Abstract.—Accurate and precise descriptions of behavioral indicators of human activities which disturb cetaceans are required to better control adverse human impacts on these animals. We hypothesize that the application of a technique used to remove a small piece of innervated tissue, a biopsy darting procedure, is likely to result in the display of such behavioral indicators. In order to describe such displays, we recorded behavior of 22 humpback whales *Megaptera novaeangliae* before and after biopsy procedures in the southern Gulf of Maine. Reactions varied considerably among animals. Although respiratory responses were not consistent, biopsied whales generally decreased their ratio of surface to dive time and their net movement rate. Hard tail flicks occurred as an immediate reaction in approximately half the cases. Although 31 behaviors were tested for variation, only hard tail flicks significantly increased in either the number of animals that displayed them or the overall frequency of occurrence during postbiopsy reaction periods. While not statistically significant, some increase was noted in the frequency of trumpet blows and tail slashes, while slow swimming and apparent investigative behavior were noted to decrease. The strongest reactions, observed in two cases, occurred when the dart and retrieval line briefly snagged the whale's flukes. These findings complement and extend other studies on the response of baleen whales to human activity at sea.

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Behavioral reactions of humpback whales *Megaptera novaeangliae* to biopsy procedures

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The humpback whale *Megaptera novaeangliae* is an endangered species that has been protected from commercial catches since the mid-1960s. Protection from hunting in the North Atlantic portion of its range extends back to 1955. The most recent population estimates suggest close to 10,000 animals remain worldwide, with 5500 in the western North Atlantic (Johnson and Wolman 1985).

The endangered status of this species as well as its affinity for nearshore habits has brought increasing concern that the collective effects of industrial development, resource exploitation, and rapid increase in the whale watching industry could result in displacement, habitat degradation, and behavior modification. It thus has become important to determine whether human activity that is not directly lethal to individual whales could still have deleterious effects on the recovery of this species.

To assess potential deleterious effects of artificial stimuli on the normal behavior of a whale, definitions of disturbed behavior must be clarified. Disturbed behavior can be defined as behavior that results from a noxious stimulus that would not otherwise have occurred. Previous observations have been made under conditions of potential disturbance, such as the presence of boats or divers or the production of underwater noise (Baker and Herman 1982, Malme et al. 1983 and 1985, Bauer and Herman 1985, Richardson et al. 1985). However, a cause-and-effect relationship between the stimulus and a whale's response has been difficult or impossible to achieve, since baseline data on behavioral reactions to clearly noxious stimuli are almost entirely lacking.

Since 1979, humpback whales have been studied intensively in the southern Gulf of Maine to evaluate the demographics, behavior, and ecology of a group of annually-returning individuals (Mayo et al. 1985, Weinrich 1985 and 1986, Clapham and Mayo 1987). In 1983, the University of Florida began studies to determine the genetic characteristics and sex of known individuals in this group of whales. To obtain the tissue samples for this project, a projectile biopsy dart was used (Lambertsen 1987, Lambertsen and Duffield 1987, Lambertsen et al. 1988).

In an attempt to better understand the disturbance response of large whales, the present study was undertaken to assess the behavioral reaction of humpback whales to the biopsy procedures previously described (Lambertsen 1987). We reason that since the biopsy dart removes a small piece of innervated tissue, it is likely to be perceived by the whale as a noxious stimulus and should cause some observable response. Our results compare the behaviors of the whales before and after exposure to this relatively short-term, moderate-level stressful stimulus.

Materials and methods

Cruises to collect biopsy samples took place each year from 1983 to 1985 on Jeffrey's Ledge or Stellwagen Bank in the Gulf of Maine. In 1983 and 1984 various types of small power vessels (≤12.8 m) were used, including an 11.6 m sportfishing vessel equipped with one Detroit Diesel 671 engine, and a 12.8 m pilot boat with two Detroit Diesel 671 engines. In 1985, a 6.1 m runabout with a 175 hp outboard engine and an 11 m sailing sloop were used simultaneously. The use of two vessels allowed one (the 6.1 m runabout) to be dedicated to collection of behavioral data in the methodology described below. The immediate response of whales to biopsy darting was recorded on two days in 1983, two in 1984, and six in 1985.

The biopsy apparatus used in this study consisted of a tethered retrievable biopsy dart, aimed at the flank below the dorsal fin, fired from a 68 kg crossbow (Lambertsen 1987). A small biopsy punch fitted with internal prongs and attached to the tip of the dart shaft removed the tissue from the animal. A small tissue sample, including both epidermis and dermis, was thus obtained by a cutting action on penetration, and tearing on rebound. Upon penetration of the dart into the whale, a rebound was forced by a 2.5 cm diameter flange set 2 cm back from the tip of the biopsy punch.

In the first 2 years of the study, emphasis was placed on collecting as many biopsies as possible during brief periods at sea. Behavioral observations were collected opportunistically to provide qualitatively classified data on immediate reactions. For comparative purposes, these observations were ranked in a manner similar to that used by Mathews (1986), who studied the reactions of eight gray whales *Eschrichtius robustus* to a similar biopsy procedure. Categories used in this initial analysis included:

No reaction The whale continued its prebiopsy behavior with no detectable change.

Low-level reaction The animal modified its behavior, but displayed none of the overtly forceful behaviors listed as moderate or strong reactions (e.g., immediate dive).

Moderate reaction The animal modified its behavior in a more forceful manner (trumpet blows, hard tail flicks), but gave no prolonged evidence of behavioral disturbance.

Strong reaction The animal modified its behavior to a succession of forceful activities (continuous surges, tail slashes, numerous trumpet blows).

To test statistical differences in reaction levels among age-classes, non- and low-level reaction frequencies were combined, as were moderate and strong reactions. This was necessitated by the low expected values of the frequencies in a chi-square table based on the data in Table 1.

All data from 1983–85 were used to categorize immediate response levels; in 1985, a 30-min prebiopsy **control** period and a 30-min postbiopsy **response** period were defined to standardize a paired data set of respiratory and other surface behaviors. This approach used the vessel dedicated to behavioral data collection to institute a focal sampling technique (Altmann 1974) to allow quantitative comparison of the pre- and postbiopsy focal periods as "paired samples." Upon sighting a group of whales, each individual was distinguished through 7×50 binoculars using distinctive natural markings on the dorsal fin or the ventral surface of the tail flukes (Katona and Whitehead 1981). Because of the necessity of identifying individual respirations and behaviors within the group, dorsal fin shape was used whenever possible during focal samples; permanent identification came from fluke photographs taken during the approach for the biopsy strike. Once individuals were distinguished from one another, a 30-min "control" (i.e., pretreatment) focal sample was then initiated. During this period the engines of the observation vessel were shut down to eliminate engine noise. No approaches closer than 100 m were made prior to the onset of, or during, the 30-min control period. If the whale moved farther than 1000 m from the research vessel, making data collection difficult, the engine was started and approach was made slowly to within ~300 m of the whale. At the conclusion of the 30-min "control" focal sample the whale was then approached at close range (3–40 m) for the biopsy attempt. The same protocol was followed after the biopsy for a comparable "experimental" (i.e., post-treatment) data set, which started at the moment of impact by the dart.

During focal samples, data were collected on four respiratory variables: (1) number of respirations ("blows") during a given surface interval, (2) time between each respiration ("blow interval"), (3) time the animal spent at the surface ("surface interval"), and (4) time spent below the surface during each dive ("dive time"), defined as the period of submergence following typical sounding behavior (i.e., a prominent, high arching of the back and tail, often followed by bringing the tail flukes above the water surface). The surface interval during which the biopsy strike took place was excluded from our analyses of both the pre- and postbiopsy respiratory variables. We determined (using chi-square goodness-of-fit tests) that the distribution of the observed data was not significantly different from a normal distribution, thus differences between the pre- and postbiopsy values were compared using two-tailed paired t-tests (Zar 1984). Surface-interval to dive-time ratios were calculated for each whale during pre-biopsy and postbiopsy focal samples, and compared using a Wilcoxon sign rank test (Zar 1984). The surface-interval/dive-time ratio integrates several respiratory values into a single measure of the respiratory "strategy" for each individual.

LORAN-C positions, which have an error of ~30 m in the study area (Day 1983), were used in estimating the net rate of movement of a whale as defined by its surfacings. LORAN positions were recorded at the start and end of each focal sample. With each LORAN-C reading, the bearing to the whale to the nearest 5 degrees and the visually-estimated distance from the vessel to the whale were also recorded. This information was used to correct the LORAN-C data if the whale was >30 m from the vessel, which was likely given the limitations of vessel movement during focal samples described above. To estimate the net movement rate, the distance between the first and last calculated whale positions within each focal sample period was divided by the elapsed time (30 minutes), yielding a net movement rate in knots. Actual swimming speed could not be determined due to uncertainty about the direction of a whale's movement underwater or the linearity of its track. Results between pre- and postbiopsy periods were compared using a Wilcoxon sign rank test (Zar 1984).

Photographs of the dorsal fin and tail flukes of individual whales were taken upon approach for biopsy. Each whale was identified using the catalog of Gulf of Maine humpback whales kept at the Cetacean Research Unit, where individuals are assigned a two-letter, one-number file code. If the animal could not be identified in the catalog, it was assigned a three-digit code. When possible, each animal was assigned to one of the following age groups: juvenile (1–3 yr), adolescent (4–6 yr), or adult (>6 yr). Age classifications were based on previous and/or subsequent repeated annual observations of the same individuals by the authors from calf year (used for juveniles and adolescents), sightings of the individual as an initially small and subsequently larger animal (juveniles and adolescents), or annually repeated sightings of an individual with no appreciable growth over several years (adults). If an animal was sighted only during the year in which it was biopsied, it was not classified by age-class.

During focal samples collected in 1985, a total of 30 behavior types were observed and analyzed. Behavior types were defined using an ethogram for humpback whales developed by the Cetacean Research Unit prior to this study (unpubl. data). The probability that any given behavior was displayed by more or fewer animals in the pre- vs. postbiopsy focal samples was tested using the binomial distribution (with the probability of each period containing an occurrence of the behavior assumed as 0.5), while the change in frequency of each behavior in individuals, given that the behavior was observed at all, was compared using Wilcoxon signed rank tests (Zar 1984). All 30 behaviors were tested for variation. Many of the behaviors did not show any variation between control and response periods, and therefore are not described in detail. These were belly-up rolls, breaches, bubble clouds (bubble clouds followed by obvious surface feeding), bubble cloud behaviors (bubble clouds not followed by obvious surface feeding), defecations, flipper flares, flipper flicks, flipper in air, flukes, half flukes, hangs, high flukes, high head-ups ("spyhops"), lobtails, logging, low flukes, quarter rolls, single bubbles, snakes (a twisting of the body), surges, tail breaches, and passing under a boat. Definitions of those behaviors which either varied significantly in frequency or showed some notable variation in the frequency of display following the biopsy procedure are the following:

Back rise Animal breaks surface while swimming, with no accompanying exhalation.

Belly-up lobtail Animal, ventral side up, elevates tail into the air, then slaps the water surface with the dorsal surface of its flukes.

Hard tail flick Animal rapidly and forcefully flexes tail up and down one time during otherwise normal swimming behavior; much spray can be thrown; flukes clear surface. The hard tail flick is faster and presents a less regular arching movement of the tail than a lob-tail.

Low head-up Animal lifts head into air at 30–45° angle to surface.

Sounding dive Animal arches its back in a typical diving posture but does not bring its tail flukes above the surface.

Tail rise Animal slowly straightens its caudal peduncle at the surface during normal swimming.

Tail slash Animal moves tail forcefully from side to side, flukes at or just below the surface; creates white water frothing.

Trumpet blow Loud, broad-band, wheeze-like sound made during exhalation at the surface.

In addition, the following behaviors showed a variation in frequency during the special case of SI4's reaction (discussed below):

Belly-up Animal rolls so that the whale has its ventral surface exposed above the surface (often for longer than a second).

Half fluke Animal rolls on its side exposing one fluke above and perpendicular to the surface.

Table 1

Qualitative ranking of intensity of behavioral responses in humpback whales *Megaptera novaeangliae* to biopsy procedures. NR = No reaction.

	NR	Low	Moderate	Strong	Total
Juveniles (1–3 yr)	2	3	10	3	18
Adolescents (4–6 yr)	0	4	6	0	10
Adults (>6 yr)	3	12	23	1	39
Unclassified	0	0	4	0	4
Total	5	19	43	4	71

Results

Immediate behavioral reactions, or the absence thereof, to the biopsy procedure were recorded for 71 biopsy strikes during the period 1983–85. Of these, 22 (recorded in 1985) are paired samples including a 30-min prebiopsy and 30-min postbiopsy focal sample. Two cases contained clearly unusual reactions, including one from the 1985 paired samples. These cases are discussed separately. This leaves 21 paired samples of behavioral data; however, in five cases some respirations could not be accurately assigned to a whale within the focal group, leaving 16 paired samples of complete respiratory data for analysis.

Immediate behavioral response

Of the 71 total biopsy attempts for which immediate behavioral reactions were recorded, 7.0% involved no behavioral reaction, 26.8% involved a low-level reaction, 60.6% involved a moderate reaction, and 5.6% involved a strong reaction (Table 1). All the strong reactions involved snagging of the flukes by the monofilament line attached to the biopsy dart.

Immediate dives were the most common response to the biopsy dart striking the animal, observed in 35 (49.2%) cases, hard tail flicks were present in 34 (47.8%), and trumpet blows were observed in 31 (43.6%) cases. Less than 20% of all reactions involved immediate surges or visually detectable increases in swimming speed.

Although an immediate dive was a frequently observed response, this may have been due to the time it took to approach the whale for the biopsy strike, i.e., the whale would have taken a dive at that point regardless of the biopsy attempt. However, the mean number of blows (4.89) during the surfacing interval in which the biopsy dart was fired was significantly lower than in the accompanying complete surfacings immediately prior to the biopsy attempt (7.17) (paired t-test: t −2.76, 15 df, p 0.015),suggesting those dives which occurred immediately after the strike of the biopsy dart were initiated as a response to the biopsy procedure.

Study animals could be categorized by age-class in 68 of the 71 trials. There was no significant difference in the intensity of reactions by age-class (χ^2 2.88, 3 df, p 0.41) (Table 1). However, 3 of the 4 reactions we ranked as strong were from juveniles. Also, strong reactions were always associated with a snagging of the retrieval line on the animals' flukes.

Respiratory and dive variables

There were no significant differences between pre- vs. postbiopsy focal samples for any of the four respiratory variables (paired t-tests (15 df): blow interval t 0.82, p 0.42; number of blows/surfacing interval t −0.93, p 0.36; surfacing interval t 1.65, p 0.11; dive time t 0.61, p 0.55). There was a significant decrease in the surface-interval/dive-time ratios during postbiopsy focal samples (Wilcoxon signed rank test, Z −2.11, p 0.03).

Substantial individual variation was found in respiratory variables. Seven animals (43.8%) showed a decrease in their mean blow interval following the biopsy procedure, and eight (50.0%) showed an increase (Table 2). Eleven individuals (68.8%) showed a decrease in the number of blows per surfacing, while in only four (25.0%) did it increase (Table 3). Similarly, eleven whales (69.0%) reduced their surface interval in the postbiopsy period, while in five (32.0%) this variable increased (Table 4). Finally, eight of the 16 individuals (50.0%) were found to decrease their dive times during the postbiopsy period, while in the other eight (50.0%) it increased (Table 5). The surface-interval/dive-time ratio also showed a decrease in 9 of the 16 animals (57.0%), while in 5 (32.0%) there was no change and in 2 (13.0%) there was an increase (Table 6). Based on binomial distribution, any case with 9 or more, or 2 or less, animals showing a change in a particular direction would be significant at p 0.07 (based on 11 samples, ignoring the 5 that showed no change), indicating a significant decrease in the surface-interval/dive-time ratio in the sample.

To determine whether the immediate behavioral response to the biopsy dart affected subsequent

Table 2

Mean blow intervals (s) and standard deviations in humpback whales *Megaptera novaeangliae* during focal samples before and after biopsy procedures. Each individual whale is represented by a two-letter one-number code or a three-number code.

	Prebiopsy			Postbiopsy			
Animal	N	$\bar{x}$	SD	N	$\bar{x}$	SD	Difference
CO7	18	27.9	35.9	17	22.0	14.8	-5.9
SE6	11	49.2	43.2	15	46.3	45.9	-2.9
ZE1	19	36.4	23.7	23	26.8	23.4	-9.6
547	23	34.7	27.2	18	38.2	18.5	3.5
SW1	26	23.3	24.0	21	32.8	16.5	9.5
TH6	22	28.3	15.8	16	28.9	22.5	0.6
LA5	17	22.3	12.1	25	22.3	15.5	0.0
TR2	23	19.3	20.5	26	24.5	14.4	5.2
KE2	12	83.7	74.3	11	51.7	37.1	-32.0
ME1	22	14.5	2.6	19	16.0	2.0	1.5
CO9	22	16.3	5.3	21	15.9	4.0	-0.4
ST1	19	34.6	23.8	30	33.3	26.4	-1.3
OC1	38	30.4	20.4	36	32.6	27.1	2.2
SM1	32	27.8	22.8	21	33.7	27.1	5.9
CI1	23	14.3	5.1	17	18.3	13.3	4.0
RA6	12	31.8	20.3	10	19.3	7.1	-12.5
Sample		30.9	16.8		28.9	9.5	-2.0

Table 3

Mean number of blows per surface interval in humpback whales *Megaptera novaeangliae* during focal samples before and after the biopsy procedure. Each individual whale is represented by a two-letter one-number code or a three-number code.

	Prebiopsy			Postbiopsy			
Animal	N	$\bar{x}$	SD	N	$\bar{x}$	SD	Difference
CO7	4	4.4	1.1	4	8.6	4.2	4.2
SE6	2	8.3	6.0	5	3.7	1.9	-4.6
ZE1	3	10.0	6.0	5	5.0	3.7	-5.0
547	5	4.7	2.9	5	4.0	2.0	-0.7
SW1	3	5.8	1.0	4	5.8	1.7	0.0
TH6	5	6.5	2.4	6	6.0	2.9	-0.5
LA5	8	4.2	2.8	5	2.8	2.2	-1.4
TR2	3	8.7	3.1	4	7.3	3.0	-1.4
KE2	4	4.0	2.5	5	3.2	2.3	-0.8
ME1	7	4.1	1.9	2	8.0	2.8	3.9
CO9	5	5.4	2.5	11	2.9	1.6	-2.5
ST1	7	3.9	1.5	5	7.0	6.6	3.1
OC1	4	10.5	6.4	5	8.2	6.8	-2.3
SM1	6	6.3	4.3	5	5.2	4.8	-1.1
CI1	6	4.4	1.8	2	2.3	1.5	-2.1
RA6	4	2.3	1.7	4	3.5	1.3	1.2
Sample		5.8	2.4		5.2	2.1	-0.6

Table 4

Mean surface interval length (s) in humpback whales *Megaptera novaeangliae* during focal samples before and after the biopsy procedure. Each individual whale is represented by a two-letter one-number code or a three-number code.

	Prebiopsy			Postbiopsy			
Animal	N	$\bar{x}$	SD	N	$\bar{x}$	SD	Difference
CO7	4	117.7	135.2	4	196.4	123.2	78.7
SE6	2	399.0	291.9	5	140.8	156.1	-258.2
ZE1	3	369.3	251.4	5	110.3	119.0	-259.0
547	5	158.0	115.7	5	121.0	58.5	-37.0
SW1	3	115.5	49.3	4	199.5	87.0	84.0
TH6	5	206.6	96.2	6	135.8	94.8	-70.8
LA5	8	62.5	71.5	5	46.3	21.6	-16.2
TR2	3	154.0	104.5	4	168.0	106.6	14.0
KE2	4	256.8	177.2	5	135.4	106.5	-121.4
ME1	7	61.1	47.2	2	122.0	42.4	60.9
CO9	5	76.0	41.4	11	29.6	19.6	-46.4
ST1	7	113.9	104.6	5	222.4	244.1	108.5
OC1	4	390.3	292.3	5	264.4	282.4	-125.9
SM1	6	216.8	157.3	5	138.6	163.8	-78.2
CI1	6	57.5	35.8	2	36.1	49.9	-21.4
RA6	4	64.5	21.5	4	32.0	14.4	-32.5
Sample		176.2	120.1		131.1	70.0	-45.1

Table 5

Mean dive interval length (s) in humpback whales *Megaptera novaeangliae* during focal samples before and after the biopsy procedure. Each individual whale is represented by a two-letter one-number code or a three-number code.

	Prebiopsy			Postbiopsy			
Animal	N	$\bar{x}$	SD	N	$\bar{x}$	SD	Difference
CO7	4	92.2	84.6	4	194.8	112.7	102.6
SE6	2	171.3	94.7	5	177.2	79.8	5.9
ZE1	3	181.0	23.5	5	145.4	87.0	-35.6
547	5	146.7	113.6	5	314.0	76.7	167.3
SW1	3	394.7	149.8	4	145.8	121.8	-248.9
TH6	5	127.5	22.9	6	200.0	88.3	72.5
LA5	8	254.5	35.4	5	216.5	48.9	-38.0
TR2	3	412.5	9.2	4	391.3	253.4	-21.2
KE2	4	228.5	90.6	5	125.0	90.6	-103.5
ME1	7	250.5	125.5	2	565.5	38.9	315.0
CO9	5	161.0	129.9	11	120.3	62.9	-40.7
ST1	7	122.0	107.1	5	204.6	251.3	82.6
OC1	4	153.8	75.7	5	124.3	127.8	-29.5
SM1	6	96.7	57.7	5	199.0	109.2	102.3
CI1	6	170.3	35.0	2	113.6	76.9	-56.7
RA6	4	324.8	115.4	4	355.0	167.5	30.3
Sample		205.5	98.9		224.5	18.4	19.0

responses in respiratory variables, we examined separately those animals that reacted to the biopsy strike with an immediate hard tail flick (*n* 9), the most obviously forceful immediate response to the biopsy strike. This subset would therefore eliminate those animals who may have not been affected by the biopsy strike. However, variation among individuals during the postbiopsy period was not appreciably different from that portion of the sample where no hard tail flick was observed (binomial test). Hence, the occurrence of an immediate forceful response to the biopsy procedure does not appear to be associated with subsequent changes in respiratory variables.

Net movement rate

For 11 of the 21 animals, LORAN-C fixes allowed a calculation of the animal's net movement rate in pre- and postbiopsy focal samples. During the prebiopsy sample, only two animals showed values >1 kn. During the postbiopsy period, the average rate did not increase significantly (Wilcoxon signed rank test, Z −1.82, p 0.07). However, only three animals had rates <1 kn, and a generally increasing trend was recorded (Table 7).

Other behavioral responses

To consider changes in behavior elicited by the biopsy procedure, the possibilities of introducing new behaviors or altering display rates of regularly observed behaviors were both considered. The former was examined using the number of pre- and postbiopsy focal samples during which each behavior type was observed, while the latter was examined using the direction and magnitude of changes in observed behavior types within individual paired samples (Tables 8, 9). Only one of the 30 tested behavior types showed significant differences between the pre- and postbiopsy period.

Eleven of the 21 (52.3%) postbiopsy focal samples contained a hard tail flick, while the behavior was not observed in the prebiopsy focal samples (binomial distribution, $p<0.001$). Only once was a hard tail flick observed more than one time after a biopsy strike. This also was the only case in which the hard tail flick was not an immediate response to the biopsy dart. The percentage of biopsy strikes where the reaction included a hard tail flick among paired samples was not significantly different from that of the larger 1983–84 sample, where 34 of 50 animals displayed the hard tail flick (χ^2 1.54, 1 df, p 0.21).

As was the case in the number of 30-min samples in which a behavior was displayed, only hard tail flicks showed a significant increase in frequency during the postbiopsy period (binomial distribution, p 0.001). While results were not significant, one or more animals also showed notable increases in the numbers of trumpet blows, tail slashes, and belly-up lobtails following the biopsy procedure; similar nonsignificant but notable decreases were seen in back rises, tail rises, and low head-ups (Tables 8, 9). The latter three behaviors are associated with slow, unhurried travel, resting, or interest in nonessential environmental stimuli (e.g., boats, seaweed).

Table 6

Mean surface-interval/dive-time ratio in humpback whales *Megaptera novaeangliae* during focal samples before and after the biopsy procedure. Each individual whale is represented by a two-letter one-number code or a three-number code.

Animal	Prebiopsy	Postbiopsy	Difference
CO7	1.3	1.0	−0.3
SE6	2.3	0.8	−1.5
ZE1	2.0	0.8	−1.2
547	1.1	0.4	−0.7
SW1	0.3	1.4	1.1
TH6	1.6	0.7	−0.9
LA5	0.2	0.2	0.0
TR2	0.4	0.4	0.0
KE2	1.1	1.1	0.0
ME1	0.2	0.2	0.0
CO9	0.5	0.2	−0.3
ST1	0.9	1.1	0.2
OC1	2.5	2.1	−0.4
SM1	2.2	0.7	−1.5
CI1	0.3	0.3	0.0
RA6	0.2	0.1	−0.1
Sample	1.1	0.7	−0.4

Table 7

Net movement rate (kn) in humpback whales *Megaptera novaeangliae* during focal samples before and after the biopsy procedure. Each individual whale is represented by a two-letter one-number code or a three-number code.

Animal	Prebiopsy	Postbiopsy	Difference
CO7	0.5	1.7	1.2
SW1	0.7	1.5	0.8
TR2	0.8	2.0	1.2
ME1	0.8	4.5	3.7
ST1	0.5	0.6	0.1
OC1	0.9	0.6	−0.3
SM1	0.8	1.3	0.5
RA6	3.8	1.5	−2.3
BI1	1.5	3.8	2.3
PE4	0.5	1.1	0.6
SI4	0.5	0.7	−0.2
Sample	1.0 (SD 0.9)	1.7 (SD 1.3)	0.7

Table 8

Frequency of various behavior types in humpback whales *Megaptera novaeangliae* before the biopsy procedure ("control period"). Each individual whale is represented by a two-letter one-number code or a three-number code.

	CO7	SE6	ZE1	547	SW1	TH6	LA5	TR2	KE2	ME1	CO9	ST1	OC1	SM1	CI1	RA6	BI1	CR1	SI4	FL2	PE4	Total
Half fluke				2								1		1				1				5
Quarter roll						1																1
Bubble cloud																					2	2
Single bubble																						0
Breach									1					1								2
Back rise	9	3	11	2	1	1	2					1	7	3			1	1				42
Tail breach									2													2
Belly up												1										1
Belly-up lobtail																						0
Cloud behavior												2									8	10
Defecation																					1	1
Flipper flick																						0
Flipper in air												2										2
Flipper flare																						0
Fluke	3		2	2	2	1	3	2		4	4	3	2	1	4	3		3	4	5	7	55
Hang																						0
High fluke												1										1
High head-up	1											1										2
Hard tail flick																						0
Low fluke				1		1								1			1		1	3	1	9
Low head-up		3	4			1						1										9
Log (min)		9.3	9.5																			19
Lobtail														1				1	1			3
Snake																			1			1
Sounding dive	4	2		2	2			1	2	1	3	1	3	3	2		1	1			1	29
Surge					2											1	1					4
Tail rise		2		3		3	1		3	1		2	1	2	1		11	1	7		2	44
Trumpet blow				4	1	2				2	2	3	2	1		2	4	5	3	4		38
Tail slash				1		1				1										7		3
Under boat												1						1				2

Feeding behavior was observed with equal frequency in both the pre- and postbiopsy samples. Those animals engaged in feeding activity showed virtually no reaction to the biopsy attempt. A hard tail flick was never observed from an animal engaged in feeding activity, although it was observed during all other prebiopsy behavioral modes. Logging (resting) behavior was also displayed equally in both sample periods; however, whales logging when biopsied were observed to temporarily interrupt their logging period immediately following the biopsy.

Special cases

Two special cases of behavior modification were noted in conjunction with the biopsy procedure. Both involved the monofilament retrieval line becoming briefly snagged around one of the flukes of the whale. These represent the most vigorous and prolonged reactions to the biopsy procedure we observed.

In one case, for a period of time after the biopsy strike (~16 min) the line remained looped around the tip of one fluke of the tail and the animal behaved abnormally, swimming at elevated speeds (6–7 knots) in a roughly S-figured course. Although visually estimated, this speed appears higher when compared with values reported above. Another whale accompanied this animal in its vigorous swimming.

SI4 exhibited another unusual reaction after a biopsy (at a different time than the reaction reported for the same individual in Table 9). This whale had been associated with CR1 during the day of the biopsy effort; 40 min prior to the first strike of SI4, CR1 was sampled with little reaction. When SI4 was first struck by the biopsy dart its reaction was also minimal, but a tissue sample was not obtained. The next shot (29 min later) missed the whale, but involved a momentary snag of the line on the animal's tail stock. In response, the animal started to trumpet blow with increasing frequency but remained stationary and was easily approached. A third firing of the biopsy dart 11 min later was successful in obtaining a tissue sample.

Table 9

Frequency of various behavior types in humpback whales *Megaptera novaeangliae* following the biopsy procedure ("reaction period"). Each individual whale is represented by a two-letter one-number code or a three-number code.

	CO7	SE6	ZE1	547	SW1	TH6	LA5	TR2	KE2	ME1	CO9	ST1	OC1	SM1	CI1	RA6	BI1	CR1	SI4	FL2	PE4	Total
Half fluke				3	1	2							1	1							1	9
Quarter roll			1			1															1	3
Bubble cloud																					2	2
Single bubble			2																			2
Breach																						0
Back rise	1	1	1	1	3	1	1		3			1	3	4								20
Tail breach									2													2
Belly up				1																		1
Belly-up lobtail									20													20
Cloud behavior																					7	7
Defecation																						0
Flipper flick														1								1
Flipper in air																						0
Flipper flare												1										1
Fluke	4	2	2	1	2	4	1	2	3	2	7	2	2	2	3	2		1	2	4	7	55
Hang												1										1
High fluke				1						1		1			1	1						5
High head-up																						0
Hard tail flick				1	1	1	1	1	1				1	2	1		1	1				12
Low fluke			1				1				1	1	1	1	2		1	2		1	2	14
Low head-up			1											1								2
Log (min)													3	3				13				19
Lobtail									4		1				1						1	7
Snake																						0
Sounding dive		4	6		2		3	1	5		2	2	1	4	5		1			3	5	44
Surge					1								1	1								3
Tail rise	3		2	2	3	2		1	1		1	1	1	4	3		2	2	2	1		31
Trumpet blow	1	1		3	2	8	1	1	1		1	1	4	9	5			4		5	10	57
Tail slash		1				2		1						1					1			11
Under boat																			1		1	2

Following the final biopsy attempt, SI4 started a series of stereotypic actions. Every 45–60 s, the animal would trumpet blow loudly, then tail slash or low-lobtail (a quick, low version of lob-tailing behavior), surge forward, and roll sideways with great force, often rolling ventral-side-up and spiraling underwater. Periods of submergence were <30 s in all cases. The swimming path was erratic, but the animal was never >100 m from the vessel. It passed immediately below the vessel twice, repeatedly surfacing on alternating sides of the boat. Swimming speed appeared greater than normal, although net movement in any one direction was minimal. During the same period CR1 appeared placid, although it did trumpet blow three times. After 14 min, the vigorous behavior of SI4 suddenly ended, and both animals started logging side by side. At this point, they were within 25 m of the vessel. Logging behavior continued for at least 15 min at which point the observation was terminated.

In order to compare the intensity of SI4's reaction with the sample analyzed above, we compared the rate at which it displayed various behavior types in the postbiopsy focal sample with the larger paired sample (n 21). To obtain a mean number of occurrences of each behavior type in the postbiopsy period, the total number of observations of each behavior type was divided by the number of paired samples (Table 10). From these data, it is clear that unusually high numbers of tail rises, trumpet blows, half flukes, belly-ups, lobtails, tail flicks, and tail slashes occurred in SI4's response.

Discussion

The results of this study indicate that behavioral reactions of individual whales to the biopsy procedure are detectable but do not appear to be severe. Immediate reactions (hard tail flicks) took place in >50% of 71 biopsy strikes, which is especially noteworthy given the rarity of this behavior in any other context. However, no significant difference was seen in most of the 30 observed behaviors in 30-min pre- and postbiopsy

Table 10

Frequency of various behavior types observed in a humpback whale *Megaptera novaeangliae* (animal SI4) subjected to repeated strikes of the biopsy dart compared with mean for the entire study population (not including SI4). Values given represent average over the 30-min postbiopsy focal sample. *N* of study population = 21.

Behavior	Study population	SI4
Half fluke	0.42	19
Quarter roll	0.14	2
Bubble cloud	0.09	0
Single bubble	0.09	1
Breach	0.00	0
Back rise	0.95	7
Tail breach	0.08	1
Belly up	0.04	12
Belly-up lobtail	0.95	0
Cloud behavior	0.33	0
Defecation	0.00	0
Flipper flick	0.04	1
Flipper in air	0.00	3
Flipper flare	0.04	2
Fluke	2.61	5
Hang	0.04	0
High fluke	0.23	0
High head-up	0.00	1
Low fluke	0.65	0
Low head-up	0.09	4
Log (min)	0.90	0
Lobtail	0.33	26
Snake	0.00	0
Sound	2.09	0
Surge	0.14	6
Tail flick	0.57	11
Tail rise	1.47	13
Trumpet blow	2.71	29
Tail slash	0.52	11
Under boat	0.09	2

behavioral focal samples. A significant decrease in the ratio of surface interval to dive time followed the biopsy procedure. Although not statistically significant, increases in trumpet blows and, to a lesser extent tail slashes and sounding dives, were noted following biopsy strikes, as were decreases in the amount of slow swimming and some nonessential behaviors.

Two of the behavior types that were noted to increase, trumpet blows and tail slashes, have been previously suggested to be agonistic (Baker and Herman 1984, Watkins and Wartzok 1985). A tail slash may be used by a humpback whale as a means of aggression against another whale in what has been interpreted as courtship battles (Baker and Herman 1984). Norris and Reeves (1977) identify "tail swishing" (our "tail slashing") as one of the more common behavioral responses to harassment.

The behaviors elicited by the biopsy procedure in most cases are not intrinsically different from those behaviors which occur naturally in this species. Thus we emphasize that it may be the change in frequency of behaviors that should be viewed as indicative of "affected" behavior, rather than the occurrence of such displays *per se*. The one notable exception is the hard tail flick, which rarely has been observed other than in response to the biopsy procedure.

The possibility exists that the hard tail flick reaction we observed is a reflex response. This reaction typically occurred at the instant of dart impact and thereafter was rarely repeated. Moreover, in some individuals a single hard tail flick at the time of the biopsy was followed by a period during which no other behavioral change was observed. A reflex response is consistent with our finding of no correlation of respiratory variation with the occurrence of this reaction.

While some of the hard tail flicks may have been purely reflexive, the same behavior was seen once in response to an extremely close vessel approach when no physical contact was made. Further, a similar reaction was reported by Watkins (1981), who labeled it a "startle response." Hence it is uncertain whether this behavior is reflexive or cognitive. It may have both components.

In other studies of whale disturbance in response to noxious stimuli, both Watkins (1981) and Mathews (1986) mention the approach of the vessel as contributing to the reaction of the animal. We made every effort to diminish vessel effects. Both previous studies were conducted from power-driven vessels approaching at moderate to rapid speeds. In over half of our paired samples, data were collected from the relatively silent approach of a sailboat. Those approaches made under power in paired samples were done at slow speed. Further, we limited movement of the research vessels near whales, except in the brief approach for the biopsy, to lessen effects of vessels. While the effect of the vessels was minimized, this approach is a necessary part of the biopsy procedure and need not be considered separately in an analysis of responses.

Our results are comparable with those found by Mathews (1986), who examined the response of eight gray whales to a similar biopsy procedure. Both studies established great variability in the reaction of whales to biopsy procedures. One clear difference is that the blow interval of gray whales showed a significant increase in the postbiopsy period, while that of the humpback whales we studied did not. Even so, four of the eight gray whales studied by Mathews (1986) showed a reduction in their surface-interval/dive-time ratios, as did 57.0% of the larger sample; only one gray whale showed an increased surface-interval/dive-time ratio.

There have been other studies of the response of humpback whales to human-induced stimuli. In Alaska, 17 humpback whales exhibiting "affected" behavior associated with the proximity of vessels increased their mean and maximum dive intervals, while their mean blow interval decreased (Baker and Herman 1982). In comparison, although the whales in our study did not consistently increase the length of their dives following the biopsy, blow intervals decreased slightly. In both studies, whales decreased surface-interval/dive-time ratios on average. The whales in our study and in that of Baker and Herman (1982) also responded with an increased rate of net movement.

Our results generally agree with other studies of the reactions of baleen whales to a variety of human-induced stimuli. Richardson et al. (1985) found that bowhead whales *Balaena mysticetus* respond to a variety of man-made stimuli (drillships, vessels, aircraft) by reducing their surface-interval/dive-time ratios. Swimming speeds increased in response to vessel traffic. Migrating gray whales, by comparison, have been reported to slow down as their migration route took them toward simulated offshore industrial activity (Malme et al. 1983, 1985). Bauer and Herman (1985) found that humpback whales on Hawaiian breeding grounds reduced their surface interval as vessels approached closely. The blow interval decreased as either the proximity or the number of vessels increased. Similarly, pod speed increased as vessels approached. Hence, the net effect in all these studies was the same as we have found; namely, that the animal avoids the source of the stimulus.

It is important to note that the reactions we describe in most cases were elicited by a noxious stimulus of brief duration and low-to-moderate amplitude. On this basis, our findings likely underestimate the effects of a more prolonged noxious stimulus, or one of greater force. For example, extreme responses, including escape, hard tail flicks, and immediate submergence, has been documented in harpooned right whales *Eubalaena glacialis* (Scammon 1874) and fin whales *Balaenoptera physalus* (Lambertsen and Moore 1983).

In the context of current management problems, the response of a whale to a prolonged sublethal noxious stimulus is a critical issue, as habitat intrusion may establish conditions of continuing, if not constant, exposure to diverse noxious stimuli. Recognizing this, Bauer and Herman (1985) considered the relationship of stimulus amplitude and duration (expressed as the number of whale-watching vessels and the length of time a whale group was in close proximity to whale-watching vessels) to elicited responses in their study of the effects of vessel traffic on humpback whales. In both cases, their data indicate a graded response in strenuous episodes of breaching, lobtailing, and flippering behavior and in movement away from the path of vessels.

Although our present study was not designed to evaluate the effects of increasing stimulus duration, including that approximated by stimulus repetition, the special case of SI4 is illuminating. Its progressively increasing reaction to repeated biopsy strikes was dramatic. After the first strike, the whale seemed unperturbed. After the second, it appeared, from its trumpet blowing and stationary position, to be annoyed but passive. After the third, it reacted with great intensity and subsequently appeared exhausted.

Based on these observations we conclude that adverse responses to rapidly repeated or prolonged noxious stimuli in whales may be incorrectly modeled as a linear function. Given the lack of any detectable response to the biopsy procedure in some animals, there seems to be a threshold for stressor amplitude below which no response will occur. Further, this threshold of tolerance may be dependent upon the specific activity in which the animal is engaged immediately prior to the time the stressor is applied; e.g., in our study animals engaged in feeding were unlikely to react to the strike of a biopsy dart. There likely are also individual differences in this threshold, as suggested by the wide variation in reactions observed.

Moreover, although one evidently can expect a graded response in the disturbance of the animal above its tolerance threshold, such gradation might be better modeled as an exponentially increasing stimulus-response function. As such, continuous or rapidly repeated moderate-level noxious stimulation could potentially lead to a general somatic alarm reaction, with endocrinologic consequences (Selye 1936, 1946). Thus, one of the important implications of this study for current management strategies to promote the recovery of endangered whale populations is that uncontrolled increases in the level or frequency of noxious intrusion into cetacean habitat may, suddenly and unexpectedly, have serious deleterious effects.

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