

LIFE HISTORY, FORAGING ECOLOGY, METABOLIC RATES AND
BIOENERGETICS OF THE BROWN STINGRAY, *DASYATIS LATA*

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We certify that we have read this dissertation and that, in our opinion, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Zoology.

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ABSTRACT

Life history characteristics, diet, nursery habitat use and metabolic rates of brown stingrays, *Dasyatis lata*, were evaluated to determine their ecological impacts in Kāneʻohe Bay, Oahu, through a bioenergetics model. The maximum age based on analysis of vertebral samples (28 years) was among the oldest for any dasyatid aged to date. Edge and marginal increment analyses, as well as recapture of two stingrays previously marked with oxytetracycline, verified deposition of a single growth band per year. Growth characteristics varied between sexes, with females attaining larger sizes and exhibiting lower growth coefficients. Brown stingray life history characteristics are similar to other elasmobranchs in that they are long lived, grow slowly and mature at a late age.

Stomach content, bulk and amino acid stable isotope analyses were used to assess the diet and habitat use of juvenile brown stingrays in Kāneʻohe Bay. A shift in stingray diet and an increase in relative trophic position were apparent with increasing size. Results indicate stingrays foraged within their Kāneʻohe Bay nursery for the majority of their juvenile lives before shifting to offshore habitats with the onset of sexual maturity. Underestimates of trophic position from amino acid analyses suggest that urea retention in elasmobranchs may have important implications for estimates of absolute trophic position determined using this method. Potential prey resources were partitioned between stingrays and sympatric juvenile scalloped hammerhead sharks, *Sphyrna lewini*.

Standard metabolic rates were estimated for juvenile brown stingrays through respirometry. Temperature and mass had significant effects on metabolic rates. The energy budget of juvenile stingrays was heavily weighted towards metabolism, which

accounted for 68% of total consumed energy. A large metabolic demand coupled with very slow growth indicated potential limitation of food resources. Daily rations estimated from the bioenergetics model declined from a high of 2.72 %BW/d to 1.23 %BW/d with age. Results suggest the potential for strong top-down effects on prey populations due to stingray predation. Use of Kāneʻohe Bay as a nursery habitat for brown stingrays appears to be a trade-off between increased juvenile survival and a late age at first maturity due to slow growth rates.

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CHAPTER I

GENERAL INTRODUCTION

Anthropogenic impacts and nursery habitat use

Anthropogenic factors such as overfishing and habitat alteration (including pollution) have contributed substantially to declines of a number of elasmobranch populations globally (Cortés et al. 2002, Robbins et al. 2006, Ferretti et al. 2008, Jennings et al. 2008, Pierce and Bennett 2010). The most significant contributor to population declines has been overfishing through both targeted and non-targeted fisheries (Clarke et al. 2006, Zeeberg et al. 2006). Many elasmobranchs are particularly susceptible to overfishing due to life history characteristics such as slow growth, late age of sexual maturity and low fecundity (Branstetter 1990, Hoenig and Gruber 1990, Pratt and Casey 1990). Population declines may be underestimated due to severe under-reporting of the global catch of elasmobranchs (Bonfil 1994, Barker and Schluessel 2005, Clarke et al. 2006). Further, there is a general lack of biological data on the majority of elasmobranch species (Fowler et al. 2005). The combination of low productivity, under-reporting and lack of data has hampered the ability of regulatory agencies to implement sound management policies. Relative to sharks (particularly carcharhinids), there is a distinct lack of life history data for batoids (White and Dharmadi 2007) despite representing > 50% of all elasmobranch species.

The benthic nature of many batoid species (Chondrichthyes: Batoidea) makes them especially vulnerable to demersal fishing gear (e.g. bottom trawls). Multiple skate species have disappeared or have become restricted to deep water fishing refuges in the

North Atlantic in response to bottom trawl fisheries (Brander 1981, Casey and Myers 1998, Dulvy et al. 2000, Kulka et al. 2002). In the Indo-Pacific, increasing demand from luxury leather markets, which process stingray skin into leather for products such as wallets, pens and boots, fuel directed fisheries for some species in the Indo-Pacific (Pauly et al. 2005).

Habitat alteration occurs in direct proportion to the proximity of land, with the greatest impacts on freshwater and estuarine environments and this poses a significant threat to some elasmobranch species that use nearshore habitats (Musick et al. 2000). There are two general models concerning the use of nearshore environments by elasmobranchs, primarily differentiated by life history characteristics. One model encompasses species which reach a relatively small maximum size, have rapid growth, mature at an early age and are relatively short lived (e.g. blacknose sharks, *Carcharhinus acronotus*; Knip et al. 2010). Use of nearshore areas may occur for the duration of their life span, with multiple life history stages using the same area. These species are generally wider ranging which may decrease their vulnerability to localized impacts (Knip et al. 2010). The second model is typical of species which reach a large maximum size, have slow growth rates and long life-expectancies (e.g. sandbar sharks, *C. plumbeus*; Springer 1967). In this model, young are born in nearshore areas where they remain until sexual maturity before joining adult populations offshore. In this case, nearshore environments may serve as nursery habitats (Heupel et al. 2007), the benefits of which may include increased access to prey resources and refuge from predation (Simpfendorfer and Milward 1993, Morrissey and Gruber 1993). However, the importance of these nursery habitats in terms of whether they function to promote early

growth or decrease predation appears to be species specific and can vary between populations of the same species. For example, growth rates were slower for juvenile lemon sharks, *Negaprion brevirostris*, in a Bahamas nursery compared to a nursery in Brazil (Freitas et al. 2006). The Brazil nursery lacks protective cover such as mangrove habitat which would provide protection from predators and faster growth rates of individuals in this population may be an adaptation to reach a size less vulnerable to predation more quickly (Freitas et al. 2006). Generally, slow growing species are either born at relatively large sizes or use protected nursery habitats, whereas faster growing species rely on growth rates to minimize predation from larger sharks (Branstetter 1990, Carlson 2002). Regardless of the specific drivers of nearshore habitat use, habitat alteration can have significant impacts on these species. For example, reduction of mangrove habitat due to increased coastal development was correlated with a 23.5% decrease in juvenile survival of lemon sharks (Jennings et al. 2008). Because overall population growth is often linked to juvenile survival (Heppel et al. 1999, Cortés 2002, Pierce and Bennett 2010), identification and conservation of nursery habitats can buffer against the negative population level effects of anthropogenic impacts.

Community effects of elasmobranch predation

Marine ecosystems are dynamic environments, influenced by numerous biotic and abiotic variables and the interactions between them. As a result, there is a burgeoning interest in ecosystem based management and a move away from single species management plans (Link 2002). This new approach requires a thorough understanding of ecosystem processes including species composition, life history characteristics,

abundance, distribution, and especially trophic relationships among constituent organisms. Apex predators such as elasmobranchs are thought to have an important role in marine ecosystems due to a disproportionate influence on ecosystem structure relative to species in lower trophic positions (Heithaus et al. 2008). Although few studies have estimated the ecosystem impacts of elasmobranchs, contradictory results from ecosystem models which include sharks suggest their impacts are complex and unpredictable (e.g. Stevens et al. 2000, Kitchell et al. 2002, Bascompte et al. 2005). For example, community response to reef-shark removal was primarily dependent on the trophic complexity and incidence of omnivory in a Caribbean reef system (Bascompte et al. 2005). Therefore, the impacts of elasmobranch population declines on ecosystem structure will be species and system specific. Only after a significantly larger range of species and systems have been studied can potential patterns and generalizations be developed.

Bioenergetics

The ecological impacts of a population are related, either directly or indirectly, to individual consumption rates and population size (Libralato et al. 2006, Heithaus et al. 2008). Consumption rates can be estimated through field based methods, which require information on the amount of food in the stomach of captured animals, as well as gastric evacuation dynamics of consumed prey (Wetherbee and Cortés 2004). Consumption rates can also be estimated through bioenergetics models which require information on energy used in growth and metabolism and energy lost through wastes (Brett and Groves 1979). Although gastric evacuation methods have been most frequently used to estimate

consumption rates in elasmobranchs (Wetherbee and Cortés 2004), bioenergetics models also provide information on an animal's energy budget and the partitioning of energy devoted to growth and metabolism relative to total consumption. This additional metric enables bioenergetics models to evaluate the energetic strategy of a population as well as estimate the effects of predator consumption on prey populations (e.g. Drazen 2002). There are relatively few studies which have developed energy budgets for elasmobranchs. The most detailed description of an elasmobranch energy budget was developed for juvenile lemon sharks, with each individual component independently estimated (Gruber 1984).

Dasyatis lata (Myliobatiformes)

Stingrays are composed of nine families with over 185 species. They are found circumglobally in all tropical and temperate seas, but are most prevalent in subtropical to tropical waters. Many are euryhaline, capable of living in waters with a wide range of salinities. Because of this, they are commonly found in estuaries and bays during some stage of their life history. As benthic predators, stingrays occupy an ecologically important niche in bays and estuaries (Smith and Merriner 1985). They can have a direct impact on ecosystem dynamics through consumption of organisms or an indirect impact through competitive feeding behavior and habitat modification (Thrush et al. 1991).

Dasyatid stingrays represent the most speciose family of stingrays with > 60 species and are the dominant batoid family on continental shelves of tropical and subtropical regions. As a result, this group of cartilaginous fishes is highly exploited by targeted and non-targeted fisheries, particularly in Indo-Pacific regions (Pauly et al. 2005,

White and Dharmadi 2007). Large scale removal of dasyatid stingrays could potentially lead to dramatic and long-lasting impacts in ecosystem structure (Stevens et al. 2000, Bascompte et al. 2005) due to their generally high trophic position (e.g. Stergiou and Karpouzi 2002).

The brown stingray, *Dasyatis lata*, is a large benthic stingray and the only elasmobranch endemic to Hawai‘i. Juvenile stingrays are abundant in near-shore waters and adults have been reported to depths up to 232 m (Cartamil et al. 2003, Randall 2007). Although *D. lata* is the only benthic stingray commonly found in Hawai‘i’s coastal ecosystems, little is known about its general biology, behavior, feeding ecology, or ecosystem impacts.

Study objectives

The overarching goal of this study was to evaluate the ecological impacts of juvenile brown stingrays in Kāne‘ohe Bay, the largest embayment in the Hawaiian Islands (46 km², Smith et al. 1981), through the use of a bioenergetics model. The bay supports a large population of juvenile brown stingrays and large adults have been captured in deeper waters immediately outside the bay’s barrier reef (Cartamil et al. 2003, D. Grubbs unpublished data). The bay is also a nursery ground for the scalloped hammerhead shark, *Sphyrna lewini*, and previous studies have indicated a high degree of overlap between habitat utilized by *D. lata* and juvenile *S. lewini* (Holland et al. 1993, Cartamil et al. 2003, Lowe 2002). Therefore, Kaneohe Bay also offers an opportunity to study the ecological interactions between two sympatric elasmobranch species. This is especially interesting since previous studies suggest that juvenile *S. lewini* may be

starving due to limited prey availability and/or slow development of foraging skills (Bush and Holland 2002, Lowe 2002, Duncan and Holland 2006). Due to the general lack of biological and ecological data concerning brown stingrays, input parameters for the bioenergetics model (i.e. age composition and growth rates, feeding habits and habitat use, metabolic rates) were directly estimated as part of this study.

Vertebral analysis was used to estimate the age and growth characteristics of brown stingrays in and immediately adjacent to Kāneʻohe Bay. Age estimates were based on sagittally thin-sectioned vertebral centra and the timing of band deposition was verified using edge and marginal increment analysis as well as recaptures of stingrays marked with oxytetracycline. Multiple growth models were fitted to observed size-at-age data and evaluated based on statistical fit and biological relevance. Maturity status was estimated from macroscopic examination of reproductive organs providing an estimate of the median size and age at first maturity.

Nursery habitat use and the food habits of juvenile brown stingrays in Kāneʻohe Bay were evaluated through stomach content, bulk and amino acid stable isotope analyses. The relative importance of individual prey items to their diet and the isotopic signatures of the stingrays were used to test for ontogenetic shifts in diet and habitat use. The use of three independent methods to quantify their food habits allowed for cross-validation between results. Amino acid stable isotope analysis was also used to evaluate competing hypotheses generated from bulk stable isotope methods and provided significant insight into the relative contribution of Kāneʻohe Bay to the early life history of brown stingrays. Raw data reanalyzed from (Bush 2003) on the food habits of juvenile scalloped hammerhead sharks in Kāneʻohe Bay (and associated trophic position

estimates), were compared to those of juvenile stingrays to test for dietary overlap. A mechanism is proposed which may explain why trophic position estimates based on individual amino acids bias estimates of absolute trophic position for some elasmobranchs.

Respirometry was used to estimate the standard metabolic rates of juvenile brown stingrays. Experimental trials were run in a 650 l closed-system recirculating respirometer on seasonally acclimated stingrays. A linear model was developed to estimate the combined effects of mass and temperature on standard metabolic rate.

Finally, estimates of age, growth, diet and metabolism obtained during this study were used as input parameters in a bioenergetics model to develop an energy budget for juvenile brown stingrays in Kāneʻohe Bay. Consumption rates were estimated for an average individual of each age-class and a range of population size estimates were used to evaluate the ecosystem level impacts of stingray foraging. Population consumption rates were combined with those of juvenile hammerheads to estimate the impacts of a multi-species complex on a common prey resource. Relative partitioning of the energy budget provided insight into the potential life history trade-offs concerning the use of Kāneʻohe Bay as a nursery habitat.

CHAPTER II

AGE, GROWTH AND MATURITY

ABSTRACT

Age, growth and maturity estimates were determined for the brown stingray, *Dasyatis lata*, from Oahu, Hawai'i. Age estimates based on vertebral analysis were obtained for 202 stingrays and ranged from 0 to 28 years for females (n = 114) and 0 to 25 years for males (n = 88). Annual growth band deposition was verified through marginal increment and centrum edge analysis, as well as recapture of two stingrays marked with oxytetracycline. Logistic, Gompertz and von Bertalanffy growth models were fitted to both disk width (DW) and weight-at-age data and evaluated for biological realism and statistical fit (Akaike's Information Criterion). Growth characteristics varied between sexes, with females attaining larger sizes and exhibiting lower growth coefficients (k), and males reaching maximum growth rates at a smaller size and younger age than females. Out of the set of candidate models, the logistic growth functions based on DW and weight-at-age data best described the growth of male and female brown stingrays, providing the best statistical fits and most realistic biological parameters, whereas essentially no support was found for the von Bertalanffy growth models. Median size and age at first maturity was estimated to be 104.9 cm DW (15 years) for females and 74.9 cm DW (8.3 years) for males. Brown stingray life history characteristics are similar to other elasmobranchs in that they are long lived, grow slowly and mature at a late age.

INTRODUCTION

The majority of age and growth studies concerning elasmobranchs have been initiated in response to exploitation in commercial or artisanal fisheries where they are either directly targeted or captured as bycatch (Musick 2004). Size-specific age information forms the basis for estimates of growth, mortality and productivity rates, age at first maturity and longevity. These parameters are integral to estimating a population's status and assessing risks associated with exploitation (Cortés 1998). Many elasmobranch species exhibit life-history characteristics such as slow growth, late age at first maturity and low fecundity which make them particularly susceptible to overexploitation (Stevens et al. 2000). However, implementation of management plans at the inception of elasmobranch fisheries is often hindered by a lack of species-specific life-history data, leading to rapid stock declines and extensive recovery times (Anderson 1990, Hoff and Musick 1990, Musick 2004). This trend is especially problematic as elasmobranchs are increasingly targeted due to depletions of more commercially valuable teleost species (e.g. Gburski et al. 2007). Increased priority on obtaining baseline data on the life-history characteristics of elasmobranchs prior to fisheries development is required to facilitate the timely implementation of management plans.

Many elasmobranchs are considered top level predators in the ecosystems in which they occur (Cortés 1999, Ebert and Bizzaro 2007), yet their trophic role in these ecosystems is poorly understood. Large declines in elasmobranch populations due to fisheries exploitation could lead to trophic cascades and restructuring of community assemblage in impacted ecosystems (Stevens et al. 2000, Bascompte et al. 2005, Myers et al. 2007). Although age and growth studies are commonly aimed at informing fisheries

management, life-history parameters are also vital inputs for ecosystem models such as EcoPath and bioenergetics models (Gamito 1998). These models can be used to understand a species role in the flow of energy in an ecosystem, evaluate changes in ecosystem composition and assess effects of habitat loss or degradation (e.g. Lowe 2002, Dowd et al. 2006a, Neer et al. 2007). The life-history characteristics which make many elasmobranch species vulnerable to overexploitation also limit their ability to withstand rapid habitat changes caused by human activity (Fowler et al. 2005). Coastal bays and estuaries are commonly used by elasmobranchs and these habitats have historically undergone significant degradation due to their close proximity to land (Lotze et al. 2006). However, little is known about how habitat degradation affects elasmobranch populations or marine food webs.

Despite the prevalence of dasyatid stingrays in tropical and subtropical nearshore estuaries where risk of exploitation and habitat degradation is high due to limited management efforts (Smith et al. 2007, White and Dharmadi 2007, White and Sommerville 2010), few studies have investigated their life-history traits. The brown stingray, *Dasyatis lata*, is a large (up to 133 cm Disk Width, 66 kg) benthic predator endemic to Hawai'i and there are currently no commercial or recreational fisheries for this species. Juveniles are abundant in shallow bays and estuaries which serve as nursery habitats, whereas adults are often found in deep offshore waters at depths of up to 232 m (Cartamil et al. 2003, Randall 2007, Chapter III). Despite their abundance in nearshore estuaries and potential for strong impacts on the trophic dynamics of these regions (VanBlaricom 1982, Thrush et al. 1994, Peterson et al. 2001), little is known about their general biology. Struhsaker (1973) postulated size at first maturity at 95 cm and 110 cm

disk width (DW) for males and females respectively based on 14 individuals. Based on the observation of four gravid females, litter size was estimated at 3 to 4 young. The timing of mating and parturition is unknown, but parturition is thought to occur in summer due to the presence of gravid females in the winter months. However, these estimates are based on small sample sizes and require further investigation. Thus, the goals of this study were to estimate growth rates, age and size at first maturity, and longevity for brown stingrays in Hawai‘i to provide baseline data on their life-history characteristics in the absence of fishing impacts. Multiple growth functions were fitted to DW and weight-at-age data based on vertebral analysis.

METHODS

Sample collection and preparation

Brown stingrays were collected using demersal longlines in and outside of Kāne‘ohe Bay, Oahu, Hawai‘i (Fig. 2.1) between August 2006 and December 2010. Inshore longlines were set at least 5 m away from patch reefs to avoid entanglement on coral (average depth 13 m). Offshore longlines were set at approximately 80 m based on catch records from a shark survey conducted in this area (D. Grubbs, unpublished data). All longlines were baited with tuna, *Thunnus spp.*, and/or squid, *Loligo spp.* Gangions consisted of a stainless-steel snap-clip attached to 3 m of monofilament followed by a 1 m stainless-steel leader attached to a circle hook. To ensure all sizes of stingray were captured, two sizes of gangions were used. Smaller gangions consisted of 136 kg monofilament, 1.6 mm stainless-steel leader and 11/0 circle hooks baited with squid or tuna. Larger gangions consisted of 250 kg monofilament, 1.6 mm stainless-steel leader

and 14/0 circle hooks baited with tuna. Hooks were allowed to soak for 3 hours before being retrieved. Captured stingrays were landed, DW, outer and inner clasper length measured to the nearest 1 mm, weighed to the nearest 0.1 kg and euthanized if needed for samples. Vertebral samples were removed from the region posterior to the cranium above the abdominal cavity and frozen until processing. Stingrays not needed for vertebral analysis were injected with oxytetracycline (OTC, 25 mg kg⁻¹ body weight), tagged with external ID tags and released for age-validation purposes.

Vertebral samples were thawed, cleaned and separated into individual centra. Individual centra were sagittally sectioned through the focus of the centrum using a Buehler Isomet rotary diamond saw. Sections were mounted on a microscope slide and polished with wet fine grade sand paper until rings were distinguishable using a dissection microscope with transmitted light. Vertebrae of recaptured stingrays were examined under ultraviolet light for OTC marks. The relationship between DW and centrum radius (CR) was estimated to assess the appropriateness of using vertebrae as an ageing structure. There were no significant differences in the relationship between DW and CR between sexes (ANCOVA: $F_{1,201} = 0.19$, $P = 0.667$), therefore log-transformed data from all samples were combined to generate the following relationship: $\log DW = 1.36 + 0.835 \cdot \log CR$ ($P < 0.001$, $r^2 = 0.95$, $n = 203$).

Size at First Maturity

Reproductive status was determined using macroscopic methods following Neer and Thompson (2005) and Smith et al. (2007). Maturity criteria for males included: (1) calcified claspers, (2) highly coiled vas deferens and (3) enlarged and lobed testes.

Maturity criteria for females included: (1) vitellogenic ova > 10 mm diameter present in the left ovary (right ovaries and uteri are typically non-functional in Dasyatidae) and (2) enlarged oviducal glands and uteri well developed with trophonemata present. Median DW at first maturity (DW_{50}) was estimated by fitting a logistic model $Y = [1 + e^{-(a + bX)}]^{-1}$ to binomial maturity data (0 = immature, 1 = mature) (Mollet et al. 2000) for males and females separately, where Y = maturity status and X = DW in centimeters. DW_{50} was then calculated from the logistic equation as $-a/b$ (Mollet et al. 2000). Median age at first maturity (A_{50}) was calculated by substituting age in years for DW in the above equation (e.g. Harry et al. 2010).

Age Estimation

Alternating opaque and translucent bands were evident in all samples (Fig. 2.2). The birth mark was determined from an angle change in the intermedialia distal to the focus. For age determination, the narrow bands were counted and all centra were counted twice without knowledge of size or sex by the senior author. If band counts between the first two readings did not agree, a third reading was conducted. If the third count did not agree with one of the first two counts, the sample was removed from analysis. Precision between age determinations were evaluated using the index of average percent error (APE, Beamish and Fournier 1981), coefficient of variation (CV) (Chang 1982) and percent agreement between counts $\pm i$ years (Cailliet and Goldman 2004). Percent agreement was summed overall and calculated for 5 cm DW size classes to assess sources of count variation (Cailliet and Goldman 2004).

Annual periodicity of band deposition was verified using edge and marginal increment analysis (Conrath et al. 2002, Cailliet et al. 2006, Smith et al. 2007). Edge analysis was conducted following Smith et al. (2007). Each centrum was assigned to one of four qualitative categories based on extent of band deposition on the centrum edge: translucent narrow (TN), translucent broad (TB), opaque narrow (ON), opaque broad (OB). Mean monthly marginal increment ratios (MIR) were used as a quantitative assessment of band deposition (Conrath et al. 2002): $MIR = MW/PBW$, where MW is the margin width or width of the outer most forming band and PBW is the width of the penultimate band pair. Young of the year rays were excluded from analysis as they have no fully formed increments. The frequency of centrum edge types and MIRs were then plotted against month of capture to determine periodicity of band formation. Significant differences in MIRs between months were tested using a one-way analysis of variance (ANOVA) and Tukeys pairwise comparisons. All centrum measurements were conducted using Image Tools Version 3 Software Package (Department of Dental Diagnostics Science, University of Texas Health Center, Austin, Texas)

Growth Models

Four growth models were fitted to the observed size-at-age data separately for male and female stingrays using the Gauss-Newton algorithm for non-linear regression in R (R Development Core Team 2009). A three-parameter von Bertalanffy growth function (VB3, von Bertalanffy 1938) was calculated using the equation:

$$DW_t = DW_\infty(1 - e^{-k(t-t_0)}),$$

where DW_t is the mean disk width at age t , DW_∞ is the theoretical average asymptotic disk width, k is the growth coefficient, t is the estimated age and t_0 is the theoretical age at zero DW. A modified version of the VB3 using the size-at-birth intercept rather than the theoretical age at zero DW (VB2, Fabens 1965) was calculated using the equation:

$$DW_t = DW_\infty - (DW_\infty - DW_0)e^{-kt},$$

where DW_0 is the observed size at birth and the remaining parameters are as previously defined. Mean size at birth was estimated from regressions of centrum radius against DW (Smith et al. 2007). A weight based form of the VB3 was calculated using the equation (Fabens 1965, Ricker 1979):

$$w_t = W_\infty(1 - e^{-k(t-t_0)})^3,$$

where w_t is the mean weight at age t , W_∞ is the theoretical average asymptotic weight and the other parameters are as described above. A Gompertz growth model (Ricker 1975) was calculated using the equation:

$$DW_t = DW_\infty e^{-e^{-k(t-t_0)}},$$

where t_0 is the inflection point of the curve and the remaining parameters are as previously defined. Finally, a logistic growth model (Ricker 1975) was also fitted using the equation:

$$DW_t = DW_\infty / (1 + e^{-k(t-t_0)}),$$

where t_0 is the inflection point of the curve and the remaining parameters are as previously defined. For the Gompertz and logistic functions, weight-at-age data was substituted for size-at-age data to obtain weight-based models (Ricker 1979). Analysis of residual sums of squares (ARSS) was used to determine whether growth models differed between sexes (Chen et al. 1992). Theoretical longevity was estimated as the age at

which 95% of DW_{∞} is reached for each growth function following the methods of Taylor (1958) (e.g. Natanson et al. 2006).

Models were assessed based on biological reality and statistical fit (Cailliet et al. 2006). Goodness-of-fit was assessed using bias-corrected Akaike's Information criteria (AIC_c) (Burnham and Anderson 2002). The model with the lowest AIC_c value was considered the best model. Differences between candidate models and the model with the smallest AIC_c value (Δ_i) were used to rank remaining models relative to the best model (Burnham and Anderson 2002). Akaike weights (Aw_i) were calculated as the weight of evidence in favor of a model being the best model in the set of candidate models (Burnham and Anderson 2002).

RESULTS

Sample Collection

A total of 544 rays were captured during the course of this study. Vertebral samples were collected from 203 rays (88 males, 115 females) and the remainder were measured, tagged, injected with OTC and released. Sizes of rays from which vertebral samples were collected ranged from 35.0 to 111.0 cm DW and 35.0 to 133.0 cm DW for males and females respectively (Fig. 2.3). Rays were collected from all calendar months, although not in all months every year. The DW – weight relationship for all rays combined was best described by the power function: $\text{weight (kg)} = 1.82 \times 10^{-5} \text{ DW (cm)}^{3.0978}$ ($r^2 = 0.99$, $n = 203$).

Age estimation

Agreement of age estimates was reached for 202 of the 203 vertebral samples collected. Age estimates for females ranged from 0 to 28 years. The largest female measured 133.0 cm DW and also had the greatest estimated age. Age estimates for males ranged from 0 to 25 years. The largest male measured 111.0 cm DW with an estimated age of 23 years, whereas the maximum estimated age was for a 110.0 cm DW male. Precision estimates for blind readings were 3.68% (APE) and 5.55% (CV). Percent agreement between the first two band counts was 58.4% (± 0), 86.8% (± 1) and 95.1% (± 2). Higher relative agreement in band counts was found for smaller compared to larger size classes (Fig. 2.4).

Edge and marginal increment analysis

Variation observed in both centrum edge and marginal increment analysis suggests one band pair is produced each year consisting of a broad opaque zone and a narrow translucent zone (Fig. 2.5). The proportion of edge types varied seasonally. Narrow translucent bands were observed in winter months between November and March and broad translucent bands decreased in proportion from January to May. Narrow opaque bands were first observed in April and decreased in proportion until October, whereas the proportion of broad opaque bands was greatest in September and October and decreased through January. Trends observed in mean monthly MIR values closely followed those seen in centrum edge analysis. Pooled mean monthly MIR values were lowest in March and peaked in September and significant differences were found between months (ANOVA, $F_{11,156} = 9.81$, $P < 0.001$). Primarily, mean MIR values from September and October were significantly greater than those from December through

April ($P < 0.03$). Cumulatively, centrum edge and marginal increment analysis indicate formation of translucent and opaque bands begins in November and April respectively.

Annual periodicity of band formation was also supported by two recaptured rays marked with OTC at liberty for 365 (R1) and 821 (R2) days. Stingray R1 was initially tagged in April 2009 and recaptured in April 2010. Peripheral to the OTC mark, the centrum showed a broad opaque band with a broad translucent band at the edge. Stingray R2 was initially tagged in May 2008 and recaptured in August 2010. Peripheral to the OTC mark, the centrum for R2 displayed 2 translucent bands and a broad opaque band at the edge (Fig. 2.6). Banding patterns for R1 and R2 were consistent with the respective times at liberty.

Growth models

There were significant differences in growth characteristics between females and males for all models considered (ARSS, $P < 0.001$). Therefore, models were subsequently analyzed separately for female and male DW and weight-at-age data. Out of the set of candidate models, the logistic growth model provided the best statistical fit for DW and weight-at-age data for both females and males, and in 3 out of 4 cases had Aw_i values $> 97\%$ (Table 2.1). Gompertz models provided the second highest Aw_i values for all models ($Aw_i = 0.9\% - 23\%$, Table 2.1). The two-parameter and three-parameter von Bertalanffy growth models had essentially no support, with the majority of Aw_i values < 1 (Table 2.1).

Estimates of DW_∞ from the logistic model for females (144 cm DW) and males (116 cm DW) were similar to and slightly larger than, respectively, the largest rays

examined in this study (Females: 133 cm DW, Males: 111 cm DW). Gompertz models also predicted asymptotic widths for females (162 cm DW) and males (126 cm DW) within reasonable range of the largest rays captured. Estimated asymptotic widths were largest and had the widest confidence intervals for the two-parameter and three-parameter von Bertalanffy models for both sexes (Table 2.1). Males achieved maximum growth rates at a smaller size and earlier age than females (Fig. 2.7). Theoretical longevity estimates were lowest for the logistic model (Females: 32.1 years, Males: 25.6 years) and were similar to the maximum estimated ages (Table 2.2). All three-parameter models produced estimates of size at birth (DW_0) similar to, but slightly greater than, the smallest free-swimming rays in this study (35.0 cm DW) and mean size at birth estimated from vertebral measurements (35.8 cm DW).

Weight based growth models followed a similar trend compared to DW based models for both sexes, with logistic models providing the best fit and von Bertalanffy models the worst fit out of the set of candidate models based on AIC_c values (Table 2.1). Weights of the largest rays sampled (Female: 65.7 kg, Males: 36.7) were slightly larger than asymptotic weights estimated by the logistic model (Females: 63.7 kg, Males: 35.6 kg), but fell within the 95% confidence intervals for asymptotic weights (Table 2.1). Consistent with DW based growth, males reached maximum growth rates at a smaller size and younger age than females (Fig. 2.7). Theoretical longevity estimates for the weight based logistic model were 27.4 years for females and 23.4 years for males (Table 2.2).

Reproduction

Reproductive status was determined for all 203 rays examined for this study. Female brown stingrays reached sexual maturity at a larger size than males. Median DW at first maturity (DW_{50}) for females was 104.9 cm (Fig. 2.8). The smallest mature female was 99.1 cm DW and the largest immature female was 113.8 cm DW. Median age at first maturity (A_{50}) was 15 years (Fig. 2.8). A lack of ovary or uterine development in the right reproductive tract of all females collected indicates only the left reproductive tract is functional in brown stingrays. No gravid females were collected, thus we were unable to assess fecundity or timing of parturition. Median DW at first maturity for males was 74.9 cm DW (Fig. 2.8). The smallest mature male was 66.3 cm DW and the largest immature male was 77.5 cm DW. Median age at first maturity was 8.3 years (Fig. 2.8).

DISCUSSION

Accuracy of age estimates plays an important role in fitting growth functions to observed size at age data (Campana 2001). Smith et al. (2007) reported an APE of 8.4% for the diamond stingray, *Dasyatis dipterura*, and suggested the value may be inflated due to storage of vertebral centra in alcohol. Centra for this study were stored frozen until analysis and APE was 3.7%, similar to that found for cownose rays in the Gulf of Mexico (Neer and Thompson 2005). Overall percent agreement between readings was high ($95\% \pm 2$ years), but agreement was most variable for larger size classes which was primarily due to bands becoming more tightly grouped, and therefore more difficult to distinguish, at the outer edge of the centra (Cailliet and Goldman 2004, Cailliet et al. 2006).

Multiple lines of evidence provided support for the assumption that one growth band is deposited per year. Marginal increment ratios indicated a single peak in the winter and a single minimum in the spring, with significant differences between seasons, indicating narrow bands are formed in the winter months. Results from centrum edge analysis were in close agreement with those of marginal increment analysis, indicating that translucent bands begin forming in November whereas opaque bands begin forming in April. Recapture of two individuals tagged with OTC provided an additional validation of annual growth band deposition. Both individuals showed banding patterns consistent with time at liberty and date of tagging/recapture. Although direct validation of annual growth band formation should be conducted for all age classes (Campana 2001), close agreement of each method supports our assumption of a single growth band per year for brown stingrays of all ages. Validation of annual growth band deposition for other myliobatiform rays (Neer and Thompson 2005, Smith et al. 2007, Hale and Lowe 2008, Pierce and Bennett 2009) suggests the assumption is generally appropriate for this group (Pierce and Bennett 2009).

Maximum observed ages in this study were 28 and 25 years for females and males respectively and are similar to the oldest age estimates for other dasyatid species (Smith et al. 2007, Jacobsen and Bennett 2011). Out of the set of candidate models, the logistic growth functions based on DW and weight-at-age data best described the growth of male and female brown stingrays, providing the best statistical fits and most realistic biological parameters. Conversely, the von Bertalanffy growth functions had virtually no statistical support and substantially overestimated asymptotic size and theoretical longevity compared to maximum observed sizes and estimated ages. The von Bertalanffy growth

function has historically been the only growth function applied in elasmobranch age and growth studies (Cailliet et al. 2006), yet an increasing number of recent studies have demonstrated that alternative growth functions may better describe these parameters for some species. For example, Gompertz growth functions were found to best describe growth for cownose rays, *Rhinoptera bonasus* (Neer and Thompson 2005) and captive pelagic stingrays, *Pteroplatytrygon violacea* (Mollet et al. 2002). Although less commonly applied, the logistic growth function was found to provide the best fit for length-at-age data for big skates, *Raja binoculata* (Zeiner and Wolf 1993) and weight-at-age data for blacktip sharks, *Carcharhinus limbatus* (Killam and Parsons 1989) and spinner sharks, *Carcharhinus brevipinna* (Carlson and Baremore 2005).

The von Bertalanffy growth function has been criticized in part for its limited ability to reflect early growth (Moreau 1987, Gamito 1998, Lester et al. 2004, Cailliet et al. 2006). This limitation is particularly apparent when early growth is relatively slow, as the von Bertalanffy growth function assumes a constant decrease in growth rate with increasing size preventing detection of an inflection point (Ricker 1979, Moreau 1987). Slow growth for both sexes of brown stingrays early in life may be caused by higher mass specific metabolic rates coupled with an inability to capture sufficient prey required for significant somatic growth. For example, in a recent diet study the smallest size class of juvenile brown stingrays also had the highest proportion of empty stomachs compared to larger size classes (Chapter III). Poor foraging skill has previously been hypothesized to explain weight loss in newborn juvenile hammerhead sharks, *Sphyrna lewini*, in Kāneʻohe Bay (Lowe 2002, Bush 2003, Duncan and Holland 2006). Intraspecific competition may also impact the feeding success of small stingrays due to the occurrence

of multiple age classes in Kāneʻohe Bay. Although diet composition was significantly different between size groups of juveniles in the bay (Chapter III), access to high value sub-habitats may still be limiting if there is spatial overlap in prey populations. Large brown stingrays held in captivity have been observed to act aggressively towards smaller conspecifics when presented with food (J. Dale Personal Observation). Additional studies on the effects of ontogeny on the long-term habitat use of juvenile brown stingrays within Kāneʻohe Bay are required.

Sigmoid growth functions such as the Gompertz and logistic functions may better reflect lifetime growth for some elasmobranch species (such as batoids) which increase in mass at a greater rate than width or length (Cailliet and Goldman 2004, Neer and Thompson 2005) or for species where growth may occur in two phases (Carlson and Baremore 2005, Braccini et al. 2007). Two phase growth may occur due to ontogenetic shifts in habitat use, feeding behavior or energy allocation (shift from energy devoted to somatic growth to reproduction) (Araya and Cubillos 2006, Braccini et al. 2007). Support for the energy allocation hypothesis was found for spinner sharks (Carlson and Baremore 2005) and piked spurdogs (*Squalus megalops*, Braccini et al. 2007), where the change in growth rate corresponded with size at maturity. In this study, the inflection point (t_0) of the weight based logistic growth function was 14.6 for females and 11.6 for males. The inflection point coincides very closely with median age at first maturity for females ($A_{50} = 15$ yrs), but occurs slightly later than the median age at first maturity for males ($A_{50} = 8.3$). Brown stingrays forage within Kāneʻohe Bay for extended periods of their juvenile lives, shifting to offshore habitats in close association with the onset of sexual maturity (Chapter III). However, this shift occurs at a DW slightly larger than

median DW at first maturity for males and slightly smaller for females. Cumulatively, these results support an energy re-allocation hypothesis for females and an ontogenetic shift in resource use hypothesis for males as explanations for two phase growth.

Based on the observation of 14 individuals, Struhsaker (1973) suggested that female brown stingrays attain sexual maturity between 110 and 125 cm DW whereas males reach sexual maturity at 95 cm DW. In this study, the smallest mature female was 99 cm DW and the largest immature female was 113 cm DW similar to, but slightly lower than suggested by Struhsaker (1973). Although our estimate of size at maturity for males is smaller than that estimated for females, DW_{50} and the size of the largest immature male was significantly lower than that reported by Struhsaker (1973). The small number of samples examined by Struhsaker (1973) probably explains the differences between studies, but several studies have noted geographical variation in life-history characteristics within species (e.g. Driggers et al. 2004, Neer and Thompson 2005, Carlson et al. 2006). All of the samples examined for this study came from within or immediately offshore of Kāneʻohe Bay whereas Struhsaker (1973) sampled from the North Shore of Oahu. There is currently no information concerning population structure or movement patterns of brown stingrays beyond the immediate area of Kāneʻohe Bay although they are found throughout the Main Hawaiian Islands (Randall 2007). Consequently, additional studies from other regions of the islands are required to further evaluate whether there is geographical variation in the life-history characteristics of brown stingrays.

Due to a lack of gravid females in this study, we were unable to estimate fecundity or the timing of parturition for brown stingrays. Previous studies have noted

that embryos are commonly aborted due to stress of capture (Struhsaker 1969, Snelson et al. 1988, Smith et al. 2007) and this has been observed to occur in brown stingrays (K. Holland Personal Observation). Aborted embryos may explain our lack of gravid females. Longline sampling was used to collect samples in this study and rays could have been hooked for up to three hours before being retrieved. Based on gravid brown stingrays collected from bottom trawl surveys, Struhsaker (1973) estimated litter size to be 3-4 offspring which is similar to observed fecundity of other Dasyatid species (1-4; Snelson et al. 1988, Villavicencio Garayzar et al. 1994, Smith et al. 2007). The collection of the two smallest brown (both 35.0 cm DW) in May and June supports the hypothesis of summer parturition (Struhsaker 1973), but further studies on the reproductive characteristics of brown stingrays are required to confirm this hypothesis.

Male brown stingrays mature at a substantially smaller size and age compared to females (~30 cm DW smaller and ~7 years younger), similar to other Dasyatid stingrays (males 10 – 30 cm DW smaller than females; Devadadoss 1978, Snelson 1989, Villavicencio Garayzar et al. 1994, Smith et al. 2007), and consistent with the fact that female brown stingrays grow to larger sizes and older ages than males. In general, elasmobranchs attain sexual maturity at about 70% of their maximum size and 38% of their maximum age (Frisk et al. 2001, Dulvy and Forrest 2010). Female brown stingrays reached sexual maturity at 78% of their maximum observed size and 53% of their maximum estimated age, falling in the higher end of this range and similar to the Alaska skate, *Bathyraja parmifera* (Matta and Gunderson 2007). Male brown stingrays on the other hand, reach sexual maturity at about 67% of their maximum observed size and 33% of their maximum estimated age, slightly lower than the mean values for elasmobranchs.

However, for both female and male brown stingrays, median size and ages at first maturity are consistent with a late age at maturity found for many elasmobranchs.

This study provides baseline information on the life-history characteristics of brown stingrays without the confounding effects of fisheries exploitation. These life history characteristics indicate that brown stingrays are long lived, slow growing and mature at a late age, similar to other elasmobranchs that have been characterized as extremely vulnerable to overexploitation (Musick 1999). Should a fishery for brown stingrays open in Hawai'i, significant caution should be exercised in management of the fishery. However, in the absence of fisheries exploitation, the use of nearshore nursery habitats by this species indicates that habitat alteration may be the main threat to brown stingray populations, especially since it is in these areas that the most rapid growth occurs.

Table 2.1 Growth parameter estimates (standard error) and goodness of fit measures from disk width and weight based models for female and male brown stingray data. VB: von Bertalanffy growth function (2-parameter and 3-parameter); S_{∞} : Asymptotic size; S_0 : Size at birth; AIC: Akaike's information criterion, values based on absolute differences between models (Δ_i) and a value of 0 indicates the 'best' model, $Aw_i\%$: Akaike weight

Model	S_{∞} (s.e.)	$\pm 95\%$ CI	S_0	k (s.e.)	t_0 (s.e.)	AIC _c (Δ_i)	$Aw_i\%$
DW Based							
<i>Female</i>							
VB2	207.5 (11.8)	40.81		0.03 (0.01)		19.05	0.01
VB3	231.8 (24.0)	47.58	37.9	0.03 (0.01)	-6.84 (0.59)	20.33	0.00
Gompertz	161.7 (5.7)	11.29	39.3	0.07 (0.01)	4.67 (0.47)	7.03	2.89
Logistic	144.1 (3.1)	6.20	40.6	0.12 (0.01)	7.61 (0.40)	0	97.10
<i>Male</i>							
VB2	136.5 (4.7)	14.11		0.06 (0.01)		6.12	3.37
VB3	152.4 (9.5)	18.81	38.8	0.04 (0.01)	-6.78 (0.05)	8.12	1.24
Gompertz	126.2 (3.7)	7.39	39.7	0.09 (0.01)	1.59 (0.29)	2.24	23.47
Logistic	116.6 (2.3)	4.62	40.5	0.14 (0.01)	4.52 (0.31)	0	71.92
Weight Based							
<i>Female</i>							
VB3	98.4 (7.0)	13.86	0.2	0.07 (0.01)	-1.83 (0.48)	25.07	0.00
Gompertz	79.5 (3.4)	6.63	0.8	0.11 (0.01)	13.86 (0.48)	8.25	1.59
Logistic	63.7 (1.4)	2.73	2.1	0.23 (0.01)	14.61 (0.28)	0	98.41
<i>Male</i>							
VB3	54.3 (4.5)	8.99	0.8	0.08 (0.01)	-3.69 (0.56)	21.24	0.00
Gompertz	44.2 (2.2)	4.44	1.2	0.12 (0.01)	10.9 (0.55)	9.53	0.85
Logistic	35.6 (0.9)	1.83	1.9	0.25 (0.01)	11.65 (0.33)	0	99.15

Table 2.2 Theoretical longevity estimates (years) from disk width and weight based data for female and male brown stingrays. VB: von Bertalanffy growth function (2-parameter and 3-parameter)

Model	Female	Male
Maximum Estimated	28	25
DW Based		
VB2	93.6	44.9
VB3	93.0	68.1
Gompertz	47.1	34.6
Logistic	32.1	25.6
Weight Based		
VB3	56.4	47.3
Gompertz	40.6	35.4
Logistic	27.4	23.4

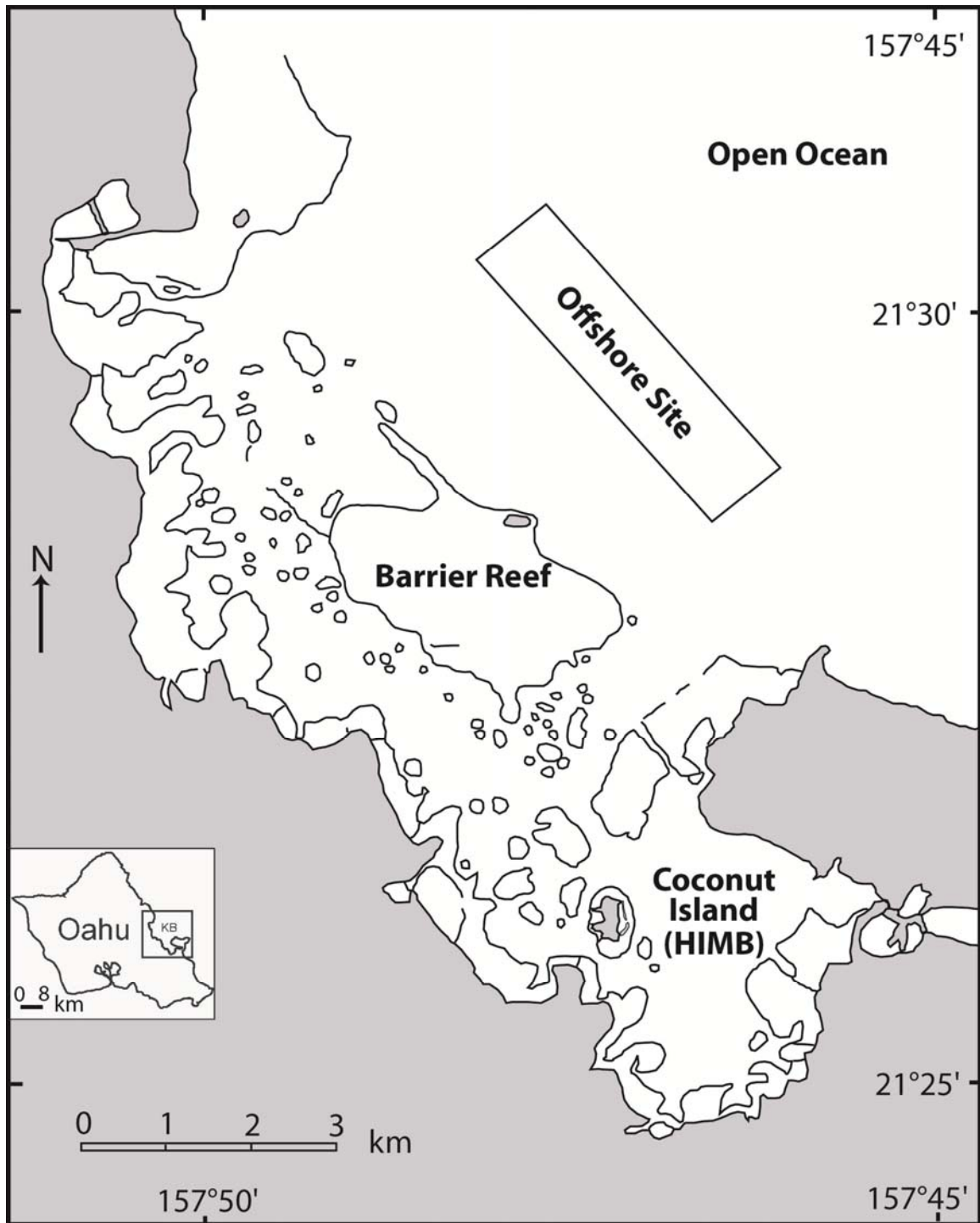


Figure 2.1 Map of Kāneʻohe Bay showing outline of patch reefs and barrier reef. Inset: location of Kāneʻohe Bay on Oahu, Hawaiʻi. HIMB: Hawaiʻi Institute of Marine Biology.

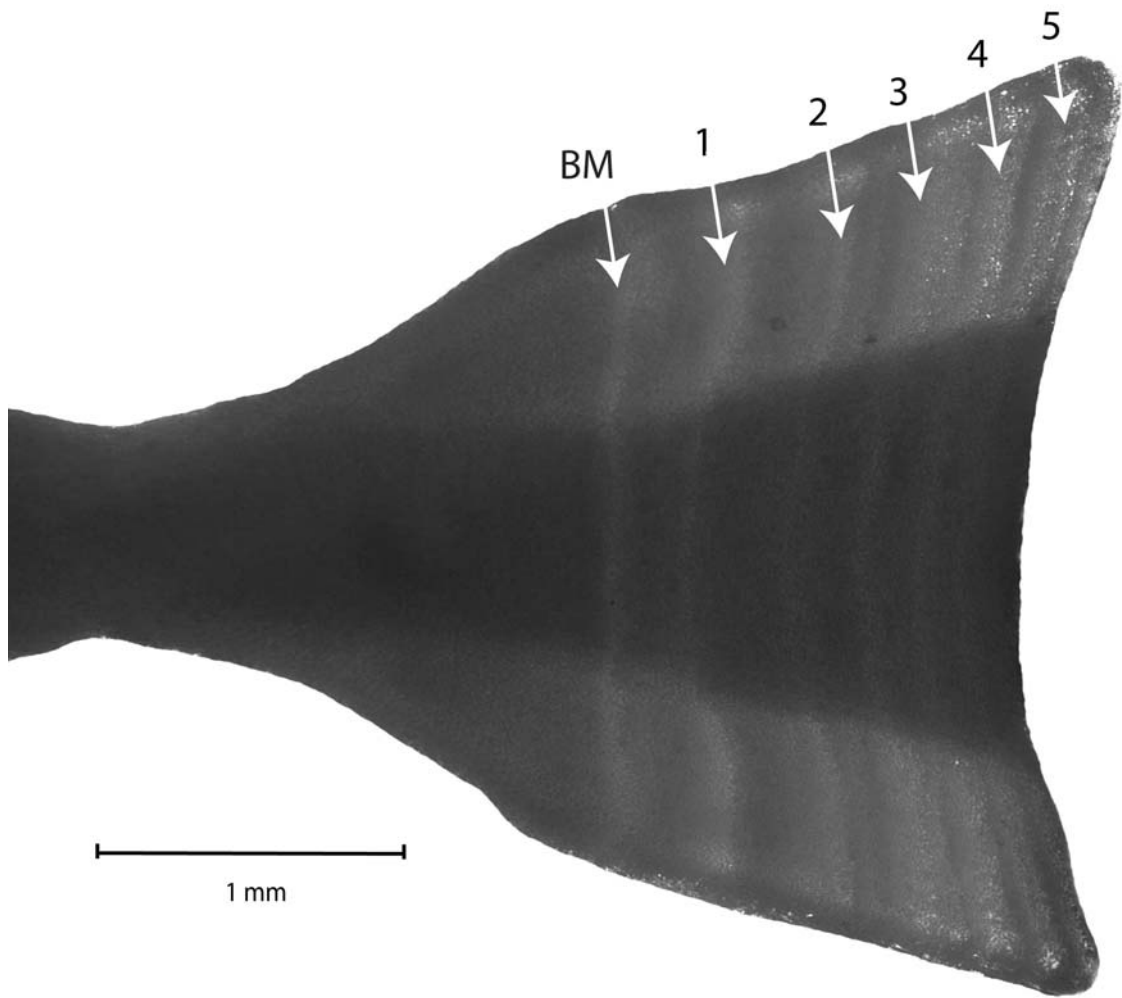


Figure 2.2 Sagittal thin section of a brown stingray vertebral centrum estimated to be 5 years in age. BM: birth mark.

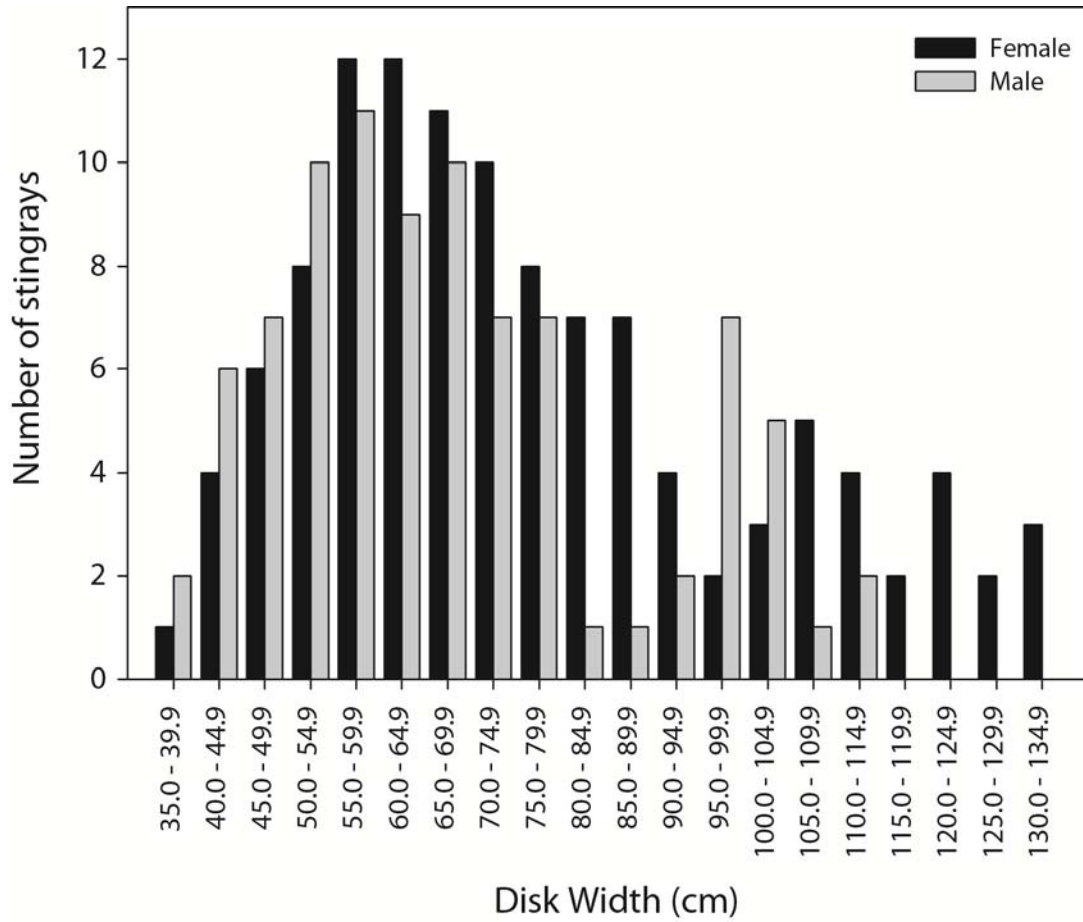


Figure 2.3 Length frequency histogram of brown stingrays retained for age estimation for this study (Females =115, Males = 88).

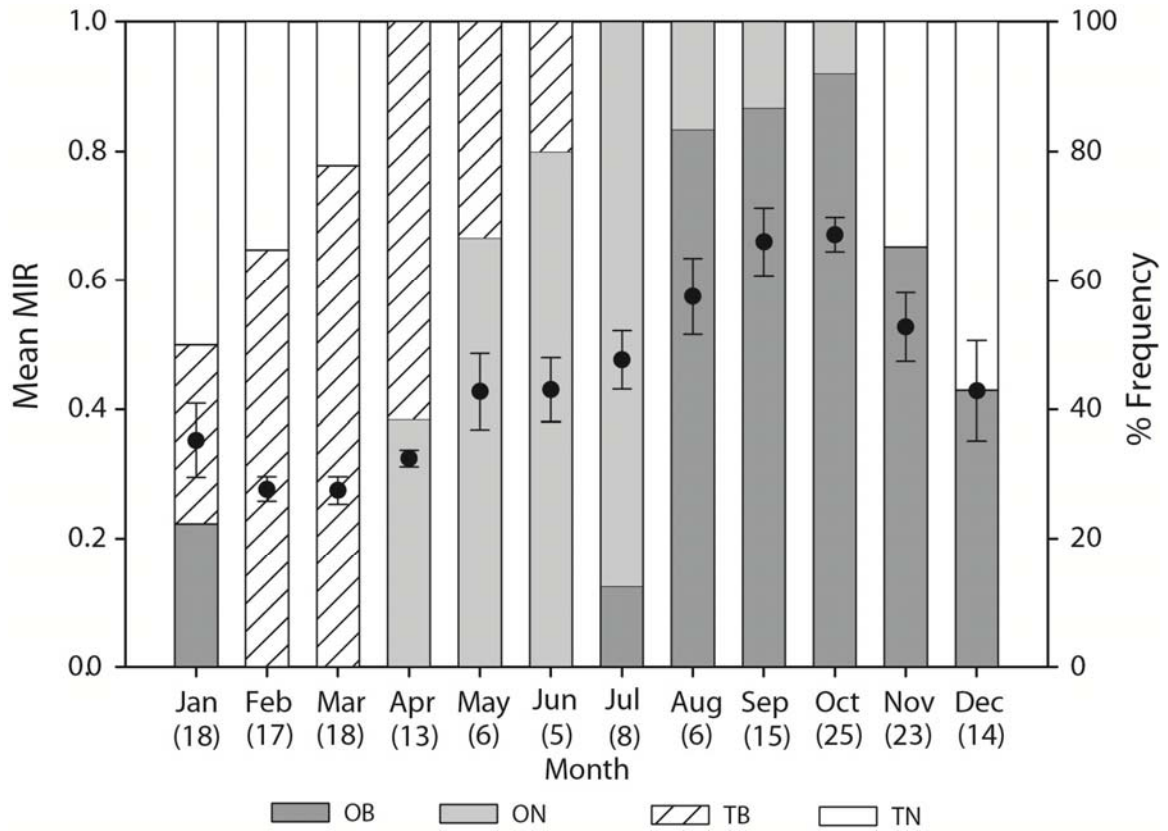


Figure 2.4 Monthly variation in centrum edge and mean marginal increment ratios (MIR) ± 1 standard error determined from pooled sexes and size classes. Values listed in parentheses below the x-axis are the sample sizes for each month. OB: opaque broad edge; ON: opaque narrow edge; TB: translucent broad edge; TN: translucent narrow edge.

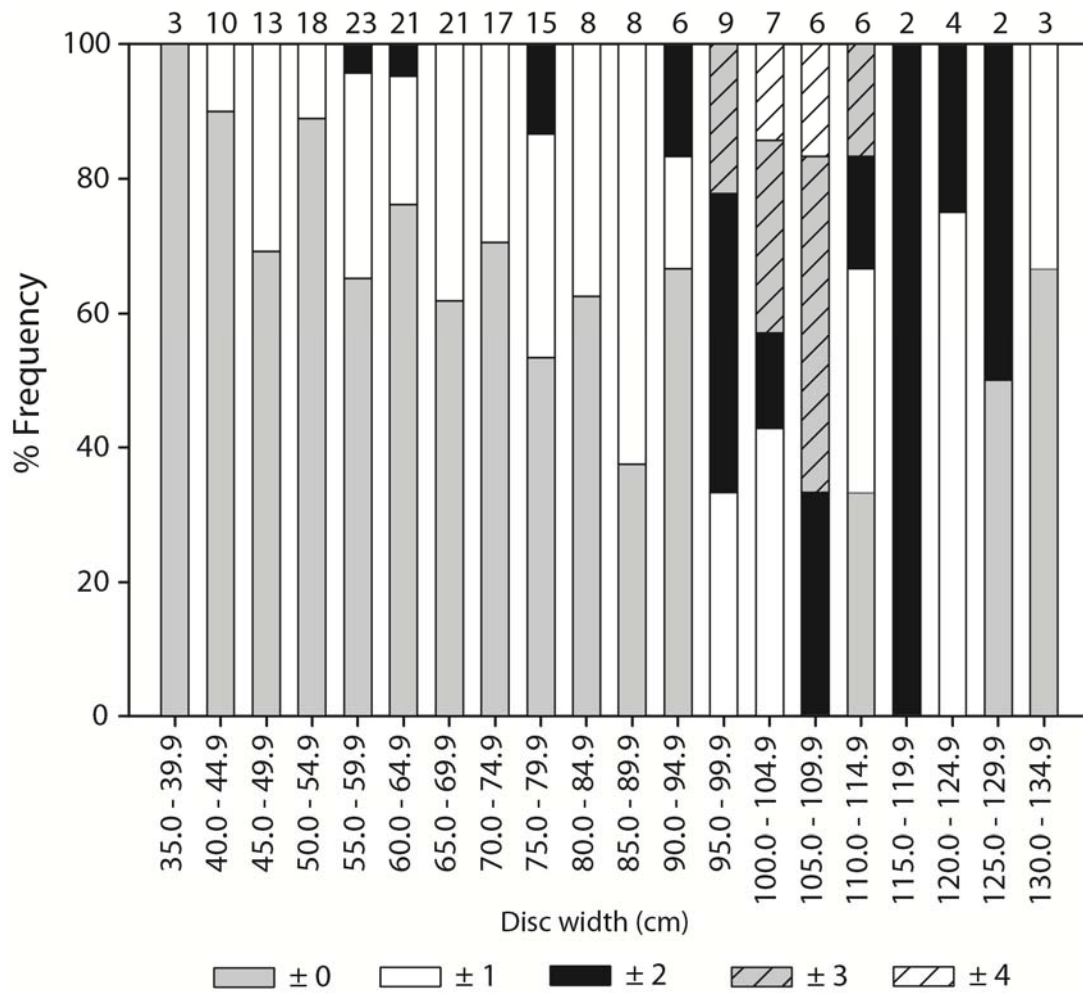


Figure 2.5 Percent agreement plotted by 5 cm disk width classes. Values above bars are the sample sizes for each disk width class.

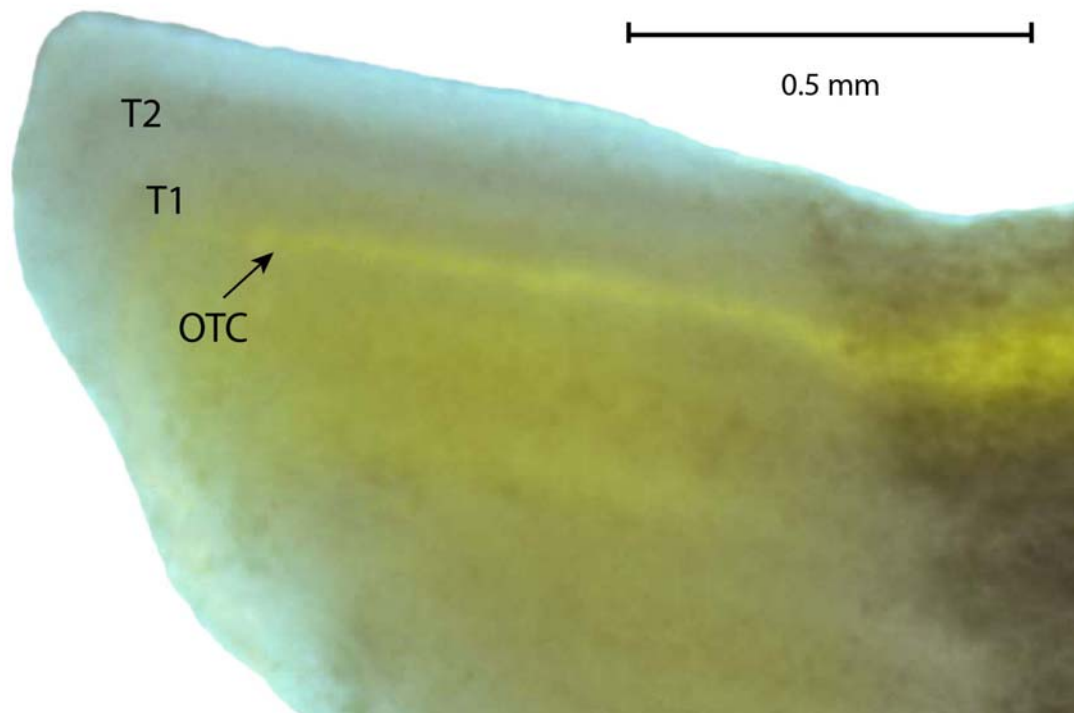


Figure 2.6 Edge of a sagittal thin section of a vertebral centrum from a oxytetracycline (OTC) injected brown stingray (R2). Initial injection was May 2008 and the animal was recaptured in August 2010. Two translucent bands (T1 and T2) are present following the OTC mark and a broad opaque band is present on the centrum edge.

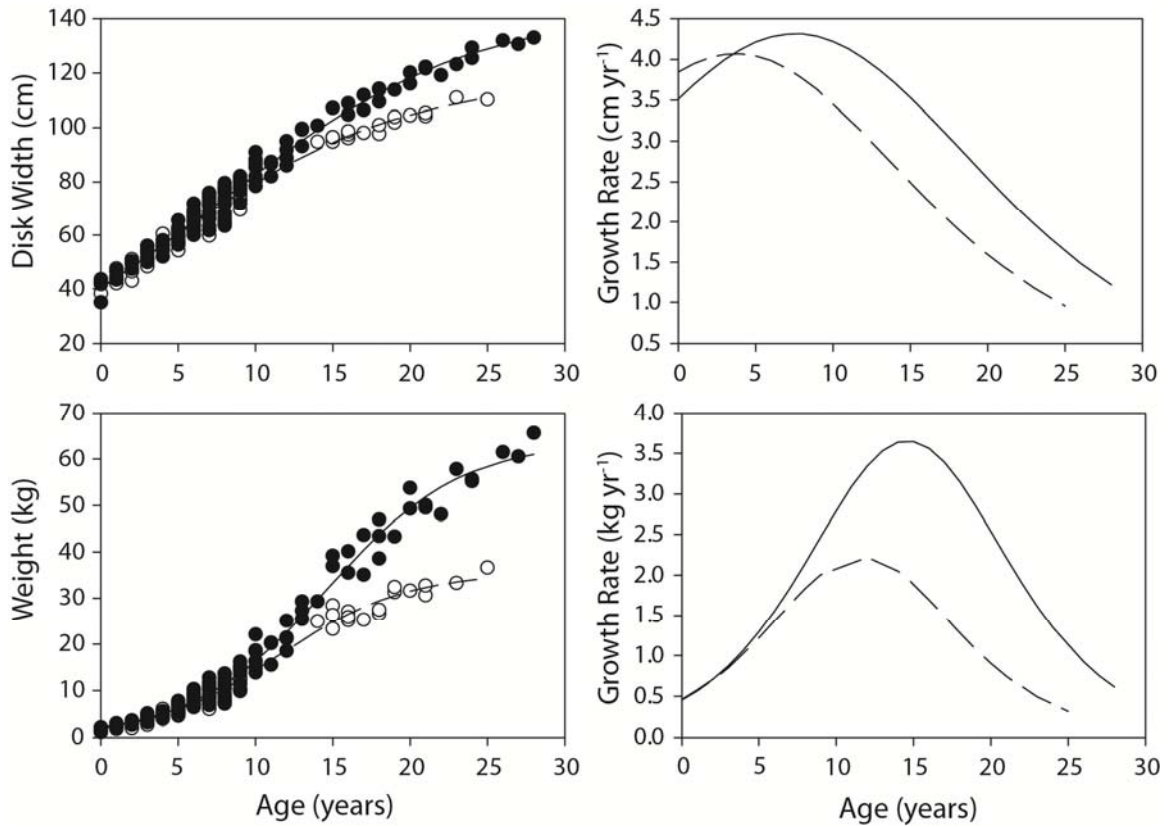


Figure 2.7 Logistic growth function fits and growth rates for (A, B) disk width and (C, D) weight at age estimates for male ($n = 88$) and female ($n = 114$) brown stingrays. Females: filled circles, solid lines; Males: open circles, dashed lines.

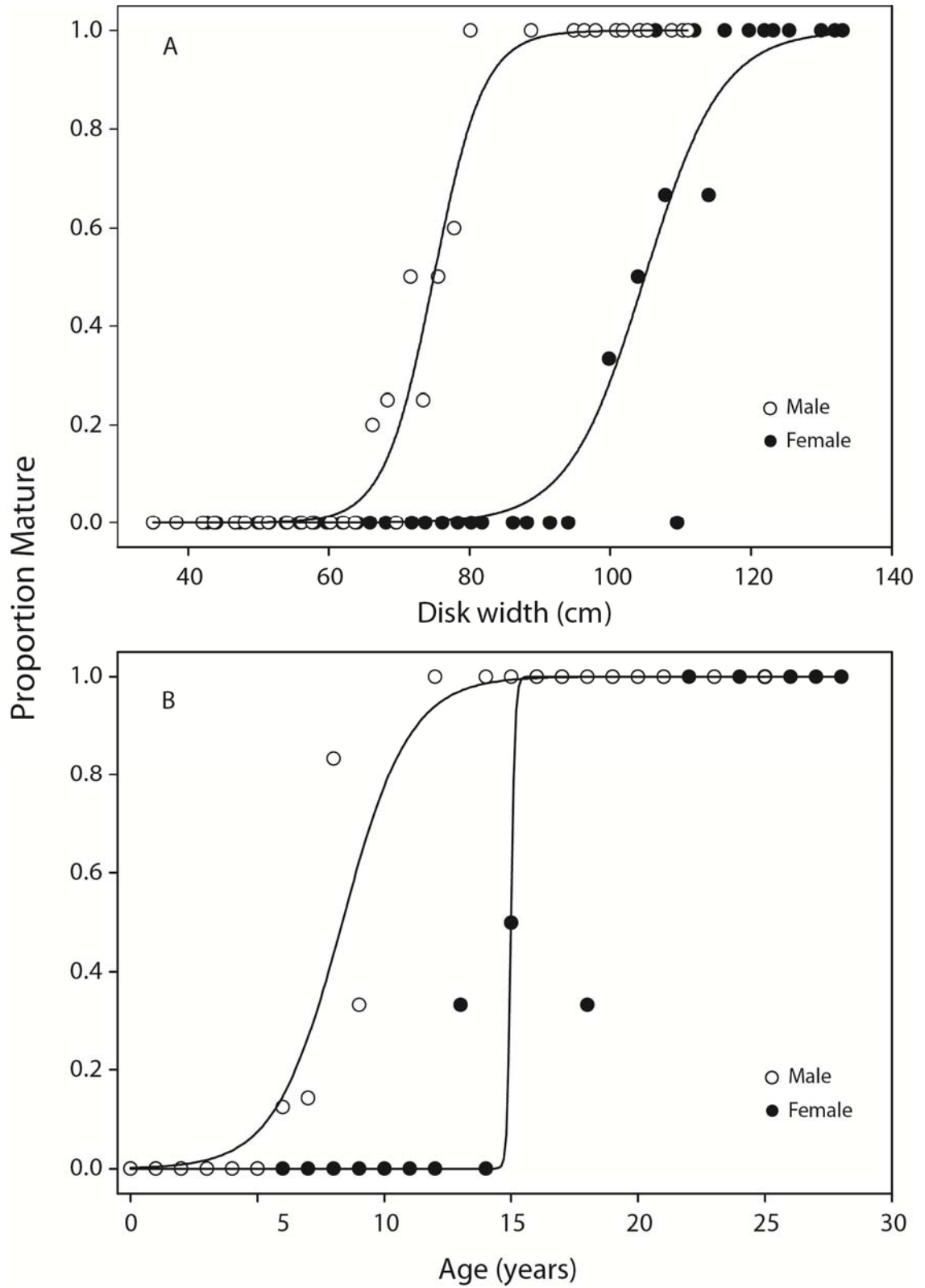


Figure 2.8 Relationship between maturity status and (A) disk width and (B) estimated age for male (n = 88) and female (n = 115) brown stingrays.

CHAPTER III

NURSERY HABITAT USE AND FORAGING ECOLOGY

Note: In press in Marine Ecology Progress Series under the following citation:

Dale, J.J., Wallsgrove, N.J., Popp, B.N., Holland, K.N. In press. Nursery habitat use and foraging ecology of the brown stingray, *Dasyatis lata*, determined from stomach contents, bulk and amino acid stable isotopes. DOI: 10.3354/meps09171.

ABSTRACT

Identification of nursery habitats and knowledge of the trophic ecology and habitat use of juveniles within these habitats are fundamental in developing sound management and conservation strategies. The brown stingray, *Dasyatis lata*, is a large benthic predator which inhabits the coastal waters of Hawai‘i. Although abundant in these ecosystems, little is known about its basic ecology. Stomach content, bulk and amino acid stable isotope analyses were used to assess diet and habitat use of juvenile brown stingrays and to examine the possibility of competitive interactions with juvenile scalloped hammerhead sharks (JSH), *Sphyrna lewini*, that are sympatric with brown stingrays in Kāne‘ohe Bay, Oahu. Based on stomach contents, stingrays fed almost exclusively on crustaceans. An ontogenetic shift in stingray diet and an increase in relative trophic position (TP) were apparent from stomach content and stable isotope analyses. Stingray bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values indicated long term foraging fidelity to sub-regions of the bay. Use of Kāne‘ohe Bay as a nursery habitat was supported by nitrogen isotopic analysis of individual amino acids from stingray muscle samples. Our

results clearly demonstrated that stingrays foraged within the bay for the majority of their juvenile lives then shifted to offshore habitats with the onset of sexual maturity. Trophic enrichment factors used to estimate TPs from amino acid analysis in previous studies may underestimate TPs in elasmobranchs due to urea retention for osmoregulation. Potential prey resources were partitioned between stingrays and JSHs and TP estimates from each analytical method indicated that JSHs forage on higher TP prey than juvenile stingrays. These results show that the study of foraging ecology and habitat use of marine animals can greatly benefit from integrating traditional stomach content and bulk stable isotopic analyses with nitrogen isotopic analyses of individual amino acids.

INTRODUCTION

Coastal ecosystems such as bays and estuaries are generally characterized by high productivity and a diverse range of habitats, offering an abundant prey base for faunal constituents and refuge from predation (Shulman 1985, Beck et al. 2001). Because of these characteristics, coastal ecosystems commonly serve as nursery habitat for a wide range of fishes, including elasmobranchs (Beck et al. 2001, Heupel et al. 2007). These ecosystems are highly susceptible to degradation from anthropogenic impacts (Lotze et al. 2006) which can have substantial effects on juvenile survivorship (e.g. Jennings et al. 2008). Because juvenile survivorship is often one of the most important factors regulating overall population size (Heppel et al. 1999, Cortés 2002), identification of nursery habitats and knowledge of the trophic ecology and habitat use of juveniles within these habitats are fundamental in developing sound management and conservation strategies.

The trophic ecology of elasmobranchs has traditionally been studied through stomach content analysis (SCA) (Hyslop 1980). However, bulk tissue stable isotope analysis (SIA) has increasingly been used to compliment SCA (e.g. Graham et al. 2007). Stable isotope analysis is based on the observation that the ratio of carbon isotopes ($^{13}\text{C}/^{12}\text{C}$) in an animal's tissues primarily reflects the isotopic signatures of the primary producers at the base of its food chain and nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$) can indicate the trophic position (TP) of the animal (Post 2002). Thus, stable isotope analyses provide a complimentary method to SCA for detecting ontogenetic shifts in diet and foraging habitat, and calculation of TP (Post 2002, Fisk et al. 2002, Cocheret de la Morinière et al. 2003, MacNeil et al. 2005). Calculation of TP using bulk tissue SIA can require knowledge of the habitat-specific nitrogen isotopic composition of primary producers (Post 2002). These values may be difficult to obtain in habitats such as coral reefs which have a diverse range of primary producers, or in deep water habitats which are not easily sampled. Recent studies have shown that analysis of the isotopic composition of individual amino acids (as opposed to bulk tissue) taken from a consumer can circumvent these challenges (Uhle et al. 1997, Fantle et al. 1999, McClelland & Montoya 2002, Chikaraishi et al. 2007, Popp et al. 2007, Chikaraishi et al. 2009, Hannides et al. 2009, Lorrain et al. 2009, Chikaraishi et al. 2010). This is because the $\delta^{15}\text{N}$ values of some amino acids, such as phenylalanine, retain the $\delta^{15}\text{N}$ values of primary producers. Other amino acids, such as glutamic acid, undergo large, consistent enrichments ($\sim 7.6\%$) with each successive trophic position. The advantage of this approach is that the baseline $\delta^{15}\text{N}$ values of a consumer's foraging habitat as well as the consumer's TP can be determined by analyzing $\delta^{15}\text{N}$ values of individual amino acids in the consumer's tissues. Trophic

positions have been estimated with this method for a variety of consumers across a range of TPs (Pakhomov et al. 2004, Schmidt et al. 2004, Popp et al. 2007, Chikaraishi et al. 2009, Hannides et al. 2009, Lorrain et al. 2009), yet TP has only been estimated for a single elasmobranch (Chikaraishi et al. 2010).

The brown stingray, *Dasyatis lata*, is a large (up to 133 cm Disk Width, 66 kg; Chapter II) benthic predator endemic to Hawai‘i. Juveniles are abundant in shallow bays and estuaries, whereas adults are often found in deep offshore waters (Cartamil et al. 2003, Randall 2007, Chapter II). Despite their abundance in near-shore estuaries and potential for strong impacts on the trophic dynamics of these regions (VanBlaricom 1982, Thrush et al. 1994, Peterson et al. 2001), little is known about their general biology, foraging ecology and habitat use. The habitat use and activity patterns of brown stingrays actively tracked for short durations (days) overlapped significantly with that of juvenile scalloped hammerhead (JSH) sharks, *Sphyrna lewini*, that are sympatric with brown stingrays in Kāne‘ohe Bay, Oahu, Hawai‘i (Holland et al. 1993, Lowe 2002, Cartamil et al. 2003). These results suggest that competition for prey resources between these two sympatric species may contribute to high rates of starvation found for neonate JSH sharks within their Kāne‘ohe Bay nursery (Bush and Holland 2002, Lowe 2002, Duncan and Holland 2006).

The goals of this study were to quantify the foraging ecology and habitat use of brown stingrays in Kāne‘ohe Bay and evaluate the extent of ecological interactions between brown stingrays and JSH sharks. Specifically, we used SCA and SIA to address the following questions; (1) What are the food habits of brown stingrays?, (2) Do their food habits change through ontogeny?, (3) Is Kāne‘ohe Bay utilized as a nursery habitat

by brown stingrays?, (4) Do isotopic analyses of individual amino acids accurately reflect the TP of brown stingrays and JSH sharks?, and (5) Is there evidence of competition between brown stingrays and JSH sharks for prey resources? We found that the combined use of stomach content, bulk tissue and individual amino acid stable isotope analyses allowed for validation of results from each individual method and provided significant insight into the early life history and ecological relationships of brown stingrays. These results highlight the advantages of integrating multiple approaches in the study of foraging ecology and habitat use.

METHODS

Study Site and Sample Collection

Kāneʻohe Bay, located on the east coast of Oahu, covers an area of approximately 46 km² and is bounded on its seaward side by a barrier reef (Fig. 3.1). The inner bay, landward of the barrier reef, consists of deep lagoon (average depth 13 m) interspersed with numerous patch reefs (Smith et al. 1981). Spatial differences in diet composition and habitat use were evaluated by dividing the bay into three zones: South, Mid and North (Fig. 3.1). Zones were based on previous studies examining environmental and ecological processes within the bay (Smith et al. 1981, Duncan and Holland 2006). Brown stingrays were collected using standard demersal longlines in and outside of Kāneʻohe Bay. Inshore longlines were set at least 5 m away from patch reefs to avoid entanglement on coral. Offshore longlines were set at depths of approximately 80 m based on catch records from a shark survey conducted in this area (D. Grubbs unpublished data). All longlines were baited with tuna, *Thunnus spp.*, and/or squid,

Loligo spp. Lines were soaked for 3 hours before being retrieved. Captured stingrays were landed, disk width (DW) measured to the nearest 1 mm and euthanized. Maturity state (juvenile/adult) was assessed as part of a concurrent study on the age and growth of brown stingrays (Chapter II).

Stomach Content Analysis

Stomach contents were removed and preserved in 70% ethyl alcohol. Upon analysis, stomach contents were rinsed and individual prey items were identified to the lowest possible taxa. The total number and wet weight of each taxon was recorded and prey items were then dried to a constant weight at 60°C. The contribution of each prey taxon to the diet of brown stingrays was quantified using several metrics of dietary composition. Percent abundance ($\overline{\%N}$, (number of individuals in a prey category/total number of individuals among all prey categories)*100) and percent weight ($\overline{\%W}$, (weight of individuals in a prey category/total weight of individuals among all prey categories)*100) were calculated for each sample to provide mean and variability estimates (Bizzarro et al. 2007, Chipps and Garvey 2007). The percent frequency occurrence (%FO) was calculated as the number of stomachs containing a prey taxon/total number of stomach containing prey*100 (Hyslop 1980). Individual metrics were also combined into a composite metric, the index of relative importance (IRI), for an additional description of diet composition (Pinkas et al. 1971), defined as:

$$IRI = (\overline{\%N} + \overline{\%W}) * \%FO.$$

The IRI values were expressed as a percentage ($\overline{\%IRI}$) to facilitate comparisons between prey taxon (Cortés 1997). Percent IRI values were calculated for each sample to provide mean and variability estimates for each prey taxon (Bizzarro et al. 2007).

There was no significant effect of sex or location on gravimetric diet data based on ANOSIM procedures (see below) therefore, for ontogenetic comparisons, data were pooled. Three size classes were identified by cluster analysis using the group average linkage method (PRIMER v.6): small (35.0 – 54.9 cm DW), medium (55.0 – 69.9 cm DW) and large (70.0 – 94.9 cm DW). Stingrays were separated by DW into 5 cm bins, and the average $\overline{\%W}$ for each prey item was calculated. Bin averages were required because individual stomachs were too variable for meaningful interpretation. To compare dietary composition between identified size classes, non-metric multidimensional scaling ordination plots (MDS) were generated and analysis of similarities (ANOSIM) used to test for significant differences between size groups (PRIMER v.6). The resultant global R-statistic ($-1 > R < 1$) describes the amount of similarity between each pair in the ANOSIM analysis. A zero value indicates no difference while a value close to $|1|$ indicates that the two groups are entirely separate. P-values generated from the R-statistic < 0.05 were considered statistically significant. Multivariate dispersion (MVDISP) calculated the degree of dispersion between samples within size classes and similarity percentages (SIMPER) identified which dietary categories contributed most to the dissimilarities between size classes (PRIMER v.6). For each size class, stingrays were randomly sorted into groups of four (i.e. dietary samples) and mean values for each prey taxon determined in order to overcome the problem of low prey diversity in the stomachs of individual stingrays (Platell and Potter

2001). Gravimetric diet data were square root transformed prior to multivariate analysis and when appropriate, used to construct a Bray-Curtis similarity matrix. Only prey items representing $>5\% \overline{W}$ were included in order to reduce effects of rare prey. Prey diversity was calculated for each size class using the Shannon-Wiener diversity index (H') (Krebs 1999). Trophic positions were calculated for each size class following Cortés (1999). Trophic position estimates for prey categories were taken from the *Sea Around Us* Project database (see www.seaaroundus.org) and Ebert and Bizzarro (2007).

To evaluate the possibility of competitive interactions, diet composition was compared between brown stingrays and JSH sharks. Raw data from Bush (2003) were reanalyzed to generate $\overline{\%W}$ values for each individual JSH sample. Dietary data were grouped into broad categories to eliminate biases in comparisons based on variable levels of taxonomic identification (Cortés 1997). Differences between hammerhead and stingray diets were analyzed following the procedures described for stingray size class comparisons (i.e. ANOSIM, MDS).

Bulk Stable Isotope Analysis

A preliminary study conducted in September 2009 examining the food web structure of Kāneʻohe Bay found red macroalgae to be the primary nitrogen source for benthic predators (mean $\delta^{15}\text{N} \pm \text{S.D.}$: $3.3 \pm 0.7\text{‰}$, $n = 6$, J. Dale unpublished data). Macroalgae were collected by hand from sites throughout the bay, washed in distilled water and visually inspected for sources of contamination. Stingray and JSH epaxial white muscle tissue ($\sim 1 \text{ cm}^3$) was removed from each sample and frozen until further analysis. Samples were dried at 60°C and ground into a fine powder with a mortar and

pestle. Lipid extraction was not performed due to the low molar C:N ratios of stingray (mean \pm S.D.: 2.97 ± 0.10) and JSH (3.00 ± 0.12) muscle samples, indicating low lipid concentrations in tissues and little variation between individuals (Post et al. 2007). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of white muscle tissue were determined using a carbon-nitrogen analyzer coupled with an isotope ratio mass spectrometer (Finnigan Conflo II/Delta-Plus, Bremen, Germany). Isotope values are reported as δ -values in ‰ relative to V-PDB and atmospheric N_2 standards for carbon and nitrogen respectively. Accuracy of isotopic measurements was determined through repeated analyses of a sample of glycine isotopically well characterized by long-term (>10 years) analyses using multiple instruments in different laboratories and interspersed with samples. Average accuracy and precision of all stable isotopic analyses determined by replicate analysis of glycine and samples was less than $\pm 0.1\text{‰}$ (1 S.D.).

A general linear model (GLM) was used to test for differences in stingray bulk isotopic compositions between bay zones and sexes, with stingray DW as a covariate. If significant differences between zones or sex were found, a subsequent GLM was used to test for differences in isotopic values between size classes while controlling for variability due to significant factors, which were included as fixed effects. Trophic positions for each stingray size class and JSHs were calculated using the following equation:

$$TP_{bulk} = \frac{\delta^{15}N_{consumer} - 3.3}{2.7} + 1,$$

where 2.7‰ is the assumed trophic enrichment factor (TEF) (Vanderklift and Ponsard 2003) and 3.3‰ is the average nitrogen isotopic value for red macroalgae abundant in Kāne‘ohe Bay (J. Dale unpublished data, Stimson et al. 2001).

Stable Nitrogen Isotope Analysis of Individual Amino Acids

Prior to analysis, stingray and JSH muscle tissue was subjected to acid hydrolysis, esterification of the carboxyl terminus and trifluoroacetylation of the amine group (Macko et al. 1997, Popp et al. 2007). Muscle tissue (~5 mg) was hydrolyzed at 150°C for 70 minutes using sequanal grade 6 N hydrochloric acid (HCl) in a culture tube that was flushed with nitrogen gas (N₂) and fitted with a Teflon-lined cap. Glutamine is converted to glutamic acid during hydrolysis (Metges et al. 1996) so $\delta^{15}\text{N}$ values of glutamic acid include glutamine. The HCl was evaporated to dryness using a Thermo Savant Speed Vac concentrator coupled with a UVS400 at 55°C for 1.5 hr. The residue was re-dissolved in 1 ml 0.01 N HCl and purified by filtration (0.45 μm hydrophilic filter), and the filter washed with 1 ml 0.01 N HCl. Amino acids were further purified by cation exchange (~ 5 cm Dowex 50WX8-400 in a Pastuer pipette). The filtered hydrolysate was added to the ion exchange column in 0.01 N HCl and amino acids eluted with 4 ml ammonium hydroxide and evaporated to dryness under a stream of N₂ at 80°C. The samples were re-acidified by adding 0.5 ml of 0.2 N HCl, the vials were flushed with N₂, heated to 110°C for 5 minutes and then dried using the Speed Vac concentrator for 1.5 hr at 55°C. The hydrolyzed muscle samples were esterified using 2-3 ml of 1:4 acetyl chloride:isopropanol in N₂-flushed vials heated to 110°C for 60 minutes. Excess solvent was removed under a stream of N₂ at 60°C. Trifluoroacetylation of the amine group was accomplished by adding 3:1 methylene chloride:trifluoroacetic anhydride (TFAA) to each vial and heating to 100°C for 15 minutes. The samples were further purified by solvent extraction following Ueda et al. (1989) using 2 ml of P-buffer (KH₂PO₄ +

Na₂HPO₄ in milli-Q water, pH 7). The acylated amino acids were partitioned into chloroform, the chloroform evaporated to dryness and the trifluoroacetylation step repeated to ensure full derivitization. Samples were stored at -20°C in 3:1 methylene chloride:TFAA for up to one month until isotope analysis.

Just prior to isotope analysis, the 3:1 methylene chloride:TFAA was evaporated under a stream of N₂ at room temperature. Samples were re-dissolved in 100 µl of ethyl acetate. The stable N isotope composition of the amino acids were determined using either a Delta XP or Delta V Plus mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (650°C), and liquid nitrogen cold trap. The samples (1-2 µl) were injected (split/splitless injector, 10:1 split ratio) onto a BPx5 *forte* capillary column (30m x 0.32mm x 1.0 µm film thickness) at an injector temperature of 180°C with a constant helium flow rate of 1.4 ml min⁻¹. The column was initially held at 50°C for 2 minutes and then increased to 190°C at a rate of 8°C per minute. Once at 190°C, the temperature was increased at a rate of 10°C per minute to 300°C where it was held for 7.5 minutes. Internal reference compounds, amino adipic acid and norleucine of known nitrogen isotopic composition, were co-injected with samples and used to normalize the measured δ¹⁵N values of unknown amino acids. All samples were analyzed at least in triplicate. Reproducibility associated with isotopic analysis of glutamic acid and phenylalanine averaged ±0.44‰ (1 S.D) and ranged from ±0.06‰ to ±0.85‰. The accuracy of each measurement was determined by using the known δ¹⁵N value for amino adipic acid to determine the measured δ¹⁵N value of norleucine as an unknown, and vice versa. We have found that the combustion reactor on the GC-C III is susceptible to rapid failure when used for

nitrogen isotopic analyses of amino acids. Co-injection of aminoadipic acid and norleucine allows monitoring of combustion reactor degradation and provides an internal check on the accuracy of each sample injected. The accuracy averaged $\pm 1.5\%$ (1 S.D.) and ranged from $\pm 0.36\%$ to $\pm 2.4\%$.

Determination of Trophic Position Using Amino Acid Isotope Analyses

The fractional trophic position of brown stingray and JSH shark samples was calculated using the measured $\delta^{15}\text{N}$ values of glutamic acid and phenylalanine as described by Chikaraishi et al. (2009):

$$TP_{Glu/Phe} = \frac{(\delta^{15}N_{Glu} - \delta^{15}N_{Phe}) - 3.4}{7.6} + 1,$$

where $TP_{Glu/Phe}$ is the trophic position determined using glutamic acid (Glu) and phenylalanine (Phe), 3.4 is the isotopic difference between glutamic acid and phenylalanine in the primary producers (β), and 7.6 is the TEF. A conservative error associated with the trophic position calculation was determined by propagation of error (e.g., Gelwicks and Hayes 1990) using the uncertainty in β and the TEF in Chikaraishi et al. (2009), as well as the measured reproducibility for glutamic acid and phenylalanine for each sample.

RESULTS

Stomach Content Analysis

A total of 156 juvenile brown stingrays were sampled from Kāneʻohe Bay (size range 35.0-94.9 cm DW), of which 20 (12.8%) had empty stomachs. The number of empty stomachs varied significantly between size classes, with the greatest percentage

occurring in small rays (22%), followed by large (17%) and medium (5%) rays ($\chi^2 = 6.4$, $df = 2$, $P = 0.04$). Prey from stomach contents of brown stingrays were separated into 16 prey categories (Table 3.1). Crustaceans, represented by alpheid shrimps, portunid crabs and stomatopods, were the most important prey group for all size classes ($\overline{IRI} \pm S.D. = 93.25 \pm 18.30$, small; 86.46 ± 25.52 , medium; 84.67 ± 23.32 , large), with minor contributions from polychaete worms and teleosts (Table 3.1). Gobies were the most important teleost component and only one other teleost family was identified (Table 3.1). With increasing stingray size, alpheid shrimps and polychaete worms declined in importance whereas the portunid crab *Podophthalmus vigil* and gobies increased in importance (Table 3.1). Another portunid crab, *Libystes villosus*, increased in importance from small to medium sized individuals, but then decreased from medium to large sized individuals (Table 3.1). There was a positive, linear relationship between stingray size (DW) and stingray mouth gape ($R^2 = 0.86$, $P < 0.001$) as well as stingray size and carapace width of *P. vigil* consumed ($R^2 = 0.51$, $P < 0.001$). Based on calculation of TP from prey in stingray stomachs, juvenile stingrays were classified as secondary consumers ($TP < 4$) and TP increased with increasing stingray size (range: 3.3 – 3.7, Table 3.1).

Gravimetric diet composition differed significantly overall between the three size classes (ANOSIM, Global R statistic = 0.428, $P = 0.001$) and also for each of the pairwise comparisons between size classes. The R statistic values for pairwise comparisons were greatest between small and large size classes (0.856, $P = 0.001$) and comparably lower between small and medium (0.291, $P = 0.004$) and medium and large (0.298, $P = 0.004$) size classes. Ordination of the mean gravimetric dietary data showed a

gradual dietary transition in small to large stingrays (Fig. 3.2). Sample dispersion was similar between the three size classes (MVDISP value range = 0.906 – 1.054). Although dietary samples overlapped between size classes, no samples from the small size class overlapped with samples from the large size class (Fig. 3.2). *Alpheus malabaricus* and *P. vigil* were identified as the prey items most responsible for differences in dietary compositions between size classes by SIMPER analysis. Dietary breadth was lowest for small stingrays ($H' = 0.563$) and greatest for large stingrays ($H' = 0.708$) (Table 3.1).

Diet composition of JSH sharks was significantly different than that of stingrays in Kāneʻohe Bay (ANOSIM, Global R-statistic = 0.842, $P = 0.001$) and all pairwise comparisons between hammerheads and stingray size classes were also significant ($P = 0.001$). Ordination of the dietary data revealed two distinct clusters with no overlap, one composed of JSH samples and the other composed of stingray samples (Fig 3.3). SIMPER analysis identified teleosts and portunid crabs as contributing most to the dissimilarity between JSHs and all stingray size classes. Hammerheads consumed larger amounts of teleosts and smaller amounts of portunids by weight compared to stingrays (Fig. 3.4). Alpheid shrimps also contributed strongly to the dissimilarity between JSHs and large stingrays, with hammerheads consuming larger amounts of alpheids by weight compared to large stingrays (Fig. 3.4). Trophic position based on stomach contents for JSHs was 4.0, approximately 0.5 TPs higher than the average TP for all juvenile stingrays.

Bulk Stable Isotope Analysis

White muscle tissue from 44 juvenile stingrays collected within Kāneʻohe Bay, ranging from 42.1 to 93.1 cm DW, were analyzed for bulk carbon and nitrogen isotopic compositions. These stingrays were a subset of the 156 individuals examined for SCA and represented the entire geographic distribution within Kāneʻohe Bay, full size range and sex of juvenile stingrays. There was no significant effect of sex on bulk $\delta^{13}\text{C}$ ($P = 0.578$) or $\delta^{15}\text{N}$ ($P = 0.774$) values (Table 3.2). Disk width and location explained 51% and 52% of the variance for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values respectively. Of the total explained variance, 17% and 25% was explained by size and 33% and 26% was explained by location for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values respectively (Table 3.2, Fig. 3.5). Stingrays captured in the south part of Kāneʻohe Bay were on average more depleted in ^{13}C (1.4‰, $P < 0.001$) and more enriched in ^{15}N (1.2‰, $P < 0.002$) compared to stingrays captured in the mid and north parts of the bay (Table 3.3, Fig. 3.5). There were significant differences between size classes for $\delta^{13}\text{C}$ values (GLM, $F_{2,39} = 4.17$, $P = 0.023$) and $\delta^{15}\text{N}$ values ($F_{2,39} = 4.74$, $P = 0.015$). Small stingrays were more depleted in ^{13}C ($P = 0.018$) and ^{15}N ($P = 0.013$) compared to large stingrays (Table 3.3, Fig. 3.5). There were no significant differences between small and medium stingrays and between medium and large stingrays. For TP calculations, $\delta^{15}\text{N}$ values of stingrays captured in the south part of the bay were normalized by 1.2‰ to correct for spatial variation. Trophic positions for stingray size classes based on bulk nitrogen isotopic values were in close agreement to those based on SCA and increased with increasing stingray size (range: 3.3 – 3.6, Table 3).

Differences in dietary composition between JSH sharks and juvenile stingrays were also supported by bulk SIA. Juvenile hammerhead sharks were significantly more

depleted in ^{13}C (mean $\delta^{13}\text{C} = -16.41 \pm 0.34$; ANOVA, $F_{3,48} = 89.94$, $P < 0.001$) and more enriched in ^{15}N (mean $\delta^{15}\text{N} = 11.83 \pm 0.60$; ANOVA, $F_{3,48} = 15.61$, $P < 0.001$) compared to all stingray size classes (stable isotopic values of stingrays and JSHs caught in the south bay normalized for spatial variation). The TP of JSHs based on bulk nitrogen isotopic values was 4.1, approximately 0.6 TPs higher than juvenile stingrays (south bay samples normalized for spatial variation).

Stable Nitrogen Isotope Analysis of Individual Amino Acids

An additional 23 muscle samples were collected from very large (>95 cm DW) stingrays captured both within and outside Kāneʻohe Bay. Inclusion of these samples revealed a dramatic decrease in bulk tissue $\delta^{15}\text{N}$ values, and onset of this decrease varied by sex (Fig. 3.6). There are two competing hypotheses which could explain the observed decrease in $\delta^{15}\text{N}$ values; (1) stingrays of all sizes are feeding in habitats that are isotopically similar and the observed decrease in $\delta^{15}\text{N}$ values is due to very large stingrays changing to food that is on average 0.7 TPs lower than that of large (70 – 94.9 cm DW) juvenile stingrays, or (2) very large stingrays are feeding in a habitat with basal nitrogen isotopic values distinct (lower) from those within Kāneʻohe Bay. In order to test these hypotheses, we analyzed the $\delta^{15}\text{N}$ values of individual amino acids from 11 individuals representing the entire DW range that was used to determine the TP of stingrays. Based on this analysis, TP increased in very large stingrays compared to juvenile stingrays. This result is contrary to the trend in bulk tissue results and unambiguously supports hypothesis (2). Namely, very large stingrays are feeding in a habitat isotopically different from Kāneʻohe Bay (Fig. 3.7).

Calculations of TP from amino acid nitrogen isotopic analyses based on a TEF of 7.6‰ and a $\Delta\delta^{15}\text{N}_{\text{glu-phe}}$ of 3.4‰ consistently underestimated absolute TP for stingrays by ~1 (9 out of 11 samples had a TP <3) and JSHs by ~0.7 compared to independent TP estimates based on SCA and bulk SIA (Tables 3.1, 3.3 and 3.4). Based on the close agreement of TP estimates from SCA and bulk SIA, TP estimates from amino acid analysis were increased by 1 (stingrays) and 0.7 (JSHs) TPs and a new TEF for each individual sample was calculated with a $\Delta\delta^{15}\text{N}_{\text{glu-phe}}$ of 3.4‰. New TEFs ranged from 4.1‰ to 5.9‰ with an overall mean of 5.0‰ ($\pm 0.6\%$, 1 SD, Table 3.4) which is 2.6‰ less than the 7.6‰ found by Chikaraishi et al. (2009). Trophic positions calculated with the new TEFs ranged from 3.2 for a 43.6 cm DW stingray to 4.2 for a 110.0 cm DW stingray and 3.9 to 4.1 for JSHs (Fig. 3.7, Table 3.4).

DISCUSSION

Foraging Ecology

The diet of juvenile brown stingrays was dominated by crustaceans, particularly alpheid shrimps and portunid crabs. Brown stingrays primarily use the deep lagoonal portions of Kāneʻohe Bay which are characterized by muddy substrate (Smith et al. 1981, Cartamil et al. 2003). Previous studies suggest that these mud habitats are of low productivity and faunal diversity, and are dominated by alpheids, polychaetes, gobies and portunids (Harrison 1981, Smith et al. 1981, Smith and Kukert 1996, Bush 2003). Collectively, these results suggest that juvenile brown stingrays are opportunistic benthic foragers which utilize the most abundant prey resources of the Kāneʻohe Bay benthos.

These findings are consistent with dietary studies of dasyatid rays in other locations (Hess 1961, Struhsaker 1969, Gilliam and Sullivan 1993, Taniuchi and Shimizu 1993, Ismen 2003). Alpheids are a common prey item for dasyatids from Florida and the eastern Mediterranean (Snelson and Williams 1981, Yeldan et al. 2009), whereas portunids were common in the diets of dasyatids from the central Bahamas and western Atlantic (Struhsaker 1969, Gilliam and Sullivan 1993). Site-specific differences in prey abundances likely contribute to a lower importance of crustaceans for some dasyatid rays. For example, polychaetes were important prey items for dasyatids from West and South Africa in habitats where polychaetes were abundant (Devadoss 1978, Ebert and Cowley 2003). Additionally, teleosts contributed significantly to the diets of dasyatids from the central Bahamas, West Africa and Japan (Devadoss 1978, Gilliam and Sullivan 1993, Taniuchi and Shimizu 1993).

The dietary composition of juvenile brown stingrays varied with stingray size. Although crustaceans were the dominant prey for all size classes, the relative contribution of different prey items varied. Small stingrays primarily fed on alpheids, with minor contributions from the portunid crab, *Libystes villosus*, and polychaetes. These are all small prey items, likely easy prey for small stingrays. As brown stingrays increase in size, the contribution of alpheids decreases substantially (<10% IRI for large stingrays), with a corresponding increase in the contribution of portunids, particularly *P. vigil* (>50% IRI for large stingrays). Larger stingrays also fed more evenly on potential prey items as indicated by an increase in dietary breadth with increasing size. The ontogenetic dietary shift indicated by stomach content analysis was supported by bulk and compound-specific stable isotope analyses. Both bulk tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and the TP based

on amino acid $\delta^{15}\text{N}$ values increased with increasing size, consistent with a dietary shift from smaller, lower trophic level prey (alpheids and polychaetes) to larger, higher trophic level prey (*P. vigil* and gobies). Ontogenetic diet shifts are common for elasmobranchs and these shifts have been linked to morphological, behavioral and physiological characteristics (Scharf et al. 2000, Wetherbee and Cortés 2004, Lucifora et al. 2006). In this study, a positive correlation between the size of *P. vigil* consumed and stingray morphology suggests that the abundance of small prey items in the diet of small stingrays is largely due to gape limitations. Ingestion of less abundant but larger, more energetically valuable prey may decrease the amount of time and energy larger stingrays spend foraging (Scharf et al. 2000) and would limit competition with smaller conspecifics. Lucifora et al. (2006) suggested that small sharks should have higher consumption rates, and therefore a lower proportion of empty stomachs, due to higher mass-dependent metabolic rates. However, small brown stingrays had the largest percentage of empty stomachs in this study, potentially due to slow development of foraging skills as has been suggested for newborn JSH sharks in Kāneʻohe Bay (Lowe 2002, Bush 2003).

Trophic position calculations based on stomach content and bulk SIA were in close agreement, classifying juvenile brown stingrays as secondary consumers. In order to calculate stingray TP from bulk $\delta^{15}\text{N}$ values, we assumed a TEF of 2.7‰. However, several studies have indicated significant variation in TEFs due to variables such as species, tissue type, diet, environment, method of excretion and biochemical composition (Pinnegar and Polunin 1999, Vander Zanden and Rasmussen 2001, McCutchan et al. 2003, Vanderklift and Ponsard 2003, MacNeil et al. 2006, Barnes et al. 2007, Caut et al.

2009). Although TEFs have not yet been experimentally determined for stingrays, Hussey et al. (2010a) determined a TEF of $2.3 \pm 0.2\%$ for three adult sand tiger (*Carcharias taurus*) and one sub-adult lemon shark (*Negaparon brevirostris*) in a long-term (12 month) controlled feeding experiment. However, the concordance of TP estimates between stomach content and stable isotope methods in our study supports the assumed ecosystem-level TEF of 2.7%. Stomach content and bulk tissue SIA both indicated an increase in TP with increasing size for juvenile stingrays, although these increases were generally small (0.3 - 0.4 TPs). This small increase is predominantly driven by stingrays shifting to larger, higher trophic level prey while retaining crustaceans as their primary prey resource. There is little information on TPs for other stingray species. A TP of 3.7 was estimated for marbled stingrays (*Dasyatis marmorata*) from the Mediterranean and skates primarily feeding on decapods had TPs ranging from 3.5 – 3.9, similar to those observed in this study (Stergiou and Karpouzi 2002, Ebert and Bizzarro 2007).

Habitat use

Bulk SIA indicated a significant effect of capture location on stingray $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Stingrays captured in the southern part of Kāneʻohe Bay were consistently depleted in ^{13}C and enriched in ^{15}N compared with stingrays caught in the mid and north bay. A lack of spatial variation in juvenile stingray diet composition suggests stable isotopic differences are due to variability in baseline values between bay zones. Variation in baseline values is supported by the independence of stingray bulk tissue $\delta^{15}\text{N}$ values and TPs calculated from amino acid isotopic analysis, which indicates stingray

bulk tissue $\delta^{15}\text{N}$ values are controlled primarily by variations in baseline $\delta^{15}\text{N}$ values within the bay. The south part of Kāneʻohe Bay is characterized by relatively long water residence times (13 d vs. 8 d for the remainder of the bay), receives 30% of the stream runoff entering the bay, and is bordered by relatively high levels of human population (Smith et al. 1981). In addition, sediments may still be heavily impacted from treated sewage dumped into the south bay from 1951 to 1978. Recent analyses showed that high rates of dissolved inorganic nitrogen efflux continue to support macroalgae growth within the south bay (Smith et al. 1981, Stimson et al. 2001). Cumulatively, these factors likely contribute substantially to the low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ values found in stingrays captured in the south part of the bay because freshwater and anthropogenic inputs have been shown to decrease $\delta^{13}\text{C}$ and increase $\delta^{15}\text{N}$ values respectively in coastal ecosystems (McClelland and Valiela 1998, Vizzini et al. 2005). These results highlight the importance of understanding spatial variability of basal stable isotopic values for foraging ecology studies, even in relatively small ecosystems. Our results show that incorporation of stable isotopic analysis of individual amino acids can provide the information necessary for gaining insight into the underlying causes of variations in bulk tissue $\delta^{15}\text{N}$ values.

Stable isotope analysis has been used to determine residence time and detect small scale migration within and between habitats for a variety of fishes (e.g. Cocheret de la Morinière et al. 2003). Despite relatively long nitrogen isotopic turnover times for muscle in elasmobranchs (>1 yr; MacNeil et al. 2006, Logan and Lutcavage 2010), we were able to detect both site fidelity within and recent migrations between bay zones. Capture location for the majority of stingrays sampled during this study could be

identified by their bulk stable isotopic values, indicating long term foraging in that area. In a prior study, stingrays actively tracked in the south part of Kāneʻohe Bay remained in the area for the duration of the study (>3 days, Cartamil et al. 2003). However, some stingrays displayed stable isotopic values distinctly different from their capture location suggesting recent migration between bay zones. Alternative methods such as passive acoustic tracking and/or SIA utilizing tissues with faster turnover times (e.g. blood, liver) might be required to more accurately describe the movement patterns of stingrays throughout the bay.

Analysis of individual amino acids in stingray muscle samples allowed us to evaluate competing hypotheses generated from bulk SIA and calculate TPs for brown stingrays (including very large stingrays from offshore habitats) and JSH sharks without prior knowledge of the $\delta^{15}\text{N}$ values of primary producers. Sampling of all potential primary producers in offshore habitats would have been challenging due to the depths at which very large stingrays typically occur (>40 m). Trophic positions based on recalculated TEFs (stingrays range: 3.2 – 4.2; JSHs range: 3.9 – 4.1) were consistent with those based on stomach content and bulk SIA. The increase in stingray TP based on amino acid analysis was independent of bulk $\delta^{15}\text{N}$ values (especially evident for very large stingrays) and confirmed a foraging habitat shift from inside Kāneʻohe Bay to offshore waters. The onset of this shift coincides closely with the onset of sexual maturity. Males mature and migrate to offshore habitats at a smaller size than females (Fig. 3.6, Chapter II).

Heupel et al. (2007) proposed 3 criteria for defining an area as a nursery: (1) juveniles are more commonly encountered in the nursery than other areas. In our case,

juvenile brown stingrays were rarely captured during ongoing longline fishing surveys outside Kāneʻohe Bay (J. Dale unpublished data); (2) juveniles have a tendency to remain or return for extended periods. Results from SIA suggest that juvenile brown stingrays forage within Kāneʻohe Bay for the majority of their juvenile lives; and (3) the habitat is repeatedly used across years. Juvenile stingrays were captured across multiple years during this study indicating repeated use of the bay habitat. Thus, based on the criteria proposed by Heupel et al. (2007), Kāneʻohe Bay is an important nursery habitat for brown stingrays.

The relative ontogenetic and interspecific variation in TPs calculated from amino acid analysis, regardless of the TEF used, was consistently represented for brown stingrays and JSHs in this study and further demonstrates the value of this method for understanding the foraging ecology of elasmobranchs. All elasmobranchs are carnivores, requiring a minimum TP of 3.0 (Wetherbee and Cortés 2004). However, in this study, initial calculations of absolute TPs systematically underestimated TP by ~1 TP (stingrays; range: 2.2 – 3.2) and ~0.7 TPs (JSHs; range: 3.2 – 3.4). Assuming the $\Delta\delta^{15}\text{N}_{\text{glu-phe}}$ of 3.4‰ is correct (Chikaraishi et al. 2009), we estimated an average TEF of $5.0 \pm 0.6\text{‰}$ for brown stingray and JSH shark muscle. A previous study utilizing stable isotopic compositions of amino acids found a TEF of 3.6‰ more accurately represented penguin TPs (Lorrain et al. 2009). That difference was attributed to the use of blood samples for isotopic analysis, whereas earlier studies had used whole animal or muscle tissue (McClelland and Montoya 2002, Chikaraishi et al. 2007, Popp et al. 2007, Chikaraishi et al. 2009, Hannides et al. 2009).

The use of muscle in the current study precludes tissue biochemical effects as a viable explanation for the lower observed TEF from amino acid analysis. Alternatively, lower observed TEFs in brown stingray and JSH shark muscle may be due to lower reaction rates involving glutamic acid as a consequence of urea retention for osmotic regulation (Evans et al. 2004). This has been suggested from elasmobranch bulk tissue isotopic analysis (e.g., Fisk et al. 2002, Hussey et al. 2010b) and is consistent with retention of ^{15}N -depleted waste products. Trophic enrichment of glutamic acid in elasmobranchs is more difficult to explain but likely results from its frequent use in transamination and deamination reactions (Gannes et al. 1998, Chikaraishi et al. 2007). Glutamate can be converted to α -ketoglutarate by glutamate dehydrogenase (GDH) for use in the citric acid cycle (Ballantyne 1997) or converted to glutamine by glutamine synthetase to provide nitrogen to the ornithine-glutamine-urea pathway (Anderson 1991). Activity levels of GDH provide a measure of importance of amino acids as an oxidative substrate and a strong inverse relationship was found between hepatic GDH and urea levels in elasmobranch muscle (Speers-Roesch et al. 2006). Increased importance of the glutamate-glutamine-urea pathway could result in lower glutamate catabolism (Speers-Roesch et al. 2006) and reduced ^{15}N enrichment in glutamic acid in muscle tissue. Elasmobranchs use a unique carbamoyl phosphate synthetase (CPSase III) that utilizes glutamine in the pool of free amino acids in liver mitochondrial cells as the nitrogen-donating substrate rather than ammonia for urea formation (Julsrud et al. 1998). We speculate that reduced hepatic glutamate catabolism resulted in lower ^{15}N enrichment of glutamic acid in muscle tissue of stingrays and JSHs. Nitrogen isotope fractionation associated with cleavage of the amide functional group of glutamine should be smaller

than that associated with cleavage of the single amine group of glutamate because glutamine has two nitrogen atoms and only one nitrogen bond is broken. Therefore we suggest that ^{15}N enrichment should be lower in glutamic acid exiting the liver to form muscle protein in elasmobranchs with high muscle urea concentrations and perhaps in other animals that excrete urea (or uric acid) as a waste product.

These results suggest that urea retention in elasmobranchs can have important impacts on metabolic pathways involving glutamic acid which could influence TPs determined using amino acid compound-specific isotopic analyses. Consequently, TP estimates based on a TEF of 7.6‰ estimated from non-ureosmotic species would result in an underestimation of absolute stingray and JSH shark TPs. However, in the only other study to use nitrogen isotopic analysis of individual amino acids to estimate TP for an elasmobranch, Chikaraishi et al. (2010) estimated a TP of 4.8 for a *Squalus sp.* using a TEF of 7.6‰. This is 0.6 – 0.9 TPs higher than TPs estimated from stomach content studies for this genus (3.9-4.2; Cortés 1999). It is known that amino acids rather than lipids can be important oxidative substrates in elasmobranch muscle tissue (Ballantyne 1977), thus in addition to ureagenesis, TEF could vary with relative animal activity. For example, the higher TEF we found for JSHs could be due to lower urea production in the liver or higher muscle activity in JSHs relative to brown stingrays. These contradictory results emphasize the need to further investigate the relationship between urea content in elasmobranchs, hepatic GDH activity, animal activity and the extent of ^{15}N enrichment in glutamic acid for white muscle tissue. In order to adapt to hypotonic environments, freshwater elasmobranchs retain low levels of urea (Thorson et al. 1967, Tam et al. 2003) and express higher levels of hepatic GDH activity than marine elasmobranchs (Speers-

Roesch et al. 2006). Therefore, future experimental studies should also focus on both marine and freshwater species to evaluate the potential effects of urea on amino acid TEFs.

Ecological interactions

Significant differences exist in the foraging ecology of brown stingrays and JSH sharks, suggesting available prey resources are partitioned between these two species in Kāneʻohe Bay. Differences between diets were primarily due to a larger contribution of teleosts to the diet of JSHs and a larger contribution of portunids to the diet of juvenile stingrays. A larger contribution of teleosts to the JSH diet is also supported by bulk tissue and amino acid stable isotope analysis. Hammerhead bulk muscle tissue was enriched in ^{15}N by $\sim 1.5\text{‰}$ compared to juvenile stingray muscle and the amino acid based TP was higher by ~ 0.5 indicating that hammerheads are feeding higher in the food web. These dietary differences can potentially be explained by differences in mouth morphology and prey capture behavior between species. Brown stingrays have larger mouths than JSH sharks which would allow them to forage on larger prey such as portunids. The prey capture behavior of brown stingrays is similar to that described for the blue stingray (*Dasyatis chrysonota*, Ebert and Cowley 2003). Once a prey item is located, the stingray quickly settles upon the substrate, trapping prey under its body, followed by suction of prey into the mouth (J. Dale pers. obs.). Although this foraging mechanism is well suited for benthic prey, it may limit the stingray's ability to catch faster, more mobile prey such as teleosts. In contrast, the use of ram feeding by JSHs would facilitate capture and ingestion of teleosts. Similar mechanisms were suggested to

explain low dietary overlap between a rhinobatid ray and sharks in an Australian bay (White et al. 2004).

Conclusions

Brown stingrays use Kāne‘ohe Bay as a nursery ground and, while there, tend to show restricted movements within sub-sections of the bay. Stingrays move out of the bay when they become sexually mature although some adults are occasionally found within the bay. Juvenile brown stingrays are generalist benthic predators whose diets reflect the low diversity of potential prey items in Kāne‘ohe Bay. Ontogenetic shifts in dietary composition are correlated with mouth size, with larger individuals able to forage on larger, more energetically valuable prey. Future studies focusing on the growth rates and energetics of brown stingrays would provide further insight into their role in ecosystem energy flow and impact on prey populations (e.g. Lowe 2002). This study demonstrated that analysis of the nitrogen isotopic composition of individual amino acids is a viable method for detecting ontogenetic foraging habitat shifts and determining relative trophic positions for elasmobranchs. However, our results suggest that the TEF used to calculate absolute TP in previous studies requires adjustment for elasmobranchs, potentially due to retention of urea as an osmolyte and its affect on biochemical reactions involving glutamic acid. Controlled experiments are required to better understand the effect of urea on the metabolic pathways of amino acids, particularly glutamic acid. Prey resource partitioning between brown stingrays and JSHs in Kāne‘ohe Bay indicate competition for prey resources is not a factor contributing to high mortality rates of hammerhead pups.

Table 3.1 Mean and standard deviation (S.D.) of percent number ($\overline{\%N}$) and percent weight ($\overline{\%W}$), percent frequency occurrence (%FO) and mean and S.D. of percent index of relative importance ($\overline{\%IRI}$) of prey consumed by juvenile brown stingrays in Kāneʻohe Bay. Sample size, trophic level and Shannon-Wiener Index given at the bottom of each size class.

Prey	35.0 - 54.9 cm DW							55.0 - 69.9 cm DW							70.0 - 94.9 cm DW						
	$\overline{\%N}$	S.D.	$\overline{\%W}$	S.D.	%FO	$\overline{\%IRI}$	S.D.	$\overline{\%N}$	S.D.	$\overline{\%W}$	S.D.	%FO	$\overline{\%IRI}$	S.D.	$\overline{\%N}$	S.D.	$\overline{\%W}$	S.D.	%FO	$\overline{\%IRI}$	S.D.
Crustacea	86.51	22.40	87.73	23.86	96.97	93.25	18.30	79.01	27.16	78.57	31.45	93.65	86.46	25.52	74.95	27.43	81.55	30.38	97.50	84.67	23.32
Alpheidae																					
Alpheus malabaricus	68.05	27.44	55.50	34.42	93.94	78.37	26.26	38.63	30.81	20.40	29.72	77.78	40.58	31.49	13.64	20.97	2.64	6.60	40.00	8.58	15.16
Unidentified alpheid	0.52	2.29	0.08	0.42	6.06	0.02	0.10	0.49	2.86	0.00	0.01	3.17	0.02	0.13							
Portunidae																					
Podophthalmus vigil	3.76	7.56	9.50	22.32	24.24	2.78	6.72	15.38	26.92	20.83	35.16	38.10	16.63	29.44	36.36	36.54	47.11	43.36	70.00	50.70	39.58
Portunus granulatus															2.50	15.81	2.50	15.81	2.50	2.50	15.81
Portunus longispinosus	0.18	1.02	0.86	4.94	3.03	0.02	0.14	0.29	2.29	0.14	1.10	1.59	0.02	0.16	1.25	7.91	1.87	11.82	2.50	0.22	1.38
Libystes villosus	10.95	21.45	17.39	28.95	42.42	11.03	22.24	16.25	21.49	27.48	34.21	50.79	23.21	27.85	8.76	16.14	12.49	25.44	30.00	8.96	16.78
Unidentified Portunidae								0.45	2.52	0.41	2.35	3.17	0.09	0.57							
Unidentified crab	2.12	9.27	4.18	17.45	6.06	0.51	2.33	3.96	14.45	4.42	16.25	12.70	2.36	12.75	4.82	12.48	6.73	18.97	17.50	4.04	10.58
Stomatopoda																					
Oratosquilla oratoria								1.36	7.68	0.76	5.36	3.17	0.27	1.82	0.86	3.84	1.57	6.95	5.00	0.15	0.64
Gonodactylaceus falcatus								0.34	2.70	0.35	2.75	1.59	0.06	0.49							
Pseudosquilla ciliata								1.33	6.38	2.54	12.46	4.76	0.62	2.82	3.01	15.07	3.80	17.54	5.00	1.77	9.41
Unidentified mantis	0.93	3.05	0.22	0.71	9.09	0.09	0.30	0.52	3.28	1.24	6.95	3.17	0.08	0.47	3.75	14.69	2.83	15.58	10.00	2.35	13.70
Polychaeta	9.33	20.84	6.69	18.24	36.36	5.50	17.70	7.06	18.65	6.01	19.49	26.98	4.47	17.72	1.80	6.97	1.44	7.70	7.50	0.28	1.31
Unidentified Polychaeta	9.33	20.84	6.69	18.24	36.36	5.83	17.76	7.06	18.65	6.01	19.49	26.98	5.27	17.96	1.80	6.97	1.44	7.70	7.50	0.43	2.01
Osteichthyes	2.34	7.25	1.25	4.11	15.15	0.34	1.05	9.50	17.51	8.82	20.56	39.68	6.15	15.65	20.76	24.23	13.88	24.24	55.00	14.26	22.04
Gobiidae	2.20	7.25	1.24	4.11	12.12	0.38	1.24	8.81	17.65	7.37	19.16	33.30	6.85	17.17	17.96	24.68	12.24	23.78	45.00	17.73	27.80
Ophichthidae								0.18	1.40	1.04	8.27	1.59	0.04	0.30							
Unidentified teleost	0.14	0.79	0.01	0.05	3.03	0.00	0.02	0.52	2.42	0.41	3.15	4.76	0.05	0.28	2.80	8.16	1.64	7.92	12.50	1.08	3.54
Miscellaneous	1.89	5.43	4.46	16.87	15.15	0.95	3.98	4.42	14.06	6.60	18.14	22.22	2.92	12.92	2.49	6.38	3.13	9.71	15.00	0.78	2.33
Unidentified remains	1.89	5.43	4.46	16.87	15.15	1.00	4.07	4.42	14.06	6.60	18.14	22.22	3.86	13.96	2.49	6.38	3.13	9.71	15.00	1.49	4.26
Sample size (empty)				42 (9)							66 (3)							48 (8)			
Trophic Level				3.3							3.5							3.7			
Shannon-Wiener Index				0.563							0.691							0.708			

Table 3.2 Results of GLM evaluating differences in juvenile brown stingray bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between bay zones (Location) and sex, with size (Disk Width) as a covariate.

Source	DF	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$			
		SS	MS	F	P	SS	MS	F	P
Disk Width	1	9.296	11.737	17.33	< 0.001	14.469	5.612	8.04	0.007
Location	2	18.154	9.077	13.40	< 0.001	15.053	7.526	10.78	< 0.001
Sex	1	0.849	0.213	0.31	0.578	0.276	0.058	0.08	0.774
Error	39	26.420	0.677			27.229	0.698		
Total	43	54.719				57.026			

Table 3.3 Mean and standard deviation (S.D.) of juvenile brown stingray muscle bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the three size classes by bay zone. Trophic position estimates of each size class for all zones combined provided.

Size Class (cm)	South Bay				Mid Bay				North Bay				Trophic Position ^a
	$\delta^{13}\text{C}$	S.D.	$\delta^{15}\text{N}$	S.D.	$\delta^{13}\text{C}$	S.D.	$\delta^{15}\text{N}$	S.D.	$\delta^{13}\text{C}$	S.D.	$\delta^{15}\text{N}$	S.D.	
35.0 - 54.9	-13.45	0.73	10.37	0.68	-11.65	1.39	9.6	1.38	-11.63	0.45	9.37	0.64	3.3
55.0 - 69.9	-12.66	0.34	11.91	0.47	-11.48	0.43	9.64	0.61	-10.33	0.04	10.68	0.54	3.5
70.0 - 94.9	-11.85	0.96	11.62	0.18	-11.49	0.91	10.57	0.35	-10.61	1.12	10.35	0.89	3.6

^aTrophic position calculations based on corrected bulk $\delta^{15}\text{N}$ values for stingrays captured in the south bay (see Results).

Table 3.4 Summary of $\delta^{15}\text{N}$ values of glutamic acid (Glu) and phenylalanine (Phe) for 11 stingrays (S1-S11) and 3 juvenile scalloped hammerhead sharks (JSH1-JSH3). Sizes are reported as disk width for stingrays and fork length for JSHs. TP1: Trophic position estimated from $\delta^{15}\text{N}$ values Glu and Phe using the trophic enrichment factor (TEF) of 7.6‰ from Chikaraishi et al. (2009), see Methods for details. TP2: Revised trophic position estimated by increasing TP1 by 1 (stingrays) and 0.7 (JSHs) trophic positions; see Results for details. TEF2: Recalculated TEF for each individual sample based on their corresponding TP2 value.

Sample ID	Size (cm)	Bulk $\delta^{13}\text{C}$	Bulk $\delta^{15}\text{N}$	$\delta^{15}\text{N}$ Glu (± 1 SD)	$\delta^{15}\text{N}$ Phe (± 1 SD)	TP1 _{Glu/Phe} (± 1 SD)	TP2 _{Glu/Phe}	TEF2
S1	43.6	-13.9	10.4	18.1 \pm 0.3	6.0 \pm 0.3	2.2 \pm 0.1	3.2	4.1
S2	43.7	-13.9	11.3	18.1 \pm 0.3	3.8 \pm 0.5	2.4 \pm 0.2	3.4	4.5
S3	74.1	-12.5	11.8	22.1 \pm 0.3	5.8 \pm 0.1	2.7 \pm 0.1	3.7	4.8
S4	104.6	-15.7	8.8	20.2 \pm 0.7	2.4 \pm 0.4	2.9 \pm 0.2	3.9	5.0
S5	106.4	-16.7	8.8	20.6 \pm 0.7	2.5 \pm 0.1	2.9 \pm 0.2	3.9	5.0
S6	42.1	-12.3	8.5	18.5 \pm 0.1	5.8 \pm 0.2	2.2 \pm 0.1	3.2	4.2
S7	60.0	-11.7	10.7	21.6 \pm 0.8	3.0 \pm 0.5	3.0 \pm 0.3	4.0	5.1
S8	110.0	-15.9	8.0	19.1 \pm 0.3	-1.2 \pm 0.6	3.2 \pm 0.2	4.2	5.2
S9	130.6	-13.7	11.1	21.9 \pm 0.6	3.3 \pm 0.6	3.0 \pm 0.3	4.0	5.1
S10	63.5	-11.0	9.9	19.1 \pm 0.2	1.9 \pm 0.8	2.8 \pm 0.3	3.8	4.9
S11	88.7	-16.1	9.1	20.5 \pm 0.5	2.8 \pm 0.4	2.9 \pm 0.2	3.9	5.0
JSH1	36.0	-15.9	13.6	26.1 \pm 0.3	4.4 \pm 0.8	3.4 \pm 0.3	4.1	5.9
JSH2	37.5	-15.9	12.3	25.2 \pm 0.4	5.2 \pm 0.9	3.2 \pm 0.3	3.9	5.8
JSH3	38.5	-16.7	13.0	25.1 \pm 0.2	3.2 \pm 0.4	3.4 \pm 0.2	4.1	5.9

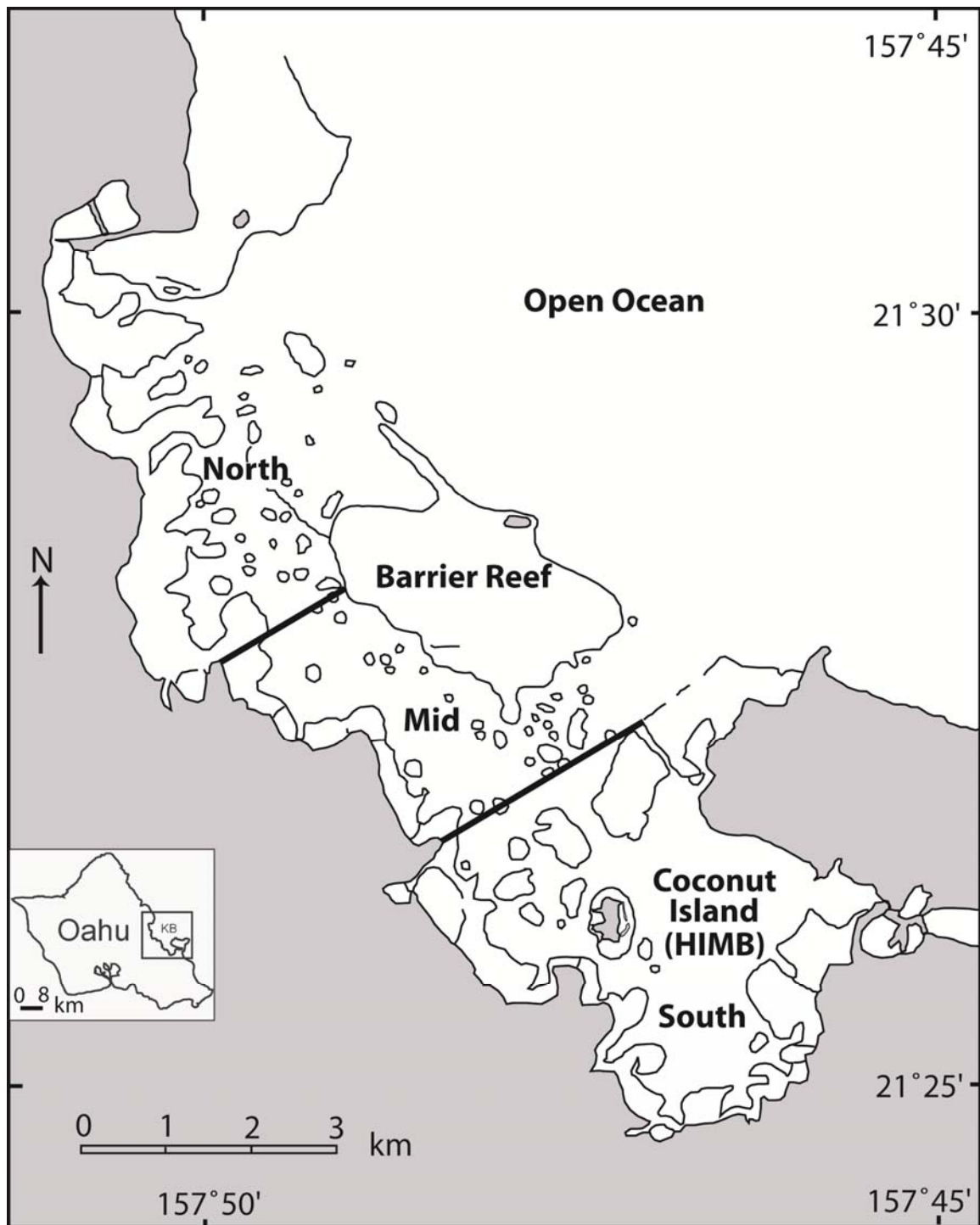


Figure 3.1 Map of Kāneʻohe Bay showing outline of patch reefs and bay zones (South, Mid and North). Inset: location of Kāneʻohe Bay on Oahu, Hawaiʻi.

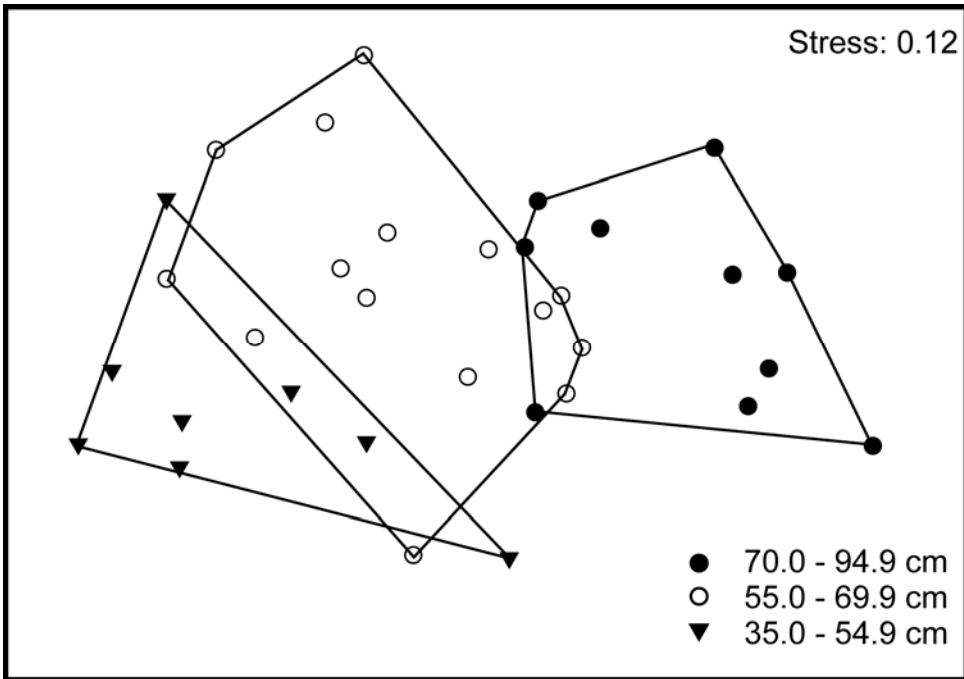


Figure 3.2 Non-metric multidimensional scaling of the mean gravimetric dietary data for the three size classes of juvenile brown stingrays.

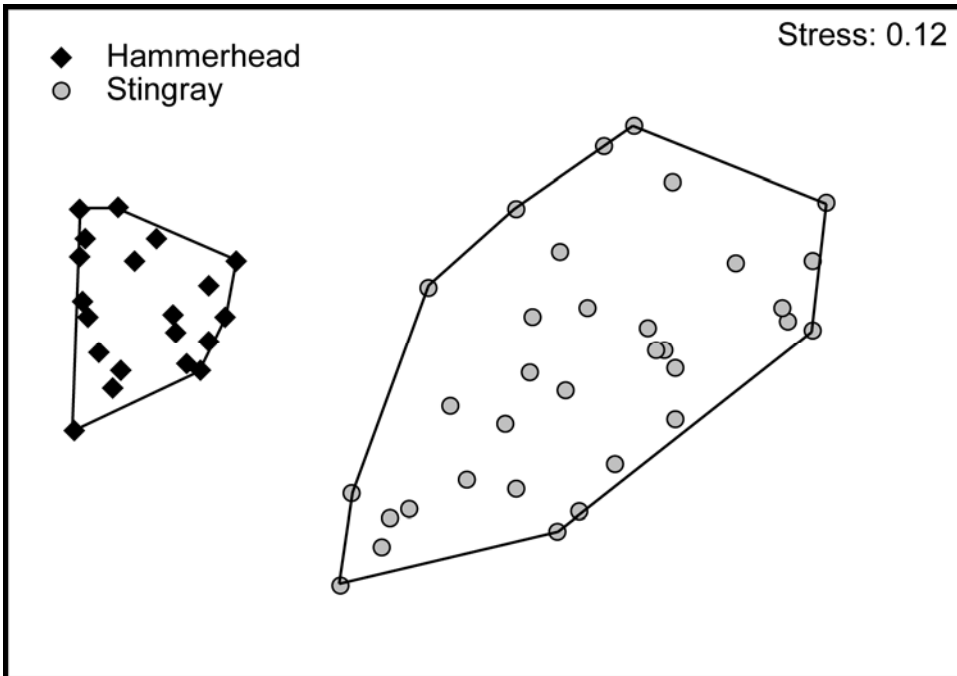


Figure 3.3 Non-metric multidimensional scaling of the mean gravimetric dietary data for juvenile brown stingrays and juvenile scalloped hammerhead sharks. Juvenile scalloped hammerhead dietary data reanalyzed from Bush (2003).

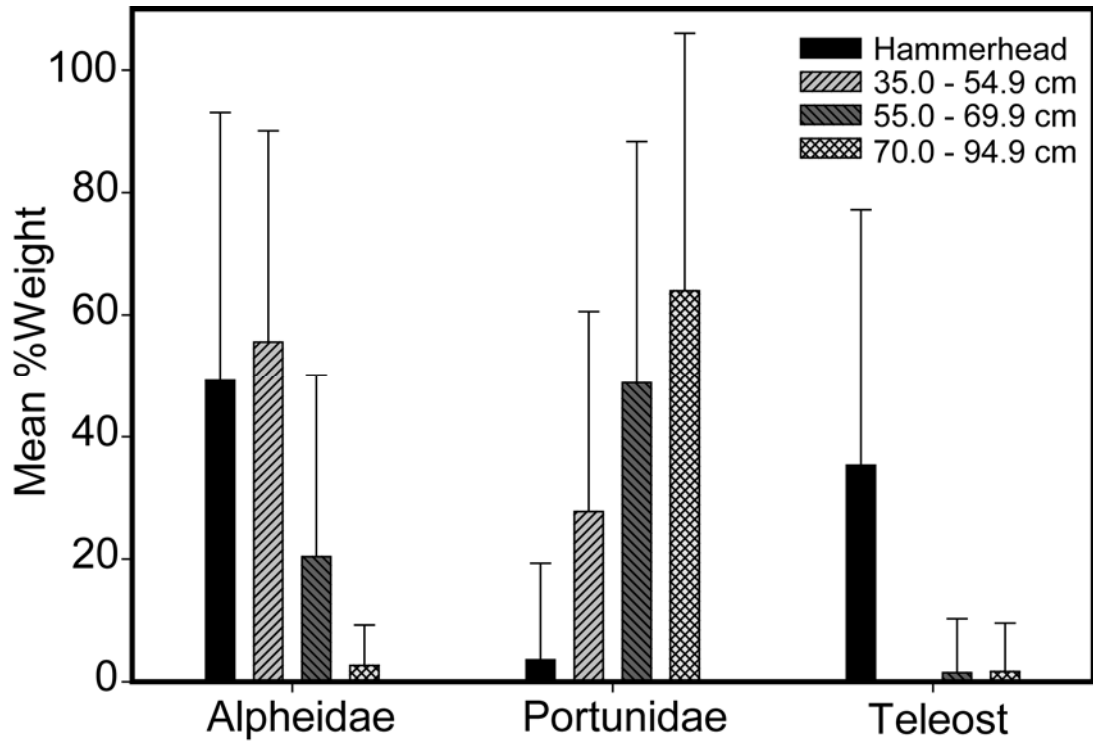


Figure 3.4 Gravimetric contribution (± 1 S.D.) of the prey groups primarily responsible for differences in dietary composition between the three size classes of brown stingrays and juvenile scalloped hammerhead sharks. Juvenile scalloped hammerhead dietary data reanalyzed from Bush (2003).

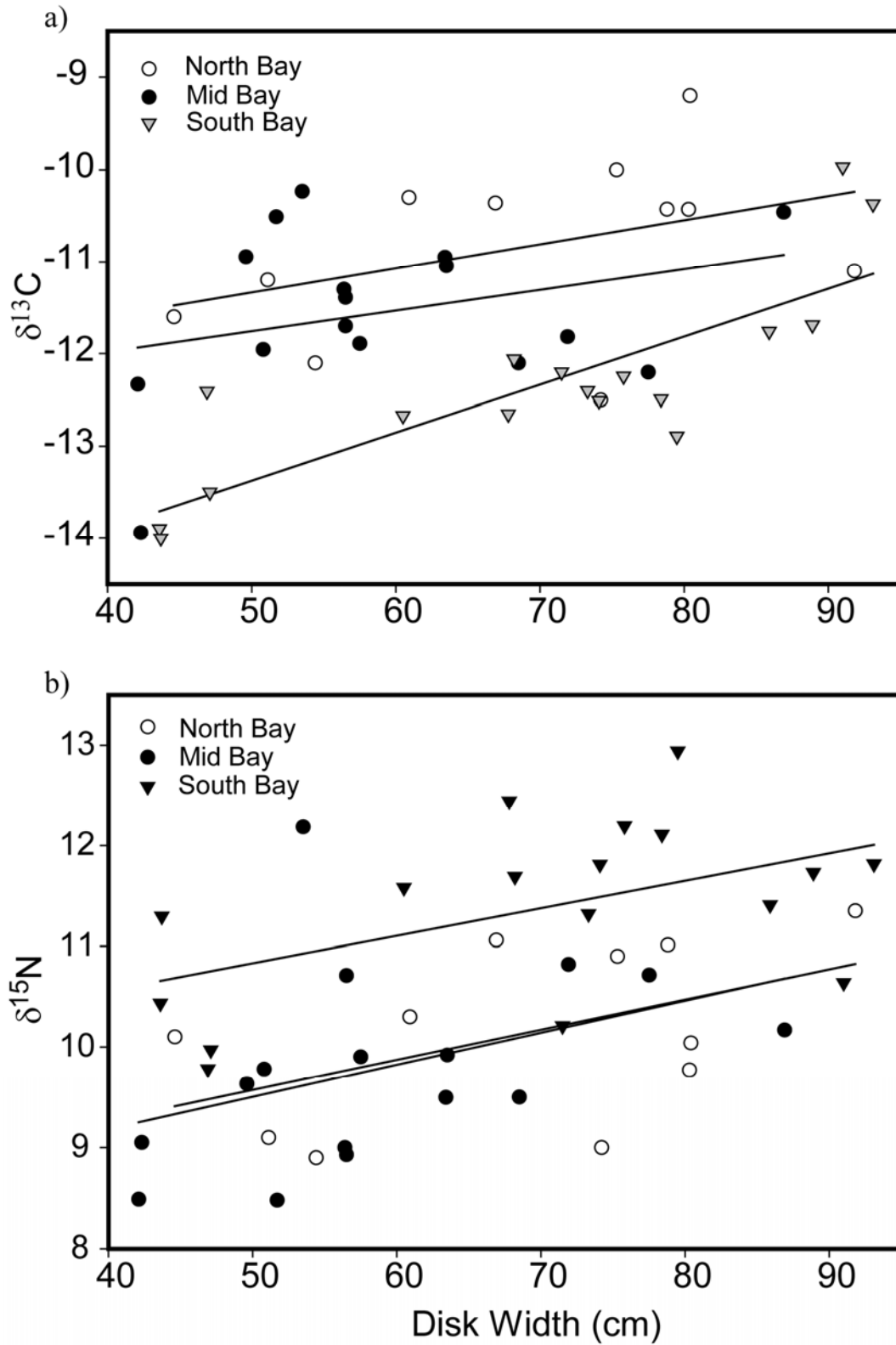


Figure 3.5 Effect of size and capture location of juvenile brown stingrays on bulk (a) $\delta^{13}\text{C}$ values and (b) $\delta^{15}\text{N}$ values.

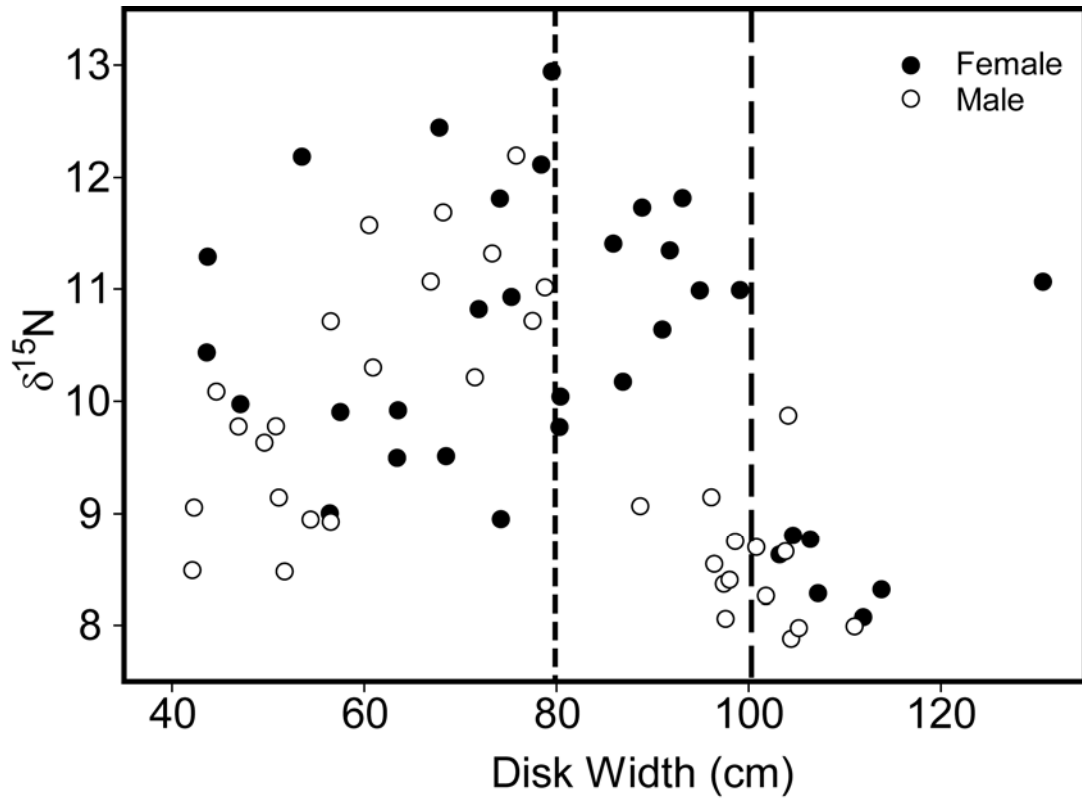


Figure 3.6 Effect of size on bulk $\delta^{15}\text{N}$ values for juvenile and adult brown stingrays captured both within and outside Kāneʻohe Bay. Dashed lines represent approximate size of sexual maturity (J. Dale unpublished data, males: short dash, females: long dash).

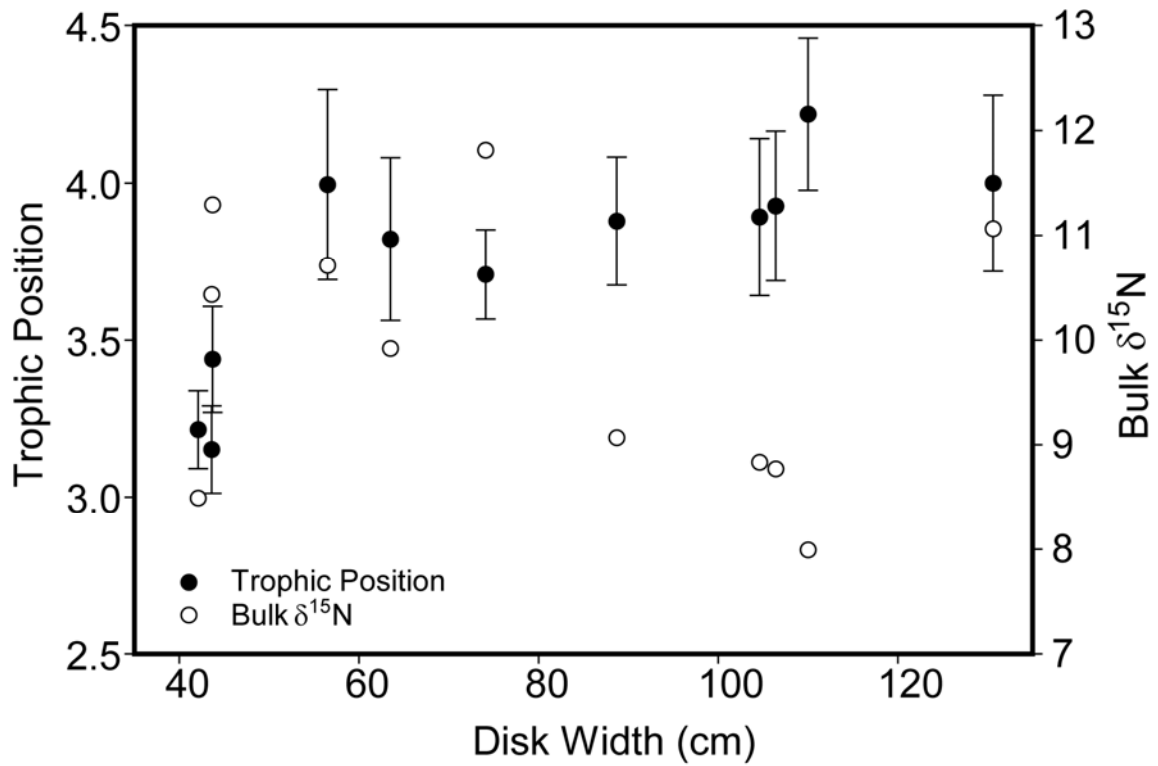


Figure 3.7 Relationship between size, amino acid-based trophic position (± 1 S.D. of propagated error) and bulk $\delta^{15}\text{N}$ values for juvenile and adult brown stingrays captured both within and outside Kāneʻohe Bay and analyzed for the nitrogen isotopic composition of individual amino acids. Trophic position estimates based on recalculated trophic enrichment factors for each individual (see Results).

CHAPTER IV

METABOLIC RATES AND BIOENERGETICS

ABSTRACT

Standard metabolic rates (MO_2) were estimated for juvenile brown stingrays (JBS), *Dasyatis lata*, through respirometry and used as input parameters for a bioenergetics models to predict consumption rates. Stingrays used in respirometry were seasonally acclimated to experimental temperatures (22.5 to 27.3°C) and ranged in mass from 1.03 to 9.85 kg. Estimates of mass-specific MO_2 ranged from 53.06 $mgO_2\ kg^{-1}\ h^{-1}$ for a 9.85 kg individual at 23°C to 115.99 $mgO_2\ kg^{-1}\ h^{-1}$ for a 1.16 kg animal at 27°C. Temperature and mass had significant effects on mass-independent metabolic rates. Standard metabolic rates increased with temperature at a Q_{10} (22-27°C) of 1.8 and increased with mass following the allometric equation: $MO_2\ (mgO_2\ h^{-1}) = 105 \times M^{0.78}$. The energy budget of JBS was heavily weighted towards metabolism, which accounted for 68% of total consumed energy. The growth component of the energy budget accounted for only 4% of the energy budget, indicating very slow growth and potential limitation of food resources. Daily ration estimated from the bioenergetics model declined from a high of 2.72 %BW/d for an age-2 stingray down to 1.23 %BW/d for an age-14 stingray. Population consumption rates based on a range of population estimates suggest the potential for strong top-down effects on prey populations due to stingray predation. The use of Kāne‘ohe Bay as a nursery habitat for JBS appears to be a trade-off between increased juvenile survival and a late age at first maturity due to slow growth rates.

INTRODUCTION

Many studies have estimated the food habits of elasmobranchs, which collectively suggest they are high trophic position predators in marine ecosystems (Cortés 1999, Ebert and Bizzaro 2007). As high trophic position predators, elasmobranchs may have important impacts on prey populations and food web dynamics (Heithaus 2004). However, food habit studies only provide information on the relative contribution of prey items to the predator's diet. In order to evaluate the ecosystem impacts of elasmobranch predation, consumption rates must also be quantified. Understanding the trophic impacts of elasmobranchs has become increasingly important due to the effects of fisheries removal and habitat alteration on elasmobranch populations (Cortés 2004, Jennings et al. 2008, Pierce and Bennett 2010). Because biological systems conform to the laws of thermodynamics, information on energy used in growth and metabolism and energy lost through wastes can be incorporated into bioenergetics models to estimate consumption rates (Brett and Groves 1979). Although relatively uncommon, several recent studies have used bioenergetics models to estimate consumption rates for elasmobranchs (Lowe 2002, Schindler et al. 2002, Dowd et al. 2006a, Bethea et al. 2007).

Metabolic rates are typically the largest and most variable components of an organism's daily energy budget (Boisclair and Sirois 1993, Lowe 2001), having the greatest effect on consumption rates estimated from bioenergetics models (e.g. Bartell et al. 1986). Due to the difficulties of measuring metabolic rates for elasmobranchs, bioenergetics models often lack species-specific data for metabolism (Stillwell and Kohler 1982, 1993, Medved et al. 1988, Schindler et al. 2002), limiting the accuracy of

consumption rate estimates. For example, estimates of daily ration for juvenile sandbar sharks, *Carcharhinus plumbeus*, which incorporated species-specific metabolic rates (Dowd et al. 2006a) were 60-70% higher than previous estimates which used metabolic rates of spiny dogfish (*Squalus acanthias*, Stillwell and Kohler 1993, Medved et al. 1988). Such inconsistencies could create significant bias when evaluating the trophic impacts of elasmobranchs in marine ecosystems. Clearly, obtaining species-specific estimates of metabolic rates should be of high priority when estimating daily ration from bioenergetics models.

Several demographic studies have shown that survivorship of juvenile age classes contributes significantly to elasticity analyses, and minimizing negative impacts on juveniles is one of the most effective ways of stabilizing populations (Heppel et al. 1999, Cortés 2002, Pierce and Bennett 2010). Juveniles of many elasmobranch species use coastal estuaries as nursery habitats (Heupel et al. 2007) where the potential for anthropogenic impacts is high (Lotze et al. 2006, Jennings et al. 2008). Estimates of consumption rates could provide a means of evaluating the effects of anthropogenic impacts on juvenile populations within these nursery habitats. For example, an individual based bioenergetics model predicted a 12% increase in consumption rates for cownose rays (*Rhinoptera bonasus*) due to an increase in water temperature (Neer et al. 2007). Kāneʻohe Bay (Oahu, Hawaiʻi) is one such nursery habitat, used by juvenile brown stingrays (JBS), *Dasyatis lata*, and juvenile scalloped hammerhead sharks, *Sphyrna lewini*. Juvenile brown stingrays forage within Kāneʻohe Bay for the majority of their juvenile lives before shifting to offshore habitats with the onset of sexual maturity (~8 and 15 years for males and females respectively; Chapter II, III). With a lack of fisheries

exploitation for this species in Hawai‘i, habitat alteration may have the greatest impacts on juvenile populations (e.g. Jennings et al. 2008). This bay is relatively small (46 km²) and provides an excellent system to evaluate the trophic role of an abundant benthic predator. The objectives of this study were to estimate the standard metabolic rates of JBS through respirometry and incorporate previous estimates of food habits and growth (Chapter II, III) to develop an energy budget and estimate their age-specific daily ration with a bioenergetics model. A similar study conducted on juvenile scalloped hammerhead sharks in Kāne‘ohe Bay (Lowe 2002), provides an opportunity to evaluate the combined effects of two elasmobranch species on prey populations in this system.

METHODS

Sample Collection

Juvenile brown stingrays were captured within Kāne‘ohe Bay (21°26.1’N, 157°46.6’W) using standard demersal longlines. Upon capture, stingrays were transported back to the Hawai‘i Institute of Marine Biology (HIMB, located within Kāne‘ohe Bay) where they were maintained in a 7 m diameter tank with flow-through seawater. Stingrays were fed ad libitum and maintained for a minimum of 7 days. Prior to experimental trials, stingrays were starved for 96 h to ensure that they were in a post-absorptive state (Lowe 2001).

Respirometry

Measurements of oxygen consumption were conducted on JBS in a rectangular 650 l closed-system, recirculating respirometer (107 x 107 x 57 cm, acrylic). Seawater

for the respirometer was taken from Kāneʻohe Bay adjacent to HIMB at ambient temperature and salinity and no manipulation of water temperature was conducted (i.e. experiments were run on seasonally acclimated rays). The respirometer could also be operated in flow-through mode allowing stingrays to acclimate to the respirometer for 24 h prior to experimental trials. During acclimation, fresh seawater was continuously pumped into the respirometer to maintain ambient oxygen concentrations. The respirometer was isolated with shade cloth to minimize visual disturbance.

Immediately prior to experimental trials, the inflow water was shut off and the respirometer switched to closed-circuit. Oxygen concentration and water temperature were measured once per minute with a fiber optic oxygen sensor (Aanderaa Data Instruments, oxygen optode 3830) interfaced with a notebook computer and salinity was measured at the end of each trial. All equipment was calibrated to the manufacturer's specifications. Trials were run until oxygen concentration in the respirometer dropped to 70% saturation. Activity of the ray was monitored with an infrared video camera mounted above the respirometer. Only trials where the ray remained quiescent were used for calculation of oxygen consumption. At the conclusion of each experimental trial, the respirometer was run without animals to measure background respiration, which was accounted for in final MO_2 calculations (Lowe 2001). Additionally, the disk width of each ray was measured to the nearest 0.1 cm, weight measured to the nearest 0.1 kg, and the animal was subsequently released back into Kāneʻohe Bay following resumption of feeding in the holding tank.

Data analysis

Oxygen consumption rate was calculated using the slope of the change in O₂ concentration over the course of the experimental trial (Lowe 2001) and normalized by mass for mass-specific metabolic rates (mgO₂ kg⁻¹ h⁻¹). The decrease in water volume due to stingray displacement was measured and accounted for in final calculations. Multiple regression analysis was used to estimate the effects of stingray mass and water temperature on mass-independent metabolic rates (mgO₂ h⁻¹). In order to meet the assumptions of parametric testing, data were log transformed prior to analysis.

Proximate Chemical Analysis

Proximate chemical analysis was conducted on five JBS to determine their energy density. For each stingray, disk width, total mass and liver mass was measured and a sample of white muscle and liver tissue was analyzed for percent dry matter, ash, crude protein and crude fat (Ag Diagnostic Services, University of Hawai‘i, Honolulu). Energy densities of muscle and liver samples were estimated using a conversion factor of 5.7 kcal/g for protein, 8.7 kcal/g for lipid and 4.19 kJ/kcal (McNeill and Lawton 1970, Drazen 2007). Total energy density of the stingrays was estimated by multiplying the tissue specific energy density by the relative contribution of that tissue to total mass assuming the energy density of all non-liver tissue was approximated by the energy density of white muscle. Energy densities of three whole crabs, *Podophthalmus vigil*, an important component of the stingray diet and commonly found in Kāne‘ohe Bay, were also estimated from proximate chemical analysis using the same conversion factors.

Bioenergetics Model

A bioenergetics model was used to develop an energy budget and estimate age-specific daily energy consumption (kJ/day) of individual JBS in Kāneʻohe Bay, expressed as:

$$C = \text{RMR}(T,M) + G(M) + \text{SDA} + W \quad (1)$$

where $\text{RMR}(T,M)$ = energy used in daily routine metabolic rate as a function of temperature and individual mass, $G(M)$ = energy used for daily growth as a function of individual mass, SDA = daily metabolic costs of specific dynamic action, W = daily energy lost as waste and is the sum of energy lost through feces and urine (Brett and Groves 1979). The model used a daily time step and ran for one year beginning July 1. Because SDA and W were represented as fractions of consumption (see below), equation (1) was rearranged and solved for C as follows:

$$C = \frac{\text{RMR} + G}{(1 - \text{SDA} - W)} \quad (2)$$

Model Parameters

Daily standard metabolic rates were calculated for each individual in the model using a regression equation relating mass-independent standard metabolic rates (SMR) to mass and temperature for JBS (see Results);

$$\text{SMR} = aM^bT^c \quad (3)$$

where a = intercept, b = mass scaling coefficient, c = temperature scaling coefficient, M = mass and T = temperature. There are currently no quantitative estimates relating routine metabolic rates to standard metabolic rates for batoids. I used the relationship between MO_2 and swimming speed for blacknose sharks (*Carcharhinus arconotus*, Carlson et al. 1999);

$$\log \text{MO}_2 = 0.007U + 2.38 \quad (4)$$

where U = swimming speed (cm/s), with the average rate of movement for JBS actively tracked in Kāneʻohe Bay (6.78 cm/s, Cartamil et al. 2003) as an approximation of the increase in standard metabolic rate due to activity. Based on this relationship, the mean ratio of RMR/SMR was 1.11 which was the initial estimate of the activity multiplier (ACT) to generate field metabolic rates (Schindler et al. 2002). In comparison, this ACT value is lower than those estimated for juvenile sandbar sharks, *Carcharhinus plumbeus* (1.6, Dowd et al. 2006b) and juvenile scalloped hammerhead sharks (1.4, Lowe 2002), both obligate ram ventilators, and subadult lemon sharks, *Negaprion brevirostris* (1.3, Sundström and Gruber 1998), but similar to that of a dorsoventally flattened teleost, *Platichthys flesus* (1.1, Stevens et al. 2006). Daily metabolic rates ($\text{mgO}_2 \text{ day}^{-1}$) were converted to daily metabolic energy consumption (kJ day^{-1}) using the oxycalorific coefficient $13.59 \text{ J mgO}_2^{-1}$ (Jobling 1994).

The growth component of the bioenergetics model is composed of somatic and reproductive growth. Reproductive growth was assumed negligible as the model only included juvenile stingrays. Somatic growth was based on growth rates obtained from a logistic growth function fit to observed weight-at-age data (Chapter II), which were used to calculate daily growth increments (kg day^{-1});

$$\frac{dM}{dt} = \frac{kM(W_{\infty} - M)}{W_{\infty}} \quad (5)$$

where M = mass, k = growth coefficient, W_{∞} = asymptotic size. Growth in mass was then converted to growth in energy (kJ day^{-1}) using the energy density of JBS determined from proximate chemical analysis.

Specific dynamic action represents energy expended on pre- and post-absorptive activities, including gastrointestinal muscle contraction, production of digestive acids and

enzymes, nutrient absorption and the synthesis of compounds from absorbed nutrients (Jobling 1981, Secor et al. 2007), and has only been estimated for a few elasmobranchs (range: 0.6 – 0.17C; Du Preez et al. 1988, Sims and Davies 1994, Duffy 1999, Ferry-Graham and Gibb 2001). A nominal value of 0.1C was used to estimate the fraction of consumed energy attributed to SDA in the model (*sensu* Schindler et al. 2002, Dowd et al. 2006a).

Absorption efficiency has only been estimated for a single elasmobranch species, *N. brevirostris*, and ranged from 61.9 – 83.1%, with the greatest absorption efficiencies occurring at the highest ration levels which approached feeding rates in the field (Wetherbee and Gruber 1993). Based on these values and mean waste values (F+U) for carnivorous teleosts, a total waste component of 27% of consumed energy is generally accepted (Brett and Groves 1979, Lowe 2002, Schindler et al. 2002, Dowd et al. 2006a, Bethea et al. 2007, Neer et al. 2007). Energy lost as feces and urine accounts for 20% and 7% respectively (Brett and Groves 1979, Wetherbee and Gruber 1993).

To calculate consumption rates for individuals across the observed size range, starting weights for an individual of each age class were estimated by a logistic growth function based on observed weight-at-age data (Chapter II);

$$M_t = \frac{W_\infty}{(1 + e^{-k(t-t_0)})} \quad (6)$$

where t_0 is the inflection point of the curve and the other parameters are as previously defined. Water temperature data from 2009 and 2010 were taken from a NOAA weather station located in Kāneʻohe Bay and daily water temperatures for the model were averaged between years and ranged from 22.2° to 28.3°C. Diet composition data was taken from a recent diet study of JBS in Kāneʻohe Bay (Chapter III). Dietary data were

represented as the gravimetric contribution (%Weight) of individual prey items and observed ontogenetic shifts were incorporated into calculations of consumption rates (Table 4.1). Estimated daily energy rations (kJ/d) were converted to daily ration (%Body Weight (BW) day⁻¹) using the relative gravimetric contribution of prey items to the stingray diet and the energy density of prey items (Table 4.1). Energy densities of prey items were obtained from the literature (Table 4.1) with the exception of portunid crabs which were approximated by the energy density of *P. vigil*. The relative abundance and sex ratio of JBS age classes in Kāneʻohe Bay were taken from longline surveys conducted from 2006-2010 (J. Dale unpublished data). Sex ratios were not significantly different from 1:1 ($\chi^2 = 2.9$, $df = 1$, $P = 0.09$) for stingrays < 9 years old (all stingrays > 8 years old were female).

Error analysis

A Monte Carlo simulation was used to evaluate the sensitivity of consumption rate estimates to variation in input parameters (Bartell et al., 1986, Dowd et al. 2006a, Bethea et al. 2007). Probability density functions were used to generate variation within model parameters (Table 4.2). Normal distributions were used for metabolic scaling exponents estimated from the relationship between mass, temperature and SMR ($SMR_a =$ intercept, $SMR_b =$ mass coefficient, $SMR_c =$ temperature coefficient), daily water temperature, age-specific starting mass, waste and stingray energy density. The ACT and SDA were represented by triangular distributions due to the limited amount of data for these variables. Asymptotic size (W_∞) and the growth coefficient (k) were estimated from a bivariate normal distribution based on the covariance between W_∞ and k (Chapter

II). Parameters SMR_a , SMR_b , SMR_c , W_∞ , and k were considered individual traits and were assigned to each individual at the start of a simulation run and then held constant. The remaining parameters were considered environmental traits and were allowed to vary on a daily basis. For each run of the simulation, parameter values were randomly chosen from the assigned probability density functions. An average daily value over the one year simulation was calculated for each parameter in each age class. This process was repeated 2,000 times, providing frequency distributions, medians and confidence intervals (2.5th and 97.5th percentiles) for parameter estimates. Monte Carlo simulations were run in R (R Development Core Team 2009). Individual parameters were ranked in importance by their relative contribution to the variance of daily consumption rates using relative partial sum of squares (RPSS, Bartell et al. 1986). This method estimates the sensitivity of consumption rate estimates to modeled variation in individual parameters. A sensitivity analysis using individual parameter perturbations (IPP) was also conducted (Bartell et al. 1986). Age-specific consumption rates were estimated from a deterministic model using nominal input parameter values. Each parameter was then increased or decreased by 10% with the remaining parameters fixed at their nominal values. The percent change in consumption rate estimates due to a $\pm 10\%$ change in parameter values was then calculated to assess the sensitivity to variation in individual parameters due to model structure.

RESULTS

Metabolic Rates

Standard metabolic rates were calculated for 22 JBS ranging in mass from 1.03 to 9.85 kg at temperatures ranging from 22.5 to 27.3°C. Estimates of mass-specific MO_2 ranged from 53.06 $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ for a 9.85 kg individual at 23°C to 115.99 $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ for a 1.16 kg animal at 27°C (mean \pm S.D.: $76.98 \pm 15.31 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at 25 °C) (Fig 4.1). Both log-transformed mass and temperature had significant effects on mass-independent MO_2 ($P < 0.001$), explained 98% of the variance in MO_2 values and was best represented by the equation (SE): $\log \text{MO}_2 (\text{mgO}_2 \text{ h}^{-1}) = -0.05 (0.32) + \log \text{Mass} * 0.78 (0.02) + \log \text{Temp} * 1.48 (0.22)$ (Fig 4.2). This equation was used to standardize the mass of all animals to 6.0 kg in order to calculate a Q_{10} value, which was 1.8 (22-27°C). Similarly, temperature was standardized to 25°C in order to estimate the allometric relationship between mass and MO_2 , which was best represented by the equation: $\text{MO}_2 = 105 * \text{Mass}^{0.78}$.

Bioenergetics

Average energy density of JBS (mass range: 2.8 – 9.9 kg) determined from proximate chemical analysis was $6.03 \pm 0.40 \text{ kJ g}^{-1}$ and ranged from 5.58 kJ g^{-1} for a 2.8 kg individual to 6.55 kJ g^{-1} for a 7.3 kg individual. Average water, crude protein and crude fat content were 76.9 ± 1.8 , 21.8 ± 1.1 and $2.3 \pm 1.0\%$ respectively. There was a slight increase in energy density with increasing size, however this relationship was insignificant ($F = 7.0$, $df = 4$, $P = 0.08$), likely due to the small sample size. Average water, crude protein, crude fat and energy density of the three *P. vigil* individuals were 65.9 ± 6.4 , 10.7 ± 1.9 , $0.3 \pm 0.2\%$ and $2.67 \pm 0.56 \text{ kJ g}^{-1}$ respectively.

The energy budget of JBS was heavily weighted towards metabolism (RMR + SDA) which represented $68 \pm 0.002\%$ of total consumed energy. Routine metabolic rate alone represented $57 \pm 0.002\%$ of total consumed energy. Growth on the other hand was a relatively small fraction of the energy budget representing $4 \pm 0.003\%$ of total consumed energy (Fig 4.3). Metabolism initially increases at a greater rate than growth but decreases at a greater rate with increasing age. Thus, the percentage of total energy expenditure devoted to growth relative to metabolism remained stable at $\sim 6\%$.

There was significant variation in consumption rates within age classes due to the use of stochastic input parameters (Table 4.3). Median daily energy rations increased from 180 kJ/d for age-0 (mean BW = 2.1 kg) individuals to 1269 kJ/d for age-14 (mean BW = 31.8 kg) individuals. Relative to BW, daily ration decreased from a high of 2.72 %BW day⁻¹ for age-2 individuals to 1.23 %BW day⁻¹ for age-14 individuals (Table 4.3). Gross conversion efficiency, the fraction of consumed energy incorporated into growth, was $\sim 4\%$ for all age classes. Maintenance ration, the consumption rate at zero growth, was $\sim 95\%$ of total energy consumption.

Total yearly consumption ranged from 20 (935% mean BW) to 143 kg year⁻¹ (449% mean BW) (Table 4.3) and total yearly energy consumption ranged from 65,550 (516% total stingray energy content) to 463,330 kJ year⁻¹ (241% total stingray energy content) for age-0 and 14 individuals respectively. Based on the relative abundance of juvenile age classes, population consumption rates were highest for the 4 and 5 year age classes and decline with increasing age (Fig 4.4). This trend is due to individual consumption increasing with size, coupled with a corresponding decrease in the relative abundance of older age classes.

Individual parameter perturbations indicated that the model was highly sensitive to variation in the scaling coefficients related to metabolism (SMR_a , SMR_b , SMR_c) (Table 4.2). The response of consumption rate estimates to variation in these parameters was nonlinear, with a 10% increase resulting in an average decrease in consumption rate estimates of 35% for SMR_a , and an average increase of 87 and 52% for SMR_b and SMR_c respectively. A 10% decrease in these parameters resulted in an average increase in consumption rate estimates of 61% for SMR_a and an average decrease of 42 and 32% for SMR_b and SMR_c respectively. Consumption rate estimates were linearly sensitive to the remaining parameters and a 10% change resulted in a change in consumption rate estimates $< 10\%$. Of these remaining parameters, consumption rate estimates were most sensitive to variation in the ACT parameter with a 10% change resulting in an 8% change in consumption rate estimates. Error analysis with RPSS indicated the scaling coefficients SMR_a and SMR_c contributed most to the variance of estimated consumption rates for all age classes (Table 4.2). The percentage of variance due to SMR_a ranged from 17% for age-12 individuals to 36% for age-4 individuals and SMR_c ranged from 18 to 31% for age-12 and age-5 individuals respectively. There was no correlation between age class and the relative contribution of either parameter. The relative contributions of the remaining parameters to the variance of estimated consumption rates were all negligible.

DISCUSSION

Metabolic Rates

Mass-specific metabolic rates for JBS in this study were similar to those reported for other myliobatiform stingrays when corrected for differences in mass and temperature (Table 4.4). The metabolic rate of a 5 kg JBS at 20°C was most similar to those of the bull ray (*Myliobatis Aquila*, Du Preez et al. 1988), pelagic stingray (*Pteroplatytrygon violacea*, Ezcurra 2001) and cownose ray (*Rhinoptera bonasus*, Neer et al. 2006) despite significant differences in their autecology. The pelagic stingray, cownose and bull ray are specialized for active swimming (McEachran 1990) whereas brown stingrays are primarily benthic and relatively sedentary (Cartamil et al. 2003). Metabolic rates of the pelagic stingray and cownose ray were notably higher than that of the bull ray which has the lowest metabolic rate of any previously studied myliobatiform stingray. Adaptations for active swimming could include large gill surface area and greater cardiac and swimming muscle capacity, which would increase SMR (Brill 1996). Due to the potential morphological and physiological adaptations, metabolic rates of these active stingrays might be expected to be closer to that of the bat ray (*Myliobatis californica*, Hopkins and Cech 1994), whose metabolic rates are more similar to active sharks (Carlson et al. 2004). Alternatively, the metabolic rates of the pelagic stingray, cownose and bull rays may be representative of stingrays specialized for active swimming and the metabolic rates of brown stingrays and bat rays are unusually high. However, due to the low number of myliobatiform species for which metabolic rates have been experimentally determined, the effects of active swimming specialization can not yet be evaluated.

Mass and temperature explained the majority of variance in mass-independent metabolic rates. The mass scaling exponent was 0.78 and falls within range of scaling

exponents estimated for other elasmobranchs (e.g. 0.68 – 0.85; Du Preez et al. 1988, Sims 1996, Dowd et al. 2006b). The Q_{10} of 1.8 in this study is among the lowest value yet reported for a batoid species. However, comparisons of Q_{10} between studies may be misleading due to differences in experimental procedures. For example, experimental temperatures for the bat ray were increased by 0.5°C/h until desired temperatures were reached (Hopkins and Cech 1994), resulting in a mean Q_{10} of 3.0. Such rapid changes in temperature could increase the effects of temperature on metabolic rates, resulting in inflated Q_{10} values (McNab 2002). In contrast, temperatures for the bull ray were increased by 1°C/24h and animals were allowed to acclimate to experimental temperatures for eight days prior to trials resulting in a Q_{10} of 1.87 (Du Preez et al. 1988). Acclimation to warmer temperatures is generally quicker compared to cooler temperatures (McNab 2002) and eight days may have been sufficient for complete acclimation to occur. Q_{10} values may also vary between temperature ranges (Gillooly et al. 2001). A pattern of decreasing Q_{10} values with increasing temperature was found for the bull ray and was lowest at the highest temperature range ($Q_{10} = 1.61$, 20 – 25°C, Du Preez et al. 1988). The bat ray had a Q_{10} value of 6.81 at a temperature range of 14 – 20°C, but Q_{10} was notably lower at higher and lower temperature ranges (Hopkins and Cech 1994). The bat ray Q_{10} at a temperature range of 20 – 26°C was 1.85, similar to the value for JBS in this study at a temperature range of 22 – 27°C. The Q_{10} of juvenile scalloped hammerhead sharks in Kāneʻohe Bay was also relatively low (1.34) at a temperature range of 21 – 29°C (Lowe 2001). Because Q_{10} is also temperature dependant, metabolic rates will depart from a purely exponential relationship with temperature at high temperatures (Gillooly et al. 2001). The low Q_{10} values for JBS, bull

rays, bat rays and juvenile scalloped hammerhead sharks are likely due to the relatively high temperatures at which they were estimated.

Bioenergetics

Metabolism was the largest component of the energy budget, accounting for 68% of total energy consumed. This value is relatively high in comparison to estimates for other elasmobranch species and carnivorous teleosts which range from 44 (Brett and Groves 1979) to 60% (Gruber 1984, Dowd et al. 2006a, Chen et al. 2008). Growth was a much smaller component of the energy budget accounting for 4% of total consumed energy, which is substantially lower than those estimated for other elasmobranchs and carnivorous teleosts (20 – 30%, Brett and Groves 1979, Gruber 1984, Dowd et al. 2006a, Chen et al. 2008). However, these estimates are remarkably similar to estimates for juvenile scalloped hammerhead sharks in Kāneʻohe Bay which exhibited positive growth ($M = 69\%$, $G = 5\%$, Lowe 2002). For both of these species, 93-95% of their total energy consumption was allocated to maintenance and routine activity.

Daily ration estimates (as %BW) decreased with increasing age primarily due to associated decreases in mass-specific metabolic rates. Consumption rates for age-0 to age-5 brown stingrays were comparable to those estimated for similarly aged sandbar sharks (2.2 – 1.3% BW/d) in Chesapeake Bay (Dowd et al. 2006a). Although sandbar sharks are a relatively active species with higher mass-specific metabolic rates (Dowd et al. 2006b), modeled water temperatures in Chesapeake Bay were on average 2-3°C cooler than those used in this bioenergetics model. Daily ration was markedly lower than estimates for juvenile scalloped hammerhead sharks in Kāneʻohe Bay which showed

positive growth (3.9% BW/d, Lowe 2002). These differences can be attributed to the significantly higher metabolic rates required to sustain activity rates of the juvenile scalloped hammerhead sharks (Lowe 2001, 2002). However, gross conversion efficiency was similar between the two species (~4%, Lowe 2002, Duncan 2006), but lower than juvenile sandbar sharks (10 – 16%, Dowd et al. 2006a).

Gross conversion efficiency generally increases with decreasing temperature due to the effects of temperature on metabolic rates (Mateo 2007), which may partially explain differences between studies. Conversion efficiencies are also linked to prey density and quality (Stewart and Ibarra 1991). Low conversion efficiencies may be an adaptation to an abundant prey base, maximizing foraging at the expense of efficiency (Mateo 2007). However, Kāneʻohe Bay is an oligotrophic system represented by a low diversity of benthic fauna (Laws and Allen 1996, Smith and Kukert 1996) and the very low growth rates of JBS suggests food is limiting. Conversion efficiency has also been shown to decrease with decreasing protein content of consumed energy (e.g. Pérez-Casanova et al. 2009). The diet of JBS is dominated by crustaceans which are generally of low protein and energy content relative to teleosts (Bush 2002, Lowe 2002, Goodman-Lowe et al. 1999). For example, crude protein content of *P. vigil* and *Alpheus malabaricus*, two common prey items of JBS in Kāneʻohe Bay, were 31 and 50% (dry weight) respectively (Bush 2002, Lowe 2002), significantly lower than the crude protein content of Hawaiian teleosts (mean = 63% dry weight, Goodman-Lowe et al. 1999). The combination of low gross conversion efficiency, relatively high metabolic requirements, low prey quality and warm subtropical waters of Kāneʻohe Bay likely explains the very low growth rates of JBS.

Individual parameter perturbations indicated that several input parameters had minimal impacts on predicted consumption rates. Fortunately, these low-impact parameters included those parameters for which the least amount of empirical data were available (e.g. waste, SDA). This analysis also indicated that uncertainty in input parameters associated with metabolism (SMR_a , SMR_b , SMR_c and to a lesser extent ACT) can have important impacts on consumption rate estimates, consistent with sensitivity analyses in other bioenergetics studies (Bartell et al. 1986, Dowd et al. 2006a, Bethea et al. 2007). The two input parameters identified by RPSS analysis as contributing most significantly to the variance in consumption rate estimates were associated with metabolism (SMR_a and SMR_c). Values used for these parameters were experimentally determined for JBS in this study, and these results likely represent the contribution of natural variation to the variability in consumption rate estimates. However, the relationships between SMR and mass and temperature were only calibrated for stingrays up to 10 kg, whereas juveniles up to 30 kg were modeled. Extrapolation of these relationships to larger stingrays may have introduced additional error to the model. Although the ACT used to estimate RMR had a negligible contribution to the variance of consumption rates, it may have introduced significant variance through its multiplicative effects on the error associated with RMR. The relationship between metabolic rate and swimming speed was based on data for blacknose sharks (Carlson et al. 1999). However, this assumes that these two species have similar body shapes and swimming kinematics and may overestimate the effects of activity on standard metabolic rates. Additionally, estimates of rate of movement for juvenile brown stingrays were point-to-point estimates (Cartamil et al 2003), which tend to underestimate actual rates of movement (Gruber et

al. 1988) and would therefore underestimate RMR. These results highlight the need for quantitative data on energy expenditures in the field for stingrays (e.g. Lowe 2002).

Population consumption rates were highest for the 4 and 5 year age classes due to their high abundance relative to other age classes. A number of factors can affect the relative catchability of different age classes such as hook and bait size, variation in foraging behavior and competition with conspecifics (e.g. Godø et al. 1997, Dale et al. 2011). To minimize gear effects, multiple gear configurations were concurrently used during longline surveys (see Chapter II for additional information on gear configurations). However, the influence of foraging behavior and competitors on stingray catchability could not be accounted for and may have led to underestimates of relative abundance for the smallest age classes.

In order to evaluate the ecosystem impacts of JBS within Kāneʻohe Bay, a range of population sizes between 500 and 3,000 individuals was modeled. Five hundred is approximately the number of animals which were captured during longline surveys. The majority of these animals were tagged with external ID tags and released. Very low numbers of recaptures (< 1%) suggests either a transient population or a population size larger than the number of tagged animals. A stable isotope study revealed that JBS forage within Kāneʻohe Bay for the majority of their juvenile lives (Chapter III). Therefore, a minimum population size of 500 stingrays is likely a conservative estimate. The upper value of 3,000 individuals is based on population estimates for juvenile scalloped hammerhead sharks in Kāneʻohe Bay (Duncan and Holland 2006) and the ratio of JBS to juvenile scalloped hammerhead catch-per-unit effort (CPUE) from longline survey data. The use of CPUE data to estimate population size assumes this metric

accurately reflects the actual abundance of these two species and the catchability of these two species are equivalent. Although there are currently no empirical estimates of population size for JBS to compare with CPUE data, juvenile scalloped hammerhead CPUE based on longline data closely follows the relative seasonal trends in actual juvenile scalloped hammerhead population size (Duncan and Holland 2006).

Additionally, the density of JBS in Kāneʻohe Bay based on a population size of 3,000 individuals (0.0002 stingrays m^{-2}) is similar to that of similarly sized giant shovelnose rays (*Glaucostegus typus*, 0.00014 rays m^{-2}) in Sharks Bay, Australia (Vaudo and Heithaus 2009). Therefore, 3,000 stingrays is likely a reasonable upper limit for JBS population size.

A population ranging between 500 and 3,000 stingrays within Kāneʻohe Bay would consume between $\sim 30,000$ and $182,000$ kg of benthic prey per year (Fig 4.5). Consumption rates were highest on portunid crabs ($\sim 16,500 - 100,000$ kg/yr) followed by alpheid shrimps ($\sim 6,000 - 37,000$ kg/yr). *Podophthalmus vigil* is the most common portunid crab consumed, with consumption rates ranging between $\sim 10,500 - 63,500$ kg/yr. The average *P. vigil* consumed by juvenile brown stingrays weighs ~ 9 g and an average alpheid shrimp weighs ~ 0.4 g (Bush 2002). These weights would translate to $\sim 1.2 \times 10^6 - 7.1 \times 10^6$ *P. vigil* and $\sim 1.5 \times 10^7 - 9.2 \times 10^7$ alpheid shrimps consumed per year. The most recent estimates of population density for *P. vigil* and *Alpheus malabaricus* are 1.27 and 3.62 m^{-2} respectively (Bush 2003). The mud floor of Kāneʻohe Bay covers 14.9×10^6 m^2 (Smith et al. 1981). Assuming these prey are uniformly distributed, total population sizes for these species may be 1.9×10^7 crabs and 5.4×10^7 shrimps. Therefore, the stingray population would consume $\sim 6 - 37$ and $28 - 170\%$ of the

crab and shrimp population by number respectively, without replacement, over the course of one year. However, the productivity of *A. malabaricus* was estimated to be $0.669 \text{ g m}^{-2} \text{ year}^{-1}$ ash free dry weight (Bush and Holland 2002) which amounts to $47,636 \text{ kg year}^{-1}$ wet weight (13.3% ash, 25% water, Bush 2002) and the stingray population would consume ~13 - 78% of the estimated productivity. Based on estimates of daily ration, population size and mortality rates for juvenile scalloped hammerhead sharks in Kāneʻohe Bay, which are sympatric with JBS, their population consumption rate would be $4,658 \text{ kg year}^{-1}$ (Bush and Holland 2002, Lowe 2002, Duncan and Holland 2006). They would consume $658 \text{ kg of alpheids year}^{-1}$ (Bush 2003) or 1.4% of alpheid productivity, and the combined consumption by these two elasmobranchs would be 14.4 – 79.4% of alpheid productivity. Assuming the true stingray population size in Kāneʻohe Bay is in the low thousands as suggested by low recapture rates and CPUE data, the population would have a moderate to strong impact on these two species, and their impacts on the shrimp population is significantly greater than that of juvenile hammerhead sharks. These results suggest that slow growth rates for JBS may be due to insufficient prey resources to support higher consumption rates and therefore, higher growth rates. Food limitation is supported by growth rates of two juvenile stingrays held in captivity at HIMB (J. Dale unpublished data). These stingrays were fed ad libitum daily and gained 14 and 11 kg in mass over the course of one year. These growth rates are substantially higher than field based rates which peak at 3.7 and 2.3 kg/yr for females and males respectively (Chapter II) and demonstrate the capacity for faster growth with sufficient dietary intake. Additionally, other factors such as habitat complexity, anti-predator behavior or interspecific competition with carnivorous teleosts such as jacks

may decrease encounter rates or capture efficiency, further limiting growth in the field (Laprise and Blaber 1992, Meyer et al. 2001, Bush and Holland 2002).

Conclusions

An increased forage base and/or refuge from predation are generally the two main factors proposed to explain the advantages of nursery habitat use. The high energetic requirements of JBS and low energetic quality of their prey suggests that their slow growth rates in Kāneʻohe Bay are a trade-off between increased juvenile survival and a late age at first maturity resulting in delayed recruitment to adult populations (Chapter II, Chapter III). Survival would be enhanced through decreased predation from larger predators such as tiger sharks, which are less abundant in Kāneʻohe Bay compared to surrounding waters (Crowe et al. 1996). Stomach content analysis indicated the youngest age classes of JBS had the highest proportion of empty stomachs relative to older age classes (Chapter III). Slow development of foraging skills may explain the especially low relative growth rates of the youngest age classes as has been suggested for juvenile scalloped hammerhead sharks (Bush and Holland 2002, Lowe 2002, Duncan and Holland 2006). Estimated consumption rates suggest that JBS can have a strong impact on their prey populations. These results are in agreement with previous studies which suggest that some elasmobranchs may have a substantial top-down ecosystem role (e.g. Stevens et al. 2000, Bascompte et al. 2005). Estimates of prey population densities have decreased in recent years due to the diversion of treated waste water which was dumped into the bay between 1951 and 1978 (Smith et al. 1981). These changes in habitat quality due to anthropogenic impacts, although generally beneficial for a coral reef ecosystem,

may have decreased stingray consumption rates resulting in the current slow growth rates. Although no data exists concerning growth rates for JBS concurrent with elevated prey densities in the field, accelerated growth rates in captivity suggest that bottom-up effects could have important implications on the life-history of brown stingrays (e.g. Jennings et al. 2008).

Table 4.1 Gravimetric contribution of individual prey items to the diets of juvenile brown stingray age classes. Age class divisions are based on observed ontogenetic diet shifts from Chapter III. Energy density of Stomatopoda based on the average energy density of benthic decapods from Thayer et al. (1973). Energy density of unidentified prey estimated as the average energy density of all prey types.

Prey	% Gravimetric Contribution by Age Class			Energy Density (kJ/g)	Source
	0-3	4-7	7-14		
Alpheidae	55.58	20.4	2.64	3.6	Bush 2002, Lowe 2002
Gobiidae	1.25	8.82	13.88	4.64	Bush 2002, Lowe 2002
Portunidae	31.93	53.28	70.71	2.67	This Study
<i>Podophthalmus vigil</i>	9.50	20.83	47.11	2.67	This Study
Stomatopoda	0.22	4.89	8.2	5.2	Thayer et al. 1973
Polychaeta	6.69	6.01	1.44	3.55	Thayer et al. 1973
Unidentified	4.46	6.6	3.13	3.93	

Table 4.2 Parameters, probability distributions, nominal values and variability used in the bioenergetics model. Results of sensitivity analyses are represented as the mean value for all age classes. IPP: Individual parameter perturbation ($\pm 10\%$). Values represent the percent change in consumption rate estimates due to a 10% change in nominal parameter values. Single values represent linear sensitivities. RPSS: Relative residual sum of squares. Values represent the contribution of each parameter to the variance of consumption rate estimates. Energy density parameter represents the energy density of brown stingrays. See text for remaining parameter definitions.

Parameter	Distribution	Nominal value	SE or range	IPP	RPSS	Source
SMRa	Normal	-0.05	0.34	-35.4, 61.1	28.2	This Study
SMRb	Normal	0.78	0.02	86.7, -42.5	1.8	This Study
SMRc	Normal	1.48	0.22	51.9, -32.1	23.5	This Study
ACT	Triangular	1.11	1.06-1.16	8.4	0.2	Carlson et al. (1999), Cartamil et al. (2003)
SDA	Triangular	0.1	0.06-0.17	< 0.1	0.1	DuPreez et al. (1988), Sims and Davies (1994), Duffy (1999), Ferry-Graham and Gibb (2001)
Wi and k				0.4, 1.6	0.2, 0.1	Chapter II
Female	Bivariate Normal	63.7, 0.23	-0.008			
Male	Bivariate Normal	35.6, 0.25	-0.008			
Waste	Normal	0.27	3	0.7	0.2	Brett and Groves (1979), Wetherbee and Gruber (1993)
Energy density	Normal	6.03	0.4	< 0.1	0.2	This Study

Table 4.3 Median daily energy ration (DER), daily consumption relative to mass and total prey consumption over the course of one year for an average juvenile brown stingray individual of each age class estimated from Monte Carlo simulations of the bioenergetics model. Confidence intervals (CI) are represented as the 2.5 and 97.5 percentiles.

Age Class	DER (KJ/d)	Consumption Rate (%BW/d)		Consumption Rate (kg/y)		
		CI	CI	CI	CI	
0	180	70 - 737	2.56	1.00 - 10.51	20	8 - 81
1	208	115 - 461	2.63	1.46 - 5.82	23	13 - 51
2	297	188 - 521	2.72	1.73 - 4.78	33	21 - 57
3	359	260 - 538	2.62	1.89 - 3.92	39	28 - 59
4	430	329 - 592	2.53	1.94 - 3.49	48	37 - 66
5	521	410 - 747	2.40	1.89 - 3.44	58	45 - 83
6	605	428 - 889	2.27	1.61 - 3.34	67	47 - 99
7	708	478 - 1069	2.25	1.52 - 3.39	80	54 - 120
8	870	557 - 1418	2.12	1.36 - 3.46	98	63 - 160
9	920	543 - 1574	2.01	1.19 - 3.43	104	61 - 177
10	1096	517 - 3031	1.82	0.86 - 5.03	123	58 - 341
11	1154	520 - 2712	1.69	0.76 - 3.98	130	59 - 306
12	1202	374 - 5529	1.39	0.43 - 6.39	135	42 - 623
13	1233	328 - 5785	1.30	0.35 - 6.1	139	37 - 652
14	1269	224 - 7388	1.23	0.22 - 7.16	143	25 - 832

Table 4.4 Comparison of mass-specific metabolic rates among myliobatiform stingrays. When necessary, metabolic rates have been corrected to 20°C and 5 kg using species-specific temperature and mass scaling coefficients when available or assuming a Q₁₀ of 2.3 and a mass coefficient of 0.8.

Species	N	MO₂ (mgO₂ kg⁻¹ h⁻¹)	Source
<i>Myliobatis aquila</i>	5	47.5	Du Preez et al. (1988)
<i>Myliobatis californica</i>	6	158.1	Hopkins and Cech (1994)
<i>Dasyatis americana</i>	6	93.6 ^a	Fournier (1996)
<i>Dasyatis violacea</i>	8	63.3	Ezcurra (2001)
<i>Rhinoptera bonasus</i>	19	73.0	Neer et al. (2006)
<i>Dasyatis lata</i>	22	52.8	This study

^a Routine metabolic rate

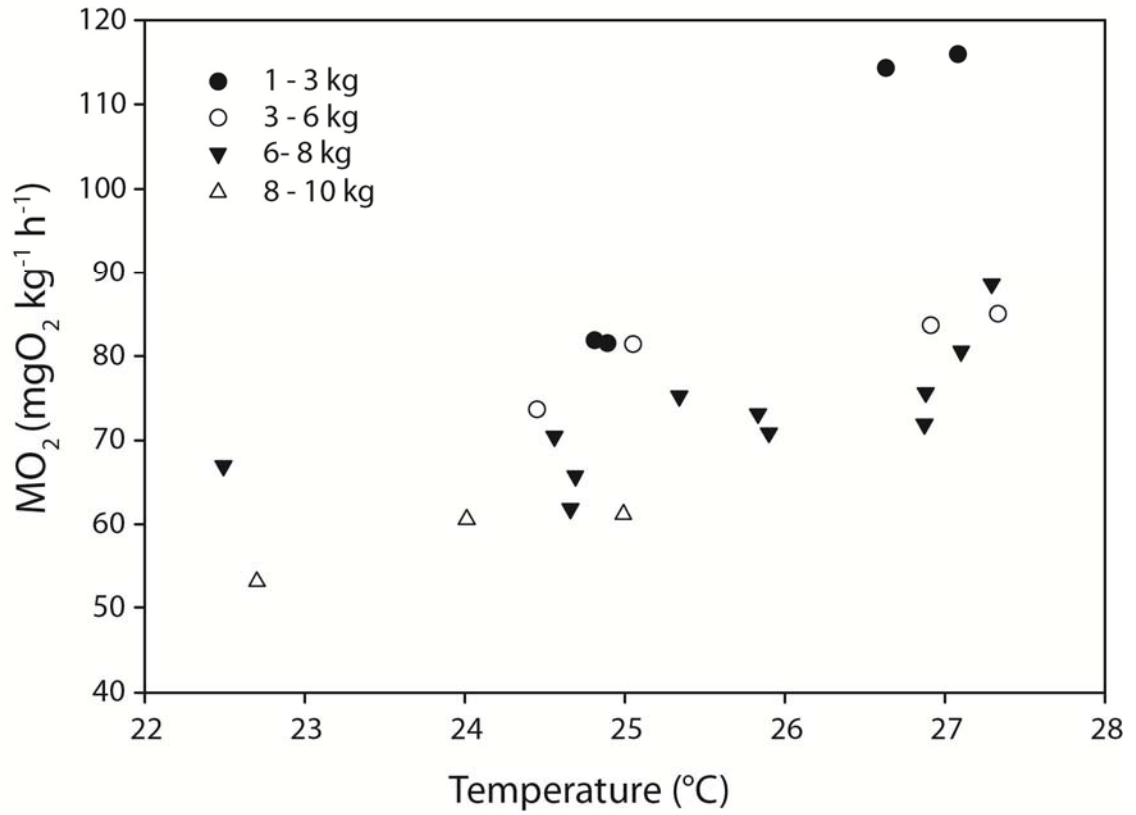


Figure 4.1 Standard mass-specific metabolic rates ($\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) as a function of temperature ($^{\circ}\text{C}$) determined for juvenile brown stingrays plotted by weight group ($n = 22$).

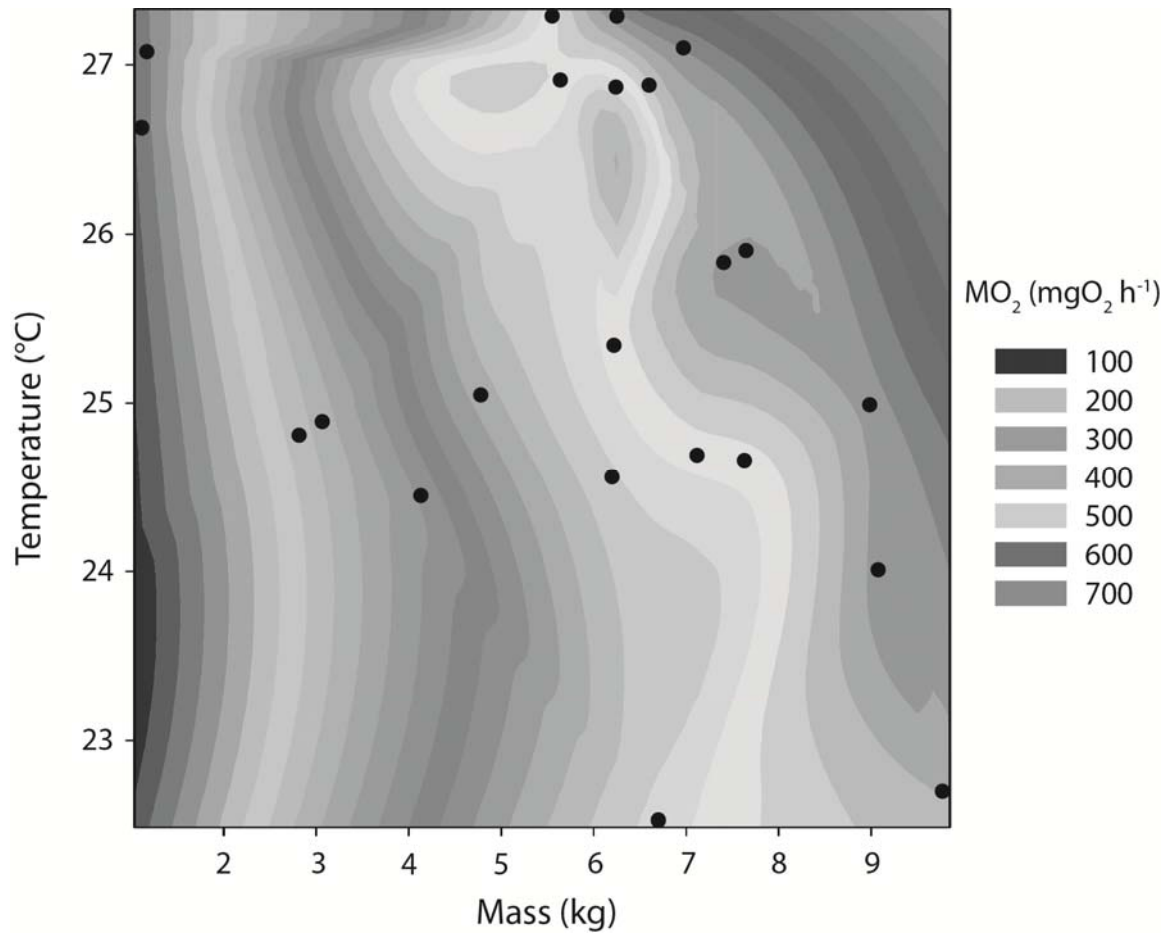


Figure 4.2 Contour map representing the effects of mass (kg) and temperature (°C) on standard metabolic rates (mgO₂ h⁻¹) for juvenile brown stingrays. Black circles represent the observed standard metabolic rates of individual stingrays from respirometry experiments (n = 22).

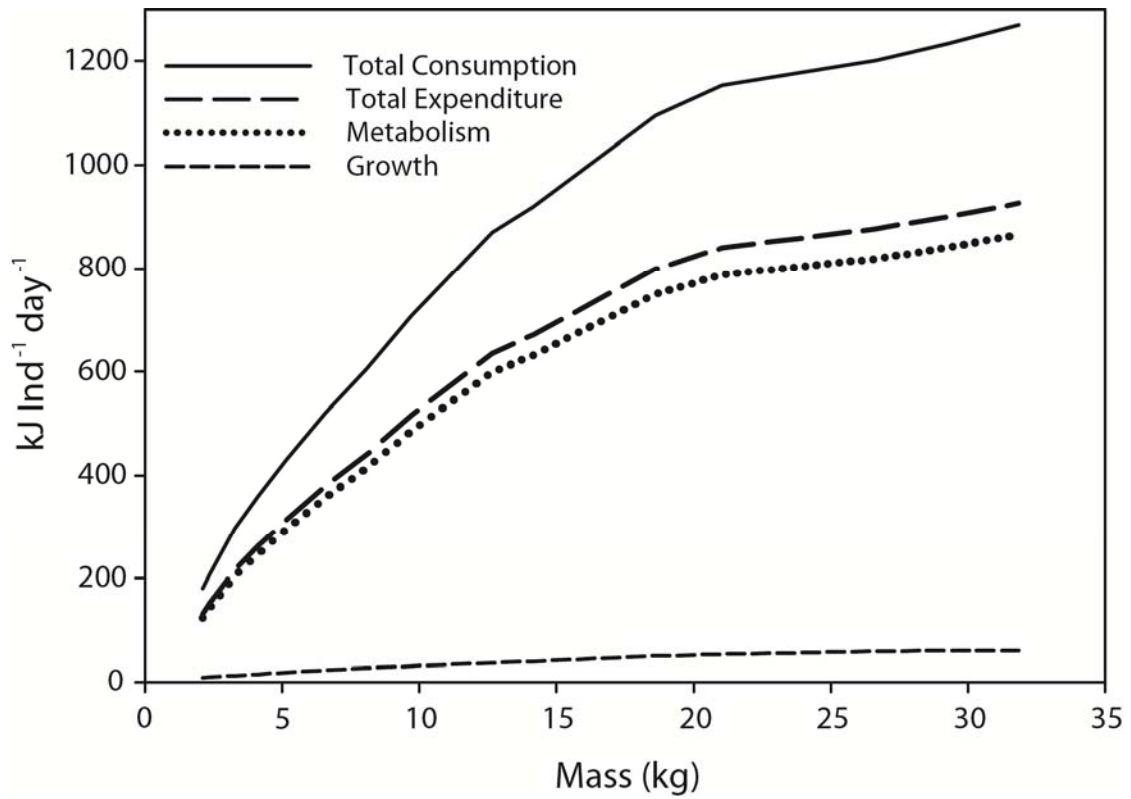


Figure 4.3 Daily energy budget (kJ individual⁻¹ day⁻¹) for individual juvenile brown stingrays as a function of mass (kg). Total expenditure is the sum of metabolism and growth. Total consumption is the sum of metabolism, growth and waste.

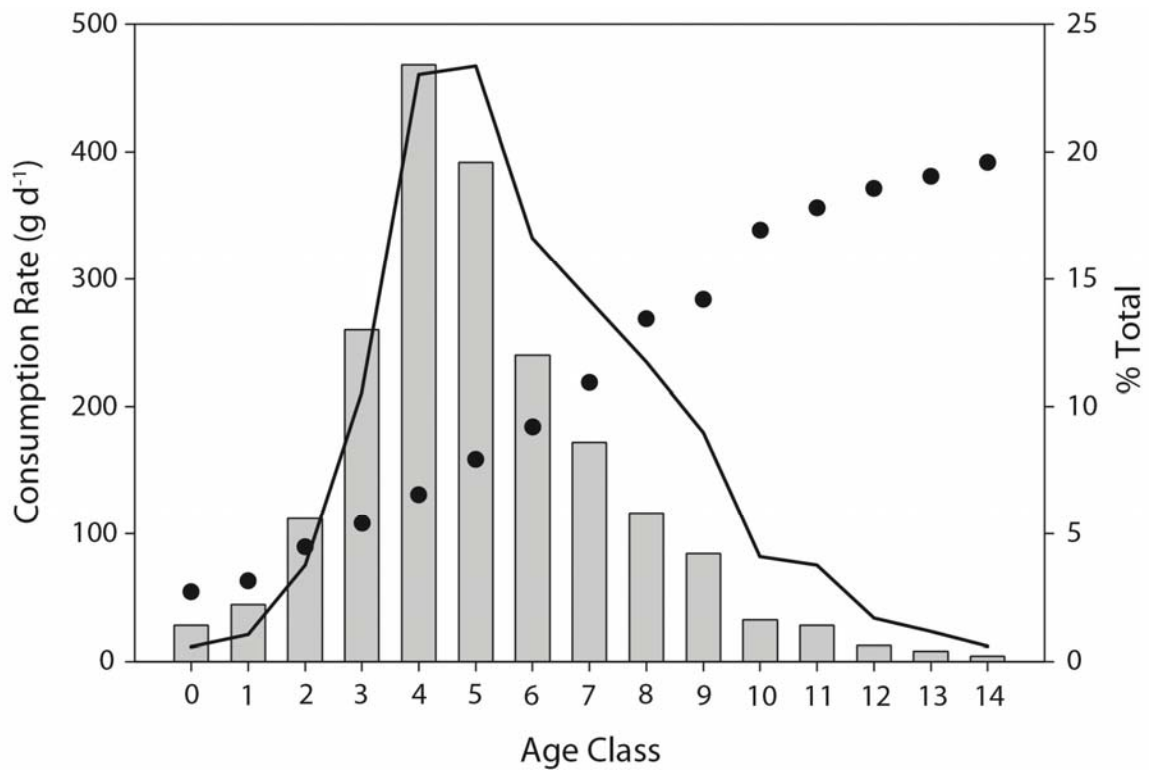


Figure 4.4 Relative abundance (% , vertical bars), age-specific consumption rate (g d^{-1} , black circles), and percent of total population consumption by age class (% , solid line) calculated from the relative abundance of each age class. Relative abundance is based on longline surveys conducted from 2006 – 2010 within Kāne‘ohe Bay (J. Dale unpublished data).

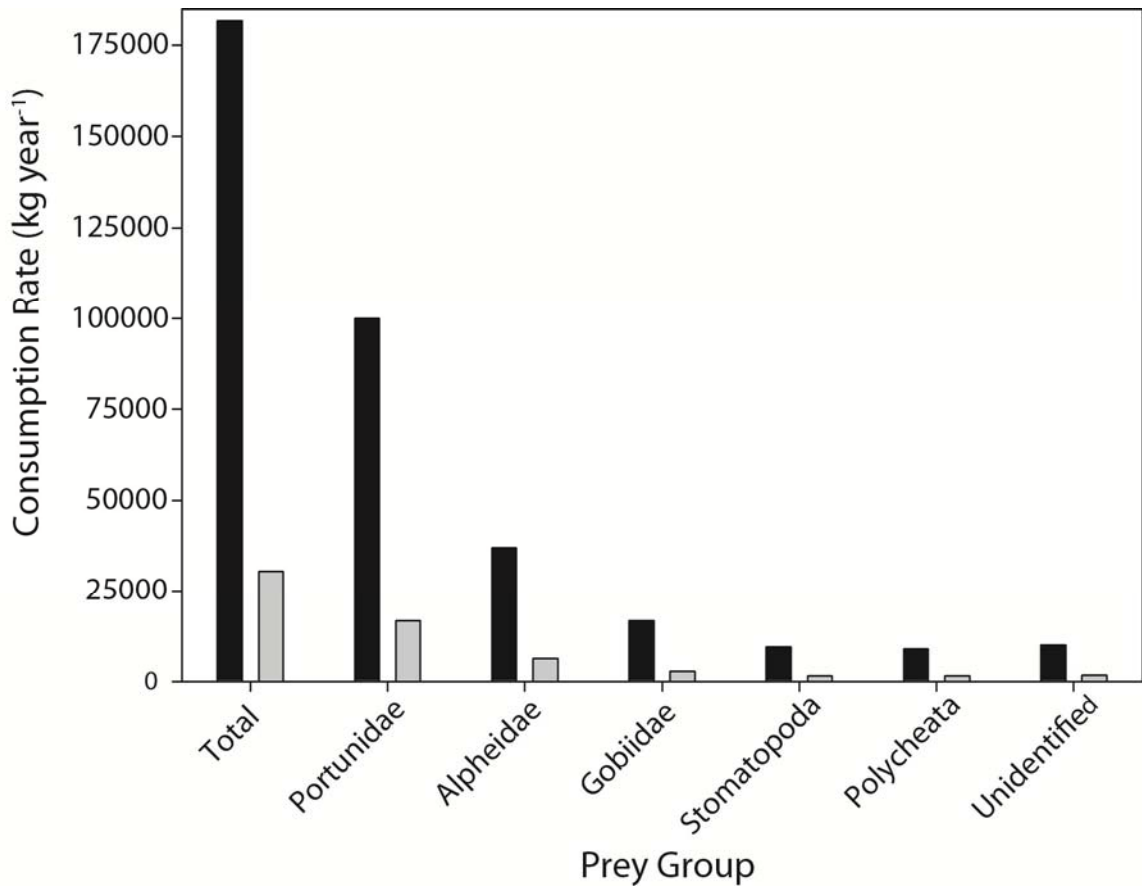


Figure 4.5 Prey specific population consumption rates by juvenile brown stingrays in Kāneʻohe Bay based on a population size of 500 (grey bars) and 3,000 (black bars) individuals.

CHAPTER V

SUMMARY AND DIRECTIONS FOR FUTURE RESEARCH

This study investigated the life-history, nursery habitat use, energetics and foraging ecology of the brown stingray, a large benthic elasmobranch predator endemic to Hawai'i. The overarching goal of this study was to evaluate the ecological impacts of these stingrays in a coastal estuary, Kāneʻohe Bay, Oahu. Kāneʻohe Bay was an ideal system for this study because: (1) juveniles are abundant, easily captured and maintained in captivity, (2) adults can be captured in deep water immediately adjacent to Kāneʻohe Bay, (3) the foraging ecology of sympatric juvenile scalloped hammerhead sharks has been intensively studied in this system, providing the opportunity to test specific hypotheses of inter-specific competition and evaluate the foraging impacts of a multi-species complex on a common prey resource and (4) the Hawai'i Institute of Marine Biology (HIMB) is located within Kāneʻohe Bay, providing easy and reliable access to the study system. A bioenergetics model, which requires species-specific data on age composition and growth rates, reproduction, habitat use, diet and metabolism, was used to estimate consumption rates of juvenile brown stingrays. Due to the paucity of biological and ecological data for this species, each of these components were directly estimated during this study to parameterize the bioenergetics model.

Summary

Vertebral analysis showed that brown stingrays follow a life-history strategy similar to many other elasmobranch species; they are long lived, have relatively slow

growth rates and mature at a late age. Stingrays aged in this study were among the oldest dasyatid species aged to date. Growth rates were relatively slow, which corresponded to a prolonged juvenile stage, reaching sexual maturity after 8 and 15 years for males and females respectively. The von Bertalanffy growth function, which is the most frequent, and often the only growth function used in age and growth studies, provided a poor fit to observed size-at-age data for brown stingrays. Logistic growth functions were most appropriate for this species and support hypotheses that sigmoid growth functions may be more applicable to batoids which increase in mass at a greater rate than width. Sigmoid growth functions may also be more appropriate for species in which growth occurs in two phases (Chapter II).

A combination of stomach content, bulk and amino acid stable isotope analyses were used to investigate the diet and nursery habitat use of juvenile brown stingrays in Kāneʻohe Bay. Their diet was dominated by crustaceans and reflected the low diversity of benthic fauna in this system. Ontogenetic differences in prey consumption were primarily due to morphological constraints. Mouth size increased with increasing stingray size allowing larger individuals to feed on larger prey such as portunid crabs. Comparison of diets between juvenile brown stingrays and juvenile scalloped hammerhead sharks revealed that prey resources are partitioned between these two sympatric species, with hammerheads feeding on a larger proportion of teleosts relative to stingrays. These results were supported by both bulk and amino acid stable isotope analyses which showed trophic positions for stingrays increase with size and hammerheads feed at a higher trophic position relative to stingrays. Competition from stingrays for prey resources does not appear to be a factor contributing to high mortality

rates for hammerhead pups. Amino acid stable isotope analysis also demonstrated the importance of Kāneʻohe Bay as a nursery habitat for brown stingrays. Juveniles forage within the bay until the onset of sexual maturity, at which point they shift to deep offshore waters. This is one of the first studies to use amino acid stable isotope analysis with an elasmobranch, and results suggest that parameters used to calculate trophic position with this method for non-elasmobranch species may not be applicable for some elasmobranchs. It is hypothesized that urea retention for osmoregulation by elasmobranchs reduces the rate of catabolism of glutamic acid compared to non-ureosmotic species, resulting in lower ^{15}N enrichment of glutamic acid in muscle tissue and subsequent underestimation of absolute trophic position (Chapter III).

Static respirometry was used to estimate standard metabolic rates of juvenile brown stingrays across a range of mass and temperature. Mass and temperature explained the majority of variation in estimated metabolic rates. Metabolic rates were similar to those of more active myliobatiform stingrays which suggests metabolic rates of brown stingrays may be inherently high relative to their level of activity (Chapter IV).

Using species-specific input parameters estimated in the previous chapters, a bioenergetics model was developed to create an energy budget and estimate age-specific consumption rates for juvenile brown stingrays in their Kāneʻohe Bay nursery. Slow growth rates are dictated by a large fraction of their energy budget devoted to maintenance and routine activity. Population consumption rates based on a range of population estimates suggest the potential for strong top-down effects on prey populations due to stingray predation (Chapter IV).

Collectively, these results indicate that juvenile brown stingrays can have a strong impact on food web dynamics in Kāneʻohe Bay. The benefits of using Kāneʻohe Bay as a nursery habitat appears to be increased juvenile survival due to decreased predation from large sharks, but this benefit comes at the expense of growth. Slow growth translates into delayed recruitment to adult populations. Because of this, increases in juvenile mortality would likely have significant impacts on the overall population. Because there are currently no directed commercial or recreational fisheries, and no significant by-catch of this species, the future status of brown stingray populations may be most impacted by other anthropogenic impacts such as habitat alteration. Conservation of habitats such as Kāneʻohe Bay which serve as nursery habitats for multiple species, should be of high priority for management agencies in Hawaiʻi.

Future Directions

The use of three independent methods to quantify trophic position (stomach content, bulk and amino acid stable isotope analyses) provided cross-validation of each estimate in this study. However, accurately characterizing the diet through stomach content analysis often requires large sample sizes (Ferry and Cailliet 1996), and for some species/sizes lethal sampling. Because stable isotope analysis provides information on the time-integrated, assimilated diet, smaller samples sizes are generally needed to accurately reflect intraspecific variation. Additionally, stable isotope techniques only require a small biopsy of tissue, minimizing negative impacts to the animal. Yet the trophic enrichment factor used to estimate trophic position must be accurately quantified without similar cross-validation. Gannes et al. (1997) emphasized the need for more

laboratory experiments concerning the physical and biological processes underlying the application of stable isotope techniques to ecological questions and Martinez del Rio et al. (2009) re-emphasized this need over a decade later. This is particularly pertinent to ecological studies concerning elasmobranchs which increasingly use stable isotope techniques. Only a single study has estimated the trophic enrichment of bulk tissue ^{13}C and ^{15}N (Hussey et al. 2010a). Observational studies typically rely on trophic enrichment factors experimentally determined for teleosts (e.g. Fisk et al. 2002), despite several studies indicating significant interspecific variation in trophic enrichment factors (e.g. Caut et al. 2009) and the unique physiology of elasmobranchs (Speers-Roesch and Treberg 2010). The abundance of brown stingrays in Kāneʻohe Bay and their ease of maintenance at HIMB makes this species an excellent candidate for manipulative experiments and future stable isotope studies with this species, and elasmobranchs in general, would greatly benefit from such.

Amino acid stable isotope analysis can provide information on both the trophic position of the animal as well as the $\delta^{15}\text{N}$ values of primary producers at the base of the food web, making this a powerful and convenient tool to investigate the foraging ecology of elasmobranchs. However, the quality of future results using this technique will likely be proportional to the amount of experimental support underlying its assumptions. In this study, bias in absolute trophic position estimates based on amino acid analyses, and the potential influence of urea retention on trophic position estimates, highlights the need for experimental studies with elasmobranchs. Natural variations in the amount of urea retained for osmoregulation between species (i.e. freshwater vs. marine) should provide a

suitable model for evaluating the effects of urea retention on amino acid trophic enrichment factors.

Bioenergetics of elasmobranch species is sorely in need of additional research. Several of the input parameters used in the bioenergetics model were directly estimated as part of this study. However, there were still parameters for which limited or no data existed for brown stingrays and are relatively unquantified for elasmobranchs in general (ACT, SDA, Waste). The metabolic cost of activity is potentially the largest and most variable component of an organism's daily energy budget (Boisclair and Sirois 1993, Lowe 2001). The ACT value used to approximate RMR in this study was based on the relationship between swimming speed and metabolic rate in an unrelated shark species (Carlson et al. 1999). In active sharks, this relationship is typically estimated in a swimming flume where swimming speed can be accurately quantified (e.g. Lowe 2001). This technique is more difficult to apply to benthic stingrays due to their resistance to forced swimming. Indirect measures of activity level such as heart rate and tail beat frequency have been applied to elasmobranch species to estimate metabolic rates of free-swimming animals in the field (e.g. Scharold et al. 1989, Scharold and Gruber 1991, Lowe 2002). Yet variability in the relationship between heart rate and oxygen consumption (Scharold et al. 1989, Scharold and Gruber 1991, Thorarensen et al. 1996), and the unique swimming kinematics of stingrays (Rosenberger 2001) limits the applicability of these techniques for this group of elasmobranchs. Three-dimensional acceleration data loggers provide a promising alternative to these techniques for estimating field based activity levels (e.g. Whitney et al. 2008) and associated metabolic rates (e.g. Gleiss et al. 2010). This technique could be easily applied to brown stingrays

and calibrated through laboratory experiments to further refine estimates of daily consumption.

Slow field based growth rates of juvenile brown stingrays observed in this study, coupled with significantly higher growth rates of captive stingrays fed ad libitum, suggests that food is limiting in Kāneʻohe Bay. Several physiological and behavioral factors other than prey availability can affect consumption rates such as meal size and frequency, gastric evacuation rate, prey detection and capture success (Wetherbee and Cortés 2004, Krebs 2001). Although these parameters are inherently difficult to measure for elasmobranchs, emergent technologies such ‘ecology tags’ (e.g. Papastamatiou et al. 2007, Nakamura et al. 2010) could help fill these knowledge gaps. Finally, empirical estimates of juvenile brown stingray population size and sub-habitat use would further benefit our understanding of their ecological impacts within this important nursery system.

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