Project Number:	1640-512-5261
Project Title:	Use of soy-based products in practical diets for white seabass (*Atractoscion nobilis*) and California yellowtail (*Seriola lalandi*) & U.S. Soybean Industry/HSWRI Cooperative Ocean Cage Aquaculture Study.
Organization:	Hubbs-SeaWorld Research Institute
Principal Investigator Name:	Mark Drawbridge

Project Status - What key activities were undertaken and what were the key accomplishments during the life of this project? Please use this field to clearly and concisely report on project progress. The information included should reflect quantifiable results (expand upon the KPIs) that can be used to evaluate and measure project success. Technical reports, no longer than 4 pages, may be included in this section.

Use of soy-based products in practical diets for white seabass (*Atractoscion nobilis*) and California yellowtail (*Seriola lalandi*)

The main objective of this project was to build on past research into practical diet development for White Seabass (WSB, *Atractoscion nobilis*) and California Yellowtail (CYT, *Seriola lalandi*). The two specific objectives this year were to 1) evaluate the interaction of dose response to dietary taurine in plant based (soy) vs animal based (poultry) diets for WSB and CYT and 2) evaluate secondary limiting amino acids (AAs) and attractants in low fish meal diets.

Objective 1 - Laboratory trials to evaluate the interaction of dose response to dietary taurine in plant based (soy) vs. animal based diets in white seabass and California yellowtail.

We ran an 8 week feeding trial with WSB testing a series of nine diets consisting of either poultry (animal based) protein with supplemental taurine levels between 0-0.6g kg^{-1} or with soy (plant based) proteins with supplemental taurine levels between 0.12-0.92g kg^{-1}. Diet 8 (soy based protein with 0.72g kg^{-1} taurine) had the best feed conversion rate (FCR) while Diet 1 (poultry based with no additional taurine) had the worst. All soy based diets had a better FCR than even the best poultry FCR, suggesting that soy-protein based feeds can be well utilized by WSB in terms of growth with additional taurine bioavailability. During the final two weeks of the study, food consumption and growth declined markedly among fish fed the poultry-based diets. Diets were tested and found to be rancid. It is unclear what the source of the rancidity was and when its effects first manifested among fish in the trial. This finding limited final data analyses from this trial. We attempted to repeat the trial but ran out of time during the abbreviated 7-month study period allotted during this grant cycle.

Table 1. Diet formulation and proximates for Study 1 (g/100g as is)- Evaluating the interaction of dose response to dietary taurine in plant (soy) vs animal (poultry) based diets in WSB.

	Poultry Based				Soy Based				
Diet	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Menhaden fishmeal	-	-	-	-	-	-	-	-	-
Poultry by product	**53.97**	**53.97**	**53.97**	**53.97**	**20.00**	**20.00**	**20.00**	**20.00**	**20.00**

meal									
Corn Gluten meal	-	-	-	-	-	-	-	-	-
Soybean meal	-	-	-	-	**31.38**	**31.38**	**31.38**	**31.38**	**31.38**
Soycomil SPC	-	-	-	-	-	-	-	-	-
Nutrivance SPC	-	-	-	-	-	-	-	-	-
Pepsoygen	-	-	-	-	-	-	-	-	-
Empyreal 75	-	-	-	-	10.00	10.00	10.00	10.00	10.00
Lysto	-	-	-	-	-	-	-	-	-
Casein	-	-	-	-	-	-	-	-	-
Gelatin	-	-	-	-	-	-	-	-	-
Menhaden fish oil	2.52	2.52	2.52	2.52	5.97	5.97	5.97	5.97	5.97
Soy oil	-	-	-	-	-	-	-	-	-
Cotton seed oil	-	-	-	-	-	-	-	-	-
Corn Starch	16.18	15.98	15.78	15.58	2.90	2.70	2.50	2.30	2.10
Whole wheat	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Nutribinder	-	-	-	-	-	-	-	-	-
Trace Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA_Vitamin premix w/o choline	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-dibasic	-	-	-	-	2.00	2.00	2.00	2.00	2.00
Lecithin (soy commercial)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol	-	-	-	-	-	-	-	-	-
Lysine	-	-	-	-	0.28	0.28	0.28	0.28	0.28
Methionine	0.28	0.28	0.28	0.28	0.30	0.30	0.30	0.30	0.30
Glutamic acid	-	-	-	-	-	-	-	-	-
Taurine	**-**	**0.20**	**0.40**	**0.60**	**0.12**	**0.32**	**0.52**	**0.72**	**0.92**
Cellufill	-	-	-	-	-	-	-	-	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate Comp.	g/ 100g as is								

Crude Protein	40.46	39.76	39.36	40.62	39.94	40.42	41.54	41.46	42.31
Moisture	7.35	9.08	10.18	7.66	9.11	8.29	6.64	7.15	6.30
Crude Fat	10.88	10.72	10.53	10.94	11.25	11.08	11.74	12.99	12.22
Crude Fiber	1.63	1.74	1.82	1.92	2.54	2.46	2.90	2.82	2.68
Ash	7.55	7.38	7.34	7.79	5.25	5.37	5.55	5.49	5.48

Table 2. Performance results for WSB over an 8 week period for Study 1- Evaluating the interaction of dose response to dietary taurine in plant (soy) vs animal (poultry) based diets in WSB.

Diet #	Initial wt	Biomass	Mean	Wt gain	Pgain	Survival	Feed/fish	FCR
1	5.6	185.2	18.5	12.9	229.3	100.0	30.1	**2.34**
2	5.3	216.7	22.4	17.1	321.6	96.7	32.9	**1.93**
3	5.4	226.9	22.7	17.3	316.6	100.0	33.1	**1.94**
4	5.4	195.3	21.0	15.6	286.9	93.3	32.8	**2.12**
5	5.8	236.0	23.6	17.8	310.2	100.0	31.1	**1.74**
6	5.3	242.1	24.2	18.9	354.1	100.0	30.1	**1.59**
7	5.7	263.1	26.3	20.6	362.0	100.0	33.0	**1.60**
8	5.8	287.7	28.8	23.0	398.9	100.0	34.1	**1.48**
9	5.3	256.7	25.7	20.3	382.0	100.0	31.6	**1.56**

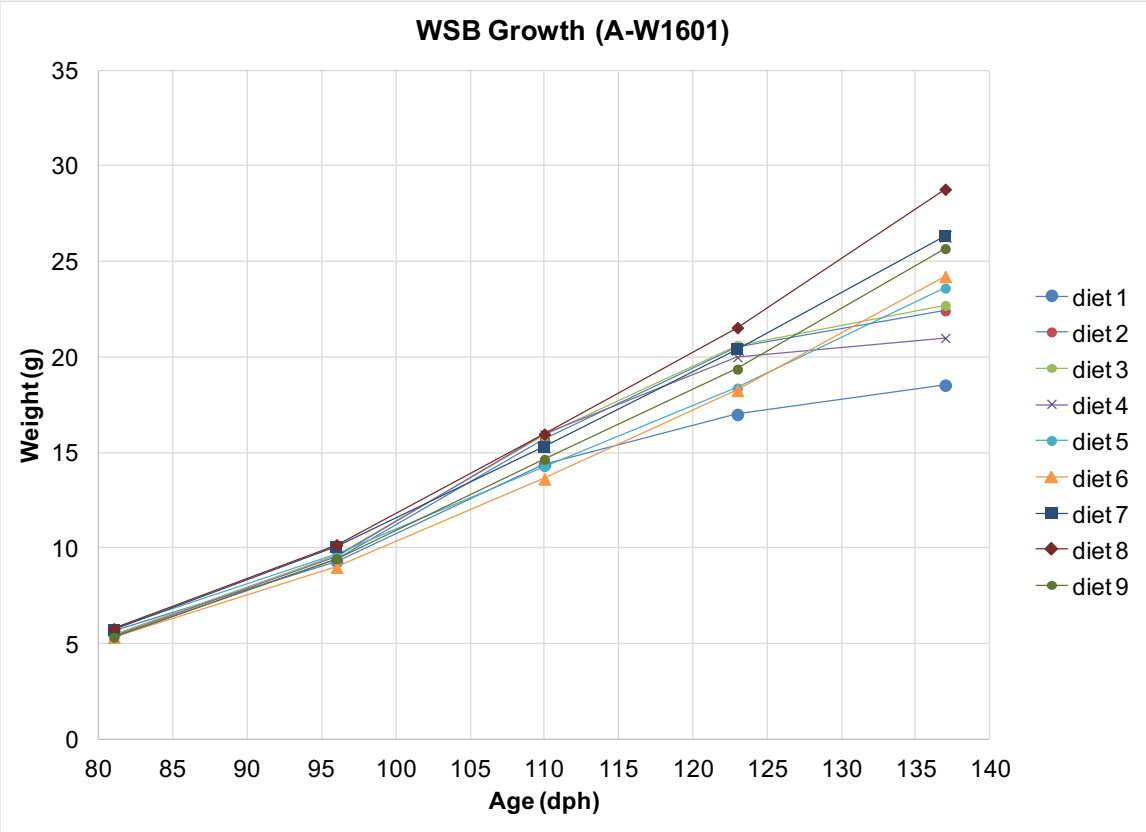

Figure 1. Growth trajectory for WSB over an 8 week period for Study 1- Evaluating the interaction of dose response to dietary taurine in plant (soy) vs animal (poultry) based diets in WSB. Growth and feed consumption declined after week 6 in the poultry-based diets, presumably due to rancidity of the diets

We conducted an 8 week feeding trial with CYT testing a series of eight diets consisting of either poultry (animal based) protein with supplemental taurine levels between 0-0.06g kg^{-1} and with soy (plant based) proteins with supplemental taurine levels between 0.06-0.12g kg^{-1}. Weight gain and FCRs improved with increasing taurine levels in each dietary series. The soy-based diet series yielded the best growth, with fastest growth occurring in diet 8.

The results of Ancova analyses of the relationship between dietary taurine and growth for both categories of diets indicated that there was a significant effect of taurine supplementation, no effect of protein source, and no significant interaction. Therefore, the effect of taurine supplementation in soy had the same effect than that in PBM-based diet.

Table 3. Diet formulation and proximates for Study 2 (g/100g as is)- Evaluating the interaction of dose response to dietary taurine in plant (soy) vs animal (poultry) based diets in CYT.

	Poultry Based				Soy Based			
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Menhaden fishmeal	-	-	-	-	-	-	-	-
Poultry by product meal (2016)	**35.00**	**35.00**	**35.00**	**35.00**	**15.00**	**15.00**	**15.00**	**15.00**
Meat & bone meal	-	-	-	-	-	-	-	-
Soybean meal (2016)	-	-	-	-	**28.63**	**28.63**	**28.63**	**28.63**
Casein	13.64	13.64	13.64	13.64	13.64	13.64	13.64	13.64
Gelatin (Spice Sage, 2016)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Menhaden fish oil	5.81	5.81	5.81	5.81	8.41	8.41	8.41	8.41
Lecithin (DV Aqua)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Corn Starch	13.90	13.88	13.86	13.84	0.61	0.59	0.57	0.55
Whole wheat	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Mineral premix (marine fish)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix (marine fish)	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
Stay C 35% active	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic	0.60	0.60	0.60	0.60	2.50	2.50	2.50	2.50
Taurine (2016)	**-**	**0.02**	**0.04**	**0.06**	**0.06**	**0.08**	**0.10**	**0.12**
Methionine (TCI 4/16)	-	-	-	-	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximates Comp.	g/ 100g as is							
Crude Protein	45.73	45.78	47.09	46.61	45.26	44.38	45.83	44.7
Moisture	6.99	6.46	7.01	5.03	6.37	8.3	5.92	8.55
Crude Fat	10.76	10.83	10.95	11.08	11.83	11.63	11.68	11.29
Crude Fiber	1.73	1.54	1.73	2	2.58	2.43	2.47	2.38
Ash	5.29	5.2	5.61	5.45	6.13	6.09	6.12	5.94

Table 4. Performance results for CYT over an 8 week period for Study 2- Evaluating the interaction of dose response to dietary taurine in plant (soy) vs animal (poultry) based diets in CYT.

Diet #	Initial wt	Biomass	No. Fish	Mean	Wt gain	Pgain	Survival	Feed/fish	FCR
1	6.7	631.3	14	46.1	39.5	589.7	94.5	61.7	**1.56**
2	6.7	722.3	15	49.9	43.2	646.5	100.0	63.9	**1.48**
3	6.7	743.8	15	51.3	44.6	668.8	100.0	64.7	**1.45**
4	6.7	772.5	15	51.5	44.8	674.2	100.0	66.2	**1.48**
5	6.7	590.4	13	46.2	39.6	592.5	94.5	61.3	**1.55**
6	6.6	567.7	13	44.4	37.8	569.8	98.1	58.1	**1.54**
7	6.7	585.8	12	50.0	43.3	649.2	89.1	59.8	**1.38**

8	6.7	688.3	13	55.1	48.3	720.3	89.0	63.4	**1.31**

Table 5. Whole fish proximate composition averages for Study 2- Evaluating the interaction of dose response to dietary taurine in plant (soy) vs animal (poultry) based diets in CYT.

	Protein	Moisture	Fat	Fiber	Ash
Initial Fish	16.18	75.33	5.46	0.07	2.87
Diet 1	18.27	74.49	4.52	0.06	2.98
Diet 2	17.41	74.84	4.78	0.07	3.10
Diet 3	17.62	74.42	4.98	0.09	3.06
Diet 4	17.96	74.17	5.24	0.06	3.00
Diet 5	18.05	75.09	3.62	0.09	3.19
Diet 6	17.50	75.61	3.84	0.10	3.29
Diet 7	18.02	74.81	4.09	0.07	3.29
Diet 8	17.90	74.73	4.14	0.10	3.33

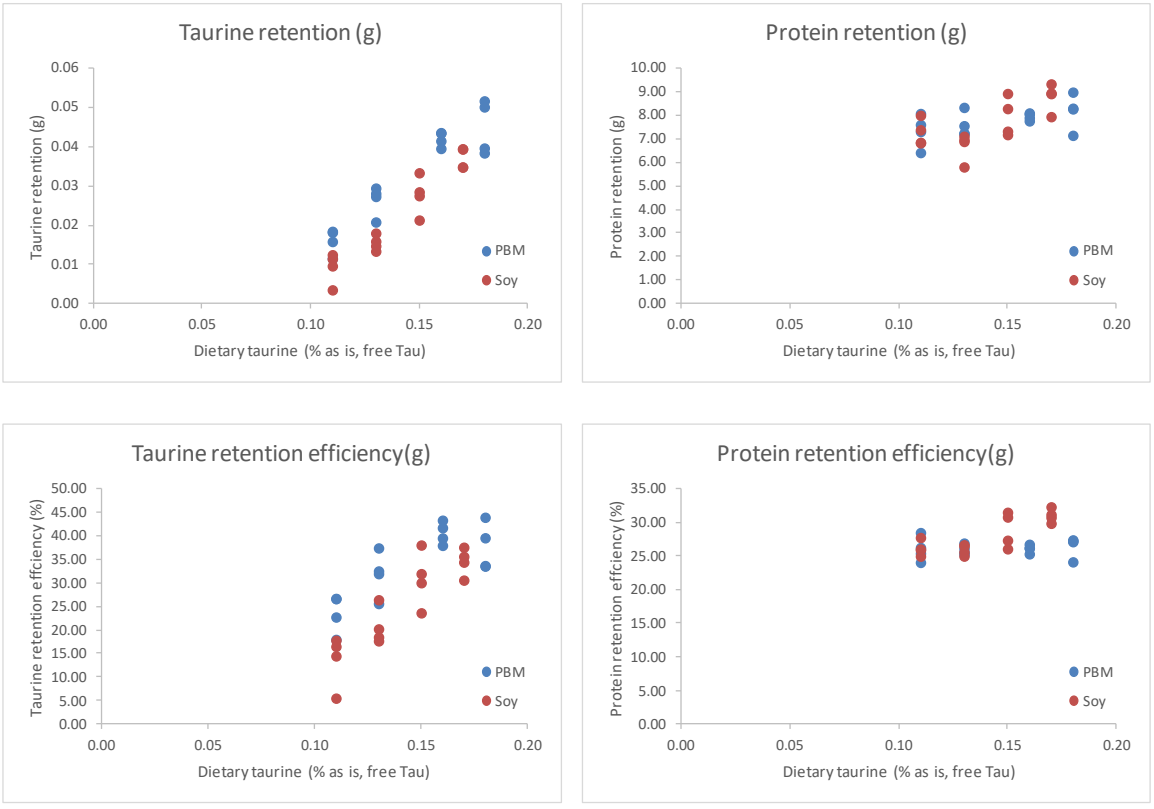

Figure 2. Taurine and protein retention and retention efficiency among - Evaluating the interaction of dose response to dietary taurine in plant (soy) vs animal (poultry) based diets in

CYT.

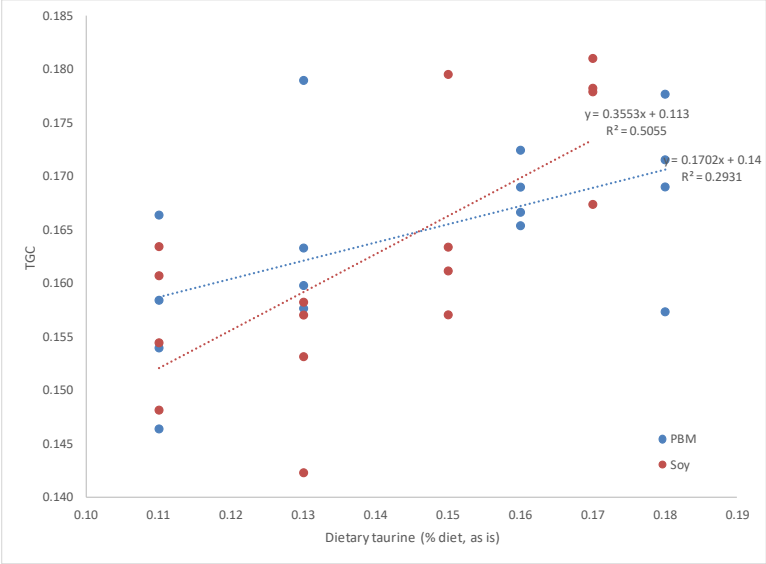

Figure 3. Relationship between dietary taurine and growth for poultry and soy-based diets tested on CYT. Slopes were not significantly different.

Objective 2 - Laboratory trial to evaluate secondary limiting nutrients and attractants in diets for white seabass.

This objective was not completed due to a failure of the chilling unit that delayed the start of the trial.

U.S. Soybean Industry/HSWRI Cooperative Ocean Cage Aquaculture Study.

Objective 1 – Evaluation of the serial curve plasma cortisol response post-acute stressor of fingerling white seabass (WSB).

Fish, average weight of 16 g, were stocked into 27 tanks and allowed to acclimate for four

weeks. Ten fish were stressed from each tank (3 per time period) by net capture and exposed to air for 30 seconds. Blood was collected at baseline and following acute stressor at several time periods. Utilizing an ELISA technique, the acute plasma cortisol response time was determined to peak at 30 minutes following acute stressor event, and returned to baseline 24 hours following the event. This information was used to determine sampling time points for Objective 2. Mean cortisol values and standard error bars are presented over time:

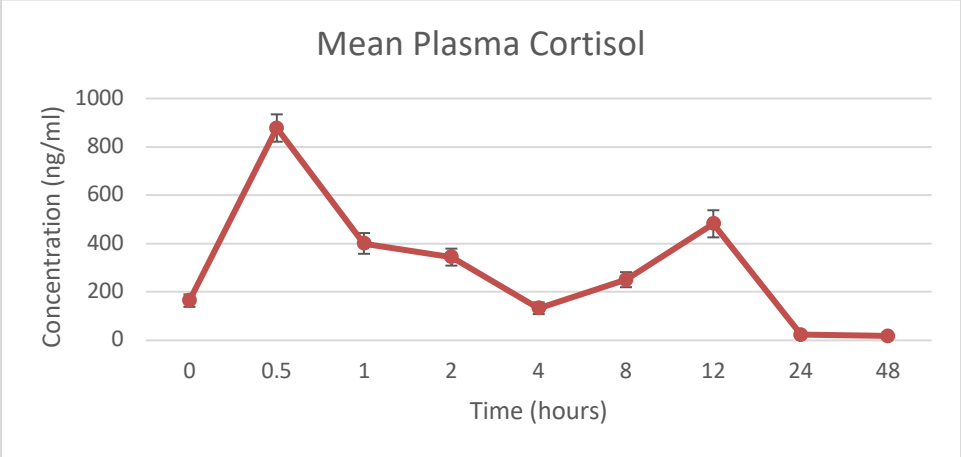

Figure 4. Relationship between plasma cortisol level and time post air-exposure stress event.

A slight peak in plasma cortisol was noted at 12 hours post-acute stressor, corresponding to an automated turning off of the lighting system in the experimental system.

Objective 2 - Evaluate acute cortisol response of fingerling WSB stocked and reared in offshore nets, both with and without surrounding predator nets.

Fish, weighing on average 28 g, were stocked in offshore nets (both with and without predator nets) in late June. Fish were allowed to acclimate for a period of six weeks prior to final sampling. Fish were stressed by pulling the containment net to the surface and fish were exposed to air for 30 seconds. Blood was collected at baseline and following acute stressor event. Mean plasma cortisol values and standard error bars are presented over time by treatment (with/without predator net):

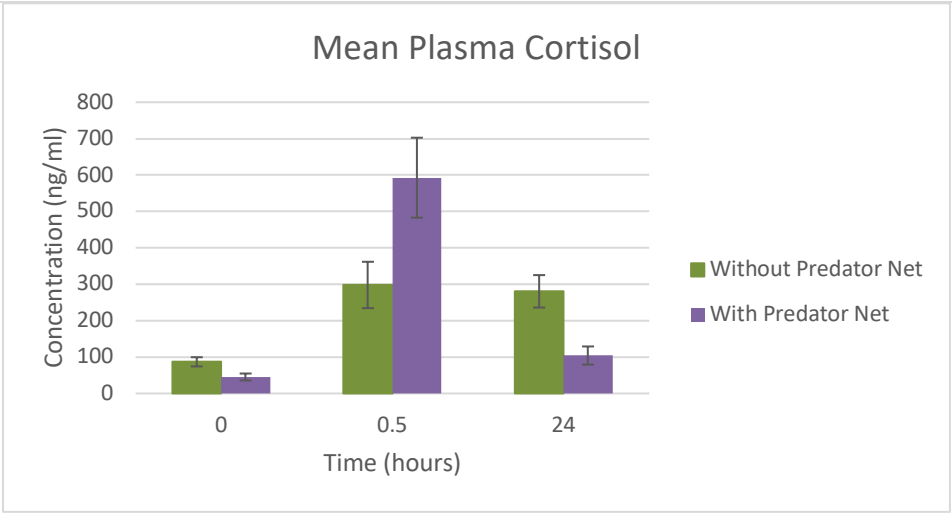

Figure 5. Relationship between plasma cortisol level and time post air-exposure stress event for two treatment groups.

Significant differences were noted between treatments at both the baseline and 24 hour post-stressor periods, with the non-predator net group having higher cortisol levels at both time periods. There were no significant differences between treatments at the 30 minute post-acute stressor time period, likely due to significant variability among stress values at this time period. In general, fish lacking a predator net had higher baseline cortisol levels and did not resolve the stress response as efficiently as fish in predator nets. Though not significantly different, fish reared without a predator net appeared to not respond as acutely to a stress event as fish reared with a surrounding predator net.

Additional stress parameters were evaluated, lactate and glucose, to augment plasma cortisol findings in juvenile white seabass. Mean and standard error bars for both glucose and lactate are as follows:

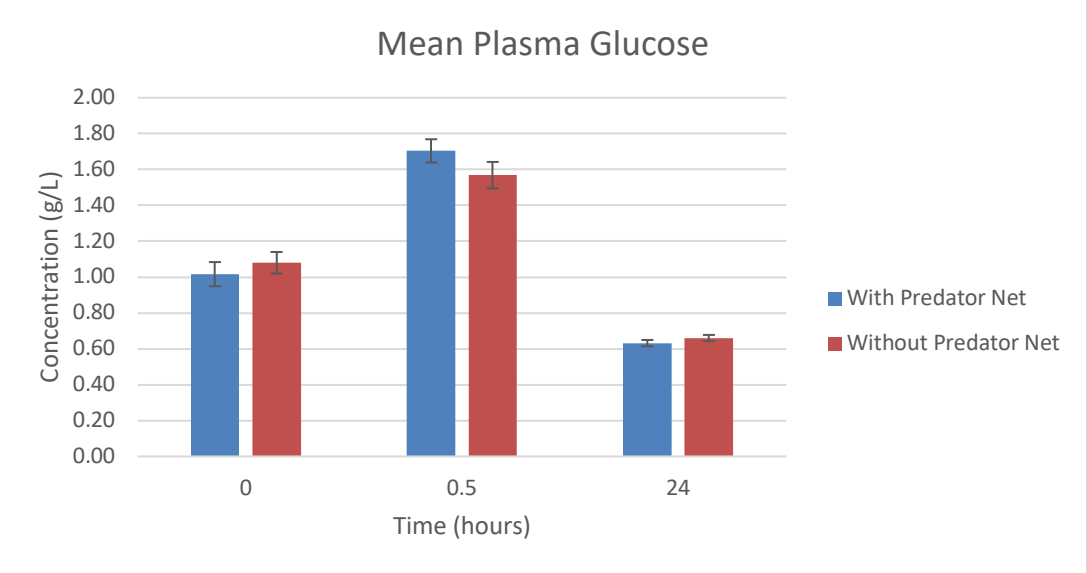

Figure 6. Relationship between plasma glucose level and time post air-exposure stress event for

two treatment groups.

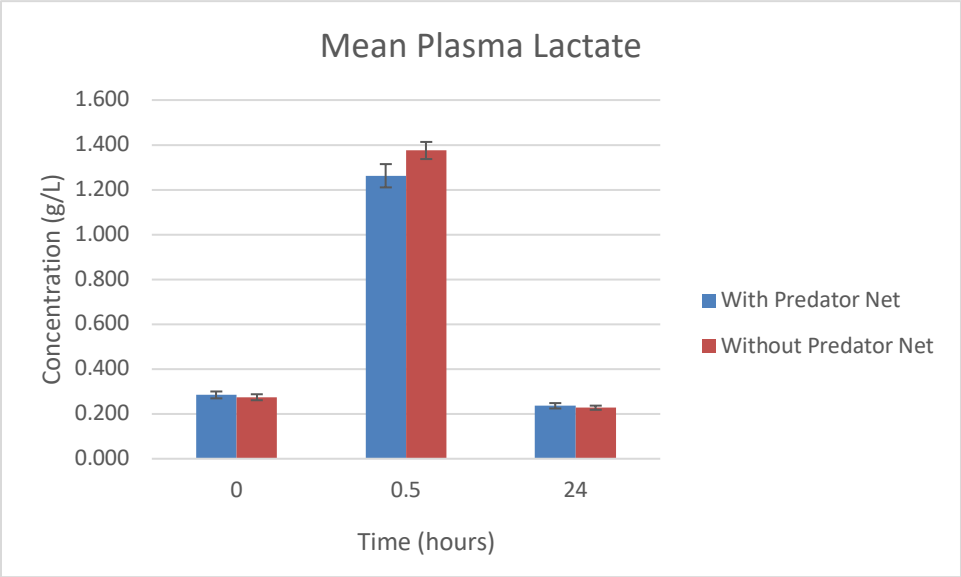

Figure 7. Relationship between plasma lactate level and time post air-exposure stress event for two treatment groups.

There were no differences between treatments at each time interval, and in general, the trend follows what is identified for the cortisol time series.

Objective 3 – Evaluate the performance of fish reared in offshore nylon nets with a surrounding predator net and those without.

The mean total length, standard length and weight of fish reared with surrounding predator nets was 242 mm, 209 mm, and 131 grams, respectively. For fish reared without predator nets, the mean total, standard length and weight was 243 mm, 211 mm, and 134 grams, respectively. The survival rate of fish reared in containment nets only was 98.7% and for fish reared in containment with a surrounding predator net was 97.9%. There were no differences when comparing morphometric data between treatments.

Did this project meet the intended Key Performance Indicators (KPIs)? List each KPI and describe progress made (or not made) toward addressing it, including metrics where appropriate.

Key Performance Indicator 1 – Determine if taurine bioavailability is affected by protein source. We determined that protein source does not affect taurine bioavailability in yellowtail under the experimental conditions used here. Weight gain and FCRs improved with increasing taurine levels in each dietary series. The soy-based diet series yielded the best growth. We were not able to definitively determine this relationship for seabass due to rancidity in the diets that most clearly affected the fish late in the study, thus confounding results.

Key Performance Indicator 2 – Determine serial cortisol curve: This key performance indicator was clearly delineated and the results from this indicator were utilized to design the trial for the

second key performance indicator. Results from this initial study were included in our bi-monthly aquaculture newsletter to inform the public of ongoing research performed at Hubbs-SeaWorld Research Institute as part of the US soybean funding.

Key Performance Indicator 3 – Evaluate acute cortisol response of fish reared with and without predator nets: Results clearly delineated differences among fish rearing practices both pre-stressor and at the 24 hour time period. The peak stress response was not determined to be different among treatments though there is a trend suggesting that fish reared without predator nets have higher levels of cortisol at rest and do not readily return to baseline levels following an acute stressor. Furthermore, additional stress parameters, including plasma lactate and glucose were able to be evaluated to substantiate a similar stress response identified as that identified in the plasma cortisol results. Updates on this project were included in our bi-monthly aquaculture newsletter that is disseminated to the public throughout the US.

Key Performance Indicator 4 – Evaluate performance of fish reared with and without predator nets: This key indicator was met through growth measures and survival statistics. Both groups had high survival for a six week period despite rearing practice.

Expected Outputs/Deliverables - List each deliverable identified in the project, indicate whether or not it was supplied and if not supplied, please provide an explanation as to why.

We identified another important finding for formulating soy-based diets for yellowtail relative to interactions between protein sources and taurine. Specifically, the protein source does not significantly affect the bioavailability of taurine. This will be used to help optimize diet formulations in the future.

We determined that seabass held in cages protected by predator nets keep stress at lower levels at baseline and they recover better from stress. There was a trend that the fish with predator nets had a more profound cortisol response when stressed. More research is needed to determine the broader consequences of these findings relative to justifying the use of predator nets in all situations.

We will disseminate the results of this research at the next WAS conference in early 2017.

Describe any unforeseen events or circumstances that may have affected project timeline, costs, or deliverables (if applicable.)

In our feeding trial for WSB we experienced rancidity among some of the experimental diets, which confounded results and will require a re-run to answer the questions definitively. We also had a malfunction in a our primary chilling unit that delayed our second WSB trial long enough so that it could not be completed in the compressed seven month contract period.

In an effort to diminish outside laboratory costs and effectively allow for more stress parameters to be tested, some of the diagnostic work was performed in-house. This resulted in an overall cost savings to the project.

What, if any, follow-up steps are required to capture benefits for all US soybean farmers? Describe in a few sentences how the results of this project will be or should be used.
The results of this research add to the existing base of knowledge on feed formulations for high value marine species. In the case of yellowtail, it is a species of global importance. Critical follow up research should involve growout trials of the best soy-based formulations. The results of this research demonstrate benefits, on a physiologic level, of predator net use in rearing fish in open water systems. Further research should include longer surveillance of the stress response in fish reared in open water systems, specifically changes in cortisol response when chronically stressed or when on differing nutrition regimes. It could be that acute stress responses are not affected in the short-term, though longer term stress situations may result in inability to produce cortisol to adapt to environmental stressors; including the potential to succumb to potential pathogens that may result in disease.
List any relevant performance metrics not captured in KPI's.