Spawning dynamics and egg production characteristics of captive *Seriola dorsalis* assessed using parentage analyses.

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Abstract

In pelagic fish species, studying reproductive behavior and spawning dynamics are challenging. In culture scenarios these parameters are difficult to investigate for broadcast spawners such as *Seriola*. Hubbs-SeaWorld Research Institute has been culturing California yellowtail (*S. dorsalis*), a species that is expected to become one of the first commercial offshore aquaculture species in Southern California. Brood fish are typically kept in a large tank and spawn volitionally throughout the spawning season. This method is successful in yielding a consistent supply of eggs, however it limits the understanding of spawning group dynamics or individual brood fish contribution, information that will be important to scaling production to commercial levels. In this study, genetic-based offspring parentage analyses were used to evaluate captive spawning dynamics in *S. dorsalis* over two consecutive spawning seasons. The analyses determined that typically one female released eggs during a spawning event, compared with an average of six males who released milt during these same spawning events. Notably, the analyses revealed that a single female dominated spawning output in both years, participating in 63 spawning events and produced 49.7 million eggs. The largest single spawn contribution by a female was 2.5 million eggs, corresponding to a batch fecundity estimate of 110,000 eggs kg^{-1}. The spawning interval for the most productive females was 5-6 days. Using genetic tools to characterize spawning events will help to improve breeding program designs for *S. dorsalis*, and this information will serve as a proxy for estimating spawning capacities of this species in the wild.

Keywords: *Seriola dorsalis*, parentage analyses, California Yellowtail, spawning interval

Abbreviations:
CYT: California Yellowtail
HSWRI: Hubbs-SeaWorld Research Institute
EtOH: ethyl-alcohol
dph: days post-hatch
YSL: yolk-sac larvae
MS-222: tricaine methanesulfonate
PCR: polymerase chain reaction
LOD: logarithm of the odds
HWE: Hardy-Weinberg equilibrium
Ne_f: effective population size of females
Ne_m: effective population size of males
N_e: effective population size (broodstock population)
$N_{f,m,e}$: effective number of spawning females, males, or both
q: proportional contribution from each female or male.
n_f: census number of females
n_m: census number of males
n_e: census number of females and males (broodstock size)

1. Introduction

Reproductive behavior and spawning dynamics are two of the most challenging biological aspects to study for pelagic fish species. In fact, for many of the commercially valuable and popular pelagic species, such as the tunas, most of what is known has only been inferred from gonadal histological analyses of wild fish. These analyses can help answer questions related to spawning readiness, spawning frequency, and overall fecundity of a species (Margulies *et al*. 2007). However, without direct observation, little can be determined regarding reproductive behavior and social interactions leading up to, or involved in the spawning events (Moran *et al.* 2007). Even where direct observation is possible (e.g., in aquaculture settings), many pelagic species exhibit group spawning behaviors, making it difficult to determine individual brood fish participation (Knibb *et al.* 2016). *Seriola*, the group of fish commonly known as yellowtail, kingfish, or amberjacks, are an example of this type of species. These fish are popular in the growing aquaculture industry due to their high value as a sashimi-grade fish (Purcell *et al.* 2015), and culture for *Seriola* is underway or in development around the world.

This includes *Seriola quinqueradiata* in Japan and Taiwan, *S. dumerili* in Japan, Saudi Arabia, Korea, and the Mediterranean, *S. rivoliana* in Hawaii and Mexico, *S. lalandi* in Chile, Australia, New Zealand, Japan, and Europe, and *S. dorsalis* in Mexico and California (Dettleff *et al*. 2020; Symonds *et al.* 2014; Aguilera *et al.* 2013; Abbink *et al.* 2012).

The California yellowtail (CYT), *Seriola dorsalis* (formerly referred to as *S. lalandi*; Martinez-Takeshita *et al.* 2015; Purcell *et al.* 2015; Baxter 1960), is expected to become one of the first commercially developed offshore aquaculture species in Southern California. Similar to other pelagic finfishes, *Seriola* are aggregate, broadcast spawners; releasing gametes in multiple synchronized events during warmer months (Stuart *et al*. 2020; Rodriguez-Barreto *et al.* 2013; Sala *et al.* 2003; Poortenaar *et al.* 2001; Sumida *et al.* 1985; Baxter 1960). Due to this spawning behavior, individual parental contribution in the wild or culture has been difficult to ascertain, and little is known about spawning dynamics in this or other *Seriola* species (Knibb *et al.* 2016).

Hubbs-SeaWorld Research Institute (HSWRI; San Diego, CA) has been culturing CYT since 2003 (Stuart & Drawbridge 2013); similar to other *Seriola* culturing techniques (Knibb *et al.* 2016; Jerez *et al.* 2006), wild-caught CYT broodstock are kept in a large tank where spawning occurs naturally during the spring and summer months. This method helps ensure a source of fertilized eggs throughout the spawning season, and minimizes handling stress involved in strip spawning, which has had mixed results in *Seriola.* (Knibb *et al.* 2016). However, volitional spawning in groups does not allow assessment of individual brood fish contribution without the use of an additional tool such as genetic-based offspring parentage assignment.

Genetic markers, such as microsatellites, have been successfully applied to a number of aquaculture species to evaluate parentage and pedigree relationships (Perez-Enriquez *et al*. 2020; Whatmore *et al*. 2013; Gruenthal & Drawbridge 2012; Gold *et al.* 2010; Norris *et al.* 2000). In aquaculture, these analyses are indispensable in developing and evaluating breeding programs (Fessehaye *et al.* 2006), minimizing inbreeding effects (Vandeputte & Haffray 2014; Sekino *et al*. 2003), and preventing genetic diversity loss due to reproductive variance (Rodriguez-Barreto *et al*. 2013; Boudry *et al*. 2002). These methods can also be used to identify unfit brood fish, or select high quality wild or F1 fish for use in subsequent broodstock populations (i.e., broodstock selection) (Jerry *et al*. 2006; Vandeputte *et al*. 2004).

In order to provide an in-depth evaluation of captive spawning dynamics of CYT, a panel of nine microsatellite markers was used to identify parental contribution for all spawning events over two consecutive spawning seasons (n= 69 in 2013, and 62 in 2014). This study represents the first attempt to genetically evaluate spawning dynamics in CYT, and it is also one of the most thorough assessments of season-long reproductive efforts for any *Seriola* species. The individual-level analyses of spawning frequencies, proportional contributions, and spawning intervals for captive male and female brood CYT, in this study, greatly advances the understanding of spawning dynamics of this species and other *Seriola* species, which, up until this point, have been poorly understood. Characterization of these spawning events will directly improve breeding program design of these fish in a culture setting, and will also importantly serve as a proxy for spawning capacities of CYT in the wild.

2. Materials and methods

Ethics Statement

The authors cite compliance with the US National Research Council's "Guide for the Care and Use of Laboratory Animals," the US Public Health Service's "Policy on Humane Care and Use of Laboratory Animals," and "Guide for the Care and Use of Laboratory Animals". The institutional animal care and use committee (IACUC) protocol used for this study was 2014-01.

2.1 Sample collection

At HSWRI, the CYT broodstock were kept in a 140 m^3 fiberglass tank that was exposed to natural light cycles (Stuart *et al*. 2020, Stuart & Drawbridge 2013). Ambient temperature seawater was filtered and recirculated in the tank at a rate of three to six turnovers per day. Broodstock fish were fed a diet of vitamin supplemented fresh fish and squid three to five times each week, as described in Stuart & Drawbridge (2013). Prior to stocking the fish into the broodstock tank, each brood fish was pit-tagged and sexed by cannulation. Fin clips (approximately 5 x 10 mm) were also collected from each fish and stored in 100% non-denatured ethyl-alcohol (EtOH) for genetic analysis. On an annual basis, the fish were measured for mass (kg), total length (mm), and standard length (mm).

CYT broodstock spawn volitionally at HSWRI without the use of hormones. During the spawning season (March – September), 500 µm mesh egg-traps were placed in the collection sump; these egg-traps receive 100% of the tank effluent stream coming from both the surface and bottom of the tank. The mesh traps were checked daily for eggs, and if present, all eggs were collected and measured volumetrically in 10 L graduated cylinders. Then, egg volumes were converted to total egg numbers using a previously determined conversion factor of 500 eggs ml^{-1} (K. Stuart, personal communication). From the collected eggs, approximately 1,500 floating eggs were stocked for hatching from each spawning event. Samples of zero-day post-hatch (dph) yolk-sac larvae (YSL) were collected upon hatching for each spawning event. The sampled

larvae were humanely euthanized in a bath containing a lethal >300 mg L^{-1} of the anesthetic tricaine methanesulfonate (MS-222), in accordance with IACUC protocol (#2014-01). Larvae were then transferred to 100% non-denatured EtOH until DNA extraction.

In 2013, the broodstock population consisted of 11 males and 8 females (average weight of 20.7 kg), (hereafter referred to as "original" or "larger" broodstock fish). Prior to the start of the 2014 spawning season, 18 smaller fish (average weight of 8.4 kg) were added to the broodstock population for a total of 18 females and 19 males (hereafter referred to as "smaller" broodstock fish). Two of these smaller fish were removed for health reasons; a final number of 17 females and 18 males comprised the 2014 broodstock population, and between March 08 and September 01, this group generated 62 spawning events.

2.2 Power Analyses

As little data was available at the time, a power analysis was conducted to determine the minimum number of YSL required to accurately characterize the genotypic representation of a given spawning event. The sample size calculator, EpiTools (Sergeant 2009), was used to account for allelic contribution. Genetic parameter inputs were conservatively chosen; allelic contribution of each fish was assumed to be equal (with an estimated proportion of 0.5), the confidence level was set at 95%, and the desired precision of the estimate was set at P = 0.05. The sample size calculator also assumed an infinite population of samples available for analysis. Using these conservative parameters, a minimum sample size of n = 385 was estimated.

As spawning participation by brood fish is rarely complete or equal (Gruenthal & Drawbridge 2012), five spawns were further analyzed that most closely met the estimated sample size criterion (n = 325-377, which become our "true" values for later binomials). Bootstrap

resampling was performed on these spawning events to model the effects of subsampling these data. Following parentage assignment, data from each of the five spawns were split into paternal and maternal parentage contributions, and these data were bootstrapped 10,000 times with final sample sizes (n-values) set to: 10, 20, 30, 40, 47, 60, 100, 150, 200, 250, and 300. From the bootstrapped data, averages and 95% confidence intervals were determined separately for males and females. Binomial tests with Bonferroni corrections were conducted to determine whether parental contribution proportions in subsamples were significantly different from "true" proportions.

2.3 DNA extraction and PCR amplification

DNA was extracted from either a 1-mm^2 fin tissue clip (for broodstock fish samples), or from whole YSL using a 10% (weight/volume) Chelex (Bio Rad) boiling protocol (Hyde *et al.* 2005). A panel of nine previously published microsatellite loci (Table 1) was selected to use for the parentage analysis, these loci were previously found to contain sufficient diversity to allow unambiguous assignment of parentage for F1 larvae produced by the HSWRI broodstock population (Purcell & Hyde, unpublished data; Purcell *et al.* 2015). Microsatellite loci were combined into multiplex polymerase chain reaction (PCR) sets based on compatible annealing temperatures and fragment sizes (Table 1). Forward primers were fluorescently labeled on their 5' end. PCR reactions were conducted using 10-20 ng template DNA in a 11 µL reaction containing 67 mM Tris-HCl pH 8.8, 16.6mM $(NH_4)_2SO_4$, 10 mM β-mercapto-ethanol, 2 mM $MgCl_2$, 800 µM dNTPs (Bioline), 0.5 mg ml^{-1} BSA, 0.15 - 0.3 µM fluorescently labeled forward primer(s), 0.15 - 0.3 µM reverse primer(s), and 0.25 units *Taq* DNA polymerase (New England Biolabs). Thermal cycling parameters were as follows: denaturation at 94 °C for 4 minutes, followed by 35 cycles of 94 °C for 30 s, annealing 53 – 59 °C (Table 1) for 35 s, and elongation

at 72 °C for 30 s, with a final extension for 5 minutes at 72 °C, followed by a 4 °C hold. Products from PCR multiplexes 1 and 2 were further pooled prior to genotyping (Table 1). Samples were analyzed on an ABI 3730XL Genetic Analyzer (Applied Biosystems) using the GeneScan™ 500 ROX™ Size Standard (Applied Biosystems). Fragment data were analyzed using GENEMAPPER 4.0 (Applied Biosystems) and scored visually.

2.4 Parentage analyses

Parentage assignments were calculated using exclusion algorithms (Jones & Arden, 2003) on Cervus v3.0.6 software (Kalinowski *et al.* 2007). Allele frequencies were calculated to assess Hardy-Weinberg Equilibrium (HWE), using a minimum expected frequency of five percent, and Bonferroni corrections to evaluate significance of HWE. Parentage simulations were assessed for 10,000 offspring using logarithm of the odds (LOD) scores with confidence set at 90% (for relaxed confidence levels), and 95% (for strict confidence levels), using a minimum of six genotyped loci. The proportion of parents sampled was set to 1.0, as all of the genotypes from the broodstock population were sampled and included in the analyses. To corroborate results of the parentage assignment, analyses were conducted using known sex data for the broodstock fish, and again without the sex data, to ensure parentage matches were consistent.

2.5 Correlation tests, fecundity estimates, and effective population size estimates

Mass (kg) of brood fish for both 2013 and 2014 spawning seasons were tested for normality using a Shapiro-Wilks test, and fulfilling the assumptions for normality; smaller broodstock and larger broodstock were compared using a two-tailed t-test. Correlations were tested for each spawning event between total number of eggs and environmental factors, between female mass, and batch/annual fecundity estimates using Spearman's rank correlation. Proportional contribution from each individual was compared to theoretical equal proportions

(e.g. each brood fish contributing equally to all spawns) using a binomial test. All bootstrapping, binomials tests, correlations, normality tests and t-tests were conducted using R v3.0.2. (R Core Team, 2013).

Estimates of effective population size, proportional contributions, fecundity estimates (female), and spawning intervals (female) were calculated as described below. Effective population sizes for females (Ne_f), males (Ne_m), and for the broodstock population (N_e) were calculated using the following equations from Gold *et al.* (2008): $N_e = (4 * Ne_f * Ne_m) / (Ne_f + Ne_m)$, where $Ne_{f/m} = 1 / (\sum q^2)$, where $n_{f,m,e}$ = number of spawning females, males, or both; and q = proportional contribution from each female or male. Values for q were calculated using the estimated contribution from the genotyped offspring from each spawn. To test method robustness, this calculation was repeated twice using the five spawning events analyzed in the power analysis. Comparisons between the standard 47 YSL and subsequently with the additional 278 – 330 YSL using a Binomial tests with a Bonferoni correction. Individual female fecundity estimates were extrapolated from the estimated number of eggs per spawn (see above for quantification method), then multiplied by the proportion of YSL attributed to that female from the same spawn event. Summary statistics for egg production and fecundity were reported using only females that spawned during the recorded season. For individual females, number of days between spawning events were reported as spawning intervals; if a female spawned only once, no interval was estimated.

3. Results

3.1 Power analysis

In testing the number of samples needed to reflect individual brood fish contributions to spawning events, it was determined that a sample size of n = 30 YSL, with 95% confidence, was

not significantly different from 'true' contributions based on bootstrap resampling and binomial analyses. Proportions considered 'true' were estimated from larger sample sizes (> 300 YSL) per spawning event. To further test the impact of sample size, fecundity estimates for these same spawning events were calculated with contribution frequencies (q) based on the smaller sample (47 YSL) and larger sample (>300 YSL). Individual brood fish contributions and contribution frequencies were not significantly different based on these different sample sizes (P = 0.019 – 1.00). Based on these results, 47 YSL per spawning event were used for the remaining analyses.

3.2 Parentage analyses and effective population sizes

From the 69 spawning events in 2013, YSL were available from 68 spawns; one spawn was not sampled due to egg hatching failure. Of the 62 spawns in 2014, two spawns were not sampled due to egg hatching failure. For the majority of spawning events, at least 47 YSL were available for parentage analyses. However, two spawns in each of the two years had fewer than 47 sampled YSL; 9 and 15 YSL in 2013, 32 and 38 YSL in 2014). For those four spawn events, analyses were completed using the available samples. For each spawn, a negative PCR control (sterile H2O) was included in the batch; no negative controls showed any contamination during fragment analysis of the PCR products. Following stringent sample quality filtering (i.e., sufficient number of genotyped loci per individual), 3170 and 2789 YSL were assigned to parental pairs from the 2013 and 2014 spawning seasons, respectively. For both years, all microsatellite loci were in HWE. Parentage analyses indicated that spawns were often dominated by a single female (59.4% of spawns for 2013 and 72.6% for 2014); male contribution was more broadly distributed, with individual males contributing between 0 and 64% per spawn (mean = 9.03% per spawn, Figures 1, 2, and 3). This sex-based skew in spawning participation was also reflected in the effective size estimates; N_{em} was estimated as 5.89 and 6.23 and N_{ef} at 1.38 and

1.21 for 2013 and 2014, respectively (Table 2). The smaller brood fish, added before the 2014 spawning season, contributed far less than the larger brood fish. During 2014, the smaller females only produced 9.6% of the offspring, and similarly, smaller males only contributed to 14.6% of the offspring. Despite the addition of the 18 fish to the 2014 broodstock population, the annual total effective population size increased by only 0.13 in females and 1.16 in males (Table 2).

3.3 Fecundity estimates and correlation tests

The mean mass of the larger females in the broodstock population was 20.7 kg (range 14.6 – 27.2 kg), whereas the smaller female fish added in 2014 were 8.4 kg (range 7.2 –10.2 kg) (Table 2). In 2013, the population produced 69 million eggs of which, we were able to extrapolate parental assignment to 66 million. All females contributed eggs, with an estimated annual production of 8.2 million eggs female^{-1}. Average batch fecundity was 460,000 eggs per spawn or 23,000 eggs kg^{-1} spawn^{-1}. The maximum spawn size from a single female in 2013 was 1.9 million eggs or 97,000 eggs kg^{-1}.

In 2014, the population produced 58 million eggs of which we were able to extrapolate parental assignment to 56 million. All eight of the original larger females spawned in 2014 and they contributed 96% of all eggs produced. Among the larger females, estimated annual production averaged 6.8 million eggs female^{-1}. Average batch fecundity was 240,000 eggs spawn^{-1} or 11,000 eggs kg^{-1} spawn^{-1}. The maximum spawn size from a single female in 2014 was 2.5 million eggs or 110,000 eggs kg^{-1}.

Among the smaller fish introduced to the population in 2014, seven of ten females spawned, with three females not spawning at all. Contribution to the total annual egg production

was only 2 million eggs, with an estimated annual production that averaged 290,000 eggs female^{-1} (Table 2). Average batch fecundity for these small fish was 21,000 eggs spawn^{-1} or 2,000 eggs kg^{-1} spawn^{-1}. The maximum spawn size from a single 9.2 kg female in 2014 was 400,000 eggs or 43,000 eggs kg^{-1}.

No correlation between female mass and average annual or average batch fecundity was detected for the 2013 spawning season (P = 0.753 – 0.739); however, both were positively correlated during the 2014 spawning year (P = 0.0003 – 0.00045, see Table 2).

3.4 Spawning frequency

The female contributing to the greatest number of spawns in 2013 (083-027-609) had a mean and median spawning interval of 5.0 and 5.0 days, respectively. The second and third most prolific spawning females in 2013 (083-103-352 and 061-621-862) had mean and median spawning intervals of 6.8 and 7.0 days and 11.4 and 11.0, respectively. Although the overall mean and median spawning interval estimates for all females in 2013 were still relatively close at 11.1 and 10.6 days, respectively (Table 2), the spawning interval means and medians calculated for the other individual females differed by more than four days.

The most prolific female brood fish in 2013 also contributed to the greatest number of spawns in 2014 (083-027-609). In 2014 her mean spawning interval was 6.3 days. When comparing the spawning intervals for the original large fish between each year, the median interval remained very similar to 2013 at 6.5 days, while the mean interval increased to 13 days. The seven smaller fish that spawned in 2014 had mean and median spawning intervals of 27 and 26 days, respectively (Table 2). This was not surprising given how infrequently the smaller fish spawned in comparison to the larger fish. No correlation was detected between batch fecundity

and the number of days between spawning events, and no significant correlations were detected between individual spawning event participation and the environmental parameters tested in this study (i.e., water temperature, lunar cycle, and day length).

During both spawning seasons, a small subset of female brood fish dominated egg production. For the largest spawning events in both years (3.65 million eggs in 2013, 3.02 million eggs in 2014), only three females contributed to the spawns. The dominant female in 2013 and 2014 (083-026-609) was identified as the maternal parent of approximately 40% of the total YSL genotyped during the two seasons (Figures 1, 2, 3), and it was estimated that this fish produced between 23 and 27 million eggs. Out of the 69 spawning events in 2013, three females (083-027-609, 061-621-862, 083-103-352) contributed to 32 (46.4%), 16 (23.2%), and 20 (29.0%) events, respectively. In 2014, the prolific female spawner again contributed to 28 of the 62 (45.2%) spawn events. The second largest contributor in 2014, female 083-026-876, contributed to 13 spawns with 14.8% of the YSL assigned to this individual. Female 061-621-862 was assigned 10.6% of the total YSL, but was estimated to contribute the second largest proportion of eggs (20.1%). Among the smaller fish, only one female (061-621-862) made a notable reproductive contribution of 8.2% of the assigned YSL, with an estimated 2.7% of the total eggs (Figure 4).

The individual male contribution in 2013 was more balanced than female contribution when analyzed by spawning events (Figure 1). Male spawning proportions did not differ significantly from equal contribution ($P = 0.05$). On average, 5.87 males (1.67-12.37), contributed during each spawn in 2013, and an average 6.24 males (2.77-9.73) participated in spawn events in 2014 (Table 2). Spawning patterns for larger male brood fish were similar in the

2013 and 2014 spawning seasons, however, the smaller males added in 2014, showed lower overall participation per spawn (Figures 3 and 4).

4. Discussion

Following simulations to determine the sample size of YSL needed to represent "true" parentage proportions, it was determined that a sample of 30 YSL was sufficient to accurately characterize parentage assignment proportions (within a 95% CI) from each spawning event. This result is similar to what has been reported in other studies (Hale *et al.* 2012). While the minimum sample size estimated by simulation (n = 30) was an order of magnitude smaller than what was predicted by the power analysis (n = 385), this is not surprising given the sex-biased skew in spawn participation, which is common in fishes. Further analyses confirmed that the estimates of fecundity for the five initial spawning events (sample size >300 YSL) were not statistically different from sub-samples of 47 YSL. The reduced costs of the smaller sample size allowed for a greater number of spawning events to be tested. Forty seven YSL were used to represent each spawn because it allowed individuals to be removed (if needed) due to quality issues while also efficiently utilizing standard genotyping protocols (i.e. standard 96-well plate to accommodate two spawns per plate and controls).

In both spawning seasons, no significant environmental correlations were detected that influenced spawning patterns or parental brood fish contributions. For both sexes, the mean effective population size was much lower than census size, but particularly for female brood fish. A similar pattern was reported in *S. lalandi* (Dettleff *et al*. 2020), *S. dumerili* (Rodriguez-Barreto *et al*. 2013), Atlantic cod (*Gadus morhua*; Herlin *et al.* 2008), red drum (*Sciaenops ocellatus*; Gold *et al.* 2010), and white seabass (*Atractoscion nobilis*; Gruenthal & Drawbridge 2012). The annual effective population sizes were closer to census numbers for both sexes; however, the

annual effective number was still considerably lower than census size for females. The spawning dynamics, leading to this pattern of small effective broodstock size, are evident by observing the relative individual fish contributions. In each spawning event, the common pattern is that multiple males (mean = 8.65 and 10.07 for 2013 and 2014, respectively) participate in the spawn, while only a single female releases the majority of the eggs for that event. In fact, for the majority of the spawning events in 2013 and 2014 (59.4% and 72.6%, respectively), only one female contributed the eggs in a given spawning event.

Based on the results of this study, it appears that *S. dorsalis* exhibits a spawning dynamic similar to what has been reported in related species *S. dumerili* (Rodriguez-Barreto *et al.* 2013) and *S. lalandi* (Dettleff *et al*. 2020, Symonds *et al.* 2014; Moran *et al.* 2007). This spawning dynamic is characterized by multiple males courting a single female to initiate spawning and synchronize the release of gametes (Gonçalves & Oliveira 2010). This spawning dynamic has also been reported in other pelagic species (Perez-Enriquez *et al*. 2020; Gruenthal *et al*. 2014; Margulies *et al.* 2007; Hutchings *et al.* 1999; Magnusson & Prescott 1996; Smith 1986). In other species, such as the yellowfin tuna (*Thunnus albacares*), a spawning event consists of several small-group spawning sessions over a short period; in this spawning dynamic, a greater total number of females contributes to the spawning event on a given day (Margulies *et al.* 2007).

While the female brood fish releasing the majority of eggs varied between spawning events, parentage analyses did reveal that one brood female (individual 083-27-609) produced approximately 40 percent of the offspring genotyped in 2013 and 2014. Unequal contribution to offspring has been previously reported in other fish species (Beldade *et al.* 2012; Gruenthal & Drawbridge 2012; Liu *et al.* 2012), but it is not clear as to why this occurs. Several possible explanations have been proposed, for example, sperm competition, variable fertilization or

variable larval survival, or spawning/social behaviors (e.g., mate choice) (Rodriguez-Barreto *et al*. 2013). For *S. dorsalis*, the unequal contribution is more pronounced in females than males, so it is unlikely that sperm competition is a strong factor. Variable larval survival is also unlikely to explain this pattern as YSL were sampled just after hatching, thereby minimizing the survival variability. It is important to note that only successfully hatched YSL were sampled for the parentage analyses in this study, therefore variability in hatching or fertilization success cannot be ruled out as a possible mechanism generating unequal spawning contributions. Variable hatching and fertilization success could very well occur between hatching and non-hatching eggs, and between fertilized and non-fertilized eggs, respectively. Overall, egg quality parameters for all spawn events were recorded over the two years of this study and has been reported in Stuart *et al*. (2020). Unfortunately the egg quality performance have not been linked to individuals and the authors hope to address this in future publications.

The spawning patterns observed in *S. dorsalis* may indicate spawning behaviors or social interactions that lead to unequal parental contributions in this species. Observations of groups of males pursuing a single female have been reported in *S. dorsalis* (K. Stuart personal communication). Social dominance, operating in both sexes, has been documented across a broad diversity of fish species (Paull *et al.* 2010). Social dominance often influences behaviors involving the largest individual of either sex (Hutchings *et al*. 1999; McCormick 1998). The prolific female in the HSWRI brood tank (individual 083-27-609) was one of the larger females (22.6 kg) from the original stock. However, a larger female (5 kg heavier) only produced 6.2% of the offspring genotyped. Although size-based spawning dominance may occur in female *S. dorsalis*, this study could not rule out other possible dominance factors such as female age or nutritional status (Hixon *et al.* 2013). Future spawning studies should address this issue through

manipulations of sex ratio and stocking density, as this behavior can affect the efficiency of culture, especially when selective breeding practices are used.

The average spawning interval for the original large females in both years ranged between 11.1 and 13.0 days, while the most prolific female spawned consistently every 5 to 6 days. This spawning interval is similar to what HSWRI has seen when one female and two male yellowtail are in a spawning tank together; spawning intervals can range from 3 to 15 days depending on the female (n=48; K. Stuart unpublished data). Other *Seriola* species have reported spawning intervals within the same range with *S. riviolana* spawning every 14 to 21 days (Quiñones-Arreola *et al*. 2015), and *S. lalandi* spawning every 2 to 5 days (Moran *et al.* 2007; Yang *et al*. 2016). Similarly, white seabass have demonstrated a broad spawning interval range, from 7 to 35 days under culture conditions (Gruenthal & Drawbridge 2012). The average intervals reported in this study are longer than spawning intervals reported in other pelagic species such as yellowfin tuna (*Thunnus albacares*), south pacific albacore tuna (*T. alalunga*), north pacific albacore tuna (*T. alalunga*), southern bluefin tuna (*T. maccoyii*), and pacific bluefin tuna (*T. orientalis*), which were between 1.1 to 4.5 days (Farley *et al.* 2015, 2013; Chen *et al.* 2010, 2006; McPherson 1991). Several of these studies found that larger females spawned more frequently within that range (Farley *et al.* 2015; Chen *et al.* 2006; McPherson 1991), similar to the pattern detected in this study. Other carangid fish, such as bigeye scad (*Selar crumenophthalmus*) and mackerel scad (*Decapturus macarellus*), spawn every three days (Clarke & Privitera 1995). It is important to note, that these values are averages based upon oocyte development from histological examinations of wild specimens, rather than actual spawning events.

Genetic parentage analyses also allow for direct estimates of batch fecundity to be made for individual brood fish. In this study, batch fecundity estimates ranged from an average of 420,000 eggs kg^{-1} for the larger females (14.6 – 27.2 kg), to approximately 32,000 eggs kg^{-1} for the smaller fish (7.15 – 10.3 kg). The maximum estimated spawn size for an individual female CYT in this study was 2.5 million eggs, which equates to 110,000 eggs kg^{-1} of body weight. Similar batch fecundity rates from *S. dorsalis* have been observed when individual females were spawned in isolation with two males. Specifically, we have observed, 26,000 – 60,000 eggs kg^{-1}, for females weighing 8 – 12 kg, with spawn sizes of 279,000 – 480,000 eggs depending on the female (K. Stuart unpublished data). Traditional estimates of batch fecundity in *S. dorsalis*, using oocyte development and ovarian weight, have ranged from 458,000 to 3,914,000 eggs $female^{-1}$ $spawn^{-1}$ (Baxter 1960). The upper range of these fecundity estimates is considerably larger than what is reported here, although this discrepancy may be in part due to the differing methods. Batch fecundity estimates for other carangid species are often greater than 100,000 eggs $female^{-1}$ $spawn^{-1}$. Reported batch fecundity estimates for two scad species were 92,000 and 136,000 eggs (Clarke & Privitera 1995), and 344,700 eggs for the Atlantic horse mackerel (*Trachurus trachurus*) (Macer 1974). In this study, both annual and batch fecundity were found to be positively correlated with female mass, a pattern that was also detected in wild CYT and in other fish species (Hixon *et al.* 2013; Beldade *et al.* 2012; Baxter 1960).

5. Conclusion

This study has provided the first detailed assessment of spawning dynamics, spawning intervals, and fecundity estimates for *S. dorsalis* under culture conditions using genetic parentage assignment. While individual brood fish contributing to offspring production varied between spawning events, the general trend of multiple males and only a single female participating in a

given event held true for the majority of spawning occurrences. Additionally, the data revealed that male brood fish contribution was roughly equal over the duration of the spawning season; however, female contribution was heavily skewed by contributions from two to three prolific females who spawned more frequently and produced more eggs over the course of the two spawning seasons. While not all possible causes of this pattern could be investigated in this study, this pattern may reflect a behavioral or chemical control of spawning that promotes particular females and inhibits spawning in the other female fish. Gaining a better understanding of this spawning dynamic in CYT will be important to optimizing broodstock management in order to enhance egg production, and to maintain genetic diversity in the offspring produced. Maintaining the genetic diversity in offspring will be critically important if, or when, a genetic breeding program is established for this species. Additionally, if there is a behavioral inhibitor for some of the female brood fish, there may be greater efficiencies for commercial scale aquaculture production if broodstock are divided into smaller spawning groups to maximize egg production (Rodriguez-Barreto *et al.* 2013), or by using rotational mating schemes (Symonds *et al.* 2014).

Genetically informed parentage approaches, coupled with measures of batch and annual fecundity, dramatically advances the understanding of spawning dynamics in *S. dorsalis*, and will help to optimize husbandry practices and culture of this species. This information can also be used to help improve estimates of these life history parameters in wild CYT populations. This information is vital for stock assessments and for the successful management of wild CYT populations, but it is exceedingly difficult or impossible to study *in vivo*. Importantly, the results of this study may also help improve life history estimates for other coastal pelagic species that are not cultured, and for which there are not opportunities to conduct this type of research.

Author Contributions

Elizabeth Schmidt: Conception and design of experiment, analysis and interpretation of data, drafting the article, revising the article for publication.

Kevin Stuart: Conception and design of experiment, culturing of yellowtail used in the experimental design, providing samples, revising the article for publication.

John Hyde: Conception and design of experiment, molecular analysis development, revising the article for publication.

Catherine Purcell: Conception and design of experiment, molecular analysis development, drafting the article, revising the article for publication.

Mark Drawbridge: Project administration, funding acquisition, conception and design of experiment, providing samples, revising the article for publication.

Conflict of Interest

The authors confirm that there is no conflict of interest to declare in submission of this manuscript to Aquaculture Research.

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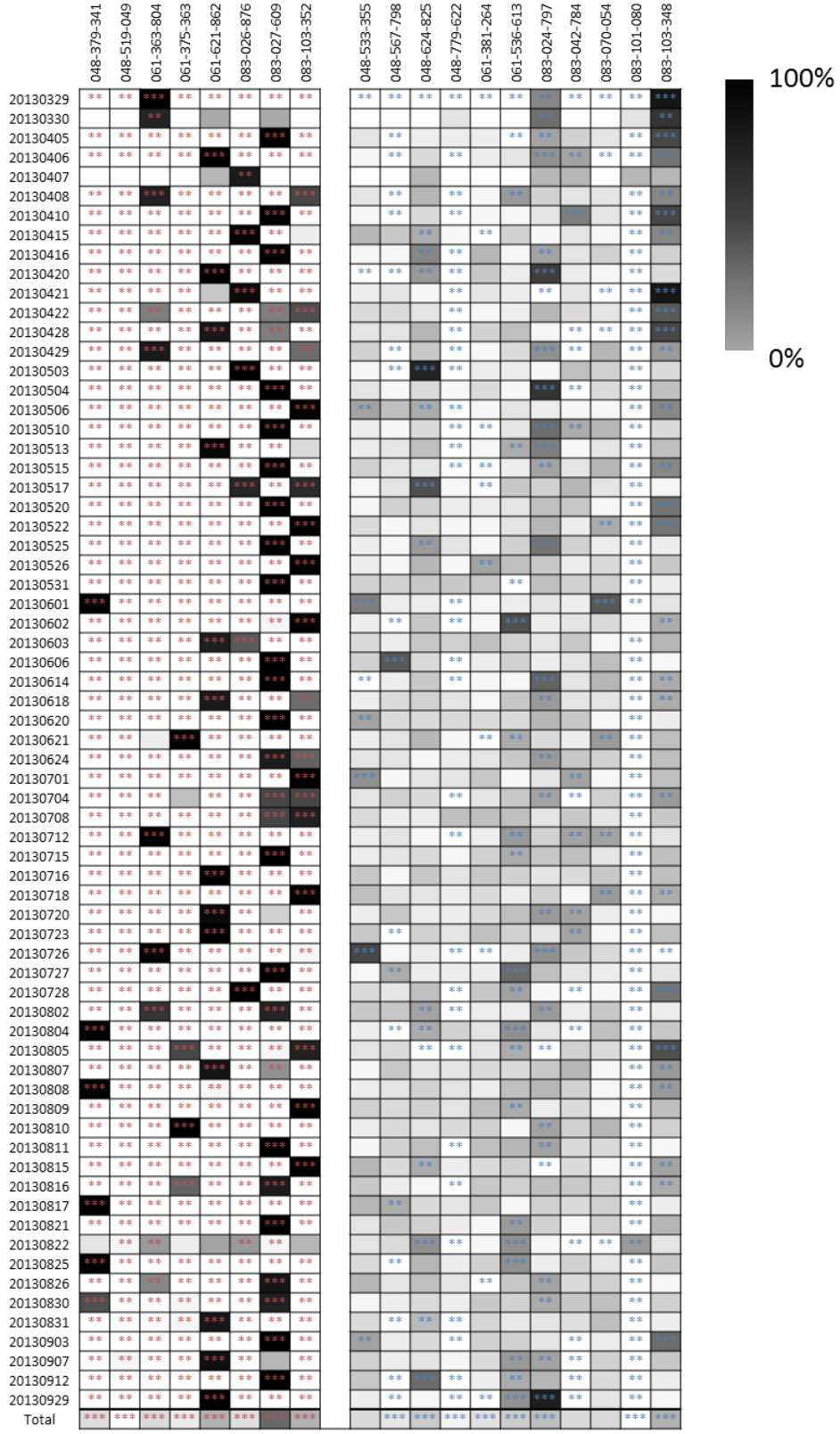
FIGURES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20130831
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Total
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0%

Figure 1. Proportion contribution to individual spawn for each fish from the 2013 spawning season, where black indicates 100% spawn contribution, and white indicating 0% contribution. Dates of spawn are given as 'yyyymmdd' (first column), and all fish are represented by pit-tag number. Symbols within each cell indicate significance from binomial tests for females (left side) and males (right side), evaluating whether observed parental contribution differs significantly from equal contribution, where blank cells are not significance, ** = $0.05 > P > 0.0001$, and *** = $P < 0.0001$.

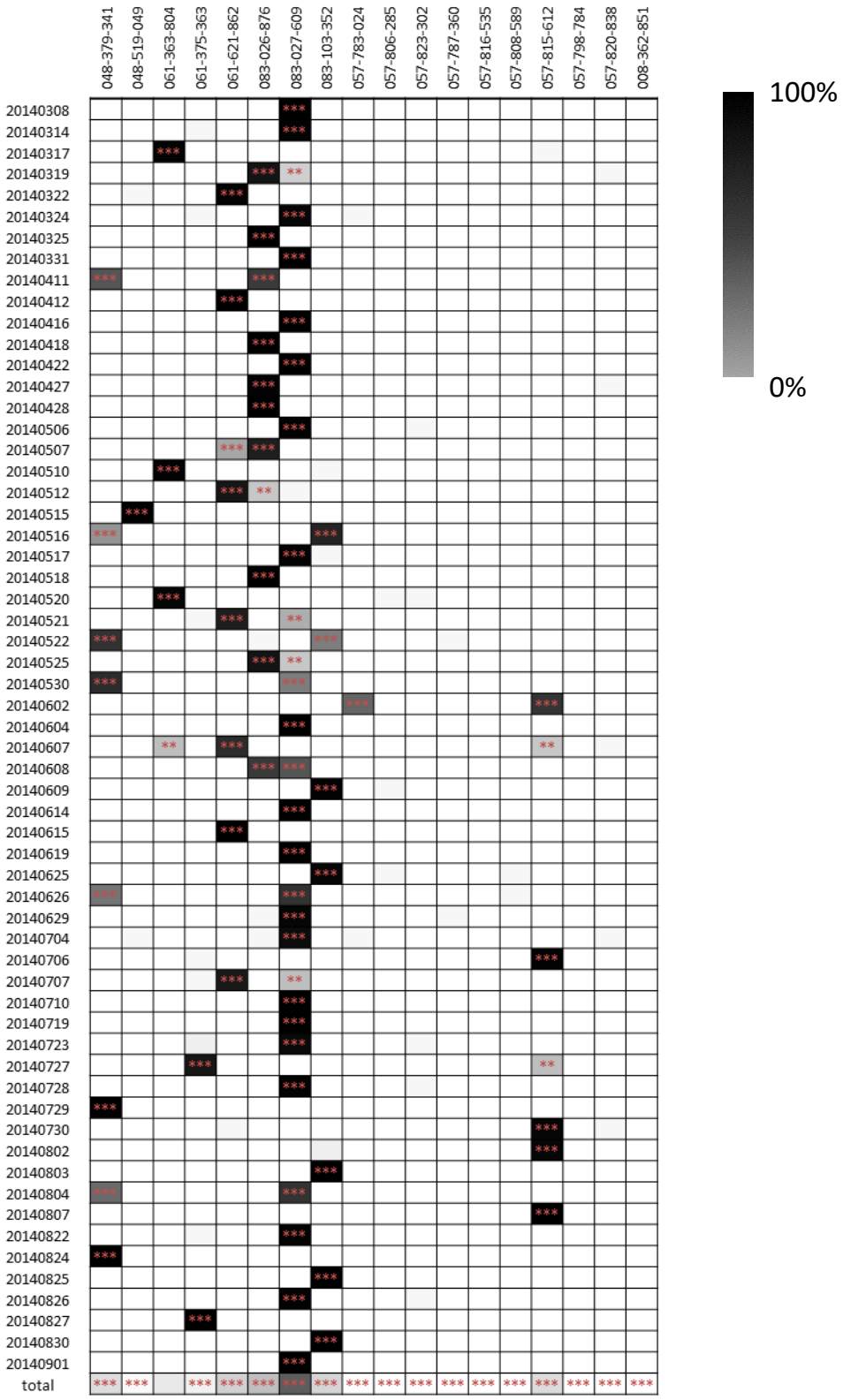

Figure 2. Proportion contribution to individual spawn for female fish from the 2014 spawning season, where black indicates 100% spawn contribution, and white indicating 0% contribution. Dates of spawn are given as 'yyyymmdd' (first column), and all fish are represented by pit-tag

number. Symbols within each cell indicate significance from binomial tests, evaluating whether observed parental contribution differs significantly from equal contribution, where blank cells are not significance, ** = $0.05 > P > 0.0001$, and *** = $P < 0.0001$.

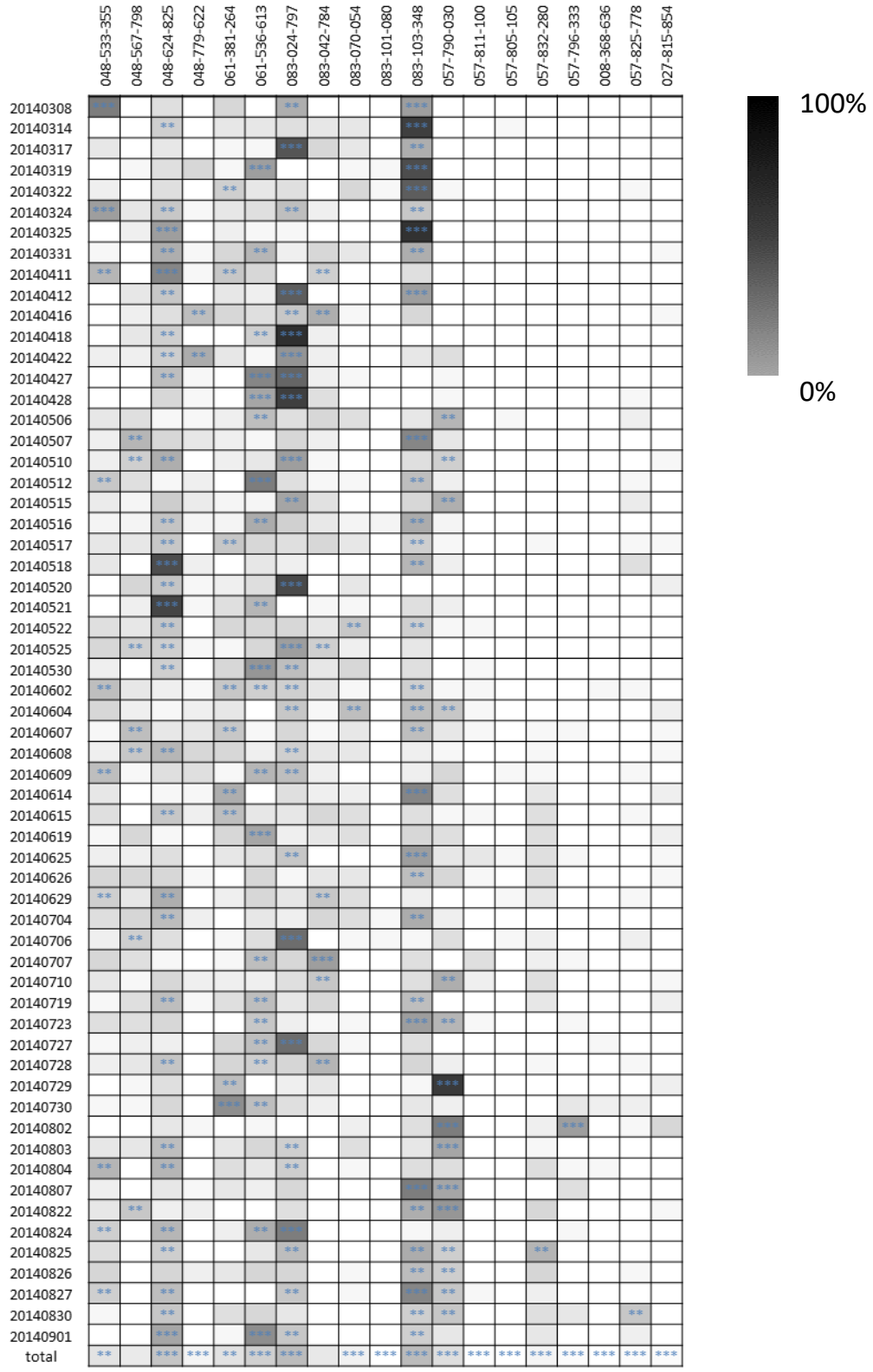

Figure 3. Proportion contribution to individual spawn for male fish from the 2014 spawning season, where black indicates 100% spawn contribution, and white indicating 0% contribution. Dates of spawn are given as 'yyyymmdd' (first column), and all fish are represented by pit-tag

number. Symbols within each cell indicate significance from binomial tests, evaluating whether observed parental contribution differs significantly from equal contribution, where blank cells are not significance, ** = 0.05 > P > 0.0001, and *** = P < 0.0001.

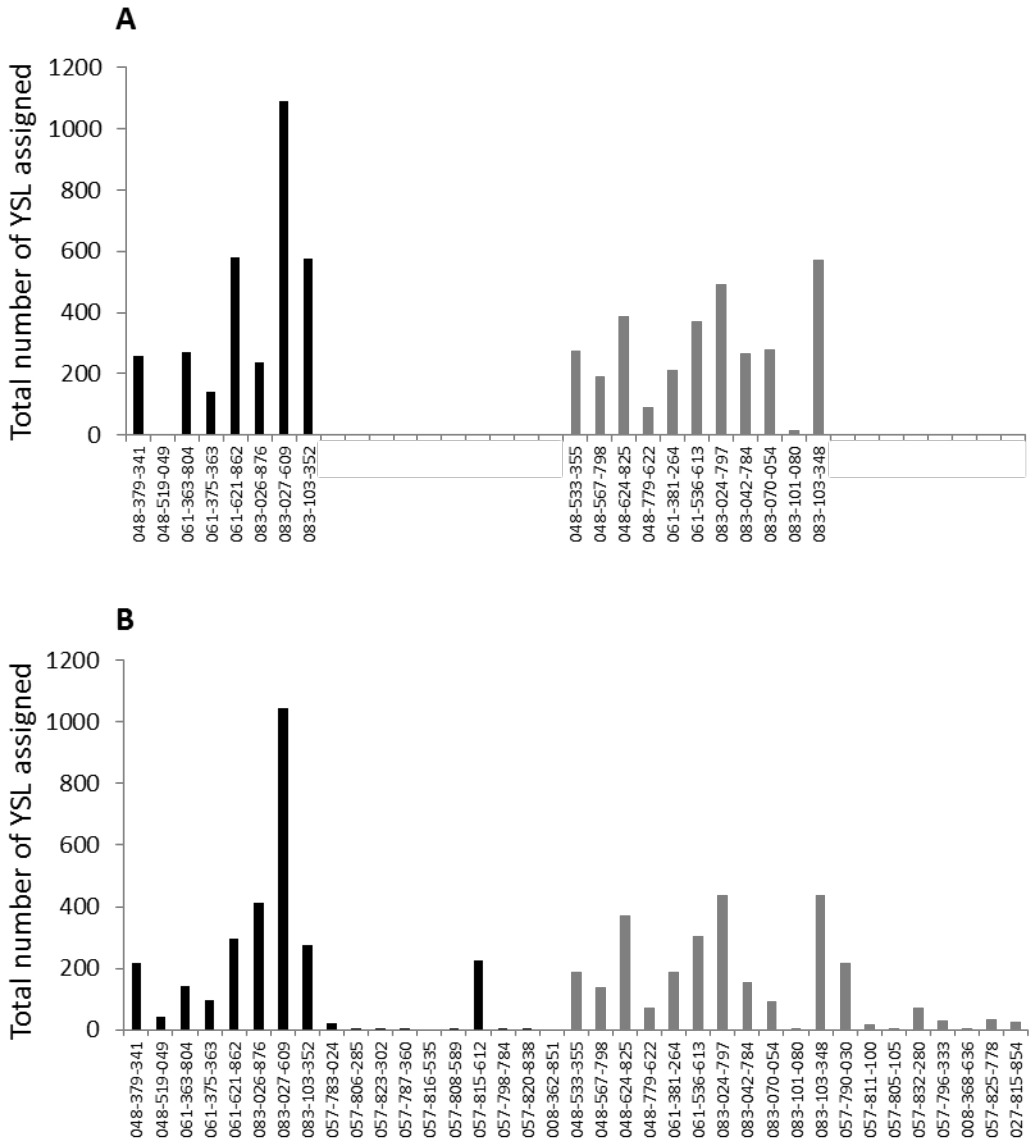

Figure 4. Total number of YSL assigned to each brood stock CYT, from the 2013 (A), and the 2014 (B) spawning season. All brood fish are listed by pit-tag number, with females in black and males in gray. For the 2014 spawning season, additional brood fish were added to the tank. In 2013, there was a total 3170 YSL assigned, and 2789 YSL from 2014.

TABLES

Table 1. Microsatellite primer pairs, annealing temperatures, PCR (1, 2, and 3) and genotyping (A, B) multiplex sets, and fragment lengths.

Locus	Forward primer	Reverse primer	Annealing temp.	PCR/ Genotyping Set	fragment length (bp)
Sequ38[1]	5HEX_CCATTACAATTTGTCTCTC	CTTATCAACACACGAGCG	53 °C	1/A	100-145
Sequ77[1]	5HEX_CCTACACATGCACATGAA	CAAGGCTGATACGTCATG	55 °C	2/A	135-190
Sdu gA3D[2]	56-FAM_CTCAACATGAGAGGCAACG	GCATGGCTTCATGGGAAGG	55 °C	2/A	140-180
Sdu 46[3]	56-TAMN_GCAGTGTGAGCCATACATTAC	CTACAGGACAAAAGCCATT	55 °C	2/A	220-260
Sdu 4[3]	56-FAM_GGAAATAGTTTGGATCACGCTGG	GGATGCTCAGTGAAGTTGTGC	55 °C	2/A	270-310
Sequ320[1]	5HEX_GACAGGGTAAGAAACGAAAC	GACAATGACCAAAGCTGCC	59 °C	3/B	90-140
Sequ230[1]	56-FAM_CTCCAGAAACGCCACATAAC	AAGCAAACCGCACAAGTAGG	59 °C	3/B	150-165
Sdu 10[3]	56-TAMN_CCAAGTCCTCCGCTACTACCAT	CCTTGTGGATGACCTGTTTG	59 °C	3/B	250-310
Sdn 06[4]	56-FAM_GGGTTTGTGCTGTGAGTG	TCCGTCTGTCTTTTCCTGT	59 °C	3/B	300-330

[1] Ohara *et al.*, 2003, 2005; [2] Porta *et al.*, 2009; [3] Renshaw *et al.*, 2006, 2007; [4.] Nugroho & Taniguchi, 1999

Table 2. Summary statistics of census and effective population sizes for females (n_f / Ne_f), males (n_m / Ne_m) and both sexes (n_e / N_e) for the 2013 and 2014 spawning season. Estimates of annual and batch fecundity, and spawning intervals for original larger females were calculated for the 2013 and 2014 spawning seasons. Data calculated from smaller female fish added in 2014 are shown in parentheses, as metrics are positively correlated with female fish mass (P = 0.0003 – 0.00045). Data for annual and batch fecundity, and spawn intervals excluded zero values from both years analyzed.

	2013 Spawning Events						2014 Spawning Events					
	Min	Mean	Median	Max	Std. Dev.	Annual total	Min	Mean	Median	Max	Std. Dev.	Annual total
n_f	1	1.63	1	8	1.06	8	1	1.9	2	5	0.93	15
Ne_f	1	1.38	1	5.25	0.69	4.87	1	1.21	1.04	2.05	0.33	5
n_m	2	89.25	9	10	1.53	11	5	10.07	10	15	2.3	19
Ne_m	1.66	5.89	5.77	12.73	1.89	8.63	2.22	6.23	6.31	9.78	2.11	9.79
n_e	3	10.28	11	16	1.79	19	6	11.97	12	19	2.77	34
N_e	2.5	4.26	3.53	10.2	1.46	12.46	2.75	3.98	3.58	6.69	1.03	13.24
Mass of female broodfish (kg)	14.63	20.75	21.42	27.16	3.97	166	14.63 (7.15)	20.73 (8.44)	21.42 (8.65)	27.16 (10.2)	3.99 (0.96)	165.8 (84.4)
Annual fecundity I (eggs / year)	55,000	8,200,000	4,700,000	27,000,000	8,800,000	66,000,000	400,000 (35,000)	6,800,000 (290,000)	3,300,000 (67,000)	23,000,000 (1,500,000)	7,700,000 (550,000)	54,000,000 (2,000,000)
Annual fecundity II (eggs / kg)	2,500	420,000	260,000	1,200,000	420,000	NA	18,000 (3,400)	330,000 (32,000)	170,000 (7,500)	1,000,000 (160,000)	350,00 (58,000)	NA
Batch fecundity I (eggs / spawn)	18,000	460,000	460,000	1,900,000	250,000	NA	50,000 (4,400)	240,000 (21,000)	180,000 (8,000)	2,500,000 (400,000)	180,000 (29,000)	NA
Batch fecundity II (eggs / spawn / kg)	850	23,000	25,000	97,000	13,000	NA	2,300 (430)	11,000 (2,000)	10,000 (1,000)	110,000 (43,000)	8,300 (3,100)	NA
Spawn intervals (days)	1	11.1	10.6	64	4.7	NA	1 (1)	13 (26.9)	6.5 (26)	58 (77)	13.3 (22.2)	NA