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A Synopsis of Methods for the Narcotisation of Marine Invertebrates

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INTRODUCTION

The successful preservation of marine invertebrates often depends on the treatment afforded them prior to fixation. Many invertebrates are highly contractile and will assume contorted postures if placed straight into fixative. To avoid contortion, rupture of the body wall, evisceration, ejection of gut contents and other undesirable side-effects of poor fixation, it is usually necessary to narcotise the animal before fixing it. After narcotisation, the animal may then be fixed in as life-like a posture as possible, with the least distress involved. This is especially important for such groups as Coelenterates and Platyhelminthes, but less so with certain arthropods and molluscs. Nevertheless, a certain amount of narcotisation usually leads to a more satisfactory end product.

This synopsis of narcotisation methods is intended to be no more than a basic guide to the most satisfactory methods for each group of invertebrates. A selection of methods is given for each group, and since many invertebrates are not constant in their reaction to a narcotic, it is wise to try several different narcotisation methods on a particular animal species. One method will usually be more effective than the others. Most of the methods given here are simple; any complex or esoteric methods have been omitted since in most cases only basic apparatus and chemicals will be available.

The synopsis is arranged following the generally-accepted systematic arrangement of the animal kingdom, and most groups are represented. The first reference given on each page refers to the original description of the method, or to a detailed account. Subsequent references, where given, refer to other users of the method. We have not had time to try out all the methods on all the groups, but comments have been added where appropriate. A space is provided at the bottom of each page for any notes the user might wish to add, thus improving the information content.

It is assumed here that the narcotisation procedure will lead to the death of the animal and to its subsequent fixation, dissection or examination. Many of these techniques may be used for anaesthetising animals and allowing their subsequent recovery, but since this is more within the realm of the physiologist, we have not attempted to provide such information here.

Phylum: PROTOZOA

Method for: GENERAL

Narcotic used: ATROPINE SULPHATE

Method:

1. Make up a solution of 1% atropine sulphate.
2. Add this solution to the water containing the protozoa in a 1:1 ratio.
3. Induction time variable: 1–10 mins.
4. A less concentrated solution (0.2%) may be used to give slow narcotisation.

Reference(s):

Kaas (1909)

User's Notes:

Phylum: PROTOZOA

Method for: GENERAL

Narcotic used: BUTACAINE

Method:

1. Prepare a 0.1% solution of butacaine.
2. Add this solution drop by drop until the animals quieten.
3. Induction time variable, normally a few minutes.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	PROTOZOA
Method for:	GENERAL
Narcotic used:	CHLOROFORM

Method:

1. Spray chloroform onto the surface of the culture water, using a fine syringe.
2. Repeat every 5–10 minutes.
3. Alternatively, diethyl ether may be used in the same manner.

Reference(s):

Kaplan (1969)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	PROTOZOA
Method for:	GENERAL
Narcotic used:	CARBON DIOXIDE

Method:

1. Using a very fine tube, CO_2 can be bubbled through the culture water. Observe periodically and cease when animals are immobile.
2. Alternatively, a soda-siphon can be filled with soda-water, the CO_2 bulb injected, and the resulting soda-water added gradually to the culture water.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum: PROTOZOA

Method for: GENERAL

Narcotic used: MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$)

Method:

1. Make up a 0.1% solution of $MgCl_2$ in sea-water.
2. Add a few drops to a few millilitres of culture. Observe. Add further $MgCl_2$ solution if necessary.
3. Induction time variable: 5–15 mins.

Reference(s):

Kaplan (1969)

Hale (1958)

Pantin (1962)

User's Notes:

Phylum: PROTOZOA

Method for: CONTRACTILE FORMS

Narcotic used: MENTHOL

Method:

1. Place specimens in a small dish with a few drops of water.
2. Add one or two small crystals of menthol to the water surface. Observe narcotisation and fix when animals extend.

Reference(s):

Kaplan (1969)

Hale (1958)

Abdel-Malek (1951)

User's Notes:

Phylum: PROTOZOA

Method for: CONTRACTILE FORMS

Narcotic used: BENZAMINE HYDROCHLORIDE

Method:

1. Make up the following solution:

Benzamine hydrochloride	1 grm.
90% ethanol	10 ml.
Distilled water	10 ml.

2. Use as a 1% solution in sea-water and slowly introduce into the water containing the specimens.

Reference(s):

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum: PROTOZOA

Method for: CONTRACTILE FORMS

Narcotic used: SODIUM IODIDE

Method:

1. Prepare a 1% solution of sodium iodide in sea-water.
2. Immerse the specimens in this solution.
3. Induction time variable. Fix when permanently extended.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	PROTOZOA
Method for:	CILIATA & CONTRACTILE FORMS
Narcotic used:	TOBACCO SMOKE

Method:

1. Fill a short tube with tobacco smoke.
2. Invert over its mouth a slide carrying the specimens in a drop of fluid.
3. Observe under low power and remove the slide as soon as narcotisation is complete (¼ – 1 minute).
4. Alternatively, bubble tobacco smoke through the water containing the specimens.

Reference(s):

Pantin (1962)

Kaplan (1969)

Hale (1958)

User's Notes:

Phylum:	PROTOZOA
Method for:	CILIATA
Narcotic used:	HYDROXYLAMINE HYDROCHLORIDE

Method:

1. Make up a 0.1% solution of hydroxylamine hydrochloride in sea-water. Neutralise with sodium bicarbonate.
2. Immerse the organisms in the 0.1% solution for 30 minutes or less. Observe.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum: PROTOZOA

Method for: CILIATA

Narcotic used: NICKEL SULPHATE

Method:

1. Dissolve 0.4 grms. of $NiSO_4$ in 1 litre of filtered sea-water.
2. Mix a drop of the above solution with an equal amount of water containing the protozoa.
3. Narcotisation is gradual. If necessary, further drops of $NiSO_4$ solution may be added.

Reference(s):

Bovee (1958)

Kaplan (1969)

User's Notes:

Phylum: PROTOZOA

Method for: CILIATA

Narcotic used: CHLORETONE

Method:

1. Make up a 0.12% solution of chloretone in sea-water.
2. Add a few drops of this solution to a few millilitres of culture water.
3. Induction time approx. 10 mins.
4. Stronger solutions may cause permanent contraction.

Reference(s):

Kaplan (1969)

Pantin (1962)

von Randolph (1900)

Hale (1958)

User's Notes:

Phylum:	COELENTERATA
Method for:	SMALL COELENTERATES
Narcotic used:	MENTHOL and CHLORAL HYDRATE

Method:

1. Allow specimens to expand in dish of sea-water.
2. Grind together in mortar 12 gm. of menthol and 13 gm. of chloral hydrate. They will form a fluid.
3. Dip a glass rod into this fluid, and allow a little loose fluid to remain on the rod.
4. Lower rod into the water containing the animals. A thin film of narcotic will spread over the surface.
5. Induction time 15–30 hours.

Reference(s):

Steedman (1976)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	BENZAMINE HYDROCHLORIDE (EUCAINE)

Method:

1. Place animals in approx. 100 ml. sea-water. Allow polyps to expand.
2. Make up 1% solution of benzamine hydrochloride.
3. Run one or two drops of this solution into the water containing the animals. Observe and add further drops if necessary.
4. Induction time 15–30 minutes.
5. Methods using less dangerous chemicals are preferable.

Reference(s):

Sartory (1936)

Kaplan (1969)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	CARBON DIOXIDE as SODA WATER

Method:

1. Allow polyps to expand in dish of sea-water.
2. Fill soda-siphon with sea-water and inject CO_2 bulb.
3. Add soda-water directly to dish containing colonies or squirt soda-water into beaker and add gradually to dish containing colony.
4. Levels of 30–50% soda-water usually effective.
5. Induction time of 10 mins. – 1 hour depending on size of colony.
6. Test touch-reaction of tentacles and fix when no withdrawal occurs.
7. Very high percentages of soda-water may reduce pH considerably.

Reference(s):

Kaplan (1969)

Hale (1958)

Pantin (1962)

Smaldon (1978)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	CHLORAL HYDRATE

Method:

1. Place colonies in dish with plenty of sea-water. Allow polyps to expand.
2. Make up 10% solution of chloral hydrate in sea-water.
3. Add very small quantities of this solution to the dish over period of three hours or more.
4. Test touch-response of tentacles periodically.
5. Alternatively, drop small crystals of chloral hydrate into dish at intervals. Observe polyps and test touch-response.

Reference(s):

Wagstaffe & Fidler (1955)

Hale (1958)

Pantin (1962)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	70% ETHANOL

Method:

1. Allow polyps to expand in small dish of sea-water. Avoid disturbance.
2. Add a drop of 70% ethanol, from a pipette, every 5 minutes or so.
3. Carefully observe animals. Test touch-reaction of tentacles with fine needle. Fix when no withdrawal of tentacles on touch.
4. Induction time of 10 mins. – 1 hour depending on size of colony.

Reference(s):

Duncan (1917)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	ETHYL CARBAMATE (URETHANE)

Method:

1. Make up a 2% solution of urethane in sea-water.
2. Place colony in this solution.
3. Observe periodically and test touch-response of tentacles.

Reference(s):

After Macklin (1976)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$)

Method:

1. Allow polyps to expand in dish of sea-water.
2. Add a few crystals of $MgCl_2$ every fifteen minutes or so.
3. Observe animals carefully and test tentacles after approx. 1 hour for touch-response.
4. Fix if no withdrawal of tentacles.
5. Induction time 3–4 hours (normal).
6. Alternatively, immerse colony in solution of 7.5% $MgCl_2$ made up with sea-water. Observe carefully. Do not leave in narcotising solution longer than necessary.

Reference(s):

Wagstaffe & Fidler (1955)

Kaplan (1969)

Hale (1958)

Pantin (1962)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_2O$)

Method:

1. Place colonies in dish of sea-water (approx. 1 litre) and allow polyps to expand.
2. Put approx. 100 grms. $MgSO_4$ in a piece of cheesecloth and tie to make into a bag.
3. Suspend this bag of $MgSO_4$ over the dish of hydroids and allow the tip of the bag to dip into the water.
4. Streams of sulphate solution will descend into the water.
5. Do not disturb the animals if at all possible.
6. Induction time 15–30 minutes.
7. Up to 250 grms. of $MgSO_4$ can be used per litre of sea-water containing hydroids.

Reference(s):

Griffin (1910)

For variations on this method, see:
Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	MENTHOL

Method:

1. Place colonies in dish with plenty of sea-water. Allow polyps to expand.
2. Sprinkle a few menthol crystals on the surface of the water. Cover dish.
3. Observe polyps occasionally & test for tentacle touch-response after 2 hours.
4. Induction time variable, depending on size of colony, amount of water and sensitivity of animal. Normally 8 hours is sufficient. Maceration may set in if animals left too long under menthol.

Reference(s):

Duncan (1917)

Kaplan (1969)

Wagstaffe & Fidler (1955)

Hale (1958)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Polyps)
Narcotic used:	MENTHOL and CHLORAL HYDRATE

Method:

1. Place colonies in dish with plenty of sea-water. Allow polyps to expand.
2. Make up mixture of menthol: chloral hydrate in ratio of 45:55. Grind crystals together in mortar with a little sea-water.
3. Add enough of this mixture to form a thin layer on the surface of the sea-water in dish with hydroids.
4. Test touch-response of tentacles periodically.
5. Induction time normally 6–8 hours.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum: COELENTERATA

Method for: HYDROZOA (Polyps)

Narcotic used: TOBACCO SMOKE

Method:

1. Place colonies in dish with plenty of sea-water. Allow polyps to expand.
2. Using a fine glass tube, slowly bubble tobacco smoke through the water. For best results, the tube should rest on the bottom of the dish. Try not to disturb polyps with bubbles.
3. Not suitable for large colonies.
4. Induction time 1–3 minutes.
5. Alternatively, fill a short tube with smoke and invert slide containing polyp over mouth of tube. Observe under microscope.

Reference(s):

Wagstaffe & Fidler (1955)

Kaplan (1969)

Pantin (1962)

User's Notes:

Phylum: COELENTERATA

Method for: HYDROZOA (Medusae)

Narcotic used: CHLOROFORM

Method:

1. Allow animals to expand in plenty of sea-water.
2. Use pipette with fine orifice to spray chloroform onto surface of water.
3. Repeat every five minutes as necessary.
4. Induction time normally 1–2 hours.

Reference(s):

Kaplan (1969)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Medusae)
Narcotic used:	ETHANOL

Method:

1. Add ethanol to sea-water containing medusae to make approx. 3% solution.
2. Fix when no touch response or movement evident.

Reference(s):

Lo Bianco (1899)

User's Notes:

Phylum:	COELENTERATA
Method for:	HYDROZOA (Medusae)
Narcotic used:	ETHYL CARBAMATE (URETHANE)

Method:

1. Prepare 10% solution of urethane in sea-water.
2. Immerse medusae in this solution.
3. Alternatively, add a few crystals of urethane to surface of the water containing the medusae and allow to stand.
4. Induction time variable, 1–4 hours or occasionally less.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	COELENTERATA
Method for:	ALCYONARIA
Narcotic used:	FORMALIN

Method:

1. Place animals in at least 50 times their volume of sea-water. Allow to expand.
2. Add a few drops of dilute formalin (3 drops of 1% formalin to each 100 ml. of sea-water containing animals).
3. Repeat at 15 minute intervals.
4. The amount of formalin may be doubled each hour.
5. Animal should eventually die with little or no contraction.

Reference(s):

Gohar (1937)

User's Notes:

Phylum:	COELENTERATA
Method for:	ALCYONARIA : *Tubipora, Acabaria, Clathraria, Sympodium & Clavularia* only
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Place specimens in plenty of clean sea-water and allow polyps to expand.
2. Place $MgSO_4$ in cheesecloth bag (150 gms. per litre of water used) and place bag in corner of dish farthest away from animal.
3. Observe periodically and test tentacle touch-response.
4. Induction time 5–24 hours.
5. Osmotic imbalance may occur due to large quantities of $MgSO_4$. Specimens often unsuitable for histology.

Reference(s):

Gohar (1937)

User's Notes:

Phylum:	COELENTERATA
Method for:	ALCYONARIA (and GORGONACEA)
Narcotic used:	MENTHOL

Method:

1. Place colony in plenty of fresh sea-water.
2. Allow all polyps to expand.
3. Sprinkle menthol crystals on the surface of the water. Use sparingly.
4. Cover dish. Observe periodically.
5. Take care not to leave under menthol for too long, or animals may macerate.
6. Not all polyps may remain extended. 100% success is seldom possible.
7. Induction time normally 12–24 hours.

Reference(s):

Duncan (1917)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	COELENTERATA
Method for:	GORGONACEA
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_2O$)

Method:

1. Place animals in dish with plenty of fresh sea-water.
2. Place $MgSO_4$ in cheesecloth bag (150 grms. per litre of water used) and place bag in corner of dish farthest away from animal.
3. Alternatively, suspend bag of $MgSO_4$ over dish so that tip of bag dips into water and $MgSO_4$ streams into water.
4. Observe periodically and test tentacle touch-response.
5. Induction time normally 15–30 minutes.

Reference(s):

Griffin (1910)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Actiniaria
Narcotic used:	CHLORAL HYDRATE

Method:

1. Place animals in dish of sea-water.
2. Allow to expand.
3. Siphon off two-thirds of the sea-water, without disturbing animals.
4. Replace this water with 0.2% chloral hydrate solution, made up in sea-water.
5. After a few minutes, pour off liquid until there is barely enough to cover animals, and fix.
6. Induction time of a few minutes.
7. Alternatively, gradually add crystals to sea-water containing animals, or slowly add a 2% solution to the sea-water. Induction time 3–24 hours.

Reference(s):

Lo Bianco (1899)

Wagstaffe & Fidler (1955)

Pantin (1962)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Actiniaria
Narcotic used:	CHLORETONE

Method:

1. Place animals in dish of sea-water.
2. Allow to expand.
3. Gradually add a few crystals of chloretone to the sea-water, at half-hourly intervals. Induction time of 5-6 hours.
4. Alternatively, immerse animals in a 0.05% solution of chloretone in sea-water.
5. Test tentacle touch response periodically.

Reference(s):

Kaplan (1969)

Pantin (1962)

Hale (1958)

von Randolph (1900)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Actiniaria
Narcotic used:	CLOVE OIL

Method:

1. Place single anemone in approx. 800 ml. of sea-water. Allow to expand.
2. Add several large drops of clove oil once an hour for three hours.
3. Test touch-response of tentacles. If retract, add more clove oil.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Actiniaria
Narcotic used:	ETHANE DISULPHONATE

Method:

1. Allow animals to expand in dish of sea-water.
2. Carefully siphon off most of sea-water and replace with 0.25% solution of ethane disulphonate made up in sea-water.
3. Induction time of 10–15 minutes.
4. Test touch-reaction of tentacles.

Reference(s):

Ehrhardt, Wissocq & Niaussat (1970)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Actiniaria (*Adamsia, Edwardsia*)
Narcotic used:	ETHANOL

Method:

1. Place animal in dish of sea-water and allow to expand.
2. Add 70% ethanol drop by drop to the sea-water (use burette).
3. Fix when tentacles exhibit no touch-response.

Reference(s):

Lo Bianco (1899)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA
Narcotic used:	FORMALIN

Method:

1. Place animals in dish of sea-water and allow to expand.
2. Add a few drops of 40% formalin to the water.
3. Repeat every 15 minutes or so, until approx. one-tenth of the total volume is formalin.

Reference(s):

Baylis and Monro (1941)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Actiniaria
Narcotic used:	FREEZING

Method:

1. Place specimens in plenty of sea-water and allow to expand.
2. Place dish in refrigerator until water becomes ice cold.
3. Place in deep-freeze and allow whole to freeze.
4. Remove as much ice as possible by allowing to melt, but do not expose animal.
5. Animal embedded in remaining ice allowed to thaw in fixative.
6. Suitable for small specimens.

Reference(s):

Gohar (1937)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$)

Method:

1. Place animals in plenty of sea-water and allow to relax.
2. Add drops of 0.81M $MgCl_2$ solution to the sea-water, allowing up to 20 ml. of $MgCl_2$ per 200 ml. sea-water (use a burette).
3. Induction time of approx. 20 hours.
4. Alternatively, place specimens in sea-water, allow to expand, then gradually replace sea-water with 7½% solution of $MgCl_2$ made up in sea-water.

Reference(s):

Fujii and Isaka (1957)

Pantin (1962)

Kaplan (1969)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Actiniaria
Narcotic used:	MAGNESIUM SULPHATE & FORMALIN

Method:

1. Place animals in dish with plenty of sea-water.
2. Allow to expand.
3. Place crystals of $MgSO_4.7H_20$ in a muslin bag (approx. 100 grms. per litre of sea-water used).
4. Put this bag of crystals in corner of the dish farthest away from animal and allow to dissolve slowly.
5. Add a few drops of 1% formalin (3 drops per 100 ml. sea-water) at intervals of about 15-minutes. The amount of formalin may be doubled each hour.
6. The animal eventually dies with little contraction.
7. Induction time 3–8 hours.

Reference(s):

Gohar (1937)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA
Narcotic used:	MENTHOL

Method:

1. Place specimens base-down in dish and cover with sea-water. Ensure sea-water is at least one inch deep above specimens.
2. Allow animals to expand fully. Do not disturb dish.
3. Sprinkle crystals of menthol on surface of the sea-water. Use sparingly.
4. Cover dish. Test sensitivity by touch-reaction after 6–8 hours.
5. Induction time of 8–18 hours.
6. Results often variable, even within the same species.

Reference(s):

Duncan (1917)

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Actiniaria
Narcotic used:	SODA – WATER

Method:

1. Fill soda-siphon with sea-water and inject CO_2 bulb.
2. Place animals in dish of sea-water and allow to expand.
3. Squirt soda-water into a beaker. Add this soda-water gradually to the dish containing the animal. Try not to disturb animal.
4. Increase soda-water amount to approx. 50% total volume of water with animals over period of 30 minutes. Add more if necessary.
5. Test touch response of tentacles. Complete success often not possible, since animals vary in reaction.

Reference(s):

Smaldon (1978)

Kaplan (1969)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	COELENTERATA
Method for:	ANTHOZOA : Especially *Adamsia* sp.
Narcotic used:	TOBACCO SMOKE

Method:

1. Remove crabs from shells bearing *Adamsia.*
2. Place shells in beakers of sea-water allowing plenty of room for expansion.
3. Place beakers in shallow tray containing a little water. Allow animals to expand.
4. Cover with bell-jar. Bottom of bell-jar must be in water.
5. Fill bell-jar with smoke via one tube, allowing air to escape via second tube.
6. Repeat fumigation after three hours and leave overnight. Test touch-reaction of tentacles.
7. If no response, place open beaker with few ml. of chloroform beside jar with anemones. Leave for 2–3 hours, then fix. If tentacles retract, repeat fumigation.

Reference(s):

Lo Bianco (1899)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	TURBELLARIA
Narcotic used:	CHILLING

Method:

1. Place animals in small dish with sea-water.
2. Surround this dish with a salt and ice mixture and place the whole in the freezing compartment of a refrigerator.
3. Check for sensitivity after 30 minutes.

Reference(s):

Kaplan (1969)

Mahoney (1966)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	TURBELLARIA
Narcotic used:	CHLORETONE

Method:

1. Place animals in dish of sea-water and allow to relax.
2. Siphon off the sea-water and replace with 0.1% solution of chloretone made up in sea-water.
3. Induction time of 3–4 hours. Fix when quiescent.

Reference(s):

Kaplan (1969)

von Randolph (1900)

Pantin (1962)

Hale (1958)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	TURBELLARIA
Narcotic used:	ETHANOL

Method:

1. Place animals in a dish of sea-water. Allow to expand and crawl.
2. Add a few drops of 10% ethanol.
3. Allow excitation to subside then add few more drops. Repeat.
4. Induction time 5 minutes – 1 hour.

Reference(s):

Pantin (1962)

Kaplan (1969)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	TURBELLARIA
Narcotic used:	HYDROXYLAMINE HYDROCHLORIDE

Method:

1. Place animals in dish of sea-water.
2. Siphon off sea-water and replace with a 1% solution of hydroxylamine hydrochloride made up in sea-water.
3. Fix when quiescent.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	TURBELLARIA
Narcotic used:	MENTHOL

Method:

1. Place animals in dish of sea-water and allow to relax.
2. Sprinkle a few crystals of menthol on the surface of the water. Use sparingly. Cover dish.
3. Observe periodically. Fix when quiescent. Do not leave too long under menthol, as maceration occurs rapidly.
4. Induction time 1–5 hours.

Reference(s):

Mahoney (1966)

Kaplan (1969)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	TURBELLARIA : Meiofaunal forms
Narcotic used:	PROPYLENE PHENOXETOL

Method:

1. Make up a stock solution of 1.5% propylene phenoxetol in warm tap-water.
2. Mix 1 part of stock solution with 10 parts of sea-water.
3. Add this solution to sand sample or filtered sample.
4. Induction time 10–15 minutes, occasionally longer.

Reference(s):

McKay and Hartzband (1970)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	MONOGENEA and TREMATODA
Narcotic used:	CHLORETONE

Method:

1. Dissolve 2 grms. of chloretone in 500 ml. of sea-water.
2. Place animals in solution and shake occasionally.
3. Individual specimens ready for fixation in 30–40 minutes. When shaking provokes no muscular responses, the worm is narcotised.
4. For mass narcotisation, gills are removed, viscera removed and opened. Place in jars with enough solution to cover. Keep in solution for 30–40 minutes, with periodic shaking (approx. 50 times).

Reference(s):

Hargis (1953)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	TREMATODA (free or *in situ*)
Narcotic used:	DISTILLED WATER

Method:

1. If trematodes have been removed from host, place them in distilled water and fix when quiescent.
2. *In situ* narcotisation: Inject freshly dead fish with distilled water through mouth and anus using 50 ml. syringe. About 100 ml. water normally sufficient for 400 grm. fish.
3. Put fish aside for 1 hour, then inject with concentrated fixative. Store in fixative.

Reference(s):

Eagle and McCauley (1965)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	TREMATODA
Narcotic used:	MENTHOL and CHLORAL HYDRATE

Method:

1. Grind together a mixture of chloral hydrate and menthol in a ratio of 55:45.
2. Place animals in a dish of sea-water.
3. Add a few crystals of mixture to the sea-water. Cover dish.
4. Induction time 30 minutes – 24 hours.
5. Add more crystals if necessary.
6. Watch specimens to ensure no maceration occurs.
7. Menthol alone can be used. Scatter a few crystals on water surface and cover dish.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	CESTODA
Narcotic used:	CHILLING

Method:

1. Place specimen in dish of sea-water.
2. Place dish in refrigerator overnight.
3. Transfer dish to larger dish containing salt-ice mixture.
4. Leave for 30 minutes – 1 hour.
5. Wind specimen around beaker and fix.

Reference(s):

Roudabush (1947)
Kaplan (1969)

User's Notes:

Phylum:	PLATYHELMINTHES
Method for:	CESTODA
Narcotic used:	MENTHOL

Method:

1. Place animals in small dish with sea-water or saline solution.
2. Powder some menthol crystals and spread on the surface of the water.
3. Test irritability after 15 minutes by probing with fine brush.
4. Alternatively, dissolve 24 grm. of menthol in 10 ml. of 95% ethanol. Add one drop of this solution for each 100 ml. of water containing animals. Acts more quickly than crystals.

Reference(s):

Abdel-Malek (1951)

User's Notes:

Phylum:	RHYNCHOCOELA
Method for:	NEMERTINES : Meiofaunal inhabitants
Narcotic used:	CHLORAL HYDRATE

Method:

1. Place sample in dish with sea-water.
2. Scatter a few crystals of chloral hydrate on water surface.
3. Add more crystals after one hour if necessary.
4. Induction time 10 minutes – 2 hours.

Reference(s):

Hulings & Gray (1971)

User's Notes:

Phylum:	RHYNCHOCOELA
Method for:	NEMERTINES : Larger species
Narcotic used:	CHLORAL HYDRATE

Method:

1. Place specimen in large glass container with sufficient sea-water (e.g. for a specimen of 5 cm., 300 ml. is sufficient).
2. Add a few crystals of chloral hydrate at 10 minute intervals.
3. If contraction occurs, and the proboscis is partly everted, remove the specimen to fresh, clean sea-water. On revival, repeat process.
4. Induction time varies with size of specimen, but is normally 30 minutes to 2 hours.
5. Urethane may be used instead. Same procedure is adopted.

Reference(s):

Kirsteuer (1967)

User's Notes:

Phylum:	RHYNCHOCOELA
Method for:	NEMERTINES
Narcotic used:	CHLORETONE

Method:

1. Place specimen in dish of sea-water.
2. Put container in dimly-lit place and allow animal to settle.
3. Over a period of 3–4 hours add a few crystals of chloretone to the water at 30 minute intervals.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum: RHYNCHOCOELA

Method for: NEMERTINES

Narcotic used: MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Make up 80 grm. of $MgCl_2$ in one litre of sea-water.
2. Immerse animals in this solution.
3. Watch carefully and avoid rupture of of body wall. Fix when flaccid to avoid maceration.
4. Induction time 1–4 hours.
5. Alternately, place animal in a dish of sea-water and slowly add an equal quantity of 14% $MgCl_2$ made up with sea-water.

Reference(s):

Ledingham & Wells (1942)

Mahoney (1966)

Pantin (1962)

User's Notes:

Phylum: RHYNCHOCOELA

Method for: NEMERTINES

Narcotic used: MENTHOL

Method:

1. Place animals in dish of sea-water. Allow to relax.
2. Sprinkle menthol crystals on surface of water. Use sparingly. Cover dish.
3. Observe at intervals to prevent over-exposure to menthol. which causes rupture of body wall and maceration.
4. Induction time of 2–8 hours.

Reference(s):

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	ROTIFERA
Narcotic used:	BENZAMINE HYDROCHLORIDE

Method:

SLOW NARCOTISATION :

1. Place rotifers in approx. 8 ml. of sea-water in flat-bottomed watch glass.
2. Add one or two drops of a 2% aqueous solution of benzamine hydrochloride and mix with a coarse pipette. Wait 20–30 minutes then add further one or two drops. Mix cautiously then fix. Process may take up to 3 hours.

RAPID NARCOTISATION :

1. Make up mixture as follows :

   3 parts of 2% aqueous benzamine hydrochloride
   6 parts water
   1 part pure cellosolve
2. For each ml. of water containing rotifers, add up to 0.5 ml. of mixture. Stir. Induction time 3–5 mins.

Reference(s):

Hanley (1949)

Wagstaffe & Fidler (1955)

Kaplan (1969)

Mahoney (1966)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	ROTIFERA
Narcotic used:	BENZAMINE LACTATE

Method:

1. Place rotifers in flat-bottomed watch glass with approx. 8 ml. of sea-water.
2. Add 2 drops of a 2% solution of benzamine lactate. Stir carefully.
3. After 20 minutes, add further 2 drops and stir carefully.
4. Induction time 1–3 hours.
5. Further additions of narcotic may be necessary.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	ROTIFERA
Narcotic used:	CHLORETONE

Method:

1. Make up a 0.1% solution of chloretone in sea-water.
2. Add this to a small watch glass containing a drop of water with rotifers.
3. Induction time variable 5–30 minutes.
4. A solution of 0.05% may sometimes be necessary for certain species.

Reference(s):

von Randolph (1900)

Pantin (1962)

Hale (1958)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	ROTIFERA
Narcotic used:	PHYSOSTIGMINUM SALICYLICUM

Method:

1. Make up a 0.005% solution of physostigminum salicylicum in sea-water.
2. Immerse rotifers in this solution.
3. Induction time approx. 25 minutes.

Reference(s):

Gliwicz (1968)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	ROTIFERA
Narcotic used:	PROCAINE and MENTHOL

Method:

1. Place rotifers in a concave watch glass in approx. 3 ml. of sea-water.
2. Make up a solution of 5% procaine in 50% methanol.
3. Add 3 or 4 drops every 5 minutes until all the rotifers lie on the bottom of the watch glass, and ciliary action has stopped.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	GASTROTRICHA
Narcotic used:	BENZAMINE HYDROCHLORIDE

Method:

1. Make up a 1% solution of benzamine hydrochloride.
2. Pipette a few drops of this solution into the small vessel containing the animals.
3. Induction time is few seconds only.

Reference(s):

Zinn & Kneeland (1966)

User's Notes:

Instituut voor Zeewetenschappelijk onderzoek
Institute for Marine Scientific Research
Prinses Elisabethlaan 69
8401 Bredene - Belgium - Tel. 059 / 80 37 15

Phylum:	ASCHELMINTHES
Method for:	GASTROTRICHA
Narcotic used:	BENZAMINE LACTATE

Method:

1. Make up a 2% solution of benzamine lactate.
2. Add one or two drops of this to a small vessel (e.g. watch-glass) containing the specimens.
3. Observe under microscope. Add further drop after 20 minutes if narcotisation has not taken place.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	GASTROTRICHA
Narcotic used:	CHLORETONE

Method:

1. Place specimens in a few ml. of water in a watchglass.
2. Add a 0.1% solution of chloretone drop by drop until motion and ciliary action cease.

Reference(s):

Kaplan (1969)
Pantin (1962)
Hale (1958)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	GASTROTRICHA
Narcotic used:	HYDROXYLAMINE HYDROCHLORIDE

Method:

1. Make up a 2% solution of hydroxylamine hydrochloride.
2. Add two drops to the small vessel containing the specimens.
3. Observe under microscope. Add further drops at periodic intervals until animals are narcotised.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	GASTROTRICHA
Narcotic used:	SODIUM ETHYLENEDIAMINETETRA ACETATE (EDTA)

Method:

1. Make up a 0.01M solution of EDTA at pH 7.2. Use at room temperature.
2. Immerse the specimens in this solution.
3. Leave until the specimens no longer move or respond.
4. Progressively dilute the solution to ensure full extension of the animals.
5. Induction time approx. 3 hours.

Reference(s):

Robotti & Lovisolo (1972)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	KINORHYNCHA
Narcotic used:	PROPYLENE PHENOXETOL

Method:

1. Make up a stock solution of 1.5% propylene phenoxetol in warm tap water.
2. Mix one part of stock solution with ten parts of sea-water. Soak sample in this mixture to anaesthetise.

Reference(s):

McKay & Hartzband (1970)

Hulings & Gray (1971)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	NEMATODA
Narcotic used:	CHLOROFORM

Method:

1. Put specimens in shallow dish with water.
2. Put chloroform in a wide-mouthed container and place next to dish containing nematodes.
3. Cover both with bell-jar and place in fume cupboard.
4. Induction time variable: 2–12 hours.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum: ASCHELMINTHES

Method for: NEMATODA

Narcotic used: DICHLORO-ETHYL ETHER

Method:

1. Put about 50 ml. of water into a small, stoppered bottle.
2. Add two drops of dichloro-ethyl ether. Shake well and allow to clear.
3. Place nematodes in this solution.
4. Nematodes will recover if placed into fresh water.

Reference(s):

Goodey (1963)

Pike & Aidley (1972)

Mahoney (1966)

User's Notes:

Phylum: ASCHELMINTHES

Method for: NEMATODA

Narcotic used: HEAT

Method:

1. Subject the sample containing nematodes to gradual increase in temperature from electric bulb or radiator.
2. Bring temperature up to approx. 30°C.
3. Paralysis results. Not suitable for work involving histological examination.
4. Alternatively, heat rapidly to 60–70°C, but remove from heat as soon as paralysis occurs.

Reference(s):

Kaplan (1969)

Pantin (1962)

Hulings & Gray (1971)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	NEMATODA
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Allow specimens to relax in a shallow dish of water.
2. Gradually add crystals of $MgSO_4$ until a 20% solution is obtained.
3. Alternatively, plunge the animals into a saturated solution of $MgSO_4$.

Reference(s):

Kaplan (1969)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	NEMATODA
Narcotic used:	MENTHOL

Method:

1. Place animals in a petri-dish with sea-water or saline solution.
2. Sprinkle a few crystals of menthol on the surface of the water.
3. Cover the dish.
4. Induction time variable: 2–12 hours.

Reference(s):

Abdel-Malek (1951)

Hale (1958)

User's Notes:

Phylum:	ASCHELMINTHES
Method for:	NEMATODA : Meiofaunal forms
Narcotic used:	PROPYLENE PHENOXETOL

Method:

1. Make up a stock solution of 1.5% propylene phenoxetol in warm tap water.
2. Mix one part of stock solution to ten parts of sea-water.
3. Immerse sample in this solution.
4. Induction time variable.

Reference(s):

McKay & Hartzband (1970)

Hulings & Gray (1971)

User's Notes:

Phylum:	PRIAPULIDA
Method for:	
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Make up a 7½% solution of $MgCl_2$ in sea-water.
2. Immerse specimens in this solution.
3. Induction time variable, 2–8 hours.

Reference(s):

Ledingham & Wells (1942)

Mahoney (1966)

Hale (1958)

User's Notes:

Phylum: SIPUNCULA

Method for:

Narcotic used: ETHANOL

Method:

1. Place specimen in dish of sea-water. Allow to relax and keep water cool.
2. Slowly add drops of 10% ethanol to the sea-water until there is a solution of 50:50 water:ethanol.
3. Induction time variable, dependent on size: 10–16 hours.
4. Fix sipuncula when introvert is extended and not withdrawn on touch.

Reference(s):

Gibbs (1977)

Lo Bianco (1899)

Baylis & Monro (1941)

Stephen & Edmonds (1972)

User's Notes:

Phylum: SIPUNCULA and ECHIURA

Method for:

Narcotic used: MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$)

Method:

1. Place specimens in glass dish with enough sea-water to cover them. Allow to relax.
2. Add crystals of $MgCl_2$ gradually, over 2 hour period, to make a solution of approx. 7½% in the sea-water.
3. Fix sipuncula when introvert is extended and not withdrawn on touch.
4. Alternatively, place specimens directly into 7½% $MgCl_2$ solution made up in sea-water. Induction period variable 1–6 hours.

Reference(s):

Mahoney (1966)

Stephen & Edmonds (1972)

User's Notes:

Phylum: SIPUNCULA and ECHIURA

Method for:

Narcotic used: MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Place specimens in dish with sea-water. Allow to relax.
2. Gradually add $MgSO_4$ to sea-water over period of 3–6 hours, until a 20% solution in sea-water is formed.
3. Fix sipuncula when introvert is extended and not withdrawn on touch.
4. Alternatively, echiuroids may be narcotised by immersing for 24 hours in a 0.17M solution of $MgSO_4$ in sea-water.

Reference(s):

Wagstaffe & Fidler (1955)

Fujii & Isaka (1957)

Kaplan (1969)

User's Notes:

Phylum: SIPUNCULA and ECHIURA

Method for:

Narcotic used: PROPYLENE PHENOXETOL

Method:

1. Make up a 1% solution of propylene phenoxetol in sea-water.
2. Immerse specimens in this solution.
3. Induction time 2–12 hours depending on size.
4. Fix sipuncula when introvert is extended and not withdrawn on touch.

Reference(s):

Stephen & Edmonds (1972)

User's Notes:

Phylum:	MOLLUSCA
Method for:	POLYPLACOPHORA : Chitons
Narcotic used:	CLOVE OIL

Method:

1. Place specimens in dish and add enough sea-water to cover them.
2. Add a few drops of clove oil to the water.
3. Observe animals, and add more clove oil after 15 minutes, if necessary.
4. Induction time 30 minutes – 1 hour.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA : General
Narcotic used:	ETHANOL

Method:

1. Place animals in a dish with enough sea-water to cover them.
2. Gradually add 10% ethanol to the sea-water, avoiding contact with the animals.
3. Test touch-reaction of the foot before fixing.

Reference(s):

Lo Bianco (1899)

Baylis & Monro (1941)

Mahoney (1966)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA : General
Narcotic used:	MENTHOL

Method:

1. Place animals in dish with enough sea-water to cover them. Allow to relax.
2. Sprinkle menthol crystals on the surface of the water. Use sparingly.
3. Cover dish. Observe periodically and fix when foot does not retract on touching.
4. This method is variable in effect.
5. Induction time 8–24 hours.

Reference(s):

Wagstaffe & Fidler (1955)

Runham et al. (1965)

Mahoney (1966)

Kaplan (1969)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA
Narcotic used:	NEMBUTAL (Sodium pentabarbitone) & MENTHOL

Method:

1. Make up a stock solution of nembutal containing 60 mgm. per ml.
2. Place animals in sea-water and add 1 ml. of stock solution per 150 ml. sea-water. Leave at room temperature for 1¼ – 1½ hours. Prevent snails from clinging to the dish or to each other.
3. Add powdered menthol to water surface. Put in refrigerator for 16–18 hours. Cover dish.
4. Test touch response of foot before fixation.
5. Method designed for freshwater gastropods but may have marine applications.

Reference(s):

McCraw (1958)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA : Muricids
Narcotic used:	SEVIN (1-naphthyl N-methyl-carbamate) and CARBON DIOXIDE

Method:

1. Immerse animals in 10 p.p.m. of Sevin and filtered sea-water in a depth at least three times the height of the animals. Allow to extend at room temperature for 1 hour. Keep snails on their backs and out of reach from each other.
2. Transfer snails to fresh Sevin solution in one atmosphere of CO_2 at room temperature. Leave for 3 hours.
3. Remove from narcotic one at a time and place the ventral or lateral surface of the foot quickly against the surface of a block of dry ice held in a deep freeze. Cover with chipped dry ice, insulate and leave for 24 hours.
4. Thaw in fixative.

Reference(s):

Carriker & Blake (1959)

Kaplan (1969)

Runham et al. (1965)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA : *Gibbula* sp.
Narcotic used:	SODA-WATER

Method:

1. Fill a soda-siphon with sea-water and inject CO_2 bulb.
2. Place animals in a dish and cover with sea-water. Allow to relax.
3. Gradually add soda-water until it makes up approx. 60% of the whole volume.
4. Induction time 30 minutes – 1 hour. Test touch response of foot before fixing.

Reference(s):

Smaldon (1978)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA : *Patella* sp.
Narcotic used:	SODA-WATER

Method:

1. Fill a soda-siphon with sea-water and inject CO_2 bulb.
2. Place animals on their backs, foot upwards, in a dish and cover with sea-water.
3. Gradually add soda-water until it makes up approx. 45% of the total volume.
4. Test touch-response of foot and ensure pallial tentacles are visible before fixation.
5. Induction time 30 minutes – 1 hour.

Reference(s):

Smaldon (1978)

User's Notes:

Phylum:	MOLLUSCA
Method for:	VELIGER LARVAE (*Patella*)
Narcotic used:	GLUTARALDEHYDE

Method:

1. Make up a 2% solution of glutaraldehyde from the stock solution, using sea-water.
2. Place veligers in sea-water in a small crystallising dish.
3. Observe veligers under binocular microscope. Add drop of 2% glutaraldehyde. Observe, add second or third drop if necessary. When fully relaxed, transfer to fixative.
4. Induction time 2–3 minutes.

Reference(s):

P.R. Smaldon (Pers.comm.)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: OPISTHO-BRANCHIA: Nudibranchia
Narcotic used:	CHILLING

Method:

1. Place animals in a dish and cover with sea-water. Allow to expand.
2. Place in a refrigerator until the water becomes cold.
3. Transfer to a deep-freeze and leave to freeze.
4. Remove, and allow as much ice as possible to melt without exposing the animals.
5. Place animals and remaining ice in fixative and allow to thaw.

Reference(s):

Gohar (1937)

Hanna (1955)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: OPISTHO-BRANCHIA: *Bulla* and *Scaphander*
Narcotic used:	DILUTE SEA-WATER

Method:

1. Place animals in dish of sea-water.
2. Add tap-water until original volume of sea-water is doubled. Dilute gradually over period of 1 hour.
3. Leave in this water until ready for fixation.

Reference(s):

Lo Bianco (1899)

Runham et al. (1965)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: OPISTHO-BRANCHIA: *Doris* sp.
Narcotic used:	ETHANOL

Method:

1. Place animals in dish and cover with sea-water. Allow to expand.
2. Add 70% ethanol a little at a time to the water. Test touch-reaction before fixation.
3. Induction time variable, normally 1–5 hours.

Reference(s):

Lo Bianco (1899)

Mahoney (1966)

Baylis & Monro (1941)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: OPISTHO-BRANCHIA: small specimens
Narcotic used:	EUCAINE (Benzamine hydrochloride)

Method:

1. Place small opisthobranchs in a shallow glass tray filled with fresh sea-water. Cover with a black cloth.
2. Leave for 1 hour. The animals usually rise to the surface.
3. Add a few drops of a saturated solution of eucaine, and leave for 30 minutes to 1 hour.
4. Menthol can be used if eucaine is not available.

Reference(s):

Smith (1961)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: OPISTHO-BRANCHIA: Nudibranchia
Narcotic used:	FORMALIN

Method:

1. Make up a solution of 1% formalin in sea-water.
2. Place animals in dish with at least 50 times their volume of water. Allow to expand.
3. Add 3 drops of the formalin per 100 ml. of sea-water.
4. Repeat at 15 minute intervals. The amount of formalin may be doubled every hour.
5. Induction time 3–5 hours.

Reference(s):

Gohar (1937)

Runham et al. (1965)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: OPISTHO-BRANCHIA: Nudibranchia
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Make up a solution of 7½% $MgCl_2$ in sea-water.
2. Immerse the animals in this solution.
3. Observe periodically to avoid maceration.
4. Fix when no touch-response.
5. Induction time 1–4 hours.

Reference(s):

Ledingham & Wells (1942)

Hale (1958)

Pantin (1962)

Kaplan (1969)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: OPISTHOBRANCHIA
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Place animals in a dish with plenty of clean sea-water.
2. Add crystals of $MgSO_4$ to the corner of the dish farthest away from the animals.
3. Alternatively, a muslin bag may be packed with $MgSO_4$ (200 grms. per litre of sea-water) and this placed in a corner of the dish.
4. Induction time varies with species, but normally 5–24 hours. Check at intervals to avoid over-narcotisation and subsequent maceration.

Reference(s):

Gohar (1937)

Wagstaffe & Fidler (1955)

Mahoney (1966)

Kaplan (1969)

Spiritosa (1964)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: OPISTHOBRANCHIA: *Limapontia*
Narcotic used:	NEMBUTAL (Sodium pentabarbitone)

Method:

1. Prepare a solution of 0.08% nembutal in sea-water.
2. Immerse the animals in this solution.
3. Induction time approx. 12 hours.

Reference(s):

Runham et al. (1965)

User's Notes:

Phylum:	MOLLUSCA
Method for:	GASTROPODA: Nudibranchia: Eolids
Narcotic used:	STOVAINE (Amylocaine hydrochloride)

Method:

1. Place animals in a dish of clean sea-water. Allow to expand.
2. Gradually add 1% solution of stovaine to the water (via burette).
3. Induction time varies, 1–3 hours.

Reference(s):

Smith (1961)

Kaplan (1969)

User's Notes:

Phylum:	MOLLUSCA
Method for:	SCAPHOPODA: *Dentalium*
Narcotic used:	CHLORAL HYDRATE

Method:

1. Place animals in shallow dish of sea-water.
2. Siphon off sea-water and replace gently with 0.2% solution of chloral hydrate made up in sea-water.
3. Induction time 10–12 hours.
4. Extra crystals of chloral hydrate may be added to the water if effect is not strong enough.
5. For certain species a solution of 5% may be required.

Reference(s):

Lo Bianco (1899)

Pantin (1962)

Kaplan (1969)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	MOLLUSCA
Method for:	LAMELLIBRANCHIA
Narcotic used:	ETHANOL

Method:

1. Place animals in a dish with plenty of sea-water. Allow them to open and start siphoning.
2. Gradually add drops of 70% ethanol to the water (via burette).
3. Continue until valves remain open.
4. Induction time 6–12 hours, depending on species.

Reference(s):

Lo Bianco (1899)

Mahoney (1966)

User's Notes:

Phylum:	MOLLUSCA
Method for:	LAMELLIBRANCHIA: *Mytilus*
Narcotic used:	ETHYL URETHANE

Method:

1. Place mussels in a dish and add sufficient sea-water to cover them. Allow valves to open.
2. Gradually siphon off sea-water and replace with 1% solution of ethyl urethane in sea-water.
3. Induction time 12–20 hours.

Reference(s):

Krogh (1914)

Pantin (1962)

User's Notes:

Phylum:	MOLLUSCA
Method for:	LAMELLIBRANCHIA
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Prepare a solution of 7½% $MgCl_2$ in sea-water.
2. Immerse specimens in this solution.
3. Check touch-response periodically.
4. Do not over-narcotise, since some species close the valves permanently.
5. Fix when no reaction to touch.
6. Induction period and reaction to this method varies between species.

Reference(s):

Pantin (1962)

User's Notes:

Phylum:	MOLLUSCA
Method for:	LAMELLIBRANCHIA
Narcotic used:	PROPYLENE PHENOXETOL

Method:

1. Place specimens in deep container and cover with 10–15 cm. of sea-water. Allow valves to open and animals to commence siphoning.
2. Shake 5 ml. of propylene phenoxetol with 20 ml. of sea-water to produce a fine emulsion.
3. Add this emulsion to the jar containing the actively-siphoning animals.
4. Alternatively, add the propylene phenoxetol directly to the jar and allow it to collect as a large globule.
5. Induction time approx. 30 minutes.

Reference(s):

Owen (1955)

McKay & Hartzband (1970)

Kaplan (1969)

User's Notes:

Phylum:	MOLLUSCA
Method for:	LAMELLIBRANCHIA: *Cardium* sp.
Narcotic used:	SODA-WATER

Method:

1. Fill a soda-siphon with sea-water and inject CO_2 bulb.
2. Place animals in dish and cover with sea-water. Allow valves to open and siphoning to commence.
3. Squirt soda-water into a beaker and gently add to the dish containing animals. Try not to disturb animals.
4. Repeat at 5 minute intervals until the soda-water is approx. 75% of the whole volume.
5. Induction time is approx. 1 hour.

Reference(s):

Smaldon (1978)

User's Notes:

Phylum:	MOLLUSCA
Method for:	VELIGER LARVAE
Narcotic used:	CHLORETONE

Method:

1. Make up a solution of one part saturated aqueous solution of chloretone to two parts sea-water.
2. Immerse veligers in this solution.
3. Induction time 3–4 minutes.
4. Prolonged exposure will lead to break-up and damage.

Reference(s):

Clement & Cather (1957)

User's Notes:

Phylum: MOLLUSCA

Method for: CEPHALOPODA

Narcotic used: CHLORETONE

Method:

1. Place animals in plenty of sea-water in semi-darkness.
2. Add crystals of chloretone to produce a solution of at least 0.05%.
3. Test touch-response of tentacles after 30 minutes. Add more chloretone if necessary.

Reference(s):

Kaplan (1969)

Pantin (1962)

Hale (1958)

User's Notes:

Phylum: ANNELIDA

Method for: POLYCHAETA

Narcotic used: CHLOROFORM

Method:

1. Place animals in dish of sea-water. Allow to relax.
2. Spray small amounts of chloroform onto the surface of the water containing the animals. Cover the container.
3. Alternatively, put some chloroform in another container and place this, plus the container with worms, under a bell-jar.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	ANNELIDA
Method for:	POLYCHAETA
Narcotic used:	ETHANOL

Method:

1. Place specimens in dish with plenty of sea-water.
2. Add 5% ethanol gradually to the sea-water (use burette).
3. Do not disturb animals if possible. Continue gradually adding ethanol at 15 minute intervals until worms cease to move and do not respond to touch. Double strength of alcohol after first hour.
4. Induction time variable, 2–12 hours.

Reference(s):

Lo Bianco (1899)

Baylis & Monro (1941)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	ANNELIDA
Method for:	POLYCHAETA
Narcotic used:	GLYCERINE/ETHANOL/ SEA-WATER

Method:

1. Make up a solution of the following :

   1 part Glycerine
   2 parts 70% Ethanol
   2 parts sea-water
2. Place animals in a dish of sea-water and allow to relax.
3. Very gradually pour a stratum of the above mixture over the surface of the sea-water. This stratum will slowly diffuse through the water, and the animals will become narcotised slowly.
4. Induction time of several hours.
5. Animals best transferred to ethanol to fix.

Reference(s):

Lo Bianco (1899)

User's Notes:

Phylum:	ANNELIDA
Method for:	POLYCHAETA
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Make up a solution of 7½% $MgCl_2$ in sea-water.
2. Immerse specimens in this solution.
3. May not be very effective for serpulid species.
4. Induction time 1–4 hours.

Reference(s):

Ledingham & Wells (1942)

Hale (1958)

Pantin (1962)

User's Notes:

Phylum:	ANNELIDA
Method for:	POLYCHAETA
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Place specimens in a dish with plenty of sea-water. Allow to relax.
2. Place magnesium sulphate crystals (approx. 200 grm./litre sea-water) in a muslin bag and suspend bag over dish. Allow tip of bag to dip into sea-water. Streams of sulphate solution will descend. Do not disturb animals.
3. Test touch-response periodically.
4. Alternatively, add crystals of $MgSO_4$ gradually to water.

Reference(s):

Griffin (1910)

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum: ANNELIDA

Method for: POLYCHAETA: small specimens

Narcotic used: PROPYLENE PHENOXETOL

Method:

1. Make up a stock solution of 1.5% propylene phenoxetol in warm tap-water.
2. Narcotise animals in a solution of 1 part stock solution to 10 parts sea-water.
3. Induction time variable.

Reference(s):

McKay & Hartzband (1970)

User's Notes:

Phylum: ANNELIDA

Method for: OLIGOCHAETA: small specimens

Narcotic used: CHLOROFORM

Method:

1. Place specimens in small dish of sea-water.
2. Add a few drops of chloroform to the water.
3. Fix when movement ceases.

Reference(s):

Mahoney (1966)

User's Notes:

Phylum:	ANNELIDA
Method for:	OLIGOCHAETA
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Make up a solution of 7½% $MgCl_2$ in sea-water.
2. Immerse specimens in this solution.
3. Induction time variable: 1–8 hours.

Reference(s):

Pantin (1962)

Hale (1958)

Kaplan (1969)

User's Notes:

Phylum:	ANNELIDA
Method for:	OLIGOCHAETA: small specimens
Narcotic used:	NICOTINE

Method:

1. Soak pipe tobacco overnight in sea-water (water : tobacco ratio of 1:4). Filter the mixture and retain filtrate.
2. Individual worms may be placed on a microscope slide in a drop of water. Add small quantities of the nicotine filtrate via a pipette.
3. Observe the narcotisation process under the microscope. Fix when any movement ceases. Induction time normally few minutes.
4. Increase amounts of water and narcotic for larger samples.

Reference(s):

Murchie (1955)

Kaplan (1969)

User's Notes:

Phylum:	ANNELIDA
Method for:	OLIGOCHAETA: Meiofaunal species
Narcotic used:	PROPYLENE PHENOXETOL

Method:

1. Make up a stock solution of 1.5% propylene phenoxetol in warm tap water.
2. Mix one part stock solution to ten parts sea-water.
3. Immerse sample in above mixture to anaesthetise.

Reference(s):

McKay & Hartzband (1970)

User's Notes:

Phylum:	ANNELIDA
Method for:	OLIGOCHAETA: Meiofaunal species
Narcotic used:	TEMPERATURE INCREASE

Method:

1. Place specimens in small dish of sea-water.
2. Heat the water rapidly to a temperature of 60–70°C.
3. Observe specimens. Fix as soon as movement ceases, otherwise some fragmentation may occur.

Reference(s):

Hulings & Gray (1971)

User's Notes:

Phylum:	ANNELIDA
Method for:	HIRUDINEA
Narcotic used:	CHLORAL HYDRATE

Method:

1. Place specimens in dish of sea-water with sufficient water to cover them.
2. Over a period of 2–3 hours, gradually add crystals of chloral hydrate to the sea-water.
3. Test touch-response periodically.

Reference(s):

Wagstaffe & Fidler (1955)

Hale (1958)

Pantin (1962)

Kaplan (1969)

User's Notes:

Phylum:	ANNELIDA
Method for:	HIRUDINEA
Narcotic used:	CHLOROFORM or DIETHYL ETHER

Method:

1. Allow animals to expand in dish of sea-water.
2. Place chloroform or ether in dish and place this dish and dish with animals under a bell-jar.

Reference(s):

Kaplan (1969)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	ANNELIDA
Method for:	HIRUDINEA
Narcotic used:	ETHANOL

Method:

1. Place animals in dish of sea-water, and allow to expand.
2. Add small quantities of 10% ethanol until animals are insensitive to touch.
3. Straighten and fix.

Reference(s):

Baylis & Monro (1941)

User's Notes:

Phylum:	ANNELIDA
Method for:	HIRUDINEA
Narcotic used:	ETHYL URETHANE

Method:

1. Place specimens in a dish of sea-water.
2. Allow to relax.
3. Siphon off sea-water and replace with 1% solution of ethyl urethane in sea-water.
4. Induction time varies depending on species. Normally 1–6 hours.

Reference(s):

Krogh (1914)

Pantin (1962)

Hale (1958)

User's Notes:

Phylum: ANNELIDA

Method for: HIRUDINEA

Narcotic used: LEMON JUICE

Method:

1. Allow animals to relax in a dish with a little water.
2. Slowly and carefully add lemon juice to the water at 5-minute intervals.
3. Continue until touch-response invokes no reaction. Straighten and lay flat before fixation.
4. Lemon juice must be fresh. PLJ is effective.

Reference(s):

Baylis & Monro (1941)

User's Notes:

Phylum: ANNELIDA

Method for: HIRUDINEA

Narcotic used: MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Place specimens in a dish of sea-water and allow to relax.
2. Make up a saturated solution of $MgCl_2$ in sea-water.
3. Add this solution slowly to the sea-water containing the animals (use burette).
4. Test touch-response periodically.

Reference(s):

Wagstaffe & Fidler (1955)

Hale (1958)

Mahoney (1966)

Pantin (1962)

Kaplan (1969)

User's Notes:

Phylum:	ANNELIDA
Method for:	HIRUDINEA
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Place specimens in dish with sufficient sea-water to cover them. Allow to relax.
2. Over a period of several hours, add small amounts of a 25% $MgSO_4$ solution.
3. If narcotic is added too quickly, the animals may contract and be of little use.

Reference(s):

Baylis & Monro (1941)

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum:	ANNELIDA
Method for:	HIRUDINEA
Narcotic used:	MENTHOL

Method:

1. Place specimens in a small dish and cover with sea-water. Allow to relax.
2. Sprinkle powdered menthol on the surface of the sea-water in excess of the amount needed for a saturated solution.
3. Induction time variable, dependent on species and on temperature.

Reference(s):

Abdel-Malek (1951)

User's Notes:

Phylum:	ANNELIDA
Method for:	HIRUDINEA
Narcotic used:	NICOTINE

Method:

1. Put leeches in minimum amount of water. Allow to relax.
2. Add a few shreds of tobacco.
3. Increase amount after 1 hour if no effects evident.
4. Test touch-response before fixation.
5. Induction time 30–60 minutes.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	ANNELIDA
Method for:	HIRUDINEA
Narcotic used:	SODA-WATER

Method:

1. Fill a soda-siphon with sea-water and inject CO_2 bulb.
2. Place animals in dish of sea-water and allow to expand.
3. Gradually add soda-water to the dish. Continue at intervals until animals insensitive to touch.
4. Levels of 60–70% soda-water may be necessary.
5. Induction time 30 minutes – 1 hour.

Reference(s):

Baylis & Monro (1941)

Wagstaffe & Fidler (1955)

Kaplan (1969)

Pantin (1962)

User's Notes:

Phylum:	TARDIGRADA
Method for:	
Narcotic used:	EDTA (Sodium ethylene-diaminetetra-acetate)

Method:

1. Prepare a 0.01 M solution of EDTA at pH 7.2.
2. Add to sample containing tardigrades.
3. Leave for three hours, then dilute slowly to bring about full extension of tardigrades.
4. Alternatively, a saturated aqueous solution of sodium pyrophosphate ($Na_4P_2O_7$) at pH 9 may be used in the same manner.

Reference(s):

Robotti & Lovisolo (1972)

User's Notes:

Phylum:	TARDIGRADA
Method for:	MARINE SPECIES
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$)

Method:

1. Make up a solution of 10% $MgCl_2$ in sea-water.
2. Add this solution to the sand sample and allow to stand for 10 minutes.
3. Agitate the container and swirl the fluid rapidly to force the tardigrades into suspension, then filter.
4. If material is to be used for permanent or semi-permanent preparations, keep first in deoxygenated sea-water to asphyxiate.

Reference(s):

Morgan & King (1976)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: BRANCHIOPODA: Cladocera
Narcotic used:	CHLOROFORM

Method:

1. Place animals in small dish of sea-water.
2. Add four drops of chloroform to each 25 ml. of sea-water containing animals.
3. Induction time of 2–3 minutes.
4. Method originally devised for fresh-water cladocera, but probably acceptable for marine forms.

Reference(s):

Gannon & Gannon (1975)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: BRANCHIOPODA
Narcotic used:	ETHANOL

Method:

1. Allow animals to relax in dish of sea-water.
2. Gradually add drops of 10% ethanol.
3. Do not allow amount of ethanol to form more than one-tenth of the total volume.
4. Fix when animals become quiescent.

Reference(s):

Baylis & Monro (1941)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: BRANCHIOPODA: Cladocera
Narcotic used:	ETHYL URETHANE

Method:

1. Place specimens in dish of sea-water and allow to relax.
2. Remove sea-water slowly and replace with 1% solution of ethyl urethane made up in sea-water.
3. Induction time 30 minutes – 2 hours, but variable.

Reference(s):

Krogh (1914)

Pantin (1962)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Ostracoda
Narcotic used:	CHLORETONE

Method:

1. Make up a solution of 0.05% chloretone in sea-water.
2. Immerse specimens in this solution.
3. Induction time variable, but normally several hours.
4. Specimens may become more transparent than usual.

Reference(s):

von Randolph (1900)

Pantin (1962)

Hale (1958)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: COPEPODA: Cyclopoida
Narcotic used:	CHILLING

Method:

1. Place specimens in a small dish of sea-water.
2. Place this dish in a larger dish containing crushed ice and allow sea-water to cool.
3. Alternatively, place dish with specimens in refrigerator for 24 hours.
4. Originally devised for fresh-water cyclopoids, but can be used with marine species if temperatures are reduced to 1 or 2°C.

Reference(s):

Onabamiro (1950)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: COPEPODA
Narcotic used:	ETHANOL

Method:

1. Place specimens in a small dish of sea-water.
2. Add a few drops of 10% ethanol at 2 minute intervals.
3. Monitor activity under binocular microscope and fix when immobile.
4. Induction time approx. 10 minutes, but depends on size of sample.

Reference(s):

Onabamiro (1950)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: MEIOFAUNAL COPEPODA
Narcotic used:	PROPYLENE PHENOXETOL

Method:

1. Make up a stock solution of 1.5% propylene phenoxetol in warm tap water. (Shake).
2. Mix one part of this stock solution to ten parts of sea-water and pour over sample.
3. Induction time up to 30 minutes.

Reference(s):

McKay & Hartzband (1970)

Hulings & Gray (1971)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: COPEPODA
Narcotic used:	SODA-WATER

Method:

1. Fill a soda-siphon with sea-water and inject CO_2 bulb.
2. Squirt soda-water into sea-water containing copepods (usually a plankton sample). Leave for 1 or 2 minutes then add more soda-water.
3. Add until animals are immobile (normally 2–5 minutes).
4. Prevents dropping of egg sacs by females, and voiding of gut contents.

Reference(s):

Gannon & Gannon (1975)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: CIRRIPEDIA
Narcotic used:	MAGNESIUM CHLORIDE / MENTHOL

Method:

1. Make up a 7½% solution of $MgCl_2 6H_2O$ in sea-water.
2. Immerse specimens in this solution.
3. Induction time variable, 1–8 hours.
4. Alternatively, place specimens in sea-water in a dish. Allow to expand and add menthol crystals to the surface of the water. Use sparingly. Cover dish. Induction time 3–8 Hours.

Reference(s):

Southward & Crisp (1963)

Pantin (1962)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Isopoda (*Idotea*)
Narcotic used:	ETHYL URETHANE

Method:

1. Make up a solution of 1% ethyl urethane in sea-water.
2. Immerse the animals in this solution.
3. Induction time variable: 6–12 hours.

Reference(s):

Krogh (1914)

Pantin (1962)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Amphipoda (*Corophium & Marinogammarus*)
Narcotic used:	TRICAINE METHANE SULPHONATE (MS222)

Method:

1. Place specimens in container with approx. 500 ml. of sea-water.
2. Add MS 222 to the sea-water until its concentration is 0.5 grm./litre.
3. Aerate and maintain at temperature of 10°C.
4. Gravid females may liberate young from the brood pouches prematurely. These young will recover if placed in fresh sea-water.
5. Induction time approx. 30 minutes.

Reference(s):

Gamble (1969)

Ahmad (1969)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Decapoda
Narcotic used:	CHLOROFORM

Method:

1. Inject a quantity of chloroform into the body cavity at the base of a limb. A large *Carcinus* requires approx. 5 ml. of chloroform.
2. Alternatively, expose the crab to chloroform vapour, or place some chloroform in the water containing the crab and agitate to prevent it settling on the bottom of the container.

Reference(s):

Baker (1955)

Gohar (1937)

Mahoney (1966)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Decapoda (*Carcinus & Portunus*)
Narcotic used:	ETHANE DISULPHONATE

Method:

1. Make up a solution of 2.5 grm./litre ethane disulphonate in sea-water for *Carcinus*. Immerse animal in this solution. Induction time of approx. 5 minutes.
2. For *Portunus*, make up a solution of 0.9 grm./litre. Induction time approx. 15 minutes.

Reference(s):

Ehrhardt, Wissocq & Niaussat (1970)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Decapoda
Narcotic used:	FRESH WATER

Method:

1. Place specimens into fresh water from tap or similar source.
2. Allow to remain in fresh water until death; remove before appendages cast off.
3. Induction time variable.
4. Method may cause osmotic distortion of tissues and should not be used if subsequent histological work is envisaged.
5. Not suitable for euryhaline species.

Reference(s):

Lo Bianco (1899)

* See also Baker (1955)
Gunter (1961)
and Baylis & Monro (1941)
for comment.

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Decapoda
Narcotic used:	HEAT

Method:

1. Place animal in dish of fresh sea-water.
2. Slowly raise the temperature to 40°C.
3. Monitor carefully. The animal should become limp.

Reference(s):

Gunter (1961)

* For debate over this and other methods see:
Baker (1962)
Schmidt-Nielson (1962)
Bernarde (1962)
and Gunter (1962)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Decapoda (Lobsters)
Narcotic used:	ISOBUTYL ALCOHOL

Method:

1. Place animal in large tank of sea-water.
2. Add isobutyl alcohol to the water in the amount of 1.5–7 ml. per litre.
3. Induction time normally 5–20 minutes.

Reference(s):

Foley, Stewart & Holley (1966)

Kaplan (1969)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Decapoda (Lobsters)
Narcotic used:	METHYL PENTYNOL

Method:

1. Fill a large tank with sea-water and add the experimental animal.
2. Add methyl pentynol to the water in the amount of 5 ml. per litre.
3. Induction time 5–20 minutes.
4. Slightly higher concentrations may be necessary in some cases.

Reference(s):

Foley, Stewart & Holley (1966)

Kaplan (1969)

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Decapoda (*Carcinus & Cancer*)
Narcotic used:	PROCAINE HYDROCHLORIDE 3%

Method:

1. Procaine hydrochloride 3% at 25 mg. per kg. is injected using 25 s.w.g. needles inserted through the coxal arthrodial membrane of a convenient posterior limb whilst restraining the crab manually.
2. Return crab to sea-water after injection.
3. Full induction time of 4 minutes, following initial period of tonic contraction of all appendages.

Reference(s):

Oswald (1977)

* See this reference for use of other specialised methods.

User's Notes:

Phylum:	ARTHROPODA
Method for:	CRUSTACEA: Decapoda (*Crangon* and *Palaemon*)
Narcotic used:	SODA-WATER

Method:

1. Fill a soda siphon with sea-water and inject the CO_2 bulb.
2. Gradually add this soda-water to the water containing the animals until there is approx. 45% soda-water. Cover dish to prevent animals jumping out.
3. Induction time 15–20 minutes.

Reference(s):

Smaldon (1978)

User's Notes:

Phylum:	PHORONIDA
Method for:	
Narcotic used:	ETHYL ALCOHOL (ETHANOL)

Method:

1. Allow animals to expand in a bowl of sea-water. Do not disturb.
2. Add a few drops of 70% ethanol every 15 minutes, avoiding any disturbance to the polyps.
3. Induction time 3–4 hours, sometimes less.

Reference(s):

Kaplan (1969)

Lo Bianco (1899)

User's Notes:

Phylum:	PHORONIDA
Method for:	
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Make up a 7½% solution of $MgCl_2$ in sea-water.
2. Place specimens in a bowl of sea-water and allow to expand. Do not disturb.
3. Carefully siphon off most of the sea-water and gently replace with $MgCl_2$ solution.
4. Induction time variable: 1–6 hours.
5. Not all polyps will expand. Test touch with fine needle.

Reference(s):

Mahoney (1966)

Hale (1958)

User's Notes:

Phylum:	BRACHIOPODA
Method for:	
Narcotic used:	ETHYL ALCOHOL (ETHANOL)

Method:

1. Place specimens in a dish of sea-water. Allow valves to open.
2. Add 10% ethyl alcohol, a few drops at a time, each 5 minutes or so.
3. Induction time 6–12 hours, occasionally less.

Reference(s):

Kaplan (1969)

Lo Bianco (1899)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: ACETONE

Method:

1. Place colonies in a bowl of clean sea-water. Allow polyps to extend. Avoid disturbance.
2. Over a period of several hours, add acetone to the water drop by drop (burette).
3. Induction time 6–8 hours.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: CHLORAL HYDRATE

Method:

1. Place animals in bowl of clean sea-water.
2. Make up a saturated solution of chloral hydrate in sea-water.
3. Add drop by drop over period of 1–3 hours.
4. Test touch-response of tentacles with fine needle.
5. Alternatively, gradually add a few crystals to the water.

Reference(s):

Lo Bianco (1899)

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: CHLORETONE

Method:

1. Place animals in a bowl of clean sea-water.
2. Allow polyps to extend. Avoid disturbance.
3. Make up a small quantity of saturated solution of chloretone in sea-water.
4. Add to specimen dish drop by drop over period of 1–3 hours.
5. Test touch-response of polyps with fine needle.

Reference(s):

Kaplan (1969)

Pantin (1962)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: CLOVE OIL

Method:

1. Place specimens in a small bowl of clean sea-water.
2. Allow polyps to extend.
3. Add a few drops of clove oil to the water each hour for 3–4 hours. Avoid disturbance.
4. Induction time 3–4 hours.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum:	BRYOZOA
Method:	*Flustra, Cellepora, Crisia, Bugula*
Narcotic used:	ETHYL ALCOHOL (ETHANOL)

Method:

1. Place specimens in a small bowl of clean sea-water. Allow polyps to extend.
2. Gradually add drops of 10% ethyl alcohol to the water. Avoid disturbance.
3. Touch tentacles and test response after 1–2 hours.
4. Induction time variable: 1–6 hours.

Reference(s):

Lo Bianco (1899)

User's Notes:

Phylum:	BRYOZOA
Method:	
Narcotic used:	EUCAINE HYDROCHLORIDE

Method:

1. Place specimens in a small bowl of clean sea-water. Allow polyps to extend.
2. Make up a 1% solution of eucaine hydrochloride in sea-water.
3. Run one or two drops into the bowl containing the animals. Repeat after 15 minutes if necessary.
4. Induction time 15–30 minutes.

Reference(s):

Sartory (1936)

Hale (1958)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: HANLEY'S SOLUTION

Method:

1. Place animals in a small bowl of clean sea-water. Allow polyps to extend.
2. Make up Hanley's solution:

   Water : 90 ml.
   Ethyl Cellosolve : 10 ml.
   Eucaine Hydrochloride : 0.3 grm.
3. Add one drop of Hanley's solution per millilitre of sea-water containing animals. Add gradually.
4. Method is most suitable for small colonies.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Place the bryozoa in fingerbowls about $\frac{2}{3}$ full of seawater.
2. Keep away from bright light and allow polyps to extend.
3. Make up a 20% solution of $MgSO_4$ in sea-water.
4. Add this solution gradually to the fingerbowls, avoiding disturbance.
5. Induction time 2–6 hours.

Reference(s):

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: MENTHOL

Method:

1. Place specimens in dish and cover with clean sea-water.
2. Allow polyps to extend. Avoid disturbance.
3. Scatter a few menthol crystals on the surface of the water. Cover the dish, preferably with piece of glass.
4. Observe periodically. Do not over-narcotise. Fix when polyps do not respond to touch. Not all polyps may extend.
5. Induction time variable, 1–12 hours.

Reference(s):

Duncan (1917)

Ryland & Hayward (1977)

Pantin (1962)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: MENTHOL / CHLORAL HYDRATE MIXTURE

Method:

1. Place colonies in a bowl of clean sea-water. Allow polyps to extend.
2. Grind together a mixture of menthol: chloral hydrate 45:55.
3. Sprinkle a few crystals of the mixture on the water surface.
4. Induction time 6–8 hours.

Reference(s):

Kaplan (1969)

User's Notes:

Phylum: BRYOZOA

Method for:

Narcotic used: STOVAINE (AMYLOCAINE HYDROCHLORIDE)

Method:

1. Place animals in clean sea-water.
2. Allow polyps to extend. Avoid disturbance.
3. Gradually add a 1% solution of stovaine to the water or scatter a few crystals of stovaine on the water surface.
4. Induction time variable. Fix when polyps do not respond to touch with fine needle.

Reference(s):

Wagstaffe & Fidler (1955)

Mahoney (1966)

User's Notes:

Phylum: CHAETOGNATHA

Method for:

Narcotic used: MENTHOL

Method:

1. Place specimens in small bowl of clean sea-water.
2. Sprinkle a few crystals of menthol on the water surface and cover the bowl.
3. Observe periodically. Fix when immobile.
4. Induction time 1–4 hours.

Reference(s):

Wagstaffe and Fidler (1955)

Mahoney (1966)

Kaplan (1969)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	LARVAE
Narcotic used:	STOVAINE (AMYLOCAINE HYDROCHLORIDE)

Method:

1. Place animals in a small bowl of clean sea-water.
2. Make up a solution of 1% stovaine in distilled water.
3. Add drops gradually until any ciliary activity ceases.

Reference(s):

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	CRINOIDEA
Narcotic used:	FRESH WATER

Method:

1. Place specimens in a bowl of fresh water until completely narcotised.
2. Remove and fix when narcotised. Distortion occurs if left too long in fresh water.

Reference(s):

Mahoney (1966)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	CRINOID LARVAE
Narcotic used:	CHLORAL HYDRATE

Method:

1. Make up a solution of 0.1% chloral hydrate in sea-water.
2. Immerse specimens in this solution.
3. Induction time 2–4 hours.
4. Alternatively, gradually add crystals of chloral hydrate to a bowl of sea-water containing the animals.

Reference(s):

Lo Bianco (1899)
Wagstaffe & Fidler (1955)
Hale (1958)
Pantin (1962)
Kaplan (1969)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	ECHINOIDEA
Narcotic used:	FRESH WATER

Method:

1. Place specimens in bowl of fresh water.
2. Leave for 15 minutes.
3. Do not leave too long as distortion may occur.
4. Not suitable if subsequent histological examination is envisaged.

Reference(s):

Granger (1889)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	ECHINOIDEA
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Make up a 7½% solution of $MgCl_2$ in sea-water.
2. Place specimens in this solution.
3. Observe periodically. Fix when tube feet are extended and do not retract on touch.
4. Induction time variable: 1–8 hours.

Reference(s):

Pantin (1962)
Fujii & Isaka (1957)
Hale (1958)
Mahoney (1966)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	ECHINOIDEA
Narcotic used:	MENTHOL

Method:

1. Place animals in large bowl of sea-water.
2. Scatter a few menthol crystals on the surface of the water. Cover the bowl.
3. Observe periodically. When tube feet remain extended on touch, fix.
4. Do not leave too long under menthol.
5. Induction time variable: 1–4 hours.

Reference(s):

Mahoney (1966)
Hale (1958)

User's Notes:

Phylum: ECHINODERMATA

Method for: OPHIUROIDEA

Narcotic used: ETHYL ALCOHOL (ETHANOL)

Method:

1. Place animals in a bowl of sea-water. Allow to settle.
2. Gradually add 10% ethyl alcohol to the water over a period of 2 hours. Avoid direct contact between animal and undiluted alcohol.
3. Fix when immobile.

Reference(s):

Mahoney (1966)

User's Notes:

Phylum: ECHINODERMATA

Method for: OPHIUROIDEA

Narcotic used: FORMALIN

Method:

1. Place specimens in a large bowl of fresh sea-water.
2. Make up a 1% solution of formalin in sea-water.
3. Add 3 drops of the formalin solution per 100 ml. of sea-water. Repeat at 15 minute intervals.
4. The amount of formalin may be doubled every hour.

Reference(s):

Gohar (1937)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	OPHIUROIDEA
Narcotic used:	FRESH WATER

Method:

1. Immerse animals in a bowl of fresh water.
2. Leave until immobile and then fix.
3. Do not leave too long, since often animals may cast off their arms.

Reference(s):

Granger (1889)

Lo Bianco (1899)

Mahoney (1966)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	ASTEROIDEA
Narcotic used:	FRESH WATER

Method:

1. Place specimens upside-down in plenty of fresh water.
2. Fix when tube feet are immobile.
3. Do not leave too long in fresh water, or distortion will occur.
4. Not suitable if subsequent histological examination is envisaged.
5. Induction time approx. 5 minutes.

Reference(s):

User's Notes:

Phylum:	ECHINODERMATA
Method for:	ASTEROIDEA
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Place animals in a bowl with plenty of clean sea-water.
2. Over a period of several hours, add crystals of $MgSO_4$ to the sea-water, until a 20% solution is obtained.
3. Induction time variable: 8–24 hours.

Reference(s):

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	ASTEROIDEA
Narcotic used:	SODA-WATER

Method:

1. Allow animals to expand in a considerable volume of sea-water, e.g. in washing-up bowl.
2. Fill a soda-siphon with sea-water and inject the CO_2 bulb.
3. Add this soda-water to the sea-water containing the animals over a period of 10 minutes, to eventually make approx. 50% soda-water.
4. Induction time 15 minutes – 1 hour.

Reference(s):

Wagstaffe & Fidler (1955)

Kaplan (1969)

Pantin (1962)

Smaldon (unpubl.)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	HOLOTHUROIDEA
Narcotic used:	HEAT

Method:

1. The specimen is placed into a plastic bag with the minimum of touching and disturbance.
2. The amount of sea-water in the bag should be approx. twice the volume of the animal.
3. Place the bag plus animal in a sink of cold water (tap-water is suitable).
4. Turn on the hot tap and gradually let the temperature of the water reach 20°C. Over an hour or so allow it to reach 50°C.
5. Fix specimens when fully relaxed. Do not leave too long.

Reference(s):

Kufferath (1963)

Pantin (1962)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	HOLOTHUROIDEA
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_20$)

Method:

1. Place animals in a bowl with plenty of clean sea-water. Allow to extend.
2. Over a period of 3–4 hours, slowly add $MgCl_2$ crystals to the sea-water until there is a solution of approx. 7% $MgCl_2$.
3. Induction period 3–6 hours. Not suitable for large specimens.

Reference(s):

Mahoney (1966)

Pantin (1962)

Hale (1958)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	HOLOTHUROIDEA
Narcotic used:	MAGNESIUM SULPHATE ($MgSO_4.7H_20$)

Method:

1. Place animals in a bowl with plenty of clean sea-water. Allow to extend.
2. Over a period of some hours, introduce crystals of $MgSO_4$ until there is a solution of approx. 20%.
3. Induction time variable: 8–24 hours.

Reference(s):

Wagstaffe & Fidler (1955)

Kaplan (1969)

Mahoney (1966)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	HOLOTHUROIDEA
Narcotic used:	MENTHOL

Method:

1. Place animals in a bowl with plenty of clean sea-water. Allow to extend.
2. Sprinkle a few menthol crystals on the surface of the water. Cover the bowl.
3. Induction time variable: 8–18 hours.
4. Suitable for large specimens.

Reference(s):

Duncan (1917)

Baylis & Monro (1941)

Wagstaffe & Fidler (1955)

Kaplan (1969)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	HOLOTHUROIDEA : *Stichopus*
Narcotic used:	PROPYLENE PHENOXETOL

Method:

1. Place animal in a bowl and cover with plenty of sea-water. Allow to extend.
2. Add propylene phenoxetol slowly so that it forms a globule of approx. 1-inch diameter on the bottom of the dish.
3. Not yet tested on whole holothurians, this method was devised for narcotising experimental portions.
4. Induction time approx. 1 hour.

Reference(s):

Hill (1966)

User's Notes:

Phylum:	ECHINODERMATA
Method for:	HOLOTHUROIDEA
Narcotic used:	SODA-WATER

Method:

1. Place animals in a bowl with plenty of clean sea-water. Allow to extend and avoid disturbance.
2. Fill a soda-siphon with sea-water and inject the CO_2 bulb.
3. Gradually add the soda-water to the bowl, avoiding disturbance if possible.
4. Continue until bowl contains approx. 50% soda-water.
5. Induction time variable: 15 minutes – 1 hour.
6. Not suitable for large specimens.

Reference(s):

Kufferath (1963)

Wagstaffe & Fidler (1955)

Smaldon (unpubl.)

User's Notes:

Phylum: HEMICHORDATA

Method for: ENTEROPNEUSTA

Narcotic used: ETHYL ALCOHOL (ETHANOL)

Method:

1. Place specimen in dish of clean sea-water.
2. Allow to relax.
3. Gradually add 10% ethyl alcohol to the water over a period of approx. 4 hours.
4. Test touch response before fixation.

Reference(s):

Lo Bianco (1899)

Mahoney (1966)

User's Notes:

Phylum: CHORDATA

Method for: UROCHORDATA : ASCIDIACEA

Narcotic used: CHLORAL HYDRATE

Method:

1. Make up a solution of 1% chloral hydrate in sea-water.
2. Place animals in a shallow dish of sea-water and allow to expand.
3. Slowly siphon off the sea-water and replace with chloral hydrate solution.
4. Alternatively, place animals directly in the 1% solution. For large species the strength of solution may be doubled.
5. Induction time 3–24 hours.

Reference(s):

Lo Bianco (1899)

Wagstaffe & Fidler (1955)

Pantin (1962)

User's Notes:

Phylum:	CHORDATA
Method for:	UROCHORDATA : ASCIDIACEA
Narcotic used:	MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$)

Method:

1. Make up a 7½% solution of $MgCl_2$ in sea-water.
2. Immerse specimens in this solution.
3. Induction time variable: 2–12 hours.

Reference(s):

Pantin (1962)

Ledingham & Wells (1942)

User's Notes:

Phylum:	CHORDATA
Method for:	UROCHORDATA : ASCIDIACEA
Narcotic used:	MENTHOL

Method:

1. Place specimens in a bowl with plenty of clean sea-water. Allow to extend.
2. Sprinkle a few menthol crystals on the surface of the water.
3. Cover bowl.
4. Induction time variable: 6–12 hours.
5. Suitable for all ascidians.

Reference(s):

Duncan (1917)

Wagstaffe & Fidler (1955)

User's Notes:

Phylum:	CHORDATA
Method for:	UROCHORDATA : Thaliacea (Salps)
Narcotic used:	ETHANOL

Method:

1. Place specimens in a dish in fresh sea-water.
2. Add a few drops of weak ethanol (10%) at 5-minute intervals.
3. Prevent flattening by blowing a few air bubbles into the cavity of the animal when fixing.
4. Induction time 1–2 hours.

Reference(s):

Lo Bianco (1899)

User's Notes:

Phylum:
Method for:
Narcotic used:

Method:

Reference(s):

User's Notes:

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