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Authors: Buser, Thaddaeus J., Kee, Victoria E., Terry, Rebecca C., Summers, Adam P., and Sidlauskas, Brian L.

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Taurus of the Tidepool? Inferring the Function of Cranial Weapons in Intertidal Sculpins (Pisces: Cottoidea: Oligocottinae)

Thaddaeus J. Buser[1,2], Victoria E. Kee[1], Rebecca C. Terry[3], Adam P. Summers[4], and Brian L. Sidlauskas[1]

From dissuading predators to gaining an edge on intraspecific rivals, animals have evolved weapons to meet various needs. Those with the most extreme weapons often use them to battle conspecifics, but some weapons defend against predation and others signal prowess to prospective mates and rivals. Many fishes have evolved armaments, but humans rarely observe these structures in action due to the inaccessibility of many weapon-bearing fish species. For example, how sculpins use the diverse horn-like spines that project from their head remains a mystery. We deduced the function of the weaponized preopercle in the 16 species of sculpins in the subfamily Oligocottinae by determining whether they exhibit three well-documented hallmarks of offensive weapons in terrestrial animals: ontogenetic change, sexual dimorphism, and fluctuating asymmetry. Geometric morphometrics of micro-computed tomography (μCT) scans show no sexual dimorphism in preopercular spine shape but reveal phylogenetically widespread ontogenetic shape change. Fluctuating asymmetry is low to moderate across species. Taken together, these results suggest that despite their varied reproductive habits, frequent territoriality, and possession of weapons that resemble bovid horns, oligocottine sculpins evolved their spines primarily to defend against predators.

WEAPONS of the animal kingdom have inspired centuries of awe and wonder about their evolutionary origins (Darwin, 1871; Geist, 1966; Gould, 1974; Emlen, 2014). Weapons tend to evolve to satisfy one of two primal needs: dominate intraspecific rivals or protect their bearers from predators. The horns of *Styracosaurus*, the antlers of Irish Elk (†*Megaloceros giganteus*), and the horns of Atlas beetles (*Chalcosoma*) are spectacular exemplars of the former (Gould, 1974; Farlow and Dodson, 1975; Emlen et al., 2005; Emlen, 2008), while the armor plating and spikes of ankylosaurs (Ankylosauria), the quills of a porcupine (Erethizontidae, Hystricidae), and the namesake of horned lizards (*Phrynosoma*) exemplify the latter (Hayashi et al., 2010; Bergmann and Berk, 2012; Stankowich and Campbell, 2016; Arbour and Zanno, 2018). Chemical weapons such as venoms or poisons may evolve to enhance or, in the case of poisons, as an alternative to the physical arsenal (Nelsen et al., 2014; Harris and Arbuckle, 2016; Smith et al., 2016). Most research on animal weapons comes from terrestrial systems, but fishes and other aquatic fauna possess a dizzying array of armaments, ranging from the lateral armor plating and fin spines of sticklebacks (Gasterosteidae) to the veritable Swiss Army Knife of weaponry possessed by scorpionfishes (Scorpaenidae), which includes bony spikes projecting from the top of their head; venomous fin spines; and a retractable, switch-blade-like weapon under each eye (Morris et al., 1956; Smith et al., 2016, 2018). Aquatic and terrestrial animals interact with their environments differently, and such divergent interactions have led to different evolutionary trajectories in the two realms (Daeschler and Shubin, 1995; Daeschler et al., 2006; Helfman et al., 2009; Price et al., 2015). Whether such disparity could extend to the evolution of weapons is unclear, especially as it relates to the dominant drivers of weaponization: offense or defense.

Many terrestrial animal weapons evolved as primarily offensive tools that aid intraspecific combat (Darwin, 1871; Emlen, 2008). For example, ungulates, "pachyderms," beetles, and tusked wasps independently evolved horn- and tusk-like structures that they use in agonistic bouts (Geist, 1966; Emlen et al., 2005; Emlen, 2008). Comparison of these diverse weapon-bearing taxa has revealed several consistent evolutionary trends. Offensive weapons tend to: (1) increase in complexity and relative size across ontogeny (i.e., positive allometry) such that they become more effective fighting instruments when the animal is sexually mature (Gould, 1974; Kodric-Brown et al., 2006; O'Brien et al., 2018), (2) exhibit larger or more ornate forms in whichever sex competes for access to the other (Caro et al., 2003; Stankowich and Campbell, 2016), and (3) randomly deviate from symmetry with higher frequency and magnitude than non-weaponized structures in the same individual (Møller, 1992; Manning and Chamberlain, 1993). This increased fluctuating asymmetry stems from directional selective pressure on the weapon, which induces genetic stress and developmental instability (Møller, 1992; Manning and Chamberlain, 1993). Sexual selection mediated through fighting also appears to drive exceptional diversification of weapon shapes, even among closely-related weapon-bearing taxa (Lundrigan, 1996; Caro et al., 2003; McCullough et al., 2014; Emberts et al., 2021). Lastly, sexually selected weapons tend to show positive evolutionary allometry with body size, thus large-bodied species tend to possess disproportionally

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[1] Department of Fisheries, Wildlife and Conservation Sciences, Oregon State University, Corvallis, Oregon; Email: (VEK) Vickee47@yahoo.com; and (BLS) Brian.sidlauskas@oregonstate.edu. ORCID: (BLS) 0000-0003-0597-4085.

[2] Present address: Department of BioSciences, Rice University, Houston, Texas; Email: Thaddaeus.buser@gmail.com. ORCID: 0000-0002-2518-1835. Send correspondence to this address.

[3] Department of Integrative Biology, Oregon State University, Corvallis, Oregon; Email: Rebecca.Terry@oregonstate.edu. ORCID: 0000-0002-9803-6292.

[4] Department of Biology and SAFS, University of Washington, Friday Harbor Laboratories, Friday Harbor, Washington; Email: Fishguy@uw.edu. ORCID: 0000-0003-1930-9748.

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larger weapons (Gould, 1973; Kodric-Brown et al., 2006). Because these trends hold true across the great phylogenetic distance separating rhinoceri from rhinoceros beetles, they have enabled inference of how extinct animals evolved and used their weapons (Knell and Fortey, 2005; Knell et al., 2013). Investigations of ontogenetic shape change, sexual dimorphism, and asymmetry have been used to infer the function of weaponized structures in taxa as disparate as trilobites, dinosaurs, and early chondrichthyans (Knell et al., 2013) and present an effective way to infer the origin and function of weapons in extant taxa whose natural behavior is difficult to observe, such as most weapon-bearing fishes.

While many fishes have weaponized one part of their bodies or another (Smith and Wheeler, 2006; Emlen, 2008; Smith et al., 2016; Kolmann et al., 2020a, 2020b), our understanding of how and why they use their weapons is based on only a small subset of weaponized structures. The information on weapon use in fishes that does exist paints a different evolutionary scenario than that described in terrestrial taxa. While land-dwelling animals like bovids and beetles appear to evolve weapons primarily for fighting conspecifics and may use them secondarily for defending themselves from predators (Emlen, 2008), fishes tend to evolve weapons primarily for defending themselves (e.g., rigid fin spines; see Morris et al., 1956; Price et al., 2015) or for maiming prey (e.g., sawfishes, billfishes; see Shimose et al., 2007; Wueringer et al., 2012a). Fishes do compete frequently for reproductive opportunities, but typically use behavioral displays to communicate fitness to prospective mates and rivals (Kodric-Brown, 1990). When fishes possess specialized structures for combat, those specializations tend to take the form of enlarged mouths and exaggerated canine teeth, particularly in males (e.g., salmonids, gymnotiforms; see Evans et al., 2018; Quinn, 2018). The function of many other fish weapons remains shrouded in mystery. For example the lachrymal saber in stonefishes (Synanceiidae) and the spiny armor plating in poachers (Agonidae) are presumed to function defensively but may also be used in intraspecific competition (Smith et al., 2018; Kruppert et al., 2020).

Defensive structures also evolve regularly among animals and, as with offensive weapons, several evolutionary trends have emerged. Predominantly defensive structures tend to: (1) evolve in animals that are not large enough to rely on body size to deter predators (Stankowich and Caro, 2009; Stankowich and Campbell, 2016) and (2) be indistinguishable in males and females because both sexes use them for the same purpose. In stickleback fishes (Gasterosteidae), defensive spines and armor show lower levels of fluctuating asymmetry in high-predation environments than in low-predation environments because asymmetric defensive structures deter predation less effectively than symmetric ones in this species (Bergstrom and Reimchen, 2003). Natural selection therefore appears to stabilize the symmetry of their defensive structures (Bergstrom and Reimchen, 2003).

The approximately 380 species of sculpins (superfamily Cottoidea) possess a modified preopercle bone that may function as such a defensive structure. This bone bears one to four spines on its posterior margin (Smith and Busby, 2014), which can be large, branched, serrated, and/or strongly curved, depending on the species (Fig. 1). These spines are thought to serve a defensive purpose because sculpins have no other obvious defensive structures: they lack the rigid fin spines found in most percomorph fishes, most are small, and outside of the poachers (Agonidae), few have protective body armor (e.g., scutes or scales; Cowan, 1969). Alternatively, the sheer diversity of preopercular spine shapes and sizes suggests that some sculpin species may use their spines agonistically, perhaps in place of defense. Widespread and strong sexual selection among sculpins lends support to the agonism hypothesis, as does marked sexual dimorphism in body size, coloration, and territoriality in some species (Bolin, 1944; Van Vliet, 1964; Downhower et al., 1983; Petersen et al., 2005). Males of some species even flare their preopercles as a threat display before battling with other males over territory, with actual combat taking the form of biting (Morris, 1955; Petersen et al., 2005). While it is unclear how widespread such combat is across sculpins, the known natural history of the group clearly admits both agonism and defense as plausible functions for their impressively varied weaponry.

If sexual selection and intraspecific agonism drive preopercle spine diversity among sculpins, we expect trends in the development and evolution of sculpin spines to mirror those in other taxa bearing offensive weapons. Specifically, the spines should (1) increase in size/complexity over ontogeny, (2) demonstrate sexual dimorphism in size and complexity, and (3) display high levels of fluctuating asymmetry. We also predict that evolutionary allometry, if present, will show a positive relationship between the average size and/or complexity of the shape of the preopercular spines and the maximum body size of a species. Alternatively, if the spines serve a primarily defensive purpose, we expect the opposite trends: (1) decreasing complexity and relative size of the preopercular spines across ontogeny, (2) no sexual dimorphism, and (3) low levels of fluctuating asymmetry. We also predict that, if spines deter gape-limited predators, evolutionary allometry will be present and show an inverse relationship between average preopercle spine size and maximum body length of a species. In other words, the spines will be most prominent in small species that cannot rely on body girth as a mechanism to avoid engulfment.

We inferred whether the preopercular spines function primarily offensively or defensively by characterizing the diversity, development, and evolution of spine shape among sculpins of the subfamily Oligocottinae, a group of 16 species that inhabit nearshore and intertidal habitats along the Pacific coast of North America. These fishes have diverse preopercle spine shapes and sizes, and they engage in a range of behaviors linked to sexual selection including territoriality, intraspecific combat, and ritualized posturing and threat displays, making this a useful group to test the potential role of sexual selection in the evolution of the shape of the preopercle (Atkinson, 1939; Morris, 1952; Ragland and Fischer, 1987; Bro-Jørgensen, 2007; Emlen, 2014). We use 3D geometric morphometrics to quantify the degree of sexual dimorphism, fluctuating asymmetry, and allometric shape change within each species. We use the average preopercle shape for each species in a phylogenetic comparative framework to test for covariation between shape and the strength of sexual selection, and between shape and the average degree of fluctuating asymmetry. We also determined whether preopercles grow allometrically or isometrically within each species and tested for evolutionary allometry across all oligocottine species.

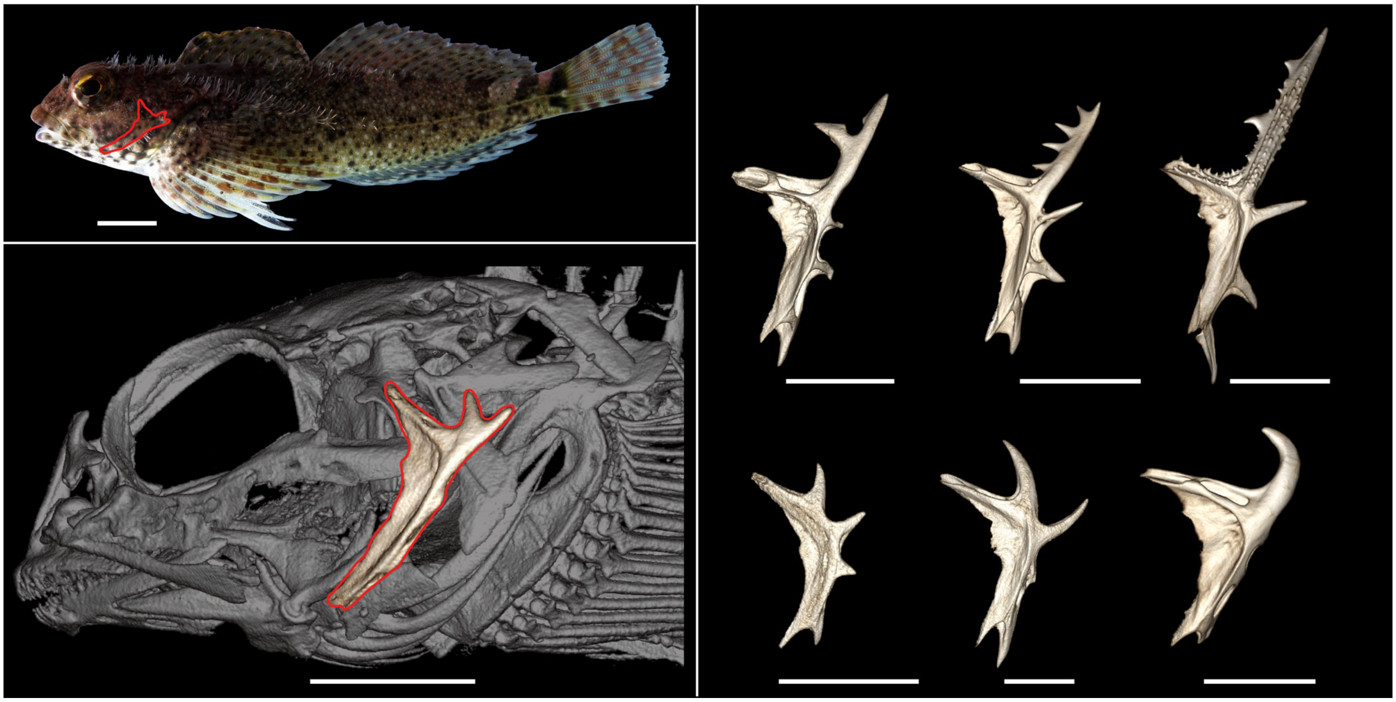

Fig. 1. Location and morphology of the preopercle (outlined in red) in *Clinocottus analis* (left panel; OS 26170, 76.55 mm SL) and diversity of preopercle shape as represented in six phylogenetically dispersed cottoid species (right panel, clockwise from top left): *Cottiusculus gonez* (SU 69887, 36.6 mm SL), *Antipodocottus galatheae* (LACM 42620-7, 48.8 mm SL), *Enophrys diceraus* (OS 15214, 67.27 mm SL), *Artediellus gomojunovi* (UW 20981, 64.0 mm SL), *Micrococttus sellaris* (OS 11467, 89.0 mm SL), *Hemilepidotus spinosus* (OS 3769, 62.0 mm SL). Scale bar is 5 mm.

MATERIALS AND METHODS

Quantifying preopercle morphology.—To represent the morphology of oligocottine preopercles, we gathered CT scan data from museum specimens representing each species in the Oligocottinae and the outgroup taxon *Chitonotus pugetensis* ($n = 8$–32 individuals per species, median 11). To ensure that our samples could capture any potential sexual dimorphism or allometric growth, we included males and females spanning a range of sizes (see intraspecific variation methods below). All CT scanning and reconstruction took place at the Karel F. Liem Bioimaging Center at Friday Harbor Laboratories (Friday Harbor, WA) using a Bruker Skyscan 1173 μCT system. Scanning parameters ranged from 50–75 kV and 100–160 μA, and resolution from 8.8 to 55 μm voxel size (Table S1; see Data Accessibility). All scans used a 1 mm aluminum filter. Raw image stacks were reconstructed using NRecon (Bruker μCT, Kontich, Belgium, 2016). We included multiple specimens in each scanning session and isolated each using DataViewer 2.1 (Bruker, Kontich, Belgium, 2010). We supplemented this dataset with CT reconstructions of oligocottine species ($n = 1$–3 individuals per species, median 1; Buser et al., 2018) downloaded from MorphoSource (https://www.morphosource.org), for a total sample size of 221 individuals across 17 species drawn from the collections of the University of Alaska Museum, University of Washington Burke Museum, Oregon State University, and Scripps Institution of Oceanography (Table S1; see Data Accessibility). Because males of most oligocottine sculpins possess an intromittent organ, it was simple to determine the sex of most individuals using external features (as described in Bolin [1944] and Mecklenburg et al. [2002]). For individuals where the sex was not clear externally, we made a small incision into the abdominal cavity and examined the gonads.

We digitally isolated the left and right preopercle of each specimen and exported them as 3D surface files using the workflow described in Buser et al. (2020), including optional steps 3.a–c, 7.b–c, 7.d, and 9.e using the programs Fiji (Schindelin et al., 2012; Rueden et al., 2017; Rueden and Eliceiri, 2019), 3D Slicer (Fedorov et al., 2012; Kikinis et al., 2014), and MeshLab (Cignoni et al., 2008). We represented the shape of each preopercle using 28 putatively homologous landmarks and a curve of 60 sliding semilandmarks (see Supplemental Methods and Fig. S1; see Data Accessibility). We recorded the 3D Cartesian coordinate positions (x, y, z) of each landmark and semilandmark for a given preopercle using IDAV Landmark Editor (Wiley et al., 2005). Preliminary results frequently showed appreciable shape differences between left and right preopercles for a single individual, so we duplicated the model of each preopercle three times and placed the landmarks on each duplicate independently to assess precision. Our dataset therefore consists of 221 individuals x 2 preopercles x 3 duplications, for a total of 1,326 digital models. The CT-image stacks of the whole skeletons as well as the 3D models of the isolated preopercles are available for each specimen on MorphoSource (Table S1; see Data Accessibility).

Summarizing evidence of sexual selection.—We summarized the known presence or absence of sexual dichromatism, sexual dimorphism in size, and secondary sexual characteristics in each oligocottine species by reviewing published accounts and recorded descriptions (Table S2; see Data Accessibility; Atkinson, 1939; Bolin, 1944; Hart, 1973; Marliave, 1981; Ragland and Fischer, 1987; Petersen et al., 2005; Lamb and Edgell, 2010). We summed the scores of the traits for each species and used this number, which we termed the "sexual selection index (SSI)" as a proxy for the prevalence of sexual selection in the biology and ecology of each species. High SSI scores indicate the presence of many traits linked to sexual selection and suggest that a species experiences high levels of sexual selection.

Geometric morphometric analyses.—All remaining analyses were performed in the R statistical environment using the program R Studio (R Studio Team, 2018; R Core Team, 2019), with base functions, custom functions from previous studies (Buser et al., 2017, 2018), and functions from various packages (Revell, 2012; Adams and Otárola-Castillo, 2013; Adams et al., 2019; Oksanen et al., 2019; Paradis and Schliep, 2019). Supplemental Data 1 (see Data Accessibility) contains pertinent raw data and an annotated script for all analyses conducted herein. Because the left and right preopercle mirror one another (i.e., matching symmetry; Klingenberg, 2015), we reflected the landmark coordinates of the right preopercle along the vertical (y) axis. Thus, each individual fish was represented twice in our dataset: once by its left side and again by its reflected right side. The reflection allows us to analyze the shape of each side in a manner that eliminates differences due solely to chirality. We quantified and analyzed the variation in preopercle shape using standard 3D geometric morphometric techniques, including Procrustes superimposition to remove non-shape variation and principal components analysis (PCA) to visualize the variance (Zelditch et al., 2012). We averaged each landmark location across the three replicates of each preopercle and analyzed left–right symmetry using the bilat.symmetry function in the R package geomorph (Mardia et al., 2000; Klingenberg et al., 2002).

Intraspecific variation.—We tested for (1) allometric growth, (2) sexual dimorphism, and (3) fluctuating asymmetry in the preopercle of each species. For the first two of these tests, we represented the preopercular shape of each specimen using the symmetric component of left and reflected right preopercle shapes, which equals their mean shape because of their matching symmetry (Klingenberg, 2015). Thus, for tests 1 and 2, a single set of landmarks represented the preopercular shape of each specimen, which we used as dependent variables (hereafter referred to as "shape variables"). We tested for allometry (test 1) using multivariate regression of the shape variables against the standard length of the specimens. A significant relationship between standard length and preopercle shape rejects the null hypothesis of isometric growth (Esquerré et al., 2017) and separates species that undergo allometric shape change from those that that do not. We tested the hypothesis that all species share a common allometric trajectory using a distance-based homogeneity of slopes (HOS) test following the procedures outlined in Drake and Klingenberg (2008) and Esquerré et al. (2017). We defined sexual dimorphism as a significant difference in mean preopercle shape between males and females and tested for dimorphism in each species (test 2) with a multivariate analysis of variance (MANOVA). We quantified the fluctuating asymmetry of each specimen (test 3) using the standard definition of fluctuating asymmetry described by Klingenberg (2015). To account for the tendency of more complex shapes to yield mathematically larger asymmetries without this necessarily corresponding to greater biologically meaningful variation, we relativized the fluctuating asymmetry by the expected variation in preopercle shape for each species (see Supplemental Methods; see Data Accessibility). We hereby define the "relative fluctuating asymmetry (rFA)" of an individual as its fluctuating asymmetry score divided by the average Procrustes distance from the mean shape of either the left or right preopercle (whichever was greater) for its species (Fig. S2; see Data Accessibility). We used this as a basis for delineating four descriptive categories of rFA: very high ($\text{rFA} > 1.0$), high ($1.0 \geq \text{rFA} > 0.90$), moderate ($0.90 \geq \text{rFA} > 0.75$), and low (rFA values ≤ 0.75).

Finally, to assess whether putative biological asymmetry could simply reflect imprecision in our landmark placement, we calculated pairwise Procrustes distances between the three replicates of each preopercle and between a given replicate and each replicate of the opposite preopercle for that individual. We compared these distances using analysis of variance (ANOVA).

Macroevolutionary hypotheses.—We used phylogenetic generalized least squares regression (PGLS) to test for evolutionary covariation of preopercle shape with the strength of sexual selection, maximum body size, and/or degree of fluctuating asymmetry. We used a previously-published phylogenetic hypothesis of oligocottine sculpins (Buser and López, 2015; Buser et al., 2017) as a framework for this analysis. The mean of the shape variables for each species provided the dependent variables. The three potential explanatory variables were: 1) SSI score, 2) maximum body size, and 3) average rFA.

RESULTS

Overall, 10 of the 16 oligocottine species match the predictions of primarily defensive spines by showing ontogenetic reductions in spine complexity and size (i.e., negative allometry), lacking sexual dimorphism in spine shape, and displaying low to moderate levels of rFA. Two additional species (*Oligocottus rubellio* and the outgroup, *C. pugetensis*) possess sexually monomorphic spines and low to moderate levels of rFA but display positive spine allometry, meaning that they match two of the three predictions of defensive primacy. No species matched all the predictions of primarily offensive spines (Table S2; see Data Accessibility).

Intraspecific variation.—Allometry in preopercle shape is common across the Oligocottinae. The preopercles of 13 of the 16 species in the subfamily change significantly across ontogeny (see Fig. 2, Table S2; see Data Accessibility). In all but one of these allometric species, small individuals have sharply angled preopercles with elongated spines, while large individuals have rounded preopercles with short spines. This ontogenetic reduction in complexity and spine length matches our prediction for spines that are used primarily defensively. Despite sharing a general reduction in size and complexity across ontogeny, there were obvious species-specific differences in morphology and allometry, especially relating to the length and/or curvature of the uppermost spine and the size of the lower three spines. The HOS test rejected the null hypothesis that all species share a common allometric trajectory ($P = 0.001$; see supplementary script for visualizations; see Data Accessibility). The exceptions to the

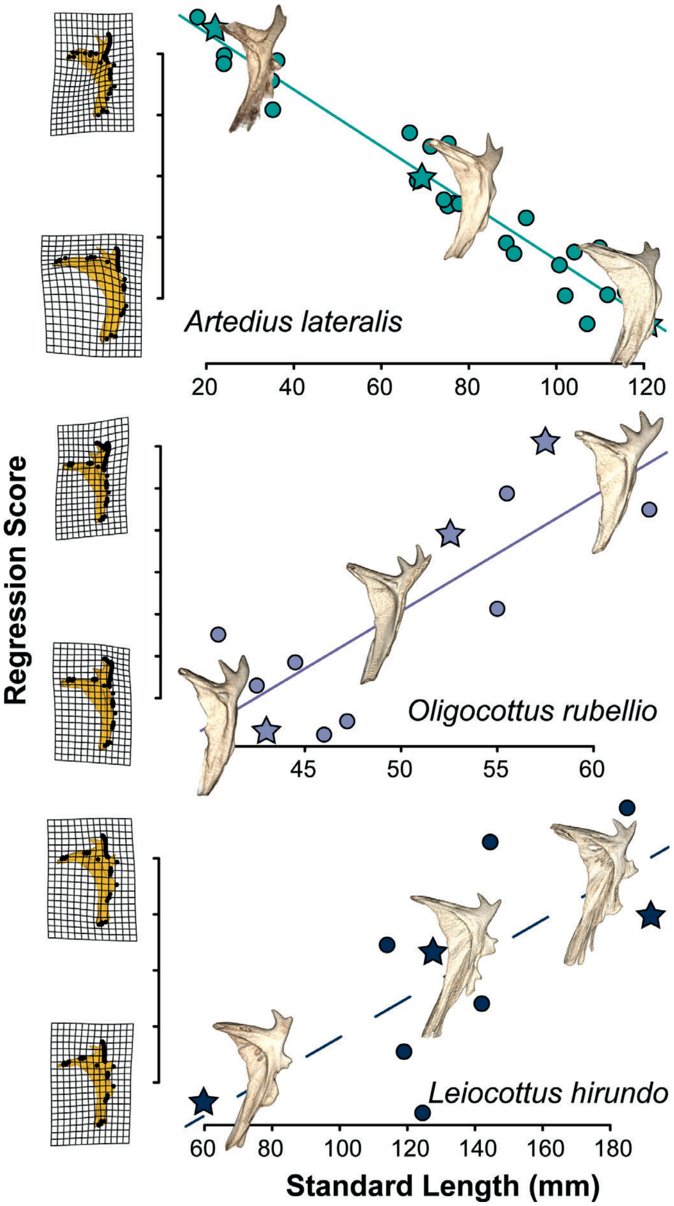

Fig. 2. Allometric trajectories of three representative oligocottine sculpins. The regression score (vertical axis) represents the axis of maximum variance of allometric shape change of the preopercle and is unique to each species. A TPS transformation grid overlayed on a model of a preopercle that has been warped to show the change in shape illustrates the extremes of variation along that axis. The species illustrated (from top to bottom) are: *Artedius lateralis*, *Oligocottus rubellio*, and *Leiocottus hirundo*. Solid lines indicate a statistically significant relationship between preopercle shape and the standard length of the specimen; dashed line indicates a non-significant relationship (e.g., isometry). Stars and points in each plot represent the symmetric component of the shape of the left and right preopercle for an individual specimen of a given species. The inset preopercles in each graph show the morphology of the left preopercle of a small, medium, and large specimen of each species (respectively, from left to right; each specimen marked by a star). The color of stars, points, and regression lines in each plot represents the allometric trajectory such that green is negative (top graph), purple is positive (middle graph), and dark blue is isometry (bottom graph).

trend of negative allometry are *Oligocottus rubellio* and the outgroup taxon *C. pugetensis*, which both showed positive allometry. In these species, relatively short, simple spines in small individuals develop into prominent, complex spines in large individuals (see Fig. 2). The observed trend for these two species follows our predictions for spines that are used primarily for intraspecific agonism. Two species, *A. notospilotus* and *Orthonopias triacis*, have elongate, complex spine shapes and a sharply angled preopercle at all sizes. This isometric growth follows neither prediction but could indicate continuously high predation pressure and/or co-option of the large spines later in life to serve as intraspecific weapons.

We found no evidence for sexual dimorphism in spine shape for any oligocottine species (see exemplar in Fig. S3; see Data Accessibility). The morphospace of males and females completely overlapped within each species, and there was no significant difference in mean shape for males vs. females within any species. These results match the prediction for primarily defensive spines.

Asymmetry was present in all oligocottine species, with most (13/16 + *C. pugetensis*) showing low to moderate levels of rFA (Table S2, examples in Fig. S4; see Data Accessibility). In all cases, the mean difference in shape between replicates of the same preopercle was far smaller than the difference in shape between the left and right preopercles of a given individual ($P < 0.0005$), indicating that the above descriptions of asymmetry represent true differences and not artifacts of precision in landmark placement.

Macroevolutionary hypotheses.—Tabulation of the SSI revealed that *Artedius harringtoni* has the greatest number of sexually dimorphic traits, followed by members of the genus *Oligocottus* (Table S2; see Data Accessibility). Most oligocottine species, however, have few or no external sexual dimorphisms other than an intromittent organ. We found no evidence that the 1) SSI score, 2) maximum body size, or 3) average rFA of a species predict the average preopercle shape (all $P > 0.3$). Because no significant evolutionary covariates of spine shape were found, these results support neither the predictions of the primarily intraspecific agonism or primarily defensive scenarios.

The PCA shows that the greatest axis of shape variance in our dataset (PC1, explaining 52% of the variance) indexes the relative length of the preopercular spines (Fig. 3). Spine length varies inversely with the relative length of the upper arm of the preopercle bone, such that relatively long spines are associated with a relatively short upper arm. The second greatest axis of variation (PC2, 21% variance) captures the 3D curvature of the uppermost preopercular spine. On this axis, high values indicate spines that curve along their entire length and reach anterodorsally toward the midline of the body, while negative values indicate posterodorsally directed straight spines with only a slight curve at their tips (Fig. 3). The remaining PC axes each capture less than 10% of the variance and can be visualized using our supplementary R script (see Data Accessibility).

DISCUSSION

Preopercular spines primarily defend oligocottine sculpins.—Our results support the defensive hypothesis for preopercular spine function in oligocottine sculpins. Species with clear

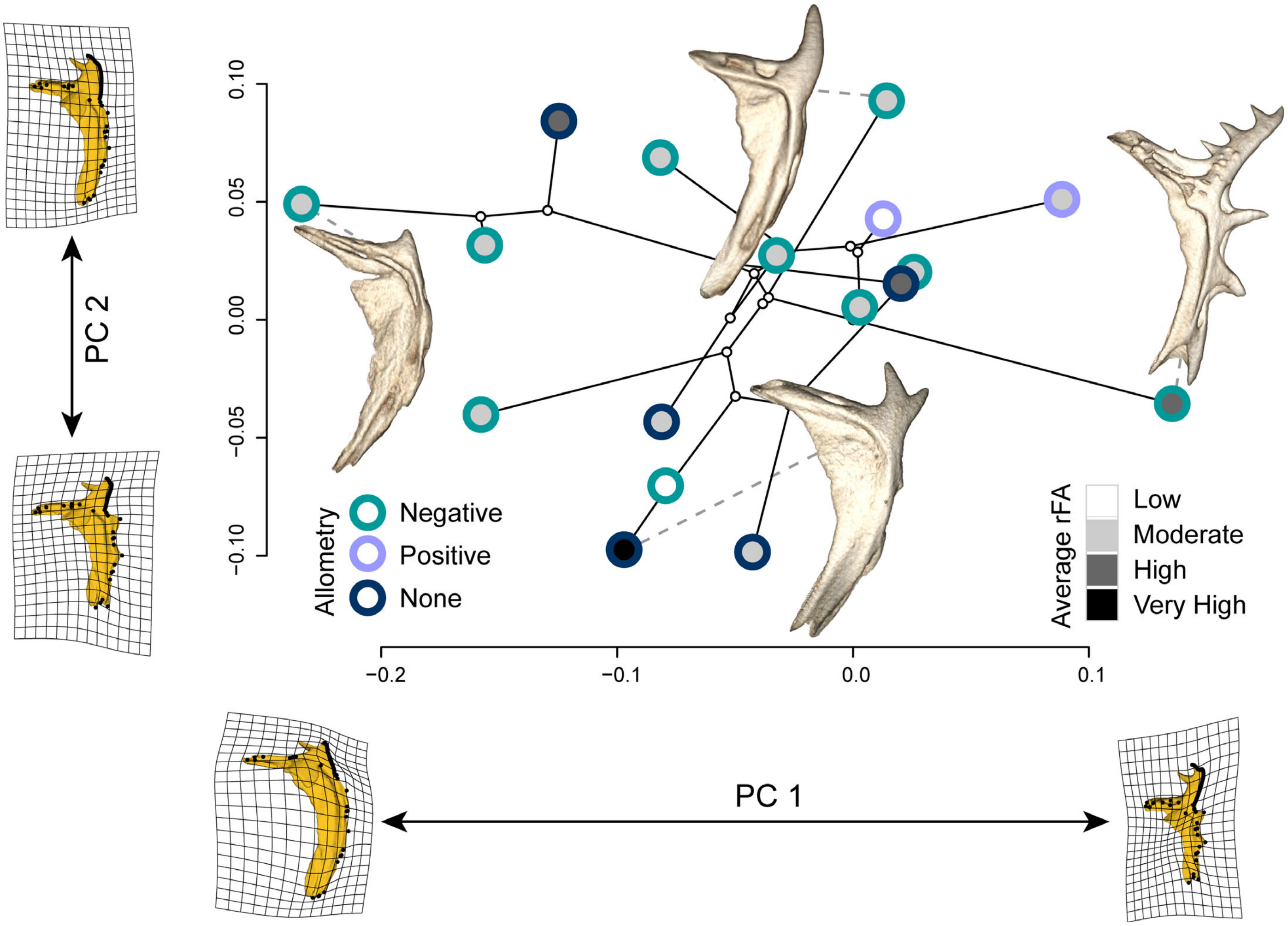

Fig. 3. Phylomorphospace of preopercle shape for Oligocottinae. Each colored dot represents the average preopercle shape for a given species. The fill color of each dot reflects the average rFA of that species. The outline color of each dot represents the kind of allometric trajectory of each species (color follows that of Fig. 2). The shape change captured by PC1 and PC2 is represented by transformation of a model of the average shape of the preopercle in Oligocottinae, with a TPS transformation grid overlaid. A CT reconstruction of the left preopercle from an average specimen of the species that occupies the extreme values of each PC axis is placed near the species location in morphospace, with a dashed gray line connecting the preopercle to the point representing its species. Clockwise, from the top, the illustrated specimens are: *Clinocottus acuticeps* (UAM 47689, 44.00 mm SL), *Chitonotus pugetensis* (OS 5269, 92.95 mm SL), *Artedius corallinus* (SIO 51-34, 59.84 mm SL), and *Clinocottus recalvus* (SIO 51-249, 59.00 mm SL).

hallmarks of spines as defensive weapons occur in each of the major lineages within the Oligocottinae (Fig. 3, Table S2; see Data Accessibility). This fundamental conclusion sets up the sculpins in the Oligocottinae as foils to the dominant pattern of weapon evolution in terrestrial mammals, in which agonistic functions dominate. We propose that this fundamental difference results from how fishes and mammals experience predation in terrestrial versus aquatic environments. The primary predators of terrestrial, weapon-bearing mammals tend to be other mammals, which almost universally dismember their prey prior to consumption. Conversely, predatory fishes tend to consume their prey whole, often using modes of suction-based predation that only work in a fluid environment. Elongate, rigid spines deter gape-limited predators effectively and occur in many fishes (Price et al., 2015). Thus, while some sculpins such as *A. harringtoni* do use the preopercles agonistically (see below), their specialization as offensive weapons may be limited by a need to maintain a shape that frustrates would-be predators that attempt to swallow the bearer whole. The dearth of additional deterrents in sculpins generally and especially in oligocottines may accentuate the need to maintain a defensive spine function. Oligocottines are universally small (maximum SL < 250 mm), have reduced or absent squamation, and lack rigid fin spines and any other defensive structures outside of the preopercular spines. (Bolin, 1944; Mecklenburg et al., 2002). If all oligocottine species experience substantial risk of engulfment even as adults, that factor may explain why we failed to find evidence for evolutionary allometry between body size and preopercular shape. In other words, we suspect that no oligocottine achieves sufficient size for length or girth alone to deter predation. If so, the relative length of the preopercular spines (i.e., PC1) would substantially affect the chances of surviving a predation event for any individual of any species at any point in its life history. While it is possible that other factors could reduce predation pressure and thereby reduce the need to maintain defensive, elongated preopercular spines in adult oligocottines (see below), we find that maximum length per se does not appear to reduce the importance of spination.

Outside of the Oligocottinae, we know relatively little about what drives the evolution of the diverse anatomical arsenal found in fishes. Enlarged spines projecting from the "cheek" area similar to those in sculpins occur in many other groups, including the Bony-eared Assfish (*Acanthonus armatus;* Ophidiidae), marine angelfishes (Pomacanthidae), armored sea robins (Peristediidae), and toadfishes (Batrachoidididae; Kawai, 2013; Nelson et al., 2016). Some fishes, such as loaches (Cobitidae) and the aforementioned stonefishes (Synanceiidae) have retractable blades that they can project from their cheeks. Among the more bizarre weapons found in adult fishes are the medial, unpaired, horn-like structures that have evolved in unicornfishes (genus *Naso*: Acanthuridae; Randall, 2002) and, less spectacularly, in some poachers (e.g., genus *Xeneretmus*; see Kanayama, 1991). In the case of the unicornfishes, the "horn" is used by males in intrasexual competition, but it isn't clear whether this is its sole function, especially since acanthurids also possess sharp, blade-like caudal barbs (Arai and Sato, 2007). The methods described in the current study could be applied to these and other groups of weapon-bearing fishes to test more broadly whether these weapons have evolved under the paradigm typifying terrestrial animals (i.e., primarily for combat) or in response to a primary and primal need to defend against engulfment, as revealed herein for oligocottine sculpins.

Preopercle display: not a toothless threat.—While we found ample evidence of strong sexual selection in several oligocottine species, we uncovered no evidence that shifts in the intensity of sexual selection affect spine shape directly. The species with the highest SSI score (*A. harringtoni*) has short, forked spines similar in shape to those of several of the species with the lowest value on the index (e.g., *Leiocottus hirundo*; see Fig. S5; see Data Accessibility). Though *A. harringtoni* does not possess large spines projecting from the preopercle, the upper arm of the preopercle is relatively elongate, particularly in larger individuals. This elongation could serve two synergistic purposes: 1) it increases the area of attachment of the *levator arcus palatini* muscle, which powers the flaring movement of the preopercle and 2) exaggerates the size of the preopercle (and thus the apparent size of the head) when the preopercles are flared. Some other sculpin species flare their preopercles in a threat display prior to fighting (Morris, 1955), with actual combat taking the form of males biting their opponent. The teeth and mouth of mature male *A. harringtoni* are greatly enlarged relative to females and juveniles (Bolin, 1944; Petersen et al., 2005), so perhaps they too use their preopercles to intimidate rivals before attacking with their teeth, akin to behavior documented in better studied groups such as salmons and South American knifefishes (Evans et al., 2018; Quinn, 2018) and even mammals (Barrette, 1977; Plavcan et al., 1995) and frogs (Emerson, 1994) that bite conspecifics to establish dominance. So, while sexual selection may not directly affect spine shape as we originally hypothesized, it may still indirectly affect preopercle morphology by promoting the evolution of large, threatening displays.

Habitat complexity as a potential influence on preopercle morphology.—Though this study focuses on sexual selection and predation pressure, nothing in nature occurs in a vacuum. Increased habitat complexity, for example, can lead terrestrial vertebrates to reduce the shape and relative size of their weapons to avoid entanglement (Caro et al., 2003). The reverse effect occurs in bovids, wherein females of species that are unable to access the relative safety of complex habitats have evolved horns for defense against predators (Stankowich and Caro, 2009). Does habitat complexity influence weapon evolution in aquatic vertebrates as well? Oligocottine sculpins universally inhabit complex rocky habitats, and while they still bear prominent spines, they are less spiny than some close relatives living in less complex habitats. The species with arguably the most complex spine shape in this study is the outgroup taxon, *C. pugetensis* (see Fig. 3), which exclusively occupies open, sandy habitats (Bolin, 1944; Mecklenburg et al., 2002). Differences in habitat could plausibly drive the differential evolution of weapon complexity among sculpins through two different mechanisms.

As one possibility, complex spines may increase the risk of entanglement, similar to how complex antlers create a liability for cervids moving through dense forests (Gould, 1974; Caro et al., 2003). If so, living in complex habitats may promote spine reduction in oligocottines by imposing an upper limit on spine complexity. Alternatively, the mechanism may involve differential predation pressure in open versus closed habitat. The large spines and positive allometry observed in *C. pugetensis* may reflect higher levels of predation pressure in the exposed habitats in which it lives. In the absence of cover for hiding, *C. pugetensis* may rely on its large and complex spines as the primary predation deterrent. In this case, perhaps the crypsis that oligocottines enjoy in their complex habitats relaxes predation pressure enough to free the preopercle from the need to maintain especially long, defensive spines. Future studies should investigate the potential effects of habitat on spine shape in greater detail and attempt to disentangle these tantalizing possibilities.

Why not simply observe weapon use?—By applying a framework that was originally developed to infer the function of weapons in extinct animals, we concluded that the preopercular spines in oligocottine sculpins evolved primarily to defend against predation. One might reasonably inquire whether this framework was necessary when, presumably, we could have observed the fishes in their natural habitat and documented their behavior. And indeed, an audience member has asked some version of that question every time we have presented this work at a seminar or conference. We acknowledge that observational studies were key to revealing the function of weapons in the extant taxa upon which our framework was constructed and that direct observation may be the most conclusive method of documenting how oligocottine sculpins use their preopercular spines today. However, such an observational study in an aquatic habitat would face serious logistical challenges and could fail to disambiguate the modern and historic uses of the preopercular spine.

In general, documenting animal behavior in the wild requires long hours of observation. Long stretches of unbroken observation create logistical challenges regardless of location, but aquatic habitats present a hard physiological constraint to human observers. The scorpionfishes mentioned above, for example, mostly live in warm, shallow waters but are highly cryptic and generally occur in low abundance (Santhanam, 2018). Even these relatively accessible fishes are only observable in their natural habitat for brief periods of time. Such challenges far exceed those presented by most terrestrial animals and are akin to searching for and observing the natural behavior of beetles in a tropical rainforest while tethered to a hot air balloon floating above the canopy, all while on a limited supply of oxygen.

Even if we could observe many species of sculpins using their spines directly, this would only tell us part of the story. Many weapon-bearing animals use their weapons for a variety of purposes, despite clear trends in the evolutionary forces that shaped those structures. Most animals that bear horn-like structures, for example, are known to use them defensively when the need arises, despite the primary function (both modern and historical) being a tool in intraspecific contests (Metz et al., 2018). Among fishes, the saw of the sawfish represents perhaps the clearest example of a multifunctional weapon, and the rostrum's electroreceptive function in prey detection almost certainly predated its cooption into a tool of prey manipulation (Wueringer et al., 2012a, 2012b), as revealed by the extensive use of electroreception during hunting in their closest living relatives, and in their inferred most recent common ancestor. Such exaptations (Gould and Vrba, 1982) thus present a kind of palimpsest of function, in which the modern use may obscure the evolutionary pressures that shaped a weapon. Since we are interested in macroscale processes, the framework presented herein provides a more direct test of our hypotheses than would an observational study, and more effectively illuminates the origins of sculpin armament.

Fighting fishes.—Our result differs from those obtained in studies of most terrestrial animals that bear horn or antler-like weapons (e.g., *Bos taurus*) that predominantly use their cranial weapons to fight conspecifics. Aquatic and terrestrial habitats challenge their inhabitants differently, and the demands of life in a fluid environment may have influenced the evolution of spine shape among sculpins in ways that have no terrestrial analog. The preponderance of suction-feeding, gape-limited predators in aquatic habitats has shaped fish evolution in many ways, and the morphology of sculpin preopercles may be one of them. The need to maintain a defensive role of the preopercle may constrain the evolution of sculpin preopercles, limiting their ability to develop sexual dimorphism or serve double-duty as intraspecific offensive weapons. Any agonistic uses of the preopercle are thus likely secondary to the primarily defensive function. This pattern runs exactly opposite to that observed among terrestrial species, who have generally evolved their horn-like structures for agonistic bouts and may use them for a secondarily defensive purpose.

The question remains of whether the trends in preopercular spine evolution observed in the Oligocottinae hold constant across the nearly 400 species of cottoids or the numerous other fish groups that bear weapons. While oligocottines are generally adapted to living in complex, shallow habitats, cottoids writ large inhabit diverse environments, and some species grow as large as a meter in length. Large-bodied species living in open habitats, such as most members of the genus *Myoxocephalus*, would provide insight into the degree to which the results presented herein follow from the small size and littoral niches of oligocottines. If even the largest cottoid species use their spines predominantly for defense, not agonism, then weaponized structures among all sculpins would have likely developed under a fundamentally different paradigm than did the cranial weapons of terrestrial animals.

DATA ACCESSIBILITY

All CT data analyzed as part of this study are available from MorphoSource.org using the MorphoSource identifiers listed in Table S1. An annotated R script that performs all analyses conducted herein and all pertinent data files are also provided in supplementary materials. Supplemental material is available at https://www.ichthyologyandherpetology.org/i2022044. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License.

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