# NTIC FILE COPY

UNCLASSIFIED SECURITY CLASSIFICATION OF THIS PAGE

UNCL	ORT SECURITY CLASSI			16. RESTRICTIVE MARKIN	VGS		
	ASSIFIED						
2a. SECU	IRITY CLASSIFICATION	AUTHORITY		3. DISTRIBUTION/AVAILA	BILITY OF REPO	AT	
2b. DECL	ASSIFICATION/DOWN	GRADING SCHEDULE					
L				Approved for public release; distribution is unlimited. 5. MONITORING ORGANIZATION REPORT NUMBER(S)			
4. PERFC	JHMING ORGANIZATIO	N REPORT NUMBER(S)		5. MUNITORING ORGANI	ZATION HEPOR	I NUMBER(S)	
6a. NAMI	E OF PERFORMING OF	IGANIZATION	6b. OFFICE SYMBOL (# applicable)	78. NAME OF MONITORING ORGANIZATION			
	cean Systems Cente		NOSC	Naval Ocean Systems Center			
6c. ADDF	RESS (Cily, State and ZIP Code)			7b. ADDRESS (City, State and	ZIP Cade)		
San Diego, CA 92152-5000				San Diego, CA 92152-5000			
82. NAME OF FUNDING/SPONSORING ORGANIZATION 80. OFFICE SYMBOL (# applicable)				9. PROCUREMENT INSTR	NUMENT IDENTIF	CATION NUMB	IER
Naval S	Sea Systems Comma	nd	NSEA				
8c. ADDRESS (City, State and ZIP Code)				10. SOURCE OF FUNDING PROGRAM ELEMENT NO		TASK NO	I AGEN
				THUGHAM ELEMENT NO	HOJECT NO.	TASK NO.	ACCESSIO
ł					}		
Washington, DC 20362				<u>63709N</u>	MM01		DN788
11. TITLE	E (include Security Classification)	}					
						CAIN A TRANS	T
	ING ATLANTIC BO	DTTLENOSE DOLPH	IINS (TURSIUPS T	RUNCATUS) FOR ART	ITICIAL INS	EMINATION	·
	Keller, Jr.				(Vest Month Own		
13a. TYPE OF REPORT13b. TIME COVEREDPresentation/speechFROM Oct 1986TO Oct 1986				14. DATE OF REPORT (Year, Month, Day) 15. PAGE COUNT			
	LEMENTARY NOTATI		10 00 1900	July 1988		L	
17 000	ATI CODES		A SUBJECT TERMAS	Continue on reverse if necessary and it	institute bicorte sumbar		
FIELD	GROUP	SUB-GROUP		www.anue.common.common.common.common.common.common.common.common.common.common.common.common.common.common.com	wany uy unuan nutinuer)	•	
• <u> </u>	- GROUP		biology				
		+					
		1					
J. ABST	TRACT (Continue on reverse i	f necessary and identify by block num	nber)			<u> </u>	
J. ABST	•	, , ,		the past eight years ha	s maintained a	an artificial in	semination
(AI) pi	The Naval Ocean rogram for Atlantic	Systems Center, Hav hottlenose dolphins, E	waii Laboratory, for Both male and fema	the past eight years ha le dolphins are trained	for blood colle	ction from ves	sels in the
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer	sels in the
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained	for blood colle ed for AI. All	ction from ves behaviors wer	sels in the
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer	sels in the
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer	sels in the
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer	sels in the
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer	sels in the
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer deep.	re trained us
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer deep.	sels in the
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer deep.	re trained us
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer deep.	re trained us
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer deep.	ssels in the retrained us CTE 7, 1988
(AI) pr flukes.	The Naval Ocean rogram for Atlantic In addition, male d	Systems Center, Have bottlenose dolphins. E olphins are trained fo	waii Laboratory, for Both male and fema r semen collection,	le dolphins are trained while females are trained	for blood colle ed for AI. All	ction from ves behaviors wer deep.	re trained us
(AI) pr flukes. operant	The Naval Ocear rogram for Atlantic In addition, male d t conditioning with f	a Systems Center, Hav bottlenose dolphins. E olphins are trained fo ish as reinforcements.	waii Laboratory, for Both male and fema r semen collection, . All test animals an	le dolphins are trained while females are traine re kept in floating pens	for blood colle ed for AI. All 20' x 20' x 12	ction from ves behaviors wer deep.	seels in the retrained us ECTE 7, 1988 E
(AI) pr flukes. operant	The Naval Ocear rogram for Atlantic In addition, male d t conditioning with f	a Systems Center, Hav bottlenose dolphins. E olphins are trained fo ish as reinforcements.	waii Laboratory, for Both male and fema r semen collection, . All test animals an	le dolphins are trained while females are trainer re kept in floating pens Conference, 27-31 Octo	for blood colle ed for Al. All 20' x 20' x 12 cr cr	ction from ves behaviors wer deep. DELE SEP ( SEP ( a	seels in the retrained us ECTE 7, 1988 E
(AI) pr flukes. operant Present	The Naval Ocear rogram for Atlantic In addition, male d t conditioning with f	a Systems Center, Hav bottlenose dolphins. E olphins are trained fo ish as reinforcements. hal Marine Animal Tr Y OF ABSTRACT	waii Laboratory, for Both male and fema r semen collection, . All test animals an All test animals an raners' Association	le dolphins are trained while females are trainer re kept in floating pens Conference, 27-31 Octo 21. ABSTRACT SECURIT	for blood colle ed for Al. All 20' x 20' x 12 cr cr	ction from ves behaviors wer deep. DELE SEP ( SEP ( a	seels in the retrained us ECTE 7, 1988 E
(AI) pr flukes. operant Present 20. DIST	The Naval Ocear rogram for Atlantic In addition, male d t conditioning with f ed at the Internation RIBUTION/AVAILABILIT CLASSIFIED/UNLIMITED	a Systems Center, Hav bottlenose dolphins. E olphins are trained fo ish as reinforcements. hal Marine Animal Tr Y OF ABSTRACT X SAME AS RPT	waii Laboratory, for Both male and fema r semen collection, . All test animals an	le dolphins are trained while females are trainer re kept in floating pens Conference, 27-31 Octo 21. ABSTRACT SECURIT UNCLASSIFIED	for blood colle ed for AI. All 20' x 20' x 12 cober 1986, Va	ction from ves behaviors wer deep. ELE SEP ( SEP ( A ncouver, Cana	ssels in the retrained us ECTE 7, 1988 E ada.
(AI) pr flukes. operant Present 20. DIST	The Naval Ocear rogram for Atlantic In addition, male d t conditioning with f	a Systems Center, Hav bottlenose dolphins. E olphins are trained fo ish as reinforcements. hal Marine Animal Tr Y OF ABSTRACT X SAME AS RPT	waii Laboratory, for Both male and fema r semen collection, . All test animals an All test animals an raners' Association	le dolphins are trained while females are trained re kept in floating pens Conference, 27-31 Octo 21. ABSTRACT SECURIT UNCLASSIFIED 22b. TELEPHONE (include)	for blood colle ed for AI. All 20' x 20' x 12 cober 1986, Va	ction from ves behaviors wer deep. DELE SEP ( SEP ( a ncouver, Cana ION 22c. OFFICE S	ssels in the retrained us E TICE TICE TICE TICE TICE TICE TICE TI
(AI) pr flukes. operant Present 20. DIST 22. NAM	The Naval Ocear rogram for Atlantic In addition, male d t conditioning with f ed at the Internation RIBUTION/AVAILABILIT CLASSIFIED/UNLIMITED	a Systems Center, Hav bottlenose dolphins. E olphins are trained fo ish as reinforcements. hal Marine Animal Tr Y OF ABSTRACT X SAME AS RPT	waii Laboratory, for Both male and fema r semen collection, . All test animals an All test animals an raners' Association	le dolphins are trained while females are trainer re kept in floating pens Conference, 27-31 Octo 21. ABSTRACT SECURIT UNCLASSIFIED	for blood colle ed for AI. All 20' x 20' x 12 cober 1986, Va	ction from ves behaviors wer deep. DELE SEP ( SEP ( SEP ( Control of the second control	ssels in the retrained us ECTE 7, 1988 E ada.

# TRAINING ATLANTIC BOTTLENOSE DOLPHINS (<u>Tursiops</u> <u>truncatus</u>) FOR ARTIFICIAL INSEMINATION Karl V. Keller Naval Ocean Systems Center, Hawaii Laboratory Kailua, HI 96734

# INTRODUCTION

The Naval Ocean Systems Center, Hawaii Laboratory, for the past eight years has maintained an artificial insemination (AI) program for Atlantic bottlenose dolphins. Both male and female dolphins are trained for blood collection from vessels, in the flukes. In addition, male dolphins are trained for semen collection, while females are trained for AI. All behaviors were trained using operant conditioning with fish as reinforcements. All test animals are kept in floating pens 20' x 20' x 12' deep.

### BLOOD COLLECTION FROM FLUKES

Both male and female dolphins are trained to present their flukes for blood sampling to measure the seasonal fluctuations of testosterone and progesterone levels. This training is difficult because the animal must remain motionless during venipuncture. Initial training began with basic handling of the animal in the water. While the animal was stationed near the side of the pen, we reinforced the animal for allowing us to manipulate its flukes. In time, we paired manipulation of this area with a simple hand cue. After we established stimulus control, we gradually raised the flukes from the water and placed them on the pen deck while the animal remained on its side. With the flukes resting on a pad on the deck, we gradually increased manipulation of the flukes to simulate the actual blood sampling procedure. Throughout most of the training a second person observed the training and eventually assisted during the blood collection. Once good

stimulus control was established, we held the flukes about 4 minutes for actual blood collection. Initial blood sampling was short, lasting about one to two minutes; it was gradually increased over several days to the desired time interval. A consistent body position for fluke bleeding was important. Having the animal on its side and parallel to the pen with its ventral surface facing the pen deck is the best position for bleeding. If the animal's head is farther away from the pen deck, the animal twists, resulting in more fluke movement. With its ventral surface parallel to the pen deck, the animal appears to be comfortable and can see the trainers. Because the animal can breathe easily in this position, it can hold the position longer. The dolphin can be bled from either the dorsal or ventral side by changing the angle of the flukes.

To maintain the behavior over time, we practiced imitating the actual bleeding process without the venipuncture. We varied the length of holding the flukes so the animal did not anticipate the end of the behavior. We ended the fluke-holding behavior when the animal was quiet and relaxed. SEMEN COLLECTION

Because this procedure has not previously been performed on dolphins, we developed a new process. The training steps consisted of correct body position, penis erection, and semen collection. It took about 49 days to train two captive males.

Initially, the animal was trained to station horizontally with its ventrum facing the water's surface. After the proper body orientation was under stimulus control, the next phase began.

The initiation of penis erection was difficult to convey to the animal. The training sessions were about 20 minutes per day. The animal was rewarded for its movement of the genital slit opening and exposure of the penis tip. Once the exposure of the penis tip was consistent, we reinforced

this behavior after every other response. Partial erections were reinforced immediately with fish.

The erection behavior was shaped by reinforcing greater and greater exposure in a step-like manner and by using intermittent reinforcement. First, partial erections of one inch were reinforced after every other response. Partial erections greater than one inch were reinforced immediately. Then partial erections to two inches were reinforced on about every seventh response, with greater erections reinforced immediately. Finally, when partial erections of greater than two inches were consistently performed, the behavior was reinforced on about every seventh response.

After penis erection was controlled, the animal's position with respect to the pen deck was shaped to allow the trainer easy access to the genital slit area.

The final stage in this training was collection of the ejaculate. The samples were collected in glass test tubes with an attached clear plastic sleeve. The clear plastic sleeve was slipped over the tip of the penis. The trainer applied slight pressure and massage and funneled the ejaculate into the collection tube. Using these techniques semen was collected reliably for three years, adding to knowledge of the dolphin reproductive biology (Schroeder, et al., 1983).

## ARTIFICIAL INSEMINATION

The process of AI has been used with many species of terrestrial mammals, e.g., horses, cows, dogs (Gomes, 1977; Watson, 1978). At Naval Ocean Systems Center we have made the first attempts to use AI procedures in the dolphin (Schroeder, 1984). The reproductive system of the female bottlenose dolphin includes a pseudocervix with muscular folds in the

vaginal wall. In <u>T</u>. <u>truncatus</u> there are at least two of these folds, and there can be as many as 6 to 12 in certain balaenopterids (Harrison, 1969). This pseudocervix is within the spermathecal recess, where sperm is deposited during copulation. In the AI process, semen collected from a male dolphin is placed in the spermathecal recess with the aid of a fiber-optic laryngoscope (Schroeder, 1985).

Female dolphins were conditioned to handling with simulated AI procedures. The dolphins were caught by a stranding device. The animal was placed in a fleece-lined stretcher with openings for the flippers and a slit for the genital area, then laid on its side on a pen deck padded with foam rubber. After 15 minutes of occasional manipulation of the genital area, the animal was returned to the floating pen and rewarded.

During the actual AI procedure, we used a speculum and a fiber-optic laryngoscope to locate the mouth of the pseudocervix and direct the laryngoscope tip into the spermathecal recess. Three milliliters of semen were deposited in the spermathecal recess using the laryngoscope's internal channel.

#### SUMMARY

This paper has described some basic training techniques for AI of Atlantic bottlenose dolphins. Both male and female dolphins, after training, voluntarily submitted to blood collection via fluke bleeding. Male dolphins were trained for semen collection, while females were trained to submit for AI procedures.

Similar AI programs of other species of small cetaceans, if successful, could decrease our reliance on acquiring mammals from the wild. A marine mammal sperm bank also could ensure genetic variability and preserve gene pools of endangered species.

#### REFERENCES

Gomes, W. R. (1977) Artificial Insemination. In: Reproduction in Domestic Animals (H. H. Cole and P. T. Cupps, eds.), pp 257-284, Academic Press, New York.

Harrison, R. J. (1969) Reproduction and reproductive organs. In: The Biology of Marine Mammals (H. T. Andersen, ed.), pp 270-275, Academic Press, New York.

Seager, S., Wildt, D., and Platz, C. (1978) Artificial breeding of nonprimates. In: Artificial Breeding of Non-Domestic Animals (P. F. Watson, ed.), pp 207-218, The Zoological Society of London, Academic Press, London, England.

Schroeder, J.P., K.V. Keller, and V. L. Kirby (1983) Testosterone levels and sperm production in Tursiops truncatus, Fifth Biennial Conference on the Biology of Marine Mammals. (Abstacts, p. 91), Boston, USA.

Schroeder, J.P. (1984) Induced reproductive cycle events in Tursiops truncatus. In: Reproduction in Whales, Dolphins and Porpoises (W.F. Perrin, R.L. Brownell, Jr. and D.P. Demaster, eds.), International Whaling Commission, Cambridge (Special Issue 6).

Schroeder, J.P. (1985) Artificial Insemination of the Bottlenose Dolphin, Tursiops truncatus. In: Abstracts and Papers, 1985 Annual Meeting, American Association of Zoo Veterinarians, (M.S. Silberman, D.V.M. and S.D. Silberman, eds.), pp 122-124.

COPY

Acoustics for WIIS GPACE - DIIC THE Unannounced i Justification SPECTE Fy\_ Distribution/ Availability Codes Avail and/or Dist Special