

Collection and Culture Techniques for Gelatinous Zooplankton

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Abstract. Gelatinous zooplankton are the least understood of all planktonic animal groups. This is partly due to their fragility, which typically precludes the capture of intact specimens with nets or trawls. Specialized tools and techniques have been developed that allow researchers and aquarists to collect intact gelatinous animals at sea and to maintain many of these alive in the laboratory. This paper summarizes the scientific literature on the capture, collection, and culture of gelatinous zooplankton and incorporates many unpublished methods developed at the Monterey Bay Aquarium in the past 15 years.

Introduction

Gelatinous zooplankton is a generic term for transparent and delicate planktonic animals with mesoglea-like internal tissues that aid in regulating buoyancy. These animals include some radiolarians and foraminifera, as well as medusae, siphonophores, ctenophores, chaetognaths, pteropods, heteropods, appendicularians, salps, doliolids, and pyrosomes (*e.g.*, Hamner *et al.*, 1975). These taxonomic groups are widely distributed in large numbers in all the world's oceans, throughout the water column. They are the least understood of all planktonic animal groups. This is partly due to their fragility, which typically precludes the capture of intact specimens with nets or trawls. In fact, many systematic descriptions of hydromedusae and siphonophores during the past 200 years were based only on fragments of animals (*e.g.*, Mayer, 1910; Russell, 1953). Fortunately, during the past 30 years, specialized tools and

techniques have been developed that permit researchers and aquarists to collect intact gelatinous animals at sea and to maintain many of these alive in the laboratory. These new methods and technologies have allowed scientists to resolve the life cycles of many organisms whose hydroid and hydromedusa stages were previously thought to be separate species, and to conduct a variety of experimental studies in the laboratory. In addition, aquarists are now able to rear and display many species of medusae and ctenophores for the first time in public aquariums. Paffenhöfer and Harris (1979) and Strathmann (1987) provide good reviews of many gelatinous zooplankton culture studies, as well as a detailed review of culture methods for non-gelatinous organisms. The general lack of information about gelatinous zooplankton is due not only to their extreme fragility, but also to a shift of emphasis in the discipline of biological oceanography that occurred more than 100 years ago. The change led from a qualitative interest in the systematics and developmental biology of all zooplankton, to the present quantitative concern for the fisheries implications of certain components of the zooplankton as they relate to cycles of energy and material in the sea.

This dramatic shift of emphasis was advocated by Victor Hensen (1887). Ernst Haeckel and others used fine-meshed plankton nets towed slowly at the surface from small boats, or they carefully dipped individual animals from the sea surface by hand. In contrast, Hensen (1887) used large, vertically hauled plankton nets from large ships to collect fish eggs and copepods. This procedure produced quantitative information on the distribution and abundance of fish eggs, copepods, and larval forms, but it seriously under-sampled and physically damaged the gelatinous fauna. Even though Haeckel believed that Hensen's approach to oceanic

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biology was flawed, Hensen's attempt to quantify planktonic ecology has prevailed, and the most recent manual on zooplankton methodology (Harris *et al.*, 2000) is still primarily concerned with crustaceans and fish eggs.

Throughout the first half of the 20th century, systematists continued to collect individual gelatinous animals (*e.g.*, Kramp, 1965), but interest in the developmental biology of these animals diminished as Haeckel's ideas about phylogenetic recapitulation through ontogeny lost favor. In the 1950s, neurophysiologists such as Pantin (1952), Bullock (1943), and Mackie (1960) began to investigate the neurology of the so-called lower invertebrates (anemones, medusae, siphonophores, flatworms, and ctenophores), an effort that required that these animals be collected carefully at sea and also maintained alive, if only briefly, in the laboratory.

The first use of scuba to collect or view planktonic animals at sea was in the 1960s by divers who collected siphonophores in the Mediterranean (frontispiece in Totton, 1965) and by Ragulin (1969), who first viewed krill underwater in the Antarctic. Soon thereafter, use of scuba to investigate oceanic gelatinous animals became routine in the epipelagic blue waters of the Gulf Stream (*e.g.*, Gilmer, 1972; Hamner *et al.*, 1975). Research submersibles in midwater extended these *in situ* observations of gelatinous plankton from the upper 30 m of the sea to thousands of meters below the surface (*e.g.*, Larson *et al.*, 1992; Robison *et al.*, 1998; Raskoff, 2001, 2002).

In situ observations of epipelagic and midwater animals stimulated a revival of attention to all aspects of the living biology of gelatinous zooplankton. We know today that gelatinous animals are an important component of marine ecosystems, with particular significance for fisheries management (*e.g.*, Purcell, 1997; Purcell and Arai, 2001), an issue anticipated by Haeckel (1893). We now know that gelatinous organisms are often the dominant macrozooplankton of oceanic ecosystems (*e.g.*, Robison *et al.*, 1998). Recent studies with *in situ* techniques have shown that scyphomedusae, hydromedusae, siphonophores, and ctenophores are abundant, often quite large, and apt to play disproportionately important roles as top predators in their food webs (see Mills, 2001; Purcell *et al.*, 2001). Yet these animals continue to be neglected in many syntheses of biological oceanography.

Our ignorance of gelatinous plankton biology is thus partly due to the history of oceanography, partly to inadequate collection and observational technologies, and partly to the fragility of many gelatinous taxa. Although it is now possible to capture most species of gelatinous animals in good condition, it is still difficult or impossible to keep many of these taxa alive in laboratory aquaria for observation and experimentation. This paper summarizes the scientific literature on the culture of gelatinous zooplankton and incorporates many unpublished culturing and displaying

techniques developed at the Monterey Bay Aquarium during the past 15 years.

Methods

Collection

The primary goal of all collection methods for gelatinous zooplankton is to minimize handling and damage.

Surface collection. Many common species can be collected easily from surface waters by using a small boat or while snorkeling. Ocean slicks, glassy patches on the ocean's surface caused by a combination of wind and current (Haeckel's "animal roads"), are excellent sources of epipelagic species (Alldredge and Hamner, 1980; Hamner and Schneider, 1986; Larson, 1991). Smooth-rimmed glass beakers and glass jars (perhaps attached to the end of a pole) are good collecting containers, since most cnidarian tentacles do not adhere to glass as readily as to plastic. Larger specimens can be collected in plastic buckets or in plastic bags. Some hardy species can be collected with dip nets or small plankton nets. The smaller the mesh size the better, as larger meshes can cut into the soft gelatinous tissue. Knotless mesh of broad, flat strands of soft material in the shape of a gusseted bag is the most effective type of hand-held dip net. It is important not only to minimize the stress and disturbance to the animal, but also to avoid introducing air bubbles into the body cavity; air bubbles are exceptionally difficult to remove, and if left within the animal either produce tissue embolisms or cause the animal to rise to the surface, where exposure to air can damage it further.

Subsurface collection. Although many specimens can be collected in good condition at or just under the surface, others may already be damaged, particularly those garnered from surface convergences crowded with flotsam. Furthermore, for the vast array of species not routinely found at the air-water interface, collectors may need to employ other methods. Use of scuba is one possibility. When working in the disorienting, featureless, blue-water environment of the open sea, protocols for diving-safety must be followed (see Hamner, 1975; Heine, 1986). Divers who are even slightly negatively buoyant can easily sink below safe depths. Also, divers who are not connected to one another and to the support boat often come to the surface away from the boat, where they can be difficult to see in a choppy sea. However, blue-water diving techniques, properly executed, provide a safe and effective way to collect specimens and to observe the behavior of undisturbed animals in their natural habitat. Many ethological discoveries in the last 20 years have been made using these techniques (*e.g.*, Madin, 1974; Hamner, 1985; Matsumoto and Harbison, 1993).

For collection of specimens deeper than the limits of safe scuba diving, or when diving conditions are not optimum, various nets have been deployed successfully from the surface. Midwater trawls, bottom trawls, and plankton nets

can all be effective in capturing delicate living specimens (Sameoto *et al.*, 2000) if the nets are pulled slowly ($<1.0 \text{ km h}^{-1}$) for a relatively short time, and if the cod end of the net is large and without side windows, which generate turbulence in the collecting well (Baker, 1963; Reeve, 1981; Childress and Thuesen, 1993). Thermally insulated cod ends have also proved very successful in the capture of gelatinous organisms in good physiological condition (Childress *et al.*, 1978; Thuesen and Childress, 1994). The use of research submersibles and remotely operated vehicles (ROVs) has permitted gelatinous species to be collected from meso- and bathypelagic depths when the use of nets is not an option (Youngbluth, 1984; Robison, 1993). These vehicles may be equipped with large collection cylinders open at both ends, permitting the vehicle pilot to slide the collecting container over a gelatinous animal by maneuvering the entire vehicle and then gently closing the ends of the sampler. Some species are so delicate that they have never survived even these samplers: descriptions of several deep-sea species, such as *Kiyohimea usagi* (Matsumoto and Robison, 1992) and *Lampocteis cruentiventer* (Harbison *et al.*, 2001), were based on *in situ* observations and photography.

Transport of specimens

Once collected, specimens can be safely transported to the rearing facility either in their collection containers or in larger jars or tubs. If the animals are transferred to a larger container, it is important to minimize their exposure to air and to avoid pouring them roughly from one container to the other. Each animal must be dipped gently out of the collecting container with a transfer jar, which is then emptied by tipping it below the surface of the water in the transport container. Several devices have been designed for the transfer of individual gelatinous zooplankton (Acuña *et al.*, 1994; Sato *et al.*, 1999). The water in the transport containers should be free of bubbles and have the same temperature and salinity as the water in the collecting vessel; if not, small volumes of water should be exchanged between them slowly, over the course of perhaps an hour, to permit temperature and osmotic adjustment by the animals. Transport vessels can then be put into an insulated box or cooler with cold or hot packs as needed for the duration of the trip. The water can be saturated with oxygen before transport, but this is more crucial for large animals, those being shipped in warm water, or those kept in the shipping container for a long time. All air must be removed from the container before sealing because even small air bubbles can damage gelatinous specimens. With an appropriately low ratio of biomass to volume of water ($<1:2$), the animals often survive trips of 18 h or more. For small medusae and polyp cultures, air-permeable plastic fish bags are very effective.

Culture

Once organisms have been collected, cultures can be started in a number of ways. The most common method is to facilitate natural spawning by grouping both sexes together in a small controlled space. Spawning can often be induced by crowding (some scyphozoans), by leaving animals in the dark for several hours followed by periods of light (some hydrozoans), or by simply permitting the temperature of the water to slowly rise over several hours (see Mills and Strathmann, 1987). In some cnidarian genera, such as *Aurelia*, females brood their planulae on their oral arms. It is often sufficient to place the brooding female in a small volume of seawater and wait for a few hours for the planulae to be released. Alternatively, larvae may be removed from the edges of the oral arms with a pipette. A more labor intensive spawning technique involves *in vitro* fertilization. For this procedure, gonadal tissue from both males and females are incubated together in a small volume of water for several hours until the eggs are fertilized and larvae begin to develop. The sexing of zooplankton can be difficult, but the eggs can often be seen inside the female reproductive tissue. The most accurate way to determine the sex of the specimens is to remove a small piece of the gonad and examine the tissue under a compound or dissecting microscope to look for sperm and eggs. This will also help determine if the specimen is mature.

After a spawning event, it is necessary to examine the water for larvae or fertilized eggs. Collection and handling techniques for many larval taxa are summarized in Strathmann (1987). Planulae range in length from 100 to 1000 μm in hydrozoans, range from 100 to 400 μm in scyphozoans, and up to 160 μm in cubozoans (Martin and Koss, 2002). Ctenophore larvae range in length from 280 to 1000 μm (Baker and Reeve, 1974; F. Sommer, unpubl. data).

Cnidarian planulae will typically settle and attach to the substrate within a few days, often within hours if a suitable substrate is available. Planulae will settle on many types of substrate (Brewer, 1984). Glass or plastic microscope slides or cover slips are often used due to the ease of post-settlement manipulation. Some species may preferentially settle on substrates that have been "conditioned" by several days' immersion in seawater to accumulate a light microbial film (Brewer, 1984; Schmahl, 1985). Several chemicals (TPA, DAG, Cs^+ , Li^+ , NH_4^+) have been shown to positively affect larval settlement (Siefker *et al.*, 2000). Larvae typically settle to the bottom of the chamber and often are thigmotactic, tending to settle at the edges (Brewer, 1976; Orlov, 1996). Some species (*Aurelia aurita*, *Cyanea capillata*, *Ptychogena lactea*) are also light sensitive and will settle under opaque objects such as small rocks or shell (Custance, 1964; Brewer, 1978, 1984; Raskoff, unpubl. data), while others (*Clava multicornis*) are positively phototactic (Orlov, 1996). There is evidence that some species

settle preferentially in areas with a high density of conspecifics (Keen, 1987). Some larvae may also settle at the air-water interface, attaching upside down onto the surface film (Pagliara *et al.*, 2000). These can be dislodged by gently disturbing the surface tension with a drop of water, whereupon the polyps drop to the bottom and reattach to a benthic substrate. Additionally, planulae will attach to a floating substrate that is gently placed on the water surface. Larvae induced to settle on microscope slides can be raised off the bottom of the culture chamber after they have started to reproduce asexually, and inverted so the polyps hang upside down. This facilitates their feeding and allows their wastes to fall to the bottom of the tank, reducing fouling.

With consistent feeding and a debris-free environment, healthy polyps will generally grow and produce juvenile medusae. However, several treatments can be used to initiate or speed up the process. Scyphozoan polyps typically produce juvenile medusae by the process of strobilation, which can be induced in various ways. These include brief temperature increases of ≈ 5.0 °C, prolonged (4–6 weeks) reduction of water temperature by ≈ 5 –10 °C followed by a return to normal temperatures over a few days, and changes in the amount of feeding (Abe and Hisada, 1969; Calder, 1974; Cargo, 1975). Other inducers found to have some success are changes in illumination level and pH, increases in salinity, and treatment with various chemicals (iodine, thyroxine, etc.) (Spangenberg, 1971; Olmon and Webb, 1974).

When the polypoid phase begins to release juvenile medusae, it is helpful to remove them from the culture chamber and place them into a rearing tank as soon as possible. Young ephyrae and hydromedusae can be injured or eaten by other members of the polyp colony. The young medusae will often be swept out the outflow of the polyp culture tank (Fig. 1) and into the grow out tank, but transporting them *via* a large-diameter pipette is preferable because it reduces the stress on the juvenile medusae.

The medusae of most species can be placed directly into flow-through or aerated rearing tanks after release (Fig. 1), although some species such as *Pelagia colorata* and *Aequorea victoria* respond better if first placed into small dishes with still, filtered water for several days to weeks. Juvenile medusae may need to be transferred into several grow-out tanks of increasing sizes and decreasing conspecific densities throughout their development, depending on the species and size of the medusae (Spangenberg, 1965).

Once a polyp culture has been started, it is often necessary to propagate the polyps in additional culture containers. Propagated polyps can be used to set up replicate cultures for experimentation, for transfer to other researchers or aquarium facilities, or as backup in case of problems. Both hydrozoan and scyphozoan polyps can be removed from substrates by gently scraping with a small instrument, such as a plastic toothpick or a trimmed, hard-bristled paint

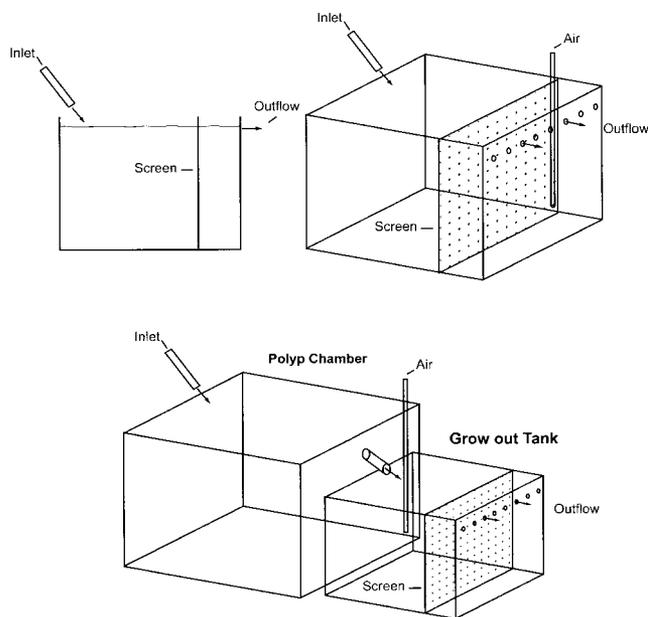


Figure 1. Flow-through culture tanks and grow-out facility. Tank sizes should allow for free, unrestricted feeding and movements of specimens. Mesh sizes should be smaller than the smallest dimension of the organism.

brush. Razor blades or narrow-tipped utility knives are helpful for scraping polyps off smooth, flat surfaces such as glass slides. Once removed, polyps are placed into separate tanks and allowed to resettle. Many species reattach quickly when simply resting on the bottom of a dish; others may take longer. One method for raising these polyps off the bottom to facilitate feeding is to tie a tight loop of small-gauge monofilament line around, or slip a small rubber band over, a glass microscope slide, and then insert the base of the polyp under the line on the flat portion of the microscope slide (Groat *et al.*, 1980; F. Boero, Universita di Lecce, Italy, pers. comm.). The tension of the monofilament line holds the polyp next to the surface of the slide without cutting through the stalk of the polyp. The microscope slides can then be inverted, allowing the polyp's tentacles to hang freely. After several days to weeks, the polyp will attach to the slide, and the monofilament can be cut and removed. Asexual reproductive bodies, such as cysts and frustules, can also be removed from the original tank to seed a replicate culture. Cysts can be removed by scraping, and the damage caused to the capsule of the cyst sometimes stimulates excystment and subsequent growth of the polyp, as can changes in temperature (Brewer and Feingold, 1991). Swimming frustules are produced in some species (hydroid example: *Craspedacusta*; scyphozoan example: certain rhizostomes such as *Cassiopeia* and *Mastigias*), and these can be pipetted into a dish where they will settle and develop into polyps. After settlement, the dish can be transferred to a flow-through tank.

The use of antibiotics to aid in the culture of gelatinous

organisms has not had much study. Strathmann (1987) lists several antibiotics and fungicides that might help fight infections. The antibiotic tetracycline has been used to treat bacterial infections on large scyphomedusae. After being placed in a 20-ppm bath for 2 h a day, 5 days in a row (B. Upton, Monterey Bay Aquarium, pers. comm.), the infected medusae improved markedly. This technique shows great promise for treating the common "bell rot" encountered with many large medusae.

Feeding

Among the types of food that can be used to feed gelatinous zooplankton are *Artemia* nauplii, krill, chopped squid and fish tissue, medusae, wild plankton (copepods, etc.), rotifers, trochophore larvae, agar-based foods, algae, bivalve hepatopancreas, and "grow-lights" for those species of medusae with zooxanthellae. *Artemia* nauplii are the most common food items used in culture of polyps and medusae and provide the backbone of most species' diets in laboratory conditions. Most species can be fed *Artemia* daily, but some very small polyps may have difficulty capturing and ingesting prey of this size (about 400 μm). Tentaculate ctenophores thrive on *Artemia*, but non-tentaculate beroid ctenophores need gelatinous prey. The lack of appropriate food items is a major stumbling block for the culture and study of many gelatinous taxa. For example, the natural food of many pteropods is other species of pteropods, which are difficult to culture in the laboratory; therefore, even if the animals themselves can be successfully maintained in tanks, providing them with adequate nutrition over long periods of time is a challenge (Conover and Lalli, 1972).

Hatching times and water temperatures vary between the different species and strains of *Artemia*, so recommendations provided by the supplier should be consulted. After hatching, the nauplii should be fed for a day or so with a food supplement (Super Selco, Algamac, algae, yeast, etc.). By enriching the content of protein and free amino acids in the nauplii acid (Helland *et al.*, 2000), these supplements contribute to the subsequent growth and health of the animals to which the nauplii are fed. The nauplius must have a mouth (2nd instar stage) before it can ingest the enrichment medium, which must be dispersed (emulsified, aerated, or otherwise kept in the water column) so that the nauplii can eat it. The "shells" of the *Artemia* cysts can be removed to reduce fouling and increase hatching efficiency. Several methods of cyst decapsulation are available on the Internet. Decapsulated cysts can be kept for extended periods, refrigerated in water, until they are needed.

Krill, squid, and other large or fleshy prey can be cut or homogenized to an appropriate size and fed to many polyps, medusae, and heteropods. A disadvantage is that these food items quickly sink to the bottom of the tank and thus are

available for capture only briefly. These foods must be removed or they rot and promote growth of fouling organisms. Live *Aurelia* and other medusae are a good and sometimes necessary dietary supplement for many medusivorous jellyfish, including *Pelagia*, *Cyanea*, *Chrysaora*, *Phacellophora*, and *Aequorea*. Smaller stages of these medusivores can be fed *Aurelia* ephyrae, finely diced adult medusae, or small hydromedusae. Small, newly released hydromedusae, such as *Aequorea*, *Eutonina*, and *Bougainvillea*, are especially important in the diet of *Pelagia colorata* ephyrae, which are difficult to raise on *Artemia* alone (Sommer, 1993). Wild-caught plankton also offer an important dietary supplement to gelatinous zooplankton in culture. Live copepods are desirable for tentaculate ctenophores and scyphomedusae. Recent research has pointed out the importance of utilizing natural prey whenever possible. The reason that at least two species of naturally bioluminescent medusae do not produce light when reared in the laboratory is a dietary deficiency of the luciferin coelenterazine (Haddock *et al.*, 2001). Thus, even seemingly healthy cultured animals may not receive all of their nutritional needs from convenient laboratory prey, and alternative or supplemental foods should be tried routinely.

Small and newly metamorphosed animals can be difficult to feed due to their diminutive size. Various live single-celled algae, such as *Tetraselmis* spp., *Isochrysis galbana*, and *Nannochloropsis* spp., can be valuable food sources for small polyps, as well as for filter-feeding salps and doliolids (Paffenhöfer, 1970, 1973; Heron, 1972; Paffenhöfer and Harris, 1979). Rotifers ($\approx 100\text{--}200\ \mu\text{m}$), such as *Brachionus plicatilis*, and oyster trochophores ($\approx 50\ \mu\text{m}$) are in the right size range for capture and consumption by polyps, which may be unable to consume the much larger *Artemia* nauplii. Rotifers can be fed on the above algae as well. All of the above prey items are commercially available. Rotifers and algae are easily cultured in tanks similar to those used for *Artemia*, and the trochophores can be purchased frozen. Another food that has been used with some success is agar-enriched medium. Homogenized food items mentioned above, as well as amino acids, lipids, and protein sources, can be mixed into heated agar and, when cooled, a gel is formed. This gel can be cut into small pieces and fed by hand to polyps, medusae, and beroid ctenophores. This is labor intensive but useful for some species that are otherwise difficult to feed. Another common feeding technique uses bivalve hepatopancreatic tissue, finely chopped and cleaned in successive changes of seawater. These pieces are then hand-fed to individual polyps. Common intertidal copepods can be cultured in shallow pans as a food source.

Several species of medusae depend on the photosynthetic products of zooxanthellae for nutrition. In addition to a normal diet of prey, these species require a strong light with an appropriate action spectrum for photosynthesis by the zooxanthellae. The type and power of the light can be

variable depending on tank size and depth. For example, at the Monterey Bay Aquarium, the scyphozoans *Mastigias papua* and *Cassiopeia xamachana* have been reared for several months in tanks with metal halide and actinic or daylight fluorescent lamps.

Tanks

Benthic stages. With careful cleaning and frequent water changes, benthic hydroids and polyps can be kept in simple jars and dishes (e.g., Miglietta *et al.*, 2000). However, when dealing with large cultures, or when flowing water is desired for efficient feeding, more complex facilities are needed. Rees and Russell (1937) designed the first successful large-scale culture system for cnidarian polyps. This consisted of rows of glass beakers that held the polyps, and vertical microscope slides attached to a rocker arm driven by an automatic pipette washer. This moved the slides gently forward and back at the top of the beaker, keeping the water stirred and aerated, and the food in suspension. This type of system has also been used to raise a variety of larvae (Strathmann, 1987). The water in the beakers was changed and the beakers were cleaned regularly. A better arrangement for polyp cultivation uses flowing seawater, and the culture tank therefore requires an incoming water line and an exit drain. Rectangular clear plastic boxes of various sizes (pet cages available from most pet stores) make ideal culture and grow-out tanks. Plastic containers can be easily modified and are inexpensive, but any small tank can suffice. Depending on the purpose of the tank, the drainage can flow into another tank to collect newly released medusae, or the drainage can be screened off with mesh (Fig. 1). If the exit drain is to be screened, the screen mesh must be smaller than the smallest medusae that will be released (mesh sizes of 120–500 μm are commonly used). In addition, the surface area of the exit screen must be maximized so that the drain pressure at any one point is low enough to prevent the medusae from being trapped against the screen. Screens are typically put across one entire side of the tank, several centimeters from the drain.

A simple way to set up a “medusa factory” using this technique is to clip a beaker or dish containing polyps to an edge of the culture chamber, suspending it slightly above the water level of the tank. Incoming water runs into the polyp beaker and spills over the side into the tank (Sommer, 1993). In this manner, newly produced medusae are washed out of the polyp beaker into the catch tank, where they will be safe from capture by the polyps (Fig. 1A). Alternatively, the polyps can be kept at the bottom of a tank without a screened-off outflow. As the medusae are produced, they tend to swim up; eventually most will go out the outflow. A second catch tank with a screened-off outflow is placed below to collect the juvenile medusae (Utter, 2001). This catch tank can be of similar design to the tanks described

above (Fig. 1B). The addition of an air line close to the screen in any catch or grow-out tank will cause bubbles to rise along the screen, and these will create a gentle upward current that encourages juvenile medusae to stay up in the water column and off the screen.

Pelagic stages. Tanks for pelagic animals offer unique challenges, but the aim is to mimic a natural environment as closely as possible. The vast majority of gelatinous zooplankton are pelagic, and their tanks must minimize contact between the animal and all tank surfaces. That being said, many gelatinous taxa have been maintained or cultured in the laboratory in nothing more than jars or aquaria of still water in temperature-controlled environments. Radiolarians (Sugiyama and Anderson, 1997) and foraminifera (Hemleben and Kitazato, 1995) have been kept for extended periods in small jars and culture dishes. Reeve (1970) and Reeve and Walter (1972) raised chaetognaths in 30-l aquaria with daily water changes. Conover and Lalli (1972) kept the pteropod *Clione limacina* “indefinitely” in small dishes and beakers with filtered water. Baker and Reeve (1974) and Martindale (1987) raised the ctenophore *Mnemiopsis mccradyi* in 30-l aquaria with gentle aeration, but had very low survival. Hirota (1972) used large jars for the culture of the ctenophore *Pleurobrachia bachei*. Heron (1972) raised the salp *Thalia democratica* in small tanks with lids that prevented the salps from encountering the air-water interface. Many researchers continue to raise small hydromedusae, ctenophores, and other gelatinous organisms in dishes, small-volume culture plates, and jars of various sizes (Rees, 1979; Mills *et al.*, 2000).

The standard pelagic tank designs used today are all variations of the planktonkreisel designed originally by Greve (1968, 1970, 1975), which was modified and re-designed for shipboard use by Hamner (1990) and for public display by the Monterey Bay Aquarium (Sommer, 1992, 1993). Paffenhöfer (1970) described a rotating culture apparatus used very successfully for copepods, appendicularians, and doliolids, which has been modified to various degrees (e.g., Sato *et al.*, 2001; Gibson and Paffenhöfer, 2000). Ward (1974) described some simple aquarium systems for maintenance of ctenophores and jellyfish. Dawson (2000) devised a horizontal mesocosm that stratified by various salinity layers and may hold promise for species that require complex water masses for development. The planktonkreisel design, however, has proved to be the most useful, and it has been modified over the years into several designs that offer more complex flow patterns and easier access to the inside of the tank and to the animals (Sommer, 1992, 1993). Despite these alterations, the basic principles of the planktonkreisel remain unchanged.

The main chamber of the tanks is circular, with curved sides and bottom and a flat back and front (Fig. 2). The water inlets and drains are designed to keep organisms from coming in contact with the screen that shields the drain.

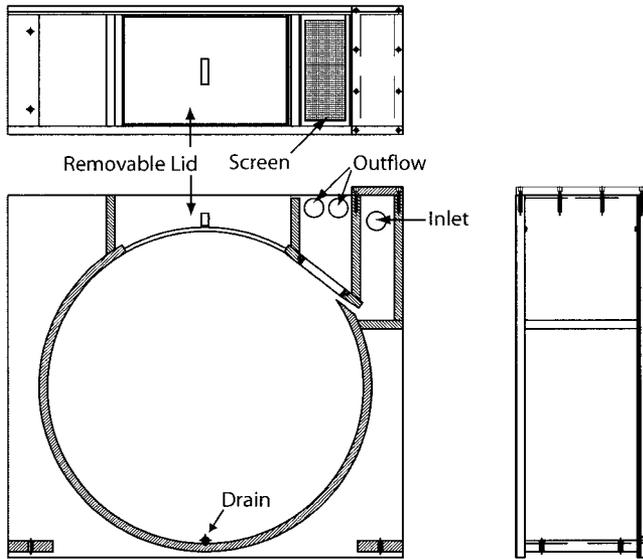


Figure 2. Contemporary planktonkreisel design showing separated inlet/outlet chamber and tank access lid. Detailed plans of this tank are available online at <http://www.mbari.org/midwater/tank/tank.htm>.

Water flows from the inlet chamber and jets in a laminar flow across the lower side of a fine-mesh screen, which separates the main tank from the drain outflow. In this way any specimen that drifts near the outflow screen will be pushed away by the incoming water. The placement of a few parallel layers of polycarbonate double-wall sheet, commonly used as greenhouse siding, into the space between the inlet chamber and the main tank will force the inlet water to enter with a smooth laminar flow. Modifications to the planktonkreisels made by the Monterey Bay Aquarium include the construction of a separate outflow and

lid, which allows animals to be put into or removed from the kreisel without danger of being sucked down the drain. A larger lid allows for easier access into the tank for cleaning and manipulation of the specimens (Sommer, 1993). For scientific purposes, a matte black back plate allows for side lighting of transparent plankton, achieving dark-field illumination (Hamner, 1990). For display aquaria, a matte translucent blue-and-white acrylic back, illuminated from behind with fluorescent lamps, can be used to create the appearance of a lifelike blue-water environment. Spotlights from the sides of the tank are used to illuminate animals for display or photographic purposes. Strong lights do not appear to bother many gelatinous species, which typically have limited visual equipment. Most gelatinous organisms can do well in planktonkreisels (see Tables 1 and 2 for a summary). Plans of a planktonkreisel developed by Kim Reisenbichler at the Monterey Bay Aquarium Research Institute (Fig. 2) are available for download at <http://www.mbari.org/midwater/tank/tank.htm>.

Another variation on the planktonkreisel design is the stretch kreisel, or Langmuir kreisel (Fig. 3). The tank has two inlet/outlet chambers that are located on each side of a rectangular tank, sending flow upward. The dimensions of the rectangular tank (still with circular ends) must be about twice as wide as tall, permitting the formation of two gyres, one of which rotates clockwise and the other counterclockwise. The top of the tank is open and the flows meet in the middle, where they are joined by water added from a horizontally positioned perforated tube, creating convergent currents that descend down the center of the tank. The two opposing circular flows result in downwelling at the center of the tank and upwelling at either end. This design works well with species that tend to swim actively into a current

Table 1

Selected culture techniques for medusae commonly used for display

Species	Diet	Temperature (°C)	Lifespan	Tanks*
Scyphozoa				
<i>Aurelia aurita</i>	<i>Artemia</i> , Krill	10–15	2–4 y+	K, PK, HP
<i>Aurelia labiata</i>	<i>Artemia</i> , Krill	10–15	2–4 y+	K, PK, HP
<i>Phacellophora camtschatica</i>	<i>Artemia</i> , Juvenile <i>Aurelia</i> , Krill	10–15	1 y+	K, PK, SK
<i>Pelagia colorata</i>	<i>Artemia</i> , Juvenile <i>Aurelia</i> , Krill	10–15	1 y+	K, SK, PK
<i>Chrysaora fuscescens</i>	<i>Artemia</i> , Juvenile <i>Aurelia</i> , Krill	10–15	2 y+	K, SK, PK
<i>Cassiopeia xamachana</i>	<i>Artemia</i> , Lighting	24–27	1 y+	RF
<i>Mastigias papua</i>	<i>Artemia</i> , Lighting	27–29	3 mo+	K, PK, HP
Hydrozoa				
<i>Aequorea victoria</i>	<i>Artemia</i> , Rotifers (hydroids), Juvenile <i>Aurelia</i> , <i>Eutonina</i>	10–15	6 mo+	K, PK
<i>Eutonina indicans</i>	<i>Artemia</i> , Rotifers	10–15	3 mo+	K, PK
<i>Polyorchis penicillatus</i>	<i>Artemia</i>	10–15	3 mo+	PK, K
<i>Craspedacusta sowerbii</i>	Wild freshwater plankton, Frozen <i>Daphnia</i>	Freshwater 27	<3 mo	K, RT
<i>Tima formosa</i>	<i>Artemia</i> , Rotifers	24–27	6 mo+	PK, K

Data summarized from Sommer (1992, 1993) for the Monterey Bay Aquarium.

* K = Kreisel; PK = Pseudokreisel; SK = Stretch kreisel; RF = Reverse flow; HP = Horizontal pseudokreisel; RT = Rectangular tank.

Table 2

Selected culture techniques of non-cnidarian gelatinous zooplankton

Species	Diet	Temperature (°C)	Lifespan	Tanks*	Reference
Ctenophores					
<i>Pleurobrachia bachei</i>	<i>Artemia</i>	10–15	2 mo+	K, PK	Sommer, 1992
	Wild-caught zooplankton	15	nd	Large jars	Hirota, 1972
<i>Pleurobrachia pileus</i>	Wild-caught copepods	15	8 mo+	Modified PK	Greve, 1970
<i>Bolinopsis infundibulum</i>	<i>Artemia</i>	10–15	8 mo+	K, PK	Sommer, 1992
	Wild-caught zooplankton	16	7 mo	Modified PK	Greve, 1970
<i>Mnemiopsis mccradyi</i>	Wild-caught zooplankton	21–31	<2 mo	30 l tanks	Baker and Reeve, 1974
<i>Beroe</i> spp.	Ctenophores, gelatin	10–15	<3 mo	K, PK	Sommer, 1992
<i>Beroe gracilis</i>	<i>Pleurobrachia pileus</i>	15	6 mo	Modified K	Greve, 1970
<i>Beroe cucumis</i>	<i>Bolinopsis infundibulum</i>	15	1 mo+	Modified K	Greve, 1970
Molluscs					
<i>Clione limacina</i>	None	10–15	2 mo+	PK, K	This study
	Wild-caught pteropods	12–14	4 mo+	Dishes	Conover and Lalli, 1972
<i>Cliopsis krohni</i>	None	10–15	6 mo+	PK, K	This study
Chaetogranths					
<i>Sagitta hispida</i>	Copepods	17–31	2 mo+	30-l tanks	Reeve, 1970; Reeve and Walter, 1972
	nd	nd	nd	Modified K	Greve, 1968
Pelagic Tunicates					
<i>Oikopleura dioica</i>	Cultivated phytoplankton	13	8–12 days	RJ	Paffenhöfer, 1973
<i>Friillaria borealis</i>	Cultivated phytoplankton	12	nd	RJ	Paffenhöfer and Harris, 1979
<i>Thalia democratica</i>	Phytoplankton	nd	8–20 days	Large jars	Heron, 1972

nd = no data.

* K = Kreisel; PK = Pseudokreisel; RF = Reverse flow; RJ = Rotating jars.

(such as *Chrysaora fuscescens*), since they will tend to congregate in the center of the tank, away from the walls (Tables 1 and 2).

Any rectangular tank can be modified into a “pseudo-kreisel,” but care must be taken to ensure that the height and width of the tank are about equal, or the water in the tank will not be able to rotate in a perfect circle and will create areas within the tank of limited flow where the animals may accumulate and contact the sides. Rectangular tanks are modified by glueing a screen across the upper corner at an angle of about 30°–40° from vertical in front of the overflow (Fig. 4). Water enters the tank through a perforated tube positioned so that the flow sweeps across the screen down towards the bottom of the tank. It is important that the tube is positioned so that the flow is parallel to the screen and covers the entire screen so that specimens are swept away rather than drawn against it. Curved plastic or vinyl inserts are glued with silicone into the bottom corners to round them into a more circular shape. Friction-fitting stiff screens can also be used to round the corners, although this option makes the tank more difficult to clean and maintain than one with solid corners.

Water

Several water quality issues are important for the successful culture and rearing of gelatinous organisms. Temperature and salinity must be kept within a range appropri-

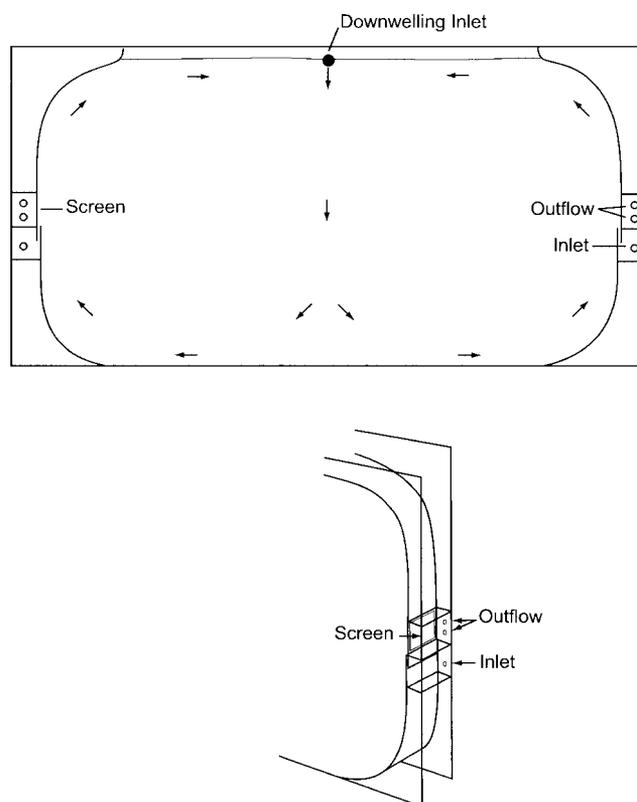


Figure 3. Stretch kreisel design showing the two rotating Langmuir cells set up by the placement of the side and downwelling inlets.

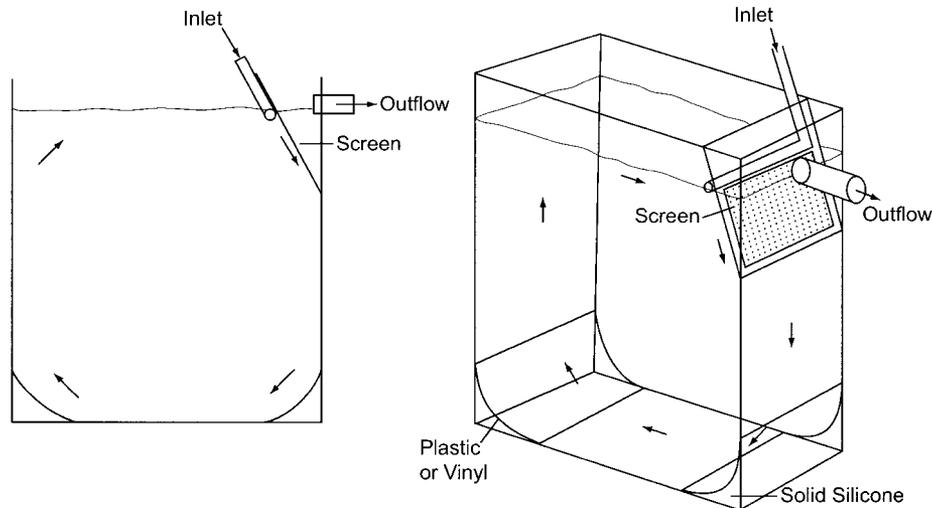


Figure 4. Pseudokreisel design made from a standard tank. Bottom corners are filled in with silicone and solid pieces of plastic or vinyl. Outflow is separated from the tank by the inlet and screen.

ate for the species being reared. The water must be relatively clean and filtered, especially if the animals are to be used for any display purpose. Small particles in the water will quickly clog the outflow screens. Filtering the water with 20- μm pleated cartridge filters is usually sufficient; however, some cultures that are very sensitive to biological fouling (such as many hydroid species) may need additional filtration to the 3 μm level. Although air bubbles can be helpful in the culture of many small gelatinous animals by increasing water circulation, they can be detrimental to larger adult sizes (>3 cm). The bubbles can be ingested and collect in the gut and radial canals of medusae and ctenophores, causing the animals to become positively buoyant, disrupting their normal swimming and feeding behaviors. A more serious problem is that these bubbles will slowly work themselves through the mesoglea, which can lead to infection. A degassing system for the water may be needed if the incoming water tends to be supersaturated. A degassing tower in which the water trickles down through small plastic balls or other material serves to degas the water before it enters the tank. Deep-sea animals may be sensitive to the high oxygen concentrations of surface waters. Reducing the oxygen concentration in tank water by bubbling nitrogen gas has been used in the past with some success, although it does not appear to be critical for most deep-sea species.

Maintenance

Throughout the course of feeding and rearing, tanks accumulate debris that should be removed regularly. The use of pipettes, small brushes, basters, and siphons for removing larger debris, including waste, uneaten *Artemia*, and other food items, will help keep the tanks clean and discourage fouling growth. Pipettes of any size and type can be used to

gently lift and collect debris. Kitchen basters work well for removing larger items because of their large reservoir volume and wide bore. Siphons are best constructed from small-bore acrylic tubes with flexible plastic tubing attached, so that the tubing may be pinched to stop flow if an animal gets too close to the suctioning tip. Additionally, siphoning the “waste water” into a temporary container allows for the retrieval of any specimen that might inadvertently be removed. To protect the insides of the tanks from scratches, it is helpful to dip the end of the acrylic tube into liquid plastic, available from most hardware stores; alternatively, a small ring of Nalgene tubing may be placed on the end of the siphon tube. Floating layers of lipid-rich materials can be removed by skimming with small jars or beakers or fine-meshed nets, or by absorbing the material onto paper towels floated on the surface of the water. The sides of the tanks can be cleaned by wiping with brushes (firm paint brushes work well) or non-abrasive pads. For larger tanks (>75 l), painting or scrub pads can be covered with nonabrasive nylon mesh fabric and attached to poles for cleaning hard-to-reach areas of the tanks. The wood or metal handles of these scrubbers can be covered in plastic tubing to reduce the adherence of tentacles. Flow to the tanks can also be temporarily shut off and the animals allowed to collect on the bottom of the tank during cleaning. Also, tanks can be cleaned just after the animals have been fed, when tentacles are typically retracted and less apt to become ensnared (C. Widmer, Monterey Bay Aquarium, per. comm.). Screens in the tanks collect debris quickly and need to be scrubbed and cleaned at regular intervals. When screens become clogged, organisms are more likely to stick to them, possibly with fatal results.

Even with proper cleaning and filtration, biofouling in

culture and rearing tanks can become a serious problem. In some cases of diatom and algae fouling, reducing the light that shines on the tank can help reduce growth, but typically, scrubbing the tanks eventually becomes necessary. When diatom, hydroid, or other fouling organisms cannot be satisfactorily removed by any of the means discussed previously, bleaching is necessary. This can be especially useful on the screens, pumps, and waterlines, which can be very difficult to clean by other means. The entire tank system may need to be bleached every 1–6 months, depending on the size and fouling rate. During bleaching, the occupants of the tank must be removed and transferred to a holding facility. The longer the tanks and lines are allowed to bleach, the more complete the fouling kill will be. Overnight is preferred, but bleaching for even an hour kills most fouling organisms. As a rule of thumb, 1 l of standard 3%–6% sodium hypochlorite (NaOCl) bleach will treat about 200 l of water (≈ 1 gallon bleach/800 gallons of water), but this amount can be increased or decreased depending on the severity of the fouling and the time available to let the tank bleach. The water level in the tank should be dropped so that there is no overflow when the bleach is added. If the tanks have self-contained pumps, these should be run at a high flow rate to mix the bleach and flush it into the pump housings.

To complete the process, the bleach must be neutralized. This can be accomplished by adding about 60 g of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) per liter of bleach used (≈ 1 cup/gallon). The sodium thiosulfate crystals may be dissolved in a bucket of water prior to adding to the tank. When the color of the water in the aquarium changes from yellow-green to clear, sufficient thiosulfate has been added for neutralization. Allow the thiosulfate several minutes to run through the entire tank and pumps. The treated water is then drained from the tank and discarded. While draining, thoroughly rinse out the tank with freshwater. Stubborn growth can be removed at this time by scrubbing. After all debris and treated water is removed, begin to refill the tank with seawater, minimizing turbulence and bubbles during the refilling since bubbles will stick to the walls of the tank and will have to be removed before gelatinous animals are returned.

Discussion

The use of the techniques described herein for the capture, culture, and rearing of gelatinous zooplankton has allowed researchers to address many important biological issues. Historically, these contributions were limited primarily to the disciplines of systematics, developmental biology, and evolution. More recently, new advances in our understanding of behavior, physiology, ecology, and oceanographic processes from the sea surface to the abyssal depths have also been possible.

Through the use of culture methodologies, laboratory-based experimentation on salps and larvaceans has begun to address important ecological questions about the role these animals play in the nutrient cycling of the oceans and their impact on the ecosystem. These organisms have some of the fastest generation times and largest nutrient turnovers in the world, and their fecal pellets and associated “marine snow” are important sources of carbon transport into the deep sea (*e.g.*, Alldredge, 1972; Silver *et al.*, 1998).

Recent laboratory studies have shown that some species of medusa have chemically-regulated feeding behaviors (Arai, 1991, 1997; Tamburri *et al.*, 2000), with several different chemical stimuli controlling the feeding and swimming of both hydrozoan and scyphozoan medusae. Tank-based studies on the vertical migration of medusae (Mackie *et al.*, 1981; Mills, 1983) and on their swimming and feeding behaviors (*e.g.*, Costello and Colin, 1995; Suchman and Sullivan, 2000) have provided much information on the physiological and behavioral components of medusa locomotion as it relates to prey selection and capture.

The interactions between gelatinous zooplankton and humans are increasing, whether from envenomation (Burnett, 2001); blooms that clog power plant intakes (Masilamoni *et al.*, 2000); interactions, both positive and negative, with fisheries (Mutlu *et al.*, 1994; Mutlu, 1999; Mills, 2001; Purcell and Arai, 2001); or the general increase in gelatinous zooplankton populations in perturbed or eutrophic environments (Mills, 1995, 2001; Arai, 2001). The opportunities for scientific studies of gelatinous zooplankton are vast and largely untouched. We hope researchers can use some of the techniques presented here to expand the research being done on these important but poorly understood marine organisms.

The public's fascination with and appreciation of gelatinous zooplankton is growing rapidly. What were once considered nasty animals that might sting or otherwise disturb beachgoers are now a major attraction in public aquaria all over the globe. The time and money spent by the aquarium industry to provide compelling exhibits on gelatinous zooplankton is a testament to their appeal. Over 3.4 million people visited the Monterey Bay Aquarium during the temporary “Planet of the Jellies” exhibit in 1992 and 1993 (Powell, 2001; J. Tomulonis, Monterey Bay Aquarium, pers comm.). Jellyfish and ctenophores were given permanent starring roles in the Outer Bay Wing, and in a new temporary exhibit, “Jellies: Living Art.” Aquarists in the United States and elsewhere are responsible for many of the techniques discussed in this paper. Aquariums around the world provide the bulk of the layperson's information on gelatinous zooplankton, and we hope that the rising public appreciation of these important and beautiful animals may lead to increased financial and societal support for their continued study.

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