

## **BACKGROUND AND OBJECTIVES**

Barred sand bass (Paralabrax nebulifer) has been a popular sport fish in southern California for decades. During the summer spawning months barred sand bass are vulnerable to harvest impacts because they form large spawning aggregations that are easily targeted by sport fishermen (Jarvis et al. 2010). Barred sand bass catch-perunit-effort has notably declined in recent years, which has raised concerns about the sustainability of the resource and has prompted research that will form the basis for evaluating the status of the stock.

Spawning fraction (the proportion of females spawning on any given day) and spawning frequency (the number of spawning events per female) are essential parameters for a future stock assessment because they are used to calculate spawning output. A previous study on the reproductive biology of barred sand bass reported a 1.6 day spawning interval (number of days between spawning events; Oda et al. 1993). The samples in their study were collected during a two-week period in July, which the authors noted was the reproductive "subseason". To determine if barred sand bass spawning varies across an entire spawning season, we quantified ovarian activity using histological crosssections from barred sand bass ovaries collected throughout the known spawning season and into September. Improved estimates of reproductive timing and parameters should enhance fisheries management of this popular sport fish.

Our objective was to quantify the following barred sand bass reproductive parameters over the entire spawning season and by month: 1) spawning fraction, interval, and frequency, 2) the proportion of daily spawners and non-spawners, and 3) the proportion of females with ovarian follicular atresia (*i.e.*, degenerating ovarian follicles).

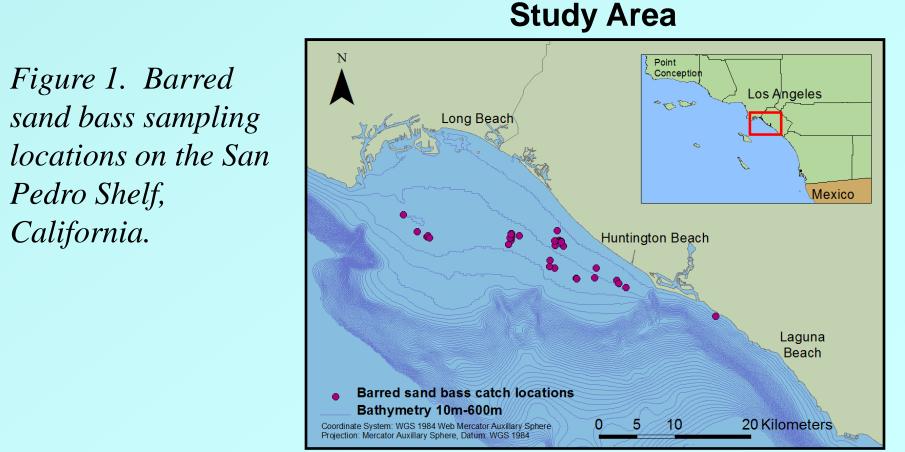
#### METHODS

Adult barred sand bass were collected at several locations on the San Pedro Shelf by hook-and-line or spear from June to September 2011 (Figure 1). For each fish we recorded standard and total length (mm), weight (0.01) kg), time of capture, and fishing location. All fish were humanely sacrificed. The gonads were removed and weighed to the nearest 0.01 g, fixed in 10% formalin for 7-10 days, and stored in 70% ethanol. Cross-sections (2-3 mm thick) were taken from the center of the gonad and embedded in paraffin wax for histological analysis. Serial sections (6µm thick) were cut on a microtome, mounted on slides, and stained using hemotoxylin and eosin (Loke-Smith et al. 2010). Oocytes were categorized into the following eight developmental stages according to Lowerre-Barbieri et al. (2011): primary growth (PG), cortical alveolar (CA), vitellogenic I, II, and III (vtg-I,II,III), germinal vesicle migration (MN), hydration (H), and postovulatory follicle (POF) (Figure 2).

Figure 1. Barred

Pedro Shelf,

California.



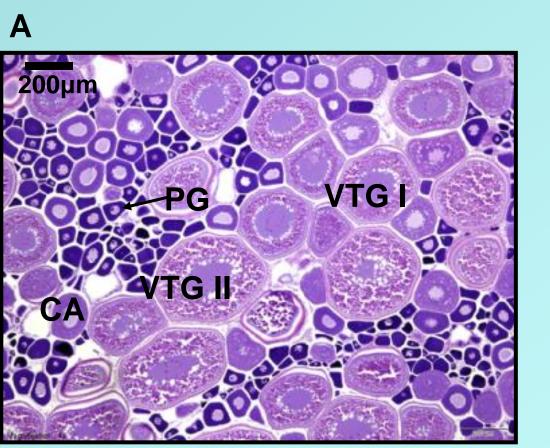
The most commonly used method for estimating spawning fraction in multiple spawning fishes is the postovulatory follicle method (Ganias et al., 2011). A barred sand bass postovulatory follicle aging key based on timed serial sacrifices (Oda et al. 1993) was generated from labeled histological slides archived at the Natural History Museum of Los Angeles. The slides were referenced to assign ages to those POFs identified in the current study (e.g., day 0 = 100 less than 4 hours old, day 1 = 4 to 24 hours old, and day 2 + = 3 greater than 24 hours old; Figure 2, 3). Criteria for our spawning fraction calculations were fish with day 0 and/or day 1 POFs. Non spawning fish were females with no evidence of new or old postovulatory follicles. Daily spawning activity was identified by the presence of at least one of the four following combinations of oocyte developmental stages according to Oda et al. (1993): day 1 POFs and MN, day 1 POFs and H, day 1 and day 2+ POFs, day 0 and day 1 POFs, and day 0 POFs and H (Figure 2, 3). The presence of ovarian follicular atresia was assigned to females having multiple atretic follicles. Monthly differences in reproductive parameters were tested using Chi Square Test of Homogeneity (alpha= 0.05) and Bonferroni multiple comparisons ad hoc. We report Adjusted Wald 95% confidence intervals (Sauro and Lewis 2005) with proportion data.

# **IMPROVED ESTIMATE OF SPAWNING FRACTION, INTERVAL, AND FREQUENCY FOR BARRED SAND BASS, AN AGGREGATE SPAWNER IN SOUTHERN CALIFORNIA**

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## RESULTS

1) Spawning condition was indicated by the presence or absence of postovulatory follicles (POFs), migratory nuclei, hydrated oocytes, and atretic follicles.



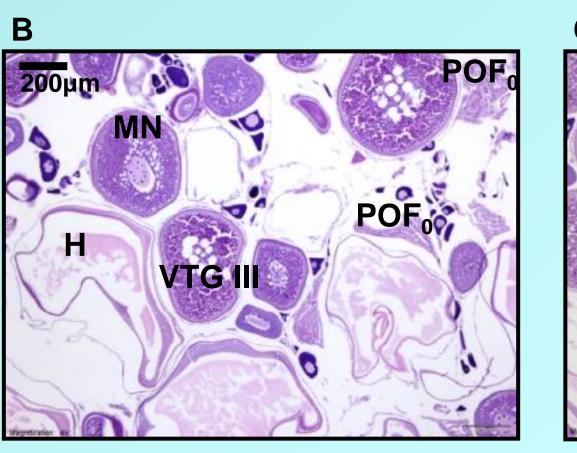
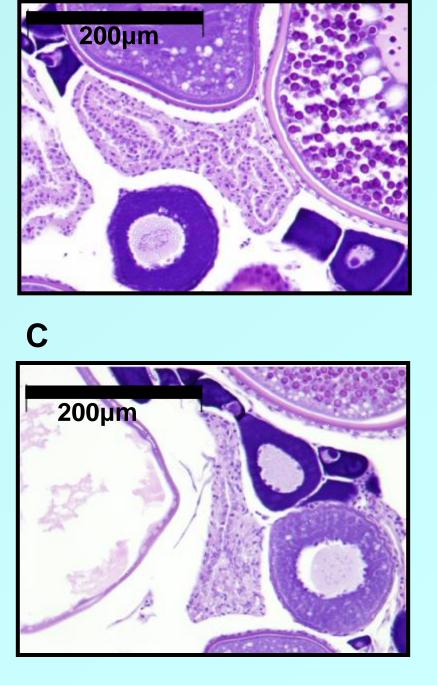


Figure 2. Images of representative ovary sections at 4x magnification for females collected in A) June (developing), B) July (spawning capable), C) August (spawning capable), and D) September (regressing). State of reproductive phases were described according to Brown-Peterson et al. (2011). Stages of follicle development labeled in figures A-D: PG=primary growth, CA=cortical alveolar, VTG(I,II,III)=vitellogenic(I,II,III), MN=migratory nucleus, H=hydrated oocyte, POF<sub>0</sub>=day 0 postovulatory follicle,  $POF_1 = day 1$  postovulatory follicle,  $POF_2 = day 2 + postovulatory follicle, A = atretic follicle.$ 

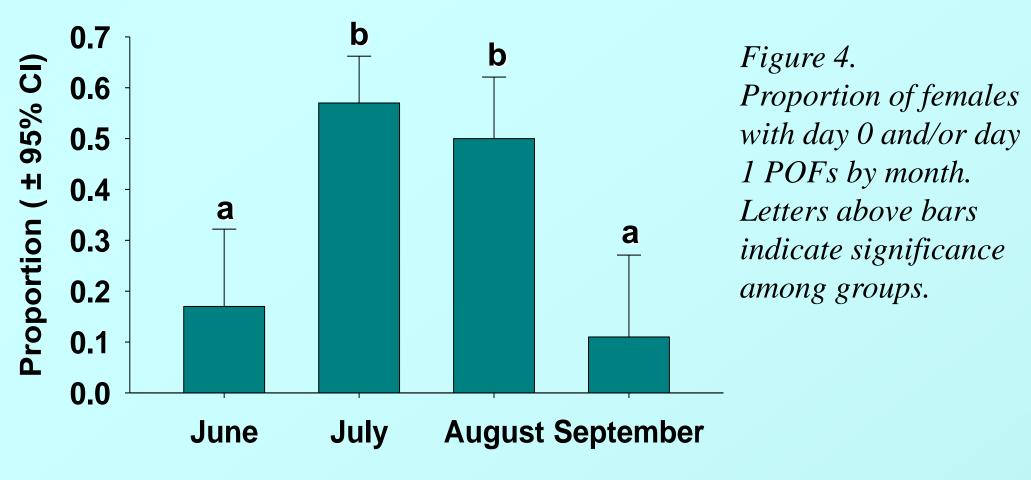
2) The age of postovulatory follicles indicated how recently females had spawned.





*Figure 3. Images of representative* ovary sections at 20x magnification for females with A) day 0 POF (spawned within the last 4 hr), B) day 1 POF (spawned between 4 and 24 hr ago), and C) day 2+POF (spawned 24 hr or more prior to collection).

#### 4) The proportion of females spawning (spawning fraction) was highest in July and August.



5) Evidence of recent spawning activity was highest in July.

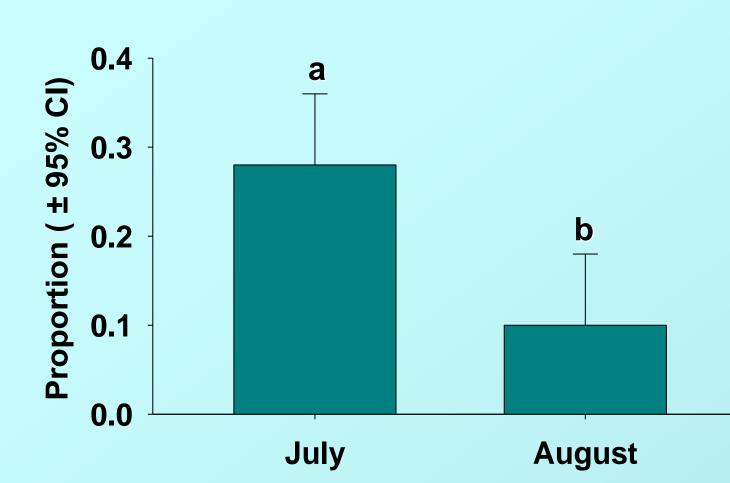
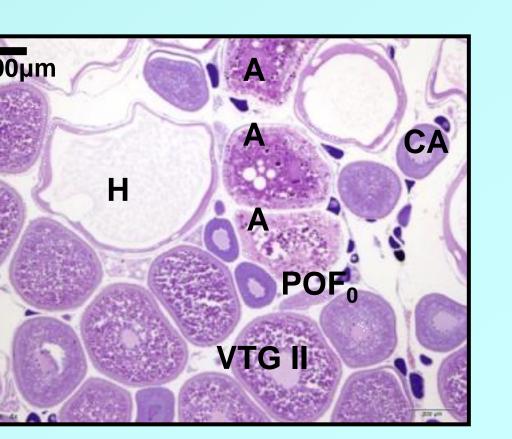
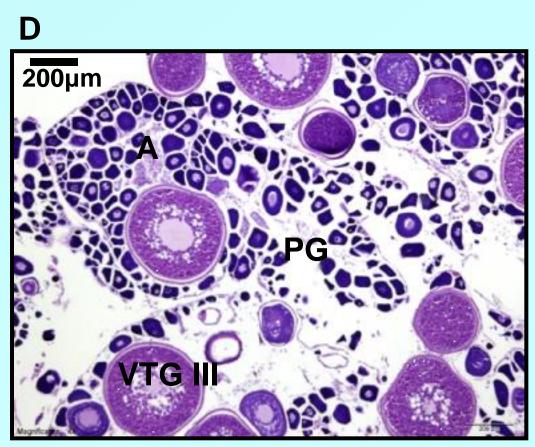


Figure 5. *Proportion of females* with day 0 POFs (age < 4 hr) during peak spawning months. Letters above bars indicate significance among groups.





#### 3) The spawning interval, frequency of spawning events, and the proportion of daily spawners varied by sampling month.

Table 1. Spawning interval and frequency, and proportion of females showing evidence of daily spawning by sampling month.

	Spawning Interval (days)	Spawning Frequency (events)	Proportion of Daily Spawners
June	6.00	5.00	0.08
July	1.74	17.80	0.44
August	2.00	15.50	0.38
September	9.00	3.33	0.00

#### 6) The proportion of non-spawning females was highest in June and September.

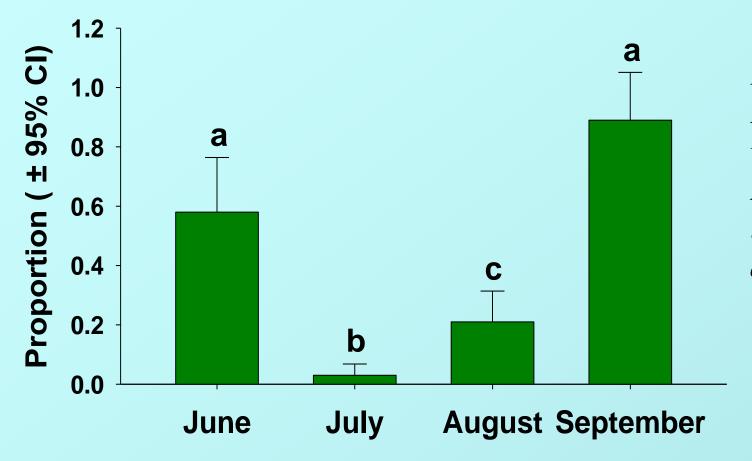


Figure 6. *Proportion of females* without POFs. *Letters above bars* indicate significance among groups.

#### 7) The incidence of follicular atresia was highest in September, indicating the end of spawning season.

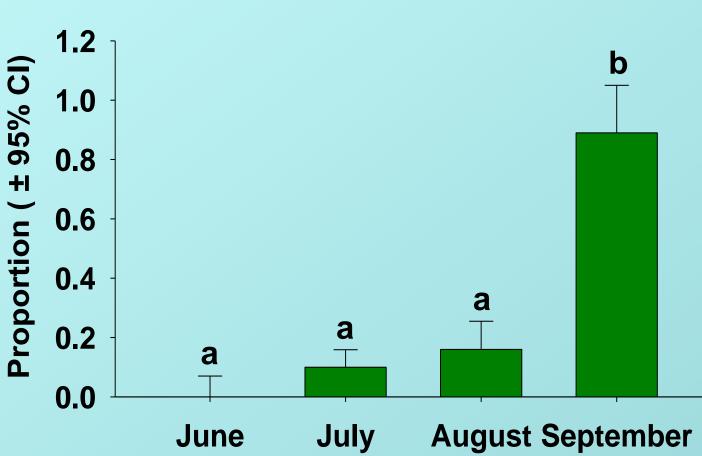


Figure 7. *Proportion of females* with atretic follicles. Letters above bars *indicate significance* among groups.

Accurate measures of barred sand bass spawning seasonality are necessary for quantifying reproductive potential; however, barred sand bass spawning seasonality in the literature ranges from three months, June-August, in Clark (1932) to six months, April-September, in Love (2011). Clark's estimate was based on gross observations of barred sand bass ovaries in commercially landed fish from May to September while other reports perhaps were anecdotal or based on the spawning seasonality of kelp bass, P. clathratus, a southern California congener. Eighty years after Clark (1932) reported her findings, our examination of barred sand bass histological ovary sections indicates similar spawning season duration.

Spawning frequency is an estimate of spawning potential that is derived using spawning interval. Our spawning interval estimates varied by month, which highlights the importance of sampling throughout the spawning season in order to obtain a realistic estimate of the total number of spawning events per individual per year.

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K. Evans, H. Gliniak, A. Helget, O. Horning, M. Kibby and K. Lakos (*R*/V Garibaldi), and the CDFW Dive Team and Natural Resource Volunteers assisted with sample collection; B. Macewicz (NOAA Fisheries) provided technical expertise on fish gonad histology; R. Feeney (Natural History Museum of Los Angeles) provided the archived barred sand bass histology slides. This study was funded by the Federal Aid in Sport Fish Restoration Act Grant #F-50-R-24.



### DISCUSSION

> Our histological examination of barred sand bass ovaries indicated females collected on the San Pedro Shelf spawned for approximately three months in 2011, and reproductive parameters varied monthly within the spawning season.

The July spawning interval (1.74) calculated in the present study is similar to the previous estimate obtained in late July (1.67) by Oda et al. (1993) suggesting the spawning fraction has not significantly changed over time. Future estimates of spawning interval should be calculated to verify its consistency over time. The annual spawning fraction of another batch spawner in southern California, Pacific sardine, Sardinops sagax, was similarly consistent over time (Macewicz et al. 1996).

Water temperature can affect POF re-absorption rates in multiple spawning fishes (Hunter and Macewicz 1985; Lowerre-Barbierrie et al. 2011). Although the POF aging key we used in the current study was based on barred sand bass collected in 1988 (Oda et al. 1993), the average sea surface temperature during our study (18.9  $\pm$  1.3 °C) was within the range of water temperatures reported in the previous study (16.9 - 19.9 °C) providing confidence in our spawning parameter estimates.

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## ACKNOWLEDGMENTS

