and D. G. Lidquist. 2003. Ingress of transformation stage gray snapper through Beaufort Inlet, N.C. Bulletin of Marine Science 72:891-908.

Walsh, J. J., J. K. Jolliff, B. P. Darrow, J. M. Lenes, S. P. Milroy, A. Remsen, D. A. Dieterle, K. L. Carder, F. R. Chen, G. A. Vargo, R. H. Weisberg, K. A. Fanning, F. E. Muller-Karger, E. Shinn, K. A. Steidinger, C. A. Heil, C. R. Tomas, J. S. Prosperso, T. N. Lee, G. J. Kirkpatrick, T. E. Whiteledge, D. A. Stockwell, T. A. Villareal, A. E. Jochens, and P. S. Botmpi. 2002. Red Tides in the Gulf of Mexico: where, when, and why? Journal of Geophysical Research. C. Oceans 11pp.

Weinstein, W. P., and K. L. Heck Jr. 1979. Ichthyofauna of seagrass meadows along the Caribbean coast of Panama and in the Gulf of Mexico: composition, structure and community ecology. Marine Biology 50:97-107.

Whaley, S. D., J. J. Burd Jr., and B. A. Robertson. 2007. Using estuarine landscape structure to model distribution patterns in nekton communities and in juveniles of fishery species. Marine Ecology Progress Series 330:83-99.

Wuenschel, M. J., A. R. Jugovich, and J. A. Hare. 2004. Effect of temperatute and salinity on the energetics of juvenile gray snapper (*Lutjanus griseus*): implications for nursery habitat value. Journal of Experimental Biology and Ecology 312:333-347.

Zimmerman, M. S., and R. J. Livingston. 1976. Seasonality and physiochemical ranges of benthic macrophytes from a North Florida estuary (Apalachee Bay, Florida). Contributions in Marine Science 20:33-45.

APPENDIX

Appendix A

Animal Care and Use Committee Approval

University of West Florida

Research and Sponsored Programs
11000 University Parkway
Pensacola, FL 32514-5750

FILE COPY

MEMORANDUM

November 16, 2007

TO: Dr. William Patterson, III
Biology Department
Building 58, Room 006

FROM: Animal Care and Use Committee
Dr. Richard S. Podemski, Associate Vice President for Research
and Dean of Graduate Studies

SUBJECT: ACUC Approval

The Animal Care and Use Committee has completed its review of your proposal titled 'Population Dynamics and Connectivity of Gray Snapper in West Florida Estuaries' and has granted provisional approval for you to proceed with your study. The committee is doing further research to establish the appropriateness of the method of euthanasia in your project. Also, the category of your project will be changed from "C" to "D." Please note the following:

- Prior ACUC approval is required for significant changes to your protocol.
- The maximum approval period is three years. Should your project continue beyond the three year period, you must request ACUC approval prior to the end of the approval period, you must request ACUC approval prior to the end of the approval period.
- Annual status reports may be required by the ACUC. These reports must include a complete description of any and all changes in your project.

Please take time to review our ACUC web page at: http://research.uwf.edu/boards-committees/acuc.htm which includes the ACUC Policies and Procedures, the PHS Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and other pertinent ACUC documents.

Good luck in your research endeavors.

CC: Ms. Cecelia Lounder
Dr. George Stewart, Chair

Phone **850.474.2824** Fax **850.474.2082**
Web **research.uwf.edu**