Copeia, 1994(3), pp. 684–690

Cleaning Symbiosis between the Wrasse, *Thalassoma duperry*, and the Green Turtle, *Chelonia mydas*

George S. Losey, George H. Balazs, and Lisa A. Privitera

A previously undocumented association between green turtles and wrasses is reported from several sites in Hawaii. Observations from videotaped encounters at common turtle aggregation sites indicated that turtles exhibited a solicitation posture for cleaning of ectoparasites by wrasses (*Thalassoma duperry*). Time-lapse activity budgets revealed at least one turtle present nearly half of the time and posing for cleaning over one-third of the time. Gut contents of wrasses captured from these cleaning stations revealed that some of the fish had specialized to feed exclusively on ectoparasitic barnacles from the skin of turtles. No barnacles were found in the gut contents of wrasses caught more than 10 m from the cleaning stations. We propose several possible explanations for the development and adaptive significance of this symbiotic relationship, particularly with respect to the recent epidemic outbreak of fibropapilloma or "tumor" infections in green turtles in Hawaii.

WE report an apparently specialized cleaning relationship between green turtles, *Chelonia mydas*, and some individuals of a carnivorous wrasse, *Thalassoma duperry* (Labridae), in Hawaii. Cleaning symbiosis is usually defined as one organism, a cleaner, feeding on the body surfaces of another and at least occasionally ingesting ectoparasites (review in Losey, 1987). Reports of fishes cleaning or grazing upon sea turtles usually document either herbivorous fishes cleaning the shells of turtles or seemingly facultative and incidental feeding on turtle skin (Limpus, 1978; Balazs, et al., 1987; Smith, 1988). Booth and Peters (1972) reported removal of parasitic barnacles from the skin of green turtles in Australia by the wrasse, *T. lunare*, but had no information on the frequency of the relationship. Our results suggest that some individual wrasses specialize both their diet and behavior to exploit parasitic barnacles on sea turtles. It is urgent to explore such relationships because an epidemic outbreak of fibropapilloma or "tumor" infections recently has occurred on turtles in Hawaii and Florida and poses a severe threat to their survival (Balazs and Pooley, 1991).

Methods

Two turtle cleaning stations about 2 km apart in Kaneohe Bay, Oahu, Hawaii, were studied in detail. Both sites were easily disrupted by divers. At site I, a remote-controlled video system was used to observe and verify cleaning. However, the turtles appeared to be wary of the camera, perhaps because of the large size of the unit that projected a meter above the bottom. Only one turtle came close to the camera for a total of 49 min over two days of monitoring. A less obtrusive miniature wide angle time-lapse video camcorder was placed at sites I and II and at three control sites that were within 50 m of site I and of similar topography. Less extensive observations and videotapes of several other sites throughout Hawaii were donated by recreational divers, especially several years of videos and field observations at Honokowai, Maui, taken by U. Keuper-Bennett.

One second of time-lapse video was taken every 15 sec between 1000 and 1500 h. An event recorder was used to make an "on-the-dot" time sample of the tapes by scoring a count of the number of time-lapse samples that had no turtle present, at least one turtle present, and at least one turtle posing for inspection by fishes. Because the lag between samples was much less than the several minutes that turtles normally remain on the station, the on-the-dot time sample should be an accurate estimate of the true time budget (Tyler, 1979).

Thalassoma duperry were speared or captured with a barrier net. Seven fish were taken within 10 m of site I and eight from site II. One was taken 20 m from site I and eight fish from 50–100 m from the station but on the same reef. Capture efforts concentrated on the largest individuals (109–164 mm SL). Their gut content items of widely disparate sizes were quantified at 60× magnification. Relative mass of different food types was estimated by counting the number of times that food items crossed (hit) a line across the center of the ocular field. Larger items were sampled by multiple hits. After each count, the dish was shaken to rearrange its contents. Counts continued until at least 100 hits had been scored. For some nearly empty guts, a complete count was made with less than 100 hits. The size of parasitic barnacles was measured with an ocular micrometer. Diet diversity was estimated by Shannon's information estimate, H. Barnacles were removed from several areas of the skin of two turtles captured in Kaneohe Bay and measured in a similar manner to establish the size distribution of the "prey" population.

Worldwide, *C. mydas* is an endangered species. Sampling that could have any effect on the turtles was limited to the minimum requirement for statistical assurance. All handling of turtles was conducted by National Marine Fisheries Service personnel (GHB).

Results

Kaneohe Bay cleaning station.—Turtles at both sites showed apparently cooperative posing behavior (fig. 4 in Booth and Peters, 1972) that typifies cleaning in fishes (Losey, 1987). Swimming ceased and flippers were usually fully extended and drooped downward in a relaxed position. The neck was often fully extended and arched upward or downward. Approach and, especially, feeding bites by fishes on the skin produced seemingly cooperative responses in which the cleaning site was more fully exposed. The single turtle in the real-time video was in cleaning posture for 58% of the time that it was visible.

Turtles were very common on the cleaning stations in the time-lapse videos and more common than in the control areas ($P < 0.05$, rank sum test; Table 1). There were up to four turtles visible at a time and at least one turtle visible about half of the time and posing one-third of the time. Individuals could not be identified. Turtles with tumors were seen at both stations by divers but could not be discriminated on the videos.

Thalassoma duperry cleaners were characterized by common inspection and feeding on the skin surfaces of the turtles. When the single turtle to approach the real-time video camera was visible (49 min of tape), from one to three *T. duperry* were inspecting or cleaning for 43% of the time. The wrasses showed forceful and singular feeding bites apparently aimed at particular items and concentrated on the skin areas (Table 2). Two of the three control sites included fish cleaning stations occupied by the Hawaiian cleaning wrasse, *Labroides phthirophagus*, but it was never observed to approach or clean passing turtles.

Herbivores, usually *Ctenochaetus strigosus*, cleaned only 6% of the time. They differed from *T. duperry* in that they fed largely on the shell surfaces and only occasionally appeared to

TABLE 1. TIME BUDGETS FOR CLEANING STATION SITES I AND II AND THREE CONTROL SITES. Data are from the time-lapse video as the percentage of the on-the-dot samples in which at least one turtle was visible, at least one turtle was posing, and the number of minutes covered by the time-lapse sample.

Location	Turtle visible (%)	Turtle posing (%)	Sample duration (min)
Site I	40	34	80
Site I	46	37	117
Site I	47	34	134
Site II	60	36	91
Control	2	0	42
Control	0	0	57
Control	5	0	64

"stray" onto areas of skin ($P < 0.01$, chi-square, 2×2 comparison of skin vs shell areas; Table 2). The acanthurids also maintained their typical algal grazing feeding pattern (tightly clustered bites) when feeding on the skin.

Five of the 15 *T. duperry* captured on the cleaning stations apparently specialized on parasitic turtle barnacles, *Platylepas hexastylos*, only known to occur on the skin of sea turtles, manatees, and dugongs (Schwarz, 1960; Monroe and Limpus, 1979; Table 3). As many as 35 barnacles were found in a single fish. Individuals captured at distances greater than 50 m from the stations differed in that they lacked barnacles ($P \cong 0.05$, chi-square, 2×2 comparison for presence of barnacles in gut contents, controls vs cleaning stations) and fed primarily on benthic invertebrates with a somewhat higher diversity of items in their gut ($0.10 > P > 0.05$, rank sum test for median H values, cleaners = 1.1, controls = 1.4).

The size of ingested barnacles over their longest axis (2.1–5.0 mm, mean = 3.78, SD = 0.69, n = 46) was similar to that sampled from living turtles (1.7–6.2 mm, mean = 5.49, SD = 0.76, n = 85). Turtles varied widely in the number of barnacles found. Three turtles sampled had 195–490 barnacles on their skin. Barnacles were not deeply embedded in the skin of the turtle and lacked any special parasitic extensions into the turtles' tissues. However, the surrounding skin usually adhered to each barnacle as it was removed. Fresh barnacles in the gut contents similarly were surrounded by turtle tissue of about equal mass to the barnacle. One fish had removed two adjacent barnacles with the associated tissues in a single bite on three occasions.

Other sites.—Observations at other sites suggest that the *T. duperry* in Kaneohe Bay are unique.

TABLE 2. DISTRIBUTION OF FEEDING BOUTS BY FISHES ON THE BODY OF A TURTLE AS A COUNT FROM UNDERWATER VIDEO RECORDINGS.

Fish species	Location of feeding bouts: Flippers	Shell	Head and neck	Anal
Thalassoma duperry	15	0	4	4
Ctenochaetus strigosus	4	14	0	0
Scarus sp.	1	0	0	1

On one occasion at a south-shore Oahu turtle "resting site," *T. duperry* were observed biting at the head and around the eyes of a turtle while various fishes were grazing on the turtle's shell. The turtle reacted strongly to the wrasses, striking at them with the front flippers and apparently trying to bite them (R. Brock, pers. comm.). Videotapes and four years of observations of turtles at Honokowai, Maui (U. Keuper-Bennett, pers. comm.), included many instances of cleaning by T. duperry. The wrasses and the sharp-nosed puffer, *Canthigaster jactator* focused their cleaning on the eyes, tumors, and other "white spots" in the head region. Apparent feeding bites by both fishes produced an obvious response by the turtle as though to a painful stimulus and were often followed by the turtle leaving the area.

Other cleaning stations also differed in having a strong predominance of cleaning by herbivorous fish that concentrate their attention on the turtles' shells. Turtles are typically cooperative at these stations. A survey of divers in the community and several videos revealed the location of many such stations. A videotape supplied by M. Rice (Hawaii Preparatory Academy) showed unusually frequent feeding on the head and neck skin in addition to the shell by the surgeonfishes, *C. strigosus*, *Acanthurus nigrofuscus*, and *Zebrasoma flavescens* on turtles at Puako, Hawaii. However, the feeding bites were typical of herbivores repeatedly scraping a small area, not the action patterns required to remove barnacles or tumor tissue. The turtles did not avoid the approaches of these fishes toward their head.

DISCUSSION

New cleaning symbiosis?—In Kaneohe Bay, T. *duperry* displays a unique turtle cleaning specialization that may impact the cleaning of turtles in general and their overall well-being. Some individual wrasses specialize in feeding almost exclusively on ectoparasitic barnacles from turtle

TABLE 3. GUT CONTENTS OF *Thalassoma duperry* COLLECTED AT CONTROL SITES AT LEAST 10 m DISTANT FROM CLEANING SITE I OR ON CLEANING SITES I AND II. Data are the counts of the number of line crossings (see text) of each type of food item, the total number of line crossings, the diversity (H) of the gut content categories, and the standard length (mm) of the fish.

Food category	Control sites								
	A	B	C	D	E	F	G	H	I
Barnacle									
Crustacean	90	80	104	114	32	57	105		31
Mollusc	37	16	3		29	1	6		3
Echinoderm							28		
Worm				4			1	1	
Nudibranch									
Fish egg					55	22			64
Fish scale		1						5	1
Algae						8			
Unknown	7	5	7	9	5	13	2	94	13
N	134	102	114	127	121	101	142	100	112
H	1.2	1.0	0.51	0.57	1.7	1.7	1.1	0.37	1.5
SL	127	131	131	138	140	150	151	164	147

	Cleaning sites														
	I	II	II	I	II	II	I	II	I	II	II	I	II	I	I
Barnacle	70							73					99	72	47
Crustacean	6	28	60	44	40	60	3		50	68	37	1			2
Mollusc		24	3	14	20	5	34	17		7	10	6		1	10
Echinoderm						23				2		73			
Worm		5		14	4		4	4			1				1
Nudibranch		10	13		17	4					13				
Fish egg													1		
Fish scale								3							
Algae		5	2		1	2			34	1					
Unknown	22	7	21	17	22	8	50			24	44	11		3	5
N	100	79	99	89	104	100	96	101	84	102	105	91	100	76	65
H	1.1	2.3	1.6	1.8	2.1	1.8	1.4	1.1	0.97	1.4	1.8	0.95	0.08	0.34	1.3
SL	109	113	114	118	118	121	125	125	129	130	132	133	136	143	143

skin. Cleaning stations are characterized by having wrasses' inspection and cleaning of turtles' skin being far more common than the usually widespread grazing on the shells by herbivores. The wrasses have probably learned to recognize the barnacles as a food supply and how to remove this food in a manner that promotes cooperation and apparent solicitation by the turtles. Turtles in Kaneohe Bay likely have learned to recognize *T. duperry* as cleaners and the location of the cleaning stations where the cleaners aggregate. The "posing" behavior by turtles is identical to their interactions with shell-cleaning herbivores and is common in fish cleaning symbioses.

This stands in sharp contrast with observations at Honokowai and on the south shore of Oahu. Here wrasses and sharp-nose puffers appear to feed on tumors and white spots on the skin and are strongly avoided by turtles. Cleaning by wrasses and other nonherbivores is rare at all sites found outside of Kaneohe Bay and, at least at some sites, includes painful biting by the cleaner.

Evidence suggests Kaneohe Bay wrasses have learned to exploit this parasitic food supply only recently and that the habit is now spreading through social transmission. The first clue is that, at least within recorded time, the barnacle food source is only recently abundant. We can make gross estimates of the availability of turtle barnacles as food for specialized cleaners. Green turtles have increased greatly in abundance in Hawaii over the last several years as a result of successful conservation efforts. In 1989, at least 500 turtles inhabited Kaneohe Bay (Balazs et al., 1993) compared to perhaps as few as 50 in 1979. Using our best estimate of the number of barnacles per turtle (380), a 30-day period for a barnacle to grow to edible size (L. Walters, pers. comm.), and a daily ration of 70 barnacles per cleaner (twice the maximum number found), it should take at least five turtles to form a sustainable food supply for a single cleaner. These numbers indicate that there are probably fewer than 100 cleaners in Kaneohe Bay, and, with far fewer turtles available in the past, these barnacles would not have formed a reliable food supply.

The opportunistic nature of *T. duperry* and the plasticity of its feeding habits argue for the likelihood of its learning to specialize on this food supply. It is a very abundant fish on Hawaiian reefs and ". . . is consistently the first fish to appear when a sea urchin has been crushed, or when a rock has been overturned and vulnerable organisms exposed" (Hobson, 1974). Gut content analyses indicated each individual as somewhat different even though they all came from the same microhabitat. The relatively large size of the barnacles and the lack of a mucus food supply on turtles make it unlikely that highly specialized cleaners such as *L. phthirophagus* would adopt turtles as hosts (Gorlick, 1980, 1984).

Geographic isolation of a habit suggests a local origin of the behavior. The two cleaning sites in Kaneohe Bay are on the same shallow reef bench, and *T. duperry* frequently migrates between reefs separated by mud bottom up to 15 m deep (Brock et al., 1979). To date, we have no evidence of similar behavior from any other location in Hawaii.

Final persuasion is provided by the ubiquitous nature of learning in cleaning symbiosis. Hobson (1971) found similar individual specialization in a California fish cleaner, the senorita, *Oxyjulis californica*. Only some individuals of this species concentrated on cleaning, and these individuals each specialized on a certain species of host fish. Even highly specialized cleaners such as *Labroides dimidiatus* appear to learn how and where to clean each species of host (Potts, 1973). Host fishes may depend largely on learning of cleaners that is reinforced by rewarding tactile stimulation provided by the cleaners (Losey and Margules, 1974; Losey, 1979).

Why is this cleaning habit rare?—It is puzzling that such cleaning behavior is not found at all underwater turtle aggregating areas. Turtles with barnacles are commonly found quiescent on the bottom at common turtle resting sites (GHB) and, if above estimates are correct, present a potentially rich resource. Why is it usually only the herbivores that feed on the turtles and then only at special sites within the aggregating area?

Some cleaning stations may lack a sufficient number of turtles to sustain specialized barnacle cleaners. We estimate that five to 10 turtles "resident" at a cleaning station are required as a stable food source for a single specialized cleaner. But we find it difficult to accept that *T. duperry* would not discover and prey heavily on such a food supply and routinely overshoot the maximum sustainable yield.

Must the prospective barnacle-feeding fish learn special methods to remove the barnacles without causing pain to their host? We doubt this possibility because the barnacles are so easily removed. However, the observation of turtles responding aggressively or with avoidance to cleaning by *T. duperry* at Honokowai suggests that cleaners might have to learn to avoid the region of the eyes and to avoid biting at tumors or white spots on the skin. We have not observed any special methods of delivering tactile reward to the turtles as is common among fish cleaners (Losey and Margules, 1974; Losey, 1979).

Do most fish fail to recognize the barnacles as food items on the mottled skin surface of the turtle? Even if this is true, it is difficult to understand why the majority of the individuals in such a highly social and opportunistic species as *T. duperry* would not have acquired the habit through social transmission. Have we only now begun to notice the relationship since turtle sightings have become far more frequent in Hawaii? The strikingly different behavior of wrasses between Kaneohe and Honokowai suggest the former: *T. duperry* are exploiting a formerly rare resource and, in geographically isolated areas, have adopted very different tactics and targets for their feeding.

Impact of cleaning on turtle survival.—On many reefs, turtles interact with herbivorous fishes in a manner reminiscent of fish-cleaning stations (Eliot, 1978), but the fishes show little specialization for cleaning. Turtles may receive a tactile reward from this behavior similar to fish cleaning hosts (Losey and Margules, 1974; Losey, 1979), but the herbivorous grazers merely interrupt their bottom feeding and shift the same behavior to the backs of turtles.

Special traditional cleaning stations are, however, common, contrary to the report by Limpus (1978). At one site off Waikiki beach, defined by a prominent coral head, the cleaning station remained in the same geographic location after the coral head had been rolled some distance away by storm seas (GHB, pers. obs.).

Removal of algae and barnacles from the shell of turtles is likely advantageous because of drag reduction. Green turtles inhabit epipelagic waters prior to taking up residency in near shore benthic habitats in Hawaii at 35–45 cm carapace length. New recruits to coastal zones differ from residents in their complete absence of fouling organisms such as barnacles and algae (GHB). Turtles lack any method for discouraging algal growth other than rubbing against objects and sweeping the dorsal and ventral surfaces with their front flippers and might quickly acquire a drag-inducing load of algae. Turtles in Honokowai with tumors that were discouraged from visiting cleaners because of their painful biting carried noticeably more algae, and their shells had a fouled appearance.

The significance of removal of skin parasites is more speculative. Choy et al. (1989) thought that visits to cleaning fish might help to control infections by a piscicolid leech that parasitizes and deposits egg masses on turtles. Unfortunately we know nothing about any irritation caused by skin barnacles or whether they cause any impediment to movement by the turtle. These barnacles lack special parasitic processes that could tap into the turtle as a food source and appear to depend on the turtle only as an attachment site. On the other hand, removal of the barnacle leaves a shallow depression that could possibly lead to secondary infections. Another barnacle, *Stephanolepas muricata*, less common on turtles in Hawaii, burrows into the anterior margins of the front and hind flippers. This barnacle is more deeply attached and, when removed, can produce a bleeding wound.

Cleaning symbionts must be included in the list of factors that should be studied in attempts to discover causes and cures for the current fibropapilloma epidemic. Causes for the epidemic outbreak of fibropapillomas or "tumor" infections in green turtles in Hawaii and Florida have eluded researchers (Jacobson, et al., 1989; Balazs and Pooley, 1991). Viral and trematode infections are among the candidates as causal agents, and cleaning fish might provide sites for infections by opening small wounds in turtles. Cleaners might even serve as a carrier or vector for the agent that causes the fibropapilloma. Hobson (1971) found that individual senorita, *O. californica*, a California cleaner fish, harbored a similar set of ectoparasites as the species of host that they had been cleaning. The importance of these questions is underscored by current tagging studies that indicate tumor infection rates as high as 50% at Honokowai and Kaneohe Bay (GHB, unpubl.).

Acknowledgments

We thank M. L. Hetland, R. L. Pyle, B. Winton, R. Miya, and S. Koga for field and laboratory assistance. Videotapes were kindly supplied by M. Rice, U. Keuper-Bennett, and J. Wilson. Support was provided by NMFS Contracts 40-JJNF-1-0212 and 40-JJNF-2-0134. Contribution no. 943 of the Hawaii Institute of Marine Biology and SOEST contribution no. 3543.

Literature Cited

Balazs, G. H., and S. G. Pooley. 1991. Research plan for marine turtle fibropapilloma. U.S. Dept. Commer., NOAA Tech. Memo. NMFS, NOAA-TM-NMFS-SWFSC-156:1–113.

———, R. G. Forsyth, and A. K. H. Kam. 1987. Preliminary assessment of habitat utilization by Hawaiian green turtles in their resident foraging pastures. U.S. Dept. Commer., NOAA Tech. Memo. NMFS, NOAA-TM-NMFS-SWFC-71:1–107.

———, R. Fujioka, and C. Fujioka. 1993. Marine turtle faeces on Hawaiian beaches. Marine Pollution Bull. 20:392–394.

Booth, J., and J. A. Peters. 1972. Behavioral studies on the green turtle (*Chelonia mydas*) in the sea. Anim. Behav. 20:808–812.

Brock, R. E., C. Lewis, and R. C. Wass. 1979. Stability and structure of a fish community on a coral patch reef in Hawaii. Mar. Biol. 54:281–292.

Choy, B. K., G. H. Balazs, and M. Dailey. 1989. A new therapy for marine turtles parasitized by the piscicolid leech, *Ozobranchus branchiatus*. Herpetol. Rev. 20:89–90.

Eliot, J. L. 1978. Hawaii's far-flung wildlife paradise. National Geographic 153:670–691.

Gorlick, D. L. 1980. Ingestion of host fish surface mucus by the Hawaiian USA cleaning wrasse, *Labroides phthirophagus*, Labridae, and its effect on host species preference. Copeia 1980:863–868.

———. 1984. Preference for ectoparasite infected host fishes by the Hawaiian cleaning wrasse, *Labroides phthirophagus*, Labridae. Copeia 1984:758–762.

Hobson, E. S. 1971. Cleaning symbiosis among California inshore fishes. Fish. Bull. 69:491–523.

———. 1974. Feeding relationships of teleostean fishes on coral reefs in Kona, Hawaii. *Ibid.* 72:915–1031.

Jacobson, E. R., J. L. Mansell, J. P. Sundberg, L. Hajjar, M. E. Reichmann, L. M. Ehrhart, M. Walsh, and F. Murru. 1989. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). J. Comp. Path. 101:39–52.

Limpus, C. J. 1978. The reef: uncertain land of plenty, p.187–222. *In*: Exploration north. H. J. Lavery (ed.). Richmond Hill Press, Victoria, Australia.

Losey, G. S. 1979. Fish cleaning symbiosis: proximate causes of host behaviour. Anim. Behav. 27: 669–685.

———. 1987. Cleaning symbiosis. Symbiosis 4:229–258.

———, and L. Margules. 1974. Cleaning symbiosis provides a positive reinforcer for fish. Science 184: 179–180.

Monroe, R., and C. J. Limpus. 1979. Barnacles on turtles in Queensland waters with descriptions of three new species. Mem. Qd. Mus. 19:197–223.

Potts, G. W. 1973. The ethology of *Labroides dimidiatus* (Cuv. & Val.) (Labridae: Pisces) on Aldabra. Anim. Behav. 21:250–291.

Schwarz, F. J. 1960. The barnacle, *Platylepas hexastylos*, encrusting a green turtle, *Chelonia mydas mydas*, from Chincoteague Bay, Maryland. Chesapeake Sci. 1:116–117.

Smith, S. H. 1988. Cleaning of the hawksbill turtle (*Eretmochelys imbricata*) by adult french angelfish (*Pomacanthus paru*). Herp. Review 19:55.

Tyler, S. 1979. Time-sampling: a matter of convention. Anim. Behav. 27:801–810.

(GSL, LAP) University of Hawaii, Hawaii Institute of Marine Biology, P.O. Box 1346, Kaneohe, Hawaii 96744; and (GHB) National Marine Fisheries Service, Southwest Fisheries Science Center, Honolulu Laboratory, 2750 Dole Street, Honolulu, Hawaii 96822-2396. Submitted: 6 Jan. 1993. Accepted: 30 July 1993. Section editor: W. J. Matthews.