05/28/03

Dear George,

Please find enclose a copy of My Research Paper. I would very Much appreciate your comments and opinion, at any moment that is convenient for you. I hope you enjoy reading it.

Thank you again for your help. It was a great research experience for me and I hope that I can one day be of any help for our turtles.

Sincerly,

Maude Tremblay

P.S. I am in Honolulu for the summer. I you need help, let me know! phone # 808-283-6664

SENIOR THESIS RESEARCH

Growth Rates of Juvenile Hawaiian Green Sea Turtles (*Chelonia mydas*) Reared in Captivity

Presented
To
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By

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ABSTRACT

Green sea turtles obtained from Hawaiian waters by Sea Life Park on Oahu in the 1960's are nesting on an artificial beach in the park and producing hatchlings every year. Six hatchlings born during the summer of 2000 were transferred to Maui Ocean Center for display as part of an educational loan program. Earlier studies have indicated that captive-reared turtles have faster growth rates than wild ones. Because of the difficulty in determining the age of living green turtles and the lack of knowledge about their life span, very little information about the growth rates of turtles in the wild is available. Morphometric measurements of the six juvenile turtles were taken from October 2002 to March 2003. Initially, the turtles were fed fish and marine invertebrates, along with vegetables. However, as of November 2002, their diet became strictly vegetarian. Upon arrival at Maui Ocean Center, the turtles measured between 33 and 39 cm in straight carapace length. A year later, the average increase in straight carapace length was 9.6 cm. As a comparison, the mean growth rates of wild Hawaiian specimens, based on spline integration, is 4-5 cm/yr for early juveniles, declining to 2cm/yr by the age of 10, and declining again to less than 1 cm/yr as they approach 30 years in age. The faster growth of captive-reared turtles may be due to the high nutritional value of their diet, the constant supply of food and the lack of migration, as observed in wild Hawaiian green sea turtles.

INTRODUCTION

Green sea turtle (*Chelonia mydas*) populations are found in almost every ocean of the world (Ehrhardt and Witham, 1992) and are protected under the Endangered Species Act of 1973. While some populations of green turtles are considered endangered, the Hawaiian *C. mydas*, known locally as Honu, is listed as threatened (Hawaii 's Marine Protected Species, NOAA, 2002). Because of the slow growth rate of green turtles and the common difficulties in determining age among reptiles, estimates of growth at a given age have not been reported for *C. mydas* (Ehrhardt and Witham, 1992). Understanding the population dynamics of a specie is essential in developing proper conservation programs to support the recovery and protection of endangered species. A fundamental aspect of the population dynamics of any species is the study of its growth rate.

The green turtle is predominantly herbivorous, feeding on either algae or seagrass but not a mixture of both, even when both seagrass and algae are available (Bjornal, 1980; Mortimer, 1981, 1982). Others studies have revealed a two-stage diet adapted to the different habitats associated with the life stage of *C. mydas*; the posthatching pelagic phase versus the juvenile/adult coastal phase (Wood and Wood, 1981, Bjorndal, 1985). The green turtle appears to be carnivorous during its first year and then gradually become herbivorous as it matures (Lewis, 1940; Hughes et al., 1967, Ferreira, 1968). In Hawaiian waters, skeletochronological age estimates indicate that juvenile green turtles remain in the pelagic phase until 4-10 years of age (Balazs et al., 2002).

Late sexual maturity of sea turtles has repeatedly been established (Bolten et al., 1992, Parham and Zug, 1998); 20 or more years is the length of time female green turtles must survive to lay their first clutch of eggs (Balazs et al., 2002). The mean size in straight carapace length (SCL) for nesting Hawaiian *C.mydas* is 92 cm, and ranges from 81-106 cm (Balazs, 1980), which implies that the female may be 30 or more years of age at first nesting. Although most Honu reside in coastal waters around the main Hawaiian Islands, reproduction and nesting take place after the turtles have migrated hundreds of miles to the Northwestern Hawaiian Islands, more specifically at the isolated French Frigate Shoals (Hawaii Marine Protected Species, NOAA, 2002). The year 2000 was an exception; the first documented nesting of a green turtle on Maui took place (Balazs et al., 2001). Turtle 5690, a tagged turtle born in 1980 at the French Frigate Shoals, was reared for one year in captivity and released in Hilo Bay, on the island of Hawaii. According to Balazs, this 20-year-old female would have grown a robust three cm/year during her 19 years of life in the wild. Determining the age and growth rates of wild green turtles is difficult. The usual capture-recapture methods used to study growth of marine organisms are inefficient due to the long life span of *C.mydas*. Additionally, the change of habitat, and the resultant change in feeding behaviors experienced by juvenile turtles, expose the green sea turtles to several different growth conditions throughout their life (Ehrhardt and Witham, 1992).

In this study, I have documented the growth rates of six juvenile Hawaiian *C. mydas* in captivity. Because these turtles were born in captivity and have been closely monitored, the growth patterns could be established in relation to their age and feeding behaviors. I also compared the growth of captive juvenile turtles to wild turtles and found the growth of captive turtles to be more rapid. This comparison was possible due to the recent development of a technique that estimates the age of sea turtles from the number of growth increments formed on the humerus (Parham and Zug, 1998). The samples used in this study, conducted by Balazs et al. (2002), were dead animals retrieved from Hawaiian waters, as it is not possible to practice this technique on live specimens. Subsequent to my findings, I have hypothesized several factors that may account for the faster growth rates of green turtles in captivity.

METHODS

The six *Chelonia mydas* in this study were hatched at Sea Life Park, a public aquarium on the island of Oahu, during the summer of 2000. The captive parents were collected from Hawaiian waters prior to being protected under the Endangered Species Act of 1973. The Hawaiian population of green sea turtles is part of the larger Indo-Pacific *C. mydas* gene pool but it also consists of a distinct genetic stock containing a unique mtDNA haplotype (Bowen et al., 1992).

The juvenile green sea turtles have been living at Maui Ocean Center since March 18, 2002. Because of the young age of the turtles, the sexes are unknown. Their habitat consists of an artificial lagoon receiving natural seawater directly from the ocean at a flow rate of approximately 677 liters per minute. The lagoon contains a total volume of 71 095 liters of water, its temperature ranges between 24- 28 degrees Celsius and the salinity is 35-36 parts per thousand. The deepest portion of the tank is 2.06 meters deep and the shallowest part is connected to a small beach, allowing the turtles to come out of the water. There are no other animals sharing the habitat of *C. mydas*.

The diet of *C. mydas* changed considerably over the study period. The long-term goal, set by the head curator of the aquarium and the turtles' veterinarian, was to feed the animals a diet that was as close as possible to their natural diet. According to Gorman (personal communication, 2002), this facilitates the successful introduction of the turtles into the wild while maintaining a growth rate that is in accordance with the specie. The turtles are fed four times a day; early morning, mid-morning, mid-afternoon and early evening. The first and the last feed of the day consist of a prepared gel diet, the content of which has been variable. The two other feeds consist of a head of romaine lettuce and about 340 grams of fresh broccoli, celery or green bell pepper- depending on availability - which is distributed between all six turtles.

The gel diet is prepared by the curatorial staff of the aquarium every three weeks. The final product is divided into blocks and kept frozen. Each turtle receives two blocks per day. The basic ingredients of this gel diet include frozen spinach, fresh vegetables, spirulina, brewers yeast and gelatin. From March until June, 2002, the turtle gel recipe also included fish, squid, anchovy, shrimp and dried krill. In July 2002, the turtles were introduced to pellets that are especially manufactured for sea turtles (Melick Aquafeed). Since the turtles did not accept the pellets themselves, they were slowly added to the original gel recipe. During the following months, the amount of seafood products in the gel diet was decreased while the amount of vegetables and pellets were increased. By the end of November 2002, the goal set by the veterinarian was accomplished and the turtles were accepting the fish-free gel diet. A nutritional analysis of the turtle gel diet was performed by a professional firm (Food Quality Labs, Oahu) and the protein content was measured to be 16.55%.

Measurements of *C.mydas* were performed in October, November and December of 2002 and in February and March of 2003. A caliper was used to measure straight carapace length (SCL) and straight carapace width (SCW). A non-stretching tape was used to determine curved carapace length (CCL) and curved carapace width (CCW). Length measurements of the carapace were taken from the mid-point of the nuchal scute to the posterior-most tip of the carapace. CCW was taken at the sixth marginal of the carapace while SCW was taken at the widest part of the turtle's carapace, in a straight line. All measurements were to the nearest mm.

A quarterly medical exam was performed by a veterinarian in order to monitor the turtles' health. During the exam, each animal was carefully inspected; the carapace, the eyes, the nares, the oral cavity, the plastron and the genitals. Additionally, a complete blood analysis was also performed in March 2003.

RESULTS

Upon arrival at Maui Ocean center, all six animals measured between 33 cm and 39 cm SCL. A year later, the increase in SCL ranged between 9.1 cm/year and 11.1 cm/year, with a mean of 9.6 cm/year. The monthly increase in SCL appears to be constant between individuals, ranging from 0.7 cm/month for the two turtles with the slowest growth (Keoki and Opihi) to 0.9 cm/month for the two fastest growing animals (Nakine and Pele). Table 1 summarizes those findings.

TABLE 1: SCL measurements of turtle's carapaces after a year at Maui Ocean Center. Data are in decreasing order of growth. Note that Nakine indicates the fastest growth, while Opihi is the slowest growing animal.

TURTLES	**March 6, 2002 SCL (cm)**	**March 5, 2003 SCL (cm)**	**GROWTH (cm/year)**	**AVERAGE GROWTH (cm/month)**
Nakine	35.1	46.2	11.1	0.9
Pele	35.7	46.7	11.0	0.9
Kupualoha	33.9	43.6	9.7	0.8
I'miloa	34.3	43.4	9.1	0.8
Keoki	35.5	43.9	8.4	0.7
Opihi	39.0	47.2	8.2	0.7
	MEAN SCL INCREASE:		**9.6 cm/year**	**0.8 cm/month**

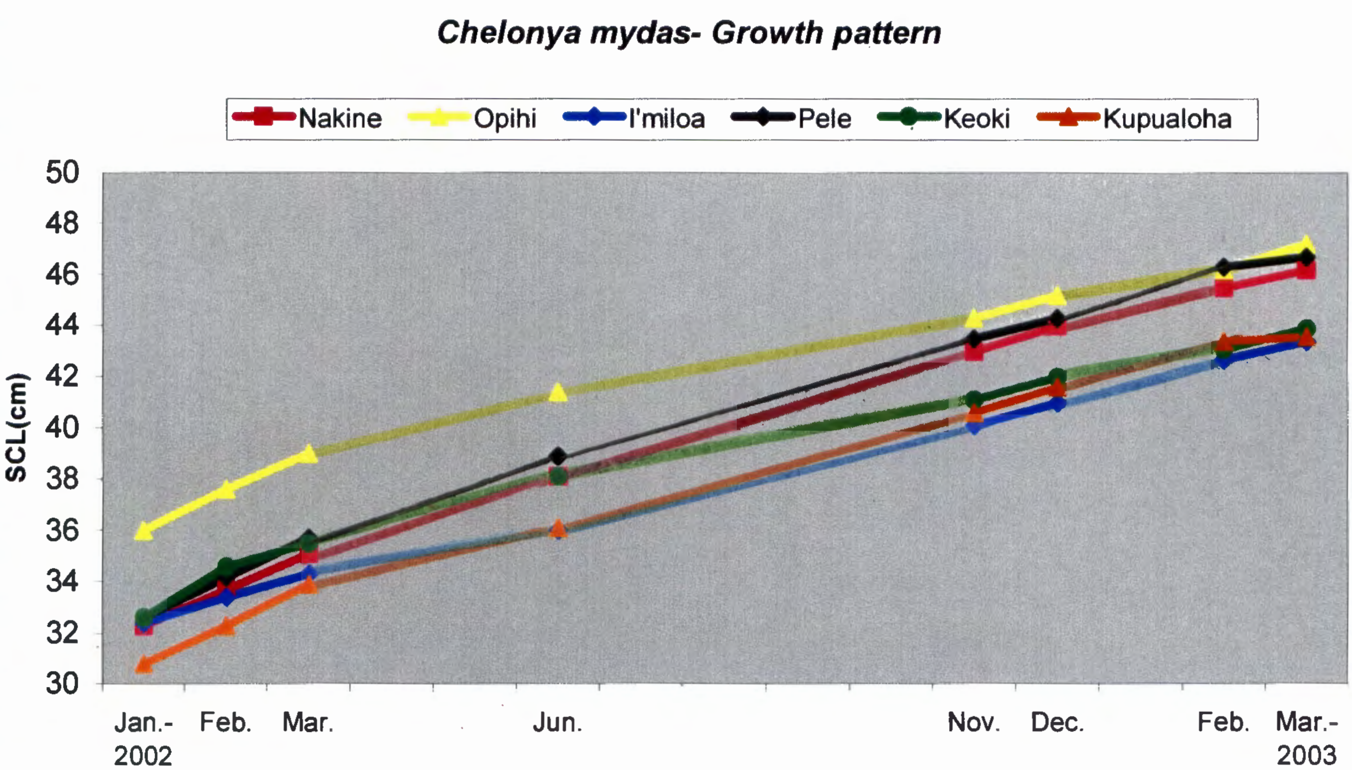
The mean increase for the total sample, for all morphometric data, including weight, are summarize in table 2. Note that the increases per month are averages based on the annual increase.

TABLE 2: Summary of growth in SCL, SCW, CCL, CCW and weight, monthly and annually. Note that SCL, CCL and CCW are respectively 0.1- 0.2 cm apart, while SCW is 1.7-1.9 cm apart from the other morphometric measurements (mean per year, excluding weight).

MEAN GROWTH	**SCL (cm)**	**SCW (cm)**	**CCL (cm)**	**CCW (cm)**	**WEIGHT (kg)**
In cm or kg / year	9.6	7.7	9.5	9.4	6.6
In cm or kg / month	0.8	0.6	0.5	0.8	0.6

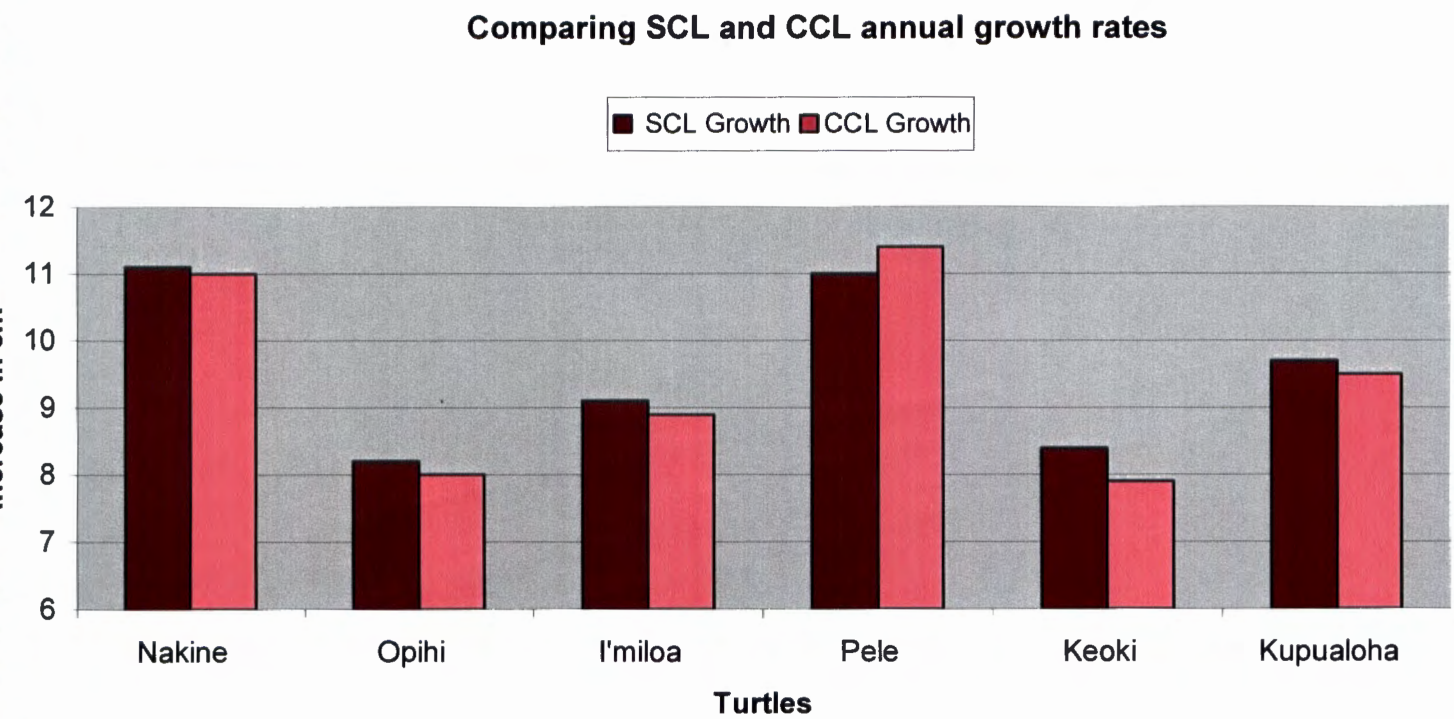
Looking at the SCL measurements recovered at different intervals from January 2002 to March 2003, it appears that all six Honu follow a similar growth pattern. Initially, in January 2002, Nakine, Pele, I'miloa and Keoki had very similar SCL values whereas Opihi was particularly bigger and Kupualoha was notably the smallest of all six. However, at the end of the study, in March 2003, the initial trend had changed and two subgroups became visible. Nakine and Pele had caught up with Opihi, together becoming the three biggest turtles in terms of SCL, while Keoki and I'miloa joined Kupualoha in the smaller turtles subgroup. Those trends are well represented in Figure 1. In fact, Opihi was initially 3.3 cm and 3.9 cm (SCL) larger then Nakine and Pele, respectively. By March 2003, Opihi was only larger then Nakine and Pele by 1 cm and 0.5 cm, respectively. Although it is clear that Opihi is the largest turtle in SCL for each measurement, from the start to the end, this turtle reveals the slowest annual growth rate (8.2 cm/year in SCL, from March 2002 to March 2003 - see table 1). Consequently, the initial size of the animal does not appear to affect its growth rate, i.e. Opihi has not grown faster due to its lager size in SCL all along.

FIGURE 1: SCL measurements for each animal, from January 2002 to March 2003. Note that two size subgroups develop over the course of the study.



When comparing annual increases in SCL and CCL, both values appear to be similar, varying only from 1-5 mm between SCL and CCL. Pele is the only animal whose CCL growth rate is higher (4mm) than the growth rate of the SCL; whereas all others have a slightly higher value for SCL growth rate. Figure 2 illustrates this pattern.

FIGURE 2: SCL growth versus CCL growth, for each turtle, from March 2002 to March 2003. Note that Pele is the only turtle whose CCL growth for the year is higher than its SCL growth.



COMPARING GROWTH RATES OF MOC CAPTIVE-REARED VS. WILD HONU

As mentioned before, green sea turtles do not have constant growth rates throughout their life, partly because of the change of diet of juvenile turtles from carnivorous to herbivorous. Therefore, the growth rates of wild or released and recaptured turtles are typically given accordingly to size-classes, referring to the SCL (Wood and Wood 1993, Balazs et al., 2001). In January 2002, the captive-raised turtles were all greater then 30 cm (SCL) and in March 2003, they all measured less then 50 cm. Hence, during that time, two different size-classes, 30-40 cm and 40-50 cm, were distinguished. Table 3 represents the growth rates of the turtles studied, based on size class. The size-class 30-40 cm corresponds to the period from January to June 2002 (6 months) while the size-class 40-50 cm is associated with the period from November-2002 to March 2003 (4 months). Note that this last period corresponded with the fish-free diet.

TABLE 3: Growth rates of each turtle, according to corresponding size-class. Note that the division of the time-periods corresponds to the change of diet.

TURTLES	GROWTH SCL SIZE-CLASS 30-40 cm (6 months)	AVERAGE CM /YEAR	GROWTH SCL SIZE CLASS 40-50 cm (4 months)	AVERAGE CM /YEAR
Nakine	5.8 cm	11.6	3.2 cm	9.6
Opihi	5.4 cm	10.8	2.9 cm	8.7
I'miloa	3.6 cm	7.2	3.3 cm	9.9
Pele	6.3 cm	12.6	3.2 cm	9.6
Keoki	5.5 cm	11.0	2.8 cm	8.4
Kupualoha	5.3 cm	10.6	3.0 cm	9.0
	MEAN GROWTH:	10.6 cm/ year	**MEAN GROWTH**	9.2 cm/year

For all turtles except I'miloa, the growth rates decreased from size-class 30-40 cm to size-class 40-50 cm; a decrease ranging from 1.6 to 2.6 cm per year. This is also the case for wild turtles (Balazs et al., 2002), showing a decrease of 1.4 cm per year, as table 4 illustrates.

TABLE #4: Growth rate statistics of Hawaiian green turtles by size-class. Note that the growth rate statistics were derived from spline-integration data (Balazs et al., 2002).

SIZE-CLASS SCL (cm)	MEAN GROWTH RATE (cm/year)	SD (cm/year)
20-30	4.4	2.2
30-40	3.5	2.7
40-50	2.1	1.2
50-60	2.3	1.0
60-70	2.2	0.9
70-80	2.1	1.0
80-90	1.3	0.5
90-100	0.6	0.3

For the size-class 30-40 cm, the average calculated growth rate of the captive-reared green turtles in this study is 10.6 cm/year, more than three times the growth rates of wild *C.mydas* (3.5 cm/year). For the size-class 40-50 cm, the growth rate of the turtles studied is 9.2 cm/year, more than four times the growth observed for wild turtles (2.1 cm/year). The difference in growth rate between captive and wild Hawaiian green sea turtles is therefore greater for the class-size 40-50 cm than for the 30-40 cm size-class.

NUTRITIONAL ANALYSIS

The following nutritional analysis of the turtle gel diet was performed by Food Quality Labs (Oahu) in February 2003.

Protein:	16.55%
Carbohydrates:	11.77%
Fat:	3.06%
Ash:	4.22%
Crude Fiber:	1.22%
Moisture:	64.40%

CALORIC CONTENT OF TURTLE GEL

The caloric content of the gel fed to the turtles was calculated based on the results from the nutritional analysis. The total calories per 100 g of gel are 141. The daily caloric intake per animal was calculated based on the average weight of one portion of gel (107.3 g). Considering that each animal gets two portion of gel food per day, the daily intake of calories is approximately 302 calories per turtle.

DISCUSSION

During their first year at Maui Ocean Center, the captive-reared turtles grew three to four times faster than wild Honu with the same corresponding carapace length. This difference in growth rates may be explained by the constant daily supply of food (turtles are fed four times a day) and by the high nutritional value of their diet (Protein, Minerals, Vitamins, Carbohydrates, Amino Acids, Fat and Fiber). Additionally, the energy that wild Honu usually spend searching for food, avoiding predators, and swimming long distances in high seas is energy that is not going toward growth. As an example, the only green turtle that was successfully tracked by satellite in the Pacific (Balazs, personal communication, 2003), Mauna Lani Honu No. 22270, was also hatched at Sea Life Park and held captive for the first years of its life. Upon released, the 3-year-old turtle traveled 3000 miles around the Hawaiian Island during its first 8 months of ocean life. According to Balazs, the turtle weighed 30 pounds when it left and 30 pounds when it returned. The fact that Honu No. 22270 did not lose weight proves that the turtle ate during its 8 months journey. Factors such as long migration, search for food and switching between pelagic and benthic habitats are missing from the life of captive-reared turtles.

When comparing the growth rates of the period during which the turtles receive fish products in their diet to the following fish-free period, a slight decrease in growth rates was noted, from 0.89 cm/month to 0.77 cm/month (table 4). As this decrease coincided with the change in the turtles food content, this decrease in growth rates may be related to the removal of fish products from the turtles' diet. Nevertheless, it is difficult to draw this conclusion because the removal of fish products was gradual and extended over a four-month period. However, according to Balazs (personal communication, 2003), the most critical factor for growth of green turtles is the protein content of their diet. Consequently, a decrease in protein level of the diet would be associated with a decrease in growth rate. A study on how various protein levels affect growth rates of captive green turtles has been done at the Cayman Turtle Farm (Wood and Wood, 1980). The study revealed a significantly different ($P<0.05$) weight gain among the 14 month-old turtles at the various protein levels (25%, 30% and 35%). The percentage of weight gain was more than double, when the protein content was increased from 25% to 35%. The author concluded that the differences in weight gain were likely due to the different protein levels.

The difference in growth rates between individual turtles in this study varies from 0.1 to 3.1 cm/year in straight carapace length (table 1). The behaviors of the turtles during feeding time is an important factor to consider when attempting to explain a difference of 3.1 cm (SCL) between the fastest growing turtle (Nakine) and the slowest growing one (Opihi). It has been often noted by the aquarium staff that Nakine appears more aggressive than the others and will even steal food out of the mouths of others. Since this behavior might indicate that Nakine received more food than the other turtles, it may account for the faster growth of Nakine. Accordingly, the animals exhibiting no sign of aggression toward each other during feeding seem to be the smallest turtles in SCL (I'miloa, Keoki and Kupualoha). Factor such as genetic variability may also explain the differences in growth rates between individual turtles in this study.

CONCLUSION

The study of growth rates of green sea turtles is an indispensable element to better understand the specie dynamics and ultimately, to offer a greater protection for the specie. However, the study of sea turtles growth based on individuals of known age is difficult because of the longevity of sea turtles, which prevents the collection of growth observations over reasonable time-periods. Moreover, green sea turtles are exposed to many growth conditions during their long life span; the change of habitat and the change of feeding behaviors with age are consequently influencing their growth patterns. Data presented in this study describe the growth rates of immature Hawaiian *C. mydas*, hatched and reared in captivity, over a year period. The results indicated that the turtles in this study grew three to four times faster than wild green turtles of corresponding sizes. It is likely that the rapid growth of captive-reared green turtles would result in the turtles reaching sexual maturity at a younger age. Age and size at first maturity are very important parameters in regards to conservation programs for endangered species; setting the critical frame during which survival is necessary to maintain reproduction of the specie. Future researches on growth rates of green turtles in parallel with the sex of the turtles, as well as the age at first sexual maturity of captive Honu, are yet to be explored. The continuous improvement of our knowledge of endangered species can only be beneficial and positive for the survival of those species.

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LITTERATURE CITED

- Balazs, G.H., Murakawa, S.K.K., Parker, D.M. and Rice, M.R. 2000. Adaptation of captive-reared green turtles released into Hawaiian coastal foraging habitats, 1990-99. Proceedings of the Twentieth Annual Symposium on Sea Turtle Biology and Conservation, February 29-March 4, 2000, Orlando, Florida. NOAA Tech. Memo. NMFS-SEFSC-477: 187-189.

- Balazs, G.H., Nakai, G. L., Hau, S., Grady, M.J. and Gilmartin, W.G.2001. Year 200 nesting of a captive-reared Hawaiian green turtle tagged and released as a yearling. In press. Proceedings of the Twenty-first Symposium on Sea Turtle Biology and Conservation, February 24-28, 2001, Philadelphia, Pennsylvania.

- Balazs, G.H., Wheterall, J.A., Zug, G.R., Parker, D.M. and Murakawa, S.K.K., 2002. Age and Growth of Hawaiian green sea turtles (*Chelonia mydas*): an analysis based on skeletochronology. Fish. Bull. 100:117-127.

- Ehrhardt, N. M. and Witham, R. 1992. Analysis of growth of the green sea turtle (*Chelonyia mydas*) in the western central Atlantic. Bull. Mar. Sci. 50(2): 275-281.

- Bjorndal, K. 1985. Nutritional ecology of sea turtles. Copeia: 736-751.

- Wood, J.R. and Wood, F. E. 1981. Growth and digestibility for the green turtle (*Chelonia mydas*) fed diets containing varying protein levels. Aqua. 25: 269-274.

- NOAA and State of Hawaii. 2002. Hawai'i's marine protected species. The laws and regulations for federally protected marine resources.11-13.