

METHODS FOR NARCOTIZING AND PRESERVING MARINE INVERTEBRATES,  
ABSTRACTED FROM WAGSTAFFE AND FIDLER, ARRANGED BY MAJOR TAXA

PROTOZOA, FORAMINIFERA, RADIOLARIA

Not narcotized, but killed, fixed and stained by various methods.

PORIFERA

Not narcotized.

- (a) Wash in clean salt or fresh water.
- (b) Immerse in 95% alcohol if small; if large, use only small piece for preserving.
- (c) Store in 95% alcohol.
- (d) Spicules
  - 1) Siliceous
    - a) Boil in nitric acid to get rid of soft parts.
    - b) Pour off acid and wash several times with distilled water, filter, dry in an oven and mount on a slide in euparal.
  - 2) Calcareous
    - a) Macerate in 10% caustic soda.
    - b) Wash as above, filter, dry and mount on a slide in euparal.
- (e) Larvae
  - 1) Killing and fixing. Place in little seawater and flood for 2 or 3 minutes with Flemming's fluid (see page 52)

CNIDARIA (Coelenterata)

- A) Hydroids
  - 1) Place in seawater and allow to extend.
  - 2) Use menthol, magnesium sulphate or chloral hydrate crystals in a 5-10% solution: Add over a period of 3 + hours until animals are unresponsive to touch.
- B) Medusae
  - 1) Place in a shallow dish with a little seawater and allow to expand.
  - 2) Use menthol, magnesium sulphate or chloral hydrate crystals in a 5-10% solution, stovaine or chloroform.
  - 3) Caution - Do not let them die before preserving them.

C) Siphonophora

- 1) Caliconectae
  - a) Kill with saturated picric acid.
  - b) Store in 10% formalin.
- 2) Physonectae
  - a) Narcotize with menthol.
  - b) Kill with 10% formalin, or a saturated solution of corrosive sublimate.
  - c) Add Flemming's fluid (see page 52) or Kleinenberg's fluid (see page 52) and leave specimens in it 24 hours.
  - d) Store in 70% alcohol.
- 3) Cystonectae
  - a) Kill with corrosive sublimate and glacial acetic acid (95 ml corrosive sublimate saturated solution in distilled water and 5 ml glacial acetic acid). When dead, place in 1/2% for 1 1/2 hours, 30% alcohol for 1/2 hour, 50% alcohol for 1 hour.
  - b) Store in 70% alcohol.
- 4) Disconectae
  - a) Kill with saturated picric acid or corrosive sublimate solution. When dead add a little chromic acid and leave for 1/2 hour. If killed with picric acid, place in 30% alcohol for 2 hours, 50% alcohol for 1 hour. If killed with corrosive sublimate, wash well with running water and pass through alcohols as above.
  - b) Store in 70% alcohol.

D) Scyphomedusae

- 1) Kill with a 5% solution of formalin in seawater. In 10 minutes add enough 5% solution of picric acid to color the formalin. Stir contents over a 24 hour period and increase the formalin to 10%.

E) Actinozoa

- 1) Alcionaria
  - a) Narcotize with menthol crystals.
  - b) Store in 70% alcohol.
- 2) Zoantharia
  - a) Allow specimens to expand in seawater. Sprinkle in a few crystals of menthol or chloral hydrate, 5-10%, use 3% alcoholized seawater or bubble tobacco smoke through specimen container. Leave over night.

- b) Killing. Draw off water but do not uncover animals and flood with 10% formalin.
- c) Store in 5-10% formalin or 70% alcohol.
- 3) Other Methods
  - a) Add magnesium sulphate crystals gradually or place them in a muslin bag with the specimens.
  - b) Poison by adding a few drops at a time of dilute formalin. Double the quantity each time.
  - c) Freeze in block of ice, chip off excess ice. Allow to melt in strong formalin.
  - d) Draw water down without uncovering animals and kill suddenly with boiling formalin.
  - e) Caution. DO UNDER FUME HOOD.
- 4) Cnidae
  - a) Scrape off ectoderm. Place it in a drop of seawater on a slide. Tease apart with needles. Drop over it a cover slip and press up and down to help break up the tissue. With a camel's hair brush transfer the dissociated tissue to clean seawater. Smear a cover slip with albumen. Place a little of the dissociated tissue on it and air-dry until tacky. Fix with Schaudinn's fluid for 5-10 minutes. Wash with 50, 70% alcohol; then 70% iodized alcohol. Put in 90% alcohol for 1 hour and stain in light green (0.5% solution in 95% alcohol) for several minutes. Rinse in clean 95% alcohol. Mount in euparal.

#### CTENOPHORA

- A) Allow to expand in standing or running seawater. Transfer from water to chromo-osmic (chromic acid 1%, 100 ml, osmic acid 1%, 2 ml) for 15-60 minutes, copper sulphate and corrosive sublimate. Pass through 30, 50, 70% alcohol.
- B) Store in 70% alcohol.
- C) Caution - DO NOT STORE IN FORMALIN.

#### PLATYHELMINTHES

- A) Turbellaria
  - 1) Narcotize by placing specimens in salt or fresh water depending on the species and add small amounts of menthol or chloral hydrate crystals to the

water for 2-3 hours or until movement is barely perceptible.

- 2) Kill by passing animals through 35, 50% alcohol.
- 3) Store in 70% alcohol.

B) Trematoda

- 1) Clean by shaking specimens in a 1% salt solution.
- 2) Kill by adding an equal (equal to the amount of salt solution) amount of hot (about 60°C) saturated aqueous solution of corrosive sublimate.
- 3) Fix in this solution for 1-2 days.
- 4) Wash in running water for 4-5 hours and pass through 50% alcohol.
- 5) Store in 70% alcohol.

C) Cestoda

- 1) Clean by washing in a 1% salt solution.
- 2) Kill by immersion in a 1% aqueous solution of chromic acid after wrapping around a glass bottle or dish. When dead, place in formol-acetic alcohol for 24 hours. Wash in 50% alcohol.
- 3) Store in 70% alcohol.

NEMERTEA

- A) Allow to expand in running or standing seawater. Narcotize in a 5-10% solution of menthol or chloral hydrate crystals.
- B) Caution - Watch carefully that specimens do not vomit proboscides.
- C) Killing.
  - a) After resting in standing seawater for 10-15 minutes, draw off most of water and flood with an aqueous solution of corrosive sublimate and leave specimens in this for 15-20 minutes.
  - b) Use almost boiling water and transfer to corrosive sublimate for 10 minutes. Wash in several changes of 50% iodized alcohol. Pass to 70% alcohol.
- D) Store in 70% alcohol.

ROTIFERA

- A) Slow Narcotization. Place specimens in 8 ml water in a watch-glass. Add 2 drops of a 2% aqueous solution of benzamine hydrochloride. Mix with a coarse pipette. After 20-30 minutes, add 1 or 2 drops of benzamine hydrochloride (as much as 6 drops may be needed if water is acid). Total time, 1-3 hours.

- B) Rapid Narcotization. Place specimens in less water than above. For every ml of water, add 3-10 drops of:
- |  |       |               |
|--|-------|---------------|
| 2% aqueous solution of benzamine hydrochloride | ....  | 3 parts       |
|  | water | ..... 6 parts |
- Pure cellosolve (ethylene glycol mono-ethyl ether)...1 part  
Total time, 3 + minutes.
- C) Killing.
- a) with 4 ml of 10% formalin.
  - b) with very hot water. Allow specimens to expand in cold water in a watch-glass; then flood with hot water and transfer to 10% formalin.

#### NEMATODA

- A) Extraction. Wash out gut with a 1% aqueous salt solution.
- B) Cleaning. Place washings in a glass jar of 1% aqueous salt solution and shake well. When nematodes sink to the bottom, decant saline and replace with new saline. Continue this process until specimens are clean.
- C) Killing. Pour off saline and add 70% alcohol for 1-2 minutes.
- D) Store in fresh 70% alcohol.

#### ANNELIDA

- A) Polychaeta
  - 1) Place in clean seawater and add 70% alcohol drop by drop until specimens no longer respond to touch. Tubicolous forms will leave tubes if menthol crystals are sprinkled on their water surface and left over night.
  - 2) Killing. Arrange parts on a dry glass plate and press behind the head to force the extension of the proboscis. Flood with formol-alcohol for 20 minutes. Place in a flat dish with formol-alcohol for 24 hours.
  - 3) Store in fresh formol-alcohol.
- B) Hirudinea
  - 1) Allow to expand in water and from time to time add crystals of chloral hydrate or magnesium sulphate.
  - 2) Killing. When specimens are unresponsive to touch, straighten out on a glass plate and flood with warm 10% formalin.
  - 3) Store in 70% formol-alcohol.
- C) Gephyra
  - 1) Add a few crystals of chloral hydrate or magnesium sulphate from time to time.

- 2) Killing. Place on a glass plate. Extend proboscis and flood with 70% formal-alcohol.
- 3) Store in 70% formal-alcohol.
- 4) Larvae. Killing and fixing. Draw off most of the water and flood with Flemming's fluid (see page 52) for 2-3 minutes.

#### CRUSTACEA

- A) Branchiopoda, Ostracoda, Copepoda.
  - 1) Kill with 5-10% formalin for 10 minutes. Transfer to 50% alcohol for 10 minutes.
  - 2) Store in 70% alcohol or 5-10% formalin.
- B) Branchiura
  - 1) Kill by dropping into caustic soda (NaOH). Transfer to 50% alcohol.
  - 2) Store in 70% alcohol.
- C) Cirripedia
  - 1) Allow to expand in water and narcotize with menthol crystals.
  - 2) Kill in 10% formalin or when no longer responsive to touch use hot (60°C) saturated solution of corrosive sublimate for 10-20 minutes and wash in 70% iodized alcohol.
  - 3) Store in 70% alcohol.
- D) Malacostraca
  - 1) Decapoda
    - a) Kill by placing specimens in cold freshwater with or without chloroform. Drop small forms into 70% alcohol.
    - b) Store in 70% alcohol with a little glycerin added.
  - 2) Larval Stages
    - a) Kill by placing animals in Bouin's fluid for 3-6 hours. Wash in several changes of 30% alcohol for 30 minutes; 50% alcohol for 30 minutes.
    - b) Store in 70% alcohol.
  - 3) Isopoda
    - a) Kill by dropping specimens into 100 ml alcohol + 4 ml glycerin. They may remain in this for 10-14 days.
    - b) Transfer and store in 70% alcohol.

#### MOLLUSCA

- A) Narcotizing substances and groups
  - 1) Acetic acid and seawater.
    - a) Tritonia

- 2) Alcohol, 70%
  - a) Chromodoris
  - b) Doris
  - c) Natica josephinia
- 3) Alcoholized seawater, 3% or alcohol added slowly.
  - a) Amphineura
  - b) Decapoda
  - c) Doridium
  - d) Fissurellidae
  - e) Gastropods
  - f) Haliotidae
  - g) Lamellibranchia
  - h) Mariona
  - i) Opisthobranichia
  - j) Patellidae
  - k) Placophora
  - l) Pleurophyllidia
  - m) Scaphander
  - n) Scaphoda
- 4) Chloral Hydrate, 0.1-0.2%.
  - a) Aplysia depilans
  - b) Aplysia limacina
  - c) Aplysia punctata
  - d) Amphinenra
  - e) Decapods
  - f) Gymnosomata
  - g) Octopoda
  - h) Scaphopoda
- 5) Chloroform added slowly.
  - a) Lamellibranchiata
- 6) Cocaine.
  - a) Chromodoris
  - b) Doris
  - c) Gastropoda
  - d) Heteropoda
  - e) Lamellibranchia
  - f) Pleurobranchia Meckeli
- 7) Freezing in a block of seawater and thawing in 10% formalin.
  - a) Nudibranchiata
- 8) Formalin, dilute. Add a few drops and double the quantity each time they are added.
  - a) Nudibranchiata
- 9) Glycerin, 20 parts; 70% alcohol, 40 parts; seawater, 40 parts and place animals in as little seawater as possible.
  - a) Tectibranchiata

- 10) Magnesium sulphate crystals, allowed to dissolve in a bag in with the animals, or free in their container.
  - a) Nudibranchiata
- 11) Menthol crystals.
  - a) Amphineura
  - b) Lamellibranchia
  - c) Scaphopoda
- 12) Seawater, standing.
  - a) Aeolidiidae
  - b) Atlantidae
  - c) Elysiidae
  - d) Heteropoda
  - e) Hyaleidae
  - f) Idalia
  - g) Philine
  - h) Pteropoda
  - i) Polycera
  - j) Tethys
  - k) Triopa
- 13) Killing and or preserving.
  - a) Alcohol. Place specimens in 50% alcohol for 48 hours and transfer to 70%.
    - 1) Amphineura
    - 2) Lamellibranchiata
    - 3) Scaphopoda
  - b) Chloroform, ether or ethyl bromide. Inject into mantle cavity.
    - 1) Cephalopoda
      - a) Preserve by placing specimen on board and pin down arms. Inject 5% formalin into mantle cavity and place animal in 10% formalin to harden. Wash in running water. Remove from board.
      - b) Store in 70% alcohol or 10% formalin.

**POLYZOA (Bryozoa)**

- A) Narcotizing. Place specimens in salt or fresh-water depending on the species. Use menthol, chloral hydrate 5-10%, stovaine or B-eucaine hydrochloride (the last, especially for freshwater forms). When polyps no longer respond to touch, draw off water and replace with 5% formalin.
- B) Store in 5% formalin.
- C) Cleaning. Transfer specimens to distilled water. Remove foreign matter with a soft camel's hair brush and wash several times in clean, distilled water.

- D) Larvae
- 1) Narcotize as above and fix in hot (60°C) Bouin's fluid. Wash in water.
  - 2) Store in 70% alcohol.

#### BRANCHIOPODA

- A) Narcotize by adding alcohol to specimen's seawater to make a 5% solution.
- B) Kill by leaving specimens in this seawater for 30 minutes.
- C) Store in 70% alcohol.

#### \* CHAETOGNATHA

- A) Narcotize by adding menthol or chloral hydrate crystals to small amount of seawater containing specimens.
- B) Killing. Straighten specimens out on a piece of glass and flood with Bouin's or Gilson's fluid.
- C) Storage. If fixed in Gilson's fluid, wash in 2-3 changes of 50% iodized alcohol. If fixed in Bouin's fluid wash in 2-3 changes of 50% alcohol and in both cases store in 5% formalin.

#### ECHINODERMATA

- A) Asteroidea
  - 1) Narcotize by adding crystals of magnesium sulphate to their seawater (narcotize with tube feet uppermost).
  - 2) Kill by placing specimens in 70% alcohol.
  - 3) Store in 70% alcohol.
- B) Ophiuroidea
  - 1) Narcotize specimens in seawater to which weak alcohol is gradually added.
- C) Echinoidea
  - 1) Kill by placing specimens quickly in 70% alcohol or strong acetic acid. If acetic acid is used, wash out well in running freshwater.
- D) Holothuroidea
  - 1) Narcotize in fresh water to which crystals of menthol or magnesium sulphate have been added.
  - 2) Kill after narcotized by immersing in 15% formalin for a few minutes and immediately inject 70% alcohol into body cavity.
- E) Crinoidea
  - 1) Killing. Place animals head down in a dish of seawater and hold in position by pressing down. Flood with 90% alcohol for 3-4 minutes.

F) Larvae

- 1) Narcotizing. Place in seawater and add a few chloral hydrate crystals or stovaine.
- 2) Killing. Draw off most of seawater and flood with Bouin's fluid. Leave for 2-3 hours and wash with water.

TUNICATA

- A) Narcotize in clean seawater to which crystals of menthol or chloral hydrate have been slowly added or 3% alcoholized seawater.
- B) Killing. Draw off most of seawater containing animals and flood with Bouin's fluid or a saturated solution of corrosive sublimate and leave for 1-24 hours.
- C) Storage. If fixed in Bouin's fluid, wash in several changes of 50% alcohol. If fixed in corrosive sublimate, wash in several changes of 50% iodized alcohol. In either case store in 70% alcohol.