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Evaluation of two anaesthetic agents and the passive integrated transponder tagging system in *Octopus vulgaris* (Cuvier 1797)

J Estefanell[1], J Socorro[1,2], J M Afonso[1], J Roo[1], H Fernández-Palacios[1] & M S Izquierdo[1]

[1]Grupo de Investigación en Acuicultura, Instituto Canario de Ciencias Marinas & Instituto Universitario de Sanidad Animal y Seguridad Alimentaria, Canary Islands, Spain

[2]IES Marítimo Pesquero de Las Palmas, Simón Bolívar, Las Palmas de Gran Canaria

Correspondence: J Estefanell, Grupo de Investigación en Acuicultura, Instituto Canario de Ciencias Marinas & Instituto Universitario de Sanidad Animal y Seguridad Alimentaria, PO Box 56, E-35200 Telde, Las Palmas, Canary Islands, Spain. E-mail: juanestefanell@iccm.rcanaria.es

Abstract

Octopus vulgaris is a species of demand in the market with the potential to diversify European aquaculture. However, this species develops complex social interactions under culture conditions, which may have negative effects on its growth, survival and profitability. In order to understand its behaviour under such conditions, individual tagging systems allow a careful evaluation of biological parameters, such as growth and longevity. The present work describes a combined protocol (anaesthetic and tagging) for implanting subcutaneous passive integrated transponder tags (PIT). The effect of two anaesthetic agents in facilitating octopus handling is assessed: clove oil at 20–40–100 mg L^{-1} and ethanol (96%) at 1–1.5–2%. The most suitable body location of PIT tags, its effect on growth and mortality, the addition of a stitch and the PIT retention rate after 2 months in floating cages were evaluated. It was concluded that immersion in seawater with 1.5% of ethanol at 22.3 ± 0.5 °C is a suitable anaesthetic for this species. The results showed that the best-selected PIT body location was the upper left arm III. No effect of the PIT tagging system was found on growth and survival when tagged and untagged octopuses were compared. It was observed after 2 months that the stitch did not induce an increase in the retention rate and 81–100% tag retention regardless of the dietary treatment.

Keywords: *Octopus vulgaris*, PIT tags, anaesthetics, retention

Introduction

The common octopus (*Octopus vulgaris*, Cuvier 1797) shows high market prices and an increasing demand in European, South American and Asian countries (Vaz-pires, Seixas & Barbosa 2004). Its rapid growth rate (Mangold 1983) and easy adaptation to culture conditions (Iglesias, Sánchez, Otero & Moxica 2000) confer this species with great farming potential (Socorro, Roo, Fernández-López, Guirao, Reyes & Izquierdo 2005; Chapela, González, Dawe, Rocha & Guerra 2006; Rodríguez, Carrasco, Arronte & Rodríguez 2006; García García, Cerezo Valverde, Aguado-Giménez, García García & Hernández 2009). Despite unsolved problems in larval rearing (Iglesias, Sánchez, Bersano, Carrasco, Dhont, Fuentes, Linares, Muñoz, Okumura, Roo, Van Der Meeren, Vidal & Villanueva 2007), a few companies based in Spain (Galicia) have been pioneers in octopus farming. Annual production has fluctuated from 16 to 32 tonnes/year from 1996 to the present day, using the floating cage technology and low-cost species as feed.

In wild populations, this species has a solitary life, mating only for reproductive purposes (Mangold 1983; Guerra 1992). Under culture conditions, *O. vulgaris* develops complex social relations and interactions, which may have a negative effect on growth and survival. It seems that the interaction between animals in group rearing tends to generate a conflict of dominance (Rey Méndez, Tuñón & Luaces-Canosa 2003; García García *et al.* 2009), and understanding this hierarchical behaviour appears to be essential to guarantee profitability of octopus culture. In this sense, physical tagging is a precise and effective method for obtaining individual data, allowing a careful evaluation of biological parameters such as growth or longevity.

An appropriate tagging system for *O. vulgaris* must have several characteristics like a high retention rate, be economical and easy to apply and have no effect on growth and survival. Several external-visual tags have been tested in cephalopods for fisheries management and ecology studies (Watanuki & Iwashita 1990; Nagasawa, Takayanagi & Takami 1993; Domain, Jouffre & Caverivière 2000; Domain, Caveriviere, Fall & Jouffre 2002; Fuentes, Otero, Moxica, Sánchez & Iglesias 2006). External systems have the advantages of being economical, easy to apply and do not require sophisticated equipment (Moffett, Crozier & Kennedy 1997) but may cause some damage to the organism (Nagasawa *et al*. 1993). External-non visual tags (sonic and radio transmitters) have been used with some success to track adult *Octopus dofleini* (Mather, Resler & Cosgrove 1985), although they are expensive, which limits the number of individuals that can be tracked. An internal-visual tag, the Visible Implant Elastomer, has been tested in squid *Sepioteuthis sepioidea* and no effect was found on growth or survival (Zeeh & Wood 2008), although this tagging system is designed to mark cohorts and individual identification is complex, especially for a huge number of animals. Regarding chemical tagging in cephalopods, Fuentes, Iglesias and Moxica (2000) described an efficient tagging system for *O. vulgaris* paralarvae. In order to tag cephalopods sub-adults, internal non-visual tags (passive integrated transponders or PIT tags) are unequivocal, easy to apply and appear to have no effect on growth and survival in *Octopus tetricus* and *Octopus maorum* (Anderson & Babcock 1999). This tagging system was used in *O. vulgaris* by Rey Méndez *et al*. (2003) to study the behaviour of this species in floating cages.

To facilitate handling, a few anaesthetics agents have been tested in *O. vulgaris*. Immersion in seawater with 1–3% of urethane (Messenger 1968) or 2% of ethanol (Andrews & Tansey 1981; O'dor, Mangold, Boucher-rodoni, Wells & Wells 1984) has proved to be effective. Cold water anaesthesia (3–6 °C) was used by Fuentes (2004) and Andrews and Tansey (1981) with an exposure time up to 5 min. Messenger, Nixon and Ryan (1985) evaluated the anaesthetic effect of magnesium chloride on this species. On the other hand, Seol, Lee, Im and Park (2007) assessed the narcotic effect of clove oil in O*ctopus minor*. Clove oil is a natural anaesthetic and has been widely used in aquaculture (García-Gómez, De la Gándara & Raja 2002; Hoskonen & Pirhonen 2004; Bilbao, Vieira, Courois De Vicose, Roo, Ferández Palácios & Izquierdo 2007; Otero, Socorro, Molina, Herrera, Villares, Monroy, Fernández Palacios & Izquierdo 2007).

However, tagging systems need to be tested for each species because of differences in susceptibility to anesthesia and manipulation, the capacity to recover, growth rate and morphology (Navarro, Oliva, Zamorano, Ginés, Izquierdo, Astorga & Afonso 2006). Thus, the present work describes the PIT tagging procedure and evaluates its effect on growth and mortality in *O. vulgaris* on two body locations and confronted with untagged individuals. Also, the addition of a stitch at the insertion point and PIT retention after 2 months in floating cages under two dietary treatments was tested. On the other hand, the effect of two anaesthetics agents was also assessed in this species: ethanol and clove oil.

Materials and methods

Capture and acclimation of the stock

Octopuses were caught at sea in Mogán (Gran Canaria, Spain). Local fishermen used cylindrical trawls (1.5 m diameter, 0.4 m height, with a metallic net of 31.6 mm mesh) located at 20–30 m depth. Octopuses were kept on board in open flow-throw seawater reservoirs and were transported by truck to the laboratory in 500 L square tanks. This operation took 60–80 min, oxygen flow was provided so that the oxygen level would not be limiting (above 12 mg L^{-1}), the mean temperature was 22.9 ± 0.7 °C and a 100% survival was recorded to handling and transport at arrival and after 24 h. Acclimatization lasted 10 days in rectangular 1.5 m^3 tanks with an open flow-through seawater system (one full renovation per hour). The PVC tubes were provided as shelters and shadowing nets (Hanlon & Messenger 1996). A control diet, based on 'discarded' bogue (*Boops boops*, L. 1758) and de-frozen crab (*Portunus pelagicus*, L. 1758), was provided on alternate days once a day six times per week. Food ration was provided to station and the photoperiod was natural. The bogue used in these experiments was supplied by local fish farms as 'discarded' species from off-shore sea bream cages. After 10 days, those octopuses that were unwounded and regularly consumed and ingested food were selected for the following experiments.

Table 1 Description of anaesthesia and recovery stages observed in *Octopus vulgaris*

Stages	Anaesthesia	Recovery
I	Hyperventilation	Resurgence of sucking activity
II	Muscle tone disappears. Flaccid arms	Recovery of chromatophore activity
III	Weak breathing and loss of sucking intensity	Recuperation of breathing movements
IV	Chromatophores relax (the skin becomes white)	Recovery of activity, regular breathing

Anaesthetic

After acclimation, octopuses where placed in 40 L aerated seawater tanks and treated with different concentrations of anaesthetics. Ethanol (96%) was tested at 1–1.5–2% and clove oil at 20–40–100 mg L^{-1}. The water temperature during the experiments was 22.3 ± 0.5 °C. Exposure time, up to 6 min, was evaluated separately in three octopuses per treatment (1268 ± 291 g.), which were immediately transferred into 500 L tanks with an open flow-through seawater system to assess the recovery time and evaluate mortality within 24 h. A description of the anaesthesia-recovery stages observed in this species is shown in Table 1. Finally, the most suitable anaesthetic agent identified was tested in five octopuses weighing between 700 and 1130 g in order to find a relationship between octopus weight and exposure time to become anaesthetized.

Tagging

Passive Integrated Transponders (Trovan, Douglas, UK) were 0.096 ± 0.001 g in weight, 2.05 × 11 mm in size and were associated with a 15-digit code detected by an ARE H5 reader (Trovan). All octopuses were anaesthetized by immersion in seawater with 1.5% of ethanol (96%) before tagging, and PIT tags were immersed previously in alcohol and then introduced at a subcutaneous level using a hypodermic needle (Trovan). Each tag was inserted away from the hypodermic insertion point to avoid tag ejection. The tagging procedure was quick and simple, with tags being implanted within 10 s. Passive integrated transponder retention rate and its effect on growth and mortality were evaluated in four experiments. Each experimental tank was provided with PVC tubes as shelters and an open flow-through seawater system was adjusted so that the oxygen levels would be above 80% saturation (Cerezo Valverde & García García 2005). Water temperature and oxygen levels were measured once a day. Food was supplied to satiation six times per week and the photoperiod was natural.

Experiment 1: the objective of this experiment was to determine a suitable body location for PIT tags in *O. vulgaris*. Twelve octopuses (2633 ± 271 g), all males, were selected and two body locations were tested. Accordingly, six animals were tagged in the upper left arm III (PIT-A) and another six individuals were tagged in between the eyes (PIT-E). The assay lasted 10 days and both tagging treatments were held separately in 2 m^3 tanks. The control diet was provided to station once a day. The mean water temperature was 19.4 ± 0.1 °C.

Experiment 2: the objective of this experiment was to determine whether PIT tagging had an effect on growth and survival confronted with untagged animals under two dietary treatments. Twenty four octopuses (442 ± 115 g) were selected. A triplicate of four octopuses for each treatment, sex ratio 1:1, was placed in 400 L tanks. One tank was PIT tagged in the upper left arm III so that biological parameters could be confronted with the remaining two untagged tanks. The assay lasted 4 weeks and PIT tags were read every week to evaluate retention. The control diet (PIT-A control diet, Untagged control diet) and a moist diet (PIT-A moist diet, Untagged moist diet) were provided to station once a day. The mean water temperature was 21.5 ± 0.7 °C.

Experiment 3: the objective of this experiment was to determine whether a stitch on the syringe insertion point could improve PIT retention. Eighteen octopuses (2207 ± 494 g.) were PIT tagged in the left arm III (PIT-A), and on half of the individuals, an 'X'-shaped stitch, made of a thin fishing line, was added on the syringe insertion point (PIT-S). A triplicate of three octopuses from each treatment, sex ratio 2:1, was placed in 1 m^3 tanks. The assay lasted 4 weeks and PIT tags were read every week to evaluate retention. Discarded bogue was provided to station once a day. The mean water temperature was 23.5 ± 0.3 °C.

Experiment 4: the objective of this experiment was to evaluate PIT tag retention in floating cages in two successive on-growing cycles under two dietary treatments. This floating cage was situated in Taliarte Harbour (Gran Canaria, Spain). It was made of galvanized stainless steel, approximately 10 m^3 in total volume (3 × 3 × 1.5 m) and with a 2 × 2 cm mesh, divided into two sub-cages. The initial biomass was 9.2–9.9 kg m^{-3} and the assays lasted 2 months. Passive integrated transponder tags were read after 30 days to evaluate retention. Dietary treatments were the same as those tested in experiment 2 and were provided to station once a day. In the first cycle, 64 octopuses (1483 ± 269 g) were selected, divided into two groups (sex ratio 1.5:1) and placed in each sub-cage (PIT-A control 1, PIT-A bogue 1). The mean water temperature was 18.3 ± 0.3 °C. In the second cycle, 42 octopuses, all males (2280 ± 305 g), were selected, divided into second groups and placed in each sub-cage (PIT-A control 2, PIT-A bogue 2). The mean water temperature was 21.0 ± 1.3 °C.

Biological parameters

All individuals were weighted at the beginning and when PIT digits were read until the end of the experimental period. The following parameters were calculated individually: Absolute growth rate: $AGR = (W_f - W_i)/t$; PIT tag retention: $R = (N_t - N_l) \times 100/N_t$, where W_f = final weight in g; W_i = initial weight in g, t = time in days, N_t is the number of animals that were initially tagged; and N_l are the number of individual who lost tags. Mortality was evaluated every day and expressed as accumulated percentage. Mortality within the first week (M_{w1}) and the final mortality (M_T) were calculated.

Statistical analysis

Means and standard deviations were calculated for each parameter measured. All data were tested for normality and homogeneity of variances. Every index from each diet fed group was subjected to an *F*-test to compare standard deviations. When $P \geq 0.05$ means were compared using a Students '*t*' and significant differences were considered at $P < 0.05$ (Sokal & Rolf 1995).

Results

Anaesthetic

Table 1 shows a description of anaesthesia and the recovery stages observed in this species. Anaesthesia starts with hyperventilation (stage I), followed by the loss of muscle tone and flaccid arms (stage II). Loss of sucking activity and weak breathing (stage III) occur before full anaesthesia (stage IV) when cromatophores relax and the skin becomes white. Once animals are placed back in clean seawater, sucking activity (stage I) and cromatophore activity (stage II) indicate that the octopus is recovering. Recuperation of breathing movements (stage III) to regular breathing and normal activity (stage IV) indicates full recovery. Clove oil, regardless of the concentration and the exposure time, hardly reached stage I of anaesthesia, showing a poor narcotic effect in *O. vulgaris* under the conditions described. On the other hand, ethanol, regardless of the concentration, always reached level IV of anaesthesia. Ethanol 2% had the lowest exposure time to full anaesthesia (Fig. 1). In all cases, recovery was quick: 3–6 min. Survival was 100% in both the ethanol and the clove oil treatments.

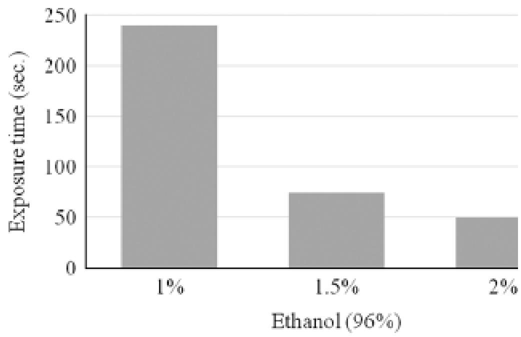

Figure 1. Exposure time (seconds) to reach full anaesthesia in seawater with different % of ethanol (96%) in *Octopus vulgaris*.

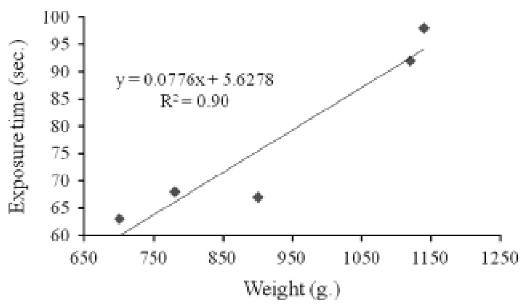

Figure 2. Relationship between exposure time in seawater with 1.5% of ethanol (96%) and octopus initial weight.

The most suitable anaesthetic agent identified was 1.5% of ethanol (96%) in seawater and was tested in octopuses ranging in weight between 700 and 1130 g. Figure 2 shows the relationship between exposure time to become anaesthetized and octopus weight. As can be observed, exposure time was positively related with octopus weight (R^2=0.9).

Table 2 Number of individuals (*N*), rearing time (*t*, days), PIT retention (%), mortality within the first week (M_{w1}), total mortality (M_T) and AGR obtained in experiments 1–4

Experiments	Tag (treatment)	*N*	*t* (days)	Retention (%)	M_{w1} (%)	M_T (%)	AGR (g day^{-1})
1	PIT-A (arm)	6	10	83.3	0	0	51.1 ± 14.7
	PIT-E (eye)	6		83.3	0	0	37.0 ± 7.8
2	PIT-A (control diet)	4	28	100	0	25.0	14.9 ± 4.1
	Untagged (control diet)	8		–	0	0	20.1 ± 6.4
	PIT-A (moist diet)	4		100	0	0	5.4 ± 1.1
	Untagged (moist diet)	8		–	0	12.5	4.7 ± 2.3
3	PIT-A	9	28	100	0	11.1	17.8 ± 8.0
	PIT-S (stitch)	9		88.9	0	0	16.1 ± 5.6
4	PIT-A control 1	32	60	84.4	0	28.0	37.3 ± 21.3
	PIT-A bogue 1	32		81.3	0	21.9	30.4 ± 12.8
	PIT-A control 2	21		100	0	35.0	47.0 ± 18.9
	PIT-A bogue 2	21		100	0	63.6	16.2 ± 12.4

PIT, passive integrated transponder; AGR, absolute growth rate.

Tagging

The results obtained in tagging experiments are summarized in Table 2.

- *Experiment 1*: One PIT tag was lost from each treatment and 100% survival was recorded after 10 days. Despite no statistical difference in growth, higher values were obtained in octopuses tagged in the left arm III.
- *Experiment 2*: Pit tag retention was 100% regardless of the dietary treatment. No statistical difference was found in terms of growth between tagged and untagged individuals. Nevertheless, one tagged octopus died in the control diet treatment and another untagged octopus died in the moist diet treatment.
- *Experiment 3*: The addition of the stitch on the syringe insertion point did not lead to an increase in the tag retention rate. In fact, only one PIT tag recorded belonged to the stitch treatment and all stitches were lost by the end of the experimental period. Besides, no difference in growth was observed between treatments. Regarding mortality, one individual tagged in the arm without the stitch died on the last day of the experimental period.
- *Experiment 4*: The PIT retention rate was 81–100% regardless of the dietary treatment. Mortality, on the other hand, occurred towards the end of the experimental period (sixth to eighth week).

Discussion

In the present experiment, octopuses showed high resistance to handling; hence, anaesthesia was required before tagging. In contrast, some authors claim that tagging in octopods (*O. tetricus* and *O. maorum*) can be performed without anaesthesia (Anderson & Babcock 1999). Immersion in seawater with 2% of ethanol (96%) generated the lowest anaesthetic time, although the drastic change to white colour in the three individuals tested suggests that its effect could be lethal if prolonged. In fact, 2% concentration has been used to anaesthetize cephalopods before dissection (Boyle 1981; O'Dor *et al.* 1984; Gleadall, Ohtsu, Gleadall & Tsukahara 1993). On the other hand, this anaesthetic agent was satisfactorily tested before surgery in *O. vulgaris* (Andrews & Tansey 1981). Cold water anaesthesia (Andrews & Tansey 1981; Fuentes 2004) and immersion in seawater with magnesium chloride (Messenger *et al.* 1985) have proved to be effective in this species, although longer exposure times to reach narcotic effects were recorded. Recently, Seol *et al.* (2007) concluded that clove oil is an effective anaesthetic agent in *O. minor* at concentrations higher than those evaluated here. In the present study, clove oil was ineffective while immersion in seawater with 1.5% of ethanol (96%) showed a rapid anaesthetic time without the drastic effects shown by the higher concentration.

Regarding the PIT tagging system, left arm III was selected as a possible PIT body location because it was opposite to the hectocotylus in males; hence, the least interference with reproductive processes was expected. On the other hand, PIT tagging between the eyes was selected because it was considered to be an easy point to locate. In the present experiment, no difference was found in terms of retention, growth or mortality between body locations, unlike Fuentes *et al.* (2006) and Domain *et al.* (2002), who found lower retention in the mantle independent of the arm tested. In the present experiment, PIT tagging in the upper left arm III is recommended because it is considered to be a safer tagging location. On the other hand, Anderson and Babcock (1999) selected the dorsal crown at the first left arm pair for tag insertion because they intended to read the PIT without removing the animal from the shelter in ecology studies.

In the present study, the tag retention rate was lower than that obtained by Anderson and Babcock (1999) with other species of octopods (*O. tetricus* and *O. maorum*), but higher than that in some other trials where external visual tags were tested in several cephalopods species (Domain *et al.* 2000, 2002; Fuentes *et al.* 2006). In Rey Méndez *et al.* (2003), the PIT retention rate was not evaluated. Regarding the PIT tagging system in other marine organisms, it has been widely used in several species of fish (Baras, Westerloppe, Mélard & Philippart 1999; Baras, Malbrouck, Houbart, Kestemont & Mélard 2000; Roussel, Haro & Cunjak 2000; Bubb, Lucas, Thom & Rycroft 2002; Navarro *et al.* 2006; Soula, Navarro, Zamorano, Roo, Real, Ginés, Hernández-Cruz & Afonso 2006) and in sea turtles (Kamekazi, Kuroyanagi & Sugiyama 1998; Godley, Broderick & Moraghan 1999), with retention rates close to 100%.

Passive integrated transponder tagging showed no effect on growth and survival when compared with untagged individuals (Anderson & Babcock 1999). In fact, mortality in all experiments was observed towards the end of the experimental period and could be related to increased size dispersion under culture conditions (García García *et al.* 2009) or a high rearing temperature (Aguado Giménez & García García 2002; Miliou, Fintikaki, Kountouris & Verriopoulos 2005; García García *et al.* 2009). In fact, in experiment 2, there was no mortality in octopuses fed the moist diet, while there was mortality in untagged octopuses fed the same diet.

In experiment 3, only one tag loss was recorded in an individual whose left arm III was wounded before tagging. It is therefore advisable to avoid tagging in wounded arms because regenerative processes may have a negative effect on PIT retention.

In conclusion, immersion in seawater with 1.5% of ethanol (96%) and PIT tagging at a subcutaneous level are simple and effective methods to facilitate handling and evaluate individual biological parameters in *O. vulgaris*, with no effect on growth and survival and retention rates of 81–100% regardless of the dietary treatment.

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