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Narcotising Sea Anemones

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Standard techniques have been reviewed and modified to improve methods for narcotising actiniarians. Experiments have been carried out on the more common species of intertidal and shallow water British sea anemones. A notable increase in the usual success rate has been achieved.

INTRODUCTION

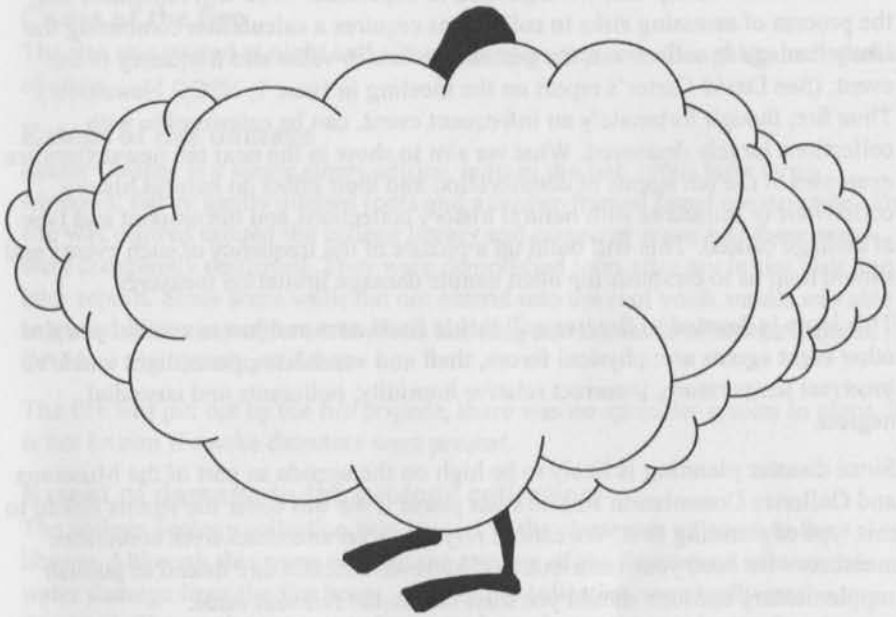
Many techniques have been devised to overcome certain problems encountered when narcotising actiniarians. From these a few have been selected as being at least partly successful. Further modifications to these narcotising methods have been introduced in order to block more effectively the nervous pathways responsible for secondary contraction reactions. Until now, perfectly expanded specimens have been achieved largely by chance. This paper records experimental results and arising problems as a contribution to a better understanding of the process and a more consistent rate of success.

NATURE OF PROBLEMS

1. Keeping an anemone in a medium so that it will not close during the induction period - until it no longer responds to food or tactile stimuli (primary narcotisation stage or PNS). Successful PNS can be achieved by studying the circadian rhythms of the anemones related to water movement for intertidal species and by introducing artificial conditions which stimulate the anemone to remain open; these are cool environment, water movement and presence of food.
   A. Cool environment - container kept in constant-temperature room at 4-10°C.
   B. Water current - to stimulate tidal motion; maintained by magnetic stirrer set between 350 and 500 rpm in 2-5 litres of seawater. This also effected an even mixture of the narcotic.
   C. Feeding with small amounts of food not only encouraged the anemone to stay open but was also necessary in assessing stages of narcotisation as response to food fell off.
2. Having successfully reached PNS, the secondary nervous pathways had to be blocked since the anemone was still responsive to chemical stimuli. Further chemical induction could lead to a gradual closure either by rapid detection of a less tolerable environment or, at a more advanced stage, by the anemone becoming 'accommodated' to the narcotic. According to Shelton *et al.* (1982,

The Ten Agents of Deterioration

An issue by issue guide to the risks facing museum collections



1. Fire

pp. 203-242) this reaction is brought about by the anemone's SS2 endodermal nervous system (see below) which partly controls mouth opening and requires Mg^{2+} cations to block the Ca^{2+} channels and prevent associated nerve endings from firing. In this blocked state the anemone will die and many start to autolyse and decompose which leads to problem 3.

3. Determination of the end point (EP) of narcotisation where a narcotised anemone no longer reacts to any stimulus but is still perfectly intact and has not started to decompose. Visible decomposition normally occurs after about 20 minutes after EP has been attained so that exact estimation or recognition of EP condition is essential. Since it is almost impossible to recognise such a narrow time parameter, coupled with the risk of causing closure by premature fixation or losing the specimen as it autolyses, the problem can only be easily overcome by placing the container of narcotised specimens into a deep freeze approximately 30 minutes before EP is estimated to be achieved. This also has the effect of enhancing narcotisation but can only be effectively carried out with pre-cooled specimens. Room temperature experiments will need to be moved to a cooler environment at least one hour before EP. Introduction of a fixative may also cause tentacle shrivelling due to osmotic syneresis especially noted in (*Anemonia viridis*). Introduction of fixative at low dilution levels has been found to be too slow to halt autolysis.

Narcotising techniques ideally need to be capable of accommodating these problems without becoming too cluttered with physical inducements to keep the specimens from closing. Workers in the field can then carry out effective narcotisation without the burden of complex equipment.

The species narcotised in these experiments are listed below in order of decreasing ease of induction to EP:

Calliactis parasitica, Adamsia carciniopados, Anemonia viridis, Edwardsia tuberculata, Corynactis viridis, Caryophyllia smithi, Cereus pedunculatus, Actinia equina, Urctina felina, Actinia fragacea, Metridium senile (see below) and *Bunodactis verrucosa*.

Specimens of *Sargatia* spp. were not found in sufficiently large numbers for experimentation and there were only enough specimens of *Metridium senile* for one experiment.

RELEVANT NERVOUS SYSTEM PHYSIOLOGY

Muscular actions in Actiniaria are controlled by the following four nervous systems that act independently or interact with each other.

1. Through-conducting nerve net (TCNN) controls fast or slow contractions, caused by stimuli throughout the entire animal.

Points to think about

- MGC standards for collection care require an automatic fire detection and alarm system to BS 5839.
- Although all UK public buildings are required to have a fire alarm, it may only be a manual system and not automatic, i.e. if there is a fire the alarm has to be set off manually and serves only to make people inside the building aware of the need to evacuate; it may not call the fire brigade. If a fire breaks out at night in a building with this kind of alarm, the fire brigade will only be summoned if someone else notices the fire and dials 999.
- Some intruder alarm systems with infra-red motion detectors may pick up the movement of smoke. Break glass detectors would presumably be activated if an arsonist broke a window.
- If you have smoke and heat detectors in your building, check that the calling system is automatic.
- If a building move is on the cards, a new wired-in automatic fire detection system (an expensive short-term investment) is radio operated and portable. Re-useable systems can be supplied by some companies.
- What sort of fire station serves your area? In remote areas, some fire stations are operated by volunteer fire crews on call and will therefore take longer to respond since the fire crew (like lifeboat crew) are called by pagers from another place of work.
- A system incorporating sprinklers is the best safeguard against fire damage. The volume of water discharged by sprinkler heads over the immediate area of the fire is far less damaging than the several hundred of gallons per minute pumped by fire hoses.
- Halon systems are also very effective but under Montreal Protocol on CFCs can no longer be installed as new.

Further information from:
MGC Standards for Biological and Geological Collections (Numbers 2 and 3) include a very thorough standard for the protection against fire with a list of sources of advice and help.

Kate Andrew

Next Issue: Flood

2. Slow system 1 (SS1), ectodermal, controls radial muscles of oral disc, longitudinal muscles of column and tentacles, detachment of pedal disc.
3. Slow system 2 (SS2), endodermal, controls opening and closing of mouth and pharyngeal protrusion.
4. Delayed initiation system (DIS). Evidence of this nervous pathway has been reported by Jackson and McFarlane (1976) in *Calliactis parasitica*. The DIS produces delayed bursts of SS1 pulses.

Excess Mg^{2+} cations (in magnesium chloride) will block the calcium pathways of the TCNN, SS1 and SS2 until recordable activity ceases (PNS). Ciliary action, however, has been observed to continue on the column of *C. parasitica* and narcotisation must therefore be continued until the EP is reached. Organic narcotics have been found to suppress the actions of the SS1 resulting in PNS but few, such as menthol, have been found that can continue narcotisation up to EP; inorganic (Mg^{2+} containing) narcotics have been used in a secondary role to reach EP.

RESPONSE MONITORING

Response to tactile stimuli was made by observing reaction to a 1mm cube of mackerel muscle. The responses were graded (in Figures) as:

1- normal feeding response;
2- nematocysts firing , therefore food adheres to tentacle, but anemone cannot transfer food to mouth and eventually drops it;
3- nematocysts barely firing, food adheres to tentacle for not longer than 2 mins;
4- nematocysts no longer firing, food not adhering to tentacle at all, anemone has no response to tactile stimuli and has reached PNS.

RESULTS

All results were obtained at room temperature unless otherwise stated . Constant improvement was noted throughout. Control specimens were kept in fresh seawater and were put alongside the specimens undergoing narcotisation to monitor the latter's state of opening (i.e. to check for any circadian rhythm induced closing).

Magnesium chloride technique (Figure 1)

Successful narcotisation results were obtained regularly with *Actinia equina* (with magnetic stirrer at 10°C), *Cereus pedunculatus, Bunodactis verrucosa* and with *Corynactis viridis* at 10°C.

Batches of specimens were pre-cooled to 10°C over two hours in two litres of seawater agitated by a magnetic stirrer at 350 rpm.

Smaller batches were used as controls in current-induced seawater, the other specimens were treated with 500ml of a saturated solution (7.5%) of magnesium chloride which was dripped into the container so that a concentration of 1.875% was eventually reached over seven hours.

Actinia equina at 10°C (Figure 1.1). Narcotised specimens were transferred directly to the freezer overnight. Next day, surplus ice was washed away and the remaining block was transferred to a container of Steedman's fixative, semi-concentrated to allow for ice-melt dilution. Final result: out of 24 specimens 18 were fully expanded, six half to three-quarters expanded (88% success). This result proved consistent throughout further experiments.

Cereus pedunculatus at 10°C (Figure 1.2). The specimens were frozen overnight. Surplus ice was washed away the next day and specimens were fixed as before. Final result: out of 15 specimens 11 were fully expanded, two half expanded (80% success).

Bunodactis verrucosa at 10°C (Figure 1.3). Narcotised specimens were frozen overnight and thawed next day in fixative. Final result: all 10 specimens were fully expanded (100% success).

Corynactis viridis at 10°C (Figure 1.4). Specimens were frozen overnight. Surplus ice washed away next day and specimens fixed as before. Final result: all 20 specimens were fully expanded (100% success).

MS-222 Sandoz (tricaine methanesulfonate) (Figure 2)

Successful results were obtained regularly with *Anemonia viridis*, *Corynactis viridis, Bunodactis verrucosa, Calliactis parasitica, Adamsia carciniopados* and *Caryophyllia smithi*. Partly successful results were also obtained from *Actinia equina, Actinia fragacea*. A weak solution of Sandoz was dripped into the narcotising container until a concentration of 0.02% or 0.05% was reached.

Anemonia viridis at 10°C (Figure 2.1). Although this anemone is unable to retract fully the numbers (open) refer to specimens that showed no signs of being affected by the narcotic such as tentacle shortening, syneresis. The EP for *Anemonia* is critical. The specimens had to be frozen fairly rapidly since the first signs of autolysis were already apparent after seven hours narcotising. Final result: out of 12 specimens five were fully expanded (42% success). Fixation of *Anemonia* was found to be difficult since tentacular syneresis occurred This coupled with fast autolysis at EP made this anemone quite difficult to process satisfactorily using this narcotic.

Corynactis viridis (Figure 2.2). Final result: out of 25 specimens 22 were fully expanded and were fixed by dripping Steedman's fixative into the container (82% success).

Bunodactis verrucosa at 4°C (Figure 2.3). Eight specimens were part-fixed during freezing due to signs of rapid autolysis. Final result: out of ten specimens eight were fully expanded (80% success).

Calliactis parasitica at 10°C (Figure 2.4). Narcotised specimens were frozen and thawed in fixative next day. Hermit crabs were removed from shells prior to fixation. Final result: all 15 specimens were fully expanded (100% success).

Adamsia carciniopados (Cloak anemone) together with its hermit crab undergoing fixation in Steedmans following successful narcotisation using chloral hydrate

Adamsia carciniopados at 10°C (Figure 2.5). Narcotised specimens were frozen overnight and thawed in fixative next day. Final result: out of eight specimens seven were fully expanded (88% success).

Caryophyllia smithi (Figure 2.6). Narcotised specimens were frozen after seven hours induction and thawed in fixative the next day. Final result: out of nine specimens eight were fully expanded (90% success).

Actinia equina at 4°C (Figure 2.7). Narcotised specimens were frozen overnight and thawed in fixative the next day. Final result: out of 15 specimens only four were fully expanded (25% success).

Actinia fragacea at 4°C (Figure 2.8). Narcotised specimens were frozen overnight and thawed in fixative the next day. Final result: out of 15 specimens nine were fully expanded (60% success).

Chloral hydrate technique (Figure 3)

Successful results obtained from *Urticina felina, Bunodactis verrucosa, Adamsia carciniopados, Edwardsia tuberculata.*

Narcotisation was carried out at either 10°C or 4°C at a concentration reaching 0.15% or 0.2%.

Urticina felina at 10°C (Figure 3.1). Specimens were gradually cooled to 10°C over three hours in three litres of seawater, gently agitated by a magnetic stirrer at 200 rpm. Narcotised specimens were frozen after eight hours induction. Final result: out of 15 specimens nine were three-quarters to fully expanded. (60% success).

Bunodactis verrucosa at 4°C (Figure 3.2). Narcotised specimens were frozen overnight and thawed the next day in fixative. Induction was rapid. Final result: all ten specimens were fully expanded. (100% success). (The chloral hydrate technique was found to be roughly 50% successful at 10°C and at a final concentration of 0.5%.)

Adamsia carciniopados (Figure 3.3). Hermit crabs were normally removed from their shells. 100 ml of fixative were dripped into the container away from the specimens which were removed to full strength fixative after one hour. Final result: all 15 specimens were fully expanded (100% success).

Metridium senile at 10°C (Figure 3.4). Unfortunately not many specimens were available for experimentation. Narcotised specimens were frozen after eight hours and thawed in fixative next day. Final result: out of six specimens four were three-quarters to fully expanded (60% success).

Edwardsia tuberculata at 10°C (Figure 3.5). Narcotised specimens were frozen overnight and thawed in fixative the next day. Final result: all ten specimens were fully expanded (100% success).

Menthol technique (Figure 4)

Successful results obtained from *Calliactis parasitica, Urticina felina, Corynactis viridis, Adamsia carciniopados, Anemonia viridis, Edwardsia tuberculata.* Partly successful results from *Actinia equina, A. fragacea.*

Menthol crystals were ground to a coarse powder and scattered on the surface of the seawater in the narcotising container. Menthol was found to be equally effective at various temperatures although lower temperatures slowed down the rate of induction.

Calliactis parasitica (Figure 4.1). Provided that the anemones were fully expanded prior to induction, this technique was invariably 90-100% successful.

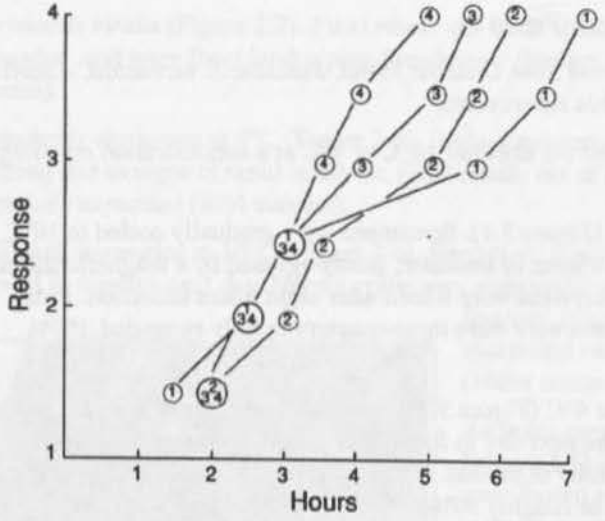


Figure 1, Magnesium chloride technique (all at 10°C). 1= *Actinia equina.* 2= *Cereus pedunculatus.* 3=*Bunodactis verrucosa.* 4=*Corynactis viridis*

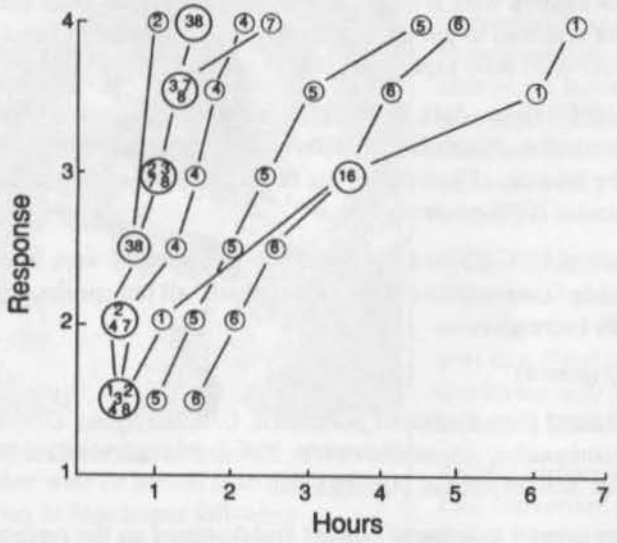


Figure 2. MS-222 Sandoz technique. 1=*Anemone viridis* at 10°C. 2.= *Corynactis viridis.* 3=*Bunodactis verrucosa* at 4°C. 4=*Cereus pedunculatus* at 10°C. 5=*Adamsia carciniopados* at 10°C. 6=. 6=Caryophyllia smithi. 7=.Actinia equina at 4°C. 8=*Actinia fragacea* at 4°C

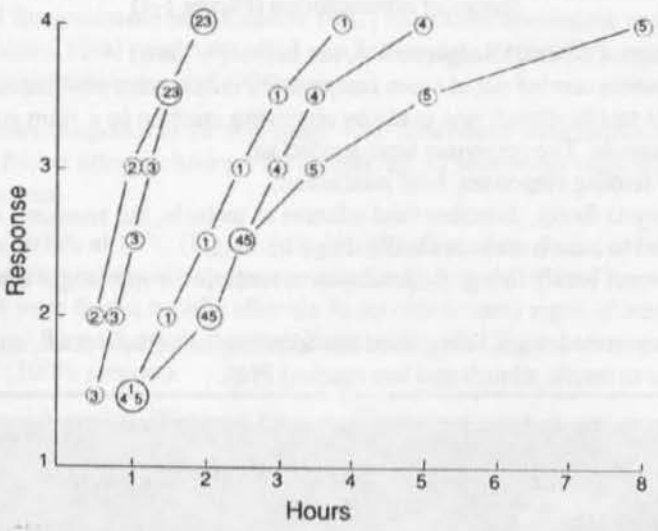


Figure 3. Chloral hydrate technique 1=*Urticina felina.* 2= *Bunodactis verrucosa* at 4°C. 3=*Adamsia carciniopados.* 4=*Metridium senile* at 10°C. 5=*Edwardsia tuberculata* at 10°C.

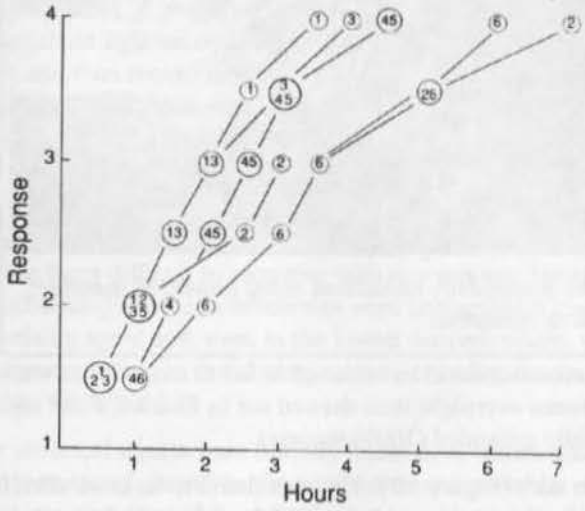
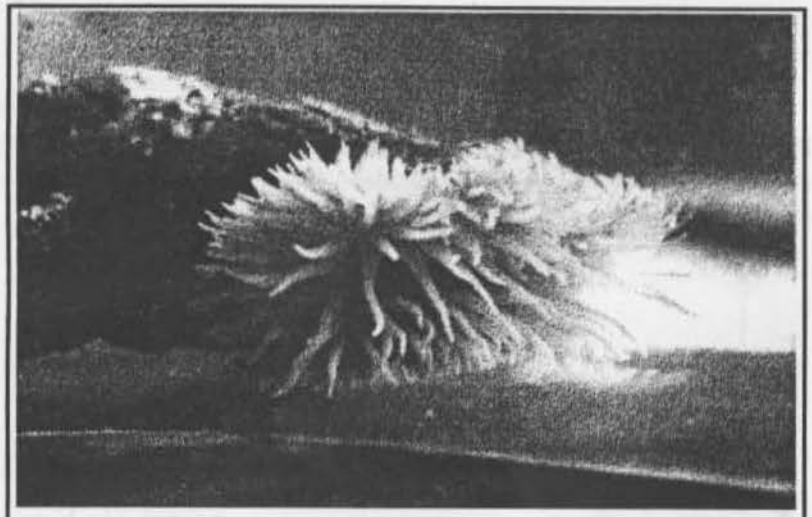


Figure 4. Menthol technique. 1=*Calliactis parasitica.* 2=*Urticina felina* at 4°C. 3=*Corynactis viridis* at 10°C. 4=*Adamsia carciniopados at 10*°C. 5=*Anemone viridis* at 10°C. 6=*Edwardsia tuberculata.*

Stages of narcotisation (Figues 1-4)

Time in hours, ('x' axis) Response 1-4, see below ('y' axis)
All experiments carried out at room temperature unless otherwise indicated.
Response to tactile stimuli was made by observing reaction to a 1mm cube of mackerel muscle. The responses were graded as:
1 - normal feeding responses, food swallowed;
2 - nematocysts firing, therefore food adheres to tentacle, but anemone cannot transfer food to mouth and eventually drops it;
3 - nematocysts barely firing, food adheres to tentacle for not longer than 2 minutes;
4 - nematocysts no longer firing, food not adhering to tentacle at all, anemone has no response to tactile stimuli and has reached PNS.



Calliactis parasitica successfully narcotised using powdered menthol, undergoing fixation in Steedman.

Hermit crabs (for this anemone) could be removed or left to narcotise as well. Narcotised specimens frozen overnight then thawed out in fixative. Final result: all 16 specimens were fully expanded (100% success).

Urticina felina at 4°C in dark (Figure 4.2). This was found to be more effective in the dark at 4°C, thawed in fixative the next day. Final result: out of 12 specimens two were fully expanded, six were three-quarters expanded and four, half expanded (79% success).

Corynactis viridis at 10°C. (Figure 4.3). A higher success rate (of full expansion) was noted for specimens narcotised at 10°C; narcotised specimens were frozen after six hours. Final result: out of 13 specimens eight were fully expanded, five were three-quarters expanded (90% success).

Adamsia carciniopados at 10°C (Figure 4.4). Specimens were narcotised at 10°C and were frozen after six hours. Final result: all 12 specimens were fully expanded (100% success).

Anemonia viridis at 10°C (Figure 4.5). Narcotisation was carried out at 10°C, the lower temperature helped to prevent rapid autolysis once EP had been reached, specimens were frozen rapidly after six hours due to early signs of autolysis and were thawed the next day in fixative. Final result: all 12 specimens were fully expanded (100% success).

Edwardsia tuberculata (Figure 4.6). Induction was carried out at room temperature. Narcotised specimens were frozen overnight and thawed the next day in fixative. Final result: out of 12 specimens nine were fully expanded (75% success).

Freezing technique

This was found to be rather unpredictable and should be used only if no narcotising chemicals are available. Fair results were obtained, however, from *Actinia equina, A. fragacea* (about 50% success rate) and especially from *Urticina felina* (about 80% success rate). Specimens were pre-cooled at 4°C for several hours and then moved to the freezer overnight. After washing away surplus ice, they were immersed in semi-concentrated fixative (to allow for dilution by the remaining ice).

Conclusions

Actiniarians have been found to be highly chemo-sensitive to adverse conditions making them difficult to narcotise with any success. Many current techniques for anaesthetising marine invertebrates were unsuccessful due to the toxicity of the narcotising agent and, even in the lowest concentrations, were detected by the anemone resulting in the gradual retraction and/or the secretion of a mucus barrier (see Table 1.).

Four chemical agents were initially found to be usable with actiniarians and these have been tested. Rapidity of narcotisation was variable: compare, for example, the narcotising time of *Bunodactis* in magnesium chloride to MS-222. The menthol technique proved to be one of the simplest and most effective techniques and is recommended for use in the field. Despite its average 25% success rate, the MS-222 Sandoz technique could also be used in the field. Unsuccessfully

expanded anemones could easily be revived within two hours by replacing them into aerated seawater.

If full expansion is required, the user must be prepared to set up the necessary simple apparatus and follow the technique through with patience. If this is done a good consistent result can be assured.

Table 1. ***Narcotising agents found to be unsuccessful in certain species***

	Chloral hydrate	Freezing	Chlorbutol Urethane	Formalin	MS-222	Menthol	$MgCl_2$
A. equina	x		x	x		x	x
A. fragacea	x		x	x		x	
U. felina			x	x	x		
B. verrucosa		x	x	x			
C. pedunculatus	x	x	x	x	x	x	
A. viridis	x	x	x	x			x
C. parasitica	x	x	x	x			x
A. carciniopados		x	x	x			x
C. viridis	x		x	x			

REFERENCES

Jackson, A.J. & McFarlane, I.D., 1976. Delayed initiation of SS1 pulses in the sea anemone *Calliactis parasitica*: evidence for a fourth conducting system. *Journal of Experimental Biology,* **65**, 539-552.

McFarlane, I.D., 1969. Two slow conduction systems in the sea anemone *Calliactis parasitica. Journal of Experimental Biology*, **51**, 377-385.

McFarlane, I.D., 1982. *Calliactis parasitica.* In *Electrical Conduction and Behaviour in 'Simple' Invertebrates* (ed. G.A.B. Shelton), pp. 243-256. Oxford: Clarendon Press.

Shelton, G.A.B. (ed.), 1982. *Electrical Conduction and Behaviour in 'Simple' Invertebrates*. Oxford: Clarendon Press.