Journal of Structural Biology 171 (2010) 318–331

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Quantitative microstructural studies of the armor of the marine threespine stickleback (*Gasterosteus aculeatus*)

Juha Song [a], Steffen Reichert [b], Ilan Kallai [c], Dan Gazit [c,f], Matthew Wund [d], Mary C. Boyce [e], Christine Ortiz [a,*]

[a] Department of Materials Science and Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, RM 13-4022, Cambridge, MA 02139, USA
[b] Department of Architecture and Planning, Massachusetts Institute of Technology, 77 Massachusetts Avenue, RM 13-4022, Cambridge, MA 02139, USA
[c] Skeletal Biotech Laboratory, Hebrew University, Hadassah Faculty of Dental Medicine, Ein Kerem, Jerusalem 91120, Israel
[d] Department of Biology, Clark University, 950 Main Street, Worcester, MA 01610, USA
[e] Department of Mechanical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
[f] Department of Surgery and Cedars-Sinai Regenerative Medicine Institute (CS-RMI), Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

ARTICLE INFO

Article history:
Received 3 March 2010
Received in revised form 16 April 2010
Accepted 20 April 2010
Available online 28 April 2010

Keywords:
Biomineralization
Micro-computed tomography
Exoskeleton
Stickleback
Natural armor
Armored fish

ABSTRACT

In this study, a quantitative investigation of the microstructure and composition of field-caught marine *Gasterosteus aculeatus* (threespine stickleback) armor is presented, which provides useful phylogenetic information and insights into biomechanical function. Micro-computed tomography (μCT) was employed to create full three-dimensional images of the dorsal spines and basal plate, lateral plates, pelvic girdle and spines and to assess structural and compositional properties such as the spatial distribution of thickness (~100–300 μm), the heterogeneous cross-sectional geometry (centrally thickened), plate-to-plate juncture and overlap (~50% of the plate width), and bone mineral density (634–748 HA/cm^3). The convolution of plate geometry in conjunction with plate-to-plate overlap allows a relatively constant armor thickness to be maintained throughout the assembly, promoting spatially homogeneous protection and thereby avoiding weakness at the armor unit interconnections. Plate-to-plate junctures act to register and join the plates while permitting compliance in sliding and rotation in selected directions. Mercury porosimetry was used to determine the pore size distribution and volume percent porosity of the lateral plates (20–35 vol.%) and spines (10–15 vol.%). SEM and μCT revealed a porous, sandwich-like cross-section beneficial for bending stiffness and strength at minimum weight. Back-scattered electron microscopy and energy dispersive X-ray analysis were utilized to quantify the weight percent mineral content (58–68%). Scanning electron microscopy and surface profilometry were used to characterize the interior and exterior surface topography (tubercles) of the lateral plates. The results obtained in this study are discussed in the context of mechanical function, performance, fitness, and survivability.

1. Introduction

The threespine stickleback (*Gasterosteus aculeatus*) has become a promising model system to investigate the genetic and ecological origins of adaptive phenotypic evolution, through the assessment of macroscopic morphology for divergent populations from differing environments (Bell et al., 1993; Foster, 1995; Hagen and Gilbertson, 1973; Östlund-Nilsson et al., 2007; Reimchen, 1994; Cresko et al., 2007; Kingsley and Peichel, 2007; Walker, 1997; Huntingford and Coyle, 2007; Baker et al., 2008). Morphological assessment of *G. aculeatus* has involved the measurement of body features (e.g., size, shape), as well as the quantification of the dimensions and geometry (e.g., length, shape, position, number) of its distinctive external bony armor components which include: a series of lateral plates, three dorsal spines, a pair of pelvic spines, and a complex pelvic girdle (Bell, 1987; Reimchen, 1983; Nelson, 1971). The dermal plates of the threespine stickleback are anisotropic in shape (oval to rectangular), conformal to the body of the fish, porous, and composed of acellular lamellar bone (Reimchen, 1983; Sire et al., 2009). They additionally interlock with the ascending branch of the pelvic girdle, the basal plate of the dorsal spines, and with each other via an articulation mechanism located along the lateral line, which is shifted towards the dorsal side of the body (Bell, 1987; Reimchen, 1983; Nelson, 1971) (Fig. 1). The pelvic and dorsal spines are attached to the pelvic girdle and basal plate, respectively, with a peg-and-socket interconnection (Bell, 1987; Reimchen, 1983; Nelson, 1971). *G. aculeatus* is known to undergo rapid and dramatic evolutionary adaptations of its armor and associated genetic isolation within as few as eight generations (Bell et al., 2004; Kristjansson, 2005). Marine threespine sticklebacks, which represent the ancestral condition (Bell and Foster, 1994), possess a continuous row of lateral armor plates

* Corresponding author. Present address: Department of Biology, The College of New Jersey, P.O. Box 7718, Ewing, NJ 08628-0718, USA. Fax: +1 617 258 6936.
E-mail address: cortiz@mit.edu (C. Ortiz).

1047-8477/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2010.04.009

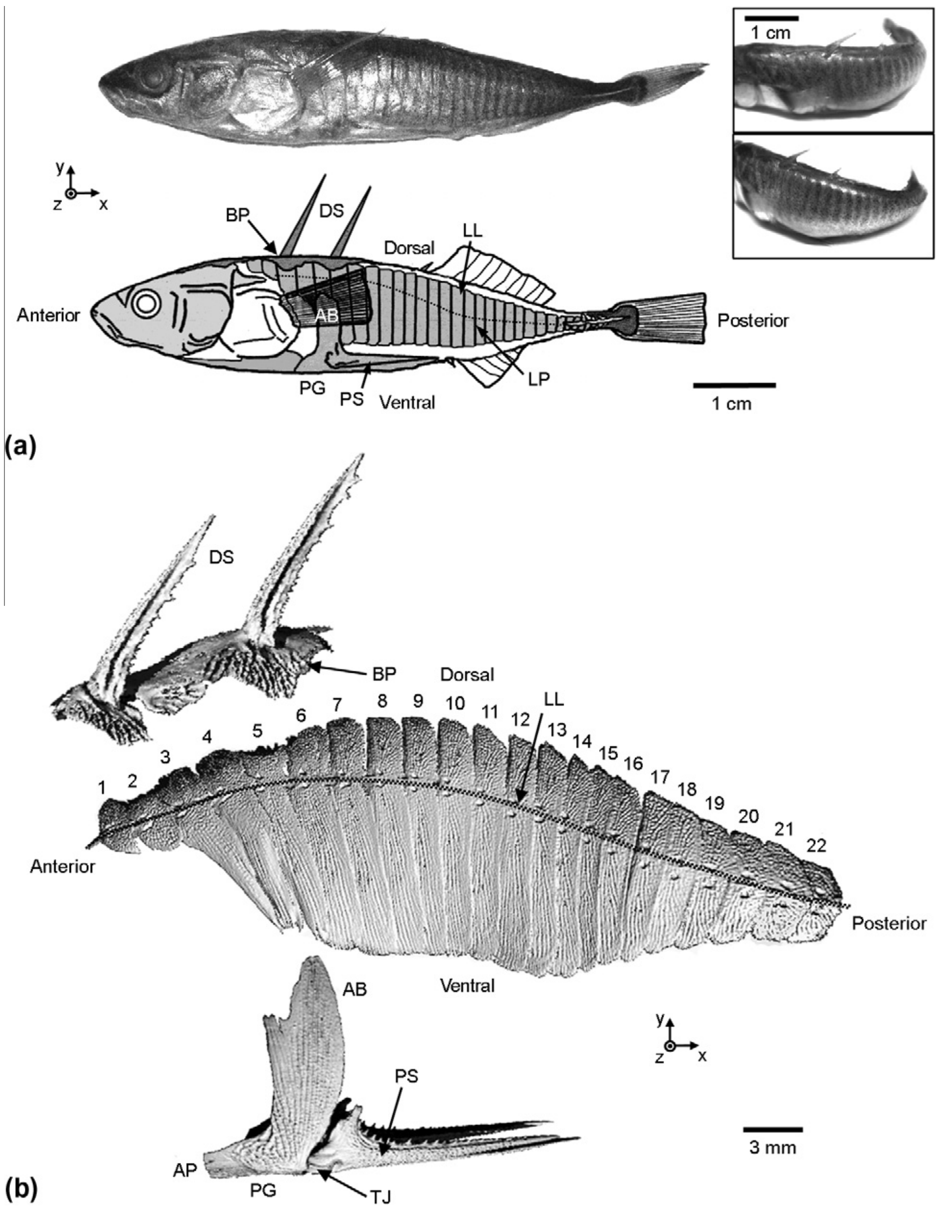

Fig. 1. Three-dimensional structure of armor plate assembly of marine *Gasterosteus aculeatus* (threespine stickleback); (a) photographs and schematic illustration and (b) μCT images of disassembled armor components with dorsal spines (*top*), lateral plates (*center*), and pelvic girdle/spines (*bottom*) in rest position. "AB" = ascending branch of the pelvic girdle, "AP" = anterior process, "BP" = basal plate, "DS" = dorsal spines, "LL" = lateral line, "LP" = lateral plates, "PG" = pelvic girdle, "PS" = pelvic spines, and "TJ" = trochlear joint.

(∼30–36, "complete morph", Fig. 1a) while evolutionarily derived, freshwater sticklebacks often exhibit a reduction in the number of plates (∼0–9, "low morph"), or, less commonly, an intermediate number of plates ("partial morph") (Hagen and Gilbertson, 1973; Bell and Foster, 1994; Bell, 1977). The loss of armor in freshwater populations has been attributed to a variety of factors: low ion concentrations which increase the mineralization "cost" of armor, a juvenile growth advantage, reduced weight and increased maneuverability affording quicker access to cover from predators, and a decreased range of predators (Bell et al., 1993; Östlund-Nilsson et al., 2007; Kristjansson, 2005; Giles, 1983; Marchinko and Schluter, 2007; Reimchen, 1995, 2000; Pennish, 2004; Bergstrom, 2002). Armor loss in freshwater populations might also be partly due to correlated selection on other traits, since the main genetic locus controlling plate reduction (*Eda*) is linked to loci involved in the regulation of salt secretion and parasite susceptibility (Marchinko and Schluter, 2007; Colosimo et al., 2005).

In this study, we present a quantitative, materials science-based approach to the investigation of the microstructure of field-caught marine *G. aculeatus* armor, which provides useful phylogenetic information and insights into the biomechanical function of the armor of both the evolutionary ancestral state, as well as subsequent morphs. From a comparative morphological perspective, this information is not only relevant to the evolution of armor within the threespine stickleback radiation (e.g., partially armored freshwater, fully armored anadromous), but also could form the basis for comparison with other stickleback species that have lateral plates (e.g., Brook stickleback, *Culaea inconstans*, ninespine stickleback, *Pungitius pungitius*) (Mattern, 2004). Micro-computed tomography (μCT) was employed to create full three-dimensional images of the dorsal spines and basal plate, lateral plates, pelvic girdle and spines and to assess structural and material properties such as the spatial distribution of thickness, the cross-sectional geometry, plate-to-plate interconnections and overlap, bone mineral density, and