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COLLECTING AND PROCESSING LEPTOSTRACANS

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INTRODUCTION

Leptostracans (order Leptostraca), or "thin-shelled shrimps," are distinguished from other crustaceans by the presence of several key anatomical features, including a movable rostrum, an antennular scale, a folded carapace that encompasses the thoracic and anterior abdominal somites, and eight phyllopodous thoracic limbs (Hessler and Schram, 1984). The abdomen, which projects from the posterior margin of the carapace, consists of eight pleonites, and a single pair of uropods arises from the terminal segment. All leptostracans possess six pleopods, the first four of which are pronounced and are the most conspicuous limbs of the animal (Martin et al., 1996).

As currently understood, leptostracans are represented by 10 genera and 43 nominal species. The actual alpha-diversity of the order is much greater. As with most crustacean taxa, leptostracans are relatively small crustaceans (often <1 cm total length), and therefore they often are underrepresented if sampling bias exists due to design and/or the interest of the investigator. Positive evidence for greater alpha-diversity of Leptostraca exists with numerous collections of undescribed species in various museum and private holdings.

ECOLOGY

For what is considered to be a small group, the leptostracans inhabit a surprisingly broad array of marine habitats. The majority of leptostracans are epifaunal. Species such as *Nebalia daytoni* Vetter, 1996, *Sarsinebalia cristoboi* Moreira, Gestoso and Troncoso, 2003 and *S. urgorrii* Moreira, Gestoso and Troncoso, 2003, have been reported from oligotrophic coarse sands and gravel (Vetter, 1996; Moreira et al., 2009). Nearly all species described to date, however, reside in areas with accumulations of organic material and more fine-grained sediments. A few species have been collected from relatively shallow waters, including the high intertidal zone. *Nebalia gerkenae* Haney and Martin, 2000, *N. kensleyi* Haney and Martin, 2005, and *N. pugettensis* (Clark, 1932), for instance, all inhabit algal mats in estuarine environments along the Pacific coast of the Americas (Haney and Martin, 2000). Species of *Nebalia* are known from seagrass communities as well (Rainer and Unsworth, 1991). *Paranebalia* Claus, 1880 is a pantropical genus that includes four nominal species, known primarily from shallow-water coral reefs. Most leptostracan taxa inhabit deeper subtidal waters; 31 of the 43 nominal species have been collected at depths greater than or equal to 5 m, and 16 of these species have been recorded from depths greater than 100 m. Species of *Sarsinebalia* Dahl, 1985 have been collected at depths ranging from 7 to 3930 m. Another deep-water species is the hydrothermal-vent endemic *Dahlella caldariensis* Hessler, 1984, so far known only from vent fields in the East Pacific Rise and Galapagos Rift, where it occurs among tube worms as deep as 2620 m (Hessler, 1984). Notable exceptions to the rule that leptostracans are benthic include the larger-bodied bathypelagic *Nebaliopsis typica* Sars, 1887 and the mesopelagic *Pseudonebaliopsis atlantica* Petryashov, 1996, which have been recorded as deep as 5124 m and 500 m, respectively (Brahm and Geiger, 1966; Petryashov, 1996). The presence of *Nebaliella caboti* Clark, 1932 and *Sarsinebalia typhlops* (Sars, 1870) in mid-water trawls suggests that these two species are pelagic as well. The monotypic genus *Speonebalia* Bowman, Yager and Iliffe, 1985 is endemic to marine caves in the western Atlantic Ocean (Bowman et al., 1985).

COLLECTION

Given their ubiquity in the marine realm, the techniques by which Leptostraca can be collected are diverse. Species of *Nebalia* Leach, 1814 can be collected by hand in intertidal environments. Leptostracans can be distinguished from similar-sized crustaceans such as gammaridean amphipods by their swimming behavior; leptostracans use their thoracic limbs and flex their abdomen in a signature movement that clearly differs them from the more direct swimming of peracarids. Additionally, leptostracans often become trapped momentarily by surface tension, whether *in situ* or in a container. Leptostracans can consequently be found simply by overturning algae, rocks, or other debris so that they emerge at the surface of small pools of water that form in depressions of the substrate when algae or rocks are lifted. Lep-

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tostracans from coastal waters are most often associated with algal mats, seagrass beds, and other eutrophic benthic environments. For instance, *Nebalia* often occurs within colonies of common algae (e.g., *Ulva*, *Gracilaria*, *Chaetomorpha*) on intertidal mudflats, and it is known from subtidal detrital mats consisting of algal and plant material (e.g., *Macrocystis*, *Egregia*, *Phyllospadix*). *Paranebalia* frequently occurs in collections of the green alga *Halimeda* on tropical coral reefs (Haney and Martin, 2004). The bulk collection of such organic material is an efficient technique, and repeated rinsing of algae or other debris in a bucket or tray can yield large numbers of specimens. Modlin (1996), using snorkeling gear, collected all material within a 0.2 m^2 quadrat using cotton bags fitted over the quadrat's PVC frame. Scuba has been used to enclose and collect entire colonies of *Halimeda* in sampling bags, which are then brought to the surface to ensure collection of all algae-associated specimens (TAH, unpublished). *Nebalia hessleri* Martin, Vetter and Cash-Clark, 1996 was collected from subtidal detrital mats using an air-powered suction device as well as steel coring devices (Martin et al., 1996). The sorting of live material is often simpler than sorting preserved material because the movements of individuals make them easier to discover; rinsing with water of ambient temperature and salinity is then recommended. Capture of most specimens can be ensured with the use of a net or sieve with 200- to 300-μm mesh.

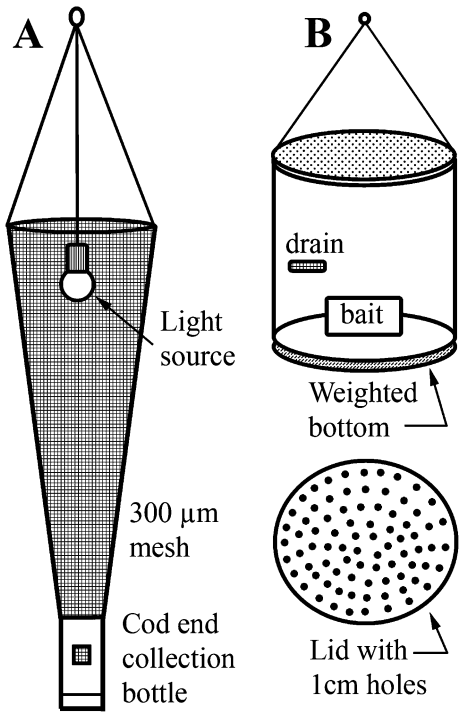

Fig. 1. A, modification of a plankton net for light trapping, vertically suspended with addition of a weight to the cod end, and an underwater lamp; B, inexpensive trap from modified bucket, or similar container.

Traps

Leptostracans have been collected in association with animals as well. Carnivory has been observed in *Nebalia* (see Martin et al., 1996), and additional indirect support for necrophagy includes collection records of *Nebalia* from the carapaces of spiny lobsters and crabs (Martin et al., 1996). Baited traps have certainly proven successful, whether baited with chicken (Biernbaum and Wenner, 1993) or fish (e.g., Olesen, 1999). The body of the trap is generally made of molded plastic, with some designs incorporating funnels and nested chambers. The design of traps, however, needs not be complicated. Piping and fittings made of polyvinyl chloride (PVC) are excellent materials for the construction of small traps; PVC is inexpensive and easy to assemble (and disassemble). A simple, highly effective trap can be made from a weighted plastic bucket with many small (approximately 1 cm) slits cut into the lid in lieu of funnels (Fig. 1). Lee and Morton (2005) reported collections using traps made from a series of baby feeding bottles, simply with the rubber nipples cut and turned inward to produce a funnel. Othman et al. (2016) collected *Nebalia* using cheesecloth-wrapped fish in a 500 ml polyethylene jar with 8 mm holes drilled into the bottle cap.

The same designs can be used for light traps. Many designs of aquatic light traps are commercially available (e.g., BioQuip Products, Rancho Dominquez, CA, USA). Most traps incorporate the use of one or more inwardly directed funnels to channel the entry of organisms while reducing the odds of escape. A light source, such as a blue or green chemical light stick (e.g., Cyalume Technologies, Springfield, MA, USA; also see Gerken, 2016) or waterproof lamps (dive flashlights), can be placed inside of a weighted plankton net or bucket (Fig. 1). Chemical light sticks hold several advantages over dive lights, being inexpensive, lightweight, long-lasting (generally 6-12 h), are available in different colors with 360° illumination, and requiring no batteries. Perhaps they can be submerged to greater depths than most underwater lights as well. An advantage of underwater dive lights or similar waterproof lamps is greatly enhanced brightness (1000 to 2000 lumens) with LED technology, ease of adding filters, and the ability to select designs with differing beam angles and color temperatures. For collecting of leptostracans and other crustaceans such as cumaceans and decapod larvae, white light or wavelengths in the middle of the visible spectrum appear to be most effective. Few studies have reported the collection of leptostracans with the use of light traps (e.g., Haney and Martin, 2004; Song et al., 2012).

While various traps have proven effective, the success of the collection effort is affected by several key factors apart from the sampling environment itself; these include the attractant, the style of the trap, ambient light as in the case of the lunar cycle and dock lights, and duration of exposure. The period of time for which a baited trap must be submerged is generally greater than that for a light trap. A light trap often produces good results after 45-90 min. of exposure, whereas baited traps have higher yields following at least 12-24 h of exposure (in part depending on the amount of bait, density of the target organism, and other factors). A longer-term trapping method and an effective collecting technique is the use of artificial structures that can be colonized by leptostracans. Zimmerman and Martin (2004) submerged Artificial Reef Matrix Structures (ARMS) in a coral reef for approximately one year; these included plastic scrubbing pads with small interstices that were colonized

by *Paranebalia*. Any matrix that can become fouled with organic material seems to be suitable for leptostracans. Some leptostracans have been collected from the atria of reef-associated sponges (*Tedania*, *Ircinia*) in tropical waters from Florida and Veracruz, Mexico to Brazil as well as Palau (e.g., Ortiz et al., 2011). Thiele (1904) reported specimens of *Paranebalia* from the base of the glass sponge *Euplectella* caught by trawling at 130-300 m.

Traps set on the seafloor appear to yield more specimens than those suspended in the water column, but the extent to which leptostracans occur higher in the water column has not been well explored. Brattegard (1970) reported the collection of a specimen of *Nebalia* at the surface at a site 5-6 m deep while using light as a lure. A single leptostracan was collected off the coast of Japan by plankton net (unpublished). The morphology of the specimen, including the presence of many unusually elongated setae, suggests that it represents a previously unknown epipelagic species.

Deep-Water Collection

Most collections of leptostracans available for study were obtained from deep-water surveys, usually sorted from material taken during trawls or from sediment collected by benthic grabs. Leptostracans have been collected with bottom trawls (bottom otter, beam) and mid-water trawls (Isaacs-Kidd) (Wakabara, 1976). Several species have been described from material collected using dredges (naturalist's dredge, Agassiz trawl, epibenthic sled) (Hessler and Sanders, 1967; Walker-Smith, 2000). Brattegard (1970) made 16 collections of leptostracans using an Ockelmann sledge (see Ockelmann, 1964). Otherwise, bottom grab samplers (Van Veen, Smith-McIntyre, orange peel) represent the most commonly employed method of obtaining specimens from the deep-water benthos (e.g., Barnard, 1970; Bocher and Zettler, 2012).

Deep-water collections of the vent-endemic *D. caldariensis* Hessler, 1984 have been made by the use of suction devices (slurp samplers) mounted on submersibles. Specimens of this species have also been recovered from shipboard washes of larger collections of tube worms.

Preservation

A large portion of the available collections of leptostracans were fixed in 5-10% formaldehyde and later transferred to ethanol. This continues to be the most common practice for material that is collected for programs of broad taxonomic sampling that are not focused upon leptostracans as the target species, such as ocean monitoring and biotic surveys. As is in other crustaceans, leptostracans can be preserved well (although not "fixed"; see Martin, 2016) in 70-95% ethanol. It is best to rinse specimens with distilled water prior to preserving to avoid precipitates that would otherwise form with the mixing of seawater and ethanol.

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