

An Examination of the Diet and Movement Patterns of the Atlantic Cownose Ray

Rhinoptera bonasus within a Southwest Florida Estuary

by

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For my parents, Bob and Susie Barker, for their unconditional love and support, and for teaching me that
dreams are simply future plans.

For my husband, Josh Collins, for his gentle nature and quiet humor – and for reminding me that it's all
small stuff.

And for all of the fishes in the sea, for letting us poke and prod, tag and track, hook and harass – so that we
can make an attempt to intelligently protect them for future generations.

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ABSTRACT

Cownose rays are benthic, suction feeders whose foraging activities have been implicated in severe damage to commercial shellfish industries and seagrass habitat. With jaws highly modified for durophagy, it has been assumed that they are crushing specialists, feeding primarily upon hard molluscan prey. In addition, *R. bonasus* are believed to be highly migratory, transient residents of coastal inshore waters. However, minimal quantitative data exist regarding *R. bonasus* feeding or movement patterns in the Gulf of Mexico. Stomach contents from 50 cownose rays caught within the Charlotte Harbor estuary between July 2003 and July 2004 were analyzed using the index of relative importance (IRI). Crustaceans, polychaetes, and bivalves were the dominant groups present, with bivalves representing the smallest proportion of the three dominant groups. High dietary overlap was observed between sexes, size groups and seasons. Shoalmates exhibited significantly more similar diets to each other than to members of other shoals. Although currently believed to be a hard prey specialist, these results suggest the cownose ray may behave as an opportunistic generalist, consuming any readily available

prey. Between July 2003 and November 2004, 21 cownose rays were tagged and tracked within Charlotte Harbor using passive acoustic telemetry. Residence time ranged between 1-102 days. No significant relationship was detected between activity patterns and tidal stage or time of day. Minimum convex polygons (MCP) and kernel utilization distributions (KUD) were calculated to demonstrate the extent of an animal's home range and core areas of use. Daily MCPs ranged between 0.01 and 25.8 km², and total MCPs ranged between 0.81 and 71.78 km². Total 95% KUDs ranged between 0.18 and 62.44 km², while total 50% KUDs were significantly smaller, ranging from 0.09 to 9.68 km². Both MCPs and KUDs exhibited a significant positive relationship with residence time and with disc width. As mobile, pelagic swimmers capable of traversing large distances, these data also demonstrated that cownose rays may remain within relatively small areas for extended periods. These diet and tracking results provide insight to *R. bonasus* use of a south Florida estuary and allow predictions regarding the impact of this species in similar environments.

Chapter 1: General Introduction

Study species

Atlantic cownose rays (*Rhinoptera bonasus*, Mitchill 1815) occur along coastlines of the western Atlantic (from New England to Brazil), Caribbean and Gulf of Mexico. Reaching disc widths greater than 100 cm, *R. bonasus* are commonly documented within Florida waters. Cownose rays exhibit schooling and shoaling behavior (Pitcher and Parrish, 1993) and have often been observed traveling along coastlines in organized groups that can number thousands of individuals (Clark, 1963; Schwartz, 1965; Blaylock, 1989; Rogers, 1990). Schools and shoals appear to form based on size, and typically contain representatives of both sexes. These massive aggregations have been suggested to form during large-scale migrations, triggered by seasonal changes in water temperature or sun orientation (Schwartz, 1965, 1990). This idea is supported in many areas by the inshore abundance of cownose rays during spring and summer (Snelson and Williams, 1981; Smith and Merriner, 1987; Blaylock, 1992) and absence during fall and winter (Grant, 1983; Hoese and Moore, 1977). Schwartz (1990) suggested that there are two separate migrating populations of *R. bonasus* within U.S. waters. He proposed that the Atlantic population migrates along the east coast from New England to Brazil, and that the population in the Gulf of Mexico travels clockwise along coastlines in a circular pattern from the Yucatan Peninsula to Florida. Alternatively, it has been suggested that cownose rays may simply move offshore during the colder months (Smith and Merriner,

1987; Rogers, 1990). While data supporting these hypotheses are lacking, *R. bonasus* is assumed to be a transient, seasonal resident of bays and estuaries.

Although pelagic swimmers, *R. bonasus* behave as benthic suction feeders (Sasko, 2000) and have been observed creating feeding pits up to 1 meter wide and 45 cm deep (Orth, 1975). Considerable evidence exists that rays (including *R. bonasus*) can significantly impact the benthic environment, either directly through consumption of organisms (Smith and Merriner, 1985; Blaylock, 1992; Peterson et al., 2001), or indirectly via invasive feeding behavior (Orth, 1975; Valentine et al., 1994, Silberhorn et al., 1996). Along the eastern coast of the United States, their feeding activities have been implicated in severe damage to the commercial shellfish industry (Smith and Merriner, 1985, Blaylock, 1992, Peterson, 2001) and seagrass habitat (Orth, 1975; Hovel and Lipcius, 2001). All previous evidence indicates that cownose rays are hard prey specialists (Smith and Merriner, 1985, Blaylock, 1992, Silberhorn, 1996, Peterson et al., 2001). With jaws highly modified for durophagy (Summers, 2000, 2003), it has been suggested that they are stenophagous in their choice of bivalve mollusks (Smith and Merriner, 1985). Due to the tendency of *R. bonasus* to travel in large groups, impacts from their feeding behavior can be concentrated in relatively small areas, magnifying potential effects.

Most of the information available regarding cownose ray ecology stems from research done along the Atlantic coast of the United States, where damage to seagrass habitat and commercial shellfish beds (Orth, 1975; Merriner and Smith, 1979; Smith and Merriner, 1986; Blaylock, 1992; Kraeuter and Castagna, 1980; Peterson et al., 2001) prompted some investigation of *R. bonasus* diet and distribution. It is important to note

that although considerable data have been gathered for *R. bonasus* along the eastern coast of the U.S., there have been no quantitative data collected regarding feeding or movements of cownose rays within inshore waters of the Gulf of Mexico. The goals of this study were to identify the diet and movement patterns of *R. bonasus* within Charlotte Harbor, a southwest Florida estuary, to help better define the ecological role of this species along the western coast of Florida.

Study site

Charlotte Harbor is an estuary on the west coast of Florida (between 27°05' and 26° 27' N latitude, 81°50' and 82° 30' W longitude). The shore of Charlotte Harbor is variable with highly developed canal systems interspersed with undeveloped, protected areas (e.g. National Wildlife Refuge). The aquatic habitat within the harbor varies from shallow sand and mud flats to seagrass beds and deep channels (Figure 1). Depths within the estuary range from zero to ten meters with a tidal range of 0.7 to 1.8 meters. Seagrass beds are typically found in areas less than 2 m deep and consist of three main species: manatee grass, (*Syringodium filiforme*), turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrightii*).

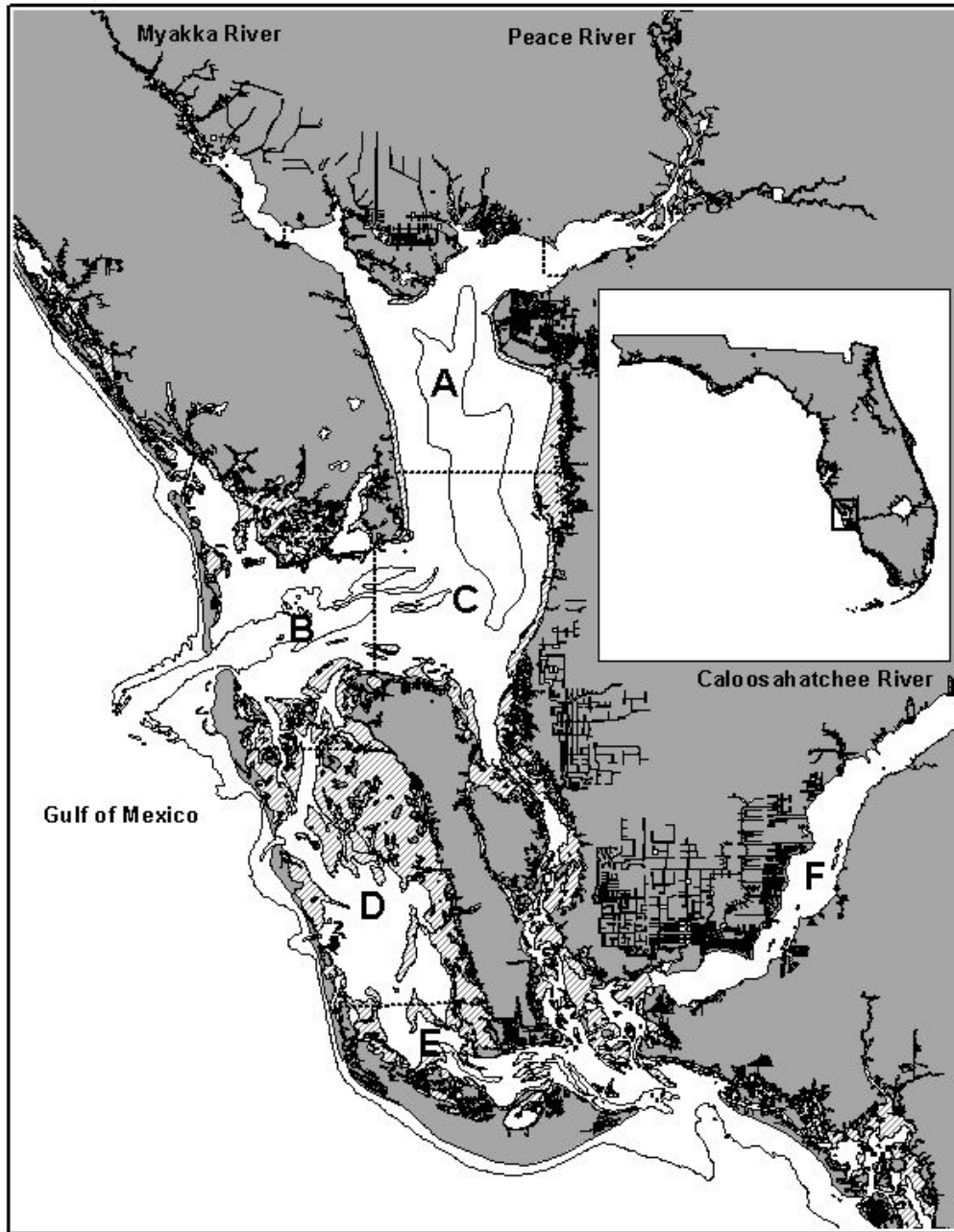


Figure 1-1. Map of Charlotte Harbor and its location along the Gulf coast of Florida. Sampling zones for stomach content analysis are indicated by dashed lines and labeled A – F. Hatched areas indicate seagrass beds and the bathymetry contour indicates depths greater than 4 meters.

Chapter 2: Feeding Ecology

Introduction

As an abundant predator that can significantly modify the benthic environment, the cownose ray has the potential to be a major determinant of community structure (Choat, 1982). Cownose rays are benthic, suction feeders (Sasko, 2000), capable of creating feeding pits up to a meter wide and 45 centimeters deep (Orth, 1975).

Rhinopterids have been shown repeatedly to feed upon bivalve mollusks (Smith, 1907; Bigelow and Schroeder, 1953; James, 1962; Blaylock, 1992; Smith and Merriner, 1985). Smith and Merriner (1985) concluded that the cownose ray is stenophagous and consistent in its consumption of bivalves. Since their jaws are highly modified for durophagy (Smith and Merriner, 1985; Summers, 2000), it has been suggested that *R. bonasus* eat hard molluscan prey “to the exclusion of all else,” (Summers et al., 2003).

Most of the information available regarding cownose ray feeding ecology stems from research done along the Atlantic coast of the United States, where the feeding activities of cownose rays have been implicated in substantial damage to the commercial shellfish industry (Orth, 1975; Merriner and Smith, 1979; Smith and Merriner, 1986; Blaylock, 1992; Kraeuter and Castagna, 1980; Peterson et al., 2001). Smith and Merriner (1985) have performed the only quantitative diet study to date, examining the stomach contents from 68 *R. bonasus* caught within Chesapeake Bay. Bivalves were the dominant prey item, making up 99.8% of the total volume examined. They observed an ontogenetic

shift in prey type, with adults consuming deeper, burrowing bivalves while juveniles ate shallower or non-burrowing bivalves. Bigelow and Schroeder (1953) described *R. bonasus* prey to include oysters, clams and other large bivalves, as well as crustaceans and gastropods. Blaylock (1992) reported considerable damage to oyster beds in Chesapeake Bay and Tiller et al. (1952) attributed the loss of “600 bushels of clams in 2 nights” to feeding cownose rays. The minimal *R. bonasus* diet data that exist for the Gulf of Mexico are qualitative, but also suggest that bivalves are the predominant prey type (Wang and Raney, 1971; Livingston, 1984; Valentine et al., 1994).

Charlotte Harbor, one of the largest and least contaminated estuaries within Florida (Pierce et al., 1986, 2003), supports significant and diverse populations of invertebrate fauna (Estevez, 1981, 1986), including commercial shellfish. Cownose rays have been documented within Charlotte Harbor throughout the year (R. Hueter, Mote Marine Laboratory, Center for Shark Research, unpublished data). Presence of *R. bonasus* within the area suggests they are using this habitat and will feed within this region throughout the year. The goals of this study were to describe the diet and distribution of cownose rays within Charlotte Harbor, giving consideration to size and seasonal differences, to better understand the ecological impact of cownose rays within estuaries along Florida’s Gulf coast.

Methods

Stomachs were obtained from *R. bonasus* captured within Charlotte Harbor zones A-F (Figure 1-1) during daylight hours over a twelve-month period (July 2003-July 2004) during routine sampling by both Mote Marine Laboratory's Center for Shark Research (Mote CSR) and Florida Fish and Wildlife Research Institute's (FWRI) Fisheries-Independent Monitoring (FIM) program. Rays were collected in entanglement nets, purse seines, or beach seines. No evidence of regurgitation was observed in any collected individuals. Animals were euthanized upon capture with an overdose of tricaine-methane-sulfonate (MS222) and placed on ice until returning to the lab. Rays were measured to the nearest centimeter (straight disc width, SDW), weighed to the nearest 0.1 kilogram, and sexed. Stomachs were removed from 50 individuals (28 adults; >70 cm disc width and 22 juveniles; <70 cm disc width) upon returning to the lab and stored frozen until transferred to 10% buffered formalin for fixation. Maturity was based on sampling data by Mote CSR (Collins, unpublished data) and judged by reproductive condition (clasper calcification in males and average minimum size at pregnancy in females). Size at maturity differs from Smith and Merriner (1986), who estimated maturity for *R. bonasus* in Chesapeake Bay to be >90 cm (females) and >84 cm (males), but conservatively agree with size at maturity for *R. bonasus* in the northern Gulf of Mexico (65 cm; Neer and Thompson, 2005). After fixation all stomach contents were rinsed and placed in 70% ethanol or isopropyl alcohol prior to sorting.

Prey items were identified to the lowest possible taxonomic level. To determine whether sufficient stomachs were collected, a cumulative prey curve was constructed by plotting total number of prey families observed against the number of stomachs examined

(Kyne and Bennett, 2002). The number of stomachs included was deemed sufficient to adequately describe the diet when the curve reached an asymptote.

Numerical and volume composition (N_c and V_c) and frequency of occurrence (F_o) were calculated for each prey type and used to determine the index of relative importance (IRI) (Pinkas et al., 1971; Hyslop, 1980):

$$IRI = (\%N_c + \%V_c) \%F_o$$

Prey that were not whole were enumerated by defining a unique part (i.e. head of crustacean, whorl of gastropod, head of polychaete) as one individual. After enumerating all prey items from an individual stomach, each prey type from that stomach was volumetrically quantified using displacement. Prey types were put into a cylinder containing a known volume of seawater, and the volume displaced was used to determine volumetric composition (V_c) of prey types within each stomach. To facilitate comparisons among prey type, percent IRI (%IRI) was calculated (Cortés, 1997). After identification to the lowest possible taxonomic level, prey items were then classified into nine major taxonomic groups: bivalves, brachiopods, crustaceans, chordates, detritus, echinoderms, gastropods, nematodes, and polychaetes.

The Shannon-Weiner diversity index (H') (Krebs, 1999) was calculated for each stomach to measure the diversity of prey items consumed. This index gives a value to the uncertainty of correctly guessing the next prey type to be found within a stomach – in other words, it quantifies the heterogeneity of prey items within a sample. H' was calculated for each stomach and averaged over all stomachs in each size class:

$$H' = - \sum_{i=1}^s (p_i)(\log_2 p_i)$$

where H' = Shannon-Weiner Index of diversity,

s = number of major taxonomic prey groups (i.e. bivalves, crustaceans...), and
 p_i = proportion of total sample (individual volume) belonging to the i^{th} group.

Evenness (E) describes the equitability of distribution of individuals among major prey groups (Krebs, 1999). For example, a stomach containing only two types of prey would have a low evenness value, while a stomach containing equal proportions of all possible prey groups would display complete evenness ($E=1.0$). Evenness allows us to compare actual diversity to the maximum possible diversity, and was calculated for each stomach and averaged over all stomachs in each size class as:

$$E = H' / H_{\max}$$

where H' = Shannon Weiner Index of diversity,

$$H_{\max} = \log_2 s, \text{ and}$$

s = total number of major taxonomic prey groups consumed by one size class of rays (Gray et al., 1997; Krebs, 1999).

To determine how stomach volume changed with ray size, individual stomach content volumes were measured for all ray stomachs containing prey, and qualitative stomach fullness (SF) values of 1-10 (10 being a completely full stomach) were assigned for each stomach. Linear regressions were performed to detect any correlations between *R. bonasus* size and stomach volume or fullness.

The size of individual prey items was estimated to the nearest millimeter. Total body length for crustaceans, nematodes, polychaetes, gastropods and chordates were recorded, while total width was measured for bivalves and brachiopods. When crushed, gastropod, bivalve and brachiopod sizes were conservatively estimated by measuring intact visceral masses. The broken condition of all observed echinoderms forced

minimum estimations of their numerical composition by counting intact mouths as one individual, and measuring echinoderm size was not possible. Detritus and seagrass were excluded from this analysis because they could not be enumerated. Prey items were measured and an average prey size value was calculated for each *R. bonasus* stomach and a linear regression was performed to determine whether prey size increased with ray disc width.

Dietary overlap was assessed for male and female *R. bonasus*, immature and mature *R. bonasus*, and between seasons, using the Simplified Morisita Index of overlap (Horn, 1966; Krebs, 1999):

$$C_H = \frac{2 \sum_i^n p_{ij} p_{ik}}{\sum_i^n p_{ij}^2 + \sum_i^n p_{ik}^2}$$

where C_H = Simplified Morisita index of overlap (Horn, 1966) between groups j and k

p_{ij} = proportion resource i of the total resources used by group j ,

p_{ik} = proportion resource i of the total resources used by group k , and

n = total number of prey groups.

A value of zero indicates no dietary overlap, while a value of one represents complete overlap. The system used by Morte et al. (2002) was followed, where overlap index values were arbitrarily classified as low (0.00 to 0.29), moderate (0.3 to 0.6) and high (>0.60).

When multiple rays from a single shoal were collected, these individuals were tested to determine whether animals shoaling together had more similar stomach contents

than those not shoaling together. This was calculated using the Simplified Morisita Similarity Index (Morisita, 1959; Krebs, 1999 p. 391):

$$C_H = \frac{\sum P_{ij}P_{ik}}{\sum [(P_{ij}^2 / N_j^2) + (P_{ik}^2 / N_k^2)]}$$

where C_H = Morisita's index of similarity between sample j and k ,

P_{ij} , P_{ik} = volumetric proportion of species (or lowest possible taxonomic group) i in sample j and k , and

N_j , $N_k = \sum P_{ij}^2$, $\sum P_{ik}^2$ = total proportion of individuals in sample j and k .

Volumetric proportions were used rather than numeric proportions because these better describe the nutritional contribution of a prey item to its predator (Hyslop, 1980). C_H values were calculated for each individual compared to all of its shoalmates, as well as to all of the other rays that were not part of its shoal. The similarity values both within and between shoals were then averaged and examined for differences.

Results

Fifty *R. bonasus* were collected from Charlotte Harbor between July 2003 and July 2004 (Table 2-1). Straight disc widths (SDW) ranged from 37 to 78 centimeters. Thirty-seven out of 50 stomachs (74.0%) contained identifiable material. Of these 37, 16 were from mature (7 females, 9 males) and 21 (8 females, 13 males) were from immature *R. bonasus*. Thirty-five of the 37 analyzed (94.6%) were collected in zones A, B or C. No rays were caught in zone D. The remaining two *R. bonasus* were captured in lower Pine Island Sound (zone E) and the Caloosahatchee River (zone F). Results from the cumulative prey curve analysis indicated 26 stomachs were sufficient to represent the diet of the cownose ray within Charlotte Harbor (Figure 2-1).

Table 2-1. Monthly numerical and sexual composition of rays caught between July 2003 and July 2004.

Month	N	Male	Female
July	3	2	1
August	2	2	0
September	3	0	3
October	7	6	1
November	7	3	4
December	1	0	1
January	5	5	0
February	6	2	4
March	6	3	3
April	0	0	0
May	4	2	2
June	6	3	3

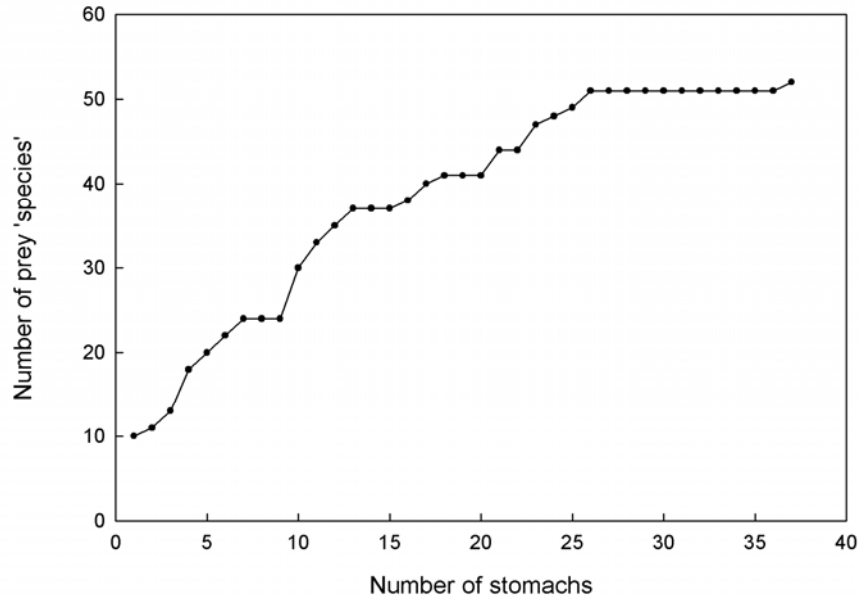


Figure 2-1. Cumulative prey curve displaying the total prey group ('species' = when prey could not be identified to species, the next lowest possible taxonomic classification was used) vs. the total number of *Rhinoptera bonasus* stomachs analyzed.

A total of 92,577 prey items were identified and fell into nine major groups: crustaceans, polychaetes, bivalves, echinoderms, gastropods, brachiopods, chordates, nematodes, and detritus (Table 2-2). Nematodes, chordates, gastropods, and brachiopods all had a %IRI of less than 1. Nematodes were the least common item, with a single organism being present in only two stomachs. Chordates also had a low frequency of occurrence, found in only five stomachs. However, when present, chordates did at times occur in high volumes. For example, one stomach contained 66% (by volume) *Branchiostoma* sp. (lancelets) and another contained 18% tunicates. Gastropods and brachiopods had a high frequency of occurrence (43.2% and 37.8%, respectively) but occurred in low numbers or volumes relative to the dominant prey groups, making their relative IRI values low (0.84% and 0.71% respectively). Echinoderms, represented

primarily by the sand dollar *Mellita* sp., occurred in 10 stomachs and had a %IRI of 2.3. Although echinoderms occurred in 27% of the examined stomachs, the %IRI was low due to minimum estimations of this group's numerical composition. Detritus occurred in 64.9% of the examined stomachs and was classified as any organic, non-animal matter (usually wood, leaves or seagrass). Detritus represented 5.8% of total volume consumed. Its IRI value was not considered because detritus could not be accurately enumerated.

Crustaceans, polychaetes, and bivalves were the dominant prey groups present across all stomachs examined (%IRI = 55.3, 25.2, and 12.6 respectively; Table 2-2), together making up 98.5% (numerically), 75.3% (volumetrically) and 93.1% of the total IRI. Overall, crustaceans had the highest volumetric composition (28.8% V_c), followed by polychaetes (26.0% V_c) and bivalves (20.5% V_c). Polychaetes and bivalves had the highest frequency of occurrence (81.1 and 75.7 %F_o, respectively), and crustaceans represented 70.3 %F_o. Numerical composition of prey items was extremely high (92,577 items) due primarily to the consumption of small cumaceans (~2mm), an epibenthic crustacean that can occur in high density swarms. A total of 71,484 cumaceans was consumed by 17 rays comprising 77.2% of the total N_c. Infaunal polychaete worms, *Pectinaria gouldii*, were the next most numerically abundant prey items with 14,068 (15.2% by number) consumed by 24 rays. These two prey items contributed to the high numerical values and comprised 92.4% of the total number of prey items recorded.

Table 2-2. Prey groups identified for all *Rhinoptera bonasus* collected from Charlotte Harbor, Florida. Frequency of occurrence (F), numerical composition (N), volumetric composition (V), Index of Relative Importance (IRI), and their respective percentages are shown for major taxa.

Prey	F	F_o (%)	N	N_c (%)	V (ml)	V_c (%)	IRI	IRI (%)
Bivalves	28	75.68	2141	2.31	116.82	20.54	1729.45	12.58
Mytiloidea	20	54.05	1520	1.64	52.24	9.22		
Mytilidae	20	54.05	1520	1.64	52.24	9.21	586.79	4.27
Veneroidea	14	37.84	513	0.55	43.08	7.60		
Crassatellidae	2	5.41	11	0.01	1.09	0.19		
Mactridae	4	10.81	17	0.02	2.40	0.42		
Solecurtidae	1	2.70	7	0.01	0.10	0.02		
Tellinidae	12	32.43	440	0.47	35.57	6.27		
Veneridae	4	10.81	16	0.02	1.15	0.20		
Veneroid family	2	5.41	22	0.02	2.77	0.49		
Arcoidea	4	10.81	47	0.05	2.61	0.46		
Glycymerididae	4	10.81	47	0.05	2.61	0.46		
Pterioda	1	2.70	8	0.01	14.00	2.47		
Ostreidae	1	2.70	8	0.01	14.00	2.47		
Unidentified bivalves	9	24.32	53	0.06	4.89	0.86		
Brachiopoda	14	37.84	666	0.72	10.06	1.78	94.42	0.69
Lingulidae	14	37.84	666	0.72	10.06	1.78		
Chordata	5	13.51	280	0.30	17.63	3.11	46.13	0.34
Branchiostomidae	2	5.41	240	0.26	15.55	2.75		
tunicates	1	2.70	11	0.01	1.90	0.34		
teleost scales	2	5.41	29	0.03	0.17	0.03		
Crustaceans	26	70.27	73561	79.46	162.95	28.76	7604.89	55.31
Isopoda	9	24.32	162	0.18	5.27	0.93		
Anthuridae	8	21.62	161	0.17	5.17	0.91		
Idoteidae	1	2.70	1	0.00	0.10	0.02		
Amphipoda	14	37.84	1047	1.13	9.99	1.76		
Ampeliscidae	2	5.41	47	0.05	0.47	0.08		
Oedicerotidae	10	27.03	572	0.62	5.59	0.99		
Unidentified amphipods	2	5.41	428	0.46	3.93	0.69		
Mysidacea	7	18.92	656	0.71	12.06	2.13		
Mysidae	7	18.92	656	0.71	12.06	2.13		
Cumacea	17	45.95	71484	77.22	133.19	23.51	4628.01	33.66
Bodotriidae	17	45.95	71484	77.22	133.19	23.51		
Decapoda	5	13.51	9	0.01	0.69	0.12		
crabs	5	13.51	8	0.01	0.65	0.11		
shrimps	1	2.70	1	0.00	0.05	0.01		
Maxillopoda	6	16.22	129	0.14	0.77	0.14		
barnacles	6	16.22	129	0.14	0.77	0.14		
Unidentified crustaceans	8	21.62	74	0.08	0.96	0.17		
Echinoderms	10	27.03	19	0.02	67.01	11.83	320.26	2.33
Holothuroidea	1	2.70	2	0.00	1.80	0.32		
Echinoidea	7	18.92	13	0.01	62.86	11.10		
Ophiuroidea	2	5.41	3	0.00	2.30	0.41		
Unidentified echinoderms	1	2.70	1	0.00	0.05	0.01		
Gastropods	16	43.24	424	0.46	12.18	2.15	112.78	0.82
Cephalaspidea	4	10.81	126	0.14	1.74	0.31		
Acteocinidae	2	5.41	107	0.12	0.83	0.15		
Haminoeidae	2	5.41	19	0.02	0.91	0.16		
Neotaenioglossa	2	5.41	2	0.00	0.08	0.01		
Assimineidae	1	2.70	1	0.00	0.03	0.01		
Strombidae	1	2.70	1	0.00	0.05	0.01		
Neogastropoda	2	5.41	2	0.00	0.10	0.02		
Columbellidae	1	2.70	1	0.00	0.05	0.01		
Nassaridae	1	2.70	1	0.00	0.05	0.01		
Unidentified gastropods	10	27.03	294	0.32	10.26	1.81		
Nemata	2	5.41	2	0.00	0.06	0.01	0.07	0.00
Polychaeta	30	81.08	15484	16.73	147.31	26.00	3464.59	25.20
Capitellidae	1	2.70	50	0.05	1.00	0.18		
Goniadidae	6	16.22	206	0.22	4.09	0.72		
Nereididae	16	43.24	133	0.14	2.59	0.46		
Pectinariidae	24	64.86	14068	15.20	125.74	22.20	2425.42	17.64
Phyllodocidae	1	2.70	1	0.00	0.10	0.02		
Spionidae	3	8.11	817	0.88	7.05	1.24		
Opheliidae	1	2.70	94	0.10	3.30	0.58		
Unidentified polychaetes	3	8.11	116	0.13	3.45	0.61		
Detritus and Grass	24	64.86	n/a	0.00	32.93	5.81		

A small number of species represented an overwhelming proportion of the three dominant prey groups (Figure 2-2). The cumaceans *Cyclaspis* sp. and *Oxyurostylis smithii* (family Bodotriidae) had a combined %IRI of 33.7 and represented 97.2% (numerically) and 81.7% (volumetrically) of all crustaceans present. *Pectinaria gouldii* was the dominant polychaete with a %IRI of 17.6, making up 90.9% (numerically) and 85.4% (volumetrically) of all polychaetes present. Of the bivalves, *Amygdalum papyrium* (paper mussel) was the most abundant, with a %IRI of 4.27, representing 71.0% (numerically) and 44.7% (volumetrically) of all bivalves consumed.

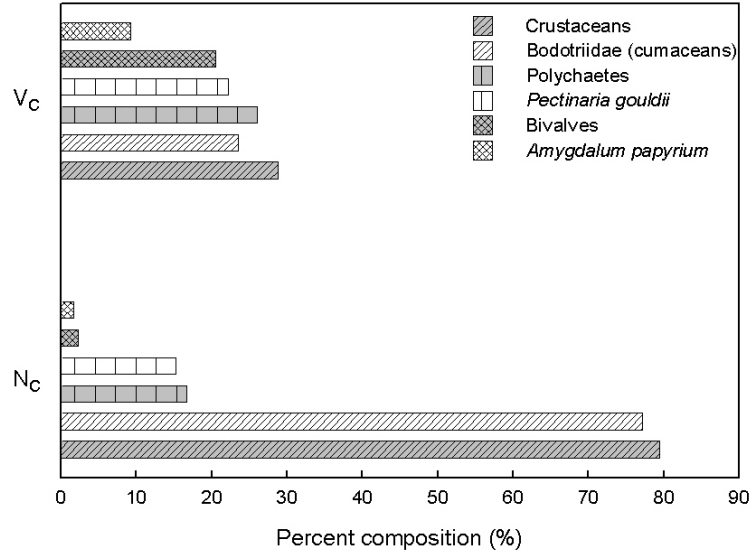


Figure 2-2. Numerical and volumetric percent composition (%Nc and %Vc, respectively) for the dominant prey groups and species comprising them. Both cumacean species identified within *R. bonasus* stomachs (*Cyclaspis* sp. and *Oxyurostylis smithii*) are in the family Bodotriidae.

The number of major taxonomic groups (i.e. polychaetes, echinoderms, etc.) per stomach ranged between one and seven, and the number of families per stomach ranged between two and 17. Although all stomachs contained multiple items, there was a tendency for individual stomachs to be dominated by a particular species. The volumetric

percent composition of the dominant prey species (or lowest possible taxonomic group) was never less than 40% of the total stomach volume (Figure 2-3). The average percent volumetric composition (%V_c) of the dominant prey item per stomach was 69.0%. Diversity (H') varied between stomachs, and values ranged between 0 and 1.9. Overall, H' was generally greater than 0.75 (mean = 0.94) (Table 2-3). No distinct pattern emerged when H' was compared to *R. bonasus* disc width (Figure 2-4), and there were no obvious differences in diversity between immature and mature rays (Table 2-3). Evenness (E) ranged between 0 and 0.66, but was generally low (overall average E= 0.33). As with H', E values did not display a distinct relationship with *R. bonasus* disc width (Figure 2-4, Table 2-3). A generalized linear model (Statistica) was used to further test for a association between H' or E and disc width. No significant relationship was found for either value when regressed against SDW (H' vs. SDW: p = 0.44; E vs. SDW: p = 0.29).

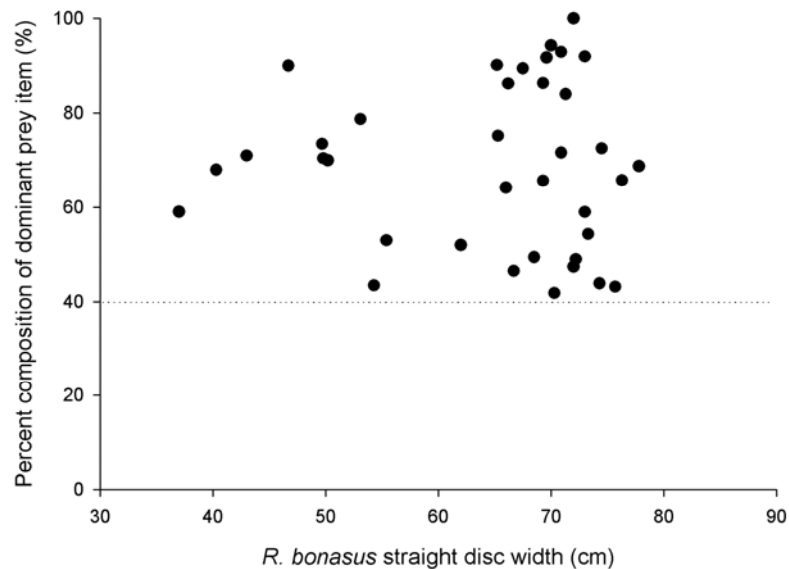


Figure 2-3. Volumetric percent composition of the single dominant prey ‘species’ (‘species’= when prey could not be identified to species, the next lowest possible taxonomic classification was used) within individual stomachs. Dotted line identifies 40% volumetric composition of a single prey type.

Table 2-3. Mean Shannon-Weiner diversity index (H') and Evenness (E) values for all, immature, and mature *R. bonasus* stomachs examined.

	H'	E
Total	0.94	0.33
Males (n=22)	0.99	0.34
Females (n=15)	0.87	0.30
Mature	0.87	0.29
Males (n=9)	1.04	0.35
Females (n=7)	0.66	0.22
Immature	0.99	0.35
Males (n=13)	0.95	0.34
Females (n=8)	1.05	0.37

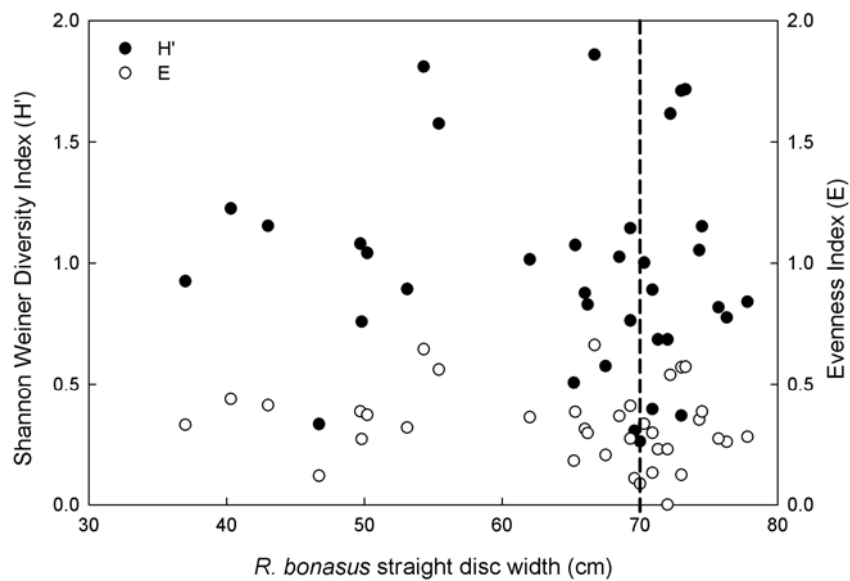


Figure 2-4. Shannon-Weiner diversity index (H', closed circles) and Evenness (E, open circles) values for all *R. bonasus* stomachs examined. Dashed line separates immature from mature rays.

Mean stomach volume (SV) of all sampled *R. bonasus* was 17.36 ml, and the average stomach fullness (SF) value was 4.8. Immature rays had lower SV values than mature rays (Figure 2-5; $r^2 = 0.64$, $p = 0.008$). Prey size ranged from < 2.0 mm to 35.0 mm, and showed a general increase with ray disc width (Figure 2-6, $r^2 = 0.88$, $p = 0.0018$).

The largest item consumed was an oyster *Crassostrea virginica* (35.0 mm), which was found in only one ray (mature female, 77.6 cm DW). Lancelets (*Branchiostoma* sp.) up to 35.0 mm long were found in large quantities in two stomachs (both mature females, 73.0 and 76.3 cm SDW). Polychaetes reached lengths of 30.0 mm, while crustaceans and gastropods fell within a size range of 2.0 - 12.0 mm.

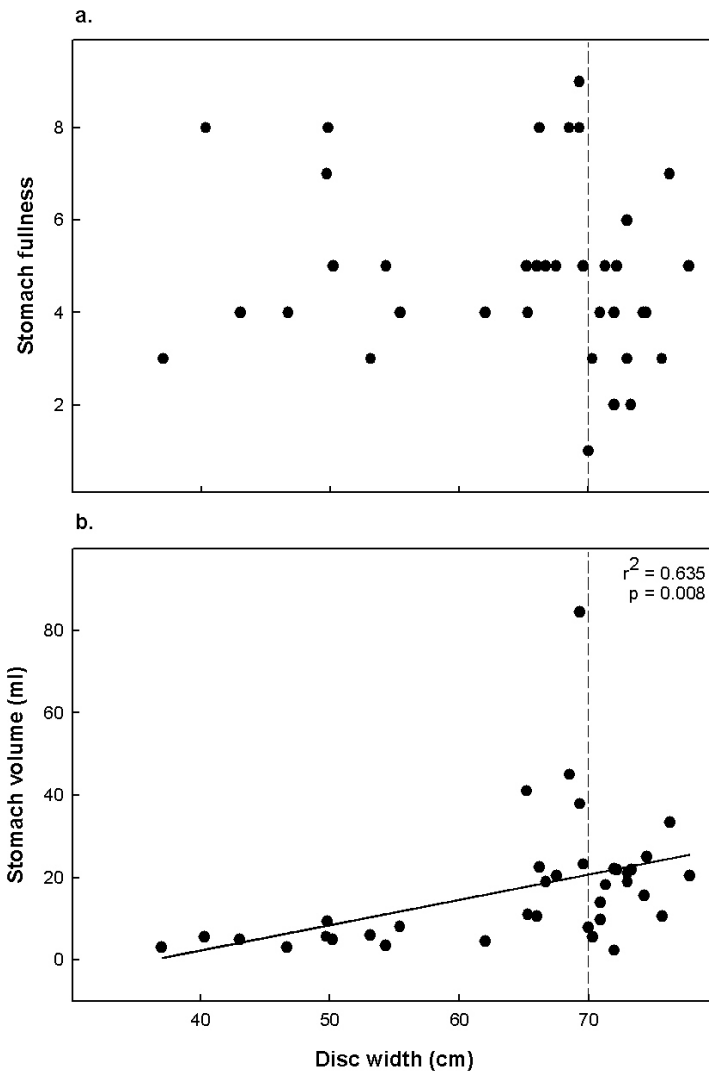


Figure 2-5. Stomach fullness, SF, (a) and stomach volume, SV, (b) based on size of individual *R. bonasus* for all stomachs analyzed. Dashed line separates immature from mature rays. There was no significant relationship between stomach fullness and disc width (linear regression, $p=0.4496$). There was a significant relationship between stomach volume and disc width (linear regression, $p=0.008^{**}$).

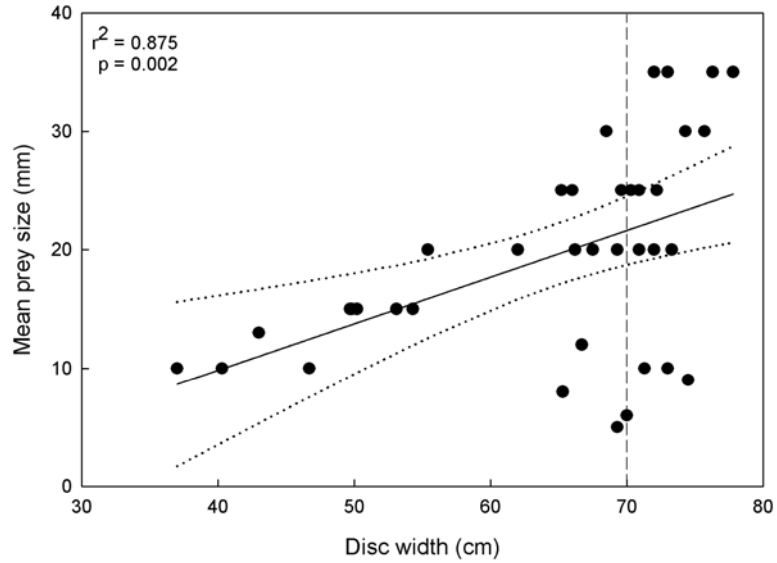


Figure 2-6. Linear regression of prey size vs. straight disc width of *R. bonasus* collected in Charlotte Harbor. There was a significant relationship between prey size and disc width (linear regression, $p=0.0018^{**}$). Dotted lines represent 95% confidence intervals. Dashed line separates immature from mature samples.

Dietary overlap between immature and mature *R. bonasus* was high ($C_H = 0.87$); Figure 2-7). For both groups, the highest %IRI values were for the same three major prey groups (crustaceans, polychaetes and bivalves). However, there were slight differences between groups. Immature rays were more likely to have detritus and grass in their stomachs ($F_o = 76.2\%$ immature, 50.0% mature). Bivalves occurred in 90.5% (F_o) of immature stomachs, but only 56.3% of mature stomachs. Crustaceans dominated numerically over both size classes ($N = 53,457$, $N_c = 77.8\%$, immature and $N = 20,104$, $N_c = 84.3\%$, mature). Crustaceans also dominated volumetrically for mature rays ($V_c = 38.1\%$), but polychaetes were present in the highest volume for immature rays ($V_c = 32.8\%$). Only mature *R. bonasus* had chordates (lancelets, tunicates or teleost scales) in

their stomachs. Teleost scales were present within two stomachs in low volume, but no other fish remains (i.e. vertebrae or muscle) were identifiable.

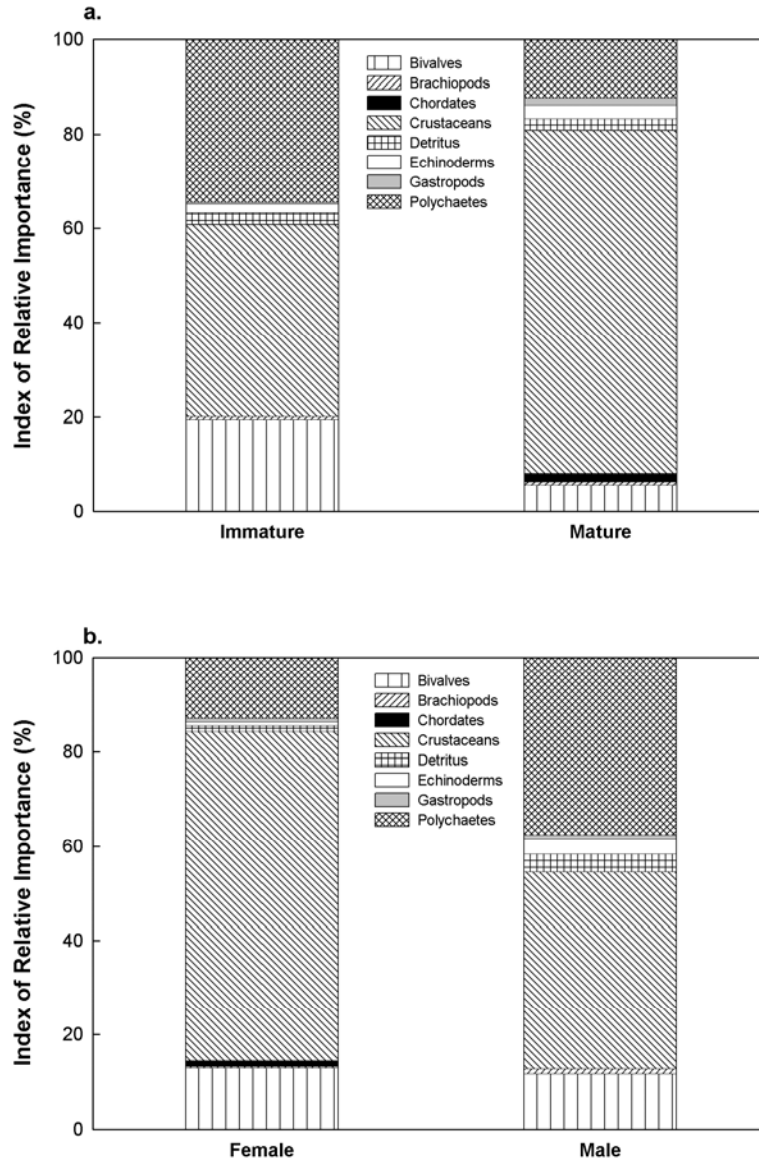


Figure 2-7. Index of Relative Importance (%IRI) for eight major prey groups consumed by mature and immature (a) and male and female (b) *Rhinoptera bonasus* collected in Charlotte Harbor for stomach content analysis. Morisita's simplified overlap index was high for both comparisons: immature vs. mature $C_H = 0.87$; female vs. male $C_H = 0.84$.

Diet comparison among male and female rays also showed high dietary overlap ($C_H = 0.84$). The only notable differences between male and female diets were that males had a higher %IRI for polychaetes (male = 37.5, female = 13.0), and females had a higher %IRI for crustaceans (female = 69.8, male = 41.8) (Figure 2-7).

Examination of seasonal differences in diet showed mixed results with all dietary overlap values falling in the medium or high categories (Table 2-4). No seasonal dietary overlap fell within the low ($C_H = 0.0$ to 2.9) category. There was high dietary overlap for all rays between spring and summer ($C_H = 0.86$), spring and fall ($C_H = 0.78$), and summer and fall ($C_H = 0.72$). Dietary overlap was classified as medium between winter and all other seasons with C_H values ranging from 0.35 – 0.56. The lowest dietary overlap was between summer and winter ($C_H = 0.35$) and was due mostly to seasonal differences in crustacean and polychaete consumption: summer polychaete %IRI = 42.1, summer crustacean %IRI = 0.15. Values for these groups were almost reversed during winter: winter polychaete %IRI = 8.3 and winter crustacean %IRI = 52.0.

Table 2-4. Seasonal dietary overlap values for all examined *R. bonasus*. Values were calculated using the Simplified Morisita measure for niche overlap (C_H). Seasonal overlap is considered high when $C_H > 0.60$, medium when C_H falls between 0.3-0.6, and low when $C_H < 0.3$. Lowest overlap values were calculated for the winter comparisons.

	Fall (n=14)	Winter (n=10)	Spring (n=9)
Summer (n=4)	0.7228	0.3497	0.8634
Spring	0.7767	0.3888	
Winter	0.5648		

Multiple individuals from the same shoal were collected on 10 separate sampling occasions. On two of these occasions, similarity values could not be calculated because all stomachs were empty, and on a third they could not be calculated because one stomach was empty. When prey items were present, dominant prey items within individual stomachs were more similar between shoalmates than between non-shoalmates (Figure 2-8). For example, examined individuals (n=3) from one shoal had each consumed >95% sand dollar (*Mellita* sp.), and all had a $C_H = 1.00$ when compared to shoalmates. Average similarity values (C_H) within a shoal ranged between 0.45 and 1.00, while average similarity values between individuals from separate shoals ranged from 0 to 0.24 (Figure 2-8).

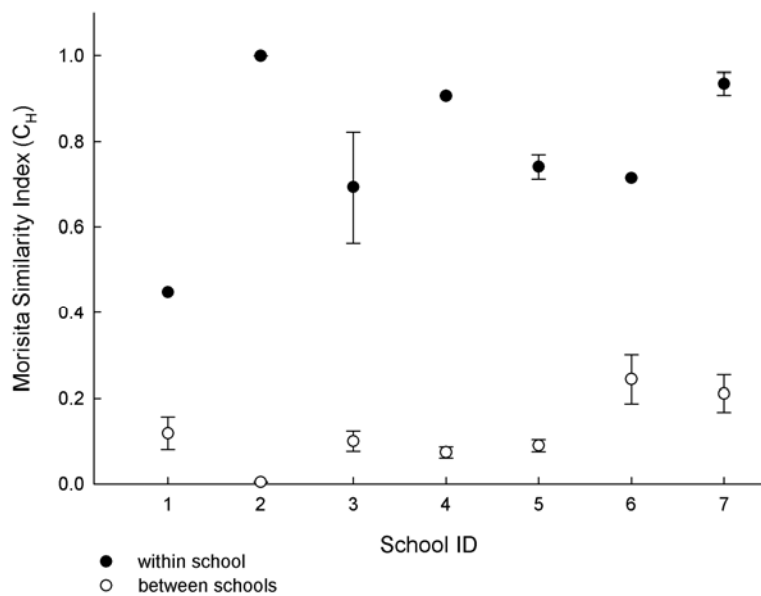


Figure 2-8. Average similarity values (Morisita’s simplified similarity Index, C_H) for individuals within shoals (closed circles) and between shoals (open circles). Error bars indicate standard deviation.

Discussion

These data indicate that *Rhinoptera bonasus* do not behave as obligate hard prey specialists within Charlotte Harbor. Although morphologically suited for durophagy (Summers 2000, 2003), *R. bonasus* within Charlotte Harbor consumed a wide variety of benthic organisms, and appear to be opportunistic generalists that feed upon prey items that are readily available. In the present study, bivalves represented only 12.6% of the total IRI. These findings differ from previously published information that *Rhinoptera* species have a restricted diet consisting mainly of bivalves (James, 1962; Smith and Merriner, 1985; Blaylock, 1992). Hard prey items (i.e. sand dollar *Mellita* sp., oyster *Crassostrea virginica*) were among identified prey types, confirming that *R. bonasus* can be durophagous in Charlotte Harbor, but these items did not dominate their diet.

Diversity (H') values represented the wide range of prey types identified across all *R. bonasus* stomachs examined (Table 2-2, Figure 2-4). Other dietary diversity values for *Rhinoptera* species are lacking; however, our values for *R. bonasus* are similar to those calculated for the bat ray *Myliobatis californica* (overall $H' = 0.73$) (Gray et al., 1997). Evenness (E) values for both *M. californica* and *R. bonasus* were consistently low, indicating that the proportions of possible prey items within individual stomachs are not equally distributed (Krebs, 1999). Overall, *R. bonasus* stomachs contained a large array of prey types, but individual stomachs were typically dominated by a single prey item. At least 40% of all stomachs consisted of a single prey species (or other lowest possible taxonomic group), suggesting that *R. bonasus* are selectively foraging in areas of high prey density, and consequently encountering and consuming dense patches of various prey species. *Rhinoptera bonasus* selectively chose habitat patches of highest prey

density when feeding on bay scallops (*Argopecten irradians concentricus*) in North Carolina (Peterson et al., 2001). Similarly, foraging eagle rays *Myliobatis tenuicaudatus* in New Zealand dramatically increased their foraging behavior above a certain prey density threshold (Hines et al., 1997). Low evenness values for *M. californica* were suggested to be attributed to ‘selective feeding and/or the relative abundances of prey items’ (Gray et al. 1997, p. 236).

Stomach volume (SV) for *R. bonasus* generally increased with ray disc width, which has also been noted for *R. bonasus* in Chesapeake Bay (Blaylock, 1992). Similar to SV, prey size also showed a general increase with *R. bonasus* disc width. Neither of these results was particularly surprising, as larger animals are usually capable of consuming higher quantities and larger sizes of prey. For example, adult *R. bonasus* in the Chesapeake Bay region ate larger bivalves than juveniles (Smith and Merriner, 1985), and larger *R. bonasus* in North Carolina were capable of eating adult bay scallops while smaller, juvenile rays were not (Peterson et al., 2001). A similar pattern was noted for *M. californica* in Humboldt Bay, with larger prey items being consumed as ray size increased (Gray et al., 1997).

Although SV increased with ray size, *R. bonasus* stomach fullness (SF) did not show any relationship to disc width. This implies that although the absolute volume of food increases with size, the rays consume approximately the same relative amount of food resulting in similar fullness. However, fullness indices must be taken with caution as these values were qualitatively assessed.

Dietary overlap was high between both size and sex groups of *R. bonasus* ($C_{H\ Imm. vs. Mat.} = 0.87$; $C_{H\ M vs. F} = 0.84$). *Rhinoptera* shoals usually form based on size (James,

1962; Blaylock, 1989; Smith and Merriner, 1987), although sexual segregation has also been noted (Smith and Merriner, 1987). Mixed-sex shoals are more often observed in Charlotte Harbor (Collins, unpublished data), so diets were expected to be similar between sexes of each size group. Previously published studies have found differences between adult and juvenile diets, as larger rays are more capable of consuming bigger and/or deeper buried prey (Smith and Merriner, 1985; Peterson et al., 2001). However, the high dietary overlap between mature and immature *R. bonasus* detected in this study was not surprising given the accessibility and small size of the predominant prey groups. Perhaps ray size does not play a major role in prey selection in Charlotte Harbor since there is such a wide array of easily captured benthic prey available (Estevez, 1986).

Both immature and mature rays were found to consume bivalves, but they were less important in mature diets (17.0% for immature rays vs. 5.7 % for mature rays). Although it appears that mature *R. bonasus* do not feed on as many bivalves as immature rays, it is possible that prey manipulation could be the reason for this deficit. For example, adults may be more proficient at rejecting shell fragments (Smith and Merriner, 1985), resulting in consumption of only visceral masses which could be digested more rapidly and escape detection. Sasko (2000) documented winnowing behavior in feeding *R. bonasus*, demonstrating that they are capable of rejecting shell fragments and other debris. The majority of bivalves represented in both immature and mature stomachs were small and thin-shelled, indicating that these are the preferred bivalve prey types, but it is possible that larger, thicker shelled bivalves that necessitate fragmentation and shell rejection were not identifiable.

Seasonal dietary overlap was high for all season comparisons except those involving winter, which had low overlap values when compared to summer and spring samples, and medium overlap values compared to fall samples. Interestingly, *R. bonasus* captured during winter months also displayed the greatest dietary diversity (mean $H' = 1.10$) (Table 5). Seasonal differences in diet could be explained by fluctuations in invertebrate fauna based on temperature, freshwater input and corresponding nutrient levels (Rubec et al., 1999; Arnold et al., 2000). Florida's rainy season typically occurs between June and October, and is characterized by lower salinities within estuaries (Estevez et al., 1984; Donaldson, 1985). The diversity of organisms within an estuary typically decreases as salinity decreases (Day et al., 1989). Estevez (1986) found that species richness of Charlotte Harbor's benthic community was highest over winter months when rainfall is less, and lowest during the summer season. The differences detected in *R. bonasus* stomach contents between seasons may simply reflect the relative abundances of prey items. If *R. bonasus* is feeding within the estuary throughout the year, then adapting to changing dynamics of available prey would be a good strategy. By eating the most common or available organisms, the opportunistic foraging behavior of cownose rays is suited to handle changing prey densities and availability that may take place within estuaries.

Examination of stomach contents demonstrated the importance of suction feeding for *R. bonasus*. *Pectinaria gouldii* is a tube dwelling polychaete that builds fragile sand cones in the sediment (Uebelacker and Johnson, 1984), and is abundant within Charlotte Harbor (Donaldson, 1985; Estevez, 1986.). Intact, whole cones up to 30 mm long were found in many stomachs. The fact that crushed echinoderms and bivalves were

sometimes found in the same stomachs, in addition to complete *P. gouldii* sand tubes, suggests that *R. bonasus* are capable of distinguishing different prey types and alternating between suction only and a combination of suction and crushing. This has also been observed for another benthic feeding elasmobranch, the nurse shark (*Ginglymostoma cirratum*) (Mattot et al., in press). Prey type probably influences this as much as prey size. Hard organisms like sand dollars and thick shelled bivalves can be fractured not only to ease swallowing but also to increase digestion rate while thin shelled and softer prey (like *A. papyrium*, cumaceans and polychaetes) can be swallowed whole. The thin-shelled paper mussel *A. papyrium* was the predominant bivalve consumed by both immature and mature *R. bonasus* in Charlotte Harbor. Similar to Smith and Merriner's (1985) findings, whole and fractured valves of *A. papyrium* were present within stomachs collected from Charlotte Harbor. However, there was little evidence of individuals preying on larger, thicker shelled mollusks. This could be due to preferential feeding on *A. papyrium* or reflect the likelihood of encountering this particular prey type. *Pectinaria gouldii*, the most dominant polychaete observed in their diets, are also common in Charlotte Harbor (Donaldson, 1985; Estevez, 1986), and although there are no reports detailing the relative abundance of cumaceans within the area, it is known that they do occur in Charlotte Harbor in high numbers (Anamari Boyes, pers. comm.). All species representing the three dominant prey groups are abundant benthic invertebrates common within estuaries of the Gulf of Mexico (Uebelacker and Johnson, 1984; Culter, 1986; Anamari Boyes and Jay Leverone, pers. comm.) and together comprised over 94% of the total numerical composition of prey identified in this study. It appears that *R. bonasus* are feeding upon the most abundant and commonly encountered prey items within Charlotte

Harbor and the fact that the dominant polychaete *P. gouldii* generally occurs in unvegetated habitat (Uebelacker and Johnson, 1984; J. Leverone, pers. comm.) indicates that cownose rays are not feeding exclusively in grass beds in Charlotte Harbor.

The shoaling tendency of *R. bonasus* should increase their likelihood of encountering these widely distributed abundant prey sources. One of the benefits of shoaling is increased foraging efficiency (Pitcher and Parrish, 1993). Traveling in a group should allow for a larger search area and therefore increased prey detection capability. Shoals of *R. bonasus* have been observed feeding synchronously (Orth, 1975; Sasko, 2000), and the results of this study demonstrate that members of a shoal have more similar diets to each other than to members of another shoal. Blaylock (1993) calculated the daily ration for *R. bonasus* in the Chesapeake Bay to be ~3% of an individual's body weight. This would mean that daily intake should range between 150 – 270 grams for cownose rays between 5 and 9 kg. Cownose rays have also been documented to consume 1.5 liters of clams in one day (Schwartz, 1990). Matern et al. (2000) suggested that high energy output is required for the consumption of large bivalves and deep burrowing invertebrates by *M. californica*. The benefit to consuming small items like epibenthic cumaceans and shallow infaunal polychaetes is that capture presumably takes less effort than deep bivalve excavation. It is notable that none of the dominant prey found in this study are deep infauna, suggesting that deep feeding pits are not necessary for *R. bonasus* in Charlotte Harbor. There may be benefits to consuming easily accessible, abundant small items vs. less abundant, larger items. Feeding on swarming or highly abundant species may reduce the amount of energy spent on prey capture.

It has been noted that foraging cownose rays may also play a beneficial, non-destructive role in structuring benthic communities by aerating benthic environments (Blaylock, 1993). Pit formation may extend the feeding activities of other organisms, such as filter feeding clams and worms (Cross and Cunan, 2000). Orth (1994) suggested that feeding cownose rays may actually enhance the dispersal of eelgrass by dislodging reproductive shoots. Cownose ray foraging can influence community structure by exposing normally obscured prey to other predators such as sharks, drum, jacks, cobia and menhaden, which associate with cownose ray schools (Rogers, 1990; Thrush et al., 1991).

Chapter 3: Movement Patterns and Residence

Introduction

Atlantic cownose rays (*Rhinoptera bonasus* Mitchill 1815) are believed to be highly migratory, transient and seasonal residents of coastal and inshore waters. They exhibit shoaling and schooling behavior, and have at times been observed traveling in groups numbering thousands of individuals (Clark, 1963; Blaylock, 1989; Rogers et al., 1990). Although large shoals are often observed traveling in coastal and inshore waters (Clark, 1963; Blaylock, 1989, 1992; Smith and Merriner, 1985) little data are available concerning the residence and movement patterns of individual *R. bonasus*. Schwartz (1990) suggested that *R. bonasus* in the Gulf of Mexico make up a separate population from those in the western Atlantic, and that each population displays a unique migration pattern in response to seasonal changes in water temperature. The Atlantic population is believed to move north/south along the coast from New England to South America. This hypothesis is based on results from a tag-recapture study where cownose rays tagged in Chesapeake Bay were recaptured as far south as Venezuela and Brazil (Schwartz, 1965). The population in the Gulf of Mexico is hypothesized to travel clockwise from the Yucatan peninsula to Florida. Beyond the data of Schwartz (1965) there are no quantitative data available to support this hypothesis. Alternately, it has been suggested that *R. bonasus* may simply move offshore to warmer water during winter (Smith and Merriner, 1987) rather than partake in extensive fall and spring migrations. In either case,

cownose rays are believed to leave shallow estuaries and bays during the winter. Currently, there are no available data on movement patterns for cownose rays in the Gulf of Mexico.

Large schools of foraging *R. bonasus* have been implicated in commercial shellfish predation as well as seagrass habitat destruction along the eastern coast of the United States (Orth, 1975; Merriner and Smith, 1979; Peterson et al., 2001). However, the lack of data regarding cownose ray movement patterns makes it difficult to define their behavior and residence within coastal estuaries. Determining and interpreting the movement patterns of cownose rays would be beneficial to understanding their life history, use of coastal habitats and role within the community.

The most common method for monitoring marine animal movements is acoustic telemetry, which is becoming more widespread as technology advances and availability increases (Voegeli, 2001). Most previous elasmobranch marine telemetry studies consisted of manually following individuals fitted with transmitters and typically provided short term (<72 hours) or intermittent behavioral data (Sciarotta and Nelson, 1977; Standora and Nelson, 1977; Klimley and Nelson, 1984; Gruber et al., 1988). While manual telemetry studies are useful in defining fine scale habitat use, they do not provide the continuous data for individuals over extended periods (weeks – months) that are necessary for a more complete understanding of residency and movement patterns. The advent of passive acoustic monitoring technology has allowed the movements of multiple animals to be continuously tracked over extended time frames (Klimley et al., 1988; Meyer et al., 2000; Vogeli, 2001; Heupel and Hueter, 2001; Heupel et al., 2004) allowing

predictions of long-term patterns, home ranges and response of individuals to environmental factors (Heupel et al., 2004).

Although sharks were among the first subjects of marine telemetry studies (Nelson and Johnson, 1970; Sciarotta and Nelson, 1977; Klimley and Nelson, 1984; Nelson, 1990; Morrissey and Gruber, 1993), batoids (skates and rays) have been relatively overlooked. As with sharks, most tracking data that are available for rays consist of short-term or intermittent manual tracking data (Blaylock, 1992, 1993; Gilliam and Sullivan, 1993; Silliman and Gruber, 1999; Matern et al., 2000; Cartamil et al., 2003). Continuous, long-term tracking data are currently limited for ray species. The objectives of this study were to investigate *R. bonasus* residency, movement patterns and habitat use using passive acoustic telemetry to better understand the ecological significance of this species within an estuary on the southwest coast of Florida.

Methods

Study area

This research was conducted in Pine Island Sound, located in lower Charlotte Harbor (Figure 3-1). The shore of Pine Island Sound is largely undeveloped including state and federally protected areas (National Wildlife Refuge). The aquatic habitat within the sound varies from shallow sand and mud flats to seagrass beds and deep channels. Depths within the sound range from zero to ten meters with a tidal range of 0.7 to 1.8 meters. Pine Island Sound experiences significant freshwater input from the Caloosahatchee River that caused salinity to vary widely (15-37 ppt) over the course of the study. Temperature within the sound ranged between 20 - 33 °C over the course of the study period (Heupel, unpublished data).

Acoustic array

An array of acoustic hydrophones (Vemco VR2 receivers) deployed within Pine Island Sound from April to December of 2003 and 2004 was used to track movements of *R. bonasus* within the study site. The main array system within Pine Island Sound (PIS) consisted of 40 hydrophones deployed in open water areas (Figure 1). The total monitoring area within PIS was approximately 184 km². Data were downloaded from hydrophones every 2-3 weeks over the course of the study period.

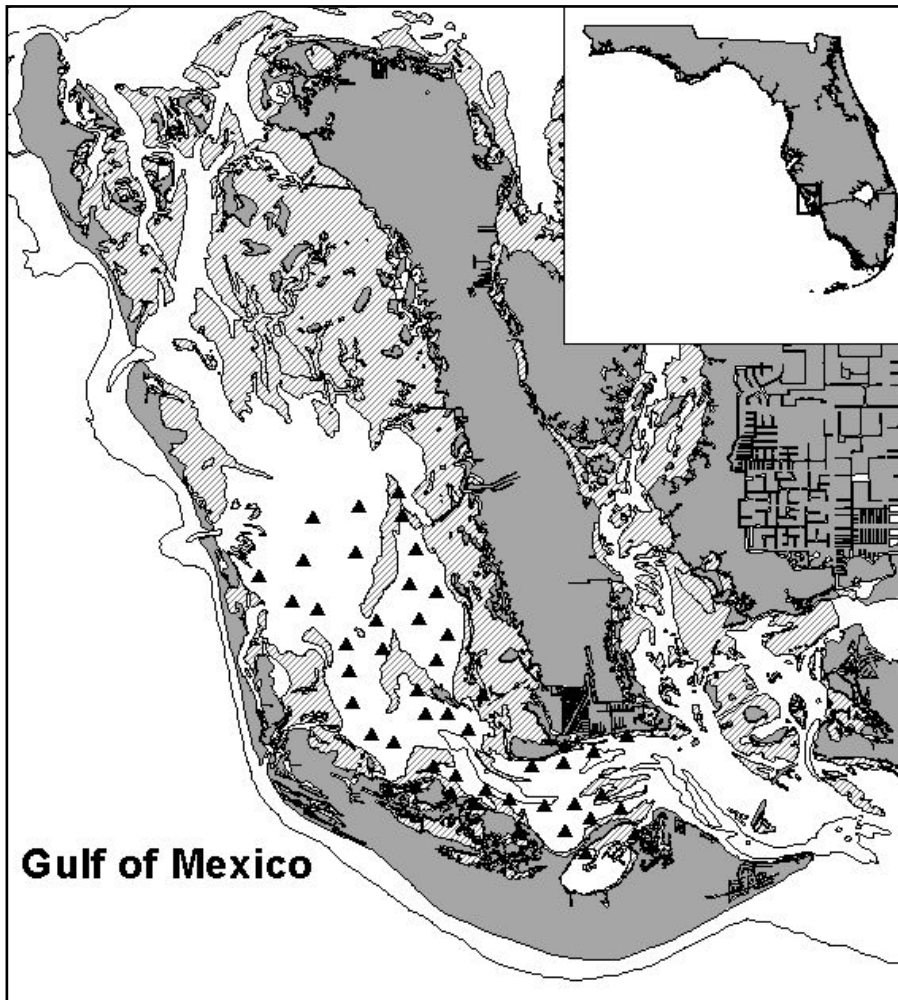


Figure 3-1. Map of Pine Island Sound and its location along the Gulf coast of Florida. Hatched areas indicate seagrass beds and bathymetry contour indicates depths > 4 meters. Triangles designate hydrophone station locations.

Study species

Cownose rays were collected in 200-m (11 or 30 cm stretch mesh) or 400-m (11 cm stretch mesh) entanglement nets. Individuals were sexed, weighed to the nearest 0.1 kilogram, and measured to the nearest centimeter (straight and curved disc width and length). Rays were defined as mature (>70 cm SDW) or immature (<70 cm) based upon degree of clasper calcification in males or minimum size at pregnancy in females within

Charlotte Harbor (Collins, unpublished data). Sizes at maturity for *R. bonasus* within Charlotte Harbor differed from those established for cownose rays in Chesapeake Bay by Smith and Merriner (1986), who recognized maturity at >90 and >84 cm for females and males, respectively. This variation may be due to latitudinal or population differences. Sizes conservatively agreed with those established for *R. bonasus* in the northern Gulf of Mexico (approximately 65 cm disc width) (Neer and Thompson, 2005). Prior to release rays were fitted with acoustic transmitters measuring 8 x 28 mm (Vemco V8, Vemco Ltd.) that had an expected battery life of 250 days and operated on 69 kHz. Transmitters were coded to allow individual identification and were set to pulse randomly once every 90-180 seconds. Random repeat rates allowed multiple individuals to be monitored simultaneously within a given area without continuous signal overlap. Detection distances for V8 transmitters within Pine Island Sound were tested within the region and determined to be 450 m (average) with a maximum detection distance of 800 m (Collins unpublished data). Wax coated transmitters were externally attached to rays by a cinch tag (Floy Tags, Seattle, WA) inserted through the spiracular cartilage. All rays were released in good condition within 500 meters of their capture location.

To determine effects of transmitter attachment and retention time, four *R. bonasus* were housed together in a circular saltwater tank (8,700 liters) at MML for 16 months. Three of the captive rays were fitted with “dummy” transmitters, equal in all physical aspects to field transmitters. One captive ray was not tagged and acted as a control. All four rays were monitored for changes in physical appearance or behavior. Ray swimming and feeding behaviors were qualitatively assessed daily, and each individual was photographed, weighed and measured monthly.

Data Analysis

The residence of cownose rays within Pine Island Sound was examined by determining the number of days individuals were present within the study site. Individuals were considered present when a minimum of two detections were recorded for that individual within a single day. Daily presence data were analyzed to determine the number of consecutive days that an individual was resident (continuous presence), as well as the total number of days that it was detected within the study area. The number of days present (both continuous and total) within the study area was tested for differences based on sex or maturity stage using *t*-tests.

The number of detections per hour was assessed for each tracked individual over its total monitoring period to define any diel differences in detection patterns. Each detection recorded for an individual was assigned to one of 24 bins based upon the hour of the detection. Assuming an equal distribution of detections over a 24 hour period, Chi-square tests were performed to determine whether the observed proportion of detections differed significantly from an expected even distribution. Significant differences from expected values would show individuals were more frequently detected at specific times of the day. This difference in detections could reflect diel changes in activity pattern or habitat use (e.g. movement into shallow regions where detection was difficult). To determine whether detection numbers were higher during day or nighttime hours, hourly detections were divided into day or night for each individual. “Daytime” fell between the hours of 06:00 and 18:59, and “nighttime” fell between 19:00 and 05:59. Day and night total detections were summed for each individual and compared using the Chi-square test

to determine whether an individual had significantly more detections during the daytime or nighttime hours.

Similar to examination of detections per hour, the number of detections at various tidal stages (in 20 cm increments of tidal height) was examined to define any effects of tidal variation on individual detections. Tide data were obtained for Galt Island, Pine Island Sound, using the program Tides and Currents (MAKER). Chi-square tests were then used to compare the frequency of detections per tidal stage with the frequency of all tidal heights during the periods individuals were monitored. Statistically significant differences in the distribution of detections would indicate that an individual was detected more frequently at specific tidal heights.

Detection data from *R. bonasus* were processed using a custom-written FORTRAN program (Simpfendorfer et al., 2002). This program used a mean position algorithm to calculate position estimates, or 'center of activity' locations, for monitored individuals every thirty minutes. Simpfendorfer et al. (2002) compared real time location data from small sharks to mean position estimates and calculated an error of approximately 225 meters using this method. Processed data were used for all of the following analyses to define individual locations and movements through time.

Calculated center of activity locations through time were used to calculate home range and activity space. Minimum convex polygons (MCP) and kernel utilization distributions (95% and 50% KUD) (Worton, 1989) were calculated for each individual using the Animal Movement Analyst Extension (AMAE) in ArcView 3.2 GIS (Hooge and Eichenlaub, 2000). Minimum convex polygons were used to demonstrate the extent of an animals range over a given period, while KUDs illustrated the utilization of space

within that range (Worton,1987). Changes in the extent of an individual's range over time were assessed by examining daily, 3-day, 7-day, 30-day and total MCPs. To detect changes in the utilization of the study site over time, KUDs were also examined over 3-day, 7-day, 30-day and total monitoring periods. Daily KUDs were not calculated because most rays did not have enough detections over a single day to calculate an accurate utilization distribution. Unpaired *t*-tests were used to determine if there were sex or size differences in home ranges within each time period (e.g. 3, 7, 30 or all days) for both estimators. To determine whether the size of an animal's range depended upon the number of days it was present, total MCPs and KUDs were compared to length of residency using linear regression. Activity space areas were also compared to straight disc width (SDW) using linear regression to determine the relationship between animal size and home range size.

Daytime and nighttime KUDs were calculated for each examined time period to determine whether individuals exhibited diel changes in home range size or distribution. Paired *t*-tests were used to detect differences in size of day vs. night activity spaces. Day and night KUDs were then compared between immature and mature *R. bonasus* using unpaired *t*-tests to determine whether maturity had an effect on the size of day and night utilization distributions.

To determine the extent of seagrass habitat utilization, total 50% KUDs (representing core areas of use over entire monitoring period) were overlaid on a map of the area's known seagrass habitat using ArcView. Total 50% KUDs were quantitatively examined for overlap by calculating the area of seagrass overlap and determining the percentage of the KUD that it represented.

The distance between consecutive centers of activity was calculated using standard geometric formulae. Distances between consecutive 30 minute centers of activity locations were used to examine linearity of movement, and define the net movement of an individual between detections. This analysis helped determine if individuals tended to remain within a confined location (small distances between consecutive locations) or moved large distances over time. Large distances between consecutive centers of activity would suggest rays were either moving very rapidly or were traveling in shallow water where detection was limited or impossible. Calculated distances were used to define the minimum distance traveled between consecutive centers of activity, and indicate whether rays were generally stationary or mobile over short periods. Linear regression was used to detect relationships between ray size and distance moved, and unpaired *t*-tests were used to detect size or sex differences in mean distances moved over time. Statistical tests were performed using Statistica (1999 edition) and Sigma Plot (version 9.0).

Results

Effect of transmitter attachment

No significant tagging effects were observed in three captive *R. bonasus* (2 mature, 1 immature) over 16 months in captivity. Transmitters were retained in captive rays for periods of 156, 187 and 457 days. Tagging sites displayed some necrosis (Figure 3-2) but transmitters were not dislodged and no negative effects on animal feeding or health were observed. Swimming and feeding behavior were considered normal based on field observations and in comparison to the control individual. All captive rays demonstrated growth and weight gain over 16 months (Appendix A). The control animal died after three months and was not included in growth analyses. Results from captive individuals suggest that wild caught individuals should retain transmitters for at least several months and not suffer any significant damage from transmitter attachment.

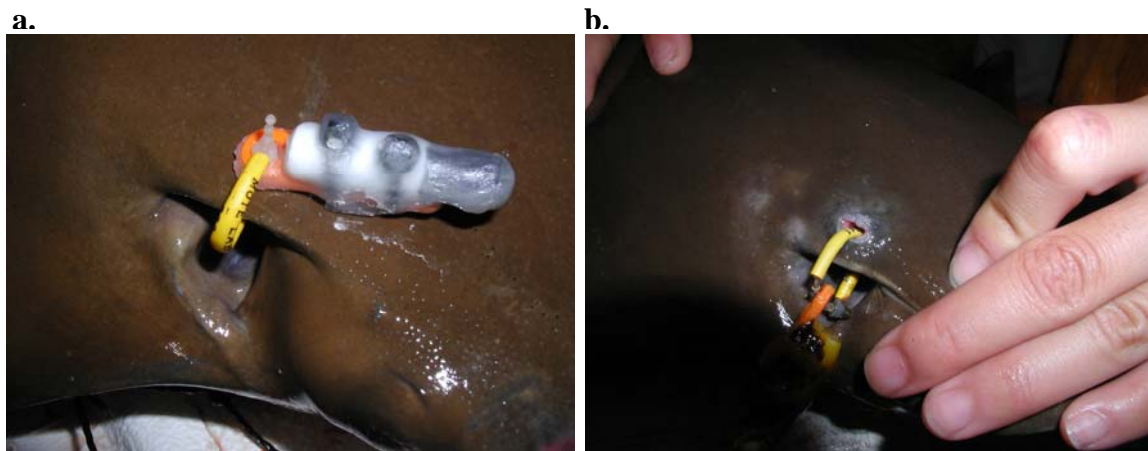


Figure 3-2. Method of attachment and site for external transmitter. Pictures illustrate captive ray on day 1 (a) and day 300 (b) after transmitter attachment.

Sampling results

Twenty one *R. bonasus* (15 male, 6 female) were monitored using passive acoustic telemetry between July 2003 and November 2004 within Pine Island Sound. Four *R. bonasus* were monitored in 2003 and 17 were monitored in 2004. Monitored *R. bonasus* ranged in size from 49 - 90 cm straight disc width (SDW) and 12 of the 21 rays were mature (8 male, 4 female). Of the 9 immature rays monitored, 7 were male and 2 were female.

Residence time

Residence time was highly variable among individuals and revealed no distinct seasonal pattern (Figure 3-3). Although some rays were resident for extended periods of time others only remained within the study site for brief periods. In 2003, all *R. bonasus* were tagged in July. One left the study area in July, two left in August, and one remained through October. In 2004, rays were captured in June, July, August and October, and left the study area in June, July, August, October and November. One ray tagged in July 2004 (no. 266) and another tagged in October (no. 512) were still in the estuary upon equipment removal from Pine Island Sound at the end of November 2004. Two *R. bonasus* fitted with transmitters in 2004 were within the study area for less than two days and were excluded from all further analyses.

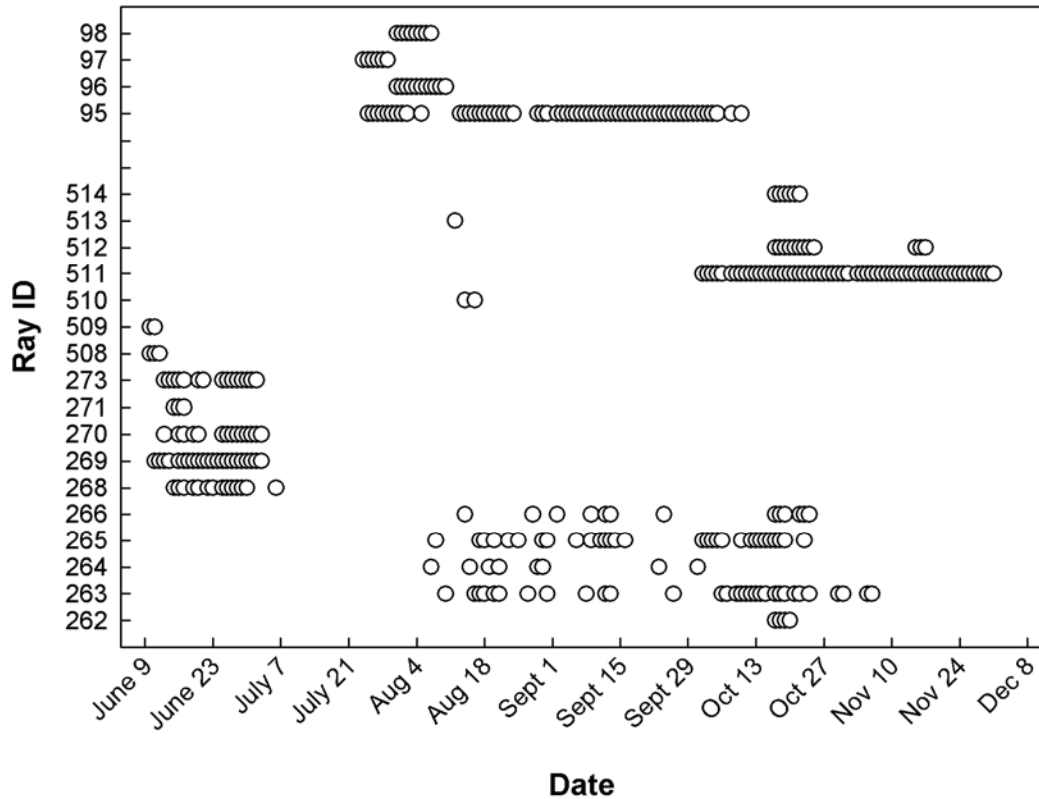


Figure 3-3. Daily presence of *Rhinoptera bonasus* monitored within Pine Island Sound between June and November of 2003 and 2004. 2003 animals (no. 95 - 98) are listed at the top and separated by a gap in the Y-axis.

Total monitoring periods ranged between one and 102 days (Figure 3-4). The mean total monitoring period for all *R. bonasus* over both years was 32 days. Total monitoring periods varied between years from 7 to 78 days in 2003 (mean=26) and 1 to 102 days in 2004 (mean=33). Females remained within the monitoring area for significantly longer periods than males (male mean = 27.7, female mean = 78.0, *t*-test, $p=0.043$). There were no significant differences between the total residence time of mature (mean = 50.7) and immature rays (mean = 30.6) (*t*-test, $p=0.398$). Periods of continuous presence ranged from 1 to 34 days in 2003 (mean = 8) and 1 to 29 days in

2004 (mean = 3). However, most periods of continuous presence were between one and three days (Figure 3-4).

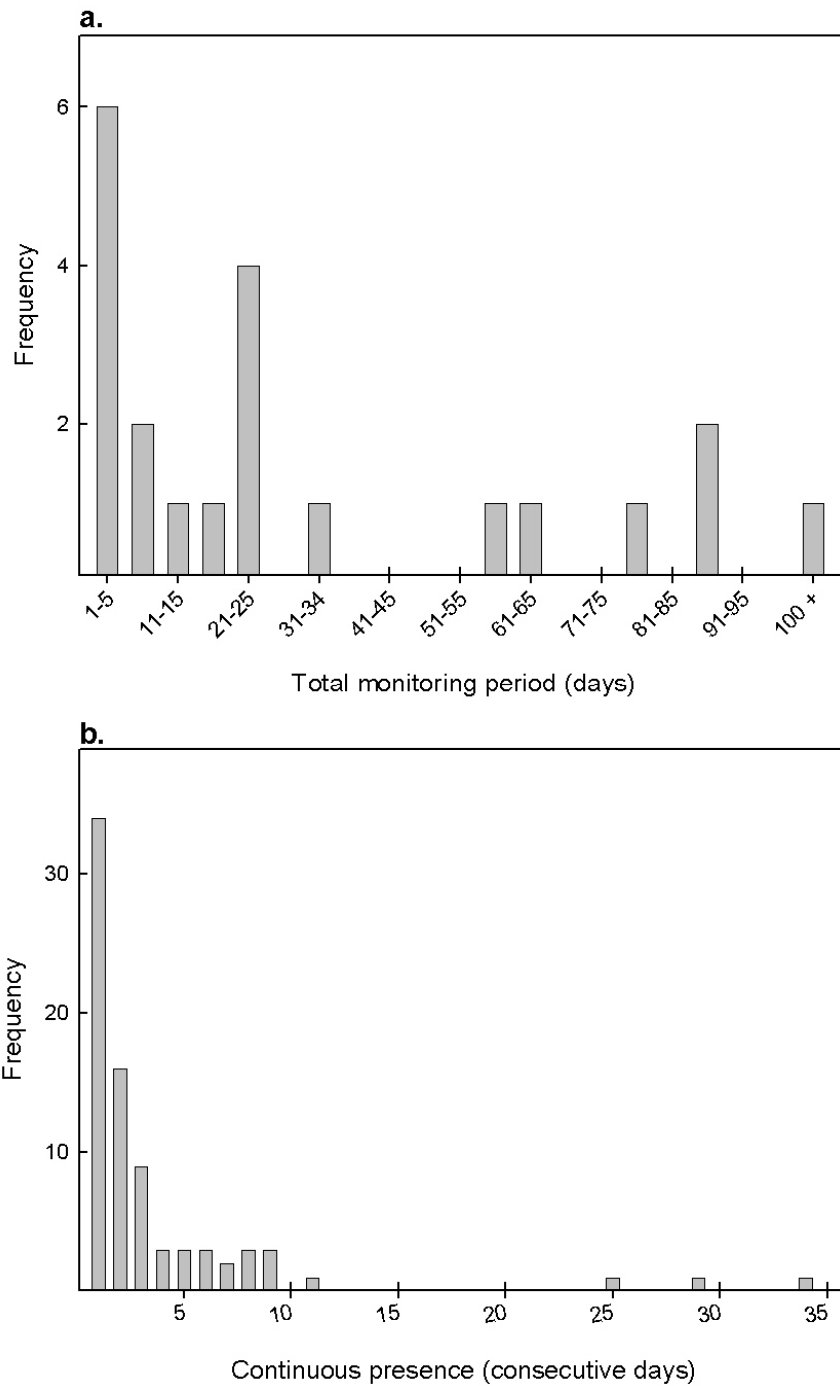


Figure 3-4. Frequency histogram of residency times for *Rhinoptera bonasus* monitored in Pine Island Sound: (a) total monitoring period, and (b) periods of continuous presence.

Activity Patterns

The variability in detection rates between individuals indicates that there were no consistent behavioral patterns that clearly demonstrate a pattern in the distribution of the numbers of hourly detections. Nineteen individuals (those present for more than two days) were tested for differences in the number of hourly detections. Observed presence by hour was significantly different from expected for 18 individuals (Chi-square test, $p < 0.05$; Table 3-1). Four juvenile rays that were tagged together in 2004 had very similar hourly detections, with substantially more detections from 06:00 to 12:00 than at other times of the day (Figure 3-5). The remaining 15 individuals did not reveal similar hourly detection patterns (Figure 3-5). Six individuals had higher detection numbers during the day, seven had higher numbers of nighttime detections, and seven showed no pattern (Table 3-1).

Analysis of detections by tidal height showed no significant differences between observed and expected detection rates for any of the monitored *R. bonasus* (Table 3-1). This result also indicates that hydrophones were capable of recording equally well at all stages of the tide.

Table 3-1. Results for Chi² analysis of the proportion of detections for *R. bonasus* within Pine Island Sound by hour, time of day (daytime vs. nighttime detections), and tidal height. Detections were expected to be equally distributed over all hours and tidal stages. *P* values < 0.05 indicate significant differences. (--) indicates there were not enough detections to test for differences.

Ray ID	Hourly differences	Day/Night differences	Highest number of detections	Tidal stage differences
	<i>p</i> value	<i>p</i> value	<i>Time of day</i>	<i>p</i> value
95	0.00000	0.00000	Night	0.19000
96	0.00000	0.00000	Night	0.87717
97	0.00000	0.60200	No pattern	0.94476
98	0.00004	0.12419	No pattern	0.99933
262	0.00000	0.01069	Night	0.90000
263	0.00000	0.16767	No pattern	0.78658
264	0.00000	0.00024	Night	--
265	0.00000	0.46894	No pattern	0.05020
266	0.00018	0.22490	No pattern	0.89325
268	0.00000	0.00000	Day	0.20587
269	0.00000	0.00000	Day	0.28716
270	0.00000	0.00000	Day	0.14818
271	0.05422	0.07343	No pattern	0.95257
273	0.00000	0.00000	Day	0.34372
508	0.00000	0.00017	Day	0.89053
509	0.00000	0.00000	Day	0.99908
511	0.00000	0.00001	Night	0.28688
512	0.00000	0.00000	Night	0.82930
514	0.00025	0.07184	No pattern	0.76041

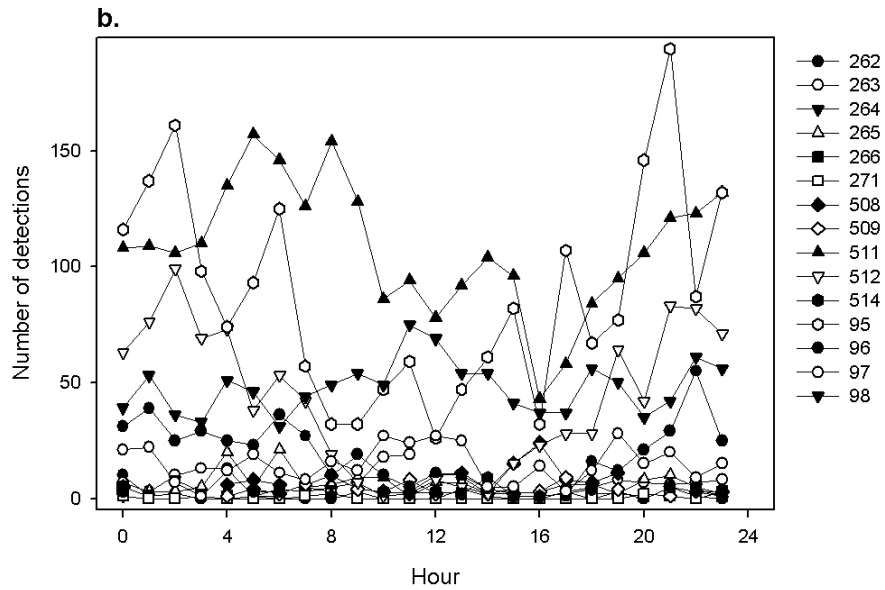
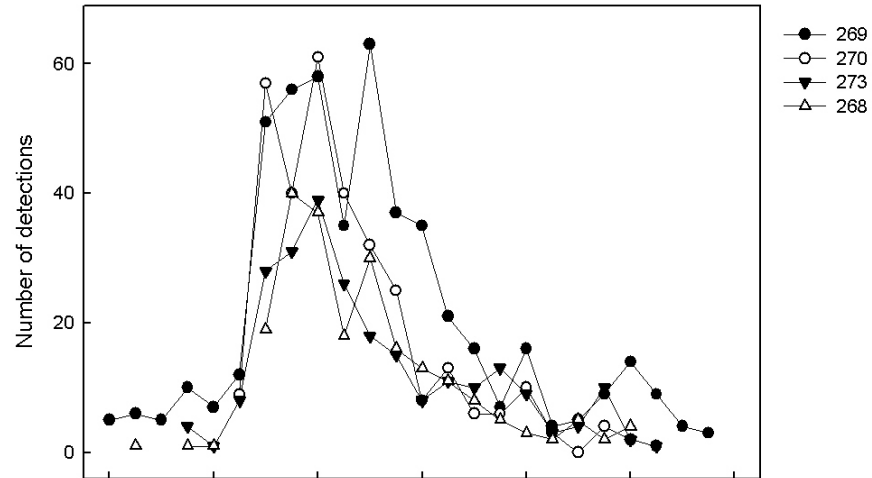


Figure 3-5. Hourly detection patterns. Similar patterns in hourly detections were observed for four immature *R. bonasus* tagged together (a) while no distinct pattern in hourly detections emerged for the remaining 17 tracked rays (b).

Home range analyses

MCPs

Daily MCPs for all rays ranged between 0.001 and 25.78 km², with a mean of 5.25 km². Most daily MCPs were less than 5 km² for both mature and immature

individuals (Figure 3-6), and did not differ significantly between size groups ($p = 0.7018$). Mature rays displayed significantly larger 3-day MCP areas and significantly smaller 30-day MCP areas (Table 3-2) (Figure 3-7). There were no significant differences between mature and immature *R. bonasus* 7-day or total MCP areas (Table 3-2) (Figure 3-7). Although a consistent significant relationship with state of maturity was not detected, largest MCP sizes were observed in individuals greater than 60 cm SDW (Figure 3-8). MCP sizes were generally larger for mature animals over all examined time frames, except the 30-day period. This discrepancy was most likely due to the small number of animals that were present within the study area for 30 consecutive days and two large 30 day ranges for immature individuals.

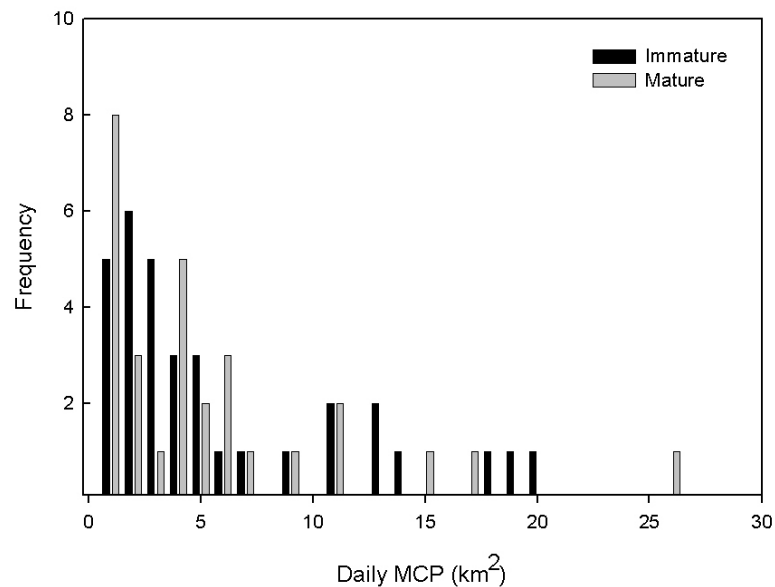


Figure 3-6. Frequency histogram representing the size of daily MCPs for immature and mature *R. bonasus*. No significant differences were detected between mature and immature daily MCPs (t -test; $p = 0.7018$).

Total MCPs for all *R. bonasus* ranged between 0.81 and 71.78 km² (mean=22.01 km²). Total MCPs showed a significant increase with longer residence ($r^2 = 0.6315$, slope = 0.2339, $p = 0.0419$). Although females remained within the study site for significantly

longer periods than males, no significant sex differences were detected in MCP size over any time frame (Table 3-3).

Table 3-2. T-test probability values (p) comparing activity space sizes between immature and mature *R. bonasus* over 3 day, 7day, 30 day and total monitoring periods. P values < 0.05 indicate significant differences. All significant values showed that mature individuals used larger areas than immature except for the 30 day MCP comparison (in italics).

Monitoring period	MCP <i>p value</i>	KUD (95%) <i>p value</i>	KUD (50%) <i>p value</i>
3 day	0.013115**	0.000373**	0.004505**
7 day	0.308216	0.047843*	0.016526*
30 day	<i>0.002652**</i>	0.451095	0.643910
Total	0.779804	0.002296**	0.000941**

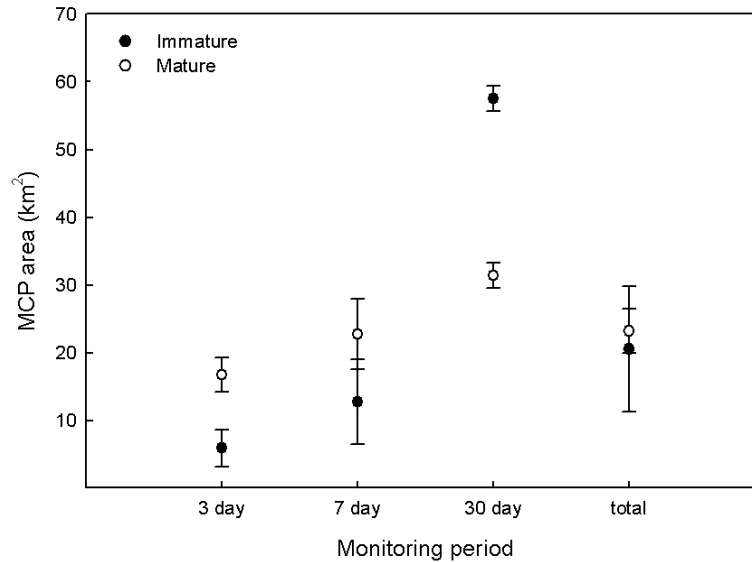


Figure 3-7. Mean MCP areas for immature (closed circles) and mature (open circles) *R. bonasus* over all monitoring periods (3 day, 7 day, 30 day and total).

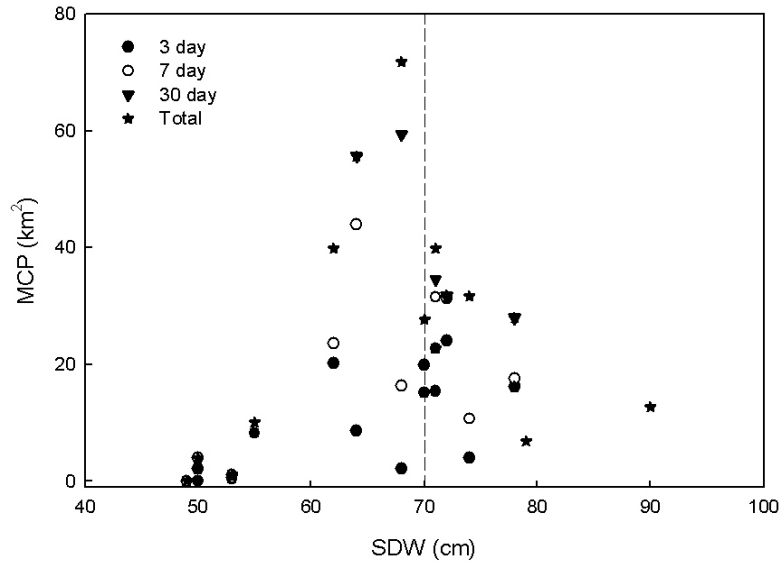


Figure 3-8. MCP areas over 3 day (closed circles), 7 day (open circles), 30 day (triangles) and total (stars) monitoring periods compared to *R. bonasus* straight disc width (SDW) (cm). Dashed line divides immature (<70 cm) from mature (>70 cm) individuals.

Table 3-3. *T*-test probability values (*p*) of *t*-tests comparing activity space sizes between male and female *R. bonasus* over 3 day, 7 day and total monitoring periods. *P* values < 0.05 indicate significant differences. Differences between 30 day monitoring periods could not be tested because only 1 female was present within the study area for 30 consecutive days.

Monitoring period	MCP <i>p</i> value	KUD (95%) <i>p</i> value	KUD (50%) <i>p</i> value
3 day	0.7288	0.2007	0.0602
7 day	0.2221	0.8014	0.0500
Total	0.1870	0.4097	0.0010**

KUDs

Mature *R. bonasus* had significantly larger KUDs (95 and 50%) than immature rays over all time frames (3-day, 7-day and total) except the 30-day period, which showed no significant difference (Figure 3-9; Table 3-2). Similar to MCP, largest KUDs occurred for individuals with disc widths greater than 60 cm (Figure 3-10). Total 95% KUDs ranged between 0.18 and 62.44 km² (mean=22.63 km²), while total 50% KUDs were significantly smaller and ranged between 0.09 and 9.68 km² (mean=3.33 km²) (Figure 3-11). Both 95% and 50% KUDs significantly increased with ray disc width ($r^2 = 0.84$, $p = 0.0046$ and $r^2 = 0.88$, $p = 0.0002$ for 95% and 50% KUDs, respectively) (Figure 3-11). Total KUD areas also increased with total monitoring periods (for 95% KUD, $r^2 = 0.67$, slope = 0.152, $p = 0.0084$; for 50% KUD, $r^2 = 0.61$, slope = 0.0281, $p = 0.0266$).

No significant sex differences were detected for either the 95 or 50% KUD areas over the 3 day or 7 day time frame (Table 3-3). Differences between 30 day KUD areas could not be calculated because only one female *R. bonasus* was present for 30 consecutive days. No difference was detected between sexes for 95% KUDs or for the 3 day and 7 day 50% KUDs, but males had significantly larger total 50% KUD areas than females (Table 3-3).

No significant differences were detected between the sizes of day and night KUDs for immature or mature rays over any time frame (Table 3-4). However, mature rays had significantly larger nighttime 95% KUDs than immature rays over all time frames except the 30 day period (Table 3-5). Nighttime 50% KUDs did not differ significantly between size classes.

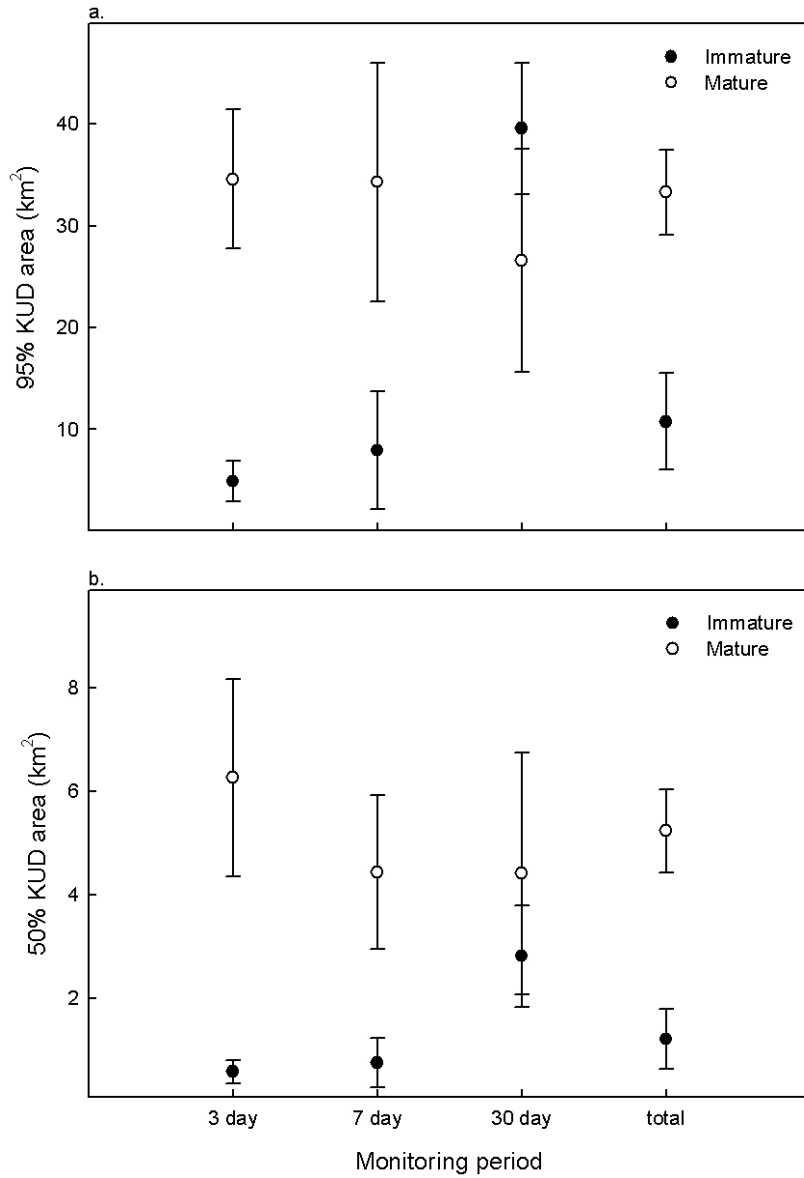


Figure 3-9. Mean 95% (a) and 50% (b) KUD areas for immature (closed circles) and mature (open circles) *R. bonasus* over all monitoring periods (3 day, 7 day, 30 day and total).

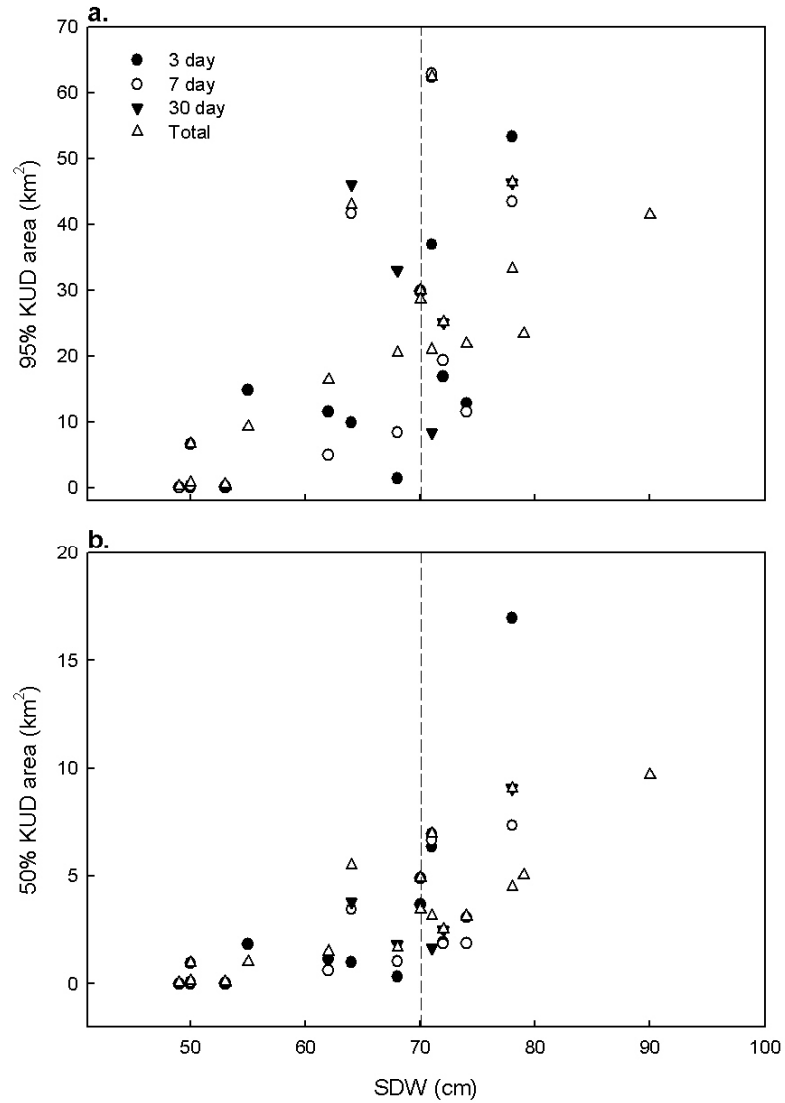


Figure 3-10. 95% (a) and 50% (b) kernel utilization distribution (KUD) areas over 3 day (closed circles), 7 day (open circles), 30 day (closed triangles), and total (open triangles) monitoring periods vs. *R. bonasus* straight disc width (SDW) (cm). Dashed line divides immature (<70 cm) from mature (>70 cm) individuals.

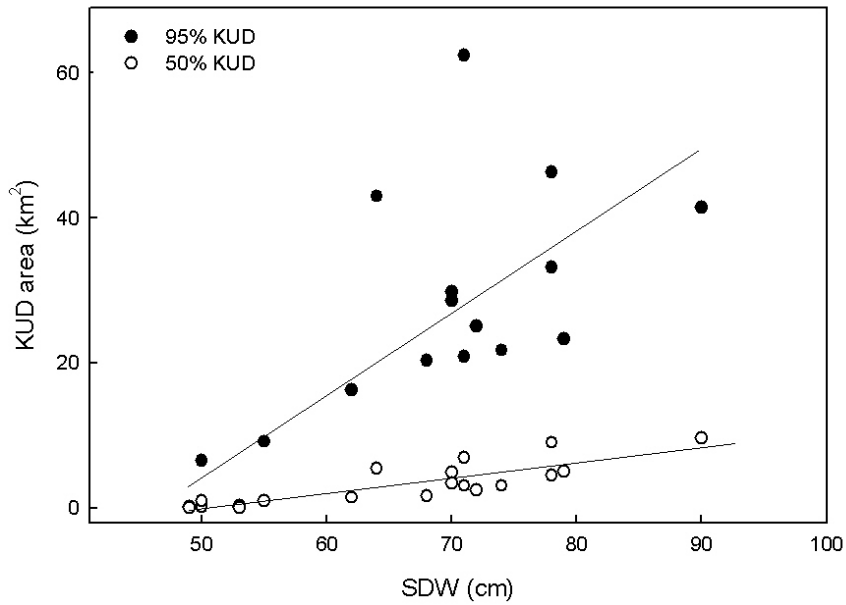


Figure 3-11. Total 95% (closed circles) and 50% (open circles) KUD areas compared to *R. bonasus* straight disc width (SDW) (cm). Both KUD areas get significantly larger as SDW increases: For 95% KUD, $r^2 = 0.84$; $p = 0.0046$ and for 50% KUD, $r^2 = 0.88$, $p = 0.0002$.

Table 3-4. *T*-test probability values (p) of *t*-tests comparing day and night KUD areas for immature and mature individuals. *P* values < 0.05 indicate significant differences.

Monitoring Period	KUD	Immature <i>p</i> value	Mature <i>p</i> value
3 day	95%	0.07108	0.63983
	50%	0.11769	0.46212
7 day	95%	0.36495	0.95589
	50%	0.41674	0.88990
30 day	95%	0.80050	0.50366
	50%	0.55939	0.66154
Total	95%	0.73829	0.1806
	50%	0.72024	0.5426

Table 3-5. *T*-test probability values (*p*) of *t*-tests comparing daytime and nighttime 95% and 50% KUDs between immature and mature animals. *P*-values < 0.05 indicate significant differences.

	3 day	7 day	30 day	Total
Day (95%)	0.29891	0.42331	0.51579	0.21303
Day (50%)	0.23751	0.36200	0.90713	0.10499
Night (95%)	0.04160*	0.03315*	0.96272	0.00682**
Night (50%)	0.14699	0.07037	0.82106	0.05058

Seagrass habitat overlap

Examination of core areas (50% KUDs) showed variability in overlap with seagrass areas. Four rays had 50% KUDs that overlapped seagrass habitat by more than 10% (range: 12% - 96%), six displayed an overlap of <5%, and nine exhibited 50% KUDs that did not overlap seagrass habitat at all (Figure 3-12).

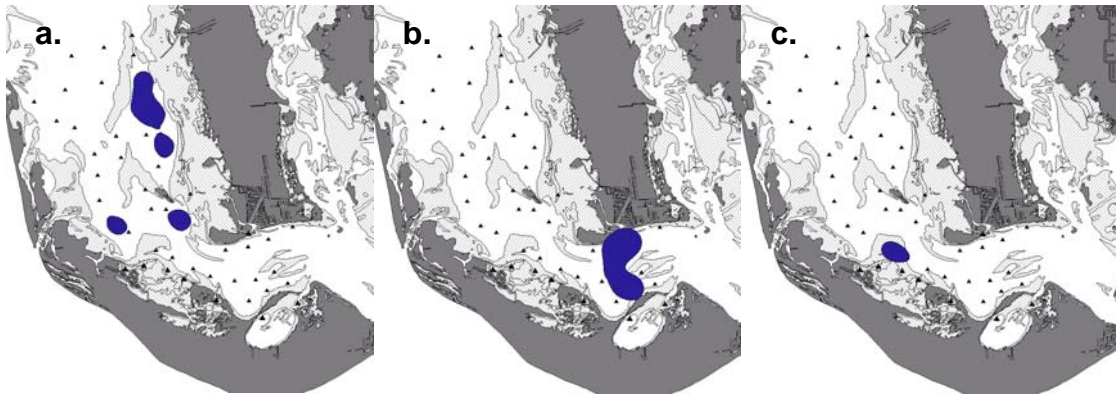


Figure 3-12. Examples of 50% KUD overlap of seagrass habitat. No overlap, (a), <5% overlap (b) and >10% overlap (c). Hatched areas represent seagrass beds and solid black circular shapes represent 50% KUD areas.

Distance between consecutive centers of activity

Analysis of the distances between 30 minute centers of activity revealed that the majority of locations were within close proximity to consecutive locations. Movement distances ranged from 0 to 13 km, but mean distances per individual ranged between 0.1 and 0.7 km (Figure 3-13). Mature *R. bonasus* displayed significantly larger distances between consecutive centers of activity (0.50 km) than immature rays (0.26 km) (t -test, $p = 0.006$), and distances generally increased with disc width (Figure 3-13; $r^2 = 0.92$, slope = 0.02, $p = 0.006$). Movements over large distances were not common, with the majority of rays traveling between 0 - 2 km within a 30 minute period (Figure 3-14).

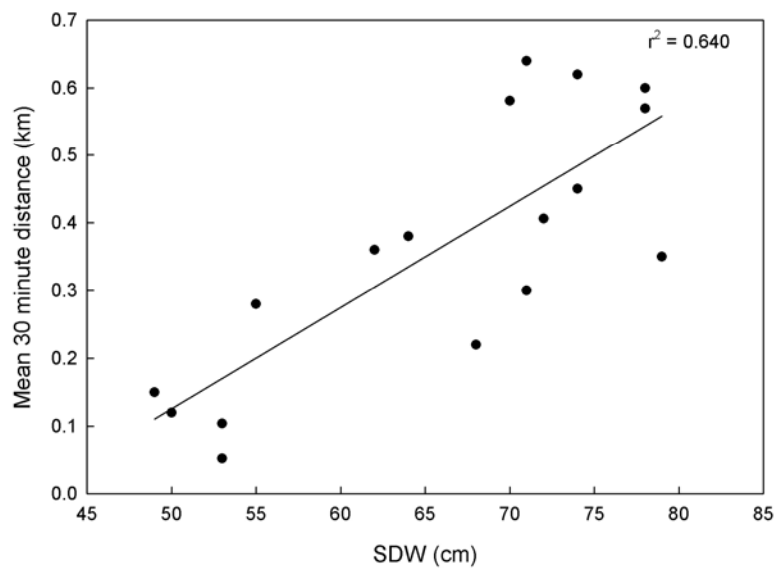


Figure 3-13. Mean distance moved over 30-minute intervals vs. ray straight disc width (SDW) (cm).

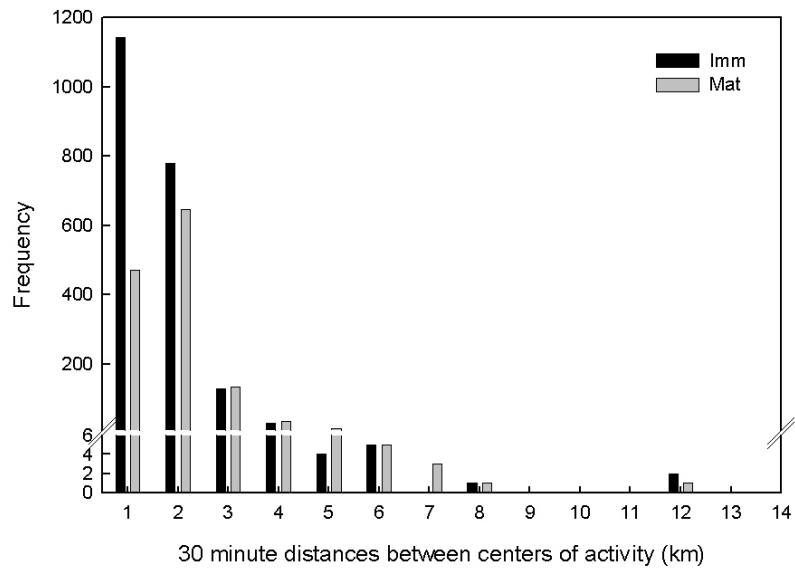


Figure 3-14. Frequency histogram of mean distance moved over 30-minute intervals for immature (black bars) and mature (gray bars) *R. bonasus*.

Discussion

Acoustic monitoring of *R. bonasus* within Pine Island Sound revealed that the residence and movement patterns of this species are complex and variable. Rays were detected within the estuary for varying lengths of time, but detection of individuals was usually not contiguous. Continuous presence data showed that most animals were not monitored for more than two consecutive days at a time. This suggests intermittent and transient use of the monitored area. Loss of contact with individuals could be the result of movement out of the study area or movement into shallow water regions where detection was improbable. Utilization of intertidal and subtidal shallow flats has been well documented for many ray species (Smith and Merriner, 1985; Snelson et al., 1988; Matern et al., 2000), so it is possible that individuals were not exiting the study site but simply moving into shallow water and out of detection range.

Although data from most individuals suggested transient use of the study site, some remained within the study area for extended periods. Seven of 21 tracked rays were monitored for at least one month, and three rays were detected within the array for over three months. This demonstrates that some *R. bonasus* do remain resident within estuaries for extended lengths of time. However, the majority of *R. bonasus* fitted with transmitters were monitored for shorter periods, leaving the study area after days or weeks and were not detected again. These data suggest that there is no consistent pattern of residency among the individuals monitored in this study.

Although there are no other continuous batoid tracking studies available for comparison, these results are similar to those from other studies of benthic feeding elasmobranchs. Bat rays (*Myliobatis californica*) in Tomales Bay, California, have been

documented within 1 km of their initial tagging site after periods of 106, 359, and 583 days during a tag-recapture study (Hopkins and Cech, 2003). However, recapture data were gathered from only three out of 257 tagged bat rays. Based on the low number of individuals recaptured during this study, the results presented by Hopkins and Cech (2003) are similar to those collected here where a small number of individuals were resident in the area while the remainder moved out of the region. Similarly, in a manual tracking study of the Pacific angel shark (*Squatina californica*), two out of nine tagged sharks were relocated after three days in the same home area that they were tagged (Standora and Nelson, 1977). Both of these studies suggest that individual variability in residence is not unusual among elasmobranchs.

As with residency patterns, there was no distinct pattern in use of the study site when examining activity pattern based on tide stage, which contrasts with existing research on cownose rays. Blaylock (1992, 1993) attached satellite tags to six *R. bonasus* in Chesapeake Bay and manually tracked individuals for 4.3 - 13.5 hours and found that all tracked individuals moved in the same general direction as tidal flow. Smith and Merriner (1985, 1987) reported that cownose rays in Chesapeake Bay were moving into intertidal zones to feed during high tide. However, if this were occurring in Pine Island Sound, the data would have reflected an absence of detections during high tide because these shallow areas are not within detection range of the hydrophone array. Similar to the present study, Matern et al. (2000) did not observe any tidal correlations while tracking bat rays in Tomales Bay, but they did notice a distinct diel pattern that they attributed to behavioral thermoregulation.

Although there was not a distinct diel activity pattern detected for cownose rays in Pine Island Sound as reported for the bat ray in Tomales Bay (Matern et al. 2000), there was a general trend for lower detection rates for *R. bonasus* between 12:00 – 18:00 over both years of this study, which could not be attributed to tidal changes. This behavior may be explained by rays moving into canal systems or onto shallow seagrass areas and thus out of detection range during this period. Movement into these areas could be for feeding or other behaviors. Movement into shallow water could also serve as a means of predator avoidance by using habitats that large sharks do not enter as frequently. In similar studies, juvenile lemon sharks (*Negaprion brevirostris*) in the Bahamas were documented to select shallower, warmer water (Morrissey and Gruber, 1993) and hammerhead (*Sphyrna lewini*) pups in Hawaii aggregated during daylight hours in more turbid parts of Kaneohe Bay (Holland et al., 1993).

Examination of home range sizes also showed that movement patterns and habitat use were not consistent across individuals. Minimum convex polygons can overestimate home range size (Kernohan et al., 2001), but are useful to illustrate boundaries and the extent to which an animal travels. Daily MCP areas between individuals varied greatly over the course of this study (<1 - 26 km²), but the majority were between 1 and 5 km² suggesting individuals generally used a small portion of the study site within the course of a single day. This is comparable to activity space estimates for bonnethead sharks (*Sphyrna tiburo*), also tracked within Pine Island Sound, which had a mean daily activity space of 8.31 km² (Heupel et al., manuscript). Like the bonnethead shark, *R. bonasus* are capable of traversing fairly large distances over the course of a 24 hour period, but tended to remain within a confined area over most days. Total MCP size significantly increased

with monitoring period, suggesting that individuals expand the areas utilized over time. The maximum total MCP recorded was 71.78 km² which falls within the range of MCPs recorded for other coastal elasmobranchs: lemon sharks displayed activity spaces ranging from 9 – 93 km² over intermittent tacking periods of 1-8 days (Gruber et al., 1988). Juvenile sandbar sharks (*C. plumbeus*) were tracked for periods between 2.5 and 70 hours, with resulting MCPs ranging between 1.1 and 333.9 km² (Rechisky and Wetherbee, 2003).

Mean MCP areas tended to increase with animal size, but significant differences based on maturity were only detected over the 3 day and 30 day time frames. Mature *R. bonasus* had significantly larger MCPs over 3 day periods and significantly smaller MCPs over 30-day periods. This result is confounding because of the correlation of MCP area with disc width. The reversal of trend for the 30 day data is likely the result of a small sample size and the large home ranges calculated for two juvenile individuals over the 30 day period. These two individuals, although nearing maturity, had much larger 30 day home ranges than any other immature or mature individual. Although female rays remained within the study area for significantly longer periods than males, there were no significant differences for MCP areas between sexes

The KUD is more descriptive than the MCP, illustrating the use of space within a home range and differentiating areas of intense use from those that are only briefly occupied (Worton, 1987). Based on KUD analysis *R. bonasus* demonstrated 95% usage areas between 0.18 and 62.44 km² over monitoring periods of 1 -102 days. These values are generally larger than those obtained for the more stationary, benthic myliobatiform *Dasyatis lata*, which exhibited 95% KUDs between 0.62 and 2.77 km² during manual

tracking over 31-74 hour periods (Cartamil et al., 2003). In contrast, the values observed for *R. bonasus* were much smaller than juvenile sandbar sharks, which exhibited 2.8 to 315.4 km² 95% KUDs over manual tracking periods up to 70 hours (Rechisky and Wetherbee, 2003). As pelagic swimmers, myliobatid rays like *R. bonasus* are typically more mobile than the predominantly sedentary dasyatid rays (Bigelow and Schroeder, 1953). However, *R. bonasus* do spend time foraging for benthic prey in one location (Smith and Merriner, 1985; Sasko, 2000), and can rest motionless on the bottom for long periods (Collins, personal observation). This behavior may produce smaller home range sizes than for a species that is continuously pelagic such as the sandbar shark (*C. plumbeus*).

Although 95% KUDs tended to expand over time, 50% KUDs appeared to be more stable. This suggested that individuals maintained a consistent small area (3.33 km²) that was used repeatedly, indicating individuals concentrated large amounts of time within these restricted core areas.

Rhinoptera bonasus monitored during this study were active diurnally and nocturnally, as daytime and nighttime activity spaces were not significantly different for any individual. This differs from patterns observed in other benthic feeding elasmobranchs. *Dasyatis lata* showed significantly larger activity spaces during the night than during the day (Cartamil et al., 2003). Similarly, both the Pacific electric ray (*Torpedo californica*) and the Pacific angel shark have been documented as nocturnal, covering much larger distances between dusk and dawn than during daylight hours (Bray and Hixon, 1978; Standora and Nelson, 1977). Both the horn shark (*Heterodontus francisci*) and the swell shark (*Cephaloscyllium ventriosum*) displayed distinct diel

patterns, increasing activity levels at night (Nelson and Johnson, 1970). Mature rays had significantly larger nighttime 95% KUDs than immature rays over all time periods, but total daytime 95% KUD comparison showed no difference between size groups. This indicates that mature animals are utilizing larger areas within their range during the nighttime hours, while immature animals remain within more confined spaces. This behavior could be attributed to increased predation risk for smaller individuals.

The increased nighttime activity space for larger individuals contributed to mature *R. bonasus* having larger home range sizes overall. The fact that mature *R. bonasus* generally used larger areas than immature rays may be explained by their increased swimming capability or by increased energetic demands and consequently larger foraging areas. Foraging area has been described as inversely related to food availability (Hamilton and Watt, 1970); thus the increased dietary requirements of larger animals could result in larger activity spaces. The difference may also be attributed to differential predation on smaller *R. bonasus* which may influence the distribution of immature animals as has been suggested for juvenile lemon sharks in Bimini, Bahamas (Morrissey and Gruber, 1993). Larger animals have often been reported to cover greater distances in given amounts of time than their smaller counterparts: lemon sharks utilize greater spaces as they grow (Sundstrom et al., 2001), longer leopard sharks (*Triakis semifasciata*) generally move faster than shorter ones (Ackerman et al., 2000) and bonnethead sharks (*Sphyrna tiburo*) have higher rates of movement as mature animals (Parsons, 1990). In the present study *R. bonasus* moved significantly larger distances over 30 minute intervals as disc width increased, and mature rays moved mean distances almost double those of immature rays (0.26 vs. 0.50 km.).

Examination of home range areas revealed that all rays had total MCP and 95% KUDs that overlapped seagrass habitat. However, only four rays had more than 10% of their core areas (50% KUD) that overlapped with seagrass habitat. Such an observation contrasted with existing evidence that *R. bonasus* utilize grass beds extensively (Orth, 1975; Peterson, 2001). The predominant use of sand and mud areas is supported by feeding data that show that the majority of *R. bonasus* prey in Pine Island Sound is found in unvegetated areas (Collins et al., manuscript). However, these results could be misleading and should be considered cautiously since there are regions of seagrass habitat within Pine Island Sound that are beyond detection range of the hydrophone array. Rays could be using this habitat during periods when detections are low or absent.

In this study, *R. bonasus* was present within Pine Island Sound throughout the summer months for the duration of the project and some individuals appear to have remained beyond the end of the study period. These results demonstrate that individual *R. bonasus* can have prolonged residency time in estuarine systems. Although some rays were only present for brief periods, there was not a distinct seasonal departure of the tagged rays as would be expected if they were undergoing seasonal migration. Rays were still present in Pine Island Sound when the hydrophone equipment was removed at the end of November 2004, and rays were detected in another portion of the estuary in January, 2005, suggesting they were not migrating out of the area for the winter even though water temperatures had declined to 16 °C (Heupel, unpublished data). In addition, *R. bonasus* were tracked in the Caloosahatchee River (adjacent to Pine Island Sound) January – May of 2004 and 2005 as part of another project (Collins, unpublished data), and eighteen *R. bonasus* were also captured in north Charlotte Harbor between November

2003 and March 2004 (Collins et al., manuscript). Along the eastern coast of the United States, seasonal fall departure and summer arrival of *R. bonasus* has been reported (Schwartz, 1965, 1990; Smith and Merriner, 1985, 1986; Blaylock, 1993), and it was assumed that cownose rays within Charlotte Harbor would follow a similar seasonal pattern. The current data do not support this hypothesis.

Hopkins and Cech (2003) noted that the departure of bat rays from coastal California during winter months was not as pronounced in more southern counties where water temperatures were warmer. Neer et al. (2005, manuscript) reported encountering cownose rays in the northeast Gulf of Mexico at temperatures between 15.5 – 33.6 °C. Based on these results it seems possible that temperatures in Charlotte Harbor do not get low enough to trigger seasonal migrations, and that individual movements may be related to other factors (e.g. prey availability, mating or predator avoidance). This concept is supported by reports of a resident population of cownose rays in Venezuela (Smith and Merriner, 1987) and year-round presence of *R. bonasus* in coastal North Carolina (Smith, 1907). It is possible that *R. bonasus* inhabiting the southwest coast of Florida, where water temperatures rarely drop below 15 °C, simply move offshore to warmer, deeper water when necessary and can easily return to inland waters when temperatures increase.

Although migratory movements, and movements outside the study area, could not be identified in the present study, data are available to show the extent of an individual's movement during the course of a day via home range estimates and consecutive movement locations. The distances between centers of activity of tracked cownose rays in Pine Island Sound ranged between 0 and 13 km per 30 minute period, and daily MCPs ranged between 0.001 – 25.78 km². The average traveling speed for *R. bonasus* has been

estimated between 3.7 - 5.6 km/hour (Blaylock, 1992) and 7.4 – 9.3 km/hour (Smith, 1980). Smith (1980) reported *R. bonasus* in the South Atlantic Bight capable of traveling 14.6 – 23.2 km in one day. As highly mobile, pelagic swimmers, *R. bonasus* have the capability to traverse large distances over relatively short lengths of time, but the current data show that they also spent long periods within a small area. The extended presence of *R. bonasus* in Pine Island Sound suggests that the estuary provides adequate resources to support their dietary and home range requirements.

Chapter 4: General Conclusions

All previous reports regarding the diet and movement patterns of *R. bonasus* have indicated that this species is a highly migratory, transient ray that feeds almost exclusively upon bivalve prey (Schwartz, 1965, 1990; Smith and Merriner, 1985, 1986, 1987). Quantative data were lacking regarding these aspects of *R. bonasus* ecology within the Gulf of Mexcio.

Contrasting with evidence from previous studies, *Rhinoptera bonasus* in Charlotte Harbor, Florida do not appear to be exclusive hard prey specialists. Bivalves represented only 12.6% of the total IRI, contrasting with existing evidence that cownose rays are stenophagous in their choice of molluscan prey. Primary prey, in decreasing order, consisted of cumaceans, the polychaete *P. gouldii*, and the bivalve *A. papyrium*, which are all common benthic invertebrates within Charlotte Harbor. The results of this study suggest that *R. bonasus* are opportunistic predators that will exploit abundant prey types within their foraging area. The high dietary overlap between immature and mature *R. bonasus*, as well as between sexes and seasons, indicate that the same prey types remain important to all groups of *R. bonasus* using the estuary. Shoal mates had significantly more similar diets than non-shoal mates, supporting group feeding hypotheses and suggesting school fidelity, at least over short periods. Most of the consumed prey were not deep infauna, indicating that deep feeding pits are not necessary for foraging in this area.

Tracking data have shown that activity patterns of *R. bonasus* within Charlotte Harbor are not significantly related to time of day or tidal stage. Tagged rays did not display a distinct seasonal departure, and tracking data provide no evidence to support theories of a massive *R. bonasus* migration in the Gulf of Mexico. Movement patterns do not appear to be predictable for this population as a whole, but indicate that Pine Island Sound provides a suitable environment for *R. bonasus* to remain resident for extended periods while also serving as a transient stopping ground for numerous individuals. As highly mobile pelagic swimmers, *R. bonasus* are capable of traveling large distances over short time periods, but they can also spend long periods within relatively small home ranges.

These results provide new insight into the feeding and movement dynamics of a population of rays along the Gulf coast of Florida. Bivalves were not the most important prey, and most organisms consumed were epifauna or shallow infauna, suggesting that cownose rays are unlikely to be responsible for commercial shellfish decline or massive modification of seagrass beds in this region. Although tracking data were produced variable results, there is no evidence to suggest a massive migration or seasonal exodus for *R. bonasus* utilizing Charlotte Harbor.

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Appendices

Appendix A.

Appendix A. Length weight data for three captive *R. bonasus* used in transmitter attachment and retention studies. Individuals were maintained at Mote Marine Laboratory between July 2003 and October 2004.

Sex	SDW (cm)		Weight (kg)		Tag retention
	start	end	start	end	(days)
F	76	77	6.1	6.6	457
F	67	72.5	3.4	5.3	187
M	72	74	4.6	5	156

Appendix B.

Appendix B. Minimum convex polygon (MCP) and kernel utilization distributions (95 and 50% KUD) in kilometers² for all tracked individuals over 3 day, 7 day, 30 day (1 month) and total monitoring periods.

Ray ID	3 day			7 day			30 day			Total		
	MCP	95% KUD	50% KUD	MCP	95% KUD	50% KUD	MCP	95% KUD	50% KUD	MCP	95% KUD	50% KUD
95	8.65	9.83	1.00	43.97	41.71	3.45	55.63	46.02	3.80	55.63	43.00	5.50
96	3.96	12.82	3.07	10.69	11.48	1.89				31.64	21.79	3.12
97	8.24	14.83	1.82							10.07	9.22	1.02
98	20.24	11.49	1.14	23.57	4.97	0.63				39.83	16.30	1.48
262	22.77	62.44	6.96							22.77	62.44	6.96
263	16.21	53.30	16.96	17.52	43.48	7.35	27.99	46.35	9.07	27.99	46.35	9.07
264										6.88	23.32	5.04
265	15.41	36.98	6.37	31.54	62.96	6.67	34.52	8.35	1.66	39.83	20.94	3.13
266										16.20	33.22	4.50
268		0.01	0.00	0.00	0.02	0.01				0.08	0.22	0.05
269	0.06	0.01	0.00	3.93	0.27	0.09				4.02	0.67	0.13
270		0.00	0.00	0.65	0.09	0.03				0.81	0.18	0.05
271	2.10	6.58	0.97							2.10	6.58	0.97
273	0.35	0.03	0.01	1.03	0.16	0.05				1.10	0.37	0.09
508	15.19	29.86	4.89							15.19	29.86	4.89
509										12.71	41.47	9.68
511	2.19	1.35	0.33	16.37	8.35	1.03	59.41	33.09	1.84	71.78	20.38	1.65
512	24.03	16.86	1.94	31.28	19.35	1.86	31.68	25.11	2.51	31.77	25.11	2.51
514	19.83	29.80	3.69							27.72	28.56	3.44