United Soybean Board
Final Report Form – Technical Bulletin

Project # and Title	Project # 1340-512-5261 Use of soy-based products in practical diets for white seabass, California yellowtail, and striped bass.
Organization & Project Leader	Organization: Hubbs-SeaWorld Research Institute Project Leader: Mark Drawbridge
Reporting Period	3/1/13-2/28/14

Introduction: Statement on the rationale and background for the studies

The overall objective of this project is to build demand for U.S. soy in aquaculture markets by developing soy based feeds for White seabass (WSB; *Atractoscion nobilis*) and California yellowtail (YT; *Seriola lalandi*) and striped Bass (SB; *Morone saxatilis*).

WSB and YT are highly valued commercial and sport fish in southern California and are considered excellent food fish. SB is a highly valued sport fish along the east and west coasts of the United States and is considered an excellent food fish. WSB commonly occur from northern Baja California, Mexico to Point Conception, California, USA. WSB are currently cultured by Hubbs- SeaWorld Research Institute (HSWRI) for stock enhancement. The hatchery in Carlsbad, California is capable of producing an excess of fingerlings required for the stock enhancement program and there is great potential and interest in the commercial culture of the species in offshore net cages. Within the existing stock enhancement project, WSB are cultured to an average size of 20-25 cm using a commercially available diet. YT are in the family carangidae or jacks – a group that typically requires high levels of high quality fish-based protein in the diet. HSWRI has cultured YT to market size in offshore cages in northern Baja California, Mexico. Commercial culture of hybrid SB (*Morone saxatilis* x *Morone chrysops*) is widespread throughout the U.S. However, aquaculture of pure SB is limited. HSWRI has cultured SB on an experimental scale, worked with cage farmers in Baja Mexico and has test-marketed seawater-reared SB in various U.S. markets with very positive feedback.

Great potential exists to expand commercial culture of all three species in both northern Baja California, Mexico and Southern California. The use of alternate ingredients such as soy protein and lipid could greatly improve profitability, while simultaneously addressing issues associated with the long term sustainability of fish meal and fish oil resources. A good opportunity exists to demonstrate the effectiveness of diets based on soy protein in the rapidly developing offshore aquaculture industry in this region.

Research funded by RFP Numbers SB8461 & SB 9463 conducted in 2008 and 2009 consisted of a series of growth trials with WSB and YT. Initial trials were designed to develop a basic practical diet that could then be utilized to identify upper limits for the inclusion of soy products and minimal levels of fish meal. Using the initial formulations we identified practical levels for lipid and dietary protein. This was then followed by studies designed to evaluate the replacement of fish meal with soy products and identify limiting nutrients. Hence, we have been able to develop a practical research diet formulated to contain 40% protein and 10% lipid for WSB and 48% protein and 14% lipid for YT. Using the developed diet, we have identified that we can reduce fish meal from 55% of the diet to around 10-20% of the diet for both species using soy as the replacement protein. We identified taurine as a limiting amino acid and methionine as potentially limiting for both species.

Research funded by RFP Number SB0463 in 2010 was designed to determine the actual requirement for taurine, determine if methionine is limiting and if so determine the actual requirement for both WSB and YT. Two WSB trials were run testing taurine levels from 0-1.6% of the diet. Combining the data sets, the requirement appears to be between 0.4 and 0.6% of the diet depending on the model used to estimate the requirement. A YT trial testing taurine inclusion from 0.25-1.25% showed no differences between treatments with regards to performance indicating that a taurine inclusion rate of 0.25% is adequate. A YT trial testing methionine inclusion from 0.0-0.24% showed no significant difference in performance between treatments indicating that in these feeds methionine was not limiting.

Research funded by RFP Number SB1463 in 2011 included a long term (10 month) grow-out trial with both WSB and YT. In each of these trials a soy-based diet performed equal to or better than commercial diets. Laboratory trials included a trial that showed WSB require a methionine supplementation of at least 0.2% in soy based diets (12% fish meal). We ran a trial showing that attractants did not improve palatability in fish meal free diets for YT. Previous research has shown a greater level of fish oil-sparing is possible with saturated fatty acid-rich, hydrogenated soy oil (SFA) than with C18 polyunsaturated fatty acid-rich, standard soy oil (STD). To confirm this, and to assess whether dietary supplementation with DHA (22:6n-3) would influence the results, we assessed the performance of juvenile WSB fed diets containing fish oil, or SFA or STD with or without the addition of an algal DHA supplement. The SFA-based feeds yielded equivalent growth performance regardless of DHA supplementation; growth performance was impaired among fish fed the STD, but the addition of DHA corrected this effect. In 2011 HSWRI also started utilizing the same methodology used on WSB and YT in to develop practical soy-based diets for SB. In the fall of 2011 we ran two trials with SB. The first trial tested graded levels of soy protein replacing fish meal from 40% to 0% and the second tested various combinations of supplements at 0% fish meal. Palatability problems in diets below 20% fish meal resulted in the early termination of these trials.

Research funded by RFP Number SB2463 in 2012 included the initiation of a commercial scale grow out trial with WSB testing a high soy diet developed through this research vs. a high fish meal commercial diet. We ran a trial showing that, as with YT, the use of attractants does not improve palatability in diets for WSB. We ran a trial with WSB to further evaluate the replacement of fish oil with soy oil supplemented with long chain-polyunsaturated fatty acids (LC-PUFA) and to confirm previous results. This trial showed that SFA soy oil performed similarly to a fish oil control with regards to growth and fillet fatty acid profile and that SFA soil oil-based diet supplemented with DHA increased fillet enrichment over a fish oil-based diet. We also ran a trial to evaluate the importance of LC-PUFA for YT which showed the essentiality of DHA and ARA in soy oil-based feeds. We also ran a trial to further evaluate the protein requirement of YT showing that growth reached a plateau at 44% protein. With SB we tested graded levels of either soybean meal or soy protein concentrate replacing fish meal from 40% to 20% and the second tested an attractant and taurine individually and in combination in a 30% fish meal diet. We saw no palatability problems with any of these diets nor did we find any significant differences between any of the treatments. Furthermore we ran the diets from the two trials run in 2011 (40-0% fish meal) on the same fish to test whether or not larger fish would find lower fish meal diets more palatable and found the same results. The SB did not find diets below 20% fish meal palatable.

There still was a need to optimize soy-based diets for WSB and YT with regards to proximate composition, carbohydrates, lipids and other soy products. Consequently, we geared research in 2013 towards laboratory trials designed to further optimize soy-based diets for WSB and YT by fine tuning protein, lipid and energy requirements, investigating carbohydrate tolerance and utilizing various soy oils and fermented soybean meal. We also continued to evaluate soy-based formulations in long term grow out trials. Our focus in with SB in 2013 was to attempt to hone in on the minimal fish meal level that SB find palatable.

Studies completed: -

Objective 1 – Laboratory Trials to Further Optimize Soy-Based Diets for WSB and YT (HSWRI/Auburn)

Study 1 - Refinement of optimum dietary protein level for WSB.
An 8 week trial tested series of 9 diets with 34-46% protein and 5-12% lipid.

Study 2 - Refinement of optimum dietary lipid level of YT.
An 8 week trial tested a series of 5 diets with 6-21% lipid and 44% protein.

Study 3 - Evaluation of lipids and carbohydrates as energy sources for YT.
A 9 week trial tested a series of 5 diets with 15-5% lipid and 10-31% carbohydrate.

Study 4 - Evaluation of lipids and carbohydrates as energy sources for WSB.
An 8 week trial is testing a series of 9 diets with 3-18% lipid and 16-38% carbohydrate.

Objective 2 – Laboratory Trial Using Soy Oil (HSWRI/SIU)

Study 5 - Evaluation of soy oil in diets for YT.
A 10 week a laboratory trial was conducted to evaluate soybean oil (rich in C18 polyunsaturated fatty acids, PUFA), partially hydrogenated soybean oil (rich in monounsaturated fatty acids, MUFA), fully hydrogenated soybean oil (rich in SFA), or blends of

these soybean oils supplemented with LC-PUFA in feeds for YT.

Objective 3 – Laboratory Trials Using Fermented Soybean Meal (HSWRI/SIU)

Study 6 - Evaluation of fermented soybean meal in diets for WSB.
An 8 week trial was conducted to evaluate PepSoyGen® vs. traditional soybean meal as fish meal alternatives in feeds for WSB.

Study 7 - Evaluation of fermented soybean meal in diets for YT.
An 8 week trial was conducted to evaluate PepSoyGen® vs. traditional soybean meal as fish meal alternatives in feeds for YT.

Objective 4 – Growout Verification Trial (HSWRI/SIU)

Study 8 - Growout verification trial of soy oil-based diet for WSB.
A long term verification trial is being conducted in $4m^3$ cages at an offshore facility operated by HSWRI. A soy oil-based diet that has been developed through this research will be tested vs. a conventional commercial diet.

Objective 5 –Commercial Growout Trial (HSWRI/Auburn/Pacifico Aquaculture)

Study 9 - Commercial grow out trial evaluating soy-based diet for WSB.
Commercial farmers of Pacifico Aquaculture conducted a 15 month trial testing a promising soy-based diet for WSB developed through our laboratory trials vs. a commercial diet high in fish meal.

Objective 6 – Publication of Results (HSWRI/Auburn)

Objective 7 – Laboratory Trials to Determine Minimum Inclusion of Fish Meal in a Soy-Based Diet for striped bass (HSWRI/Auburn)
For this objective we tested a series of diets with a graded reduction of fish meal replaced by soybean meal and select amino acid supplements.

Study 10 - Graded replacement of fish meal with soy and inclusion of various attractants and supplements at the minimum inclusion levels of fish meal.
A trial was attempted with a series of 12 diets with 20-8% fish meal and various attractants and supplements in 12% and 8% fish meal diets

Study 11 - Graded replacement of fish meal with soy and poultry meal.
A 6 week trial was run with a series of 12 diets. Six diets had a graded replacement of fish meal

(40-15%) with soy and six had a graded replacement of fish meal (30-5%) with poultry meal.

Study 12 - Graded replacement of fish meal with soy and inclusion of various attractants and supplements at low levels of soy.
A 6 week trial was run with diets from Study 10 (20-12%) fish meal to determine if larger fish would find the diets more palatable than smaller fish used in Study 10.

Study 13 - High soy diets and east coast strain SB.
Several 8% fish meal diets from Study 10 were sent to a lab in North Carolina to determine if a different strain of SB could utilize them.

Study 14 - Weaning of SB on to soy based feeds.
We attempted to wean striped bass on to a 30% and 5% fish meal diet from Study 11.

Objective 8 – Laboratory Trials to Determine Limiting Nutrients at a Minimum Inclusion of Fish Meal in a Soy-Based Diet for striped bass (HSWRI/Auburn)
For this objective we tested a series of diets with various combinations of potentially limiting nutrients in a basal diet with a minimum level of fish meal.

Studies were combined with those reported under Objective 7

Objective 9 - Test the effectiveness of small mesh copper netting in and offshore cage with juvenile WSB
For this objective, we tested the efficacy of small copper mesh netting for nursery production of marine fish as means of reducing dependency of culturing these fish in coastal areas.

Results

Objective 1 – Laboratory Trials to Further Optimize Soy-Based Diets for WSB and YT (HSWRI/Auburn)

Study 1 - Refinement of optimum dietary protein level for WSB.
In an eight week trial we tested a series of nine diets with 34-46% protein and 5-12% lipid. Results were consistent with previous trials and growth was reduced in the diet with less than 40% protein. However, satisfactory growth was obtained with lower protein levels when lipid levels were also reduced. This indicates that WSB may be able to utilize carbohydrates as an energy source. Proximate composition of whole fish showed a significant decrease in dry matter and lipid in diets with less than 10% lipid while maintaining growth and protein utilization, indicating that levels superior to 10% are excessive. At each protein level (46, 40 and 34%) looking at pair wise comparison reducing the lipid content by 4, 4 and 2%, respectively did not result in major shifts in weight gain or nutrient retention within each

protein level. This indicates that at each of the protein levels energy was not limiting and again indicates that carbohydrates were probably well utilized as an energy source.

Table 1. Diet formulations for Study 1 - Refinement of optimum dietary protein level for WSB.

	%Protein / Lipid								
	46/12	43/11	40/10	37/8	34/7	31/6	46/8	40/6	34/5
Ingredient	g/100g as is								
Menhaden Fishmeal	30.00	28.04	26.09	24.13	22.17	20.22	30.00	26.09	22.17
Soybean meal	31.80	29.40	27.00	24.70	22.40	20.00	31.80	27.00	20.00
Soy protein conc.	10.00	9.35	8.70	8.04	7.39	6.74	10.00	8.70	9.80
Corn Gluten meal	4.00	3.74	3.48	3.22	0.00	2.70	4.00	3.48	2.96
Menhaden Fish Oil	7.87	6.85	5.82	4.79	3.76	2.73	3.87	2.64	1.42
Corn Starch	0.18	6.07	11.97	17.77	23.67	29.57	0.18	11.97	23.67
Whole wheat	13.60	13.60	13.60	13.60	13.60	13.60	13.60	13.60	13.60
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.00	0.40	0.80	1.20	1.50	1.90	0.00	0.80	1.50
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Taurine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cellufill	0.00	0.00	0.00	0.00	0.00	0.00	4.00	3.18	2.33
Proximate comp.	g/100g as is								
Dry Matter	93.26	90.85	91.14	90.66	90.67	91.05	92.48	92.75	94.45
Protein	47.73	43.24	39.94	37.28	34.96	32.04	47.07	42.15	36.50
Lipid	13.02	11.29	10.00	8.63	7.46	6.10	8.66	6.86	5.12
Fiber	2.13	1.93	1.78	1.55	1.72	1.38	3.55	3.32	2.67
Ash	8.92	8.46	8.52	7.73	7.50	7.68	8.71	8.45	8.30

Table 2. Performance of WSB reared for eight weeks in Study 1 - Refinement of optimum dietary protein level for WSB.

P/L	Initial wt (g)	Final wt (g)	% Gain	% Survival	FCR	PRE	PER
46/12	8.6	38.4[ab]	345[ab]	100	1.56[ab]	21.97[f]	1.30[g]
43/11	8.8	38.5[ab]	338[ab]	100	1.56[ab]	22.53[ef]	1.38[ef]
40/10	8.9	38.8[a]	336[ab]	100	1.58[ab]	23.91[d]	1.48[d]
37/8	8.5	35.8[ab]	324[ab]	98	1.62[ab]	25.33[c]	1.57[c]
34/7	8.6	35.2[b]	311[b]	100	1.65[a]	26.35[bc]	1.64[b]
31/6	8.9	37.0[ab]	315[b]	100	1.65[a]	28.94[a]	1.79[a]
46/8	8.4	38.9[a]	364[a]	100	1.53[b]	21.92[f]	1.32[fg]
40/6	8.6	37.0[ab]	333[ab]	98	1.61[ab]	23.59[de]	1.42[de]
34/5	8.4	36.7[ab]	336[ab]	83	1.59[ab]	27.36[b]	1.66[b]

Table 3. Whole body proximate composition (g/100g as is) of WSB in Study 1 - Refinement of optimum dietary protein level for WSB.

P/L	Protein	Dry matt	Lipid	Fiber	Ash
46/12	16.74	23.36[ab]	2.79[abc]	0.40	3.73
43/11	16.26	23.36[ab]	3.02[ab]	0.41	3.76
40/10	16.19	23.50[a]	3.11[a]	0.47	3.9
37/8	16.17	22.95[bc]	2.69[bc]	0.34	3.83
34/7	16.08	22.99[bc]	2.47[cd]	0.40	3.93
31/6	16.16	22.69[cd]	2.24[d]	0.43	4.06
46/8	16.47	22.82[cd]	2.30[d]	0.37	3.87
40/6	16.53	22.89[c]	2.28[d]	0.44	3.82
34/5	16.43	22.40[d]	1.90[e]	0.42	4.03

Study 2 - Refinement of optimum dietary lipid level of YT.

In an eight week trial we tested a series of five diets with 6-21% lipid and 44% protein. Results indicated no performance gain above 12% lipid. Whole body protein decreased and dry matter increased among fish fed diets with more than 12% lipids indicating that high lipid diets do not offer an advantage and that moderate levels of lipids yield the best performance.

Table 4. Diet formulations for Study 2 - Refinement of optimum dietary lipid level for YT.

	% Lipid				
	21	15	12	9	6
Ingredient			g/100g as is		
Menhaden Fishmeal	27.00	27.00	27.00	27.00	27.00
Soybean meal	20.00	20.00	20.00	20.00	20.00
Soy protein conc.	23.00	23.00	23.00	23.00	23.00
Empyreal 75	3.10	3.10	3.10	3.10	3.10
Menhaden Fish Oil	17.38	11.38	8.38	5.38	2.38
Corn Starch	0.87	6.87	9.87	12.87	15.87
Whole wheat	5.70	5.70	5.70	5.70	5.70
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25
ASA Vitamin premix	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.50	0.50	0.50	0.50	0.50
Lecithin	1.00	1.00	1.00	1.00	1.00
Taurine	0.40	0.40	0.40	0.40	0.40
Proximate comp.			g/100g as is		
Dry Matter	96.48	95.32	95.32	93.74	93.53
Protein	46.25	46.30	46.77	47.04	46.58
Lipid	21.21	15.20	12.23	8.67	6.19
Fiber	3.69	3.89	3.30	3.65	2.62
Ash	9.13	9.18	9.24	8.62	8.73
Starch	5.91	10.74	13.40	16.27	15.53

Table 5. Performance of YT reared for eight weeks in Study 2 - Refinement of optimum dietary lipid level for YT.

% Lipid	Initial wt (g)	Final wt (g)	% Gain	% Survival	FCR
21	6.8	154.6[ab]	2162[abc]	98	1.15
15	6.8	168.0[a]	2359[a]	98	1.08
12	6.9	164.3[a]	2293[ab]	98	1.08
9	6.9	153.5[ab]	2135[bc]	100	1.18
6	6.9	146.9[b]	2039[c]	100	1.24

Table 6. Whole body proximate composition (g/100g as is) of YT in Study 2 - Refinement of optimum dietary lipid level for YT.

% Lipid	Protein	Dry matt	Lipid	Fiber	Ash
21	18.10[c]	29.50[a]	4.80	0.93	2.55
15	18.82[b]	28.90[a]	4.54	1.66	2.65
12	18.95[b]	28.61[a]	4.32	2.32	2.43
9	19.27[ab]	27.48[b]	4.42	1.23	2.86
6	19.52[a]	27.20[b]	4.69	1.62	2.89

Study 3 - Evaluation of lipids and carbohydrates as energy sources for YT.

In a nine week trial we tested a series of five diets with 15-5% lipid and 10-31% carbohydrate. Results showed that growth performance was significantly reduced only in fish fed the diet with the highest (15%) lipid and lowest (10%) carbohydrate indicating that YT can utilize carbohydrates. Whole body protein decreased and dry matter increased as dietary lipid level increased and carbohydrate decreased indicating that YT can utilize carbohydrates as an energy source. There were no differences among treatments with regards to HSI or hematology.

Table 7. Diet formulations for Study 3 - Evaluation of lipids and carbohydrates as energy sources for YT.

	% Lipid:Carbohydrate				
	15:10	12.5:15	10:20	7.5:26	5:31
Ingredient	g/100g as is				
Menhaden Fishmeal	35.80	35.80	35.80	35.80	35.80
Soy protein conc.	20.20	20.20	20.20	20.20	20.20
Empyreal 75	10.00	10.00	10.00	10.00	10.00
Menhaden Fish Oil	11.30	8.80	6.30	3.80	1.30
Corn Starch	0.00	6.00	12.00	18.00	24.00
Whole wheat	5.00	5.00	5.00	5.00	5.00
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.50	0.50	0.50	0.50	0.50

Lecithin	0.50	0.50	0.50	0.50	0.50
Taurine	0.40	0.40	0.40	0.40	0.40
Cellufill	15.25	11.75	8.25	4.75	1.25
Proximate comp.			g/100g as is		
Dry Matter	90.85	94.22	90.48	93.29	93.77
Protein	44.77	46.28	45.03	46.36	46.59
Lipid	14.50	12.41	9.48	7.65	4.80
Fiber	14.48	11.86	8.87	7.04	5.02
Ash	8.90	9.26	9.03	9.36	9.49
Starch	1.95	7.41	13.61	17.03	24.35

Table 8. Performance of YT reared for nine weeks in Study 3 - Evaluation of lipids and carbohydrates as energy sources for YT.

% Lipid:Carbohydrate	Initial Wt (g)	Final Wt (g)	% gain	% Survival	FCR
15:10	5.2	96.3[b]	1752[b]	98	1.42
12.5:15	5.2	108.0[ab]	1969[ab]	97	1.30
10:20	5.2	110.9[a]	2039[a]	100	1.24
7.5:26	5.2	101.9[ab]	1879[ab]	98	1.25
5:31	5.2	108.0[ab]	1970[ab]	98	1.26

Table 9. Whole body proximate composition (g/100g as is) of YT in Study 3 - Evaluation of lipids and carbohydrates as energy sources for YT.

% Lipid:Carbohydrate	Protein	Dry matter	Lipid	Fiber	Ash
15:10	18.21[c]	27.48[a]	4.38	2.44	2.12
12.5:15	18.49[bc]	27.32[ab]	4.61	3.08	2.14
10:20	18.82[abc]	27.04[ab]	4.81	3.47	2.33
7.5:26	18.93[ab]	26.40[ab]	4.36	2.11	2.35
5:31	19.11[a]	26.22[b]	4.29	2.78	2.38

Table 10. Hematological parameters of YT in Study 3 - Evaluation of lipids and carbohydrates as energy sources for YT.

% Lipid:carbohydrate	HSI	Glucose (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb:Glob
15:10	1.2	116.9	3.9	2.3	1.6	1.4
12.5:15	1.2	114.1	3.9	2.4	1.5	1.7
10:20	1.2	118.3	4.0	2.5	1.5	1.7
7.5:26	1.2	115.8	3.9	2.3	1.5	1.6
5:31	1.2	119.8	3.7	2.4	1.3	1.9

Study 4 - Evaluation of lipids and carbohydrates as energy sources for WSB.
In an eight week trial we are testing a series of nine diets with 3-18% lipid and 16-38% carbohydrate. This trial is currently underway.

Table 11. Diet formulations for Study 4 - Evaluation of lipids and carbohydrates as energy sources for WSB.

	% Lipid:Carbohydrate								
	12:16	10:20	8:25	7:29	5:34	3:38	7:35	12:30	18:25
Ingredient	g/100g as is								
Menhaden Fishmeal	14.50	14.50	14.50	14.50	14.50	14.50	14.50	14.50	14.50
Soybean meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soy protein conc.	15.70	15.70	15.70	15.70	15.70	15.70	15.70	15.70	15.70
Empyreal 75	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Menhaden Fish Oil	9.77	7.97	6.17	4.37	2.57	0.77	9.77	4.37	15.74
Corn Starch	0.00	5.00	10.00	15.00	20.00	25.00	16.08	21.48	10.11
Whole wheat	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Taurine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cellufill	16.08	12.88	9.68	6.48	3.28	0.08	0.00	0.00	0.00
Proximate comp.	g/100g as is								
Dry matter	96.17	95.77	95.17	94.37	94.33	93.76	94.16	94.84	95.14
Protein	40.18	41.19	40.59	40.96	40.82	41.35	40.98	40.38	41.32
Lipid	13.18	11.21	9.7	7.67	5.9	3.74	7.81	13.19	19.3
Fiber	12.42	9.76	8.83	6.76	4.51	2.26	2.66	2.29	2.34
Ash	6.87	6.77	6.88	6.75	6.87	6.89	6.88	6.85	6.8
Starch	4.6	8.63	25.7	18.77	24.52	28.46	26.8	22.26	16.43

Objective 2 – Laboratory Trial Using Soy Oil (HSWRI/SIU)

Study 5 - Evaluation of soy oil in diets for YT.
We conducted a 10 week laboratory trial to evaluate soybean oil (rich in C18 polyunsaturated fatty acids, PUFA), partially hydrogenated soybean oil (rich in monounsaturated fatty acids, MUFA), fully hydrogenated soybean oil (rich in SFA), or blends of these soybean oils supplemented with LC-PUFA in feeds for YT. There were no significant differences among any of the diets with regards to performance.

Table 12. Diet formulations for Study 5 - Evaluation of soy oil in diets for YT.

	FISH OIL	FULL HYDRO SOY	PARTIAL HYDRO SOY	STANDARD SOY	SOY BLEND 1	SOY BLEND 2
Ingredient			g/100g, as fed basis			
Menhaden fish meal	47.37	47.37	47.37	47.37	47.37	47.37
Soy protein concentrate	24.00	24.00	24.00	24.00	24.00	24.00
Menhaden fish oil	9.51	0.00	0.00	0.00	0.00	0.00
Fully hydrogenated soybean oil	0.00	7.54	0.00	0.00	2.84	1.47
Partially hydrogenated soybean oil	0.00	0.00	7.54	0.00	4.71	1.89
Standard soybean oil	0.00	0.00	0.00	7.54	0.00	4.17
Corn starch	7.95	7.95	7.95	7.95	7.95	7.95
Wheat feed flour	5.00	5.00	5.00	5.00	5.00	5.00
Corn gluten meal	3.25	3.25	3.25	3.25	3.25	3.25
Soy lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin premix	0.12	0.12	0.12	0.12	0.12	0.12
Mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Stay C	0.10	0.10	0.10	0.10	0.10	0.10
ARA concentrate	0.00	0.04	0.04	0.04	0.04	0.04
DHA concentrate	0.00	1.03	1.03	1.03	1.03	1.03
EPA concentrate	0.00	0.89	0.89	0.89	0.89	0.89
Proximate composition		g/100g, dry matter basis (except Dry matter)				
Dry matter	95.8	95.2	95.2	94.6	94.8	94.2
Protein	53.4	51.3	51.6	51.4	51.4	51.4
Lipid	16	15.5	15.4	15.5	15.6	15.6
Ash	11	10.5	10.5	10.5	10.2	10.4

Table 13. Performance of YT reared for ten weeks in Study 5 - Evaluation of soy oil in diets for YT.

	FISH OIL	FULL HYDRO SOY	PARTIAL HYDRO SOY	STANDARD SOY	SOY BLEND 1	SOY BLEND 2
Initial weight (g)	6.2	6.2	6.2	6.3	6.2	6.3
Final weight (g)	96.2	95.5	97.5	91.5	99.2	98.4
Weight gain (%)	1454	1431	1470	1351	1488	1457
FCR	1.30	1.26	1.27	1.33	1.26	1.29
SGR (% body weight/day)	3.86	3.84	3.87	3.76	3.90	3.87
Feed intake (% body weight/day)	7.02	6.82	6.93	7.0	6.94	7.12
HSI	0.9	0.9	1.1	1.1	1.1	0.9
VSI	7.1	7.1	6.8	7.0	7.1	6.9

Objective 3 – Laboratory Trials Using Fermented Soybean Meal (HSWRI/SIU)

Study 6 - Evaluation of fermented soybean meal in diets for WSB.
We conducted a 10 week trial to evaluate PepSoyGen® vs. traditional soybean meal as fish meal alternatives in feeds for WSB. Fish fed the 0% FM SBM and 0% PSG diets showed poor feeding response and growth performance and these treatments were discontinued after four weeks. After 10 weeks FM replacement was successfully achieved with treatments containing 24% and 12% FM with both SBM and PSG; while complete FM replacement with feeds containing PBM impaired production performance. Growth performance was significantly affected by dietary treatment, which was best among fish fed 24% FM PSG and poorest among fish fed 0% FM Poultry Algae and 0% FM Poultry PSG. Survival, FCR, feed intake and HSI were not significantly affected by dietary treatments.

Table 14. Diet formulations for Study 6 - Evaluation of fermented soybean meal in diets for WSB.

	48% FM CONTROL	24% FM SBM	24% FM PSG	12% FM SBM	12% FM PSG	0% FM SBM	0% FM PSG	0% FM PBM & ALGAE	0% FM PBM & PSG
Ingredient				g/100g, as is					
Menhaden fish meal	48.00	24.00	24.00	12.00	12.00	0.00	0.00	0.00	0.00
Soybean meal	10.00	15.00	0.00	25.00	0.00	41.12	0.00	0.00	0.00
PepSoyGen	0.00	0.00	15.00	0.00	25.00	0.00	47.72	0.00	45.00
Spirulina algal meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.00	0.00
Corn protein concentrate	3.67	11.99	11.79	15.64	15.46	15.77	17.21	20.00	2.02
Soy protein concentrate	8.00	18.60	17.50	20.00	18.00	21.50	12.00	0.00	4.00
Wheat flour	18.46	13.78	14.93	8.15	10.07	0.00	0.92	13.04	3.28
Poultry byproduct meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.00	30.00
Menhaden fish oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	2.14	3.93	3.96	4.83	4.88	5.84	5.86	0.92	3.13
Dicalcium phosphate	0.00	2.22	2.24	3.59	3.62	4.80	4.87	2.02	1.88
Taurine	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Methionine	0.06	0.22	0.24	0.30	0.33	0.41	0.50	0.11	0.43
Lysine	0.25	0.85	0.94	1.08	1.23	1.14	1.50	1.49	0.84
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin premix	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Mineral premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Stay-C	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Carboxymethyl cellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Proximate Composition				g/100g, dry matter (except Dry Matter)					
Dry Matter	93.40	93.60	94.00	93.90	94.90	93.50	95.60	92.20	94.50
Protein	51.10	50.80	50.60	50.80	50.20	50.70	48.60	56.30	51.90
Lipid	17.70	15.50	15.50	14.10	14.00	16.10	16.60	17.40	13.70
Ash	12.70	10.20	10.10	8.10	8.60	8.90	7.80	7.50	9.00

Table 15. Performance of WSB reared for ten weeks in Study 4 - Evaluation of fermented soybean meal in diets for WSB.

	48% FM CONTROL	24% FM SBM	24% FM PSG	12% FM SBM	12% FM PSG	0% FM SBM	0% FM PSG	0% FM PBM & ALGAE	0% FM PBM & PSG
Initial weight (g)	11.9	11.7	11.7	11.8	11.7	11.6	11.6	11.7	11.7
After 34 Days									
Survival (%)	100	100	100	98	100	100	100	100	100
Final weight (g)	32.7^{x}	31.9^{xy}	32.4^{x}	31.5^{xy}	28.1^{y}	12.2^{z}	15.4^{z}	29.1^{xy}	30.6^{xy}
Weight gain (%)	176^{x}	173xy	176^{x}	167^{xy}	140^{y}	5^{z}	33^{z}	148^{xy}	161^{xy}
FCR	0.91	0.93	0.90	0.91	1.05	1.20*	6.30**	1.01	1.00
SGR (% body weight/day)	2.98^{x}	2.96^{x}	2.99^{x}	2.88^{x}	2.57^{x}	0.14^{z}	0.80^{y}	2.67^{x}	2.82^{x}
Feed intake (% body weight/day)	1.54	1.56	1.54	1.5	1.55	1.63	1.57	1.53	1.56
HSI	NA	NA	NA	NA	NA	2.5	1.7	NA	NA
After 68 days									
Survival (%)	100	100	100	98	93	NA	NA	100	100
Initial weight (g)	11.9	11.7	11.7	11.8	11.7	NA	NA	11.7	11.7
Final weight (g)	62.4^{wxy}	63.4^{wx}	64.0^{w}	60.2^{wxyz}	56.0^{xyz}	NA	NA	53.1^{z}	55.3^{yz}
Weight gain (%)	427^{xy}	442^{xy}	446^{x}	410^{xyz}	379^{xyz}	NA	NA	352^{z}	373^{yz}
FCR	1.00	1.00	1.00	1.03	1.07	NA	NA	1.10	1.10
SGR (% body weight/day)	2.68^{xy}	2.73^{x}	2.74^{x}	2.62^{xyz}	2.53^{xyz}	NA	NA	2.43^{z}	$2.50y^{z}$
Feed intake (% body weight/day)	3.00	2.97	3.03	3.00	3.00	NA	NA	2.97	3.10
HSI	2.0	1.9	2.0	1.6	2.2	NA	NA	2.1	2.2

*average value of -17.5, 7.5 and 13.5

**average value of 13.8, 2.3 and 2.7

Study 7 - Evaluation of fermented soybean meal in diets for YT.

We conducted an eight week trial to evaluate PepSoyGen® vs. traditional soybean meal as fish meal alternatives in feeds for YT. FM replacement was successfully achieved with treatments containing 20% FM with SBM, SPC and PSG although performance was reduced in the 20% FM PSG treatment. Growth performance, FCR and feed intake were significantly affected by dietary treatment, and were best among fish fed the 40% FM control and poorest among fish fed all four 0% FM diets.

Table 16. Diet formulations for Study 7 - Evaluation of fermented soybean meal in diets for YT.

	40% FM CONTROL	20% FM SPC	20% FM SBM	20% FM PSG	0% FM SBM	0% FM PSG	0% FM PBM & ALGAE	0% FM PBM & PSG
Ingredient				g/100g, as fed				
Menhaden fish meal	40.00	20.00	20.00	20.00	0.00	0.00	0.00	0.00
Soybean meal	18.00	0.00	35.00	0.00	42.27	0.00	0.00	0.00
PepSoyGen	0.00	0.00	0.00	46.25	0.00	52.10	0.00	50.00
Spirulina algal meal	0.00	0.00	0.00	0.00	0.00	0.00	19.63	0.00

Corn protein concentrate	0.00	7.50	10.41	5.00	16.47	11.50	19.96	3.00
Soy protein concentrate	0.00	36.15	12.00	7.00	20.00	14.87	0.00	5.00
Wheat flour	16.63	19.06	5.78	4.77	0.00	0.00	13.00	1.79
Poultry byproduct meal	6.32	0.00	0.00	0.00	0.00	0.00	33.30	23.45
Menhaden fish oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	2.24	4.45	4.29	4.53	5.81	6.05	0.92	3.80
Blood meal	7.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dicalcium phosphate	0.00	2.64	2.46	2.29	4.81	4.67	2.51	2.44
Taurine	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Methionine	0.20	0.32	0.26	0.35	0.41	0.50	0.12	0.50
Lysine	0.20	0.47	0.38	0.39	0.82	0.90	1.15	0.60
Choline	0.60	0.60	0.60	0.60	0.60	0.60	3.60	0.60
Vitamin premix	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Mineral premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Stay C	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Carboxymethyl cellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Proximate Composition	g/100g, dry matter (except Dry Matter)							
Dry Matter	94.3	93.6	93.8	96	95.5	96.4	94.6	95.7
Protein	50.9	48.8	50.2	49.3	49.4	49.8	57.5	52.3
Lipid	14.5	13.9	13.9	13.6	13.3	13.4	15.2	14.3
Ash	10.9	10	10.3	10.2	9.1	9.9	8.5	9.6

Table 17. Performance of WSB reared over an 8 week period in Study 7 - Evaluation of fermented soybean meal in diets for YT.

	40% FM CONTROL	20% FM SPC	20% FM SBM	20% FM PSG	0% FM SBM	0% FM PSG	0% FM PBM & ALGAE	0% FM PBM & PSG
Survival (%)	100	100	100	100	93	100	98	98
Initial weight (g)	6.8	6.8	6.8	6.8	6.7	6.7	6.8	6.8
Final weight (g)	87.8^{x}	81.9^{xy}	77.8^{xy}	74.6^{y}	57.2^{z}	51.8^{z}	62.0^{z}	55.2^{z}
Weight gain (%)	1193^{x}	1109^{xy}	1048^{xy}	997^{y}	752^{z}	667^{z}	815^{z}	712^{z}
FCR	1.17^{z}	1.21^{yz}	1.22^{yz}	1.30^{y}	1.48^{wx}	1.60^{w}	1.42^{x}	1.53^{wx}
SGR (% body weight/day)	4.65^{w}	4.53^{wx}	4.43^{wx}	4.35^{x}	3.89^{yz}	3.70^{z}	4.02^{y}	3.81^{yz}
Feed intake (% body weight/day)	7.07^{yz}	7.01^{yz}	6.86^{z}	7.09^{y}	6.91^{yz}	7.01^{yz}	6.96^{yz}	6.92^{yz}
VSI	6.2	5.8	6.4	5.5	6.2	7.4	6.8	7.1

Objective 4 – Growout Verification Trial (HSWRI/SIU)

Study 8 - Growout verification trial of soy oil-based diet for WSB.
We are conducting a long term verification trial in $4m^3$ cages at an offshore facility operated by HSWRI at Santa Catalina Island. A soy oil-based diet that was developed through our previous research is being tested against a conventional commercial diet and an experimental fish oil-based control. So far both the soy-oil and fish oil control diet are performing similarly to the commercial control diet.

Table 18. Diet formulations for Study 8 - Growout verification trial of soy oil-based diet for WSB.

Ingredient (g/100 g as is	Fish Oil Control Diet	SFA Soybean Oil Diet
Menhaden fish meal	40.00	40.00
Soy protein concentrate	19.00	19.00
Corn starch	11.88	11.88
Wheat flour	15.00	15.00
Corn gluten meal	3.00	3.00
Menhaden fish oil	7.10	0.00
Hydrogenated soybean oil flakes	0.00	7.10
Soy lecithin	1.00	1.00
Carboxymethyl cellulose	2.00	2.00
Choline chloride	0.60	0.60
Stay-C (35%)	0.20	0.20
Vitamin premix	0.12	0.12
Mineral premix	0.10	0.10

Objective 5 –Commercial Growout Trial (HSWRI/Auburn/Pacifico Aquaculture)

Study 9 - Commercial grow out trial evaluating soy-based diet for WSB.
Commercial cage farmers of Pacifico Aquaculture in Mexico conducted a 15 month trial testing a promising soy-based diet for WSB developed through our laboratory trials against a commercial diet high in fish meal. Survival was higher in the commercial control group at 94% compared to the soy diet at 86%. However, most of the mortality occurred after the fish were treated for gill flukes in the final third of the study period. Prior to that, survival was high (>98%) in both groups. Without replication in the study design, it is unclear if the differential survival between the two groups represented a differential level of robustness. Overall the growth performance was not different between the two dietary treatments. Hematocrit values were within the range of normal (20-45%) for marine finfish. Glucose was low in both treatments at both sampling periods but this may have been a product of sampling stress. Although there were no significant differences between treatments with regards to total protein, there was an increase between the first and second sampling periods with both treatment groups. This may have been an indication of inflammation of tissues, infection or liver problems. The albumin:globulin ratio was low in both treatment groups at both sampling periods. Presumably this was due to an increased production of globulin indicative of an inflammatory response. In general these results indicated that the fish in both treatment groups were experiencing compromised health either for nutritional reasons or some other environmental influence. The fish were treated for external parasites the week before the second sampling was done so the results were probably affected by this as well. Pending histology of gut and liver could provide more insight.

Table 19. Diet formulations for Study 9 - Commercial growout trial evaluating soy-based diet for WSB.

Ingredient	Ziegler USB-WSB custom g/100g as is	EWOS Dyna Seabream
Soybean meal	26.00	
Whole wheat	24.98	
Soy protein conc.	17.02	
Menhaden fish meal	12.00	
Corn gluten (60% P)	9.20	
Fish oil	4.60	
Soy oil	2.00	
Dical	1.80	
Lecithin	1.00	
Taurine	0.70	
Choline CL	0.20	
mold inhibitor	0.15	
Mineral premix	0.15	
Vitamin C.	0.10	
Vitamin premix	0.10	
Proximate comp.	g/100g as is	Guaranteed minimum
Dry Matter	90.54	
Protein	40.60	47
Lipid	9.86	12
Fiber	2.95	
Ash	7.27	
Starch	14.99	

Table 20. Water quality and performance of WSB during a 15 month growout cycle in Study 9 - Commercial grow out trial evaluating soy-based diet for WSB.

Diet	Initial wt. (g)	Final wt. (g)	Weight gain	% gain	Feed/ fish	% Survival	FCR		Temp (°C)	D.O. (mg/L)
EWOS	60	510.4	450.4	751	859.1	94	1.91	Average	16.4±2.0	8.2±0.5
SOY	60	505.3	445.3	742	761.1	86	1.71	Min.	12.2	5.7
								Max.	21.9	10.3

Table 21. Hematology of WSB during and at the end of Study 9 - Commercial grow out trial evaluating soy-based diet for WSB.

	1st Sampling (10 months)		2nd Sampling (15 months)	
	T-1 EWOS	T-2 Soy	T-1 EWOS	T-2 Soy
Hematocrit (%)	22.1 ±6.9	21.6 ±4.4	23.4 ±3.4*	27.0 ±6.5*
Hemoglobin (g/dl)	10.2 ±2.21*	8.6 ±1.7*		
Glucose (mg/dl)	60.27 ±24.5	60.3 ±23.4	53.40 ±16.4	60.1 ±28.3
Total protein (g/dl)	1.31 ±0.33	1.50 ±0.30	2.3 ±0.33	2.2 ±0.50

Albumin (g/dl)	0.46 ±0.13*	0.71 ±0.17*	0.82 ±0.23	0.71 ±0.27
Globulin (g/dl)	0.81 ±0.27	0.78 ±0.31	1.52 ±0.20	1.53 ±0.41
Alb:Gl	0.55 ±0.11*	1.10 ±0.59*	0.55 ±0.20	0.53 ±0.49
Weight (g)	316.8 ±66.8	296.2 ±51.4	485 ±114.3* ł	413.5 ±87.3* ł
Cond fact			0.9 ±0.2	0.8 ±0.1
HSI*			1.8 ±0.4*	1.6 ±0.4*

* designates samples significantly different by t-test (P<0.05) between treatment groups within the same sampling period

ł These weights were done on the fish from which blood and tissue were sampled and are independent of the final weights done by the farm at the conclusion of the study

HSI=liver weight/fish weight*100

Cond. fact=fish weight/total length^3*100

Objective 6 – Publication of Results (HSWRI/Auburn)

- *Published*

Jirsa, D.O., G.P. Salze, F.T. Barrows, D.A. Davis, M.A. Drawbridge. 2013. First-limiting amino acids in soybean-based diets for white seabass *Atractoscion nobilis*. Aquaculture 414-415, 167-172.

Jirsa, D.O., D.A. Davis , F.T. Barrows, L.A. Roy, M.A. Drawbridge. 2013. Response of White Seabass *Atractoscion nobilis* to practical diets with varying levels of protein. North American Journal of Aquaculture, 76:1, 24-27.

Trushenski, J.R., B. Mulligan, D. Jirsa, M. Drawbridge. 2013. Sparing fish oil in feeds for white seabass: effects of inclusion rate and soybean oil composition. North American Journal of Aquaculture, 75:2, 305-315.

Jirsa, D.O., G.P. Salze, D.A. Davis, and M.A. Drawbridge. 2014. Taurine requirement of white seabass, *Atractoscion nobilis*, in a practical soy based diet. Aquaculture 422-423, 36-41.

- *Accepted with revision*

Trushenski, J.R., A.N Rombenso, M. Page, D. Jirsa, and M. Drawbridge. Traditional and fermented soybean meals as ingredients in feeds for White Seabass and California Yellowtail. North American Journal of Aquaculture.

- *Submitted/external review (sent to a journal for publication and is with the editor and/or reviewers for comment)*

Jirsa, D.O., K.R. Stuart, G.P. Salze, D.A. Davis, and M.A. Drawbridge. Limiting amino acids in soy-based diets for California yellowtail, *Seriola lalandi*. Journal of the World Aquaculture Society.

Salze, G.P., D.O. Jirsa, D.A. Davis, and M.A. Drawbridge. Methionine requirement of white seabass, *Atractoscion nobilis*, in a practical soy based diet. Aquaculture.

Jirsa, D.O., K.R. Stuart, G.P. Salze, M. Rhodes, D.A. Davis, and M.A. Drawbridge. Protein requirement of California yellowtail, *Seriola lalandi* based on practical diets. Aqua Nut.

- *In prep (in the process of being written and edited by the author(s))*

Jirsa, D.O., K.R. Stuart, G.P. Salze, K.R.B. Oliveira, D.A. Davis, M. Drawbridge. Use of Feed Attractants in Fishmeal-Free Diets for California yellowtail Seriola Lalandi Juveniles.

Rombenso, A.N., J.T. Trushenski, D. Jirsa, and M. Drawbridge. DHA and ARA are essential to meet LC-PUFA requirements for juvenile California Yellowtail.

Objective 7 – Laboratory Trials to Determine Minimum Inclusion of Fish Meal in a Soy-Based Diet for striped bass (HSWRI/Auburn)

For this objective we tested a series of diets with a graded reduction of fish meal replaced by soybean meal and select amino acid supplements.

Study 10 - Graded replacement of fish meal with soy and inclusion of various attractants and supplements at the minimum inclusion levels of fish meal.

We initiated a feeding trial for SB with a series of 12 diets with 20-8% fish meal and various attractants and supplements in 12% and 8% fish meal diets. No feeding response was observed in any of the treatments and the trial was discontinued after two weeks. It should be noted that the higher fishmeal diets were designed based on diets from the previous year in which there were no palatability or acceptance problems for the diets.

Table 22. Diet formulations for Study 10 - Graded replacement of fish meal with soy and inclusion of various attractants and supplements at the minimum inclusion levels of fish meal.

	FM20	FM16	FM12	FM12 squid	FM12 fish	FM12 AA	FM12 T
Ingredient				g/100g as is			
Menhaden Fishmeal	20.00	16.00	12.00	12.00	12.00	12.00	12.00
Poultry by product meal	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Soybean meal	26.10	31.80	37.60	35.90	36.10	35.40	37.60
Empyreal 75	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Menhaden Fish Oil	6.75	7.00	7.25	7.28	7.18	7.29	7.25
Corn Starch	10.90	8.95	6.90	7.57	7.47	8.06	6.30
Whole wheat	10.50	10.50	10.50	10.50	10.50	10.50	10.50
ASA Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	1.60	1.60	1.60	1.60	1.60	1.60	1.60
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Squid meal	0.0	0.0	0.0	1.00	0.0	0.0	0.0
Fish protein concentrate	0.0	0.0	0.0	0.0	1.00	0.0	0.0
AA mix	0.0	0.0	0.0	0.0	0.0	1.00	0.0
Taurine	0.10	0.10	0.10	0.10	0.10	0.10	0.70

	FM8	FM8	FM8 fish	FM8 AA	FM8 T

		squid			
Ingredient			g/100g as is		
Menhaden Fishmeal	8.00	8.00	8.00	8.00	8.00
Poultry by product meal	12.00	12.00	12.00	12.00	12.00
Soybean meal	43.30	41.60	41.90	41.10	43.30
Empyreal 75	10.00	10.00	10.00	10.00	10.00
Menhaden Fish Oil	7.50	7.53	7.43	7.54	7.50
Corn Starch	4.95	5.62	5.42	6.11	4.35
Whole wheat	10.50	10.50	10.50	10.50	10.50
ASA Trace Mineral premix	0.25	0.25	0.25	0.25	0.25
ASA Vitamin premix	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	1.60	1.60	1.60	1.60	1.60
Lecithin	1.00	1.00	1.00	1.00	1.00
Squid meal	0.0	1.00	0.0	0.0	0.0
Fish protein concentrate	0.0	0.0	1.00	0.0	0.0
AA mix	0.0	0.0	0.0	1.00	0.0
Taurine	0.10	0.10	0.10	0.10	0.70

Study 11 - Graded replacement of fish meal with soy and poultry meal.
We conducted a six week feeding trial with a series of 12 diets. Six diets had a graded replacement of fish meal (40-15%) with soy and six had a graded replacement of fish meal (30-5%) with poultry meal. Initially feeding response was poor on these diets but after approximately one week the fish began feeding and the feeding response increased gradually from that point on. Although survival was excellent (98-100%) at the end of the trial, the fish showed only a very meager 50% weight gain and FCR's were very poor ranging from 6.5-8.4. There were no significant differences among any of the treatments with regards to performance.

Table 23. Diet formulations for Study 11 - Graded replacement of fish meal with soy and poultry meal.

			Plant proteins			
	P FM 40	P FM 35	P FM 30	P FM 25	P FM 20	P FM 15
Ingredient			g/100g as is			
Menhaden Fishmeal	40.00	35.00	30.00	25.00	20.00	15.00
Poultry by product meal	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal	0.00	2.60	5.20	7.70	10.50	12.90
Soy protein conc.	0.00	3.25	6.50	9.63	13.13	16.13
Empyreal 75	7.00	7.00	7.00	7.00	7.00	7.00
Menhaden Fish Oil	5.58	5.97	6.35	6.74	7.12	7.51
Corn Starch	9.18	7.90	6.64	5.30	3.08	1.77
Whole wheat	26.00	26.00	26.00	26.00	26.00	26.00
ASA Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25

ASA Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.00	0.00	0.00	0.30	0.80	1.30
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Taurine	0.19	0.23	0.26	0.29	0.32	0.35

	Animal proteins					
	A FM 30	A FM 25	A FM 20	A FM 15	A FM 10	A FM 5
Ingredient	g/100g as is					
Menhaden Fishmeal	30.00	25.00	20.00	15.00	10.00	5.00
Poultry by product meal	0.00	4.90	9.60	14.50	19.20	24.00
Soybean meal	10.50	10.50	10.50	10.50	10.50	10.50
Soy protein conc.	13.13	13.13	13.13	13.13	13.13	13.13
Empyreal 75	7.00	7.00	7.00	7.00	7.00	7.00
Menhaden Fish Oil	7.84	7.49	7.18	6.84	6.53	6.20
Corn Starch	2.85	3.04	3.38	3.56	3.89	4.15
Whole wheat	26.00	26.00	26.00	26.00	26.00	26.00
ASA Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25
ASA Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35%	0.10	0.10	0.10	0.10	0.10	0.10
CaP-diebasic	0.35	0.60	0.85	1.08	1.35	1.60
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Taurine	0.29	0.30	0.32	0.34	0.35	0.37

Study 12 - Graded replacement of fish meal with soy and inclusion of various attractants and supplements at low levels of soy.

We conducted a six week trial with 20% and 12% fish meal diets from Study 10 (FM20, FM12, FM12 squid, FM12 fish, FM12T) to determine if larger fish (38g) would find them more palatable than smaller fish (7g) used in Study 10. The larger fish did eat the diets and survival was excellent (99-100%) but the fish only showed 30-40% weight gain and FCR's were poor ranging from 2.5-3.2. There were no significant differences among any of the treatments with regards to performance.

Study 13 - High soy diets and east coast strain SB.

We sent several of the 8% fish meal diets from Study 10 (FM8, FM8 squid, FM8 fish, FM8AA) to a research facility in North Carolina to determine if their east coast strain of SB would perform better on them. The SB exhibited a good feeding response throughout the eight week trial and showed almost a 200% weight gain, which was considered to be reasonable growth based on other research with these fish.

Study 14 - Weaning of SB on to soy based feeds.

Instead offering experimental feeds abruptly, we conducted a trial to gradually transition striped bass from a commercial diet to the A FM30 and A FM5 diets from Study 11. Fish were weaned to these diets from a commercial diet over a period of four days but the feeding response was as poor as it was at the beginning of Study 11 and the fish did not transition on to either experimental diet.

Objective 8 – Laboratory Trials to Determine Limiting Nutrients at a Minimum Inclusion of Fish Meal in a Soy-Based Diet for striped bass (HSWRI/Auburn)
For this objective we tested a series of diets with various combinations of potentially limiting nutrients in a basal diet with a minimum level of fish meal.

The results for this objective were covered under Objective 7

Objective 9 - Test the effectiveness of small mesh copper netting in and offshore cage with juvenile WSB and YT

For this objective, we tested the efficacy of small copper mesh netting for nursery production of marine fish as means of reducing dependency of culturing these fish in coastal areas.

All work was conducted at the Hubbs-SeaWorld Research Institute's Leon Raymond Hubbard, Jr. Marine Fish Hatchery (CLD) and the adjacent Agua Hedionda Lagoon (AHL) in Carlsbad California. Three sets of two-cage rafts were assembled each containing one copper-alloy cage and one standard nylon net. Copper-alloy cages constructed of 6.35mm mesh were assembled on land and deployed using a crane aboard a floating barge (Figure 1). Care was taken to use only copper-alloy hardware for the assembly of the copper-alloy cages and to not allow for any other metals to come in contact with the material. Trex composite decking material was used as a platform for each of the rafts and Styrofoam-filled HDPE dock floats were used for buoyancy. Nylon nets each required a pendant weight (approximately 10kg ea) at each of the four bottom net corners in order to keep the cage from collapse during ebbing and flowing tides. No such weights were necessary for the copper-alloy cages due to the rigidity of the material. Semi-rigid plastic mesh lids were made for all cages to prevent bird predation. Cage rafts were moored to existing WSB juvenile cages within the AHL. Once installed, each cage provided a total of 4 m^3 of water volume.


Figure 1 – Cages being deployed.


Figure 2 – Cages being stocked with fish.

Cages were stocked on 9/25/13 with 1200 WSB juveniles weighing an average of 6.4 grams each (Figure 2). During the 11-week trial duration, juveniles were initially fed with Otohime EP2 diet (2mm), transitioned onto 3mm EWOS marine juvenile diet on 10-days into the study and finally onto 4 mm EWOS marine fish diet 50-

days into the study. Daily temperature and dissolved oxygen readings were taken in each cage and mortalities were removed whenever present. Weight and length (SL and TL) measures were taken from 50 fish in each cage at weeks 4 and 8. A final sampling of 200 fish from each cage was taken at the termination of the trial. Fish from each cage were also processed for gill, muscle and liver copper-concentration analysis. Results from these analyses are still being analyzed.

Water samples for copper concentration analysis were collected 1 meter upstream (n=3) and downstream (n=3) of a copper cage during a 0.12-0.2 meter/second incoming tide (Figure 3). Samples were collected using methods described by EPA 1669. Samples were sent to Calscience Environmental Laboratories and were analyzed for total and dissolved copper using methods described by EPA 1640.

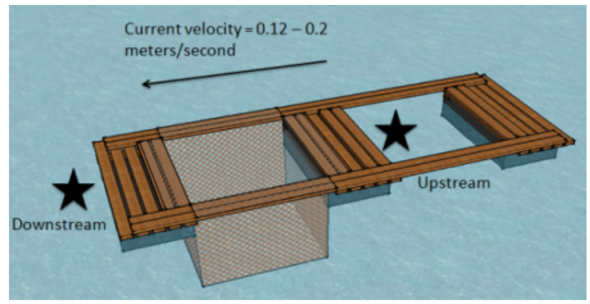

Figure 3 - Diagram of water sampling locations used to test for upstream/downstream copper concentrations

Juvenile performance – although both exhibited minimal mortality, juveniles reared in copper-alloy cages had significantly higher survival rates

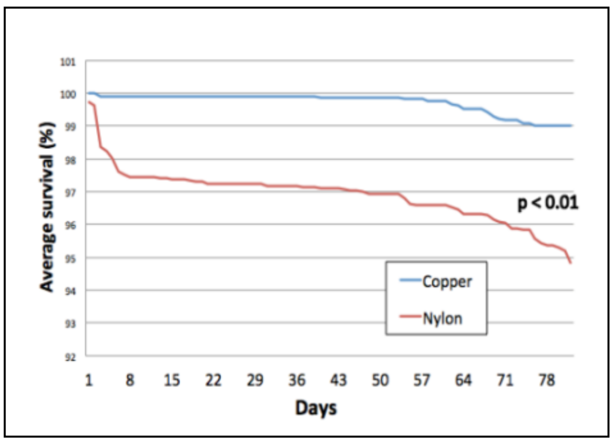

Figure 4. Average survival of juvenile WSB reared in copper-alloy and nylon mesh cages

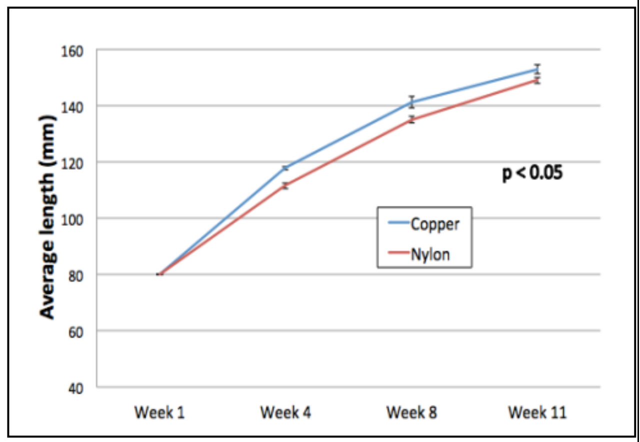

Figure 5. Average length increase of juvenile WSB reared in copper-alloy and nylon mesh cages

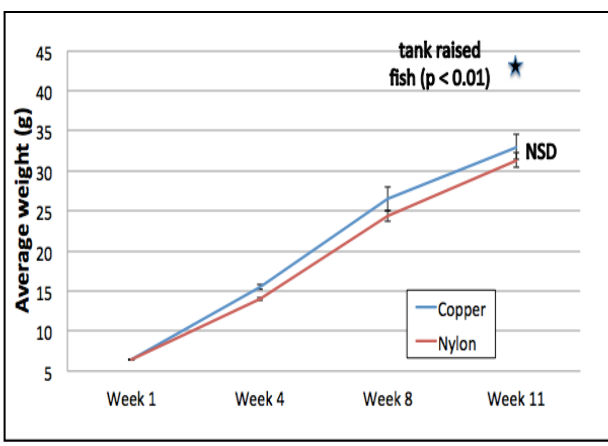

Figure 6. Average weight increase of juvenile WSB reared in copper-alloy and nylon mesh cages

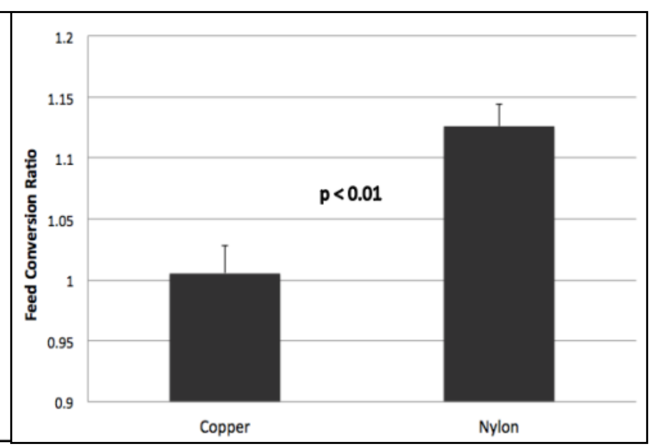

Figure 7. Average food conversion rates of juvenile WSB reared in copper-alloy and nylon mesh cages

than those reared in standard nylon net cages (Figure 4). Significantly better growth (in total length) was also observed within the copper-alloy cages (Figure 5), but no such difference was seen in weight gain between cage treatments. Due to higher culture water temperatures, tank-reared fish, however, did exhibit a significantly higher weight gain than either cage treatment (Figure 6).

Feed conversion ratios (FCR) also significantly differed between cage treatments (Figure 7). Copper-alloy reared juveniles had a significantly lower FCR than those reared in nylon nets. It is possible that this is due to the more striking contrast of the feed to the copper-alloy material; thereby making it easier for the feed technician to ration diet and avoid overfeeding.

Cage performance – The most obvious difference between cage types was the complete absence of biofouling on the copper-alloy material through the study period; the nylon material, on the other hand, exhibited relatively rapid growth of biofouling. Due to the low biomass held in the cages during this study, no obvious water quality differences were detected between cage treatments. Regardless, fouled nylon nets were swapped out for clean nets at week 7 of this study.

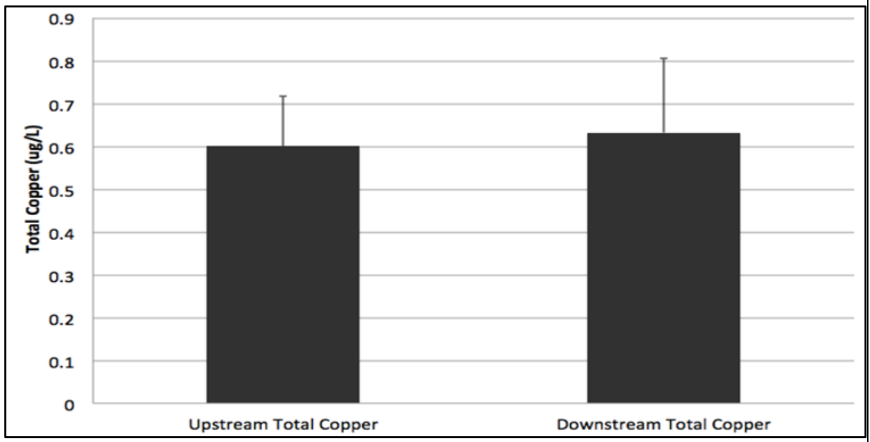

Figure 8. Total seawater copper concentrations 1 meter upstream and 1 meter downstream of copper cage during a flood tide event

Results from upstream/downstream seawater total or dissolved copper concentration analyses demonstrated no significant differences (Figures 8 and 9). Moreover, neither upstream nor downstream samples came close to the California chronic saltwater copper standard of 3.1 micrograms/liter.

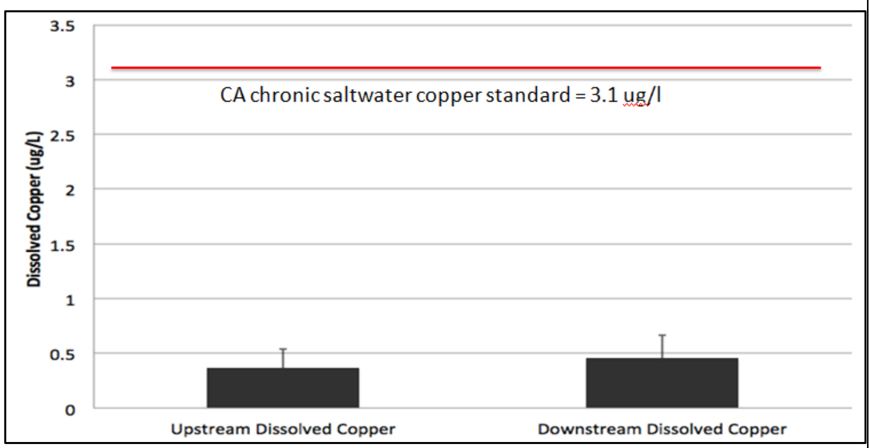

Figure 9. Dissolved seawater copper concentrations 1 meter upstream and 1 meter downstream of copper cage during a flood tide event

Other observations – juvenile WSB from both copper-alloy and nylon cage treatments exhibited a notable percentage of individuals with abraded snouts (64% and 49%, respectively). It is likely that this is caused by the fish striking the cage walls when disturbed. In fact, the fish in the copper-alloy cages could sometimes be heard striking the cage when technicians neared the raft. Also, although no obvious predation events were observed, resident wild kelp bass were often seen hovering below the cages and may have contributed to mortality in the nylon net treatment.

Conclusions - summarize overall value of research results and application opportunities by industry

Results of our research continue to be very encouraging with regards to the development and commercialization of soy based diets for WSB and YT. We continue to demonstrate a range of possibilities relative to soy-based formulations, including refinements to AA profiles. Long term growout trials have shown that soy-based diets perform comparably or better than commercial formulations. There is very limited work on SB and reports are quite variable in terms of tolerance of low fish meal diets. Based on industry input and what has been shown with hybrid striped bass, we expected SB to be more tolerant of reduced fish meal diets. However, this is not proving to be the case. We are also becoming increasingly aware that there may be strain or even cohort differences with regards to acceptability of reduced fish meal diets. We will need to determine if this is the case as well as identify other factors that may affect the minimum level of fish meal that SB will accept and then define ways to reduce fish meal from that level as we have with the other species.

This nutrition information will be valuable to show the feed manufacturing industry that soy-based diets are cost effective for each of these species. In fact, feeds used in the commercial growout trial in Mexico were less expense than the commercial control when both were provided by commercial mills. Feed manufacturers can also use the data to identify the maximum level of inclusion of soybean meal and soy oil in production diets. The project results will also target fisheries managers interested in stock replenishment of marine finfish, as well as commercial farmers who collectively will benefit from access to economically and environmentally sustainable diets. It will also target a developing commercial offshore aquaculture industry in northern Baja California, Mexico as well as what we hope will be a new commercial offshore aquaculture industry in southern California in the near future by demonstrating that the fish can be grown to marketable size using relatively high levels of soybean meal and soy oil in the diet.

The copper-alloy cage project reported here shows very promising results for developing cage-based nurseries in areas where land is very expensive or coastal waters are polluted. The demonstration cages we tested were mechanically robust and supported good biological fish performance without environmental degradation.