

Functional morphology of an adaptive radiation – sailfin silversides from Sulawesi as a model system

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Contents

Summary	1
Chapter 1: General introduction	5
1.1 Evolutionary radiations.....	6
1.2 Geometric morphometrics	9
1.3 Feeding modes in fishes	13
1.4 The Malili Lakes of Sulawesi – a hotspot for biodiversity	16
1.5 Aims of this thesis.....	20
Chapter 2: Do we need the third dimension? Quantifying the effect of the z-axis in 3D geometric morphometrics based on sailfin silversides (Telmatherinidae)	21
2.1 Summary and personal contributions	22
Chapter 3: Sexual dimorphism in an adaptive radiation: Does intersexual niche differentiation result in ecological character displacement?	25
3.1 Summary and personal contributions	26
Chapter 4: Functional morphology of prey capture in stream-dwelling sailfin silversides (Telmatherinidae) based on high-speed video recordings.....	30
4.1 Summary and personal contributions.....	31
Chapter 5: General discussion and prospects.....	35
5.1 General discussion	36
5.2 Prospects	41
References	44
Supplementary material.....	58
Acknowledgements.....	59
Appendix	60

Summary

The extent of global biodiversity is simply astonishing. Until today, between 1.5 and 2 million different species have been described but the majority remains to be discovered. These high numbers raise a central question: how could this rich biodiversity evolve? Theory predicts that a large proportion of this biodiversity is an outcome of adaptive radiations. These are defined as a rapid diversification of an ancestral lineage into an array of adaptive forms which occupy different ecological niches.

The radiation of sailfin silversides – atheriniform fishes closely related to the rainbowfishes from Australia and New Guinea – serves as a valuable model system for studying adaptive divergence within the confined space of geologically old freshwater lakes. In total, 16 sailfin silverside species have been described from Sulawesi, of which eight are endemic to ancient Lake Matano in the central highland of the island. These eight species are composed of two phylogenetic lineages, the predominantly pelagic “roundfins” and the epibenthic “sharpfins”. They occupy a variety of ecological niches and have evolved distinct morphological adaptations to exploit their habitats. Because these adaptations are presumably facilitated by ecologically-based divergent selection and since the different morphospecies hybridize occasionally, it seems plausible that ecological speciation processes are their causal root and continue to take place. However, this morphological and ecological variation is not limited to an interspecific level but has also been documented between sexes. In fact, the three roundfin morphospecies were the first case in which sexual niche partitioning was demonstrated in an adaptive fish radiation. The impact of this sexual differentiation on the adaptive spectrum of the radiation remained however unclear. Sailfin silverside species diversity is unevenly distributed across the different freshwater ecosystems of Sulawesi. While 14 species inhabit the lakes of the Malili Lakes system, only two species – *Marosatherina ladigesii* and *Telmatherina bonti* – predominantly occur in flowing habitats like rivers and streams. In contrast to the roundfins and sharpfins of Lake Matano, the ecology and morphology of these two riverine species has been barely studied.

The present thesis has three main goals which are targeted in chapters 2, 3 and 4: i) To evaluate whether 3D geometric morphometrics (GM) offers any significant benefits

compared to 2D GM performed on laterally compressed fishes. ii) To test if the sexual niche differentiation identified in roundfins results in ecological character displacement. iii) To investigate whether the two stream-dwelling sailfin silversides *M. ladiges* and *T. bonti* use alternative feeding modes and if their feeding biomechanics differ from roundfins.

Chapter 1: In this chapter, I provide insight into evolutionary radiations and the intersexual variation within them, followed by a general introduction into GM and feeding modes in fishes. Finally, I give an overview into the radiation of sailfin silversides from Sulawesi.

Chapter 2: Geometric morphometrics are a well-established method to study morphological variation among organisms since they are particularly powerful in detecting small-scaled differences in shape. Although an increased distortion and a certain loss of information seems obvious when using 2D images to describe 3D objects, 2D GM based on standardized photographs or X-ray images are still widely used. 2D methods are often favored since they are substantially less time, effort and cost intensive than 3D methods. This preference is particularly true for ichthyological studies which is presumably due to the flattened body shape of many fish species. Nevertheless, excluding the third dimension from the analysis has an impact on GM results. However, this effect has not been quantified in laterally compressed fishes so far. In this chapter, I use the three roundfin morphospecies from Lake Matano to assess the benefits and disadvantages of 2D and 3D GM for morphological studies on laterally compressed fishes. In detail, I compared the repeatability, information content, species and sex discrimination ability of both methods in the light of the substantial gap in effort. We did not detect any significant differences in accuracy or measurement error between both analyses. Although the third axis contained a significant amount of shape information in the 3D approach, this additional variance was not beneficial for distinguishing species or sexes. The 2D and 3D analyses both identified significant differences in shape between species but not between sexes of a single species. This limited additional value of the 3D data set was accompanied by a substantially higher effort, processing time and data size compared to the 2D data set. Taken together, the 2D GM analysis was more efficient than the 3D GM analysis for discriminating roundfins based on head shape. However, in order to project these findings on laterally compressed fishes in general, further studies incorporating a broad range of taxa are necessary. Meanwhile, we suggest using a

subsample in future studies to test for congruence between 2D GM and 3D GM. In case this congruence is high, we recommend using a 2D approach because of the substantially lower processing time and data storage space required.

Chapter 3: The emergence of adaptive radiations is highly facilitated by ecological opportunity and biotic interactions (e.g. resource competition) are a large component of ecological opportunity. Competition for limited resources is especially high between males and females of a single species because they generally show resembling trophic ecologies. In theory, this intersexual competition could lead to alternative modes of resource use that would reduce ecological niche overlap. Consequently, this might drive the evolution of ecological character displacement between both sexes. Sexual niche partitioning has been documented in several adaptive radiations such as in *Anolis* lizards or sticklebacks, but its effect on adaptive processes and the degree of intersexual variation remains unresolved. In this study, I test if the sexual niche differentiation demonstrated in roundfins has induced adaptations in several morphological traits of the head. We identified significant sexual dimorphism in both morphospecies that show sexual niche partitioning while sexual variation was absent in the only morphospecies that does not show sexual niche differentiation. The revealed sexual differences in head morphology are connected to the ecological niches and match the degree of sexual niche differentiation of each morphospecies. Since all analyzed structures are ecologically relevant and partly situated internally, it seems plausible that sexual selection only has a minor impact on the variation. It rather appears likely that ecologically-based divergent selection has led to the evolution of the identified sexual dimorphism. These morphological adaptations presumably reduce ecological niche overlap and minimize intersexual competition in roundfins. I conclude that ecological character displacement between males and females contributes to the adaptive spectrum of roundfins and could be crucial for limiting intraspecific competition in adaptive radiations in general.

Chapter 4: Although ray-finned fishes (Actinopterygii) include more than 30.000 species, all of their prey capture strategies can be assigned to at least one of three main feeding modes: suction feeding, ram feeding and manipulation feeding. Manipulation feeders are generally distinct in their feeding behavior and morphology since they rely on a high bite force.

Suction and ram feeders, however, both depend on a high velocity transfer. Thus, the distinction between both feeding modes is often subtle and transitional. Previous work has revealed alternative functional adaptations and feeding modes, ranging from suction to ram feeding, in lake-dwelling roundfin *Telmatherina*. On the contrary, the functional morphology of stream-dwelling sailfin silversides is unexplored so far. In this work, I test if the stream-dwelling sailfin silversides, *Telmatherina bonti* and *Marosatherina ladigesii*, use different feeding modes and whether they differ from roundfins in their feeding biomechanics. The results of the prey capture kinematics indicate that *T. bonti* is a ram feeder while *M. ladigesii* is a suction feeder. Intersexual variation in kinematics was significant in both species. Contradicting these findings, feeding biomechanics did not reveal any clear indications for alternative feeding modes or sexual dimorphism in both analyzed species. In comparison to roundfins, feeding biomechanics of *T. bonti* and *M. ladigesii* are less distinct. In conclusion, my findings suggest that *T. bonti* primarily uses ram feeding whereas *M. ladigesii* predominantly uses suction feeding. The lack of differentiation in biomechanics might be explainable by contrasting trophic niches and variable environmental conditions. However, this hypothesis needs to be treated with caution until the trophic ecology of both stream-dwelling species has been quantified.

All in all, this thesis uses the radiation of sailfin silversides to show that: i) 2D GM analyses might be beneficial over 3D GM approaches for laterally compressed fishes. ii) Sexual niche differentiation and the resulting ecological character displacement could be key factors for reducing intraspecific competition in adaptive radiations. iii) Feeding biomechanics in sailfin silversides are not entirely linked to prey capture kinematics and might be affected by environmental conditions too. Nonetheless, my work also reveals that further research is necessary. For instance, more studies investigating sexual niche differentiation in radiations are needed to evaluate its relevance for speciation processes and more ecological data on stream-dwelling sailfin silversides is required to fully understand their functional feeding morphology. However, these findings demonstrate how valuable the radiation of sailfin silversides is as a model system for adaptive radiation research.

Chapter 1

General introduction

1.1 Evolutionary radiations

Planet Earth is thought to harbor between 1.5 and 2 million described species (Costello et al., 2012, 2013; Pimm et al., 2014) and estimates predict that more than 6 million are yet to be discovered (Mora et al., 2011; Pimm et al., 2014; Stork, 2018). One of the great questions in evolutionary biology is: How could this enormous biodiversity arise? One possible explanation might be the occurrence of evolutionary radiations – the rapid and extraordinary species diversification within a single clade (Bouchenak-Khelladi et al., 2015; Naciri & Linder, 2020). In the past, evolutionary radiations and adaptive radiations – the latter being defined as rapid adaptive divergence of a single lineage into various ecological niches – have largely been considered as synonyms (Simões et al., 2016). But in recent years, the view has emerged that indeed all adaptive radiations are evolutionary radiations but not vice versa (Erwin, 1992; Kagawa, 2022).

1.1.1 Adaptive radiations

Adaptive radiations are by far the most studied form of evolutionary radiations (Kagawa, 2022; Simões et al., 2016). Probably the most prominent examples are the Darwin's finches from the Galapagos Islands, the cichlid radiations from the East African Great Lakes and the *Anolis* radiations from the Caribbean (Gavrilets & Losos, 2009; Losos, 2010). Schluter (2000) defined four criteria which can be used to identify an adaptive radiation: (i) Members of the radiation share a common ancestor, (ii) A fit between the environment and the phenotype of the component species (phenotype-environment correlation), (iii) Occurrence of phenotypic traits that increase the fitness of the different taxa within a radiation (trait utility), (iv) One or more “bursts” of speciation events (rapid speciation). A key factor for permitting these features is ecological opportunity, the availability of various different types of resources which are little used by competing species (Stelbrink et al., 2020; Yoder et al., 2010). This can be enabled in four different ways: the emergence of new resources, the extinction of competitors, the colonization of new environments with abundant resources or the evolution of novel traits (key innovations) that enable the exploitation of resources differently (Gavrilets & Losos, 2009; Losos, 2010; Martin & Richards, 2019). If at least one of these prerequisites is met, the evolution of phenotypic adaptations can facilitate the exploitation of different ecological resources (Colombo, 2017; Schluter, 2000). As a result,

members of a single lineage are able to coexist in the same environment by occupying alternative ecological niches, eventually leading to reproductive isolation (Gavrilets & Losos, 2009; Losos, 2010; Yoder et al., 2010). Such speciation events can occur through two main mechanisms: mutation-order speciation and ecological speciation (Nosil & Flaxman, 2011; Rundle & Rowe, 2018). Mutation-order speciation is defined as the evolution of reproductive isolation by the accumulation and fixation of several mutations under resembling selection pressures (Schluter, 2009; Schluter & Conte, 2009). Contrarily, in ecological speciation, barriers of gene flow arise between populations by adaptation to alternative ecological niches through divergent natural selection (Rösch et al., 2013; Rundle & Nosil, 2005; Schluter, 2001). A major difference between both mechanisms is that natural divergent selection is favored in ecological speciation, whereas it occurs randomly under the assumption of mutation-order speciation (Rundle & Rowe, 2018; Schluter & Conte, 2009). Thus, in ecological speciation the fixation of mutations is driven by natural selection acting divergently on contrasting environments, while in mutation-order speciation these fixed mutations would be beneficial in both environments (Schluter, 2009; Schluter & Conte, 2009). Repeated ecological speciation events are often regarded as a potential cause for the phenomenon of early bursts in adaptive radiations (Martin & Richards, 2019, Rundle & Price, 2009).

1.1.2 Intersexual niche differentiation and its effect on adaptive speciation processes

Ecological speciation is predominantly driven by ecologically-based divergent selection (Martin & Richards, 2019; Rösch et al., 2013). Divergent selection is ecologically-based when it emerges due to biotic factors including mutualism, predator/prey interactions or resource competition (Rundle & Nosil, 2005; Schluter, 2001). If the environments inhabited by populations of a species differ, ecologically-based divergent selection can act in contrasting directions on both populations (Rundle & Nosil, 2005; Schluter & Conte, 2009). In the case of resource competition, this will eventually result in intraspecific niche differentiation which reduces the ecological competition and leads to ecological character displacement between both populations (Losos, 2000; Schluter, 2000; Stuart & Losos, 2013). This mechanism has been reported as a key factor for permitting adaptive processes, for instance, in the cichlid radiations of East Africa (Burres, 2015; Ford et al., 2016). The various cichlid species show distinctive morphological adaptations to different diets such as algae, insects or other fishes

(Burres, 2015; Ford et al., 2016). In theory, ecologically-based divergent selection might not only act on different populations but also on males and females within a species (De Lisle, 2019; De Lisle & Rowe, 2015; Roy et al., 2013; Shine, 1989). This mechanism could facilitate sex-specific feeding ecologies that may decrease intersexual competition and thus lead to ecological character displacement between sexes (De Lisle, 2019; Butler et al., 2007; Roy et al., 2013). Recent work has demonstrated that ecological character displacement between sexes and ecological speciation can arise at the same time (De Lisle & Rowe, 2015, 2017). However, the influence of intersexual niche partitioning on speciation processes remains largely understudied (De Lisle & Rowe, 2017; Ronco et al., 2019). It has been documented for a few adaptive radiations including *Anolis* lizards, sailfin silversides or sticklebacks (Butler, 2007; Pfaender et al., 2011; McGee & Wainwright, 2013), but whether intersexual niche partitioning enhances or rather restrains adaptive speciation processes is open to debate (Bolnick & Doebeli, 2003; Butler, 2007; De Lisle & Rowe, 2015). On the one hand, ecological intersexual variation might promote adaptive radiations by facilitating males and females of a species to reach their sex-specific phenotypic optima (De Lisle & Rowe, 2017). This will increase the competitive strength of the respective species and thus enable the successful establishment in its environment (De Lisle & Rowe, 2017). On the other hand, the traditional hypothesis is that sexual dimorphism in traits of ecological relevance retards adaptive speciation since intersexual variation is assumed to evolve prior to speciation and reduces ecological opportunity (Bolnick & Doebeli, 2003; De Lisle & Rowe, 2015). This theory is supported by the fact that sexual dimorphism is usually more pronounced in species-poor radiations than in species-rich radiations (Butler et al., 2007; Herler et al., 2010). Taken together, the impact of intersexual niche differentiation on adaptive radiations remains to be understood (De Lisle & Rowe, 2017; Ronco et al., 2019; Pfaender et al., 2011).

1.1.3 The drivers of sexual dimorphism

Sexual dimorphism is a pervasive type of intraspecific morphological variation and has been identified in various adaptive radiations (McGee & Wainwright, 2013; Ronco et al., 2019; Roy et al., 2013). The most prevalent forms are sexual size dimorphism, sexual color dimorphism and sexual shape dimorphism (Tsuboi et al., 2012). Theory predicts that the evolution of sexual dimorphism can be driven by three different adaptive mechanisms: sexual selection, dimorphic niches induced by intrinsic differences between males and females or intersexual

food competition (De Lisle, 2019; Hedrick & Temeles, 1989; Herler et al., 2010). However, these three mechanisms do not necessarily exclude each other and may act side by side (Bolnick & Doebeli, 2003; Temeles et al., 2000). Originally, sexual selection was regarded as the sole causation for the occurrence of sexual dimorphism (Hedrick & Temeles, 1989; Shine, 1989). However, for structures which are cryptic to the outer appearance of a species and/or traits which are highly relevant for resource exploitation, this explanation appears to be unlikely (Bolnick & Doebeli, 2003; Ronco et al., 2019). In fishes, these include mouth orientation, dentition, oral and pharyngeal jaws, gape width, gill rakers, the opercle, the gut and the buccal cavity (Albouy et al., 2011; Keppeler et al., 2020). In these traits, intersexual ecological competition for limited resources might instead be the main driver for the evolution of sexual dimorphism (Bolnick & Doebeli, 2003; De Lisle & Rowe, 2017; Roy et al., 2013). This has been documented, for instance, in three-spined sticklebacks (Albert et al., 2008; McGee & Wainwright, 2013).

1.2 Geometric morphometrics

The anatomy and morphology of organisms is typically quantified using morphometric approaches, which includes a great variety of methods (Marcus et al., 2013; Rohlf and Marcus, 1993). Traditional morphometrics are based on one-dimensional distance data in order to describe the length, width or height of certain organisms and their structural components (Adams et al., 2004; Marcus et al., 2013; Slice, 2005). A major drawback of this method is that linear measurements are highly correlated with size whereas the geometry of the respective specimen or structure is lost (Adams et al., 2004; Rohlf and Marcus, 1993). In contrast, geometric morphometrics (GM) focus on shape which is defined as geometric information after mathematically excluding location, orientation and scale (Kendall, 1977; Lawing & Polly, 2010). The first morphometric approaches that targeted the shape of certain structures were outline methods (Adams et al., 2004; Lawing & Polly, 2010). Since the bounding edges of a structure can be considered as homologous between specimens, outline methods (e.g. the Fourier analysis) can be used to quantify the shape of organisms with rather limited equipment (Adams et al., 2004).

1.2.1 The workflow of landmark-based geometric morphometrics

Nowadays, the most common form of GM are landmark-based approaches (Kaliontzopoulou, 2011; Loy, 2007; Tatsuta et al., 2018). In this case, 2-dimensional (2D) or 3-dimensional (3D) cartesian coordinates of landmark locations are used to describe the shape of organisms (Adams et al., 2004; Lawing & Polly, 2010; Mitteroecker & Gunz, 2009; Rohlf and Marcus, 1993). Thus, the first step of a landmark-based GM approach is the definition of landmarks (Curran, 2018; Kaliontzopoulou, 2011). In order to achieve a sufficient coverage of the region of interest, landmarks have to be placed at homologous and biologically meaningful points which can be located in all specimens of the respective dataset (Curran, 2018; Zelditch et al., 2012).

Landmarks can be classified according to three different categories: Type I, Type II and Type III (Bookstein, 1991; Loy, 2007; Slice, 2005). Type I landmarks are placed at distinct anatomical points such as intersections of cranial sutures (Bookstein, 1991; Slice, 2005). They are considered as highly repeatable, easy to locate and thus as the ideal option (Curran, 2018). Type II landmarks are intermediate in repeatability and locatability (Curran, 2018). They are placed at maxima or minima of processes, pits and curves (Bookstein, 1991). Type III landmarks are generally located at the end points of traditional distance measurements, i.e. lengths, widths and heights of organisms (Bookstein, 1991; Slice, 2005). Since they are defined by relating them to other structures, Type III landmarks are the least repeatable and least reliable landmark category (Curran, 2018; Slice, 2005). Thus, it is advisable to focus on Type I and Type II landmarks in GM approaches and to avoid Type III landmarks if possible (Curran, 2018).

Once the landmarks on all specimens of the respective dataset have been digitized, the next step is to mathematically remove information on size, orientation and position from the analysis (Adams et al., 2004; Kaliontzopoulou, 2011). This alignment is achieved by a Procrustes superimposition which rotates, translates and scales the landmarks, creating a dataset that exclusively contains shape information (Adams et al., 2004; Mitteroecker & Gunz, 2009). The resulting shape variables are commonly analyzed with principal component analysis (PCA) which ordines specimens according to the major axis of shape variation (Lawing & Polly, 2010). Multivariate statistical tests, for instance a MANOVA, can then be

used to identify shape differences between groups of specimens based on the PCA scores (Curran, 2018; Lawing & Polly, 2010). The last step of a GM analysis is the graphical visualization of the results (Adams et al., 2004; Slice, 2005).

1.2.2 Advantages of geometric morphometrics

The graphical visualization is in fact one of the greatest strengths of GM methods compared to traditional morphometrics because the geometry of the studied specimens is retained throughout the analysis (Adams et al., 2004; Curran, 2018; Kaliontzopoulou, 2011). Thus, interpolation functions such as thin-plate splines are able to depict shape deformations between two or more specimens (Loy, 2007; Marcus & Corti, 1996). In 2D, this is commonly achieved by transformation grids which use the bending of a wire mesh to visualize shape differences between specimens (Curran, 2018; Kaliontzopoulou, 2011; Mitteroecker & Gunz, 2009). In 3D GM approaches, the results are often visualized by warping, a procedure that transforms the shape of one specimen into another (Mitteroecker & Gunz, 2009). A further advantage of GM methods in comparison with linear measurements is the far higher number of variables in the analysis; which make GM methods considerably more powerful than traditional morphometrics (Kaliontzopoulou, 2011; Lawing & Polly, 2010; Rohlf and Marcus, 1993). This additional information enables morphologists nowadays to quantify very discrete shape variation between specimens which had to be analyzed qualitatively in the past (Curran, 2018). Despite their high analytical power, 2D GM methods are cost-efficient, fast and only require limited preparation time (Buser et al., 2018; Lawing & Polly, 2010). These approaches can be based on standardized photographs that only require a suitable digital camera and a rather simple camera setup which allows one to collect large datasets rapidly (Buser et al., 2018; Cardini, 2014). The available software for performing complex multivariate analyses of 2D GM data can be mastered quickly and is mainly free of charge (Cardini, 2014; Lawing & Polly, 2010). However, this is solely true for GM approaches based on 2D data like digital photographs or X-ray images (Cardini & Chiapelli, 2020; Curran, 2018, Navarro & Maga, 2016).

1.2.3 Drawbacks of 3D geometric morphometrics and potential solutions

GM applications based on 3D data are, in contrast, far more sophisticated and thus consume substantially more resources, including time, effort and financial investment (Adams et al.,

2004; Gould, 2014; Navarro & Maga, 2016). Devices for capturing 3D data, for instance, μ -computed tomography (μ -CT) scanners or surface laser scanners are considerably more expensive than an X-ray machine or an adequate digital camera (Cardini & Chiapelli, 2020; Cardini, 2014; Curran, 2018). Analyzing and post-processing of the produced 3D data requires cost-intensive computational hardware and rendering software which can be challenging to handle (Abel et al., 2012; Gould, 2014). At the same time, collecting and analyzing 3D data consumes a manifold of time compared to 2D data (Abel et al., 2012; Curran, 2018; Gould, 2014; Navarro & Maga, 2016). Thus, it is still common practice in comparative morphological studies to use 2D GM instead of 3D GM (Adams et al., 2004; Cardini, 2014; Navarro & Maga, 2016). This is particularly the case for fishes (Buser et al., 2018; Higham et al., 2017; Jamniczky et al., 2015), presumably because many taxa have a laterally compressed overall body shape. Nevertheless, the vast majority of animals or biological structures are obviously 3D objects (Cardini & Chiapelli, 2020; Cardini, 2014; Curran, 2018). Using a 2D GM approach to quantify 3D objects will therefore consequently result in a certain inaccuracy and information loss (Buser et al., 2018; Cardini, 2014; Navarro & Maga, 2016).

One way to reduce the time and effort consumption of 3D GM is to omit the manual definition and placement of landmarks (Mitteroecker & Schaefer, 2022). In recent years, several “homology-free” or “landmark-free” morphometric methods have been developed (Gonzalez et al., 2016; Polly & MacLeod, 2008; Pomidor et al., 2016). Instead of using cartesian coordinates, these approaches are based on automatically generated points on outlines or surfaces (Mitteroecker & Schaefer, 2022). These methods are particularly useful when dealing with rather featureless structures such as braincases or otoliths where homologous landmarks are difficult to locate (Polly & MacLeod, 2008; Pomidor et al., 2016). Landmark-free approaches are also less time- and effort-consuming than landmark-based methods because no landmarks have to be defined and placed manually by the researcher (Gonzalez et al., 2016; Mitteroecker & Schaefer, 2022; Pomidor et al., 2016). However, it is not advisable to use landmark-free approaches on complex structures like vertebrate skulls since the used surface points are randomly selected by the respective software (Gonzalez et al., 2016; Polly & MacLeod, 2008). In this way, biologically meaningful information might be

lost during the analysis or weighed differently than intended by the operator (Gonzalez et al., 2016; Mitteroecker & Schaefer, 2022; Pomidor et al., 2016).

The availability of μ -CT and laser scanners has increased over the past decade and the required computational power to run these has become affordable for many institutions (Abel et al., 2012; Buser et al., 2018; Lawing & Polly, 2010). Thus, it is worthwhile testing if the additional information content provided by the third dimension offers any benefits in discriminating laterally compressed fishes. One aim of the present thesis therefore was to evaluate the species and sex discrimination ability of 2D and 3D GM in the light of costs, processing time and data storage space.

1.3 Feeding modes in fishes

In order to capture food items, the over 30.000 species of ray-finned fishes (Actinopterygii) use a great variety of different feeding strategies (Camp et al., 2015; Day et al., 2015). Despite this high diversity of methods, all of them can be classified into three main feeding modes: suction feeding, ram feeding and manipulation feeding (Ferry-Graham et al., 2001a,b; Norton & Brainerd, 1993; Wainwright & Bellwood, 2002). Nevertheless, many fishes are not restricted to one of these modes and rather use a combination of them (Ferry-Graham et al., 2001a; Porter & Motta, 2004). Functional feeding morphology, diet and prey capture of fishes are often strongly correlated with the respective feeding mode (Sonnefeld et al., 2014). Suction feeding is the most common and presumably the most primitive prey capture mode within fishes (Camp et al., 2015; Wainwright & Bellwood, 2002; Westneat & Olsen, 2015). By rapidly expanding the buccal cavity, suction feeders generate a water flow that drags food items into the mouth opening (Day et al., 2015; Ferry-Graham et al., 2001a,b; Norton & Brainerd, 1993). The suction performance benefits from a small mouth aperture and a short time to peak gape (Day et al., 2015; Higham et al., 2017). Thus, suction feeding is often performed by fishes with a reduced maximum gape width and powerful jaw-opening muscles (Sonnefeld et al., 2014). Their diet typically consists of mostly small, non-elusive prey like zooplankton, crustaceans and insects, organisms that can be approached rather slowly (Day et al., 2015; Ferry-Graham et al., 2001b; Sonnefeld et al., 2014). The alternative feeding mode used by fishes to capture free swimming prey is ram feeding

(Ferry-Graham et al., 2001b; Wainwright, 2001). Ram feeders use rapid forward locomotion to approach their prey and engulf it in their buccal cavity (Norton & Brainerd, 1993; Porter & Motta, 2004; Wainwright & Bellwood, 2002). This strategy is supported by a fast and powerful jaw-closing ability accompanied by a large gape which increases the strike accuracy (Higham et al., 2017; Sonnefeld et al., 2014). Ram feeding is typically performed either by fast swimming predatory fishes like pikes or plankton feeders like herrings (Porter & Motta, 2004; Wainwright & Bellwood, 2002). This feeding mode is especially efficient for capturing swarms of planktonic prey or large, free swimming, evasive prey like squids and other fishes (Ferry-Graham et al., 2001b; Porter & Motta, 2004; Sonnefeld et al., 2014). In contrast to the first two feeding modes, manipulation feeders use their jaws and teeth to bite, scrape or clip stationary prey from the substrate (Ferry-Graham et al., 2001a,b; Norton & Brainerd, 1993). Since their prey is often firmly attached and immobile, like mollusks, crustaceans, algae or sponges, the feeding apparatus of manipulation feeders is typically characterized by sturdy oral jaws, a small mouth, and a slow but powerful jaw-closing ability (Sonnefeld et al., 2014). In comparison to suction and ram feeding, manipulation feeding is less common within teleost fishes (Liem, 1980; Wainwright & Bellwood, 2002). However, it is a characteristic trait of coral reef fish assemblages (Wainwright & Bellwood, 2002).

1.3.1 Differentiating between ram and suction feeding

Since manipulation feeding is the only prey capture mode that is based on a high force transmission, morphology and feeding behavior of manipulation feeders is mostly very distinct (Wainwright & Bellwood, 2002; Wainwright & Richard, 1995). Contrarily, suction and ram feeders both depend on a high kinematic transmission (Ferry-Graham et al., 2001b; Wainwright & Richard, 1995). Thus, differentiation between these two feeding modes is often fine-scaled and rather transitional (Norton & Brainerd, 1993; Wainwright et al., 2001; Wainwright & Richard, 1995). However, suction and ram feeders are distinguishable by comparing the amount of forward locomotion of the predator with the movement of the prey through suction pressure during a prey capture event (Wainwright & Bellwood, 2002). Specialized ram feeders use rapid bursts of forward locomotion to engulf prey items which remain stationary during the strike (Ferry-Graham et al., 2001b; Wainwright et al., 2001). In contrast, extreme suction feeders use a negative pressure gradient to accelerate prey items into their buccal cavity while they remain still (Camp et al., 2015; Norton & Brainerd, 1993;

Day et al., 2015). Nevertheless, specialized suction and ram feeders are positioned at opposite ends of the ram/suction feeding continuum and are therefore rather rare (Wainwright et al., 2001; Wainwright & Bellwood, 2002; Wainwright & Richard, 1995). The majority of fishes which feed on mobile prey use a combination of suction and ram feeding (Ferry-Graham et al., 2001b; Porter & Motta, 2004; Wainwright & Bellwood, 2002).

1.3.2 High-speed videos and biomechanical models

One possible tool for distinguishing ram and suction feeders is comparing forward locomotion, speed of mouth opening and maximum gape width based on high-speed videos (minimum 60 frames per second). In a typical approach, the captive held specimens are filmed while feeding in front of a grid which serves as a scale (Higham et al., 2007; Westneat & Olsen, 2015). Two prerequisites are that the fishes are fully visible in lateral view and are recorded in close proximity to the grid during the complete prey capture strike (Ferry-Graham et al., 2001b; Higham et al., 2017; Wainwright et al., 2001). Since high-speed videos facilitate an accurate quantification of maximum gape width, forward locomotion and prey capture velocity, they are highly efficient in depicting the kinematics of prey capture (Ferry-Graham & Lauder, 2001). Thus, they have been frequently used in the past years in order to investigate the functional feeding morphology of fishes (e.g., Copus & Gibb, 2013; Day et al., 2015; Ferry-Graham et al., 2002; Konow et al., 2013; Van Wassenbergh & De Rechter, 2011).

A second common method for discriminating between ram and suction feeding is the use of biomechanical models. Contrarily to high-speed videos, biomechanical models target the force of fundamental biological movements such as the opening and closing of the jaw apparatus (Hulsey & Wainwright, 2002; Westneat, 2004). There are several biomechanical models which are widely used to study the biomechanics of the feeding apparatus of fishes (Gidmark et al., 2019). These include the opercular linkage, hyoid linkage, maximum jaw protrusion and the suction index (Anker, 1974; Hulsey et al., 2005; Muller, 1987; Wainwright et al., 2007; Westneat, 1990). Two of the most common models are the simple lever system of the lower jaw and the complex four-bar linkage system of the anterior jaw (Pfaender et al., 2011; Wainwright & Richard, 1995; Westneat, 1990). These represent functional trade-offs between the kinematic and force transmission of jaw movement (Cooper & Westneat, 2009; Wainwright & Richard, 1995; Westneat, 2004) which can be used to calculate different

kinematic ratios: the lower jaw ratio (LJR) for jaw opening, the LJR for jaw closing and the maxillary kinematic transmission coefficient (MKT) (Wainwright & Richard, 1995; Westneat, 1994). A low LJR accompanied by a high MKT is indicative for a jaw apparatus adapted to high velocity but low force transfer (Hulsey et al., 2006; Pfaender et al., 2011). This state is characteristic for suction feeding fishes (Higham et al., 2017; Sonnefeld et al., 2014). In contrast, a high LJR along with a small MKT suggests a powerful but slowly moving oral jaw which is typical for manipulation feeders (Cooper & Westneat, 2009; Wainwright & Richard, 1995). Ram feeders are usually intermediate in their velocity and force transfer (Sonnefeld et al., 2014; Wainwright & Bellwood, 2002) and thus in their LJR and MKT values.

In summary, ray-finned fishes use at least one, but more commonly a combination of three different feeding modes to capture prey items (Ferry-Graham et al., 2001a; Porter & Motta, 2004). While manipulation feeding is easily distinguishable, the distinction between ram and suction feeding is transitional (Wainwright & Bellwood 2002; Wainwright & Richard 1995). Two widely used methods for discriminating the latter two feeding modes are high-speed videos and biomechanical models. Due to their high efficiency in detecting fine-scaled differentiation in functional feeding morphology, both were used in the present work to discriminate the feeding modes of stream-dwelling sailfin silversides.

1.4 The Malili Lakes of Sulawesi – a hotspot for biodiversity

The Indonesian island Sulawesi belongs to the four Greater Sunda Islands along with Borneo, Java and Sumatra (Stelbrink et al., 2012). Due to its position in the Wallacea between the Sunda and the Sahul Shelf (Figure 1), it harbors a unique fauna with a high percentage of endemism (Stelbrink et al., 2012; Whitten & Henderson, 2012). This also applies to the various freshwater environments on Sulawesi, especially to the Malili Lakes system in the central highland of the island (Brooks, 1950; Herder et al., 2006b; Vaillant et al., 2011; von Rintelen et al., 2012; Whitten & Henderson, 2012). It is the only example of a hydrologically interconnected ancient lake system on Earth (Vaillant et al., 2011), consisting of three main lakes which are directly connected, and two satellite lakes (Herder & Schliewen, 2010; Vaillant et al., 2011; von Rintelen et al., 2012). The uppermost of the three major lakes, Lake Matano, is an extremely deep, substantially isolated graben lake of tectonic origin (Crowe et

al., 2008; Haffner et al., 2001; Herder & Schliewen, 2010; Vaillant et al., 2011; von Rintelen et al., 2012). It is connected to the much smaller and shallower Lake Mahalona by the extraordinary steep and short River Petea (Brooks, 1950; Herder & Schliewen, 2010; von Rintelen et al., 2012). Lake Mahalona is drained by the Tominanga River into the largest lake on Sulawesi, Lake Towuti, which then finally flows into the Gulf of Bone via River Lontoa (Brooks, 1950; Haffner et al., 2001; Herder & Schliewen, 2010; von Rintelen et al., 2012). The Malili Lakes system is estimated to be between one and four million years old but more precise assumptions are still lacking (Vaillant et al., 2011; von Rintelen et al., 2004, 2012). Since the lakes are widely isolated from other aquatic systems, offer a broad range of habitats and harbor endemic species flocks of gastropods, bivalves, crustaceans and fish, they are considered as aquatic biodiversity hotspots (Brooks, 1950; Herder et al., 2006b, 2008; Herder & Schliewen, 2010; Schwarzer et al., 2008; von Rintelen et al., 2007, 2012; Whitten & Henderson, 2012).

1.4.2 The adaptive radiation of sailfin silversides

One of the endemic fish species flocks in the Malili Lakes is the adaptive radiation of sailfin silversides (Telmatherinidae), which has been used as a model system for sympatric speciation processes (Herder et al., 2008; Pfaender et al., 2010, 2011). These small, often brightly colored, atheriniform freshwater fishes are characterized by a strong sexual dimorphism and a conspicuous male color polymorphism (Herder et al., 2006a, 2008; Herder & Schliewen, 2010; Kottelat, 1990, 1991; Pfaender et al., 2011, 2013; von Rintelen et al., 2012). Sailfin silversides occur with overall 16 species on Sulawesi of which 15 are endemic to the Malili Lakes system and eight exclusively to ancient Lake Matano (Herder et al., 2006b; Kottelat, 1990, 1991; Pfaender et al., 2016). These eight species of Lake Matano can be assigned to two different phenotypic clades: the rather pelagic “roundfins” consisting of three morphospecies and the more diverse, epibenthic “sharpfins” composed of five morphospecies (Herder et al., 2006a,b; Herder & Schliewen, 2010; Kottelat, 1991; Pfaender et al., 2016). Members of both groups are distinguishable by the shape of the second dorsal and anal fin in males (Herder et al., 2006a; Kottelat, 1991). According to distance-based divergence estimates and molecular clock analyses, the initial divergence of roundfins and sharpfins occurred around 1.9 million years ago but might have happened even later (Herder & Schliewen, 2010; Stelbrink et al., 2014).

All eight sailfin silverside species of Lake Matano show conspicuous morphological adaptations to different ecological niches, most likely a result of ecologically-based divergent selection and competition for limited resources, indicating ongoing ecological speciation (Pfaender et al., 2010, 2011, 2016). The three roundfin morphospecies (Figure 1), which are one focus group of this work, show significant variation in functional morphology, body and head shape according to their species specific ecological niche (Herder et al., 2008; Pfaender et al., 2011). Roundfins were the first adaptive fish radiation in which intersexual niche differentiation was identified (Pfaender et al., 2011). Two roundfin morphospecies, *T. antoniae* “large” and “small”, show intersexual niche differentiation in trophic ecology: male *T. antoniae* “small” feed to a higher extent on terrestrial insects than females while male *T. antoniae* “large” take a higher portion of mollusks compared to females (Pfaender et al., 2011). However, fine-scaled intersexual differences in functional morphology and shape were demonstrated in all three roundfin morphospecies (Pfaender et al., 2011). Although roundfins and sharpfins mostly mate assortatively, reproductive isolation among them is incomplete, suggesting an early stage of speciation (Herder et al., 2008; Herder & Schliewen, 2010; Schwarzer et al., 2008; von Rintelen et al., 2012). This is particularly the case for the more complex sharpfins which hybridize frequently and are heavily introgressed by stream-dwelling sailfin silversides (Herder et al., 2006a; Herder & Schliewen, 2010; Schwarzer et al., 2008; von Rintelen et al., 2012).

In contrast to the high species diversity in the lacustrine habitats, only two sailfin silverside species have been described in rivers and streams on Sulawesi: *Marosatherina ladigesii* and *Telmatherina bonti* (Figure 1; Herder et al., 2006b; von Rintelen et al., 2012). *Marosatherina ladigesii* is the only Telmatherinid from Sulawesi known from outside the Malili Lakes system and is considered as the sister group to all other sailfin silversides (Herder et al., 2006b; Stelbrink et al., 2014; von Rintelen et al., 2012). *Telmatherina bonti* inhabits most of the rivers and streams of the Malili Lakes system and comprises all its stream-dwelling *Telmatherina* populations (Herder et al., 2006b; von Rintelen et al., 2012). These populations show a high phenotypic and genotypic diversity according to different localities suggesting the occurrence of at least three different morphospecies (Herder et al., 2006b; von Rintelen et al., 2012). However, detailed ecological and morphological data of both stream-dwelling sailfin silverside species is still lacking.

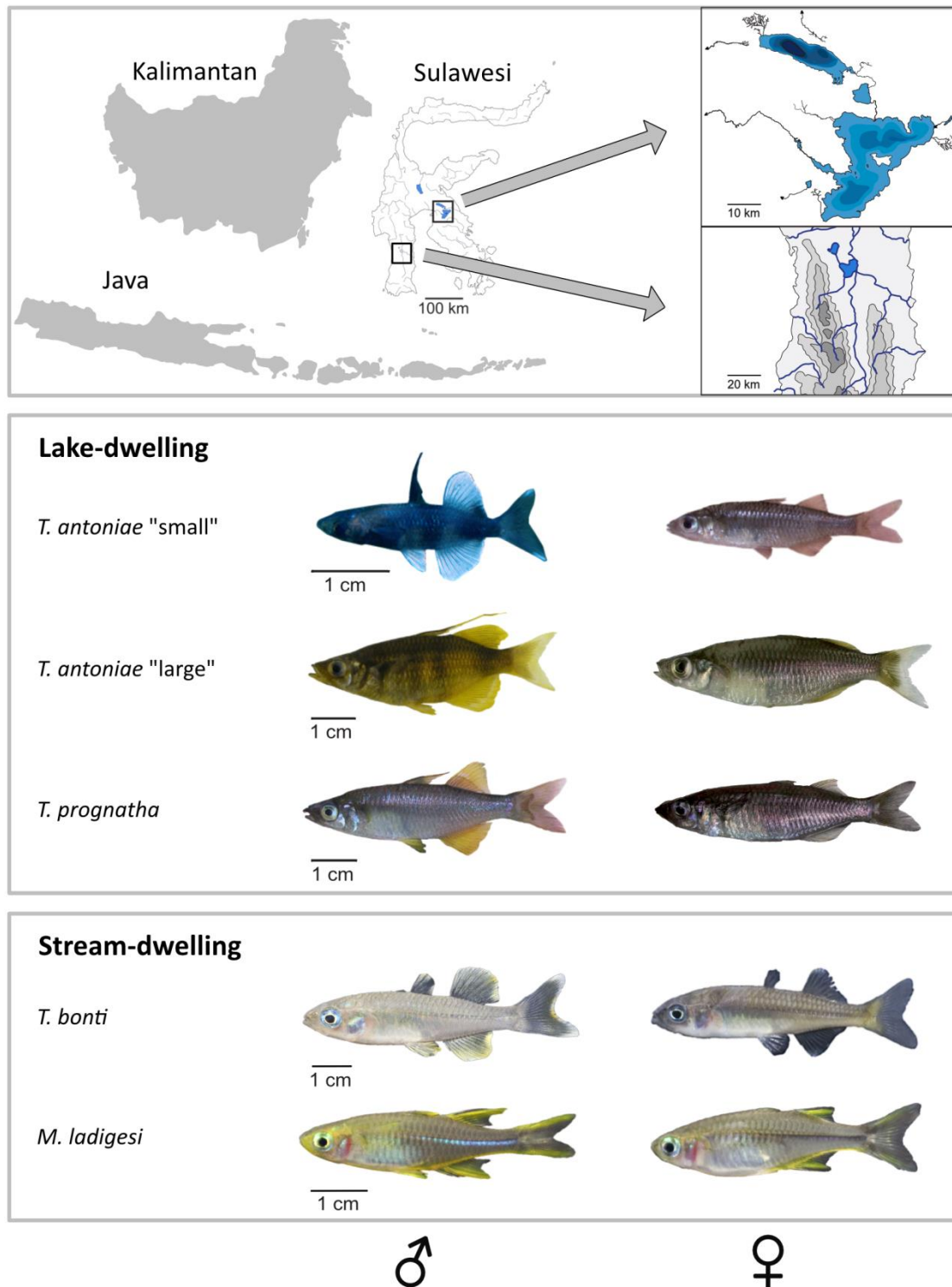


Figure 1: Indonesia, Sulawesi, the Malili Lakes system and the Maros Karst area (top box). Adult, reproducing males and females of the three lake-dwelling roundfins *T. antoniae* "small", *T. antoniae* "large" and *T. prognatha* (middle box) and the two stream-dwelling sailfin silversides *T. bonti* and *M. ladigesi* (lower box) are pictured. Figure modified from Wasiljew et al. (2021, 2022). Map by T. von Rintelen, modified (with permission).

1.5 Aims of this thesis

In this thesis, I use the adaptive radiation of sailfin silversides - endemic to Sulawesi in Indonesia - as a model system to test hypotheses on the evolution of functional and ecomorphological adaptations in respect to ecology. For this purpose, I employed a variety of different imaging methods including X-ray photography, μ -CT scanning and high-speed videos. Thus, one aim of this thesis was to evaluate the advantages and disadvantages of 2D and 3D imaging techniques for ichthyological studies. Chapter 2 therefore focuses on the impact of the third dimension on geometric morphometrics performed on laterally compressed fishes by using the roundfins of Lake Matano as study objects. Here, I compare the repeatability, information content and discrimination ability of 2D and 3D geometric morphometrics while discussing the benefits and drawbacks of both methods in regard to time, effort and financial investment. Chapter 3 deals with the question whether the sexual niche differentiation documented in two of the three roundfin morphospecies has led to ecological character displacement in their ecomorphology. In order to test this hypothesis, I quantify the shape and size of three different ecologically relevant structures by using traditional and geometric morphometrics conducted on 3D μ -CT scans of the head. In chapter 4, I assess whether two stream-dwelling sailfin silverside species use alternative feeding modes, with the help of high-speed video recordings and biomechanical models. I analyze if the prey capture kinematics and feeding biomechanics of both stream-dwelling species differ from the lake-dwelling roundfins.

Chapter 2

Do we need the third dimension? Quantifying the effect of the z-axis in 3D geometric morphometrics based on sailfin silversides (Telmatherinidae)

This chapter is published in the following article (open access):

Benjamin D. Wasiljew, Jobst Pfaender, Benjamin Wipfler, Ilham Vemandra Utama, Fabian Herder (2020). Do we need the third dimension? Quantifying the effect of the z-axis in 3D geometric morphometrics based on sailfin silversides (Telmatherinidae). *Journal of Fish Biology*, 97(2), 537-545. DOI: 10.1111/jfb.14410

Original article is attached.

2.1 Summary and personal contributions

The development of geometric morphometrics (GM) has fundamentally changed the way in which morphological information can be accessed, often termed as the “morphometric revolution” (Adams et al., 2004; Rohlf & Marcus, 1993). GM methods are powerful tools for quantifying the shape of organisms and enable the identification of rather discrete morphological variation (Adams et al., 2004; Kaliontzopoulou, 2011). Thus, they have become a standard approach in studies targeting the morphology of organisms (Curran, 2018). The majority of GM studies use 2D datasets based on standardized photographs or X-ray images (Adams et al., 2004; Cardini, 2014; Navarro & Maga, 2016). The lack of studies using 3D GM approaches based on μ -computed tomography (μ -CT) scans, is a consequence of the substantially higher efforts required for collecting and processing 3D data compared to 2D data (Cardini & Chiapelli, 2020; Navarro & Maga, 2016). These higher efforts include an increased financial investment, time expenditure, data size and workload for the operator (Abel et al., 2012; Adams et al., 2004; Gould, 2014). Due to the laterally compressed body shape of many fish taxa, 2D GM approaches are particularly frequently used in ichthyological studies (Higham et al., 2017; Jamniczky et al., 2015). However, a certain loss of accuracy and information content appears plausible when using 2D methods to study 3D objects (Buser et al., 2018; Cardini, 2014; Navarro & Maga, 2016).

The present study was designed by my supervisor PD Dr. Fabian Herder, Dr. Jobst Pfaender and myself. It compares the discrimination abilities of 2D and 3D GM on laterally compressed fishes by using the roundfins of Lake Matano as a model while relating the results to the gap in efforts between both methods. We predicted that the 3D analysis would provide a higher repeatability and higher information content which would help to discriminate between species and sexes better than the 2D analysis.

I obtained roundfin specimens from material which was collected in 2002 from three sample sites around Lake Matano's shoreline. I then μ -CT-scanned five males and five females of each roundfin morphospecies, *T. antoniae* "small", *T. antoniae* "large" and *T. prognatha*. For the 2D dataset, I used horizontally aligned maximum intensity projections created in the software Drishti (Limaye, 2012) while the 3D dataset consisted of 3D surface renders modeled in the software Checkpoint. Both datasets were created from μ -CT scans and

focused on the head of the specimens. I then placed ten landmarks at the same locations and in the same order on the cranial bones of the 30 scanned specimens in 2D with the software tpsUtil (Rohlf, 2015) and in 3D with the software Checkpoint to compare both methods. All μ -CT scans, their preparation and post-processing as well as the landmark placement were conducted by myself. I additionally measured the time expenditure and data storage space used for the 3D workflow to quantify the effort for the researcher. For the 2D pipeline, we used the data from the study of Pfaender et al. (2011). In order to remove the parameters of size, position and orientation from the analysis, we performed a Procrustes superimposition on the landmark coordinates. The resulting Procrustes coordinates were then used, firstly, to calculate the intraclass correlation coefficient to quantify the repeatability (Fruciano, 2016) and, secondly, to calculate a principal component analysis including a cross-validated correct classification test (Schlager, 2016) to identify significant inter- and intraspecific differences in both datasets. The statistical analyses were carried out by Dr. Jobst Pfaender and myself in the software packages PAST (Hammer et al., 2001) and R (Ihaka & Gentleman, 1996).

We did not identify any significant differences in measurement error or repeatability between the 2D and 3D GM analysis. Both methods were highly accurate. The z-axis harbored a significant amount of variance in the 3D dataset which was, however, negligible for discriminating between species or sexes. Both approaches detected significant interspecific but no intersexual shape differences within the three roundfin morphospecies.

Taken together, the 3D GM approach did not provide any substantial benefits in accuracy or discrimination ability compared to the 2D GM approach in the present study. Nevertheless, the 3D workflow consumed a manifold of time, effort and data storage space than the 2D pipeline. We therefore conclude that the 2D GM approach is the more effective method for distinguishing roundfins based on shape. This conclusion might also be applicable to flattened animals in general. However, additional studies using a wide selection of laterally compressed species are needed in order to support this hypothesis. For future GM approaches, we currently suggest testing for repeatability and congruence between 2D GM and 3D GM on a subsample of specimens. In the case where there is no substantial difference in repeatability or discrimination ability between both methods or if the 2D

dataset even performs better, we recommend using 2D GM because of its higher efficiency. I wrote the initial draft of this publication with contributions from PD Dr. Fabian Herder, Dr. Jobst Pfaender and Dr. Benjamin Wipfler. The final figures were created by Dr. Jobst Pfaender, Dr. Benjamin Wipfler and myself using Adobe Photoshop CS6 and Adobe Illustrator CS6. All authors critically revised the manuscript before submission.

Chapter 3

Sexual dimorphism in an adaptive radiation: Does intersexual niche differentiation result in ecological character displacement?

This chapter is published in the following article (open access):

Benjamin D. Wasiljew, Jobst Pfaender, Benjamin Wipfler, Mariam Gabelaia, Ilham Vemandra Utama, Letha Louisiana Wantania, Fabian Herder (2021). Sexual dimorphism in an adaptive radiation: Does intersexual niche differentiation result in ecological character displacement? *Ecology and Evolution*, 11(21), 14615-14629. DOI: 10.1002/ece3.8137

Original article is attached.

3.1 Summary and personal contributions

The process of adaptive radiation, the most prominent type of evolutionary radiation (Kagawa, 2022; Simões et al., 2016), is discussed as one important driver of species diversity on a global scale (Gavrilets & Losos, 2009; Naciri & Linder, 2020; Nosil, 2012; Simões et al., 2016). In such radiations, an ancestral lineage diversifies into an array of different species which show adaptations to diverse ecological niches (Gavrilets & Losos, 2009; Losos, 2010; Rundell & Price, 2009). One of the four criteria for identifying an adaptive radiation is rapid species diversification at the beginning, often termed as an “early burst” (Schluter, 2000). Theory predicts, that early bursts are facilitated by repeated ecological speciation events (Martin & Richards, 2019, Rundell & Price, 2009). Ecological speciation is driven by biotic interactions such as mutualism, predator-prey relationships or resource competition (Rundell & Nosil, 2005; Schluter, 2001). Competition is particularly strong between both sexes of a species since they rely on similar resources (McGee et al., 2020). Intersexual niche partitioning may reduce intraspecific competition, which eventually leads to ecological character displacement between males and females of the same species (De Lisle, 2019; Butler et al., 2007; Roy et al., 2013). This phenomenon has been documented e.g. in *Anolis* lizards, salamanders, and sticklebacks (Butler, 2007; De Lisle & Rowe, 2017; McGee & Wainwright, 2013). But the impact of intersexual niche differentiation on ecological speciation processes has only received limited attention so far (De Lisle & Rowe, 2015; Ronco et al., 2019). For instance, it is still unclear whether it reinforces or rather impedes adaptive radiations (Bolnick & Doebeli, 2003; Butler, 2007; De Lisle & Rowe, 2017). However, previous work has demonstrated that ecological speciation and ecological character displacement between males and females can act concurrently (De Lisle & Rowe, 2015, 2017).

I conceptualized this study together with my supervisor PD Dr. Fabian Herder and Dr. Jobst Pfaender. The aim of this work was to test whether intersexual niche differentiation has an impact on multiple ecologically relevant traits in the roundfin radiation of Lake Matano. We assumed that the three investigated morphological structures – the opercle, the pharyngeal jaw and the buccal cavity – have adapted according to the species- and sex-specific

ecological niches in roundfins. In addition, we hypothesized that the extent of sexual dimorphism would match the extent of sexual niche partitioning in each morphospecies.

For the present study, I obtained formalin-fixated roundfin specimens that were gathered in 2002 from three locations around the Lake Matano shoreline. Five males and females of each roundfin morphospecies, *T. antoniae* "small", *T. antoniae* "large" and *T. prognatha* were used for μ -CT scanning in an unstained and stained condition. For the morphometric analyses of the opercle and the pharyngeal jaw, I used 3D surface renders that I created in the software Checkpoint. I quantified the size and shape of both structures with linear, circumference and surface measurements, semi-landmarks along the outlines, as well as homologous landmarks. In addition, I counted the number of teeth on the pharyngeal jaw. I assessed buccal cavity size and shape based on 3D surface renders that I created with the semiautomatic segmentation tool within the software package Amira (Stalling et al., 2005). Buccal cavity size was quantified with linear and volume measurements. Since the shape of the buccal cavity can – due to its featureless structure – not be quantified using classical landmark-based methods, we used a landmark-free approach based on surface scans to quantify its shape (Pomidor et al., 2016; Slice, 2013). I performed all μ -CT scans, their pre- and post-processing, the measurements and the landmark placement for this study. Dr. Mariam Gabelaia and I carried out the landmark-free approach in order to quantify the buccal cavity shape.

In order to correct for size, I conducted bivariate linear models for all absolute measurements. I then used the calculated residuals to perform one-way ANOVAs with Tukey's pairwise tests to identify potential interspecific and intersexual differences in the dataset. For statistical analyses of the opercle and pharyngeal jaw shape data, I removed size, orientation and position from the analysis by carrying out a Procrustes superimposition. For the buccal cavity, this was done through an iterative closest point algorithm. The aligned coordinates were used to perform an elliptic Fourier analysis for opercle shape, a principal component analysis for pharyngeal jaw shape and generalized Procrustes surface analysis for buccal cavity shape. Based on the resulting scores of the analyses, I conducted MANOVAs and Tukey's pairwise tests to test for interspecific and intersexual shape differences in all three structures. The statistical analyses of the opercle and pharyngeal jaw data was

performed by Dr. Jobst Pfaender and myself using PAST (Hammer et al., 2001) and R (Ihaka & Gentleman, 1996). I conducted the statistical analyses of the buccal cavity data in PAST with contributions from Dr. Mariam Gabelaia.

We detected significant interspecific variation in size and shape within all three analyzed morphological structures among roundfin morphospecies. Following our predictions, the opercle, the pharyngeal jaw and the buccal cavity have adapted according to the specific ecological niche of each morphospecies (Herder et al., 2008; Pfaender et al., 2011). In addition to the interspecific differentiation, we also identified a sexual dimorphism in two of the three roundfins. Intersexual variation was most prominent in *T. antoniae* “large” which coincides with the pronounced sexual niche differentiation in this morphospecies (Pfaender et al., 2011). Males and females of this morphospecies differed significantly in all three analyzed structures. Sexes of *T. antoniae* “small”, however, only differed in buccal cavity size and shape but not in any of the opercle or pharyngeal jaw traits. In contrast, we did not detect any intersexual variation between males and females of *T. prognatha* in any of the analyzed morphological traits. This finding corresponds with the lacking sexual niche partitioning in this morphospecies (Pfaender et al., 2011).

As expected, the extent of sexual dimorphism differed between morphospecies, matching the different degrees of intersexual niche partitioning in roundfins (Pfaender et al., 2011). Since the identified sexual dimorphism in *T. antoniae* “large” and *T. antoniae* “small” is linked to prey capture, prey processing and habitat use, we conclude that these morphological differences are presumably adaptations to sex-specific ecological niches. Due to the ecological relevance of the three analyzed structures and the internal location of the pharyngeal jaw and buccal cavity, it seems improbable that sexual selection is the driver of the intersexual variation detected here (Bolnick & Doebeli, 2003; De Lisle, 2019; De Lisle & Rowe, 2017; Ronco et al., 2019). We rather infer that ecologically-based divergent selection is the most plausible explanation, leading to ecological character displacement and thus reducing intersexual competition between sexes of *T. antoniae* “large” and *T. antoniae* “small” (De Lisle, 2019; De Lisle & Rowe, 2017). The present study indicates that intersexual ecological character displacement could be an important factor for reducing sexual niche overlap in emerging radiations. However, further studies on other young radiations are

necessary to understand the impact of intersexual niche differentiation on adaptive speciation processes in general. I wrote the first draft of this manuscript with contributions from PD Dr. Fabian Herder, Dr. Jobst Pfaender, Dr. Benjamin Wipfler and Dr. Mariam Gabelaia. All figures were initially created by myself in Affinity Photo and Affinity Designer. Dr. Benjamin Wipfler contributed to the design and made final adjustments in Adobe Photoshop CS6 and Adobe Illustrator CS6. All authors critically revised the manuscript before submission.

Chapter 4

Functional morphology of prey capture in stream-dwelling sailfin silversides (Telmatherinidae) based on high-speed video recordings

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Original article is attached.

4.1 Summary and personal contributions

Grasping the link between ecology and the evolution of phenotypic adaptations is a central goal in organismic biology (Sonnefeld et al., 2014; Wainwright & Richard, 1995). One potential approach is to measure the speed, force and kinematics of motion in order to quantify the mechanics of elemental tasks of animals including feeding, locomotion or defence against predators (Cooper & Westneat, 2009; Westneat, 1994, 2004). Although ray-finned fishes show a high diversity of different mechanisms to capture food items (Day et al. 2015), they still use at least one of three main feeding modes (Ferry-Graham et al., 2001a,b; Sonnefeld et al., 2014). These are suction feeding, ram feeding and manipulation feeding (Norton & Brainerd, 1993; Porter & Motta, 2004). Manipulation feeding can generally be detected easily since it is the only feeding mode that relies on a high force transmission (Wainwright & Bellwood, 2002; Wainwright & Richard, 1995). But the discrimination between suction and ram feeders can, in contrast, be challenging since both depend on a high kinematic transmission (Norton & Brainerd, 1993; Sonnefeld et al., 2014; Wainwright & Richard, 1995). However, distinguishing between these two feeding modes is possible by relating the degree of forward locomotion of the predator with the degree of suction pressure exerted on the prey (Norton & Brainerd, 1993; Wainwright & Bellwood, 2002; Wainwright et al., 2001). In addition, ram feeders are generally characterized by a higher gape width and more powerful but slower moving jaws compared to suction feeders (Higham et al., 2017; Sonnefeld et al., 2014). These fine-scaled differences between suction and ram feeders are detectable by using high-speed videos and biomechanical models (Norton & Brainerd, 1993; Wainwright et al., 2001). While high-speed videos enable the quantification of prey capture kinematics, biomechanical models facilitate the assessment of force and kinematic transmission (Copus & Gibb, 2013; Westneat, 1994).

My supervisor PD Dr. Fabian Herder, Dr. Jobst Pfaender and I designed this study. Our goal was to test if the two riverine sailfin silversides *Telmatherina bonti* and *Marosatherina ladigesii* use different feeding modes. Based on their phenotypic appearance and aquarium observations (Kottelat, 1990; Reckel, 2001; Reckel et al., 2002; Reckel & Melzer, 2003; Sterba, 1987), we predicted that *T. bonti* is predominantly a ram feeder while *M. ladigesii* is rather a suction feeder. If this is the case, we hypothesized that both species should be

adapted in their functional feeding morphology according to their respective feeding mode. Since *T. bonti* and *M. ladigesii* both show a pronounced sexual dimorphism in outer morphology (Kottelat, 1990; Nasyrah et al., 2019; Omar et al., 2020), we also considered possible intersexual variation in functional feeding morphology, as previously shown in the related lake-dwelling roundfin *Telmatherina* (Pfaender et al., 2011). A second aim of this study was to test whether the two stream-dwelling species studied here, *T. bonti* and *M. ladigesii*, show different functional adaptations compared to the lake-dwelling roundfins. The conditions in flowing habitats like rivers and streams differ substantially from those in lake habitats. These differences include an increased water flow and more variable conditions accompanied by a reduced degree of isolation (Brinsmead & Fox 2002; King et al. 2021). Thus, we predicted that the feeding biomechanics of *T. bonti* and *M. ladigesii* should be less distinct in comparison to roundfins.

I used mature, captive held specimens of *T. bonti* and *M. ladigesii* for high-speed video analysis. I recorded prey capture events of 25 male and 25 female individuals of each species during feeding on living *Artemia salina* nauplia with a GoPro Hero7 Black camera at 120 and 240 frames per second (fps). The recordings filmed at 120 fps were used for quantifying the maximum gape width. I took screenshots of the frame in which the gape width was at its maximum extent with the software Wondershare Filmora Video Editor. These screenshots were then used to measure the standard length and the maximum gape width of every individual with the software tpsDig (Rohlf, 2015). The quantification of prey capture time was based on the high-speed videos recorded at 240 fps. I assessed the overall prey capture time, the duration until peak gape width was reached and the duration until the mouth was completely shut again (Porter & Motta, 2004) with the software Wondershare Filmora Video Editor. For quantifying the feeding biomechanics, I obtained ten male and ten female formalin-fixated specimens of *T. bonti* and *M. ladigesii*, respectively, from the LIB ichthyology collection (see Herder et al. 2006). These 40 individuals were X-rayed in lateral view with the X-ray scanner Faxitron LX-60. In order to calculate the maxillary kinematic transmission coefficient (MKT) and the lower jaw ratio (LJR), I used the resulting X-ray images to perform a biomechanical analysis based on the maxillary 4-bar linkage lever system and the simple lower jaw lever system. A high MKT and low LJR indicates a fast but forceless moving jaw while a low MKT and high LJR suggests a slow but powerful oral jaw (Cooper & Westneat,

2009; Hulsey & García de León, 2005; Wainwright et al., 2004; Wainwright & Richard, 1995; Westneat, 2004). For statistical analysis, I regressed the measurements of the maximum gape width and prey capture time with standard length. I then used the size corrected values to conduct one-way ANOVAs with Tukey's pairwise tests in the software PAST (Hammer et al. 2001) in order to identify potential interspecific and intersexual variation. This procedure was repeated for the absolute values of the MKT and LJR. I recorded the high-speed videos and performed all the measurements for this approach. The statistical analysis was also conducted by myself with support from Dr. Jobst Pfaender.

The high-speed video analysis revealed that *T. bonti* and *M. ladiges* differ substantially in maximum gape width and prey capture velocity. *T. bonti* have a significantly higher maximum gape width but a lower prey capture velocity than *M. ladiges*. These findings suggest a ram feeding mode used by *T. bonti* and a suction feeding mode used by *M. ladiges* (Day et al., 2015; Ferry-Graham et al., 2001b; Higham et al., 2006a,b, 2017; Sonnefeld et al., 2014; Wainwright et al., 2007). Following our predictions, we could also identify significant intersexual variation in prey capture kinematics within both analyzed species. Female *M. ladiges* have a significantly higher maximum gape width than males while male *T. bonti* show a significantly higher prey capture velocity than females. These results might indicate alternative trophic niches between sexes of both species, resembling the sexual niche partitioning detected in roundfins by Pfaender et al. (2011). Contrasting the substantial differentiation identified in prey capture kinematics, *T. bonti* and *M. ladiges* did not show any significant interspecific or intersexual differences in MKT and LJR for opening. The LJR for closing was the sole biomechanical parameter that differed between both species but in contrast to our predictions, the LJR for closing was significantly higher in *M. ladiges* than in *T. bonti*. This finding indicates that *M. ladiges* has a more powerful but slower moving lower jaw than *T. bonti* which contradicts the hypothesis of a suction feeding mode in *M. ladiges* and a ram feeding mode in *T. bonti* (Cooper & Westneat, 2009; Pfaender et al., 2011; Wainwright & Richard, 1995).

Taken together, prey capture kinematics support our hypothesis that *M. ladiges* is a suction feeder and *T. bonti* is a ram feeder while feeding biomechanics challenge this theory. These discrepancies might be due to the trophic ecology of both analyzed species. Field

observations and unpublished ecological data suggest that *T. bonti* predominantly feeds on elusive prey like aquatic insects, crustaceans and small fishes whereas *M. ladigesii* rather feeds on less mobile prey including terrestrial insects and zooplankton (Andriani, 2000; Bach & Herder, unpublished data; Nasyrah et al., 2019, 2020; Reckel, 2001; Reckel et al., 2002; Reckel & Melzer, 2003). A high kinematic transmission is indicative for fishes feeding on mobile prey while a high force transmission is typical for species feeding on prey that lack strong escape responses (Alfaro et al., 2005; Hulseley & Wainwright, 2002; Parnell et al., 2008; Wainwright & Richard, 1995). Thus, a high MKT and low LJR might be advantageous for *T. bonti* whereas a low MKT and high LJR could be beneficial for *M. ladigesii*. However, as the knowledge on the trophic ecology of *T. bonti* and *M. ladigesii* is limited, this hypothesis needs to be tested by quantifying the trophic niches of both species, for instance, by analyzing stomach contents.

The minor variation in feeding biomechanics detected in both stream-dwelling sailfin silversides contrasts strongly with the distinct inter- and intraspecific biomechanical differences documented in the lake-dwelling roundfins (Pfaender et al., 2011). These contradicting results could be a consequence of the different abiotic conditions of stream and lake environments (Brinsmead & Fox, 2002; King et al., 2021). In comparison to the rather stable lake habitats, rivers and streams are more variable, less isolated, more strongly connected to terrestrial habitats and go through catastrophic events more often (Brinsmead & Fox, 2002; King et al., 2021; McLaughlin & Grant, 1994). Thus, the limited biomechanical differentiation in *T. bonti* and *M. ladigesii* compared to roundfins might be explainable by the more variable conditions in their habitat. However, the maxillary 4-bar linkage lever system and the simple lower jaw lever system are only two of several biomechanical models available to quantify the transmission of force and motion in the jaw of fishes (Anker, 1974; Hulseley et al., 2005; Muller, 1987; Wainwright et al., 2007; Westneat, 1990). In order to critically test the less pronounced feeding biomechanics in *T. bonti* and *M. ladigesii*, additional biomechanical parameters need to be included in future studies. The initial draft of the present manuscript was written by myself together with my supervisor PD Dr. Fabian Herder. I took all photos and created all the figures for this manuscript in Affinity Photo and Affinity Designer. Dr. Benjamin Wipfler added final adjustments in Adobe Photoshop CS6 and Adobe Illustrator CS6. All authors critically revised the manuscript before submission.

Chapter 5

General discussion and prospects

5.1 General discussion

The discovery of the adaptive radiation of Galapagos finches by Charles Darwin was crucial for the development of his theory of evolution (Losos, 2010; Yoder et al., 2010). Nowadays, adaptive processes are recognized as relevant drivers of diversification on earth (Gavrilets & Losos, 2009; Naciri & Linder, 2020). Species flocks such as the cichlid systems in East African graben lakes allow researchers to test hypotheses on adaptive divergence and ecological speciation (Gavrilets & Losos, 2009; Losos, 2010). In contrast to these well studied examples, the species flock of Sulawesi's sailfin silversides provides the advantage of limited size and complexity (Herder et al., 2006a,b). Notably, the documented sexual niche differentiation (Pfaender et al., 2011) offers the opportunity to investigate the impact of intraspecific competition on adaptive speciation processes.

In this work, I analyzed segregation in ecomorphology within the adaptive radiation of sailfin silversides; firstly, to compare advantages and drawbacks of 2D and 3D geometric morphometrics and secondly, to test how ecology shapes the evolution of functional adaptations in emerging radiations. In Chapter 2 of this thesis, I used the lineage of roundfin *Telmatherina* endemic to Lake Matano, to assess whether 3D geometric morphometrics (GM) provide any substantial benefits over 2D GM in accuracy, information content and discrimination ability performed on laterally compressed fishes. Chapter 3 focused on the effect of intersexual niche differentiation on ecologically relevant traits in "roundfins". In Chapter 4, I tested if the two stream-dwelling sailfin silversides of Sulawesi differ in prey capture kinematics and functional feeding morphology. In the following, I will discuss my findings, summarize my conclusions and finally provide prospects for potential future studies.

5.1.1 The inefficiency of 3D geometric morphometrics

Geometric morphometric methods are, so far, predominantly performed on 2D images (Adams et al., 2004; Cardini, 2014; Navarro & Maga, 2016). Contrary to 3D GM, 2D GM approaches are fast, inexpensive, easy to handle and require a limited amount of data storage space (Buser et al., 2018; Cardini, 2014; McWhinnie & Parsons, 2019). However, technological advances in 3D imaging devices, such as the increasing availability of μ -computed tomography (μ -CT) scanners or surface laser scanners, have made the collection

of 3D data more affordable and straightforward to use in recent years (Abel et al., 2012; Gould, 2014; Lawing & Polly, 2010). That aside, including the third dimension in GM, even when applied on flattened animals, provides an increased detail and information content which is lost in 2D GM (Buser et al., 2018; Cardini, 2014; Navarro & Maga, 2016). Thus, it is of crucial importance for future morphological studies to critically evaluate advantages and disadvantages of both approaches.

In Chapter 2, I showed that 2D and 3D GM do not differ significantly in measurement error and repeatability when applied to study shape traits in roundfin *Telmatherina*. Despite the fact that the third dimension contained a substantial amount of variance, 3D GM did not perform better than 2D GM in distinguishing between roundfin species or sexes. These findings match results of previous work focusing on flattened objects (Cardini, 2014; Hedrick & Dodson, 2013; McWhinnie & Parsons, 2019) whereas studies on more three-dimensional structures detected – as expected – a higher discrimination ability in 3D GM analyses (Buser et al., 2018; Cardini, 2014). The results of this study therefore support the hypothesis that 3D GM is more powerful in discriminating highly three-dimensional structures but that 2D GM is at least equivalent when performed on animals with a limited body width as it is the case in roundfins. The limited benefits of the 3D GM approach in the present study are strongly contrasted by its considerably higher effort, time expenditure and data size compared to the 2D GM workflow. In addition, 3D imaging methods generally require cost-intensive software and hardware that are more challenging to operate than those used for 2D imaging (Abel et al., 2012; Gould, 2014; McWhinnie & Parsons, 2019). Thus, I conclude that 2D GM is more efficient for distinguishing entities of species and sex in the present case of laterally compressed fishes.

Further research is required to critically test if this result can be generalized, among fishes, and also across a wider range of animal taxa. In the meantime, I advise future studies to use a subsample to test for a potential congruence in accuracy and discrimination ability between 2D and 3D GM. If the 3D approach does not provide any significant benefits in these preliminary analyses, I recommend using a 2D dataset because of the substantially lower time expenditure and data storage space required (Cardini & Chiapelli, 2020). These conclusions of chapter 2 will enable morphometricians to increase the sample size in future

GM based studies, by making them more time- and cost-efficient. It should, however, be understood that the choice of method for studying quantitative shape traits depends on the specific goal of the respective study. It is also important to point out that there are several cases where 3D GM cannot be replaced by 2D GM. This can be true, for instance, in studies targeting fluctuating asymmetry or in studies where only certain structures of a specimen are analyzed. In case these are asymmetrically shaped or located internally, the use of 3D GM is inevitable (Attard et al., 2018; Hedrick et al., 2019; McWhinnie & Parsons, 2019; Shi et al., 2018).

5.1.2 From intersexual niche differentiation to ecological character displacement

Sexual niche partitioning is not an uncommon phenomenon in the animal kingdom. It has been documented in various groups of animals such as salamanders, snakes and hummingbirds (De Lisle & Rowe, 2015; Shine, 1989; Temeles et al., 2000). However, how this sexual niche differentiation affects ecological speciation processes, for instance, in adaptive radiations, remains largely unexplored (Butler, 2007; Butler et al., 2007; De Lisle & Rowe, 2017; De Lisle, 2019; Pfaender et al., 2011). Lake Matano's roundfins were the first adaptive fish radiation in which sexual niche partitioning was detected (Pfaender et al., 2011); since then, it was also demonstrated in radiations of stickleback and cichlid fishes (McGee & Wainwright, 2013; Ronco et al., 2019). But it is still unclear what impact sexual niche differentiation has on the evolution of sexual dimorphism in ecologically relevant traits and if it predominantly accelerates or stalls ecological speciation processes (Bolnick & Doebeli, 2003; Butler, 2007; De Lisle & Rowe, 2015, 2017).

Chapter 3 of this thesis revealed that sexual dimorphism in ecologically relevant traits was evident in both roundfin morphospecies that show sexual niche differentiation whereas it was absent in the only morphospecies that lacks sexual niche differentiation. Interestingly, the degree of intersexual variation was linked to the degree of intersexual niche differentiation within each morphospecies. As the extent of intersexual variation corresponds with adaptive patterns identified in other fish radiations (Burress et al., 2016, 2018; Carroll et al., 2004; Cook, 1996; Hellig et al., 2010; Hulsey et al., 2006; Wilson et al., 2013a,b, 2015), I conclude that the detected sexual dimorphisms are adaptations to alternative ecological niches. These morphological adaptations are beneficial for exploiting

different ecological resources by enhancing the efficiency in prey capture, prey processing and habitat use (Burress, 2016; Burress et al., 2016, 2018; Cook, 1996; Kimmel et al., 2012; Wilson et al., 2013b).

In contrast to studies focusing on sexual size dimorphism, sexual color dimorphism or sexual shape dimorphism, the impact of sexual selection can be considered as negligible in the present case because I focused on ecologically relevant structures which are predominantly situated internally (Ronco et al., 2019). Thus, as they are not visible externally, these traits are unlikely to be related to sexual selection, e.g. in terms of display for potential mates or competitors (Bolnick & Doebeli, 2003; Ronco et al., 2019). Instead, it seems plausible that ecologically-based divergent selection explains the identified sexual dimorphism in *T. antoniae* “small” and *T. antoniae* “large”. This conclusion is supported by the lack of sexual dimorphism in ecologically relevant traits detected in *T. prognatha*, the only roundfin morphospecies in which sexual niche partitioning is absent (Pfaender et al., 2011). The revealed intersexual variation can therefore be regarded as ecological character displacement which presumably reduces ecological niche overlap and thus intraspecific competition between males and females (De Lisle & Rowe, 2017; De Lisle, 2019; Slatkin, 1984). Contradicting the traditional view, this finding suggests that intersexual niche differentiation and the resulting sexual dimorphism are crucial for facilitating both sexes to reach their phenotypic optima and thus, the establishment of emerging species in adaptive radiations (De Lisle & Rowe, 2017). Previous studies on *Drosophila*, salamanders and three-spine sticklebacks have led to similar conclusions (De Lisle & Rowe, 2017; De Lisle, 2023; McGee & Wainwright, 2013) following Lande’s (1980) model of the evolution of sexual dimorphism. In summary, intersexual ecological character displacement driven by sexual niche differentiation contributes to the adaptive diversity of roundfins. Future studies on several other emerging radiations are necessary in order to fully understand the effect of intersexual niche differentiation on adaptive speciation processes.

5.1.3 The functional morphology of stream-dwelling sailfin silversides

Despite their high species richness in the lakes of the Malili Lakes system, merely two species of sailfin silversides occur in Sulawesi’s rivers and streams (Herder et al., 2006b; von Rintelen et al., 2012). Similar to the roundfins of Lake Matano, both of the stream-dwelling sailfin

silverside species, *Telmatherina bonti* and *Marosatherina ladigesii*, differ significantly in their overall body shapes and show a pronounced sexual dimorphism (Kottelat 1990; Nasyrah et al. 2019; Omar et al. 2020; Sterba 1987). But in contrast to roundfins (Pfaender et al., 2011), the functional feeding morphology of *T. bonti* and *M. ladigesii* has remained unstudied up until the present work. *Telmatherina bonti* resembles *T. prognatha* in its fusiform body shape (Pfaender et al., 2011), suggesting the use of a ram feeding mode (Arbour & Lopez-Fernandez 2014, 2016). In contrast, the flattened body of *M. ladigesii*, which is similar to *T. antoniae* (Pfaender et al., 2011), is indicative for a suction feeding mode (Arbour & Lopez-Fernandez 2014, 2016). However, in terms of water flow, variability and degree of isolation, rivers and streams are very different from lake ecosystems (Brinsmead & Fox, 2002; King et al., 2021; McLaughlin & Grant, 1994). These alternative environmental conditions of their riverine habitats might have led to different functional adaptations in *T. bonti* and *M. ladigesii* than in the roundfins of Lake Matano (Brinsmead & Fox 2002; Collin & Fumagalli 2011; Theis et al. 2014).

The findings of chapter 4 demonstrated that *T. bonti* primarily uses ram feeding while *M. ladigesii* rather uses suction feeding. Sexual dimorphism in prey capture kinematics was identified in both species, indicating a potential sexual niche differentiation, as documented in the two *T. antoniae* morphospecies. But this hypothesis remains tentative as long no detailed ecological data is available for *T. bonti* and *M. ladigesii*. Interestingly, feeding biomechanics contradict the results of the prey capture kinematics. Unlike roundfins (Pfaender et al., 2011), both stream-dwelling sailfin silversides only showed limited interspecific and no intersexual differentiation in the biomechanical parameters analyzed.

These findings challenge the hypotheses that alternative feeding modes and intersexual niche partitioning are present in *T. bonti* and *M. ladigesii*. However, there are two possible explanations for these discrepancies. Firstly, the contradicting prey capture kinematics and feeding biomechanics of *T. bonti* and *M. ladigesii* might be a result of alternative feeding ecologies. Despite the fact that the trophic niches of both stream-dwelling species have not been quantified so far, field and aquarium observations as well as preliminary studies indicate that *T. bonti* primarily feeds on highly elusive prey whereas *M. ladigesii* predominantly feeds on non-mobile prey (Andriani 2000; Bach & Herder, unpublished data;

Nasyrah et al. 2019, 2020; Reckel 2001; Reckel et al. 2002; Reckel & Melzer 2003; Sterba 1987). Prey mobility can strongly influence the feeding biomechanics of predators, as has been documented in previous studies (Alfaro et al. 2005; Hulsey & Wainwright 2002; Parnell et al. 2008; Wainwright & Richard 1995). Thus, different diet compositions might have driven their feeding biomechanics to evolve in opposing directions resulting in a limited distinction between both species. Nonetheless, these theories need to be tested by quantifying the trophic niches of both stream-dwelling species. Secondly, the disparity between the strong biomechanical variation documented in lake-dwelling *Telmatherina* and the limited differentiation revealed in stream-dwelling sailfin silversides might be caused by their different habitats. Due to their continuous flow of water, rivers and streams are generally less stable and less isolated than lake ecosystems (Brinsmead & Fox 2002; King et al. 2021; McLaughlin & Grant 1994). These unreliable conditions may have led to the evolution of less distinct feeding biomechanics in *T. bonti* and *M. ladigesii*. These findings suggest that feeding biomechanics of sailfin silversides are not exclusively influenced by feeding mode but also by their trophic ecology and habitat usage.

5.2 Prospects

In this thesis, I have shown that the adaptive radiation of sailfin silversides, although thoroughly studied in recent years, remains a model system of inestimable value. This is not only the case for the field of evolutionary biology but also for other disciplines including biomechanics and methodology.

By using the roundfins of Lake Matano in chapter 2, I have shown that 2D GM might be more efficient than 3D GM for discriminating laterally compressed fishes. This conclusion might also be applicable to other rather 2-dimensional shaped organisms or structures such as chameleons, vertebrate mandibles and theropod dinosaur skulls. In order to corroborate this hypothesis, a further study which includes representatives of various different flattened taxa is necessary. If this prediction holds true, morphologists will be able to save a considerable amount of time, money and effort in future studies.

In chapter 3, I demonstrated that the documented sexual niche differentiation in roundfins has led to sexual dimorphism in ecologically relevant traits which presumably reduces

intraspecific niche overlap. However, this study focused on cranial structures which are mainly responsible for food acquisition (Burress et al., 2016, 2018; Carroll et al., 2004; Hellig et al., 2010; Ronco et al., 2019; Wilson, et al., 2013a,b). It would be interesting to test if roundfins also show intersexual niche differentiation in habitat use and if so, whether this has resulted in intersexual variation in traits used for locomotion. This could, for instance, be analyzed using computational fluid dynamics. Another intriguing question is whether intersexual niche differentiation occurs in other species flocks of Sulawesi's sailfin silversides and if this is the case, whether this has led to sexual dimorphism in trophic morphology as well. This is particularly true for the *Paratherina* radiation from Lake Mahalona and Lake Towuti which has remained largely understudied (Pfaender, 2013). Future studies on the *Paratherina* species flock could use the same morphological structures that were analyzed in chapter 3 since I have shown that these have adapted in response to resource exploitation in roundfins. For example, quantifying the size, volume and shape of the buccal cavity has rarely been utilized to discriminate fish species and sexes so far (Herler et al., 2010; Ronco et al., 2019). Thus, I recommend considering the buccal cavity as an ecologically relevant and adaptive trait in future studies.

Chapter 4 has shown how urgently ecological data for stream-dwelling sailfin silversides is required. Although the findings of chapter 4 are supported by aquarium and field observations, detailed trophic data for *T. bonti* and *M. ladigesii*, which could be achieved by analyzing stomach contents in future studies, would further corroborate these results. This would be particularly interesting for *T. bonti* since previous work has documented local and diverse morphological differentiation in this species (Herder et al., 2006a,b; von Rintelen et al., 2012). If ecological data revealed alternative trophic ecologies, it would be intriguing to test whether these ecotypes show local differentiation in functional feeding morphology. Finally, the high-speed video analysis used in chapter 4 could also be applied to investigate the prey capture kinematics of Lake Matano's roundfins in relation to their trophic morphology and feeding biomechanics. Since both phenotypic groups are challenging to keep in an aquarium, this approach would have to be transferred to the field. However, future fieldwork will have to be conducted as soon as possible before it becomes unfeasible. Unfortunately, a number of factors including nickel mining, pet trade and the introduction of invasive fish species pose a threat to the biodiversity of the Malili Lakes system (Herder et

al., 2012; von Rintelen et al., 2012). If these risks are not addressed quickly, several of Sulawesi's endemic fish species, including the radiation of sailfin silversides, may face extinction shortly (Herder et al., 2006b). Possible solutions could be to prohibit stocking, to initiate a monitoring program and to increase the awareness of the endemic fauna within the local population (Herder et al., 2012; von Rintelen et al., 2012).

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Supplementary material

Chapter 2

Supplementary material for this study can be found under <https://onlinelibrary.wiley.com/doi/10.1111/jfb.14410> in the supporting information section.

Chapter 3

Supplementary material for this study is deposited at Dryad digital repository and can be found under <https://doi.org/10.5061/dryad.0gb5mkm1x>.

Chapter 4

Supplementary material for this study can be found under <https://link.springer.com/article/10.1007/s00435-022-00570-8> in the supplementary information section.

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Appendix

Benjamin D. Wasiljew, Jobst Pfaender, Benjamin Wipfler, Ilham Vemandra Utama, Fabian Herder (2020). **Do we need the third dimension? Quantifying the effect of the z-axis in 3D geometric morphometrics based on sailfin silversides (Telmatherinidae).** *Journal of Fish Biology*, 97(2), 537-545. DOI: 10.1111/jfb.14410

Benjamin D. Wasiljew, Jobst Pfaender, Benjamin Wipfler, Mariam Gabelaia, Ilham Vemandra Utama, Letha Louisiana Wantania, Fabian Herder (2021). **Sexual dimorphism in an adaptive radiation: Does intersexual niche differentiation result in ecological character displacement?** *Ecology and Evolution*, 11(21), 14615-14629. DOI: 10.1002/ece3.8137

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Do we need the third dimension? Quantifying the effect of the z-axis in 3D geometric morphometrics based on sailfin silversides (Telmatherinidae)

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Abstract

This study investigated the impact of the third dimension in geometric morphometrics (GM) using sailfin silversides (Telmatherinidae) from the Malili Lakes of Sulawesi (Indonesia). The three morphospecies of the monophyletic “roundfin” radiation are laterally compressed and vary in shape traits. The results of 2D and 3D GM were compared and quantified to discuss the advantages and disadvantages of both methods for closely related species and their sexes. This approach focused on the head because it is far more complex and three-dimensionally structured than the trunk or the caudal region. The results revealed no significant benefit concerning repeatability and measurement error in 3D GM compared to 2D GM. The z-axis contributed substantially to the variance of the 3D data set but was irrelevant for discrimination of species and sexes in the approach. Limited gain in information was contrasted by substantially higher effort for 3D compared to the 2D analyses. The study concluded that 2D GM is the more efficient shape analysis approach for discriminating roundfins. Broader studies are needed to test which of the two methods is more efficient in distinguishing laterally compressed fishes in general. For future studies, due to the high investment required, this study recommends carefully evaluating the necessity of 3D GM. If in doubt, this study suggests testing for congruence between 2D and 3D GM with a subsample and consequently applying 2D GM in the case of high congruence.

KEYWORDS

geometric morphometrics, Malili Lakes, μ -CT imaging, skull morphology, *Telmatherina*, 2D/3D analysis

1 | INTRODUCTION

Geometric morphometric (GM) methods are widely used to study shape variation in fishes (Higham *et al.*, 2017; Jamniczky *et al.*, 2015; Pfaender *et al.*, 2011). GM methods are highly efficient in detecting

especially small-scaled differentiation (Adams *et al.*, 2004; Kaliontzopoulou, 2011) and provide tools for the quantification of traits, *e.g.*, in evolutionary ecology applications (Gould, 2014).

Traditional GM approaches are based on 2D images like standardized photographs or X-ray images (Lawing & Polly, 2010; Pfaender

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et al., 2011; Postl et al., 2008; Zelditch et al., 2004). 3D imaging techniques, such as μ -computed tomography (μ -CT), increasingly enable the efficient acquisition of data that describe all three dimensions, with sufficient resolution to also detect the subtle variation relevant for a broad range of evolutionary and ecological studies (Buser et al., 2018). Nonetheless, efforts for collecting and analysing data for 3D models remain substantially higher compared to those required for 2D imaging (Cardini & Chiapelli, 2020; Navarro & Maga, 2016), and GM applications based on 3D data are therefore comparatively scarce in studies targeting quantitative variation among species, populations and sexes (Cardini, 2014; Gould, 2014). Increased efforts include the time required for scanning and processing the data, as well as the substantial costs for μ -CT devices and the analytical software (Abel et al., 2012; Gould, 2014). Nevertheless, it seems obvious that 3D GM has benefits over 2D GM in terms of capturing of relevant structures, accuracy, reduced distortion and, in some cases, also invasiveness (Buser et al., 2018). Once a μ -CT scan has been performed, the data can also be used for multiple further analyses forming a morphological library (Shi et al., 2018).

In vertebrate morphology 3D imaging techniques are currently widely used for qualitative analyses of inner anatomy. Modern volume and surface rendering software allows detailed analyses of morphological structures (Wake, 2012). Nonetheless, quantitative comparative analyses like GM remain rare in 3D. Vertebrate palaeontology is one of the few exceptions in which 3D GM is becoming a standard approach (Curran, 2018; van Heteren et al., 2016). This lack of 3D GM is especially true for fishes (Higham et al., 2017; Jamniczky et al., 2015), which might be due to the comparatively limited body width in many taxa. Although many fish species are laterally compressed, the exclusion of the z-axis expectedly effects GM results (Buser et al., 2018). Nonetheless, the contribution of the z-axis to the total morphological variation has rarely been quantified in fishes. Thus, 2D methods are still the norm in studies targeting morphological variation in fishes (Buser et al., 2018).

The species flock of sailfin silversides endemic to the Malili Lakes of Sulawesi (Indonesia) is used for studying processes of adaptive radiation and sympatric speciation (Herder, Nolte, et al., 2006; Herder & Schliewen, 2010; Pfaender et al., 2016; Walter et al., 2009). Roundfin *Telmatherina* are a monophyletic group within the radiation and endemic to ancient Lake Matano (Stelbrink et al., 2014; von Rintelen et al., 2012). They consist of three morphospecies, *Telmatherina antoniae* "small," *Telmatherina antoniae* "large" and *Telmatherina prognatha* Kottelat, 1991 (Herder, Schwarzer, et al., 2006). Based on lateral 2D X-ray images, the variation in body and head shape, as well as that of single bones, has extensively been studied (e.g., Herder et al., 2008; Herder, Nolte, et al., 2006; Pfaender et al., 2011). Variation among morphospecies is significant in both, body and head shape, complemented by sexual dimorphism (Pfaender et al., 2011). Nonetheless, the variation in the third dimension has not been studied so far.

This study assessed the effects of the z-axis by comparing species and sex discrimination in 2D and 3D landmark-based GM and discussed the results with regard to costs, processing time and data size. 2D GM was based on maximum intensity projections of μ -CT scans in lateral view and 3D GM on μ -CT surface renders of Lake Matano's

roundfin *Telmatherina*. This study focused on the skull because it is far more complex and three-dimensionally structured than the rest of the skeleton. The study quantified the additional information of the third axis and the difference in the results of 3D GM and 2D GM performed on the same data set. It was hypothesized that the 3D approach should have a higher repeatability compared to the 2D analysis. Further, it was expected that the z-axis harboured a substantial amount of information that helps to discriminate the three roundfins with respect to morphospecies and sexes more strongly than without the third dimension.

2 | MATERIALS AND METHODS

2.1 | Materials and imaging techniques

Roundfin *Telmatherina* specimens were available from collection material that was obtained in the dry season of 2002 from three locations around Lake Matano's shoreline using gillnets (Supporting Information Table S1). Fishes were fixed in 4% formalin and transferred to 70% ethanol for long-term storage. Because the specimens used for this study were obtained from museum collection material, no living animals were sampled, killed, harmed or treated in any other way for this paper. Therefore, an ethics permit is not required for this paper.

The heads of five male and five female specimens of each morphospecies, *T. antoniae* "small", *T. antoniae* "large"

T. prognatha, were used for μ -CT scanning using Skyscan 1272 and Skyscan 1173 μ -CT scanners (Bruker, Billerica, MA). Specimens were scanned in 70% ethanol without prior staining. The 3D resolution ranged between 11 and 19 μ m depending on the size of the specimen. Selected rotation steps varied between 0.3° and 0.4° over 180°. The chosen voltage ranged between 60 and 80 kV and the current between 111 and 166 μ A. Detailed scanner settings for each individual are provided in Supporting Information Table S1. The projections were reconstructed using NRecon version 1.7.1.0 (Bruker). Data size was then reduced using the software Dataviewer version 1.5.2.4 by Bruker and ImageJ version 1.51f by NIH (Schindelin et al., 2015). Segmentation and volume rendering of the resulting 3D models was accomplished using the software packages Drishti version 2.6.4 by ANU Vizlab (Limaye, 2012), Amira version 6.5.0 by Thermo Fisher Scientific (Stalling et al., 2005) and VG Studiomax 3.2 by Volume Graphics. Surface rendering was performed using the commercial software package Checkpoint version 17.04.21 (Stratovan Corporation). Final plates were arranged using Adobe Photoshop CS6 and Adobe Illustrator CS6.

2.2 | Geometric morphometrics

GM was performed in 2D based on horizontally aligned maximum intensity projections of μ -CT scans in lateral view to achieve images which resemble 2D X-rays (Supporting Information Figure S1). 3D GM was based on μ -CT-based surface-rendered 3D models. Ten

landmarks were placed in the same order on the cranial skeleton of the 30 μ -CT-scanned specimens in 2D using tpsUtil version 1.76 and tpsDig version 2.31 (Rohlf, 2015) and 3D with Checkpoint version 17.04.21 (Stratovan). The lower jaw was excluded from the analyses because pilot analyses showed the effects of variation depending on different degrees of mouth opening. The locations of the 10 landmarks were adjusted slightly in Checkpoint due to the differences in the methodology and visual perspective between 2D and 3D. See Figure 1 for specific landmarks.

2.3 | Statistical analyses

Procrustes superimposition, PCA and thin plate spline interpolation of the landmark points were performed using the software PAST version

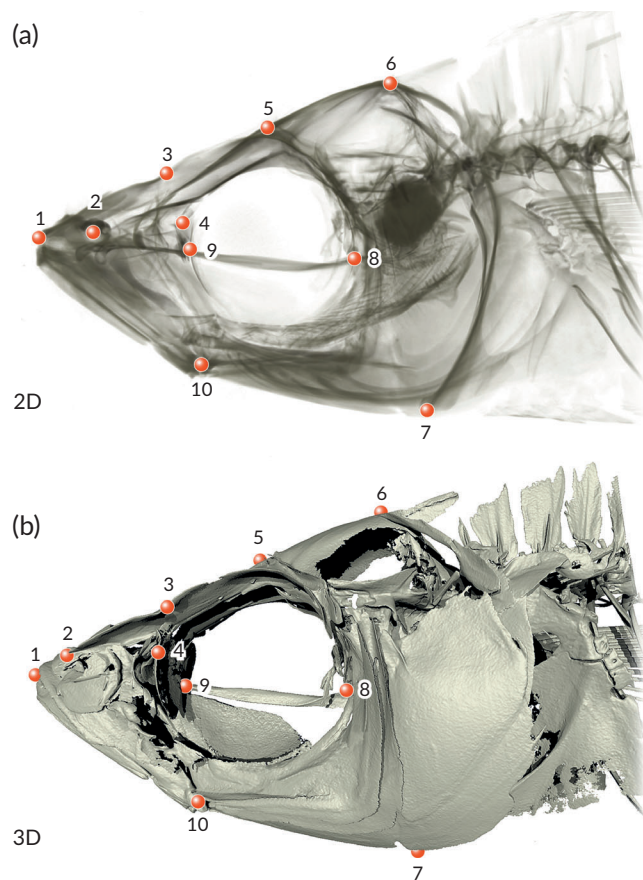


FIGURE 1 (a and b) Locations of the 10 homologous landmarks placed on the heads of μ -computed tomography-scanned roundfin specimens of *Telmatherina antoniae* "small", *Telmatherina antoniae* "large" and *Telmatherina prognatha*: (a) 2D landmark configuration; (b) 3D landmark configuration. Head shape is described by 10 landmarks: 1, anterior tip of premaxilla; 2, nasal/maxilla joint; 3, nasal/neurocranium joint; 4, lacrimal process; 5, dorsal neurocranium process; 6, posterior dorsal point of neurocranium; 7, posterior ventral end of articular; 8, most posterior-ventral point of eye socket; 9, most anterior-ventral point of eye socket; 10, posterior ventral end of articular

3.22 (Hammer *et al.*, 2001) and R version 3.5.1 (Ihaka & Gentleman, 1996). The intraclass correlation coefficient to quantify the measurement error in two and four repeated measurements was calculated following Fruciano (2016) based on the mean squares obtained from Procrustes ANOVA (procD.LM function in the R-package "geomorph") (Adams & Otárola-Castillo, 2013). Procrustes superimposition was performed using the gpagen function of the geomorph R-package. Resulting Procrustes coordinates were used to calculate a PCA, using the PCA function from the FactoMineR R-package (Lê *et al.*, 2008). The contribution of the single variables from the x-, y- and z-coordinates in single principal components (PCs) was evaluated based on the Cos2 values. The Cos2 values represent the square loadings of the variables. The sum of the Cos2 values equals one for all PCs. The percentage contribution of a variable to a certain PC was then calculated as $(\text{VariableCos2} \times 100) / (\text{total Cos2 of the respective PC})$ (Lê *et al.*, 2008). A between-group PCA with implemented cross-validated correct classification [groupPCA function of the R-package "morpho" (Schlager, 2016) with 100.000 replicates] was calculated following Cardini and Polly (2020) to test for significant differences between species and sexes in the 2D such as the 3D data set.

2.4 | Time expenditure and data sizes

Time expenditures for sample preparation, post-processing, scanning, reconstruction and digitizing landmarks, as well as the data sizes, were quantified for the 3D-based pipeline (Supporting Information Table S1). Time for X-ray stabilization and warming up was included. Reconstruction time was measured using the software NRecon version 1.7.1.0 (Bruker), and cropping duration of the resulting image stacks was measured using the software Dataviewer version 1.5.2.4 by Bruker. Time expenditures for all the other tasks performed by the researcher himself, such as sample preparation or digitizing of landmarks, were measured with the help of a standard stopwatch. Time expenditures for sample preparation, post-processing, scanning, reconstruction and digitizing landmarks, such as data sizes for 2D X-ray imaging, were gathered from an earlier project (Pfaender *et al.*, 2011).

3 | RESULTS

3.1 | 2D and 3D results

The landmark configuration of the 2D data set ($R = 0.97$, Figure 2a) and 3D data set ($R = 0.99$, Figure 2b) was highly repeatable. Although the repeatability of the landmark configuration in the 3D data set exceeded that of the 2D data set, differences in repeatability were minor.

The first three PCs of the PCA explained, in sum, 84.5% of the variance captured by the 2D data set (Table 1a). In the 3D data set, the first three PCs of the PCA explained, in sum, 73.4% of the variance (Table 1a). The z-axis contributed substantially to the variance

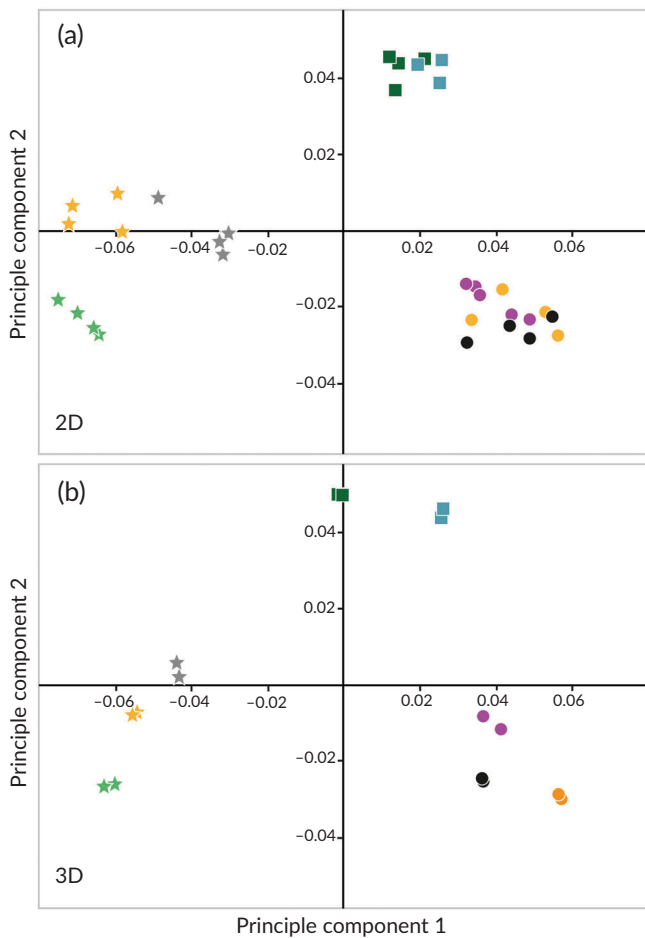


FIGURE 2 (a and b) PCA plot of a sub-set of both landmark data sets visualizing the comparability of repeated digitizing of landmarks ($n = 8$): (a) 2D landmark data set, digitized four times; (b) 3D landmark data set, digitized twice. Each colour represents one individual that was digitized multiple times. Morphospecies are symbol coded: *Telmatherina antoniae* "small" – star; *Telmatherina antoniae* "large" – square; *Telmatherina prognatha* – dot

(25.5%), which is explained by the single PCs (Table 1b). From PC3 to PC5 the z-axis contributed more to the variance than the x-axis or the y-axis (PC3: 41.0%; PC4: 70.2%; PC5: 71.8%).

T. prognatha was most distinct from the other two roundfin morphospecies in the 2D analyses; the clusters comprising the specimens of both *T. antoniae* "small" and "large" were less separated and distinctly overlapped (Figure 3a).

The 3D results show a similar pattern to the 2D results but a less distinct separation between *T. prognatha* and the other two morphospecies, which is contrasted by a more distinct separation of *T. antoniae* "small" and "large" (Figure 3b). The deformations of the 2D and 3D approaches showed a high variance in the x-axis, explained by PC1, and a lower variance in the y-axis, mainly explained by PC2 (Figure 4). The z-axis hardly contributed to the deformations of the 3D analysis in the first two PCs.

The cross-validated correct classification test performed on species level revealed significant differences between all three species in

TABLE 1 (a) Sum of variance in percentage contributed by each PC in the 2D and 3D analysis of *Telmatherina antoniae* "small", *Telmatherina antoniae* "large" and *Telmatherina prognatha* ($n = 10$). (b) Sum of variance in percentage contributed by the single axes x, y and z to each PC in the 3D analysis of *T. antoniae* small, *T. antoniae* large and *T. prognatha* ($n = 10$). (c) Sum of variance in percentage contributed by the single axes x and y to each PC in the 2D analysis of *T. antoniae* "small", *T. antoniae* "large" and *T. prognatha* ($n = 10$)

(a) Variance per method					
	PC1 (%)	PC2 (%)	PC3 (%)	PC4 (%)	PC5 (%)
3D	46.5	17.2	10.1	5.6	4.6
2D	57.9	17.9	8.5	3.9	3.1
(b) Variance per axis in 3D					
	PC1 (%)	PC2 (%)	PC3 (%)	PC4 (%)	PC5 (%)
x	44.6	30.9	39.9	5.8	13.2
y	36.8	38.0	19.1	24.1	15.1
z	18.6	31.1	41.0	70.2	71.8
(c) Variance per axis in 2D					
	PC1 (%)	PC2 (%)	PC3 (%)	PC4 (%)	PC5 (%)
x	55.9	41.6	51.9	36.7	37.4
y	44.1	58.4	48.1	63.3	62.6

Note. PC: principal component.

both, the 2D and 3D data sets (Table 2). The Euclidean distances between *T. antoniae* "small" vs. "large" and *T. antoniae* "small" vs. *T. prognatha* were higher in the 3D data set compared to the 2D data set. In contrast, *T. antoniae* "large" vs. *T. prognatha* had a higher Euclidean distance in the 2D data set than in the 3D data set.

The cross-validated correct classification test performed on sexes revealed no significant differences between males and females within all three species in 2D and 3D (Table 3). The Euclidean distances were very low within all three species.

3.2 | Time expenditures and data sizes

The overall processing time of the 3D analysis from sample preparation to digitizing of landmarks was between 50 and 70 times longer, and the actual handling time for the scientist was between 10 and 12 times longer than for a 2D analysis based on X-ray images (Supporting Information Table S1). The data sizes in 3D μ -CT imaging were on average ca. 2000 times larger than those of 2D X-ray imaging (Supporting Information Table S1).

4 | DISCUSSION

4.1 | Repeatability

Landmarks were highly repeatable in both the 2D ($R = 0.97$) and 3D ($R = 0.99$) data sets, with an R value of 1 being the highest possible

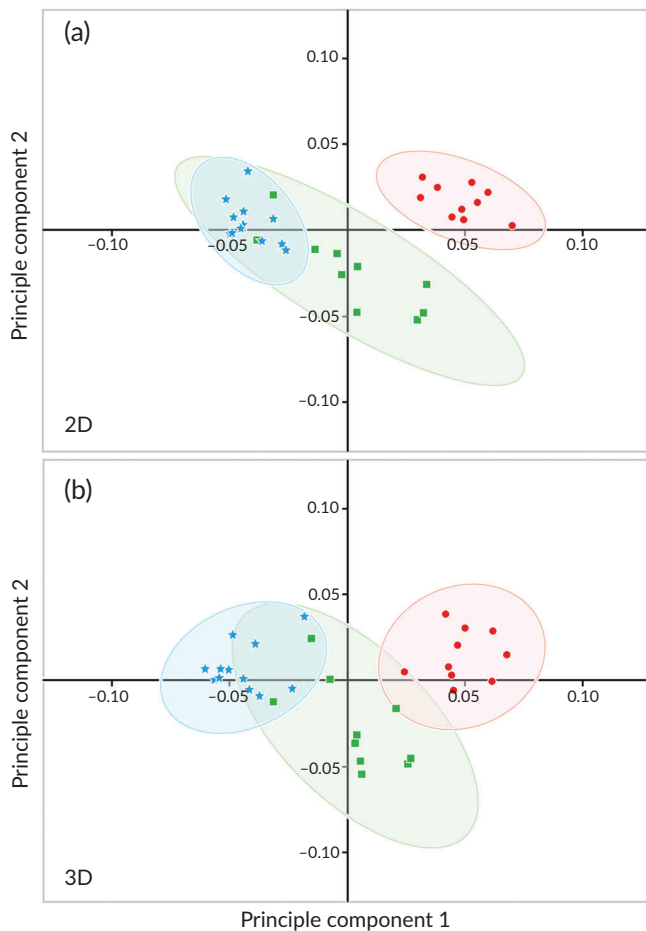


FIGURE 3 (a and b) PCA plot of both landmark data sets with point clusters of *Telmatherina antoniae* "small", *Telmatherina antoniae* "large" and *Telmatherina prognatha* ($n = 10$): (a) 2D landmark data set; (b) 3D landmark data set. Morphospecies are colour- and symbol coded: *T. antoniae* "small" – blue star; *T. antoniae* "large" – green square; *T. prognatha* – red dot

repeatability (Arnqvist & Martensson, 1998; Fruciano, 2016). Thus, in this specific approach, the 3D analysis did not have any substantial benefits over the 2D analysis concerning accuracy and measurement error. These results are consistent with the findings of Courtenay *et al.* (2018) based on cut marks which contain only a low amount of variation in the third dimension. This study's results and those of Courtenay *et al.* (2018) thus disagree with the general assumption that the high resolution, and the option of turning the specimen in all dimensions, ensures higher accuracy and that landmarks are easier to locate in 3D, consequently resulting in a lower measurement error (Cardini & Chiapelli, 2020; Roth, 1993). Nonetheless, it should be pointed out that this study and the study of Courtenay *et al.* (2018) placed only external landmarks. It is important to note that many internal or asymmetric structures can be reliably landmarked only in 3D and not in 2D (Attard *et al.*, 2018; Hedrick *et al.*, 2019; McWhinnie & Parsons, 2019). Therefore, this study's results on accuracy cannot be projected onto studies investigating small-scaled inner or asymmetric structures. Measurement error regarding the placing of the

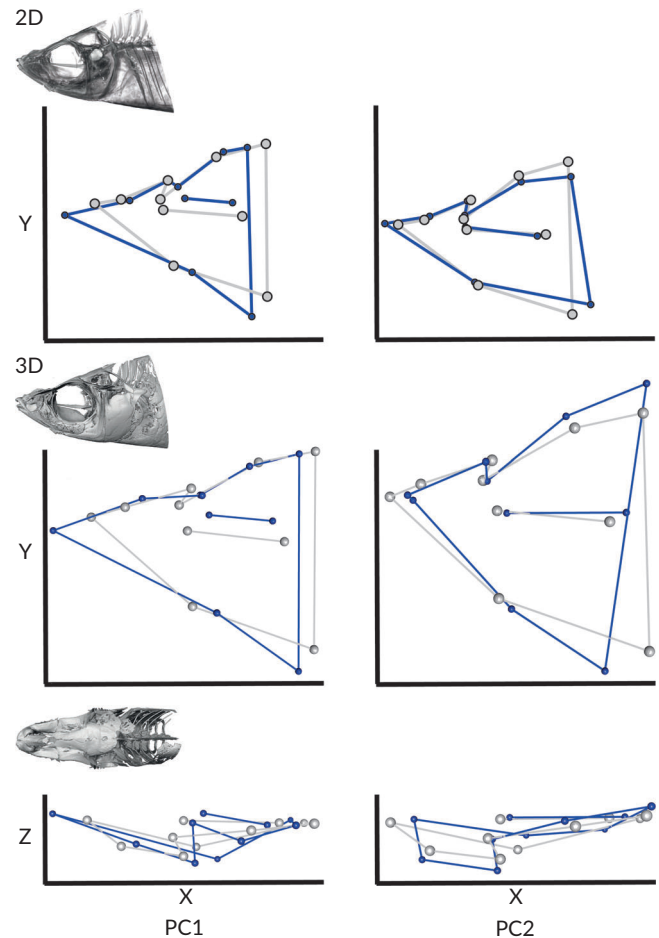


FIGURE 4 Deformations of the two first principal components in 2D and 3D for the combined data set of *Telmatherina antoniae* "small", *Telmatherina antoniae* "large" and *Telmatherina prognatha* in lateral view for 2D as well as lateral and dorsal view for 3D ($n = 10$). Mean shape is coloured in grey. Deformations are coloured in blue

TABLE 2 Results of discriminant analyses to test for morphological differences between the three roundfin morphospecies *Telmatherina antoniae* "small", *Telmatherina antoniae* "large" and *Telmatherina prognatha* ($n = 10$)

		<i>T. prognatha</i>		<i>T. antoniae</i> "large"	
		P-value	ED	P-value	ED
<i>T. antoniae</i> "small"	3D	0.0000	0.0930	0.0003	0.0626
	2D	0.0001	0.0653	0.0100	0.0466
<i>T. antoniae</i> "large"	3D	0.0003	0.0649	-	-
	2D	0.0000	0.0913	-	-

Note. Pairwise Euclidean distance means and P-values among roundfin species from the cross-validated correct classification rates of the between-group PCA are shown for the 2D and 3D approaches. ED: Euclidean distance means of the between-group averages.

specimen was also excluded from the analysis because the 2D data set was created out of 3D μ -CT scans and not *via* traditional X-ray imaging.

TABLE 3 Results of discriminant analyses to test for morphological differences between roundfin sexes within *Telmatherina antoniae* "small", *Telmatherina antoniae* "large" and *Telmatherina prognatha* ($n = 5$)

	<i>T. antoniae</i> "small" m/f		<i>T. antoniae</i> "large" m/f		<i>T. prognatha</i> m/f	
	P-value	ED	P-value	ED	P-value	ED
3D	0.5999	0.0290	0.1724	0.0470	0.8887	0.0240
2D	0.5184	0.0260	0.7327	0.0230	0.8604	0.0200

Note. Pairwise Euclidean distance means and *P*-values within roundfin species from the cross-validated correct classification rates of the between-group PCA are shown for the 2D and 3D approaches. ED: Euclidean distance means of the between-group averages; m: male; f: female.

4.2 | Information content

The variance in the 3D analysis was distributed more evenly over the PCs compared to the 2D analysis (Table 1). The first three PCs explained almost 85% of the overall variance in the 2D analysis but only about 75% in the 3D analysis. This is in agreement with the results of Cardini (2014) and McWhinnie and Parsons (2019), who also found lower variance in the first two PCs in their 3D data sets. This pattern might be driven by the fact that the 3D data set consist of a higher number of variables than the 2D data set. The variance was distributed similarly between the x-axis and the y-axis in the 2D and 3D data sets, which is also supported by the deformations (Figure 4). With 25.5% of the overall information content, the z-axis contributed substantially to the overall variance in the 3D data set. Nonetheless, the z-axis contributed primarily to the variance between PC3 and PC5 but not primarily to the variance between PC1 and PC2. This could explain why the z-axis hardly contributed to the deformations within the 3D data set. This study's results confirm the hypothesis that the third dimension contains a high amount of variance within roundfin skull shape, which cannot be projected by the 2D data set. Similar results were also found in the study of Buser *et al.* (2018) that analysed sculpins, which have much broader heads than the roundfins in the present study. In marmot skulls, Cardini (2014) found similarly high information content in the cranium but a distinctly lower one in the rather flat mandibles. Thus, the information content of the z-axis appears to be highly dependent on the object studied and cannot be generalized for all species or objects.

4.3 | Species discrimination

Despite the high information content of the z-axis, the 3D data set did not improve the discrimination between species or sexes among the roundfin *Telmatherina* studied. The morphospaces of the 2D and 3D PCA allowed a distinct separation of *T. prognatha* and did not allow a clear separation between both *T. antoniae* morphospecies. Therefore, the morphospaces of both approaches resembled each other to a great extent. Thus, the 3D approach did not have any overall benefits in discriminating between the three roundfin species by the clusters of the PCA. Previous studies demonstrated that the three roundfin morphospecies differ in body size, eye size and position, mouth position and overall body height and depth (Herder, Schwarzer,

et al., 2006; Kottelat, 1991; Pfaender *et al.*, 2011). *T. prognatha* is distinguished from the other morphospecies by its elongated snout and its fusiform body. *T. antoniae* "large" is characterized by its deeper and higher overall body, whereas *T. antoniae* "small" is discriminated by its small body size and the large, further caudal-located eyes (Herder, Schwarzer, *et al.*, 2006; Kottelat, 1991; Pfaender *et al.*, 2011). These traits are consistent with this study's results from both approaches and are well covered by the 2D and 3D landmarks selected here. Therefore, the results match the 2D results of Herder *et al.* (2008) and Pfaender *et al.* (2011). This is highly plausible because both studies are based on digital X-ray images, which correspond to the maximum intensity projections applied here (Supporting Information Figure S1). High similarity in morphospaces between the 2D and 3D analyses was also present in the sculpin heads investigated by Buser *et al.* (2018), the marmot mandibles analysed by Cardini (2014) and the cichlid mandibles examined by McWhinnie and Parsons (2019). In contrast, the cross-validated correct classification tests allowed a clear separation between the three morphospecies in both, 2D and 3D ($P = <0.01$). The ability to discriminate between *T. antoniae* "small" vs. "large" and *T. antoniae* "small" vs. *T. prognatha* was slightly stronger in the 3D analysis than in the 2D analysis, but it was slightly stronger in the 2D analysis between *T. antoniae* "large" and *T. prognatha*. This might imply that the z-axis contained information which was relevant for species discrimination in the case of *T. antoniae* "small" vs. "large" and *T. antoniae* "small" vs. *T. prognatha* but which was not present in the case of *T. antoniae* "large" vs. *T. prognatha*. Nonetheless, because the z-axis explained only *ca.* 25% of the overall variance, the additional discriminating ability appears very limited. These results match with the findings of Álvarez and Perez (2013) and McWhinnie and Parsons (2019), who also did not find any major differences in discriminating between species based on mandibles in 2D and 3D.

4.4 | Sex discrimination

In contrast to the interspecific analyses, the clusters of the sexes strongly overlapped within all three morphospecies and did not allow a clear discrimination. The cross-validated correct classification test allowed no discrimination among sexes within all three groups in 2D or 3D ($P = >0.1$). The results imply that none of the two methods is better suited to distinguish between male and female roundfins. A similar result was retrieved by McWhinnie and Parsons (2019), who

studied laterally compressed cichlid mandibles. Interestingly, this study's results do not agree with those of Pfaender *et al.* (2011), who studied the same species complex based on 2D X-ray images. They found no differences within the two *T. antoniae* morphospecies and significant differences in *T. prognatha*. Supporting Information Figure S2 shows that these differences are not associated with the different modes of 2D data generation in these studies (X-ray vs. maximum intensity projections). Methodological differences also do not account for them, as the same analytical procedure was performed. Two possible explanations appear plausible: the comparatively low sample size in the present study and the selection of landmarks. Pfaender *et al.* (2011) placed 12 landmarks on the head, compared to 10 used in the present study. The two landmarks omitted here were the quadrate/articular joint and the maxilla/articular joint to limit possible effects of different degrees of mouth opening. Therefore, this study strongly emphasizes the importance of landmark placement and configuration for all future studies.

5 | CONCLUSIONS

This study compared the repeatability, information content and discriminating ability of 2D and 3D GM for the first time on laterally compressed fishes. The results show that, although the z-axis harboured a substantial amount of information, 3D GM provided no substantial benefits in accuracy or in the discriminating ability compared to the 2D GM approach. Roundfins are laterally compressed like many other fishes, which might be the reason that the z-axis did not significantly contribute to the ability of distinguishing between morphospecies or sexes. Similar results were also found in other flattened specimens and body structures like marmot mandibles, dinosaur skulls and cichlids (Cardini, 2014; Hedrick & Dodson, 2013; McWhinnie & Parsons, 2019), but studies using strongly 3D objects such as marmot crania or sculpin skulls retrieved better discrimination results with 3D approaches (Buser *et al.*, 2018; Cardini, 2014). Thus, this study's results could further corroborate the hypothesis that 3D approaches might be advantageous only in strongly 3D objects but that 2D approaches are equally or even better suited in flattened animals such as many fishes. Nonetheless, these interpretations are based on a few studies with strongly varying animals and therefore should be considered as preliminary and tentative. This assumption has to be tested by future studies which include several different laterally compressed fishes and other clade taxa. There is clearly a potential for nonbiological variation in the analyses. Examples include the use of two different μ -CT scanners, different settings or the placement of the specimens. Nonetheless, the present study is based on the assumption that these variations are negligible compared to the variation between the 2D and 3D data sets or between single specimens. The impact of scale effects within the z-axis is also unknown and has to be evaluated systematically. In cases where landmarks are placed in obscure spots, on asymmetric samples (Attard *et al.*, 2018; Hedrick *et al.*, 2019; McWhinnie & Parsons, 2019), or when only parts of the actual specimen are studied (Shi *et al.*, 2018), 3D data are

required to be independent from the discriminating ability of the approach used. In all other cases, it is currently recommended to test for congruence between the two methods on a sub-sample. If the repeatability is high in both methods and the 3D approach allows no better discrimination in these pilot studies, this study suggests applying 2D data due to the much lower data acquisition and processing time (Cardini & Chiapelli, 2020). In the present study, generating and processing the 3D data took 50–70 times as long, 10–12 times as much actual working time and on average about 2000 times as much storage space as for the 2D data (Supporting Information Table S1). The much larger 3D data sets require considerably longer times to load and process in the respective software packages, and several additional steps are necessary in the workflow of 3D GM that further consume time and effort (Abel *et al.*, 2012; Gould, 2014; Zhao *et al.*, 2009). Nonetheless, these explicit values concerning time and data size should be treated with caution as they are strongly dependent on the applied parameters and the equipment used. Nevertheless, 3D approaches consume a manifold of time, effort and financial investment compared to 2D analyses (Abel *et al.*, 2012; Adams *et al.*, 2004; Cardini & Chiapelli, 2020; Navarro & Maga, 2016; Zhao *et al.*, 2009) and are therefore worthwhile only when 3D approaches promise better results (Fruciano, 2016; Fruciano *et al.*, 2017).

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AUTHOR CONTRIBUTIONS

B.D.W., J.P. and F.H. designed the study. B.D.W. was responsible for data acquisition and performed μ -CT scans such as digitizing of landmarks. B.D.W. and J.P. analysed the data. B.D.W. wrote the manuscript, with contributions from J.P., B.W., I.V.U. and F.H. B.W. was responsible for visualization. F.H. supervised the study. All authors contributed critically to the drafts and gave final approval for publication.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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RESEARCH ARTICLE

Sexual dimorphism in an adaptive radiation: Does intersexual niche differentiation result in ecological character displacement?

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Abstract

Evolutionary radiations are one plausible explanation for the rich biodiversity on Earth. Adaptive radiations are the most studied form of evolutionary radiations, and ecological opportunity has been identified as one factor permitting them. Competition among individuals is supposedly highest in populations of conspecifics. Divergent modes of resource use might minimize trophic overlap, and thus intersexual competition, resulting in ecological character displacement between sexes. However, the role of intersexual differentiation in speciation processes is insufficiently studied. The few studies available suggest that intersexual niche differentiation exists in adaptive radiations, but their role within the radiation, and the extent of differentiation within the organism itself, remains largely unexplored. Here, we test the hypothesis that multiple morphological structures are affected by intersexual niche differentiation in “roundfin” *Telmatherina*, the first case where intersexual niche differentiation was demonstrated in an adaptive fish radiation. We show that sexes of two of the three morphospecies differ in several structural components of the head, all of these are likely adaptive. Sexual dimorphism is linked to the respective morphospecies-specific ecology and affects several axes of variation. Trait variation translates into different feeding modes, processing types, and habitat usages that add to interspecific variation in all three morphospecies. Intrasexual selection, that is, male–male competition, may contribute to variation in some of the traits, but appears unlikely in internal structures, which are invisible to other individuals. We conclude that intersexual variation adds to the adaptive diversity of roundfins and might play a key role in minimizing intersexual competition in emerging radiations.

KEYWORDS

3D geometric morphometrics, adaptive radiation, ecological character displacement, intersexual niche differentiation, sexual dimorphism, *Telmatherina*

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1 | INTRODUCTION

The concept of evolutionary radiation, evolutionary divergence of a single lineage into a variety of different adaptive forms, is one plausible explanation for the rich biodiversity on Earth (Naciri & Linder, 2020; Nosil, 2012; Simões et al., 2016). Some of the best-studied examples of evolutionary radiations are adaptive radiations (Gavrilets & Losos, 2009; Losos, 2010; Naciri & Linder, 2020; Simões et al., 2016), which are driven by the evolution of ecological divergence and the accumulation of reproductive isolation (Martin & Richards, 2019; Rundle & Nosil, 2005; Schluter, 2009; Schluter & Conte, 2009). The evolution of morphological adaptations that enable alternative modes of ecological resource use may facilitate coexistence of closely related species in different ecological niches (Losos, 2010; Martin & Richards, 2019; Nosil, 2012; Schluter, 2000; Yoder et al., 2010).

Ecological competition is expectedly highest in populations of conspecifics (McGee et al., 2020). Divergent selection has been shown to play a key role in interspecific adaptive processes (Rundle & Nosil, 2005; Schluter, 2009) and might even be an important factor for divergence between sexes (De Lisle, 2019; De Lisle & Rowe, 2017; Roy et al., 2013). Divergent modes of resource use minimize intersexual competition for limited trophic resources, resulting in ecological character displacement between males and females (De Lisle, 2019; De Lisle & Rowe, 2017; Roy et al., 2013). Although the role of intersexual variation in speciation processes has gained little attention so far (De Lisle, 2019; De Lisle & Rowe, 2017; Ronco et al., 2019), the few studies available suggest that intersexual niche differentiation is present in adaptive radiations (De Lisle & Rowe, 2017; Pfaender et al., 2011; Ronco et al., 2019). It has been demonstrated, for instance, in *Anolis* lizards, salamanders, and sticklebacks (Butler, 2007; De Lisle & Rowe, 2017; McGee & Wainwright, 2013). Whether intersexual niche differentiation rather retards or promotes adaptive radiations is still an ongoing discussion (Bolnick & Doebeli, 2003; Butler, 2007; De Lisle & Rowe, 2015, 2017), but recent studies have shown that ecological speciation and ecological character displacement can occur simultaneously (De Lisle & Rowe, 2015, 2017). However, the actual role of intersexual niche differentiation in species flock formation remains largely unexplored (De Lisle, 2019; De Lisle & Rowe, 2017; Pfaender et al., 2011; Ronco et al., 2019).

Sexual dimorphism is widespread in adaptive radiations (Herler et al., 2010; McGee & Wainwright, 2013), most commonly as sexual size dimorphism or sexual color dimorphism (Herler et al., 2010; Tsuboi et al., 2012). It can either be induced by sexual selection, by intrinsic differences between males and females, or by intersexual competition (De Lisle, 2019; Hedrick & Temeles, 1989; Herler et al., 2010), whereby these drivers may interact in many cases of sexual dimorphism (Bolnick & Doebeli, 2003; Temeles et al., 2000). Sexual selection mechanisms provide plausible explanations for many of the spectacular cases, but cannot account for intersexual phenotypic variation in general (De Lisle, 2019; Hedrick & Temeles, 1989; Tsuboi et al., 2012). This is especially true for ecologically relevant traits and

internal structures (Bolnick & Doebeli, 2003; De Lisle, 2019; Ronco et al., 2019). In these cases, it seems rather plausible that intersexual competition for ecological resources is a main cause for the development of sexual dimorphism (Bolnick & Doebeli, 2003; Ronco et al., 2019). Examples of sexual dimorphism in ecologically relevant traits include stick insects, hummingbirds, *Anolis* lizards, salamanders, cichlids, and sticklebacks (Albert et al., 2008; Butler, 2007; De Lisle & Rowe, 2017; Herler et al., 2010; Hulseley et al., 2015; McGee & Wainwright, 2013; Ronco et al., 2019; Roy et al., 2013; Temeles & Kress, 2003).

The cranial region of fishes contains key traits for food acquisition, ranging from size and shape of the skull to variation in gill rakers, oral and pharyngeal jaws, opercle, and the buccal cavity (Burruss et al., 2016, 2018; Carroll et al., 2004; Hellig et al., 2010; Ronco et al., 2019; Rösch et al., 2013; Wilson, et al., 2013; Wilson, et al., 2013). These structural components have been identified as ecologically relevant and likely adaptive traits toward feeding mode, habitat, and prey items in fish radiations (Burruss et al., 2016, 2018; Carlig et al., 2018; Carroll et al., 2004; Cook, 1996; Hellig et al., 2010; Hulseley et al., 2006; Wilson, Colombo, et al., 2013; Wilson et al., 2015; Wilson, Furrer, et al., 2013). For instance, previous studies on cichlids, sticklebacks, and catfishes have shown that the shape and size of the opercle can be highly correlated with lifestyle and feeding mode (Stange et al., 2016; Wilson, Colombo, et al., 2013; Wilson et al., 2015; Wilson, Furrer, et al., 2013). The opercle pump helps to create a pressure gradient at the mouth opening and a current across the gills supporting the respiratory system (Kimmel et al., 2012; Wilson, Colombo, et al., 2013; Wilson et al., 2015; Wilson, Furrer, et al., 2013). A large opercle is beneficial for suction feeding performance and respiration performance of benthic living fishes; these typically live at stationary bottom waters and are usually less mobile (Kimmel et al., 2012; Wilson, Colombo, et al., 2013; Wilson et al., 2015; Wilson, Furrer, et al., 2013). The shape and dentition of the pharyngeal jaw has been shown to be strongly adapted to different prey types in several cichlids and sailfin silversides (Burruss, 2016; Burruss et al., 2016, 2018; Hellig et al., 2010; Pfaender et al., 2010). Species feeding on hard-shelled prey tend to have a sturdy pharyngeal jaw with enlarged bones and teeth adapted to crushing (Burruss, 2016; Burruss et al., 2016, 2018; Grubich, 2003; Hulseley et al., 2006; Wainwright, 2005), while fish-feeding species typically have elongated and slender pharyngeal jawbones with few, large teeth adapted for grasping (Burruss, 2016; Burruss et al., 2016, 2018; Hellig et al., 2010; Pfaender et al., 2010). Previous studies on nototheniids, centrarchids, and cottid fishes have shown that buccal cavity size can differ according to prey size and mobility (Carlig et al., 2018; Carroll et al., 2004; Cook, 1996). The buccal cavity is crucial for generating a suction pressure that draws prey items through the mouth opening, and its diameter limits the maximum prey size (Carlig et al., 2018; Carroll et al., 2004; Cook, 1996; Mihalitsis & Bellwood, 2017). A large buccal cavity is advantageous for suction feeders feeding on elusive prey because it can produce a higher pressure gradient, which is effective over distance. Although these adaptive patterns have been identified in

several fish radiations, they have mainly been investigated on an interspecific level. However, in radiations where intersexual niche differentiation is documented, these patterns might also be detectable on an intersexual level.

“Roundfin” *Telmatherina* are a small monophyletic group within the radiation of sailfin silversides and are endemic to ancient Lake Matano located in the central highlands of Sulawesi (Figure 1) (Herder, Nolte, et al., 2006; Herder et al., 2006, 2008; von Rintelen et al., 2012). Roundfins consist of three morphospecies, *Telmatherina antoniae* “small”, *Telmatherina antoniae* “large”, and *Telmatherina prognatha* (Kottelat, 1991). According to distance-based divergence estimates and molecular clock analyses, the initial divergence of these morphospecies occurred around 1 My (Stelbrink et al., 2014). However, AFLP genotyping revealed that reproductive isolation among the three morphospecies is substantial but incomplete (Herder, Nolte, et al., 2006; Herder Pfaender & Schliewen, 2008; Herder & Schliewen, 2010; Herder, Schwarzer, et al., 2006). Roundfins show significant interspecific differences in body and head shape, and a pronounced sexual dimorphism (Pfaender et al., 2011; Wasiljew et al., 2020). All three morphospecies occupy different microhabitats and specific trophic niches (Figure 1) (Herder et al., 2008; Herder & Schliewen, 2010; Pfaender et al., 2011): *T. antoniae* “small” is a pelagic, predominantly planktivorous suction feeder; *T. antoniae* “large” is a predominantly benthic, mollusk-eating suction feeder; and *T. prognatha* is a semipelagic, mainly fish eating ram feeder. Roundfin *Telmatherina* were also the first case where intersexual niche differentiation was demonstrated in an adaptive fish radiation (Pfaender et al., 2011). Two of the three morphospecies (*T. antoniae* “large” and “small”) show intersexual niche differentiation in trophic ecology, while *T. prognatha* does not (Pfaender et al., 2011). Male *T. antoniae* “small” take a significantly higher portion of terrestrial insects compared with females, which are more specialized on copepods. Male *T. antoniae* “large” consume a higher percentage of mollusks than females, which take a higher amount of terrestrial insects (Figure 1) (Pfaender et al., 2011). Other dietary components are rather negligible in these two morphospecies (Pfaender et al., 2011). However, these intersexual niche differences have not been linked to morphological structures relevant for prey processing and habitat usage so far.

Here, we test the hypothesis that multiple morphological structures are affected by intersexual niche differentiation in roundfin *Telmatherina*. Variation in three structural components—the opercle, the pharyngeal jaw, and the buccal cavity—which are directly linked to prey capture, prey processing, and habitat usage in fishes, was studied with μ -CT Imaging. This technique enables one to investigate particularly small-scaled variation of internal structures with great detail and precision without damaging the samples (Adams et al., 2004; Kaliontzopoulou, 2011; Wake, 2012; Wasiljew et al., 2020). Interspecific and intersexual variation was analyzed using different three-dimensional morphometric approaches ranging from classical measurements of distances to landmark-free geometric, morphometric analyses. We hypothesized that the opercle, the pharyngeal jaw, and the buccal cavity are adaptive in roundfins,

with specific adaptations to resource use in the respective species and sexes. Further, we predicted that the degree of intersexual variation should coincide with the degree of intersexual niche differentiation in each morphospecies.

2 | MATERIALS AND METHODS

2.1 | Material and μ -CT imaging

The present study was based on formalin-fixed roundfin *Telmatherina* specimens that were available from collection material. These were obtained in the dry season of 2002 from three locations around Lake Matano's shoreline, using gill nets (Figure 1). Since the specimens used in this study were gathered from museum collection material, no living animals were sampled, killed, harmed, or treated in any other way for this paper.

The skulls of 13 specimens of each morphospecies *T. antoniae* “small”, *T. antoniae* “large”, and *T. prognatha* were used for 3D μ -CT analyses. μ -CT scanning was performed with Skyscan 1272 and Skyscan 1173 scanners (Bruker). All specimens were scanned in 70% ethanol. Five male and five female specimens per species were stained with 0.3% phosphotungstic acid (PTA) in advance. Five male and five female specimens per species were scanned without any prior staining. The resolution ranged between 11 μ m and 23 μ m depending on the size of the specimen. Selected rotation steps varied between 0.2, 0.3, and 0.4 degrees over 180°. The chosen voltage ranged between 60 kV and 100 kV and the current between 80 μ A and 166 μ A. Detailed scanner settings for each individual can be viewed in Dryad. The projections were reconstructed with NRecon ver. 1.7.1.0 (Bruker). Data size was then reduced with the software Dataviewer ver. 1.5.2.4 by Bruker and ImageJ ver. 1.51f by NIH (Schindelin et al., 2015). Segmentation and volume rendering of the resulting 3D models were accomplished with the software packages Amira ver. 6.5.0 by Thermo Fisher Scientific (Stalling et al., 2005) and VG Studio 3.2 by Volume Graphics. Surface rendering was performed with the software packages Checkpoint ver. 17.04.21 (Stratovan Corporation) and Amira ver. 6.5.0 by Thermo Fisher Scientific (Stalling et al., 2005). Final plates were arranged with Adobe Photoshop CS6 and Adobe Illustrator CS6.

2.2 | Classical and geometric morphometrics

To identify variation in the opercle bone and the pharyngeal jaw, linear morphometric measurements and geometric morphometric analyses were conducted based on surface-rendered 3D models created by the software Checkpoint ver. 17.04.21 (Stratovan Corporation) out of μ -CT tiff image stacks. The following traits of the cranial skeleton were quantified by linear measurements: skull length, left opercle height, left opercle length, left opercle circumference, left opercle surface area, lower right pharyngeal jaw length, lower right pharyngeal jaw width, lower right pharyngeal jaw height,

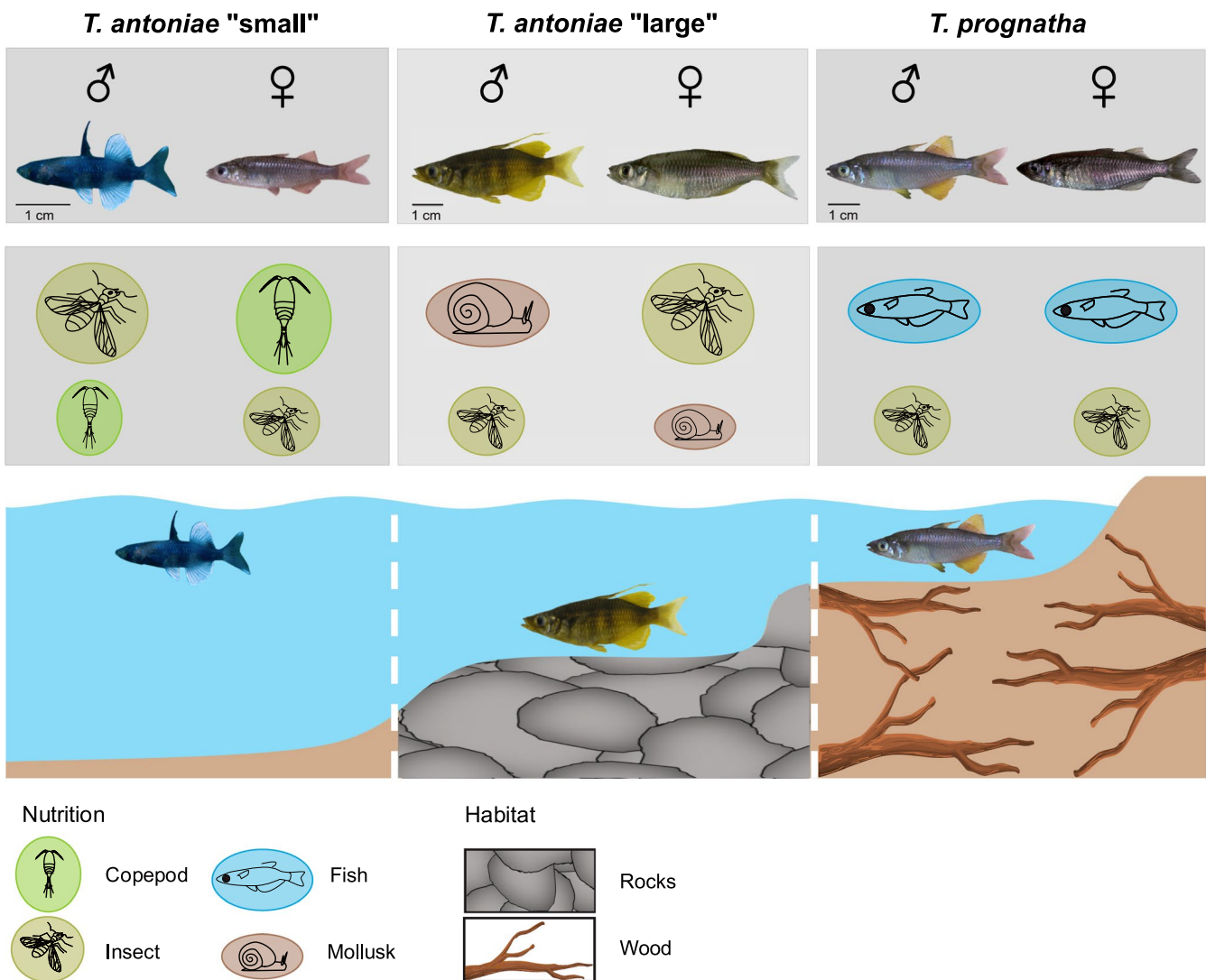
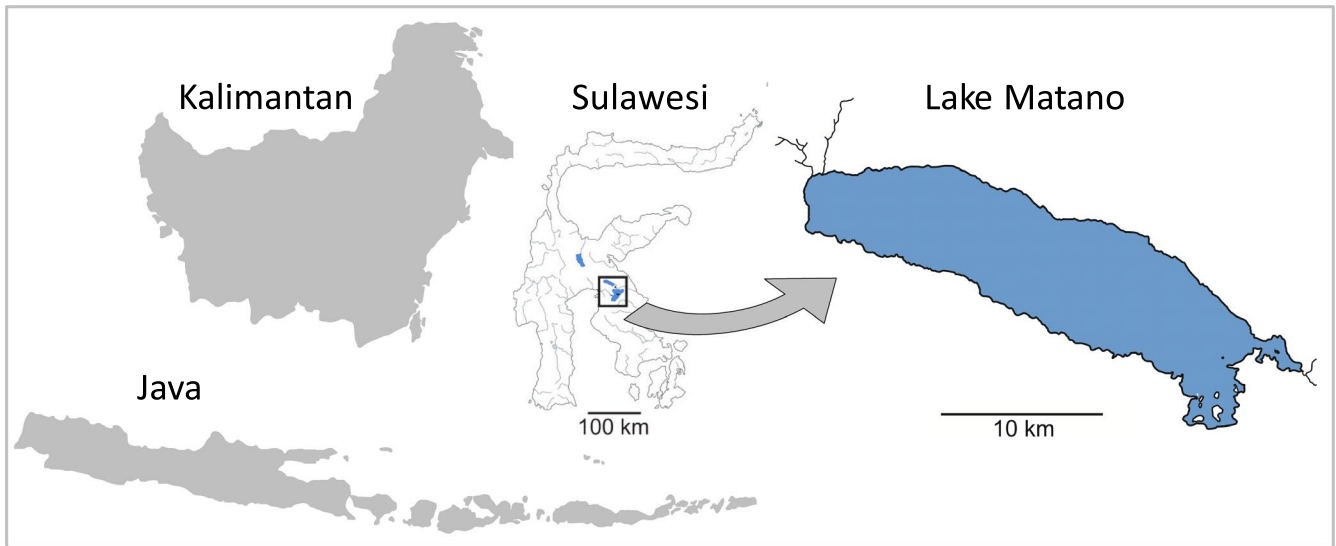


FIGURE 1 Indonesia, Sulawesi, and Lake Matano with the three endemic roundfin morphospecies *T. antoniae* "small", *T. antoniae* "large", and *T. prognatha*. Adult, reproducing males and females are pictured with key aspects of their ecological differentiation. Nutrition size corresponds to the respective relevance in diet composition. Map by T. von Rintelen, modified (with permission). This figure has been designed using resources from Freepik.com

and lower right pharyngeal jaw circumference. The number of teeth on the right lower pharyngeal jaw was counted. All measurements were carried out with the software Checkpoint.

In order to test for interspecific and intersexual shape differences, 14 landmarks were placed at homologous points on the pharyngeal jaws of the 30 unstained μ -CT scanned specimens (Figure 2a). The outline shape and circumference of the left opercle (Figure 2b) and the pharyngeal jaw were analyzed with 80 semilandmarks. Patches were used to measure the surface area of the opercle in order to quantify its overall size between species and sexes.

2.3 | Buccal cavity measurements

All classical morphometric measurements and geometric morphometric analyses of the buccal cavity were based on surface-rendered 3D models created by Amira ver. 6.5.0 by Thermo Fisher Scientific (Stalling et al., 2005) out of μ -CT tiff image stacks. In order to quantify interspecific and intersexual variation in buccal cavity size and shape, the 30 stained specimens were used for creating volume-rendered models of the cranial region with the software Amira. Surface-rendered models of the buccal cavity were created with the help of the semiautomatic segmentation tool of Amira (Figure 2c,d). Background artifacts were removed by applying the “remove islands” and “fill holes” options of Amira on the segmented 3D model. The length, width, height, and volume of the buccal cavity were measured for every prior-stained specimen. Two female specimens of *T. prognatha* were removed from the analysis because they showed deformations of the buccal cavity due to a slightly opened mouth.

Due to the rather featureless structure of the buccal cavity, surface scans were used instead of landmarks to describe its shape. A landmark-free shape analysis of the buccal cavity was performed by the Generalized Procrustes Surface Analysis (GPSA) software package in Java executable (version 20200722 provided by B. J. Pomidor upon personal request) (Pomidor et al., 2016; Slice, 2013). Surface renders were superimposed through iterative closest point (ICP) algorithm. After the superimposition, the homologous point coordinates were subjected to dimension reduction and the principal axis scores were calculated for the further analysis (Pomidor et al., 2016).

2.4 | Statistical analyses

Bivariate linear models were performed for the absolute measurements of the opercle, pharyngeal jaw, and buccal cavity in order to control for size in each trait. The absolute measurements of the opercle and the buccal cavity were regressed with skull length. The absolute measurements of the pharyngeal jaw were regressed with overall pharyngeal jaw width. The absolute number of pharyngeal teeth was regressed with pharyngeal jaw circumference. In order to test for significant differences between species and sexes, the resulting residuals of each bivariate linear model were used to perform one-way ANOVAs with Tukey's pairwise tests in the software PAST ver. 3.22 (Hammer et al., 2001). Species and sexes were tested simultaneously, resulting in six groups per model and trait.

The shape data of the opercle and pharyngeal jaw were analyzed with Procrustes superimposition followed by an elliptic Fourier analysis (EFA) respectively principal component analysis (PCA) and

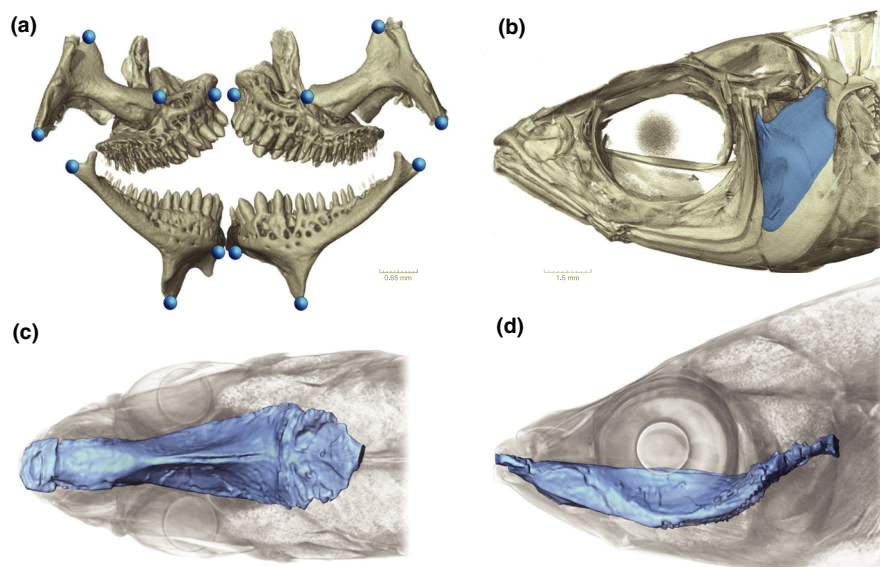


FIGURE 2 (a–d) Three analyzed structural components of roundfin *Telmatherina*. (a) Surface-rendered 3D model of the pharyngeal jaw of *T. prognatha*. Locations of 14 homologous landmarks placed on the pharyngeal jaws of μ -computed tomography-scanned specimens. (b) Surface-rendered 3D model of the head of *T. prognatha*. Location of the opercle (colored in blue) of roundfin specimens ($n = 10$). The outline was used for quantifying the circumference and the shape of the opercle. (c, d) Surface-rendered 3D model of the buccal cavity (in blue) is shown within a volume render (in gray) of a previously stained *T. antoniae* “small”. (c) Dorsal view; (d) lateral view. The surface render was used for the quantification of size and volume of the buccal cavity

thin-plate spline interpolation performed in the software PAST ver. 3.22 (Hammer et al., 2001) and R ver. 3.5.1 (Ihaka & Gentleman, 1996). The resulting scores of the PCA and EFA in the three first axes were used to perform a MANOVA and a Tukey's pairwise test with PAST in order to test for significant differences between species and in the dataset. In order to test for intersexual shape differences, this procedure was repeated for each species dataset individually, instead of a pooled-species dataset. This was done to avoid the disproportionate influence of more variable species on the principal axes over the less variable ones.

3 | RESULTS

The skulls of *T. antoniae* "large" (mean: 18.18 mm) and *T. prognatha* (mean: 19.49 mm) were significantly larger than the skulls of *T. antoniae* "small" (mean: 10.58 mm; $Q = 20.83$; $p < .01$), meeting the documented size ranges by Herder et al. (2008). Intersexual differences in skull length were only detectable in *T. antoniae* "large" ($Q = 5.91$; $p < .01$). Male *T. antoniae* "large" (mean: 19.62 mm) had significantly larger skulls than females (mean: 16.72 mm).

Size variances for different structures and parameters were unequally portioned between species and sexes. All the absolute and the majority of relative size measurements were more divergent interspecifically than intersexually. The exceptions were the relative height and surface area of the opercle, the relative circumference of the pharyngeal jaw, and the relative height of the buccal cavity. In these parameters, intersexual variance exceeded the variance among species. Detailed size variance ratios are provided in Dryad. All analyzed structures differed significantly in at least one size parameter between morphospecies. Interspecific size variation was most distinct for the pharyngeal jaw, while intersexual size variation was most distinct for the opercle. Shape variation was most distinct for the buccal cavity among species and sexes. Intersexual size differences were significant in both *T. antoniae* morphospecies but not in *T. prognatha*. Intersexual differences in shape were present in all three morphospecies.

3.1 | Opercle

Relative opercle size differed substantially between species. Following the predictions based on the species-specific niches, it was highest in *T. antoniae* "large", followed by *T. antoniae* "small", and *T. prognatha* (Figure 3a,b; $Q = 5.19$; $p < .05$). Absolute opercle size was significantly lower in *T. antoniae* "small" in comparison with *T. antoniae* "large" and *T. prognatha* (Figure 4a,b; $Q = 17.62$; $p < .01$). No significant differences in opercle size were identified between the latter two morphospecies. The shown values of relative length and circumference also reflect the patterns for the remaining not visualized parameters. In contrast, the morphospaces of the opercle shape EFA showed a large overlap of all three morphospecies without any significant differences in shape within the first three axes

(Figure 5a). However, the opercle outline of *T. antoniae* "small" could be distinguished from the other morphospecies by its round shape ($F = 4.62$; $p < .05$). *Telmatherina antoniae* "large" and *T. prognatha* shared a rather triangular-shaped opercle (Figure 6).

This discrepancy between size and shape was also detected among sexes. Male and female *T. antoniae* "small" and *T. prognatha* did not differ in relative or absolute opercle size (Figures 3a,b and 4a,b; $Q = 1.57$; $p > .1$). Consistent with the higher percentage of mollusks in their diet (Figure 1), males of *T. antoniae* "large" had significantly higher relative and absolute values than females (Figures 3a,b and 4a,b; $Q = 7.64$; $p < .05$). In contrast, intersexual variation in shape was not substantial ($F = 0.66$; $p > .05$). The morphospaces of the EFA comprising the sexes distinctly overlapped within all morphospecies (Figure 5a).

3.2 | Pharyngeal jaw

Relative pharyngeal jaw size differed significantly in height, width, and relative number of teeth ($Q = 19.25$; $p < .05$) but not in length or circumference between species (Figure 3c,d). The shown values of relative width and number of teeth were the most discriminative parameters. In line with the proportion of mollusks in its diet (Figure 1), *T. antoniae* "large" was characterized by a sturdy pharyngeal jaw with a high relative number of teeth. As predicted for a piscivorous predator (Figure 1), *T. prognatha* had a gracile pharyngeal jaw with a low relative number of teeth. Planktivorous *T. antoniae* "small" was intermediate in size, shape, and relative number of teeth (Figures 3c,d and 7). Absolute pharyngeal jaw size was lowest in *T. antoniae* "small," highest in *T. antoniae* "large," and intermediate in *T. prognatha* (Figure 4c,d; $Q = 23.30$; $p < .01$). Pharyngeal jaw shape differed substantially between morphospecies and was distinctly separated by the morphospaces of the PCA in the first three axes (Figure 5b; $F = 10.63$; $p < .05$).

Intersexual variation was absent in relative pharyngeal jaw size in all morphospecies (Figure 3c) but present in the relative number of teeth within *T. antoniae* "large" (Figure 3d; $Q = 7.86$; $p < .01$). Sexes of *T. antoniae* "small" and *T. prognatha* did not differ in absolute pharyngeal jaw size, while males of *T. antoniae* "large" had significantly larger pharyngeal jaws than females (Figure 4c; $Q = 6.22$; $p < .05$), consistent with the higher proportion of mollusks in their diet (Figure 1). Sexual dimorphism in pharyngeal jaw shape was present within all three morphospecies. The morphospaces of male and female specimens were distinctly separated (Figure 5b), but shape variation was only significant in *T. antoniae* "large" ($F = 15.45$; $p < .05$).

3.3 | Buccal cavity

Morphospecies differed in relative buccal cavity volume, but not in any of the relative linear measurements (Figure 3e,f). In line

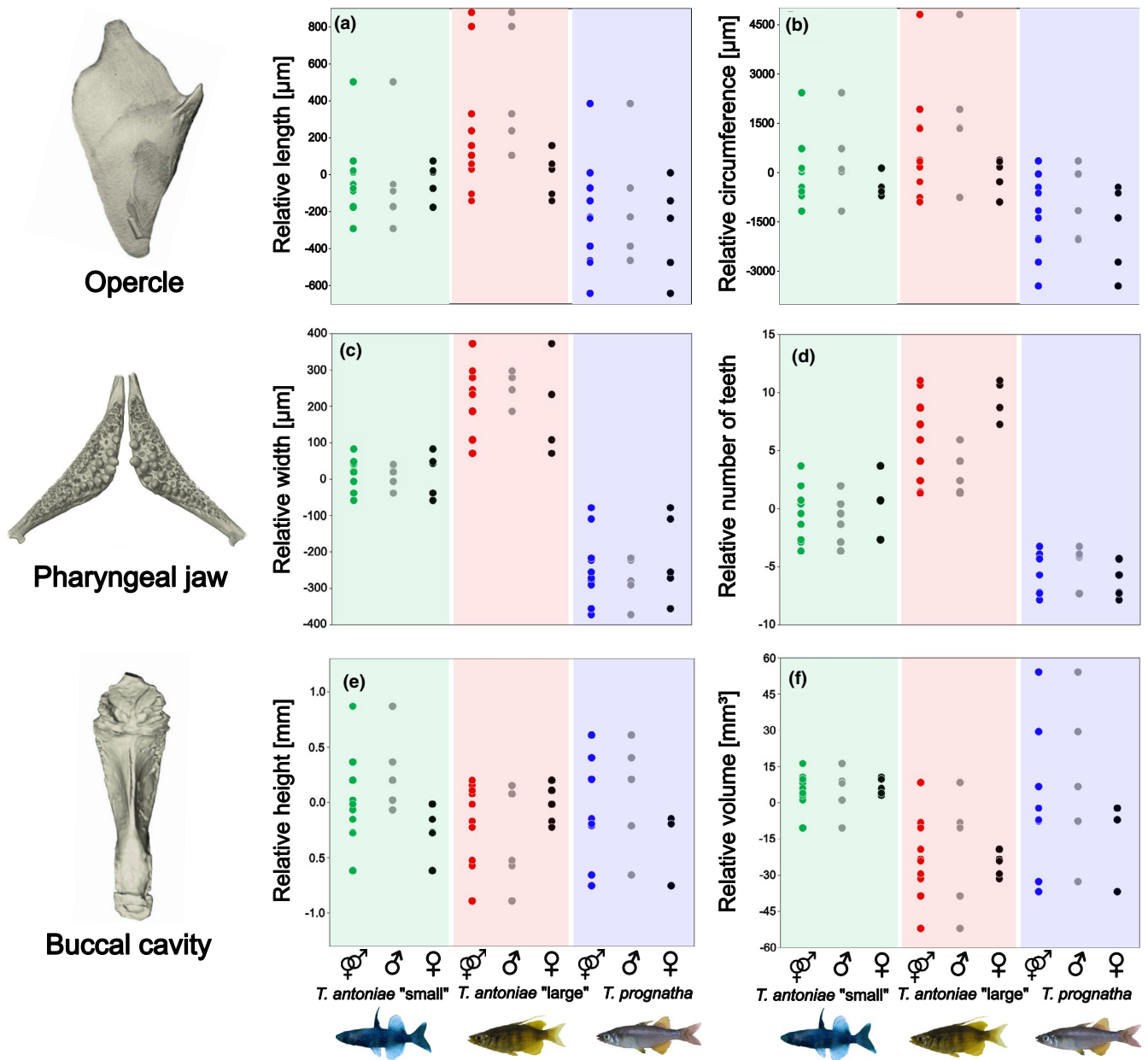


FIGURE 3 (a–f) Interspecific and intersexual variation in relative (a) opercle length, (b) opercle circumference, (c) pharyngeal jaw width, (d) number of pharyngeal teeth, (e) buccal cavity height, and (f) buccal cavity volume of roundfin *Telmatherina* (species $n = 10$; sex $n = 5$). Dots visualize single individuals. Morphospecies and sexes are color-coded: *T. antoniae* “small” combined—green; *T. antoniae* “large” combined—red; *T. prognatha* combined—blue; male—gray; and female—black. Two female specimens of *T. prognatha* were removed from the buccal cavity analysis because they showed deformations due to a slightly opened mouth

with the predictions according to feeding mode and diet composition (Figure 1), *T. antoniae* “small” had the largest, *T. antoniae* “large” the smallest, and *T. prognatha* an intermediate relative buccal cavity volume (Figure 3f; $Q = 4.66$; $p < .05$). The visualized values of relative height and volume were the most discriminative parameters. Absolute buccal cavity size was lowest in *T. antoniae* “small”, highest in *T. prognatha*, and intermediate in *T. antoniae* “large” (Figure 4e,f; $Q = 22.25$; $p < .01$). Buccal cavity shape differed substantially between morphospecies (Figure 8), while the morphospaces of the PCA were significantly separated in the first three axes (Figure 5c; $F = 14.54$; $p < .01$).

Intersexual variation in relative and absolute buccal cavity size was significant within *T. antoniae* “small” and *T. antoniae* “large”. Compared with females, relative buccal cavity height was higher in male *T. antoniae* “small”, consistent with the higher percentage of copepods in their diet (Figure 1), but lower in male *T. antoniae* “large” (Figure 3e; $Q = 3.98$; $p < .05$), in line with the higher proportion of mollusks in their diet (Figure 1). Absolute size was higher in male *T. antoniae* “small” and *T. antoniae* “large” than in females (Figure 4e,f; $Q = 5.01$; $p < .05$). Sexual dimorphism in shape was most distinct for the buccal cavity. The morphospaces of the PCA comprising the sexes were distinctly separated within

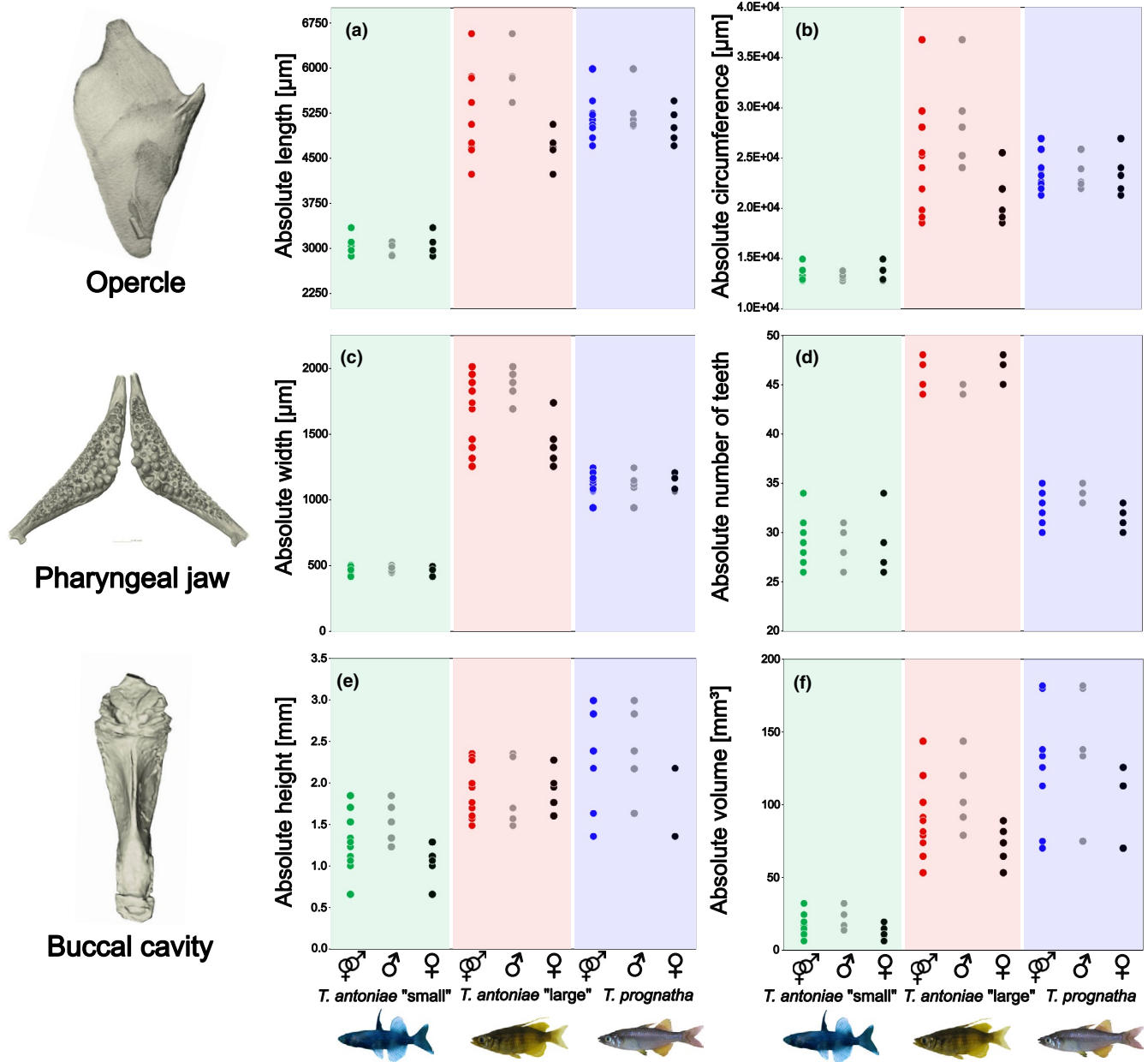


FIGURE 4 (a–f) Interspecific and intersexual variation in absolute (a) opercle length, (b) opercle circumference, (c) pharyngeal jaw width, (d) number of pharyngeal teeth, (e) buccal cavity height, and (f) buccal cavity volume of roundfin *Telmatherina* (species $n = 10$; sex $n = 5$). Dots visualize single individuals. Morphospecies and sexes are color-coded: *T. antoniae* "small" combined—green; *T. antoniae* "large" combined—red; *T. prognatha* combined—blue; male—gray; and female—black. Two female specimens of *T. prognatha* were removed from the buccal cavity analysis because they showed deformations due to a slightly opened mouth

all morphospecies (Figure 5c), but shape variation was only significant in *T. antoniae* "small" and "large" ($F = 13.95$; $p < .05$).

4 | DISCUSSION

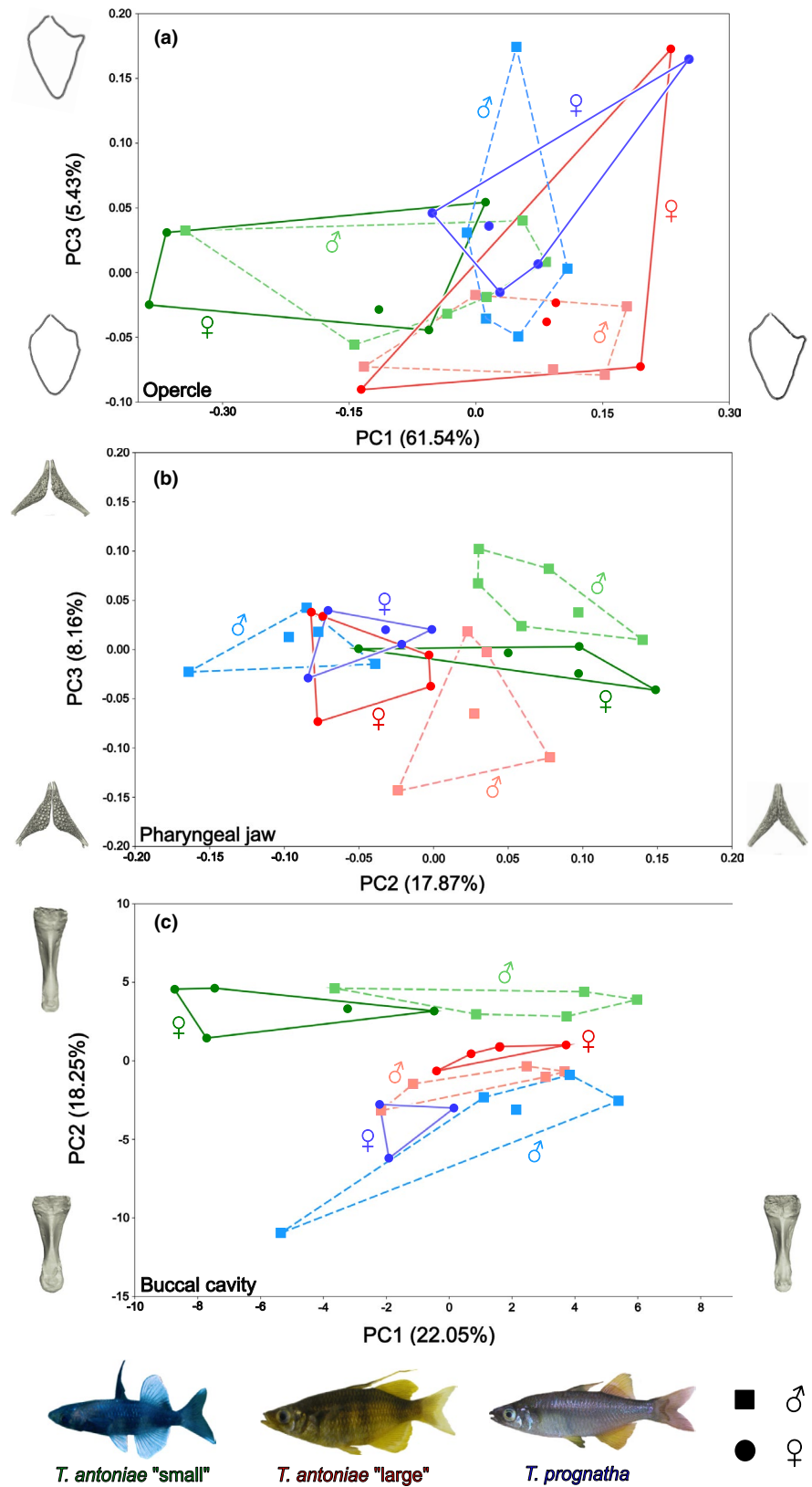
4.1 | Ecologically relevant traits in roundfins

The present study revealed significant differences between roundfin sailfin silverside morphospecies in three structural components of the head region. All of these are ecologically relevant and are

considered adaptive in other fish radiations (Burruss et al., 2016, 2018; Carlig et al., 2018; Carroll et al., 2004; Cook, 1996; Hellig et al., 2010; Hulsey et al., 2006; Wilson, Colombo, et al., 2013; Wilson et al., 2015; Wilson, Furrer, et al., 2013).

As expected for predominantly benthic, mollusk-eating, suction-feeding fishes (Burruss et al., 2016, 2018; Cook, 1996; Muller et al., 1982; Wainwright, 2005; Wilson, Colombo, et al., 2013; Wilson, Furrer, et al., 2013), *T. antoniae* "large" shows a large, triangularly shaped opercle (Figures 3a,b, 5a, and 6), a wide, sturdy-shaped pharyngeal jaw with a high relative number of teeth (Figures 3c,d, 5b and 7), and a small buccal cavity (Figures 3e,f

FIGURE 5 (a–c) Interspecific and intersexual variation in shape of the (a) opercle, (b) pharyngeal jaw, and (c) buccal cavity of roundfin *Telmatherina* with visualizations of the mean shapes for each species (species $n = 10$; sex $n = 5$). (a) Elliptic Fourier analysis plot of the opercle semilandmark data set with point clusters of species and sexes. (b) Principal component analysis plot of the pharyngeal jaw landmark data set with point clusters of species and sexes. (c) Principal component analysis plot of the buccal cavity shapes data set with point clusters of species and sexes. Morphospecies are color-coded, sexes are symbol-coded (*T. antoniae* “small”—green; *T. antoniae* “large”—red; *T. prognatha*—blue; male—square; female—dot). Two female specimens of *T. prognatha* were removed from the buccal cavity analysis because they showed deformations due to a slightly opened mouth



and 5c). The semipelagic, mainly fish eating ram feeder *T. prognatha* is characterized by having a small, triangularly shaped opercle (Figures 3a,b, 5a and 6), a narrow, gracile pharyngeal jaw with a low relative number of teeth (Figures 3c,d, 5b and 7), and

an intermediate-sized buccal cavity (Figures 3e,f and 4c). These findings are characteristic for pelagic, ram feeding predators (Burruss et al., 2016, 2018; Carroll et al., 2004; Hellig et al., 2010; Wilson, Colombo, et al., 2013; Wilson et al., 2015; Wilson,

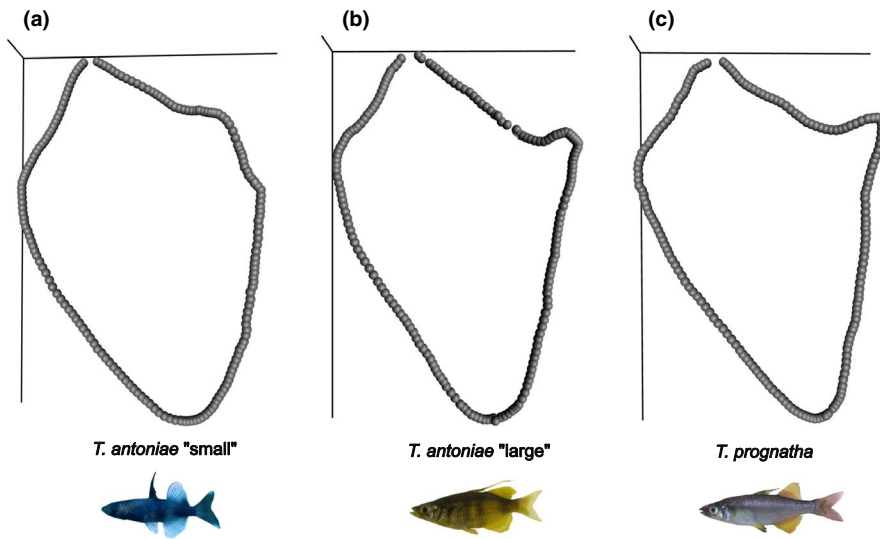


FIGURE 6 (a–c) Mean outline shape of the opercle of (a) *T. antoniae* "small"; (b) *T. antoniae* "large"; and (c) *T. prognatha*

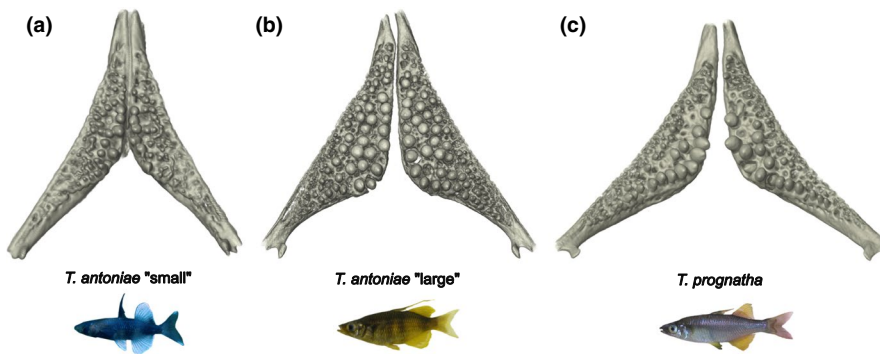


FIGURE 7 (a–c) Pharyngeal jaws in dorsal view of (a) *T. antoniae* "small"; (b) *T. antoniae* "large"; and (c) *T. prognatha*

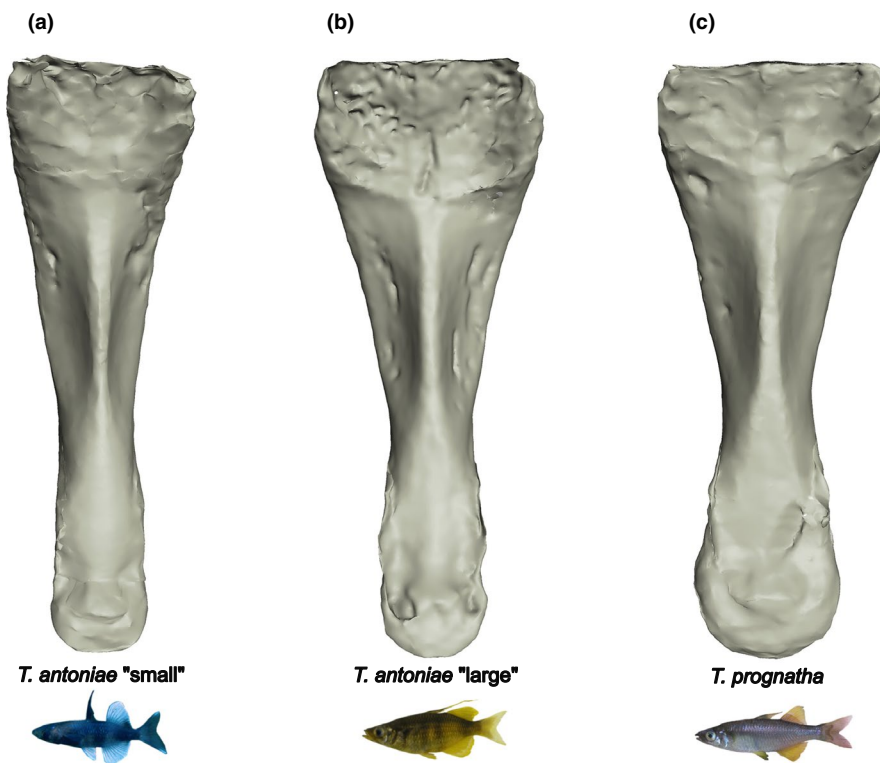


FIGURE 8 (a–c) Mean buccal cavities in dorsal view of (a) *T. antoniae* "small"; (b) *T. antoniae* "large"; and (c) *T. prognatha*

Furrer, et al., 2013). *Telmatherina antoniae* “small” shows characteristic patterns for a suction-feeding fish with a pelagic lifestyle and a planktivorous diet (Cook, 1996; Hulsey et al., 2006; Pfaender et al., 2010; Pfaender et al., 2011; Wilson et al., 2015) with its intermediate-sized and roundly shaped opercle (Figures 3a,b, 5a and 6), gracile pharyngeal jaw (Figures 3c,d, 5b and 7), and relatively large buccal cavity compared with *T. antoniae* “large” and *T. prognatha* (Figures 3e,f and 5c).

4.2 | Intersexual trait variation meets predictions derived from ecology

Sexual dimorphism may affect feeding ecology, and intersexual niche differentiation might minimize intraspecific competition in radiations (De Lisle, 2019; Pfaender et al., 2011; Roy et al., 2013). Intersexual variation in ecological adaptive traits has been reported in various animal groups (Butler, 2007; Cooper et al., 2011; De Lisle & Rowe, 2017; Maan & Seehausen, 2011), but most studies focus on size or color dimorphism, which can be induced by sexual selection rather than ecological divergent selection (De Lisle, 2019; Hedrick & Temeles, 1989; Herler et al., 2010; Tsuboi et al., 2012). In order to identify ecological-based intersexual divergent selection, it is of major interest to investigate ecologically relevant traits in well-documented cases of intersexual niche differentiation (Bolnick & Doebeli, 2003; De Lisle, 2019; De Lisle & Rowe, 2017; Ronco et al., 2019).

Among Lake Matano's roundfins, sexual dimorphism in ecologically relevant traits is most pronounced in *T. antoniae* “large”. Males have relatively larger opercles (Figure 3a,b), a lower number of teeth, more sturdy pharyngeal jaws (Figures 3d and 5b), and flatter buccal cavities than females (Figures 3e,f and 5c). This coincides with sex-specific trophic profiles: Males feed more pronounced on mollusks than females, while females feed to a higher extent on insects than males (Pfaender et al., 2011). The conspicuously sturdy pharyngeal jaws of males with a low number of large teeth (Figures 3c,d and 5b) are considered advantageous for dealing with hard-shelled prey (Burruss, 2016; Burruss et al., 2016, 2018; Hellig et al., 2010), while a large buccal cavity, as it occurs in female *T. antoniae* “large”, is considered advantageous for catching elusive prey via a suction feeding mode (Carroll et al., 2004). The relatively larger opercle of male *T. antoniae* “large” is discussed to be an adaptation to benthic suction feeding (Wilson, Colombo, et al., 2013; Wilson et al., 2015; Wilson, Furrer, et al., 2013), enhancing active ventilation of the gills, which is considered advantageous for reduced motility in static waters (Kimmel et al., 2008; Wilson, Colombo, et al., 2013; Wilson, Furrer, et al., 2013). This fits the more benthic lifestyle of male *T. antoniae* “large”, compared with female conspecifics (Pfaender et al., 2011), and matches findings of an adaptive sexual dimorphism in sticklebacks, which likewise differ in the use of both benthic versus limnetic habitats and opercle traits (Albert et al., 2008; McGee & Wainwright, 2013).

In contrast to *T. antoniae* “large”, male and female *T. antoniae* “small” differ in size and shape of the buccal cavity (Figures 3e and 5c), but not in opercle or pharyngeal jaw traits (Figures 3a-d and 5a,b). Males have significantly higher and deeper buccal cavities than females (Figures 3e and 5c). Again, these findings match the sex-specific trophic niches: Male *T. antoniae* “small” feed to a larger extent on insects than females, which are predominantly zooplanktivores (Pfaender et al., 2011). Both prey types differ significantly in size (Pfaender et al., 2011; Wainwright & Bellwood, 2002), which matches the differences detected in buccal cavity size and shape (Figures 3e and 5c). The diameter of the buccal cavity generally limits both the maximum prey size and the suction pressure (Carlig et al., 2018; Carroll et al., 2004; Cook, 1996; Mihalitsis & Bellwood, 2017). Therefore, large buccal cavities are advantageous for fish species feeding on large prey items (Carroll et al., 2004; Mihalitsis & Bellwood, 2017). Male *T. antoniae* “small” might benefit from a larger buccal cavity compared with females since they take a higher percentage of insects, which are substantially larger than zooplankton (Pavlov & Kasumyan, 2002; Pfaender et al., 2010, 2011; Wainwright & Bellwood, 2002). The studies of Herler et al. (2010) and Ronco et al. (2019) reported sexual dimorphism in the buccal cavity of mouth-brooding cichlid fishes. However, they linked intersexual variation to parental care but not to different trophic niches. The present case is hence, to the best of our knowledge, the first study documenting sexual dimorphism in buccal cavity size and shape in a non-mouth-brooding fish radiation. This finding might support the ecological relevance of the buccal cavity in fish radiations. The absence of intersexual variation in opercle and pharyngeal jaw traits (Figures 3a-d and 5a,b) may be explained by the generally similar requirements for taking insects and zooplankton (Pavlov & Kasumyan, 2002; Pfaender et al., 2010; Wainwright & Bellwood, 2002): Both prey types occur in the pelagic zone and share a similar texture (Pfaender et al., 2010, 2011; Wainwright & Bellwood, 2002). Thus, this trophic niche partitioning most likely does not affect intersexual variation in the opercle and pharyngeal jaw, which are linked to habitat usage and prey processing (Burruss, 2016; Burruss et al., 2016, 2018; Hellig et al., 2010; Kimmel et al., 2008; Wilson, Colombo, et al., 2013; Wilson et al., 2015; Wilson, Furrer, et al., 2013).

Conspicuously, the present study did not reveal indications for morphological differentiation among male and female *T. prognatha* (Figures 3 and 5). However, morphology also meets predictions derived from trophic ecology in this species (Pfaender et al., 2011). The absence of intersexual variation appears plausible since both sexes share similar trophic and habitat niches (Pfaender et al., 2011).

4.3 | Does intersexual niche differentiation result in ecological character displacement?

The degree of sexual dimorphism detected here largely meets predictions derived from niche segregation in male and female

roundfins (Figures 1, 3, and 5). It ranges from the absence of differences in ecologically relevant traits in *T. prognatha*, the species lacking intersexual niche differentiation, to *T. antoniae* "large", where both intersexual trophic niches and trait segregation are most pronounced (Pfaender et al., 2011).

Sexual dimorphism can follow ecological-based divergent selection or sexual selection (Hedrick & Temeles, 1989; Herler et al., 2010). Sexual selection can either affect display for potential mates (intersexual selection) or competitive advantages over other males (intrasexual selection) (Hedrick & Temeles, 1989; Herler et al., 2010; Tsuboi et al., 2012). Alternatively, intersexual variation can evolve by ecological selection pressure acting differentially on both sexes and thus favoring dimorphic niches (De Lisle, 2019; Hedrick & Temeles, 1989; Herler et al., 2010). An ecological cause for intersexual variation appears more plausible, if it occurs in traits likely affecting resource exploitation (Bolnick & Doebeli, 2003). The present analyses suggest that all three analyzed structures are likely ecologically adaptive in roundfins. Likewise, sexual dimorphism in the internal structures pharyngeal jaw and buccal cavity has probably evolved under ecological selection pressure, since these structures are of relevance for food acquisition (buccal cavity) and processing (pharyngeal jaw). In contrast to the majority of morphological traits analyzed in roundfins so far (Herder et al., 2008; Pfaender et al., 2011), both are nonvisible and thus unlikely to serve in signaling for potential mates or competitive males (Bolnick & Doebeli, 2003; Ronco et al., 2019). Nevertheless, we cannot exclude the possibility that sexual selection affects these structures in a nonvisual way, that is, through other signaling pathways or through allometric effects of sexual size dimorphism. However, as patterns of size and shape variation in the opercle, pharyngeal jaw, and buccal cavity detected in sticklebacks, cichlids, and other fish radiations (Albert et al., 2008; Burress, 2016; Burress et al., 2016, 2018; McGee & Wainwright, 2013) are also present in roundfins, it seems plausible that the identified variation is predominantly a result of ecological-based divergent selection.

Intersexual ecological character displacement in both *antoniae* morphospecies might minimize trophic and habitat overlap (De Lisle, 2019; De Lisle & Rowe, 2017). For instance, the intersexual variation in the buccal cavity of *T. antoniae* "small" (Figures 3e and 5c) corresponds to different diet compositions between males and females (Carroll et al., 2004; Cook, 1996; Mihalitsis & Bellwood, 2017). Sexual dimorphism in all three investigated structures within *T. antoniae* "large" (Figures 3 and 5) affects both habitat and diet composition between sexes (Burress, 2016; Burress et al., 2016, 2018; Hellig et al., 2010; Stange et al., 2016). Consequently, these morphological adaptations might reduce intersexual competition for ecological resources when resources are limited (De Lisle, 2019; De Lisle & Rowe, 2017), as in the case of this ultraoligotrophic lake (Herder & Schliewen, 2010; von Rintelen et al., 2012). The absence of intersexual variation in *T. prognatha* (Figures 3 and 5), the only roundfin species without any reported intersexual niche differentiation (Pfaender et al., 2011), further supports this theory.

5 | CONCLUSIONS

We demonstrate that roundfin morphospecies and sexes differ significantly in multiple ecologically relevant traits affecting prey capture, prey processing, and habitat use. As interspecific and intersexual variations meet patterns of niche differentiation reported in roundfins (Herder et al., 2008; Pfaender et al., 2011), these differentiations are likely adaptations to different ecological niches. Since the analyzed structural components are ecologically relevant and, in the case of the pharyngeal jaw and the buccal cavity, are invisible for other individuals, it seems unlikely that sexual selection is responsible for the intersexual variation documented here (Bolnick & Doebeli, 2003; De Lisle, 2019; De Lisle & Rowe, 2017; Ronco et al., 2019). It rather appears plausible that ecological-based intersexual divergent selection is the main driver for the revealed intersexual variation in roundfins. The intersexual ecological character displacement in *T. antoniae* "small" and *T. antoniae* "large" likely minimizes trophic and habitat overlap and thus intersexual competition for ecological resources. Intersexual morphological differentiation adds to the adaptive diversity of roundfin *Telmatherina* and might play a key role in minimizing intersexual competition in emerging radiations. Further research is also needed on other systems to deepen our knowledge of the role of intersexual niche differentiation in speciation processes.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS





Benjamin D. Wasiljew: Conceptualization (equal); data curation (lead); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (equal); software (equal); visualization (equal); writing-original draft (lead); writing-review & editing (lead). **Jobst Pfaender:** Conceptualization (equal); formal analysis (equal); supervision (supporting); writing-original draft (supporting); writing-review & editing (supporting). **Benjamin Wipfler:** Methodology (equal); software (equal); supervision (supporting); visualization (equal); writing-original draft (supporting); writing-review & editing (supporting). **Mariam Gabelaia:** Formal analysis (equal); methodology (equal); software (equal); writing-original draft (supporting); writing-review & editing

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DATA AVAILABILITY STATEMENT

Sampling locations, detailed scanner settings, absolute measurements, variance ratios, and landmark coordinates are provided at Dryad digital repository (<https://doi.org/10.5061/dryad.0gb5mkm1x>). All μ -CT scans are deposited at our institute and can be requested by contacting the third author. If required, μ -CT data can be uploaded at MorphoBank.

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Functional morphology of prey capture in stream-dwelling sailfin silversides (Telmatherinidae) based on high-speed video recordings

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Abstract

Understanding how ecology shapes the evolution of morphological traits is a major goal in organismal biology. By quantifying force of motion, hypotheses on the function of fundamental tasks of animals like feeding can be tested. Ray-finned fishes use various feeding strategies, classified into three main feeding modes: suction, ram and manipulation. While manipulation feeders are usually distinct in morphology and feeding behavior, differentiation between suction and ram feeders is often fine-scaled and transitional. Previous studies have identified different feeding modes and biomechanical adaptations on interspecific and intersexual levels in lake-dwelling sailfin silversides, species of a Sulawesi freshwater radiation. Functional feeding morphology of stream-dwelling species remained in contrast unstudied. We hypothesized that different requirements of riverine habitats favor the evolution of alternative functional adaptations in stream-dwelling sailfin silversides. To test this hypothesis, we investigated feeding of two phenotypically distinct riverine species, *Telmatherina bonti* and *Marosatherina ladigesi*, and their sexes, by high-speed videos and biomechanical models. The kinematic approaches identify *T. bonti* as ram feeder and *M. ladigesi* as suction feeder. Surprisingly, the biomechanical models of the jaw apparatus provide contradicting results: only one out of three studied parameters varies between both species. Contrarily to lake-dwelling *Telmatherina*, sexes of both species do not differ in feeding biomechanics. We conclude that *T. bonti* predominantly uses ram feeding while *M. ladigesi* primarily uses suction feeding as its main hunting strategy. Feeding biomechanics of stream-dwelling sailfin silversides are less distinct compared to lake-dwelling species, likely due to different trophic ecologies or less stable ecological conditions.

Keywords Feeding biomechanics · Feeding mode · High-speed video analysis · Force transmission · Prey capture kinematics · Sulawesi

Introduction

Understanding the connection between ecology and the evolution of morphological traits is a major goal in organismal biology (Sonnefeld et al. 2014; Wainwright & Richard 1995). The quantification of velocity, force and kinematic transmission of movements can be used to test hypotheses on the function of fundamental biological tasks of animals such

as feeding, locomotion and respiration (Cooper & Westneat 2009; Westneat 1994, 2004).

The > 30.000 species of ray-finned fishes (Actinopterygii) use a great variety of feeding strategies to capture food items (Camp et al. 2015; Day et al. 2015). Prey capture modes can be assigned to three major categories: suction feeding, ram feeding and manipulation (Ferry-Graham et al. 2001a,b; Norton & Brainerd 1993; Porter & Motta 2004). Suction feeding generates a pressure gradient that sucks food items into the mouth by rapid expansion of the oral cavity (Camp et al. 2015; Day et al. 2015; Porter & Motta 2004), and is the most common feeding mode among ray-finned fishes (Camp et al. 2015; Higham et al. 2006b; Westneat & Olsen 2015). This feeding mode is supported by a small maximum gape width, a high jaw opening and a low jaw closing velocity (Day et al. 2015; Higham et al. 2017). It enables efficient feeding on mostly small, elusive or non-elusive prey like

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zooplankton, shrimps or insects (Ferry-Graham et al. 2001b; Sonnefeld et al. 2014). In contrast, ram feeding fishes capture their prey by overtaking them in forward locomotion (Ferry-Graham et al. 2001a, b; Norton & Brainerd 1993; Wainwright & Bellwood 2002). They are characterized by a large maximum gape width, a rather low jaw closing velocity and an intermediate transmission of force (Higham et al. 2017; Sonnefeld et al. 2014). Swarms of planktonic prey or single larger elusive organisms, like other fishes, are the typical prey of ram-feeding fishes (Porter & Motta 2004; Sonnefeld et al. 2014; Wainwright & Bellwood 2002). Finally, manipulation is the direct application of jaws on prey by scraping or biting (Ferry-Graham et al. 2001a, b; Norton & Brainerd 1993). Manipulation feeders typically have a small gape width, and jaws that open and close at low velocity while transmitting high biting forces (Sonnefeld et al. 2014; Wainwright & Bellwood 2002; Wainwright & Richard 1995). The main prey of manipulation feeders is either hard shelled or attached to substrate like mollusks, crustaceans, algae or sponges (Sonnefeld et al. 2014; Wainwright & Bellwood 2002).

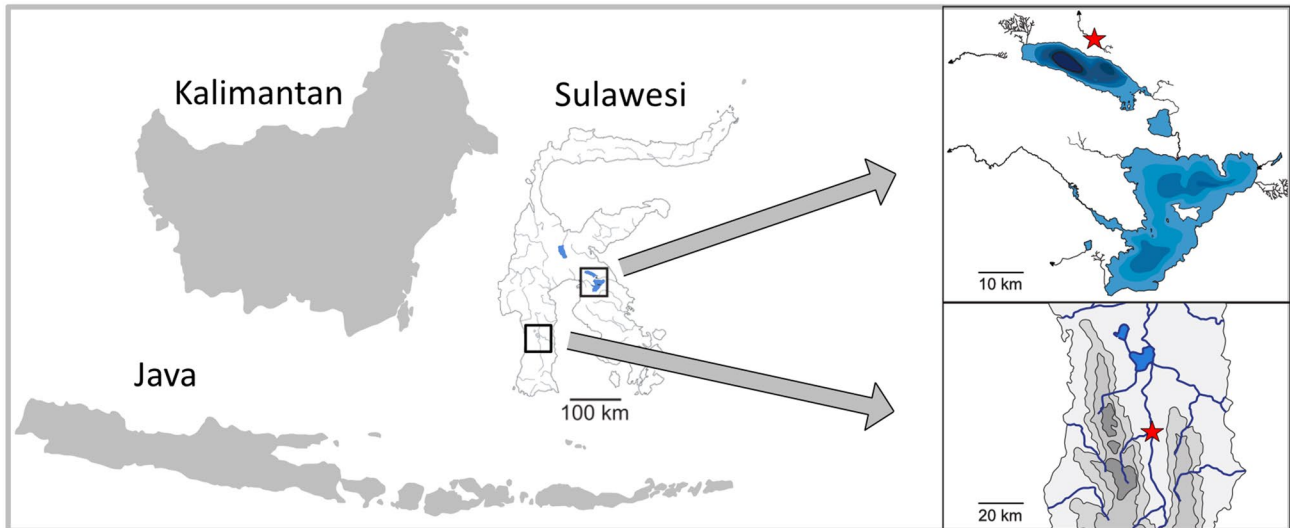
While morphology and feeding behavior of manipulation feeders is usually easily distinguishable from the other two major feeding modes because they depend on bite force, the distinction between suction and ram feeders is often more discrete and fine scaled since both feeding modes rely on speed of motion (Wainwright & Bellwood 2002; Wainwright & Richard 1995). Nevertheless, distinction between suction and ram feeding is facilitated when incorporating the extent of forward locomotion of the predator versus the acceleration of prey items through suction pressure (Wainwright & Bellwood 2002; Wainwright et al. 2001). During a specialized suction strike, the prey moves while the predator does not, contrasted by the moving predator that overtakes static prey in a pure ram strike (Norton & Brainerd 1993). However, specialized suction and ram feeding represent ends of a continuum and most fishes use a combination of both modes (Norton & Brainerd 1993; Wainwright & Bellwood 2002; Wainwright et al. 2001).

High-speed videos, i.e. videos recorded at more than 60 frames per second (fps), and biomechanical models are efficient tools for investigating the kinematics of prey capture. High-speed videos enable the precise assessment of speed and prey capture duration, and are extensively used in studies targeting the functional morphology of the feeding apparatus of fishes (e.g. Copus & Gibb 2013; Day et al. 2015; Ferry-Graham et al. 2002; Konow et al. 2013; Van Wassenbergh & De Rechter 2011). Biomechanical models, in contrast, focus on force and kinematic transmission, like those involved in opening and closing of fish jaws. Two widely used models are the simple lever system of the lower jaw and the complex four-bar linkage system of the anterior jaw (Pfaender et al. 2011; Wainwright & Richard 1995; Westneat

1990). Both models represent functional trade-offs between speed and force of jaw motion (Cooper & Westneat 2009; Wainwright & Richard 1995; Westneat 2004). These can be quantified by two kinematic ratios: the lower jaw ratio (LJR) and the maxillary kinematic transmission coefficient (MKT). High LJR and low MKT values indicate a slow but forceful motion, while small LJR and high MKT values indicate a fast but less forceful movement (Cooper & Westneat 2009; Hulsey & García de León, 2005; Hulsey & Wainwright 2002; Parnell et al. 2008; Pfaender et al. 2011; Wainwright & Richard 1995).

The Indonesian island Sulawesi harbors species flocks of sailfin silversides (Telmatherinidae) (Herder & Schliewen 2010; von Rintelen et al. 2012). These serve as a model system for investigating processes underlying adaptive divergence (Herder & Schliewen 2010; Pfaender et al. 2016; Walter et al. 2009). The study of Pfaender et al. (2011) revealed alternative feeding modes among closely related “roundfin” *Telmatherina*, a lineage endemic to ancient graben-lake Matano. Ranging from ram to suction feeding, the species show substantial biomechanical adaptations according to their respective feeding niche (Pfaender et al. 2011; Wasiljew et al. 2021). So far, two sailfin silversides have been described from Sulawesi’s rivers and streams: *Telmatherina bonti* (Weber & Beaufort 1922) and *Marosatherina ladigesi* (Ahl 1936). Both have substantially different overall body shapes (Kottelat 1990; Sterba 1987; Online Resource 1), and different feeding strategies appear plausible. The fusiform body of *T. bonti* suggests a ram feeding mode while the deeper, laterally compressed body of *M. ladigesi* (Online Resource 1) rather indicates a suction feeding mode (Arbour & Lopez-Fernandez 2014, 2016). However, the biotic and abiotic factors of a riverine habitat differ thoroughly from the conditions in a lacustrine habitat (Brinsmead & Fox 2002; King et al. 2021). Theory predicts that the different requirements in comparison to a lacustrine habitat might favor the evolution of alternative functional adaptations in both stream-dwelling sailfin silversides compared to lake-dwelling roundfins (Brinsmead & Fox 2002; Collin & Fumagalli 2011; Theis et al. 2014). Thus, the aim of the present study was to evaluate whether *T. bonti* and *M. ladigesi* also use alternative feeding modes and if so, show different biomechanical adaptations compared to the lake-dwelling roundfins.

Here, we test whether the two stream-dwelling sailfin silversides *T. bonti* and *M. ladigesi* differ in functional feeding morphology using high-speed video recordings and biomechanical models of the oral jaw apparatus. While *M. ladigesi* is endemic to karst of south-west Sulawesi (Hadiaty 2007; Nasyrah et al. 2019), *T. bonti* occurs around the Malili Lakes system in Central Sulawesi (Fig. 1; Herder et al. 2006; Kottelat 1990). Molecular clock analyses estimate the split between the ancestors of both species between 12.9 and



Telmatherina bonti



Marosatherina ladigesii

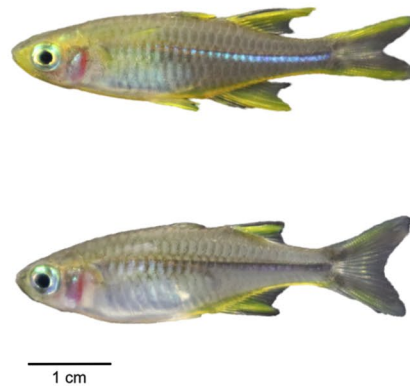


Fig. 1 Indonesia, Sulawesi, the Malili Lakes system (top right corner) and the Maros Karst area (bottom right corner) with the two endemic stream-dwelling sailfin silversides *Telmatherina bonti* and

Marosatherina ladigesii. Red stars indicate the approximate collection locality. Map by T. von Rintelen, modified (with permission)

42.9 Mya (Stelbrink et al. 2014). Since *M. ladigesii* and *T. bonti* occupy similar macrohabitats, i.e. clear hillstreams to medium-sized rivers of the Sulawesi highlands (Gray 2007; Hadiaty 2007; Nasyrah et al. 2019; von Rintelen et al. 2012), similar functional adaptations with respect to feeding ecology would appear plausible. However, aquarium observations and their overall different body morphology (Kottelat 1990; Reckel 2001; Reckel et al. 2002; Reckel & Melzer 2003; Online Resource 1) imply the use of differing feeding modes.

Based on observations (Reckel 2001; Reckel et al. 2002; Reckel & Melzer 2003) and the identified differences in overall body shape (Kottelat 1990; Online Resource 1), we hypothesized that *T. bonti* predominantly use ram feeding whereas *M. ladigesii* primarily use suction feeding. In this

case, we would expect both species to show specific functional adaptations towards the respective feeding mode (Day et al. 2015; Higham et al. 2017; Pfaender et al. 2011; Sonnefeld et al. 2014; Wainwright & Richard 1995). We used four estimators to quantify the feeding mechanics of both species: maximum gape width, prey capture velocity, LJR and MKT. We predicted that *T. bonti* has a large gape, intermediate prey capture velocity, low MKT and high LJR, as expected for a ram feeder (Ferry-Graham et al. 2001b; Pfaender et al. 2011; Porter & Motta 2004; Sonnefeld et al. 2014; Wainwright & Richard 1995). Likewise, we assumed that *M. ladigesii* has a smaller gape, high prey capture velocity, high MKT and low LJR, meeting the predicted patterns of a suction feeder (Ferry-Graham et al. 2001b; Higham et al. 2017; Pfaender et al. 2011; Sonnefeld et al. 2014; Wainwright &

Richard 1995). So far, available information on the trophic ecology of both studied species remains rather limited. Field and aquarium observations suggest that *M. ladigesii* predominantly feed on terrestrial insects complemented by aquatic insects and zooplankton (Andriani 2000; Nasyrah et al. 2019; 2020; Reckel et al. 2002; Reckel & Melzer 2003) while preliminary ecological data indicates that the diet of *T. bonti* is dominated by aquatic insects, complemented by larger and mobile organisms such as crustaceans and small fishes (Bach & Herder, unpublished data). The identification of two different feeding modes would support the assumption of different trophic ecologies in *M. ladigesii* and *T. bonti* (Ferry-Graham et al. 2001b; Porter & Motta 2004; Sonnefeld et al. 2014). Both species also show conspicuous sexual dimorphism in color, size and ornamental features (Kottelat 1990; Nasyrah et al. 2019; Omar et al. 2020). Studies on the related roundfin *Telmatherina* have documented that intersexual variation can translate into alternative diet compositions, which likely reduce competition for ecological resources (Pfaender et al. 2011; Wasiljew et al. 2021). In contrast to ornamental features, which are most likely induced by sexual selection, body size and functional feeding morphology can be affected by ecological factors (Bolnick & Doebeli 2003; Ronco et al. 2019) and thus, might influence maximum gape width, prey capture velocity, MKT and LJR (Pfaender et al. 2011; Wasiljew et al. 2021). We therefore tested for sexual dimorphism in these ecologically relevant traits.

Materials and methods

Materials and fish keeping

Adult captive bred individuals of *T. bonti* and *M. ladigesii* were used for high-speed video analysis. Specimens of *T. bonti* were bred from offspring that was obtained from field research on Sulawesi (Indonesia) in 2010. Individuals of *M. ladigesii* were purchased at an aquarium trade center. Fishes were housed and filmed in two individual 100 and 220 L glass aquaria at a water temperature of 25 °C. Observation boxes of 2.4 L were inserted in both aquaria that contained a sheet of scaled paper on the rear cover. Fishes were fed daily ad libitum at 10:00 a.m. with living *Artemia salina* nauplia within the observation boxes.

High-speed video recording

Recording of high-speed videos was performed daily during feeding with a GoPro Hero7 Black at 120 and 240 frames per second (fps) at a resolution of 960p and 1440p. Two different framerates and resolutions were selected to ensure an optimum quality for distance and velocity measurements. The

camera was placed at about 5 cm distance to the aquarium. The *A. salina* nauplia were inserted into the observation boxes in intervals of about 30 s. Prey captures of respectively 25 male and 25 female specimens of both species were recorded (total $n = 100$). To reduce measurement error, prey capture events were only used when they happened in an estimated distance of less than 1 cm to the scaled paper and if the specimens were fully visible in lateral view during the complete event (Fig. 2a, b; Ferry-Graham et al. 2001a).

Prey capture kinematics

Video recordings with 120 fps at a resolution of 1440 p were used for the analysis of the maximum gape width. Screenshots of the moment when maximum gape width was achieved were taken with the video editing software Wondershare Filmora Video Editor ver. 9.2.1.10. On the base of these screenshots measurements of the maximum gape width and the standard length of each specimen were performed with tpsUtil ver. 1.76 and tpsDig ver. 2.31 (Rohlf 2015). Specimens of *T. bonti* (mean: 51.45 mm) were significantly larger (t test, $p < 0.01$) than specimens of *M. ladigesii* (mean: 39.41 mm), following the documented size ranges of both species (Aarn et al. 1998; Kottelat 1990). Gape width was defined as the distance from the tip of the premaxilla to the tip of the dentary (Pfaender et al. 2011). To compare the measured gape width of the video recorded specimens with the maximum gape width, respectively ten male and ten female specimens of both species, which were available from collection material, were photographed with an Olympus E-330 digital camera with their mouth held open.

Video recordings at 240 fps and at a resolution 960 p were used for the analysis of the prey capture time. Time measurements for the duration of the overall prey capture event, such as for the time until the maximum gape width and complete closure was achieved (Motta & Porter 2004), were performed with Wondershare Filmora Video Editor ver. 9.2.1.10.

Feeding biomechanics

Biomechanical analyses were based on specimens of *T. bonti* and *M. ladigesii* housed at the LIB fish collection, available from earlier collections, including stream Nuha north of Lake Matano (see Herder et al. 2006).

The biomechanics of the oral jaw apparatus were analyzed using the complex maxillary 4-bar linkage lever system and the simple lower jaw lever system (Fig. 3a, b). Ten male and ten female specimens of *T. bonti* and *M. ladigesii*, respectively, were X-rayed with the X-ray scanner Faxitron LX-60 to quantify both lever systems. The four physical links of the maxillary 4-bar linkage system were measured following Pfaender et al. (2011)

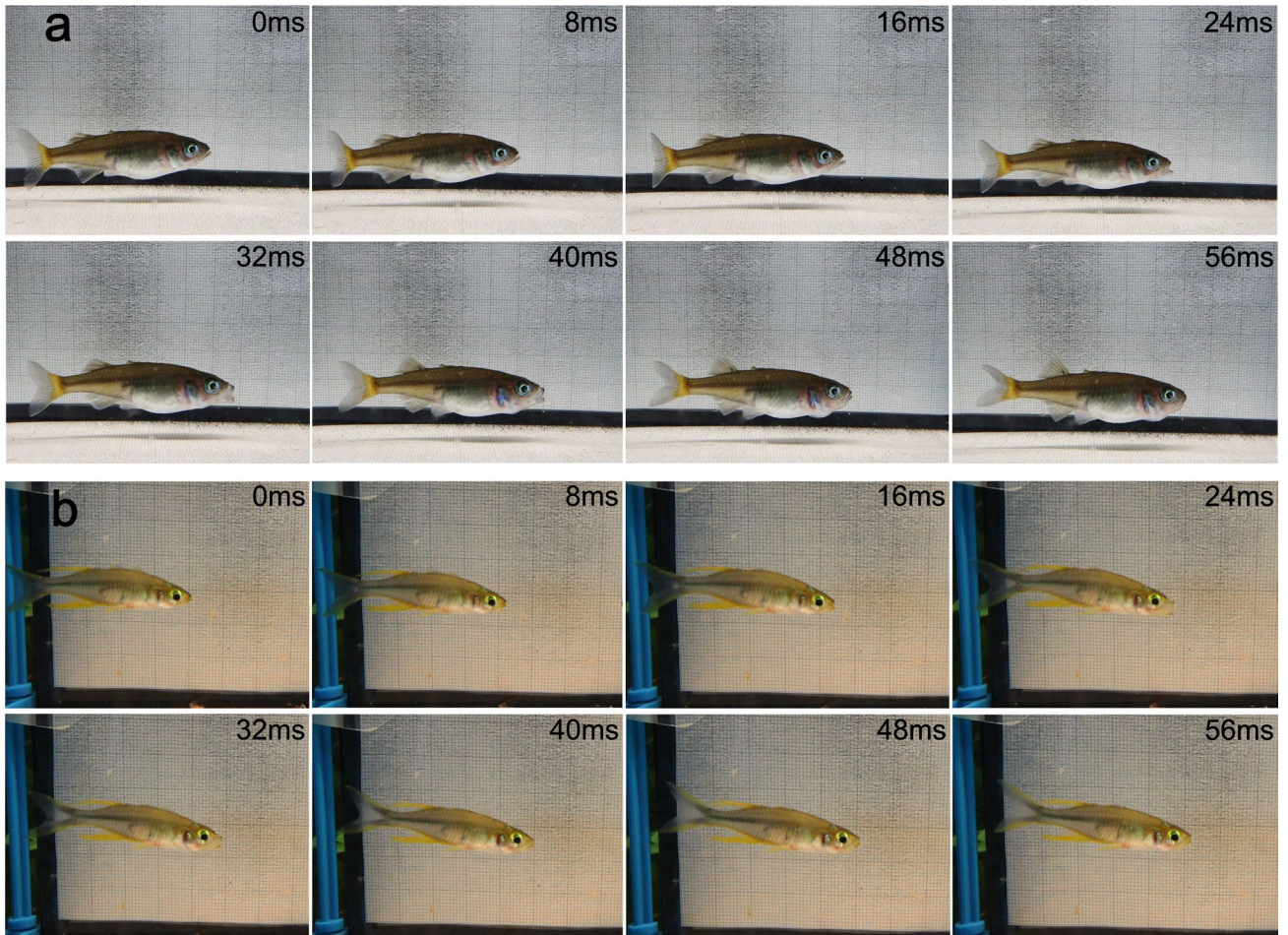


Fig. 2 Examples for a kinematic sequence of prey capture of **a** *Telmatherina bonti* and **b** *Marosatherina ladigesii* in 8 ms steps

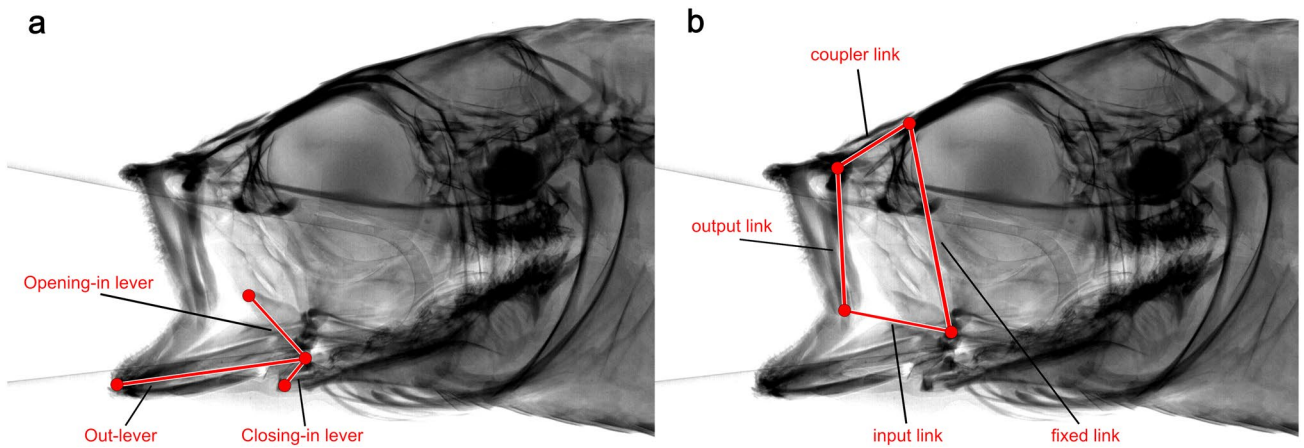


Fig. 3 X-ray images of the head of *Telmatherina bonti*. Biomechanical models used for the quantification of speed and force of oral jaw motion are visualized in red. **a** The simple lever system of the lower

jaw consisting of three levers: Out-lever, Closing in-lever and Opening in-lever. **b** The complex maxillary 4-bar linkage system consisting of four links: fixed link, input link, output link and coupler link

using the software packages tpsUtil ver. 1.76 and tpsDig ver. 2.31 (Rohlf 2015). These four links are the fixed link (defined as the distance between the contact point of the neurocranium and nasal to the coronoid process), the input link (the distance between quadrate/articular joint and maxilla/articular joint), the output link (the distance between the maxilla/articular connection and the nasal/maxilla connection) and the coupler link (measured as the length of the nasal) (Hulsey & García de León, 2005; Hulsey & Wainwright 2002; Pfaender et al. 2011; Westneat 1990). To calculate the maxillary kinematic transmission coefficient (MKT), specimens were scanned twice: once with their mouth closed and once with their mouth held open. The MKT is defined as the ratio of the maxillary output rotation to input rotation. Since the maxillary 4-bar linkage has only one degree of freedom, all angles in the system can be calculated at any point of movement if one angle is given (Hulsey & García de León, 2005; Hulsey & Wainwright 2002; Pfaender et al. 2011; Westneat 1990). The starting angle between the fixed and the input link was defined at 33° by measuring a subsample of both species for this study. Thus, all angles of the maxillary 4-bar linkage could be calculated using the law of cosines: $\text{Cos}(\text{angle}) = (A^2 + B^2 - E^2)/(2AB)$. The input rotation of the lower jaw was determined at 26° by subtracting the starting angle from the opening angle of the input link. The output rotation of the maxilla was calculated for every specimen using the maxillary 4-bar linkage model. The MKT of each specimen was then calculated by dividing the output rotation by the input rotation of 26° . A high MKT indicates a high motion transmission while a low MKT suggests a high force transmission (Cooper & Westneat 2009; Hulsey & García de León, 2005; Hulsey & Wainwright 2002; Parnell et al. 2008; Pfaender et al. 2011).

The simple lower jaw lever system consists of three levers: the Opening in-lever (distance from the jaw joint to the attachment of the interopercular mandibular ligament on the angular bone), the Closing in-lever (distance from the jaw joint to the attachment of the adductor mandibulae on the coronoid process of the articular bone) and the Out-lever (distance from the jaw joint to the anterior tip of the lower jaw) (Wainwright et al. 2004; Wainwright & Richard 1995; Westneat 2004). The measurements of these three links were performed correspondent to the measurements of the maxillary 4-bar linkage to calculate the lower jaw ratio (LJR). The LJR for opening was calculated by dividing the Opening in-lever by the Out-lever. Accordingly, the LJR for closing was calculated by dividing the Closing in-lever by the Out-lever. Contrary to the MKT, High LJR indicates a forceful and slow movement while low ratios indicate a fast but weak motion of the lower jaw (Pfaender et al. 2011; Wainwright et al. 2004; Wainwright & Richard 1995; Westneat 2004).

Statistical analyses

Bivariate linear models and a multiple linear regression were performed for the absolute measurements of maximum gape width, prey capture time and standard length. For the bivariate linear models, maximum gape width and prey capture time were paired with standard length, respectively. The resulting residuals of each bivariate linear model and the absolute values of MKT and LJR were used to perform one-way ANOVAs with Tukey's pairwise tests in the software PAST ver. 3.22 (Hammer et al. 2001) to test for differences between species and sexes.

Results

Maximum gape width

Absolute maximum gape width ranged from 4.3 mm to 7.3 mm in *T. bonti* (mean: 6.1 mm) and from 2.8 mm to 5.7 mm in *M. ladigesi* (mean: 4.2 mm). Specimens of *T. bonti* had a significantly higher absolute gape width ($p < 0.01$) than specimens of *M. ladigesi*, matching the difference in body size. Maximum gape width was linearly correlated with standard length in both species (Fig. 4a; $p < 0.01$). Relative maximum gape width was significantly higher in *T. bonti* compared to *M. ladigesi* (Fig. 5a; $p < 0.01$) and did not differ significantly between the specimens used for recording and the collection specimens used for biomechanical measurements ($p > 0.1$). Sexual dimorphism was significant in *M. ladigesi* but not in *T. bonti*. Male *M. ladigesi* were significantly larger ($p < 0.01$) and had a higher absolute but lower relative maximum gape width than females ($p < 0.05$).

Prey capture velocity

Absolute prey capture time ranged from 46 to 108 ms in *T. bonti* (mean: 72 ms) and 33 ms to 108 ms in *M. ladigesi* (mean: 57 ms). *Marosatherina ladigesi* opened and closed their jaws significantly faster than *T. bonti* ($p < 0.01$). In *M. ladigesi* there was no significant difference between jaw opening and closing time whereas jaw opening in *T. bonti* was significantly faster than jaw closing (Fig. 5c; $p < 0.01$). Overall prey capture time was linearly correlated with standard length (Fig. 4b; $p < 0.01$). In relation to standard length, *M. ladigesi* had a significantly higher prey capture velocity than *T. bonti* (Fig. 5b; $p < 0.01$). Intersexual variation in absolute prey capture velocity was not detectable in either species ($p > 0.05$). In relation to standard length, male *T. bonti* showed a significantly higher relative prey capture

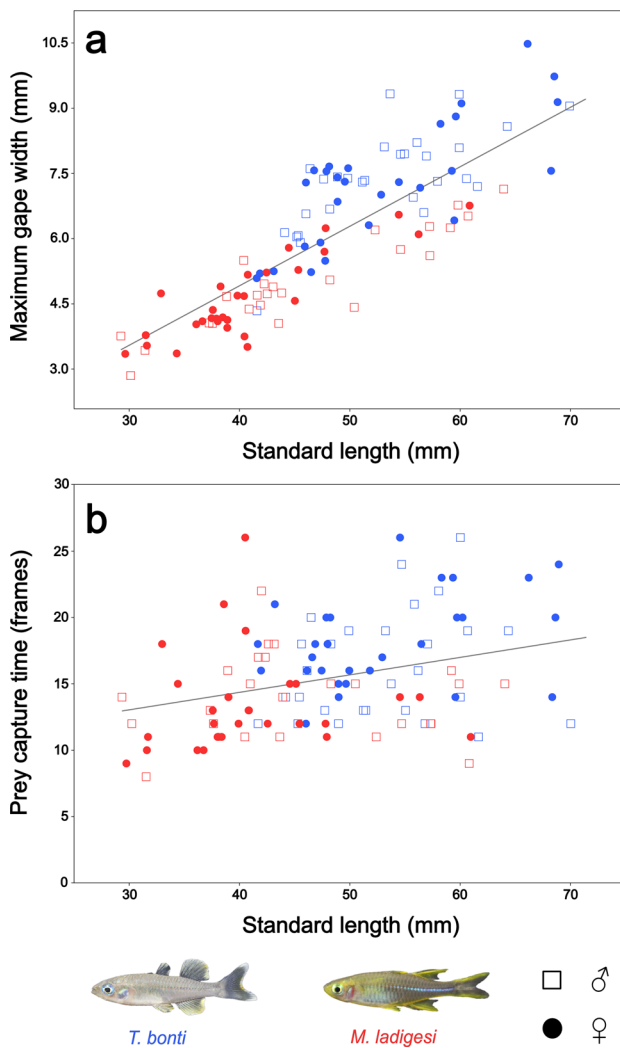


Fig. 4 Bivariate linear models of absolute **a** maximum gape width and **b** prey capture time in relation to standard length between *Telmatherina bonti* and *Marosatherina ladiges*. Morphospecies are color coded, sexes are symbol coded: *Telmatherina bonti*—blue; *Marosatherina ladiges*—red; male—square; female—dot

velocity than females ($p < 0.05$). Contrarily, there was no significant difference between sexes of *M. ladiges* ($p > 0.1$).

Feeding biomechanics

The lower jaw ratio (LJR) for opening did not differ significantly between species or sexes (Fig. 5d; mean: 0.14; $p > 0.1$) while the LJR for closing was significantly lower in *T. bonti* (mean: 0.31) compared to *M. ladiges* (Fig. 5e; mean: 0.5; $p < 0.01$). Significant intersexual differences in LJR for closing were not detectable in both species ($p > 0.1$). *Telmatherina bonti* (mean: 0.98) showed a slightly higher maxillary kinematic transmission (MKT) than *M. ladiges* (mean: 0.85), but the difference was not significant (Fig. 5f;

$p = 0.08$). Both species showed no sexual dimorphism in MKT ($p > 0.1$).

Discussion

Prey capture kinematics provide indications for different feeding modes

Interspecific differentiation in maximum gape width

The results of the prey capture kinematics support the hypothesis that feeding modes of *T. bonti* and *M. ladiges* differ substantially. Relative maximum gape width is significantly lower in *M. ladiges* compared to *T. bonti*. A small gape is considered advantageous for suction feeders because it enhances the pressure gradient that sucks prey items into the mouth opening (Day et al. 2015). Thus, the low maximum gape width detected in *M. ladiges* supports the hypothesis that this species primarily uses suction feeding to capture prey items (Day et al. 2015; Sonnefeld et al. 2014; Wainwright et al. 2007). In contrast, the high values measured in *T. bonti* rather suggest ram feeding, where a large gape is considered beneficial as it enables predators to engulf larger prey items, reduces the effect of the bow wave and increases the strike accuracy (Higham et al. 2017; Wainwright & Bellwood 2002). Likewise, the significantly higher maximum gape width of *T. bonti*, compared to *M. ladiges*, supports the theory of predominant ram feeding in *T. bonti* (Ferry-Graham et al. 2001b; Higham et al. 2017; Sonnefeld et al. 2014). Our hypotheses are supported by the findings of Higham et al. (2017) who identified a correlation between ram speed and maximum gape width in three-spined stickleback populations. Similar findings were also documented in Neotropical cichlids by Arbour & López-Fernández (2014, 2016).

Sexual dimorphism in maximum gape width within *M. ladiges*

In intraspecific comparison, we found a higher relative gape width in female *M. ladiges* compared to conspecific males, whereas this trait is not dimorphic in sexes of *T. bonti*. As the maximum gape width limits the maximum prey size a fish is able to consume (Higham et al. 2007; Wainwright & Bellwood 2002), the larger gape of female *M. ladiges* might have an effect on their diet composition, enabling them to capture slightly larger prey items than males. Sexual niche differentiation in line with sexual dimorphism in feeding morphology has been rarely documented in fishes (McGee & Wainwright 2013). It was reported for lake-dwelling roundfin sailfin silversides, where *T. antoniae* males have a larger gape and feed to

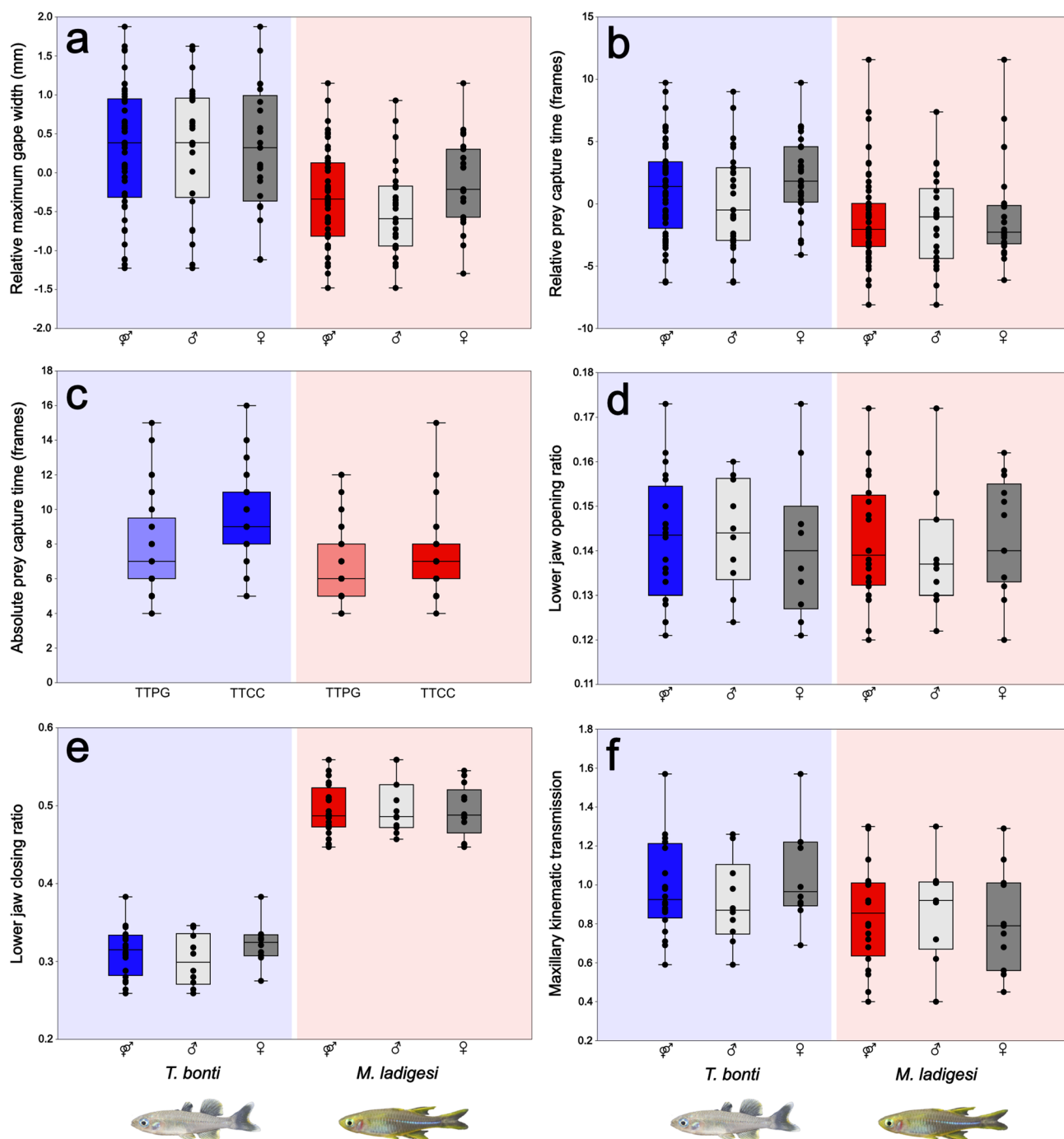


Fig. 5 Interspecific and intersexual variation in **a** relative maximum gape width, **b** relative prey capture time, **c** time to peak gape (TTPG) vs. time to complete closure (TTCC), **d** lower jaw opening ratio, **e** lower jaw closing ratio, **f** maxillary kinematic transmission of *Tel-*

matherina bonti and *Marosatherina ladiges*. Dots visualize single individuals. Morphospecies and sexes are color coded: *Telmattherina bonti* combined—blue; *Marosatherina ladiges* combined—red; male—white; female—grey

a higher extent on larger prey organisms than females (Pfaender et al. 2011). Another potential explanation for the sexual dimorphism in *M. ladiges* might be intrasexual selection, as male *M. ladiges* tend to bite opponents as part of their competition behavior (Hadiaty 2007). Since

a reduced maximum gape width is correlated with an increased bite force, a smaller mouth might be beneficial in intrasexual competition; similar patterns were reported for three-spined sticklebacks (McGee & Wainwright 2013) or blenniid fishes (Rico-Guevara & Hurme 2019). However,

without further details about the biology and ecology of *M. ladigesi*, this hypothesis remains to be tested.

Interspecific differentiation in prey capture velocity

The significant difference between the measured values of the prey capture velocity in *M. ladigesi* and *T. bonti* also corroborate the hypothesis of two different feeding modes. Prey capture events of *M. ladigesi* are significantly shorter compared to *T. bonti*. A particularly high opening velocity is considered beneficial for suction feeding because it increases the subambient buccal pressure and thus accelerates the water flowing into the oral cavity (Higham et al. 2006a, b, 2017; Wainwright et al. 2007), while a low prey capture velocity with the associated higher reliance on ram speed is indicative for ram feeding (Higham et al. 2017). The fast prey capture kinematics identified in *M. ladigesi* therefore support the theory of a suction feeding mode chiefly used by this species and match with the general pattern of a suction feeder (Day et al. 2015; Sonnefeld et al. 2014; Wainwright et al. 2007). In contrast, the low prey capture velocity of *T. bonti* supports the hypothesis of a ram feeding mode primarily used by this species and meets with the generally documented prey capture kinematics of ram feeding fishes (Higham et al. 2017; Sonnefeld et al. 2014).

Intersexual variation in prey capture velocity in *T. bonti*

Prey capture velocity of male *T. bonti* is significantly higher compared to females. Velocity is likely of high adaptive value as it directly affects the efficiency to feed on elusive prey: fast moving jaws, typical for species specialized on elusive prey, result in lower prey capture times compared to fishes feeding on immobile prey (Hulsey & Wainwright 2002; Parnell et al. 2008; Wainwright & Bellwood 2002). This has been documented for example in cichlids and labrid fishes (Arbour et al. 2020; Hulsey & Wainwright 2002; Wainwright et al. 2004). Thus, the lower prey capture time of male *T. bonti* compared to females would suggest a higher proportion of elusive prey in their diet. However, since ecological data for *T. bonti* is restricted to preliminary studies (Bach & Herder, unpublished data), it remains speculative if the intersexual variation in prey capture velocity is associated with dietary differences. The same applies to the hypothesis that a faster moving oral jaw in male *T. bonti* could also serve as display for potential mates or competing males and thus be affected by sexual selection. However, in contrast to maximum gape width, there are, to the best of our knowledge, no reported cases of a positive relationship between prey capture velocity and display in fishes.

No indications for different feeding modes based on biomechanics

In contrast to our results of the prey capture kinematics, feeding biomechanics contradict the hypothesis that *T. bonti* and *M. ladigesi* use different feeding modes. Based on previous research (Pfaender et al. 2011), we hypothesized that *T. bonti* has a lower maxillary kinematic transmission (MKT) and higher lower jaw ratio (LJR), resulting in a slower but more powerful jaw motion compared to *M. ladigesi*. However, neither the LJR for opening nor the MKT revealed any significant inter- or intraspecific differences. The only parameter where we found significant differences was the LJR for closing. But in contrast to the raised hypothesis, *M. ladigesi* showed a higher LJR for closing than *T. bonti*, which suggests a more powerful but slower closing lower jaw (Cooper & Westneat 2009; Pfaender et al. 2011; Wainwright & Richard 1995). Based on the assumption that *M. ladigesi* mainly uses suction feeding and *T. bonti* primarily uses ram feeding, this finding is surprising: ram feeders expectedly benefit from a stronger jaw grip to grasp their prey (Sonnefeld et al. 2014; Wainwright & Bellwood 2002). However, these opposing results of the LJR for closing are in accordance with discrepancies in previous studies on cichlids and sailfin silversides (Arbour et al. 2020; Arbour & López-Fernández 2016; Pfaender et al. 2011), which also identified a lower LJR for closing in ram feeding species compared to a higher value in suction feeding species. Thus, the LJR for closing might have also been affected by other parameters than feeding mode, which were not assessable with the present study.

Different trophic ecologies might explain the opposing biomechanics

A possible explanation for our supposedly contradicting results of the prey capture kinematics and the feeding biomechanics could lie in the trophic ecology of the focal species. Fishes feeding on elusive prey are expectedly characterized by a high MKT and low LJR, whereas fishes feeding on immobile prey usually show opposite values (Alfaro et al. 2005; Hulsey & Wainwright 2002; Parnell et al. 2008; Wainwright & Richard 1995). The surprisingly high MKT and low LJR for closing in *T. bonti* could therefore indicate a higher proportion of elusive prey in their diet compared to *M. ladigesi*. Although quantitative analyses of trophic ecology are lacking for both species, there are documented observations and preliminary data which support this theory (Andriani 2000; Bach & Herder, unpublished data; Nasyrah et al. 2019, 2020; Reckel 2001; Reckel et al. 2002; Reckel & Melzer 2003). For instance, preliminary ecological data suggests that *T. bonti* mainly feed on aquatic insects, crustaceans and small fishes (Bach & Herder, unpublished data),

which usually show very strong escape responses (Hulsey & García de León, 2005; Hulsey & Wainwright 2002). Thus, *T. bonti* would benefit from a combination of a high MKT, low LJR and fast moving jaws (Alfaro et al. 2005; Arbour et al. 2020; Hulsey & Wainwright 2002; Parnell et al. 2008; Wainwright & Richard 1995). Contrarily, Reckel et al. (2002) and other authors (Andriani 2000; Nasyrah et al. 2019, 2020; Sterba 1987) reported that *M. ladigesii* predominantly feeds on terrestrial insects accompanied by aquatic insects and zooplankton. These prey categories generally lack strong escape responses (Pfaender et al. 2011), meaning that a high MKT, low LJR and fast moving jaws would not be advantageous for *M. ladigesii*. Taken together, potential differences in diet composition might provide a possible explanation for the lacking biomechanical indications for different feeding modes. However, this assumption is tentative as long as no quantitative analysis of trophic ecology has been performed on both studied species.

Less pronounced biomechanical adaptations could be due to contrasting habitats

The limited biomechanical differentiation identified in the present study is in contrast to the substantial biomechanical adaptations to alternative feeding profiles revealed in roundfin *Telmatherina* (Pfaender et al. 2011), with significant inter- and intraspecific differences in MKT and LJR between the suction feeding *T. antoniae* and the ram feeding *T. prognatha* (Online Resource 2; see also Fig. 7 in Pfaender et al. (2011) for detailed kinematic and biomechanical results). This discrepancy in biomechanics between lacustrine and riverine sailfins silversides might be explained by the contrasting conditions of stream and lake habitats (Brinsmead & Fox 2002; King et al. 2021). Besides of the continuous flow and close integration with terrestrial ecosystems, stream environments are more variable, less isolated and affected by a higher frequency of catastrophic events compared to the generally more stable lake environments (Brinsmead & Fox 2002; King et al. 2021; McLaughlin & Grant 1994). The less pronounced biomechanical adaptations of *T. bonti* and *M. ladigesii* with respect to feeding mode might therefore be the result of less stable environmental conditions.

Conclusions

In summary, prey capture kinematics suggest that *T. bonti* is predominantly a ram feeder, whereas *M. ladigesii* is primarily a suction feeder. Sexual niche partitioning provides a possible explanation for the identified sexual dimorphism in *T. bonti* and *M. ladigesii*. Sexual selection might also affect the intersexual variation in maximum gape width but appears less likely for the differentiation in prey capture velocity. The

results of the biomechanical analyses contradict the prey capture kinematics as well as previous findings detected in the related roundfin radiation. In contrast to the lacustrine sailfin silversides, *T. bonti* and *M. ladigesii* do not show substantial biomechanical differences according to their respective feeding mode. We conclude that this lack of differentiation might be explainable by the more variable conditions of a flowing habitat and by different diet compositions of both species. However, it has to be kept in mind that ecological data for *T. bonti* and *M. ladigesii* is based on limited sample sizes and raw observations. It therefore has to be corroborated by future research and additional ecological data on both investigated species. Investigating additional biomechanical systems and parameters used to quantify force and kinematic transmission in the jaw of fishes, such as the opercular linkage, hyoid linkage, maximum jaw protrusion and the suction index (Anker 1974; Hulsey et al. 2005; Muller 1987; Wainwright et al. 2007; Westneat 1990) would be interesting to critically test the limited biomechanical differentiation identified in the present study.

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Author contributions BDW: performed the high-speed video recordings and collected the data. FH, JP and BDW: designed the study, BDW and JP: analysed the data. BW: aided morphological analyses. BDW: and FH: wrote the manuscript, with contributions from all other authors. All authors gave final approval for publication.

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Data availability statement The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no financial interest to declare. Benjamin Wipfler is a member of the editorial board of *Zoomorphology*.

Ethical approval No approval of research ethics committees was required to accomplish the goals of this study because no experiments on living animals were conducted. Living fishes were only filmed during the regular daily feeding procedure.

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