PROJECTILE BIOPSY SAMPLING OF CETACEAN SPECIES

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Abstract

Projectile biopsy samples are collected on all Southwest Fisheries Science Center research cruises and used in molecular genetic studies of cetacean species phylogeny, population structure and social behavior. Although we continue to improve our techniques to increase the number of species we can sample, our methodologies were essentially standardized by 1991. We have collected 1,670 biopsy samples from 5 mysticete species and 20 odontocete species during 16 research cruises in the eastern Pacific Ocean between 1991 and 1999. This paper describes our sampling methods and presents the number of samples collected by species.

INTRODUCTION

Increasing use of molecular genetic techniques to study cetacean species prompted the Southwest Fisheries Science Center (SWFSC) to implement a biopsy sampling program on all research cruises. Because the primary objective of most SWFSC cruises is to collect data for estimating population abundance using line transect methodology or for studying particular aspects of a cetacean species ecology, all biopsy sampling is conducted opportunistically. Practically speaking, this means that the species sampled depends on the species composition of the study area designated for each cruise as well as prevailing weather conditions and time available for sampling.

At the same time many researchers were developing projectile biopsy methods (see Palsboll et al. 1991), we were also experimenting with techniques to obtain skin samples from cetacean species on SWFSC cruises. The goal of our program was to develop a projectile biopsy method that could be easily and reliably used to sample all cetacean species encountered on our cruises. While several different delivery systems and biopsy tips were tried, we found that a biopsy tip, with internal barbs for sample retention, fired from a crossbow was the most consistently reliable technique for collecting skin samples from many species. Although our methods have been essentially standard since 1991, our sampling equipment and techniques continue to be improved and refined to enable sampling more species at greater range.

This report summarizes the number of biopsy samples collected by species on SWFSC research cruises between 1991 and 1999.

MATERIALS AND METHODS

Biopsy samples were collected on 7 line transect survey cruises and 9 other cruises conducted by the SWFSC between 1991 and 1999. These cruises were conducted in tropical and temperate waters of the eastern Pacific Ocean primarily aboard National Oceanic and Atmospheric Administration (NOAA) research vessels *David Starr Jordan* and *McArthur*. However an additional platform, the University National Oceanographic Laboratory System (UNOLS) research vessel *Endeavor*, was used during the 1998 eastern tropical Pacific Ocean survey cruise, and the Bering Sea right whale surveys were conducted aboard the NOAA research vessel *Miller Freeman* in 1997 and the U. S. Coast Guard cutter *Sweetbriar* in 1999. Individual cetaceans were sampled while riding the bow of these ships or from small inflatable boats (approximately 6m) launched from the research vessels. All biopsy samples were collected using crossbows to deliver a tip that cuts through the skin and removes a plug of skin and blubber from the dorsal surface of an animal.

In the early days of our program, we made our own tips and experimented with several different designs. Now we only use the biopsy tips made by Dr. Finn Larsen. These tips are stainless steel cylinders with an inside diameter of approximately 7mm and a sharpened edge for cutting skin. Three barbs are soldered to the inside of the cylinder, which ensures sample retention. The design of the tips is further described in Larsen (1998). We use 2 different tip lengths for sampling. To sample smaller odontocete species, a 25mm long tip is used, while larger odontocete species and all mysticete species are usually sampled with a 40mm tip. For sampling some species, a small piece of rubber vacuum hose is added to the tips as a bumper to reduce the depth of tip penetration and to increase the probability that the tip will rebound. An approximately 10mm length of tubing is used on the 25mm tips when sampling the smaller dolphin species from bow of a research vessel. However the length of tubing used on the 40mm tips varied from approximately 10mm to 25mm depending on the species being sampled and the sampling platform (i.e., the research vessel or small boat).

We use both the Barnett Wildcat crossbow and the Barnett Quad 300 compound crossbow to propel the sampling tips. Both crossbows have 150 lb (approximately 68kg) draw weight, but while the Wildcat crossbow propels an arrow at 72 m/sec, the Quad 300 shoots an arrow at 92 m/sec. The compound bow was added to our sampling equipment in 1997. This crossbow extended the range at which samples could be successfully obtained and has proved particularly useful for sampling large whales from the research vessel when sea conditions were too rough to launch a small boat. The addition of a red-dot sight (Tasco ProPoint Sight) to the compound bow in 1998 improved the accuracy of shots made with this bow and extended the useful operating range even farther. Currently, the maximum range documented for collection of a biopsy sample with the QUAD 300 compound bow fitted with a sight was measured at 70m in a vertical aerial photograph taken of a right whale (*Eubalaena glacialis*) being sampled in the Bering Sea (pers. comm. W. Perryman, SWFSC).

Biopsy tips are threaded on an adapter fitted to the crossbow bolt. We use bolts made of carbon fiber (Carbon Impact Ultra H 7500 arrows) in the Wildcat crossbow and of aluminum (Easton XX75 2220 Big Game arrows) in the Quad300 compound bow. A float molded on the front of the bolt provided flotation for later retrieval. However, bolts without

floats are used to sample individual cetaceans riding the bow of the ship. We use a tether on these bolts so that the research vessel does not have to stop to retrieve the bolt.

RESULTS AND DISCUSSION

We collected 1,501 biopsy samples from 4 mysticete and 20 odontocete species during 7 line transect survey cruises. An additional 169 samples were collected on the 9 other cruises, which added *Eubalaena glacialis* to our list of species sampled (Table 1).

The study area and primary objective of each cruise influenced the species encountered and the ability to collect biopsy samples. Specifically, fewer species are likely to be encountered in temperate waters, and the probability of good weather decreases when vessels are working farther offshore. However when we reviewed our collection of biopsy samples, what was evident is that we now sample many more species than we did in 1991. In part this reflects an increased emphasis on collecting biopsy samples. That is, in the early days we primarily sampled only species on which molecular research was currently being conducted. But now that our biopsy techniques have proven to be versatile and reliable, and molecular genetic techniques are becoming increasingly important for studies of cetaceans, we emphasize the collection of biopsy samples from any species encountered simply to enlarge our collection. This was a recent change for us and is most noticeable when the number of species sampled was compared pre- and post- 1998 cruises (Table 2).

When biopsy samples were collected, the number of attempts or shots made to collect a sample was recorded. The categories of attempts recorded were (1) a sample was successfully obtained, (2) a shot was made but completely missed the animal targeted, or (3) a shot hit an animal but no sample was obtained. For all mysticete species, samples were successfully retained on 84.0% of shots made that hit an animal, but only 45.3% of all attempts (i.e., all shots fired whether they hit or missed an animal) made to collect a sample were successful. By species, the success rate calculated from all attempts made to collect samples ranged from 36.0% for *Balaenoptera edeni* to 91.7% for *Eubalaena glacialis*. Published sampling success rates of 37% and 49% for *Eubalaena glacialis* sampled in the North Atlantic during two field seasons, 1988 and 1989, respectively (Brown *et al*., 1991) were comparable to our average rates for sampling mysticete species (i.e., 45.3%) but were lower than our rate for the same species sampled in the North Pacific. Similarly, for odontocete species, 68.4% of shots that hit an animal retained a sample and 44.3% of all shots fired resulted in the successful collection of a skin sample. By species, the success rates for odontocetes sampled ranged from 31.2% for *Stenella longirostris* to 73.1% for *Pseudorca crassidens* (Table 3). In part, these rates reflect the relative ease with which particular species can be sampled. A published estimate of 65% success rate for biopsy sampling *Orcinus orca* (Barrett-Lennard *et al.*, 1996) was better than our rate of 35.3%. However, our sampling conditions were not comparable to those of the Barrett-Lennard *et al.* (1996) study, because we were primarily attempting to sample offshore, transient whales rather than resident whales.

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Table 1. The total number of biopsy samples collected by species for each cruise type: line-transect survey or other. The survey cruises are of longer duration and cover a larger study area than the others, and therefore, they provide most of the sampling opportunities. The "unidentified cetacean" category includes only specimens for which there was a data recording error in the field that is currently unresolved.

Suborder/Species		Line-Transect Cruise Samples	"Other" Cruise Samples
Mysticeti			
	Balaenoptera edeni	10	2
	Balaenoptera musculus	40	32
	Balaenoptera physalus	9	9
	Eubalaena glacialis	0	11
	Megaptera novaeangliae	13	3
	Mysticeti Total	**72**	**57**
Odontoceti			
	Delphinus capensis	77	7
	Delphinus delphis	178	1
	Feresa attenuata	5	0
	Globicephala macrorhynchus	86	14
	Globicephala sp.	16	0
	Grampus griseus	8	4
	Lagenorhynchus obscurus	1	0
	Lagenorhyncus obliquidens	53	17
	Lissodelphis borealis	19	0
	Orcinus orca	11	4
	Phocoena phocoena	2	0
	Phocoenoides dalli	29	11
	Physeter macrocephalus	88	11
	Pseudorca crassidens	19	0
	Stenella attenuata	177	11
	Stenella attenuata graffmani	99	0
	Stenella attenuata subsp.	61	0
	Stenella coeruleoalba	10	0
	Stenella longirostris centroamericana	18	0
	Stenella longirostris orientalis	102	4
	Stenella longirostris subsp.	58	0
	Steno bredanensis	9	2
	Tursiops truncatus	297	26
	Unidentified dolphin or porpoise	3	0
	Odontoceti Total	**1426**	**112**
	Unidentified cetacean	3	0
	Grand Total	**1501**	**169**

Table 2. Summary of the research objectives and study area for cruises conducted by the Southwest Fisheries Science Center between 1991 and 1999. Biopsy samples were collected opportunistically on each of these cruises. The *David Starr Jordan*, *McArthur* and *Miller Freeman* are NOAA research vessels, the *Endeavor* is a UNOLS vessel and the *Sweetbrier* is a US Coast Guard cutter. The last column is the number of cetacean species with biopsy samples collected on each cruise.

Year	Type	Objective	Cruise #	Study Area	Vessel	Days at sea	# of species
1991	Line-Transect	Abundance estimate of California cetacean species	1426	30 - 42° N 117 - 131°W	*McArthur*	88	6
1992	Behavior	Behavioral study of *Stenella attenuata*	none	Eastern Tropical Pacific: coastal waters of Mexico	*McArthur*	30	2
1992	Line-Transect	Abundance estimate of *Delphinus* sp.	1467 1468	2 - 17°N 105 - 101°W	*McArthur* *David Starr Jordan*	90 90	11
1993	Behavior	Behavioral study of *Stenella attenuata*	none	Eastern Tropical Pacific: coastal waters of Mexico	*McArthur*	30	
1993	Line-Transect	Abundance estimate of *Delphinus* sp.	1508 1509	20 - 40°N 105 - 130°W	*McArthur* *David Starr Jordan*	84 84	13
1995	Behavior	Dive patterns of deep diving cetacean species	1601	20 - 29°N 105 - 155°W	*McArthur*	60	13
1995	Line-Transect	Abundance estimate of *Phocoena phocoena*	1602	Coastal CA	*McArthur*	18	4
1995	Ecology	Ecology of *Balaenoptera musculus*	1600	33.3-35.5°N 119 - 121°W	*McArthur*	28	3
1996	Ecology	Ecology of *Balaenoptera musculus*	1603	32 - 35° N 117 - 123°W	*David Starr Jordan*	24	4
1996	Line-Transect	Abundance estimate for cetacean species from the coast to 300mi offshore CA, OR, WA	1604 1605	30 - 49°N 117 - 131°W	*McArthur* *David Starr Jordan*	90 60	14
1997	Behavior	Dive time correction factors for *Physeter macrocephalus*	1606 1609	Coastal CA	*McArthur* *David Starr Jordan*	30 15	4
1997	Line-Transect	Abundance estimate of *Physeter macrocephalus*	1607	45 - 20°N 117 - 131°W	*McArthur*	90	7
1997	Population distribution	Location and distribution of *Eubalaena glacialis*	RW97	Southeast Bering Sea	*Miller Freeman*		4
1998	Line-Transect	Abundance estimate for *Stenella attenuata, Stenella longirostris, Delphinus* sp.	1610 1611 1612	Eastern Tropical Pacific 10°S - 40°N 70 - 158°W	*McArthur* *David Starr Jordan* *Endeavor*	120 120 120	22
1999	Line-Transect	Abundance estimate for *Stenella attenuata, Stenella longirostris, Delphinus* sp.	1613 1614	Eastern Tropical Pacific 15°S- 35°N 70 - 158°W	*McArthur* *David Starr Jordan*	120 120	21
1999	Population distribution	Location and distribution of *Eubalaena glacialis*	RW99	Southeast Bering Sea	*Sweetbrier*		3

Table 3. The "percentage of successful attempts" was calculated as the number of samples collected divided by the total number of attempts (i.e., shots fired) made to collect a sample. The "unidentified species″ categories listed are for specimens with currently unresolved data recording errors made in the field. For these specimens, molecular genetic techniques will be used to identify the species.

SPECIES	Number of Biopsy Samples Collected	Percentage of successful attempts
Suborder Mysticeti		
Balaenoptera edeni	9	36.0
Balaenoptera musculus	67	43.5
Balaenoptera physalus	18	45.0
Eubalaena glacialis	11	91.7
Megaptera novaeangliae	16	44.4
Sub-Total	121	45.3
Suborder Odontoceti		
Delphinus capensis	74	58.3
Delphinus delphis	176	40.1
Feresa attenuata	5	41.7
Globicephala macrorhynchus	86	50.3
Globicephala sp.	15	60.0
Grampus griseus	11	42.3
Lagenorhynchus obscurus	1	33.3
Lagenorhyncus obliquidens	66	40.2
Lissodelphis borealis	13	44.8
Orcinus orca	12	35.3
Phocoenoides dalli	29	39.7
Physeter macrocephalus	94	45.2
Pseudorca crassidens	19	73.1
Stenella attenuata	178	39.3
Stenella attenuata graffmani	99	49.8
Stenella attenuata subsp.	60	52.2
Stenella coeruleoalba	10	32.3
Stenella longirostris centroamericana	18	41.9
Stenella longirostris orientalis	105	41.5
Stenella longirostris subsp.	58	31.0
Steno bredanensis	10	41.7
Tursiops truncatus	300	51.4
Unidentified dolphin or porpoise	3	42.9
Sub-Total	1442	44.4
Unidentified cetacean	3	42.9
GRAND TOTAL	1566	44.4