Rethinking the status of *Albula* spp. biology in the Caribbean and western Atlantic

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INTRODUCTION

Worldwide, bonefishes (*Albula* spp.) are ecologically and economically important constituents of tropical, shallow-water systems. Bonefishes support economically important recreational fisheries in numerous locations in the Caribbean (e.g., Bahamas, Belize, Mexico, and Venezuela among the most notable). In southern Florida and the Florida Keys, often credited as the birth place of "flats fishing", bonefishes are an important component of the recreational fishery (Crabtree et al., 1996). Because of their ecological and economic importance, sustainable bonefish fisheries are of particular importance and knowledge of their ecological requirements is essential to successful management.

Unfortunately for managers, the taxonomy of bonefishes is still being unraveled. Bonefish were once classified as a single circumtropical species (*Albula vulpes* Linnaeus). Robins et al. (1986) noted two species of bonefish occur on the continental shelf and upper slope of the Atlantic Ocean. Recent genetic research, however, has suggested that at least eight species in the *Albula* genus exist worldwide (Colborn et al., 2001; Bowen et al., this volume), and revisions of the genus continue. Until recently (Colborn et al., 2001) the western Atlantic bonefish pursued by recreational anglers were assumed to be a single species *Albula vulpes*. (An ecologically distinct second species (*Albula nemoptera*) that reaches about half the maximum size of Albula vulpes occurrs in a limited geographic range and in depths too great for recreational angler interest (Robins et al. 1986).) Colborn et al. (2001) identified a third genetically distinct lineage of *Albula* in the Caribbean: a currently undescribed species referred to as *Albula* species B (popularly known as *Albula garcia*). Hereafter, *Albula* spp. refers to *A. vulpes* and *A*. sp. B, as addressed in this study.

Little is known about the biology and ecology of these species (but see Ault et al., this volume), or their relative contributions to recreational fisheries. Published information is on *A. vulpes* from the Florida Keys and Bahamas and mostly limited to the adult life stage: age, growth and mortality (Crabtree et al., 1996), maturation and reproduction (Crabtree et al., 1997), diet (Colton and Alevizon, 1983; Crabtree et al., 1998), movement (Colton, 1983; Humston et al., 2005), or a combination of these topics (Bruger, 1974; but also see Mojica et al., 1995 for larval duration and temporal abundance patterns). However, since these studies were conducted prior to Colburn et al.'s (2001) identification of *Albula* sp. B, verification of these findings may be required. Additionally, timing and location of spawning are not well described for *Albula* spp.,

and species composition of the fishery has not been quantified. Given the dearth of data on *Albula* spp. in the Caribbean and western Atlantic, studies that contribute additional information on these species are needed.

This paper presents results of sampling to examine three aspects of post-larval and juvenile *Albula* spp. biology and ecology: (1) spatial and temporal habitat use; (2) species composition of mature *Albula* spp. captured in regional recreational fisheries; and (3) a comparison of age and growth estimates of *A. vulpes* from several Caribbean locations to published results from the Florida Keys. These findings will contribute to better understanding of *Albula* spp. in the Caribbean and western Atlantic and provide direction for future research.

MATERIALS AND METHODS

Larval (leptocephalus) and juvenile sampling: Sampling was conducted in the Florida Keys, USA (Figure 1) during 2003 through 2005. Sampling in the Florida Keys occurred every other month from October/November 2003 through January 2005 from Key West to Elliot Key by seine (23 m x 1.2 m, 3.1 mm mesh center bag seine (for small juveniles) and 45.5 m x 1.8 m, 9.5 mm mesh center bag seine (for larger juveniles)). In October/November 2003 and January 2004, six habitat types (windward and leeward sandy beach; windward and leeward beachrock shoreline; windward and leeward mangroves) were sampled for small juveniles with the 23 m seine. Sampling effort was reduced to only sandy beach and beachrock shoreline for March, May, July, and November 2004, and January 2005 because of null catches in all habitats except sandy beach and beachrock shoreline in previous samples, and results of similar sampling conducted in the 1990s (Crabtree et al. 2003). Exploratory sampling was also conducted in shallow (< 3 m), sandy-bottom open bays on the Florida Bay side of the Florida Keys. The 23 m (Florida Keys) seine was set perpendicular to shore with one end at or on shore, pulled parallel to shore for 15 m, and either hauled onto shore (sandy beaches – average sample area = 575 m^2) or pursed offshore (all other habitat types – average sample area 166 m^2). For shore sets using the 45.5 m seine, the net was set perpendicular to shore, as above, the outer end pulled in an arc to shore, and the bag end hauled to shore (average sample area = 875 m^2). For offshore sets where the net could not be pulled against shore, the net was pulled for 15 m and pursed (sample area = 166 m^2). All bonefishes were measured (standard length), and tissue samples taken for genetic analysis.

In April 2006, a center bag seine (15 m x 1.2 m, 3.1 mm mesh) was used to sample six sandy beaches at Turneffe Atoll, Belize. Sampling was conducted as for the 23 m seine in Florida (average sample area = 225 m^2).

Tissue samples and genetic analyses: To determine species composition of juvenile *Albula* spp., tissue samples were taken from a subsample of larval and juvenile bonefishes captured in seines. Larval and juvenile bonefishes < 80 mm SL were retained whole. For juveniles ≥ 80 mm SL a triangle (12 mm x 12 mm x 12 mm) was cut from the soft ray tissue at the rear of the dorsal fin. Whole fish were placed in plastic bags on ice, and then transferred into individual vials containing 95% ethanol. Tissue samples were either placed in individual plastic bags on ice and then transferred to ethanol vials, or placed in ethanol vials on site. Sample location and date were recorded for each collection. Tissue samples from 299 (of 662 total) leptocephalus and juvenile bonefishes captured in the Florida Keys were retained for genetic analysis to identify species. Tissue samples were taken from all months and locations in which leptocephali and juveniles were captured (**Table 2**).

Similarly, to examine species composition of the recreational fishery in the Florida Keys

and Caribbean through genetic analysis, tissue samples were obtained from bonefish captured by recreational anglers in 10 locations in the Caribbean and in the Florida Keys. Fin clips were treated as described above. For samples from Puerto Rico (obtained from C. Caldow, NOAA/NOS), otoliths were ground and DNA was isolated from the ground material as described below. DNA was obtained from 17 otoliths, resulting in genetic identification for 48 adult bonefishes.

Total genomic DNA was isolated from all specimens using the Puregene® DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, Minnesota). Genetic-species-identification (GSI) assays were based on diagnostic nucleotide differences occurring in the mitochondrial DNA (mtDNA) cytochrome-*b* gene (Colborn et al., 2001). Initially, a subsample of bonefishes (N=60) that included both *A. vulpes* and *A.* sp. B specimens was sequenced for the cytochrome-*b* region (with no *a priori* knowledge of individual species identification). Representative voucher specimens are catalogued at Florida Fish and Wildlife Research Institute. Polymerase chain reaction (PCR) assays were conducted on Hybaid® thermocyclers using ALBA-1, ALBA-2, and ALBA-3 primers (Colborn et al., 2001), Applied Biosystems (ABI) BigDye® Terminator v1.1, and Taq polymerase (Promega Corp., Madison, Wisconsin). PCRs were run under the following profile: an initial cycle of 94° denaturation for 1 min, 50° annealing for 30 s, 72° extension for 1 min followed by 36 cycles of 94° for 30 s, 55° for 30s, 72° for 1 min 30 s, and a final 72° extension of 8 min. The PCR products were purified using the Quickstep2 kit (Edge Biosystems, Gaithersburg, Maryland), and the purified products prepared for forward and reverse sequencing with the following thermal profile: 35 cycles of 30 s 95° denaturation, 15 s 55° annealing, and 4 min 60° extension. Cytochrome sequences were aligned in ClustalX (Thompson et al. 1997), and analyzed in MEGA version 2.1 (Kumar et al. 2001) to determine sequence divergence between *A. vulpes* and *A.* sp. B. Example sequences of *A. vulpes* and *A.* sp. B were also compared to the cytochrome sequences submitted by Colborn et al. (2001) in Genbank (http://www.ncbi.nlm.nih.gov/). After confirmation of diagnostic nucleotide sites, new species-specific primers were developed: Avu-CytB F, Avu-CytB R, Aga-CytB F, and Aga-CytB R (**Table 1**). The two forward primers were labeled with dissimilar fluorescent dyes to allow rapid GSI assay of all specimens. Species-specific fragments were amplified under the following conditions: an initial cycle of 94° denaturation for 1 min, 50° annealing for 30 s, and 72° extension for 1 min, followed by 30 cycles of 94° for 30 s, 61° for 30 s, 72° for 1 min 30 s, and a final 72 ° extension for 8 min. All sequencing and GSI assays were conducted on automated genetic analyzers (ABI models 310 and 3100). The limited amount of DNA obtained from otoliths (17 specimens from Puerto Rico) required that these specimens be fully sequenced in lieu of the diagnostic marker assay.

Otolith samples: Otoliths were extracted from a subsample of adult fish captured in the recreational fishery in the Caribbean for age and growth comparisons with published data from the Florida Keys (Crabtree et al., 1996). Sex was determined during field dissection. Two to four 1 mm to 2 mm thick transverse sections containing the otolith core were cut with a Buehler Isomet low-speed saw with a diamond blade. The sections were mounted on a microscope slide with thermaplastic glue. Annuli were counted three times by each of three independent readers with reflected light at magnifications of 8-25x. After readers completed reading all otoliths, otoliths with different counts were re-examined. In all cases, differences were reconciled and an age assigned to the otolith.

RESULTS

Seven-hundred fifty samples were completed at 30 locations in the Florida Keys. A total of 628 juvenile bonefishes were captured along windward sandy beaches (Higgs Beach (N = 307) and Airport Beach (N = 126), Key West; Bahia Honda State Park (N = 173); Elliot Key (N = 15), Biscayne National Park) and along leeward beachrock shorelines (Elliot Key (N = 7)). Thirty-four leptocephalus larvae were captured (Table 2a), all along windward sandy beaches. Windward sandy beaches are intertidal sand shorelines with subtidal sand bottom immediately adjacent, and seagrass beginning approximately 4 to 9 m offshore of the beach. Beachrock shorelines are consolidated limestone at the intertidal zone with sand or mixed sand-seagrass bottom immediately adjacent. Juvenile or leptocephalus bonefishes were captured in all months except January and November 2004, and were in greatest abundance March through July (Table 2a). Lengths ranged from 19 – 360 mm SL. No juvenile bonefishes were captured in shallow, sandy-bottom open bays on the Florida Bay side of the Florida Keys.

A total of 35 seine samples were also conducted along six sandy beaches at Turneffe Atoll, and 35 juvenile and three lepteocephalus bonefish ranging from 24-56 mm SL were captured at two beaches on the central eastern side of Turneffe (Calabash and Rope Walk).

To identify species, tissue was taken from a subsamples of the juvenile and leptocephalus captured in the Florida Keys (299 of 662) and Turneffe Atoll (35 of 38). Tissue samples were taken from all months and locations in which leptocephali and/or juveniles were captured (**Table 2b**). During the initial cytochrome sequencing of Florida bonefishes, we found an approximate 9% sequence difference between the two species. This is slightly lower than the 12-15% difference reported in Colborn et al. (2001) and may be attributable to our larger sample sizes. The majority (93.97%) of Florida juveniles and leptocephali assayed with the cytochrome *b* diagnostic marker were identified as *A.* sp. B. Only 16 *A. vulpes* were collected from two sites; Biscayne National Park (BNP) and Bahia Honda. All *A. vulpes* juveniles occurred in July 2004 (from 2 seine hauls in BNP and 1 seine haul at Bahia Honda). The BNP July collection contained only *A. vulpes*, while the Bahia Honda collection was mixed *A. vulpes* and *A.* sp. B. All juveniles and leptochalus larvae captured at Turneffe Atoll were identified as *A.* sp. B. Thus, the findings reported here for juveniles are most applicable to *A.* sp. B.

Fin clips were obtained from 138 adult bonefishes captured at six locations: Turneffe Atoll, Belize (N = 54); Eleuthera, (7); Exuma (9), Berry Islands (5),and Mayaguana (3), Bahamas; St. Croix, U.S. Virgin Islands (1); Little Cayman, British West Indies (3); Anegada, British Virgin Islands (2); and Chetumal (7), and Punta Allen (4), Mexico (**Figure 1**). Fish ranging from 205 to 711 mm FL were either captured by anglers or by seine nets on flats frequented by guides and anglers. Based upon age estimates from otoliths of a sub-sample of 31 *A. vulpes* from the 138 above, ages at given lengths differed among locations (**Table 3, Figure 2**). Bonefish from Puerto Rico (n = 17) were similar in lengths at a given age to those reported for bonefish from the Florida Keys (Crabtree et al., 1996). All *A. vulpes* from all other Caribbean locations (n = 14) appeared to exhibit slower growth rates than the Florida Keys.

DISCUSSION

Our results revealed that: (1) most juveniles along sandy beaches appear to be *A.* sp. B; (2) juvenile habitats for *A. vulpes* remain largely unknown; (3) fish captured in the recreational fishery appear to be *A. vulpes*; and, (4) *A. vulpes* growth rates appear to differ among locations. Combined, these findings indicate that additional information is needed to ensure a successful conservation and management strategy for *Albula* spp. in the Caribbean and Western Atlantic.

The findings reported here raise questions about many aspects of the conventional wisdom of *Albula* in the Caribbean.

That very few juveniles of *A. vulpes*, the species that appears to support the recreational fishery, were captured is disconcerting. Although the decline, and/or lack of recovery, of adult stocks of many species has been blamed on overfishing, it is becoming increasingly apparent that loss or degradation of habitats may also limit species abundances (e.g., Turner et al., 1999). This generally occurs because essential juvenile habitats and/or connections between juvenile and adult habitats are lost or severely degraded. Since the extent to which different juvenile habitats contribute fishes to the adult population is essential information for successful conservation of fish populations (Beck et al., 2001), it is imperative that juvenile habitats of *A. vulpes* are determined. Only then can we determine whether juvenile habitat loss has impacted *A. vulpes* populations, and design effective conservation (or even restoration) strategies.

Nonetheless, this research has contributed to knowledge of A. sp B. The temporal occurrence of juveniles suggests that *A*. sp. B spawning occurs primarily in winter. In this study, small *A*. sp. B were in greatest abundance in March and May, and in lesser and roughly equal abundance in late January and July. If it is assumed that larval duration of *A.* sp B is not notably longer than the maximum 72 days for *A. vulpes*, then spawning occurred during fall through early spring, similar to that reported for *A. vulpes* by Crabtree et al. (1997). Concurrent spawning by *A*. sp. B and *A. vulpes* is also supported by the occurrence of juvenile *A.* sp. B in the Florida Keys in this study coinciding with the occurrence of *Albula* leptocephali in the Bahamas in 2004 (C. Dahlgren, pers. com.). The Bahamas leptocephali were subsampled, and all were genetically identified as *A. vulpes*.

The occurrence of juvenile A. sp. B along sandy beaches suggests a distinct ontogenetic habitat shift. Information from professional fishing captains indicates that adult A. sp. B reside in deeper water, whereas this study documents the occurrence of juveniles in shallow shoreline habitats. In contrast, adult Albula vulpes use a variety of mostly shallow coastal habitats, While it appears that juveniles only rarely used shallow shoreline habitats, the bulk of the resource may be outside the current sampling domain. Sampling in this study and other research suggests that *A. vulpes* juveniles use deeper habitats than were sampled during this study. For example, juvenile bonefishes were not captured in shoreline samples in St. Croix, U.S. Virgin Islands (A. Adams, unpublished data; Mateo and Tobias 2004), nor in extensive sampling with multiple gears in the Florida Keys and Florida Bay (D. Snodgrass, NOAA, pers. com.). These findings indicate that additional research elucidating species-specific spatial and temporal patterns of juvenile habitat use is necessary to provide sound ecological information that will enable sound bonefishes fishery management.

The findings on regional variation in growth rates of adult A. vulpes should be treated with great caution because of the low sample size, but do suggest that significant research is needed to specifically address the issue. For example, minimum size at maturity appears to be smaller in the Caribbean than the Florida Keys: a 342 mm FL female at Little Cayman was sexually mature, whereas 50% sexual maturity is 488 mm FL (95% CI 472 – 504 mm) for females in the Florida Keys (Crabtree et al., 1997). Although also preliminary, ongoing research in Los Roques, Venezuela, suggests differences in growth rate between Venezuela and the Florida Keys (J. Posada et al., this volume). In addition, *A. glossodonta* shows differences in growth and maximum size among locations in the Pacific (A. Friedlander et al., this volume).

These findings show a need to reassess conventional wisdom on bonefish biology in the Caribbean, and suggest directions for additional research. The potential for considerable

differences in growth rates requires research to verify these finding and to understand the underlying mechanisms. For example, Florida Keys and Puerto Rico coastal habitats receive terrestrial nutrient inputs from rivers that might increase productivity relative to other insular oceanic islands where most other *A. vulpes* were collected in this study. Alternatively, similar latitudinal differences in growth rate have been observed in other species (e.g., Murphy & Taylor, 1990), and may indicate counter-gradient variation (Edwards, 1984; Conover, 1990).

Additionally, the formation of annuli on Caribbean bonefishes otoliths needs to be verified. There was a possibility bonefish ages in this study were misclassified because otolith increments for young fish tend to be obscured in lower latitudes due to reduced seasonality, making clear interpretation of annuli difficult (Victor, 1982; Caldow, 2000). However, annuli were readily apparent for most individuals in this study, and since otoliths in this study were collected in lower latitudes (Belize, British Virgin Islands, Puerto Rico, Cayman Islands) or locations with less temperature variation than the Florida Keys (i.e., Bahamas), we would expect that the bias would have been to underestimate age.

Within *A. vulpes*, research should also be conducted to verify the apparent regional differences in growth and size at maturity. Higher sample sizes of a wider range of sizes are needed from all locations to test the preliminary conclusions of this study. Data should include length, weight, gonadal stage, meristic information, and genetic identification.

Although thus far *A*. sp. B have not been documented in the recreational fishery, considerably higher sample sizes are greatly needed to verify a single species fishery. Genetic analysis will continue to be a powerful tool in future research of *Albula*, as underscored in this study. This is especially true for differentiation of *A. vulpes* and *A.* sp. B, since *A.* sp. B morphometric information is lacking for all life stages, and limited information indicates significant overlap of morphological characteristics (Crabtree et al. 2003) drawing into question some of the conclusions based on genetic data alone. Genetic analysis may prove useful in examining species geographic ranges and population connectivity, and whether these two sympatric species might hybridize.

Finally, although the documentation of a new *Albula* species in the Caribbean has introduced a new aspect into research and conservation of bonefish in the region, this research also raises questions that are specific to *A. vulpes*. These questions include aspects of both habitat use and regional variations within the species. To the extent that additional research contributes to knowledge on these issues, the research, conservation and management frameworks for Caribbean bonefish may require modification.

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Table 1. Summary data for cytochrome-*b* mtDNA markers developed for species identification of bonefish (*Albula vulpes* and *A.* sp. B).

Marker	Primer Sequence (5′→ 3′)	Species	Fragment Size (bp)
Avu-CytB F	CCACTGTACCAATGCATCG	*A. vulpes*	169
Aga-CytB F	ATCCACTGTACTAACGCATCC	*A.* sp. B	171
Avu-CytB R	GTATCTTTACATGGAGACATG	*A. vulpes*	
Aga-CytB R	TTATCTTTACATGGAGACGTG	*A.* sp. B	

Table 2. Results of seine sampling for juvenile *Albula* spp in the Florida Keys. (A) Temporal patterns of juvenile and leptocephalus *Albula* captured in seine samples in the Florida Keys. July 2004 is the only month in which *Albula vulpes* individuals were captured.

		Month					
Year		**January**	**March**	**May**	**July**	**August**	**November**
	No. Samples	-	-	-	-	-	73
2003	No. Juveniles	-	-	-	-	-	1
	No. Leptocephali	-	-	-	-	-	2
	No. Samples	87	128	123	155	-	72
2004	No. Juveniles	0	149	144	100	-	0
	No. Leptocephali	0	9	12	0	-	0
	No. Samples	9	23	53	-	27	-
2005	No. Juveniles	54	17	161	-	2	-
	No. Leptocephali	5	6	0	-	0	-
	Total Samples	96	151	176	155	27	145
	Total Bonefish	59	181	317	100	2	3
	No. Bonefish/Sample	0.6146	1.1987	1.8011	0.6452	0.0741	0.0207

Table 2 (cont.). Results of seine sampling for juvenile *Albula* spp in the Florida Keys. Genetic analysis of juvenile and leptocephalus *Albula* spp. captured in seine sampling in the Florida Keys in 2003 and 2004. Only months in which *Albula* were captured and tested are shown. See Figure 1 for sample locations. *Elliot Key samples were collected in early February 2005.

Year/Month	Sample Location	Number Analyzed	Number Identified	
			A. vulpes	*A.* sp. B
2003				
November	Elliott Key	3	0	3
2004				
March	Key West	55	0	55
	Bahia Honda	2	0	2
May	Key West	50	0	50
	Bahia Honda	23	0	23
July	Key West	33	0	33
	Bahia Honda	15	4	11
	Elliott Key	11	11	0
October	Bahia Honda	1	0	1
2005				
January	Key West	10	0	10
	Elliott Key*	8	1	7
March	Key West	3	0	3
	Elliott Key	10	0	10
May	Key West	2	0	2
	Bahia Honda	71	2	69
August	Key West	1	0	1
	Bahia Honda	1	0	1
	Totals	**299**	**18**	**281**

Table 3. Summary of otolith-based age estimations for Caribbean bonefish *Albula vulpes*, captured in recreational fisheries as with expected values from the Florida Keys for comparison. See Figure 1 for sample locations. [a] (M) male, (F) female, (-) not determined. * Expected ages from Crabtree et al. (1996).

Location	Fork Length (mm)	Age (yr)	Sex[a]	Expected Age*
Puerto Rico	289	1	M	1
Puerto Rico	292	1	-	1
Puerto Rico	308	1	-	1
Puerto Rico	312	1	M	1
Puerto Rico	316	1	-	1
Puerto Rico	335	1	F	1
Puerto Rico	340	1	M	1
Puerto Rico	341	1	M	1
Puerto Rico	345	1	M	1
Puerto Rico	385	1	M	2
Little Cayman, BWI	279	2	M	1
Puerto Rico	330	2	M	1
Puerto Rico	372	2	M	2
Puerto Rico	397	2	F	2
Puerto Rico	411	2	M	2
Puerto Rico	417	2	M	2
Puerto Rico	352	3	M	2
Puerto Rico	357	3	M	2
Eleuthera, Bahamas	300	4	F	1
Eleuthera, Bahamas	320	5	M	1
Eleuthera, Bahamas	355	5	M	2
Little Cayman, BWI	342	6	F	2
Turneffe Atoll, Belize	410	8	-	2
Turneffe Atoll, Belize	411	8	-	2
Turneffe Atoll, Belize	420	8	-	3
Turneffe Atoll, Belize	426	8	-	3
Exuma, Bahamas	461	8	M	4
Exuma, Bahamas	472	8	F	4
Turneffe Atoll, Belize	423	9	-	3
Turneffe Atoll, Belize	411	10	-	2
Anegada, BVI	560	16	F	6

Figure 1. Map showing locations of sampling for larval and juvenile (Turneffe Atoll, Belize, and Florida Keys, Florida, USA) and adult collections (Turneffe Atoll, Belize; Eleuthera, Exuma, Berry Islands, and Mayaguana, Bahamas; St. Croix, U.S. Virgin Islands; Little Cayman, British West Indies; Anegada, British Virgin Islands; and Chetumal and Punta Allen, Mexico). For Florida Keys locations (Key West = Higgs Beach, Airport Beach; BNP = Biscayne National Park; BH = Bahia Honda State Park). The results of juvenile sampling are listed in Table 2. All values reference sampling of adults, all identified as *Albula vulpes*: the first value is number of adults providing tissue samples; the second value is the number of adults providing otoliths.

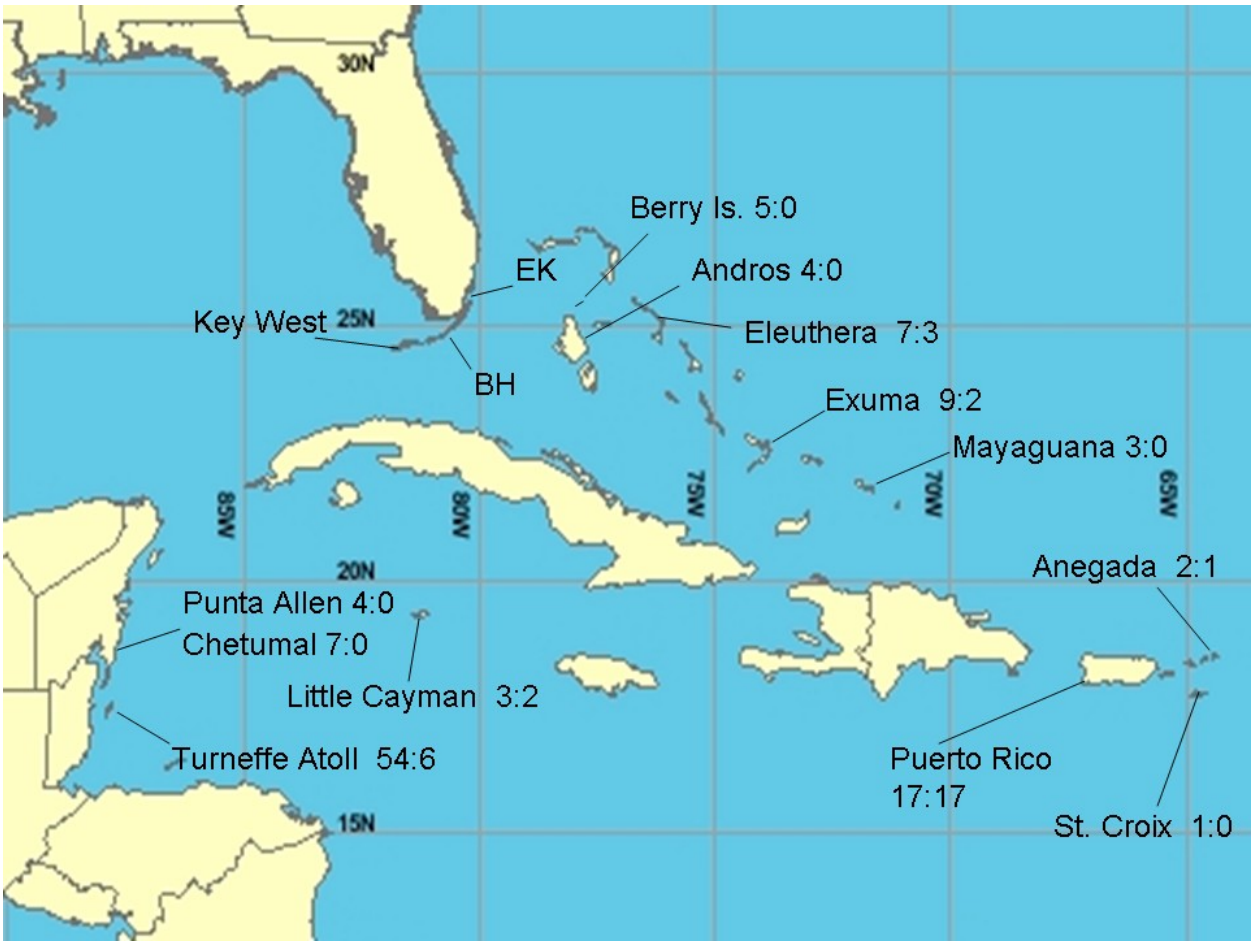

Figure 2. Observed lengths from Caribbean bonefishes identified as *Albula vulpes* (open symbols: □ Puerto Rico, ○ all other Caribbean locations) and predicted lengths of *A. vulpes* from the Florida Keys, Florida, USA (closed symbols: ■ female, ♦ male). Florida Keys values calculated from Crabtree et al. (1996).