Aquatic Mammals 2019, *45*(5), 500-506, DOI 10.1578/AM.45.5.2019.500

Short Note

A Method to Replace Whale Gingival Tissue for Long-Term Study or Exhibition of Full Baleen Racks

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The oral filter of mysticetes is made of a unique tissue: baleen. It grows throughout life, suspended from each side of the palate in paired racks of ~300 transversely oriented triangular plates, like vertical blinds spaced ~1 cm apart (Werth, 2017). As with other mammalian integumentary specializations (e.g., hair, claws, nails, and hooves), baleen is made entirely of alpha-keratin, a fibrous, rod-like scleroprotein with constituent amino acids tightly coiled and cross-linked in dimeric α-helices (Fraser et al., 1976; Marshall et al., 1991; Szewciw et al., 2010; McKittrick et al., 2012; Wang et al., 2016). As with all keratinous tissues, baleen is stiff and impervious to small amounts of water as are other keratinous tissues (Bertram & Gosline, 1987; Kitchener & Vincent, 1987; Feughelman, 1997; Taylor et al., 2004; Greenberg & Fudge, 2012). Conversely, baleen is hydrophilic and becomes more flexible when submerged in water (Werth et al., 2016, 2018). Because of its resistance to water and decay, baleen can be kept in air for years without deteriorating (Peever, 1982; Von Endt & Jessup, 1986), although baleen will crack and delaminate if conditions are too dry and warm. Additionally, baleen will often curl into a deformed curve (Figure 1) if allowed to dry rapidly after absorbing water, even from humid air. Aside from this warping or splitting, baleen is a durable tissue (Lauffenberger, 1993; Minnesota Historical Society, 2013; Brogan, 2017; Werth et al., 2018)—not only *in vivo* but also for educational display.

The rubbery gingival (gum) tissue in which baleen develops and emerges (Figure 1) is also referred to as *zwischensubstanz*, from the German word for "in between substance," referring to its location between baleen plates (Fudge et al., 2009; Pinto & Shadwick, 2013). Unlike baleen, gingiva's keratinized, stratified epithelium decomposes rapidly. Its flexibility makes it an ideal basement layer to anchor hundreds of long (50 cm to 4 m) baleen plates to each other and to the skull where they withstand strong hydrodynamic forces in a biomechanically demanding, dynamic environment (Goldbogen et al., 2017; Werth, 2018). However, gingiva's high fat and oil content means that it quickly decomposes with a rancid stench of decay (Poisson et al., 2005; Guilminot et al., 2011, 2014; Ososky, 2012). This is not a problem if individual baleen plates are pulled or cut from a rack with adhering soft tissue removed (Stevenson, 1907; Lee, 1983; O'Connor, 1987). However, if plates are to be kept together in a full rack for long-term study (e.g., flow experiments in which precise plate orientation and spacing must be maintained) or museum exhibition, the gingival tissue which connects plates presents a major obstacle.

Traditionally, baleen racks were mounted for museum display (McAlpine & Camus, 1995) by cutting plates at the gumline, then gluing or stitching the plates to strips of rubber, wood, or cardboard/fiberboard to replicate full racks (Figure 1C). Alternatively, gums were trimmed as much as possible, then allowed to desiccate and mummify. However, this approach can contort plate position and spacing and potentially attract pests. Further, mummified racks are unsuitable for studies requiring submersion because the thin gingival tissue easily tears when rehydrated. Chemically fixing gums is not a good option. The many small, tight spaces between plates are difficult to access; and, even if properly preserved, gums become heavy and wet, dripping residue or off-gassing harmful vapors (Barclay, 1989; Pelé et al., 2015). Educators and researchers often use single plates, but because baleen is an integral part of the mysticete body, fully mounted skeletons should include complete racks (McAlpine & Camus, 1995). To preserve full racks for research or display, a method is needed to connect hundreds of plates together in a permanent, non-toxic way that will not decompose or release

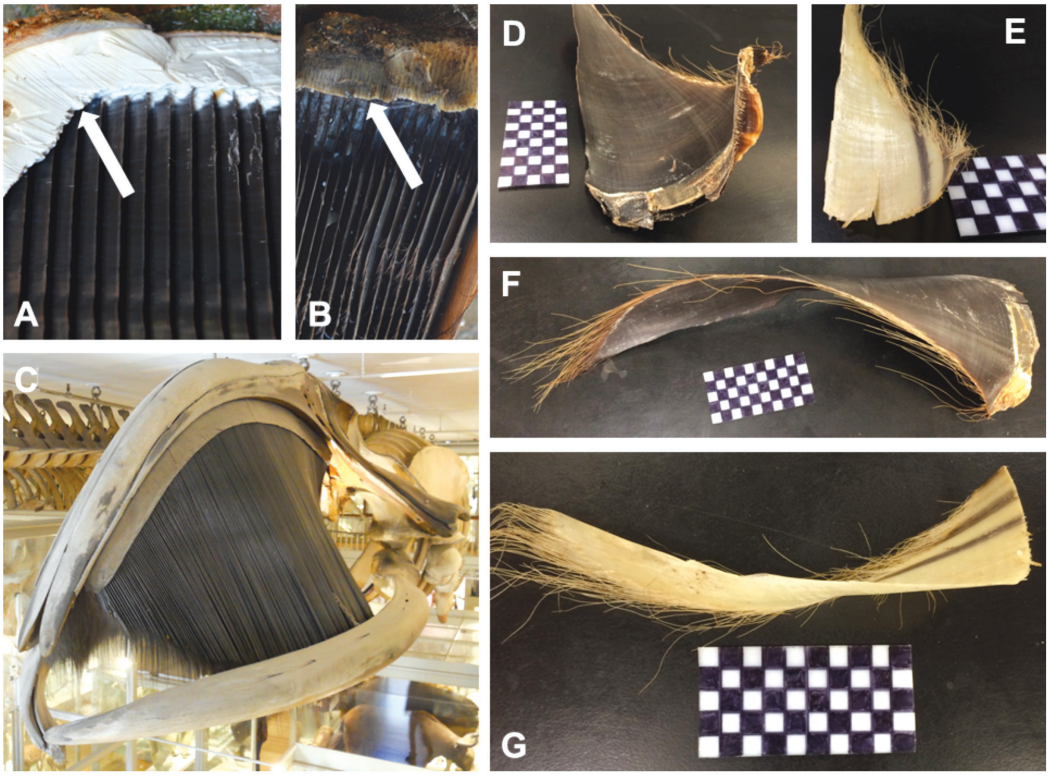

Figure 1. Mysticete gums or *zwischensubstanz*—white tissue in (A), denoted by arrow, seen in lateral view in a North Atlantic right whale (*Eubalaena glacialis*); and grayish-brown tissue in (B), denoted by arrow, seen in dorsomedial view in a bowhead whale (*Balaena mysticetus*)—are rubbery and pliant and tightly bind baleen plates to the palate. The gum tissue in (B) is slightly decomposed but also preserved with formalin, hence its darker color. Traditionally, whole mounted racks involve baleen plates individually affixed to strips of wood, pressboard, or rubber ([C] shows *E. glacialis* on exhibit in the Harvard Museum of Natural History/Museum of Comparative Zoology, Cambridge, MA, USA). When removed from the gums, individual plates typically curl along the mediolateral axis—shown with baleen from a humpback whale (*Megaptera novaeangliae*) (D) and fin whale (*Balaenoptera physalus*) (E)—and also show torsional twisting along the longitudinal or dorsoventral axis (humpback [F] and fin [G]), especially if they dry quickly or are kept in arid conditions. Scale bar shows squares of 1 cm^2.

an unpleasant stench, and that will maintain precise plate spacing and orientation.

Herein, we present a novel, multi-step method (summarized in Table 1) to preserve full baleen racks in natural position via complete replacement of the gingiva with a dual-component polymer rubber (Figure 2). The proposed rubber gingiva is strong and does not decompose if submerged, even after long-term storage. (We have monitored samples for nearly 3 years of storage with no deterioration.) It is appropriate for studies of baleen anatomy, physiology, and biomechanics given that the Shore hardness (a measure of material resistance and viscosity) is similar to that of natural mysticete gingiva (i.e., Shore A = 40 to 60). Our method involves a sequential protocol (Table 1) to secure the plates in position while gingival tissue is macerated (broken down and removed) and replaced by a tough yet elastic rubber which simulates the palatal matrix.

We undertook serial comparative assessments of materials and procedures to solve (1) how best to remove the gingival tissue; (2) how to secure plates during gingival removal without harming baleen and while maintaining plate geometry; and (3) what safe, inexpensive material best preserves the hardness and biomechanical response of natural gingival tissue. These trials are summarized in Table 2. Note that we did not attempt one potential step listed in Table 2 (i.e., use of dermestid beetle larvae to remove gums). We used the same baleen rack for many trials—for example, extending the maceration period or altering the maceration temperature.

Table 1. Summary of all steps in recommended procedure (see Figure 2)

1. Prepare to secure baleen:	Place baleen rack in watertight container.
2. Secure baleen plates:	Fill container with plaster up to gum line.
3. Prepare to remove gums:	Place rack upside down in maceration tank.
4. Finish gum removal:	Physically remove any residual gum tissue.
5. Prepare to replace gums:	Place gumless rack (plates still encased in plaster) in another watertight container.
6. Replace gums:	Pour polyurethane rubber to replace missing gums.
7. Release bound plates:	When rubber hardens, remove plaster by hammering/vibration.
8. Clean baleen:	Remove plaster residue by brushing and water/silicon spray.
9. Mount as desired:	Attach to jaws or framework for display or research.

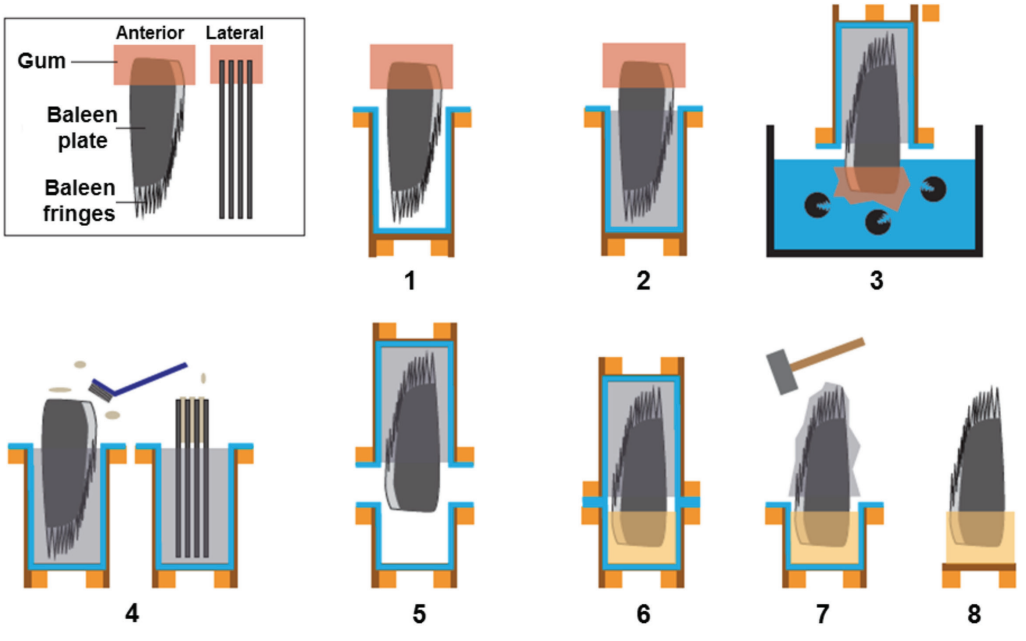

Figure 2. Schematic diagram of the sequential steps in our baleen rack preservation protocol: (1) The rack is placed in a watertight container (e.g., wood, plastic, or metal) lined with a waterproof plastic bag; (2) the bag is filled with plaster, gypsum, or other material that hardens and dries around baleen plates but not the gums; (3) the rack is placed upside down in a tank filled with macerating water, dermestid beetle larvae, or other means of removing the gums; (4) remaining gum tissue, if any, is mechanically removed by brushing; (5 & 6) the gumless baleen (with plates still immersed in dried plaster) is again inverted in a new container in which polyurethane rubber or similar material is poured; (7) after the rubber hardens, the plaster is broken by vibration or hammering; and (8) plaster residue is removed, and baleen is cleaned by brushing or other mechanical means, or by spraying or soaking in water.

Removal of the natural gingival tissue is the most challenging step. It is made particularly difficult by the close (1 cm or less) serial spacing of baleen plates (Figure 1) and the way plates often fit together with sinuous curves, which makes gum trimming problematic. The simplest approach was to trim excess gingiva and then submerge the plate base in a sealed maceration tank of 38°C water for 6 to 8 wks. Higher temperatures reduce maceration time yet tend to break down the baleen.

Of course, plates will fall out of the anchoring tissue if they are not secured—at least for the final

Table 2. Comparative assessment of materials and procedures, including all experimental parameters, tested options, and recommended solutions (italicized)

Problem	Options	Trials	Results	Solution
A. Preparatory steps?	Trim gums severely	1	Easier/faster maceration (or other gum removal) but rack can lose shape	Use intermediate trimming (neither too much nor too little)
	Trim gums slightly	1	Harder/longer maceration but rack maintains shape	*Intermediate trimming*
	Remove plates from gums completely	1	Can bypass many steps and go straight to synthetic gums but difficult to re-create rack	Leave plates in rack with gums
	Coat baleen plates with silicon before securing them	1	Intended to make plaster removal easier but plaster does not stick to baleen as well	Do not coat baleen with silicon prior to adding plaster
B. Temporary baleen fixation material?	Gypsum plaster	3	Inexpensive, lightweight, and easy to apply; adheres well to smooth baleen; antifungal	*Gypsum plaster (e.g., plaster of Paris) is clearly the best*
	Cement plaster	1	Inexpensive, heavy, and harder to mix and apply; does not adhere well to baleen; strong but cracks readily	Not recommended; heavy and works poorly
	Rubber	1	More expensive; harder to mix and apply; adheres (too) well to baleen; hard to remove	Not recommended; cannot be removed easily
C. Gum removal process?	Maceration tank	4	Requires big tanks plus lots of space, time, and heat	*Recommended, especially if tanks already present on site*
	Beetle larvae	0	Larvae might consume baleen after gums; risk of larvae escaping	Not recommended unless facility has good larval colony
D. Maceration duration/ conditions?	Lower temperature (< 30°C)	1	Less energy use; less risk of damaging baleen but requires much more time	Not recommended
	Intermediate temperature (~40°C)	2	Not too fast or slow and best tissue preservation outcome	*Recommended temperature of ~40°C*
	Higher temperature (> 40°C)	2	Faster removal of gum tissue but can distort/ damage baleen	Not recommended
	Rapid maceration (< 6 wks)	2	Requires higher temperatures which can damage baleen	Not recommended
	Slower maceration (6+ wks)	3	Requires more time but preserves baleen tissue and plate orientation/spacing	Recommended duration of 6 to 8 wks
E. Synthetic gum replacement material?	2-part polyurethane rubber (PUR)	3	Good elastomeric strength and flexibility; easy to acquire and use	*PUR recommended*
	2-part kneadable baking mold silicone rubber	1	Flexible but too bendy; weight of baleen can distort rack into unnatural position	Not recommended
	Plaster	1	Easy to use but inflexible	Not recommended
F. Plaster removal?	Hammering	3	Rubber mallet works well	*Combination of hammering and vibration best*
	Vibration	2	Removes small clinging bits	
	Brushing	5	Use coarse and soft brushes	*Combination of brushing/ spraying works*
	Spraying	5	Silicon spray helps cleaning	
G. Mounting?	Attach to jaws	1	We temporarily attached to bone; worked well and best/most natural appearance	*Recommended for museum display*
	Attach to wood or metal frame	2	Easily achieved for flow tank or other experimentation	*Recommended for research*
	Submerge in water	3	Synthetic gum holds well	*Recommended for display/ research*

1 to 3 wks of the maceration process. We experimented with several inert materials (Table 2) that withstand high-temperature maceration without degrading baleen plates and while retaining their natural orientation and serial spacing. One series of trials involved a soft, kneadable, two-component baking mold silicon, but the best solution was a basic plaster of Paris or gypsum plaster (calcium sulfate hemihydrate), or other quick-drying, readily available, multipurpose plaster. The baleen rack was placed into a box-like wood or medium density fiberboard container, with or without a waterproof plastic bag liner, and plaster was poured into the container and allowed to set around the baleen (Figure 2).

The plaster temporarily secures the baleen in position while residual gingival tissue is removed and the permanent replacement material is added (Figure 2). Once the baleen plates are secured, all residual gum tissue must be physically removed by brushing, scraping, or hydraulic jetting. Gum tissue can be removed before, during, or after bouts of maceration.

A practical replacement gingiva must be made of an inert, non-toxic, water-resistant material that will firmly attach to the keratinous baleen yet be releasable from a mold. Also, it must be a viscoelastic elastomer with the same rubbery hardness (Shore ~40 to 60) as the original gums. After testing different options (Table 2), we found that a tacky, pourable polyurethane rubber, mixed from two components (polyol 40A and isocyanate 40 to 80), worked best. We recommend that silicone sealant be applied to the mold to fill any cracks before the polyurethane (PUR) elastomer is poured; this is important because PUR curing is an exothermic reaction that causes PUR to expand. Pigment can be added to the PUR or it can be left transparent (Figure 3). Slowly pouring and carefully mixing the PUR eliminates air bubbles which could be unsightly or hinder the binding of the elastomer to the keratin. The replacement PUR "gingiva" has no odor; and if all natural gingival tissue has been removed during maceration, the entire assembly should be odor-free.

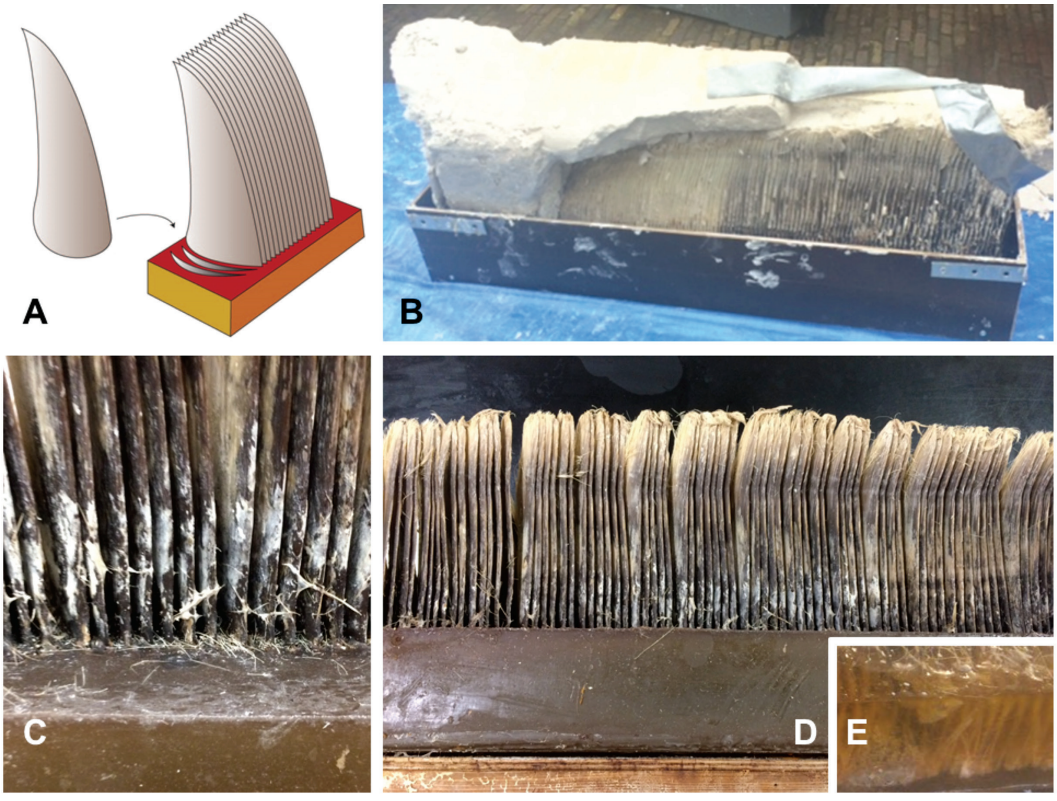

Figure 3. Rubber "gums" can be poured around a whole rack (Figure 2); alternatively, individual plates could be placed into rubber as it dries (A). (B) shows a full humpback rack as plaster encasing the plates is removed; (C) shows a close-up of plates (still being cleaned) emerging from the rubber matrix; (D) shows a full humpback rack before the rubber is trimmed; and inset (E) shows plates bound within a transparent rubber.

Once the PUR dries, the plaster is broken by vibration or hammering. Any remaining plaster residue is removed, and baleen is cleaned by brushing or other mechanical means, or by spraying or soaking in water. Application of silicon spray facilitates the removal of residual plaster dust.

The artificial rubber "gums" can be trimmed for remounting to whale jaws for museum display or attached to experimental equipment (made of wood/MDF, metal, plastic, or other material) for flow tank studies. Permanent or temporary submersion in water would also be appropriate for exhibition at a museum or other educational institution seeking to show baleen's natural function in filter feeding (Table 2).

Because full baleen racks are large and heavy, our procedure requires the efforts of several people as well as a winch or gantry to raise and lower the rack into the maceration tank and the temporary mold containers for the plaster and synthetic gingival material. Considerable space is also needed for maceration and setup/use of the molds into which the plaster and polyurethane are poured. Although initially more costly and time-intensive than traditional methods of preserving and mounting full baleen racks, this procedure (Table 1) pays off for long-term display or research as it is the only way to ensure natural baleen plate position, spacing, and biomechanical response.

Acknowledgments

We thank many people involved in procuring, preparing, and transporting the baleen specimens needed for this study, especially Guido Keijl, Pepijn Kamminga, and Wendy van Bohemen at the Naturalis Biodiversity Center, and Ton Horsten, Erwin Evertsen, Matthijs Barentsen, and Marc Beusenberg at Windesheim University of Applied Sciences. Many people assisted with materials or procedural suggestions, including Joop van Tuil, Kristof Debaetselier, Hoefmederij Diemer Tigchelaar, Cindy Sturris, Menno Wijcherson, and SilliCreations.nl. We are grateful to Shasta McClenahan of the National Oceanic and Atmospheric Administration/National Marine Fisheries Service, Pat Nutter of the Smithsonian Institution, and Caroline Harlow of the U.S. Fish and Wildlife Service for CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and other marine mammal permit approval for transporting and possessing baleen. We thank two anonymous reviewers for numerous helpful suggestions that greatly improved this short note.

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