Part I: Summary

PROJECT TITLE: **Determination and practical application of egg quality measures toward reliable culture of high-value marine finfish species**

REPORT GIVEN IN YEAR 2017

REPORTING PERIOD: (January 1, 2014 to August 31, 2017)

AUTHOR: Kevin Stuart

FUNDING LEVEL: $294,068 (YR1 - $102,370; YR2 - $75,534; YR3 – $116,164)

PARTICIPANTS:

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Principal Investigator: Ronald B. Johnson, PhD, NOAA NMFS Northwest Fisheries Science Center (NWFSC), Environmental and Fisheries Science (EFS) Division, 2725 Montlake Blvd. East, Seattle, WA 98112

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Principal Investigator: Gordon Murdoch, PhD, Animal and Veterinary Science, University of Idaho, 975 Perimeter Dr. MS 2330, Moscow, ID 83844-2330

Principal Investigator responsible for Outreach: Paul Olin, PhD, California Sea Grant (CASG) Extension Program, University of California San Diego, 9500 Gilman Dr. #0232, La Jolla, CA 92037

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REASON for TERMINATION: Objectives completed

PROJECT OBJECTIVES:

Objective 1: Measure intrinsic seasonal variation in egg characteristics produced by the broodstock populations.

Objective 2: Determine how broodstock dietary manipulation impacts egg quality.

Objective 3: Assess whether different spawning methods affect egg quality.

PRINCIPLE ACCOMPLISHMENTS:

Objective 1 (Years 1, 2, and 3): Measure intrinsic seasonal variation in egg characteristics produced by the broodstock populations

Task 1a. Quantification of basic egg and larval characteristics.

The quantification of basic egg and larval characteristics were completed for sablefish (SF; 2013 only), California halibut (CH) and California yellowtail (CYT). The SF spawning events were synchronized with Ovaplant, and spawn events in 2013 (which occurred in two spawning pulses) were recorded. Both fertilization and cell symmetry (symmetry of cell division) percentages were assessed. The average number of spawn events and average cell symmetry between the two pulses were not significantly different, however the average fertilization in pulse 1 (80%) was significantly higher than in pulse 2 (61%). Spawn events for CH and CYT in 2014, 2015, and 2016 were collected from volitional spawning events and basic egg and larval characteristics were quantified including: hatch rates, egg diameter, oil diameter, survival to first feeding, yolk sac notochord length, and yolk sac volume. For CYT, egg diameter was positively correlated with day to starvation for all years, and egg diameter decreased as the season progressed for all years. However for CH no correlations among these variables were consistent year to year.

Task 1b. Proximate compositional (PC) and fatty acid (FA) analysis

Proximate composition (PC) and fatty acid analysis (FA) from CYT spawn events were completed for three years, 2013, 2014, 2015. Analysis on CH eggs has not yet been completed, but should be done by October 2017. PC and FA were completed for 48, 36, and 24 spawning events for years 2013-2015, respectively. Spawn events from both the wild and F_1 broodstock populations were analyzed in 2013. Egg samples were partitioned into good (70 – 100% viable) and poor spawns (0 – 30% viable). In 2014 and 2015 only eggs from the wild population were analyzed and spawns were partitioned into good, fair (31-69% viable), and poor spawns.

In 2013, no significant differences in proximate composition were observed between good and poor quality eggs, or within the brood type. However, good quality F_1 eggs had more protein and less moisture than poor quality eggs, albeit the difference was not statistically significant. The greatest difference was observed in oleic acid (18:1 n-9) levels between wild and F_1 eggs. Wild eggs had nearly 5% more oleic acid than F_1 eggs. Overall, good eggs had more linoleic acid than those of poor quality. Although palmitic acid (16:0) was not significantly different across brood types, good quality eggs of each brood had significantly less palmitic acid than poor quality eggs, though the difference is less than 1.0% on average.

The PC analysis for the 2014 spawn events showed differences in levels of moisture, lipid and ash among egg batches of different quality. Poor spawns had significantly more protein than eggs from fair spawns. Significant differences in FA composition attributable to quality were detected in egg samples from 2014. The proportion of ARA in eggs from fair spawns was 20% higher than eggs from good spawns (viability of >69%) and 14% higher than eggs from poor spawns. Compared to good and poor spawns, fair spawns also had a lower ratio of EPA to ARA.

Last, fair spawns had a higher proportion of DPA than good spawns, but were not different from poor spawns.

The PC analysis for 2015 spawn events, showed no differences between quality categories for moisture, lipid, protein, or ash. Similarly, the FA analysis showed no differences between quality categories.

Task 1c. Parentage analysis

Parentage assignment from preserved yolk sac larvae of CYT was successful for all years of the project. Individual spawn events were typically dominated by one or two females, in contrast to the males who tended to contribute in fairly equal proportions. Parentage analysis from 2013, 2014, and 2015 led to identification of one dominant female who was producing low quality eggs. From this information we removed the female from the population prior to the 2016 spawning season and there was a subsequent improvement in egg quality, although spawning frequency decreased slightly (see Task 1a). Additional data analyses for this task area are pending.

Task 1d. Juvenile grow-out trials

Grow-out trials for CH were not completed in 2014 or 2016 due to inconsistent egg production from the resident broodstock population. One run was completed in 2015 and survival from egg to 60 dph juvenile was 21.0%. Along with survival, performance measures such as growth and malformation rates were recorded. Results are reported in the detailed section of this report.

For each year of the project (2014 – 2016) CYT grow-out trials were completed. Survival rates from egg to 60 dph juvenile ranged 8.2 to 35.0%. Along with survival, performance measures such as growth and malformation rates were recorded. Results are reported in the detailed section of this report.

Objective 2 (Years 1, 2, and 3): Determine how broodstock dietary manipulation impacts egg quality

This objective was completed in 2015 but final analysis was completed in 2016. We supplemented arachidonic acid (ARA) in the diet for CYT, to determine if ARA would impact egg and larval quality. Two formulated experimental diets were offered – 1) the control diet (CON) consisting of a commercial premix without ARA supplementation, and 2) the ARA diet consisting of the commercial premix with 1.0% ARA oil added.

Fish in the CON treatment spawned 53 times for a total of 18 million eggs. Fish in the ARA treatment tanks spawned 30 times for a total of 13 million eggs. The ARA treatment yielded significantly greater egg viability, hatch rates, and diameter than the control. The FA analysis of the diets showed a significantly greater concentration of ARA in the ARA treatment as expected and that same increase was measured in the eggs from the ARA treatment broodstock. We demonstrated that CYT will spawn successfully in small breeding tanks of 10 m^3, which facilitates manipulative studies of broodstock nutrition as reported here.

Objective 3 (Years 2 and 3): Assess whether different spawning methods affect egg quality.

We investigated alternative gonadotropin releasing hormone (GnRH) regimes for spawning induction in SF. We tested the effects of GnRH implant dose (high (~50ug/kg) and low (~25 µg/kg)) and primer injections of 5 µg/kg (GnRH) prior to GnRH implants. These investigations were conducted over two spawning pulses. Implants with a lower GnRH dose (25 µg/kg) seemed to do well compared with the high dose and, if coupled with a pre-injection, might be the best treatment option.

In 2016 we began to investigate the use of human chorionic gonadotropin (HCG) on spawning of CYT. For CYT, we intended to apply three different treatment dosages - 250, 500, and 1000 IU/kg. After injecting several fish we suspended the work due to health concerns for the CYT population. Specifically, the stress caused by handling was exacerbated by warm summer water temperatures leading to mortality in some fish. In 2017 we ran a trial using Aqui-S 20E to determine the appropriate dosage level for CYT in the hopes of using this anesthetic over MS-222.

OUTREACH PLAN:

Outreach and extension of information generated through this research has been accomplished through personal engagement by the PI responsible for outreach and project PIs at HSWRI, NWFSC, SWFSC and the University of Idaho. Outreach involved coordination with individuals involved in cultivation of marine finfish, and interactions with professional organizations. This includes information exchanges through the National Aquaculture Extension Steering Committee, and by sharing information with international colleagues through bilateral exchanges via the joint U.S.-Japan Natural Resources Panel on Aquaculture and U.S.-Korea JPA Aquaculture Research Panel. Finally, we are currently working on a product that will serve as a guide to husbandry and breeding of *Seriola dorsalis*

IMPACTS:

1. We found that selecting CYT eggs at the start of the season should give growers a slight advantage over stocking eggs produced at the end of the spawning season. However, eggs throughout the season are generally adequate to produce commercial levels of high quality fingerlings using the protocols we employed.

2. We used parentage analyses to determine that one dominant female in the CYT broodstock population was producing low quality eggs. Based on these results the female was removed from the population prior to the 2016 spawning season. After the removal of the female, egg quality improved for the overall population, although spawning frequency decreased slightly. Parentage analyses can make a direct impact on the industry by optimizing broodstock management and being able to focus on fish that are contributing to good spawn events. Growers should replenish brood fish with young fish periodically – e.g. for CYT use fish less than 10 years old.

3. As hypothesized, we demonstrated that ARA is an important factor in broodstock nutrition for CYT, which will ultimately help define essential nutrients for the species and foster improved production. Increasing ARA in the brood diet improved egg quality

and egg production for CYT, although we did not determine the optimum level of inclusion. Our work represents a baseline towards defining essential nutrients for this marine species

4. We also successfully demonstrated that CYT will spawn in smaller (10 ton) tanks, which will facilitate future broodstock nutrition studies, including the ability to replicate. This impact is already being realized in a collaborative nutrition study with Auburn University looking at taurine and its impact on both egg and larval quality as well as maternal contributions to the larvae and its impacts on larval performance through the rearing process.

5. We demonstrated that a preferred hormone treatment regime for SF, was a pre-injection of 5 µg GnRH/kg followed by a dose of 25 µg/kg of Ovaplant. The pre-injection appears to tighten the timing of the spawning events and produced better quality eggs based on cell division symmetry.

RECOMMENDED FOLLOW-UP ACTIVITIES:

Follow-on research should include development of broodstock nutrition for marine finfish, starting with an open formula broodstock reference diet which could then be developed into custom formulation for specific marine species. Also, when looking at egg quality characteristics, proximate composition and fatty acid analysis, as well as parentage, it would be useful to look at all fractions of the spawn event (floating, neutral, and sinking). That would give a more complete picture of egg quality and parental contribution. Finally, in order to effectively determine if egg quality impacts larval and juvenile production, more than three runs per year need to be carried out. Having the ability to rear multiple spawn events throughout a spawning season would show seasonal trends in both egg quality and larval performance, giving growers an idea of best times to use eggs for juvenile production.

SUPPORT: Use the format shown below to indicate all sources of funding and additional other support, federal and non-federal, for this project. Specify the name of the "other" sources as a footnote to the table.

Year	WRAC-USDA Funding	OTHER SUPPORT				Total Support
		University	Other Federal	Other	Total	
2014-2017	$ 294,068	$-	$ 213,000	$ 84,024	$ 297,024	$ 591,092
Total	$ 294,068	$-	$ 213,000	$ 84,024	$ 297,024	$ 591,092

"Other" = HSWRI matching

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

HSWRI bimonthly newsletter – March 2014, August 2014, January 2015, March 2015, November 2015

Hatchery International – July 2014

ORAL PRESENTATIONS:

Armbruster, Lisa, Kevin Stuart, Mark Drawbridge, Ronald B. Johnson. 2016. California yellowtail *Seriola dorsalis* egg quality and chemical composition. Aquaculture America 2016, Las Vegas, NV, February 22-26.

Armbruster, Lisa, Kevin Stuart, Mark Drawbridge, Ronald B. Johnson. California yellowtail *Seriola dorsalis* egg quality and chemical composition. US-Korea Joint Coordination Panel for Aquaculture Meeting. Seattle, WA. March 23, 2016.

Armbruster, Lisa, Kevin Stuart, Mark Drawbridge, Ronald B. Johnson. Egg chemical composition and quality are affected by feeding California Yellowtail (*Seriola dorsalis*) broodstock supplemental arachidonic acid. NOAA Northwest Fisheries Science Center Symposium. Seattle, WA. April 5, 2016.

Stuart, Kevin, Ronald B. Johnson, Lisa Armbruster, and Mark Drawbridge. 2016. Manipulation of arachidonic acid in the diet of adult California yellowtail (*Seriola dorsalis*). Aquaculture America 2016, Las Vegas, NV, February 22-26.

Stuart, Kevin, Ronald B. Johnson, Lisa Armbruster, and Mark Drawbridge. In Review. Arachidonic acid in the diet of adult California yellowtail *Seriola dorsalis* and its effect on egg quality. North American Journal of Aquaculture.

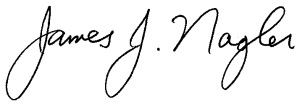
POSTER PRESENTATIONS:

Armbruster, Lisa, Kevin Stuart, Mark Drawbridge, Ronald B. Johnson. Egg chemical composition and quality are affected by feeding California Yellowtail (*Seriola dorsalis*) broodstock supplemental arachidonic acid. NOAA Aquaculture Science Review. Seattle, WA. July 26, 2016

9/12/17

SUBMITTED BY: _____

Title: PI/Date



APPROVED: 09-12-2017

Project Monitor/Date

Part II: Detail

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TECHNICAL SUMMARY AND ANALYSIS

Objective 1 is a multi-task, multi-year objective that recurs in each year of the three year project: Measure intrinsic seasonal variation in egg characteristics produced by the broodstock populations

Task 1a. *[NWFSC/HSWRI]* (Quantification of basic egg and larval characteristics)

[*NWFSC - Manchester*]

Egg quality for SF was quantified for one year of this project in 2013. The number of SF spawns, volume of fertilized eggs and fertilization and cell symmetry were quantified for 75 spawning events recorded over two spawning pulses (spawning events synchronized with Ovaplant). Since SF are batch spawners, these spawning events originated from 18 females; 10 in pulse 1 and 8 in pulse 2. The average number of spawn events/female averaged 4 in both pulses but ranged from 1-10 (Figure 1). The average number of eggs/spawning event was 437 in pulse 1 and 534 in pulse 2 (Figure 2) and were not significantly different. Of the 75 spawning events, 52 were fertilized and the fertilization and cell symmetry (symmetry of cell division) percentages were assessed. In pulse 1 the average fertilization percent was 80 ± 16% and in pulse 2 was 61 ± 21% (Figure 3) and these percentages were significantly different. The average cell symmetry percentages were 60 ± 22% and 58 ± 20% in pulse 1 and 2 (Figure 4), respectively and were not significantly different.

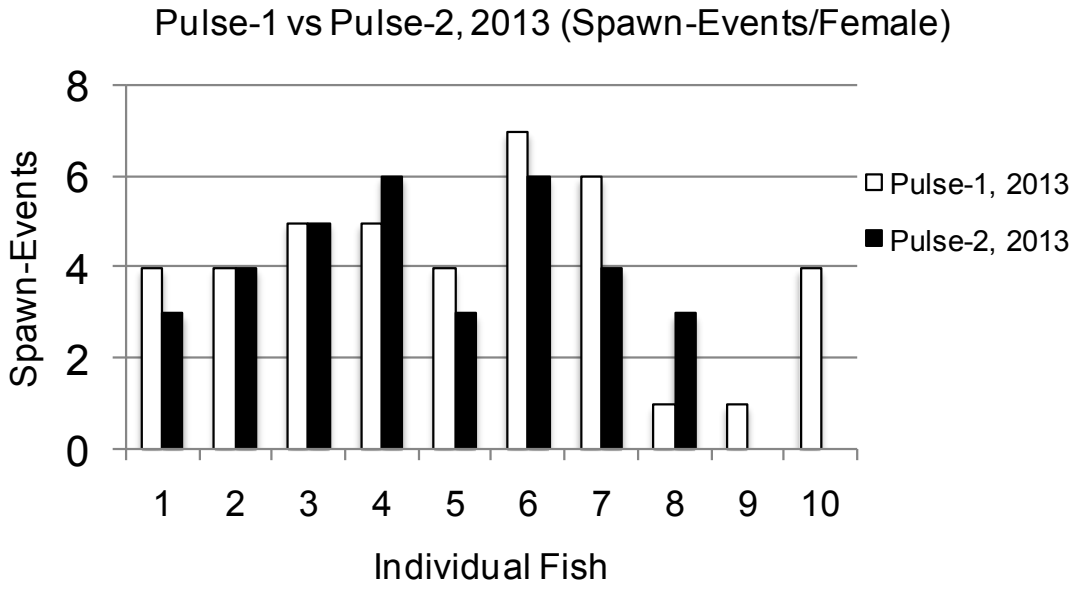

Figure 1. Spawn events/female for SF in two reproductive pulses.

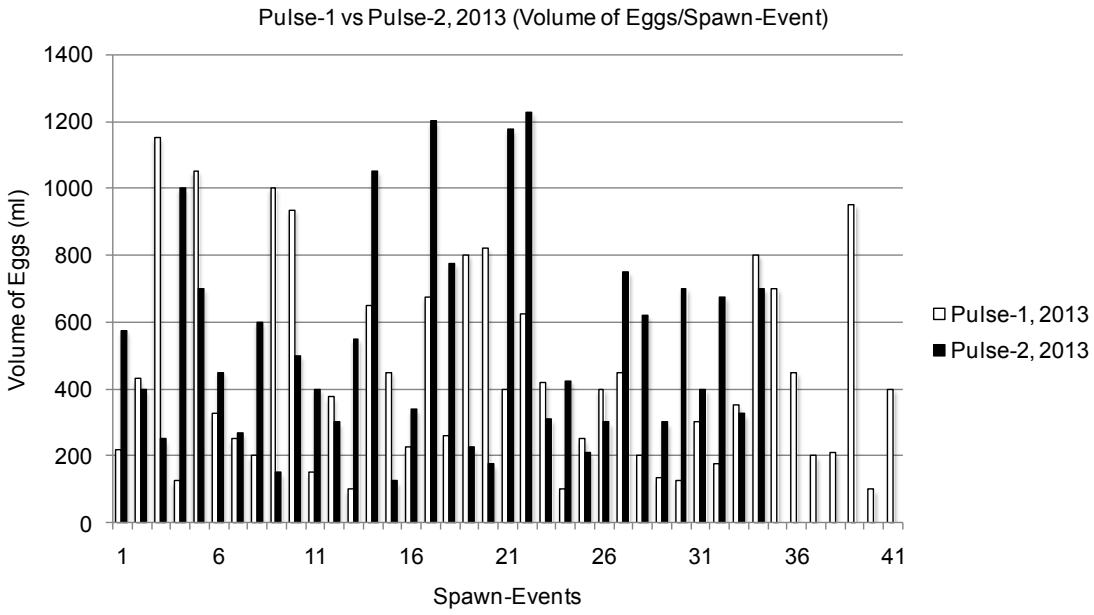

Figure 2. Egg volumes/spawning event for SF in two reproductive pulses.

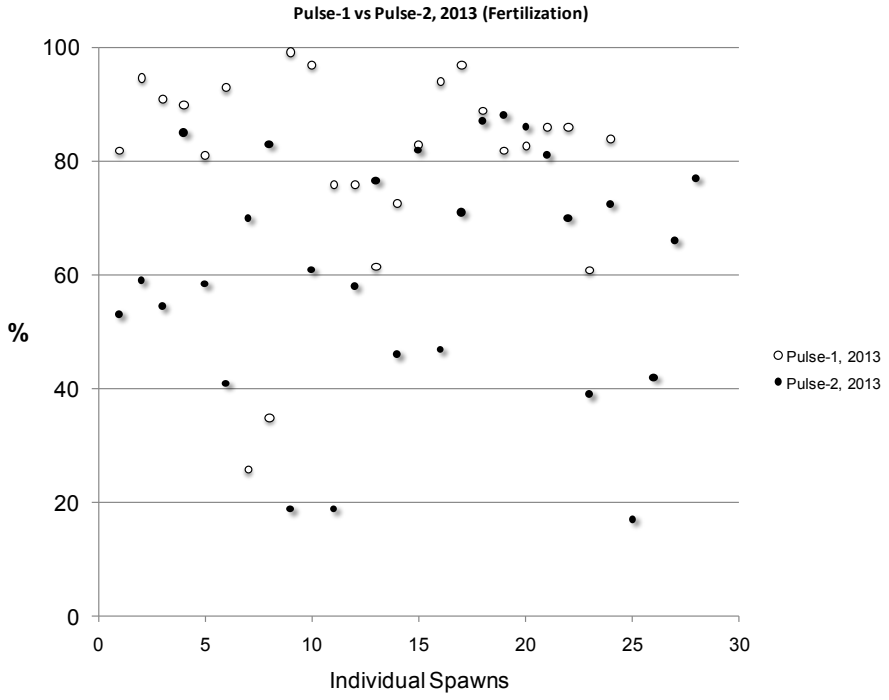

Figure 3. Fertilization rates for eggs obtained from SF in two reproductive pulses.

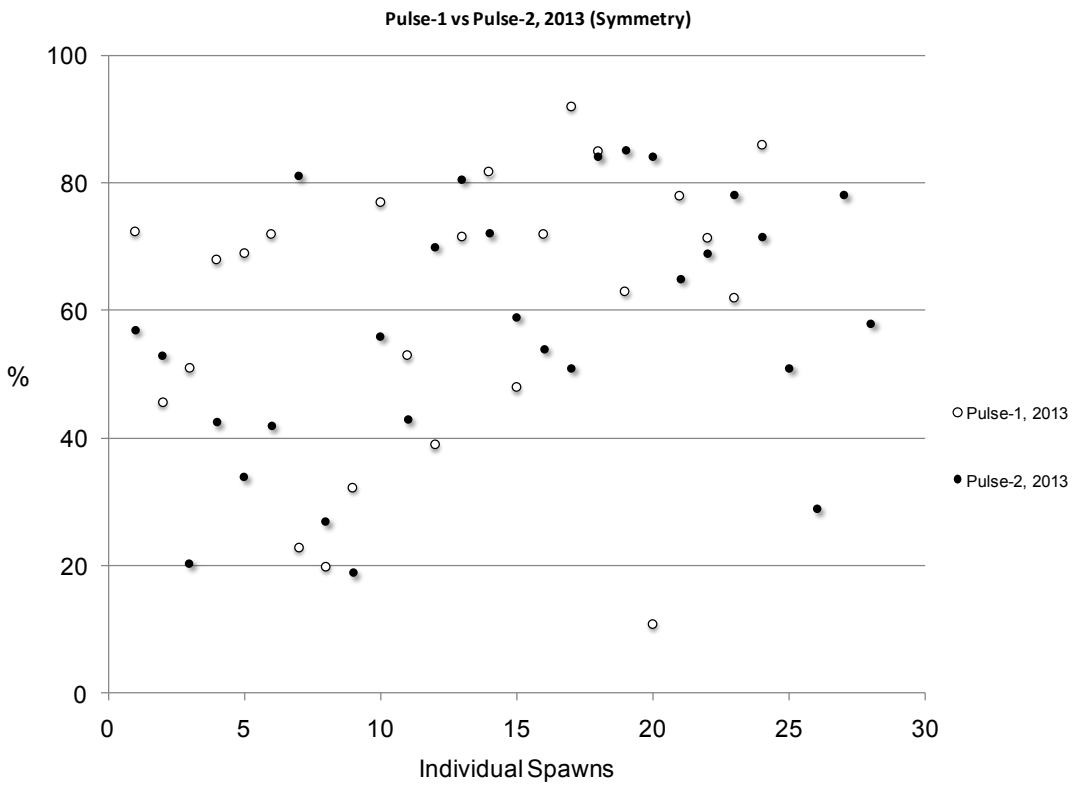

Figure 4. Cell symmetry rates for eggs fertilized from SF in Figure 3 from two pulses.

[*HSWRI*]

Both wild populations of CH and CYT spawn naturally at HSWRI, without hormone manipulation. The CH population spawned from March thru June in all years except for 2016, when the population began spawning in April. In each year the total number of females was different but the sex ratio was similar (1 to 1, female to male). The increase in females in 2015 increased the number of spawn events as well as the total egg production. In 2015 an improvement in viability, hatch rates, and larval survival to first feeding was observed as compared to 2013 (Table 1). In 2016 however, removal of fish disrupted the spawning dynamics and the population only produced four spawn events over the course of the spawning season (Table 1, Figure 5). The information from this population shows that the number of fish as well as the behavior of those fish during the spawning season are important for consistent egg production and egg quality.

Table 1. Comparison of CH spawns collected in 2014, 2015, 2016.

Variable	2014	2015	2016
Number of Spawns	57	85	4
Number of Females	3	6	4
Female Biomass (kg)	41	53	40
Total Eggs Produced	66,136,961	125,165,125	2,724,407
Percent Viability (Range)	33.1 ± 28.8	47.3 ± 31.5	17.8 ± 18.3
Percent Hatch (Range)	61.1 ± 16.8	74.5 ± 17.5	61.5 ± 16.8
Percent Survival to First Feeding (Range)	66.4 ± 15.5	68.5 ± 17.6	74.3 ± 18.1

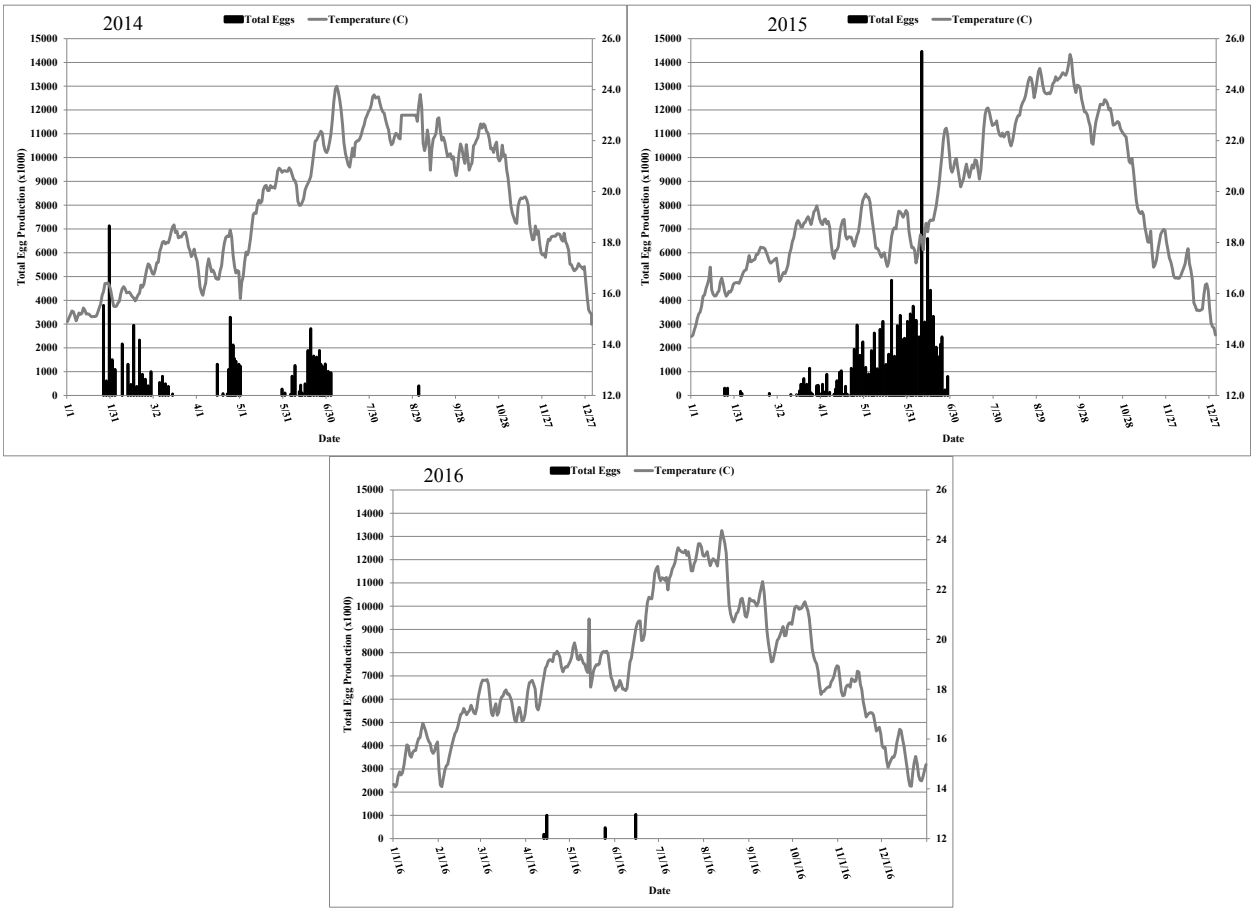

Figure 5. Relationship between temperature and egg production for wild CH held at HSWRI in 2014, 2015, 2016.

The wild caught CYT broodstock population was also different year to year, the population changed between 2014 and 2015 due to unexpected mortality events, as well as addition of new fish in 2014. However, prior to 2016 the population was changed based on parentage analysis results (Task 1c) from 2013, 2014, and 2015. Information from Task 1c showed that a single female was contributing to a majority of the spawning events and most of those events were of

poor quality. Based on those results we decided to remove the female from the population to try to increase the overall production of higher quality eggs. After that change, prior to the 2016 spawning season, the population consisted of 29 adults, 13 females and 16 males. For all years CYT spawned from March through August (Figure 6). In 2014 and 2015 spawn event sizes ranged from 40,000 to 2,600,000 eggs per spawn (Figure 6). However in 2016, spawn event size was reduced with spawns ranging from 97,000 – 1,600,000 eggs (Figure 6), the difference in egg production can be linked to the removal of the female prior to the spawning season. The 2016 egg quality measures were different from 2014 and 2015, with mean percent floating eggs, percent viability, and hatch rates improving (Table 2). Egg quality measures were similar in all three years with egg and oil diameters ranging from 1.20 to 1.42 mm and 0.27 to 0.33 mm respectively, percent oil volume varied from 0.97 to 1.36%. Larval notochord lengths ranged from 3.51 to 4.50 mm yolk sac volume at hatch ranged from 0.06 to 0.72 mm^3. The day to starvation ranged from 5 to 9 dph.

A spearman rank order correlation was performed on the egg quality data from the CYT for all three years. Different egg quality parameters correlated from year to year however, there were a few that were similar between the years. Egg diameter was positively correlated to oil diameter which shows that as the egg diameter increases so does the oil diameter (Table 3). Egg diameter and egg volume were positively correlated to survival to first feeding, showing that a more robust egg improves larval survival to first feeding. A positive relationship was also seen between oil volume and egg diameters (Figure 7 and 8). There was one seasonal trend for all years with egg diameters decreasing as the season progressed (Figures 8).

Table 2. Comparison of CYT spawns collected in 2014, 2015, and 2016.

Variable	2014	2015	2016
Number of Spawns	63	78	46
Number of Females	17	17	13
Female Biomass (kg)	206	262	234
Total Eggs Produced	58,060,386	52,410,967	31,123,593
Percent Viability (Mean ± SD)	58.5 ± 25.2	49.9 ± 24.8	68.0 ± 18.0
Percent Hatch (Mean ± SD)	62.1 ± 18.3	60.7 ± 17.4	69.4 ± 18.9
Percent Survival to First Feeding (Mean ± SD)	67.4 ± 20.3	66.9 ± 16.7	63.7 ± 26.1

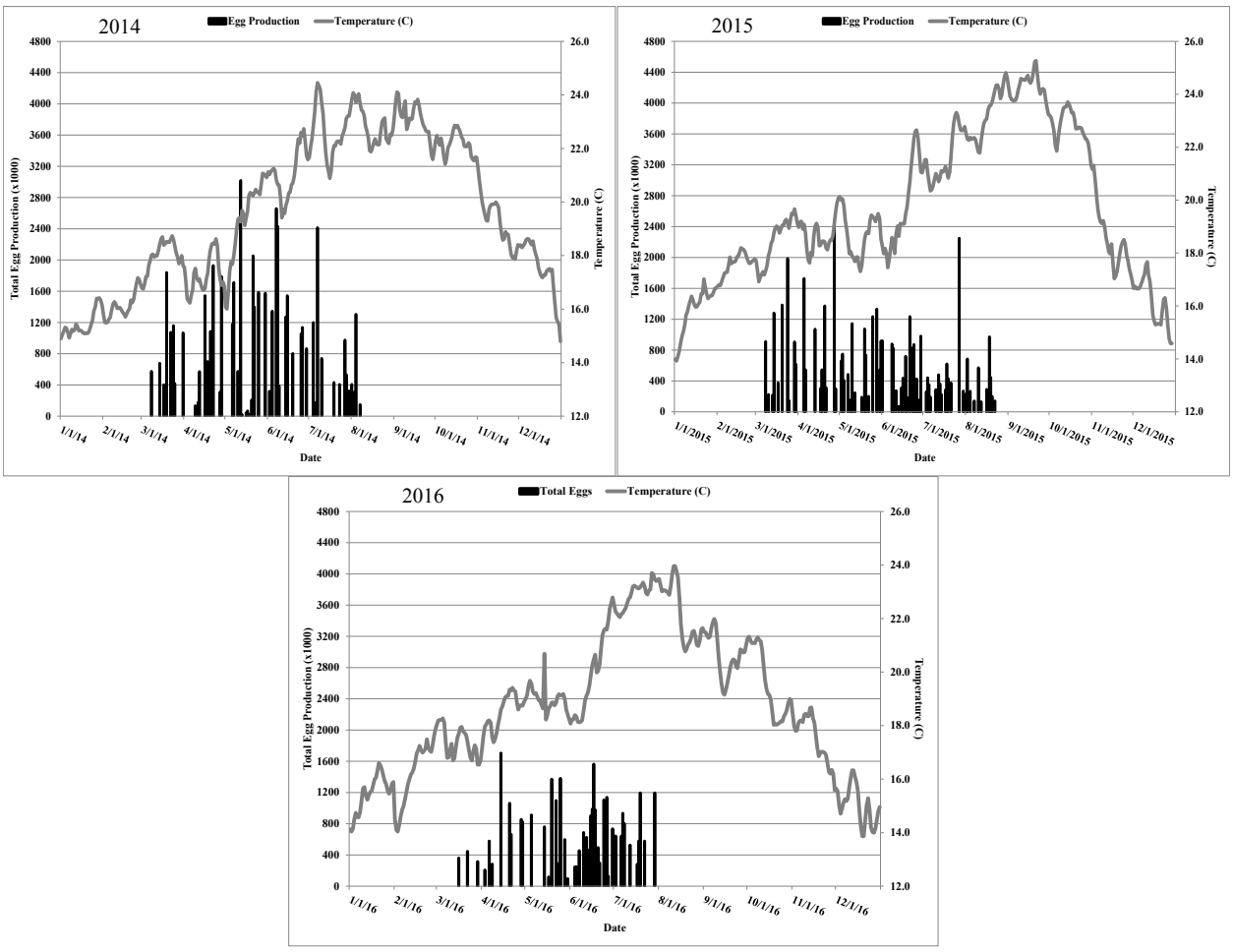

Figure 6. Relationship between temperature and egg production for wild CYT held at HSWRI in 2014, 2015, 2016.

Table 3. Spearman rank order correlations for CYT egg quality measurements for all 2014, 2015, and 2016. Significant correlations that were similar are labeled as positive.

Egg Quality Measure	Total Eggs Collected	Viability	Egg Diameter	Egg Volume	Oil Diameter	Oil Volume	Percent Oil Volume	Notochord Length	Yolk Sac Volume	Dry Weight	Hatch Rate	Survival to First Feeding	Day to Starvation
Total Eggs Collected	-	-	-	-	-	-	-	-	-	-	-	-	-
Viability	-	-	-	-	-	-	-	-	-	-	-	-	-
Egg Diameter	-	-	-	**Positive**	**Positive**	**Positive**	-	-	-	-	-	-	**Positive**
Egg Volume	-	-	**Positive**	-	**Positive**	**Positive**	-	-	-	-	-	-	**Positive**
Oil Diameter	-	-	**Positive**	**Positive**	-	**Positive**	-	-	-	-	-	-	-
Oil Volume	-	-	**Positive**	**Positive**	**Positive**	-	**Positive**	-	-	-	-	-	-
Percent Oil Volume	-	-	-	-	-	**Positive**	-	-	-	-	-	-	-
Notochord Length	-	-	-	-	-	-	-	-	-	-	-	-	-
Yolk Sac Volume	-	-	-	-	-	-	-	-	-	-	-	-	-
Dry Weight	-	-	-	-	-	-	-	-	-	-	-	-	-
Hatch Rate	-	-	-	-	-	-	-	-	-	-	-	-	-
Survival to First Feeding	-	-	**Positive**	**Positive**	-	-	-	-	-	-	-	-	-

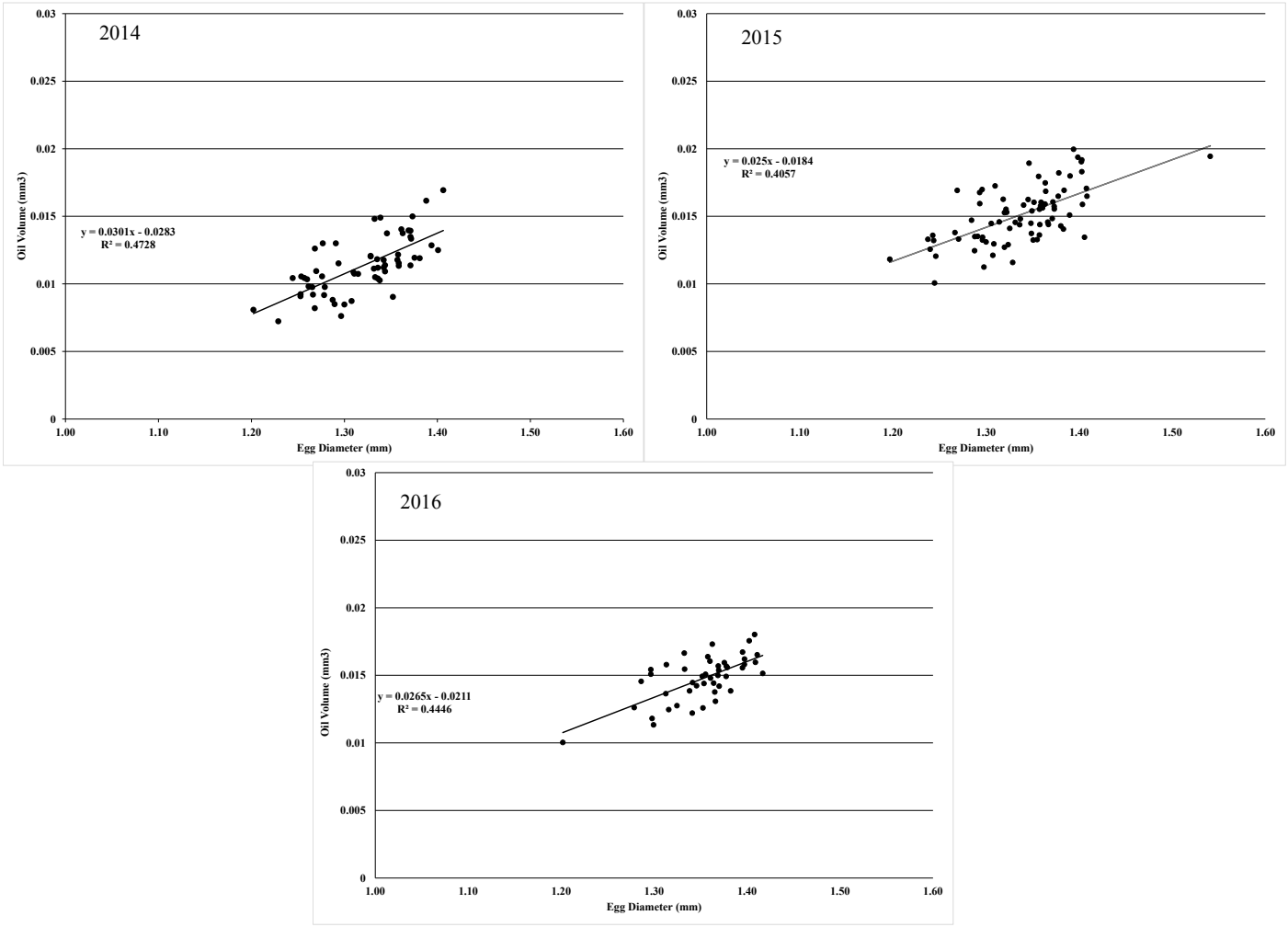

Figure 7. Relationship between oil volume and egg diameter for CYT in 2014, 2015, and 2016.

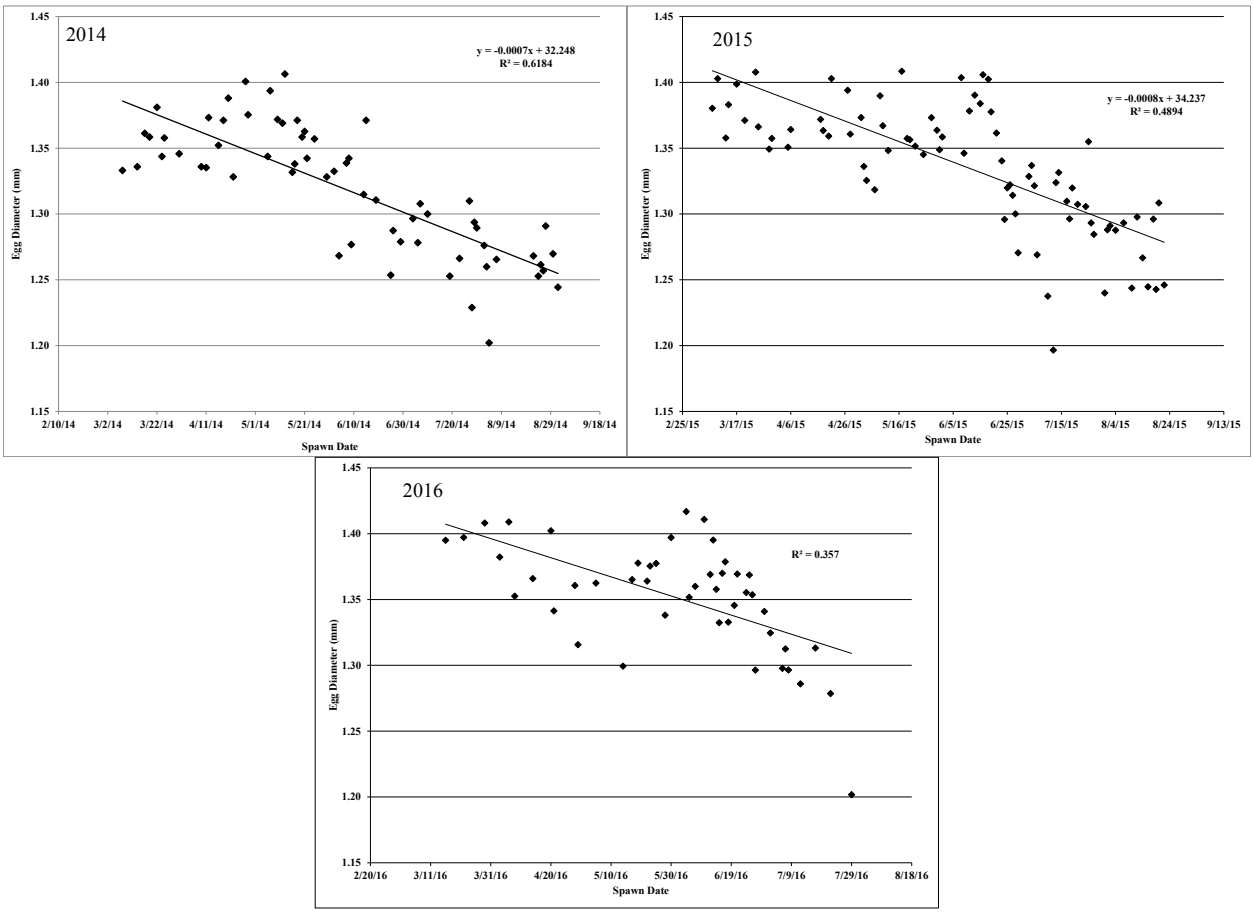

Figure 8. Egg diameters from eggs produced by CYT in 2014, 2015, and 2016.

Task 1b. *[NWFSC]* (Proximate compositional (PC) and fatty acid (FA) analysis)

Proximate composition (PC) and fatty acid analysis (FA) from CYT was completed for 48 spawning events from the 2013 spawning season, 36 spawn events were selected from the 2014 season, and 24 spawn events were selected from the 2015 season. In 2013 an equal number of spawning events from wild and F_1 broodstock populations were selected. In 2014 and 2015, however, only spawn events from the wild broodstock were analyzed. The 2013 samples were taken from each broodstock type and were chosen to represent good (70-100% viable) or poor (0-30% viable) quality. Egg samples for biochemical analyses were collected from the floating fraction of the spawn whenever possible. When there were too few floating eggs available to meet the sample requirements (100g wet weight), samples were supplemented with eggs from the neutral spawn fraction. All statistical analyses were performed using SAS 9.4 software. For the 2014 and 2015 samples the spawning events were selected for processing to represent the entire range of spawn quality, divided into three categories: good (70-100% viable), fair (31-69% viable) or poor (0-30% viable). Egg PC and FA data were statistically analyzed with a 1-way-ANOVA using the GLM procedure. Differences between means were identified by Tukey's post hoc test for multiple comparisons. Analysis on CH eggs have not yet been completed, but should be done by October 2017.

Proximate composition:

In 2013, there were no significant differences in egg PC attributable to egg quality (Table 4). Significant interactions between egg quality and broodstock type were evident in moisture and protein levels. Eggs from wild broodstock have slightly less lipid than those from F_1 broodstock. In 2014, differences were detected in spawn quality among levels of moisture, lipid or ash (Table 5). Poor spawns had significantly more protein than eggs from fair spawns, however, it is uncertain whether a tenth of a percent by wet weight is a biologically significant difference. However, the PC analysis of the 2015 spawn events showed no differences between quality categories for moisture, lipid, protein, or ash (*alpha* = 0.005; Table 6).

Table 4. Proximate composition of CYT eggs from wild or F_1 broodstock in 2013. (Results (mean ± SD) are expressed in grams per 100g (wet weight basis). Type III P values are reported, and significant P values are bolded.

	Wild Brood		F_1 Brood		*P (α = 0.05)*		
Nutrient	Good	Poor	Good	Poor	Quality	Brood	Interaction
Moisture	92.1 ± 0.6 *(n = 13)*	91.9 ± 0.5 *(n = 11)*	91.9 ± 0.4 *(n = 12)*	92.2 ± 0.6 *(n = 12)*	0.2078	0.6605	**0.0053**
Lipid	1.6 ± 0.2 *(n = 13)*	1.5 ± 0.2 *(n = 11)*	1.7 ± 0.2 *(n = 12)*	1.6 ± 0.2 *(n = 12)*	0.0965	**0.0348**	0.6782
Protein	4.6 ± 0.3 *(n = 13)*	4.9 ± 0.4 *(n = 11)*	4.9 ± 0.4 *(n = 12)*	4.5 ± 0.5 *(n = 10)*	0.3083	0.5915	**0.0060**
Ash	1.3 ± 0.3 *(n = 13)*	1.3 ± 0.3 *(n = 11)*	1.3 ± 0.2 *(n = 12)*	1.4 ± 0.2 *(n = 8)*	0.6126	0.3282	0.9585

Table 5. Proximate composition of Wild 2014 CYT eggs from good, fair and poor spawns. Results (mean ± SD) are expressed as grams per 100g (wet weight basis). Values within the same row assigned different superscript letters are significantly different. Significant *P* values are bolded.

Nutrient	Good *(n =9)*	Fair *(n = 14)*	Poor *(n = 13)*	*P (α = 0.05)*
Moisture	92.4 ± 0.2	92.5 ± 0.2	92.3 ± 0.4	0.1354
Lipid	1.4 ± 0.1	1.4 ± 0.2	1.4 ± 0.1	0.8993
Protein	4.2 ± 0.2 ^{ab}	4.1 ± 0.2 ^{a}	4.3 ± 0.2 ^{b}	**0.0185**
Ash	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.2	0.3133

Table 6. Proximate composition of Wild 2015 CYT eggs from good, fair and poor spawns. Results (mean ± SD) are expressed as grams per 100g (wet weight basis).

Nutrient	Good *(n =8)*	Fair *(n = 8)*	Poor *(n = 8)*	*P (α = 0.05)*
Moisture	92.5 ± 0.5	92.4 ± 0.4	92.5 ± 0.3	0.841
Lipid	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	0.316
Protein	4.2 ± 0.2	4.3 ± 0.1	4.1 ± 0.2	0.110
Ash	1.5 ± 0.4	1.4 ± 0.4	1.6 ± 0.1	0.525

Fatty Acid:

Egg FA results were statistically analyzed with a 2-way-ANOVA using the GLM procedure. From 2013 spawn events, significant differences in FA composition attributable to both brood type and egg quality were detected after arcsine transformation and Bonferroni adjustment for multiple comparisons (*alpha* = 0.0025; Table 7). Eggs from wild broodstock had less 14:0, saturated fatty acids, 16:1, and 20:5n-3 (eicosapentaenoic acid; EPA), but more 18:1, monounsaturated fatty acids, and 18:2n-6 (linoleic acid; LA) than F_1 broodstock eggs. Of these fatty acids, a significant interaction was detected between egg quality and brood origin for EPA. An additional interaction is present in the ratios of EPA to arachidonic acid (ARA) and docosahexaenoic acid (DHA) to ARA. With interactions present, any interpretation of differences attributable to brood type should be made with caution, if at all. Last, and in contrast to multiple fatty acids with significant brood type effects, only one fatty acid was found to be significantly different between good and poor quality eggs in 2013. Good eggs tended to have more LA than poor quality eggs. Only LA was different by quality and brood.

Table 7. Fatty acid composition of Wild and F_1 CYT eggs in 2013. Results (mean ± SD) are expressed in grams per 100g of total fatty acids (LA = linoleic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid). Significant P values are bolded. Type II P values were reported when no interaction was present, Type III P values were reported when significant interaction was present.

	Wild Brood		F1 Brood		*P (α = 0.0025)*		
Fatty Acid	Good *(n=13)*	Poor *(n=11)*	Good *(n=12)*	Poor *(n=12)*	Quality	Brood	Interaction
14:0	1.2 ± 0.3	1.2 ± 0.2	1.8 ± 0.2	2.3 ± 0.6	0.0092	**< 0.0001**	0.1275
16:0	15.7 ± 1.5	16.6 ± 0.9	16.5 ± 1.0	16.9 ± 1.0	0.0647	0.0848	0.4821
18:0	4.1 ± 0.4	4.5 ± 0.4	4.3 ± 0.3	4.5 ± 0.2	0.0060	0.2355	0.3754
Σ SFA[a]	21.4 ± 1.8	22.9 ± 1.3	23.2 ± 1.4	24.2 ± 1.5	0.0109	**0.0011**	0.5388
16:1	3.1 ± 0.6	2.9 ± 0.4	3.3 ± 0.3	4.7 ± 1.6	0.0383	**0.0005**	0.0027
18:1[b]	20.7 ± 3.0	21.7 ± 2.7	17.5 ± 0.6	16.2 ± 1.2	0.7479	**< 0.0001**	0.0607
Σ MUFA[c]	24.5 ± 3.2	25.2 ± 2.9	21.4 ± 0.6	21.4 ± 0.7	0.5817	**< 0.0001**	0.5647
18:2 n-6 (LA)	5.9 ± 1.2	5.2 ± 0.5	5.1 ± 0.3	4.1 ± 1.2	**0.0021**	**0.0007**	0.3939
20:4 n-6 (ARA)	2.4 ± 0.3	2.5 ± 0.4	2.7 ± 0.1	2.4 ± 0.3	0.5388	0.3103	0.0247
Σ n-6[d]	8.5 ± 1.3	8.0 ± 0.5	8.1 ± 0.4	6.7 ± 1.6	0.0029	0.0065	0.1048
18:3 n-3	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.0414	0.3921	0.9271
18:4 n-3	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.8 ± 0.4	0.6567	0.0510	0.0747
20:5 n-3 (EPA)	5.5 ± 1.3	5.0 ± 0.4	5.6 ± 0.5	8.2 ± 2.9	0.0353	**0.0007**	**0.0024**
22:5 n-3	2.5 ± 0.3	2.3 ± 0.3	2.4 ± 0.2	2.5 ± 0.3	0.8991	0.7992	0.0948
22:6 n-3 (DHA)	28.7 ± 2.5	27.4 ± 2.3	29.1 ± 1.2	28.6 ± 3.8	0.2110	0.2940	0.7170
Σ n-3[e]	40.4 ± 4.1	38.4 ± 3.2	41.3 ± 1.5	43.2 ± 3.3	0.9247	0.0032	0.0374
Σ PUFA[f]	50.0 ± 4.7	47.6 ± 3.6	50.7 ± 1.7	50.9 ± 3.2	0.2466	0.0558	0.1874
EPA:ARA	2.3 ± 0.4	2.0 ± 0.2	2.1 ± 0.2	3.6 ± 1.8	0.0375	0.0123	**0.0010**
DHA:ARA	12.2 ± 1.0	10.9 ± 1.2	10.9 ± 0.3	11.9 ± 0.9	0.6470	0.5507	**0.0002**
DHA:EPA	5.4 ± 0.8	5.5 ± 0.4	5.3 ± 0.6	4.0 ± 1.7	0.0375	0.0091	0.0154

a Sum of saturated fatty acids (SFAs); 14:0, 16:0, 18:0, 20:0, 22:0, 24:0.
b Sum of n-9 and n-7 isomers.
c Sum of monounsaturated fatty acids (MUFAs): 14:1, 16:1, 18:1, 20:1, 22:1, 24:1.
d Sum of n-6 fatty acids: 18:2, 20:2, 20:3, and 20:4.
e Sum of n-3 fatty acids: 18:3, 18:4, 20:3, 20:4, 20:5, 21:5, 22:5, 22:6.
f Sum of polyunsaturated fatty acids (PUFAs): 16:2, 16:3, 16:4, 18:2, 18:3, 18:4, 20:2, 20:3, 20:4, 20:5, 21:5, 22:5, 22:6.

Significant differences in FA composition attributable to quality were also detected in egg samples from 2014 wild broodstock spawning events after arcsine transformation and Bonferroni adjustment for multiple comparisons (*alpha* = 0.0025; Table 8). The proportion of ARA in eggs from fair spawns was 20% higher than eggs from good spawns and 14% higher than eggs from poor spawns. Compared to good and poor spawns, fair spawns also had a lower ratio of EPA to ARA. Last, fair spawns had a higher proportion of 22:5 n-3 than good spawns, but were not different from poor spawns. However, no significant differences were shown in the FA composition analysis for the 2015 spawn events (*alpha* = 0.0025; Table 9).

Table 8. Fatty acid composition of Wild 2014 CYT eggs. Results (mean ± SD) are expressed in grams per 100g of total fatty acids (LA = linoleic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid). Significant *P* values are bolded.

Fatty acid	Good *(n= 9)*	Fair *(n= 14)*	Poor *(n=13)*	*P (α= 0.0025)*
14:0	1.2 ± 0.2	1.6 ± 0.3	1.4 ± 0.3	0.0251
16:0	13.8 ± 0.5	14.4 ± 1.1	14.3 ± 0.9	0.3135
18:0	4.1 ± 0.4	4.0 ± 0.4	4.1 ± 0.5	0.8686
Σ SFA[a]	19.3 ± 0.8	20.3 ± 1.6	20.0 ± 1.2	0.3574
16:1	3.3 ± 0.4	3.0 ± 0.5	3.2 ± 0.4	0.3504
18:1[b]	16.8 ± 0.8	15.7 ± 0.9	15.7 ± 1.4	0.0440
Σ MUFA[c]	20.7 ± 0.7	19.4 ± 1.2	19.4 ± 1.6	0.0356
18:2 n-6 (LA)	4.9 ± 0.4	4.6 ± 0.4	4.7 ± 0.7	0.4650
20:4 n-6 (ARA)	2.0 ± 0.2 [a]	2.4 ± 0.2 [b]	2.1 ± 0.3 [a]	**0.0024**
Σ n-6[d]	7.2 ± 0.4	7.3 ± 0.4	7.2 ± 0.9	0.8290
18:3 n-3	0.9 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	0.1447
18:4 n-3	1.3 ± 0.2	1.1 ± 0.3	1.1 ± 0.1	0.1780
20:5 n-3 (EPA)	10.5 ± 2.4	7.8 ± 1.4	9.6 ± 2.1	0.0078
22:5 n-3	1.7 ± 0.2 [a]	2.8 ± 0.3 [b]	3.0 ± 0.4 [ab]	**0.0011**
22:6 n-3 (DHA)	29.6 ± 2.5	32.6 ± 2.4	31.0 ± 3.1	0.0370
Σ n-3[e]	47.4 ± 1.5	46.4 ± 1.8	47.1 ± 1.7	0.4535
Σ PUFA[f]	55.5 ± 1.1	54.9 ± 1.7	55.1 ± 2.3	0.7777
EPA:ARA	5.4 ± 1.6 [b]	3.3 ± 0.7 [a]	4.7 ± 1.7 [b]	**0.0022**
DHA:ARA	14.8 ± 0.9	13.7 ± 1.1	14.7 ± 1.1	0.0179
DHA:EPA	3.0 ± 1.1	4.3 ± 0.9	3.5 ± 1.1	0.0140

[a] Sum of saturated fatty acids (SFAs); 14:0, 16:0, 18:0, 20:0, 22:0, 24:0.
[b] Sum of n-9 and n-7 isomers.
[c] Sum of monounsaturated fatty acids (MUFAs): 14:1, 16:1, 18:1, 20:1, 22:1, 24:1.
[d] Sum of n-6 fatty acids: 18:2, 20:2, 20:3, and 20:4.
[e] Sum of n-3 fatty acids: 18:3, 18:4, 20:3, 20:4, 20:5, 21:5, 22:5, 22:6.
[f] Sum of polyunsaturated fatty acids (PUFAs): 16:2, 16:3, 16:4, 18:2, 18:3, 18:4, 20:2, 20:3, 20:4, 20:5, 21:5, 22:5, 22:6.

Table 9. Fatty acid composition of Wild 2015 CYT eggs. Results (mean ± SD) are expressed in grams per 100g of total fatty acids (LA = linoleic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid).

Fatty acid	Good *(n= 8)*	Fair *(n= 8)*	Poor *(n=8)*	*P (α= 0.0025)*
14:0	1.4 ± 0.3	1.5 ± 0.2	1.4 ± 0.2	0.679
16:0	15.0 ± 0.9	15.2 ± 0.6	15.7 ± 0.6	0.116
18:0	4.1 ± 0.3	3.8 ± 0.5	4.1 ± 0.4	0.224
Σ SFA[a]	20.9 ± 1.3	20.8 ± 1.1	21.7 ± 0.7	0.266
16:1	2.9 ± 0.4	3.0 ± 0.2	3.0 ± 0.2	0.635
18:1[b]	17.2 ± 1.0	16.6 ± 1.1	18.7 ± 2.1	0.027
Σ MUFA[c]	20.6 ± 0.9	20.1 ± 1.1	22.2 ± 2.0	0.016
18:2 n-6 (LA)	5.8 ± 0.6	5.8 ± 0.7	5.6 ± 1.1	0.814
20:4 n-6 (ARA)	2.0 ± 0.3	1.9 ± 0.1	2.0 ± 0.2	0.728
Σ n-6[d]	8.0 ± 1.0	8.0 ± 0.8	7.8 ± 1.1	0.877
18:3 n-3	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	0.102
18:4 n-3	0.8 ± 0.2	0.9 ± 0.1	0.7 ± 0.2	0.112
20:5 n-3 (EPA)	6.7 ± 0.6	6.6 ± 0.4	6.1 ± 1.2	0.332
22:5 n-3	2.4 ± 0.2	2.3 ± 0.2	2.4 ± 0.2	0.361
22:6 n-3 (DHA)	32.2 ± 1.3	32.9 ± 1.2	30.9 ± 1.6	0.024
Σ n-3[e]	46.2 ± 1.7	46.9 ± 1.6	43.9 ± 2.3	0.011
Σ PUFA[f]	55.2 ± 2.1	55.9 ± 2.0	52.7 ± 2.1	0.014
EPA:ARA	3.5 ± 0.6	3.5 ± 0.4	3.2 ± 0.8	0.414
DHA:ARA	16.7 ± 2.3	17.5 ± 1.3	15.9 ± 1.8	0.234
DHA:EPA	4.9 ± 0.5	5.0 ± 0.2	5.2 ± 1.0	0.588

[a] Sum of saturated fatty acids (SFAs); 14:0, 16:0, 18:0, 20:0, 22:0, 24:0.
[b] Sum of n-9 and n-7 isomers.
[c] Sum of monounsaturated fatty acids (MUFAs): 14:1, 16:1, 18:1, 20:1, 22:1, 24:1.
[d] Sum of n-6 fatty acids: 18:2, 20:2, 20:3, and 20:4.
[e] Sum of n-3 fatty acids: 18:3, 18:4, 20:3, 20:4, 20:5, 21:5, 22:5, 22:6.
[f] Sum of polyunsaturated fatty acids (PUFAs): 16:2, 16:3, 16:4, 18:2, 18:3, 18:4, 20:2, 20:3, 20:4, 20:5, 21:5, 22:5, 22:6.

Canonical Discriminant Analysis using FA:

The Canonical Discriminant Analysis (CANDISC) procedure was applied as a dimension reduction technique to graphically separate spawns by known group assignments using linear combinations of FAs and was used only on 2013 and 2014 data. Each linear combination is called a canonical variable (CV). The discriminating ability of a FA determines the weight of its

coefficient and contribution to group separation. Loadings of canonical coefficients can be interpreted as importance in descriptive power (Ludwig et al, 2008).

CANDISC by brood and quality using 2013 FAs listed in Table 7 separated spawns by brood on the x axis by CV1. CV1 alone explains 83% of the total variation (Figure 9). Spawns from wild origin broodstock (circles) have larger CV1 values than F_1 spawns (triangles). Pooled sample standardized coefficients show the three fatty acids important to CV1 brood differences were negative loadings of the sum of n-3 FAs and positive loadings of PUFAs and MUFAs (Table 10). Of these dominating variables, ANOVA previously detected a difference by brood in MUFA (Table 8). On the y-axis, CV2 poorly separates eggs by quality and explains the other 20% of variation in the data. The degree of separation achieved by CV2 was better for wild spawns than for F_1 spawns, indicating different quality discriminators may exist for each brood type. Maximal separation between good and poor 2013 Wild spawns was achieved when F_1 spawns were omitted from the dataset (Figure 10). The major FA variables contributing to separation of good and poor quality spawns by CV1, which explained 100% of the variation, were negative loadings of DHA and the sum of n-6 FAs, and positive loading of ARA (Table 11).

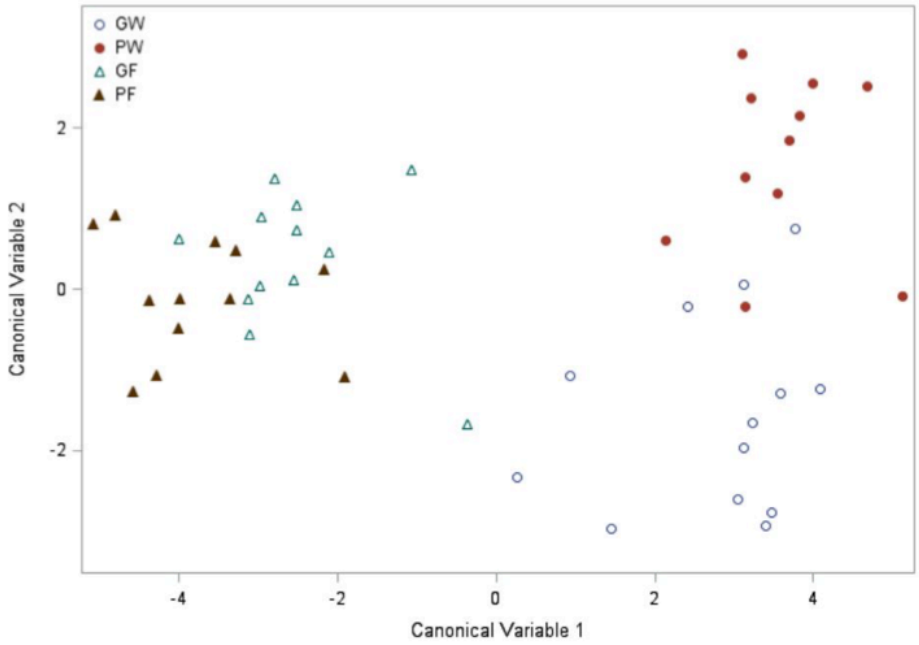

Figure 9. Canonical discrimination of arcsine transformed 2013 egg fatty acids. Each point represents an egg sample. Symbol shape differentiates brood type (Wild, circles; F_1, triangles) while symbol fill differentiates quality (Good, open; Poor, filled). GW = Good Wild, PW =Poor Wild, GF = Good F_1, PF = Poor F_1.

Table 10. Canonical coefficient loadings of top three discriminators by brood in 2013 spawns.

	Total Sample standardized coefficients	Pooled Sample standardized coefficients	Raw coefficients
CV1 (83%)	− Σ n-3	− Σ n-3	− Σ n-3
Eigenvalue = 11.6	+ Σ PUFA	+ Σ PUFA	+ Σ PUFA
	+ Σ MUFA	+ Σ MUFA	+ Σ MUFA

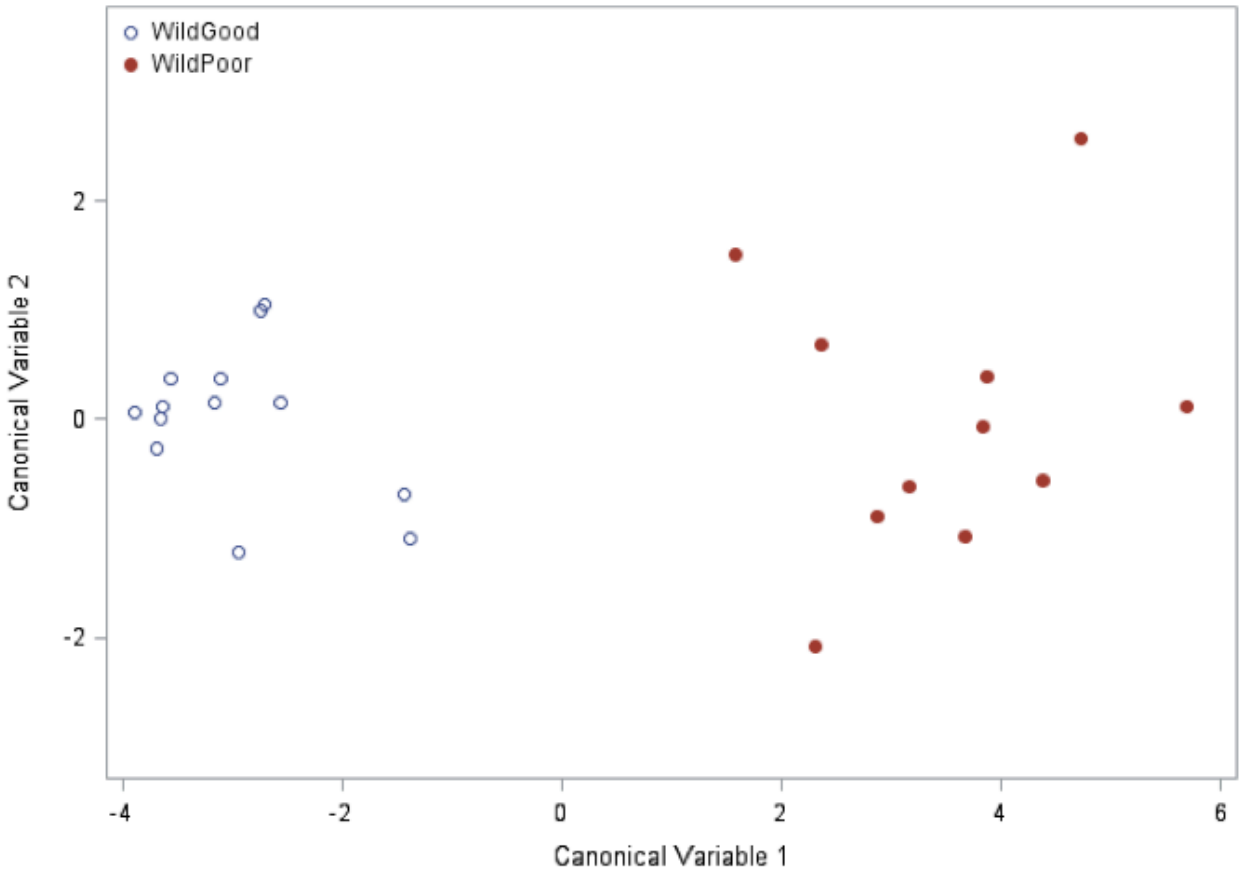

Figure 10. Canonical discrimination of arcsine transformed 2013 fatty acids from Wild spawns.

Table 11. Canonical coefficient loadings of top three quality discriminators for Wild spawns in 2013

	Total Sample standardized coefficients	Pooled Sample standardized coefficients	Raw coefficients
CV1 (100%)	− DHA	− DHA	+ ARA
Eigenvalue = 11.3	+ ARA	+ ARA	− Σ n-6
	− Σ n-6	− Σ n-6	− DHA

Wild 2014 good spawns (circles) were separated from all others via CV1 (Figure 10). Positive loadings of EPA:ARA and ARA and a negative loading of EPA were the three most important factors to CV1, which explained 80% of the variation in the data. CV2 explained the remaining 20%, and began to separate fair spawns (squares) from poor spawns (triangles) on the y axis. The dominant 3 fatty acid variables contributing to CV2 were positive loadings of DHA:EPA and EPA:ARA, and negative loading of total saturated fatty acids (Table 12).

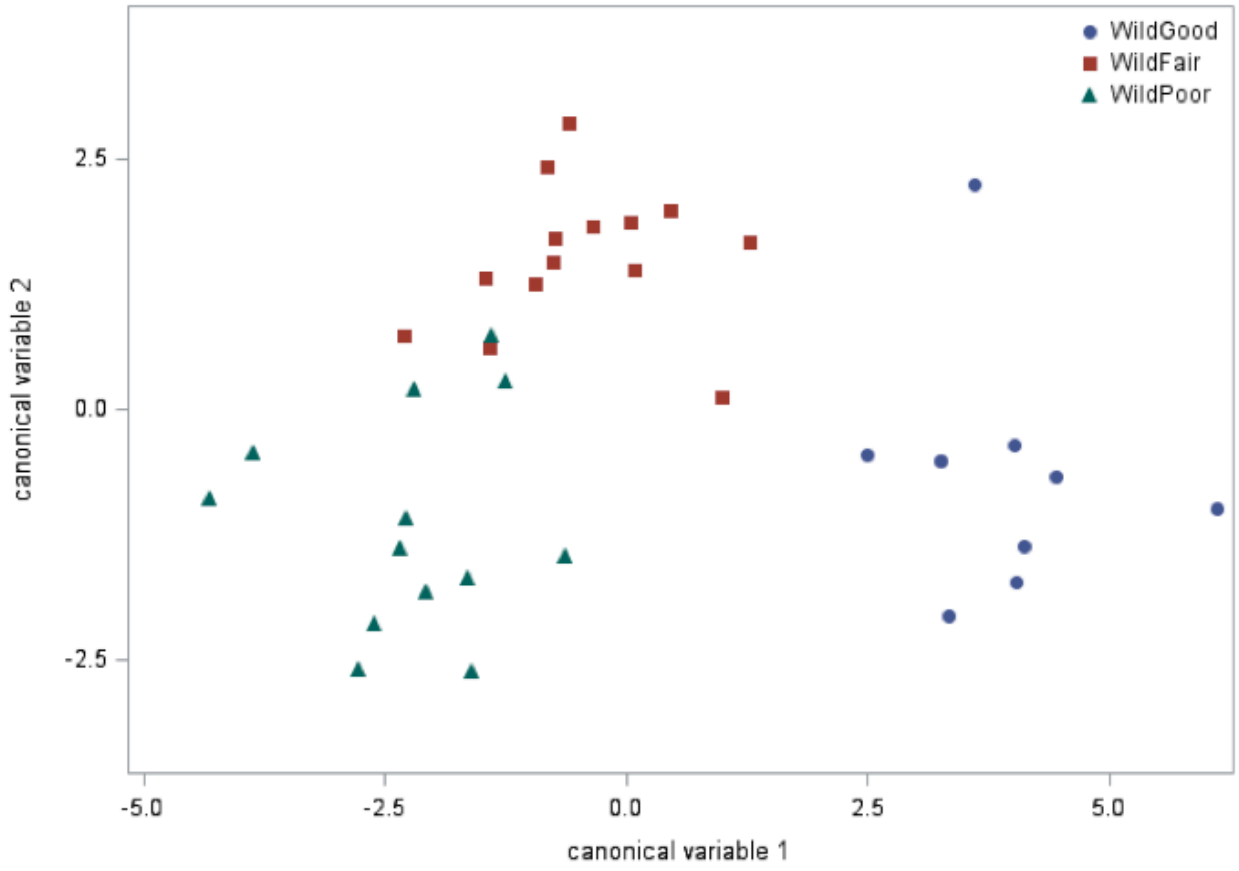

Figure 11. Canonical discrimination of arcsine transformed egg fatty acid data from 2014 Wild spawns. Each point represents one spawn event/egg sample. Symbol shape designates spawn quality (circles = good, squares = fair, triangles = poor).

Table 12. Canonical coefficient loadings for the top three quality discriminators of Wild spawns in 2014.

	Total Sample standardized coefficients	Pooled Sample standardized coefficients	Raw coefficients
CV 1 (80%)	+ EPA : ARA	+ EPA : ARA	+ ARA
Eigenvalue = 6.27	- EPA	- EPA	+ EPA : ARA
	+ ARA	+ ARA	- Σ MUFA
CV 2 (20%)	+ DHA : EPA	+ DHA : EPA	+ ARA
Eigenvalue = 1.61	+ EPA : ARA	- Σ SFA	+ 18:3 n-3
	- Σ SFA	+ EPA : ARA	- Σ PUFA

Correlation Coefficients PC and FA:

Egg PC and FA data was examined to identify associations with several quality related, independent and continuous egg variables recorded upon sample collection at HSWRI. Egg quality variables examined include: date, total spawn volume, percent floating eggs, percent non-sinking eggs (neutral and positively buoyant eggs), total egg number, egg diameter, oil diameter, % oil volume, notochord length, larval dry weight, hatch rate, percent survival to first feeding, and days to starvation (Tables 13 and 14). All variables except notochord length (not shown) had at least one relevant correlation ($|r| > 0.5$) with a nutrient variable in any year/dataset. Pearson correlation coefficients were calculated in SAS with the CORR procedure and the PEARSON option using these quality related variables with arcsine transformed PC and FA variables. Proportional variables were arcsine transformed prior to analysis, including all nutritional variables and those quality metrics designated with an asterisk (*).

Correlations for all 2013 spawns (Table 13) and Wild 2013 spawns (Table 14) were calculated separately in order to compare correlations found in 2013 and 2014 by brood type. Several differences in the 2013 datasets exist, as illustrated by the strong positive correlations (bold highlighted cells) and strong negative correlations (italics highlighted cells). These differences provide further evidence supporting the idea that each brood type may have different quality indicators.

Table 13. Pearson correlation coefficients (|r| > 0.5) for egg nutrients and quality related variables for 2013 combined Wild and F_1 spawns. Correlations are highlighted in grey with negative correlations (r < -0.5) in italics and positive correlations (r > 0.5) bolded.

Nutrient	Date	Total Spawn Volume	% Floating* (viability)	% Non-sinking*	Total Egg Number	Egg Diameter	Oil Diameter	% Oil Volume*	Larval Dry Weight	% Hatch *
N	48	48	48	48	48	47	47	48	44	47
Date	1.000	-0.175	-0.197	-0.487	-0.226	*-0.830*	*-0.660*	-0.168	*-0.516*	-0.452
14:0	**0.739**	-0.292	-0.237	*-0.652*	-0.349	*-0.648*	-0.423	-0.118	-0.276	-0.398
16:0	0.112	0.112	-0.309	-0.339	0.103	-0.153	-0.018	-0.016	-0.040	-0.038
18:0	0.084	0.106	*-0.533*	*-0.529*	0.107	-0.221	-0.228	0.008	-0.089	-0.240
Σ SFA	0.316	0.027	-0.397	*-0.534*	0.005	-0.337	-0.201	-0.060	-0.124	-0.162
16:1	**0.520**	-0.225	-0.189	*-0.552*	-0.262	-0.393	-0.087	-0.001	-0.151	-0.150
18:1	*-0.671*	**0.507**	0.003	0.470	**0.567**	**0.541**	**0.600**	0.161	0.313	0.403
Σ MUFA	*-0.539*	0.462	-0.073	0.301	**0.516**	0.455	**0.634**	0.175	0.291	0.417
18:2 n-6 (LA)	*-0.680*	0.149	0.397	**0.618**	0.170	**0.653**	0.353	0.024	0.293	0.273
20:4 n-6 (ARA)	0.295	-0.045	0.052	0.139	-0.053	-0.290	*-0.533*	-0.202	-0.164	-0.262
Σ n-6	*-0.518*	0.123	0.383	**0.606**	0.142	0.492	0.150	-0.046	0.219	0.202
18:3 n-3	-0.383	-0.149	0.477	0.292	-0.173	0.460	0.226	0.003	0.214	0.261
18:4 n-3	0.496	-0.270	0.115	-0.257	-0.307	-0.353	-0.075	0.013	-0.049	-0.068
20:5 n-3 (EPA)	**0.628**	-0.374	-0.210	*-0.581*	-0.401	*-0.513*	-0.330	-0.020	-0.245	-0.249
22:5 n-3	0.340	-0.284	-0.025	-0.166	-0.289	-0.249	-0.337	-0.017	-0.186	-0.227
22:6 n-3 (DHA)	0.082	-0.286	0.221	0.128	-0.310	-0.090	-0.277	-0.030	-0.133	-0.387
Σ n-3	0.485	*-0.501*	0.076	-0.250	*-0.539*	-0.405	-0.455	-0.046	-0.278	-0.456
Σ PUFA	0.296	-0.443	0.216	-0.026	-0.474	-0.227	-0.398	-0.063	-0.198	-0.340
EPA:ARA	0.437	-0.305	-0.214	*-0.556*	-0.323	-0.340	-0.104	0.059	-0.160	-0.125
DHA:ARA	-0.374	-0.206	0.132	-0.061	-0.213	0.361	**0.512**	0.276	0.129	0.017
DHA:EPA	*-0.575*	0.258	0.247	**0.559**	0.275	0.466	0.260	0.047	0.173	0.117
Moisture	-0.022	-0.246	-0.021	-0.113	-0.247	0.121	0.226	0.239	-0.089	-0.240
Lipid	0.343	-0.056	0.177	0.028	-0.091	-0.447	-0.376	-0.096	-0.132	-0.079
Protein	0.191	0.275	0.037	0.271	0.283	-0.292	-0.358	-0.260	0.041	-0.129
Ash	-0.179	0.070	-0.120	-0.339	0.048	0.190	0.055	-0.072	0.052	-0.047

Table 14. Pearson correlation coefficients (|r| > 0.5) for egg nutrients and quality related variables for 2013 Wild spawns. Data for yolk sac volume was not available for 2013 Wild spawns. Correlations are highlighted in grey with negative correlations (r < -0.5) in italics and positive correlations (r > 0.5) bolded.

Nutrient	Date	Total Spawn Volume	% Floating* (viability)	% Non-sinking*	Total Egg Number	Egg Diameter	Oil Diameter	% Oil Volume*	Larval Dry Weight	% Hatch*
N	24	24	24	24	24	24	24	24	21	23
Date	1.000	-0.073	-0.164	-0.086	-0.029	*-0.837*	*-0.632*	-0.282	*-0.566*	-0.332
14:0	**0.549**	0.092	-0.128	-0.002	0.147	*-0.535*	-0.249	-0.020	0.045	-0.072
16:0	-0.194	0.402	-0.304	-0.205	0.430	0.031	0.187	0.183	0.193	-0.001
18:0	-0.131	0.475	*-0.536*	*-0.609*	0.491	0.033	-0.177	-0.265	-0.083	-0.158
Σ SFA	-0.089	0.468	-0.418	-0.355	**0.504**	-0.054	0.025	0.027	0.135	-0.043
16:1	0.027	-0.060	0.266	0.379	-0.060	0.005	0.401	0.498	0.263	0.192
18:1	-0.396	0.469	-0.193	-0.121	0.447	0.273	**0.523**	**0.528**	0.050	-0.014
Σ MUFA	-0.359	0.423	-0.122	-0.038	0.403	0.253	**0.556**	**0.580**	0.096	0.033
18:2 n-6 (LA)	*-0.655*	-0.226	0.352	0.262	-0.268	**0.657**	0.308	-0.008	0.363	0.230
20:4 n-6 (ARA)	**0.761**	-0.173	-0.198	-0.182	-0.138	*-0.643*	*-0.750*	*-0.578*	-0.455	-0.321
Σ n-6	-0.280	-0.288	0.271	0.182	-0.308	0.333	-0.049	-0.262	0.168	0.139
18:3 n-3	*-0.609*	-0.371	**0.551**	0.406	-0.407	**0.640**	0.342	0.049	0.485	0.364
18:4 n-3	0.178	-0.151	0.486	**0.599**	-0.160	-0.078	0.173	0.267	0.327	0.339
20:5 n-3 (EPA)	**0.563**	-0.494	0.192	0.157	-0.465	-0.451	-0.378	-0.232	-0.157	-0.066
22:5 n-3	0.479	-0.419	0.133	0.021	-0.429	-0.283	-0.461	-0.399	-0.443	-0.198
22:6 n-3 (DHA)	0.273	*-0.552*	0.226	0.158	*-0.563*	-0.163	-0.373	-0.392	-0.218	-0.117
Σ n-3	0.406	*-0.577*	0.248	0.182	*-0.578*	-0.271	-0.413	-0.377	-0.223	-0.097
Σ PUFA	0.314	*-0.574*	0.280	0.200	*-0.579*	-0.177	-0.384	-0.394	-0.165	-0.057
EPA:ARA	-0.076	-0.385	0.355	0.313	-0.381	0.088	0.247	0.243	0.202	0.183
DHA:ARA	*-0.784*	-0.209	0.422	0.340	-0.263	**0.718**	**0.677**	0.433	0.419	0.298
DHA:EPA	*-0.530*	0.239	-0.065	-0.056	0.185	0.478	0.275	0.079	0.062	0.010
Moisture	-0.185	-0.321	0.261	0.398	-0.354	0.238	0.334	0.268	0.170	0.142
Lipid	**0.523**	-0.024	0.182	0.132	-0.024	-0.430	-0.356	-0.171	-0.368	-0.180
Protein	**0.588**	0.205	-0.299	-0.294	0.262	*-0.617*	*-0.612*	-0.419	-0.360	-0.293
Ash	-0.423	0.213	-0.123	-0.326	0.200	0.365	0.128	-0.059	0.210	0.120

Pearson correlation coefficients were calculated for 2014 Wild spawns as previously described. Date, egg diameter, oil diameter and days to starvation were associated with several fatty acid variables (Table 14). There were no spawn, egg, or larval metrics associated with proximate components. When comparing correlations from all 2013 and 2014 data the only consistent associations were a negative correlation between 14:0 and egg diameter, as well as a positive correlation between 14:0 and spawn date. When considering Wild spawns, there was a consistent negative correlation between ARA and egg diameter in 2013 and 2014, however this correlation was not seen in 2015 (Figure 12). This was also accompanied by a positive association between ARA and spawn date in years 2013 and 2014.

Table 15. Pearson correlation coefficients (|r| > 0.5) for egg nutrients and quality related variables for 2014 Wild spawns. Correlations are highlighted in grey with negative correlations (r < -0.5) in italics and positive correlations (r > 0.5) bolded.

Nutrient	Date	Total Spawn Volume	% Floating* (viability)	% Non-sinking*	Total Egg Number	Egg Diameter	Oil Diameter	% Oil Volume*	Larval Dry Weight	% Hatch *
N	36	36	36	36	36	36	36	36	36	36
Date	1.000	-0.330	0.096	-0.005	-0.325	*-0.831*	*-0.507*	-0.111	*-0.684*	-0.346
14:0	**0.590**	-0.065	-0.108	-0.232	-0.060	*-0.568*	-0.470	-0.257	-0.085	0.096
16:0	0.153	0.095	-0.238	-0.321	0.105	-0.161	-0.330	-0.350	0.006	-0.387
18:0	-0.224	0.187	-0.108	-0.246	0.196	0.109	-0.249	-0.340	0.171	-0.103
Σ SFA	0.214	0.112	-0.272	-0.414	0.126	-0.257	*-0.501*	-0.492	0.024	-0.301
16:1	*-0.707*	0.278	-0.011	0.009	0.267	**0.705**	***0.509***	0.165	0.338	-0.268
18:1	-0.312	0.122	0.167	0.249	0.117	0.360	0.245	0.104	0.120	-0.057
Σ MUFA	-0.479	0.176	0.161	0.234	0.169	**0.517**	0.372	0.154	0.214	-0.086
18:2 n-6 (LA)	-0.162	-0.037	0.273	0.307	-0.046	0.164	0.347	0.307	-0.033	0.034
20:4 n-6 (ARA)	**0.699**	-0.236	0.142	0.165	-0.222	*-0.598*	-0.356	-0.096	-0.238	0.263
Σ n-6	0.247	-0.182	0.334	0.366	-0.185	-0.207	0.113	0.233	-0.204	0.159
18:3 n-3	0.222	-0.272	0.408	0.352	-0.273	-0.158	-0.028	0.041	0.044	0.424
18:4 n-3	*-0.712*	0.121	0.120	0.081	0.110	**0.635**	0.388	0.088	0.316	0.020
20:5 n-3 (EPA)	*-0.922*	0.226	-0.090	-0.100	0.214	**0.771**	0.469	0.113	0.291	-0.249
22:5 n-3	*-0.798*	0.125	0.087	0.152	0.111	**0.721**	**0.605**	0.314	0.251	-0.192
22:6 n-3 (DHA)	**0.822**	-0.249	0.042	0.085	-0.241	*-0.730*	-0.372	0.007	-0.290	0.359
Σ n-3	-0.220	-0.084	0.055	0.106	-0.094	0.172	0.290	0.299	0.034	0.201
Σ PUFA	-0.041	-0.172	0.171	0.231	-0.181	0.021	0.246	0.317	-0.058	0.266
EPA:ARA	*-0.889*	0.254	-0.128	-0.153	0.241	**0.746**	0.439	0.097	0.284	-0.275
DHA:ARA	-0.247	0.127	-0.221	-0.212	0.115	0.171	0.158	0.142	0.082	-0.060
DHA:EPA	**0.937**	-0.256	0.097	0.103	-0.245	*-0.790*	-0.452	-0.067	-0.318	0.263
Moisture	0.177	0.007	0.181	0.265	0.014	-0.092	-0.004	-0.039	0.367	0.165
Lipid	-0.076	-0.211	0.114	-0.059	-0.206	0.078	-0.006	0.001	-0.311	-0.148
Protein	-0.173	-0.053	-0.285	-0.359	-0.059	-0.012	-0.138	-0.086	-0.393	-0.135
Ash	0.091	0.043	0.110	-0.010	0.049	-0.015	0.165	0.253	-0.007	0.038

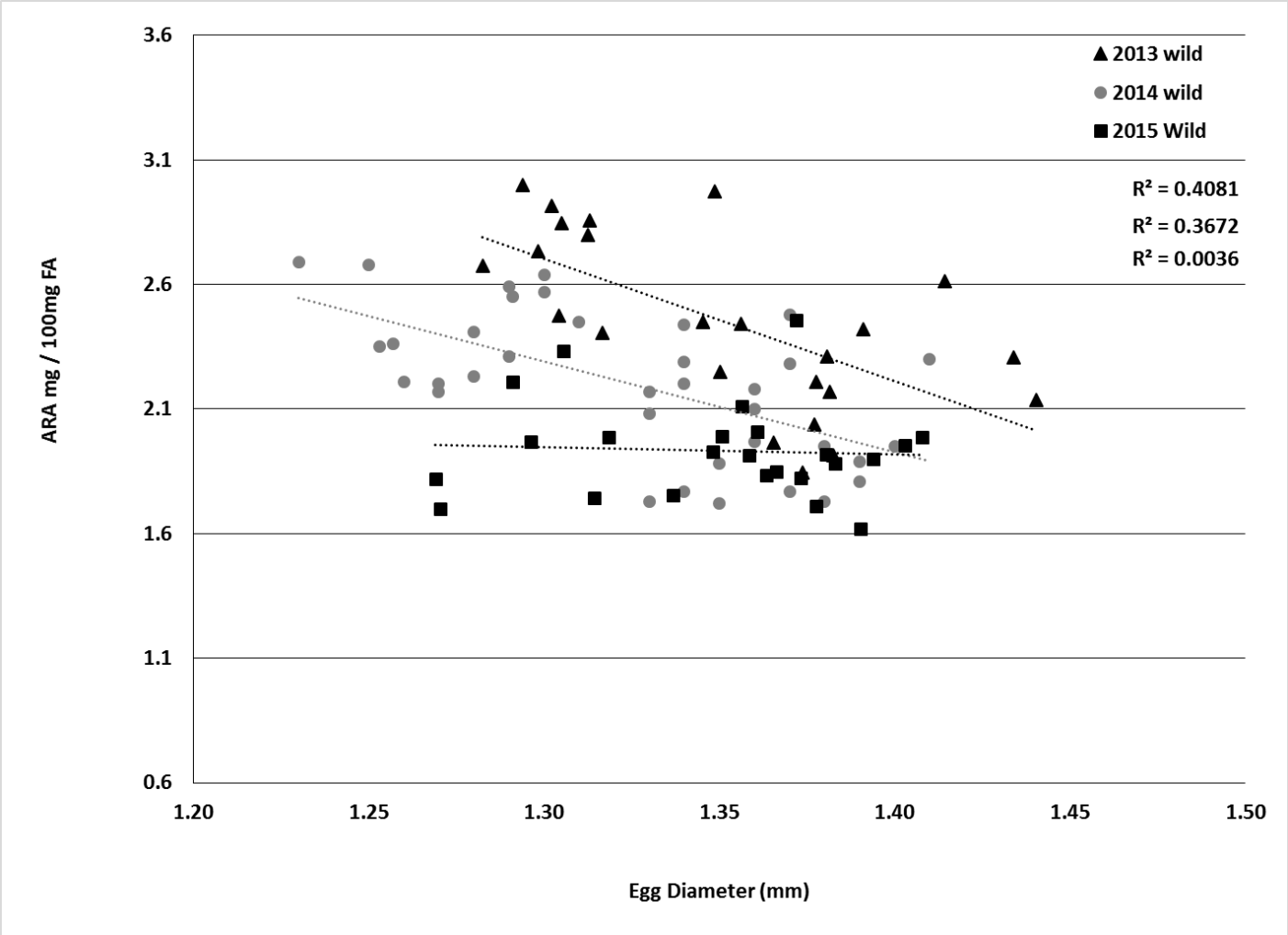

Figure 12. Linear regression of ARA and egg diameter for wild spawns by year.

Task 1c. *[SWFSC]* (Parentage analysis)

All broodstock from 2013 to 2016 were genotyped at 15 microsatellite loci and these data were used for genetic parentage for all spawns that occurred during those years. The 2013 broodstock population contained 19 individuals (8 females, 11 males) with additional broodstock added to the 2014 population for a total of 37 individuals (18 females, 19 males). The broodstock population remained the same for the 2015 season, however in 2016 the population was reduced to 29 individuals (13 females, 16 males). For each spawn event, parentage of yolk larvae was determined using the program Cervus v 3.06. For all years, individual spawns were typically dominated by one or two females (Figures 13 and 14) with roughly equal contributions by males.

From 2013 – 2015 spawn events were dominated by one main female (083-027-609), this fish contributed to over 40% of the spawn events over the three year span (Figure 13, Table 16). When looking at egg production and viability numbers attributed with spawns from that female, total annual fecundity and percent viability decreased each year from 2013 to 2015. Based on these results the female was euthanized in the winter of 2015 prior to the 2016 spawning season.

Female contribution from the older group of fish (fish held at HSWRI since 2003) in 2015 showed three dominant females (083-026-876, 083-027-609, 083-103-352; Figure 13), in 2016 fish 083-027-609 was euthanized and fish 083-026-876 became the dominant female from the older group of fish. Female contribution from the newer group of fish (fish added to the broodstock population in 2013) showed little contribution in 2014 and consistent contribution in 2015 and 2016 (Figure 14). None of the newer females contributed as much as 083-026-876, however most of the newer females contributed to over 20% of the spawn events in 2015. This trend was not seen in 2016, when only one of the newer females contributed to over 30% of the spawn events (Figure 14).

Based on the parentage analysis, removal of females has a significant impact on spawning behavior and egg production for *Seriola*. Parentage analyses can make a direct impact on the industry by optimizing broodstock management and being able to focus on fish that are contributing to good spawn events. Growers should replenish brood fish with young fish periodically – e.g. for CYT use fish less than 10 years old.

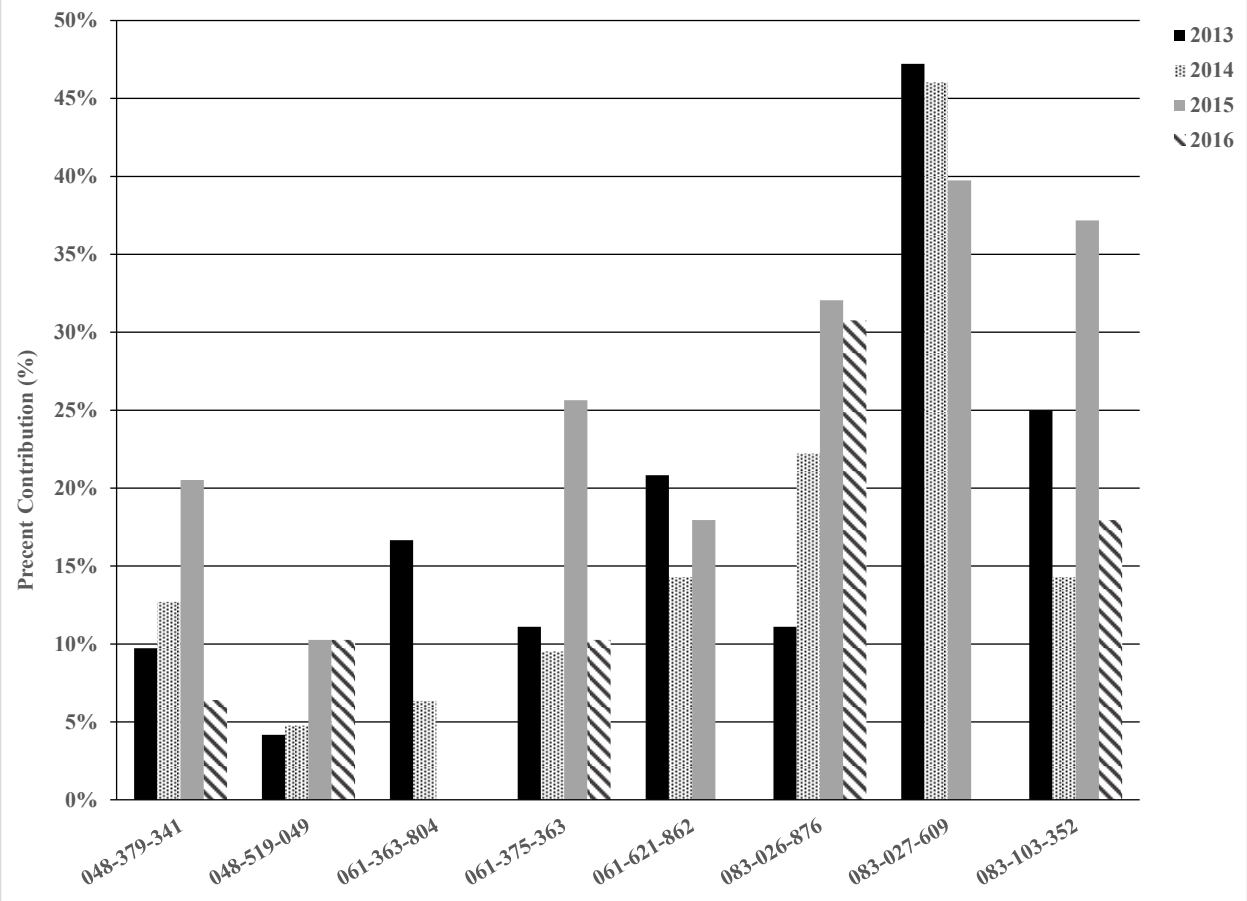

Figure 13. Female contribution from spawn events from 2013 – 2016 from female CYT that have been at HSWRI since 2003.

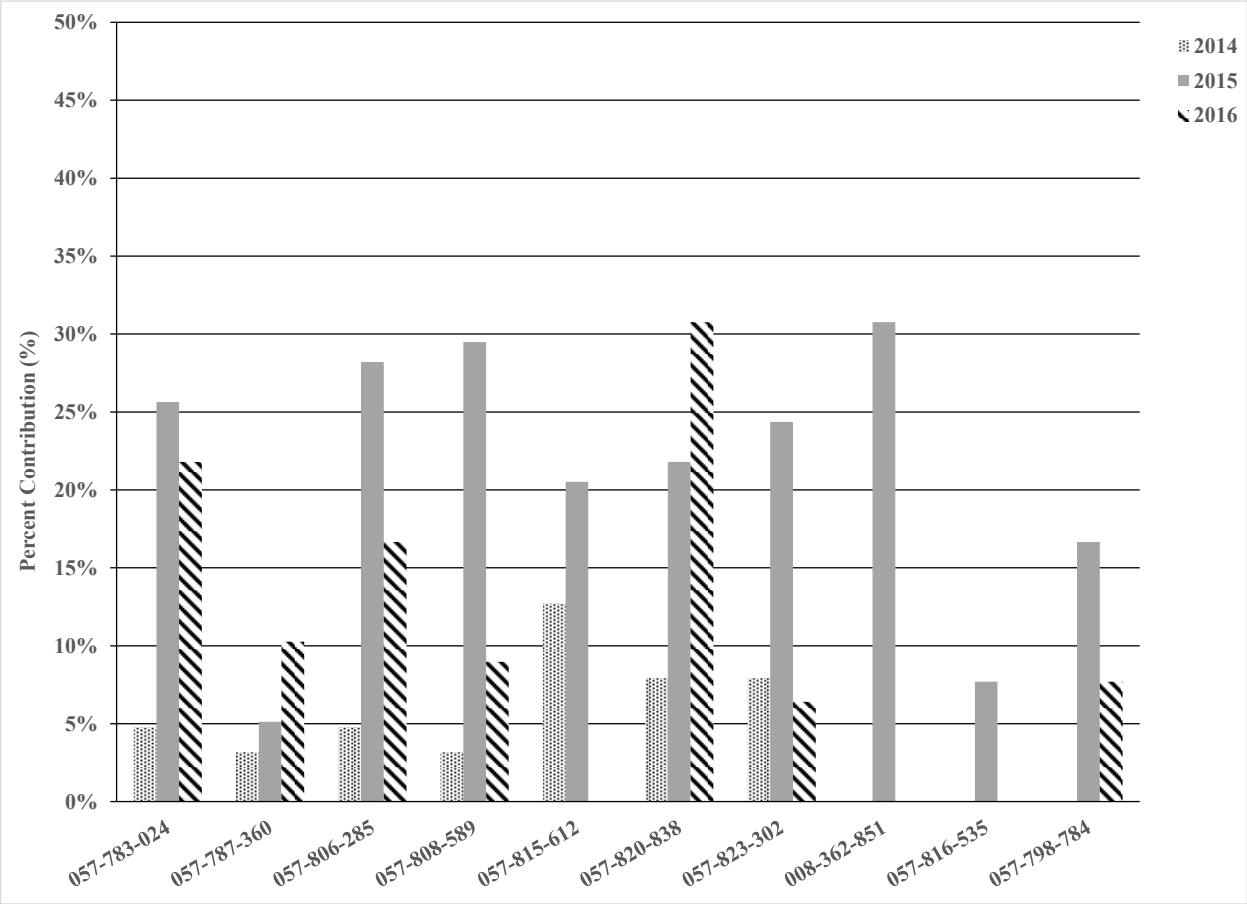

Figure 14. Female contribution to spawn events from 2013 – 2016 from CYT that were introduced into HSWRI's breeding population in 2014.

Table 16. Individual egg production numbers for female CYT (083-026-709), based on parentage analysis.

Year	Individual Spawn Events (Number)	Multiple Spawn Events (Number)	Contribution (Percent)	Mean Viability (Percent ± SD)	Mean Fecundity (Eggs/kg)	Total Annual Fecundity (Eggs/kg/yr)
2013	18	16	47.2	59.9 ± 24.4	34,752 ± 18,088	1,181,577
2014	11	19	47.6	34.5 ± 25.4	36,308 ± 19,491	1,052,921
2015	7	24	39.7	35.8 ± 20.8	16,649 ± 17,837	511,376

Task 1d. *[NWFSC/HSWRI]* (Juvenile grow-out trials)

[*NWFSC – Manchester*]

A major question with SF is whether the sequential spawns from an individual female will have varying egg quality that affects fertilization/cell symmetry, and subsequently larval survival,

growth and deformities. The hypothesis is that egg batches early and late in the cycle result in eggs and larvae with reduced quality and, thus, survival, though this has never been experimentally addressed. While we have begun to address the variation in fertilization and cell symmetry within different batches of SF eggs (Table 17), those batches were not carried through to the larval and juvenile phase. In addition, we have now developed the methods for SF sperm cryopreservation (Immerman, D. and Goetz, F.W. 2014. The activation and cryopreservation of SF (*Anoplopoma fimbria*) sperm Aquaculture 430:211-217) so that the same sperm with identical quality can be used with a female when fertilizing over different egg batches in the future.

Table 17. Fertilization and cell symmetry rates for sequential batches of eggs from 4 females of SF

Spawn Event	Female ID	Male ID	Fert. %	Symm. %		
1	6C00010305	3D9.1BF0EC5CA1	53	57		
2	6C00010305	7110262238	59	53		
3	6C00010305	3D9.1BF0EC5CA1	57	17	AVG Fert.	AVG Symm.
4	6C00010305	3D9.1BF0EC5CA1	49	24	54.5	20.5
	Female ID	Male ID	Fert. %	Symm. %		
1	6C00010655	7110262238	84	45		
2	6C00010655	7110262238	86	40		
3	6C00010655	3D9.1BF0EC5CA1	53	31	AVG Fert.	AVG Symm.
4	6C00010655	3D9.1BF0EC5CA1	64	37	66.3	46
5	6C00010655	3D9.1BF102041D	41	42		
6	6C00010655	3D9.1BF0FF6797	70	81		
1	Female ID	Male ID	Fert. %	Symm. %		
2	6C00010658	6C00011009	83	27		
3	6C00010658	3D9.1BF0FF6797	65	21	AVG Fert.	AVG Symm.
4	6C00010658	3D9.1BF0FF6797	84	17	19	19
5	6C00010658	7110262238	61	56		
	Female ID	Male ID	Fert. %	Symm. %		
1	6C00011052	3D9.1BF102041D	19	43		
2	6C00011052	3D9.1BF0EC5CA1	58	70		
3	6C00011052	7110262238	73	72	AVG Fert.	AVG Symm.
4	6C00011052	7110262238	80	89	76.5	80.5
5	6C00011052	3D9.1BF102041D	46	72		

[*HSWRI*]

One grow-out trial was completed with CH, and was done in 2015. Unfortunately, over the duration of the project spawning was intermittent and at times poor quality. However, for this run eggs were stocked from a spawn collected on 5/20/15. Survival from egg to 60 dph juvenile was 21.0%, however there was a low rate of properly pigmented fish (24.8%).

A total of 8 CYT juveniles grow-out trials were completed over the three year project. Growth, survival, swim bladder inflation, and malformation were recorded for each trial. Growth was consistent year to year and trial to trial. Survival and swim bladder inflation rates were higher in 2016 than previous years (Table 18). The improvement in survival and swim bladder inflation could be attributed to the improvement in egg quality along with the fact that these grow out trials were run in a newly constructed larval rearing system that employed self-cleaning tanks. There were no clear indications that egg quality impacted the production numbers within the years or between years. This question could better be answered in future projects if more spawn events could be reared throughout a given season.

Table 18. Grow-out trial information on three trials of CYT in 2014, 2015, and 2016 grown to 60 dph.

2014	Survival (%)	Swimbladder Inflation (%)	Malformation (%)
1	9.6	33.3	25.1
2	8.2	6.9	20.3
3	23.7	8.5	22.8
2015			
1	22.0	12.4	26.9
2	35.2	5.4	15.5
3	16.0	9.3	14.8
2016			
1	30.4	70.5	14.5
2	38.2	42.2	12.6
3	NA	NA	NA

Objective 2 is a single task *[NWFSC/HSWRI]*, multi-year objective. Determine how broodstock dietary manipulation impacts egg quality.

Under this objective, we manipulated the concentrations of arachidonic acid (ARA) in CYT F_1 broodstock to assess both the biochemical composition of the eggs and the egg quality from each spawn event. The experimental tanks used for this trial (3.6 m diameter x 1.2 m deep fiberglass flat bottom tanks) were put in place in 2014, and fish were stocked into the tanks at the end of 2014. The experimental diets included a control feed made up of a mash premix (Bio Vita Starter Mash; Bio-Oregon, Longview, WA) and an experimental feed containing the same mash premix with an ARA oil supplementation at 1.0%. Experimental diets were made using a Hobart mixer to form the diets into a sausage. Both diets included 23% water to help form the sausage (3.0 cm diameter cut to 8.0 cm in length). The ARA diet additionally contained 1% Refined Arachidonic Acid Oil (Reed Mariculture, Campbell, CA, USA). Refined Arachidonic Acid Oil is produced by Cargill, Incorporated (Minneapolis, MN, USA), and is derived from the fungus *Mortierella alpina*. The oil contained a minimum of 40% (wet weight) ARA. Each dietary treatment was offered to two replicate tanks containing four F_1 CYT (2 males, 2 females). The experimental diets were offered at the beginning of 2015, three months prior to the spawning season. The fish were fed 3-5% body weight per day for five days of the week. Fish began spawning in April of 2015 and ended in September of 2015. Significant differences were seen in viability, hatch rates, and egg diameter, all being higher in the ARA treatment (Table 19). The treatment tanks offered the ARA supplemented diet spawned a total of 30 times and produced over 13 million eggs, while the control treatment (CON) tanks spawned 53 times and produced over 18 million eggs. This difference between treatments might be notable given that the female biomass among tanks was very similar. However, it might also simply be a function of variation

in acclimation rates among the individual fish. With more resources, the study design would benefit from one or more of the following – 1) more replicates, 2) repetition of the trial in the following year, and 3) parentage analyses to link egg production to individual fish.

The PC analysis showed no significant differences between the ARA and CON diets (Table 20). The egg samples between both treatments showed a slight difference in the lipid composition, CON eggs (1.5 g $100g^{-1}$ wet weight) were slightly higher in lipid than ARA eggs (1.4 g $100g^{-1}$ wet weight) at a P value ≤ 0.05. Fatty acid analysis for both diets and eggs is shown in Table 21. There was no significant difference in the saturated fatty acids (SFA) between the diets, however the C14:0 and C16:0 were significantly higher in the control diet and the C18:0 was significantly higher in the ARA diet. ARA was significantly higher in the ARA diet and the sum of the n-6 fatty acids were also significantly higher in the ARA diets. Eicosapentaenoic acid (EPA) and the sum of the n-3 fatty acids were significantly higher in the CON diets, and the polyunsaturated fatty acids (PUFA) were significantly higher in the ARA diet. Docosahexaenoic acid (DHA) was not significantly different between the diets. Finally, the EPA:ARA and DHA:ARA ratios were significantly higher in the CON diets. Egg analysis showed significantly higher SFA in the CON eggs, with C14:0 and C16:0 being significantly higher in the CON eggs. Monounsaturated fatty acids (MUFA) were also significantly higher in the CON eggs but linoleic acid (LA) was significantly higher in the ARA eggs. ARA was also significantly higher in the ARA eggs, as was the sum of n-6 fatty acids. EPA, EPA:ARA ratio, and DHA:ARA ratio were significantly higher in the CON eggs, however the DHA:EPA ratio was significantly higher in the ARA eggs. ARA content in the eggs increased throughout the spawning season in the ARA treatment (Figure 15). In contrast, ARA content in the CON treatment eggs remained relatively constant throughout the spawning season.

Table 19. Egg quality parameters collected from CYT broodstock (*Seriola dorsalis*) when offered two experimental diets. The number and size of females were similar for both groups.

Parameter	ARA	CON
Number of Spawns	30	53
Total Eggs Produced	13,158,126	18,615,111
Viability (% ± SD)	72.1 ± 23.7^{a}	33.7 ± 39.6^{b}
Egg Diameter (mm ± SD)	1.34 ± 0.05^{a}	1.29 ± 0.03^{b}
Oil Diameter (mm ± SD)	0.31 ± 0.01	0.29 ± 0.01
Percent Oil Volume (% ± SD)	1.19 ± 0.10	1.21 ± 0.13
Notochord Length (mm ± SD)	3.91 ± 0.17	3.80 ± 0.27
Yolk Sac Volume (mm^3 ± SD)	0.32 ± 0.20	0.31 ± 0.12
Hatch Rate (% ± SD)	52.4 ± 18.8^{a}	25.6 ± 30.6^{b}

Table 20. Experimental diet and egg nutritional composition. Results are expressed as mean ± SD. Proximate composition values are reported as g $100g^{-1}$ wet weight. SD < 0.1 are reported as "0.0".

	Diets *n*=5		Eggs *n*=18		*P*[a]	
	ARA	CON	ARA	CON	Diets	Eggs
Nutrient						
Protein	42.8 ± 1.1	43.4 ± 1.9	4.4 ± 0.2	4.5 ± 0.1		
Lipid	16.4 ± 1.4	16.3 ± 2.2	1.4_6 ± 0.0_7	1.5_1 ± 0.0_8		*
Ash	6.6 ± 0.9	6.7 ± 1.1	1.3 ± 0.4	1.4 ± 0.4		
Moisture	25.4 ± 0.9	25.4 ± 0.7	92.3 ± 0.4	92.2 ± 0.5		

Table 21. Experimental diet and egg nutritional composition. Results are expressed as mean ± SD. Proximate composition values are reported as g $100g^{-1}$ wet weight. Fatty acid composition values are expressed as g fatty acid $100g^{-1}$ total fatty acids. (ND = not detectable, LA = linoleic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid). SD < 0.1 are reported as "0.0".

	Diets *n*=5		Eggs *n*=18		*P*[a]	
	ARA	Control	ARA	Control	Diets	Eggs
Fatty Acid						
C14:0	6.0 ± 0.3	6.4 ± 0.4	1.9 ± 0.2	2.1 ± 0.2	*	**
C16:0	18.5 ± 0.2	19.7 ± 0.2	15.1 ± 0.5	15.5 ± 0.5	***	*
C18:0	4.3 ± 0.1	4.1 ± 0.0	4.3 ± 0.3	4.3 ± 0.3	***	
SFA[b]	30.6 ± 0.3	30.7 ± 0.5	21.6 ± 0.7	22.2 ± 0.8		*
C16:1	6.9 ± 0.3	7.4 ± 0.4	4.9 ± 0.4	5.5 ± 0.3	*	***
C18:1[c]	15.6 ± 0.5	16.3 ± 0.6	23.3 ± 0.9	24.1 ± 0.6		**
C20:1[c]	1.7 ± 0.3	2.1 ± 0.3	0.6 ± 0.1	0.9 ± 0.1		
C22:1[c]	2.3 ± 0.3	2.5 ± 0.3	0.0 ± 0.1	0.1 ± 0.2		
MUFA[d]	26.9 ± 0.6	28.8 ± 0.5	28.9 ± 0.6	30.5 ± 0.5		***
C18:2n-6 (LA)	6.6 ± 1.4	6.3 ± 1.4	7.7 ± 0.9	7.0 ± 1.0		*
C20:3n-6	0.5 ± 0.0	*ND*	0.5 ± 0.0	0.2 ± 0.1	**	***
C20:4n-6 (ARA)	4.7 ± 0.6	1.4 ± 0.1	4.8 ± 0.4	1.9 ± 0.1	***	***
n-6 [e]	11.8 ± 2.0	7.8 ± 1.5	13.1 ± 1.4	9.2 ± 1.1	**	***
C18:3n-3	1.3 ± 0.1	1.5 ± 0.1	0.9 ± 0.1	1.0 ± 0.1		
C18:4n-3	1.5 ± 0.2	1.6 ± 0.2	0.6_9 ± 0.0_4	0.7_4 ± 0.0_6		*
C20:4n-3	0.5 ± 0.0	0.6 ± 0.0	0.4 ± 0.1	0.6 ± 0.1		**
C20:5n-3 (EPA)	11.1 ± 0.4	12.0 ± 0.3	6.5 ± 0.3	7.9 ± 0.3	**	***
C22:5n-3	1.5 ± 0.1	1.6 ± 0.2	2.5 ± 0.1	2.7 ± 0.1		***
C22:6n-3 (DHA)	9.6 ± 0.7	10.5 ± 0.6	19.9 ± 1.5	20.0 ± 1.1		
n-3[f]	25.6 ± 1.2	27.8 ± 0.9	31.4 ± 1.3	33.2 ± 1.1	*	
PUFA[g]	38.6 ± 1.1	36.7 ± 1.0	45.1 ± 1.0	43.1 ± 0.6	*	
EPA:ARA	2.4 ± 0.4	8.6 ± 0.5	1.4 ± 0.1	4.2 ± 0.3	***	***
DHA:ARA	2.1 ± 0.4	7.6 ± 0.9	4.2 ± 0.7	10.6 ± 0.8	***	***
DHA:EPA	0.9 ± 0.1	0.9 ± 0.1	3.1 ± 0.3	2.5 ± 0.2		***

[a] P – value notation is as follows: * ≤ 0.05; ** ≤ 0.01; and *** ≤ 0.001.

[b] Sum of saturated fatty acids (SFAs); 14:0, 16:0, 18:0, 20:0, 22:0, 24:0.

[c] Sum of *n*-7, *n*-9 and *n*-11 isomers.

[d] Sum of monounsaturated fatty acids (MUFAs): 14:1, 16:1, 18:1, 20:1, 22:1, 24:1.

[e] Sum of *n*-6 fatty acids: 18:2, 20:2, 20:3, and 20:4.

[f] Sum of *n*-3 fatty acids: 18:3, 18:4, 20:3, 20:4, 20:5, 21:5, 22:5, 22:6.

[g] Sum of polyunsaturated fatty acids (PUFAs): 16:2, 16:3, 16:4, 18:2, 18:3, 18:4, 20:2, 20:3, 20:4, 20:5, 21:5, 22:5, 22:6.

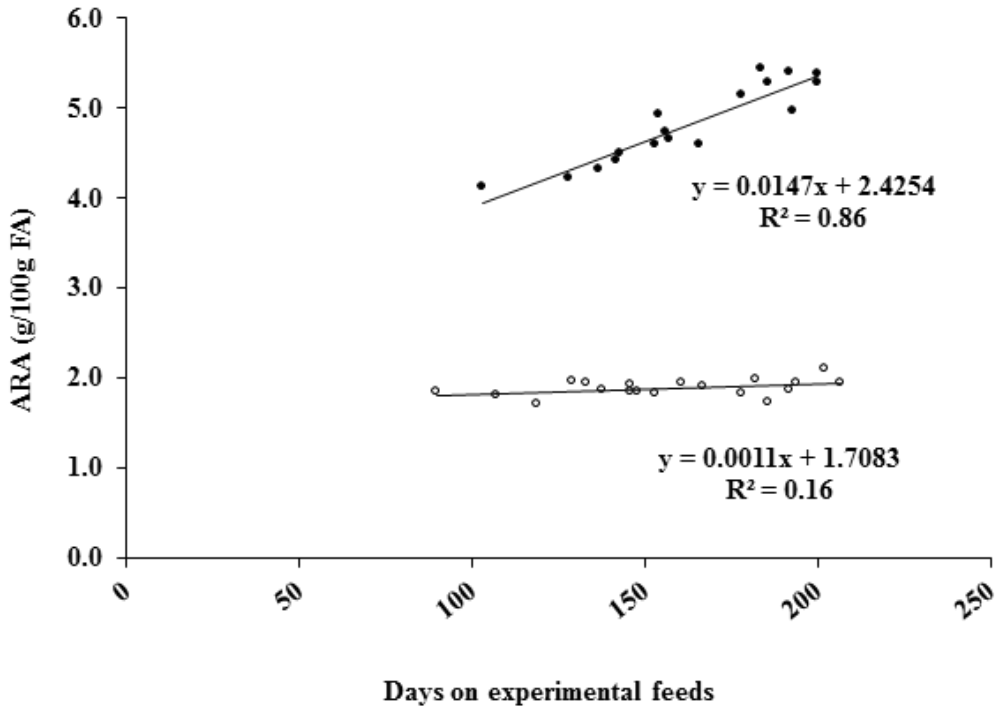

Figure 15. Arachidonic acid (ARA) content in eggs from CYT (*Seriola dorsalis*) over a spawning season where the broodstock where fed two experimental diets. The black circles represent ARA levels in eggs from the ARA treatment and empty circles represent ARA levels in eggs from the CON treatment.

Objective 3 is a single task *[NWFSC/HSWRI]*, multi-year objective. Assess whether different spawning methods affect egg quality.

In this objective we investigated alternative GnRH regimes for spawning induction of SF. We tested the effects of GnRH implant dose (high (~50 µg/kg) and low (~25 µg/kg)) and primer injections of 5 µg/kg (GnRH) prior to GnRH implants. These investigations were conducted over 2 spawning pulses in 2015.

In pulse 1, we looked at the effects of injecting a 10 fold lower dose (5 µg/kg) of GnRH one week prior to Ovaplant at ~50 µg/kg; the dose that we have used routinely. The results (Table 22) suggest that pre-injection results in better quality eggs based on fertilization and cell symmetry at early division. It also appeared that fish began spawning earlier with the pre-injection regime.

In the pulse 2, we again tested the effects of a pre-injection of 5 µg GnRH/kg prior to implant but we also tested the effects of a lower dose (~25 µg/kg) of Ovaplant alone (not in combination with pre-injection). Based on cell division symmetry, it appeared again that a pre-injection of GnRH resulted in better quality eggs (Table 23). In this pulse, pre-injection did not hasten the time to spawning. However, looking more closely at the timing of spawning events in all of the treatments revealed that pre-injection appeared to tighten the timing of the spawns (Figure 16). Both implant treatments in the absence of a pre-injection appeared to have two pulses of spawning that might reflect the disposition of the ovaries prior to implant. In contrast, pre-

injection may have made the initial ovarian state of all fish similar prior to the implant thus synchronizing the fish.

Implants with a lower GnRH dose (25 µg/kg) seemed to do quite well compared with the high dose and, if coupled with a pre-injection, might be the best treatment option.

Table 22. Effects of a pre-injection of GnRH at 5 µg/kg on sablefish spawning induced by a 50 µg/kg implant

	Implant-only	Pre-injected
total egg volume (in pulse)	1703.5	2190.0
total egg & fluid volume (in pulse)	1836.0	2383.2
egg volume (per spawn)	389.4	382.4
egg & fluid volume (per spawn)	419.7	416.1
fert % (weighted by volume of eggs)	57.9	74.5
symmetry (weighted by volume of eggs)	63.8	65.0
fert % (un-weighted)	61.3	73.4
symmetry (un-weighted)	62.6	66.0
Days until first spawn	20.5	18.3
number of fish in treatment	8.0	11.0
# of spawns	4.4	5.7

Table 23. Effects of a pre-injection of GnRH at 5 µg/kg on sablefish spawning induced by a 50 µg/kg implant and effects of 50 and 25 µg/kg implants alone.

	~50 ug/kg implant	~25 ug/kg implant	Pre-injection
total egg volume (pulse)	7200	14680	9665
total egg & fluid volume (pulse)	7580	15690	10100
egg volume (per spawn)	514.3	419.4	483.3
egg & fluid volume (per spawn)	541.4	448.3	505.0
fert % (weighted by volume of eggs)	62.2	75.7	66.7
symmetry (weighted by volume of eggs)	52.7	62.7	77.4
fert % (un-weighted)	63.5	75.8	64.8
symmetry (un-weighted)	52.4	62.9	75.8
Mean Days until first spawn	12.6	12.8	13.9
Median Days until first spawn	16.0	16.5	15.0
number of fish	5.0	10.0	8.0
mean # of spawns > 100 ml	2.8	3.5	2.5

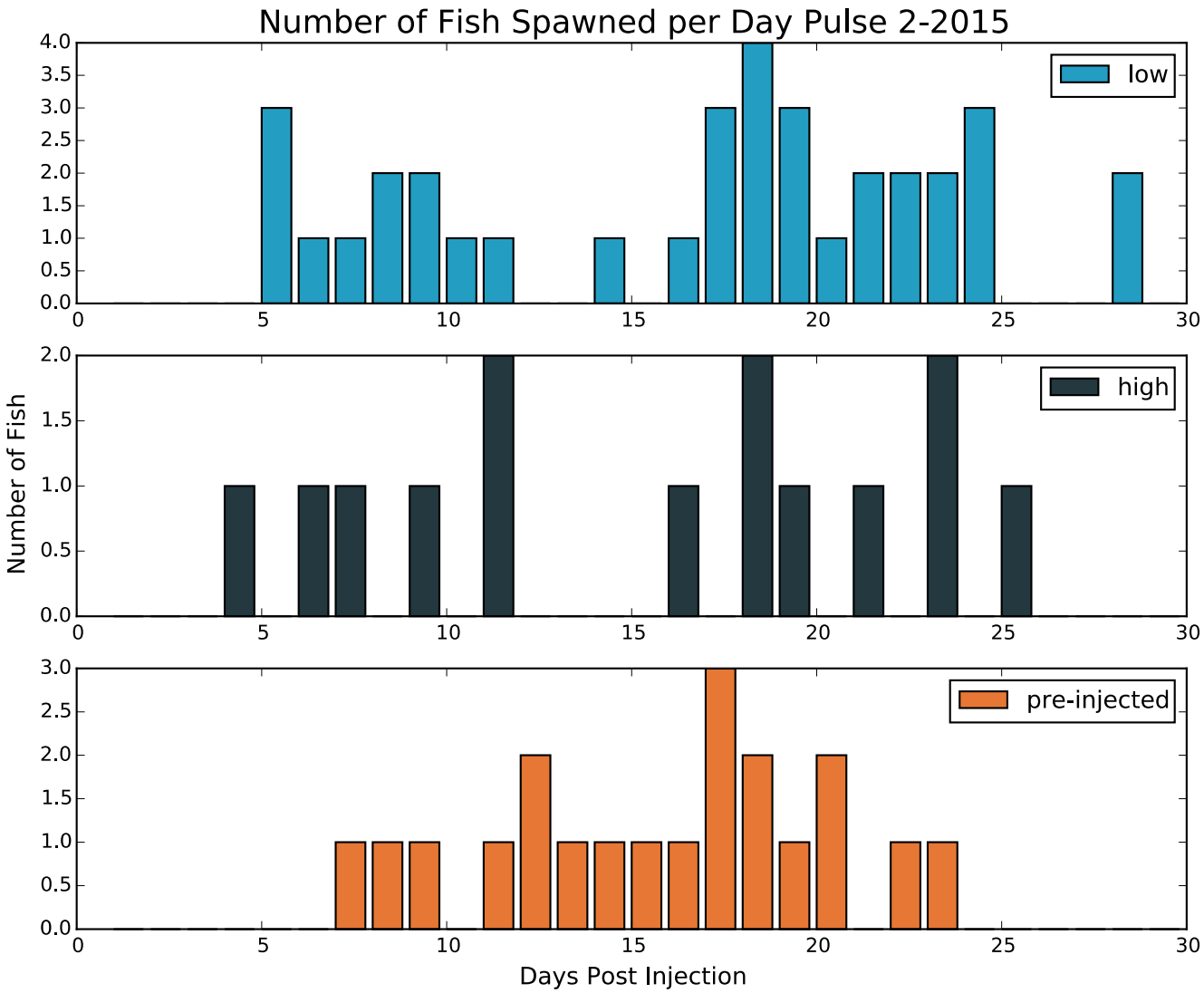

Figure 16**.** Number of fish spawning/day following an Ovaplant alone at ~50 µg/kg (high), ~25 µg/kg (low), and a pre-injection of GnRH (5 µg/kg) followed by an ~50 µg/kg Ovaplant.

In 2016 a trial was designed to use 18 month old F_1 CYT and induce these fish to spawn with Human chorionic gonadotropin (HCG). Three treatment dosages were planned based on the literature; females: 250, 500, and 1000 IU/kg; males: 125, 250, 500 IU/kg. The trial was started in July 2016 but only one of the groups of fish (four males and two females) was injected. No spawning occurred post-injection, in fact females appeared to regress after hormone induction (Figure 18). The process of handling during hormone induction also caused a lot stress and physical damage to the fish and resulted in six mortalities. Because of this we decided to postpone the trial until a gentler, less stressful handling method could be developed for this application.

In 2017 we ran a study using Aqui-S 20E, for the potential to be used an alternative anesthetic to MS-222. The treatments for this trial were as follows; 15 ppm Aqui-S 20E, 25 ppm Aqui-S 20E,

and 75 ppm MS-222. Twelve F_1 CYT were used per treatment. For each fish, opercular rate was recorded each minute, time to sedation was recorded as well as time to recovery. The sex of each fish was determined through use of coelomic ultrasound as well as through cannulation. Finally, lengths and weight were recorded. Fish were tracked for 24 hrs after the trial. Results from this trial showed that the 25 ppm Aqui-S 20E treatment fish had a lower time to sedation (1:53±0:24) while the 15 ppm Aqui-S 20E treatment had the longest time to sedation (4:40±1:30). The shortest time to recovery was the 75 ppm MS-222, while both Aqui-S 20E treatments had similar recovery times (Table 24). Slight to moderate excitation or agitation was noted upon induction of anesthesia in all treatment groups, however more of the fish (8 of 12) in the MS-222 treatment and the 25 ppm Aqui-S 20E treatment showed this behavior. In the 15 ppm Aqui-S 20E treatment, ten fish recovered in < 15 min, and two fish recovered in 17:27 and 21:21. In the 25 ppm Aqui-s 20E treatment all fish recovered, however two fish had an extended recovery time of 25 minutes. Finally, in the 75 ppm MS-222 treatment, all fish recovered from anesthesia, though three fish had prolonged recoveries that lasted longer than 15 minutes.

Overall, the 15 ppm Aqui-S 20E dose is the best treatment for anesthesia for CYT. Fish anesthetized with this dose generally maintained an appropriate and steady opercular rate throughout the entire 10 min holding period and experienced less excitation as compared with the 25 ppm Aqui-S 20E and 75 ppm M-222 groups. However, longer average sedation times and recovery times were appreciated at this dose. The longer sedation times associated with the 15 ppm dose truncated the time allowed for performance of procedures. Fish were removed from the anesthetic bath after 10 min regardless of how long they had been at a handleable plane of anesthesia. In a non-research setting without a capped time of 10 min in the anesthetic bath, this longer induction time would be less deleterious as procedures could continue for a longer period in the anesthetic if warranted.

Table 24. Time to sedation and time to recovery for CYT under three different anesthia treatments.

Treatment	Time to Sedation	Time to Recovery
15 ppm Aqui-S 20E	4:40 ± 1:30	11:16 ± 5:07
25 ppm Aqui-S 20E	1:53 ± 0:24	11:42 ± 7:37
75 ppm MS-222	2:53 ± 0:35	8:48 ± 6:07

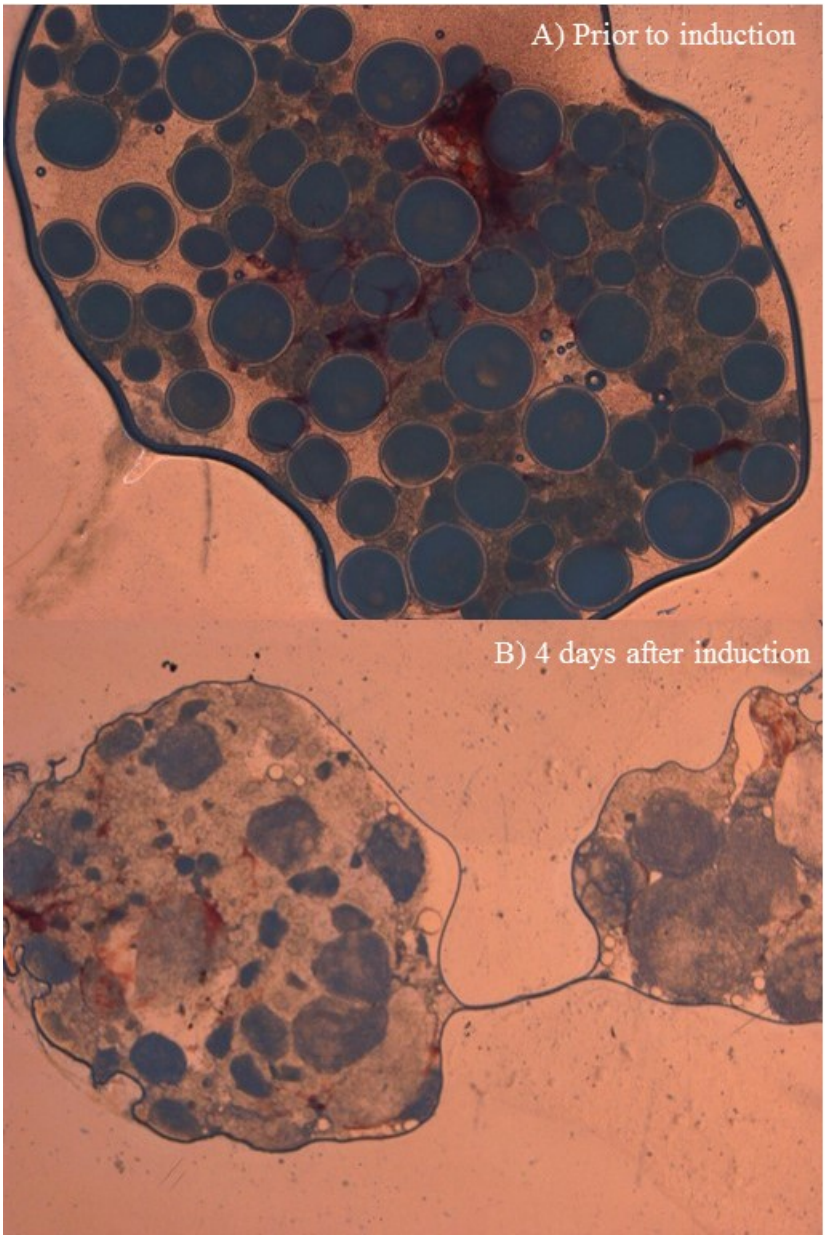

Figure 17. Ovarian samples from a CYT induced with 250 IU/kg of HCG. A) Depicts the oocytes prior to induction, showing all tertiary oocytes, B) depicts the oocytes 4 days after hormone induction, showing attritic oocytes and a lack of development.

Outreach Objectives:

Outreach and extension of information generated through this research has been accomplished through personal engagement by the PI responsible for outreach and project PIs at HSWRI, NWFSC, SWFSC and the University of Idaho. Outreach involved coordination with individuals involved in cultivation of marine finfish, and interactions with professional organizations. This includes information exchanges through the National Aquaculture Extension Steering Committee, and by sharing information with international colleagues through bilateral exchanges via the joint U.S.-Japan Natural Resources Panel on Aquaculture and U.S.-Korea JPA

Aquaculture Research Panel. Finally, we are currently working on a product that will serve as a guide to husbandry and breeding of *Seriola dorsalis*

IMPACTS

Title: Determination and practical application of egg quality measures toward reliable culture of high-value marine finfish species

Relevance:

There is increasing global awareness of the need for sustainable aquaculture. Marine finfish farming is a fledgling industry in the United States but with great promise given the available ocean waters and highly marketable native species. Hatchery technologies have been developed over the last two decades for various marine species around the country. However, without the outlets to grow the fish out, most of this work has been done on an experimental scale. In order to be immediately successful, commercial companies will need to rely on mass production of high quality juvenile fish to start the grow out process. A key limiting factor in the development of consistent juvenile production is the optimization of egg and larval quality. In the absence of high quality eggs, it is not possible to optimize husbandry practices because larval performance is substandard under typical culture conditions, such as high stocking densities, aggressive weaning regimes, and grading or other handling procedures. Unfortunately, identifying simple indicators of egg quality has been difficult as no individual metric is universally applicable within and among species. Therefore, the purpose of this project was to identify easy-to-use indicators as well as document pre and post spawning factors that affect egg quality. The results of this project are expected to have applicability to other fishes (e.g. freshwater) that are reared intensively.

Response: We assembled an expert team of scientists with experience in various facets of broodstock husbandry, physiology, and nutrition. We partitioned the project into discreet areas of focus for broodstock management in order to determine factors directly affecting egg and larval quality.

Results: We were able to show that egg diameters are an indicator for improved quality in CYT. We found larger diameter eggs are positively correlated to improved larval survival after hatch. We also demonstrated that arachidonic acid (ARA) is a critical nutrient for CYT broodstock nutrition and that the addition of ARA to the broodstock diet improved egg quality metrics as well as egg production. We showed that CYT will spawn successfully in small breeding tanks (10 m^3). This will facilitate manipulative studies on broodstock nutrition but is also of importance to all marine finfish hatcheries, since smaller breeding tanks could reduce the footprint necessary for space in a hatchery. We showed an appropriate hormone treatment for SF that will improve egg quality as well as give growers the ability to synchronize spawn events between females. Finally, we described the importance of parental analysis through genetic testing. This work confirmed that a small number of females contribute to a large proportion of the eggs produced. We also demonstrated that removal of dominant females will have direct impacts on overall egg quality and egg production. This tool will improve management and

productivity of broodstock populations and has the potential to significantly increase hatchery production and the quality of marine finfish eggs and larvae.

Impact: As appropriate, research results were applied directly into broodstock management protocols for California yellowtail, California halibut, and sablefish and will lead to more efficient egg and larval production and more consistent larval survival and quality.

Collaborators: Hubbs-SeaWorld Research Institute, National Oceanic and Atmospheric Administration, University of Idaho, California SeaGrant.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

HSWRI bimonthly newsletter – March 2014, August 2014, January 2015, March 2015, November 2015

Hatchery International – July 2014

ORAL PRESENTATIONS:

Armbruster, Lisa, Kevin Stuart, Mark Drawbridge, Ronald B. Johnson. 2016. California yellowtail *Seriola dorsalis* egg quality and chemical composition. Aquaculture America 2016, Las Vegas, NV, February 22-26.

Armbruster, Lisa, Kevin Stuart, Mark Drawbridge, Ronald B. Johnson. California yellowtail *Seriola dorsalis* egg quality and chemical composition. US-Korea Joint Coordination Panel for Aquaculture Meeting. Seattle, WA. March 23, 2016.

Armbruster, Lisa, Kevin Stuart, Mark Drawbridge, Ronald B. Johnson. Egg chemical composition and quality are affected by feeding California Yellowtail (*Seriola dorsalis*) broodstock supplemental arachidonic acid. NOAA Northwest Fisheries Science Center Symposium. Seattle, WA. April 5, 2016.

Stuart, Kevin, Ronald B. Johnson, Lisa Armbruster, and Mark Drawbridge. 2016. Manipulation of arachidonic acid in the diet of adult California yellowtail (*Seriola dorsalis*). Aquaculture America 2016, Las Vegas, NV, February 22-26.

Stuart, Kevin, Ronald B. Johnson, Lisa Armbruster, and Mark Drawbridge. In Review. Arachidonic acid in the diet of adult California yellowtail *Seriola dorsalis* and its effect on egg quality. North American Journal of Aquaculture.

POSTER PRESENTATIONS:

Armbruster, Lisa, Kevin Stuart, Mark Drawbridge, Ronald B. Johnson. Egg chemical composition and quality are affected by feeding California Yellowtail (*Seriola dorsalis*) broodstock supplemental arachidonic acid. NOAA Aquaculture Science Review. Seattle, WA. July 26, 2016

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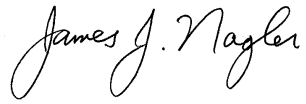
SUBMITTED BY: _____

Title: PI/Date



APPROVED: _____ 09-12-2017

Project Monitor/Date