NAMMCO
SCIENTIFIC PUBLICATIONS

Online Early Version

Evaluation of etorphine reversed by diprenorphine for the immobilisation of free-ranging Atlantic walrus (*Odobenus rosmarus rosmarus* L.)

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ABSTRACT

To date no problem-free method exists for the immobilisation of free-ranging walruses (*Odobenus rosmarus*). In the period 1989-2001, 69 immobilisations with etorphine HCl were performed by remote darting of 41 individual free-ranging adult Atlantic walruses (*O. r. rosmarus*), with body masses 633 - 1883 kg, as a prerequisite for the attachment of radio tracking and dive recording instruments, and for studies of metabolism. Ten individuals were immobilised several times. We present data on these 69 immobilisations and evaluate the method. Full immobilisation was achieved in 58 cases (84 %). The animals were insufficiently restrained in 6 cases (9 %) and 5 animals died (7 %) following the immobilisation. The animals were fully immobilised and approachable after 5 min (n = 38, range = 1.9 - 12.4 min, SD = 2.2) with a dose of etorphine of 6.1 µg/kg (range 2.4 - 12.6 µg /kg, SD = 2.4). Induction time was negatively correlated with the dosage of etorphine. Etorphine-induced apnoea lasted 13.7 min (n = 36, range 17.0 - 26.7 min, SD = 5.1) and was reversed by multiple doses of the antagonist diprenorphine HCl. The first dose of antagonist of 12.2 mg (n = 39, range 6.0 - 21.0 mg, SD = 3.5) was administered 8.4 min (n = 38, range 4.7 - 18.0 min, SD = 2.8) after injection of the agonist. The total dose of diprenorphine per animal ranged between 7.7 and 41.7 µg/kg (n = 31, mean = 17.2 µg/kg, SD = 7.5). For some animals blood pH values were measured following the apnoea and reached low levels (min pH 6.8). For animals that were immobilised several times there were no indications of changed sensitivity to etorphine as reflected in unchanged induction times. Mortalities could neither be related to the doses of agonist and antagonist, nor to the times of administration of the drugs. From this (n = 69) and other (n = 103) studies involving etorphine immobilisation of walruses (both Atlantic and Pacific) the overall success rate is 83 % (8 % casualty rate). We conclude that the combination etorphine-diprenorphine is suitable for both single and multiple immobilisations of walruses provided that (a) a casualty rate of 7-8% is acceptable (b) the antagonist diprenorphine is administered fast and well into a tissue with good blood irrigation, and (c) the animal is promptly intubated endotracheally to facilitate the restoration of breathing after drug-induced apnoea.

Mario Acquarone, Erik W Born, David Griffiths, Lars Øyvind Knutsen, Øystein Wiig and Ian Gjertz (2014) Evaluation of etorphine reversed by diprenorphine for the immobilisation of free-ranging Atlantic walrus (*Odobenus rosmarus rosmarus* L.) *NAMMCO Scientific Publications*, Volume 9. doi: http://dx.doi.org/10.7557/3.2944

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INTRODUCTION

Chemical immobilisation is typically necessary in studies of pinnipeds that require contact with the animals for the attachment of instruments or physiology experiments. This is the case when working in the field with large and potentially dangerous species like walrus (*Odobenus rosmarus*). Captive and free-ranging Atlantic (*O. r. rosmarus*) and Pacific (*O. r. divergens*) walruses have been occasionally immobilised for surgery or for research purposes using a number of different protocols and drugs in the quest of a reliable method that is safe to the animals and the researchers. A series of anaesthetic agonist agents have been employed, alone or in combinations, for surgery of captive walrus. These include, for example, ketamine (Hagenbeck *et al.* 1975), or meperidine sulfate and thiamylal (Cornell and Antrim 1987). On free-ranging walruses, several drugs, alone or in combinations, have been employed: phencyclidine and acepromazine (DeMaster *et al.* 1981), tiletamine and zolazepam (Griffiths *et al.* 1993, Hills 1992, Stirling and Sjare 1988), medetomidine and ketamine (Lydersen *et al.* 1992), etorphine (Born and Knutsen 1992a, Griffiths *et al.* 1993, Hills 1992), carfentanil (Hills 1992, Lanthier *et al.* 1999, see Table 1). However, the descriptions of the effects and the response of the animals to the different drugs are fragmentary, and more important, none has proven optimal for this species.

Remote, intramuscular administration of immobilising agents (by dart or extension syringe) is preferred when working with large or dangerous species in the wild (Lynch *et al.* 1999). Only the potent synthetic opioids carfentanil and etorphine simultaneously satisfy the requirements of a minimum injection volume, rapid induction, and the existence of an antagonist drug to control the reversal of the effects of the agonist drug. The use of carfentanil alone and antagonised with naltrexone has been described for only four Pacific walruses (Hills 1992) and for six Atlantic walruses (Lanthier *et al.* 1999). The paucity of data for carfentanil does not allow a thorough evaluation of this immobilising agent, while the relatively more extensive use of etorphine (172 immobilisations) provides a good basis for an evaluation.

Etorphine antagonized with diprenorphine was first used in 1988 for immobilisation of Pacific walrus in Alaska (Hills 1992), and in 1989 for Atlantic walrus in Greenland (Born and Knutsen 1990, Knutsen and Born 1994). In these and subsequent field studies of walrus (*e.g.* Griffiths *et al.* 1993) the number of animals treated per season has been relatively low. Therefore the accumulation of data for evaluation of the feasibility of using a particular drug for immobilisation of walrus has been a slow process. More recently walruses have been immobilised with etorphine in the

Table 1. Summary of the immobilisations of walruses reported in the literature and in the present study. The drug employed and the number of attempts, partial immobilisations and casualties are reported with an indication of the geographical area and the references for each group.

Drug	Immobili-sation Attempts	Partial Immobili-sations	Casualties	Location
Ketamine	1			Captivity (Hagenbeck *et al.* 1975)
Ketamine	1	1		Alaska (DeMaster *et al.* 1981)
Phencyclidine	1	1		Alaska (DeMaster *et al.* 1981)
Phencyclidine and Acepromazine	7	2	2	Alaska (DeMaster *et al.* 1981)
Meperedine Sulfate and Thiamidal	1			Captivity (Cornell and Antrim 1987)
Tiletamine and Zolazepam	10	3	1	Canada (Stirling and Sjare 1988)
	7	1	3	Alaska (Hills 1992)
	3		1	Svalbard (Griffiths *et al.* 1993)
Medetomidine and Ketamine	1			Svalbard (Lydersen *et al.* 1992)
Carfentanil	4			Alaska (Hills 1992)
	6			Canada (Lanthier *et al.* 1999)
Etorphine	59	8	5	Alaska (Hills 1992)
	38	2	1	Svalbard (Griffiths *et al.* 1993)
	6		2	Svalbard (Griffiths unpublished)
	69	6	5	This study

Norwegian Arctic but unfortunately no data on these procedures is available. Preliminary data from some of the early work have been partially presented in technical reports (Born and Knutsen 1990, Born and Knutsen 1992a, Born and Wiig 1995, Knutsen 1993), which are difficult to obtain. In this paper we report on more recent immobilizations in Greenland and Franz Josef Land and use them and previous studies to summarise and evaluate the experience acquired to date in the use of etorphine for the immobilisation of free-ranging Atlantic walruses.

MATERIALS AND METHODS

During the open water season in June-August 1989-92, August 1994, and July-August 1999-2001 a total of 69 immobilisation attempts were performed using etorphine on 41 individual adult Atlantic walruses (37 males, 4 females). The animals were darted at different locations in Greenland (Kane Basin in NW Greenland, Young Sound and Dove Bay in NE Greenland) and Franz-Josef Land (Apolonov, Kuhn, Hayes and Hooker Island) both on land and on ice floes. Ten out of the 37 males were treated repeatedly both within the same season and in different years (Table 2).

Table 2. List of multiple immobilisations of the same individual walruses (ID) by etorphine/ diprenorphine in NE Greenland between 1989 and 2001. The figures represent the number of immobilisations of the same individual within the same summer season.

ID	1989	1990	1999	2000	2001
1989-3	2	1		1	2
1989-4	1	1			
1999-1			1	1	1
2000-1				4	
2000-2				3	3
2000-3				2	
2000-4				3	
2000-6				3	2
2000-8				2	3
2001-9					2

Immobilisation protocol

The effect of etorphine was reversed by the antagonist diprenorphine. The drug concentrations used for immobilisation at Franz Josef Land were 9.8 mg/ml etorphine and 12 mg/ml diprenorphine (Cyprenorphine, C-Vet Ltd., Bury St. Edmunds, Suffolk, U.K.). In Greenland the concentrations used were: 4 mg/ml (EtorfinVet. Pharmacia, Denmark) and 9.8 mg/ml (either "M99" Vericore Ltd., Dundee, Scotland or Etorfin Vet., Pharmacia, Denmark) for etorphine and 6 mg/ml (Diprenorfin Vet., Pharmacia, Denmark) and 12 mg/ml ("M5050", 12 mg/ml, Vericore Ltd., Dundee, Scotland) for diprenorphine respectively. As the actual weight of the animals was unknown, dosage was based on experience and only on adult walruses in good condition were immobilised.

The animals did not receive any pre-anaesthetic medication. The drug was usually delivered after the animals had settled down on the terrestrial haulout and were resting. The etorphine was delivered in 3 ml plastic darts equipped with a 10 cm-long needle plain (Vario dart, Telinject USA, 9316 Soledad Canyon Rd., Saugas, California 91350, U.S.A.) which were shot using a 11 mm-gauge CO_2 powered darting gun (Daninject, Børkop, Denmark, and Telinject, Römerberg, Germany). Diprenorphine was administered by hand-injection. Syringes with 2 mm-gauge and 8 or 10 cm-long, plain needles were used to secure the deposition of the antagonist in the muscle tissue under the thick skin and blubber layer.

All time measures reported in this paper are relative to the time of injection of etorphine. Important events during the immobilisation were:

a) Induction time: Time elapsed between impact of the dart (injection of the drug) and time of approach (*i.e.* the animal was unable to move and typically had stopped breathing 30-60 sec before approach)

b) Apnoea: Time elapsed between cessation of breathing (estimated to 30 sec before approach if not directly observed) and first, deep breath measured with a digital chronometer (Casio, Japan).

The immobilisation protocol employed was initially developed in Greenland in 1989, adjusted in subsequent studies and was basically similar to the one described in Griffiths *et al.* (1993) and Lanthier *et al.* (1999).

Most animals were immobilised during afternoons and evenings when walruses prefer to haul out (Born and Knutsen 1997). Furthermore, during the afternoons the herd of walruses was less tightly packed (Born and Knutsen 1990). It then became easier to select a suitable animal for darting (*i.e.* ideally an animal with tusks that were large enough to carry instruments and that

presented calm behaviour), thus causing a minimum of disturbance to the other animals. When working in the pack ice it was preferred to select animals that appeared relaxed and were not associated with calves and other sub-adults that, if frightened, might cause acute disturbance. Furthermore the floes used for hauling out had to be large enough for the handling of the drugged animal (floe size > 50 m^2). The darts were shot from a distance of 15 - 40 m. The point of aim was chosen to ensure maximum penetration of the needle, based on experience and confirmed by visual inspection, and optimal drug delivery in the muscular tissue with minimal disturbance. We also attempted to hit a body location out of the animal's field of sight because viewing the dart might have induced a reaction in the darted subject. Dart impact sites during immobilisation events were: in the upper dorsal region (19); the lower dorsal region (19); the flank (11); ventral region (4); a limb (1); not recorded (15).

Etorphine can induce respiratory depression (Alford *et al.* 1974), and in walrus drug-induced apnoea is an unavoidable side-effect of the administration of etorphine (Griffiths *et al.* 1993). To minimize the duration of the apnoeic period and therefore the risk of death by suffocation, the antagonist diprenorphine was administered immediately after approach. The first dose of antagonist was either administered intramuscularly in the shoulder or lumbar region (n = 51), intravenously (n = 5) or in the highly vascularized tongue (n = 7) if muscle tonus in the jaw region did not prevent opening the mouth.

In 2000 and 2001 the walruses were subjects of physiology studies that involved taking repeated blood samples for an extended period while the animals were on land. To extend the period when the animals were tractable after they had regained regular breathing, following the injection of diprenorphine, 6 of the 32 animals were given small amounts of medetomidine ("Domitor Forte", 10 mg/ml, Orion Pharma, Turku, Finland; Griffiths *et al.* 2014). The medetomidine mean delivery time was 45 min from the etorphine injection (n = 6, range 30 – 78 min, SD = 15). Physiological and behavioural data for these animals are included in this analysis only up to the delivery of medetomidine. Data sampled after the administration of medetomidine were excluded from the analysis of the characteristics of the recovery from immobilisation by etorphine alone and are included in Griffiths *et al.* (2014).

In 1999-2001 the animals were routinely intubated with an endotracheal tube (Cook Large Animal Veterinary Products, 38-mm internal diameter). The intubation, in each case, was first attempted blindly and if this proved unsuccessful, the tube was guided by the operator's hand down the throat of the animal. In this case the operator's fingers helped locate and open the

epiglottis, and facilitated the insertion of the tube into the trachea. Inflating the cuff was normally unnecessary as airtightness was ensured by the muscular compression of the tube.

Animal monitoring during immobilisation and recovery
In 23 cases breathing rate was recorded prior to immobilisation. Breathing rate was also routinely recorded during immobilisation.

Heart rate of immobilised animals was monitored with an electronic pulse meter (Exersentry, Respironics Instruments, Inc., Monroeville, Pennsylvania 15146, U.S.A.) equipped with four sensors connected to 6 cm long needles inserted, as far as possible from each other, through the skin.

Body temperature was measured in two places with an electronic thermometer (DM852, Ellab, Copenhagen, Denmark): a blunt probe was inserted approximately 20 cm in the rectum and a needle probe was inserted in a hind limb 2 – 4 cm below the surface of the skin, the latter to monitor body surface temperature. The temperature values were not recorded systematically and were used only as a warning of overheating.

In 1999-2001 a catheter (Becton-Dickinson Secalon-T, 2.0 mm * 160 mm, Medisinsk Utstyr AS, Oslo, Norway) was inserted into the epidural vertebral venous sinus (n = 43) which provided access for blood sampling and intravenous drug delivery. In five animals blood pH-values were measured in the field by use of i-Stat Portable Clinical Analyzer (i-STAT Corporation, 104 Windsor Center Dr., East Windsor, NJ 08520, USA).

Standard and zoological body length and axillary girth were measured in the field and were subsequently used to estimate total body mass (TBM) according to methods in Born *et al.* (2003). For three walruses which were immobilised on pack ice, TBM was estimated by comparing tusk dimensions with a TBM-at-age curve established from walruses sampled from the Greenlandic subsistence catch in north-western Greenland (Born unpublished data). In 8 cases estimates of TBM were not available.

Statistical analysis
Time and temperature data were log-transformed when necessary to obtain equality of variances and homoscedasticity assumed in parametric tests. The assumed significance level in all the tests was $p = 0.05$.

The data were subsequently sorted into two groups: group ***A*** includes animals immobilised only once (31 individuals) and the first immobilisation for animals treated more than once (10 individuals) giving a total of 41 immobilisations; and group ***B*** comprising only the animals that were

subjected to repeated immobilisations (10 individuals, 38 immobilisations including the first).

Differences between males and females for dose of etorphine administered, induction time, duration of apnoea, antagonist injection time, antagonist first dose and time of first breath after induction were examined using a Mann-Whitney *U*-test. In this and subsequent tests on group ***A*** data we excluded one animal which had an unusually long induction time. In this walrus the dart only penetrated 4 cm into the animal and presumably the agonist was injected into the blubber.

On group ***A,*** linear regression analysis was employed to evaluate the effects of the dose of agonist on the induction time and the duration of apnoea and of the time between the administration of the agonist and the antagonist on the duration of apnoea. Due to missing data, the differences in pulse and body temperature between the apnoeic and post-apnoeic period were tested by use of unpaired *t*-tests.

The cumulative effect of consecutive immobilisations of the same individual within the same season (group ***B***) was investigated. The relative dose of etorphine on the log transformed induction time and log transformed apnoea duration was tested with and without the effect of the interaction between the two independent variables (*i.e.* relative dose of etorphine and time for etorphine administration and time from the previous immobilisation). Similarly, the cumulative effect of the time for diprenorphine administration was tested on the log transformed apnoea duration with and without the interaction between the two independent variables (*i.e.* time between etorphine and diprenorphine administration and time from the previous immobilisation). A spatial Gaussian correlation model for unequally spaced repeated measures including the random effect of the individual walrus (SAS® PROC MIX procedure) was used (Littel *et al.* 1996) for this purpose.

The circumstances of mortality events were investigated by bootstrapping 100 times and tested against etorphine dosage, diprenorphine dosage and time of delivery with respect to etorphine administration. Chi-square tests were employed to test the effect of the researcher in charge on the failure and mortality rate.

The statistical packages StatView® (version 5.0.1, SAS Institute Inc.) and SAS® (version 8.2, SAS Institute Inc.) were employed for data analysis.

RESULTS

Environmental conditions

In Greenland immobilisations were performed on calm (n = 54, $\bar{x}$= 2.3 m/s, range 0 – 12 m/s, SD = 2.1) and relatively warm days (n = 54, $\bar{x}$ = 4.9 °C, range –1.0 - 12.3 °C, SD = 2.8); at Franz Josef Land it was usually calm (0 – 6 m/s) and temperatures ranged between –8 °C and +3 °C.

Improvement of the immobilisation protocol

Behavioural responses during induction followed generally the sequence described by Griffiths *et al.* (1993) and Lanthier (1999). These corresponded to (1) mild and brief alertness upon darting, (2) return to resting position, (3) resumed mild alertness with gradual impairment of the ability to raise the head, and finally (4) twitching and lumbar muscle spasms at which point the animal was considered safe to approach and administration of the antagonist. In addition to the protocol described by Griffiths *et al*. (1993) and Lanthier *et al*. (1999) and early seasons described in this study, in 1999-2001 after the administration of the antagonist the walruses were routinely intubated to prevent asphyxia due to etorphine-induced muscle contractions around the airways. This operation was usually initiated relatively late during apnoea. Due to contraction of the muscles around the jaw, glottis and pharynx it was sometimes difficult to insert the tube and forced, opening of the glottis by hand was required. Forced ventilation using an inflatable boat, double action, hand pump (4 L capacity) seemed useful for testing the correct placement of the tube and during the initial part of the recovery phase. Emergency hand pump ventilation was also attempted in three of the five mortalities. In general, routine intubation alone, by ensuring opening of the glottis, seemed very to be useful in helping the animals regain full spontaneous ventilation.

Differences between males and females

There was no significant difference in the relative total dose of etorphine administered to males and females (Mann-Whitney *U*-test, P = 0.06). Likewise no significant difference between males and females was observed for apnoea start (Mann-Whitney *U*-test, P = 0.11), apnoea duration (Mann-Whitney *U*-test, P = 0.43), time of first injection of diprenorphine (Mann-Whitney *U*-test, P = 0.79), amount of diprenorphine administered as a first dose (Mann-Whitney *U*-test, P = 0.42) or time of first breath after etorphine administration (Mann-Whitney *U*-test, P = 0.08).

First time immobilisations (group *A*)

The estimated total body mass of the animals in group ***A*** ranged from 633 to 1883 kg (Table 3). A total of between 4 and 10 mg etorphine was administered per individual walrus at an estimated mean dose of 6.0 µg/kg (Table 3). Higher etorphine doses had shorter induction times ("log-induction time in

min" = 1.068 - 0.55 * "etorphine dose in µ/TBM in kg", R^2 = 0.275, p = 0.004, n = 28).

Apnoea was induced in all animals at a mean of 5.0 min after injection of etorphine (Table 3). Apnoea lasted 13.7 min (Table 3). The antagonist diprenorphine was administered 8.4 min after darting (Table 3) at a mean first dose of 12.2 mg (n = 39, range 6.0 - 21.0 mg, SD = 3.5). The total dose of diprenorphine delivered to the animals that were successfully immobilised ranged from 7.7 to 41.7 µg/kg (Table 3). For the same individuals the recovery phase, beginning from the first breathing act after the apnoeic period, started between 11.5 and 34.0 min from the injection of etorphine (n = 30, $\bar{x}$ = 18.7 min, SD = 6.0).

The duration of apnoea and the dose of etorphine were apparently not correlated ("log-duration of apnoea in min" = 1.211 - 0.136 * "log-µg/kg etorphine", R^2 = 0.03, p = 0.40, n = 27). However, if the antagonist was injected relatively late, the apnoeic period became longer. There was significant positive correlation between the time of injection of the first dose of antagonist and the duration of apnoea ("log-duration of apnoea in min" = 0.697 - 0.451 * "log-time of injection of antagonist in min", R^2 = 0.20, p = 0.011, n = 33).

Table 3. Summary of first-time immobilisations (**group *A***) presented in this study. "Induction time" represents the time between etorphine administration and complete immobilisation. "Diprenorphine Dose" represents the total dose of diprenorphine administered. And "Diprenorphine time" represents the time for the first dose.

	Body mass (kg)	Etorphine dose (µg/kg)	Induction time (min)	Diprenorphine		Duration of apnoea (min)
				Dose (µg/kg)	Time (min)	
Mean	1310	6.0	5.0	17.2	8.4	13.7
Range	633 - 1883	2.4 - 12.6	1.9 - 12.4	7.7 - 41.7	4.7 - 18.0	17.0 - 26.7
SD	294	2.4	2.2	7.5	2.8	5.1
n	34	33	38	31	38	36

Respiration rate of undisturbed resting walruses was 3.9 breaths/minute (n = 23, SD = 0.82). The breathing rate following apnoea was 9 breaths/min (n = 29, range 1 – 32 breaths/minute, SD = 6) up to 30 min from induction. During the following 175 min, the average breathing rate decreased (breath/min = 17.34 - 2.48 ∗ "log-min after induction"; R^2 = 0.094). In particular, after 60 min from induction it was 6 breaths/min (n = 16, range 2 – 20 breaths/minute, SD = 4).

The pulse of one undisturbed animal before it received a dose of etorphine was observed from movements of the ventral skin at 55 beats/min (similarly two other hauled-out animals, not subsequently drugged, showed a pulse of 48 and 52 beats/min respectively). A separate study (Bertelsen *et al.* 2006) measured the pulse of undisturbed, un-drugged, adult walruses from the same area to average 36 beats/min (SD = 3.7, n = 10). After drug delivery, during the apnoeic phase the pulse of this animal had risen to 60 beats/min. For all animals (n = 10) pulse rate during apnoea averaged 54 beats/min (range 40 – 80 beats/min, SD = 13.6). Generally there was no significant difference (*t*-test, t = 1.830, p > 0.05, df = 32) between the pulse of the animals in the apnoeic and the post-apnoeic phase (n = 24, $\bar{x}$ = 63 beats/min, range 38 – 94 beats/min, SD = 13.8). The pulse of 8 animals was measured both during apnoea and during the recovery phase. Also in this case there was no significant difference between pulse during the two periods (*t*-test, t = 1.233, p = 0.257, df = 7).

Rectal temperature during the apnoeic period measured 36.7 °C (n = 6, range 36.2 – 37.5 °C, SD = 0.51). During the same period peripheral temperature was 35.2 °C (n = 6, range 34.1 – 36.4 °C, SD = 0.88). In the recovery phase internal temperature was still 36.7 °C (n = 15, range 35.3 – 38.4 °C, SD = 0.82), whereas peripheral temperature was 30.1 °C (n = 13, range 13.9 – 36.1 °C, SD = 6.11). For 4 animals internal temperature was recorded both during apnoea and the recovery period, but no significant difference was found between the two (paired *t*-test, *t* = 0.960, p = 0.408, df = 3). Likewise, peripheral temperature measured at the hind flipper showed no significant difference between the same two periods (paired *t*-test, *t* = 1.143, p = 0.34, df = 3).

Of the 41 animals immobilised in this group, full immobilisation was achieved in 32 cases, failure to restrain the animal occurred on five occasions and four animals died. Two of the casualties (see section Mortality) occurred during or immediately after handling.

Blood pH-values were measured in five animals between 20 min and three hours from induction. Values ranged between 6.8 and 7.2 (Fig. 1).

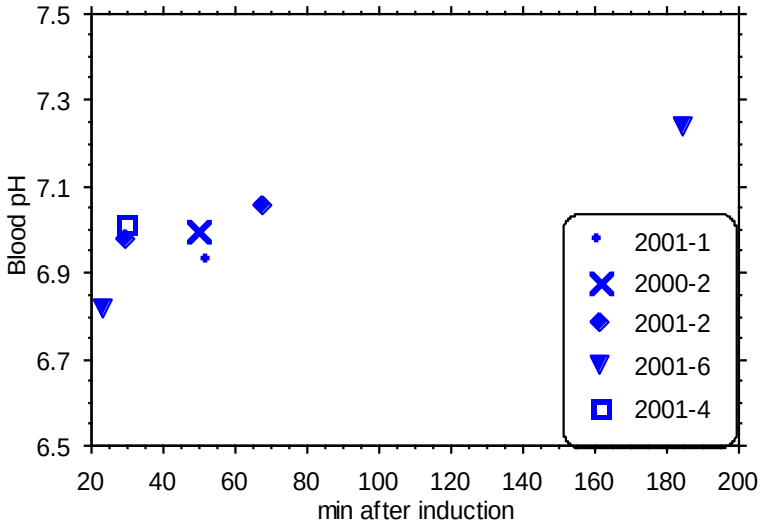

Fig 1. pH-values in blood of five Atlantic walrus (*Odobenus rosmarus rosmarus*) (Greenland 2001) during apnoea and the post-apnoeic period following immobilisation with etorphine.

Multiple immobilisations (group *B*)

This group was administered the agonist dose under the same conditions as for group ***A***. The estimated total body mass of the animals in group ***B*** ranged from 1050 to 1600 kg (Table 4). A total of between 4 and 9.8 mg etorphine was administered per individual walrus at an estimated mean dose of 6.4 µg/kg (Table 4).

Apnoea was induced in all animals 4.1 min (Table 4) after injection of etorphine and lasted 14.6 min (Table 4). A first dose of 11.3 mg (n = 35, range 3.0 - 21.0 mg, SD = 3.3) of the antagonist diprenorphine was administered at a mean of 7.8 min after darting (Table 4). The total dose of diprenorphine delivered to the animals that were successfully immobilised ranged from 7.7 to 32.2 µg/kg (Table 4).

The analysis of the effect of repeated anaesthesia on the induction time did not show any significant correlation between the different immobilisations of the same individual. Neither induction time nor apnoea duration were significantly different when ($p > 0.05$) compared with subsequent immobilisations, irrespective of the relative dose of the agonist or antagonist or the time incurring between the administrations of the two substances. This suggests that repeated immobilisation of an animal did not have any noticeable effect on induction time (*i.e.* no habituation or sensitisation to etorphine).

Table 4. Summary of multiple-time immobilisations (**group *B***) of 10 walruses presented in this study. Dose and time data include all immobilisations where data were available. "Induction time" represents the time between etorphine administration and complete immobilisation. "Diprenorphine Dose" represents the total dose of diprenorphine administered, and "Diprenorphine Time" represents the time for the first dose.

	Body mass (kg)	Etorphine dose (µg/kg)	Induction time (min)	Diprenorphine		Duration of apnoea (min)
				Dose (µg/kg)	Time (min)	
Mean	1220	6.4	4.1	15.6	7.8	14.6
Range	1050 - 1600	3.4 - 9.3	0.9 - 9.0	7.7 - 32.2	2.9 - 13.1	4.8 - 35.2
SD	177	1.9	1.7	7.0	2.2	5.3
n	10	36	36	35	35	36

One animal (***1989-3,*** Table 2) was immobilised with etorphine twice in 1989 and once in 1990 and then treated again once in 2000 and twice in 2001. This ca. 31 year old animal died during the last immobilisation after it apparently had regained consciousness and was breathing autonomously.

In 2000 and 2001, a subsample of the animals that were captured and immobilised several times were included in a study of body water content, body water turnover and energy metabolism involving the use of stable isotopes (deuterium oxide dilution and doubly-labelled water; Acquarone *et al.* 2006; Acquarone and Born 2007). It was essential for the study that the animals were kept sedated on the beach for repeated sampling of blood through the catheter during the time required for the equilibration of the injected fluids with the body water pool. In 32 cases it was possible to handle the animals taking advantage of their drowsiness (up to 2 hrs.) while they were recuperating from the etorphine / diprenorphine immobilisation. In 6 cases it was necessary to administer medetomidine (reversed with Antisedan) to prolong the period in which they were tractable (Griffiths *et al.* 2014). When the animals were sufficiently lethargic as a side-effect of the immobilisation with etorphine, blood could be sampled up to 6.5 hours after darting.

Mortality

The overall mortality rate was 7 % (5 dead of 69). The five animals died during somewhat different circumstances: In 1989, one walrus had resumed

controlled and regular breath but was found dead 230 min after having regained consciousness (first breath). In 2001, two animals had apparently started breathing after injection of the antagonist. However, breathing never became deep and regular and instead it became increasingly shallow until it stopped. Walrus ***1989-3*** (Table 2) had been immobilised several times between 1989 and 2001. Another animal became apnoeic after injection of etorphine and never breathed again. A fifth animal resumed regular breathing and went into the water after having been treated. It was found dead, 8 days after immobilisation, and stranded in the tidal zone about 9 km from the haul-out.

Mortality was tested against etorphine dose, diprenorphine dose and timing of injection of the antagonist by bootstrap analysis, but there was no significant result for any of the factors ($p > 0.05$).

Another side effect experienced was inability to fully immobilise an animal. In six cases the walruses were not sufficiently immobilised to allow for treatment. In one case in 1989 the animal (***1989-3***, Table 2) never became fully immobilised whereas in the in five other failed immobilisations the subject woke up during the initial phases of the treatment. These latter animals escaped into the water where they were apparently able to swim and dive in a coordinated fashion.

The rates of mortality and failure to fully immobilise the animals apparently differed between the researchers in charge of the drugging (Table 5). However, these differences were not statistically significant (rate of mortality: $\chi^2 = 6.82$; $p = 0.08$; $df = 3$); failure to fully immobilise an animal plus mortality: $\chi^2 = 7.44$; $p = 0.06$; $df = 3$).

In this study immobilisation was attempted 69 times. It was possible to obtain full immobilisation 58 times (84 %), in six cases (9 %) immobilisation failed and the animals were never completely restrained, while five individuals (7 %) died from the treatment for no apparent reason. Similarly Hills (1992) attempted immobilisation 59 times, resulting in eight (14 %) incomplete immobilisations and five (9 %) casualties in Pacific walruses. Griffiths *et al*. (1993) experienced 2 partial immobilisations (5 %) and one casualty (3 %) during 38 immobilisations of walruses in Svalbard. During six immobilisations at Svalbard in the subsequent years, two more casualties were experienced (Griffiths unpublished). Overall mortality was 7 % at Svalbard as well. Mortality rates experienced in these studies do not differ significantly (χ^2-test, $p > 0.05$). Hence it can be concluded that etorphine reversed with diprenorphine may result in an overall mortality of 8 % (13 died of 172 handled) in walruses.

Table 5. Rates of mortality and failure to fully immobilise walruses in this study in relation to individual researchers in charge of the drugging.

Researcher	Total Immobilis-ations	Succeeded	No. failed (%)	No. died (%)	Year
1	17	15	1 (5.8)	1 (5.8)	1989, 1990, 1994
2	9	9	0 (0)	0 (0)	1991, 1992
3	22	22	2 (9.1)	0 (0)	2000
4	21	14	3 (14.3)	4 (7.3)	2001
All	69	60	4 (8.7)	5 (7.3)	1989-2001

DISCUSSION

The mean induction time of 5.0 min reported in this study for etorphine doses between 2.4 and 12.6 µg/kg does not differ statistically ($t = 1.429$; $p > 0.05$; $df = 71$) from the average induction time of 5.7 minutes, reported by Griffiths *et al.* (1993), where 3.3 to 9.6 µg/kg etorphine was used on Svalbard on Atlantic walruses, which were of similar size to those in Greenland ($t = 1.567$, $p > 0.05$, $df = 67$).

In male Pacific walruses given etorphine the mean induction time reported was 15.4 min (n = 25, range 5.5 - 35.8 min, SD = 8.1) after administration of 7.3 µg/kg of the drug (n = 21, range 4.0 - 2.7 µg/kg, SD = 0.39; Hills 1992). Although there was a significant difference in body size between the animals in the present study and the ones reported in Hills (1992; *t*-test, $t = 4.951$, $p < 0.001$, $df = 53$), the relative dose of etorphine administered was similar (*t*-test, $t = 2.453$, $p < 0.05$, $df = 52$). When analysing the data in Hills (1992), similar to this study, it was not possible to find any correlation between dose of etorphine and induction time (linear regression analysis of dose – log-induction time; $p = 0.42$, $R^2 = 0.13$, $n = 51$). However, mean induction time in Pacific walrus was significantly longer than found here (*t*-test, $t = 7.532$, $p < 0.001$, $df = 61$). The surprisingly long induction time experienced in Hills (1992) may be ascribed to the shorter needles (6 – 8 cm) used for darting larger animals (some of the agonist may have been deposited in the skin and blubber) or to differences in the definition of "induction time". In both studies, induction time is defined as the "number of minutes from darting until the animal is safe to approach," however the parameter "safe to approach" is subjective and depends on the experience of the operator.

Etorphine depresses respiration by raising the threshold of the reflex of inspiration and by affecting stretch receptors in the lungs (Kock *et al.* 1987). Generally apnoea is induced at markedly lower levels of anaesthesia in marine mammals than in terrestrial mammals (Hammond and Elsner 1977). The drug-induced muscle tonus and apnoea observed in this study have also been reported when etorphine was used for immobilisation of phocidae (Haigh and Stewart 1979, Parry *et al.* 1981). The duration of apnoea in the present study was significantly longer than in Pacific walruses (Hills 1992; $\bar{x}$ = 11.8 min, range 5.5 - 24.0 min, SD = 4.8, n = 22; *t*-test, $t = 1.407$, $\underline{P} > 0.05$, df = 56) and Atlantic walruses on Svalbard (Griffiths *et al.* 1993; $\bar{x}$ = 11.9 min, range 5.6 – 20.8 min, SD = 3.8, $\underline{n} = 35$; *t*-test, $t = 1.778$, $\underline{P} > 0.05$, df = 69). The differences observed between this study and the others only reinforce the point stated above that "beginning of apnoea" is a very subjective parameter to be utilized with care in the analysis.

Aerobic dive limit in Atlantic walruses has been estimated at between 9.8 and 10.5 min for 1100 to 1500 kg animals, respectively (Nowicki *et al.* 1997, Wiig *et al.* 1996). Observations of diving walruses in the wild indicate that this limit is rarely exceeded (Nowicki *et al.* 1997, Wiig *et al.* 1996). However, syntheses on body mass and diving capacity in pinnipeds (Boyd and Croxall 1996, Schreer and Kovacs 1997) indicate that maximum aerobic dive duration in a 1500 kg walrus may be close to 25 min which has been confirmed by observations in the wild (Gjertz *et al.* 2001). In the present study, in seven cases the animal did not breathe for over 20 min with a maximum registered apnoea of 35 min. The markedly increased respiration rate during the recovery phase observed in all immobilisations indicates that the animals may be compensating for oxygen deficiency built up during narcosis. Furthermore Griffiths *et al.* (1993) noted that walruses immobilised with etorphine remained lethargic during the recovery phase and suggested that the prolonged apnoea produced metabolic acidosis. This supposition is confirmed by the measures of blood pH reported here. The generally smooth recovery and apparently unaffected *long-term* post-handling behaviour observed both in previous (Born and Knutsen 1992b, Born and Knutsen 1997) and in the present study indicate a large tolerance towards apnoea, hence low blood pH, in walruses during immobilisation. They suggest that respiratory and circulatory mechanisms serving long dives are maintained during anaesthesia with etorphine.

Etorphine induces alterations in the number and distribution of μ-opioid receptors in the rat cerebral cortex if administered alone, but no apparent change was visible if etorphine administration was accompanied by the antagonist naloxone (Melone *et al.* 2000). With this in mind changes in the number and distribution of opioid receptors may accompany variations in the tolerance of the animals to the drug.

The analysis of repeated immobilisations of 10 individuals, both within and across the years, did not indicate any change in the effect of the drugs on the subjects. The fact that the animals in this study were subject to several immobilisation during a relative short time frame indicates a substantial tolerance to etorphine and perhaps more noteworthy to one of its side-effect: prolonged apnoea. However, accurate monitoring of physiological parameters, with particular attention to internal temperature, pulse, blood pH and glycaemia, during immobilisation is here advocated to acquire a deeper understanding of immobilisation by this and other techniques.

A pulse of about 64 beats/minute during apnoea and early recovery was observed in this study. For comparison, during a study at Svalbard, Griffiths *et al.* (1993) noted a resting pulse which ranged between 52 and 66, whereas during deep narcosis the pulse was between 22 and 48 beats per min. Griffiths *et al.* (1993) stated, without presenting the details, that heart rates remained steady, but varied somewhat with the dose of drug received and that animals receiving a high etorphine dose showed lowest pulse rates. In a study of non-drugged walruses Bertelsen *et al.* (2006) found a resting heart rate of 36 (SD = 3.7; range: 29–43). Although higher than the resting heart rate, the pulse during etorphine anaesthesia does not seem to present any threat to the animals.

Etorphine is known to interfere with thermoregulation, causing hyperthermia or hypothermia dependent of ambient conditions and activity levels (Alford *et al.* 1974). However, the internal temperatures measured here were within the normal range reported for pinnipeds (Sweeney 1974, Whittow 1987) indicating that thermoregulation was not severely affected during immobilisation. Also in other studies where walruses were immobilised with etorphine or carfentanil there have been no apparent thermoregulatory problems. Reported body temperatures in other studies ranged between 34.2 and 37.9°C (Griffiths *et al.* 1993, Hills 1992, Lanthier *et al.* 1999). The fall in peripheral body temperature reported in this study from 35°C to 28°C indicates that peripheral circulation control is not impaired by etorphine.

The blood pH-value measured during apnoea for one animal (***2001-6***, Fig.1) was 6.8 which increased to 7.2 two hours after induction (corresponding to 2.7 hrs. of active ventilation). A second animal (***2001-2***, Fig.1) presented a blood pH of 6.9 at the end of apnoea, which increased to 7.1 half an hour later. The low values of in blood pH at the end of the apnoeic period are probably to be ascribed to the hypercapnia and reflect immobilisation-induced stress. However, the blood pH became normal relatively shortly after resumption of respiratory function.

The dosage of etorphine varies widely for all walrus studies mainly due to differences in animal TBM. The latter is a difficult parameter to assess at a distance in a field situation. However, after having experienced a case of under-dosage (4 mg corresponding to 2.4 μg/kg) in 1989, a total injection of between 6 and 10 mg etorphine irrespective of the size of the animals was routinely used. In most cases this resulted in successful immobilisation of the animal. This confirms that it is advisable to administer the maximum dose of etorphine, rather than the minimum effective dosage, and then reverse promptly with an appropriate dose of antagonist (Alford *et al.* 1974, Booth 1988), because under-dosage may cause severe physiological and behavioural reactions (Alford *et al.* 1974) such as hyperexcitability, hyperventilation and subsequent alkalosis leading to death at a later time in the recovery phase.

Re-narcotisation has been suggested to explain the death of walruses immobilised with carfentanil and subsequently antagonized with naloxone (Hills 1992). In other mammals, re-narcotisation has been observed from 2 to 72 hrs. post-immobilisation (Allen 1990, Haigh *et al.* 1983, Jacobson *et al.* 1988, Jessup *et al.* 1985, Seal *et al.* 1985). The present study suggests that re-narcotisation could serve as an explanation for cause of death in the three cases in which animals had regained breath and apparently had come out of the immobilisation. In these cases sufficient amounts of diprenorphine were given to bring the animals to full ventilation. However, as doses were routinely injected in more than one site to secure absorption, it might be that some of it was deposited in blubber. The deposition of antagonists with longer half-life such as naltrexone, alone or in combination with diprenorphine, was not tested and it might be considered for future occasions.

The researchers who were in charge of the immobilisations prior to 2001 all had several seasons of experience with using this drug combination for immobilising walrus. This is in contrast with the situation in 2001 when a relative high mortality and failure-to-immobilise rate was experienced. Familiarity with the effects of etorphine and diprenorphine in walrus and with walrus anatomy is in our opinion crucial. The secure administration of the antagonist into muscle or other metabolically active tissues is important to prevent suffocation. On the other hand injecting the antagonist directly into a vein or a blood vessel sinus may result in the animals waking up prematurely.

In this study short-term monitoring by visual observation showed no sign of adverse effects of the immobilisation treatment. However, the rates of mortality and premature awakening were relatively high. Furthermore the induction of prolonged apnoea with associated acidosis is an undesirable side effect of this immobilisation technique. It is here recommended that etorphine reversed with diprenorphine for immobilisation of walrus should be used only

for short term handling, and that experiments in search for a more suitable agent for long-term immobilisation should be effectuated.

ACKNOWLEDGMENTS

All walrus work including immobilisations has been effectuated under previous permit from the relevant authorities (Project approval of the Greenland Home Rule, file: 28.40.10). Aage V. Jensen's Foundation financed parts of this study. The Geological Survey of Greenland (Denmark), the Murmansk Biological Institute (Russia) and the Institute of Oceanology (Poland) are thanked for various help and support during the study. The Greenland Institute of Natural Resources, the National Environmental Research Institute of Denmark (now Aarhus University), the Danish National Science Foundation, the Commission for Scientific Research in Greenland (KVUG) and the late Danish Polar Center (DPC) provided financial and logistic support. Finally we would like to thank our colleagues Jonas Teilmann, Thomas Thymann Nielsen and Frank Riget for their constructive advice on statistics and data handling.

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