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REVIEW

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Review of the methods used for calculating physiological cross-sectional area for ecological questions

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Abstract

This review examines literature that used physiological cross-sectional area (PCSA) as a representative measure of an individual muscles maximal isometric force production. PCSA is used to understand the muscle architecture and how a trade-off between muscle force and muscle contractile velocity reflect adaptations of the musculoskeletal system depending of functional demands. Over the decades, differing methods have been developed to measure muscle volume, fascicle lengths, and pennation angle to calculate PCSA. The advantages and limitations of these methods (especially the inclusion/elimination of pennation angle) are discussed frequently; however, these are scattered throughout the literature. Here, we reviewed and summarized the different methods of collecting and recording muscle architectural properties to subsequently calculate PCSA. By critically discussing the advantages and limitations of each methodology, we aim to provide readers with an overview of methods to assess muscle architecture. The review may serve as a guide to facilitate readers searching for the appropriate techniques to calculate PCSA and measure muscle architecture to be applied in ecomorphology research.

RESEARCH HIGHLIGHTS

Discuss the theories behind PCSA in a synthesised review that researchers 32 can reference to make their own decisions on PCSA methodology.

KEYWORDS

fascicle length, muscle force, muscle mass, pennation angle

1 | INTRODUCTION

Movement powered by muscles is one of the characteristic features of animals. In vertebrates, the action of skeletal muscles on the (principally) bony endoskeleton accomplishes movement of body segments and the body as a whole, as an animal interacts with the environment. As would be expected, the muscle properties (physiology and anatomy) throughout an animal's body vary according to the functional demands of the animal's behaviours (Bergmann & Hare-Drubka, 2015). Together, the muscle properties with the arrangement of the skeleton will then determine the nature of locomotion and other movements such as food manipulation and processing, and thus will reflect animal ecology (i.e., if prey or predator; Higham, Korchari, & McBrayer, 2011).

Muscle function and performance are correlated with muscle anatomy. Striated muscle consists of long and slender muscle fibres, which are bundled together into fascicles and subsequently muscle bellies (Bergmann & Hare-Drubka, 2015). Muscle contractions occur when there is an activation of the tension-generating sites within the muscle fibre. Repeating units of interdigitating thick myosin and thin actin protein filaments, called sarcomeres, are the site of muscle contraction. Shortening of each sarcomere occurs via

the formation of cross-bridges between myosin heads and actin molecules. The cycle of cross-bridge formation, contraction, detachment and reattachment is powered by the energy release of ATP (Hildebrand, Bramble, Liem, & Wake, 1985; McNeill, 2003). A muscle's contractile property is determined by the number of fascicles, the fascicle length, the angle of the fascicles relative to the axis of force production (pennation angle), and the size and types of muscle fibres. Longer fascicles have more sarcomeres in a series, which results in a greater contractile velocity, and thus a greater potential for speed and movement (Hildebrand et al., 1985; Lieber & Fridén, 2000; Sacks & Roy, 1982). Force production capacity is then a function of how many sarcomeres are within a muscle (i.e., number of fibres within a muscle), which will determine the number of cross-bridges that are formed and, in-turn, increases the force that the muscles can exert (Gans & de Vree, 1987; Wickiewicz, Roy, Powell, & Edgerton, 1983). The cross-sectional area of the muscle therefore reflects capacity for force production (Gans & de Vree, 1987). Physiological cross-sectional areas (PCSAs) is a representative measure of "muscle force" while fascicle length reflects the "length excursion" of the muscle; both measures are used to determine how much work (force x distance) can be done by an individual muscle and how much power (work/time) can be produced (Allen et al., 2014; Allen, Elsey, Jones, Wright, & Hutchinson, 2010; Payne, Hutchinson, Robilliard, Smith, & Wilson, 2005). For a given muscle volume, both muscle force and working range cannot be maximised at the same time (due to force–velocity relationship); therefore, specialisation in accordance with functional demands will occur (Rosin & Nyakatura, 2017).

PCSA is applied extensively in ecological research. As PCSA is proportional to maximum isometric force production, and is used to compare muscle force production between species, as well as to investigate the trade-off between force generation (PCSA) and capacity for shortening length changes. Various aspects of animal biology, including bite force (Becerra, Echeverría, Casinos, & Vassallo, 2014), running (Leischner et al., 2018; Michilsens, Vereecke, D'Août, & Aerts, 2009), digging (Lagaria & Youlatos, 2006; Martin, Warburton, Travouillon, & Fleming, 2019; Moore, Budny, Russell, & Butcher, 2013), diet (Taylor & Vinyard, 2004), and flight (Maniakas & Youlatos, 2012; Yang, Wang, & Zhang, 2015), have been investigated by applying PCSA as a quantitative estimate of muscle force. In the discipline of anthropology, comparative PCSA has been applied to questions of hominoid evolution, to assess bite force in relation to diet (Strait et al., 2009; Strait et al., 2010), and hindlimb adaptation in the evolution of bipedal locomotion (Payne et al., 2006; Thorpe, Crompton, Gunther, Ker, & McNeill, 1999). The calculation of PCSA appears incongruous with the interpretation of fossils; however, modelling of muscle fibre arrangement and volume has been facilitated through comparative approaches between living and extinct species (Perry, 2018; Perry, St Clair, & Hartstone-Rose, 2015). This review explores and summarises different methods used to calculate PCSA to assist researchers to make a logical and informed decision on applications of PCSA for their research.

2 | ISSUES OF TISSUE PRESERVATION: FRESH OR FIXED SPECIMENS

Before determining methods with which to measure muscle architecture and calculate PCSA, the preservation of specimen tissue needs to be considered. Preservation of specimens influences the decisions of later methods, particularly muscle mass. The decision to fix specimens in formaldehyde (here after formalin) will depend where the specimen was sourced and its condition.

Formalin is a reliable chemical fixative; however, it is known to cause morphological changes in the tissue (Fox, Johnson, Whiting, & Roller, 1985) that are relevant to the calculation of PCSA. Mass is known to decrease (Cheng & Scott, 2000) and fascicles shrink (shorten) as part of the fixation process. Some studies have shown the decrease in muscle mass to be insignificant (Cutts, 1988), while other studies found up to a 7% decrease in mass (Cheng & Scott, 2000; Schremmer, 1967). Correction factors are available to counteract the shrinkage and produce a PCSA score from fixed material. Kikuchi and Kuraoka (2014) suggest that muscle mass should be adjusted to compensate for a decrease of 14%, while fascicle length should be adjusted for a decrease in length of 9–13% after formalin fixation. An alternative to formalin, and a fixative often used in histology or in preparation for digital imaging is Bouin's fluid as it minimises shrinkage of the specimens (Metscher, 2009; Schenk et al., 2013; Siebert et al., 2015; Sombke, Lipke, Michalik, Uhl, & Harzsch, 2015).

The benefit of fixing species is to preserve specimens that may be too damaged to dissect fresh (e.g., roadkill in Martin, Warburton, et al., 2019). Often specimens have been used for other studies and may already be embalmed in formalin; therefore, for consistency, all specimens used within the study should be preserved in the same manner. For comparative studies, if all specimens are preserved in the same manner, then the decrease in mass and length will be similar across all muscles, although fixation caused shrinkage needs to be considered when functional interpretations are made on the basis of PCSA values in comparison to results in the literature.

Another factor to be aware of when using cadavers is the influence of rigor mortis on joint angles (Kawakami, Ichinose, & Fukunaga, 1998). Rigor mortis causes slow contractions in muscle fascicles which is not released due to a lack of ATP to break the binding (Bendall, 1951; Davies, 1963), and thus depending on the timing of embalming/dissection, muscles may be fixed in a state of partial muscle contraction (Martin et al., 2001). Rigor mortis may be difficult to control, unless the specimen is fresh frozen immediately after death, and therefore needs to be taken into account when measuring fascicle length (i.e., freezing specimens in a consistent position immediately after death) or the potential removal of pennation angle in the calculation.

3 | THE THREE COMPONENTS USED TO CALCULATE PCSA

PCSA as a representative measure for muscle force was established in quantitative muscle architecture studies by Gans and Bock (1965) and

Gans and de Vree (1987). The measures used: (a) muscle volume, (b) fibre length, and (b) pennation angle.

$$\text{PCSA}\left(\text{cm}^2\right) = \frac{\text{Muscle mass (g) x Pennation angle } (\cos\theta)}{\text{Muscle density } \left(\frac{\text{g}}{\text{cm}^3}\right) \text{x fascicle length(cm)}}$$

To estimate the maximum isometric force (F_{max}) and instantaneous power of a muscle (both are key indicators of muscle functional performance), PCSA is multiplied by the maximum isometric stress. Two alternative maximum tetanic tensions for mammalian muscle are available; 22.5 N/cm^2 (Lieber & Fridén, 2000; Powell, Roy, Kanim, Bello, & Edgerton, 1984) calculated from guinea pig hindlimbs (Rodentia) or 30 N/cm^2 (Medler, 2002; Wells, 1965; Woledge, Curtin, & Homsher, 1985) calculated from rats (Rodentia).

Measuring these variables differs according to specimen type and researcher preferences, as well as the time and facilities available. Below, we discuss the differing methods and present the advantages and limitations for the different methodology (summarised in Table 1).

3.1 | Muscle volume (derived from muscle mass)

As the potential force production of a muscle is dependent on the number of fascicles working together in parallel, the total muscle volume will be a reflection of muscle force production, to the extent that large muscles will generally have a larger number of muscle fascicles. Muscle volume is usually calculated from the mass of muscles, which is easier to measure. ~~The muscle density constant (1.0597 g/cm^3) determined by Mendez and Keys (1960) for fresh dog and rabbit skeletal muscle or the density constant (1.0564 g/cm^3) by Murphy and Beardsley (1974) using cat skeletal muscle, has been used extensively throughout the literature to convert muscle mass to muscle volume.~~ The density constant is often simplified to 1.06 g/cm^3 in the literature (Figure 1).

Either dry muscle mass or wet muscle mass is sufficient when calculating PCSA, providing a consistent approach is undertaken throughout the study to avoid variation due to the state of hydration. Consistency is vital to ensure comparisons between muscle groups or between individual animals are valid.

TABLE 1 Summary of alternative methods that can be utilised to calculate PCSA

Component of PCSA	Alternative methods	Advantages	Limitations	Examples
Preservation of specimen	Fresh	- Time efficient - No shrinkage of architecture	- Potential for dehydration - Freezer burn? - Fresh muscles fragile	1, 2
	Formalin	- Preserves damage to specimens - Use of museum preserved specimens	- Shrinkage of muscle mass and fibre length - Expense of formalin and ethanol	3, 4
Muscle mass (mm)	Wet muscle mass	- Minimal equipment needed - Most common therefore more comparable in literature	- Extra requirements of spraying throughout dissection - Potential variation in hydration	5, 6
	Dry muscle mass	- Accuracy: no effect of hydration	- Time consuming - Require extra equipment (oven)	7, 8
FL	Muscle belly length	- Time efficient - Minimal equipment required	- Inaccurate for majority of muscles - Too simplified	8
	Incision along plane of fibres	- Time efficient - Minimal equipment required	- Potential damage to muscle - Difficult to see the whole FL - Bias of "random selection"	9, 10
	Acid to digest connective tissue	- Loosen fibre connection to visualise whole fascicle - Random selection of fascicles	- Expenses of acid - Time consuming	11, 12
	Digital dissection	- Accurate measure of FL - Non destructive	- Expensive - Time consuming	13
Pennation angle (θ)	Pennation angle	- Significant effect on PCSA if angle above 30 degrees	- Bias of "random selection" - Potential in introduce error	14, 15
	Angle set to zero	- Time efficient - Generally <30$^\theta$ thus cosθ approx. 1 - Minimal effect on PCSA	- Under-estimating PCSA	16, 17

~~Note:~~ 1: (Cuff et al. 2016), 2: (Dick & Clemente, 2016), 3: (Böhmer, Fabre, Herbin, Peigné, & Herrel, 2018), 4: (Martin, Warburton, et al., 2019), 5: (Marchi et al. 2018), 6: (Olson et al. 2017), 7: (Lagaria & Youlatos, 2006), 8: (Langenbach & Weijs, 1990), 9: (Lamas, Main, & Hutchinson, 2014), 10: (Rose et al. 2016), 11: (Hartstone-Rose, Perry, & Morrow, 2012), 12: (Leischner et al., 2018), 13: (Dickinson, Basham, Rana, & Hartstone-Rose, 2019), 14: (Allen et al., 2010), 15: (Böhmer et al., 2018), 16: (Myatt et al. 2012), 17: (Thorpe et al., 1999).

Abbreviations: FL, fibre length; PCSA, physiological cross-sectional area.

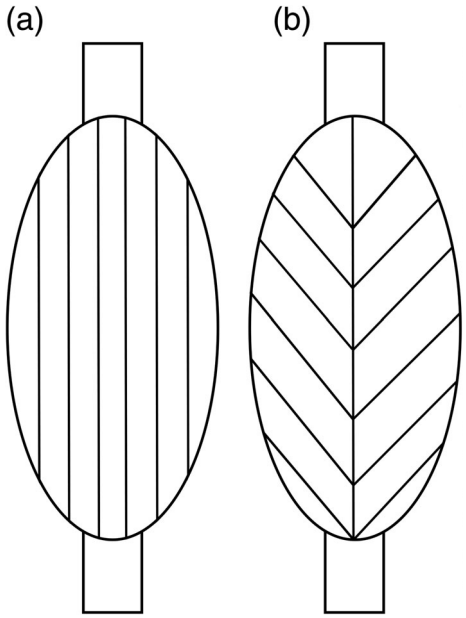

FIGURE 1 Simplified representation depicting the difference between (a) parallel architecture with fewer fascicles; however, longer fascicles and (b) pennate architecture with more fascicles; however, shorter fascicles

The dry muscle mass technique involves drying isolated muscles in a low temperature oven to remove all ~~the~~ moisture ~~content~~ (Lagaria & Youlatos, 2006; Langenbach & Weijs, 1990). This method has the benefit of ensuring that the moisture content of all muscles is consistent and is beneficial when specimens are at varying levels of hydration (i.e., older specimens may have dried out over time). The drying period often takes between 24 and 48 hr (Warburton, Grégoire, Jacques, & Flandrin, 2013), and requires an air-drying oven that can maintain a constant temperature over a long period of time. Using dry muscle mass also limits the comparisons that can be made with PCSA scores calculated with wet muscle mass, as dry mass is lighter than wet mass.

Wet weights, however, seem to be preferred and the most common method in the literature. The measurement of wet muscle mass requires periodically moistening of the specimen (either phosphate buffered saline [PBS] or water) during dissection to avoid desiccation that would lead to inconsistent results and the immediate weighing of each muscle after removal to avoid dehydration (Cheng & Scott, 2000; Moore et al., 2013; Olson, Womble, Thomas, Glenn, & Butcher, 2016). ~~The wet method can lead to inconsistencies in muscle masses, especially when using specimens of varying states of preservation and dehydration.~~ An alternative is to fully rehydrate muscles before weighing by soaking them in PBS then gently blotting dry with paper towel before weighing (Burkholder, Fingado, Baron, & Lieber, 1994; Eng et al., 2008), or storing muscles in 70% ethanol immediately after dissection (Böhmer et al., 2018). Using wet mass does allow for a wider comparison of the literature compared to dried mass.

3.2 | Fascicle length

In the calculation of PCSA, fascicle length is used to represent the number of sarcomeres in a series within the muscle fibres (Michilsens et al., 2009). If comparing two muscles of equal mass, but different length fascicles, the muscle with longer fascicles will have a faster shortening velocity as there are more sarcomeres in series, and therefore a greater contractile velocity (~~Partial chemical digestion of the muscle belly is by far the most 1;~~ Azizi, Brainerd, & Roberts, 2008; Kikuchi, 2010; Lieber & Fridén, 2000).

Five methods are used to measure fascicle and fibre length in the literature: ~~(a) measuring the muscle belly length (origin to insertion), (b) making an incision down the muscles line of action/fascicle plane (depending on fascicle architecture) to measure individual fascicles, (c) layer-wise fascicle dissection, (d) using partial chemical digestion to allow the fibres to separate for measurement, and (e) digital dissection to measure fascicles without destroying the specimen.~~

(a) Historically, total muscle belly length was used as the estimate of fascicle length. This is unlikely to be a true representation of fascicle length, especially for larger muscles, as few fascicles run the entire length of the muscle (Lagaria & Youlatos, 2006; Moore et al., 2013; Pasi & Carrier, 2003). Several architectural investigations on human upper and lower limb muscles have shown that even the most parallel orientated muscles (muscle composed of fascicles extending parallel to the muscle force generating axis) have fibres that only extend around 60% of the muscle length (Lieber & Fridén, 2000). The use of total muscle belly length is therefore a significant over-estimation of fascicle length. While this method provides a quick measure of muscle length, it is not recommended for measuring fascicle length due this over-estimation.

(b) Direct measurement of fascicle length via an incision along the plane of the fascicles within a muscle belly provides a relatively quick method of collection and reasonably accurate measure of fascicle length (Bribiesca-Contreras, Parslew, & Sellers, 2019; Lamas et al., 2014; Moore et al., 2013; Oishi, Ogihara, Endo, & Asari, 2008; Rose, Sandefur, Huskey, Demler, & Butcher, 2013; Rupert, Rose, Organ, & Butcher, 2015). A limitation of this method is that making incisions in the muscle belly ultimately destroys fascicles, and therefore it can be difficult to measure intact fascicles. This method requires precision dissecting skills to ensure fascicles are not destroyed and accurate fascicle lengths can be measured. For strap muscles, careful incision from the origin to insertion along the muscle belly can reveal complete muscle fascicles, which then are measured using callipers, ruler or digitally using photography. For pennate-fibred muscles, the incision must be made along the plane of the fascicles to expose the internal fascicle lengths (Figure 2a). An incision from origin to insertion of a pennate muscle would result in bisecting the fascicles, resulting in much shorter fascicle length measurements.

(c) Layer-wise fascicle dissection involves digitising the paths of individual muscle fascicles using a Microscribe (handheld 3D-digitiser) to produce a 3D-muscle model. Individual fascicles are removed carefully, then the groove that remains after fascicle removal is digitised

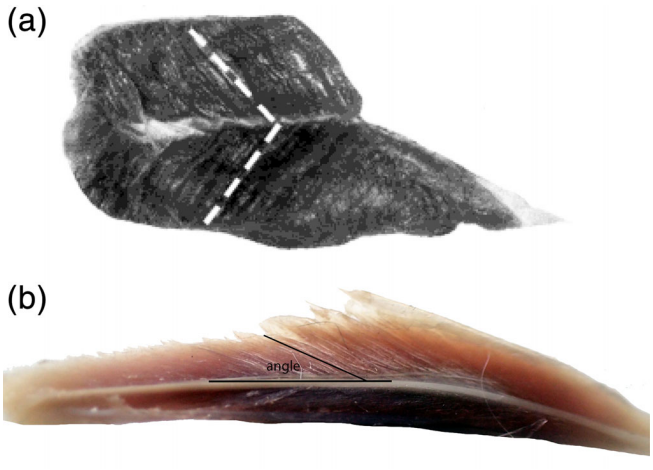

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FIGURE 2 Photographs of methods of measuring fascicle length and pennation angle. (a) Modified from Cheng and Scott (2000); measurement of internal fascicles lengths (dotted lines) of *Macca mulatta* triceps long head. (b) flexor carpi ulnaris of the Quenda (*Isoodon fusciventer*). Thick line representing the line of action, and small line is the orientation of the fascicle. Pennation angle is measured between the two intersecting lines

for the length and orientation (Nyakatura & Stark, 2015; Rosin & Nyakatura, 2017; Stark, Fröber, & Schilling, 2013; Sullivan, McGechie, Middleton, & Holliday, 2019). To monitor potential bone movement throughout the dissection process, screws are placed in palpable bony landmarks as a reference for the 3D-reconstruction (Nyakatura & Stark, 2015; Stark et al., 2013), or the specimen is pinned to a surface with metal pins which are digitised for reference (Rosin & Nyakatura, 2017). This method of fascicle measurement produces a high-quality 3D-model of the muscle without the use of micro-CT or diceCT; however, this method requires a substantial amount of time (especially for larger muscles) and dissection skills (especially for the small muscles).

(d) Partial chemical digestion of the muscle belly is by far the most commonly used method in the literature for the measurement of fascicle length (e.g., Fabre, Herrel, Fitriana, Meslin, & Hautier, 2017; Herrel, De Smet, Aguirre, & Aerts, 2008; Leonard et al., 2019; Lowie et al., 2019; Lowie, Herrel, Abdala, Manzano, & Fabre, 2018); however, it must be noted this method provides a measurement of fibre length rather than fascicle length. Isolated muscle bellies are placed in nitric or sulphuric acid (10–30%) or acetic acid (when the other acids are unavailable; Leischner et al., 2018), which dissolves the intervening collagenous connective tissue and facilitates the separation of individual muscle fibres (Hartstone-Rose et al., 2012; Hermanson & Cobb, 1992; Hermanson & Hurley, 1990). The typical digestion period is 24–48 hr (Santana, Dumont, & Davis, 2010) with a minimum of 45 min to 12 hr (Bergmann & Hare-Drubka, 2015; Dickinson et al., 2019) or maximum 3–10 days (de Souza Junior et al., 2018; Law, Young, & Mehta, 2016). Digestion time is variable due to the concentration of acid used and the size and thickness of the muscles. Digesting muscles requires close attention to ensure muscles are not over-digested and therefore become too soft to use. Digestion is commonly left at room temperature (often an assumption, as many studies do not state temperature), although a few studies have stated they heat the muscles and acid at 60–70°C (Deutsch et al., 2019; Hartstone-Rose, Hertzig, & Dickinson, 2019). Following digestion, muscles are rinsed and placed in 50% aqueous glycerine solution for 1–2 hr before the muscle can be teased apart and individual muscle fascicles measured (Herrel et al., 2008). The disadvantages of using chemical digestion to measure fascicle lengths is that digestion can be a time-consuming process and that nitric/sulphuric acid is an expense to the study.

(e) Digital dissection using high-resolution diffusible iodine-based contrast enhanced computer tomography (DiceCT) or high-resolution micro-CT techniques can also be used to measure fibre lengths. While dissection or partial chemical digestion is destructive and irreversible, digital imaging technologies provide a method of non-destructively visualising the reconstructing fascicle architecture (Dickinson et al., 2019; Nyakatura, Baumgarten, Baum, Stark, & Youlatos, 2019). Specimens are first soaked in 10% sucrose dissolved in distilled water to minimise deformation, then immersed in a low concentration iodine solution (≤1.0–10%) (Dickinson et al., 2019; Gignac et al., 2016; Sullivan et al., 2019), which increases the density of the muscular tissues and increases the contrast between the muscles and the connective tissues. Soaking times can be extensive and vary substantially, depending on specimen size and the concentration of the staining solution. Invertebrates and vertebrate embryos with a low staining concentration ~~could~~ exhibit excellent contrast overnight (<24 hr), in comparison to larger vertebrates which take several weeks to months to stain with a low concentration of stain solution (Gignac et al., 2016), ~~with the~~ average soaking time between 4 and 7 weeks. After soaking specimens are CT scanned (Dickinson et al., 2019; Dickinson, Stark, & Kupczik, 2018; Fahn-Lai, Biewener, & Pierce, 2019). There are numerous programs that can be used to segment, visualise and quantify digital scans; free software such as 3Dslicer, ParaView, Trackvis, Cloud2, or licenced software such as Amira or Avizo. Software programs are available that digitally discriminate between individual fascicles (e.g., Amira, VGMax, BitPlane) and calculate fascicle lengths. Studies comparing gross dissection and digitally dissected fascicle lengths have found comparable results, with some muscles (e.g., more complex muscles such as temporalis) showing more variability than others (Dickinson et al., 2018; Kupczik et al., 2015). Free software tools are available for segmentation and visualisation; however, digital imaging still requires access to specialised equipment, can be expensive (access to machines and high-quality software) and is potentially time-consuming for large specimens due to the preparation process in comparison to other methods of collecting fascicle length via dissection. Digital scans are beneficial as the muscle topology is kept intact and the specimens are able to be returned to a wet collection and used for further research.

For methods b–e, typically a minimum of 5 to 10 fascicles/fibres are measured from different sites throughout the muscles to account for variation (Allen et al., 2014; Cox & Baverstock, 2016; Lamas et al., 2014; McGowan, Skinner, & Biewener, 2008; Moore et al., 2013; Payne et al., 2006; Rose et al., 2013; Williams, Wilson,

Rhodes, Andrews, & Payne, 2008). Ideally, superficial fascicles/fibres can be avoided as they tend to be longer than the deeper fascicles (Cheng & Scott, 2000; Kikuchi, 2010). Once fascicles are removed, the lengths are measured using a ruler or callipers (Martin, Travouillon, Sherratt, Fleming, & Warburton, 2019), or digitally measured after photography using programs such as ImageJ (Boettcher et al., 2019; Cox & Baverstock, 2016).

Arguably, either fascicle length by making an incision to expose deep fascicles, or by partial digestion to measure fibre lengths, are acceptable methods of collecting fascicle/fibre length. Large muscles are time consuming to digest and require large amounts of acid; therefore, dissection along the plane of the fascicles may be more economical. Layer-wise fascicle dissection is an extremely time-consuming method, therefore may only be suited to studies focussing few specific muscles, in comparison to an entire limb of muscles. Smaller muscles are easily digested and results in less damaged fibres, therefore digestion may be the best option for fibre length collection. Digital dissection is an excellent method to calculate PCSA without destroying specimens utilising iodine staining and micro-CT/ DiceCT, however, is remains expensive at this point in time and the soaking times for visualisation can be extensive, especially for larger specimens.

3.3 | Pennation angle

Pennation describes the arrangement of muscle fascicles attaching obliquely to the muscle tendon, compared to strap muscles where fascicles are arranged in parallel to the long axis of the muscle belly. The advantage of a pennate arrangement is increased numbers of fascicles within a muscle of a given volume (Azizi et al., 2008; Lee et al., 2015; McNeill, 1968). "Pennation angle" refers to the angle at which the muscle fascicles lies relative to the axis of force production, or "force-generating axis" (Gans & Bock, 1965) (see Figure 2b). Pennation angle has two main physiological effects on the relationship between force production and shortening velocity. An increase in the pennation angle results in greater PCSA by accommodating a greater cross-sectional area of muscle fascicles attaching to a tendon (i.e., more fascicles packed into an area). At the same time, more obtuse pennation angles will result in shorter fascicles (due to the arrangement of muscle origins and insertions) and consequently the muscle will have a slower maximum shortening velocity (Gans & Bock, 1965; Lieber & Fridén, 2000; Lorenz & Campello, 2012).

Measuring pennation angle once the muscles has been dissected involves measuring the angles from either high quality photographs (Channon, Günther, Crompton, & Vereecke, 2009; Martin, Warburton, et al., 2019; Olson et al., 2016), or using a protractor over the surface of the muscle belly (Allen et al., 2014; Butcher et al., 2019; de Souza Junior et al., 2018; McGowan et al., 2008; Rupert et al., 2015). This method of collecting pennation angle reduces the pennation angles to one plane. Multiple individual measures of angle are required to account for the internal variation of the muscle and therefore minimise measurement error (Scott & Winter, 1991). Measuring pennation angle from photographs has the advantages that it can be measured at a later date (i.e., it is not time dependent), and that it may utilise computer programs such as Image J (RRID:SCR_003070; freeware; Rueden et al., 2017), to measure from a high-quality photograph. Taking photographs that distinctly show the angles can be difficult, and also requires the muscle belly to be fully exposed and all covering fascia removed. The protractor on the surface of the muscle allows for quick measurement of the angle but may be less precise.

Many authors choose to exclude pennation angle from their PCSA calculation (e.g., Fabre et al., 2017; Furuuchi, Koyabu, Mori, & Endo, 2013; Goh et al., 2017; Hartstone-Rose et al., 2019; Hartstone-Rose, Deutsch, Leischner, & Pastor, 2018; Lowie et al., 2018). First, excluding pennation was justified as an angle below 30° has little effect on the calculation potential force as the cosine of the angle is typically close to 1: cos(10°) = 0.98, cos(20°) = 0.94, cos(30°) = 0.87 (Payne et al., 2006; Thorpe et al., 1999). However, Channon et al. (2009) recorded a maximum of 39° pennation in gibbon hindlimb muscles, and exclusion of pennation angle would therefore have resulted in a reduction of approximately 22% in the PCSA.

Second, measurements of pennation angle only capture the angle at a fixed time (i.e., resting pennation angle). As such, the cosine of the angle only captures the component of force that is acting in the direction of the principle line of action, as some force is not transmitted along the muscle axis (Blanco & Patek, 2014). This is dependent on the position the muscle was fixed in when measured.

Third, multipennate muscles present complex pennation measurements, and some authors have stated the musculature is too complex to accurately measure pennation (Furuuchi et al., 2013). For example, muscles change pennation angle dramatically across different gape angles, and this is also true for other complex muscles (Anapol & Barry, 1996). This causes a complicated variation imparted in the rotational complex which is difficult to resolve in comparison to a simpler linear contractile muscle found in other areas of the body. Therefore, to calculate the pennation angle in one plane is not an adequate measure for masticatory muscles, and it has been argued to be more accurate to calculate PCSA without pennation angle (Hartstone-Rose et al., 2018; Hartstone-Rose et al., 2019). Scott and Winter (1991) suggested the importance of a dynamic pennation angle (measuring pennation on moving muscle), which encompasses the angle of pennation through the range of movement of the muscle. This would be the most accurate measure of pennation, and therefore force production. in vivo recordings of the pennation angle at specific joint angles (muscle tensions and lengths) are now available via ultrasound sonography (Aagaard et al., 2001), although incorporation of these measurements is complex, and this approach is unavailable for cadaver or fixed samples.

The decision to include a measure of pennation angle in the calculation of PSCA is largely dependent on the specific muscle and the applications of the data. For muscles with a high pennation angle (i.e., greater than 30°), omission is likely to introduce relatively large error into the calculation and therefore pennation angle is an important component of the PCSA calculation. Complex muscles (e.g., masticatory muscles) should ~~also~~ have pennation omitted from

the PCSA calculation as the three dimensionalities of the system cannot be accounted for.

4 | PCSA IN MODELS VERSUS IN VIVO MEASURES OF FORCE

Though PCSA is widely used, few studies that have directly compared PCSA values calculated from cadaver specimens with in vivo measures of muscle force. This is partly because in vivo forces are difficult to measure, especially in live non-human specimens. Bite force is the most commonly reported in vivo force measures, but assumes that the animal is exerting the maximum force possible. Wild-caught specimens often aggressively snap at an object approaching them; however, this may not be the case in all species (Becerra, Echeverría, Vassallo, & Casinos, 2011; Davis, Santana, Dumont, & Grosse, 2010). Gape angle is the other limiting factor with in vivo bite forces, with wider gape angles expecting to generate lower bite forces in mammals (Santana, 2016).

Studies comparing PCSA via dissection and in vivo bite forces commonly find that PCSA is a good predictor of in vivo bite force (e.g., Davis et al., 2010; Ginot, Herrel, Claude, & Hautier, 2018; Herrel et al., 2008; Huber, Dean, & Summers, 2008; Kolmann, Huber, Motta, & Grubbs, 2015; Mara, Motta, & Huber, 2010; Meyers, Nishikawa, & Herrel, 2018; Santana et al., 2010; Van Daele, Herrel, & Adriaens, 2009), although a few studies obtained greater values for PCSA via dissection than in vivo measurements (Becerra et al., 2011; Becerra et al., 2014). To mimic the in vivo bite data, three-dimensional bite models or static bite force models are used to calculate the bite force from the PCSA via dissection. These models use 3D coordinates of the origin and insertion of the muscles, their PCSA, and the 3D coordinates of the point of application of the bite (Davis et al., 2010; Herrel, Aerts, & De Vree, 1998; Santana et al., 2010). These types of models allow for many iterations to be run to generate the optimum bite force, similar to when in vivo bite forces are measured at varied gape angles (Santana, 2016).

Limb systems are also commonly modelled in the literature to integrate the musculature and skeleton. These models can be musculoskeletal models which use muscle moment arms to investigate the effectiveness of a muscle(s) to contribute to a particular motion over a range of configurations, that is, muscle effectiveness in different movements of locomotion (Sherman, Seth, & Delp, 2013). These models can be built using PCSA values collected by physical dissection (Charles, Cappellari, Spence, Wells, & Hutchinson, 2016; Goh et al., 2017), or via digital dissection from micro-CT, CT, or magnetic resonance imaging (Blemker & Delp, 2005), or more commonly they are built using 3D scans or from published descriptions of the origins and insertions of muscles, and then estimated muscle forces are generated from the computer models (Regnault & Pierce, 2018). Finite element analysis predicts the performance of bone (and other structures) by quantifying the distribution of stresses and strains within a morphological structure (i.e., within the limb) that are caused by external forces (muscles; Dumont, Grosse, & Slater, 2009). The benefits of such modelling techniques can then be applied to skeletal remains of extinct species, and further, that these can be integrated with functional studies of extant species to provide a deeper understanding of the mechanical basis of their locomotion (Orr, 2016).

5 | PCSA BETWEEN SPECIES

The benefit of PCSA is that it can be used for intraspecific or interspecific studies to investigate variation, functional morphology, or ecomorphology. PCSA calculated using the same methodology means that direct comparisons between species can be made. As PCSA calculations are dependent on size (as muscle volume is a large component of the PCSA calculation) animals with a larger body masses and absolute larger muscles will have greater PCSAs. Implementing a correction for body mass can remove the effect of body mass and allow direct comparison of the relative distribution of musculature forces between species to investigate functional adaptation to their environment or ecology. A few methods of body mass correction are used in the literature. First is the use of residual values from a regression between the muscle properties (i.e., muscle mass, fascicle length or PCSA) and total body mass (Böhmer et al., 2019; Leischner et al., 2018). Second, data can be normalised to geometric similarity (i.e., lack of change in muscle properties with ontogenetic increase in body mass) by scaling the muscle properties by body mass (m_b); muscle mass by $m_b^{1.0}$, fascicle length by $m_b^{0.33}$, and PCSA by $m_b^{0.67}$ (Allen et al., 2010; Butcher et al., 2019; Dick & Clemente, 2016; Nyakatura et al., 2019; Olson, Glenn, Cliffe, & Butcher, 2018; Rupert et al., 2015). PCSA can also be used to produce a functional space plot, which is PCSA plotted against fascicle length (Allen et al., 2010; Böhmer et al., 2018; Butcher et al., 2019; Dick & Clemente, 2016; Olson et al., 2018; Payne et al., 2005). This will separate out the muscles that are specialised for different actions (i.e., force specialised: large PCSA and short fascicles; displacement specialists: small PCSA and long fascicles; powerful: large PCSA and long fascicles; and generalists: small PCSA and short fascicles) and is used to investigate the structure–function trade-off between PCSA and fascicle length for a muscle with a given volume (Böhmer et al., 2018). These functional space plots can be used to compare the function trade-offs between species of differing postures or locomotor behaviour.

6 | CONCLUSIONS AND RECOMMENDATIONS

This review summarises methods used to calculate PCSA. The method chosen to calculate PCSA is likely to depend on the preservation of the specimen (which will influence whether dry or wet mass is able to be measured) and the size of the target muscles (which will dictate the necessity to digest the intramuscular connective tissue or more simply to incise along the plane of the fascicles to expose the fascicles). Pennation angle is entirely dependent on the area of the body the muscle is taken from and therefore inclusion of pennation angle in PCSA

calculations should be considered; pennation angles should be included for muscles with simple linear contractile properties, but pennation angles below 30° make little difference to force estimates. Reviewing PCSA methods will help ensure researchers understand benefits and limitations of different approaches and can make informed decisions about their methods. This will ensure that PCSA calculations are comparable between studies and with as little error possible. If not using standard methods, sufficient detail needs to give in methodology to ensure researches can repeat your methods and be aware of the assumptions that have been made.

AUTHOR CONTRIBUTIONS

Meg L. Martin performed the literature search and led the writing of the manuscript. All authors conceived the idea of the review and contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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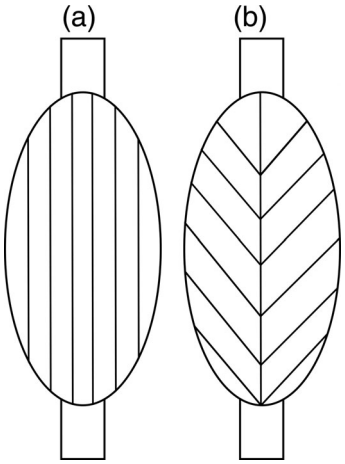

Discuss the theories behind physiological cross-sectional areas (PCSA) in a synthesised review that researchers can reference to make their own decisions on PCSA methodology.