Maternal reproductive exhaustion in a broadcast spawning marine finfish cultured for conservation

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ABSTRACT

Reproductive exhaustion can be defined as a decline in reproductive potential or output over time. We studied seasonal maternal reproductive exhaustion in a large, freely mating captive population of white seabass (*Atractoscion nobilis*), a marine finfish cultured in southern California for conservation. Using genetic parentage data, we identified 34 single-maternity spawns produced during the 2008 spawning season and quantified four physical egg characteristics for each spawn, including egg volume (EV), oil volume (OV), percent oil volume (POV), and the number of oil globules (NOG). The mean batch egg measures decreased between the beginning and end of the spawning season by 19%, 45%, 33%, and 12%, respectively. OV and POV declined linearly, whereas the trend in NOG was parabolic and negatively correlated to water temperature and light duration, which reach apices mid-season. EV displayed a similar, though not significant, trend. We also evaluated the egg measures within and among six dames that solo-spawned two or more times. The seasonal trends were similar to those observed at the population-level, but the relative measures varied significantly among dames, indicating factors other than the common broodstock diet and holding conditions may influence egg production in individual *A. nobilis* females. In terms of species conservation, assessing these egg characteristics, some of which may be associated with larval quality in *A. nobilis*, in conjunction with estimating genetic diversity for each spawn is important when choosing spawns to rear for stock supplementation, but more research into their relationship with subsequent offspring quality is warranted.

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1. Introduction

Reproductive exhaustion can be defined as a decline in reproductive potential or output over time. Aspects of the phenomenon, such as physiological and biochemical changes, sex-specificity, and timescale, have been broadly noted across taxa, from plants (Ehrlén, 1997) to invertebrates (Brokordt et al., 2000; Müller et al., 2001; Palacios et al., 1998), fish, birds, and mammals, including humans (Edwards et al., 1998; Jordan and Brooks, 2010; Neal-Perry et al., 2010; Ribeiro et al., 1996; Wise, 1994; Wise et al., 1997). In iteroparous species, the decline in reproductive output can manifest as fewer or smaller spawns or clutches produced by adults, decreasing hatch rates and survival of progeny, or increasing genetic and morphological mutation rates in progeny (e.g. Jordan and Brooks, 2010; Ribeiro et al., 1996). Hence, reproductive exhaustion impacts not only the reproductive success of breeding adults in a population but also offspring fitness.

Although often assessed in terms of aging over a lifetime (e.g. Fisher, 1930; Kirkwood, 1977), reproductive exhaustion can occur on much shorter timescales. It has been reported during the course of a single spawning season or just a few spawns in shrimp (*Penaeus* spp.; Palacios et al., 1999), anchovy (*Engraulis* spp.; Ribeiro et al., 1996), and striped trumpeter (*Latris lineata*; Bransden et al., 2007). At an extreme, sockeye salmon (*Onchorynchus nerka*) spawn, senesce, and die over a period of 11–23 days (e.g. *O. nerka*; Healey et al., 2003; McPhee and Quinn, 1998; Shrimpton et al., 2005).

Here, we focused on reproductive exhaustion in a broadcast spawning marine finfish called the white seabass (*Atractoscion nobilis* Ayres 1860). *A. nobilis* is a typical member of the family Sciaenidae native to rocky bottom and kelp bed habitat along the west coast of North America (CDFG, 2002). During the latter half of the last century, *A. nobilis* experienced severe declines in abundance off southern California due to an unfortunate blend of regulatory and environmental circumstances (Allen et al., 2007). Because the species supported (and continues to support) a regionally-important commercial and recreational fishery, it was chosen in 1983 for a pilot experimental stock replenishment program still in operation today (CDFG, 2002), where juvenile fish are produced in a hatchery and released to supplement the wild population.

The *A. nobilis* breeding program is well-suited for studying intrinsic reproductive behaviors and characteristics. Relatively large groups

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of up to 50 wild-caught fish are allowed free mate choice within mesocosm-like breeding pools. *A. nobilis* is iteroparous and a batch spawner, releasing gametes multiple times over a spawning season for multiple years (Gruenthal and Drawbridge, 2012; Moser et al., 1983), and broadcast spawning in captivity is induced by photothermal control that mimics seasonal changes in water temperature and light duration (Bartley et al., 1995).

We used genetic parentage analysis and quantified changes in basic physical egg characteristics, some of which may be associated with egg and larval quality (hatch rates and larval survival and viability; Brooks et al., 1997; Gauger, 2010; Kjørsvik et al., 2003), both non-invasively and within and among female brood fish to assess the presence of seasonal maternal reproductive exhaustion in *A. nobilis*. Because egg and larval quality is an important limiting factor in developing culture techniques for many species (Bromage, 1995; Kjørsvik et al., 1990), we discuss the knowledge gained about reproductive exhaustion in *A. nobilis* in terms of developing juvenile propagation protocols, particularly for species cultured for conservation.

2. Materials and methods

2.1. Broodstock population

This study focused on one breeding group composed of 25 male and 25 female reproductively-mature wild-caught fish as described by Bartley et al. (1995) and Gruenthal and Drawbridge (2012). Fish were held in a 48 m^3 tank maintained on a separate recirculating seawater system. The recirculating flow rate in the brood pool was 560 L min^{-1}, and complete turnover occurred every 82 min. Ozonated makeup water was supplied to each recirculating system at 4–8 L min^{-1}. Spawning brood fish were fed five days per week a ration of 1% tank biomass, consisting of fresh market squid and fish (sardine, capelin, or anchovy, depending on availability) injected with a vitamin–oil mixture (vitamin premix: Ziegler Bros., Inc., Gardners, PA; Stay-C, thiamine, soy lecithin, crude menhaden oil: TPi, Madera, CA). Water temperature and photoperiod were controlled to mimic natural conditions. The non-spawning "winter" season was simulated at 13 °C and 10 h (h) of light, while spawning was achieved between 14 °C and 11 h light and a maximum of 18 °C and 14 h light. Spawning conditions were offset from the calendar year such that a total of 84 spawns occurred from 15 Jan through 14 Jun 2008 (Gruenthal and Drawbridge, 2012). Spawning typically occurred in early evening and all eggs were collected passively in a 500 μm mesh bag (egg trap) placed in the sump of the recirculation loop. Eggs were removed the following morning between 0800 h and 1000 h using an 800 μm mesh dip net and placed in 1 L graduated cylinders for volumetric estimation of spawn size. The number of eggs was estimated according to a predetermined average of 585 eggs mL^{-1} (CDFG, 2010).

2.2. Laboratory methods

A random subset of fertilized (floating) eggs from all 84 spawns was collected for gross assessment at 5× magnification of cell division stage and quality (e.g. morphology, size, lipid content) using a Digital Microscope Camera Model DMC1 (Polaroid, Minnetonka, MN). Twenty to 24 eggs were then digitally photographed at 2× magnification for archival purposes. Parent–offspring relationships (sires and dames) were genetically determined by Gruenthal and Drawbridge (2012) for 71 of the 84 spawns, and a single dame was assigned as parent to ≥95% of 1–2 day post hatch larvae for 36 spawns. Data from these spawns were retained for assessing maternal reproductive exhaustion (Table 1), since inclusion of multiple maternity spawns would make it impossible to attribute the photographed eggs to any particular dame. Archived digital photographs were available for 34 of the 36 spawns; images were unavailable (file corrupted) for 15 Jan and 26 Apr. Photographs were visualized using Image-Pro Plus software (Media Cybernetics, Inc.,

Bethesda, MD), and specific egg characteristics, including egg volume (EV), total oil volume (OV), percent oil volume (POV), and the number of oil globules (NOG), were measured for 15–20 eggs in full view from each spawn. Egg and oil globules were assumed to be spherical, and volumes were calculated from single measurements of diameter according to the formula for the volume of a sphere (i.e. $V = \frac{4}{3}\pi r^3$). Volumes were summed across all oil globules to obtain OV.

2.3. Statistical analyses

Basic statistics for the batch (spawning event) egg characteristics, including ranges, means, and standard deviations, were calculated using Excel 2010 (Microsoft Corp., Redmond, WA). Regression coefficients of determination (R^2) were calculated based on the best fit trendlines for each distribution. Pairwise Spearman rank-order correlation coefficients (ρ) were estimated between each of the egg characteristics and dame age, body mass, and time in captivity (Table 2), as well as water temperature and light duration, using R-based statistics software developed by Wessa (2012). Additional statistics were estimated with STATISTICA v10 (StatSoft, Inc., Tulsa, OK). The Shapiro–Wilk *W* statistic was used to test for normality within spawns, and based on the results, non-parametric Kruskal–Wallis one-way analyses of variance (ANOVA) by ranks (*H*-tests) were performed to detect significant differences in the batch egg characteristics within and among spawns and dames (except 503). Data resulting in significant *H* values were further assessed with Mann–Whitney *U*-tests to identify the values responsible for the differences. Dame 503 contributed to two spawns and the data were analyzed by *U*-test only. To detect Type I errors, the results were sequential Bonferroni corrected at the table-wide $\alpha = 0.05$ level (Rice, 1989).

3. Results

3.1. Trends in batch egg characteristics

Egg characteristics were measurable (i.e. eggs were in full view) for 20 eggs from 31 spawns, 17 eggs from 12 Feb and 15 Feb, and 15 eggs from 17 Jan. Mean batch EV, OV, POV, and NOG ranged from 0.88 to 1.56 mm^3, 0.011 to 0.029 mm^3, 1.05 to 2.22%, and 1.2 to 7.2, respectively (Table 1). OV and POV exhibited simple linear declines, whereas mean NOG and, marginally, EV displayed second-order polynomial (parabolic) regressions (Fig. 1). EV declined overall but exhibited a subtle positive trend in the latter third of the season. Mean EV, OV, POV, and NOG decreased by 19%, 45%, 33%, and 12%, respectively, as measured by the overall change in the regression line. Most, but not all, of the data were normally distributed, so further analyses performed using *H*-tests rather than standard ANOVAs revealed that comparisons among spawns were significant [H (33) ≥ 283.95, $P < 0.001$; Table 3]. EV, OV, and POV were not significantly correlated with the photothermal regime [$-0.17 \leq \rho$ (32) ≤ 0.31, $P > 0.07$], but NOG was negatively correlated with both water temperature and light duration [temperature: ρ (32) $= -0.57$, $P = 0.001$; light: ρ (32) $= -0.34$, $P = 0.05$].

3.2. Trends within and among dames

Six dames (503, 522, 525, 526, 528, and 548; Table 1) contributed to two or more single-maternity spawns, accounting for 27 of the 34 spawns analyzed. The measures declined overall, despite minor increases in EV and NOG toward the end of the season, but only mean OV and POV decreased significantly by an average of 32% and 19%, respectively. The negative trend estimation was least error prone for dames 525 and 528, who were primary contributors to the largest numbers of spawns at eight and seven, respectively (Fig. 2). The changes in batch egg characteristics as the season progressed were statistically significant within dames ($P < 0.01$; Table 3), except POV and NOG for

Table 1
Egg quality characteristics for 34 single-maternity spawns produced in 2008 in one brood pool. Chronological spawn date, total eggs spawned, water temperature, light duration, dame ID, and number of eggs measured (n) at left. Egg volume (EV), oil volume (OV), percent oil volume (POV), and number of oil globules (NOG) reported as mean plus/minus one standard deviation (S).

Spawn	Date	Water temp.	Light	Total eggs	Dame	n	EV (±S)	OV (±S)	POV (±S)	NOG (±S)	N_f
		(°C)	(h)	($\cdot 10^6$)			(mm³)	(mm³)	(%)		
1	16-Jan	15	12	1.81	528	20	1.129 (0.036)	0.019 (0.002)	1.67 (0.14)	7.2 (2.2)	1.00
2	17-Jan	15	12	1.11	525	15	1.250 (0.072)	0.024 (0.002)	1.91 (0.16)	4.2 (2.3)	1.03
3	29-Jan	16	13	0.99	528	20	1.117 (0.044)	0.019 (0.001)	1.68 (0.12)	4.8 (1.2)	1.00
4	7-Feb	16	13	1.70	526	20	1.195 (0.043)	0.021 (0.001)	1.75 (0.09)	3.3 (1.1)	1.03
5	8-Feb	16	13	2.22	536	20	1.114 (0.051)	0.018 (0.001)	1.58 (0.10)	3.7 (1.4)	1.00
6	9-Feb	17	14	0.94	523	20	1.200 (0.052)	0.021 (0.002)	1.76 (0.15)	1.9 (1.1)	1.03
7	10-Feb	17	14	1.35	525	20	1.555 (0.080)	0.029 (0.002)	1.89 (0.11)	2.5 (1.4)	1.00
8	12-Feb	17	14	0.59	549	17	1.033 (0.046)	0.017 (0.001)	1.61 (0.12)	3.8 (1.4)	1.00
9	14-Feb	17	14	1.29	528	20	1.105 (0.056)	0.018 (0.001)	1.60 (0.07)	3.9 (1.1)	1.00
10	15-Feb	17	14	0.76	522	17	1.160 (0.053)	0.015 (0.001)	1.28 (0.11)	3.6 (1.1)	1.00
11	23-Feb	18	14	1.81	525	20	1.068 (0.063)	0.020 (0.001)	1.91 (0.11)	2.7 (1.3)	1.00
12	24-Feb	18	14	1.05	548	20	1.138 (0.057)	0.025 (0.001)	2.22 (0.13)	1.9 (0.8)	1.11
13	8-Mar	18	14	2.75	525	20	1.057 (0.069)	0.016 (0.002)	1.55 (0.15)	2.2 (1.0)	1.00
14	12-Mar	18	14	1.58	528	20	1.091 (0.052)	0.014 (0.001)	1.24 (0.13)	3.1 (0.9)	1.03
15	14-Mar	18	14	1.46	522	20	0.914 (0.058)	0.013 (0.001)	1.47 (0.11)	1.2 (0.5)	1.00
16	15-Mar	18	14	1.23	525	20	1.002 (0.063)	0.017 (0.002)	1.72 (0.14)	1.9 (0.9)	1.00
17	23-Mar	18	14	2.05	548	20	1.115 (0.040)	0.023 (0.001)	2.05 (0.15)	1.3 (0.6)	1.11
18	26-Mar	18	14	2.22	528	20	1.116 (0.064)	0.017 (0.002)	1.53 (0.16)	2.7 (1.4)	1.11
19	2-Apr	18	14	0.97	526	20	1.004 (0.051)	0.016 (0.001)	1.61 (0.11)	1.6 (1.0)	1.07
20	5-Apr	18	14	3.16	541	20	0.881 (0.038)	0.017 (0.001)	1.92 (0.12)	1.6 (0.7)	1.07
21	9-Apr	18	14	1.11	525	20	0.937 (0.037)	0.013 (0.001)	1.43 (0.07)	2.3 (0.9)	1.00
22	11-Apr	18	14	1.40	522	20	0.935 (0.045)	0.012 (0.001)	1.28 (0.07)	3.7 (1.6)	1.00
23	7-May	17	14	1.23	528	20	1.018 (0.061)	0.012 (0.001)	1.20 (0.06)	4.1 (1.1)	1.03
24	12-May	17	14	1.05	525	20	1.130 (0.046)	0.016 (0.001)	1.39 (0.07)	3.0 (1.6)	1.03
25	17-May	16	13	4.97	525	20	0.980 (0.069)	0.015 (0.001)	1.49 (0.11)	3.3 (1.2)	1.11
26	18-May	16	13	1.29	522	20	0.998 (0.054)	0.012 (0.001)	1.22 (0.12)	3.5 (1.7)	1.07
27	19-May	16	13	1.35	544	20	1.011 (0.046)	0.011 (0.001)	1.07 (0.13)	3.7 (1.5)	1.03
28	20-May	16	13	1.87	545	20	1.044 (0.041)	0.012 (0.001)	1.11 (0.13)	4.2 (1.9)	1.11
29	21-May	16	13	0.64	503	20	1.231 (0.091)	0.013 (0.001)	1.04 (0.11)	4.9 (2.1)	1.03
30	25-May	16	13	1.87	550	20	0.990 (0.052)	0.014 (0.001)	1.43 (0.11)	2.4 (1.0)	1.11
31	27-May	16	13	0.59	503	20	1.034 (0.050)	0.011 (0.001)	1.06 (0.13)	4.4 (2.1)	1.00
32	7-Jun	15	12	5.85	528	20	0.889 (0.075)	0.011 (0.002)	1.25 (0.15)	3.1 (1.4)	1.03
33	11- Jun	15	12	2.87	548	20	1.089 (0.047)	0.016 (0.002)	1.49 (0.14)	2.5 (1.5)	1.03
34	21-Jun	14	11	4.10	526	20	0.964 (0.030)	0.014 (0.002)	1.50 (0.22)	3.2 (1.6)	1.07

dame 503. Because 503 contributed to only two single-dame spawns, interpretation of the data was compromised by the small sample size.

Despite similar overall trends, the relative measures of the egg characteristics varied significantly among dames [H (5) > 50, $P < 0.001$; Table 3]. To illustrate, 522 produced eggs with lower total volume and lipid volume and more oil globules than 548 on average

(EV = 1.00 mm³, OV = 0.013 mm³, POV = 1.31, and NOG = 3.0 versus EV = 1.11 mm³, OV = 0.021 mm³, POV = 1.92, and NOG = 1.9). Known differences among the dames, including age, body mass, and time in captivity, exhibited no significant correlations with any egg characteristic [$-0.49 \leq \rho$ (4) $\leq 0.54, P \geq 0.22$].

4. Discussion

We documented and quantified seasonal reproductive exhaustion among spawns and, significantly, within and among breeding females (dames) in a broadcast spawning marine finfish cultured for conservation. Data on four physical egg characteristics were gathered and attributed to specific dames in a relatively large and freely-mating group of *A. nobilis* using genetic parentage analysis. Evaluating the egg data in terms of parentage allowed us to quantify changes and identify seasonal trends both non-invasively (without disrupting the spawning season, cannulation, or sacrifice) and within and among individual dames. Although there was significant inter-dame variation, *A. nobilis* did not maintain egg size and lipid content during the spawning season. EV, OV, POV, and NOG decreased overall, despite a minor upswing in EV and a more marked increase in NOG as the spawning season progressed. These changes were generally consistent with those reported in earlier research (Gauger, 2010) and occurred in the absence of changes to broodstock diet or holding conditions, outside gradual variation in the photothermal regime to mimic natural seasonal conditions. Because these results document a decrease in reproductive output in *A. nobilis* over the course of the spawning season, they indicated some level of seasonal reproductive exhaustion.

Table 2
A. nobilis dames responsible for single-maternity spawns produced during the 2008 spawning season. Fish listed by Dame ID. All fish collected at Santa Catalina Island between 15 Nov 1995 and 14 Jun 2003. Information includes time in captivity and von Bertalanffy growth function-estimated age and body mass as of 15 Jan 2008 at spawning season inception. Summary statistics listed in bottom rows in bold italics.

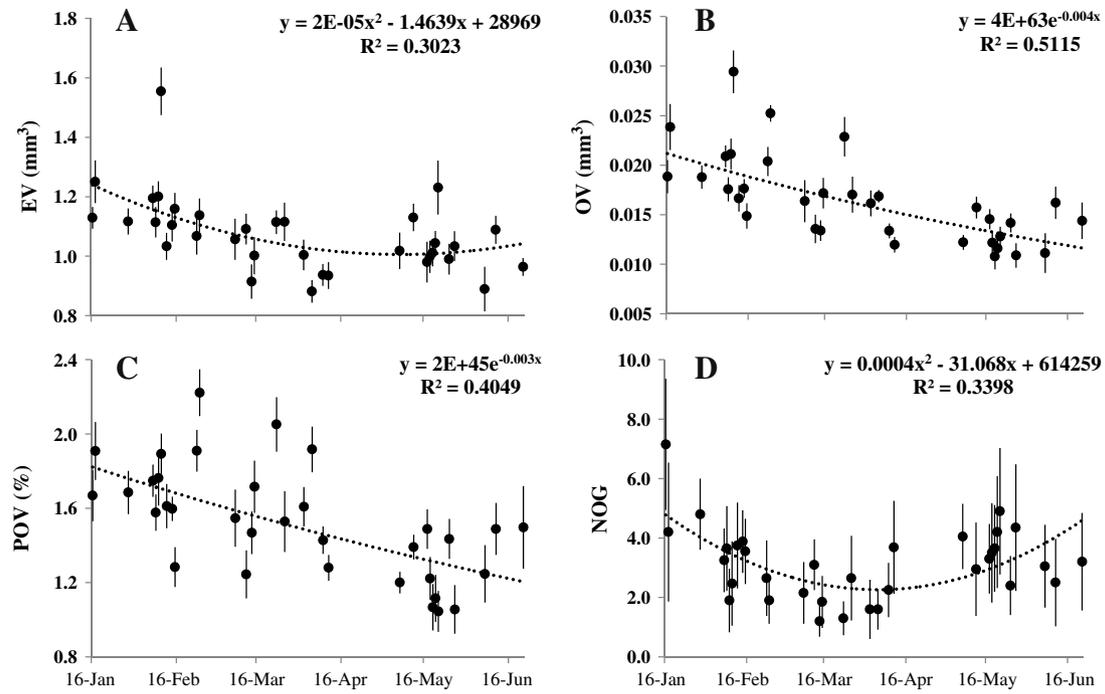| Dame	Age (y)	Time in captivity (y)	Body mass (kg)
503	15.7	11.7	18.6
522	12.4	8.8	16.4
523	13.2	8.8	17.0
525	14.5	8.8	18.0
526	13.6	8.8	17.4
528	12.2	6.7	16.2
536	12.8	8.8	16.7
541	12.9	8.8	16.8
544	12.4	7.8	16.4
545	11.7	7.8	15.7
548	12.0	7.8	16.0
549	8.5	4.6	11.7
550	8.5	4.6	11.7
Mean	*12.3*	*8.0*	*16.0*
Median	*12.4*	*8.8*	*16.4*
Standard deviation	*2.0*	*1.9*	*2.1*

Fig. 1. Egg volume (EV; A), oil volume (OV; B), percent oil volume (POV; C), and number of oil globules (NOG; D) during the spawning season. Spawn date on x-axis. Mean egg characteristics plus/minus one standard deviation for each spawn shown as black dots and whiskers, respectively. Dotted line represents best-fit trendline. Coefficient of determination [R^2 (32) ≥ 0.30, all $P < 0.001$] and trendline equations displayed above graphs.

Reproduction can carry a significant cost to the breeding individual. Shorter-term (e.g. seasonal) declines in reproductive performance, as in *A. nobilis*, have frequently been attributed to physiological resource limitation or depletion (Cerda et al., 1994; Palacios et al., 1998; Wise, 1994). Oogenesis and vitellogenesis, in particular, exact a physical toll because females put significant physiological resources into developing eggs.

Bransden et al. (2007) reported that *L. lineata* not only experienced decreased egg lipid volume over a spawning season but also lower triacylglycerol and monounsaturated fatty acid concentrations, potentially resulting from the demands of vitellogenesis. Decreases in somatic biochemical reserves, such as lipid and protein, due to egg production have been documented in many terrestrial and aquatic taxa (Boucard et al.,

Table 3
Non-parametric test statistic estimates for comparison of egg characteristics between and among spawns and within, between, and among dames. Characteristics include egg volume (EV), oil volume (OV), percent oil volume (POV), and number of oil globules (NOG). Test statistic (*U* or *H*) and associated *P*-value (in parentheses) listed per egg characteristic at each hierarchical level. Significant values listed in bold after sequential Bonferroni correction at the $\alpha = 0.05$ level.

| Statistic	Dame ID	df	EV	OV	POV	NOG
Mann–Whitney			*U* (*P*)	*U* (*P*)	*U* (*P*)	*U* (*P*)
Between spawns	503	1	**391 (<0.001)**	**362 (<0.001)**	218 (0.64)	231 (0.41)
Between females	503, 522	1	**2537 (<0.001)**	**2267 (<0.001)**	**3021 (<0.001)**	**2323 (<0.001)**
	503, 525	1	3666 (0.04)	**5882 (<0.001)**	**6080 (<0.001)**	**4716 (<0.001)**
	503, 526	1	**1653 (0.001)**	**2287 (<0.001)**	**2386 (<0.001)**	**1849 (<0.001)**
	503, 528	1	3451 (0.02)	**4284 (<0.001)**	**4923 (<0.001)**	3410 (0.02)
	503, 548	1	1214 (0.92)	**2237 (<0.001)**	**2394 (<0.001)**	**2071 (<0.001)**
	522, 525	1	**8264 (<0.001)**	**10,829 (<0.001)**	**10,977 (<0.001)**	6614 (0.2)
	522, 526	1	**3143 (0.001)**	**4220 (<0.001)**	**4307 (<0.001)**	2588 (0.35)
	522, 528	1	**7356 (<0.001)**	**7785 (<0.001)**	**7271 (<0.001)**	**6773 (0.002)**
	522, 548	1	**3843 (<0.001)**	**4667 (<0.001)**	**4499 (<0.001)**	**3324 (<0.001)**
	525, 526	1	5062 (0.21)	4906 (0.39)	4692 (0.74)	4587 (0.95)
	525, 528	1	10,538 (0.86)	**13,049 (<0.001)**	**14,291 (<0.001)**	**14,137 (<0.001)**
	525, 548	1	**5666 (0.006)**	**6404 (<0.001)**	**6792 (<0.001)**	**6078 (<0.001)**
	526, 528	1	4530 (0.25)	4966 (0.02)	**5727 (<0.001)**	**5513 (<0.001)**
	526, 548	1	**2471 (<0.001)**	**2859 (<0.001)**	**2715 (<0.001)**	**2367 (0.002)**
	528, 548	1	**5226 (0.002)**	**6823 (<0.001)**	**6838 (<0.001)**	**6698 (<0.001)**
Kruskal–Wallis			*H* (*P*)	*H* (*P*)	*H* (*P*)	*H* (*P*)
Within females	522	3	**55 (<0.001)**	**45 (<0.001)**	**33 (<0.001)**	**39 (<0.001)**
	525	7	**117 (<0.001)**	**128 (<0.001)**	**116 (<0.001)**	**25 (<0.001)**
	526	2	**42 (<0.001)**	**42 (<0.001)**	**19 (<0.001)**	**19 (<0.001)**
	528	6	**82 (<0.001)**	**101 (<0.001)**	**82 (<0.001)**	**68 (<0.001)**
	548	2	**12 (0.003)**	**46 (<0.001)**	**45 (<0.001)**	**11 (0.004)**
Among females		5	**51 (<0.001)**	**202 (<0.001)**	**235 (<0.001)**	**86 (<0.001)**
Among spawns		33	**526 (<0.001)**	**585 (<0.001)**	**559 (<0.001)**	**284 (<0.001)**
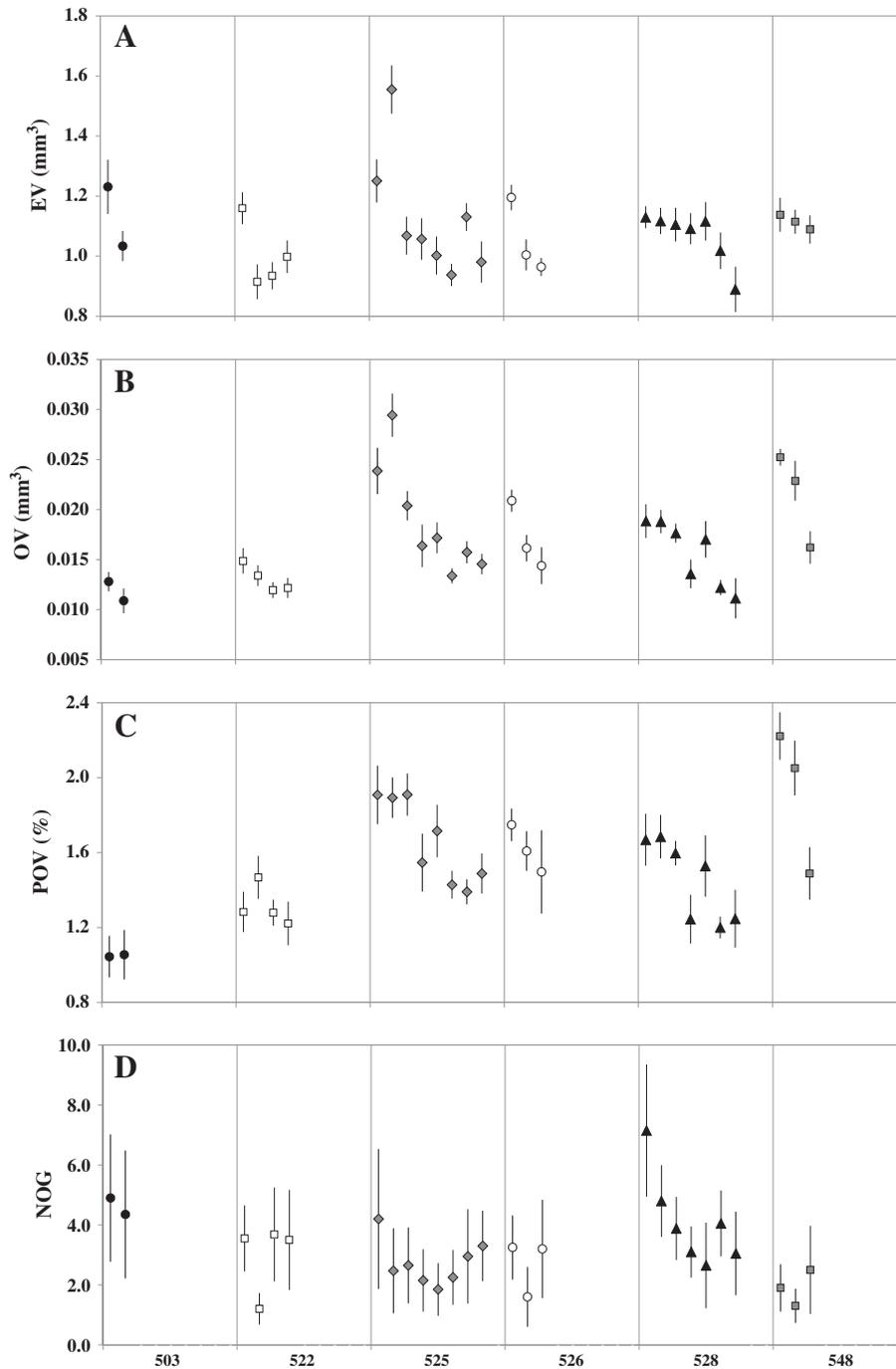
Fig. 2. Egg volume (EV; A), oil volume (OV; B), percent oil volume (POV; C), and number of oil globules (NOG; D) for specific dames during the spawning season. Dame ID shown on x-axis. Mean egg characteristics plus/minus one standard deviation for each dame shown as markers and whiskers, respectively. Spawns arranged chronologically from left to right; see Table 1 for dates.

2004; Hettyey et al., 2009; Müller et al., 2001; Palacios et al., 1998; Peréz-Jar et al., 2006; Zaviezo and Mills, 2000). Diminished biochemical stores have been further implicated in multiple negative effects for the breeding female, including decreased muscle metabolism (e.g. reduced mitochondrial oxidative capacity; Brokordt et al., 2000), depleted glycogen reserves, and impaired immune response (e.g. reduced phenoloxidase and hemoagglutinating activity; Peréz-Jar et al., 2006).

In addition to resource availability, egg characteristics may be influenced by many other factors, including but not limited to genetics, maternal effects (size, age, condition, etc.), pathogens, spawning time, spawning method, and environmental parameters like temperature,

salinity, and water quality (Chambers et al., 1989; Berkeley et al., 2004; Buckley et al., 1991; Gauger, 2010; Jerez et al., 2012; Rideout et al., 2005; Trippel and Neil, 2004). For example, temperature and light duration correlated with NOG in *A. nobilis*, perhaps due to induced changes in maturation or ovulation (Unuma et al., 2011) or the effect temperature has on the density and viscosity of lipids, but they cannot account for the changes in the other egg measures. Particularly in light of the common holding and feeding conditions, the inter-dame differences in the egg characteristics suggest that factors intrinsic to each fish, such as feeding behavior, genetics, or maternal effects, were involved in the seasonal variation we observed. Two common

contributors, fish age and body mass, did not correlate with any egg characteristic or egg production in general. More work is needed to identify possible causative factors.

Similar patterns in egg measures have been associated with variation in egg quality in other species. Moreover, egg quality is a known predictor of subsequent larval viability, quality, and stress resistance (Brooks et al., 1997; Kjørsvik et al., 2003), although no individual metric has been universally applicable within and among species. The results from previous research on *A. nobilis* indicated that certain egg characteristics (particularly OV and POV) were correlated with larval and juvenile growth, stress resistance, and survival (Gauger, 2010). Meanwhile, a combination of egg production factors (e.g. spawn date, fecundity, hCG induction response time) was predictive of larval survival in red snapper (*Lutjanes campechanus*; Bourque and Phelps, 2007), whereas Unuma et al. (2011) and Żarski et al. (2011) report negative correlation between NOG and egg quality in Japanese eels (*Anguilla japonica*) and Eurasian perch (*Perca fluviatilis*), respectively.

In terms of management, it is economically beneficial to a hatchery program if decisions are made early in the culture process regarding which spawns are best for juvenile production, ideally focusing efforts on higher-quality eggs that presumably develop into higher-quality fish. As previously mentioned, a lack of knowledge in what optimizes egg and larval quality limits aquaculture development for many species (Bromage, 1995; Kjørsvik et al., 1990). When natural population abundances are augmented with hatchery fish, higher egg quality should theoretically help increase survival rates in the wild, but it cannot be the sole factor evaluated for production purposes. Maintaining genetic diversity in the mixed population (wild and captive-bred) is also important because genetic variation is a means by which a population can persist in the face of stochastic environmental change. Hatchery stocking has been linked to reduced genetic diversity, inbreeding, and outbreeding depression in supplemented populations (Allendorf, 1986; Araki and Schmid, 2010; Ryman and Laikre, 1991; Tringali and Bert, 1998; Utter, 1998; Waples et al., 2012).

Yet, egg quality and genetic diversity can appear to work at cross-purposes in aquaculture. The highest hatch rates (~95%) and larval survival and viability (based on starvation testing) were associated with POV ≥ 1.8 in *A. nobilis* (Gauger, 2010). However, our data revealed that only two of six dames produced any single-maternity spawns with POV ≥ 1.8. Lower POV values must be considered acceptable for production to ensure that eggs are chosen from more than a small subset of "high-performing" dames, which would considerably limit genetic diversity in offspring reared for release in the wild. For instance, five of six dames produced at least one spawn with POV ≥ 1.5.

It should also be noted that these changes in the EV, OV, POV, and NOG may not significantly affect overall hatchery reproductive success, wherein large numbers of offspring often survive early life stages (i.e. in contrast to a natural population; e.g. Gold, 2004; Gold et al., 2008). Indeed, fish are often culled due to program capacity limitations rather than quality concerns. Gauger (2010) reported that starvation resistance in *A. nobilis* larvae associated with POVs from 1.5 to 1.8 differed by <14% and other measures were still adequate (e.g. hatching was still >85% at POV = 1.5). Hence, until a definitive link is established with egg and larval quality, we recommend primarily focusing current production efforts for stock replenishment on larger volume spawns (those produced by multiple dames; see Gruenthal and Drawbridge, 2012) to boost genetic diversity and secondarily considering egg measures, such as maximizing POV.

In conclusion, we conducted a simple study using genetic parentage data to document seasonal changes in physical egg characteristics in a relatively large, breeding population of *A. nobilis* in captivity. Due to the nature of these changes, including significant declines in egg size and lipid content among spawns and within and among specific dames, we posit that they are indicative of seasonal (short-term) maternal reproductive exhaustion, or a decline female reproductive output over time. While the reason(s) for the declines in egg measures

is(are) unknown for *A. nobilis*, they occurred in the absence of changes to diet or holding conditions and were not correlated with female body mass, age, or time in captivity. Because *A. nobilis* is cultured for stock replenishment purposes, we related our results to how fish may be best produced, given that conflict may arise between maximizing egg quality and maintaining genetic diversity when choosing spawns to rear for release. However, more research into the relationship between these egg characteristics and offspring quality is needed before any particular egg measure might be regarded as more than a secondary consideration for *A. nobilis* juvenile production.

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