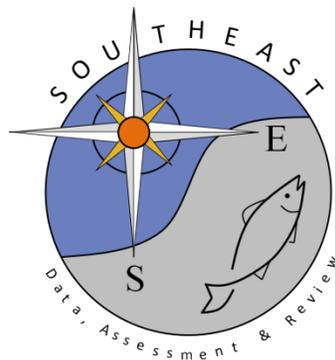


Report on the status of U.S. Caribbean stoplight parrotfish *Sparisoma viride* age, growth, and reproductive biology for the SEDAR84 Stock Assessment

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SEDAR84-AP-01

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2024



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HIGHLIGHTS OF RELEVANT BACKGROUND

Ecology and Biology of Parrotfishes

-  Parrotfishes are a dominant group of reef fishes that perform top-down role in the control of macroalgae and removal of coral skeletal material (Bruggemann et al. 1996, McAfee and Morgan 1996, Mumby 2006, Francini et al. 2010, Dromard et al. 2015).
-  Recent research on diet indicates parrotfish are not generalist consumers on algae; different species likely feed selectively on high protein filamentous cyanobacteria obtained by scraping and grazing on algal turf, sediments, and calcareous elements (Nicholson and Clements 2023).
-  In general, parrotfishes have complex socio-sexual systems important to their life histories and demographic structures.
-  Sequential protogynous hermaphrodites; dominant reproductive mode is to start out life as female with the capacity to transition to male later in life.
-  Two types of males occur in some species: primary males = individuals that do not first sexually matures as a female; secondary males = individuals derived from mature females.
-  Three color-based morphological phases: juvenile phase (JP), initial phase (IP), and terminal phase (TP)
-  Juvenile color phase does not differ from the IP in many of the Atlantic/Caribbean parrotfish species; JP consists of newly recruited individuals that are sexually immature.
-  Shortly after post-settlement, juveniles transition into IP, the majority of which are female.
-  After reproductive maturation, IP fish are capable physiologically of transitioning into the TP which consists of mature males usually derived from sex-changed females.

Stoplight Parrotfish

-  Stoplight parrotfish *Sparisoma viride* supports local fisheries in some regions in the western Atlantic.
-  Geographic range encompasses Florida, Bermuda, Bahamas in the north, throughout the greater Caribbean to waters of the southern Caribbean (Robertson and Van Tassell 2019).

 In terms of genetic population structure of stoplight parrotfish, a study looked at mitochondrial and nucleotide DNA regions of individuals collected from 34 localities throughout the Greater Caribbean and concluded that stoplight parrotfish exhibit high genetic connectivity suggesting a panmictic population (Loera-Padilla et al. 2022); study concluded that the lack of genetic population structure is due to the relatively long duration of stoplight pelagic larvae enables extensive dispersal through currents combined with high reproductive output and year-round spawning.

 A recent study on stoplight parrotfish conservation genomics sampled fish from throughout the U.S. Caribbean and Florida Keys and also documented high population connectivity among island platforms and between U.S. Caribbean and Florida (Rivera Hernandez, unpublished data).

 Stoplight parrotfish is a large (maximum length of around 500 mm TL) reef herbivore (Randall 1967) that exhibits full sexual dichromatism.

 The social mating structure of stoplight parrotfish can be describe as dynamic in which there are multiple mechanism for reproduction (van Rooij et al. 1996).

 The general mating behavior of stoplight parrotfish is described as harem; one dominant male and multiple females defend a territory; a second mating system observed in this species consists of males that do not hold permanent territories but make use of temporary territories in depths greater than 20 m for spawning events with females.

 Stoplight IP males exhibit streaking behavior, defined as “rushing in to join at the climax” and releasing sperm during a spawning event between a territorial TP male and IP female (van Rooij et al. 1996).

 van Rooij et al. (1996) documented that stoplight parrotfish spawn daily, often multiple times within a day and year-round; a territorial TP male spawns on average every 90 minutes and some females within a territory spawned multiple times within a day.

 Also of note, van Rooij et al. (1996) determined that in stoplight parrotfish sexual transition from female to male does not occur when a “dominant” female transitions to male when the territorial TP male of a harem is removed. Rather, females that undergo sexual transition leave their harems and join groups of roving TP and IP individuals.

 At least two additional studies reported that stoplight parrotfish spawn year-round (Robertson and Warner 1978, Figuerola et al. 1998).

OVERALL GOAL AND SPECIFIC OBJECTIVES

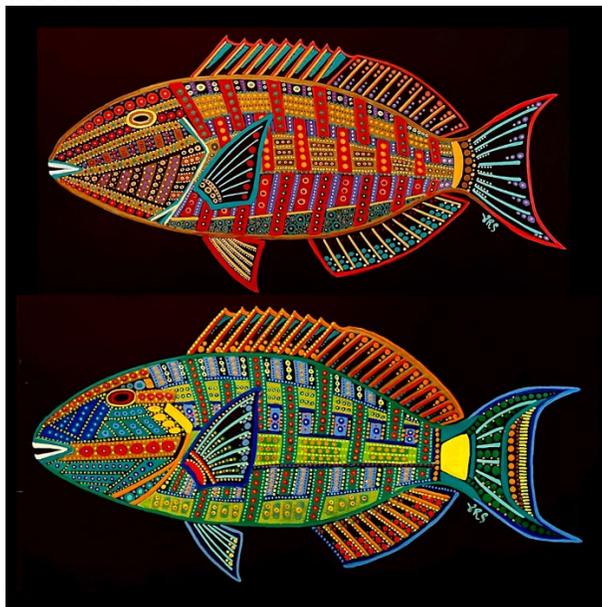
The overall goal of the current study to provide essential life history information in support of more effective fishery management for an important reef fish fisheries species in the U.S. Caribbean.

Utilization of region-specific and current information on life history information is key for assessing the local stock of stoplight parrotfish

Age and growth estimates are fundamental to reliably estimating biological reference points and are required to facilitate the transition to age-based stock assessments.

The specific objectives of this study were:

- 1) Document length-length and length-weight relationships for stoplight parrotfish from the U.S. Caribbean
- 2) Characterize attributes of population size and age structure
- 3) Determine reproductive patterns and describe reproductive seasonality
- 4) Document size and age at sexual maturity for stoplight parrotfish in the U.S. Caribbean
- 5) Document size and age at sexual transition for stoplight parrotfish in the U.S. Caribbean
- 6) Briefly compare results to selected studies from other regions and time periods to emphasize the importance of local and current information on U.S. Caribbean species in conducting stock assessments



METHODS

Sample Collection



Monthly; 2013-2023



Fisheries-dependent (FD) samples were obtained directly from fishers in USVI and Puerto Rico (2013-2023) and divided into two FD sample types:

- FD-random were collected by randomly selecting all fish from one side of a cooler containing the day's catch or by purchasing all stoplight parrotfish landed by a fisher on the day/night of sampling;
- FD-nonrandom were collected by haphazardly selecting a subsample of stoplight parrotfish from a fisher at market which meant that catch may have been combined from multiple trips and an unknown portion of fish may have been sold prior to our sampling



Fisheries-independent: Fish/Fisheries Conservation Lab collections for 2015-2023 from USVI and Puerto Rico

- Collaborative efforts to fill in temporal, spatial, and size gaps with the help of fishers using hook gear
- Castnet, spear, and trap sampling by PIs to ensure collection of small juvenile samples

Processing



For each sample we noted date of collection, gear of capture, collection location, depth (if available)



Obtained length (SL/FL/TL mm) and whole weight (g) of samples



Gonads weighed (0.01 g) and preserved for reproductive histology



Otoliths obtained for age estimation for all FD and FI samples processed by the Fish/Fisheries Conservation Lab



Collected genetic tissue sample, muscle sample, stomach, and eyes for additional research

Length-Length and Length-Weight Conversions



IMPORTANCE: Long-term, consistent, and widespread fish length data are limited for Caribbean reef fish species like stoplight parrotfish. Conversions of length-type (i.e., SL, FL, TL) serve as a helpful tool to bridge gaps in scientific sampling and measuring between studies (Jones et al. 2021a)

 Due to logistical or physical reasons, different studies may utilize differing measurement methods; for example, one study may report SL, while another primarily utilizes FL. The creation of accurate conversions of length improves upon the amount of available data for Caribbean fisheries species and promotes the sharing of data across different researchers and managers who had previously used differing measurement methodologies.

 Regression equations based on a large sample size of yellowtail snapper were calculated to create length-length and length-weight conversions

 The length-weight regressions were in the form of $W = a L^b$; where W = weight (g), L = length (mm), and a and b are the intercept and slope parameters, respectively

Evaluation of trends related to fish length

 A two factor ANOVA was used to test for significant differences in mean fish length among sexes (females, transitioning individuals, and males)

 Separate pairwise Kolmogorov-Smirnov (K-S) tests were used to determine if significant differences occurred in length frequency distributions among sexes (females, transitioning individuals, and males)

Age estimation and evaluation of trends related to fish age

 To obtain age estimates for understanding population demographics and computing growth estimates, two independent readers with 10+ years of experience assessed increment counts for each yellowtail snapper otolith without knowledge of fish size or date of collection

 In cases of between-reader increment count disagreements, the two readers concurrently evaluated the otolith section together and reached a consensus age estimate

 Average percent error (APE) between age estimates obtained by readers using the equation of Beamish and Fournier (1981)

 Separate pairwise K-S tests were used to compare the age frequency distributions among sexes (females, transitioning individuals, and males)

 An ANOVA was used to determine if mean age differed significantly among sexes (females, transitioning individuals, and males)

Growth parameter estimation

 Fork length-at-age data, von Bertalanffy growth functions (VBGF) were fit with the least squares method

 von Bertalanffy growth functions (VBGF) were computed using length-at-age data stoplight parrotfish to obtain growth parameter estimates and 95% confidence intervals (CI) for the parameters; VBGF were computed for each of the following: 1) Length type FL; 2) Length type FL; fixed t_0 value of -0.06 following the recommendations from previous parrotfish growth studies (Paddack et al. 2009, Taylor and Choat 2014); 3) Length type SL (for comparison with other studies that used SL); 4) Length type SL; fixed t_0 value of -0.06 following the recommendations from previous parrotfish growth studies (Paddack et al. 2009, Taylor and Choat 2014).

Gonad histology

 Gonads removed from each parrotfish sample and each gonad was fixed in 11% seawater-buffered formalin, Davidson's fixative, or polyethylene glycol–ethyl alcohol–glycerol–acetic acid (PAGA) fixative for up to 2 weeks and then transferred to 70% alcohol

 Gonad samples were processed using standard histological procedures for tropical fish species; tissue samples were vacuum-infiltrated and embedded in paraffin wax; at least three transverse sections (~7 μm thick) were cut using a rotary microtome, mounted on glass slides, stained with double strength Gill hematoxylin, and counter-stained with eosin-y

 Stained sections were viewed using a compound microscope to determine sex and reproductive phase, assessed according to the histological criteria described in Supplemental Table 1 for Caribbean parrotfishes

 Two readers examined each of the gonad histology slides and independently assigned sex and reproductive phase without knowledge of the capture date, specimen length, or specimen age; if differences in the assignment of reproductive phases occurred, readers examined the slide simultaneously to obtain a consensus phase assignment

Spawning season and spawning frequency

 The proportion of spawning-capable females relative to the total number of mature females in developing, regressing, and regenerating reproductive phases for each month was plotted to document overall spawning season and peak spawning period for the U.S. Caribbean region

 Spawning fraction, the proportion of actively spawning females relative to the total number of mature females, was calculated.

 Spawning interval was calculated using the early postovulatory follicle method (Rivera Hernández et al. 2019); to estimate spawning interval (number of days between spawning events) for mature yellowtail snapper samples overall, by length class, and by age class, the following equation was used: spawning interval = $1/\text{spawning fraction}$.

 Spawning frequency was computed to estimate the number of times females could spawn within a year by dividing 365 days (the number of days within the spawning season of U.S. Caribbean yellowtail snapper females; Rivera Hernández et al. 2019) by spawning frequency.

Sexual maturity and sexual transition



Size (LM_{50}) for SL and FL and age (AM_{50}) at median sexual maturity of females was computed using separate logistic regressions.



Additional size and age at maturity values were also computed for sizes and ages at which 90% (L_{90} and A_{90}) and 95% (L_{95} and A_{95}) of individuals were sexually mature.



Size (LT_{50}) for SL and FL and age (AT_{50}) at median sexual transition from female to male was computed using separate logistic regression;



For logistic regression analyses, maturity (not mature versus mature) and sex (not male versus male) were treated as binomial response variables (Jones et al. 2021b); logistic regressions were conducted using the logit function transformation and the generalized linear model procedure in R (Ogle, 2013).

RESULTS

Fish length and age trends

A total of 1801 samples were collected and measured for 2013-2023 from across the U.S. Caribbean.

Table 1. Sample summary of stoplight parrotfish across the main islands of the U.S. Caribbean.

	Overall	Puerto Rico	St. Thomas	St. Croix
Total fish sampled	1801	627	500	674
Fisheries-dependent	1592	511	431	650
Initial color phase	769	237	166	366
Transition color phase	4	-	4	-
Terminal color phase	819	274	261	284
Female	667	208	120	339
Transition	62	3	36	23
Male	846	291	270	285
Unknown	16	9	4	3
Fisheries-independent	209	116	69	24
Initial color phase	146	68	56	22
Terminal color phase	63	48	13	2
Female	124	64	39	21
Transition	11	1	9	1
Male	70	49	19	2
Unknown	4	2	2	-

Table 2. U.S. Caribbean stoplight parrotfish length-length and length-weight conversion relationships derived from regression analyses.

Category	n	Regression equation	R ²
SL→Wt	1488	$W = (2 \times 10^{-4}) SL^{2.69}$	0.93
FL→Wt	1716	$W = (4 \times 10^{-5}) FL^{2.90}$	0.95
TL→Wt	1706	$W = (3 \times 10^{-4}) TL^{2.51}$	0.95
FL→SL	1488	$SL = 0.90FL - 14.11$	0.98
FL→TL	1711	$TL = 1.28FL - 47.38$	0.97
TL→SL	1488	$SL = 0.70TL + 22.33$	0.97

Table 3. Summary of fork length (FL), standard length (SL), and age information obtained from U.S. Caribbean stoplight parrotfish samples overall ("All fish"), by sex, and by color phase x sex. Samples of unknown sex were not included beyond the "All fish" group.

Species	Group	N measured/ aged	FL range (mean) mm	SL range (mean) mm	Age range (mean) y
Sex	All fish	1801/1714	73-433 (281)	60-376 (240)	0-20 (5.4)
	Female	791/754	73-433 (259)	60-376 (218)	0-20 (5.2)
	Male	917/874	127-399 (304)	103-355 (261)	1-16 (5.7)
	Transition	73/70	183-366 (258)	148-315 (217)	2-15 (4.5)
	Unknown	20/16	135-293 (241)	114-237 (199)	2-9 (5.0)
Initial Phase	All	917/869	73-433 (258)	60-376 (217)	0-20 (5.1)
	Female	791/754	73-433 (259)	0-376 (218)	0-20 (5.2)
	Male	34/30	127-298 (238)	103-241 (198)	1-7 (4.2)
	Transition	72/69	183-366 (258)	148-315 (217)	2-15 (4.5)
	Unknown	20/16	135-293 (241)	114-237 (199)	2-9 (5.0)
Transitioning Color Phase	All	4/4	250-318 (290)	213-282 (247)	4-8 (5.5)
	Male	3/3	291-318 (303)	242-282 (258)	5-8 (6.0)
	Transition	1/1	250	213	4
Terminal Phase	Male	880/841	210-399 (306)	175-355 (264)	2-16 (5.7)

-  Mean length of males (304 mm FL) was significantly larger than mean length of females (259 mm FL) and transitioning (258 mm FL)
-  Between reader precision for age estimation: APE = 4.1%
-  Mean age of males (5.7 y) was significantly older than females (5.2 y), but only by 0.5 y; mean ages of males and females were significantly older than transitioning individuals (4.5 y)
-  Females attained an older maximum age (20 y) compared to males (16 y)

Table 4. ANOVA analyses results for mean length and mean age among sexes (female, transitioning, and male)

Source	df	Sum of Squares	Mean Square	F	P
Length (FL mm)					
Sex	2	892734	446367	300.29	< 0.001
Error	1778	2642932	1486		
Age (y)					
Sex	2	175	87	18.12	< 0.001
Error	1695	8176	5		

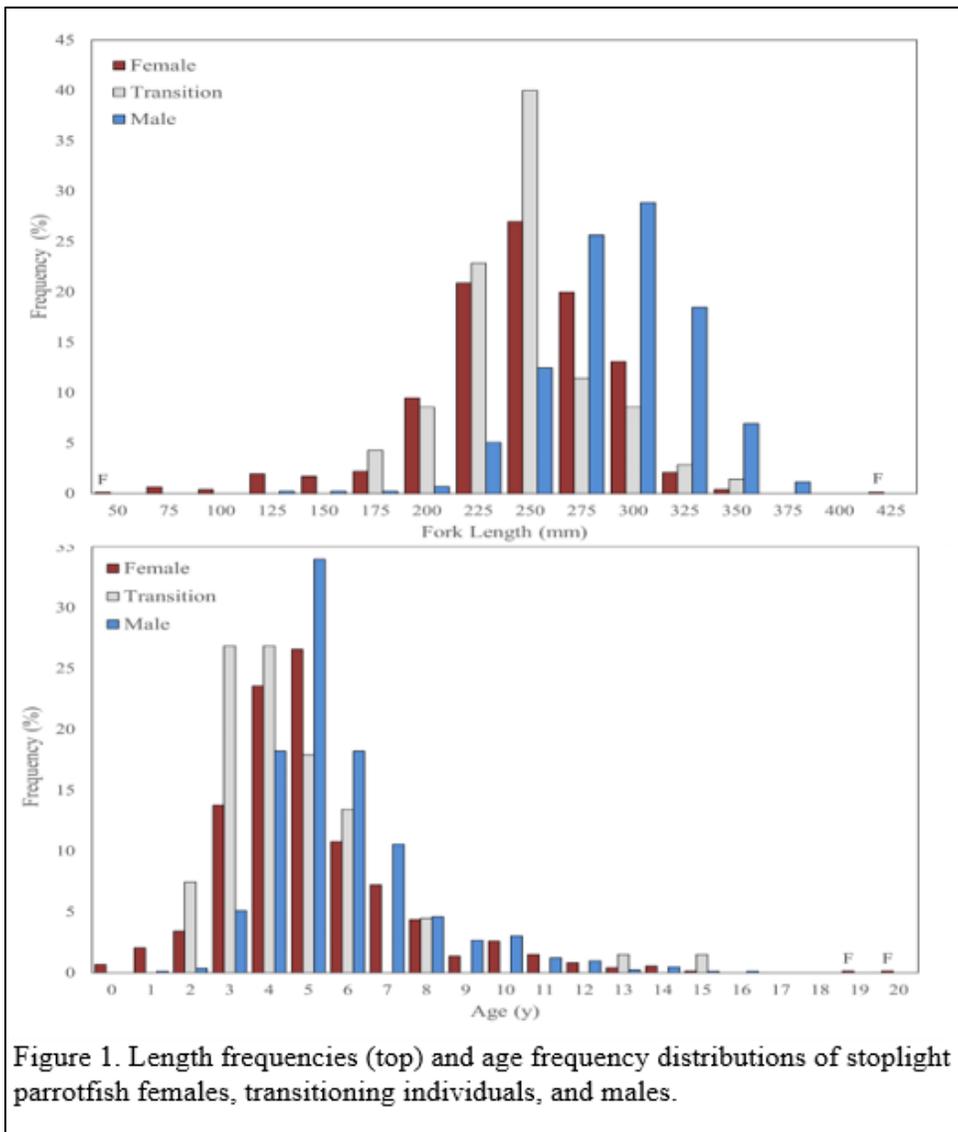


Figure 1. Length frequencies (top) and age frequency distributions of stoplight parrotfish females, transitioning individuals, and males.

Table 5. Results from pairwise Kolmogorov-Smirnov tests assessing differences in length and age frequencies.

Comparison	N	D	P
Length (FL mm)			
Initial v terminal	897 + 880	10.66	< 0.001
Female v transition	791 + 73	1.29	0.072
Female v male	791 + 917	9.58	< 0.001
Transition v male	73 + 917	4.92	< 0.001
Age (y)			
Initial v terminal	853 + 841	4.25	< 0.001
Female v transition	754 + 70	1.60	0.062
Female v male	754 + 874	3.53	< 0.001
Transition v male	70 + 874	3.03	< 0.001

 A total of 1,649 fish with size at age data were included in the growth models.

 Asymptotic length was larger ($L_{\infty} = 338$ mm FL/297 mm SL; $t_0 = -0.52$) and K was lower (0.33) when t_0 was not fixed using an a priori value

Table 6. VBGF parameter estimate results for stoplight parrotfish.

Model	N	L_{∞} (mm)	K	t_0
FL mm t_0 -fixed	1649	332 (328 - 335)	0.39 (0.35 - 0.41)	-0.06*
FL mm	1649	338 (328 - 335)	0.33 (0.31 - 0.36)	-0.52 (-0.72 - -0.35)
SL mm t_0 -fixed	1649	287 (282-290)	0.38 (282-290)	-0.06*
SL mm	1649	297 (286-300)	0.33 (0.31-0.36)	-0.40 (-0.59 - -0.23)

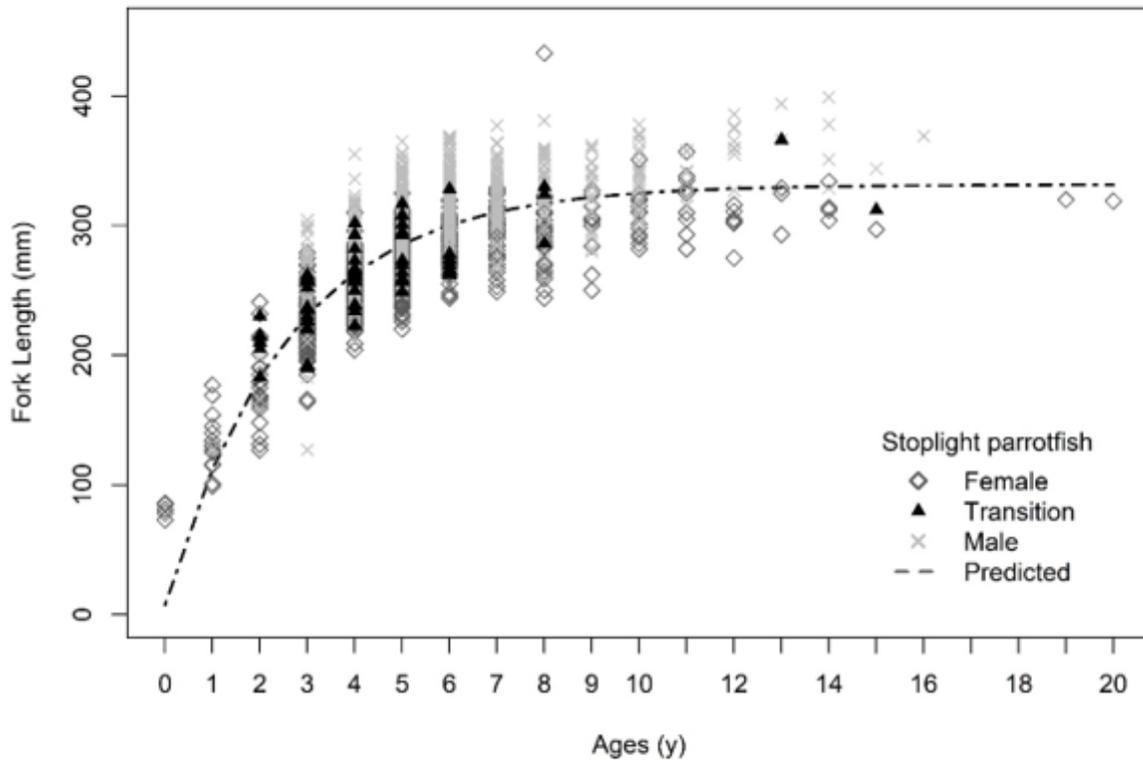


Figure 2. von Bertalanffy growth and sizes-at-age for stoplight parrotfish from waters of the U.S. Caribbean

Reproductive Biology



A total of 1,765 stoplight parrotfish gonads provided information on sexual maturity and reproductive phase.



A high proportion of both females and males were in the spawning capable phase during every month of the year indicating year-round spawning activity.

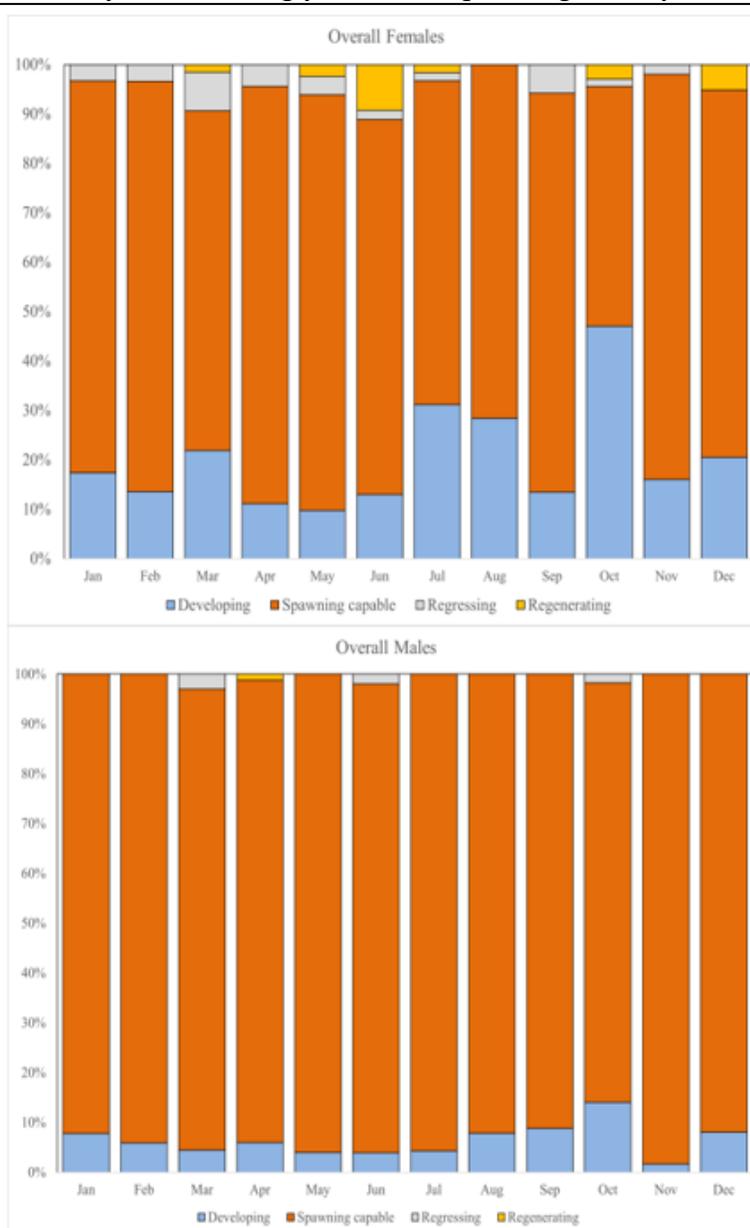


Figure 3. Reproductive seasonality for stoplight parrotfish females (above) and males (below) in the U.S. Caribbean. Monthly percentages of individuals in each reproductive phase are presented.



Spawning fraction overall for stoplight females was 0.52.



Overall spawning interval, defined as the number of days between spawning events in a female, was 1.9 d



When examining trends in spawning fraction, interval and frequency by length, spawning frequency increased with increasing length class; females in the smallest FL class had an estimated spawning frequency of 33 times a year, while females in the second to largest FL class had an estimated spawning frequency of 332 times a year. Similar increases in spawning frequency occurred when examined by age classes.

Table 7. Female stoplight parrotfish estimates for spawning fraction, spawning interval, and spawning frequency summarized overall, by length classes, and by age classes. Spawning fraction is the proportion of actively spawning females relative to the total number of mature females. Spawning interval is the estimated number of days between spawning events. Spawning frequency was computed to estimate the number of times females could spawn within a year.

Group	N of mature females	Spawning fraction	Spawning interval	Spawning frequency
Overall	732	0.52	1.9 d	190/y
FL class (mm)				
≤ 200	23	0.09	11.1 d	33/y
201 – 250	226	0.43	2.3 d	157/y
251 – 300	359	0.56	1.7 d	204/y
301 – 350	115	0.71	1.4 d	332/y
≥ 351	4	0.50	2.0 d	183/y
Age class (y)				
≤ 2	20	0.05	20 d	18/y
3-4	264	0.46	2.1 d	168/y
5-6	274	0.57	1.7 d	209/y
7-8	84	0.65	1.5 d	237/y
9+	56	0.70	1.4 d	261/y

 The length at median sexual maturity for stoplight parrotfish from the U.S. Caribbean was 153 mm FL; the smallest mature fish was a 115 mm FL female; the largest immature fish was a 218 mm FL female.

 The age at median sexual maturity for stoplight parrotfish from the U.S. Caribbean was 1.6 y.

 Females ranged in size from 73-433 mm FL, males range in size from 127-399 mm FL, and fish with gonads that were sexually transitioning ranged in size from 183-366 mm FL. These data indicate that females and males overlap across almost the full range of lengths studied.

 Females ranged in age from 0-20 y, males range in age from 1-16 y, and fish with gonads that were sexually transitioning ranged in age from 2-15 y. These data indicate that females and males overlap across almost the full range of ages studied.

 The size and age at median sexual transition for stoplight parrotfish was 279 mm FL and 4.5 y, respectively.

Table 8. Size and age at median sexual maturity and transition.

Parameter	n	Estimate
Sexual Maturity		
LM ₅₀	768	153 (140 - 165)
AM ₅₀	731	1.6 (1.4 - 1.9)
Transition		
LT ₅₀	1637	279 (275 - 282)
AT ₅₀	1558	4.5 (3.4 - 5.3)

DISCUSSION AND RECOMMENDATIONS

Maximum size observed (and verified/documentated in searchable records after removal of outliers) in the U.S. Caribbean for this species is below the maximum size noted for stoplight parrotfish. Our largest observed fish was 433 mm FL; maximum size of stoplight parrotfish from the St. Croix, USVI, visual census work was 42 cm FL (J Blondeau, NOAA, personal communication), which was similar to maximum size sampled in the current life history study.

Maximum size for this species increases with latitude so it makes sense that maximum size in Florida/Bahamas/Bermuda is bigger. However, we should not use Florida stoplight parrotfish maximum size trends to drive any assumptions about what people ****think**** is an appropriate value for L_{∞} for the U.S. Caribbean (this was done in a previous stock assessment for U.S. Caribbean stoplight parrotfish at a time when U.S. Caribbean size-at-age data did not exist). Rather, we should rely on the results from extensively documented size-at-age information for stoplight parrotfish in the U.S. Caribbean.

High variability in size-at-age has been documented for this species from other studies – females spawning daily, year-round, which means females may invest less energy in somatic growth and more energy in reproductive output (van Rooij et al. 1995a, b, van Rooij et al. 1996, van Rooij and Videler 1997). Our study supports this trend – the two oldest fish we collected were both females (19 y and 20 y) of medium lengths (319 and 320 mm FL).

Not all females appear to transition to males for the stoplight parrotfish population of the U.S. Caribbean; although the general trend for the proportion of females and males with increasing size (mm FL) is an overall decline in females such that females are mostly absent at 351 mm FL and larger. However, the trend in the proportion of females and males with increasing ages indicates that females are more dominant through age-3, then the proportion of females and males oscillates around 0.50/0.50 from ages 4-15 y.

Length (mm FL)	Total number		Proportion	
	Female	Male	Female	Male
50-75	1		1	0
76-100	5		1	0
101-125	3		1	0
126-150	15	2	0.88	0.12
151-175	13	2	0.87	0.13
176-200	17	2	0.89	0.11
201-225	71	6	0.92	0.08
226-250	160	47	0.77	0.23
251-275	205	108	0.65	0.35
276-300	169	228	0.43	0.57
301-325	110	269	0.29	0.71
326-350	18	184	0.09	0.91
351-375	3	59	0.05	0.95
376-400		10	0	1
426-450	1		1	0

Age (y)	Total number		Proportion	
	Female	Male	Female	Male
0	5		1	0
1	15	1	0.94	0.06
2	25	3	0.89	0.11
3	101	51	0.66	0.34
4	177	166	0.52	0.48
5	198	292	0.40	0.60
6	82	153	0.35	0.65
7	56	89	0.39	0.61
8	32	39	0.45	0.55
9	12	25	0.32	0.68
10	20	25	0.44	0.56
11	12	11	0.52	0.48
12	7	10	0.41	0.59
13	5	3	0.63	0.37
14	4	4	0.50	0.50
15	1	1	0.50	0.50
16		1	0	1
17				
18				
19	1		1	0
20	1		1	0

Stoplight parrotfish in the U.S. Caribbean were characterized by a medium asymptotic length compared to L_{∞} values reported for the species from other regions. K was relatively lower for U.S. Caribbean fish (0.39) compared to other studies (0.45, however we sampled across a broad range of depths utilizing a variety of gears while the other studies had low samples sizes, were limited to depths < 15 m and mainly caught stoplight parrotfish using spears.

Table 9. Comparison of studies reporting on growth and age related information of stoplight parrotfish

Study Location N (sample source)	Size range (mm) Age range	L_{∞} (mm)	K	t_0 (fixed)	Collection details and age estimation validation/verification methods
Current study					
U.S. Caribbean 1649 (FI+FD)	60-377 SL 73-433 FL 0-20 y	287 SL 332 FL	0.39	-0.06	Depth: 0-40 m Gear: cast net, seine, spear, trap; age estimation method successfully validated via bomb radiocarbon analysis
Choat et al. (2003)					
Bahamas 108 (FI)	32-379 SL 0-9 y	357 SL	0.45	-0.06	Depth: 2-15 m Gear: spear; sampled across size range present in a region; also focused on obtaining large terminal color phase Age estimation validation not successful – only examined one chemically-marked individual recaptured after 1.2 y
Venezuela 118 (FI)	61-312 SL 0-9 y	281 SL	0.60	-0.06	
Barbados 109 (FI)	72-332 SL 0-9 y	275 SL	0.71	-0.05	
Panama 82 (FI)	48-306 SL 0-7 y	264 SL	0.82	-0.05	
Paddack et al. (2009)					
FL Keys Combined 176 (FI)	11-303 SL 0-8 y	269 SL	0.84	-0.06	Depth: 2-7 m Gear: spear and net No validation of accuracy of age estimation method, but did examine relationship between otolith mass and increment counts
FL Keys Inshore 78 (FI)	11-254 SL 0-4 y	246 SL	0.90	-0.06	
FL Keys Offshore 98 (FI)	12-303 SL 0-8 y	270 SL	1.00	-0.06	

Our study is the first to provide comprehensive reproductive biology information for the species and our study included a large sample size of fish from FD and FI source caught with multiple gear types. Past studies were limited by sample number, only sampled with one gear at shallow depths, did not use gonad histology, only analyzed fish from one source (FD), and lacked age information.

Table 10. Comparison of study results with information reported on reproductive biology of stoplight parrotfish

Location (# of samples) Citation	Size range mm	Smallest Mature mm	Largest Immature mm	LM_{50} mm AM_{50}	LT_{50} mm AT_{50}	Method
U.S. Caribbean n = 1736 Current study	60-376 SL 73-433 FL	92 SL 115 FL	182 SL 218 FL	153 FL 1.6 y	279 FL 4.5 y	Histology
Panama n = 264 Robertson and Warner 1978	80-330 SL	NR	160 SL	NR	NR	Histology
Turks and Caicos n = 310 Koltes 1993	100-370 SL	170 SL	270 SL	NR	NR	Macroscopic/ Gonad scraping
Puerto Rico n = 304 Figuerola et al. 1998	153-390 FL	188 FL	240 FL	205 FL	NR	Histology

Recommendations

Continued monitoring of population demographics

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Supplemental Table 1. Reproductive phases and histological indicators for Caribbean wrasses/parrotfishes. Abbreviations are as follows: PG = primary growth oocytes, CA = cortical alveolar oocytes, Vtg = vitellogenic, OM = oocyte maturation, GVM = germinal vesicle migration, YC = yolk coalescence, GVBD = germinal vesicle breakdown, POF = postovulatory follicle, Sg1 = primary spermatogonia, Sg2 = secondary spermatogonia, Sc1 = primary spermatocyte, Sc2 = secondary spermatocyte, St = spermatid, Sz = sperm,

Reproductive phase	Histological features
Immature female	Gonads generally small; no atresia observed; muscle bundles absent; no signs of previous Vtg activity; only oogonia and PG oocytes present; PG oocytes tightly packed; ovarian wall thin
Developing female	PG, CA, Vtg1 and Vtg2 oocytes present; atresia relatively uncommon; Vtg3 oocytes may occur but no GVM; *Early developing ovaries contain mainly PG and CA oocytes
Spawning capable female	Gonad area relatively large; Vtg3 oocytes and early GVM present in addition to PG, CA, and Vtg1 and Vtg2 oocytes; minor signs of atresia may be present; *Actively spawning ovaries contain GVM and GVBD in addition to PG, CA, and Vtg oocytes; hydrated oocytes and POF common
Regressing female	Ovaries relatively large, but appear flaccid, blood vessels prominent. Atresia prominent and POFs present. Some CA and/or Vtg1, Vtg2 oocytes can occur
Regenerating female	Ovarian wall thick; PG; minor signs of atresia; gonad area relatively large in cross section; muscle bundles present in addition to signs of vascularization
Transitioning	Majority of gonad is PG oocytes and/or atretic vitellogenic oocytes. No Vtg 1, Vtg2, Vtg3, GVM, GVBD, or HO. Early spermatogenesis evident with presence of Sg and/or spermatogenic cysts (Sc1/Sc2, no Sz); Neither an active male nor female.
Developing male	Proliferation of Sg. Cysts of spermatocytes may be extensive. Early stages of spermatogenesis (i.e. Sc1/Sc2) prevalent. No pooling of Sz in lumen of lobules or ducts.
Spawning capable male	All stages of spermatogenesis (especially later ones) may be present. Significant pooling of Sz in lumen of lobules and ducts.
Regressing/Regenerating male	Lumen of lobules and ducts may be empty with minimal residual Sz. Proliferation of Sg possible with few/no cysts of spermatocytes.
**Immature male	**Only present in some species; testes area small, only Sg1 present; no lumen in lobules