

## Sex structure and potential female fecundity in a *Epinephelus guttatus* spawning aggregation: applying ultrasonic imaging

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Ultrasonic imaging was used to determine the spawning population structure and develop a fecundity estimation model for a red hind *Epinephelus guttatus* spawning aggregation within the Red Hind Bank Marine Conservation District, St Thomas, U.S.V.I. The spawning population showed considerable within-month and between-month variation in population size- and sex-structure. In the spawning season studied, males appeared to arrive at the aggregation site first in December although females represented a large proportion of the catch early in the aggregation periods in January and February. Spawning occurred in January and February, and size frequency distributions suggested that an influx of small females occurred during the second spawning month. An overall sex ratio of 2.9:1 (female:male) was recorded for the whole reproductive season. The sex ratio, however, differed between months and days within months. More females per male were recorded in January than in February when the sex ratio was male biased. Fecundity estimates for this species predicted very high potential fecundities ( $2.4 \times 10^5$ – $2.4 \times 10^6$  oocytes). The ultrasound model also illustrated a rapid increase in potential female fecundity with total length. Ultrasonic imaging may prove a valuable tool in population assessment for many species and locations in which invasive fishing methods are clearly undesirable.

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Key words: fecundity; protogyny; sex ratio; spawning aggregation; ultrasonography.

### INTRODUCTION

Many commercially important groupers (Serranidae) form large spawning aggregations at specific sites at which spawning is concentrated within 2 months each year *e.g.* red hind *Epinephelus guttatus* (L.) and Nassau grouper *Epinephelus striatus* (Bloch) (Colin *et al.*, 1987), tiger grouper *Mycteroperca tigris* (Valenciennes) (Sadovy *et al.*, 1994a) and coral trout *Plectropomus areolatus* (Rüppell) (Johannes *et al.*, 1999). The concentrated nature and short duration of these spawning aggregations render these species especially susceptible to heavy

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fishing pressure, and many aggregations have disappeared or decreased in size (Olsen & LaPlace, 1978).

In addition, most groupers are protogynous hermaphrodites, in which individuals function first as a female and then change sex to male (Shapiro, 1987). This creates a size-structured stock of smaller females and larger males and may render these species even more susceptible to overfishing (Huntsman & Schaaf, 1994). Fishing mortality may result in the differential loss of larger males and a sex ratio biased towards females, e.g. gag grouper *Mycteroperca microlepis* (Goode & Bean) (McGovern *et al.*, 1998), and a decrease in the average size of both males and females, e.g. *E. guttatus* (Beets & Friedlander, 1992). Such changes in the population structure may dramatically reduce the potential reproductive output of the spawning aggregation through sperm limitation or decreased egg production (Bannerot *et al.*, 1987).

The potential effects of male loss have been modelled for several grouper populations by exploring the effects of exploitation on operation of sex change mechanisms, e.g. graysby *Cephalopholis cruentata* (Lacapède) (Huntsman & Schaaf, 1994) and *Plectropomus leopardus* (Lacapède) (Armsworth, 2001). The ephemeral nature of spawning aggregations, however, has limited collection of empirical evidence. In addition, a decrease in the average size of females may substantially reduce egg production and thus the potential number of new recruits. The relationship between female length and fecundity, however, is typically hard to determine accurately. By necessity, most studies have relied on small numbers of individuals suffering mortality or invasive fishing methods to determine sex ratios and potential fecundities. Since sex ratios may vary markedly during the course of an aggregation period (Sadovy *et al.*, 1994b) and fecundity may change significantly with female size (Sadovy, 1993), more accurate population assessment requires significantly larger sample sizes.

In this study, a new non-invasive method of determining the sex of large numbers of red hind is employed. Ultrasound technology has been successfully used to both sex individuals and develop a model of potential female fecundity in striped bass *Morone saxatilis* (Walbaum) (Blythe *et al.*, 1994; Will *et al.*, 2002). Ultrasound has more commonly been used in aquaculture (Martin-Robichaud & Rommens, 2001), and the striped bass studies are currently the only examples of ultrasound technology applied to a wild fish population.

Red hind is a commercially important species in the U.S. Virgin Islands (USVI) (Cummings *et al.*, 1997). In 1999, following 9 years of seasonal closures, the Red Hind Bank Marine Conservation District (MCD) was established as the first no-take fishery reserve in the USVI. Recent evidence suggests that the closure of the Red Hind Bank has been successful in protecting this spawning subsection of the population. By 1997 the average size of spawning red hind had increased by >6 cm (Beets & Friedlander, 1998). Even more impressively, the number of spawning individuals increased dramatically from 4.5 fish per 100 m<sup>2</sup> in January 1997 to 23 fish per 100 m<sup>2</sup> in January 2001 (Beets & Friedlander, 1998; Nemeth, 2005). Fecundity estimates for this species have been developed using a linear equation relating fish length to potential fecundity (Sadovy, 1993). This relationship was derived from individuals from Puerto Rico, Jamaica, and the Bahamas since the complete size range of females was not available in each individual sample. Life-history characteristics, however, may

vary between populations of the same species independently of fishing pressure (Begg *et al.*, 1999) and may have decreased the accuracy of the estimated potential fecundities.

Understanding the relationships between aggregation protection and changes in reproductive characteristics are critical for evaluating the success of protective measures. The aim of the present study was to use traditional fishing methods combined with ultrasound technology to non-invasively sample large numbers of individuals, to determine the spawning population sex structure and to develop a model for fecundity estimation for the red hind spawning aggregation in the Red Hind Bank MCD, St Thomas, USVI.

## MATERIALS AND METHODS

### FIELD SITE AND STUDY SPECIES

Fieldwork was conducted in the Red Hind MCD located 12 km south of St Thomas, U.S.V.I. This marine reserve contains the largest known red hind spawning aggregation in the region (Beets & Friedlander, 1998; Nemeth, 2005). In 2003, the primary aggregation site extended from 18°12'20" N; 65°0'10" W to 18°12'20" N; 65°06'00" W and covered an area *c.* 0.25 km<sup>2</sup> (Nemeth, 2005). The depth on the aggregation site ranged from 33 to 45 m.

Red hind migrate to the spawning site and form an aggregation 5–7 days prior to the full moon in December, January and February (Shapiro *et al.*, 1993; Nemeth, 2005). Spawning typically occurs within 3 days of the full moon. Most fish rapidly disperse from the spawning site between spawning months and all fish depart from the spawning aggregation within a week following the full moon in February (E.A. Whiteman, pers. obs.).

### SPAWNING POPULATION CHARACTERISTICS

Red hind at the spawning aggregation site were sampled using traditional fishing techniques. Fishing was conducted on 16 and 18 December 2002, 13–18 January 2003 and 11–14 February 2003. Sampling in January and February was scheduled to begin 5 days prior to the full moon in each month to correspond to the arrival of the fish on the aggregation site. Red hind were captured using hook and line and baited Antillean fish traps fished for between 3 and 18 h. Expansion of air within the swimbladders of individuals occurred as the fish were brought to the surface; therefore, a 14 × 1.5" hypodermic needle inserted into the swimbladder next to the vent was used to extract gas from the over-inflated bladders and restore buoyancy to the fish. Each fish was then measured and total length ( $L_T$ ) recorded to the nearest mm, tagged and released. Fish captured using hook and line were larger than those captured in traps but by <1 cm (mean ± s.d.: hook and line, 38.4 ± 4.7 cm  $L_T$  and trap, 37.9 ± 3.7 cm  $L_T$ , Mann–Whitney *U*-test,  $n = 1299$ ,  $P = 0.03$ ). In addition, 88% of fish were captured in traps (1145 of 1299). All fish were therefore pooled together.

A portable ultrasound machine (Pie Medical Scanner) with a 3.5–5.0 Hz linear array probe was used as a non-invasive method to sex individuals captured on the aggregation. In a pilot study carried out on 5 and 8 January 2001, 25 red hind were captured for sex determination. Each fish was scanned with the ultrasound and then the sex confirmed by internal macroscopic examination. The sex of all 25 individuals was accurately predicted based on ultrasonic images.

During December 2003, trials using ultrasound images indicated that sexes could only be reliably determined with this method during the final stages of gonad maturation. Gonad images were not clearly displayed by the ultrasound and macroscopic examination of eight sacrificed fish revealed they had small resting gonads. Fish could therefore

not be reliably sexed in this month. By comparison, males and females were clearly distinguishable during the days immediately preceding spawning in January and February. During these months this method was used to determine the sex of red hind captured during fishing. Each individual was held motionless in a clear vinyl sling positioned over an 80 l tank containing sea water. The ultrasound probe was then positioned perpendicular and parallel to the body of the fish to display cross-sectional and longitudinal images of the gonads on the monitor. Thirty seven fish which suffered mortality in the field on the first day of fishing in January were used to validate sex identification. All individuals were correctly identified as male or female. Results of ultrasound sexing of individuals are therefore presented for January and February.

## FECUNDITY MODEL

A model to estimate female fecundity was constructed using ultrasonic images to estimate ovarian volume and samples of ovarian tissue to determine oocyte density.

### *Ovary volume*

Twenty seven female red hind which suffered mortality through air embolisms in the field on 17 and 18 January and 11–13 February 2003 were transported to the laboratory. Each female was scanned and the length of one ovary in the fish was estimated by using the ultrasound probe to locate the anterior point of inception of the ovary and then using a flexible ruler to measure from the probe to the vent along the ventral surface of the fish. In addition, four cross-sectional images of the ovary, recorded at uniform intervals from the vent to the anterior end, were recorded and saved with a digital camera. Maximum and mean cross sectional ovary areas were subsequently calculated using image analysis software (Image Pro from Media Cybernetics). These fish were then dissected, and total ovary volume (ml) was recorded by calculating the volume of water displaced when the whole ovary was added to a graduated cylinder filled with water.

A step-wise multiple regression analysis was used to determine which of the three variables (mean cross sectional ovary area, maximum cross-sectional ovary area and ovary length) or combination of variables could be used to best predict total ovary volume. Variables were retained in the model if they met an  $\alpha = 0.15$  significance level. A *t*-test was then used to compare measured ovary volume with model predictions of ovary volume. Measured ovary volume was significantly related to  $L_T$  ( $r = 0.70$ ,  $n = 27$ ,  $P < 0.001$ ). Fish were categorized into three size classes (28.0–32.9, 33.0–36.9 and 37.0–41.9 cm) and the model fit also tested for each size class separately. Data met all the assumptions of parametric testing.

### *Oocyte density*

A 0.1 ml sample of ovarian tissue was extracted from 24 of the fish used for ovarian ultrasonic imaging using a catheter and preserved in 10% formalin. The total number of eggs in each sample was counted using a dissection microscope. In this species, development of ovarian tissue is homogenous throughout the length of the ovary and between ovaries (Shapiro *et al.*, 1993). All cannular samples were taken from fish in the final stage of oocyte maturation prior to hydration and spawning.

### *Fecundity estimation*

Fecundity estimates were generated by integrating predicted ovary volumes with oocyte densities from cannular samples. Red hind may spawn during more than 1 month in a single spawning season (Sadovy *et al.*, 1994b; E.A. Whiteman, pers. obs.), however, all vitellogenic oocytes that have recruited into the ovary at the beginning of the spawning season are likely to be spawned in the ensuing season (Sadovy *et al.*, 1994b). Estimated fecundities are therefore assumed to represent the annual potential fecundity. Predicted fecundities were then compared across size classes and to previously published estimates for this species (Table I).

TABLE I. Fecundity estimates of *Epinephelus guttatus*. Since fecundity estimates are published in different formats, where possible these were applied to females of two sizes (31.0 and 41.0 cm total length) for direct comparisons. Applied estimates are taken from published numbers where available or by integrating ovary masses recorded in this study with published oocyte densities

Location	<i>n</i>	Fecundity estimate ( <i>F</i> )	Applied estimate for $L_T$ of		References
			31.0 cm	41.0 cm	
U.S. Virgin Islands	27	$F = 136628L_T$ (cm) - 4035628	199 840	1 566 120	This study
Puerto Rico, Jamaica and Bermuda	40	$\text{Ln } F = 9.201 + 0.0107 \text{Ln } L_F$ (mm)	10 534	10 565	Sadovy (1993)
U.S. Virgin Islands	73	3.70 oocytes per mg ovary	127 724	353 091	Olsen & LaPlace (1978)
Jamaica	6	305-740 eggs per g body mass	139 070	379 350	Thompson & Munro (1978)
Bermuda	-	90 000-3 365 000	-	-	Burnett-Herkes (1975)
	1	233 237	-	-	Smith (1961)

$L_F$ , fork length.

## RESULTS

### SPAWNING POPULATION CHARACTERISTICS

A total of 1383 fish were caught and measured during December 2002, January and February 2003. Evidence of spawning was not recorded in December. By comparison, in January and February, females were observed with visibly distended abdomens 3 days prior to the full moon and flaccid spent ovaries the following day. The primary spawning event is therefore estimated to have occurred 3 days prior to the full moon in both months (16 January and 14 February). Gravid females, however, were observed after this date in both months (E.A. Whiteman, pers. obs.).

Ultrasound images were used to sex 820 of the fish captured in January and February 2003. Females were identified on the basis of visible ovaries which appeared as oval-shaped structures lying along the ventral surface of the fish with a homogenous granular internal appearance [Fig. 1(a), (c)]. By comparison, the testes of males were typically smaller and parallel to, but not touching, the ventral surface of the fish [Fig. 1(b), (d)]. Males could be identified best by the heterogeneous internal structure of the testis visible in a longitudinal ultrasound image [Fig. 1(d)].

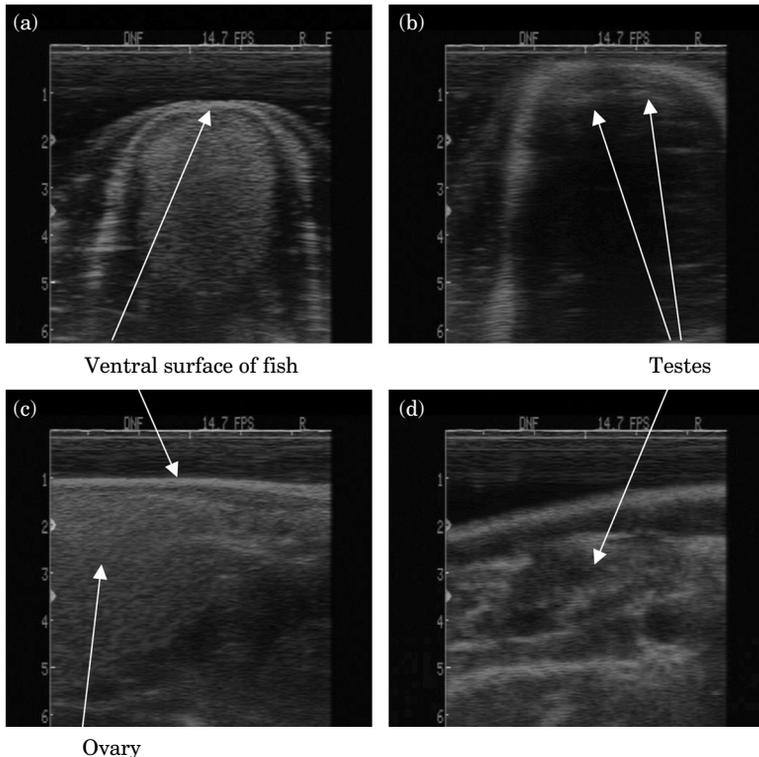


FIG. 1. Cross-sectional and longitudinal images of red hind ovaries (a), (c) and testis (b), (d) as displayed by ultrasound imaging.

Across the entire reproductive period, males were significantly longer than females (mean  $\pm$  s.d.: males,  $42.0 \pm 3.2$  cm and females,  $36.7 \pm 3.3$  cm; *t*-test, d.f. = 818,  $P < 0.001$ ). In addition, the average  $L_T$  of males was significantly higher in February than January (mean  $\pm$  s.d.: January,  $40.8 \pm 3.2$  cm and February,  $43.1 \pm 2.7$  cm; *t*-test, d.f. = 207,  $P < 0.001$ ), while the reverse was true for females (January,  $37.0 \pm 3.0$  cm and February:  $35.0 \pm 4.3$  cm; *t*-test, d.f. = 609,  $P < 0.001$ ). Size frequency distributions for each sex across the 2 months illustrated a decline in overall frequencies of females but also an increase in numbers of females  $< 30.0$  cm  $L_T$  between January and February [Fig. 2(b), (c)]. By comparison, there were more males  $> 42.0$  cm  $L_T$  during February than January [Fig. 2(d), (e)]. Comparison of the size frequency

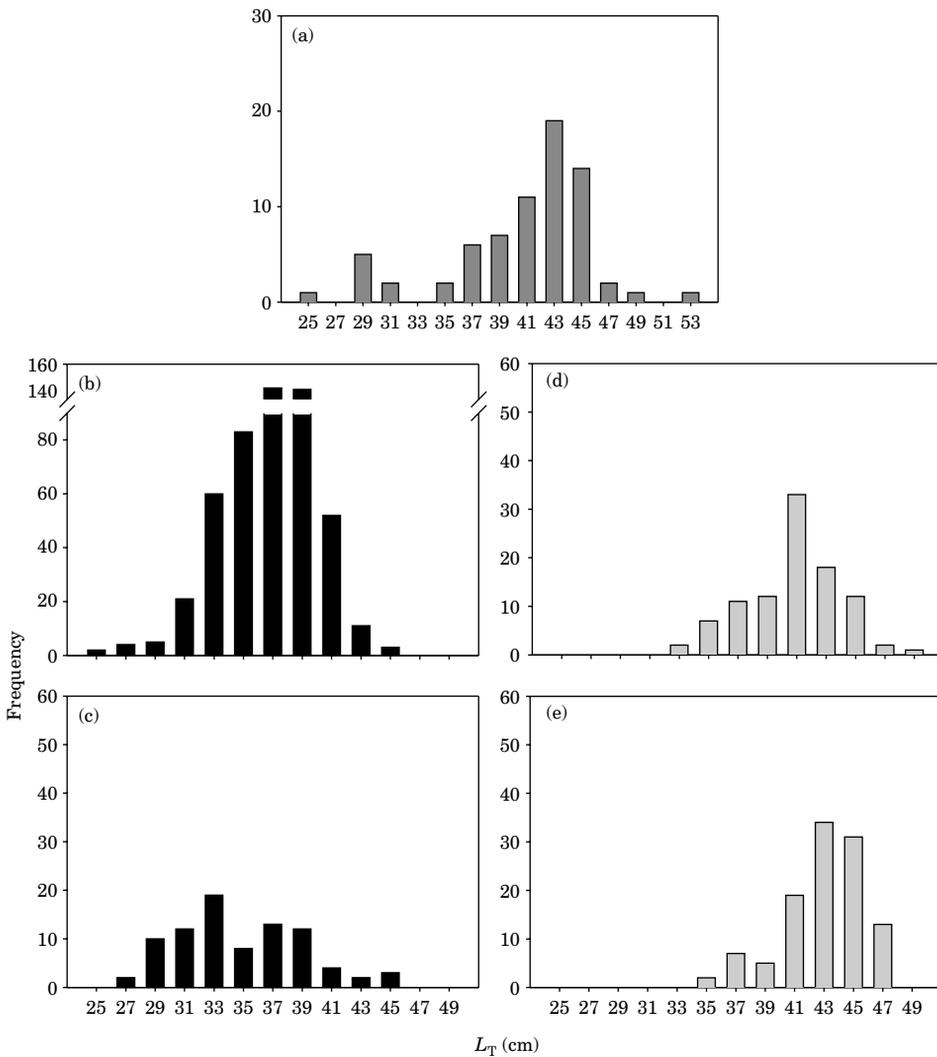


FIG. 2. Size frequency distributions for (a) all individuals during December 2002, females during (b) January and (c) February 2003, and males during (d) January and (e) February 2003.

distribution of all fish captured in December with the data from January and February indicated that many of these fish were probably males [Fig. 2(a)].

An overall sex ratio of 2.9:1 (female:male) was recorded (611 females:209 males) for the whole reproductive season. The sex ratio, however, differed between months and days within months. More females per male were recorded in January than in February when the sex ratio was male biased (Table II). Females represented a greater proportion of the catch each day early in the aggregation periods in both months. In January the number of females per male declined following spawning but did not change in February (Table II).

## FECUNDITY MODEL

### Ovary volume

Twenty seven female red hind ranging from 28.7 to 41.4 cm  $L_T$  were used to develop the model of ovary volume. Step-wise regression analysis revealed that ovary volume could be best predicted using two variables: average ovary cross-sectional area ( $A_{av}$ ) and total ovary length ( $L$ ) (ANOVA,  $F_{2,24}$ ,  $P < 0.001$ ). Maximum cross-sectional area did not meet the 0.15 significance level. Predicted ovary volume ( $V$ ) therefore resulted in the following model:  $V = 3.805A_{av} + 15.44L - 79.716$  ( $r^2 = 0.845$ ,  $A_{av}$ :  $b = 0.65$ ;  $L$ :  $b = 0.35$ , where  $b =$  standard, second regression coefficients that indicate the relative importance of each variable in predicting ovary volume). Predicted ovary volumes did not significantly differ from measured ovary volumes when all fish are considered together (Fig. 3; paired- $t$ -test, d.f. = 26,  $P > 0.99$ ) and for each size class separately (paired- $t$ -tests,  $P > 0.05$  in all cases). There were, however, significant differences between size classes in both measured and predicted ovary volumes (ANOVA, measured: d.f. = 2, 25,  $P < 0.01$ ; predicted: d.f. = 2, 25,  $P < 0.01$ ). Ovary volumes were significantly higher in fish  $> 38$ cm than those  $< 38$ cm (Tukey HSD,  $P < 0.05$ ).

TABLE II. Sex ratio and the proportion of females each day captured from the *Epinephelus guttatus* spawning aggregation, St Thomas, USVI, in the 5 days preceding the full moon in January and February 2003. The dotted line indicates the timing of spawning. Data collection ended 2 days before the full moon in February

Days before full moon	January			February		
	Sex ratio (F:M)	Per cent females	$n$	Sex ratio (F:M)	Per cent females	$n$
-5	11.3:1.0	91.9	37	1.5:1.0	59.4	32
-4	14.4:1.0	93.5	200	0.9:1.0	48.8	41
-3	2.7:1.0	72.7	33	0.4:1.0	28.6	63
-2	4.5:1.0	81.9	11	0.9:1.0	46.7	60
-1	4.1:1.0	80.2	197	—	—	—
0	3.6:1.0	78.1	146	—	—	—
All	5.4:1.0	84.3	624	0.8:1	43.4	196

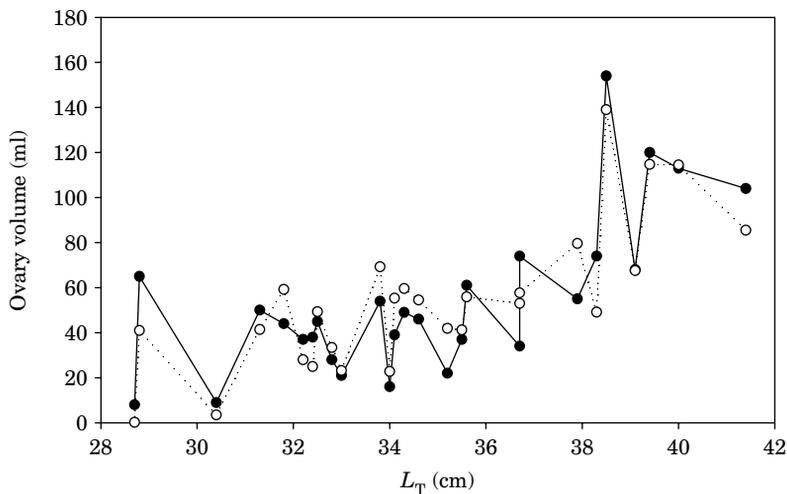


FIG. 3. Measured (—●—) and model predicted (· · ·○· · ·) ovary volumes for 27 red hind collected from the Red Hind Bank MCD in January and February 2003.

#### *Oocyte density*

Cannular samples were taken from 12 females over 2 days in each of January and February. Oocyte densities did not significantly differ between days before the full moon (*t*-tests,  $P > 0.05$  in both months). Across both months, oocyte density increased with  $L_T$  ( $r = 0.57$ ,  $n = 24$ ,  $P = 0.004$ ). In January, oocyte densities were significantly higher than expected given the  $L_T$  of the fish (one-sample *t*-test on standardized residuals, d.f. = 11,  $P = 0.01$ ). Oocyte densities in February, however, were not significantly different from predicted values based on the relationship between  $L_T$  and oocyte densities (one-sample *t*-test on standardized residuals, d.f. = 11,  $P = 0.2$ ).

#### *Fecundity estimation and model application*

Estimates of fecundity were calculated using mean oocyte densities recorded for each size class of fish. The average potential fecundity for a female within the spawning aggregation was 978 620 oocytes (based on an average female size of 36.7 cm  $L_T$ ). Fecundity estimates for the red hind, however, differed significantly between size classes (ANOVA, d.f. = 2, 24,  $P < 0.001$ ; Fig 4). Fish  $> 37.0$  cm had significantly higher potential fecundities than those  $< 37.0$  cm (Tukey HSD,  $P < 0.05$ ). This model based on ultrasound-derived data and oocyte densities predicted fecundities  $> 10$  times higher in fish 26–30 cm and 100 times higher in fish  $> 40$  cm than those developed from the length-fecundity relationship of Sadovy (1993) (Table I). These results therefore also predict a significantly higher rate of fecundity increase with fork length (comparison of regression coefficients, d.f. = 50,  $P < 0.001$ ).

## DISCUSSION

The structure of the red hind spawning aggregation varied both within and between months. Males appeared to arrive at the aggregation site first in

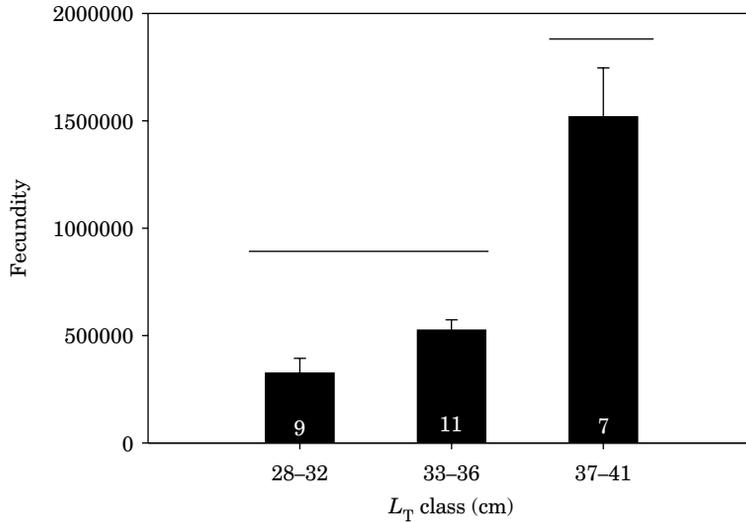


FIG. 4. Estimated fecundity of 27 red hind across three size classes. Sample sizes are given inside each bar. Lines connecting bars indicate size classes that were not significantly different from each other (Tukey HSD,  $P < 0.05$ ).

December although females represented a large proportion of the catch early in the aggregation periods in January and February. Spawning occurred in January and February but size frequency distributions suggested turnover of individuals between months. An influx of small females occurred during the second spawning month and the average size of males also increased from January to February, although this may have resulted from both arrival of large males and departure from the aggregation of smaller males. Sex ratios were biased towards females in January but more males were captured in February. Fecundity estimates for this species predicted very high potential fecundities. Even more importantly, the ultrasound model illustrated a rapid increase in potential female fecundity with  $L_T$ .

Variation in individual sizes and sex ratios within and between spawning aggregation periods appears to characterize spawning aggregations for a wide range of grouper populations. Males have been observed to arrive first at aggregation sites in *Epinephelus polyphkadion* (Bleeker) (Rhodes & Sadovy, 2002), *P. areolatus* (Johannes, 1989) and *P. leopardus* (Samoilys, 1997), and the results suggest that the same pattern is repeated in red hind. The data also suggest that turnover of females may occur within a spawning season. In general, competition among females in a harem may limit spawning opportunities for small females, e.g. angelfish *Centropyge potteri* (Jordan & Metz) (Lutnesky & Kosaki, 1995). A similar effect may operate on a larger, aggregation scale and may explain the shift towards smaller females observed during the second spawning month for red hind. The reduction in average oocyte densities from January to February, however, may also indicate that some females spawn during both months. The extent to which these patterns are repeated annually unfortunately cannot be determined from the data. The results, however, are

consistent with previous anecdotal observations, which suggest turnover, particularly of females, within a spawning season (E.A. Whiteman & R.S. Nemeth, pers. obs.). Indeed, some variation between years may help to explain the apparent male bias to the spawning population observed during February.

Such dynamics within aggregations have rarely been elucidated but may have a considerable effect on the total reproductive output from the aggregation in a single year. Moreover, changes in the internal structure of the aggregation highlight the importance of re-examining spawning aggregations at the same time, season, lunar phase and even daily times as previous observations. Frequently grouper aggregation studies present a single aggregation sex ratio and yet the timing of observations can have drastic effects on the data obtained. Results from this study indicate that red hind undergo a three-fold change in the aggregation sex ratio within a single month.

The model developed using ultrasound images to predict potential fecundities resulted in values that were significantly greater than previously estimated with a length-fecundity relationship for this species (Sadovy, 1993). Oocyte development within ovaries is homogenous through the length of the ovary, however, the presence of oocytes at different stages of development in individual females may have biased the volumes of eggs recorded from cannular samples. Yet, the derived fecundity estimates fall within the ranges of other published estimates for this species (Table I) and are consistent with estimates published for related species, e.g. *E. polyphkadion* (Tamaru *et al.*, 1996; Rhodes & Sadovy, 2002).

It is possible that life-history characteristics of fish from different geographical areas vary as a result of fishing pressure or natural population differences. Indeed, fish from Puerto Rico, although only slightly smaller, had significantly lower calculated fecundities than those from Bermuda and Jamaica (Sadovy, 1993). Using otolith ageing techniques Sadovy (1993) also demonstrated that equivalently aged fish were >20 mm longer in St Thomas than Puerto Rico. Whether these growth differences reflect environmental differences or stock differences is unknown. Finally, comparisons between the ultrasound model in this study and the previous fecundity estimates for red hind in Puerto Rico also suggest that the potential fecundity for a female from Puerto Rico is less than an equivalently sized female from the St Thomas aggregation. Whether these differences are the result of natural population differences and fishing pressure is also unclear. Nonetheless, the lower potential fecundities in fish from Puerto Rico may further exacerbate the population level consequences of exploiting these spawning aggregations.

Understanding how high potential fecundities recorded from red hind in the U.S. Virgin Islands may be translated into an increased reproductive output from the spawning aggregation necessarily requires observations of spawning behaviour and assessments of the number of females that are able to spawn successfully. Even without such observations, however, it is evident that protection of the spawning aggregation and an increase in female size could have significant positive effects on stock recruitment. Not only did the ultrasound fecundity model predict high absolute fecundities but also a very rapid increase in fecundity with fish size. Protection of red hind within the MCD in St Thomas has resulted in an increase in the average size of females by 3 cm over 4 years between 1997 and 2001 (R.S. Nemeth, pers. obs.). These changes in individual

size may increase stock recruitment through increased larval production. Tracking larvae from the red hind aggregation and assessing recruitment into juvenile habitats can link spawning aggregation changes with local stock abundances and will determine the ultimate success of this marine protected area.

Finally, ultrasound technology has helped to clarify the effects of protection on the reproductive characteristics of red hind at a spawning aggregation. Many grouper and snapper aggregations are threatened or declining (Domeier *et al.*, 2002). Population size and sex structures as well as potential fecundities provide managers with valuable information for informed management decisions. Ultrasonic imaging may prove a valuable tool in population assessment for many species and locations in which invasive fishing methods are clearly undesirable.

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