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Lunar and seasonal patterns in fecundity of an indeterminate, multiple-spawning surgeonfish, the yellow tang *Zebrasoma flavescens*

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Reproduction was investigated in relation to lunar and annual cycles in a population of yellow tang *Zebrasoma flavescens*, a popular aquarium species commercially harvested in Hawaii. Lunar periodicity was determined to be an inherent characteristic of reproduction; peaks in mean daily egg production, female gonado-somatic index (I_G) and the fraction of females with eggs were observed at the full moon of each sampled month. An increase in the fraction of late-stage vitellogenic oocytes within the ovaries was also observed at the full moon. Reproductive effort peaked in the late spring and summer as indicated by high values of mean daily egg production, female I_G and the recorded incidence of females spawning for at least two consecutive days. Mean daily egg production and I_G of monthly samples were lowest in November to February, although some level of egg production continued throughout the year. Large individual variation in batch fecundity was observed, with a range from 44 to >24 000 eggs per female produced on a single sampling date. Smaller females, 80–120 mm standard length (L_S), produced limited numbers of eggs, while females ≥120 mm L_S were capable of maximal egg production (>20 000 eggs per batch). In contrast to trends observed in many fish species, no significant relationship between batch fecundity and adult L_S > 120 mm was observed in female *Z. flavescens*. An estimate of annual fecundity (mean ± s.e. 1 055 628 ± 120 596 eggs) was also generated using a simple model of the lunar variability in egg production. This study illustrates the importance of accounting for potential variation in egg production over time, especially with respect to diel and lunar cycles, in the design of reproductive studies of multiple-spawning fishes. Greater insight into the environmental factors that regulate reproductive activity may be gained by determining the relative reproductive investment allocated at each spawning event. The ability to estimate annual fecundity for more multiple-spawning species will facilitate examination of the effects of fishing on the reproductive characteristics of these populations and permit examination of life-history evolution across a broader suite of fishes.

Key words: Acanthuridae; annual and batch fecundity; coral-reef fish; lunar cycle; oocyte maturation; reproduction.

INTRODUCTION

Estimating seasonal or annual fecundity for indeterminate, multiple-spawning fishes is more complicated than making such estimates for determinate, single-spawning

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species with synchronous ovarian development (Hunter *et al*., 1992; Murua *et al*., 2003). The ovaries of determinate-spawning species contain a fixed number of oocytes that remains essentially unchanged throughout the breeding season up until the time of spawning (de Vlaming, 1983). This standing stock of oocytes matures simultaneously, so annual fecundity can be assessed at any point in the life cycle of the fish before spawning by simply counting all oocytes within the ovary, after effects of atresia have been accounted for (McEvoy & McEvoy, 1992; Murua *et al*., 2003). Since most species of coral-reef fishes are multiple-spawners, however, few reliable estimates of annual fecundity are available for these species (Sadovy, 1996).

In the ovaries of indeterminate, multiple-spawning fishes with asynchronous oocyte development, such as the present focal species, *Zebrasoma flavescens* Bennet, oocytes are recruited continually from primary stages. Oocyte batches mature at different rates throughout the season, with the female releasing the most mature batch at each spawning (Wallace & Selman, 1981; Murua *et al*., 2003; Bushnell, 2007). As a result, a count of the standing stock of oocytes from the ovary of an individual indeterminate-spawning fish gives little or no information about the seasonal or annual fecundity of that individual. Batch-fecundity can be determined by counting mature, hydrated oocytes (*i.e.* those ready to be spawned) from a fish that has been sampled just before the next spawning. These batch fecundity measurements can then be used to produce an estimate of annual fecundity when combined with a thorough understanding of the patterns in their variability (*e.g.* lunar and seasonal cycles). Annual fecundity is an important metric that has been used by fisheries scientists to examine intraspecific geographic variability between populations of interest (Hunter *et al*., 1989) and used more broadly to examine life-history evolution among fishes (Rochet, 2000).

Zebrasoma flavescens (Acanthuridae) is an herbivorous, gonochoristic, tropical reef fish, which makes up *c*. 80% of the fishes caught along the west coast of Hawaii Island for the aquarium trade. The total catch of *Z. flavescens* has recently approached half a million individuals per year (Williams *et al*., 2009) as the fishery has expanded greatly over the past two decades. This expansion led to increased conflict among stakeholders in the late 1990s; in response, the State of Hawaii created a network of marine protected areas (MPA) in 1999 to manage this fishery. To date, the MPA management strategy has been viewed as a success based on increases in fish populations coinciding with stable or increased yield to the fishery (Tissot *et al*., 2004; Williams *et al*., 2009). Very little is known, however, about the reproductive output of this valuable species. Accurate information regarding reproductive potential, which is a key to modelling MPA effectiveness (Gerber *et al*., 2003), will allow managers to understand how increases in fish abundance translate into increased reproductive output for MPA within this newly formed network. Furthermore, information concerning the reproductive patterns (*e.g*. lunar and seasonal) of female *Z. flavescens* can be useful for implementing additional management strategies, such as size restrictions, and will be particularly valuable for ongoing aquaculture programmes that aim to produce this species for commercial sale.

Lunar periodicity among repetitive spawning species has been established for many marine tropical fish species (Lobel, 1978; May *et al*., 1979; Robertson *et al*., 1990; Mizushima *et al*., 2000; Soyano *et al*., 2003; Takemura *et al*., 2004; Vagelli & Volpedo, 2004) and has been suggested for some acanthurids (Randall, 1961*a*, *b*; Thresher, 1984; Colin & Clavijo, 1988). Randall (1961*a*) observed a greater number

of ripe female *Acanthurus triostegus* (L.) around the full moon among populations in Oahu, similar to patterns observed for female *Zebrasoma scopas* (Cuvier) in the Society Islands (Randall, 1961*b*), and Colin & Clavijo (1988) observed peak spawning of *Acanthurus coerulus* Bloch & Schneider between 3 and 8 days after the full moon in Puerto Rico. Preliminary data from field sampling of *Z. flavescens* in 2005 (Claisse & Bushnell, unpubl. data) and from fish held in captivity (Laidley, unpubl. data) suggested that a lunar cycle of reproductive activity might exist for *Z. flavescens* in Hawaii as well, with increased egg production around the full moons.

The few specific studies of reproductive seasonality within the Acanthuridae have shown substantial differences in annual spawning patterns among species. *Acanthurus nigrofuscus* (Forsskål), found on coral reefs all over the world and abundant in Hawaii, exhibits a clear demarcation between on and off-season spawning months in the Red Sea (Fishelson *et al*., 1987). Randall (1961*a*) suggested that *A. triostegus* in Hawaii has a distinct spawning season, based on trends in the gonado-somatic index (I_G) of males and females sampled over 1 year. In contrast, *A. triostegus*, *Acanthurus guttatus* Forster and *Acanthurus lineatus* (L.) were found to spawn year-round in American Samoa, with periods of more intense reproductive effort in the austral summer months (Craig, 1998). Based on observations of adult spawning behaviour (Lobel, 1989) and observations of recently settled individuals throughout the year (Walsh, 1987; J. T. Claisse & M. E. Bushnell, pers. obs.), it is likely that *Z. flavescens* in Hawaii reproduce continually as well. Captive fish harvested from Oahu and Hawaii Island have been found to spawn during all months of the year (Laidley, unpubl. data).

The main goal of this study was to investigate the temporal variability in egg production in a *Z. flavescens* population located on the west coast of Hawaii Island. Specific objectives addressed in the study were to: (1) confirm the presence or absence of lunar periodicity of reproduction, and if observed, quantify changes in both daily and seasonal egg production; and (2) produce an estimate of annual fecundity of this important coral-reef fishery species, incorporating daily and monthly fluctuations in egg production.

MATERIALS AND METHODS

SAMPLING

Sampling took place on the west coast of Hawaii Island (19° 42′ N; 156° 03′ W). Collections were made by scuba diving using pole spears, with a target goal of at least 20 female fish per sampling occasion, although environmental conditions sometimes led to smaller sample sizes. Fish were collected in adult daytime habitat, 3–10 m in depth along the outer drop-off of the shallow pavement zone (Walsh, 1984; Claisse, 2009). The specific dates of collection are presented in Table I.

Zebrasoma flavescens spawning behaviours have been observed in the wild occurring within a distinct window of time, inclusive of the 1 h before sunset in Hawaii (Walsh, 1984) and between 1600 and 1800 hours at Johnston Atoll (Sancho *et al*., 2000). In addition, recent histological investigation of ovaries and oocyte maturation has offered further support for evening spawning of Hawaiian populations (Bushnell, 2007). Therefore, all sampling dives were conducted between 1 and 2 h before sunset to ensure that egg release had not yet occurred, and that any oocytes to be spawned that day had either already ovulated [remaining within the ovarian lumen (Fig. 1)] or had at least reached the final hydration stage. This also

TABLE I. Sample sizes of adult *Zebrasoma flavescens* group M (those individuals collected within 1 day of the full moon each month) and group S (those individuals collected during the 65 day summer sampling period) fish

Month	*N* (month)	Day of sampling period (summer 2006)	*N* (day)	*N* (histo)
Group M fish		Group S fish		
May 2006	12	0	6	—
June 2006	16	4	12	5
July 2006	7	6	17	—
August 2006	—	9	15	5
September 2006	17	13	11	5
October 2006	9	18	11	—
November 2006	14	24	14	—
December 2006	17	28	11	5
January 2007	19	31	10	—
February 2007	21	33	16	5
March 2007	18	35	16	5
April 2007	19	38	14	—
		42	11	5
		48	11	5
		51	16	—
		58	16	5
		61	11	5
		63	7	—

N (month), number of female fish caught in the corresponding monthly collection; *N* (day), number of females caught on the corresponding day of the summer sampling period; *N* (histo), number of females examined histologically for vitellogenic oocyte–size-frequency distributions in Fig. 5. Cells shaded in grey, the same sample of fish used in both groups.

standardized the effect of egg hydration on ovary mass, since a positive relationship between time of day and ovary mass had been noted previously (Bushnell, 2007).

The sex of *Z. flavescens* cannot be determined visually from a distance during underwater collection; however, a consistent difference in the genital openings of male and female *Z. flavescens* was noted early in the study. Twenty-three adult fish collected in May 2006 were examined post-collection, before dissection, and were identified as having either a female or a male genital opening (Fig. 2). Subsequent dissection and macroscopic examination of the gonads established a 100% correlation between the initial visual identification of sex and the actual sex of the individuals in this collection and in all subsequent collections of fish in which this protocol was followed.

Intensive sampling (*i.e. c.* every 4 days) was performed during the period of the year that had been hypothesized to be the peak of the spawning season, based on previous studies in Kona (Walsh, 1987; Lobel, 1989), as well as earlier research on Oahu (Laidley, unpubl. data). For a 65 day period lasting from 8 May to 10 July 2006, sampling occurred *c.* every 4 days for a total of 18 collections over the summer months (group S fish collections; Table I).

Monthly sampling (group M fish collections) continued for the remainder of the calendar year (May 2006 to April 2007) within 1 day of the full moon for every month except August (Table I). Data from the 65 day summer sampling period were applied to the remainder of the year-round sampling effort in order to maximize sampling of reproductively active adults. No collection was made in August 2006 due to weather conditions. In addition to group M and S fish collections, 10 smaller female fish (72–96 mm standard length, L_S) were collected from the primarily juvenile habitat in deeper water (10–20 m; Walsh, 1984; Claisse, 2009) in April 2007.

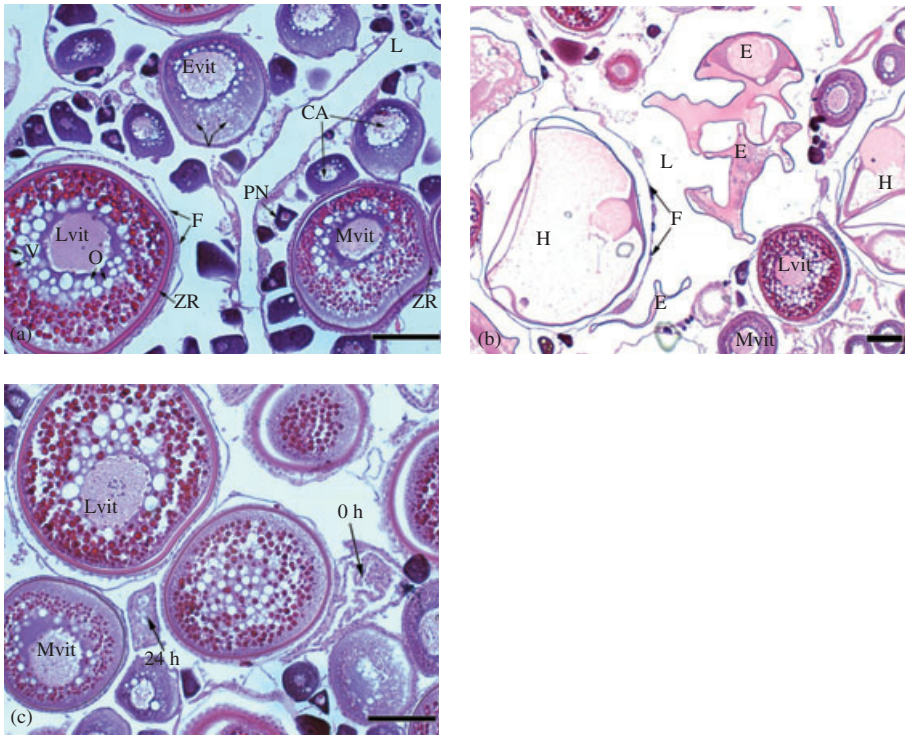

FIG. 1. Asynchronous oocyte development in *Zebrasoma flavescens* ovaries. (a) The three stages of vitellogenic oocytes. Early vitellogenic stage oocytes (Evit) were distinguished by the first observation of vitellin (V) particles. Middle vitellognic stage oocytes (Mvit) were characterized by larger and more numerous vitellin particles, larger oil droplets (O), a wider zona radiata (ZR) and overall increase in cell diameter. Late vitellognic stage oocytes (Lvit) were characterized by an enlarged ZR width, larger and more numerous vitellin particles and oil droplets and larger oocyte diameter. L, Ovarian lumen; PN, perinucleolar stage oocyte; F, follicle; CA, cortical alveolar stage oocyte. A more detailed treatment and specific definitions of ovarian stages in this species can be found in Bushnell (2007). (b) Hydrating oocytes (H) still within the follicle in the same ovary as ovulated eggs (E) in the ovarian lumen. Any hydrated oocytes within an ovary were assumed to be part of the same spawning batch as the ovulated eggs. (c) A 0 h POF (0 h) and a 24 h POF (24 h) within the same ovary. All fish were sampled within 1·5 h before evening spawning. Scale bars = 100 μm.

After spearing, fish were euthanized by decapitation in accordance with animal use protocols of the University of Hawaii (Protocol No. 03-54) and immediately placed on ice until processing. Within 12 h of collection, fish were weighed (g), sexed and measured (mm) for L_S, and all gonads were removed in their entirety from the body cavity. Gonads were blotted dry, weighed and placed either on ice for daily egg production counts or into Dietrich's fixative (Gray, 1954) for histological processing. I_G was calculated for each individual following Crim & Glebe (1990) and condition factor following Froese (2006).

HISTOLOGICAL PROCESSING

Histological processing was conducted on one lobe of the ovary from every sampled fish noted in Table I. Ovaries processed for histology were kept for at least 1 month in Dietrich's fixative and then embedded in paraffin wax and sectioned at 6 μm. Paraffin-wax samples were

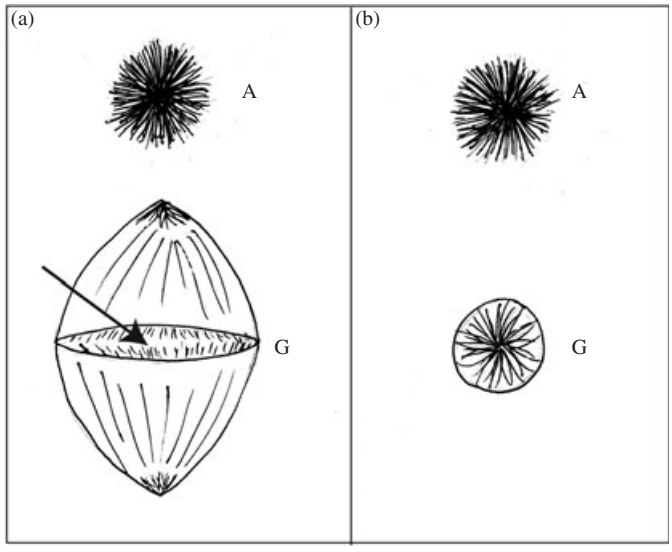

FIG. 2. Diagrams of adult (a) female and (b) male genital openings of *Zebrasoma flavescens* drawn to the same scale. The anus (A) is anterior to the genital (G) opening in both sexes. The arrow denotes the opening (slit) from which eggs are released. Sperm are released from the centre of the male genital opening.

stained with commercially obtained Harris haematoxylin and eosin-Y according to standard protocols.

Ovaries were analysed histologically for one of two reasons: first, to acquire a more complete understanding of the oocyte maturation process and timing over the lunar cycle (individual group S fish listed in Table II), and second, to note any histological evidence of consecutive-day spawning (group M fish).

To explore the timing of vitellogenic maturation over the monthly cycle, ovaries of five randomly selected individual females from 11 of the 18 summer sampling days of group S fish (Table II) were analysed in the following way: individual diameters of all vitellogenic oocytes sliced through the nucleus were measured from a cross-section of each ovary using QCapturePro software (available from Q Imaging; www.qimaging.com). For each individual fish, a size-frequency distribution based on the diameter of oocytes in each of the three vitellogenic stages [early, middle and late, as defined by Bushnell (2007); Fig. 1] was calculated. In this way, the fraction of vitellogenic oocytes in each of the three vitellogenic stages was determined for each individual female (Table II).

The fraction of the female population spawning for at least two consecutive days was determined by histological analysis of gonads from nine to 12 females sampled at the full moon of each month (group M fish collections). The presence of 24 h old postovulatory follicles (POF) in addition to hydrated oocytes within an ovary was taken as an indication that the individual had spawned the day before sampling and was prepared to spawn the day of sampling (Fig. 1). For a more detailed account of these methods, see Bushnell (2007). In brief, female *Z. flavescens* harvested from the wild before spawning were held captive for 48 h. Starting at time 0 (spawning), three or four females were sampled at each 6 h interval, processed as described previously and the ovaries retained for histological processing. The age of POF was determined by describing cell degeneration of POF at each of the eight time intervals using traditional histological techniques.

ESTIMATES OF DAILY EGG PRODUCTION

Batch fecundity was defined as the total number of ovulated eggs and hydrated oocytes within an ovary because female *Z. flavescens* sampled within 1 to 1·5 h of spawning were

TABLE II. The fraction of oocytes within the early, middle and late vitellogenic stage in female *Zebrasoma flavescens* sampled over the summer (group S fish from Table I). The ovaries of five individuals were analysed for 11 of the 18 sampled days. The mean percentage of oocytes within the late vitellogenic stage over the summer is illustrated in Fig. 5 (values of shaded cells; $P < 0{\cdot}001$)

Day of summer sampling period	*N* (oocytes measured)	Fraction of vitellogenic oocytes in each stage			Individual fish identification
		Early	Middle	Late	
0	255	0·169	0·424	0·408	ZF516
0	150	0·248	0·269	0·483	ZF520
0	189	0·291	0·412	0·296	ZF532
0	199	0·197	0·421	0·382	ZF533
0	152	0·069	0·423	0·508	ZF527
Day 0 mean		**0·195**	**0·390**	**0·415**	
4	61	0·115	0·393	0·492	ZF568
4	160	0·081	0·525	0·394	ZF569
4	86	0·045	0·489	0·443	ZF571
4	136	0·037	0·404	0·559	ZF572
4	137	0·007	0·423	0·569	ZF575
Day 4 mean		**0·057**	**0·447**	**0·491**	
9	77	0·291	0·468	0·215	ZF582
9	223	0·287	0·430	0·283	ZF586
9	99	0·303	0·424	0·273	ZF588
9	51	0·226	0·415	0·321	ZF595
9	134	0·193	0·444	0·348	ZF597
Day 9 mean		**0·265**	**0·442**	**0·293**	
14	196	0·296	0·439	0·265	ZF599
14	327	0·239	0·367	0·394	ZF607
14	150	0·247	0·387	0·367	ZF609
14	106	0·302	0·264	0·434	ZF613
14	166	0·271	0·398	0·331	ZF615
Day 14 mean		**0·271**	**0·371**	**0·358**	
28	138	0·210	0·326	0·464	ZF649
28	302	0·195	0·440	0·364	ZF650
28	132	0·235	0·318	0·447	ZF656
28	194	0·182	0·370	0·448	ZF657
28	102	0·343	0·441	0·216	ZF658
Day 28 mean		**0·233**	**0·379**	**0·388**	
33	102	0·324	0·333	0·343	ZF674
33	219	0·174	0·297	0·530	ZF677
33	217	0·249	0·378	0·373	ZF680
33	159	0·245	0·245	0·509	ZF681
33	144	0·271	0·313	0·417	ZF687
Day 33 mean		**0·252**	**0·313**	**0·434**	
35	43	0·05	0·37	0·58	ZF760
35	166	0·07	0·40	0·53	ZF765
35	125	0·06	0·35	0·58	ZF770

TABLE II. Continued

Day of summer sampling period	*N* (oocytes measured)	Fraction of vitellogenic oocytes in each stage			Individual fish identification
		Early	Middle	Late	
35	135	0·04	0·32	0·64	ZF771
35	152	0·08	0·30	0·63	ZF774
Day 35 mean		**0·060**	**0·347**	**0·593**	
42	78	0·308	0·372	0·321	ZF800
42	56	0·161	0·482	0·357	ZF804
42	134	0·172	0·537	0·291	ZF805
42	180	0·383	0·367	0·250	ZF807
42	95	0·463	0·295	0·242	ZF809
Day 42 mean		**0·297**	**0·411**	**0·292**	
48	68	0·206	0·603	0·191	ZF820
48	110	0·182	0·418	0·400	ZF821
48	120	0·267	0·433	0·300	ZF822
48	114	0·377	0·500	0·123	ZF829
48	86	0·256	0·372	0·372	ZF830
Day 48 mean		**0·257**	**0·465**	**0·277**	
58	45	0·267	0·378	0·356	ZF862
58	59	0·136	0·458	0·407	ZF864
58	57	0·193	0·474	0·333	ZF865
58	31	0·452	0·323	0·226	ZF867
58	46	0·174	0·239	0·587	ZF868
Day 58 mean		**0·244**	**0·374**	**0·382**	
61	159	0·08	0·42	0·50	ZF876
61	224	0·20	0·45	0·35	ZF877
61	170	0·19	0·42	0·38	ZF880
61	160	0·20	0·53	0·27	ZF882
61	179	0·22	0·53	0·26	ZF883
Day 61 mean		**0·178**	**0·470**	**0·352**	

N (oocytes measured), the number of oocyte diameters measured from a cross-section of the ovary.

observed to retain both ovulated eggs within the lumen of the ovary and fully hydrated oocytes within their respective follicles (Fig. 1). These hydrated oocytes were not yet ovulated but were clearly part of the most mature, spawnable batch (under a dissecting microscope); hydrated oocytes were visually distinct from oocytes in earlier stages of development by size and clarity. In addition, these hydrated oocytes were easily separated from ovarian tissue during counting. Batch fecundity counts were performed on every female with ovulated eggs and hydrated oocytes. The daily egg production of each female was reported as either the batch fecundity or zero (in the case of females with no hydrated oocytes or ovulated eggs in the ovary).

Batch fecundity estimations were accomplished using established gravimetric techniques (Bagenal, 1971) from one ovarian lobe per individual female. The other lobe was stored in Dietrich's fixative for histology. One sub-sample per unfixed lobe, weighing between 10% (for the largest ovaries) and 100% (for ovaries weighing 1·0 g or less), was weighed, and every hydrated oocyte and ovulated egg within the sub-sample was counted. The following equation was used to calculate batch fecundity (F_B) of each individual female: $F_B = N$

$(M_{OL} + M_{OR})M_{SU^{-1}}$, where N = number of eggs counted in sub-sample, M_{OL} = mass of left ovary (g), M_{OR} = mass of right ovary in (g) and M_{SU} = mass of sub-sample (g).

LUNAR PERIODICITY AND ANNUAL FECUNDITY ESTIMATE

Periodic regression of daily egg production, I_G, and the fraction of vitellogenic oocytes in the late vitellogenic stage over the two intensively sampled summer months (May to July 2006) were used to test for the presence or absence of a lunar cycle of spawning. The following periodic regression equations from Cryer (1986) and deBruyn & Meeuwig (2001) were used: $Y = b_0 + b_1(\cos\theta) + b_2(\sin\theta)$, where Y is the dependent variable (*e.g.* egg production or I_G), b_0 is the mean level of Y and b_1 and b_2 are model coefficients which together define the phase shift and amplitude of the sine wave, and $\theta = 2\pi t_n f^{-1}$, where t_n is the original time variable (day of sample) and f is the frequency of the expected pattern. For the calculations, $f = 29{\cdot}5$, the number of days in a lunar month.

Previous studies of annual fecundity in multiple-spawning species have multiplied spawning fraction by batch fecundity estimates (usually averaged across a seasonal or annual period) to estimate the total number of eggs produced per female (Murua *et al*., 2003). Because the focus was to examine fine-scale (daily and lunar) patterns of reproductive output, sampling was accomplished on a similarly fine scale throughout the summer months (Table I). Due to restrictions on the total number of harvested individuals allowed on site, however, daily sample sizes were too small to provide suitable estimates of both daily batch fecundity and daily spawning fraction. Therefore, the mean daily egg production measurements taken at the peak of each of the 11 sampled months were used to create an estimate of annual fecundity per individual female.

By assuming that the cyclic variations in daily egg production observed during the 65 day summer sampling period continued to follow a sine curve (represented by the periodic regression model in Fig. 3) throughout the lunar year, a rough estimate of annual fecundity for an average adult female was obtained. Mean daily egg production (P_{DM}) at the monthly peak (collected within 1 day of each full moon) was sampled, and this value was used to estimate the maximal daily egg production (P_D) for each lunar month (P_{DM}) (Table III). The P_{DM} was assumed to be the apex of the sine wave for that lunar month. Based on this relationship, the P_{DM} divided by 2 (as per the sine wave function) and multiplied by 29·5 days in the lunar month resulted in an estimate for the average number of eggs produced per female (P_T) for that lunar month (M) (monthly total egg production, P_{TM}) from $P_{TM} = 14{\cdot}75\ P_{DM}$. By shifting the amplitude of the sine function according to the P_{DM}, P_{TM} was determined for 11 months of the year (Table III). No collection was made in August 2006, so values for P_{DM} and P_{TM} were interpolated for August based on the midpoint values between July and September 2006.

The P_{TM} were summed across all months to compute annual fecundity (F_A). The F_A is therefore a partial annual estimate, since the total number of days of egg production calculated is equal to 12 × 29·5 or 354 days. The variance (σ^2) of F_A was computed using the S.E. of P_T ($P_{S.E.}$) from each month (M) ($P_{S.E.M.}$):$\sigma^2 = 14{\cdot}75^2 \sum_{1}^{12} (P_{S.E.M.})^2$.

RESULTS

LUNAR CYCLE

Lunar periodicity was observed in both daily egg production and female I_G during the period of intensive sampling in the summer of 2006 (daily egg production: periodic regression, d.f. = 2, 219, $P < 0{\cdot}001$; I_G: periodic regression, d.f. = 2, 225, $P < 0{\cdot}001$). Peaks of mean daily egg production appeared on or just before the full moon for each of the three lunar months sampled. For May, June and July, mean daily egg production of days sampled nearest to the full moon was 11 804,

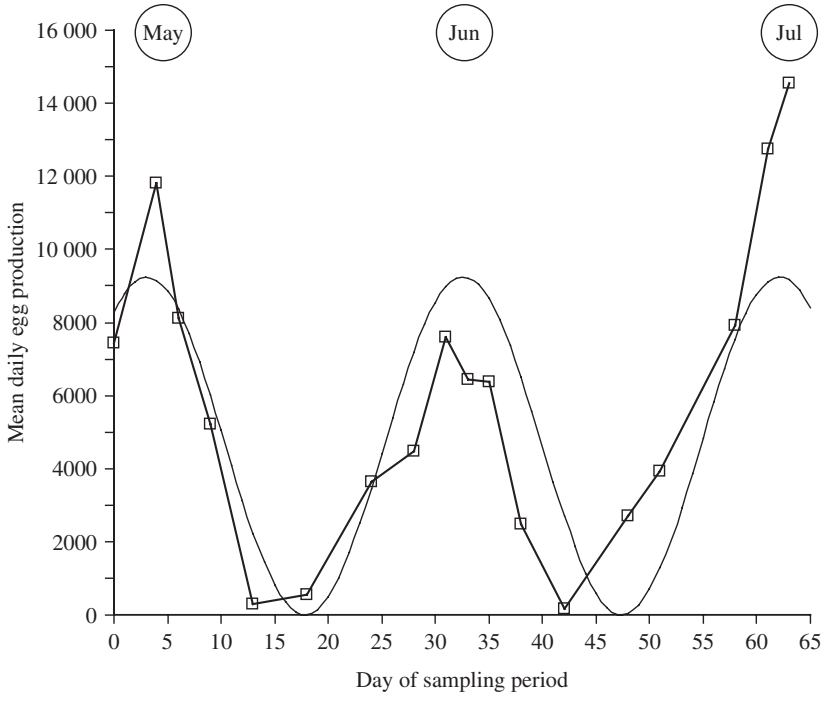

FIG. 3. Mean daily egg production of *Zebrasoma flavescens* (□) over the 65 day summer sampling period (group S fish) and sine function from the periodic regression analysis (—). The sine function represents the best-fit curve for the time-dependant relationship of mean daily egg production to sampling date. Periodic regression values (on the *y*-axis) also indicate the average egg production of the average female over time within this population. Full moons are indicated (○) at days 4, 34 and 63.

6504 and 12 445 eggs, respectively (Fig. 4). Peaks of mean I_G values appeared on the day of the full moon in May (5·53) and 2 to 4 days before the full moon for the months of June (4·29) and July (5·25) (Fig. 4). A similar pattern was also noted in the proportion of females on each day sampled that contained hydrated oocytes and eggs in their ovaries (periodic regression, d.f. = 2, 15, $P < 0{\cdot}001$) and by the increase in late-stage vitellogenic oocytes at the full moon (periodic regression, d.f. = 2, 52, $P < 0{\cdot}001$; Fig. 5). Taken together, these patterns suggest that lunar periodicity is an inherent characteristic of egg production in this population.

ANNUAL SEASONALITY AND FECUNDITY ESTIMATE

From the monthly samples taken within 1 day of the full moon, a seasonal peak in reproductive output in spring and summer months is suggested by both I_G and mean daily egg production (Fig. 6). The proportion of females producing eggs at the full moon each month did not decrease below 0·5 for the entire year (Fig. 7), and even though mean daily egg production was lower in the autumn and winter, some level of reproduction continued throughout the year (Figs 6 and 7). Additionally, a proportion of females in every month sampled showed evidence of spawning for at least two consecutive days, with fewer instances of 2 day spawning suggested in January and February 2007 (Fig. 7), the 2 months also exhibiting the lowest mean

TABLE III. Monthly and annual fecundity estimates of *Zebrasoma flavescens*

Month	*N*	P_{DM}	0·5 P_{DM}	P_{TM}
May 2006	12	11 804	5902	174 109 ± 2525
June 2006	15	6504	3252	95 934 ± 1580
July 2006	7	12 445	6223	183 564 ± 3669
August 2006*	—	8381	4190	123 620 ± 5604
September 2006	17	4316	2158	63 661 ± 1358
October 2006	9	5816	2908	85 786 ± 1561
November 2006	14	3239	1620	47 775 ± 1345
December 2006	17	2741	1371	40 430 ± 1125
January 2007	19	1271	636	18 747 ± 725
February 2007	21	1113	557	16 417 ± 772
March 2007	18	5802	2901	85 580 ± 950
April 2007	18	8136	4068	120 006 ± 1931
F_A ± S.E.				1 055 628 ± 120 596

N, number of females sampled; P_{DM}, monthly peak of mean daily egg production; P_{TM} ± S.E., monthly total egg production; F_A, annual fecundity.

*P_{DM} and P_{TM} ± S.E. values were interpolated for the month of August 2006 due to lack of sampling data.

daily egg production. Female condition did not change significantly over the course of the year sampled (one-way ANOVA, $F_{10,163}$, $P > 0{\cdot}05$).

The F_A was estimated to be 1 055 628 ± 120 596 eggs per average adult per year (Table III).

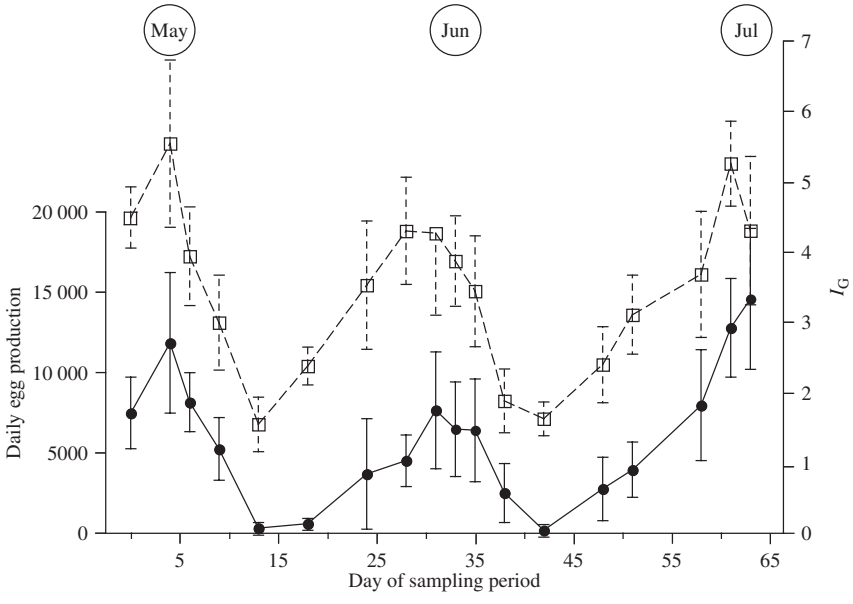

FIG. 4. Mean ± S.D. daily egg production (●) and gonado-somatic index (I_G) (□) of female *Zebrasoma flavescens* over the 65 day summer sampling period (group S fish). Full moons are indicated (○) at days 4, 34 and 63.

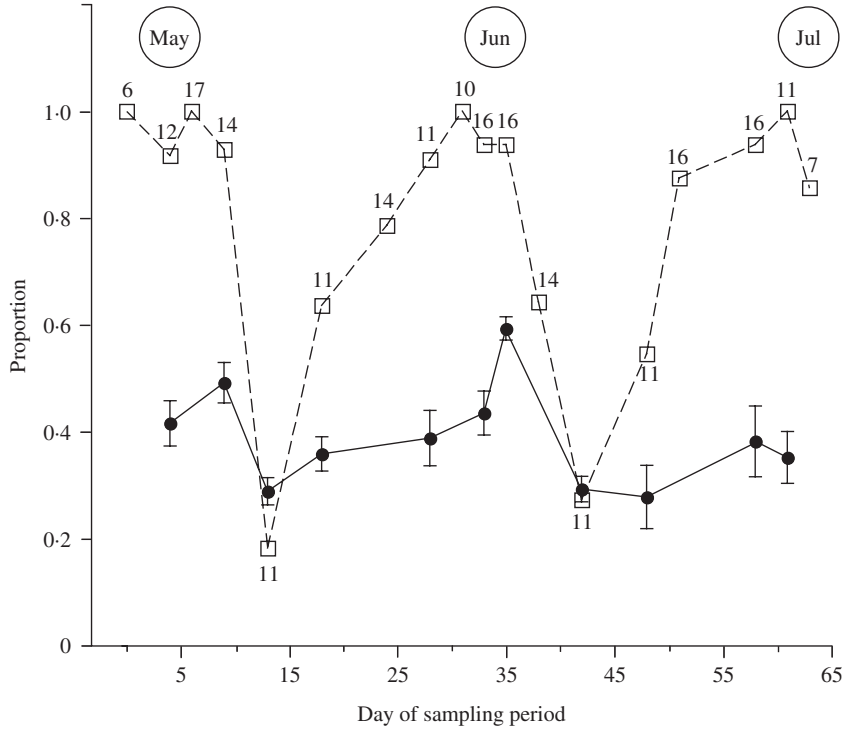

FIG. 5. The proportion of female *Zebrasoma flavescens* with ovulated eggs and hydrated oocytes inside the ovary (□) and the mean ± S.E. fraction of vitellogenic oocytes in the late vitellogenic stage (●) over the 65 day summer sampling period (group S fish). The number above the sampling date denotes the number of females sampled (N) per day. For each of the vitellogenic oocyte counts, $N = 5$. Full moons are indicated (○) at days 4, 34 and 63.

SIZE-FECUNDITY RELATIONSHIP

To reduce variability in batch fecundity values due to temporal influences of the lunar cycle, only fish sampled during the peak of reproductive output each month (*i.e.* within 1 day of the full moon) were included in this regression. Nevertheless, a large amount of variation in individual batch fecundity was observed, with a range as great as 44 to >24 000 on a single sampling date. The data indicate a size threshold *c*. 120 mm L_S, above which the largest batch fecundities (>20 000) are observed (Fig. 8). No significant relationship between L_S and batch fecundity was found (linear regression, $n = 262$, $r^2 = 0{\cdot}1\%$, $P > 0{\cdot}05$) in females >120 mm. The smallest female with eggs and hydrated oocytes inside the ovary (batch fecundity = 182) was 78 mm L_S.

DISCUSSION

In this study, lunar periodicity was observed in daily egg production, female I_G, the fraction of females with eggs in the ovary and the fraction of late-stage vitellogenic oocytes inside ovaries, with peaks for each occurring on or just before the full moon

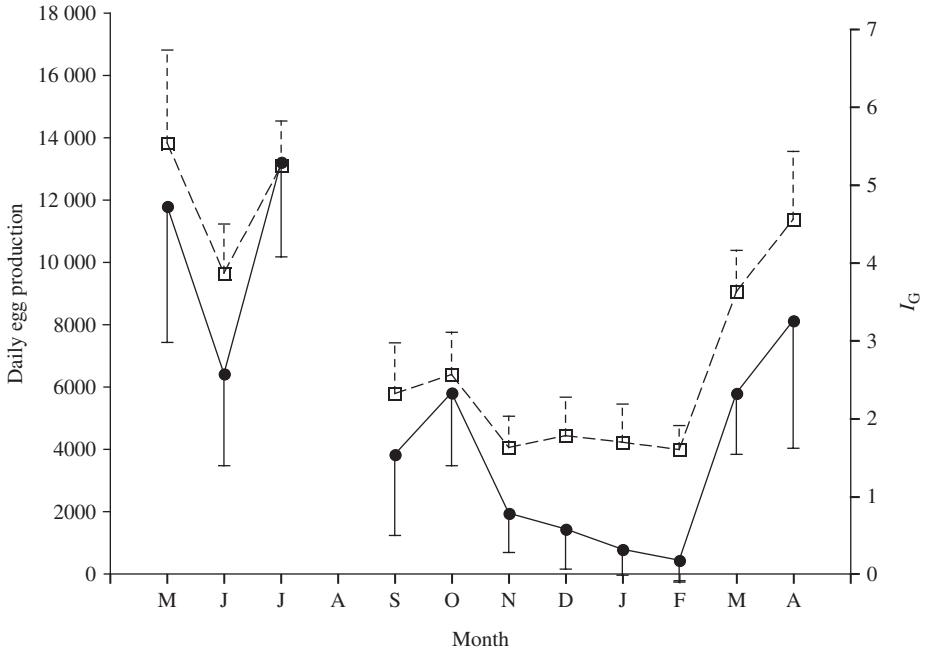

FIG. 6. Mean ± S.E. daily egg production values (●) and gonado-somatic index (I_G) (□) of female *Zebrasoma flavescens* each month ($n = 11$) over the year sampled (group M fish). All samples were collected within 1 day of the full moon of each month. No sampling occurred in August.

(Figs 4 and 5). In order to discern the lunar pattern of reproduction more accurately, minimization of another source of variability, ovarian mass, was accomplished by sampling at a consistent time of day in relation to the time of spawning. Preliminary studies in this population suggested that ovarian mass and, therefore I_G, increased throughout the day due to hydration of eggs (Bushnell, 2007), a trend that was also noted in *A. nigrofuscus* by Fishelson *et al*. (1987). Had the present daily sampling occurred within a larger window of time each day, the difference in mean I_G between days within the lunar cycle might not have been observed. The magnitude of seasonal patterns might also have been masked if the time of day and the relationship to the lunar cycle had not been accounted for in the sampling protocol.

Two previous studies of *Z. flavescens* (in which fecundity was not measured) found trends in I_G similar to those observed in this study, with reproductive activity peaking in spring and summer (Lobel, 1989; Laidley, unpubl. data). The peak of female reproductive output (at the full moon), however, was probably underrepresented by sampling protocols used in these studies, resulting in diminished differences between the high and low season. For example, in the study conducted by Lobel (1989), the highest I_G values of females ranged from *c*. 2 to 3·5 for fish sampled along the same coastline as the present study and peak I_G values of females were measured up to only 4·5 on Oahu Island (Laidley, unpubl. data). In contrast, the I_G of individual females measured in the current study at the lunar peak (July 2006) ranged from 4·0 to 9·1. Neither Lobel (1989) nor Laidley (unpubl. data) directly considered either the effects of a lunar cycle or the time of day of sampling on their measurements of female I_G.

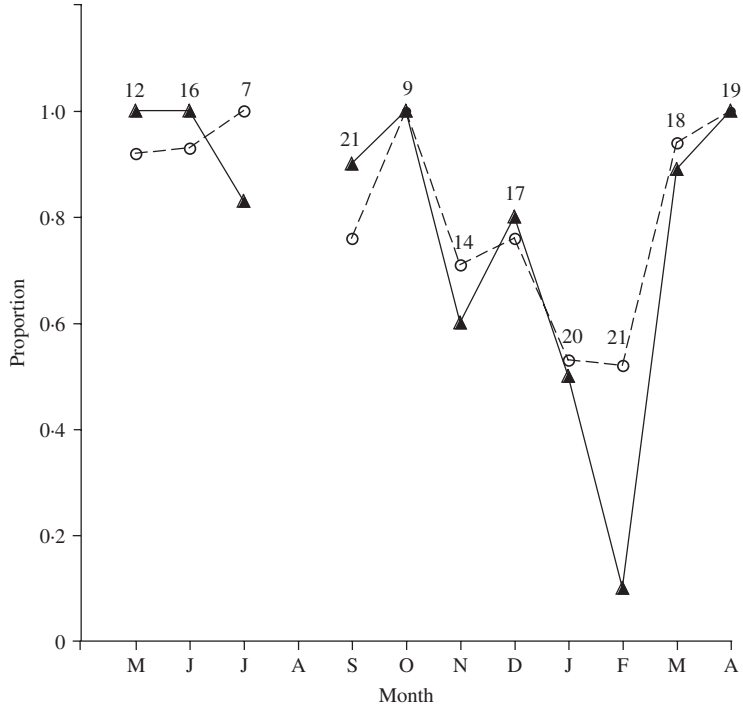

FIG. 7. The fraction of female *Zebrasoma flavescens* with ovulated eggs and hydrated oocytes in the ovary (○) and the fraction of females with evidence of spawning for at least two consecutive days (▲) over the year sampled (group M fish). Evidence of spawning two consecutive days was inferred from females with both ovulated eggs and hydrated oocytes and 24 h old postovulatory follicles in histological samples. All samples were collected within 1 day of the full moon of each month. No sampling occurred during the month of August. The number above the sampling date denotes the number of females sampled (N) per month.

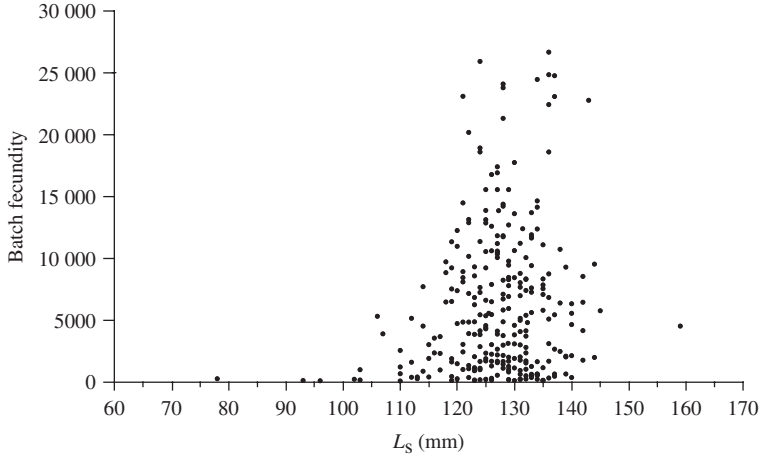

FIG. 8. Scatterplot of batch fecundity and standard length (L_S) for female *Zebrasoma flavescens*. Only females collected at the peak of reproduction of each month (within 1 day of the full moon) with ovulated eggs and hydrated oocytes within the ovary are included.

In both studies, sampling either was accomplished during a wider window of time [between 1200 and 1700 hours (Lobel, 1989)] or was not performed at a consistent time of day or month (Laidley, unpubl. data). While seasonal effects of reproduction in other species may be strong enough to overcome daily fluctuations in ovarian mass or lunar variability in daily egg production similar to that observed here, it is important to account for the possibility of such variability in future studies, where sample sizes may be limited and lunar or seasonal patterns may not be as strong.

Fine-scale measurements of batch fecundity (*i.e.* daily, or at least several times per month) are rarely assessed in studies of coral-reef fishes (Sadovy, 1996). Previous studies of this and other coral-reef species (Walsh, 1984; Sancho *et al*., 2000) have instead relied upon observations of spawning behaviour to quantify reproductive activity. The present study illustrates that *Z. flavescens* females vary reproductive investment among spawning events following environmental cues (the lunar cycle), probably independent of any observable difference in spawning behaviour. In future studies of multiple-spawning coral-reef fishes, greater insight into the environmental factors that regulate reproductive activity may be gained by determining the relative reproductive investment in each spawning event.

While the cyclic trends in *Z. flavescens* reproduction were statistically relevant and visually obvious, a large amount of temporal and individual variability in each of the reproductive measurements was noted, especially in batch fecundity. No significant linear relationship between body size and batch fecundity was observed in adult *Z. flavescens*. This is in contrast to many other perciform species, where the number of eggs per batch is positively correlated with size (Davis & West, 1993; Roumillat & Brouwer, 2004). It has been shown that wide-bodied fishes (*e.g.* groupers) are capable of attaining larger ovary mass and therefore producing more eggs per g body mass than some laterally compressed fishes (*e.g.* butterflyfishes or angelfishes) (Sadovy, 1996). Surgeonfishes have a strongly laterally compressed body shape and a sharply asymptotic growth curve (Choat & Axe, 1996), both of which contribute to a relatively small range of adult body size and ovary size. This limited size range of adults, combined with the high variability in batch fecundity in Z. *flavescens*, probably contributed to the lack of a positive relationship between body size and batch fecundity in this species. Smaller fishes with little body cavity space can only produce large number of eggs by spawning frequently over the course of a lifetime, in contrast to larger fishes with the ability to spawn much fewer, but also much larger batches (sometimes with up to millions of eggs) (Robertson, 1991). While the present study found that *Z. flavescens* spawn frequently, it was not possible to address whether larger (or older) females spawn more often, are able to produce more large egg batches over a more protracted spawning season, or whether they may contribute higher quality eggs than smaller (or younger) females, all of which are characteristics that have been shown to relate positively with body size or age in other fish species (Bagenal, 1971; DeMartini & Fountain, 1981; Lambert, 1987; Quinn *et al*., 1995; Berkeley *et al*., 2004; Trippel & Neil, 2004; Abdoli *et al*., 2005).

The size at maturation is another characteristic of reproduction often estimated by fisheries scientists, as it is used in the development of management strategies of harvested species, usually with the goal of minimizing capture of individuals before they begin reproducing. Also referred to as size at first reproduction (SFR), this value is sometimes defined as the minimum length ($L_{\min}$) at which sexual maturity is observed in the population, but more often, it is defined by the length at which 50%

of the individuals have attained sexual maturity (L_{50}) (Sadovy, 1996). In this study, the smallest *Z. flavescens* found to have ovulated eggs in its ovary (L_{min}) was 78 mm L_S. Since there was limited sampling for smaller individuals, L_{50} was not estimated. Given the amount of variability in batch fecundity at a given length during the peak reproductive period each month (Fig. 8) and the lack of a positive L_S and fecundity relationship in fish >120 mm, the use of SFR defined as L_{50} may not be particularly informative or useful in developing management strategies for this or similar species. A more appropriate designation may be the size at which maximal egg production becomes possible (here, *c*. 120 mm L_S). In this species, this is also approximately the size at which females make an ontogenetic shift in daytime feeding habitat from deeper, coral-rich habitat (10–20 m) to shallower pavement habitat (3–10 m) (Claisse *et al*., 2009). Changes in food availability associated with this habitat shift may contribute to increased reproductive capabilities for larger fish, although further research is warranted.

Because sampling throughout the year always occurred at the apparent monthly peak of mean daily egg production (within 1 day of the full moon), employment of traditional methods to estimate annual fecundity in multiple-spawning fishes by multiplying mean batch fecundity by spawning frequency over time (Hunter & Macewicz, 1985; McBride & Thurman, 2003; Murua *et al*., 2003) would have created an overestimate of monthly reproductive output for *Z. flavescens* in the population studied. To account for the lunar cyclic fluctuations in mean daily egg production, the periodic regression model based on a sine function was used to approximate this monthly variability and produce an estimate of annual fecundity for an average adult (Fig. 3). This method does not distinguish between spawning and non-spawning females, as it represents only the mean number of eggs produced by an individual on a given day of the monthly cycle.

The use of the periodic regression model in the annual fecundity estimate also assumes maintenance of the lunar cycle for the entire year. At present, there is no evidence to suggest that this cyclic pattern of spawning does not continue throughout all months of the year, since all other reproductive activities of females in the wild [(*e.g*. production of eggs; Fig. 5), participation in spawning behaviours (Lobel, 1989), evidence of spawning at least 2 days in succession (Fig. 6)] persisted, albeit at lower levels in winter months. In addition, data from spawning of captive fish suggest a year-round lunar spawning pattern (Laidley, unpubl. data).

Understanding temporal variability in egg production is an essential component of the reproductive biology of multiple-spawning fishes and can have important applications for the management of fished populations. When fecundity is used as a metric to evaluate the effectiveness of marine protected areas (Evans *et al*., 2008), the potential for temporal variability over short-time scales (*e.g*. diel and lunar) should be considered during sampling and calculations of reproductive output. Egg production variability could be quantified in future studies using a temporally fine-scaled sampling approach similar to what was employed in the present study. For west Hawaii *Z. flavescens*, estimation of annual fecundity was predicated upon understanding the lunar pattern in egg production. The ability to estimate annual fecundity for more multiple-spawning fishes will facilitate examination of the effects of fishing on the reproductive characteristics of these populations (Hunter *et al*., 1989; Rochet, 1998; LaPlante & Schultz, 2007) and will permit examination of life-history evolution across a broader suite of fishes (Rochet, 2000).

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