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REVIEW

Integrating biomechanics in evolutionary studies, with examples from the amphidromous goby model system

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ABSTRACT

The functional capacities of animals are a primary factor determining survival in nature. In this context, understanding the biomechanical performance of animals can provide insight into diverse aspects of their biology, ranging from ecological distributions across habitat gradients to the evolutionary diversification of lineages. To survive and reproduce in the face of environmental pressures, animals must perform a wide range of tasks, some of which entail tradeoffs between competing demands. Moreover, the demands encountered by animals can change through ontogeny as they grow, sexually mature or migrate across environmental gradients. To understand how mechanisms that underlie functional performance contribute to survival and diversification across challenging and variable habitats, we have pursued diverse studies of the comparative biomechanics of amphidromous goby fishes across functional requirements ranging from prey capture and fast-start swimming to adhesion and waterfall climbing. The pan-tropical distribution of these fishes has provided opportunities for repeated testing of evolutionary hypotheses. By synthesizing data from the lab and field, across approaches spanning high-speed kinematics, selection trials, suction pressure recordings, mechanical property testing, muscle fiber-type measurements and physical modeling of bioinspired designs, we have clarified how multiple axes of variation in biomechanical performance associate with the ecological and evolutionary diversity of these fishes. Our studies of how these fishes meet both common and extreme functional demands add new, complementary perspectives to frameworks developed from other systems, and illustrate how integrating knowledge of the mechanical underpinnings of diverse aspects of performance can give critical insights into ecological and evolutionary questions.

KEY WORDS: Performance, Locomotion, Adhesion, Feeding, Fish

INTRODUCTION

Nature is a demanding place to make a living. For animals to survive, they must, at a minimum, secure food and other resources, avoid being eaten, and withstand physical and physiological pressures from their environment (Waterman, 1999, 2001). The stakes of success are high because, for species to persist, individuals must survive long enough to reproduce. Relative reproductive output of individuals (i.e. fitness), which can determine the evolutionary trajectories of species, is thus intimately related to functional performance across a wide range of tasks (Arnold, 1983; Irschick et al., 2008).

Comparative biomechanics contributes diverse, foundational tools for measuring functional performance and its underlying mechanisms (Lauder, 1991; Biewener, 1992, 2002; Drucker and Lauder, 1999; Ashley-Ross and Gillis, 2002; Westneat, 2003; Brainerd et al., 2010; Vogel, 2013; McCullough et al., 2014; McInroe et al., 2016; Ilton et al., 2018). By evaluating the capacity of organisms (or their structures) to produce or resist forces that contribute to movement, either by parts of the body or of the whole body through the environment, biomechanical studies provide opportunities for critical insights into why some individuals (or species) execute tasks better or worse than others under specified conditions. These insights provide a key link in understanding variation in ecological distributions and fitness (Arnold, 1983; Koehl, 1996).

Although there is a strong foundation for a general connection between biomechanical performance, ecology and evolution, the nature of such relationships in specific systems can be complicated by several factors. Survival depends on the successful performance of multiple tasks that sometimes impose conflicting demands, such that particular body designs may incur tradeoffs in functional performance (Walker, 2007): strong designs may be slow (Kemp et al., 2005), maneuverable bodies may be unstable (Fish, 2002), and designs emphasizing speed may sacrifice endurance (Vanhooydonck et al., 2001). Moreover, the demands placed on individuals commonly change as they mature and grow (Carrier, 1996; Herrel and Gibb, 2006; Heers, 2016), migrate through different habitats (Ebenman, 1992; Diamond et al., 2019), or experience fluctuations in environmental conditions (Gibbs and Grant, 1987; Grant and Grant, 2002). In addition, multiple structural designs can sometimes exhibit equivalent functional performance, or 'many-to-one mapping' (Alfaro et al., 2005; Wainwright et al., 2005). Such issues can complicate efforts to resolve relationships between biomechanics, ecology and evolution. However, they also represent an opportunity for comparative biomechanists, providing a framework for developing hypotheses to test the relative significance of these factors, and their interactions, in generating functional diversity.

In the context of the issues highlighted above, understanding how biomechanical performance contributes to fitness by allowing animals to meet environmental demands requires the examination of multiple functional components, across multiple life stages and suites of environmental conditions. Despite the substantial effort required for such comparisons, several biological systems have undergone focused research seeking to span many of these axes of

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variation. For example, studies of Galápagos finches have associated weather fluctuations that impact the relative availability of food types across years with beak shape (Grant and Grant, 2002) and with mechanical performance of the jaw bones and muscles in relation to both feeding and song production, linking biomechanics to survival and reproductive isolation (Podos, 2001; Podos et al., 2004; Herrel et al., 2005, 2009; Soons et al., 2015). Biomechanical approaches have also garnered evolutionary insights in studies of *Anolis* lizards. For example, though hindlimb length correlates positively with running and jumping performance in many *Anolis* species (Losos and Sinervo, 1989; Losos, 1990a, 1990b), longer hindlegs also incur greater aerodynamic drag that may be selected against during extreme weather events such as hurricanes (Donihue et al., 2018, 2020; Dufour et al., 2019; Debaere et al., 2021). Fishes have also received considerable attention in efforts to link biomechanics and evolution (Langerhans and Reznick, 2010; Higham et al., 2016). For instance, studies of *Gambusia* (mosquitofish) have shown that populations of *G. affinis* with different intensities of predation exhibit differences in body shape and flexibility that contribute to superior performance in either sustained or escape swimming (Langerhans, 2009a); moreover, *G. hubbsi* from habitats with predators have caudal shapes that correspond with higher average velocity and peak acceleration during fast-start escape responses (Langerhans, 2009b). Studies of Trinidad guppies (*Poecilia reticulata*) have also tested complex links between biomechanical performance, ecology and evolution. For example, experiments with guppies have provided rare tests of a common assumption that faster escape responses enhance the chance of surviving encounters with predators (Walker et al., 2005). In addition, populations from high-predation localities show morphological traits that contribute to elevated escape performance, but pregnant females from these locations experience steeper declines in many aspects of performance as gestation proceeds (Ghalambor et al., 2004). Moreover, increased reproductive output that has been selected for in these females yields smaller, less skeletally mature neonates with inferior escape performance, indicating that selection for maternal fecundity can supersede selection on juvenile performance (Dial et al., 2016).

These examples illustrate how diverse model systems have contributed a range of insights into the role of biomechanics in ecology and evolution. In this context, studies of additional, carefully selected systems can lead to new questions and further distinctive insights that deepen understanding of the complex relationships between form and function, as well as factors that cause those relationships to change. This Review synthesizes findings from such a system: amphidromous goby fishes inhabiting streams of oceanic islands. Several aspects of the life history, geographic distribution and diversity of these species make them advantageous subjects for testing how multiple functional mechanisms contribute to survival across diverse, challenging and variable conditions. By drawing together these findings and noting comparisons with other systems, we demonstrate how multiple aspects of biomechanics that underlie performance, including some that have received limited attention in other systems, contribute to ecological and evolutionary diversity. We also suggest hypotheses for further testing in other systems, identifying new opportunities for integrative biomechanical studies.

Biology of amphidromous gobies: features that facilitate studies of functional diversity and evolution

The complex life cycles of amphidromous gobies (Keith, 2003; McDowall, 2003, 2004; Watanabe et al., 2014) exhibit many features that facilitate integration of biomechanical and evolutionary studies (Fig. 1). Gobies are a diverse group (>1000 species) of small-bodied fish (most <10 cm in length) (Thacker and Roje, 2011). Though most are strictly marine, numerous amphidromous species have penetrated freshwater habitats in the streams of circumtropical oceanic islands (Keith, 2003; Keith et al., 2015). In this life cycle, adults live and spawn in freshwater but, upon hatching, larvae are swept downstream to nearshore or ocean habitats where they grow for up to 10 months before returning to freshwater (Radtke and Kinzie, 1996; Nishimoto and Kuamo'o, 1997; Radtke et al., 2001; Hoareau et al., 2007; Teichert et al., 2016), though not necessarily to their natal streams (Moody et al., 2015). Post-larvae then migrate upstream to their eventual adult habitat, where they can live and breed for several years (Fitzsimons and Nishimoto, 1990; Leonard et al., 2012; Teichert et al., 2013a). Such migrations expose amphidromous gobies to a series of pressures that can exert opposing functional demands (Fig. 1). For example, returning post-larvae must first evade predators and then, for species with ranges further upstream, often overcome instream barriers such as massive waterfalls and/or dams that must be climbed to reach breeding habitats (Holmquist et al., 1998; Blob et al., 2010; Lagarde et al., 2020). Stream reaches above waterfalls are mostly free from predators in some island systems, but predators persist in others (Diamond et al., 2019, 2021); moreover, upstream reaches in which fish hold station to feed and establish reproductive territories typically experience faster flow than lower reaches where post-larvae first enter freshwater (Maie et al., 2009a, 2009b). The pan-tropical distribution of goby subpopulations and species across streams (and islands) with different physical and biological environments allows for replicate tests of biomechanical tradeoffs, using comparisons across systems in which different demands predominate and different aspects of performance could be favored (Blob et al., 2010; Moody et al., 2015, 2017, 2019; Lagarde et al., 2018, 2021; Diamond et al., 2019, 2021). Such replicate testing of evolutionary hypotheses across disparate island systems can reduce the influence of unique local environmental conditions as confounding factors in efforts to draw general conclusions.

Several distinctive aspects of goby functional systems also promote opportunities for comparative biomechanical studies. All gobies possess an adhesive disc (Fig. 1D,E) formed by developmental fusion of the pelvic fins (Budney and Hall, 2010) that allows them to attach to substrates by suction (Maie et al., 2012). Adhesion provides a novel framework for biomechanical comparisons (e.g. Irschick et al., 2006; Russell et al., 2019), such as testing for differences in performance and the contributions of different sucker components in climbing versus non-climbing species (Maie et al., 2012; Palecek et al., 2021, 2022). In addition, waterfall-climbing likely had a single evolutionary origin (Fig. 2), but climbing species use one of two distinct mechanisms that reflect different physiological strategies (Fig. 1B,C): 'powerburst' and 'inching'. Powerburst climbing is likely ancestral (Blob et al., 2019) and uses rapid pectoral fin adduction and cycles of axial undulation to propel fish upwards between periods of sucker attachment to the substrate (Schoenfuss and Blob, 2003). In contrast, inching evolved in the genus *Sicyopterus* and involves alternating attachment and advancement of the pelvic sucker and an additional oral adhesive structure formed from a velum in the upper lip, which pressure recordings have demonstrated also attaches to surfaces via suction (Schoenfuss et al., 1997; Maie et al., 2012). Inching movements are slower than those of powerburst climbing, with limited axial undulation or adduction of the pectoral fins (Schoenfuss and Blob, 2003). How such strategies of fast versus slow movement are
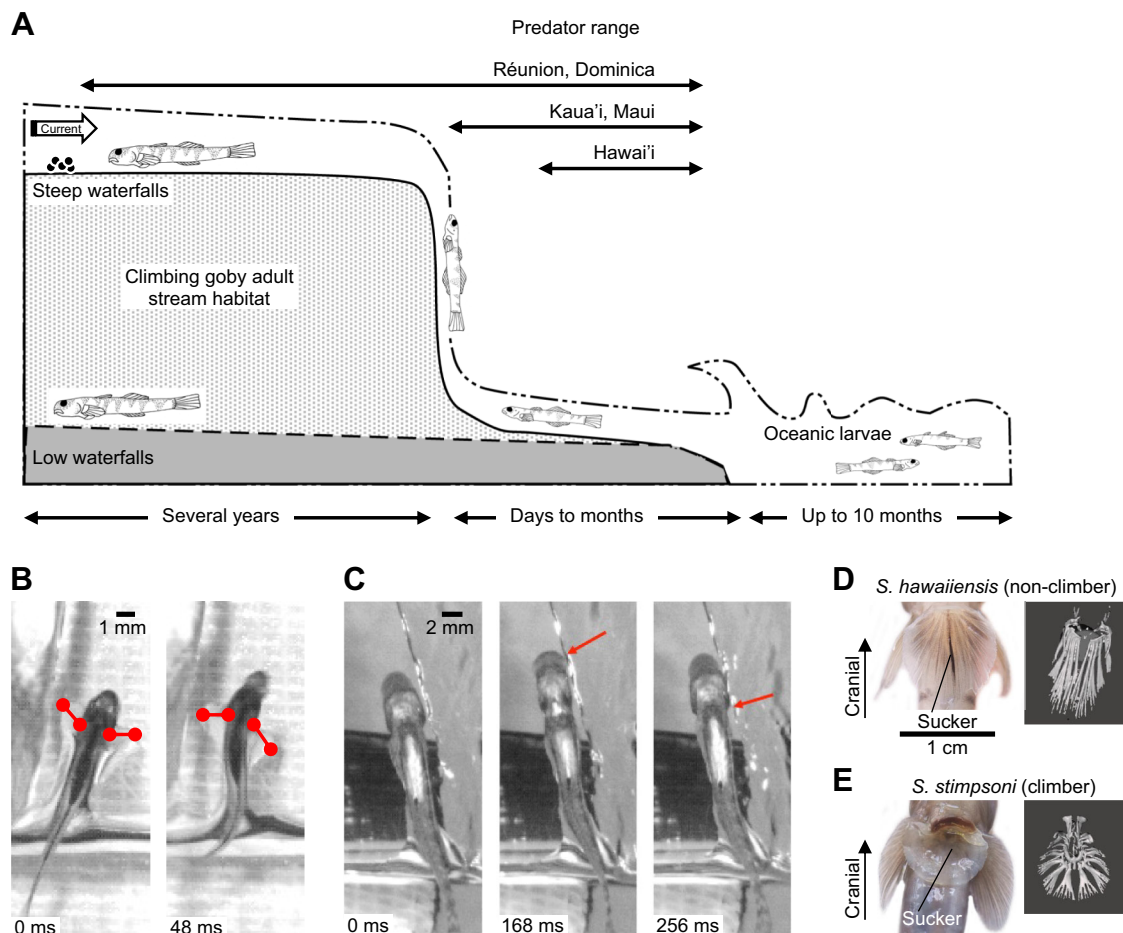
Fig. 1. Life cycle and anatomy of amphidromous gobies. (A) Adults lay eggs upstream. Currents carry hatched larvae to the ocean. After several months, larvae return to freshwater, metamorphose, and migrate upstream to adult habitats. Streams on different islands vary in the severity of waterfall barriers, and whether predators are found above waterfalls. (B) Images from ventral-view, high-speed video of a powerburst-climbing post-larva. Red lines highlight pectoral fin adduction. (C) Images from ventral-view, high-speed video of an inching post-larva. Red arrows indicate upward advance of oral (168 ms) and pelvic (256 ms) suckers. (D,E) Ventral photos (left) and micro-computed tomography (microCT; right) reconstructions of pelvic suckers from non-climbing (D, *Stenogobius hawaiiensis*) and climbing (E, *Sicyopterus stimpsoni*) species. Adapted from Schoenfuss and Blob (2003); Blob et al. (2010) and Palecek et al. (2022).

facilitated by underlying traits, and how they impact climbing performance, represent further frameworks of biomechanical comparisons that can clarify the diversity and evolution of a novel functional ability. In addition, the high reproductive output of gobies and their tendency to migrate into streams in pulses of thousands of individuals enables experimental approaches that require large sample sizes, such as tests of selection on performance (Blob et al., 2008, 2010; Kawano et al., 2013; Moody et al., 2017).

In the context of these distinctive features, the next sections of our Review highlight examples across different aspects of performance that illustrate how the integration of biomechanical approaches has enabled broader ecological and evolutionary insights for gobies, providing new perspectives for studies of other systems.

Integrating mechanisms of performance in ecological and evolutionary contexts
Feeding
The availability of appropriate food is a major factor determining whether species can survive in a particular habitat (Schoenfuss et al., 2004; Julius et al., 2005). Species of amphidromous gobies typically use one of three broad feeding strategies: detritivory (e.g. *Stenogobius hawaiiensis*); suction feeding on insects and other food

floating in the water (e.g. *Lentipes concolor*); and grazing diatoms from rock substrates (e.g. *Sicyopterus stimpsoni*) (Kido, 1996; Julius et al., 2005). Evaluations of the mechanisms underlying feeding performance in amphidromous gobies have revealed multiple factors that facilitate food acquisition and could influence survival, species distributions and evolution.

In the Hawaiian Islands, the suction-feeding species *Awaous stamineus* and *Lentipes concolor* show considerable overlap in diet (Kido, 1996), but typically inhabit different regions of streams, with *A. stamineus* in lower, slower flowing reaches and *L. concolor* further upstream in faster flow that might necessitate faster feeding strikes (Kinzie, 1988; Tate, 1997). Kinematic measurements of feeding in these species show that *L. concolor* achieve larger gapes than *A. stamineus*, which might be expected to decrease the suction pressure they can produce (Fig. 3A). However, *L. concolor* also open their jaws more quickly, which mathematical models show compensates for the impact of gape size on suction pressure, and likely facilitates capture of floating prey in fast-flowing, upstream habitats (Maie et al., 2009a). Specializations for rapid feeding also extend to the jaw musculature (Fig. 3B), as histochemical data show that *L. concolor* have a greater proportion of fast oxidative-glycolytic fibers in both jaw-opening (sternohyoideus) and
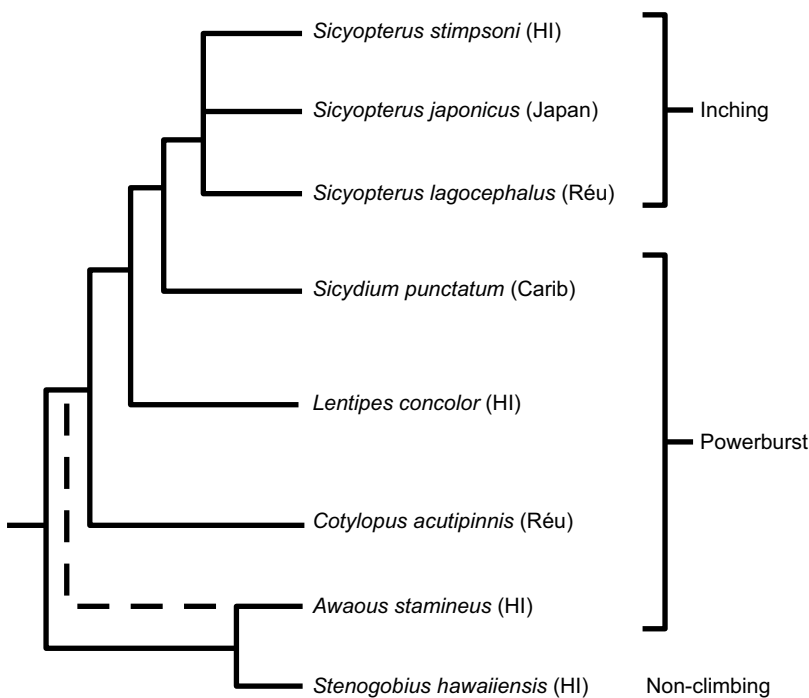
Fig. 2. Phylogenetic and geographic relationships of goby taxa in which climbing has been evaluated. HI, Hawai'i; Réu, Réunion; Carib, Caribbean (Dominica). Climbing styles are indicated next to species names. Dashed line indicates possible relationships for *Awaous*. Data support a single origin of climbing, with powerbursting ancestral. Based on Taillebois et al. (2014) and Blob et al. (2019).

jaw-closing (adductor mandibulae) muscles than *A. stamineus* and the other three gobioid species that live further downstream (Maie et al., 2011). Considered in the context of the climbing ability of these species, these results suggest a novel interaction between feeding and locomotion (Higham, 2007; Kane and Higham, 2011, 2014), as the ability of *L. concolor* to climb further upstream above high waterfalls imposes additional functional demands for capturing food in fast flow (Maie and Blob, 2021) that must be met via changes in feeding biomechanics. Moreover, musculoskeletal models (Fig. 3C) show that a diverse range of goby species exhibit functional differentiation across portions of the jaw-closing muscle adductor mandibulae, such that the a. m. rictomalaris subdivision is better suited for forceful closing and the a. m. stegalis subdivision is suited for rapid closing (Maie et al., 2009b). These results suggest an ability to modulate feeding mechanics (Wainwright et al., 2001, 2008), a capacity that could help gobies accommodate changing functional demands across different portions of streams and might have facilitated the original penetration of upstream habitats.

Biomechanical evaluations of other feeding modes have provided further evolutionary insights. Comparisons of feeding kinematics between algal grazing in Hawaiian *Sicyopterus stimpsoni* and suction feeding in other gobies show significant differences in the maximum values of many variables (e.g. mandible and hyoid retraction) but similar patterns of movements, suggesting that the transition to the novel feeding mode of grazing was achieved largely through changes in the extent of cranial movements, rather than the types or sequence of movements (Cullen et al., 2013). Premaxilla movements are an exception to this pattern, extending much further and with a different timing compared with other motions during algal grazing than in suction feeding by other species. However, as an inching climber using an oral sucker, movements of the premaxilla and other cranial structures in *S. stimpsoni* (Fig. 4) are similar between feeding and climbing (Cullen et al., 2013). These results show another novel interaction between feeding and climbing mechanics (Kane and Higham, 2014); moreover, kinematic similarities between these behaviors suggest that the

novel climbing mechanism of inching gobies may have evolved via exaptation of feeding mechanics (Gould and Vrba, 1982; Kingsolver and Koehl, 1985; Cullen et al., 2013).

The feeding mechanics of predators on gobies also provide insight into factors impacting their evolution. In the Hawaiian archipelago, returning post-larvae of the inching climber *S. stimpsoni* are ∼50% larger than post-larvae of powerburst species such as *A. stamineus* and *L. concolor* (Schoenfuss and Blob, 2003). High-speed recordings of feeding strikes on post-larvae by their main predator, the suction-feeding fish *Eleotris sandwicensis*, show that post-larvae of the larger species can be captured from significantly greater predator–prey distances (Maie et al., 2014). This aspect of predator performance, with greater success capturing larger prey, could provide a biomechanical basis for the strong selection against large body size that predators impose on post-larval gobies (Blob et al., 2010).

Fast-start escape responses

Upon re-entering streams from the ocean, juvenile gobies face intense predatory pressure (Corkum, 2002; Blob et al., 2010; Maie et al., 2014). Gobies use fast-start escape responses (Fig. 5A) to avoid capture (Eaton et al., 1977; Domenici and Blake, 1997; Turesson et al., 2009); however, the stream habitats to which post-larvae return often require escapes to be performed against strong currents (Donaldson et al., 2013). Such currents might directly impact fast-start performance, as well as the ability of gobies to detect flow stimuli produced by predators (Stewart et al., 2013, 2014), reducing their chance of escape. In fast-start trials of post-larval *S. stimpsoni* conducted in a flow tank, using a water jet-pulse as a stimulus, *S. stimpsoni* showed lower response rates to attacks parallel to the water flow compared with attacks against the flow (Fig. 5B), suggesting that flow environments might produce a 'blind spot' to mechanical stimuli from predator strikes (Diamond et al., 2016). However, high-speed videos of natural predatory encounters in streams show that *E. sandwicensis* do not preferentially attack from directions that take advantage of such blind spots; in fact, attacks succeed more frequently when performed against stream
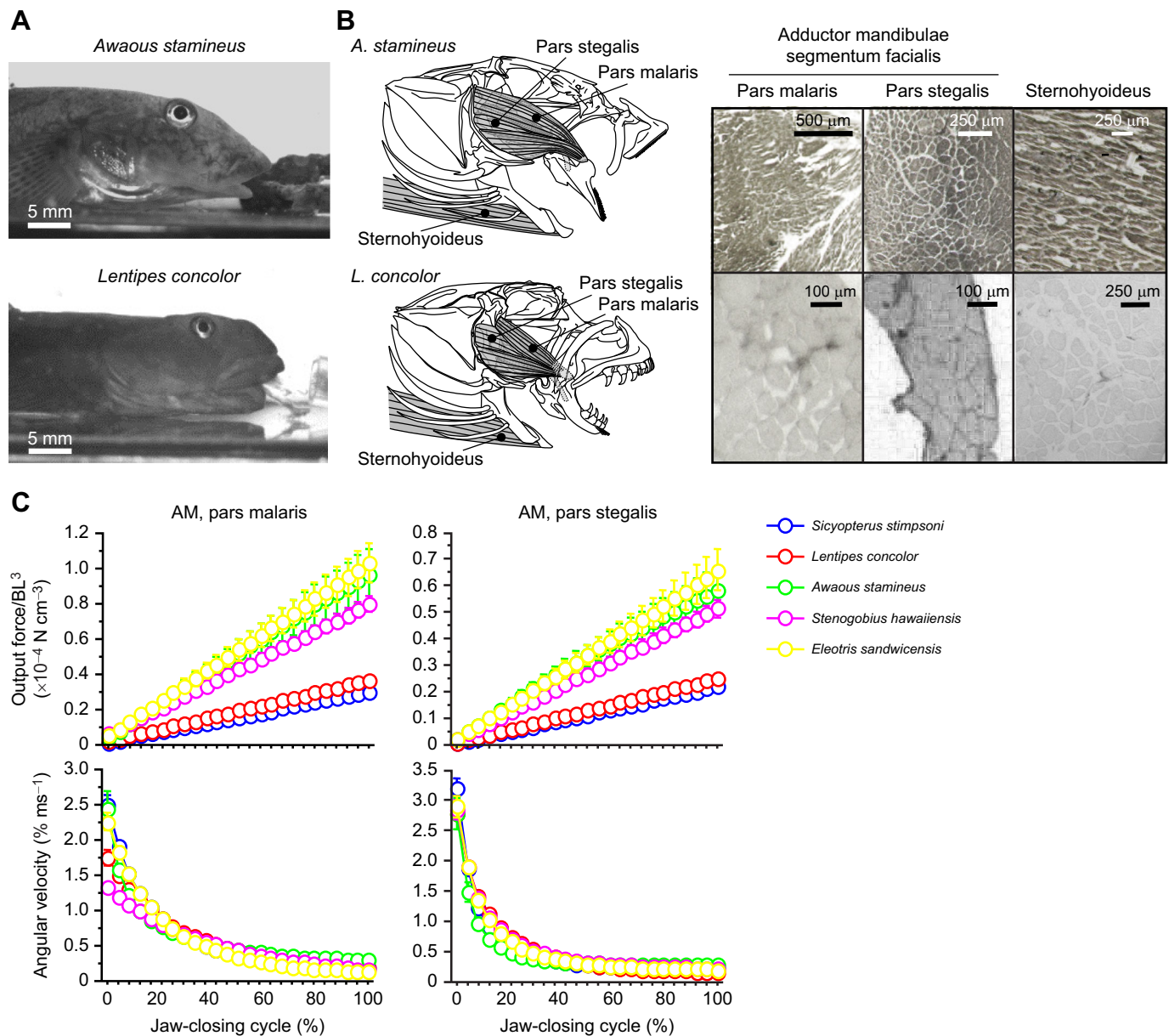
Fig. 3. Feeding performance and underlying mechanisms in amphidromous gobies. (A) Images from high-speed video of food capture by *Awaous stamineus* (lower flow habitat) and *Lentipes concolor* (higher flow habitat) at peak gape. Images have been mirror-flipped from the original for consistency with B. (B) Illustration of jaw closing (adductor mandibulae subdivisions) and opening (sternohyoideus) muscles with representative histochemical sections, illustrating the greater proportion of white fibers in *L. concolor*. (C) Force output (per body length cubed, BL^3) and velocity for subdivisions of the jaw-closing adductor mandibulae (AM) muscle for five gobioid species, showing greater force output for pars malaris and greater closing speed for pars stegalis for all taxa. Adapted from (Maie et al., 2009a,b, 2011).

flow, potentially because of the slower escape speeds by prey or the action of current moving prey toward the predator (Schnieder et al., 2021). These results underscore the utility of field-based tests of lab-based conclusions regarding biomechanical impacts on interactions between species (Combes et al., 2012; Moore and Biewener, 2015; Freymiller et al., 2019).

Some species of amphidromous gobies (e.g. *Stenogobius hawaiiensis*) re-enter streams but do not climb waterfalls, leaving them at risk of predation throughout their life cycle (Schoenfuss and Blob, 2007). Moreover, on islands in the Indian Ocean and Caribbean, predation risk persists after post-larval gobies ascend waterfalls because of the presence of climbing predators (Schoenfuss et al., 2011; Lagarde et al., 2015). In locations such as the Hawaiian archipelago, however, individuals that successfully climb waterfalls reach habitats devoid of most predators (Schoenfuss and Blob, 2003; Schoenfuss et al., 2013). This range of environmental settings guides predictions for how fast-start performance might vary ontogenetically across species and geographic locations varying in predation pressure. For example, post-larvae have higher response frequencies and faster accelerations than adults of the same species, matching predictions based on both scaling (Hale, 1999; Domenici, 2001) and relative predation risk; in addition, adults from taxa that climb out of the range of predators have lower escape performance than species exposed to predators as adults (Fig. 5C) (Diamond et al., 2019). However, species that use climbing styles with different
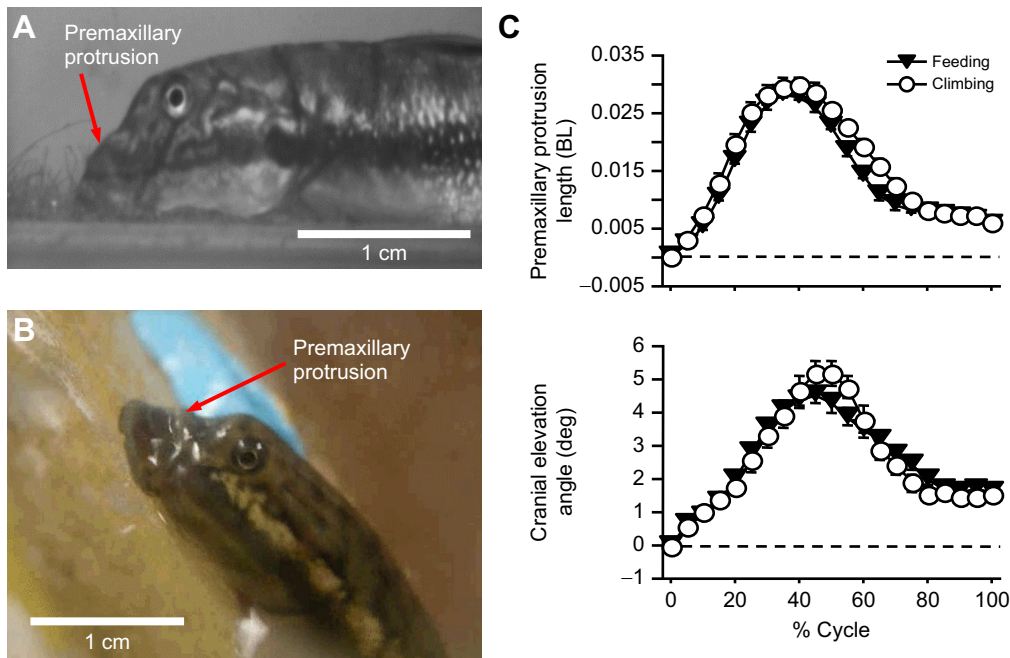
Fig. 4. Kinematic similarity of cranial movements between feeding and climbing in the inching goby *Sicyopterus stimpsoni*. (A,B) Lateral views of *S. stimpsoni* during algae grazing (A) and inching climbing (B), showing similar premaxillary protrusion. (C) Average kinematic profiles for *S. stimpsoni* in feeding and climbing for premaxillary protrusion (top) and cranial elevation (bottom). Adapted from Cullen et al. (2013).

demands for rapid movement (e.g. slow inching versus rapid powerbursting) do not differ significantly in fast-start performance; moreover, closely related species do not have the most similar performance (Diamond et al., 2021). Thus, fast-start acceleration shows little evidence of evolutionary constraint, and selection for competing pressures, such as demands of slow versus fast climbing mechanics, need not limit escape performance (Diamond et al., 2021).

Adhesion and climbing

Located ventrally, just posterior to the opercula, the goby adhesive disc enables attachment to diverse submerged and subaerial surfaces. In many amphidromous species, the disc can adhere to vertical surfaces and assist in climbing rocky substrates of waterfalls up to hundreds of meters tall (Schoenfuss and Blob, 2003; Blob et al., 2006), facilitating upstream migration by post-larvae and repopulation of streams denuded by floods (Fitzsimons and Nishimoto, 1995; Nishimoto and Fitzsimons, 1999), as well as in-stream movements and station holding by adults (Fukui, 1979; Fitzsimons et al., 1997; Blob et al., 2007; Maie, 2022). The two distinct climbing mechanisms used across goby species have dramatically different speeds of movement (Fig. 6A), with video of climbing on lab-simulated waterfalls showing that post-larvae from inching species have size-normalized speeds roughly half as fast as those of post-larvae from powerburst species during single bouts (Schoenfuss and Blob, 2003; Schoenfuss et al., 2011; Blob et al., 2019). However, when climbing extended distances, inching species spend approximately half of their time moving, whereas powerburst species spend <25% of their time in motion and >75% attached to surfaces, resting between bouts (Fig. 6B) (Blob et al., 2006, 2019; Schoenfuss et al., 2011). These alternative strategies are facilitated by structural and physiological differences. For example, the evolutionarily derived slow movements of inching climbers are accompanied by significantly greater fractions of slow oxidative (red) fibers in their axial muscles, which could promote sustained activity (Cediel et al., 2008). In contrast, the ratio of time spent in movement versus rest by powerburst climbers is typical of species using intermittent locomotion, allowing metabolic recovery that can

delay fatigue and extend travel distances (Weinstein and Full, 1992, 1998, 1999, 2000; Weinstein, 2001). Climbing ability is sustained through ontogeny to varying degrees among goby species, with the kinematics of powerburst and inching species becoming more similar with increasing size (Blob et al., 2007; Christy and Maie, 2019; Maie and Blob, 2021).

Although factors such as mucous production and surface structure might also contribute to attachment (Denny and Gosline, 1980; Wainwright et al., 2013; Ditsche et al., 2014), *in vivo* pressure transducer measurements during climbing show that the adhesion of goby pelvic discs is achieved substantially through suction (Fig. 7A) (Maie et al., 2012). Climbing species show pressure differentials between their suckers and the ambient atmosphere that are greater than those of non-climbing species, and greater than expected if suction were strictly a passive function of disc area (Maie et al., 2012). Climbing species also show large safety factors and positive allometry of adhesive strength through ontogeny (Maie et al., 2012; Christy and Maie, 2019; Maie and Blob, 2021), facilitated by greater cross-sectional areas of pelvic retractor muscles and/or lever ratios for these muscles that are advantageous for force production (Fig. 7B) (Maie et al., 2013). In the inching climber *S. stimpsoni* from the Hawaiian Islands, sustained adhesion also appears to be facilitated by elevated proportions of red fibers in muscles of the pelvic disc (~80%) compared with the pectoral fins (<60%), helping to maintain increased volume (and decreased pressure) of the chamber formed when the sucker seals over a surface (Schoenfuss et al., 2013). Moreover, the proportion of red fibers in pelvic disc muscles increases among individuals collected from sites above successively higher waterfalls, whereas measurements of muscle lever arms remain consistent. This suggests that differences in muscle fiber proportions, rather than lever mechanics, may facilitate higher climbing and attachment in faster stream reaches (Schoenfuss et al., 2013).

In further studies of sucker biomechanics, measurements of passive adhesive shear forces during pull-off tests of freshly euthanized specimens (Fig. 7C) show that, among the four native Hawaiian goby taxa, species with the ability to climb furthest
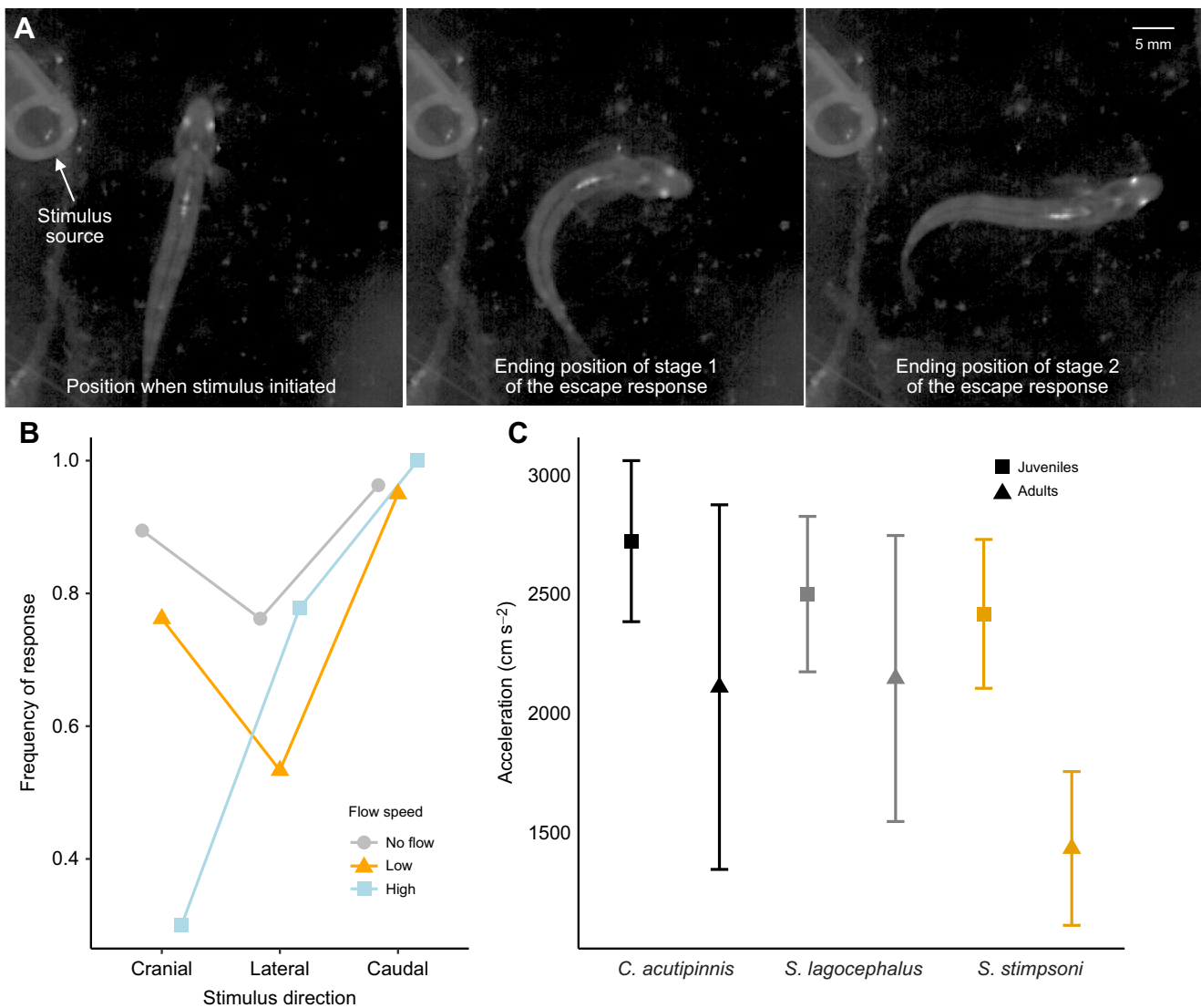
Fig. 5. Comparative fast-start performance in amphidromous gobies. (A) Representative fast-start stages in post-larval *Sicyopterus stimpsoni*. (B) Response frequencies of post-larval *S. stimpsoni* stimulated from three directions in three flow conditions, illustrating low response frequencies for cranial stimulations (parallel to flow) in high flow speeds. (C) Peak accelerations for post-larvae and adults of species from Hawai'i (*S. stimpsoni*) and Réunion (inching *Sicyopterus lagocephalus*, powerbursting *Cotylopus acutipinnis*), illustrating faster performance by post-larvae in all species, and in taxa from habitats where predators overlap with adult ranges (*C. acutipinnis* and *S. lagocephalus*). Error bars indicate 95% confidence intervals. Adapted from Diamond et al. (2016, 2019).

upstream exhibit suckers with the greatest tenacity (pull-off force per unit of sucker area; Palecek et al., 2021). Moreover, sucker tenacity for all species improved on rough substrates, paralleling patterns from other fishes (e.g. clingfish; Wainwright et al., 2013; Ditsche et al., 2014), as well as data that show improved performance on rough substrates during *in vivo* climbing trials (Blob et al., 2006). Attachment to rough surfaces may be facilitated by flexibility of the sucker that could help close gaps between fins and the substrate, increasing friction and preventing leakage at the sucker margin (Wainwright et al., 2013; Wicaksono et al., 2016; Ditsche and Summers, 2019). Supporting this hypothesis, mechanical property measurements from goby lepidotrichia show that fin rays from the pelvic sucker are more flexible than those from the tail in all tested species; in addition, pelvic lepidotrichia are more flexible in the suckers of species that are better climbers (Taft et al., 2017). Amphidromous gobies also show prominent morphological differences across species in the skeletons of their pelvic suckers

(Taft et al., 2017). However, pull-off tests using biomimetic suction cups in which silicone was overmolded (Huie and Summers, 2022) onto different model skeletons, each based on the pelvic anatomy of gobies with different climbing abilities but made from the same material, indicate that external sucker shape likely has a larger impact on tenacity than interior pelvic structure (Palecek et al., 2022). These results suggest that material specializations of the sucker skeleton may contribute more to differences in adhesive performance across species than variation in skeletal morphology. The extent of material specialization of the skeleton related to differences in functional demands has been debated (Currey, 1979, 1999; Erickson et al., 2002; Blob and Snelgrove, 2006; Horton and Summers, 2009; Blob et al., 2014). Findings from gobies add to studies of other fishes noting functionally correlated evolutionary diversity in fin ray stiffness (Taft and Taft, 2012; Aiello et al., 2017, 2018), which might have a material as well as morphological basis.
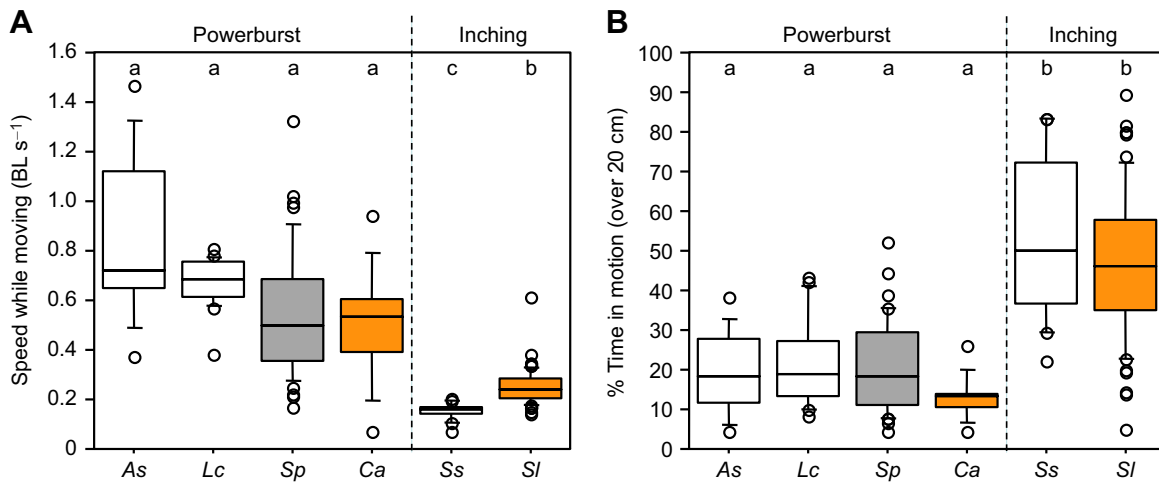
Fig. 6. Comparisons of goby climbing performance. Box plot comparisons of speed while moving (A) and fraction of time in motion (B) across post-larvae of six species, including four powerburst climbers and two inchers. Boxes show 25th percentile, median and 75th percentile; whiskers show 10th and 90th percentiles; circles indicate outliers. Colors indicate localities (white, Hawai'i; gray, Dominica; orange, Réunion). Different letters above boxes denote significant differences between groups (Kruskal–Wallis, Dunn's *post hoc* tests). *As*, *Awaous stamineus*; *Lc*, *Lentipes concolor*; *Sp*, *Sicydium punctatum*; *Ca*, *Cotylopus acutipinnis*; *Ss*, *Sicyopterus stimpsoni*; *Sl*, *Sicyopterus lagocephalus*. Adapted from Blob et al. (2019).

Evolutionary insights through integration of biomechanics with an environmental context

Habitat gradients across the Hawaiian archipelago

Organisms perform as an integrated whole, but the performance demands of some tasks can be at odds with each other (Ghalambor et al., 2003, 2004), producing the potential for tradeoffs that can shape the diversity and evolution of species across varying conditions (Walker, 2007). In amphidromous gobies, the functions of predator escape and climbing might impose conflicting demands, with escape responses likely aided by deep bodies that could improve thrust production, whereas climbing could be facilitated by streamlined bodies that reduce drag from water flowing down waterfalls (Walker, 1997; Langerhans, 2008). One system inhabited by amphidromous gobies, the Hawaiian archipelago, shows a gradient of habitats from its youngest to oldest islands that influences the predominance of predation versus climbing as functional demands. Whereas the youngest island (Hawai'i) has short estuaries, steep stream gradients and many tall waterfalls close to the shore, most waterfalls on the oldest island (Kaua'i) have eroded far inland, leading to streams with long, shallow-gradient reaches (Blob et al., 2008, 2010; Moody et al., 2015, 2017, 2019). This environmental gradient provides a foundation for testable predictions of evolutionary divergence within goby species, informed by understanding of their biomechanics.

Complementary selection trials that exposed cohorts of post-larvae of the inching species *S. stimpsoni*, collected as new recruits into stream estuaries, to either predation or climbing (Fig. 8A,B) support the presence of a tradeoff between these demands: predation survivors had deeper bodies than cohort means prior to selection, and successful climbers had more streamlined bodies than unsuccessful climbers (Blob et al., 2008, 2010). These patterns broadly parallel morphological divergence between individuals from Kaua'i (where the pressures of predation are stronger) and Hawai'i (where the pressures of climbing are stronger), even though larvae from different source islands can mix during their oceanic phase, and genetic data show that larvae do not necessarily recruit to their natal island (Moody et al., 2015). This correspondence between the results of selection trials and observed morphological variation across an environmental gradient supports a conclusion that selection on biomechanical performance acts as a mechanism contributing to diversification, complementing findings in other systems such as the Galápagos finches (Gibbs and Grant, 1987; Herrel et al., 2009), *Anolis* (Donihue et al., 2018), and mosquitofish (Langerhans, 2008). Among the Hawaiian gobies, the range of morphologies and performance capacities generated through oceanic mixing of larvae from different source islands may help to ensure variation that enables at least some portion of returning larvae to survive in most types of streams, or in the event of fluctuations in environmental conditions.

Moving across the archipelago, comparisons of selection results for *S. stimpsoni* post-larvae between Kaua'i and Hawai'i indicate that the predominant pressure on each island (predators on Kaua'i, climbing on Hawai'i) tends to impose less significant selection, and thus may be less effective in promoting morphological changes (Fig. 8C) (Moody et al., 2017). One possible explanation for this pattern is that variation in functionally significant traits for each of these environments may already have been reduced by the past action of directional or stabilizing selection (Moody et al., 2017). Explanations for observed divergence patterns were further explored using individual-based biophysical models of larval dispersal across the archipelago, in which the strength of each selective pressure could be varied (Moody et al., 2019). These analyses showed much greater effects on goby body shape from varying the intensity of predation pressure, compared with varying the intensity of selection from climbing (Moody et al., 2019). These results indicate that, even with the evolutionary emergence of climbing as a novel performance requirement, differences in functionally relevant aspects of goby morphology between the islands may be driven primarily by variation in the intensity of selection by predators (Moody et al., 2019). The importance of predator evasion is corroborated by differences in other factors contributing to performance across individuals from these islands. For example, post-larval *S. stimpsoni* from Kaua'i (where predation pressure predominates) have a greater proportion of white (fast glycolytic) fibers in the axial muscles that power fast-starts compared with juveniles from Hawai'i (Blob et al., 2020). However, no differences in fiber-type proportions were observed
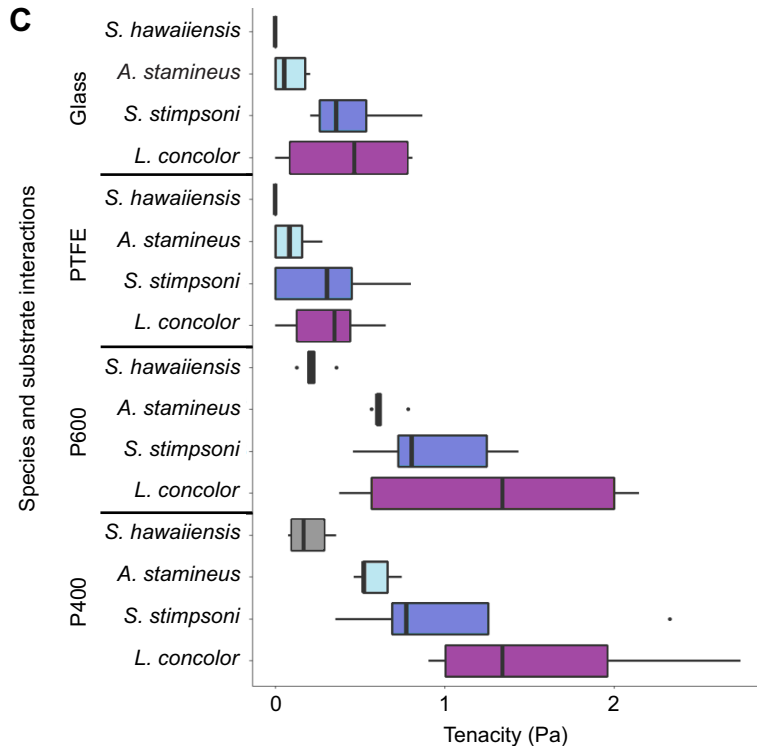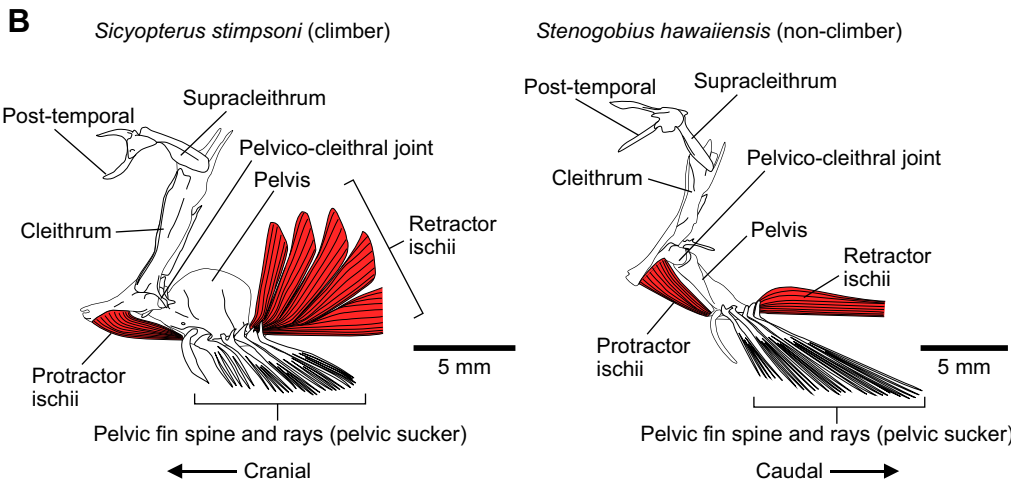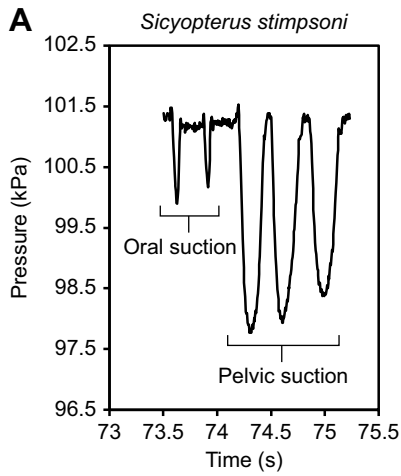
Fig. 7. Adhesive mechanisms and performance in amphidromous gobies. (A) Representative pressure differentials for oral and pelvic suckers of inching *Sicyopterus stimpsoni*. (B) Illustration of muscles controlling the sucker between climbing (*S. stimpsoni*) and non-climbing (*Stenogobius hawaiiensis*) taxa. (C) Box plot comparisons (format follows Fig. 6) of sucker tenacity from shear pull-off tests across surfaces of different roughness for non-climbing *S. hawaiiensis* and species that climb sequentially further upstream (*Awaous stamineus*, *S. stimpsoni* and *Lentipes concolor*), showing greater tenacity for rougher surfaces (from top to bottom) and better climbing taxa. Adapted from Maie et al. (2012, 2013) and Palecek et al. (2021).
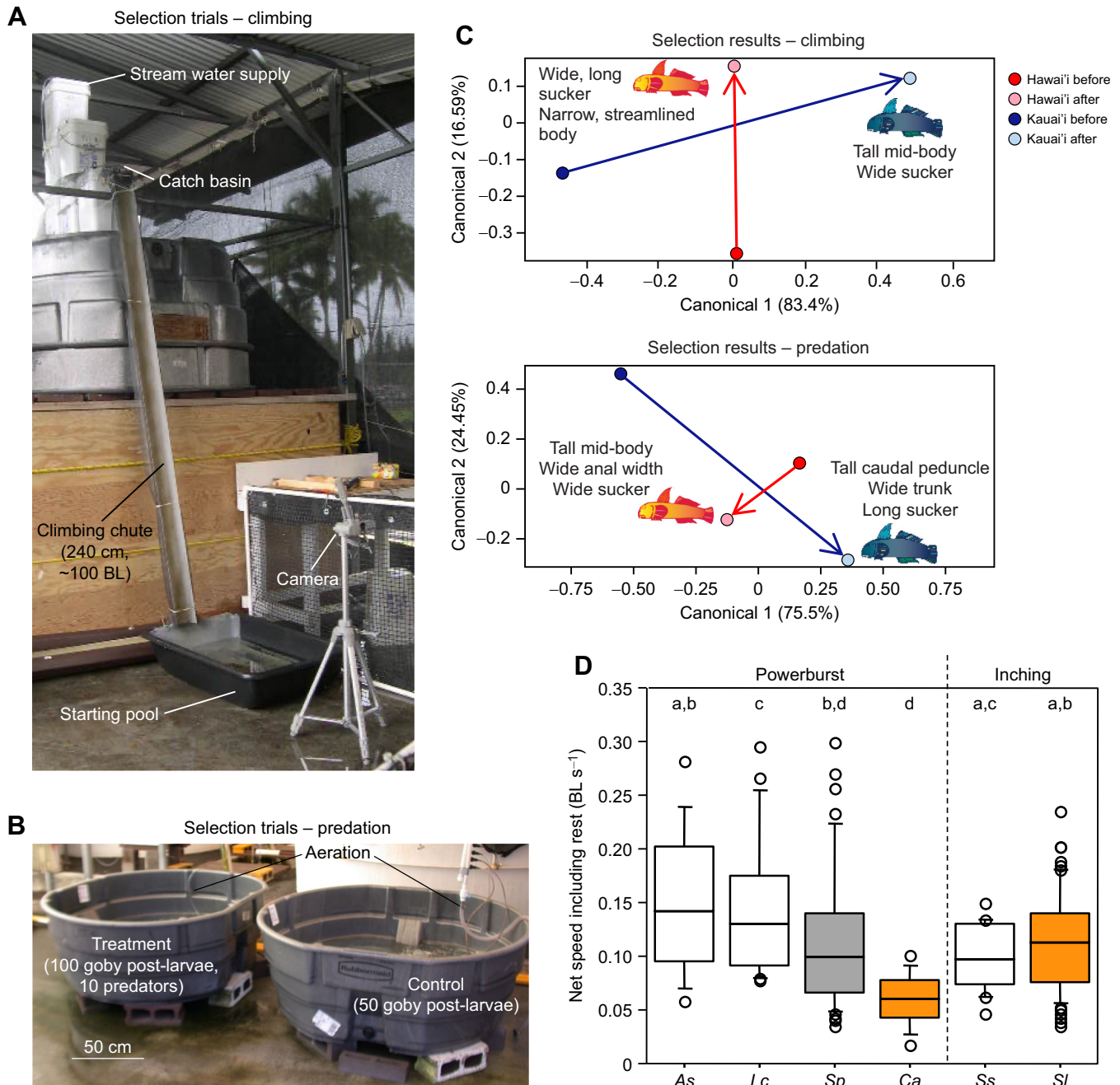
Fig. 8. Selection in post-larval *Sicyopterus stimpsoni*, with comparisons of net climbing performance across species. (A) Climbing selection: groups of ~20 fish in the starting pool were stimulated to climb by stream water flowing down the chute; the morphology of fish reaching the catch basin was compared with that of fish that did not. (B) Predator selection: goby post-larvae were placed in treatment and control tanks; treatments were exposed to 10 predatory *Eleotris sandwicensis* until ~50% of post-larvae were eaten (36 h), after which all fish were collected and morphology was compared between survivors and control fish. (C) Mean canonical axis scores from discriminant function analyses of post-larval *S. stimpsoni*, comparing successful and unsuccessful fish from climbing trials (top) and individuals before and after predation selection trials (bottom), from the islands of Hawai'i (red) and Kaua'i (blue). Corresponding traits are illustrated and arrows indicate the direction of shape difference between the centroids of each group: successful versus unsuccessful climbers, and before predation (control group) versus survivor (post-predation). Gobies from the islands of Hawai'i and Kaua'i show opposing selection imposed by climbing and predation pressures. (D) Box plot comparison (format follows Fig. 6) of net climbing speeds (including both motion and rest) across post-larvae of six goby species, including four powerburst climbers and two inchers. *As*, *Awaous stamineus*; *Lc*, *Lentipes concolor*; *Sp*, *Sicydium punctatum*; *Ca*, *Cotylopus acutipinnis*; *Ss*, *Sicyopterus stimpsoni*; *Sl*, *Sicyopterus lagocephalus*. Adapted from Blob et al. (2008, 2019) and Moody et al. (2017).

between successful and unsuccessful climbing individuals of this species, suggesting that climbing imposes little selection on this trait (Blob et al., 2020). Thus, even in the context of an extreme competing demand like climbing, amphidromous gobies parallel

other fish systems such as sticklebacks (Walker, 1997) and mosquitofish (Langerhans, 2009a, 2009b) in the significance of predator evasion performance shaping evolutionary diversification. For amphidromous gobies, this trend may relate to their sequential

order of evolutionary pressures: climbing imposes selection only after fish have been exposed to predators, potentially constraining the diversity on which selection from climbing can act.

Comparisons across broad geographic and phylogenetic scales

The geographic and phylogenetic diversity of amphidromous gobies provides opportunities for further comparisons of biomechanical performance that can give insight into the evolution of functional diversity. For example, selection trials on climbing post-larvae from a species using powerburst mechanics, *Sicydium punctatum* from the Caribbean, show impacts on different sets of morphological traits and, on average, stronger magnitudes of both directional and correlational selection than were observed in the inching climber *Sicyopterus stimpsoni* (Kawano et al., 2013). Greater selection strength in *S. punctatum* may relate to the different requirements of climbing mechanics in this species, in which fin and body axis movements must be coordinated to a greater extent than during inching kinematics. Thus, patterns of selection may be impacted by specific biomechanical patterns, such as locomotor kinematics, that species use to perform a behavior.

Because most species of fishes do not climb waterfalls, the evolution of waterfall climbing among gobies can be viewed as a novel functional ability. The role of such novelties in evolutionary diversification has been debated (Liem, 1973; Wainwright, 2007). By opening opportunities to use new resources or habitats, novelties may contribute to the diversification or radiation of taxa in a lineage (Konow et al., 2008). However, in some cases, the evolution of novel functions has had limited effects (Price et al., 2010; Wainwright and Price, 2016) or even reduced functional diversity in a group (Higham et al., 2015a). One factor that could influence the impact of novel traits on functional diversity is time: older traits might show more variation than more recently evolved ones, because they have had more time for changes to accrue (Wainwright and Price, 2016). Comparisons of climbing kinematics and performance across juveniles of multiple inching and powerburst taxa show similar net climbing speeds among inching climbers, suggesting this more recently evolved behavior may show less variation, as predicted (Blob et al., 2019). However, different inching taxa in which climbing has been measured produce similar performance through different pathways: species from the Hawaiian Islands move more slowly, but spend less time resting, than species from Réunion in the Indian Ocean (Blob et al., 2019). This pattern of multiple functional pathways producing similar performance parallels the concept of many-to-one mapping developed for structure–function relationships (Alfaro et al., 2005; Wainwright et al., 2005). In addition, because of tradeoffs between speed of motion and time spent moving observed across inching versus powerburst taxa, species that use these alternative mechanisms show little difference in net climbing speed (i.e. performance that accounts for time spent both moving and resting when traveling a given distance: Fig. 8D) (Schoenfuss et al., 2011; Blob et al., 2019). In the context of understanding the evolution of functional novelties, such similarity in net performance between powerburst climbing and inching suggests an intriguing conclusion that selection on locomotor performance may not have been a major factor promoting the evolution of a new locomotor behavior (Blob et al., 2019). This conclusion parallels interpretations of the origins of other functional novelties, such as the evolution of flight in insects (Kingsolver and Koehl, 1985), potentially suggesting a widespread pattern in the evolution of novel functions.

Conclusions and future directions

Animals in nature must secure food, shelter and mates in habitats that can include hazards ranging from hungry predators and physical barriers to dangerous weather and changing climate patterns. Evaluating how animals perform tasks in the face of such challenges is critical for understanding their current diversity, how they evolved, and how they may be impacted by environmental change. Hypotheses about which functional systems and levels of organization show variation linked to differences in performance must be tested (Mykles et al., 2010; Schoenfuss et al., 2013). In this context, systems that are amenable to diverse tests provide opportunities for new insights (Losos, 1990a; Ghalambor et al., 2003; Soons et al., 2015). Comparative biomechanical studies of amphidromous gobies have shown that functional specializations that help meet environmental demands are ubiquitous across multiple anatomical systems and levels of organization (Schoenfuss et al., 2013), from whole-body shape (Maie et al., 2007; Moody et al., 2015; Lagarde et al., 2018; Diamond et al., 2021) to lever systems (Maie et al., 2009b, 2013), muscle fiber types (Cediel et al., 2008; Maie et al., 2011; Schoenfuss et al., 2013) and bone mechanical properties (Taft et al., 2017; Palecek et al., 2022). However, important aspects of performance can be similar across species despite being generated via divergent mechanics (Cullen et al., 2013; Blob et al., 2019); some prominent functional demands may have less influence on diversification than expected (Moody et al., 2019); and some sources of variation may have little impact on performance (Schnieder et al., 2021; Palecek et al., 2022). Achieving such understanding of functional contributions to biodiversity requires the coordination of lab and field studies (Diamond et al., 2016; Schnieder et al., 2021) and the integration of biomechanical approaches (e.g. performance measurements: Schoenfuss and Blob, 2003; Maie et al., 2012) with perspectives from ecology (e.g. individual-based modeling: Moody et al., 2019) and evolution (e.g. selection trials: Blob et al., 2010; Kawano et al., 2013; Moody et al., 2017; population connectivity: Moody et al., 2015). Examining diverse functional components from varied perspectives provides a framework of questions and approaches that can be applied to studies of functional evolution across many systems.

Several additional approaches could be applied to deepen understanding of how functional performance diversifies. For example, neurobiological data indicate that the pectoral fins of round gobies (*Neogobius melanostomus*) can detect fine details of contacted surfaces, providing input to adjust fin position in response (Hardy and Hale, 2020; Hale et al., 2022). Experiments to test whether such abilities differ between climbing and non-climbing goby species, and for their presence in pelvic fins fused into suckers, would evaluate how yet another level of organization contributes to the evolution of novel functions and provide an example for other systems. In addition, tests of escape behaviors motivated by means other than physical stimuli to the lateral line system, such as vision and odor cues (Domenici, 2002; Hale, 2002; Kelley and Magurran, 2003; Ylönen et al., 2007; Chicoli et al., 2014), could improve understanding of critical aspects of performance because such modes of detection might be differentially impaired by changing stream conditions, such as increases in turbidity after heavy rains (Higham et al., 2015b). Additional contexts for performance measurements would also be valuable. For example, return migrations of goby post-larvae into streams typically take place over multiple days with numerous individuals each day, during which time stream conditions can change (Smith and Smith, 1998). Knowledge of whether early migrants are typically superior in

aspects of performance could have wide implications for diverse migratory species (Diamond, 2019). Links between functional performance and adult reproductive success also warrant study. After post-larvae ascend waterfalls, the demands imposed by adult habitats may diverge between males and females as fish grow large enough to breed (Maie and Blob, 2021). Males of many species actively court females by displaying to them in areas exposed to high flow (Fitzsimons and Nishimoto, 1990; Fitzsimons et al., 1993; Teichert et al., 2013b). Female fish often favor larger and deeper-bodied males (Kozak et al., 2008; Ward and McLennan, 2009), shapes that may incur higher drag and greater energetic costs in flowing water. Measuring the mechanical costs of different body shapes under a range of flow conditions could reveal conflicts between natural and sexual selection, and whether such conflicts vary across environmental gradients (Basolo and Alcaraz, 2003; Oufiero and Garland, 2007; Clark and Dudley, 2009).

Considering findings from amphidromous gobies in a broader context suggests additional questions and testable hypotheses about relationships between form and function, and how those relationships change, that can be extended to other systems. For example, in systems in which multiple components contribute to functional capacity (e.g. skeletal morphology, skeletal material properties, muscle physiological properties), are there predictable patterns for which components (or levels of biological organization: Lauder and Reilly, 1996) show more or less variation related to differences in performance? Variation in the morphology of skeletal lever systems is well documented in systems such as the jaws of fishes (Alfaro et al., 2005; Wainwright et al., 2005), but seems less prevalent across the lever systems of goby suckers, where performance variation relates more clearly to differences in muscle fiber-type proportions (Schoenfuss et al., 2013) and the mechanical properties of fin rays (Taft et al., 2017). The extension of variation into these particular functional components might relate to the extreme demands of climbing encountered by amphidromous gobies; however, the ways in which extreme demands impact such patterns will be difficult to evaluate until comparable muscle and bone property data are more widely available for lineages that experience more typical demands. The impact of the evolution of novel climbing behaviors on the diversification of species and body shapes in gobies also remains be formally tested. Given the contrasting impacts of evolutionary novelty on diversity observed in other lineages (Konow et al., 2008; Price et al., 2010; Higham et al., 2015a; Wainwright and Price, 2016), analyses from gobies could improve understanding of the circumstances in which evolutionary novelties promote or constrain diversification.

The diversity of study systems that have linked biomechanics with ecology and evolution has facilitated understanding of many factors contributing to how biodiversity is structured (Koehl, 1996; Lauder, 2003; Wainwright, 2007; Muñoz et al., 2017, 2018). An especially distinctive feature of amphidromous gobies is their global distribution of species across distant islands, providing multiple settings for measuring biomechanical performance in varied contexts. Comparisons across nearby islands within archipelagos have led to several insights at a microevolutionary scale (Moody et al., 2017; Blob et al., 2020), but other analyses have required comparisons across much greater geographic distances (Kawano et al., 2013; Blob et al., 2019; Diamond et al., 2021). Comparisons at broad geographic scales have only recently begun, with biomechanical performance of species from many localities (e.g. West Africa, South Pacific) yet to be evaluated. With available phylogenetic data (Thacker and Roje, 2011; Taillebois et al., 2014; McCraney et al., 2020), wider sampling of biomechanical

performance would enable broader formal testing of macroevolutionary hypotheses through comparative phylogenetic methods (Anderson et al., 2014; Anderson and Patek, 2015; Price et al., 2015; McGee et al., 2016; Friedman et al., 2021; Hodge et al., 2021). Further, advancements in next-generation sequencing and genomic bioinformatics could enable quantification of genome-to-phenome variation and evolutionary trajectories (Wang and Guo, 2019; Ronco et al., 2021). Such opportunities should open further avenues to enrich understanding of how biomechanics contributes to evolutionary diversity.

ECR Spotlight

This article has an associated ECR Spotlight interview with Amanda Palecek.

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