

## SHORT DURATION IMMOBILIZATION OF ATLANTIC WALRUS (*ODOBENUS ROSMARUS ROSMARUS*) WITH ETORPHINE, AND REVERSAL WITH NALTREXONE

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**Abstract:** Forty adult, male Atlantic walrus (*Odobenus rosmarus rosmarus*) were successfully immobilized for the attachment of global positioning system loggers on their tusks and collection of various biologic samples. A standard dose of 7.8 mg etorphine was used for each animal, regardless of body size. All animals were reversed with an iv or im injection of 250 mg naltrexone, immediately after tag attachment. Twenty-seven of the animals were intubated and ventilated with 100% oxygen during the recovery period. The induction time was, on average, 4 min 51 sec  $\pm$  1 min 46 sec. Several animals had venous pH, and P<sub>CO</sub><sub>2</sub> levels that indicated severe acidosis and hypercarbia. All animals recovered within an average of 5 min 16 sec  $\pm$  2 min 47 sec after reversal. The total time from darting to recovery was 15 min 23 sec  $\pm$  3 min 33 sec. The use of naltrexone is recommended for reversal of etorphine immobilization in adult, male walrus, and the use of positive-pressure ventilation with oxygen is highly encouraged.

**Key words:** Etorphine, immobilization, naltrexone, *Odobenus rosmarus rosmarus*, ventilation, walrus.

### INTRODUCTION

The Atlantic walrus (*Odobenus rosmarus rosmarus*) is the largest pinniped in the North Atlantic Ocean, with adult males attaining body masses in excess of 1,500 kg and adult females weighing up to 900 kg. This subspecies has a distribution from the eastern Canadian Arctic eastward to Greenland and beyond, to Svalbard and Franz Josef Land. The Svalbard–Franz Josef Land population was hunted almost to extinction in the early 1900s; the species became protected from hunting in this area in 1952.<sup>12</sup> The population seems to be showing strong signs of recovery during the past decade, with the Svalbard fraction currently consisting of approximately 4,000 animals.

Several drugs have been used for immobilization of free-ranging walrus,<sup>1,4,7,14,15,16</sup> but all drugs or drug combinations used so far have been associated with a relatively high risk of mortality. However, walrus are too strong to be physically restrained, and they normally haul-out close to the water. If an animal moves into the water under

the influence of sedative or anesthetic drugs, this could pose a major risk of drowning. Thus, immobilization of walrus is a prerequisite for attachment of various instruments to the animals' tusks and for obtaining many types of biologic samples.

Dissociative anesthetics, such as ketamine or tiletamine, have been used in a few walrus for immobilization, with variable results.<sup>7,14,19</sup> There are no reversal agents for these drugs, and the duration of their effects is relatively long, posing a substantial risk for animals to move into the water during induction or recovery.

Most free-ranging walrus have been immobilized using potent opioids, such as etorphine or carfentanil.<sup>1,4,7,15,16</sup> These drugs have the advantage of a low dose volume and rapid and consistent induction. A major disadvantage of both of these drugs is that they cause convulsions and apnea at the time of induction. However, the effects of these drugs can be reversed by the use of opioid antagonists. In walrus, carfentanil has been reversed using naltrexone, whereas diprenorphine has mainly been used to reverse etorphine.<sup>1,7</sup> Diprenorphine is a partial opioid antagonist,<sup>5</sup> which has some agonistic properties, so overdosing may cause continued immobilization or a delay in recovery. In contrast, naltrexone is a pure opioid antagonist with no agonistic properties.<sup>5,13</sup>

Protocols using etorphine for immobilization of adult male walrus have been used on more than 170 occasions with an 83% success rate.<sup>1</sup> However, there is an overall mortality rate of approxi-

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mately 8%. The recovery period after diprenorphine reversal is relatively long, and the animals remain exhausted and disoriented for a long time after they regain consciousness.<sup>7</sup> In addition, blood pH measurements indicate substantial acidosis in many of those animals.<sup>1</sup>

The main objective of this study was to describe and evaluate the protocol used for short-time immobilization of male walrus for transmitter placement. Because of the high incidence of unexplained mortalities during walrus immobilizations, the main priority was to keep the anesthesia time to an absolute minimum. The protocol was based on the method used on Svalbard for male walrus immobilization in recent years,<sup>7</sup> with minor modifications. Notably, naltrexone was used for reversal of anesthesia, instead of diprenorphine.

### MATERIALS AND METHODS

This study was approved by the Norwegian Animal Care Authority (2013/36153-2) and the Governor of Svalbard (2014/00066-2 and 2015/00218).

Forty adult, male walrus were immobilized at three different locations within Svalbard, Norway. Twenty animals were immobilized in July 2014 at Sletteøya (76°58'48"N, 22°4'12"E), four animals at Sarstangen (78°43'48"N, 11°27'36"E) in August 2015, and sixteen at Purchasneset on Lågøya (80°22'12"N, 18°16'48"E) in August 2015. The ambient temperature varied between 5 and 12°C during the field work in the 2-yr study.

Study animals were all adult, male walrus, with estimated body masses ranging from 900 to 1,500 kg, with suitable (intact and modestly large) tusks for placement of the global positioning system (GPS) logger, and that were apparently healthy and lying toward the back of a haul-out group, farthest from the water. In addition, the animal's body position had a role in selection; animals lying in deep depressions or close to obstacles, such as a big rock or log, which would complicate rolling it over into a sternal position were rejected as study subjects. Animals were approached carefully and slowly by one person, on foot. If animals became alert and looked up, the person would stop and wait for the group to resettle. All animals were darted with a CO<sub>2</sub>-propelled Teledart RD 706 injection gun (Teledart GmbH & Co, D-67368 Westheim, Germany). Each 2-ml dart was fitted with a 12 cm, 14-ga needle, with a red sealing sleeve (Teledart). The animals were darted into the upper thigh or lower

back muscles from a distance of approximately 5–10 m.

The darts were prepared in advance, the same day they were to be used. Each contained a standard dose of 1 ml of etorphine hydrochloride 9.8 mg/ml (Captivon 98, Wildlife Pharmaceuticals Ltd, White River 1240, South Africa). The volume of anesthetic remaining in the needle was estimated to be approximately 0.2 ml. All animals thus received a standard dose of approximately 7.8 mg etorphine hydrochloride, regardless of body size. A 5-ml dart with 250 mg of naltrexone (Trexonil 50 mg/ml, Wildlife Pharmaceuticals) was kept ready in case the animal moved toward the water.

The induction time was recorded as the time from injection until the walrus had its first muscle spasm. During that time, breathing was assessed visually, and the duration of the inevitable period of apnea was recorded. When that occurred, the walrus was approached, and other walrus lying close to the anesthetized animal were driven 3–4 m away, by tapping them gently on the head with a 3.5-m-long piece of plastic tubing. The first walrus was injected with naltrexone immediately after approaching the animal on the assumption that the recovery period would allow attachment of the GPS logger and the necessary sample collection. (This was based on experience from the "old" protocol, in which diprenorphine was used as the reversal agent). However, the animal recovered so quickly and was so alert that only attachment of the GPS logger was achieved. No biologic samples were obtained. So, for the remaining 39 walrus, naltrexone was injected immediately after the GPS logger was attached (250 mg naltrexone either iv or im if iv access could not be achieved).

The iv access was obtained, using a 2.0-mm × 115-mm needle inserted in the extradural vein at the lumbar-sacral region. Blood samples were also collected from that site. The walrus was then rolled into sternal recumbency, if it was not already in that position, and intubated with a 24-mm internal diameter, cuffed, silicone endotracheal tube (Smiths Medical PM, Inc. Waukesha, Wisconsin 53186, USA) by manual palpation of the trachea. The cuff was inflated and the animal was ventilated with 100% oxygen until spontaneous breathing commenced. A demand-valve providing 160 L/min (L063, Allied Healthcare Products, Inc, St. Louis, Missouri 63110, USA) was used for positive-pressure ventilation. Ventilation was managed by giving 5–8 sec inspiratory bursts four to five times per minute. If there was



**Table 1.** Characteristics of short duration immobilization of 40 male Atlantic walrus with etorphine and reversal with naltrexone.

Characteristic	Mean $\pm$ SD	Range
Induction time	4 min 51 sec $\pm$ 1 min 46 sec	1 min 31 sec–9 min 30 sec
Duration before reversal <sup>a</sup>	5 min 16 sec $\pm$ 1 min 28 sec	1 min 31 sec–8 min
Recovery time	5 min 16 sec $\pm$ 2 min 47 sec	1 min 35 sec–15 min 15 sec
Apnea duration	10 min 32 sec $\pm$ 3 min 22 sec	5 min 31 sec–23 min 10 sec
Procedure duration <sup>b</sup>	15 min 23 sec $\pm$ 3 min 33 sec	9 min 57 sec–29 min 20 sec

<sup>a</sup> Duration before reversal was the time from when induction was complete to reversal being administered.

<sup>b</sup> Procedure duration was the time from darting until the animal awoke and started breathing.

no sign of recovery after approximately 5 min after reversal, the animal received a second injection of 250-mg naltrexone iv, if possible (otherwise, im). Once the animal resumed spontaneous breathing, the cuff was immediately deflated. The tube was left in place if the animal tolerated it, and oxygen was supplied on inspiration. Once the animal started to lift its head, the tube was removed.

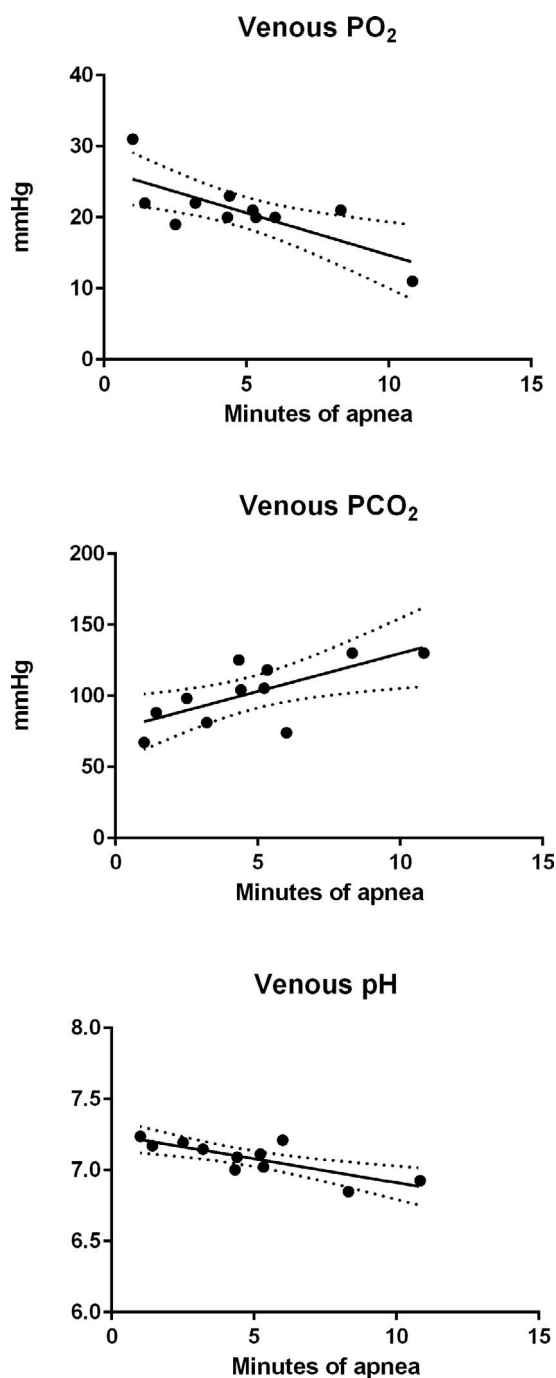
Pulse oximetry with a reflector probe (Nellcore oximax PM10N and Forehead SpO<sub>2</sub> sensor, Covidien LLC, Mansfield, Massachusetts 02048, USA) was applied on the oral mucosa or tongue for monitoring. In 2015, venous samples were analyzed from 15 animals with a VetScan ISTAT 1 hand-held analyzer (Abaxis, Inc, Union City, California 94587, USA) for pH, PCO<sub>2</sub>, PO<sub>2</sub> and lactate, using either CG4<sup>+</sup> or CG8<sup>+</sup> cartridges (Abbot Point of Care Inc, Abbott Park, IL 60064, USA). The first venous blood sample was taken as soon as venous access was achieved. If possible, a second venous sample was taken late in the recovery period; the needle was removed when the animal started to move. The animal was then left alone and observed from a distance. Venous samples for ISTAT analysis were drawn into 1.5 ml heparinized syringes (PICO70, Radiometer Medical ApS, 2700 Brønshøj, Denmark). Syringes were kept airtight and were analyzed within 25 min of collection. These venous samples were not taken at exactly the same time in relation to induction in all animals, but rather when the opportunity arose during handling. The correlation between apnea time and blood gasses was investigated using GraphPad Prism (Version 6.00 for Windows, GraphPad Software, La Jolla California USA). Because of a paucity of paired samples, no attempts at repeated-measures analysis were made. If the pCO<sub>2</sub> value exceeded the measuring capacity of the machine of 130 mm Hg, a value of 130 mm Hg was used in the calculations.

## RESULTS

Darted walrus typically lifted their heads and looked around for approximately 10 sec before lying back down. None of subject animals attempted to go to the water, although a few of the walrus walked a few steps toward the center of the group before settling down. All animals were successfully induced, and the average induction time was 4 min 51 sec  $\pm$  1 min 46 sec (Table 1). All animals showed the same characteristic signs of induction: first, there was a slight shivering in the vibrissae and twitching of the upper lip, and that was followed soon after by clonic spasms in the entire body. Those spasms consistently coincided with apnea. The spasms were strongest at induction and normally receded gradually during the next 3–4 min. The GPS loggers were attached within a mean time of 3 min, with a range of 1–6 min. Animals in sternal recumbency were left in that position. If the animals were in lateral recumbency, it was very difficult to gain venous access or to perform intubation. Therefore, once the tag was attached to animals in that position, they were rolled into sternal recumbency, and venous access was attempted if it had not already been achieved. At that time, most of the animals were relaxed, although a few still had slight muscle spasms. After injecting naltrexone iv or im, the animal's head was flexed backward so that the neck was extended, and the tusks were placed onto the ground to support the head in this position. The animals were then intubated, if possible. Thirteen animals could not be intubated because they started to wake up when we were positioning the head. Four walrus started to breathe within 1 min after placement of the tube, and two walrus started to breathe when the larynx was palpated. Apnea time was, on average, 10 min 32 sec  $\pm$  3 min 22 sec. (Table 1).

All 40 animals recovered, and recovery was fast in most of the animals. The average time from





**Figure 1.** Correlation of time between apnea and venous oxygen and carbon dioxide tension and pH in 11 immobilized male Atlantic walrus. The dotted lines represent 95% confidence limits. For *P*-values see text.

reversal with naltrexone until the animals were awake was 5 min 16 sec  $\pm$  2 min 47 sec (Table 1). Once the animal started to breathe spontaneously,

it soon lifted its head. Despite heavy breathing, the walrus was alert and responsive to their surroundings. Most of the walrus then moved toward the other animals. Total mean time from darting until the animal was awake and breathing was 15 min 23 sec  $\pm$  3 min 33 sec (Table 1).

Despite numerous attempts to measure oxygen saturation with the pulse oximeter, it was not possible to get any reliable readings. The mucus membrane color of the oral cavity and conjunctiva was monitored; in most cases, the color changed from bluish to bright red after oxygen supplementation.

Venous samples were analyzed successfully on the ISTAT from 13 animals.

Eleven venous samples were taken before intubation and positive-pressure ventilation. The results for venous PO<sub>2</sub>, PCO<sub>2</sub>, and pH and the correlation of those parameters in relation to apnea time are displayed in Figure 1. Venous CO<sub>2</sub> tension increased significantly with apnea time (*P* = 0.0177), and pH decreased correspondingly (*P* = 0.0041). Venous oxygen tension decreased significantly with apnea time (*P* = 0.0078) to levels below 20 mm Hg. In two animals from which venous samples were collected 8 and 11 min after induction, respectively, the PCO<sub>2</sub> values were above the readable limit of 130 mm Hg, and PO<sub>2</sub> values were 21 mm Hg and 11 mm Hg, respectively. After 6 min of positive-pressure ventilation, the PCO<sub>2</sub> level had decreased to 94.2 mm Hg for the first animal but was still more than 130 mm Hg for the other. However, in the walrus, PO<sub>2</sub> increased to 39 and 40 mm Hg, respectively.

Twelve venous samples, taken after positive-pressure ventilation had started, were analyzed. Of those, four were taken after less than 2 min of ventilation, six were taken after 2–4 min of ventilation, and two samples were taken after 6 min or more of ventilation.

The mean venous PO<sub>2</sub> value before ventilation was 20.9  $\pm$  4.4 mm Hg (range, 11–31 mm Hg), whereas the mean value for venous PO<sub>2</sub> after ventilation was 27.9  $\pm$  9.2 mm Hg (range, 17–40). Mean values for venous PCO<sub>2</sub> were not calculated because several samples were greater than the readable limit.

The venous pH values before ventilation had a mean value of 7.09  $\pm$  0.12 (range, 6.85–7.24). After ventilation, the mean value was 7.02  $\pm$  0.11 (range, 6.81–7.17). Lactate levels were only measured in five animals before ventilation; the mean value for those animals was 7.7  $\pm$  3.3 mM/L (range, 2.34–11.15). After ventilation the mean



value for lactate was  $10.2 \pm 3.9$  mM/L (range, 4.59–18.54) ( $n = 12$ ).

## DISCUSSION

The basic protocol used for immobilization of walrus in the present study was based on previous experience with immobilizations of walrus on Svalbard,<sup>7</sup> but several notable improvements were made during this study. The short induction time and successful immobilization of all animals, regardless of size, suggest that the dose of etorphine used might be greater than what was absolutely needed to achieve the desired effect on the animals. However, if the dose was too small, there was a high risk of incomplete immobilization and further complications related to that. Keeping the induction time short is a priority when immobilizing animals hauled out close to the water. Another priority is to keep things simple when working under field conditions. In this case, a fixed dose was used in all animals, and darts were prepared in advance to minimize potential hazards of handling ultra-potent opioids.

It should be possible to combine etorphine with a muscle relaxant for immobilization of walrus. That may provide a smoother induction and the potential for immobilization without spasms. However, although obviously not desirable, the spasms are useful in that they are a consistent and easily observed indication that induction is complete and that it is safe to approach the walrus. Walrus often lay completely still while resting, and it is difficult to even observe breathing if the animal is positioned behind another walrus. If the induction is smooth and gradual, as would generally be preferred, one might have to approach and prod the animal to evaluate the effect, which might result in the study animal fleeing for the water if it were still awake. Nonetheless, adding a sedative, such as medetomidine, would likely decrease the etorphine dose needed substantially. Historically, medetomidine reduced the dose of carfentanil needed to immobilize walrus, but the combination still caused apnea at induction.<sup>4,14</sup> It would be an advantage to intubate and ventilate the animals immediately after apnea occurs. However, the strong muscle spasms resulting from the present protocol prevented intubation at that stage; it only became possible to intubate a few minutes after the spasms had decreased in strength. A muscle relaxant might facilitate earlier intubation.

It is difficult to access the cardiovascular effects of drug combinations on walrus during immobilization. Historically, several animals have died without an obvious reason.<sup>1</sup>

The consistent apnea that occurs with all walrus under etorphine immobilization inevitably leads to hypoxemia. In this study, very low venous oxygen tensions were reached within minutes. However, like other diving mammals, walrus likely have a high hypoxemic tolerance.<sup>18</sup> Studies on elephant seals (*Mirounga angustirostris*), Weddell seals (*Leptonychotes weddellii*), and harbor seals (*Phoca vitulina*) have reported venous oxygen levels as low as 2–3 mm Hg.<sup>8,9,20</sup> The lowest venous  $PO_2$  level measured in the present study was 11 mm Hg.

The low pH measurements, coupled with high  $PCO_2$  and high lactate levels, indicate that most of the animals develop a respiratory and metabolic acidosis. Although that might contribute to mortality, there have also been recordings of a blood pH of 6.8 in a Weddell seal after a dive of 61 min,<sup>11</sup> and lactate levels of 26 mM/L in the same species.<sup>10</sup>

Walrus normally dive for relatively short periods (4–6 min)<sup>3,17,18,21</sup> and might have lower tolerance levels than pinnipeds that dive for much longer durations.

However, the tolerance of low blood pH, hypercarbia, and hypoxia in walrus has never, to our knowledge, been studied. Levels of those physiologic parameters have likely been exceeded in the cases in which immobilization has led to mortality in walrus.

Ventilation counteracts acidosis and hypercarbia and improves oxygenation. In this study, a relatively small endotracheal tube of 24-mm internal diameter was selected for ease of placement. The space available in the oral cavity of walrus is very limited, and even passing the arm down to the larynx was difficult in many cases in this study. Because the trachea is much larger, it is important to deflate the cuff immediately after spontaneous breathing occurs, such that inspiration resistance is limited. In retrospect, based on the measured  $PCO_2$  levels after ventilation, it is likely that the cuff was not sufficiently inflated in some of the animals. On the other hand, although we were not able to collect arterial samples or measure oxygen saturation with the pulse oximeter, the mucus membrane color observed indicated a good effect of oxygen supplementation, and venous measurements increased up to 40 mm Hg after ventilation. The ventilation rate of 4–5 breaths/min

only just exceeded that of the reference range for resting walrus (3 breaths/min),<sup>2</sup> and although oxygenation was improved, it would appear prudent to increase the rate in future work to reduce PCO<sub>2</sub> more rapidly.

Naltrexone reversal resulted in a quicker and more-complete recovery than reversing with diprenorphine.<sup>1</sup> The animals also appeared to be much less fatigued than when diprenorphine was used.

The primary aim of this field work was to attach GPS loggers to the walruses, and priority was, therefore, given to keeping the anesthesia time as short as possible. Failure to get venous measurements on some animals was due to the low temperature of the ISTAT analyzer, overfilling of the cartridge, or malfunction of the cartridge. The venous samples for pH, PCO<sub>2</sub>, PO<sub>2</sub>, and lactate were collected when possible but at different times in relation to anesthesia time. However, those samples did give an indication of severe acidosis, hypoxia, and hypercarbia shortly after induction. They also provided an indication that ventilation was effective in counteracting those findings. Bringing oxygen tanks to the field is cumbersome, but the results of this study suggest it is worthwhile. Additional studies incorporating blood gas analysis would be helpful to evaluate the method and its safety further.

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