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Efficacy of 2-phenoxyethanol as an anaesthetic for the musky octopus, *Eledone moschata* (Lamarck 1799), (Cephalopoda: Octopodidae)

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Abstract: The efficacy of 2-phenoxyethanol (2-PhOH) as an anaesthetic for the musky octopus, *Eledone moschata* (Lamarck, 1799), was examined. Different doses (1.0, 1.2, 1.4, 1.6, and 1.8 mL L^{-1}) of 2-PhOH were prepared in 20 L transparent plastic containers containing 10 L of continually aerated seawater (pH 8.2, O_2 8 mg L^{-1}, salinity 37.2‰ at 15.3 °C). Six individuals were used in each experiment. After quantifying the anaesthetic time for each animal, which was defined as the loss of sucking intensity (Stage A3), the specimens were transferred to a plastic recovery tank (430 L of aerated seawater), defined as regular breathing (Stage R4; 12-13 breaths min^{-1}), where they were observed for 48 h to document any mortality. There were significant differences in induction and recovery time among the various 2-PhOH concentrations (ANOVA, $P < 0.05$). The musky octopus was anaesthetised effectively at 1.2, 1.4, and 1.6 mL L^{-1} of 2-PhOH ($P > 0.05$) and no mortality was observed within 48 h. However, 50% mortality occurred at 1 mL L^{-1} 2-PhOH within 24 h after anaesthesia, and 100% mortality at 1.8 mL L^{-1} of 2-PhOH within 48 h. Based on the recovery time, a dose of 1.6 mL L^{-1} of 2-PhOH was considered the most effective dose for anaesthetising the musky octopus.

Key words: Anaesthetic, 2-phenoxyethanol, *Eledone moschata*, musky octopus

Mis ahtapot, *Eledone moschata* (Lamarck 1799), (Cephalopoda: Octopodidae) için 2-fenoksietanolün anestezik olarak etkisi

Özet: Mis ahtapot *Eledone moschata* (Lamarck, 1799) için 2-fenoksietanol (2-PhOH)'ün anestetik olarak etkisi incelendi. 2-PhOH'ün farklı dozları (1.0, 1.2, 1.4, 1.6 ve 1,8 mL L^{-1}) 20 litrelik şeffaf kabın içinde bulunan ve sürekli havalandırılan 10 litre deniz suyunda (15,3 °C sıcaklıkta, tuzluluk ‰ 37,2, pH 8,2 ve O_2 8 mg L^{-1}) hazırlandı. Her denemede altı ahtapot kullanıldı. Her hayvan için anestezi zamanı, ki vantuzların yapışma kabiliyetinin kaybolması (Safha A3) olarak tanımlandı, belirlendikten sonra ahtapotlar vakit geçirilmeden plastik ayıltma tanklarına(içinde 430 L iyi havalandırılmış deniz suyu bulunan) nakledildi, ayılma normal nefes alış verişi (Safha R4; 12-13 nefes dak^{-1}) olarak tanımlandı, burada 48 saat boyunca ölüm olup olmayacağı gözlendi. Kullanılan 2-PhOH konsantrasyonlar içinde bayılma ve ayılma zamanlarında istatistiksel olarak önemli farklar vardı (ANOVA, $P < 0,05$). Mis ahtapot, 1,2, 1,4 ve 1,6 mL L^{-1} 2-PhOH konsantrasyonlarında etkili şekilde anestezi edildi ($P > 0,05$) ve 48 saat içinde ölüm gözlenmedi. Ancak, anesteziden 24 saat sonra 1.0 mL L^{-1} 2-PhOH konsantrasyonunda % 50 ölüm ve 1,8 mL L^{-1} 2-PhOH konsantrasyonunda 48 saat içinde % 100 ölüm oldu. Ayılma zamanına bağlı olarak 1,6 mL L^{-1} 2-PhOH konsantrasyonu mis ahtapotun anestezisi için en etkili doz olarak görüldü.

Anahtar sözcükler: Anestetik, 2-fenoksietanol, *Eledone moschata*, mis ahtapot

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Introduction

During the last 4 decades, numerous cephalopod culture techniques have been developed for the common octopus *Octopus vulgaris* (Cuvier, 1797), loliginid squids, *Sepioteuthis lessoniana* (Lesson, 1830) and *Sepioteuthis sepiodea* (Blainville, 1823), cuttlefishes *Sepia officinalis* (Linnaeus, 1758), *Sepia pharaonis* (Ehrenberg, 1831), and *Sepialla inermis* (Orbigny, 1848) (1-4), and cephalopod culture has been gaining a remarkable commercial interest. Cephalopods are also used as experimental animals due to their well developed nervous system (5).

Anaesthetic agents are widely used in fisheries and aquaculture to immobilise animals for transport, vaccination, measuring, sorting and tagging, sampling for blood or gonadal biopsies, and collection of gametes, as well as to decrease stress and facilitate the performance of painful procedures (5-8). Several anaesthetics have been used in fish (6,9,10). Among them, 2-phenoxyethanol (2-PhOH) is considered very suitable for aquacultural practises, because of its ease in preparation, low price, rapid action and easy recovery (11,12), as well as bactericidal and fungicidal actions (13). The effective anaesthetic concentrations of 2-PhOH in a number of species of fish have been reported and ranged from 0.2 to 0.6 mL L^{-1} (9,10,14-21).

The musky octopus, *E. moschata*, belongs to the medium size octopus of octopodidae and is a commercially important species in coastal countries of the Mediterranean region (22). Paralarval rearing of the musky octopus was achieved by Boletzky (23) and, as pointed out by Şen (24), the musky octopus can adapt easily to controlled conditions without shelter in <10 days and no cannibalistic behaviour or food competition was observed. The musky octopus is particularly difficult to handle, as it is very quick, has a very sensitive skin, and has a habit of grabbing and holding on to things. There is no available information on appropriate anaesthetics and their doses for the musky octopus, *Eledone moschata*. The objective of the present study was to evaluate the efficacy of 2-PhOH as an anaesthetic for the musky octopus.

Materials and methods

A total of 50 musky octopuses were captured in İzmir Bay, Turkey, by bottom trawling on 15 November 2006. One month prior to the studies, the individuals were acclimatised in a polyester stock tank (2 × 2 × 1.2 m, 4000-L volume) placed in indoor facilities of the Faculty of Fisheries of Ege University (Urla, İzmir), and supplied with flow-through, filtered seawater. During the acclimatising period, the salinity, pH, dissolved O_2, and temperature were 37 ± 2‰ (508-IIW, Nippon, Japan), 8.2, 8 ± 0.5 mg L^{-1} (YSI 5750, USA), and 15.5 ± 0.5 °C, respectively. The specimens were fed to satiation by hand, with low price fish species (i.e. *Engraulis encrasicolus, Sardina pilchardus*) or discarded fish (i.e. *Lepidotrigla cavillone*) from bottom trawling. The following day, uneaten fish or remains were removed by siphoning. The mean total body weight of the individuals used in the experiment was 203 ± 55.5 g (n = 30).

Different doses (1.0, 1.2, 1.4, 1.6, and 1.8 mL L^{-1}) of 2-phenoxyethanol (2-PhOH) were prepared in 20 L transparent plastic containers containing 10 L of continually aerated seawater (pH 8.2, O_2 8 mg L^{-1} and 37.2‰ at 15.3 °C). After quantifying the anaesthetic time for each animal (n = 6), the individuals were transferred to a plastic recovery tank with 430-L of well-aerated seawater, where they were observed for 48 h due to any mortality.

The criteria for anaesthetic effects were according to Seol et al. (25) and were loss of sucking intensity under anaesthesia (Stage A3) and recovery of regular breathing (R4) (Table 1). Anaesthetising the musky octopus involved several stages, beginning with a change in body colour (Stage A1) to the loss of sucking intensity (Stage A3), at which stage the specimen was transferred to a recovery tank. Recovery time was estimated as the point at which the octopus recovered normal activity (Stage R3) and regular breathing (Stage R4; 12-13 breaths min^{-1}).

One-way analysis of variance (ANOVA) and Duncan's multiple range tests were applied to determine the statistical significance of the differences between the mean induction time and recovery times, using SPSS 11.0. The level of significance was taken at $P < 0.05$.

Table 1. Stages of induction and recovery times for 2-PhOH on the musky octopus as defined by Seol et al. (25).

Stage	Description	Remarkable behaviour
Anaesthetic		
A1	Change in body colour	Body colour becomes pale
A2	Change in mantle cavity shape	Mantle cavity becomes uneven and elliptical
A3	Loss of sucking intensity	Inactivity of arms and loss of sucking intensity
A4	Cessation of breathing (death)	Closing of funnel
Recovery		
R1	Recovery of sucking intensity	Start of unusual (slow-moving) action
R2	Recovery of mantle cavity shape	Recovery of mantle cavity, but breathing still irregular
R3	Recovery of activity	Recovery of activity, but breathing is laboured
R4	Recovery of regular breathing	Regular breathing; 12-13 breaths min^{-1}

Results

The induction and the recovery time were significantly different at different 2-PhOH concentrations ($P < 0.05$) (Table 2). The musky octopus was anaesthetised effectively at 1.2, 1.4, and 1.6 mL L^{-1} of 2-PhOH without any mortality within 48 h. On the other hand, the 1.8 mL L^{-1} 2-PhOH group had longer induction time than those of the effective ones since the musky octopuses tried to climb out of the anaesthetic solution. The musky octopuses in all groups did not violently eject ink, but they released ink under sedation. Moreover, 50% and 100% mortality occurred at 1 mL L^{-1} 2-PhOH within 48 h and at 1.8 mL L^{-1} 2-PhOH within 24 h, respectively.

Discussion

The efficiency and safety of 2-PhOH at the studied doses (1.2-1.6 mL L^{-1} 2-PhOH) were presented for the musky octopus firstly. However, the current results showed that 1.0 mL L^{-1} and 1.8 mL L^{-1} of 2-PhOH were fatal in these animals.

Andrews and Tansey (26) reported that a 3% solution of urethane (w/v seawater) and 2% ethanol (v/v in seawater) used routinely for *Octopus* spp. had some traumatic effects, such as climbing out of the anaesthetic solution and ejecting ink violently. In the present experiment, the musky octopus did not climb out of the anaesthetic solution, except for in the 1.8 mL L^{-1} group, and did not violently eject ink, but they

Table 2. The induction and recovery times for the musky octopus anaesthetised with 2-PhOH at different doses.

Dose (mL L^{-1})	N	Body weight (g)*	Induction time (s)*	Recovery time (s)*
			A3	R4
1.0	6	232.0 ± 55.9	627.2 ± 86.7[a]	750 ± 112.2[a]
1.2	6	165.3 ± 46.4	449.6 ± 107.8[b,c]	530 ± 282.5[b]
1.4	6	185.8 ± 21.6	440.0 ± 30.9[b,c]	456 ± 46.6[b]
1.6	6	220.2 ± 64.5	370.0 ± 58.9[b]	250 ± 58.9[c]
1.8	6	211.0 ± 66.4	530.0 ± 88.3[c]	Ex

*Data are given as mean ± standard deviation (n=6). ex refers to 100% mortality.
Values in the same column that do not share a common superscript are significantly different ($P < 0.05$).

released ink under sedation. Moreover, 2-PhOH is a more effective anaesthetic agent than urethane and ethanol based on anaesthetic doses. Conversely, Andrews and Tansey (26) demonstrated that immersion in cold water has no such traumatic effects and is a perfectly executable anaesthetic for octopuses. Nonetheless, the latter method has 2 disadvantages: the great contraction of the musculature caused by cold water makes biomedical operations difficult and it is also inconvenient to maintain water at around 3-5 °C when the ambient temperature is about 25 °C. Temperature control is important because if the temperature falls below 2 °C death results, while if it rises to 6 °C the reflexes are no longer abolished. According to the current study, 2-PhOH does not have the disadvantages of cold water.

Messenger et al. (27) pointed out that magnesium chloride ($MgCl_2$) is an effective anaesthetic and narcotising agent for cephalopods. However, this anaesthetic agent took longer for the induction of anaesthesia (i.e. 12-13 m for *Octopus vulgaris* at 22 °C and 15-20 m for *Eledone cirrhosa* at 14-15 °C) than of 2-PhOH for the musky octopus in this experiment. Furthermore, Seol et al. (25) noted that 50-300 mg L^{-1} of clove oil was a suitable and effective anaesthetic for *Octopus minor*. However, the authors found longer recovery times at 15 °C in all doses, when comparing to the effective doses of 2-PhOH for the musky octopus at nearly the same temperature. According to the present results, 2-PhOH is a more effective anaesthetic agent than magnesium chloride and clove oil based on induction time and recovery time, since the desirable attributes of anaesthetics used for aquatic animals include a short induction period and recovery time (9,28).

Gleadall (29) used a standard protocol to evaluate the potential of 11 anaesthetics such as urethane, magnesium chloride, MS222, metomidate, propoxate, chloral hydrate, chlorbutanol, menthol, nicotine sulphate, and 2-PhOH as well as cooling techniques for *Octopus* spp. However, the author did not succeed in obtaining a controlled anaesthesia with these agents. In contrast, controlled anaesthesia for the musky octopus in the present study was achieved with 2-PhOH.

According to the present results, concentrations between 1.2 and 1.6 mL L^{-1} of 2-PhOH did not cause any mortality or toxicity in the musky octopus during 48 h. At these concentrations, the experimental animals displayed similar induction and recovery patterns as described by Seol et al. (25). However, at 1.0 mL L^{-1} of 2-PhOH, the individuals' body colour became pale and only partial sedation was achieved. Moreover, induction and recovery times of these animals were longer than those of the other effective doses. Therefore, it seems that the 50% mortality that occurred within 48 h in the lowest dose might be due to partial toxicity of 2-PhOH because of the very long exposure time. Although the octopus in 1.8 mL L^{-1} of 2-PhOH group exhibited similar induction patterns as in the other doses, they tried to climb out of the anaesthetic solution. Therefore, this behaviour extended the induction time of the musky octopuses. For this reason, they could not recover after anaesthesia, resulting in 100% mortality within 24 h. This mortality could be the result of a toxic effect of 2-PhOH due to a high concentration.

In conclusion, 2-PhOH was demonstrated to be an effective and safe anaesthetic for the musky octopus at doses between 1.2 and 1.6 mL L^{-1} of 2-PhOH.

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