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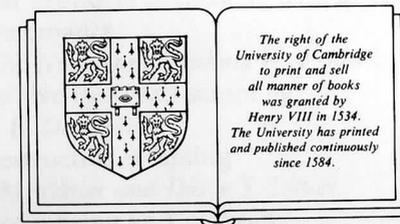
ECOLOGICAL FIELD METHODS: MACROALGAE

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4: Collection, handling, preservation, and logistics

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I. Introduction

Good ecological field work requires good taxonomy, which is dependent on the quality of voucher specimens preserved as documentation. This chapter describes methods for qualitatively assessing the macroalgal flora of a study site, whether the site is a single bay, a coastline, or the reefs around an island. The various methods of collecting, handling, and preserving specimens in the field and laboratory for later taxonomic determination, as well as logistic considerations, are described and evaluated. Publications that provide excellent descriptions of field and laboratory methods of collecting, processing, and identification are as follows: Taylor (1950, 1960, 1962), Dawson (1966), Abbott and Hollenberg (1976), and Abbott and Dawson (1978). Key points of these treatments are highlighted and summarized in this chapter.

II. Field collecting

A. Equipment and supplies

Because the primary objective in a qualitative assessment is to collect as many species as possible, that is, to obtain a representative flora of the area, very little in the way of equipment and supplies is needed. If one plans to wade in the shallow waters near shore, foot protection is necessary. High-topped, athletic rubber-soled shoes (e.g., tennis or jogger shoes) or Japanese *tabi* are comfortable and will provide protection from jagged rocks, corals, and toxic marine animals (e.g., cone shells or the toxic-spined fishes that inhabit coral reefs). A glass-bottom "look box" or diving mask is useful. To assess the macroalgae of deeper waters, skin-diving gear, for example, face mask, snorkel, and swim fins, or even SCUBA equipment may be used (see Foster et al., Chap. 10); in this case, a small boat is very convenient as a diving platform and for depositing full bags of material.

One should use judgment about protective clothing. Some individuals who work in tropical waters prefer a wet suit with weight belt;

others wear coarse jeans or knee pads to protect against sharp organisms. Hats, shirts, or a protective lotion are mandatory to prevent sunburning. Obviously, a wet suit or dry suit is essential for diving in colder waters. Hip-height waders or wet-suit boots and trousers are necessary in temperate waters for inshore collecting. A first-aid kit is a necessary item to include on collecting trips.

In most cases, the algae can be collected by hand; however, a hammer, chisel, knife or spoon, geologist's pick, or mountain climber's ice ax are helpful for collecting smaller algae attached to hard substrata, collecting parts of larger algae (e.g., kelp), and removing holdfasts. In shallow waters, Whirl-pak bags that can be drained, sealed, and stored in larger bags or buckets are convenient. In deeper waters, a fine-mesh bag to hold the algae is best, although some collectors prefer perforated or unperforated plastic bags. Plastic vials with small holes are ideal containers for small, filamentous algae. It is very difficult to place a small specimen in an unperforated bag or vial underwater without loss of some portions of the specimen. In addition, an underwater writing slate or commercially available waterproof paper (Nalge Company) attached to a clipboard is essential for taking notes on natural history during collection. Some workers (e.g., Neushul 1965) have employed tape recorders in plastic bags or waterproof housings to take rapid floristic and ecological field notes. Tape-recorded notes are then transcribed in the laboratory.

Other supplies needed after collection are commercial Formalin or 95% ethyl alcohol (as preservatives), buckets and trays (for sorting), tweezers, plant press (for drying), bottles and vials, herbarium paper, labels (100% rag), and a permanent black ink pen.

B. Methods

In regions where the tidal amplitude is great, it is best to collect during the ebbing tide, because the water is usually clearer, especially at the period nearest mean lower low water (MLLW). Beach-drift specimens are best collected last, because fresher specimens can usually be found growing nearby and it is vastly preferable to determine the precise habitat.

After selection of the study site (collection locale), it does not matter where collecting begins as long as the algae taken from the different habitats are kept in separate containers. Each container label must accurately describe the habitat of the collection site. Experience has shown that one can collect a representative flora in a short period by simply wading or snorkeling perpendicular to the shore, whereas one can survey patchiness by swimming or wading parallel to shore. During SCUBA diving in subtidal waters, it is best to begin collecting at the deepest depth and work toward the shallower

waters. Data on tidal height and zonation are very important, especially on reefs (i.e., inner reef flat, outer reef flat, reef margin, or reef front) and the deeper reef slope and terraces. The vertical ranges of species subsequently can be determined from accurate records of collection depths made on a tape recorder or with underwater writing materials, as well as photographs (see Littler and Littler, Chap. 8). The depth readings can be obtained from an accurately calibrated depth gauge during skin or SCUBA diving; time of collection must also be noted for tidal height corrections.

Ecologists frequently conduct population studies in which certain measured areas are scraped clean, and the material is sorted, weighed, acidified, dried, and reweighed (see De Wreede, Chap. 7). In sorting of the material, identification to genus is usually accomplished initially; samples should be kept for later, more specific identification. Species diversity studies also require that taxonomic vouchers of all algae be taken from measured areas. Here, it is important to have different-size classes of the same taxon, from adjacent areas if necessary, in order that a good representation of the morphological and ecological variation be understood by the person identifying the species. The small scraps of thalli frequently collected by marine ecologists in such studies are difficult to utilize even for experienced algal taxonomists.

In herbivory studies, collections within the area of the predators should be made (including crustose taxa). To reduce bulk, the gut contents of the animals may be preserved separately (using ~10% Formalin). The animals themselves are preserved differently from plants; most invertebrates require anesthetization before preservation (few zoologists will accept poorly preserved specimens for identification, and phycologists are beginning to follow suit). A preservative suitable for the whole animal is not necessarily adequate to preserve gut contents, and injection is usually required (see Vadas, Chap. 26). Time of feeding is important for collecting gut contents that can be identified. Preservation of animals varies by animal group, and *Light's Manual* (Smith and Carlson, 1975) should be consulted.

Some recommendations for collecting macroalgae are as follows. (1) When collecting epiphytes or smaller algae on solid substrata, break off the portion of the substratum on which the algae are growing; this will later provide substratum information, and there will be less chance of losing the specimens during sorting. (2) Collect the entire basal holdfast or rhizoidal portion with the specimen; these structures may greatly aid in later identification. (3) Make an effort to collect reproductive specimens, since these may also be critical for precise identification. Because the objective of the assessment and collection is to provide a qualitative checklist based on the taxonomic

vouchers taken, collection of entire specimens of larger forms (e.g., kelps) is often unnecessary unless one suspects that they may represent variants or new species.

If time is limited, a considerable area can be surveyed by slowly (less than 3 knots) towing an observer/collector with a rope well behind a small boat (e.g., inflatable boat with a 15- to 25-horsepower outboard engine). Safety precautions comparable to those recommended for towing water skiers (e.g., established hand signals) should be followed. This includes an observer in the boat during towing. Wet suits or clothing worn during towing should be designed to avoid scooping water down the neck and sleeves. Fins should always be worn for maneuverability and to aid in quick exit from the water when the curious shark or barracuda inevitably appears. This method is especially effective for locating a broad range of habitats if applied safely in clear waters, especially when two divers are towed simultaneously.

In deeper, turbid, or otherwise unsafe waters, dredging may be the most practical and safest means of obtaining an algal collection; however, a ship with the capacity to dredge is seldom available for routine algal collecting. Bottom trawls that cover a considerable amount of area are much preferable to the grab sampler, which covers a very small area. As pointed out by Taylor (1960), bottom trawls are very difficult to operate in rough seas. In addition, the bottom topography must be considered, because there is danger of losing the trawl in rocky terrain. See Holme and McIntyre (1971) for detailed methods of collecting marine benthos from surface vessels.

Since some areas of a country or even the country as a whole may have special laws or permit requirements for collecting, individuals should determine these regulations and comply before entering the water. Even in areas where there may be no official laws against collecting, local customs may dictate acquiring permission from the resident population or authorities before field activities are begun.

III. Processing

A. Field processing

On a regional scale (Tsuda and Stojkovich 1980), it may be desirable to collect a set of specimens over a 1-yr period at monthly, bimonthly, or quarterly intervals to locate seasonal or ephemeral species and to document variability adequately (phenotypic plasticity). At a minimum, collections should be undertaken during each extreme season in a highly seasonal environment. Such information as the maturity and fertility of the thallus, habitat conditions, and environmental considerations (e.g., water temperature, salinity, light, and wave

action) may prove to be important at a later time, to the chemotaxonomist (see Norris and Fenical, Chap. 6) as well as the ecologist and biogeographer (see Druehl and Footitt, Chap. 15).

When collecting is done over several days or weeks and laboratory facilities are unavailable for immediate processing, it is necessary to preserve the specimens after each day of collecting. One method is to preserve the specimens from each collection in plastic bags (e.g., Whirl-paks) or glass containers with either a 3–5% solution of commercial Formalin (= 37% formaldehyde) or ethyl alcohol diluted to 65 to 70%. Seawater must be used to make these solutions, and a buffer, such as borax, should be added to the Formalin–seawater solution until it gives a pink reaction when tested with phenolphthalein.

As described by Taylor (1960), the Formalin solution is initially best for coarse species, and the specimens should be kept out of the light. Alcohol amounting to 30% added to the Formalin solution is recommended for more delicate specimens. In general, the ethyl alcohol solution is sufficient for all species, but there is a tendency for the pigments, especially chlorophyll, to extract and bleach. Some cell shrinkage occurs when any of these preservatives are used.

If only a limited amount of preservative is available, the preservative solution can be reused. The algae should be totally immersed for two or more days; then the excess preservative can be drained off, the algal specimens being left “damp-dry,” and the preservative reused in new collections. The specimens can also be dried between newspaper (for coarse algae) or wax paper (for delicate algae) in a plant press or simply air-dried for a short period in the sun on a black plastic sheet and later placed in a shady area to complete the drying process. Also, hanging the algae in a mesh bag in a windy area will produce rapid drying; however, one must be careful to avoid clumping of the algae. Air-dried specimens, although brittle, can be resoaked and mounted on herbarium paper later.

Another method that can be used if no preservatives are available is the use of common rock salt. The process is quite similar to that used by fishermen in preserving fish. A large quantity of rock salt is added to seawater to form a strong brine solution. Taylor (1960:38) suggests that if “the brine first formed is drained and the algae relayered with fresh salt, many species, and even many rather delicate ones, may be preserved in this way for several years.” One must quickly and thoroughly mix the salt with the specimens.

If a freezer is handy, one can freeze the algae-filled plastic bags; however, considerable cell damage may occur, especially in small, delicate species.

Each plastic bag should be numbered consecutively and keyed to

its respective number in a field notebook. Short strips of herbarium paper (100% rag) make ideal labels if numbered with permanent black ink (e.g., India ink); these numbered labels are placed directly into the plastic bags. Information on the collecting site, date, habitat description, and anything else that is noteworthy should be recorded in the field notebook. It is also useful to record a general list of the visually dominant algae found in each habitat.

The plastic bags with preserved specimens and labels are then placed in a suitable shipping container (e.g., Liqui-Pak). “Ship-biscuit” aluminum 5-gal containers are also useful and can be obtained in many areas of the world. Any nonleaking container is adequate. When preparing preserved specimens for shipment (i.e., by vehicle, ship, or airplane), drain the preservative from each of the plastic bags into a suitable deep hole and bury it. Consolidate the bags into as few containers as possible. The dried specimens can be placed in plastic bags according to collection sites and shipped in cardboard boxes.

B. Laboratory processing

If the collections have been well labeled and roughly sorted as they lie in their plastic bags, they can be placed – bag and all – in large containers that have 3–5% Formalin poured over them. They should not be left in Whirl-paks that will rust over time or in containers from which the preservative evaporates. Except for certain brown algae, most marine algae will fade on standing in preservative; consequently, they should be sorted and voucher specimens prepared as soon as possible, generally within 1 to 2 mo. If previous arrangements have not been made for the care of excess materials or for their identification by colleagues and experts, it is reasonable to discard them at this time. Unless someone or some institution has announced an interest in one's algal collections, one should collect only as much as one can properly process oneself. It is a chore for someone else, and an added expense, to process collections conscientiously in order to save them rather than to study them.

1. Sorting. If the contents of plastic bags were previously rough-sorted when packed, they can be divided, for example, into groups of large and small specimens. It is usually preferable to work on the large specimens first – in order to be rid of the bulk more quickly. Place them in a bucket of seawater or fresh water, if they have been preserved in Formalin, rinsing off preservative, sand, and small animals and removing epiphytes if any are present. Many individuals are sensitive to the fumes of Formalin (it appears that such sensitivity increases with a person's age), and it may be necessary to work under

Table 4-1. Common stains, fixatives, and preservatives used for benthic marine algae, including Corallinaceae

	Reference
<i>Preservatives</i>	
3-5% formalin in seawater (treating commercial formaldehyde as 100%); ~0.06 liter borax is added to 4 liters liquid	—
35, 50, 70% ethyl alcohol, stored 70% in ethyl alcohol	—
<i>Fixatives (for future cytological studies)</i>	
FAA (Formalin-acetic alcohol)	Berlyn and Miksche (1976)
Karpetchenko (Papenfuss modification)	Papenfuss (1946)
<i>Fixatives for coralline algae</i>	
Susa solution	Suneson (1937)
Perenyi's solution	Mason (1953)
EDTA	Giraud and Cabioch (1976)
Trichloroacetic acid	Johansen (1969)
<i>Stains (aqueous, suitable for corn syrup technique)</i>	
Aniline blue ^a	Papenfuss (1937)
Delafield's hematoxylin	Sass (1958)
1% Fast green	Sass (1958)
1% Congo red	Sass (1958)
Crystal violet (glycerine method) ^b	Chemin (1929)

^a Aniline blue is not a critical stain but a good general stain. It is used mainly by students of G. F. Papenfuss.

^b Crystal violet is used by P. S. Dixon and his students. Similar results are obtained using light green or fast green in glycerine (Papenfuss 1937).

a fume hood or with specimens in running water. Use plastic or rubber gloves. Press the specimen (see Sec. III.B.2.b) or, in the case of a large, coarse brown alga such as *Sargassum* or a thick one such as *Halimeda*, place it in newspaper to dry (see Sec. III.B.2.a). Retain epiphytes and small specimens less than 4 cm tall in individual vials with appropriate labels including collection numbers and data transferred from field books. Letters are frequently added to the numbering system for individual small algae when a number of them occur epiphytically on the same host. The hosts for epiphytes should be recorded. The vials should be filled with 3 to 5% buffered Formalin (Table 4-1) or with 65 to 70% ethyl alcohol, whichever was used in the field collection. If specimens were not preserved in the field, a choice of substances is available for preservation (Table 4-

1). If specimens were previously preserved in alcohol, they are placed in a 1:1 alcohol/water solution for 15 to 20 min before pressing. Alcohol-preserved marine algae are usually less pliable and dry to a more brittle state than Formalin-preserved specimens. When a large number (50-100) of these small specimens have accumulated, put aside a few hours or a day to process them further by (1) examining under a microscope to identify at least to genus and to determine reproductive state, etc.; (2) pressing as herbarium specimens those large enough to be seen; and (3) making microscope slides of very small specimens or parts of larger specimens.

A further sorting step that will greatly facilitate retrieval can be taken at this time. In the preliminary sorting of large versus small specimens, categories of algae can be kept together by major phyla (colors of pigments) and thus taken up for identification in similar taxonomic groups. Blue-green algae (Cyanophyta), which usually constitute a small number, should be identified as soon as possible, because their gelatinous structures frequently disappear with further laboratory treatment, making it very difficult to identify them to species. If arrangements have been made for identification by an expert, his or her preferred method of preservation should be known before collections are made. Green algae (Chlorophyta) and brown algae (Phaeophyta) should be processed as herbarium specimens, except for very small taxa of browns, which should be preserved in Formalin or processed on microscope slides (see Sec. III.B.2.e). Red algae (Rhodophyta), which will be the most numerous, should be pressed immediately if the thalli are gelatinous or mucosoid; others will soon lose their color if left in preservative. A choice must be made, if time is short, between attractive specimens or material in a state that can be adequately studied. It is a generalization that, of all algal groups, red algae are the most difficult to identify from dried specimens. It is also true, however, that the ones bleached in preservative are not often in a condition to be shared and shown to others. The ideal method would be to remove small portions of red algae to vials with preservative while drying the remainder of the thalli as herbarium specimens.

2. *Preservation as voucher specimens.* Voucher specimens are those that serve as evidence or documentation that a given species is that species or was collected from a certain place. The literature is full of undocumented lists of algae that were supposedly collected at certain places. Workers that follow can never check earlier identifications, update the nomenclature, or evaluate old lists without voucher specimens. It is especially important for nontaxonomists to appreciate the value of taking voucher specimens - taxonomists keep specimens

routinely. The easiest and best way to maintain voucher specimens is to press them on herbarium sheets, but small specimens may have to be kept on pieces of mica (the best, but increasingly difficult to purchase); pieces of clear, firm plastic sheeting; firm, good-quality cards; or microscope slides. Methods for these preparations are outlined here.

a. Placing coarse specimens in newspaper to dry. When nothing else is available, dry newspaper will serve adequately to contain specimens until other facilities are available. The newspaper must be changed daily until the specimen becomes dry. If the specimen adheres to the newspaper, it is usually because the paper was not changed frequently [in the field, one of us (IAA) changes newspaper twice each day]. If necessary, the specimen can be soaked off at a later date, the newsprint and paper fibers will brush off, and the specimen can be arranged (or rearranged) on a clean herbarium sheet. Appropriate collection numbers must be transferred each time.

b. Preparing a herbarium sheet. Herbarium paper, fitted blotters, and corrugated cardboard ventilators can be purchased (Carpenter/Offutt Papers, Inc., or Turtox Biological Supplies) in standard herbarium shapes and sizes. In addition, pieces of cloth, cut or torn approximately 10 × 16 in., newspapers, wax paper, or paper towels should be available. Small paint brushes (2–5 cm wide) or an ear syringe (a rubber bulb with a pointed tip) are used to encourage branches to lie in one plane or to spread out to show their pattern. The cloth used can consist of pieces of unbleached muslin, which usually contain no sizing, are readily available, and are long lasting. Old bed sheets also make excellent herbarium cloths. If these sources are lacking, rags can sometimes be purchased in laundries or paint shops. Cloths are not mandatory; however, drying is hastened with their use.

Using a shallow pan or tray (cafeteria trays or plastic trays used by photographers) that is larger than the standard herbarium sheet (approximately 25 × 45 cm) and is 75% filled with water, insert a piece of plastic, galvanized iron, or hardware cloth (cut to fit) with a piece of herbarium paper above this firm base. Then float the specimen to be pressed out over the wet sheet of paper, slowly lift the base at an angle, straighten the specimen with branches laid flat, and finally lift the three pieces (base plus paper plus specimen) out of the pan and allow the water to drain off. Place the paper with specimen on a dry blotter that rests on a cardboard ventilator (or the side of a cardboard carton cut to fit). The advantage of a ventilator is that the corrugates will be running across the width, allowing circulating air or heat through the most openings over the shortest distance. The holes of a cardboard carton will run through

the length of the board. Cover the specimen with a cloth or, if the specimen is slippery or mucosoid, with wax paper. Place a layer of newspaper over the wax paper or cloth if the specimen is bulky; otherwise, place a dry blotter over the wax paper, followed by a dry ventilator, then a dry blotter, which will ready the press for the next sandwich of specimens. When a stack about 30–60 cm high has been accumulated, place a plant-press frame or piece of plywood cut to fit on the top and bottom of the stack. Use a woven strap around this stack, pulled as tightly as possible. Twelve to 18 h later, change all blotters, ventilators, and cloths in the stack. If waxed paper was used, insert a new piece. The specimens that have left a large amount of water on the adjacent blotters should perhaps have two layers of blotters on each side of them when the new stack is made. A weight (lead brick, large rock, many books, or sandbags) can be placed on the stack. The stack can now be left for 24 h and then reexamined. It may be possible to eliminate cloths and blotters at this change. Dry the damp blotters, ventilators, and cloths by placing them in a standard, heated plant dryer or by hanging them on a line. In places that have room heat, they may be overlapped and stood on end. Pressing cloths should be washed every few months under normal use to remove accumulated salts.

In the case of a specimen with low water content, drying should be hastened by more frequent changes or by eliminating blotters. Very slow drying encourages growth of fungi or formation of salt crystals. On the other hand, the use of plant dryers that flowering plant taxonomists routinely employ will cause the algae to dry too quickly, resulting in shrinkage of thalli or leaving them too brittle for future handling. Thin, flat blades such as *Ulva* or delicate red algae such as *Polysiphonia* will probably be dry enough to remove after the second change. Other, more bulky algae such as species of *Chondrus*, *Gracilaria*, and *Colpomenia* may take 3–5 d (with changes each day). For very thick algae such as kelps, mold may develop before the specimens are completely dry. A dilute solution of Formalin (about 10%) should be dabbed on the moldy areas, and the specimens can be finished off by brief placement in a plant dryer or in an oven at low temperatures (~100°C) for about 20 min.

c. Calcified, lumpy, or odd-shaped specimens. Nonarticulated coralline algae, many of which have been removed from their coral (animal) or rock substrata, are too bulky to process as outlined in the preceding subsections. They are usually placed in 5 to 10% Formalin for 2 to 3 d and then air-dried. Erect, articulated corallines also are frequently placed in 5 to 10% Formalin overnight, then placed between folds of newspaper and dried between blotters and ventilators, as in the case of other specimens. When dried, they should

be thoroughly glued to herbarium sheets. Coralline algae are frequently stored in shallow, flat boxes in order to keep the brittle fronds free of physical damage and to allow for easy removal for closer examination.

Crustose, noncalcified algae (e.g., *Ralfsia* among the brown algae and *Peyssonnelia* among the red algae) should be chipped as closely as possible from their substratum with a chisel or, better yet, completely detached. They are very difficult to study on rocky substrata since they dry irregularly while attached or pull away differentially from the substrata, and resulting dried specimens only approximate the natural cell arrangements. Generally, fleshy crusts are studied while fresh and microscope slides are made at that time; the remaining material becomes the voucher specimen after air-drying.

d. Small specimens (less than 2 cm long). Mica is purchased in small, multilayered sheets that are usually peeled off to one or at most two thicknesses of a size just larger than the specimen to be preserved. Mica is used like herbarium paper, except that it is not immersed in water. The specimen is positioned on the piece of mica and, by means of forceps and a wet paint brush, is laid flat. Waxed paper or cloth is placed over the specimen, and the usual drying procedure is followed. The field data or specimen number can be written directly on the piece of mica. Sheets of firm, clear plastic (of windowpane thickness) can be used for delicate and small red algae with success, but such plastic does not hold small blue-green, green, or brown algae satisfactorily. When dry, both mica-mounted and plastic-mounted specimens should be placed in an envelope or packet and glued to a herbarium sheet.

Good-quality cards of various sizes also can be used for mounting specimens that are too small for a large herbarium sheet. The usual method for a large sheet is employed. When dry, the card should be glued to a standard herbarium sheet. The practice of some herbaria is to glue several small cards containing the same species from the same or different collections to a single herbarium sheet, each specimen receiving a different accession number. In other herbaria, each card is placed on a different herbarium sheet. The saving of paper and space in a herbarium cabinet is obvious in the first practice, but the second is justifiable because of the possibility of identification errors when multiple specimens are on the same sheet. Unauthorized removal of specimens from herbarium sheets is illegal in most herbaria.

Finally, small specimens and sections for microscopic examination can be kept on glass slides. The prepared slides can in turn be stored in a slide collection and cross-indexed with the herbarium sheet

(using the same field or collecting number in every case). Also, slides can be placed in cardboard containers with depressions that fit glass slides (Clay-Adams Co.), and these can be glued to the herbarium sheet or placed in a manila envelope, which in turn is glued to the sheet. Many endophytic and small epiphytic algae are kept on slides, since they would be lost on a large sheet or difficult to locate if retained on the host specimen. Although breakage of a glass slide is always a possibility (thus making mica or plastic more favorable), it is important to preserve small algae in a manner that facilitates study at a later time.

e. Preparation of permanent microscope slides as voucher specimens. The objectives are (1) to prepare a slide that can be made quickly and (2) to be able to use the slide as a permanent record. Preparation of slides for future cytological examination, a very different objective, is discussed separately.

The corn syrup method, introduced about 30 years ago at the University of California, Berkeley, has been tested over time and is now the favored technique for most marine phycologists. Clear corn syrup (Karo brand, available in most grocery stores), with phenol preservative added, is diluted with fresh water to form a series ranging from 35, 50, 70, to 80% in which most algae will not plasmolyze severely. The specimen to be used for this method must have been preserved earlier in Formalin or some other preservative in the field or laboratory. The alga is placed on a microscope slide (if small), the branches arranged so that they are in one plane; a drop of 0.5 to 1.0% aqueous stain (see Table 4-1) is added and fixed (if necessary) with a drop of 1% hydrochloric acid. This is rinsed off by flooding with distilled water and holding the slide at an angle over a paper towel to drain. The excess stain can also be carefully removed by blotting along the edges of the thallus with a piece of paper towel or bibulous paper (American Hospital Supply Corp.). A drop of 35% corn syrup is added, allowed to stand for a few minutes (up to 30 min), and then a drop of 50% corn syrup is added. With experience, it is possible to use 50% corn syrup directly; however, until the delicate thalli are known not to plasmolyze readily, it is safer to start at the lower concentrations of sugar. A cover slip is added carefully, one edge resting on a probe or forceps, and it is gently lowered over the syrup surface. In 8 to 12 h the slides should be inspected for plasmolysis or for air bubbles due to evaporation, and more syrup of the next higher concentration should be added to the margins of the cover slip. The slides must be kept on a flat surface. When bubbles no longer appear, the cover slips can be sealed with ordinary clear nail polish. This step, however, is not necessary, for if properly done, the preparation is self-sealing. The

<p>GILBERT MORGAN SMITH HERBARIUM Hopkins Marine Station of Stanford University</p> <p>MARINE ALGAE OF THE MONTEREY PENINSULA CALIFORNIA</p> <p><u>Ceramium eatonianum</u> (Farlow) DeToni (tetrasporangial) Common in mixed turf dominated by <u>Corallina officinalis</u> var. <u>chilensis</u> at +1.0 ft tide level, middle flats of Stillwater Cove, Pebble Beach.</p> <p>Coll. I. A. Abbott 16153 Aug. 8, 1982 Det. ISABELLA A. ABBOTT</p>	<p>----Herbarium where specimen is filed</p> <p>----Geographic range of species covered</p> <p>----Binomial, including author(s)</p> <p>----Specific ecological niche</p> <p>----Collector, collector's number; date of collection</p> <p>----Person who identified the specimen</p>
<p>from Hopkins Marine Station, Pacific Grove</p> <p>MARINE ALGAE OF CALIFORNIA</p> <p><u>Porphyra perforata</u> J. Agardh Voucher for photosynthesis studies</p> <p>+3.5 ft tide level on granite rock, beach southeast of Mussel Point, Pacific Grove</p> <p>leg. Celia M. Smith Aug. 8, 1982 Det. ISABELLA ABBOTT</p>	<p>---from a herbarium (=gift)</p> <p>--- Geographic range of species</p> <p>--- Binomial</p> <p>--- Evidence or record</p> <p>---Where collected</p> <p>--- Collector; date of collection</p> <p>---Person who identified the specimen</p>
<p>ALGAE OF THE HAWAIIAN ISLANDS Oahu Island</p> <p><u>Lophocladia trichocladus</u> (C. Ag.) Schmitz New record for Hawaiian Islands</p> <p>8 km offshore in 70 m depth, using "Deepstar" on eroded and living coral shelf.</p> <p>leg. K. Agegian Aug. 8, 1982 Isabella Abbott Det.</p>	<p>--- Broad geographic range</p> <p>--- Narrow geographic range</p> <p>--- Binomial</p> <p>--- Voucher for new record</p> <p>--- Where collected</p> <p>--- Collector; date of collection</p> <p>--- Person who identified the specimen</p>

Fig. 4-1. Format for several types of labels commonly used.

slides should remain flat for at least 1 wk, after which they can be packed in standard slide boxes and stored so that the slides are still parallel to the shelf. After 1 mo, they can be stored on edge. Slides made in this way – for example, some of those of E. Y. Dawson in the U.S. National Herbarium, Smithsonian Institution, that are more than 20 years old – are as usable as if made today. A few drops of phenol or thymol should be added to dropping bottles containing the corn syrup in order to discourage the growth of fungi and these bottles should be labeled POISON.

Standard microscope slides 25 × 75 mm are available with one end ground (on which one can write) or as clear glass. Cover slips come in several thicknesses, no. 1 being the thinnest. For microscopy no other thickness should be used. Cover slips may be round, square (22 × 22 mm), or rectangular. For the majority of algae, either the round or square cover slips are used. The disadvantage of the longer ones is that very little space is left for a label. Slide labels come in perforated sheets, which are convenient to use when one is traveling, or in rolls in which the labels (22 mm²) are self-sticking on one side. The collection number of the specimen, at least the genus name, and the place of collection are the acceptable minimum for the slide label.

The voucher specimen is fully prepared when

1. it is a dried, pressed, herbarium specimen that is adequately labeled, or
2. it is a dried or wet-preserved specimen that is labeled and contained, or
3. it is a small specimen that is on a labeled and prepared microscope slide.

To complete the documentation, an adequate label must be prepared to accompany the field or collecting number that was first assigned to the specimen, and necessary cross-indexing must be added to the herbarium materials. Any preserved material or slides containing a subset of the material, for example, should be noted on the herbarium sheet. Formats for labels and kinds of information on them are given Fig. 4-1. Labels should be on 100% rag paper so as to prevent insect damage and so that they can be used in liquid as well.

IV. Identification

The assignment of species names must be based on adequate voucher specimens and not on undocumented "reports" in the literature (Tsuda and Stojkovich 1980). Although the name for a species must be tied to one herbarium sheet (i.e., the type specimen), the inter-

pretation of what the species *is* depends on a number of specimens showing a variety of morphology and, if possible, ecological differences (Abbott 1972). The latter are expressed in uniform ways in some species and in slightly to extremely variable degrees in others.

When practicable, it is advisable to have two or more complete sets of specimens, and when data on them are published, the places that they are stored should be noted, for example, the U.S. National Herbarium, Smithsonian Institution, which is abbreviated (Holmgren and Keuken 1974) US. Private individual herbarium collections should be discouraged since they are not readily available to workers from all parts of the world. When one is collecting abroad, it is not only courteous, but probably mandatory as well to leave one set of specimens for the local area. This not only fosters interest and pride, but encourages further activity and sometimes additional valuable collections.

Rough identification will facilitate the processing of the voucher specimens that have been prepared. Once they are identified to genus, the search for a species name will become easier. There are several good regional algal floras, but their effective use requires a knowledge of marine algae. However, Abbott and Dawson (1978) provide a pictured key to the most common marine algae of all coasts of continental United States, and many species that occur in Alaska and Hawaii. With a common alga in hand, making choices in the key will enable even those whose English is limited to find the correct genus. A brief assessment of useful marine floras is given in Table 4-2.

1. Procedures for the identification of individual species

1. Note the major group of algae to which the specimen might belong by judging its color: some clear shade of grass green (Chlorophyta); olive green to golden (Phaeophyta); pink, red, purple, even greenish (Rhodophyta); dark blue-green, brownish, or black (Cyanophyta).

2. Select an appropriate flora by geographical region (Table 4-2) and look up the major group (phylum or division) that you have chosen.

3. Use keys that are available in the flora. If they contain a vocabulary that is too technical, try the keys in Abbott and Dawson (1978) first; then refer again to the flora you have chosen.

4. Be sure to examine the illustrations that are referred to in the descriptions.

5. Read the description of the species that you think you have slowly and critically. Use the glossary if one is included in the book (glossaries are usually worked over extensively before they are

Table 4-2. Marine floras helpful for species identification of common macrophytes

Pacific West Coast of North America

Abbott, I. A., and Hollenberg, G. J. 1976 *Marine Algae of California*. Stanford University Press, Stanford, Calif. 827 pp. Keys to genera of green, brown, and red algae; keys to species; each species in the book illustrated.

Scagel, R. F. 1967. *Guide to Common Seaweeds of British Columbia*. British Columbia Provincial Museum, Victoria. 330 pp. Includes many more northerly species than those in California.

Waaland, J. R. 1977. *Common Seaweeds of the Pacific Coast*. Pacific Search Press, Seattle. Some ecological notes, 16 excellent colored photographs as well as black and white illustrations.

Mexico and Central America

Dawson, E. Y. 1941-1965. It would be impossible to do any critical taxonomic studies in this geographical area (and to the immediate north and south) without referring to the numerous publications of this important contributor to the understanding of marine algae. Do not neglect these studies because they are not in book form! For reference to Dawson's papers, see Abbott and Hollenberg (1976).

Caribbean and Florida

Taylor, W. R. 1960. *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas*. University of Michigan Press, Ann Arbor. 870 pp. Well-illustrated, comprehensive coverage of the warm Atlantic and Caribbean. It can be added to but never replaced as a basic flora.

Northeastern United States and Canada

Taylor, W. R. 1957. *Marine Algae of the Northeastern Coast of North America*, rev. ed. University of Michigan Press, Ann Arbor. 509 pp. Timeless in its judgment and value.

All geographical areas

Humm, H. J., and Wicks, S. R. 1980. *Introduction and Guide to the Marine Blue-green Algae*. Wiley, New York. 194 pp. Very useful keys to genera and to species. Species descriptions contain the most common synonyms under which they may be found in other references.

Umezaki, I. 1961. The marine blue-green algae of Japan. *Mem. Coll. Agr. Kyoto Univ.* no. 83, 1-149. Keys to orders, genera, and species. More technical than Humm and Wicks (1980). Excellent illustrations.

published). If the generic description fits the specimen but not the species, look in other floras suggested in the book's references.

6. At this stage, it may be obvious that you need to know more about the specimen than you can observe without a microscope. Steps that can be taken are given in the next subsection.

7. The majority of ecologists work with common species. It is likely that the steps listed here have led to a name for the specimen, tentative as the identification may be. However, the specimen can

now be retrievably filed rather than left in a stack of unidentified specimens.

8. If uncertain, pencil the species name with its author(s) on the herbarium sheet. Initial the identification. When verified, the name can be added to the label.

9. Look over the other voucher specimens to see whether there are other specimens of the same species and put them together. You may now be more certain of whether your identification was correct.

2. *Examination for details of structure in order to match descriptions.* Thin sections of vegetative or reproductive structures must be made in order to place most algae within families, genera, or species. If preserved material has been saved, the job is relatively easy but, if not, the dried specimen can be sectioned.

1. Whether the specimen is liquid-preserved or dried, place it, or a small piece of it, under a dissecting microscope. Examine for reproduction if a brown or red alga.

2. Using a new single-edged razor blade and observing through the microscope, carefully cut a small piece (about 2 mm) of the specimen off. Place the tiny piece on a clean microscope slide and remove the remainder of the specimen from the microscope.

3. Place the slide with its specimen under the microscope again, hold the specimen down with a glass slide held at an angle close to the edge of the specimen, and cut across the specimen until the angled slide is reached. Lift it, move it back, and continue to cut sections, cut as many as possible.

4. Most anatomical descriptions are based on cross sections. The sections that have been cut must be turned on their broadest faces (i.e., so that they will present a cross section). Fine needles will help to do this.

5. If sections of dried brown or green algae have been cut, they are probably very hard and the cells lie very closely together. The sections can be expanded by the addition of a drop of water or of several drops of dilute detergent (~5 ml in 250 ml of water) or by gentle heating of the wet specimen on the slide over an alcohol lamp.

6. When the material is expanded, use the previous staining and mounting procedure employing corn syrup. If the material came from liquid preservative, add stain directly to the cut sections and follow the steps for making a permanent microscope slide.

7. In order to identify coralline algae, particularly nonarticulated species, special procedures are followed. Having been placed in fixatives designed for their preservation and decalcification (Table 4-1), they are usually sectioned with a freezing microtome or by the paraffin method of slide preparation. A botanical microtechnique

reference (see Table 4-1) should be consulted for these methods. A freezing microtome is used for many algae other than corallines, but for identification purposes, a sharp razor blade, a steady hand, and patience will do a more than adequate job.

3. *Preparation of material for cytological study.* Each phycologist has special techniques for treating various algae. Only a brief explanation is needed here to indicate that, although the methods described earlier are standard and routinely accepted by most workers in the field, specialized disciplines require more attention to the microtechnical aspects demanded by individual algal species. For example, details of nuclear structure, location of pyrenoids, and other organelles will not be sufficiently preserved or retained in the Formalin or alcohol preservatives. Furthermore Karpetchenko fixative or FAA (Table 4-1) is generally better than either Formalin or alcohol but inadequate for chromosome studies. Finally, none of these can be used in the preparation of samples for electron microscopy. It will be sufficient to state here that many more specialized microtechniques exist and are required for detailed studies of marine algae, but they are beyond the scope of the present treatment. The interested reader is referred to Gantt (1980) as a source of many appropriate cytological methods.

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5: Electrophoresis

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