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Reproductive potential and spawning periodicity in barred sand bass (*Paralabrax nebulifer*) from the San Pedro Shelf, southern California

Erica T. Jarvis*, Kerri A. Loke-Smith, Kyle Evans, Ricky E. Kloppe, Kelly A. Young, and Charles F. Valle

California Department of Fish and Wildlife, Marine Region, 4665 Lampson Avenue, Suite C, Los Alamitos,CA 90720,USA (ETJ, KAL, KE, REK, CFV)

California State University Long Beach, College of Natural Sciences and Mathematics, 1250 Bellflower Boulevard, Long Beach,CA 90840,USA (REK, KAY)

**Correspondent: Ejarvis@ocsd.com*

Current address: Orange County Sanitation District, Ocean Monitoring Program, 10844 Ellis Avenue, Fountain Valley,CA 92708,USA (ETJ)

Barred sand bass (*Paralabrax nebulifer*) form large, predictable spawning aggregations that are heavily exploited in the recreational fishery, but robust reproductive estimates (i.e., essential fishery information) are lacking for this species. Barred sand bass were collected on the San Pedro Shelf during June–September 2011 to improve estimates of gonadosomatic index (GSI), spawning fraction, batch fecundity, and spawning periodicity. We calculated spawning fraction using the post-ovulatory follicle method; batch fecundity was estimated using the hydrated oocyte method. Blood plasma samples were analyzed for concentrations of 17β-estradiol (E2, n=160), 11-ketotestosterone (11KT, n=96), and progesterone (P4, n=153) to examine spawning periodicity. Spawning occurred predominantly in July and August, peaking just days before the new and full moon phases. Sea surface temperature (β=0.45) and time of capture (β=-0.35) were the most significant predictors of female E2 (R^2=0.38, $F_{(6,139)}$=9.2, P<0.001); E2 concentrations positively fluctuated with temperature and were significantly higher before noon than after noon (W=10263.5, P=0.0001). The relationship between batch fecundity (n=40, range 204 to 461 mm SL) and ovary mass was $\text{Log}_{10}y$=0.9815($\text{Log}_{10}x$)+3.1353 (R^2=0.94); batch fecundity ranged from 23,536 to 330,443 oocytes, and females were estimated to spawn 42 times. Based on our estimates of spawning frequency and batch fecundity, potential annual fecundity for female barred sand bass ranged from 0.98 to 13.9 million oocytes, and averaged 3.5±2.5 million. These newly available reproductive estimates should enhance fishery assessments and management of this popular sport fish.

Key words: barred sand bass, *Paralabrax nebulifer*, spawning periodicity, reproductive potential, batch fecundity, potential annual fecundity, spawning fraction, gonadosomatic index, 17β-estradiol, 11-ketotestosterone, progesterone

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Barred sand bass (*Paralabrax nebulifer*; Family Serranidae [BSB]) has been a popular sport fish in southern California for decades; however, BSB catch-per-unit-effort has notably declined in recent years due to fishing and suboptimal environmental conditions (Jarvis et al. 2014). Barred sand bass are primarily targeted in the summer months when they form large spawning aggregations comprised of hundreds to thousands of resident and migrant fish (Turner et al. 1969, Love et al. 1996, Jarvis et al. 2010). Each year the peak fishing season typically lasts from one to three months and fishing occurs at well-known spawning aggregation hotspots. In response to concerns over the sustainability of the resource, more restrictive harvest regulations for this fishery were implemented in 2013 (FGC 2012, Jarvis et al. 2014). Evaluating the effectiveness of these regulations is important for monitoring the fishery's sustainability and for maximizing fishing opportunities. Unfortunately, no biological reference points such as maximum sustainable yield exist for BSB, primarily due to an absence of biomass estimates and data on their reproductive potential. To evaluate the effectiveness of these regulations and the health of the fishery, a future fishery assessment will depend on the best available essential fishery information, which includes the species' reproductive biology (Phipps et al. 2010).

Barred sand bass are gonochoristic (Sadovy and Domeier 2005) and females are indeterminate serial spawners, in which oocytes (presumptive eggs) develop throughout the spawning season and are spawned in multiple batches (DeMartini 1987, Oda et al. 1993). Annual fecundity, the number of eggs produced by a female in a single year, is a measure of reproductive potential and can be used to predict stock sustainability (Pitman et al. 2013). For serial spawners, potential annual fecundity can be calculated using estimates of batch fecundity (the number of eggs released in a single batch), the spawning fraction (the proportion of females spawning per day), and spawning frequency (the number of spawning events per season) (Hunter and Macewicz 1985). The BSB spawning fraction was estimated by Oda et al. (1993); however, the samples were collected during a two-week period in July, which the authors noted was the reproductive "subseason" and may not accurately have reflected the spawning fraction over the entire spawning season. Since the spawning fraction can vary depending on when samples are collected, knowledge of spawning seasonality and sufficient temporal resolution in sampling effort is critically important to obtaining unbiased results.

Reports of BSB spawning seasonality in the literature range from three months (June–August; Clark 1933) to seven months (April–November; Allen and Hovey 2001). Clark's (1933) estimate was based on gross observations of BSB ovaries in commercially landed fish from May to September, but information upon which the other estimates were based is unclear. *Paralabrax* spp. in the Southern California Bight have a plankton larval duration of approximately one lunar month (Allen and Block 2013) and eggs or larvae occur from June through October (Moser et al. 2001); however, this group includes kelp bass (*P. clathratus*), which spawn from May to October (Erisman and Allen 2006) and spotted sand bass (*P. maculatofasciatus*), which spawn from June to August (Allen et al. 1995). Thus, there is a need to better define spawning seasonality in BSB.

An update on BSB batch fecundity estimates is also needed to better estimate annual fecundity. Previous estimates were based on small sample sizes and differed considerably from each other (DeMartini 1987, Oda et al. 1993). Batch fecundity estimates obtained from active or recent spawners might underestimate batch fecundity because ovaries from these individuals could contain partially-spawned batches (Hunter et al. 1992, Ganias et al. 2010). Thus, more accurate batch fecundity estimates might be difficult to obtain in adequate sample sizes because samples are limited to only females with ovaries that contain hydrated oocytes and no new post-ovulatory follicles (Hunter et al. 1985, Ganias et al. 2010). Batch fecundity estimates would be improved by sampling more fish over a wider size range and by increasing our understanding of how samples obtained from females with partially spawned batches affect BSB batch fecundity estimates.

Reproductive hormones such as 17β-estradiol (E2) and 11-ketotestosterone (11KT) typically fluctuate with spawning activity and may peak during spawning aggregation pulses. Individual BSB are capable of daily spawning (Oda et al. 1993), but it is unknown whether BSB spawning peaks occur with regular periodicity throughout the spawning season. Tag and recapture data suggest that formation of BSB spawning aggregations occurs at monthly intervals during spawning season (Jarvis et al. 2010), and ovarian histology suggests that BSB spawning peaks mid-day (Oda et al. 1993), but this was not confirmed by visual spawning observations or steroid hormone profiles. Within-day and within-season spawning periodicity could be driven by environmental cues that are ultimate or proximate in nature. For many animals, the ultimate environmental cue is photoperiod (Bradshaw and Holzapfel 2007). However, proximate cues, such as water temperature and lunar phase, can trigger or enhance a physiological reproductive response (Frisch et al. 2007). These cues are important for some species to synchronize spawning, and could represent optimal conditions for survival of fertilized eggs and larvae (Colin 1992, Sancho et al. 2000).

Our first objective was to investigate BSB spawning seasonality and to determine how BSB reproductive parameters vary across the spawning season. Our second objective was to determine BSB spawning frequency and batch fecundity to enable estimates of annual reproductive potential. Our final objective was to determine any within-season and within-day spawning periodicity for BSB, and the relationship between environmental factors and concentrations of E2, progesterone (P4) and 11KT in male and female BSB during the spawning season. Understanding which cues affect BSB spawning will be important for understanding how, or if, reproductive potential varies from year to year.

Materials and Methods

Study animals.—All BSB were collected during 0700–1500 or 2000–2200 by hook and line, baited trap, or spear from 21 June 2011 to 22 September 2011 along the San Pedro Shelf in southern California (Figure 1). After capture, blood was drawn from the caudal vein using an 18- or 20-gauge needle and heparinized syringe. Standard length (SL, mm) and total length (TL, mm), somatic weight (to the nearest 0.01kg), and time of capture were recorded for each fish. Fish were then euthanized by placing them on ice. The gonads were excised, placed in 10% neutral buffered formalin for 7 to 10 days, and then weighed to the nearest 0.001 g. The sex of the fish was identified macroscopically and confirmed histologically at a later date. Gonadosomatic index (GSI) was calculated for each individual as gonad mass divided by somatic mass (SM) multiplied by 100.

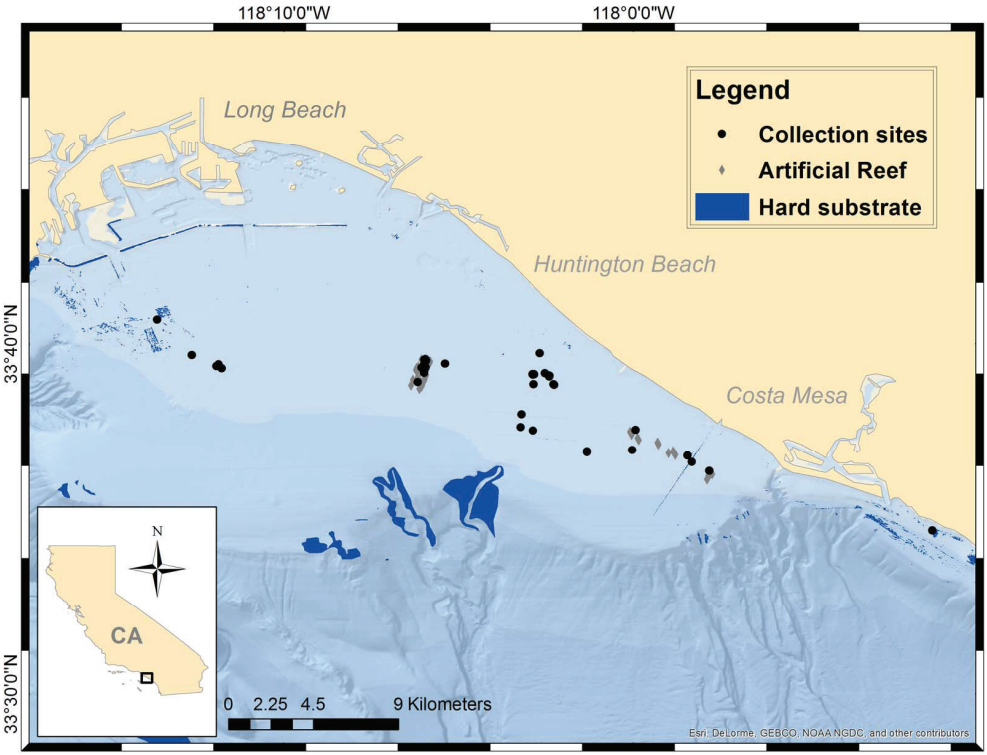

Figure 1.—Barred sand bass collection sites on the San Pedro Shelf in southern California, June–September 2011.

Histology.—Following preservation, a cross-section 2–3 mm thick was taken from the center of one of the gonad lobes of each fish, transferred to 70% ethanol, and saved for histological analysis. Gonad histology was conducted by Diagnostic Pathology Medical Group, Inc. (Sacramento, California); additional sections for select individuals were prepared at California State University, Long Beach using standard paraffin embedding, sectioning, and hematoxylin and eosin staining procedures (Loke-Smith et al. 2010). Upon examination, oocytes were categorized into one of eight developmental stages (Lowerre-Barbieri et al. 2011a: primary growth [PG], cortical alveolar [CA], vitellogenic I, II, and III [vtg-I,II,III], germinal vesicle migration [MN], hydrated [H], and postovulatory follicle [POF]; Figure 2). The spawning fraction (*S*) was estimated using the POF method to determine the proportion of spawning females (females with POFs <25 hours old). A BSB postovulatory follicle aging key based on timed serial tissue collection (Oda et al. 1993) was generated from labeled histological slides archived at the Natural History Museum of Los Angeles County. The ageing key was used to assign POF ages to fish collected for the current study (Day 0 = less than 4 hours old [POF_0], Day 1 = 4 to 24 hours old [POF_1], and Day 2+ = greater than 24 hours old [POF_2]; Figure 3). The average sea surface temperature (SST) during the current study (18.9±1.3° C) was within the range of water temperatures reported by Oda et al. (1993; 16.9–19.9° C); thus, POF absorption rates in both periods were assumed to be similar.

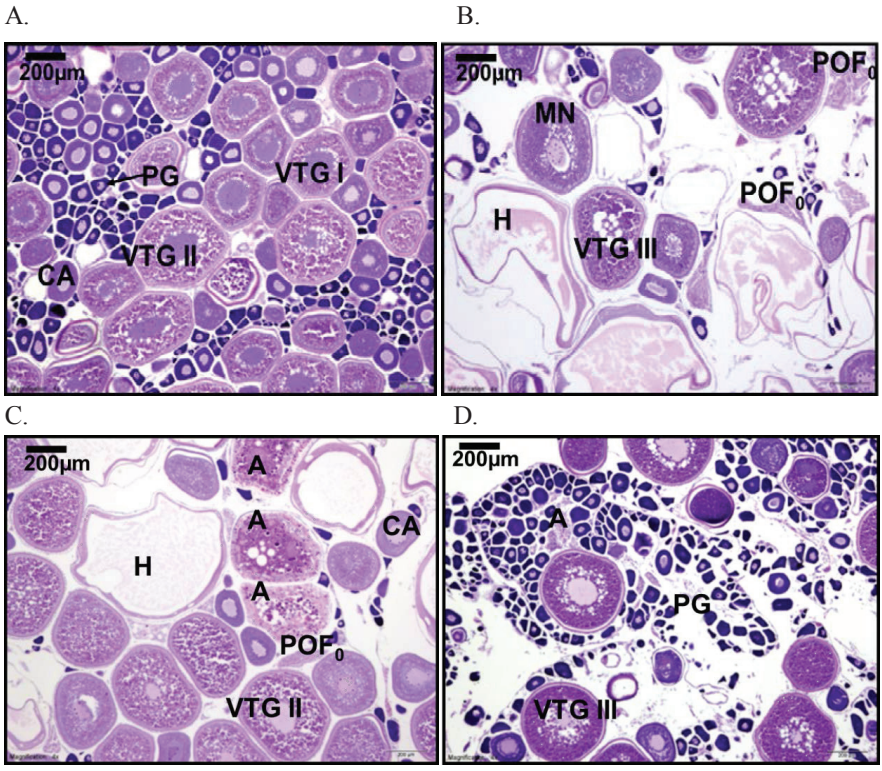

Figure 2.—Images of barred sand bass ovary sections at 4× magnification depicting oocyte development stages and follicles during various reproductive stages including developing (A), spawning capable (B and C) , and regressing (D). PG = primary growth; CA = cortical alveolar; VTG (I,II,III) = vitellogenic (I,II,III); MN = migratory nucleus; H = hydrated oocyte; POF_0 = Day 0 postovulatory follicle; A=atretic follicle.

Females with no evidence of new or old postovulatory follicles or hydrated oocytes, but having ovaries containing vitellogenic oocytes, were classified as non-spawning (Figure 2a). Daily spawning activity was identified by the presence of at least one of the five following combinations of follicle or oocyte developmental stages (Oda et al. 1993: POF_1 and MN, POF_1 and H, POF_1 and POF_2, POF_0 and POF_1, and POF_0 and H; Figure 2b, Figure 2c, Figure 3). The presence of ovarian follicular atresia was assigned to females having multiple atretic follicles.

Batch fecundity.—Ovaries identified as having hydrated oocytes or POFs were retained to estimate batch fecundity using the hydrated oocyte method (Hunter et al. 1985). The number of hydrated oocytes in a subsample of ovarian tissue was counted, whereby a tissue sample weight of approximately 0.100±0.025 g was determined to contain 100–200 hydrated oocytes for analysis. Tissue samples were removed from each ovarian lobe in a pie-shaped wedge (weighed to 0.001 g), mounted in 33% glycerol, and allowed to sit for ten minutes to loosen connective tissue before gently tapping and teasing them apart. Oda et al. (1993) and DeMartini (1987) determined that neither the location of gonad tissue sample nor the specific lobe influenced batch fecundity estimates for BSB.

Oocytes were covered with a glass cover slip and the hydrated oocytes from each lobe were counted ≥2 times under a compound microscope (Figure 4). In addition, the

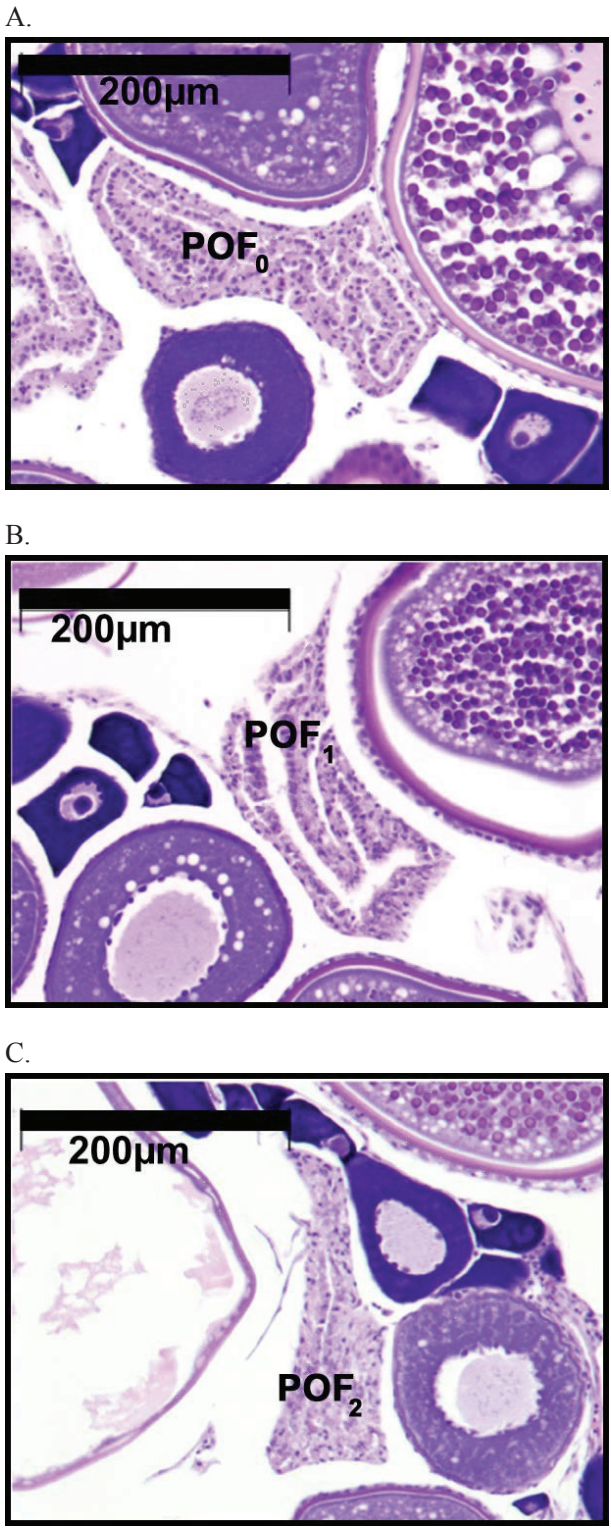

FIGURE 3.—Images of representative ovary sections at 20× magnification for barred sand bass females collected on the San Pedro Shelf in southern California, June–September 2011 with (A) POF_0 (spawned within 4 hours of collection); (B) POF_1 (spawned between 4 and 24 hours prior to collection); and (C) POF_2 (spawned greater than 24 hours prior to collection). POF = post-ovulatory follicle.

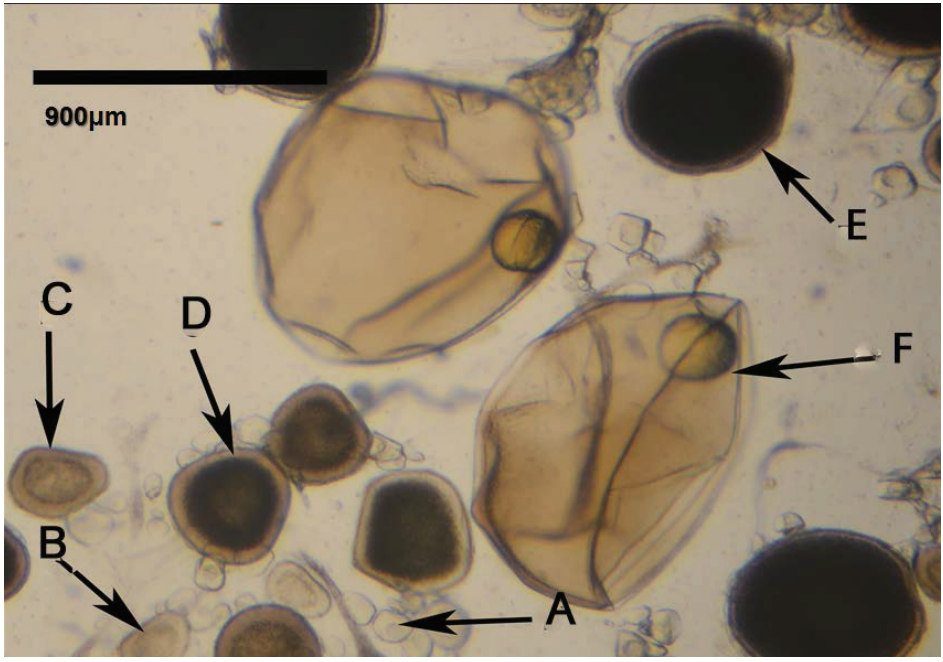

Figure 4.—Image of barred sand bass ovarian tissue wet mount highlighting the various oocyte development stages, including (A) primary growth, (B) cortical alveolar, (C) vitellogenic stage I, (D) vitellogenic stage II, (E) vitellogenic stage III, and (F) hydrated oocyte.

mean diameters of thirty oocytes of each developmental stage were measured to the nearest millimeter for each fish using a Max ERB dissecting scope with an eyepiece micrometer calibrated at 100× for hydrated oocytes and 200× for all other developmental stages. We multiplied the mean number of hydrated oocytes per gram of ovarian tissue by the total mass of the ovary (OM) to estimate the number of hydrated oocytes to be spawned in a batch.

Reproductive hormones.—Blood samples were centrifuged at 5000 revolutions/minute for 5 minutes to separate the blood plasma. Plasma was removed and stored at -80 °C until hormone assays were conducted. Female plasma E2 and P4 concentrations were measured using Cayman Chemical ACE™ competitive enzyme immunoassays (17β-estradiol EIA kit; Cayman Chemical Item 582251 and progesterone EIA kit; Cayman Chemical Item 582601), and male plasma 11KT concentrations were measured using the 11-keto Testosterone EIA kit (Cayman Chemical Item 582751).

Two dilutions of each blood plasma sample that were between 20% and 80% of B/B_0 (the ratio of sample absorbance to that of a maximum binding control) were used in hormone assays. The dilutions were analyzed in duplicate and values were averaged. Plates were read using a Powerwave XS Bio-Tek microplate spectrophotometer at 412 nm. Raw data (absorbances) were analyzed using 2006 Cayman Chemical Enzyme Immunoassay Tools software. Intra-assay coefficients of variability (CV) ranged from 8.7 to 16.3% for E2, from 9.7 to 16.1% for P, and from 4.6 to 10.1% for 11KT. Inter-assay CV was 14.8, 8.8, and 16.5% for E2, P, and 11KT, respectively.

Data analysis.—Male and female GSI data from June to September 2011 were analyzed with historic monthly BSB GSI data (collected during 1993–1995 and archived by the California Department of Fish and Wildlife) to look for seasonal patterns and to identify a best fit curve for predicting sex-specific GSI by month. Barred sand bass in the 1990s study were collected throughout southern California.

The spawning fraction (*S*) is the fraction of mature females whose ovaries contain hydrated oocytes and (or) POF_0 or POF_1 (imminent, active, or recent spawners; Lowerre-Barbieri et al. 2011a). We calculated the monthly spawning fraction, spawning interval (i.e., time lag between spawning events, $1/S_{month}$), and monthly spawning frequency (monthly spawning events [the number of days in the month divided by $1/S_{month}$]). Monthly differences in spawning fraction were tested using a Chi Square Test of Homogeneity (or Fisher's Exact Test in cases with expected values <5%), and Bonferroni multiple comparisons *ad hoc*. We report Adjusted Wald 95% binary confidence intervals with proportion data and LaPlace point estimates for proportion data equal to zero females (Sauro and Lewis 2005).

The seasonal spawning fraction was calculated as the number of imminent, active, or recent spawners divided by the total number of mature females sampled from June to September 2011; the seasonal spawning interval was the inverse of this value. The total seasonal spawning frequency was calculated as the sum of the monthly number of estimated spawning events per female.

Batch fecundity, OM, SM, ovary-free weight wet (OFWW), and SL were $\log_{10}$-transformed and batch fecundity-size relationships were examined with linear regression. Since gonad wet weights were not obtained for all fish, OFWW was calculated from the formalin preserved gonad weight, which did not significantly differ from the fresh weight for a subsample of BSB (Preserved Weight=1.0137(Wet Weight)+0.4813, n=106, R^2=0.9984). Females with ovaries showing signs of active or recent spawning (e.g., tissue counts <100 hydrated oocytes in each lobe or presence of POF_0 or POF_1) were assumed to contain partially spawned batches; therefore, batch fecundity curves were compared with and without these data. A Kruskal-Wallis test was used to determine if there was a difference in the condition factor (i.e., fish health, K) and relative fecundity (hydrated oocytes/g OFWW) among POF_2 females, POF_0 or POF_1 females, and females with low hydrated oocyte counts; this was followed by a Mann-Whitney test for pairwise comparisons. The condition factor was calculated using the equation, $K=(SM\times10^2)/SL^3$, where SM was in grams and SL was in centimeters (Moyle and Cech 1988). Potential annual fecundity was calculated as the estimated batch fecundity for POF_2 females multiplied by the total estimated spawning events per female per year (i.e., total seasonal spawning frequency).

We calculated mean hourly and daily concentrations (pg/ml) of each reproductive hormone to identify any peak(s) in hormone concentration during a 24-hr period and also throughout the spawning season. For temporal comparison, daily E2 concentrations were plotted relative to new and full moon phases and average daily values of SST for Newport Pier in Newport Beach, California (SCCOOS 2013) and tidal flux (m) obtained for Balboa Pier in Newport Beach, California (Nobeltec Tides and Currents Pro v 3.5 software). Tidal flux was calculated as the difference between the lowest and highest tide heights on the day of capture. A Wilcoxon Mann-Whitney test compared E2 concentrations in females sampled in July and August between 0700–1200 hours and 1200–1500 hours.

A standard multiple regression analysis was conducted to determine how well fish size (TL), time of capture, SST, tidal flux, chlorophyll concentration, and photoperiod predicted female E2 and male 11KT reproductive hormone concentrations; SST and chlorophyll

concentrations for each sampling date and time were obtained from SCCOOS (2013). Hormone concentrations were normalized by square-root transformation, all variables were converted to *Z*-scores, and an extreme E2 outlier was removed for one female. We did not include interactions because these variables could not be controlled in the field. All statistical analyses were performed using Minitab 16.2.2 statistical software with α=0.05. Curve-fitting for GSI and batch fecundity relationships was done using SigmaPlot 10.0.

Results

We collected 352 BSB (212 females, 138 males) over 29 sampling days (June = 8 days, July = 13 days, August = 7 days, September = 1 day). All fish were mature, ranging in size from 204 to 509 mm SL and from 0.18 to 3.40 kg.

Spawning seasonality and fraction.—A subset of 272 fish (192 females, 80 males) with associated somatic and gonadal weights was available for GSI analysis. An additional 282 BSB GSI records from the 1990s (135 females, 91 males, 56 unknown; size range: 138–474 mm SL, 0.07–2.25 kg) were obtained from CDFW archives. For individual fish, GSI values ranged from <1.0 to 18.1%. Mean monthly GSI by sex (females and males) peaked during June, July, and August (Figure 5a); this trend was the same when the 1990s data and 2011 data were examined separately. No data were available for April, October, or December; however, a non-linear best-fit curve of the data suggested GSI was low during those months (Figure 5a). Although average daily GSI was highly variable, females and males showed five coincident peaks of differing magnitudes between late June and late August of 2011 (Figure 5b).

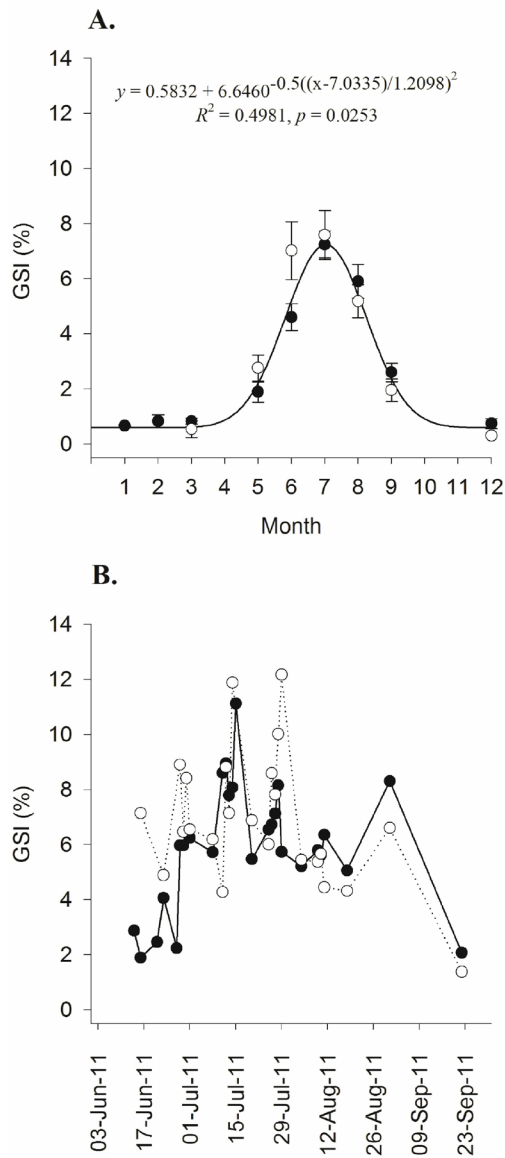

Figure 5.—Mean (A) monthly (± 2 *SE*) gonadosomatic index (GSI; males, *n*=171; females, *n*=327; 1993–1995 and 2011) and (B) daily GSI (males, *n*=80; females, *n*=192; 2011 only) for male (open circles) and female (black circles) barred sand bass collected in southern California. Non-linear fit through monthly data is based on data for all fish (*n*=553), including individuals of unknown sex.

Although female GSI was highest from June to August, the spawning fraction showed daily variability, and peaked twice in July and once in August (Figure 6a). There was a significant difference in spawning fraction by sampling month (χ^2_3, 208=23.1, P<0.001) with the proportion of spawning females being 2- to 6-fold higher in July and August when compared with June or September (Figure 6b). The spawning interval and spawning frequency likewise varied by sampling month, as did the proportion of daily spawners (Table 1). Although we found no significant difference between the July and August spawning fraction (χ^2_1, 166=0.836, P=0.361), the incidence of active spawning (i.e., females with POF_0) was significantly higher in July than August (χ^2_1, 166=6.75, P=0.009). The proportion of

Table 1.—Monthly spawning interval (days), spawning frequency (events), and proportion of daily spawners estimated for female barred sand bass collected on the San Pedro Shelf in southern California, June–September 2011.

Month	Spawning interval (days)	Spawning frequency (events)	Proportion daily spawners
June	6.0	5.0	0.08
July	1.7	17.8	0.44
August	2.0	15.5	0.38
September	9.0	3.3	0.00

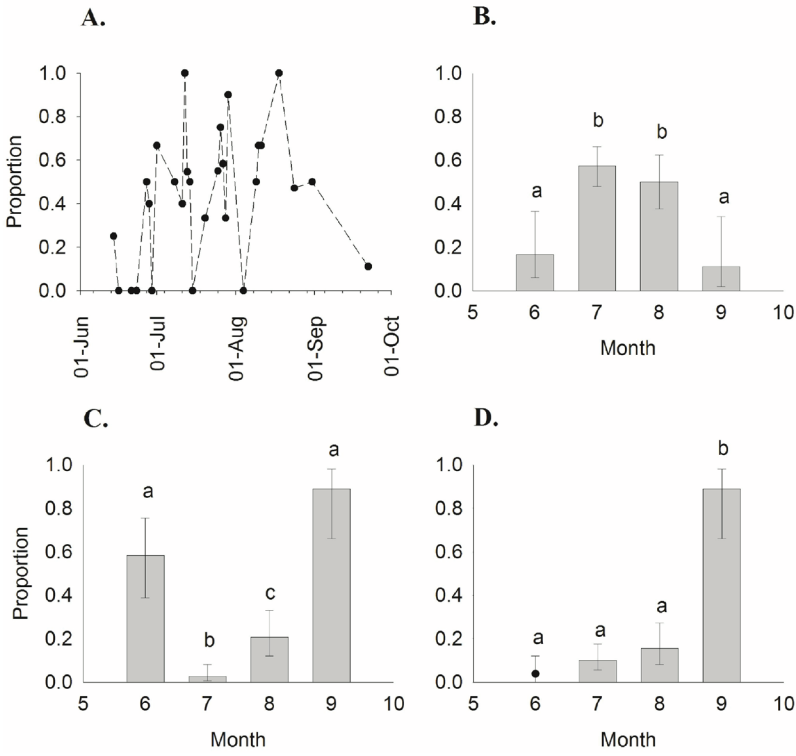

Figure 6.—Spawning fraction of barred sand bass females collected on the San Pedro Shelf in southern California (June–September 2011) by collection date (A) and by collection month (B), and the monthly proportion of females (C) in non-spawning condition and (D) with mass follicular atresia. Error bars are 95% binomial confidence intervals; the black circle represents a LaPlace point estimate. Sample sizes for June, July, August, and September were 24,108, 58, and 18, respectively.

non-spawning females also differed by sampling month (Fisher's Exact Test, P=0.008), with June and September showing the highest non-spawning fractions, and September showing the highest incidence of follicular atresia (i.e., spawning cessation; Figure 6c, Figure 6d). Females spawned approximately 42 times from June to September 2011 (Table 1).

The percent of females with hydrated oocytes steadily decreased between the sampling hours of 0700 and 1300; no females with hydrated oocytes were sampled between 1300 and 1500 hours (Figure 7). Only four females were sampled between 2000 and 2200 hours and the most advanced oocyte stages present were early vitellogenic (vtg-I,II; n=3) and advanced vitellogenic (vtg-III; n=1); these fish were sampled in mid-June and had no POFs. The mean (±*SD*) percent of non-spawners between 0700 and 1300 hours was 14±14% (n=256), while the mean between 1300 and 1500 hours was 58±13% (n=26).

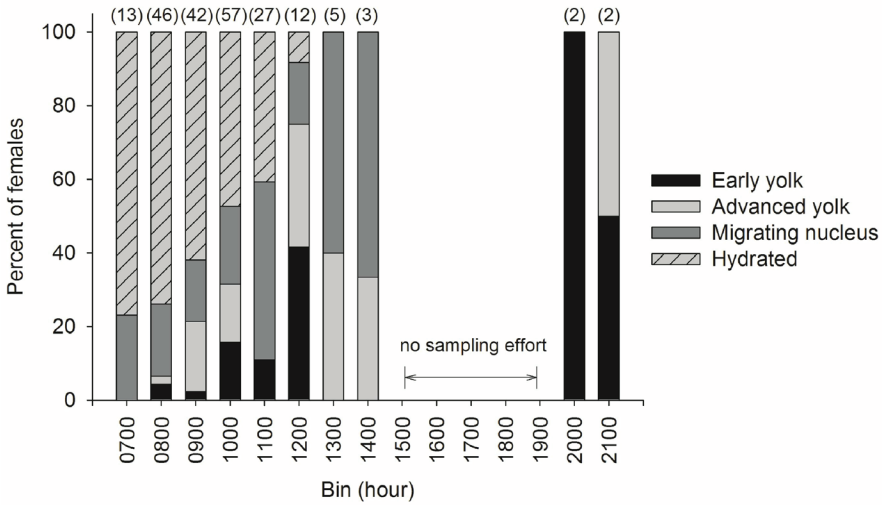

Figure 7.—Percent of barred sand bass females collected on the San Pedro Shelf in southern California (June–September 2011) grouped by the most advanced oocyte development stage present in the ovary and the hour of collection. Early yolk = vitellogenic stage I and II; advanced yolk = vitellogenic stage III. No sampling occurred between 1500 and 2000 or between 2200 and 0700.

Batch fecundity.—Oocyte development stages differed in mean diameter ($F_{5, 11594}$=135,152, P<0.001) and all pairwise comparisons were significant. The mean (±*SD*) diameters (mm) for each stage were 0.07±0.02 (PG, n=1,945), 0.14±0.02 (CA, n=1,942), 0.22±0.03 (VtgI, n=1,942), 0.33±0.04 (VtgII, n=1,944), 0.46±0.03 (VtgIII, n=1,944), and 0.89±0.06 (H, n=1,883). Batch fecundity estimates were analyzed for 63 females (size range: 204–461 mm SL, 0.18–2.85 kg; ovary weights: 13.90–200.71 g; capture dates: 28 June to 31 August 2011). The $\log_{10}$-transformed linear relationships between batch fecundity and OM, OFWW, and SL were all significant (P<0.001); however, subsequent removal of active or potential recent spawners (i.e., females with hydrated oocyte counts <100 or POF_0, POF_1) greatly improved these relationships, especially for SL (Figure 8a, Figure 8b, Figure 8c). Batch fecundity for POF_2 females (n=40; size range: 204-461 mm SL, 0.18–2.85 kg; average size: 299 mm SL, 0.77 kg; capture

dates: 28 June to 31 August 2011) ranged from 23,536 to 330,443 oocytes and averaged 84,032. Potential annual fecundity ranged from 0.98 to 13.9 million oocytes and averaged 3.5±2.5 million. The relationship between SL and OFWW for the 40 POF_2 females was defined by the function $OFWW=0.00004*SL^{2.9019}$ (R^2=0.94); the mean (±*SD*) ratio of OFWW to SM was 0.91±0.2, and the mean (±*SD*) number of hydrated oocytes per gram of ovarian tissue was 1,278±199.

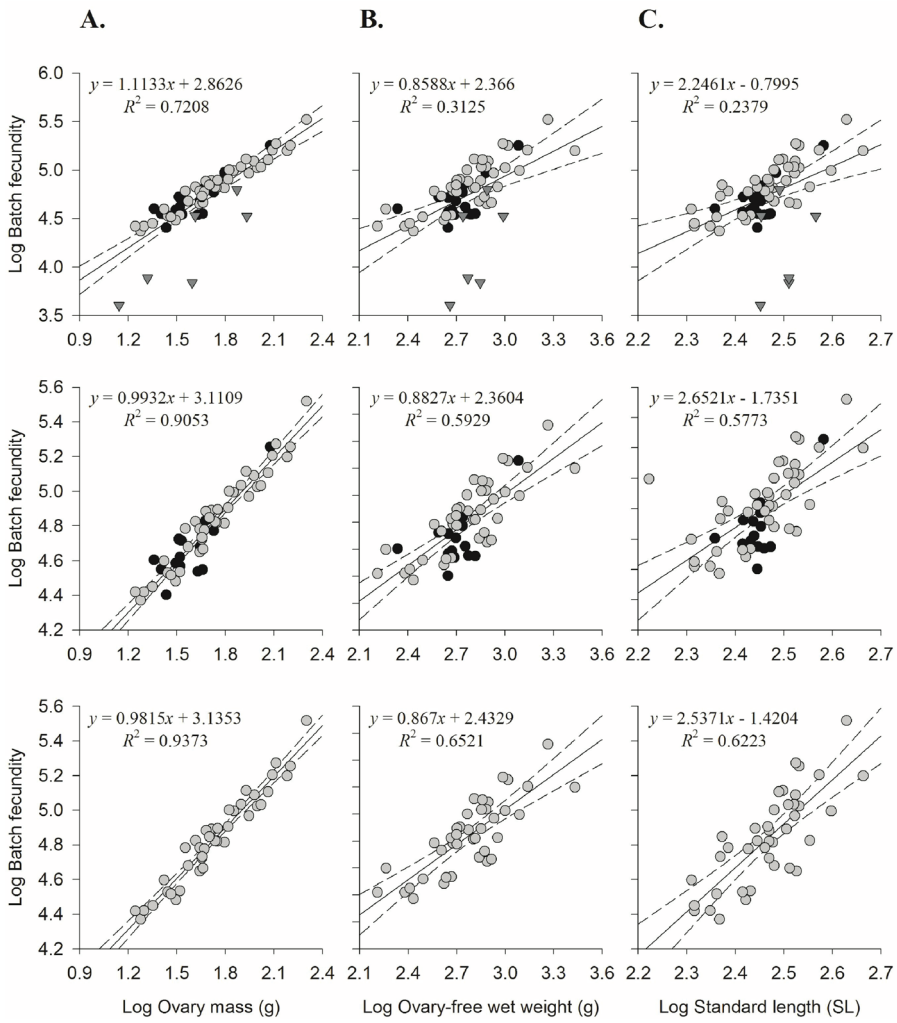

Figure 8.—Relationship between Log_{10} batch fecundity (±95% *CI*) of barred sand bass females collected on the San Pedro Shelf in southern California (June–September 2011) and (A) Log_{10} ovary mass (g); (B) Log_{10} ovary-free wet weight (g); and (C) Log_{10} standard length (SL). The top panels include females with POF_2 (gray dots, n=40) and females with recently spawned batches having POF_0 or POF_1 (black dots, n=17) or low hydrated oocyte counts (gray inverted triangles, n=6). The middle and bottom panels represent the subsequent removal of these females until all potential recent spawners are excluded.

Mean (±*SD*) relative fecundity (number of hydrated oocytes/g OFWW) was highest for POF_2 females (123.46±43.08, n=40), followed by POF_0 and POF_1 females combined (99.31±38.84, n=17) and females with low hydrated oocyte counts (34.83±30.42, n=6). Relative fecundity was different among the three groups (H=14.98, df=2, P=0.001); however, the difference between POF_2 females and females with POF_0/POF_1 was marginally nonsignificant (W=387.0, P=0.07). There was no relationship between OFWW and relative fecundity, and there was no significant difference in the condition factor among POF_2 females (median: 2.54, mean: 2.62), POF_0/POF_1 females (2.62, 2.65), and females with low hydrated oocyte counts (2.26, 2.22; H=3.74, df=2, P=0.154).

Spawning periodicity.—We assayed 160 female E2, 153 female P4, and 96 male 11KT blood plasma samples. Female E2 and male 11KT concentrations varied among individuals and across the spawning season (Figure 9). Mean daily E2 concentrations

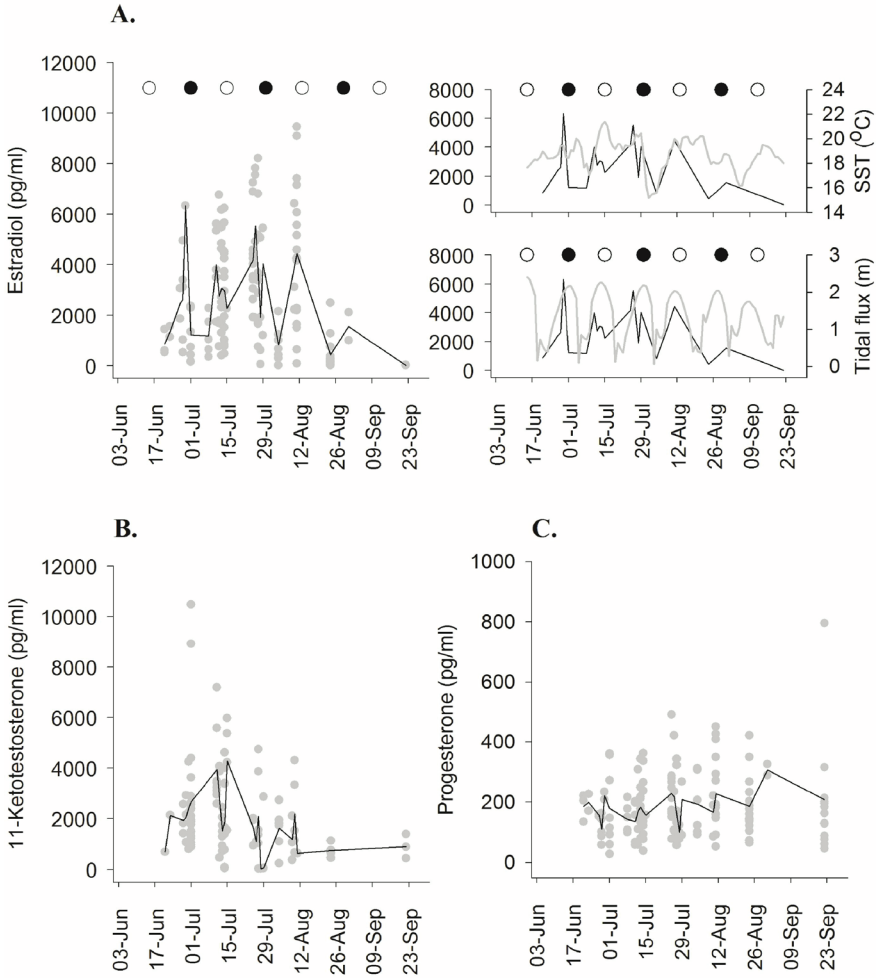

Figure 9.—Mean daily concentrations of (A) female 17β-estradiol (pg/ml, black line) relative to moon phase (full moon, open circles; new moon, black circles), SST (°C), and tidal flux (m); (B) male 11-ketotestosterone (pg/ml); and (C) female progesterone (pg/ml) sampled from barred sand bass collected on the San Pedro Shelf in southern California, June–September 2011. Gray circles represent raw data.

in female BSB blood plasma peaked in late June, mid-July, late July, and mid-August, occurring just days before the new and full moons (Figure 9a). The peaks were an average of 15 (±1 *SD*) days apart and an average of 3.3 (±1.2 *SD*) days prior to the new or full moon phases. Fluctuations in SST and tidal flux tended to correspond with these peaks, albeit with different magnitudes (Figure 9a). By mid-September, E2 concentrations measured in nine females were near zero. In contrast, mean daily concentrations of 11KT in male blood plasma peaked once in mid-July, just before the full moon (Figure 9b). Low 11KT concentrations in late June and late August were similar to values obtained on a single sampling date in mid-September. Although female P4 concentrations varied among individuals, mean daily values were relatively stable from late June to mid-August, peaking only in late August; the highest individual value occurred in September (Figure 9c). Mean hourly concentrations of E2 in females sampled in July and August remained elevated between 0700 and 1200 hours and were low between 1200 and 1500 hours (Figure 10); median E2 concentrations before 1200 and after 1200 were significantly different (W=10263.5, P<0.001). In contrast, male 11KT concentrations peaked at 0700 and decreased through 1200; no males were sampled after 1200 (Figure 10b).

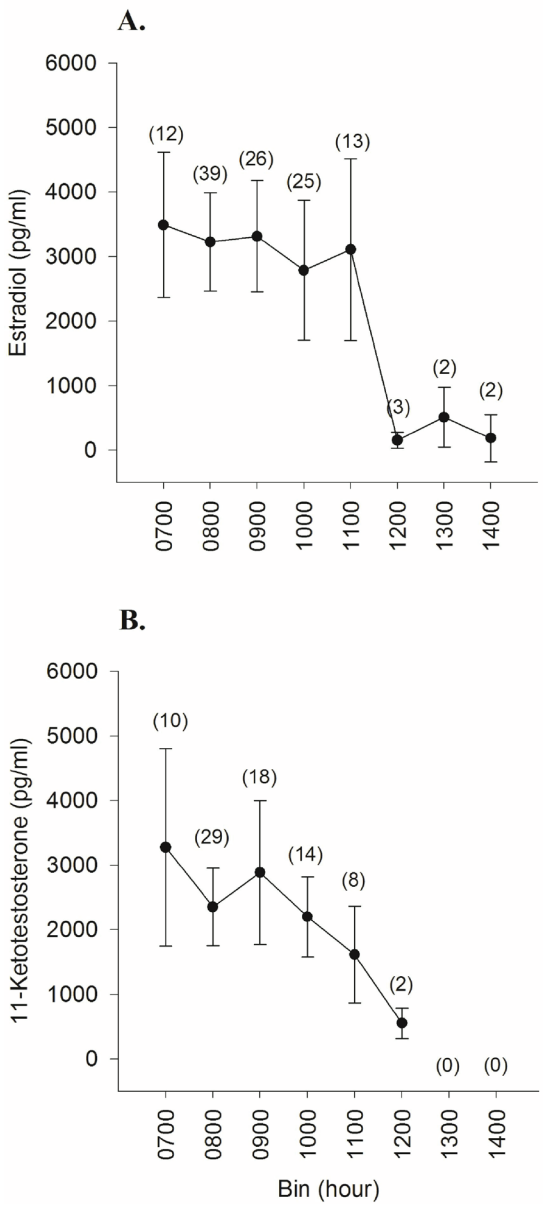

Figure 10.—Mean (±2 *SE*) hourly concentrations of (A) female 17β-estradiol (pg/ml) and (B) male 11-ketotestosterone (pg/ml) measured in barred sand bass collected on the San Pedro Shelf in southern California, June–September 2011. No sampling occurred between 1500 and 0700.

The full model for E2 concentrations in female BSB was significant (R^2=0.38, $F_{6,139}$ = 9.2, P<0.001), but the coefficient for TL was not significant; the reduced model explained 37% of the variability in E2, and SST and time of capture were the most significant predictors (R^2=0.37, $F_{5,140}$=16.5, P<0.001; Table 2). Photoperiod was the only significant predictor of BSB male 11KT concentrations (R^2=0.91, $F_{1,103}$=10.3, P=0.002).

Table 2.—Multiple regression results of the full and reduced model for 17β-estradiol concentrations in female barred sand bass collected on the San Pedro Shelf in southern California, June–September 2011. SST = sea surface temperature.

Predictor	Coefficient, β	*SE* Coefficient	*T*	*P*
Full Model				
Constant	0.0000	0.0665	0.00	1.000
Total length	-0.1091	0.0691	-1.58	0.117
SST	0.4297	0.0928	4.63	0.000
Time of capture	-0.3560	0.0819	-4.34	0.000
Tidal flux	-0.2139	0.1067	-2.00	0.047
Chlorophyll	-0.2027	0.0809	-2.51	0.013
Photoperiod	0.1776	0.0792	2.54	0.027
Reduced Model				
Constant	0.0000	0.0668	0.00	1.000
SST	0.4537	0.0920	4.93	0.000
Time of capture	-0.3510	0.0823	-4.26	0.000
Tidal flux	-0.2151	0.1072	-2.01	0.047
Chlorophyll	-0.2052	0.0813	-2.52	0.013
Photoperiod	0.1893	0.0793	2.39	0.018

Discussion

Reproductive potential.—The results of the reproductive parameters of the current study combined with historic monthly GSI data from the 1990s agree with the June to August spawning season reported for BSB by Clark (1933) over eighty years ago. By September, female E2 and male 11KT concentrations were low, and females showed a high incidence of follicular atresia and a peak in P4, both indicators of spawning cessation. Although BSB males appeared primed for spawning in June (e.g., elevated GSI), male 11KT concentrations didn't peak until July, and evidence of spawning for most BSB females occurred in July and August as elevated GSI, E2, and POFs). Unfortunately, we did not sample during the early part of June; however, GSI from fish collected during June in the 1990s was between 0.46 and 1.49%, suggesting we captured the onset of spawning from mid to late June. Using our monthly spawning fractions for June to September, female BSB were estimated to spawn approximately 42 times per year. This is in contrast to 55 times per year estimated by Oda

et al. (1993) using spawning fraction from late July, which highlights the importance of sampling throughout the spawning season.

In addition to differences in the temporal resolution of sampling effort, spawning frequency estimates can be affected by individual variability in spawning periods, including variation in spawning residence times of migrant fish and oversampling of aggregative females (i.e., only sampling spawning hot spots; Lowerre-Barbieri et al. 2011b). McKinzie et al. (2014) analyzed the fine-scale horizontal and vertical movement patterns of BSB and during spawning season found that presumed spawning individuals spent most of their time in the mid-water over sand habitat during the day and remained more closely associated with the seafloor at night, exhibiting a positive edge response (i.e., showing preference for a rock-sand ecotone; Mason and Lowe 2010). Although tag and recapture data suggest a BSB spawning ground residence time of 7 to 35 days for individual fish, only a portion of BSB on the spawning grounds appear to be migrant, and average BSB migration distances are inclusive of the area we sampled (Jarvis et al. 2010). Therefore, we feel confident in our spawning frequencies estimated during June – September 2011, since a large size range of mature individuals was sampled and because BSB were collected on reefs and within spawning aggregations over sand flats using a variety of sampling methods.

Our batch fecundity and size relationships were improved by the exclusion of females with hydrated oocyte counts less than 100 and females with POF_0 or POF_1. We assumed the six females with low hydrated oocyte counts contained partially spawned batches; these fish were captured from late June through peak spawning and atretic follicles were not more prevalent in these samples than samples without low hydrated oocyte counts. POF_0 females likely contained partially spawned batches since these fish provided evidence of active spawning (POF ages <4 hrs). The post-ovulatory follicles of POF_1 females ranged in age from new to old (from 4 to 24 hours). Thus, it is possible that at least some of these females also contained partially spawned batches, especially since removal of POF_0 and POF_1 females shifted our batch fecundity curve slightly higher. Although we found no significant difference in relative fecundity between POF_0 and POF_1 females and POF_2 females, the significance level was marginal and may have been affected by a low sample size of POF_0 and POF_1 females, in addition to the variability introduced within the POF ages themselves. For example, different POF ages may actually represent similar spawning times prior to collection, whereby a female that spawned 23 hours prior to collection would theoretically be assigned POF_1, while a female that spawned 25 hours prior to collection would be assigned POF_2.

It is important to note that if lower relative fecundity in POF_1 females is due to subsequent spawns of daily spawners being less fecund than females that spawn every 2–3 days, then excluding POF_1 females may have overestimated our batch fecundity results (batch fecundity including the 12 POF_1 females [i.e., POF_2 AND POF_1 females combined] averaged 79,156 oocytes; average size was 297 mm SL and 0.75 kg). However, it is not known whether this occurs with BSB. Without improved resolution in assigning POF ages of POF_1 females, future BSB batch fecundity estimates should attempt to exclude these females through histological analysis or by exclusively sampling fish at times of the day when they are not likely spawning.

In comparing our results with previous *Paralabrax* batch fecundity estimates for kelp bass (Oda et al. 1993) and for kelp bass and BSB combined (DeMartini 1987), our BSB estimations were higher than those determined by DeMartini (1987), but very similar to what Oda et al. (1993) reported for similarly sized fish. For example, based on our batch

fecundity estimate using OFWW, a 700-g OFWW female BSB would average about 79,000 eggs per batch, while the same size fish in Oda et al. (1993) was reported to average 81,000 and in DeMartini (1987), only 43,000. Oda et al. (1993) noted that DeMartini's results may have been influenced by temperature, since fish in that study were collected during an El Niño period. However, based on the effect of temperature on fecundity for other species, one might expect warmer temperatures to result in higher fecundity (Lambert 2008). It is also possible that DeMartini's (1987) results were influenced by females with partially spawned batches. Unlike Oda et al. (1993) and the current study, Demartini (1987) did not distinguish between females with new and old POFs and most females were collected between 0900 and 1100 hours when females were likely spawning.

Potential annual fecundity for female BSB in this study was very similar to the estimate reported for another temperate serranid, blacktail comber (*Serranus atricauda*): 0.91 to 15.5 million oocytes, with an average of 5.1±4.1 million (Garcia-Diaz et al. 2006). Monitoring annual fecundity can be useful for understanding the effects of fishing, individual variability (e.g., condition, lipid content, morphological constraints), and environmental conditions on this reproductive parameter (Lambert 2008, Pitman et al. 2013). For example, Pitman et al. (2013) found that fecundity was negatively related to the stock size of orange roughy (*Hoplostethus atlanticus*), with exploitation having a density-dependent compensatory effect on fecundity. Moreover, an increasing number of studies have shown the relationship between spawning stock biomass and stock egg production might not always be reliable (Lambert 2008). Although BSB batch fecundity can be predicted based on the size relationships provided here, obtaining actual batch fecundity estimates along with monthly spawning frequency estimates is better for monitoring potential annual fecundity. Our results from 2011 should provide a baseline from which to measure changes in BSB potential annual fecundity before and after the 2013 implementation of more restrictive harvest regulations.

Spawning periodicity.—Reproductive hormone concentrations were highly variable among individuals; however, a few trends were apparent. Between late June and mid-August, E2 in female BSB peaked with regular periodicity just days before the new and full moons, which were coincident with high tidal fluxes. These peaks also appeared to occur with increases in SST, which had the highest significant coefficient for predicting female E2 concentrations in our model. Unlike for some tropical aggregative spawners, lunar spawning synchronicity would not necessarily be expected for BSB, which are capable of daily spawning and have a protracted spawning season. For example, spawning aggregation formation or activity was not related to the lunar cycle in dusky grouper (*Epinephelus marginatus*) (Herue et al. 2006) or kelp bass (Erisman and Allen 2006), and both are temperate serranids that have protracted spawning seasons like BSB. Erisman et al. (2007) also did not find evidence for lunar synchronicity in the aggregative daily spawner, leopard grouper (*Mycteroperca rosacea*). However, evidence for lunar spawning synchronicity in these studies was measured by examining temporal changes in GSI or *in situ* with visual observations of spawning behavior or activity, rather than with analysis of individual reproductive hormone concentrations, which may provide finer-scale resolution in these relationships for some species. In addition, lag times, sample sizes, and sampling frequency can also distort possible relationships.

E2 is known to regulate vitellogenesis in many teleosts (Redding and Patino 1993); thus, although a fraction of BSB females spawned every few days in July and August, it appears there are specific times when vitellogenesis in BSB females is ramped up, and this

would result in peak recruitment of primary oocytes into vitellogenic growth (Cheek et al. 2000). Interestingly, we observed regular peaks in average daily BSB GSI over the spawning season, which upon further examination appear to coincide with the peaks in E2, which coincided with peaks in water temperature. Thus, despite the variability we observed in GSI and E2 among individuals, the overall average daily fluctuations in GSI suggest there are peak periods of gonadal growth followed by gamete release.

Optimal water temperatures could be causing an increase in hydration and subsequent ovulation that coincides with high tidal flows. Such conditions could increase egg or larval survival (Colin 1992, Sancho et al. 2000). The fine-scale vertical movements of BSB acoustically tracked during spawning season indicate that BSB are associated with the thermocline and make repeated vertical dives toward the seafloor during the day (McKinzie et al. 2014); those authors noted the thermocline association may facilitate rapid hydration and egg development. Furthermore, BSB have positively buoyant eggs, and Gadomski and Caddell (1996) reported that successful hatching of viable BSB embryos occurred only at 16–28° C, which is inclusive of typical summertime surface waters in the local region (~15–22° C). Finally, daily tidal fluxes in the region during this study were high (~3 m), which could provide swift transport of eggs or larvae away from schooling ichthyoplankton predators.

Within-day trends in periodicity were limited to the hours of our sampling effort, which was primarily from 0700 to 1500. Nevertheless, results from this study and previous studies potentially provide insight into BSB diel spawning periodicity. Hourly trends in the most advanced oocyte stage present in the BSB ovary and in female E2 and male 11KT concentrations suggest spawning ceased after 1200. Although the ovaries of the four females sampled at night (2000–2200) did not contain hydrated oocytes or POFs, these fish were collected in mid-June when the spawning fraction was low (17%), so this alone does not rule out the potential for evening spawning. Oda et al. (1993) reported a mid-day spawning peak for BSB (1200–1400); however, those authors also collected ovulating BSB females into the night (1900–2300). It is unclear what measure they used to classify an ovulating female since none of the BSB collected at that time contained hydrated oocytes or new POFs; thus, spawning was not likely occurring. In addition, McKinzie et al. (2014) noted that diving behavior of presumed spawning or courting BSB individuals occurred during daylight hours and resting (non-diving) behavior occurred at night. The one exception was a fish acoustically tracked during a full moon that exhibited diving behavior during both day and night, suggesting that spawning-related behavior at night occurs during full moon periods.

Given the results reported here and in previous studies, BSB spawning appears to begin at dawn and ceases for most females by the early afternoon. Following the drop in E2 at that time (i.e., following the peak in vitellogenesis), the process of BSB oocyte hydration likely begins. This scenario would yield an approximate 15-hour hydration period for BSB (1300–0600), as reported by Oda et al. (1993) for kelp bass; DeMartini (1989) also predicted "a long hydration period" for BSB. Prolonged diel spawning in BSB may be reserved for within-season periods of optimal environmental conditions for eggs and larvae.

The within-season trends in periodicity reported here offer an alternative, or additional, fishery management option for BSB. For example, a partial spawning season

closure that straddled a full moon and new moon phase would likely provide valuable protection during a period of the spawning season when the fish are most vulnerable and when spawning output is potentially higher due to spawning synchronicity.

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Literature Cited

Allen, L. G., and H. E. Block. 2012. Planktonic larval duration, settlement, and growth rates of the young-of-the-year of two sand basses (*Paralabrax nebulifer* and *P. maculatofasciatus*: fam. Serranidae) from Southern California. Bulletin of the Southern California Academy of Sciences 111:15-21.

Allen, L. G., and T. E. Hovey. 2001. Barred sand bass. Pages 224-225 *in* W. S. Leet, C. M. Dewees, R. Klingbeil and, E. J. Larson, editors. California's living marine resources: a status report. California Department of Fish and Wildlife, Sacramento, USA.

Allen, L. G., T. E. Hovey, M. S. Love, and J. T. W. Smith. 1995. The life history of the spotted sand bass (*Paralabrax maculatofasciatus*) within the Southern California Bight. California Cooperative Fishery Investigations Reports 36:193-203.

Bradshaw, W. E., and C. M. Holzapfel. 2007. Evolution of animal photoperiodism. Annual Review of Ecology, Evolution, and Systematics 38:1-25.

Cheek, A. O., P. Thomas, and C. V. Sullivan. 2000. Sex steroids relative to alternative mating behaviors in the simultaneous hermaphrodite *Serranus subligarius* (Perciformes: Serranidae). Hormones and Behavior 37:198-211.

Clark, F. N. 1933. Rock bass (*Paralabrax*) in the California commercial fishery. California Fish and Game 19:25-35.

Colin, P. L. 1992. Reproduction of the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae) and its relationship to environmental conditions. Environmental Biology of Fishes 34:357-377.

Demartini, E. E. 1987. Tests of ovary subsampling options and preliminary estimates of batch fecundity for two *Paralabrax* species. California Cooperative Oceanic Fisheries Investigation Reports 28:168-170.

Erisman, B., and L. G. Allen. 2006. Reproductive behaviour of a temperate serranid fish, *Paralabrax clathratus*, from Santa Catalina Island, California, USA. Journal of Fish Biology 68:157-184.

Erisman, B. E., M. L. Buckhorn, and P. A. Hastings. 2007. Spawning patterns in the leopard grouper, *Mycteroperca rosacea*, in comparison with other aggregating groupers. Marine Biology 151:1849-1861.

FGC (Fish and Game Commission). 2012. Initial statement of reasons for regulatory action [Internet]. Amend Sections 27.65 and 28.30 Title 14, California Code of Regulations Re: Basses. Available from: http://www.fgc.ca.gov/ regulations/2012/27_65isor.pdf

Frisch, A. J., M. I. McCormick, and N. W. Pankhurst. 2007. Reproductive periodicity and steroid hormone profiles in the sex-changing coral-reef fish, *Plectropomus leaopardus*. Coral Reefs 26:189-197.

Gadomski, D. M., and S. M. Caddell. 1996. Effects of temperature on the development of survival of eggs of four coastal California fishes. Fishery Bulletin 94:41-48.

Ganias, K., M. Rakka, T. Vavalidis, and C. Nunes. 2010. Measuring batch fecundity using automated particle counting. Fisheries Research 106:570-574.

Garcia-Diaz , M., J. A. Gonzalez, M. J. Lorente, and V. M. Tuset. 2006. Spawning season, maturity sizes, and fecundity in blacktail comber (*Serranus atricauda*) (Serrandidae) from the eastern-central Atlantic. Fishery Bulletin 104:159-166.

Herue, B., D. Diaz, J. Pasqual, M. Zabala, and E. Sala. 2006. Temporal patterns of spawning of the dusky grouper *Epinephelus marginatus* in relation to environmental factors. Marine Ecological Progress Series 325:187-194.

Hunter, J. R., and B. J. Macewicz. 1985. Measurement of spawning frequency in multiple spawning fishes. NOAA Technical Report NMFS 36:79-94.

Hunter, J. R., B. J. Macewicz, N. C. Lo, and C. A. Kimbrell. 1992. Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fishery Bulletin 90:101-128.

Hunter, J. R., N. C. Lo, and J. Roderick. 1985. Batch fecundity in multiple spawning fishes. NOAA Technical Report NMFS 36:67-77.

Jarvis, E. T., C. Linardich, and C. F. Valle. 2010. Spawning-related movements of barred sand bass, *Paralabrax nebulifer*, in southern California: interpretations from two decades of historical tag and recapture data. Bulletin of the Southern California Academy of Sciences 109:123-143.

Jarvis, E. T., H. L. Gliniak, and C. F. Valle. 2014. Effects of fishing and the environment on the long-term sustainability of the recreational saltwater bass fishery in southern California. California Fish and Game 100:234-259.

Lambert, Y. 2008. Why should we closely monitor fecundity in marine fish populations? Journal of Northwest Atlantic Fishery Science 41:93-106.

Loke-Smith, K. A., M. A. Sundberg, K. A. Young, and C. G. Lowe. 2010. Use of morphology and endocrinology to predict sex in California sheephead: evidence of altered timing of sex change at Santa Catalina Island, California. Transactions of the American Fisheries Society 139:1742-1750.

Love, M. S., A. Brooks, and J. R. R. Ally. 1996. An analysis of commercial passenger fishing vessel fisheries for kelp bass and barred sand bass in the Southern California Bight. California Fish and Game 82:105-121.

Lowerre-Barbieri, S. K., N. J. Brown-Peterson, H. Murua, J. Tomkiewicz, D. M. Wyanski, and F. Saborido-Rey. 2011a. Emerging issues and methodological advances in fisheries reproductive biology. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 3:32-51.

Lowerre-Barbieri, S. K., K. Ganias, F. Saborido-Rey, H. Murua, AND J. R. Hunter. 2011b. Reproductive timing in marine fishes: variability, temporal scales, and methods. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 3:71-91.

Mason, T. J., and C. G. Lowe. 2010. Home range, habitat use, and site fidelity of barred sand bass within a southern California marine protected area. Fisheries Research 106:93-101.

McKinzie, M. K., E. T. Jarvis, and C. G. Lowe. 2014. Fine-scale horizontal and vertical movement of barred sand bass, *Paralabrax nebulifer*, during spawning and non-spawning seasons. Fisheries Research 150:66-75.

Moser, H. G., R. L. Charter, P. E. Smith, D. A. Ambrose, W. Watson, S. R. Charter, and E. M. Sandknop. 2001. Distribution atlas of fish larvae and eggs in the Southern California Bight region 1951–1998. California Cooperative Oceanic Fisheries Investigation Atlas 34.

Moyle, P. B., and J. J. Cech. 1988. Fishes. An introduction to ichthyology. Second edition. Prentice Hall, Englewood Cliffs, New Jersey, USA.

Oda, D. L., R. J. Lavenburg, and J. M. Rounds. 1993. Reproductive biology of three California species of *Paralabrax* (Pisces:Serranidae). California Cooperative Oceanic Fisheries Investigation Reports 34:122-132.

Phipps, K. E., R. Fujita, and T. Barnes. 2010. From paper to practice: incorporating new data and stock assessment methods into California fishery management. University of California San Diego: California Sea Grant College Program. Managing data-poor fisheries: case studies, models and solutions 1:49-58.

Pitman, L. R., J. A. Haddy, and R. J. Kloser. 2013. Fishing and fecundity: the impact of exploitation on the reproductive potential of a deep-water fish, orange roughy (*Hoplostethus atlanticus*). Fisheries Research 147:312-319.

Redding, J. M., and R. Patino. 1993. Reproductive physiology. Pages 503-534 *in* D. H. Evans, editor. The physiology of fishes. CRC Press, Boca Raton, Florida, USA.

Sadovy, Y., and M. L. Domeier. 2005. Perplexing problems of sexual patterns in the fish genus *Paralabrax* (Serranidae, Serraninae). Journal of Zoology (London) 267:121-133.

Sancho, G., A. R. Solow, and P. S. Lobel. 2000. Environmental influences on the diel timing of spawning in coral reef fishes. Marine Ecological Progress Series 206:193-212.

Sauro, J., and J. R. Lewis. 2005. Estimating completion rates from small samples using binomial confidence intervals: comparisons and recommendations. Proceedings of the Human Factors and Ergonomics Society 49:2100-2104.

SCCOOS (Southern California Coastal Ocean Observing System). 2013. Sea surface temperatures. Available from: http://www.sccoos.org/

Turner, C. H., E. E. Ebert, and R. R. Given. 1969. Man-made reef ecology. Fish Bulletin 146:1-221.

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