Photographic Evidence for the Regression of Fibropapillomas Afflicting Green Turtles at Honokowai, Maui, in the Hawaiian Islands

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My wife, Ursula, and I spend each July and August diving in the waters of Honokowai, West Maui, Hawaii. She's a teacher and I'm a technical writer, and we spend most of our dives watching, photographing, and videotaping Hawaiian green turtles, or *honu*. We can do this because the honu are among the tamest of nature's wild creatures.

We started in 1988, when we met Clothahump, our first sea turtle. By 1990, we had explored Reef 2 and discovered the Turtle House; the two places where turtles congregate at Honokowai. Many of the turtles we saw had fibropapilloma (FP), so we reported this to George Balazs of the NMFS Honolulu Laboratory. George provided us with background information about the disease. From the material he supplied, this quote from Herbst (1994) stood out:

"The number of turtles that develop GTFP in the wild over time (incidence) and the proportions of affected turtles that develop severe disease and die (morbidity and mortality) are unknown. These data are desperately needed if we are to understand the demographic impact of GTFP on wild populations"

We were concerned about the turtles. We wanted to do something. This quote spoke to us because it meant that by turtle watching, we might be able to help.

Methods

We went on to take nearly 4000 pictures and over 150 hours of videotape. In 1997, George reminded us that it was time to quantify things, so we built a database. Then, with George's guidance and help, we set out to analyze this material.

To tell the turtles apart, we relied on the patterns formed by the scales on their faces and their profiles. This pattern allows us to identify individuals from year to year. For each individual, we recorded their profiles, their sexual maturity, and if present, their tag information.

For every year that we saw a turtle, we also recorded a count of tumours by size for external surfaces of the turtle, an overall estimate of the prevalence and severity of FP, and an estimate of the size of the turtle.

To count and evaluate tumours, we used the method developed by Balazs (1991) with the help of John Sundberg, and later refined by Work & Balazs (this volume). This system places tumours in four sizes: A is anything less than 1 cm in diameter, B includes tumours from 1-5 cm, C tumours are from 5-10 cm, and D includes anything over 10 cm.

The method also includes an overall score for the turtle. This is a subjective estimate based on the total number and size of tumours. There are three categories for tumoured turtles: light, moderate, and heavy.

We were keen to assess the impact of FP on juvenile turtles. To do this, we followed size assignments used by Balazs for honu since 1973: juvenile (post-pelagic up to 65 cm), sub-adult (65 up to 85 cm), and adult (larger than 85 cm).

Although we don't take measurements in the wild, photos do let us make estimates by comparison with objects of known size.

Because our data are gathered from images, it isn't possible to get a complete assessment for an animal. For example, we can't check whether a turtle has tumours deep inside the mouth. Sometimes we have a record of only part of a turtle. Our data, therefore, are skewed in a manner that *underestimates* the occurrence of FP.

We define regression as follows: if a tumour has gotten smaller or become undetectable in our photos and video, we call that regression. We first noticed regression in Tutu, who had a size B eye tumour in 1990. By 1993, however, her eye tumour had almost disappeared. Finally, our photos from 1997 leave no doubt that the tumour has vanished.

Since 1996, we've seen more and more animals that show some regression. The question, therefore, was how many tumoured Honokowai turtles were regressing?

Results

We identified 247 turtles with 158 (64%) having had FP at some point. 37% (91) have been seen in more than one summer. We consider these turtles to be Honokowai residents, and we call them "resights." Of the resights, 73% (66) have had FP and 32% (21) of those have regressed.

Of these 21 regressed animals, we have presumed 52% (11) are sexually mature. Three are females that were tagged while nesting at the French Frigate Shoals. The other eight are males, judged by the fact that their tails have grown beyond their hind flippers.

As judged by size, 17% (43 of 247) turtles were classed as juveniles at some point in our records. 60% (26) of these 43 had FP. Nineteen of the juvenile turtles have been resighted. So far, 74% (14) of them have eventually developed FP. We have documented regression for only one resighted juvenile.

Discussion

Course of the disease: In 81% (17 out of 21) regression cases, we saw the turtle get worse before it got better. Infected turtles had light tumours at the onset, got visibly worse in the second summer, and peaked in the third. In turtles that showed regression, tumour growth either stabilized or reversed itself after the third summer. This was usually followed by two summers of steady regression, after which tumours often became undetectable by examination of our images. We have documented this level of regression in 11 cases; all animals that had A and B sized tumours only.

This leaves 10 cases in which the tumours can still be seen. In 7 of these cases, regression is still underway. The other three cases had tumours that reached size C, however.

While these C-sized tumours have regressed significantly, it appears that they might not disappear completely.

Tumour scoring is a reliable indicator: The subjective tumour score does help to predict outcomes. Of the 66 resighted turtles that have had FP:

- 53% (n=35) were lightly afflicted. Of our 21 regression cases, 72% (n=15) were scored as light.
- 33% (n=22) were moderate. 29% (n=6) of the 21 regression cases were moderate.
- 14% (n=9) were scored as heavily afflicted. No turtle scored as heavy has ever regressed.

Moderately afflicted adults can recover: Tiamat is the most severely afflicted adult that we've seen regress. We've known her since 1992, when she was fine. For 1994, however, we classed her as moderately afflicted. Unexpectedly, by 1995 her condition had improved. In 1996, we happily added her to our list of regression cases. We didn't see her in 97, but in 98 she bore an engraved V40 on the right side of her carapace—proof that she had nested at the French Frigate Shoals in 1997.

Only one juvenile has ever regressed: Another moderate case is our most interesting and remarkable story. Kamaha'o is Hawaiian for "remarkable." This is the only juvenile turtle that we have ever seen regress. In 1994, Kamaha'o had eye and mouth tumours, and was notably emaciated. This turtle looked to be doomed. In fact, we're so accustomed to youngsters disappearing on us that we didn't immediately recognize this robust turtle, photographed in 1997, as the tumoured little beast from 1994. It was only in the preparation of this paper that we made the connection. This showed us that FP does not have to be a death sentence for juveniles.

Juveniles affected most severely: Nevertheless, our data show that regression clearly favors larger turtles. To recap: only 5% (1 of 21) regression cases was a juvenile (based on size), while 21% (14 of the 66) resighted turtles that have had FP were juveniles at some point. While our sample is small, this hint that FP cuts a deep swath through the little ones is echoed in data from Kaneohe Bay, Oahu, Hawaii. There, most turtles sampled are juveniles and the FP regression rate is only 4.5% (Balazs *et al*. 2000).

Males vs. females: At first, the regression rate for subadult and adult turtles seems encouraging. 38% (n=20) of 52 larger resighted turtles that have had FP have regressed. It's instructive, however, to look at the 11 sexually mature turtles that we identified in the regression cases: eight of them are male. If our data are typical, almost three times more males are recovering than females. This reflects work done by Koga and Balazs (1996), who report significantly higher FP in female honu based on necropsies of hundreds of stranded animals.

Potential population impact: Since we have no other data to compare, we don't know if what we are seeing is typical of the broader FP picture. We hope it isn't, but if it is, we have a disease that:

- devastates young turtles (juvenile size class) and
- in the larger size classes, exhibits some recovery but favors males by a factor of three.

The implications for the honu are troublesome.

Environmental concerns/Potential for future study: Recent findings by Landsberg *et al*. (In press) have suggested there is preliminary and provocative evidence linking high concentrations of dinoflagellates (specifically *Prorocentrum*) to a high prevalence of fibropapilloma. One of the sites they sampled for these organisms was Honokowai.

Early findings suggest the honu might be suffering from chronic exposure to okadaic acid produced by the dinoflagellates living in the seaweeds that the honu use for food. It is difficult to watch turtles eat when we know it is possible that they are slowly poisoning themselves. Still, we continue to document their foraging habits. For one thing, we hope to understand why the disease affects the little ones so profoundly.

Of course, if FP is indeed fueled by eutrophication, which in turn is caused by run-off, sewage, animal wastes, and the destruction of wetlands, we are forced to wonder about the future of any animal that is dependent on clean water.

Finally, as if the current state of the coastal waters isn't worrisome enough, a new storm cloud blew into Honokowai last summer. Tutu (Hawaiian for grandmother), known since 1990 and our first regression case, showed troubling white anomalies on her neck and shoulders.

While this might be the beginning of a dose of a new type of barnacle we've been seeing on the turtles only recently, or perhaps something else entirely, we cannot rule out the possibility that this could be the harbinger of the return of FP.

Summary

In summary, we have collected a considerable body of photo documentation of the prevalence of FP in a community of Hawaiian turtles. Through retrospective examination of this data, we have shown that about one in three diseased turtles has regressed, but a closer look at exactly which turtles regress reveals disturbing patterns: juveniles rarely regress, and recovering males outnumber females three to one. Finally, there is one last sobering thought—Tutu's condition has raised an ugly, troubling question: is regression permanent?

Acknowledgments

Dr. Larry Herbst, Richie Moretti, and Dr. Grahame Webb for their encouragement and support.

Literature Cited

Balazs, G.H. 1991. Current status of fibropapillomas in the Hawaiian green turtle, *Chelonia mydas*. In G.H. Balazs and S.G. Pooley (eds.), Research plan for marine turtle fibropapilloma, p. 47-57. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SWFSC-156.

Balazs, G.H., S.K.K. Murakawa, D.M. Ellis, and A.A. Aguirre. 2000. Manifestation of fibropapillomatosis and growth rates of green turtles in Kaneohe Bay, Hawaii. In: Proc. 18th Ann. Symp. Sea Turtle Biol. Conserv., March 3-7, 1998, Mazatlan, Mexico. U.S. Dep. Commer. NOAA Tech. Memo. NMFS-SEFSC-436.

Herbst, L.H. 1994. Fibropapillomatosis of marine turtles. Annu. Rev. Fish Dis. 4:389-425.

Koga, S.K. and G.H. Balazs. 1996. Sex ratios of green turtles stranded in the Hawaiian Islands. In J.A. Keinath, D.E. Barnard, J.A. Musick, and B.A. Bell (comps.), Proc. 15th Ann. Symp. Sea Turtle Biol. Conserv., February 20-25, 1995, Hilton Head, South Carolina, p. 148-152. U.S. Dep. Commer. NOAA Tech. Memo. NMFS-SEFSC-387.

Landsberg, J.H., G.H. Balazs, K.A. Steidinger, D.G. Baden, T.M. Work and D.J. Russell. In press. The potential role of natural tumor promoters in marine turtle fibropapillomatosis. Journal of Aquatic Animal Health 11:199-210.

Work, T.M. and G.H. Balazs. This volume, poster presentation. Quantification of tumor severity and hematology in green turtles afflicted with fibropapillomatosis in the Hawaiian Islands.

Marine Turtle Fibropapillomatosis: Hope Floats in a Sea of Ignorance

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It has been 60 years since the occurrence of fibropapillomatosis (FP) in marine turtles was first reported in the scientific literature (see Herbst 1994) and yet only in the past 10 years, as FP has become recognized as a growing threat to these endangered animals, has significant effort been focused on trying to understand the nature of this disease. Yet in this relatively short period of time a tremendous amount of progress has been made toward understanding the cause of this condition, and it is my hope that such progress can continue. Much more, however, remains to be learned about this disease before practical and effective management strategies can be developed and each answer unleashes a cascade of new questions that must be addressed. This presentation reviews what has been learned about FP from observation and experimental studies in order to establish a foundation upon which to frame future studies and management plans.

Most of what we can say conclusively about FP has been gained from a series of experimental transmission studies in green turtles (*Chelonia mydas*). We know from these controlled experiments that FP is a transmissible disease and that tumors contain an infectious agent that can be transmitted to other turtles through skin injection or scratch inoculation (Herbst *et al.* 1995). We know that this agent is very small, is found in the cell free (filterable) fraction of tumor tissue homogenate and that it is inactivated by organic solvents, which strongly suggest that the causative agent is an enveloped virus (Herbst *et al.* 1996b). We also have observed in two cases, spontaneous horizontal transmission from tumor-bearing turtles to naive turtles following co-housing involving extensive physical contact.

These transmission experiments also have implicated a novel tumor-associated herpesvirus in the disease, since herpesvirus replication and shedding was detected in tumors of donor animals and in experimentally induced tumors (Herbst 1994, Herbst *et al.* 1999). In addition, all turtles that developed experimentally induced tumors also developed anti-herpesvirus antibodies (seroconversion) (Herbst *et al.* 1998). The association of this herpesvirus with FP has been confirmed by PCR in a number of studies (see Herbst *et al.* 1999). This herpesvirus remains the main candidate for the etiology of FP and since my first reports of these findings and preliminary genetic analyses of this herpesvirus (Herbst *et al.* 1996a) a number of independent laboratories have focused their efforts on trying to isolate and characterize this virus further. Transmission of FP with purified virus remains the only one of Koch's postulates that must be fulfilled to prove that this virus is the etiologic agent of this disease.

It is evident from experiments that apparently healthy individuals are susceptible to infection and that they need not be debilitated or immune suppressed to develop tumors. It is my opinion that a virus, perhaps the FP-associated herpesvirus, is necessary and sufficient to cause FP in marine turtles. This conceptual model, however, does not preclude the involvement of a variety of cofactors in modulating the severity of the disease and affecting whether or not individuals recover or succumb to it (Herbst and Klein 1995).

If this model of FP as a viral infectious disease is accepted, then the questions that become important in developing practical management strategies to limit the impact of this disease relate to understanding the ecology of this virus. For example, whether or not the virus is shed continuously or intermittently from infected turtles or their tumors, whether or not virus can remain infectious for long periods in the environment outside the host, and whether or not there is an environmental or biological reservoir other than turtles, have tremendous implications for the success of control strategies that rely on isolation or removal of turtles with FP. Ultimately, it may be found that there is no practical way to manage this disease in wild populations short of widespread vaccination.

The prospect that it is impractical to control this disease in wild populations or that the opportunity to bring it under control has been lost, now that it is pandemic (Herbst 1994), must lead us to ponder the long term impact of this pandemic on marine turtles species. Here again, we lack sufficient epizootiologic data, information about the behavior of this disease in populations to generate predictive models. We know that this disease kills some turtles outright and we also know that some turtles recover. We also know that the disease course is prolonged and that, while they are affected with FP, turtles are more susceptible to other mortality factors such as predation, entanglement, and starvation. The long term effect of this pandemic on

NOAA Technical Memorandum NMFS-SEFSC-443

PROCEEDINGS OF THE NINETEENTH ANNUAL SYMPOSIUM ON SEA TURTLE CONSERVATION AND BIOLOGY

2-6 March 1999
South Padre Island, Texas, U.S.A.

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September, 2000