

2 September 2021 NOAA/MOCMI – Sea Turtle Rehab Program Case Evaluation

Summary

Hilo 02-Wailoa is a juvenile free-ranging Hawaiian green sea turtle (*Chelonia mydas*) who was found stranded in Hilo (Hawaiian island) in a freshwater stream (Wailoa).

Timeline/case progress:

- August 1, 2021- Stranded in Wailoa, HI
- August 3, 2021 Transported and admitted by NOAA MTBAP staff and placed in rehabilitation pool at low depth
- August 5, 2021- Mild hind end buoyancy noted. Loose fecal matter noted on bottom of pool, Mild cloacal prolapse noted
 - Empirical Therapy for buoyancy secondary to gastrointestinal stasis and gas dilated GI tract started
 - Ceftazadine IM, Metronidazole PO, Mineral Oil PO, Simethicone PO
 - Force fed 2 pieces of squid
- August 7, 2021- Mild buoyancy persists. Improved activity. Significant grass fecal output noted in pool. Cloacal Prolapse Resolved. See photos. Eating Limu and Squid on own.
 - Empirical Therapy for buoyancy secondary to gastrointestinal stasis and gas dilated GI tract continued
 - Ceftazadine IM, Metronidazole PO, Mineral Oil PO, Simethicone PO
 - LRS fluids given IC
- August 9, 2021- Mild buoyancy persists. Improved activity. Significant grass fecal output noted in pool. See photos. Eating Limu and Squid on own.
 - PCV-21% T.S- 2.4 Plasma appeared green in color. Chemistries not run.
 - Empirical Therapy for buoyancy secondary to gastrointestinal stasis and gas dilated GI tract continued
 - Metronidazole PO, Mineral Oil PO, Simethicone PO- Last dose
- August 10, 2021 Buoyancy Resolved. Improved appetite, behavior, and activity. Heavy amounts of grass fecal matter found in pool. Determined to be terrestrial salt tolerant grass.
- August 7 to August 15th- Heavy amounts of grass fecal matter noted daily in tank
 - Fed Limu, Squid and fish
 - Approx 75-100 grams of limu daily
 - Approx 100 grams of fish offered daily
 - Diet preference for Squid and Fish
- August 20,2021 Transferred to Maui Ocean Center Marine Institute for long term rehabilitation therapy
- August 20, 2021- BCS 2/9- Body weight 15.8 kg. Physical exam Findings- Soft plastron and carapace with some scute sloughage noted.
 - PCV-18% TS 2.7
 - Calcium- 6.6 mg/dl
 - Phosphorous -7.2 mg/dl
 - Uric Acid 1.3 mg/dl
 - Potassium- 5.3 meq/L



- Good appetite and good activity. Started feeding 400-500 grams squid per day
- August 24, 2021- Updated body weight 16.6 kg
 - No vitamin supplement started to date. Mazuri Sea Turtle Vitamin on site.
- August 26, 2021- Only ate 50% of squid diet offered. Rule out satiation.

It is believed that while this turtle was in the freshwater stream, it was consuming terrestrial salt-tolerant grass (*Paspalum vaginatum* (Poaceae)) submerged at high tide.

Assessment

The current diet of 400-500g squid provides this active, swimming juvenile with 2.4% BW and ~332-415 kcal/day. Energetic needs of green sea turtles depend on activity and whether animals are in or out of the water. Active swimming greens are described as needing 18.82 kcal GE*BW_{kg}^{$^{0.799}$}, whereas if out of the water, active animals need as much as 79.27 kcal GE* BW_{kg}^{$^{0.799}$} and if resting, as little as 6.65 kcal GE*BW_{kg}^{$^{0.799}$}.

In water		Out of water	
Current, Active	Calculated [†] , Active	Calculated [†] , Resting	Calculated [†] , Active
332-415 kcal ME*/day	206 kcal GE/day	73 kcal GE/day	868 kcal GE/day

*<u>Note</u>: Because gross energy (GE) calories have not been measured for squid (via bomb calorimeter), metabolizable energy (ME) was used. ME is calculated based on Atwater factors, which consider digestible calories provided by protein, fat and carbohydrates. GE will always be slightly greater than ME, because ME assumes some loss in available calories due to metabolic processes. *<u>Note</u>: Because the current body condition is too lean at score 3/9, calculated energy needs were based on a more ideal BW of 20kg (25% greater BW than current).

At this juvenile life stage, green sea turtles are transitioning from carnivory to herbivory. The fecal material containing copious amounts of intact grass indicates poor digestibility of the grasses in the freshwater stream. Green sea turtles have been seen consuming salt-tolerant terrestrial grasses and while they do have a greater capacity for fermentation/fiber digestion, the lignin content is much greater than in marine sea grasses and algae (McDermid et al 2015). Therefore, despite this turtle's strong appetite, the amount of terrestrial grass consumed, in the absence of other natural diet items, exceeded its digestibility and limited the available nutrients for absorption, leading to a caloric deficit and nutritional deficiencies.

Considering the natural diet transition while in the rehab setting, the goal is to limit seafood fed, even though it is often preferred, and encourage plant material consumption. A diet of solely squid, with no additional supplementation, provides excess protein and fat with deficient mineral concentrations, including calcium, which are necessary for repairing the soft shell.



Recommendations

- 1) <u>Feeding schedule</u> Offer smaller, more frequent meals, ideally 4x/day.
- 2) Diet items/amounts, transition, and feeding strategies

Starting diet – Offer daily:

Diet Ingredient	Amount (g)
Loligo squid	300
Limo (Gracilaria salicronia)	300
Leafy greens	300
-Romaine lettuce	
-Green leaf lettuce	
-Endive	
-Bok choy	
-Kale	
Green pepper*	50
Zucchini*	50
Cucumber*	50
Mazuri Sea Turtle vitamin (0.5g tablet)	2 tablets
Puritan's Pride Calcium 1200mg + Vit D 25 mcg	3 tablets
(240mg elemental calcium + 500 IU vit D per tablet)	
Puritan's Pride Biotin 1000mcg	1 tablet
NOW Vitamin C-1000mg	3 tablets

This diet will provide 380 kcal/day and 5.5% BW. See nutrient profile below. The protein, calcium, and vitamin D provided will support continued growth and healing. The fat content is greater than recommendations, so it would be important to monitor for over-conditioning. The fiber content is lower than recommended for an herbivorous adult sea turtle, but short-term, during this juvenile life stage, should not cause a problem. The minerals and vitamins provided in excess of recommendations are not at concerning concentrations. Additional vitamin D will support immune cell function and vitamin E will provide antioxidant support while healing and dealing with stress of being under human care. Excess water-soluble vitamins will be excreted in the urine.

Because the turtle is currently only eating squid, slowly introduce herbivorous diet items and reduce the squid offered over 10-14 days. Begin by offering the limo first, and although this analysis was based on the nutrient profile of *Gracilaria salicronia* (McDermid et al 2003), any available species can be offered. Be consistent and provide limo at every feed but offer the majority during the first 2 feeds of the day, reserving squid for the last 2 feeds of the day. If consistently eating the limo, begin offering leafy greens as well. Again, consistency is important, as the animal may reject the foreign diet items for 2 wks or more before finally giving them a try. The cut vegetables* can be added later for some variety/enrichment if desired.



Depending on how long this animal is in the rehab setting, there may be a need to switch to a more herbivorous diet. An example long-term diet to aim toward:

Diet Ingredient	Amount (g)
Loligo squid	<u>150</u>
Limo (Gracilaria salicronia)	300
Leafy greens	600
-Romaine lettuce	
-Green leaf lettuce	
-Endive	
-Bok choy	
-Kale	
Green pepper*	50
Zucchini*	50
Cucumber*	50
Mazuri Sea Turtle vitamin (0.5g tablet)	2 tablets
Puritan's Pride Calcium 1200mg + Vit D 25 mcg	2 tablets
(240mg elemental calcium + 500 IU vit D per tablet)	
Puritan's Pride Biotin 1000mcg	1 tablet
NOW Vitamin C-1000mg	3 tablets

This diet will provide 305 kcal/day and 6.3% BW. See nutrient profile below. The protein, calcium, and vitamin D provided are appropriate to support continued growth and healing. The fat content is greater than recommendations, so it would be important to monitor for over-conditioning; however, the fiber content is greater due to the contribution of more plant material, which will benefit long-term gastrointestinal health. The minerals and vitamins provided in excess of reported recommendations are not concerning. Additional vitamin D will support immune cell function and vitamin E will provide antioxidant support while healing and dealing with stress of being under human care. Excess water-soluble vitamins will be excreted in the urine.

Alternative diet items that can be trialed include the Mazuri Herbivorous Sea Turtle Gel (5B0W). If palatability is poor, the Mazuri Carnivorous Sea Turtle Gel (5B37) can be mixed with the Herbivorous formula at 50:50 ratio to see if that improves acceptance. Samples may be able to be obtained for a feeding trial if this is of interest. If one or both gels are accepted, a modified diet can be designed based on its inclusion.

3) <u>Supplementation[¥]</u></u></sup>

- a. Multivitamin supplement Begin Mazuri Sea Turtle Supplement, 0.5g tablets, as directed in the diets above, to meet nutrient recommendations for herbivorous sea turtles.
- b. Calcium/vitamin D supplement Begin a calcium + vit D supplement for support health/growth. Recommend Puritan's Pride 1200mg Calcium + Vitamin D 25 mcg, which provides 600mg calcium carbonate (or 240mg elemental calcium) and 500 IU vitamin D per tablet (<u>https://www.puritan.com/calcium-supplements-001/absorbable-calcium-1200mg-with-vitamin-d-1000-iu-006274</u>), as directed in the diets above. If this product cannot be obtained, find a similar replacement with comparable calcium/vit D concentrations.



- c. Biotin supplement Begin additional supplementation with biotin to support carapace healing. Recommend Puritan's Pride Biotin 1000mcg tablets (<u>https://www.puritan.com/biotin-421/biotin-1000-mcg-007961</u>), as directed in the diets above. If this product cannot be obtained, find a similar replacement with comparable biotin concentration.
- d. Vitamin C supplementation A supplemental source of this antioxidant prevents stressinduced immune suppression and promotes bactericidal activity. Supplementation of 2.5-10 mg ascorbic acid/kg diet 'as fed' is generally recommended for sea turtles. Although plant-based diets contain natural vitamins, when those vegetables are exposed to water, naturally occurring vitamin C is readily lost; therefore, additional supplementation is needed. Recommend NOW Vitamin C-1000mg tablets (<u>https://www.amazon.com/NOW-Vitamin-C-1000-250-</u> Capsules/dp/B00130UNK4/ref=sr 1 5?crid=LV6FUA91DPPE&dchild=1&keywords=v

Capsules/dp/B0013OUNK4/ref=sr 1 5?crid=LV6FUA91DPPE&dchild=1&keywords=v itamin+c+1000mg+capsules&qid=1630593561&sprefix=vitamin+c+1000mg%2Caps%2 C206&sr=8-5), as directed in the diets above.

*<u>Note</u>: Estimated weights were used for supplement tablets to calculate their contribution to the total diet. When decisions are made about which product will be used and those items in hand, please weigh 1 tablet of each supplement and send weights to nutritionist. Diet nutrient profiles will be updated with proper weights to make sure the number of tablets recommended for both diets are appropriate.

Monitoring/follow-up

- Diet consumption Monitor closely to ensure turtle is consuming all diet items offered. If concerns, discuss how to troubleshoot feeding strategy with veterinary team and nutritionist.
- Body weight/body condition Measure body weight and assess body condition at least every other week to determine the effect of the diet on animal health.
- Blood work monitoring Before diet changes are made, measure baseline CBC, chemistry panel, ionized calcium, and serum vitamin D. Vitamin D analysis, including circulating metabolite 25(OH)D and total vitamin D, should be performed via HPLC at a reliable laboratory, like Heartland Assays (<u>https://www.heartlandassays.com/</u>). While normal reference ranges for serum vitamin D in sea turtles are not known, wild and rehabilitated turtle data has been reported and can be used for comparison, and trends can be followed to determine effect of dietary supplementation (Stringer et al 2010).
- Diet reevaluation As budget allows, reevaluate nutrition based on body weight, body condition, health status, and blood work in 1-2 months. Re-evaluation would include updated calculations of diet nutrient profile based true consumption of diet ingredients and supplements utilized (as noted above).

References

McDermid KJ, Lefebvre JA, and Balazs GH. 2015. Nonnative seashore Paspalum, *Paspalum vaginatum* (Poaceae), consumed by Hawaiian green sea turtles (*Chelonia mydas*): Evidence for nutritional benefits. Pacific Science, 69(1): 48-57.



McDermid KJ, and Stuercke B. 2003. Nutritional composition of edible Hawaiian seaweeds. J Applied Phycol, 15: 513-524.

Stringer EM, Harms CA, Beasley JF, and Anderson ET. 2010. Comparison of ionized calcium, parathyroid hormone, and 25-hydroxyvitamin D in rehabilitating and healthy wild green sea turtles (*Chelonia mydas*). J Herp Med Surg, 20(4): 122-127.

Current Squid-only Diet Evaluation:

			Compiled Sea Turtle Nutrient
Current Squid-only Diet			Recommendations (DMB)
Nutrient Profile	AS FED ANALYSIS	DRY MATTER ANALYSIS	<u>Herbivores</u>
Calories (kcal ME/100g)	83.09	405.30	
Moisture(%)/DM(%)	79.50	20.50	
CrudeProtein(%)	16.77	81.80	6-22 (35 growth)
Crudefat(%)	1.50	7.30	0.4-1.2
Ash(%)	1.60	7.80	
CHO(%), by difference	0.64	3.10	
ADF(%)	0.49	2.40	13.00
NDF(%)	0.57	2.80	
Ca(%)	0.02	0.12	0.6-0.9 (1-4 growth)
P(%)	0.25	1.24	0.3-0.78
Ca:P	0.10	0.10	>1.1
Mg(%)	0.05	0.23	0.04-0.17
К(%)	0.27	1.31	0.22-0.56
Na(%)	0.26	1.25	0.07-0.22
CI(%)			0.17-0.33
S(%)	0.44	2.15	
Fe(ppm)	2.46	12.00	33-167
Zn(ppm)	16.61	81.00	17-117
Mn(ppm)	0.82	4.00	3-116
Cu(ppm)	29.73	145.00	3-29
Se(ppm)	0.68	3.31	0.14-0.29
Mo(ppm)	0.00	0.00	
Co(ppm)	0.01	0.07	
lodine(ppm)			0.5-1.2
VitA(retinol,IU/kg)	0.00	0.00	2200-7400
VitD3(IU/kg)	0.00	0.00	400-1250
VitE(mg/kg)	0.00	0.00	30-166
VitK(mg/kg)	0.00	0.00	
Thiamin(ppm)	0.00	0.00	0.6-9
Biotin(ppm)	0.00	0.00	0.1-1.1
Riboflavin(ppm)	0.00	0.00	3-17
Niacin(ppm)	0.00	0.00	11-31
Panthethenate(ppm)	0.00	0.00	11-33
Pyridoxine(ppm)	0.00	0.00	3-17
Folate(ppm)	0.00	0.00	0.6-2.2
VitB12(ppm)	0.00	0.00	
VitC(ppm)	0.00	0.00	17-50

*Yellow highlighted cells are nutrients provided in excess of reported recommendations; orange highlighted cells represent deficiencies compared to recommendations.



Modified Starting Diet Evaluation:

			Compiled Sea Turtle Nutrient
			Recommendations (DMB)
Starting Diet Nutrient Profile	AS FED ANALYSIS	DRY MATTER ANALYSIS	<u>Herbivores</u>
Calories (kcal ME/100g)	35.96	316.39	
Moisture(%)/DM(%)	88.63	11.37	
CrudeProtein(%)	5.41	47.63	6-22 (35 growth)
Crudefat(%)	0.58	5.11	0.4-1.2
Ash(%)	2.24	19.70	
CHO(%), by difference	2.27	19.98	
ADF(%)	0.51	4.46	13.00
NDF(%)	0.64	5.64	
Ca(%)	0.10	0.91	0.6-0.9 (1-4 growth)
Р(%)	0.09	0.78	0.3-0.78
Ca:P	1.16	1.16	>1
Mg(%)	0.03	0.30	0.04-0.17
К(%)	0.67	5.89	0.22-0.56
Na(%)	0.11	0.97	0.07-0.22
CI(%)			0.17-0.33
S(%)	0.24	2.10	
Fe(ppm)	23.69	208.47	33-167
Zn(ppm)	8.86	77.93	17-117
Mn(ppm)	9.53	83.86	3-116
Cu(ppm)	8.89	78.25	3-29
Se(ppm)	0.21	1.86	0.14-0.29
Mo(ppm)	0.01	0.13	
Co(ppm)	0.01	0.05	
lodine(ppm)	0.03	0.27	0.5-1.2
VitA(retinol,IU/kg)	586.29	5158.61	2200-7400
VitD3(IU/kg)	170.21	1497.66	400-1250
VitE(mg/kg)	30.26	266.25	30-166
VitK(mg/kg)	0.06	0.50	
Thiamin(ppm)	30.26	266.25	0.6-9
Biotin(ppm)	0.13	1.16	0.1-1.1
Riboflavin(ppm)	2.27	19.97	3-17
Niacin(ppm)	2.27	19.97	11-31
Panthethenate(ppm)	2.27	19.97	11-33
Pyridoxine(ppm)	2.27	19.97	3-17
Folate(ppm)	0.08	0.67	0.6-2.2
VitB12(ppm)	0.38	3.33	
VitC(ppm)	2948.89	25946.53	17-50

*Yellow highlighted cells are nutrients provided in excess of reported recommendations; orange highlighted cells represent deficiencies compared to recommendations.



Long-Term Rehab Diet Evaluation:

			Compiled Sea Turtle Nutrient
Long-Term Rehab Diet Nutrient			Recommendations (DMB)
<u>Profile</u>	AS FED ANALYSIS	DRY MATTER ANALYSIS	<u>Herbivores</u>
Calories (kcal ME/100g)	25.28	291.89	
Moisture(%)/DM(%)	91.34	8.66	
CrudeProtein(%)	3.00	34.65	6-22 (35 growth)
Crudefat(%)	0.39	4.48	0.4-1.2
Ash(%)	2.00	23.06	
CHO(%), by difference	2.44	28.23	
ADF(%)	0.61	7.04	13.00
NDF(%)	0.81	9.36	
Ca(%)	0.08	0.90	0.6-0.9 (1-4 growth)
Р(%)	0.05	0.62	0.3-0.78
Ca:P	1.45	1.45	>1
Mg(%)	0.03	0.33	0.04-0.17
К(%)	0.62	7.16	0.22-0.56
Na(%)	0.07	0.82	0.07-0.22
CI(%)	-		0.17-0.33
S(%)	0.16	1.83	
Fe(ppm)	23.87	275.65	33-167
Zn(ppm)	6.09	70.37	17-117
Mn(ppm)	8.96	103.50	3-116
Cu(ppm)	4.18	48.26	3-29
Se(ppm)	0.12	1.35	0.14-0.29
Mo(ppm)	0.02	0.20	
Co(ppm)	0.00	0.04	
lodine(ppm)	0.03	0.31	0.5-1.2
VitA(retinol,IU/kg)	513.80	5932.56	2200-7400
VitD3(IU/kg)	107.73	1243.92	400-1250
VitE(mg/kg)	26.52	306.20	30-166
VitK(mg/kg)	0.05	0.57	
Thiamin(ppm)	26.52	306.20	0.6-9
Biotin(ppm)	0.12	1.34	0.1-1.1
Riboflavin(ppm)	1.99	22.96	3-17
Niacin(ppm)	1.99	22.96	11-31
Panthethenate(ppm)	1.99	22.96	11-33
Pyridoxine(ppm)	1.99	22.96	3-17
Folate(ppm)	0.07	0.77	0.6-2.2
VitB12(ppm)	0.33	3.83	
VitC(ppm)	2595.71	29971.38	17-50

*Yellow highlighted cells are nutrients provided in excess of reported recommendations; orange highlighted cells represent deficiencies compared to recommendations.