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Toward responsible stock enhancement: broadcast spawning dynamics and adaptive genetic management in white seabass aquaculture

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Abstract

The evolutionary effects captive-bred individuals that can have on wild conspecifics are necessary considerations for stock enhancement programs, but breeding protocols are often developed without the knowledge of realized reproductive behavior. To help fill that gap, parentage was assigned to offspring produced by a freely mating group of 50 white seabass (*Atractoscion nobilis*), a representative broadcast spawning marine finfish cultured for conservation. Similar to the well-known and closely related red drum (*Sciaenops ocellatus*), *A. nobilis* exhibited large variation in reproductive success. More males contributed and contributed more equally than females within and among spawns in a mating system best described as lottery polygyny. Two females produced 27% of the seasonal offspring pool and female breeding effective size averaged 1.85 per spawn and 12.38 seasonally, whereas male breeding effective size was higher (6.42 and 20.87, respectively), with every male contributing 1–7% of offspring. Further, females batch spawned every 1–5 weeks, while males displayed continuous reproductive readiness. Sex-specific mating strategies resulted in multiple successful mate pairings and a breeding effective to census size ratio of ≥0.62. Understanding a depleted species' mating system allowed management to more effectively utilize parental genetic variability for culture, but the fitness consequences of long-term stocking can be difficult to address.

Introduction

The temporal and spatial aggregation of adults during broadcast spawning facilitates concentration of gametes in the water column, which helps increase fertilization rates (Pennington 1985; Oliver and Babcock 1992; Hedgecock 1994). Energetic investment is often placed in the number of gametes released, rather than quality (with offspring exhibiting classic Type III survivorship). As multiple parents are involved and clutch sizes are very large, the dynamics of broadcast spawning are best assessed through genetic determination of parentage.

Here, we used genetic parentage analyses to perform an intensive investigation into mating patterns and reproductive success in a broadcast spawning pelagic marine finfish in culture (e.g., Rowe et al. 2007; Rowe and Hutchings 2008). Furthermore, we presuppose it allowed one of the most comprehensive assessments of reproductive dynamics in a relatively large breeding group of any fish that may approximate a spawning aggregation in the wild. Our study system was the white seabass (*Atractoscion nobilis* Ayres 1860) replenishment hatchery in Carlsbad, California, USA, where four breeding groups of 50 fish each are maintained in equal sex ratio (Bartley et al. 1995; see 'Supporting information' for a history and description of the program). To induce year-round spawning, water temperature and photoperiod are controlled to mimic natural seasonal conditions and offset in each pool. No hormones or strip spawning are used, and fish freely mate within their breeding group.

Very rarely does a culture program have the operational capacity to permit free mate choice. This makes the

A. nobilis system unique, even in relation to well-known programs, such as those for red drum *Sciaenops ocellatus* (McEachron et al. 1993; Smith et al. 2003; Tringali et al. 2008), Pacific salmonids *Oncorhynchus* spp. (Waples 1994; Reisenbichler et al. 2004; Thrower and Joyce 2004), and Atlantic cod *Gadus morhua* (Jørstad 2004). Beyond its general interest to those that study marine mating systems, we anchor this research in specific context by comparing knowledge gained of *A. nobilis* spawning to that of the closely related *S. ocellatus*, arguably the most comprehensively assessed sciaenid (family Sciaenidae; drums, grunts, and croakers) in terms of genetics (e.g., Gold 2004; Gold et al. 2008, 2010).

Not only was our intent to advance understanding of the complexity of broadcast spawning in a marine finfish, we also hoped to adapt hatchery management to an exploited species' mating system (e.g., Rowe and Hutchings 2003). Genetics has become integral to stocking programs worldwide (Allendorf and Ryman 1987; Blankenship and Leber 1995; Leber 2004; Taniguchi 2004; Lorenzen et al. 2010), and *A. nobilis* is one of many such species reared for conservation. The goals of the *A. nobilis* program remain experimental in nature, however, oriented toward assessing the economic, environmental, and biologic feasibility of replenishment, and as such, have not been formally integrated into the species' management plan (California Department of Fish and Game (CDFG), Marine Region 2002). Our associated genetics research has been focused primarily on ensuring that the fish released for replenishment do not have a significant negative impact on the natural population. Here, we asked questions that had practical application toward developing best management practices, such that brood fish are semi-representative of the wild population and stockable juveniles maintain a sufficient amount of the genetic variability available in the parental generation:

1 Who is reproducing with whom and when? Estimating the proportional contribution of parents within and among spawns will help elucidate mating patterns, strategies, and systems, enabling broodstock management (e.g., census size, sex ratio) to be tailored to a species' mode of reproduction. A lack in knowledge of captive spawning dynamics was a recognized shortcoming of the original *A. nobilis* plan (Bartley et al. 1995) and many others (Rowe and Hutchings 2003).

2 What was the breeding effective size within and among spawns? Past research on *S. ocellatus* and *A. nobilis* indicated that individual spawns may have a low effective number of breeders (often less than five; Coykendall 2005; Gold et al. 2008), which would require close management to maintain effective size in the fish grown for release. Further, understanding the relationship between realized effective and census size may aid in optimizing broodstock census size, a critical factor in evaluating the inbreeding potential of a culture system (Duchesne and Bernatchez 2002).

3 Can we estimate female fecundity and spawning periodicity? It was assumed *A. nobilis* batch spawned, with females releasing small, pelagic eggs several times during a spawning season (Moser et al. 1983; Donohoe 1997), but repeat spawning was never definitively documented. Because females produce tangible indicators of spawning (eggs), estimating their reproductive potential in conjunction with breeding effective size may enhance our ability to choose and manage spawns (e.g., numbers per cohort, cohort mixing) suitable for juvenile production.

Based on answers to these questions, we present a genetic management system intended to be modifiable to fit stock enhancement programs for comparable species elsewhere. As it is known that culture-based stocking can have negative fitness impacts over very few generations (e.g., Araki et al. 2007) that can result in a mixed population less able to respond to stochastic environmental change (Tringali and Bert 1998; Taniguchi 2004), we also discuss the effects releasing these cultured fish might have had on the wild population.

Materials and methods

Sample collection

This study focused on one brood group of 25 males and 25 females designated 'B2' (Table 1). Fin clips were collected from every brood fish by clipping a 1- to 2-cm^2 section of caudal fin soft rays. Yolk sac larvae (YSL) were acquired by quasi-randomly subsampling and incubating 10 mL of floating (i.e., fertilized) eggs from each broadcast spawning event that occurred in B2 in 2008. Brood fish typically spawned during a discrete time period in early to late evening, and a spawning event or 'spawn' was defined as the sum of eggs or offspring produced during a single evening. Subsampled eggs hatched into YSL within 48 h, and random subsample of 10–15 mL of 1–2 days posthatch (dph) YSL was collected. Tissue samples were preserved with 95–100% undenatured ethanol (EtOH). Additional description of the broodstock populations, spawn induction, and YSL rearing is presented in the 'Supporting information'.

Molecular methods

Genomic DNA was extracted from ~50 mg of fin clip or whole YSL by boiling and centrifugation, using a 10% Chelex 100 resin (200–400 mesh) extraction buffer containing 1% Tween 20 and 1% Igepal CA-630. A panel of

Table 1. *Atractoscion nobilis* in Carlsbad hatchery brood group B2 during the 2008 spawning season.

	Genetic ID	Time in captivity (years)	Age (years)	Body mass (kg)
Females				
	502	12.2	16.3	18.9
	503	11.7	15.7	18.6
	510	9.7	12.3	16.3
	512	9.7	12.3	16.3
	520	9.7	13.8	17.5
	521	9.7	12.6	16.6
	522	8.8	12.4	16.4
	523	8.8	13.2	17.0
	525	8.8	14.5	18.0
	526	8.8	13.6	17.4
	527	6.7	10.4	14.3
	528	6.7	12.2	16.2
	530	8.8	12.7	16.7
	531	8.8	11.2	15.2
	532	8.8	16.2	18.9
	535	6.7	11.3	15.4
	536	8.8	12.8	16.7
	538	6.7	12.6	16.5
	541	8.8	12.9	16.8
	544	7.8	12.4	16.4
	545	7.8	11.7	15.7
	546	7.8	11.9	15.9
	548	7.8	12.0	16.0
	549	4.6	8.5	11.7
	550	4.6	8.5	11.7
Mean		*8.3*	*12.6*	*16.3*
Median		*8.8*	*12.4*	*16.4*
SD		*1.8*	*1.9*	*1.8*
Total				*407.1*
Males				
	501	11.7	13.3	14.4
	504	9.7	11.9	13.4
	505	11.7	15.6	15.7
	506	11.7	15.2	15.5
	507	11.7	13.6	14.6
	508	11.7	14.2	14.9
	509	11.7	13.7	14.6
	511	9.7	13.1	14.2
	513	9.7	12.6	13.9
	514	9.7	13.0	14.2
	515	9.7	13.1	14.3
	516	9.7	13.1	14.3
	517	9.7	13.5	14.5
	518	9.7	11.8	13.3
	519	9.7	13.1	14.2
	524	8.8	15.3	15.5
	529	8.8	12.7	14.0
	533	8.8	12.1	13.5
	534	8.8	15.4	15.6
	537	8.8	13.7	14.6
	539	8.8	13.0	14.2
	540	8.8	14.4	15.0
	542	7.8	11.3	12.9
	543	7.8	13.1	14.3
	547	7.8	11.9	13.4
Mean		*9.7*	*13.3*	*14.4*
Median		*9.7*	*13.1*	*14.3*
SD		*1.3*	*1.2*	*0.7*
Total				*359.0*

Brood fish listed by sex and genetic ID.
All fish collected at Santa Catalina Island, except female 502 from Santa Cruz Island, between Nov 15, 1995, and Jun 14, 2003.
Information includes time in captivity and VBGF-estimated age and body mass reported as of Jan 15, 2008, at spawning season inception.
Summary statistics listed in bottom rows in italics.

five *A. nobilis*-specific nuclear microsatellite loci (*Ano*A, *Ano*D, *Ano*E, *Ano*R, and *Ano*Z; Table 2) developed by Franklin (1997) was polymerase chain reaction (PCR) amplified on all 50 brood fish and 60 randomly chosen YSL per spawn. PCR was performed in 10 μL volumes containing 1 μL of template DNA, 2 pmol each primer, 0.25 U GoTaq® DNA Polymerase (Promega, Madison, WI, USA), 2 μL 5X GoTaq® Colorless Master Mix (1.5 mM $MgCl_2$), 1 μg bovine serum albumin (BSA) (except *Ano*D), and 2 μM each dNTP final concentration. Thermal cycling took place in DNA Engine (Bio-Rad Laboratories, Hercules, CA) and PE9700 (Perkin Elmer, Waltham, MA, USA) PCR machines. Conditions included an initial two-minute denaturation at 95°C, followed by 35 three-step cycles: 30-s denaturation at 95°C, 30-s annealing (48°C for *Ano*D and *Ano*Z; 54°C for *Ano*A and *Ano*E; and 58°C for *Ano*R), and 30-s extension at 72°C. Genotypes were generated on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and scored using GeneMapper v4.0 software (Applied Biosystems). Samples that could not be scored were re-amplified; those that failed a second time were excluded from analyses.

Data analyses

Genetic relatedness among brood fish was estimated using ML-RELATE (Kalinowski et al. 2006). Locus-specific statistics for the broodstock, including the number of alleles, expected and observed heterozygosity, polymorphism information content (PIC), and parental nonexclusion probability (NE), were generated with Cervus v3.0 (Kalinowski et al. 2007). PIC and NE were used to preliminarily gauge the power of each locus and the panel as a whole for assigning parentage. PIC was defined as the probability of identifying which homologue of a given parent was transmitted to an offspring or the probability

Table 2. Microsatellite summary statistics for the *Atractoscion nobilis* B2 broodstock.

Locus	Repeat	*N*	*k*	H_e	H_o	PIC	NE
Ano A	ATA_n	50	12	0.822	0.800	0.793	0.165
Ano D	CA_n	50	35	0.961	0.880	0.949	0.017
Ano E	AAT_n	50	9	0.780	0.700	0.743	0.231
Ano R	TTA_n	50	9	0.784	0.720	0.746	0.234
Ano Z	ACT/AAT_n	50	20	0.931	0.900	0.917	0.040
All loci		*50*	*17*	*0.856*	*0.800*	*0.830*	*<0.001*

General information includes locus name, repeat type, and number of brood fish genotyped (*N*).
Summary statistics estimated include number of alleles (*k*), expected (H_E) and observed (H_O) heterozygosity, polymorphism information content (PIC), and nonexclusion probabilities for parental pairs (NE).
Means and combined NE for all loci listed in bottom row in italics.

a parent is heterozygous multiplied by the probability the offspring is informative (Botstein et al. 1980). NE was defined as the probability of a random match between parent and offspring at a locus or one minus the probability an individual can be rejected as a candidate parent caused by a mismatch in alleles. Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) among loci were evaluated using GENEPOP v4.0.10 (Rousset 2008).

Parentage analyses were also performed with Cervus, which used a combination of exclusion and likelihood ratio tests to determine the most likely candidate parents for each YSL. Default settings were used: proportion of loci typed for each spawn = 1.00, minimum number of loci required for individual assignment = 2, proportion of loci mistyped = 0.01, and error in likelihood calculations = 0.01. The confidence level for the true proportion of offspring assigned parents was set at 95% during both simulations (to assess power to assign parentage) and sample analysis. The parental combination generating the top pair confidence score for each YSL relative to critical likelihood values determined in simulations was used in further analyses.

Parameter estimates (designated throughout with a '^') of proportional parental contribution; effective number of female, male, and total breeders (N_f, N_m, and N_b, respectively); batch, seasonal (annual), and maximum fecundity (f_b, f_s, and f_m, respectively); and cyclicity were performed in Microsoft™ Excel® 2007. Proportional contribution was defined as the number of YSL produced by a single parent divided by the total number of YSL sampled. Demographic (parentage-based) estimates of per spawn and seasonal breeding effective sizes (where $N \approx 60$ and 4,249 YSL, respectively; see Results) were denoted as $\hat{N}_f^d$, $\hat{N}_m^d$, and $\hat{N}_b^d$ and took into account variation in the number of contributors within and between the sexes [$N_b N_b = 4N_m N_f \cdot (N_m + N_f)^{-1}$, where N_m and $N_f = \sum_{k=1}^{n} q_k^{-2}$, n is the census number of males or females that contributed, and q is the proportion of offspring contributed by each male or female; Gold et al. 2008]. Seasonal breeding effective size was also derived genetically (denoted $\hat{N}_b^g$) from LD among the entire pool of multilocus offspring genotypes ($N = 4{,}249$) under the random mating model in LDNE v1.13 (Waples and Do 2008). The number of offspring produced per brood fish per spawn was defined as f_b, and f_s was measured as the sum of offspring produced per brood fish during the spawning season. Number of eggs kg^{-1} was determined using f_b and female body mass and age as of Jan 15, 2008, at the inception of the spawning season. Estimates of mass and age depended on known size-at-age relationships for captive and wild *A. nobilis*. Captive, sex-specific von Bertalanffy growth function (VBGF; von Bertalanffy 1938) curves were derived from multiple measurements of growth in 94 male and 79 female brood fish over 5–10 years (y) at the Carlsbad hatchery (M.A. Shane, HSWRI, unpublished data). VBGF curves for wild fish (Hervas et al. 2010) were used to estimate age prior to introduction to B2. Cyclicity was defined as the number of days (d) between serial or periodic spawns per female.

Results

Locus-specific statistics for the broodstock are presented in Table 2. All 50 DNA samples amplified at all five loci. Number of alleles ranged from nine in *Ano*E and *Ano*R to 35 in *Ano*D, with an average of 17. There was marginally significant evidence for LD between *Ano*E and *Ano*R ($P = 0.04$; all other $P > 0.14$). Our power to assign parentage appeared sufficient. Each brood fish exhibited a unique multilocus genotype, and 87% (1065 of 1225) of brood fish pairs were classified as unrelated in pairwise comparisons, regardless of sex. Mean PIC was fairly high, and NE for parental pairs was low (0.830 and <0.001, respectively; Table 2). Simulations of 10^5 offspring derived from the broodstock genotypes predicted 100% biparental assignment at the 95% confidence level.

The photothermal regime and associated egg production for B2 are shown in Fig. 1. Eighty-four spawns

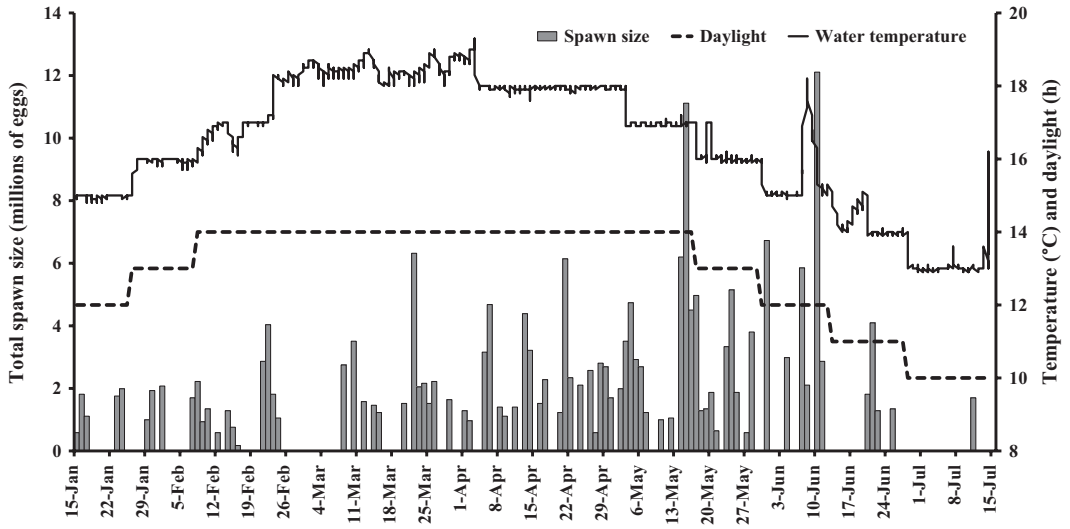

Figure 1 Spawning season profile in 2008 for *A. nobilis* in brood pool B2 at the Carlsbad 2 hatchery. Weekly chronological date is shown on *x*-axis. Photothermal cycling regime for three spawning season induction is shown by solid lines (actual water temperature in °C) and broken four lines (hours of daylight). Vertical gray bars represent total spawn size in millions of eggs. Five absence of a gray bar indicates no spawn occurred that day.

occurred between Jan 15, and Jun 11, 2008, and YSL were preserved for 71 spawns (85%); eggs were unviable for 13 spawns, and no YSL could be collected. Summed across the 71 spawns, nearly 1.8×10^8 eggs were produced. Hatch rates were >90%, and it was assumed there was no significant differential survival between the egg and YSL stages. Over 2×10^4 genotypes were generated from 4249 YSL. The loci appeared to display Mendelian inheritance in the YSL: all alleles were also present in the parental generation, and all loci were in HWE, with no evidence of homozygote deficiency or excess (all $P > 0.05$). The realized biparental assignment rate was 99–100% at the 95% confidence level. Eleven YSL (0–2 per spawn; <0.3%) failed to PCR amplify clearly or could not be assigned parents and were excluded from the data set.

Proportional contribution and breeding effective size

There was large variation in reproductive success within and between the sexes. Females 525 and 528 combined contributed 27% of offspring produced during the season, whereas 520, 521, and 527 did not effectively contribute. The remaining 20 females contributed 1–10% [mean ($\bar{x}$) = 0.04, SD (S) = 0.03] each. $\hat{N}_f^d$ per spawn was low ($\bar{x}$ = 1.85, S = 1.74) but was 12.38 (95% CI of 8.59–17.73) for the spawning season. Forty-nine spawns were the result of between one and 1.5 effective female breeders, requiring a primary female to contribute to >80% of offspring; 36 spawns had a single female assigned as parent to ≥95% of offspring ($1.00 \leq \hat{N}_f^d \leq 1.11$; e.g., 15 Jan, 24 Feb, or 7 Jun; Table 3; see also Table S1A). Occasionally, multiple females contributed more equally to a spawn (20–50% each; e.g., 22 Feb, 20 Mar, 28 Apr), but this occurred in less than one in three spawns. In contrast, all 25 males contributed 1–7% ($\bar{x}$ = 0.04, S = 0.02; see also Table S1B) of the seasonal pool of offspring, and $\hat{N}_m^d$ was higher both per spawn ($\bar{x}$ = 6.42, S = 3.04) and seasonally ($\hat{N}_m^d$ = 20.87, 95% CI of 11.97–42.48).

For the sexes combined, $\hat{N}_b^d$ per spawn was low ($\bar{x}$ = 5.31, S = 3.62), but$\hat{N}_b^d$ = 31.09 (95% CI of 20.01–50.44) when evaluated for the season. Seasonal estimates via the LD method were generally higher, although there was CI overlap: $\hat{N}_b^g$ = 43.0 [95% CI of 39.3–46.8 (parametric) or 38.5–47.7 (jackknifing over loci)] for allele frequencies down to 0.02; $\hat{N}_b^g$ = 43.7 and 53.5 for allele frequencies down to 0.01 and 0.05, respectively. $\hat{N}_b^d$ was an order of magnitude less than census size [N = 50; $\hat{N}_b^d \cdot N^{-1}$: $\bar{x}$ = 0.11, median ($\tilde{x}$) = 0.08, S = 0.07] per spawn but demographic and genetic $\hat{N}_b \cdot N^{-1} \geq 0.62$ for the season.

Batch spawning patterns

The average female contributed to 10.5 (S = 5.7) spawns. Time between batch spawns was variable within and among females and ranged from 1 to 5 weeks ($\bar{x}$ = 23.9 d, S = 15.3 d; see also Table S1A). Cycle time depended on the particular female and her progression through the spawning season. A few females started spawning (e.g., 508, 546) or spawned more frequently (e.g., 510, 530, 550) beginning in mid-May, after the photothermal height of the season (Fig. 1). No females

Table 3. Census (n) and demographic breeding effective sizes (denoted here as $\hat{N}_f$, $\hat{N}_m$, and $\hat{N}_b$) per spawn event and practical application of the data via the female equivalent (fe).

				Females				Males		Both sexes	
Spawn	Date	No. of eggs ($\times 10^6$)	Spawn vol. (L)	$\hat{n}$	$\hat{N}_f$	fe	fe–$\hat{N}_f$	$\hat{n}$	$\hat{N}_m$	$\hat{n}$	$\hat{N}_b$
1	15-Jan	0.59	1.00	1	1.00	1	0.00	13	7.53	14	3.53
2	16-Jan	1.81	3.10	1	1.00	1	0.00	8	2.41	9	2.83
3	17-Jan	1.11	1.90	2	1.03	1	−0.03	9	4.75	11	3.40
4	29-Jan	0.99	1.70	1	1.00	1	0.00	9	5.68	10	3.40
5	30-Jan	1.93	3.30	2	1.64	1	−0.64	11	6.77	13	5.29
6	1-Feb	2.08	3.55	3	1.15	2	0.85	10	6.51	13	3.90
7	7-Feb	1.70	2.90	2	1.03	1	−0.03	11	5.64	13	3.50
8	8-Feb	2.22	3.80	1	1.00	2	1.00	10	3.70	11	3.15
9	9-Feb	0.94	1.60	2	1.03	1	−0.03	13	2.09	15	2.77
10	10-Feb	1.35	2.30	1	1.00	1	0.00	9	3.80	10	3.17
11	12-Feb	0.59	1.00	1	1.00	1	0.00	11	6.57	12	3.47
12	14-Feb	1.29	2.20	1	1.00	1	0.00	12	6.95	13	3.50
13	15-Feb	0.76	1.30	1	1.00	1	0.00	4	2.52	5	2.86
14	21-Feb	2.87	4.90	3	1.35	2	0.65	15	9.38	18	4.72
15	22-Feb	4.04	6.90	11	7.73	2	−5.73	12	7.14	23	14.85
16	23-Feb	1.81	3.10	1	1.00	1	0.00	13	6.21	14	3.44
17	24-Feb	1.05	1.80	4	1.11	1	−0.11	11	4.42	15	3.54
18	8-Mar	2.75	4.70	1	1.00	2	1.00	11	6.25	12	3.45
19	10-Mar	3.51	6.00	4	1.76	2	0.24	12	4.97	16	5.19
20	12-Mar	1.58	2.70	2	1.03	1	−0.03	13	3.07	15	3.09
21	14-Mar	1.46	2.50	1	1.00	1	0.00	4	2.33	5	2.80
22	15-Mar	1.23	2.10	1	1.00	1	0.00	10	6.14	11	3.44
23	20-Mar	1.52	2.60	14	8.53	1	−7.53	9	4.69	23	12.10
24	22-Mar	6.32	10.80	10	3.47	4	0.53	21	6.75	31	9.17
25	23-Mar	2.05	3.50	3	1.11	1	−0.11	12	6.98	15	3.83
26	26-Mar	2.22	3.80	3	1.11	1	−0.11	17	13.24	20	4.08
27	2-Apr	0.97	1.65	3	1.07	1	−0.07	9	4.37	12	3.44
28	5-Apr	3.16	5.40	2	1.07	2	0.93	10	3.69	12	3.31
29	6-Apr	4.68	8.00	3	1.75	3	1.25	13	5.36	16	5.28
30	8-Apr	1.40	2.40	4	2.02	1	−1.02	10	5.14	14	5.81
31	9-Apr	1.11	1.90	1	1.00	1	0.00	16	7.69	17	3.54
32	11-Apr	1.40	2.40	1	1.00	1	0.00	9	5.44	10	3.38
33	13-Apr	4.39	7.50	4	2.71	3	0.29	11	8.33	15	8.19
34	14-Apr	3.22	5.50	3	1.26	2	0.74	14	7.44	17	4.32
35	20-Apr	1.23	2.10	4	1.42	1	−0.42	12	2.99	16	3.84
36	21-Apr	6.14	10.50	14	3.81	4	0.19	22	11.92	36	11.55
37	22-Apr	2.34	4.00	6	1.23	2	0.77	18	11.11	24	4.43
38	24-Apr	2.11	3.60	3	1.14	2	0.86	15	9.63	18	4.09
39	26-Apr	2.57	4.40	4	1.11	2	0.89	12	3.41	16	3.34
40	27-Apr	0.59	1.00	3	1.22	1	−0.22	8	4.59	11	3.86
41	28-Apr	2.81	4.80	4	2.99	2	−0.99	12	5.84	16	7.90
42	30-Apr	1.70	2.90	2	1.68	1	−0.68	11	6.50	13	5.35
43	2-May	1.99	3.40	2	1.40	1	−0.40	9	5.84	11	4.52
44	3-May	3.51	6.00	5	1.23	2	0.77	15	7.53	20	4.23
45	4-May	4.74	8.10	5	2.28	3	0.72	15	10.98	20	7.54
46	5-May	2.93	5.00	5	1.23	2	0.77	15	10.11	20	4.38
47	6-May	2.69	4.60	3	2.02	2	−0.02	14	8.04	17	6.46
48	7-May	1.23	2.10	2	1.03	1	−0.03	7	2.16	9	2.80
49	12-May	1.05	1.80	2	1.03	1	−0.03	13	6.62	15	3.58
50	14-May	6.20	10.60	6	4.74	4	−0.74	19	15.25	25	14.46
51	15-May	11.12	19.00	17	6.81	7	0.19	23	15.40	40	18.88
52	16-May	4.50	7.70	4	2.95	3	0.05	20	9.38	24	8.97
53	17-May	4.97	8.50	4	1.11	3	1.89	10	2.85	14	3.19

Table 3. Continued

Spawn	Date	No. of eggs ($\times 10^6$)	Spawn vol. (L)	Females				Males		Both sexes	
				$\hat{n}$	$\hat{N}_f$	*fe*	*fe*–$\hat{N}_f$	$\hat{n}$	$\hat{N}_m$	$\hat{n}$	$\hat{N}_b$
54	18-May	1.29	2.20	3	1.07	1	−0.07	4	2.43	7	2.97
55	19-May	1.35	2.30	2	1.03	1	−0.03	10	4.36	12	3.34
56	20-May	1.87	3.20	3	1.11	1	−0.11	10	2.59	13	3.10
57	21-May	0.64	1.10	2	1.03	1	−0.03	7	2.29	9	2.85
58	23-May	3.33	5.70	3	2.06	2	−0.06	17	7.32	20	6.43
59	24-May	5.15	8.80	6	4.09	3	−1.09	19	10.45	25	11.76
60	25-May	1.87	3.20	3	1.11	1	−0.11	13	6.10	16	3.75
61	27-May	0.59	1.00	1	1.00	1	0.00	9	3.01	10	3.00
62	28-May	3.80	6.50	4	1.95	2	0.05	10	5.29	14	5.70
63	4-Jun	2.98	5.10	6	2.04	2	−0.04	15	9.84	21	6.75
64	7-Jun	5.85	10.00	2	1.03	4	2.97	14	7.20	16	3.62
65	8-Jun	2.11	3.60	4	1.54	2	0.46	16	9.84	20	5.34
66	11-Jun	2.87	4.90	2	1.03	2	0.97	13	6.36	15	3.56
67	20-Jun	1.81	3.10	16	9.33	1	−8.33	18	10.71	34	19.94
68	21-Jun	4.10	7.00	3	1.07	2	0.93	12	7.03	15	3.71
69	22-Jun	1.29	2.20	3	1.14	1	−0.14	5	3.13	8	3.35
70	25-Jun	1.35	2.30	2	1.26	1	−0.26	12	6.62	14	4.23
71	11-Jul	1.70	2.90	5	1.32	1	−0.32	16	6.87	21	4.42
Mean		*2.51*	*4.30*	*3.70*	*1.85*	*1.72*	*−0.14*	*12.28*	*6.42*	*15.99*	*5.31*
Median		*1.93*	*3.40*	*3.00*	*1.14*	*1.00*	*0.00*	*12.00*	*6.25*	*15.00*	*3.84*
SD		*1.81*	*3.06*	*3.40*	*1.73*	*1.07*	*1.64*	*4.00*	*2.99*	*6.53*	*3.60*

Sequential spawn ID, date, and number of eggs shown at left.
The *fe* is a term used to visually judge the number of females contributing to a spawn and can be multiplied by three to generally estimate N_b, or the breeding effective size.
Spawns 34 and 42 highlighted in gray were grown out during the 2008 stocking season for replenishment.
Summary statistics listed in bottom rows in italics.

terminated spawning early in the season. In contrast, males did not show temporal rhythm. Rather, most males (except 508) participated on a daily or semi-daily basis throughout the season, averaging 34.9 (S = 10.2) successful spawns (see also Table S1B).

On 33 occasions, a female released eggs over an extended period of two or more days rather than in a single mass release. These 'extended releases' were defined liberally as two consecutive daily contributions of ≥1% from a particular female. Fifteen females participated in at least one extended release, and eight of the 15 (e.g., 512, 523, 530; Table S1A) participated in two or more, accounting for 26 of the 33 releases in this category. Most extended release contributions were <10% of the total daily spawn volume, however, and overall, different primary females contributed from one evening to the next.

Batch, annual, and maximum fecundity

Spawns used in estimation of f_b and eggs·kg^{-1} body mass were the 36 spawns where a single female was assigned as parent to ≥95% of YSL ($\hat{N}_f \approx 1$; Table 3), and f_s was estimated using data from all 25 females. VBGF mass and age are reported in Table 1. Females ranged in mass from 11.7 to 18.9 kg ($\bar{x}$ = 16.3, S = 1.8) and in age from 8.5 to 16.3 y ($\bar{x}$ = 12.6, S = 1.9). Mean $\hat{f}_b = 1.7 \times 10^6$ ($S = 1.1 \times 10^6$) eggs at 1.1×10^5 ($\bar{x} = 0.8 \times 10^5$, $S = 0.7 \times 10^5$) eggs·kg^{-1}. Mean $\hat{f}_s = 7.1 \times 10^6$ ($S = 7.4 \times 10^6$) eggs, with a strong positive skew relative to median $\hat{f}_s = 4.4 \times 10^6$. While there was a positive trend, neither body mass nor age was significantly correlated with $\hat{f}_b$ (r = 0.13, P = 0.45, and r = 0.08, P = 0.64, respectively) or $\hat{f}_s$ (r = 0.11, P = 0.60 and r = 0.09, P = 0.67, respectively), likely due to the limited ranges of body size and age represented in the breeding population.

There was a highly significant correlation between $\hat{f}_b$ and $\hat{f}_s$ (r = 0.85, P < 0.001), but the association was not perfect. The combination of cyclicity and $\hat{f}_b$ directly affected $\hat{f}_s$. For example, 528 and 546 both exhibited a mean $\hat{f}_b = 1.4 \times 10^6$ eggs. Whereas 528 contributed 14% of offspring, 546 contributed <5%, largely because 546 had a longer period between spawns at close to a month versus nine days for 528.

A tentative estimate of $\hat{f}_m$ was made using data from female 528 (16.2 kg, 12.2 y), who contributed to 19 spawns, with $\hat{f}_b = 1.4 \times 10^6$ eggs and $\hat{f}_s = 25.7 \times 10^6$ eggs.

She produced a season-high release on 7 Jun of 5.8×10^6 eggs at 3.6×10^5 kg^{-1}. This was the single greatest contribution for any female during this season. The result was originally doubted, but six additional spawns occurred in which an unusually large number ($>3.0 \times 10^6$) of eggs were produced by a single female, including another release of 3.3×10^6 eggs on 6 Apr also attributed to 528.

We could not truly assess sperm production capacity in this study. Male 'fecundity', or ability to reproduce, was likely dependent more on limitations external to the male (Bishop 1998; Levitan 2005), such as proximity to a gravid female, sperm dilution, or sperm competition. Nevertheless, we estimated the equivalent of f_b and f_s, using data from all 25 males. Mean $\hat{f}_b = 0.2 \times 10^6$ ($\bar{x} = 0.2 \times 10^6$, $S = 0.1 \times 10^6$) offspring. Mean $\hat{f}_s$ was the similar to but less variable than in females ($\bar{x} = 7.1 \times 10^6$, $S = 3.2 \times 10^6$), with little skew. Male body mass and age were estimated at 12.9–15.7 kg ($\bar{x} = 14.4$, $S = 0.7$] and 11.3–15.6 y ($\bar{x} = 13.3$, $S = 1.2$), respectively. As in females, there was a significant correlation between $\hat{f}_b$ and $\hat{f}_s$ ($r = 0.86$, $P < 0.001$), but there was no correlation between mass and age and $\hat{f}_b$ ($r = 0.14$, $P = 0.50$ and $r = 0.13$, $P = 0.54$, respectively) or $\hat{f}_s$ ($r = 0.31$, $P = 0.12$ and $r = 0.32$, $P = 0.13$, respectively).

Discussion

The *A. nobilis* study system provided for a new appreciation of the complexity of seasonal broadcast spawning dynamics in a relatively large breeding aggregation of a pelagic marine finfish (albeit in a closed space). *A. nobilis* exhibited sex-specific mating strategies and variation in reproductive success. More males contributed and contributed more equally than females within and among spawns. We do not know whether the general pattern holds in the wild, although behavioral observation of a semi-natural *A. nobilis* spawning aggregation indicated that multiple males typically surround single gravid females prior to and during spawning (Aalbers and Drawbridge 2008). This type of mating system is best described as lottery polygyny, where males mate often relative to and/or compete equally for females (Nunney 1993). It is the same mating system assumed for the closely related, well-known, and well-studied *S. ocellatus* (Turner et al. 2002; Gold et al. 2010).

Females mitigated some of the difference in reproductive success within their own sex and between the sexes by cyclically batch spawning. Mean $\hat{N}_f^d$ was 28% of $\hat{N}_m^d$ per spawn but 60% of $\hat{N}_m^d$ by cessation of the spawning season, and although $\hat{N}_b^d$ per spawn was low, seasonal $\hat{N}_s > 31$ (regardless of estimation method), with nearly 90% of female and 100% of male brood fish contributing to the seasonal pool of offspring. This was also similar to what Gold et al. (2008) found for captive *S. ocellatus*, where $\hat{N}_b^d \approx 2.6$ per spawn, but 59% of females and 89% of males contributed at least once during 13 spawns studied (though the breeding groups were not comparable in terms of census size or sex ratio).

Repeat spawning by both sexes resulted in multiple successful pairings with multiple mates, which guards against reproductive failure for individual breeders and gives rise to genetically variable offspring, while female promiscuity has been found to decrease the risk of inbreeding at the population level (e.g., Michalczyk et al. 2011). In addition, multiple paternity in females may create a sperm competition, a process seen in many species of fish (Stockley et al. 1997; Taborsky 1998), as well as other taxa, both terrestrial and aquatic (Birkhead and Møller 1992; Pearse et al. 2001; Dean et al. 2006), whereby the female is not only ensured variability but also theoretically higher-quality offspring.

We do acknowledge a significant difference from probable natural spawning dynamics: while observed captive $\hat{N}_b$ was less than N ($\hat{N}_b \cdot N^{-1} \geq 0.62$), the ratio may be orders of magnitude higher than that in the wild (e.g., $N_e \cdot N^{-1} \approx 0.001$ for *S. ocellatus* in the northern Gulf of Mexico; Turner et al. 2002). Unequal sex ratios, variance in family size, and fluctuation in population size commonly result in $N_e \cdot N^{-1}$ significantly less than one in wild populations (Frankham 1995). In a hatchery, however, adults that may never have successfully reproduced have greater opportunity because mates are readily available, rapid dilution of gametes by turbulence is lessened, and there is no natural culling of eggs or larvae because of adverse environmental conditions or predation (minus cannibalism in culture).

Implications for management

Releasing fish for stock enhancement is often dual-purpose: sustain fishery catches and supplement natural populations as hatchery fish and their offspring breed with wild conspecifics. It follows that a common concern is loss of genetic diversity in cultured offspring relative to the wild population (Taniguchi 2003), which can reduce the adaptive potential of a mixed hatchery and wild population over subsequent generations. Consequently, parentage data were collected not only in general pursuit of knowledge of broadcast spawning dynamics and complex mating systems, but also for its relevance to responsible management of the *A. nobilis* captive breeding program.

Our basic assumption was that the B2 results could be extrapolated to all four *A. nobilis* breeding groups. Observation of spawning behavior and quantization of egg production over many years indicated no significant evidence to the contrary (K. McClune, HSWRI, unpublished data),

but in the absence of multi-year among-pool parentage data, this remains a hypothesis. We also used $N_b = 74$ as a target minimum for determination of broodstock N and juvenile production per annum (not per generation interval), as it maintained historical context for the *A. nobilis* system. Bartley et al. (1995) applied binomial sampling theory to conclude that $N_b = 74$ represented at least 99% of wild genetic diversity and included rare alleles in the breeding population down to 0.02 frequency. This does constitute many fewer rare alleles than in a large population at mutation–drift equilibrium (Ryman et al. 1995; Waples and Naish 2009), but we must work within operational confines. Finally, we selected $\hat{N}_b^d$ as a factor in determining N, despite concern that it was ~25% less than that via LD. LD estimation was allele frequency-based, and LD via $\hat{r}^2$ (used in LDNE; see also Waples 2006) may be more sensitive to genotyping error than other methods (Akey et al. 2001). $\hat{N}_b^d$ was parentage-based, and while exclusion was sensitive to genotyping error, as well, in that it could not assign through candidate parent–offspring mismatches, the likelihood method in Cervus accounted for a user-defined level of error (Kalinowski et al. 2007). However, Cervus also assumed complete linkage equilibrium (i.e., physically unlinked loci), although *Ano*E and *Ano*R were marginally linked. In the absence of knowing which method was more accurate, the demographic estimate assuming lower $\hat{N}_b$ would be more conservative.

Duchesne and Bernatchez (2002) found that captive adult census population size was the most important factor when evaluating inbreeding potential in supplemented populations (maximum tested $N = 100$). Assuming $\hat{N}_b^d \cdot N^{-1} = 0.62$ for *A. nobilis*, hatchery-wide $\hat{N}$ required for $N_b = 74$ would be 120 brood fish, 40% fewer than stipulated in prior protocol (Bartley et al. 1995; see also Table S2). However, we recommend maintaining greater N in free-spawning systems (here, buffered by ≥15% at 140–200 fish), divided evenly among tanks, to account for mortality and variation in reproduction, if any, within and among fish and brood groups.

Further, broodstocks are often maintained in equal sex ratio (or may even be male-biased; e.g., Gold et al. 2008), which does not account for the limiting effect females of lottery polygynous species have on N_b. To provide opportunity for greater female contribution without making males limiting, managers might consider a sex ratio of 60% female to 40% male for enhancement of species with these mating behaviors. This equalized contributions between the sexes for *A. nobilis*, based on breeding effective to census size ratios ($\hat{N}_f^d \cdot N^{-1} = 0.50$ and $\hat{N}_m^d \cdot N^{-1} = 0.83$), should remain relatively constant. Two other requirements implemented that our data do not address include 1) collecting broodstock for conservation from the wild within the evolutionarily significant unit of interest to reduce potential for outbreeding depression and 2) replacing older brood fish with new wild fish so that allele frequencies in the breeding population remain statistically representative over time (see the 'Supplemental information' for details).

Moving from broodstock to juvenile production, we wanted to give hatchery managers a tool to help decide whether to choose a spawn for culture, in addition to egg quality metrics (e.g., general appearance, size, lipid content), quantity and quality of juveniles currently in the system, remaining rearing tank capacity, and the quantity of fish already stocked in the wild that year. $\hat{N}_b^d$ per spawn was highly correlated with $\hat{N}_f^d$ ($r = 0.94$, $P < 0.001$), and the number of eggs per spawn was correlated with $\hat{N}_f^d$ and $\hat{N}_b^d$ ($r = 0.45$ and 0.63, respectively, both $P < 0.001$). Following these relationships, we developed a concept called the 'female equivalent' (*fe*) to quickly describe the number of females contributing to a spawn within an acceptable margin of error and without using genetics. It is estimated from a volumetric measure of the number of eggs, and the volume of eggs per *fe* is adaptable to the breeding group of interest, depending on female biomass, female N, and mean eggs kg^{-1} body mass. As *A. nobilis* produces ~10^5 eggs kg^{-1} at a predetermined 585 eggs mL^{-1}, then 1 *fe* = 3 L of eggs for the B2 brood group (where $N = 25$ and biomass = 407 kg; Table 1).

To test accuracy, *fe* was estimated, rounded to the nearest integer for simplicity to indicate whole numbers of females, for all 71 spawns and compared to $\hat{N}_f^d$ (Table 3). The two metrics were significantly correlated ($r = 0.40$, $P < 0.001$), with a mean difference of −0.14 ($\bar{x} = 0.00$, $S = 1.64$) between corresponding pairs of $\hat{N}_f^d$ and *fe*. Two spawns chosen for production in 2008 (Table 3, gray rows) were both within one *fe* of $\hat{N}_f^d$, but eight (~11%) spawns did yield differences >1 *fe*, with the bulk of the error in three spawns underestimated by >5 *fe*; removing these three outliers increased the correlation between $\hat{N}_f^d$ and *fe* to 0.81 ($P < 0.001$). Although not optimal, it implied significant error in *fe* estimation was conservative, which may work in favor of a supplemented species if the true N_b of stocked fish is higher than estimated.

What the *fe* concept could also provide was an estimate of breeding effective size (and to some extent, genetic variability) for nongeneticists. The *fe* can be multiplied by three for a general idea of N_b for *A. nobilis* ($\hat{N}_b^d \cdot \hat{N}_f^{d-1} : \bar{x} = 3.12$, $S = 0.40$). Alternatively, if target $N_b = 74$ for stockable juveniles, then a minimum 23–24 *fe* should be produced annually. We recommend culturing 28–32 *fe* (equalized among brood pools) to account for error in *fe* estimation. The cyclical nature of female

spawning should ideally be addressed, as well, but repeat spawning can be difficult to identify (without genetics). External tagging and visual monitoring are being considered for *A. nobilis*, but in its absence, it is assumed that *fe* and $\hat{N}_b$ are additive across spawns and brood pools.

Fitness considerations

Culture programs, especially those that release juveniles in the wild, create a myriad possible fitness effects. We touched on avoiding inbreeding and outbreeding depression, as well as the consequences of reduced genetic variability, but other hatchery-related impacts can occur, including but not limited to domestication, genetic swamping, fragmentation, competition, and disease transmission (Taniguchi 2003; Tringali et al. 2007). We are in the midst of research into differential survival among *A. nobilis* culture stages, which may provide (i) insight into whether genetic variability (e.g., allelic richness, heterozygosity) can be maintained throughout the rearing process and (ii) a proxy for assessing unintentional domestication selection in a hatchery environment.

Postrelease, there was potential for profound fitness effects in the wild (Araki and Schmid 2010). The *A. nobilis* replenishment program has a lengthy history: it has been in operation for nearly three decades. Fortunately, genetic considerations have been integral, with direct application of theory and research in broodstock management (Bartley et al. 1995). Also, State regulation has prevented the program from becoming truly large-scale; less than two million fish have been released under an annual limit of 125–350 thousand juveniles. In contrast, Texas' *S. ocellatus* enhancement program allows releasing 20–30 million fingerlings every year (Gold et al. 2010). As a potential result, mark–recapture research using coded-wire tags indicates <0.5% of *A. nobilis* caught in the fisheries are cultured (M. Shane, HSWRI, unpublished data). Higher relative mortality of hatchery juveniles in the wild likely contributes to the low recapture rates, as well, as might a large wild N, rebounding populations, etc. In fact, between a currently favorable water temperature regime in the Southern California Bight and a ban since 1994 on nearshore gillnetting of *A. nobilis* spawners, the natural population appears to be in recovery, suggesting historical fishery declines were because of recruitment limitations brought on by a combination of adverse fisheries managerial and environmental conditions (Allen et al. 2007).

The above-mentioned factors make genetic swamping unlikely, but we do not truly know because it is difficult to evaluate the genetic impact(s) of this program in the wild (e.g., allelic replacement, Ryman–Laikre effect; Ryman and Laikre 1991; Tringali et al. 2007). $\hat{N}_b$ is insufficient for estimating loss of genetic variability over time, in part because there is no straightforward relationship between N_b and N_e for iteroparous species (i.e., N_b multiplied by the generation interval can approximate N_e; Waples 1990; e.g., Schmeller and Merilä 2007). Even so, *A. nobilis* population statistics are a black box: a stock assessment was never performed, and population estimates were never made (Leet et al. 2001; California Department of Fish and Game (CDFG), Marine Region 2002). Consequently, we do not have estimates of wild adult N or generation interval. The only estimates of wild N_e are circa 2001 from Coykendall (2005), but no one knows what N_e was at the inception of stocking or is now; the topic must be revisited in future research. Further, while *A. nobilis* mature in 2–4 years and fish entering the fisheries are generally ≥5 years of age, we do not know whether hatchery fish successfully reproduce in the wild. Released fish are not genetically tagged, and the large number of genetic combinations arising from a free-mating culture system makes targeted genetic identification of hatchery signatures (e.g., familyprinting or mixed stock analysis; Pella and Milner 1987; Letcher and King 1999) in the F_1 or later generations computationally intensive, if not impossible.

In conclusion, *A. nobilis* captive breeding provided one of the best operational systems to date for an intensive study of broadcast spawning dynamics and mating strategies in a pelagic marine finfish. The data gathered then allowed us to modify broodstock and juvenile production protocols based on the species' mating system. However, while maintenance of breeding effective size was the primary focus for management in the hatchery, more research is needed in order to empirically assess the fitness impact of these cultured fish once released in the wild. Managers of new and existing stock enhancement programs must consider the availability of biologically relevant information on a species of interest if associated genetic work is to be comprehensive, addressing pre- and postrelease concerns. Ideally, wild stock structure, genetic diversity, historical and contemporary census and effective population sizes, and generation interval, as well as hatchery diversity, breeding effective size, and intended and realized contribution rates to the wild, would be known or inferable. For *A. nobilis*, performing a stock assessment could provide critical biologic data. In the absence of pertinent information (e.g., stock size, generation interval), the *A. nobilis* replenishment program (among others) cannot yet fully understand the potential genetic effects of releasing large numbers cultured juveniles on natural populations.

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Data archiving statement

Microsatellite data underlying the main results in this paper are available in the Dryad repository: doi:10.5061/dryad.6n391t06

Literature cited

Aalbers, S. A., and M. A. Drawbridge. 2008. White seabass spawning behavior and sound production. Transaction of the American Fisheries Society **137**:542–550.

Akey, J. M., K. Zhang, M. Xiong, P. Doris, and L. Jin. 2001. The effect that genotyping errors have on the robustness of common linkage disequilibrium measures. American Journal of Human Genetics **68**:1447–1456.

Allen, L. G., D. J. Pondella II, and M. A. Shane. 2007. Fisheries independent assessment of a returning fishery: abundance of juvenile while seabass (*Atractoscion nobilis*) in the shallow nearshore waters of the Southern California Bight, 1995-2005. Fisheries Research **88**:24–32.

Allendorf, F. W., and N. Ryman. 1987. Genetic management of hatchery stocks. In N. Ryman, and F. Utter, eds. Population Genetics and Fishery Management, pp. 141–159. University of Washington Press, Seattle.

Araki, H., and C. Schmid. 2010. Is hatchery stocking a help or harm? Evidence, limitations and future direction in ecological and genetic surveys Aquaculture **308**:S2–S11.

Araki, H., B. Cooper, and M. S. Blouin. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science **318**:100–103.

Bartley, D. M., D. B. Kent, and M. A. Drawbridge. 1995. Conservation of genetic diversity of white seabass enhancement program in southern California. American Fisheries Society Symposium **15**:249–258.

von Bertalanffy, L. 1938. A quantitative theory of organic growth. Human Biology **10**:181–213.

Birkhead, T. R., and A. P. Møller. 1992. Sperm Competition in Birds. Evolutionary Causes and Consequences. Academic Press, San Diego.

Bishop, J. D. D. 1998. Fertilization in the sea: are the hazards of broadcast spawning avoided when free-spawned sperm fertilize retained eggs? Proceedings of the Royal Society B **265**:721–735.

Blankenship, H. L., and K. M. Leber. 1995. A responsible approach to marine stock enhancement. American Fisheries Society Symposium **15**:167–175.

Botstein, D., R. L. White, M. Skolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in the man using restriction fragment length polymorphisms. American Journal of Human Genetics **32**:314–331.

California Department of Fish and Game (CDFG), Marine Region. 2002. White Seabass Fishery Management Plan. The Resources Agency, Sacramento, CA.

Coykendall, D. K. 2005. Population structure and dynamics of white seabass (*Atractoscion nobilis*) and the genetic effect of hatchery supplementation on the wild population. Ph.D. dissertation, University of California Davis, Davis, CA.

Dean, M. D., K. G. Ardlie, and M. W. Nachman. 2006. The frequency of multiple paternity suggests that sperm competition is common in house mice (*Mus domesticus*). Molecular Ecology **15**:4141–4151.

Donohoe, C. J. 1997. Age, growth, distribution, and food habits of recently settled white seabass, *Atractoscion nobilis*, off San Diego County, California. Fisheries Bulletin **95**:709–721.

Duchesne, P., and L. Bernatchez. 2002. An analytical investigation of the dynamics of inbreeding in multi-generation supportive breeding. Conservation Genetics **3**:47–60.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genetical Research **66**:95–107.

Franklin, M. P. 1997. An investigation into the population structure of white seabass (*Atractoscion nobilis*) in California and Mexican waters using microsatellite DNA analysis. Ph.D. dissertation, University of California Santa Barbara, Santa Barbara, CA.

Gold, J. R. 2004. Stock structure and effective size of red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico and implications relative to stock enhancement and recruitment. In K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svåsand, eds. Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities, 2nd edn. pp. 353–370. Blackwell Publishing Ltd, Oxford.

Gold, J. R., L. Ma, E. Saillant, P. S. Silva, and R. R. Vega. 2008. Genetic effective size in populations of hatchery-raised red drum released for stock enhancement. Transactions of the American Fisheries Society **137**:1327–1344.

Gold, J. R., M. A. Renshaw, E. Saillant, and R. R. Vega. 2010. Spawning frequency of dams and sires in a marine fish stock-enhancement hatchery. Journal of Fish Biology **77**:1030–1040.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In A. Beaumont, ed. Genetics and Evolution of Aquatic Organisms. pp. 1222–1344. Chapman and Hall, London.

Hervas, S., K. Lorenzen, M. A. Shane, and M. A. Drawbridge. 2010. Quantitative assessment of a white seabass (*Atractoscion nobilis*) stock enhancement program in California: post-release dispersal, growth and survival. Fisheries Research **105**:237–243.

Jørstad, K. E. 2004. Genetic studies in marine stock enhancement in Norway. In K. M. Leber, S. Kitada, H. L. Blankenship, and

T. Svåsand, eds. Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities, 2nd edn. pp. 339–352. Blackwell Publishing Ltd., Oxford.

Kalinowski, S. T., A. P. Wagner, and M. L. Taper. 2006. ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes **6**:576–579.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology **16**:1099–1106.

Leber, K. M. 2004. Marine stock enhancement in the USA: status, trends, and needs. In K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svåsand, eds. Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities, 2nd edn. pp. 11–24. Blackwell Publishing Ltd, Oxford.

Leet, W. S., C. M. Dewees, R. Klingbeil, and E. J. Larson 2001. California's Living Marine Resources: A Status Report. CDFG. The Resources Agency, Sacramento, CA. Pub SG01–11.

Letcher, B. H., and T. L. King. 1999. Targeted stock identification using multilocus genotype 'familyprinting'. Fisheries Research **43**:99–111.

Levitan, D. R. 2005. The distribution of male and female reproductive success in a broadcast spawning marine invertebrate. Integrative and Comparative Biology **45**:848–855.

Lorenzen, K., K. M. Leber, and H. L. Blankenship. 2010. Responsible approach to stock enhancement: an update. Reviews in Fisheries Science **18**:189–210.

McEachron, L. W., C. W. McCarty, and R. R. Vega 1993. Successful enhancement of Texas red drum (*Sciaenops ocellatus*) population. In Interactions between Cultured Species and Naturally Occurring Species in the Environment: Proceedings of the 22nd U.S.-Japan Aquaculture Panel Symposium, Homer. pp. 53–56.

Michalczyk, Ł., A. L. Millard, O. Y. Martin, A. J. Lumley, B. C. Emerson, T. Chapman, and M. J. G. Gage. 2011. Inbreeding promotes female promiscuity. Science **333**:1739–1742.

Moser, H. G., D. A. Ambrose, M. S. Busby, J. L. Butler, E. M. Sandknop, B. Y. Sumida, and E. G. Stevens. 1983. Description of early stages of white seabass, *Atractoscion nobilis*, with notes on distribution. CalCOFI Report **24**:182–193.

Nunney, L. 1993. The influence of mating system and overlapping generations on effective population size. Evolution **47**:1329–1341.

Oliver, J., and R. Babcock. 1992. Aspects of the fertilization ecology of broadcast spawning corals: sperm dilution and measurements of fertilization. Biological Bulletin **183**:409–417.

Pearse, D. E., R. J. Janzen, and J. C. Avise. 2001. Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature. Behavioral Ecology and Sociobiology **51**:164–171.

Pella, J. J., and G. B. Milner 1987. Use of genetic marks in stock composition analysis. In N. Ryman, and F. Utter, eds. Population Genetic and Fisheries Management. pp. 247–276. University of Washington Press, Seattle.

Pennington, J. T. 1985. The ecology of fertilization of echinoid eggs – the consequences of sperm dilution, adult aggregation and synchronous spawning. Biological Bulletin **169**:417–430.

Reisenbichler, R., S. Rubin, L. Wetzel, and S. Phelps. 2004. Natural selection after release from a hatchery leads to domestication in steelhead, *Oncorhynchus mykiss*. In K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svåsand, eds. Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities, 2nd edn, pp. 371–383. Blackwell Publishing Ltd, Oxford.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources **8**:103–106.

Rowe, S., and J. A. Hutchings. 2003. Mating systems and the conservation of commercially exploited marine fish. Trends in Ecology and Evolution **18**:567–572.

Rowe, S., and J. A. Hutchings. 2008. A link between sound producing musculature and mating success in Atlantic cod. Journal of Fish Biology **72**:500–511.

Rowe, S., J. A. Hutchings, and J. E. Skjæraasen. 2007. Nonrandom mating in a broadcast spawner: mate size influences reproductive success in Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences **64**:219–226.

Ryman, N., and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. Conservation Biology **5**:325–329.

Ryman, N., F. Utter, and L. Laikre. 1995. Protection of intraspecific biodiversity of exploited fishes. Reviews in Fish Biology and Fisheries **5**:417–446.

Schmeller, D. S., and J. Merilä. 2007. Demographic and genetic estimates of effective population and breeding size in the amphibian *Rana temporaria*. Conservation Biology **21**:142–151.

Smith, T. I. J., W. E. Jenkins, M. R. Denson, and M. R. Collins. 2003. Stock enhancement research with anadromous and marine fishes in South Carolina. UJNR Technical Report **30**:175–189.

Stockley, P., M. J. G. Gage, G. A. Parker, and A. P. Møller. 1997. Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. The American Naturalist **149**:933–954.

Taborsky, M. 1998. Sperm competition in fish: 'bourgeois' males and parasitic spawning. Trends in Ecology and Evolution **13**:222–227.

Taniguchi, N. 2003. Genetic factors in broodstock management for seed production. Reviews in Fish Biology and Fisheries **13**: 177–185.

Taniguchi, N. 2004. Broodstock management for stock enhancement programs of marine fish with assistance of DNA marker (a review). In K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svåsand, eds. Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities, 2nd edn. pp. 329–338. Blackwell Publishing Ltd, Oxford.

Thrower, F. P., and J. E. Joyce. 2004. Effects of 70 years of freshwater residency on survival, growth, early maturation, and smolting in a stock of anadromous rainbow trout from southeast Alaska. American Fisheries Society Symposium **44**:485–496.

Tringali, M. D., and T. M. Bert. 1998. Risk to genetic effective population size should be an important consideration in fish stock-enhancement programs. Bulletin of Marine Science **62**:641–659.

Tringali, M. D., T. M. Bert, F. Cross, J. W. Dodrill, L. M. Gregg, W. G. Halstead, R. A. Krause *et al.* 2007. Genetic Policy for the Release of Finfishes in Florida. Florida Fish and Wildlife Conservation Commission. Florida Fish and Wildlife Research Institute, Publication No. IHR-2007-1, St. Petersburg.

Tringali, M. D., K. M. Leber, W. G. Halstead, R. McMichael, J. O'Hop, B. Winner, R. Cody *et al.* 2008. Marine stock enhancement in Florida: a multi-disciplinary, stakeholder-supported, accountability-based approach. Reviews in Fisheries Science **16**: 51–57.

Turner, J. F., J. P. Wares, and J. R. Gold. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-depended marine fish (*Sciaenops ocellatus*). Genetics **162**:1329–1339.

Waples, R. S. 1990. Conservation genetics of Pacific salmon. II. Effective population size and the rate of loss of genetic variability. Journal of Heredity **81**:267–276.

Waples, R. S. 1994. Genetic considerations in recovery efforts for Pacific salmon. Conservation Biology **8**:884–886.

Waples, R. S.. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci*. Conservation Genetics **7**:167–184.

Waples, R. S., and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources **8**:753–756.

Waples, R. S., and K. A. Naish 2009. Genetic and evolutionary considerations in fishery management: research needs for the future. In J. Beamish, and B. J. Rothschild, eds. The Future of Fisheries Science in North America. pp. 427–451. Springer, Dordrecht.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Parental contribution to *A. nobilis* offspring across the 2008 spawning season in the Carlsbad hatchery brood group B2.

Table S2. Former and revised broodstock and juvenile production management plans for the *A. nobilis* stock replenishment program.

Appendix S1.

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