REGULAR PAPER

Sex and male breeding state predict intraspecific trait variation in mouth-brooding fishes

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Abstract

Sex-specific reproductive roles contribute to sexual dimorphic morphological trait variations. In uniparental mouth-brooding fishes, the mouth performs a reproductive function in addition to its key roles in feeding and respiration, resulting in the potential for sex-specific functional performance trade-offs. Trait differences related to parental care may occur when the individual matures or be restricted to periods when the parent is mouth-brooding. This study explored sexual dimorphism and morphological trait adaptations related to feeding, breeding, respiration and locomotion performance in two paternal mouth-brooding freshwater fishes (*Glossamia aprion* and *Neoarius graeffei*). Eight morphological traits were evaluated for sexual dimorphism (non-brooder males *vs.* females) and male breeding state differences (brooders *vs.* non-brooders). Male breeding state was a significant predictor of trait variation in both species. Brooders differed in buccal volume and in several feeding and locomotory traits compared to non-brooder males. Non-brooder males had bigger buccal volumes and relative eye diameters (*G. aprion*) and larger relative gape sizes (*N. graeffei*) compared to females, a potential response to both mouth-brooding and feeding requirements. Although there were clear trait differences between brooder and non-brooder males, further research is required to confirm whether individuals return to their former morphology once mouth-brooding has ceased or if trait differences are maintained post-brooding. This study highlights the importance of considering the potential impacts of intraspecific trait variation on the performance of critical life functions, such as feeding, respiration and locomotion across the life history.

KEYWORDS
freshwater, functional ecology, northern Australia, parental care, sexual dimorphism

1 | INTRODUCTION

Sexual dimorphism typically reflects trait adaptations in males and females that enhance species' lifetime reproductive success (Hedrick &

Temeles, 1989; Lande, 1980; Shine, 1989). Teleost fishes exhibit a range of secondary sexually dimorphic traits (*i.e.*, character traits that are not directly used in reproduction) to complement their different reproductive styles, mating behaviours and life histories (Amundsen, 2003; Parker, 1992). Sexual size dimorphism and colouration (sexual dichromatism) may increase female fecundity and male mating opportunities through sexual selection, respectively (Blanckenhorn, 2005; Kodric-Brown, 1998; Parker, 1992). Species may also exhibit sexual dimorphism to avoid resource competition between males and females through niche divergence (Hedrick & Temeles, 1989; Lande, 1980; Shine *et al.*, 2002). Whereas sexual size dimorphism and dichromatism are well studied (*e.g.*, Kodric-Brown, 1998; Marshall *et al.*, 2019; Parker, 1992), dimorphism related to post-mating reproductive strategies, such as parental care, has received less attention (but see Barnett & Bellwood, 2005; Hoey *et al.*, 2012; Tkint *et al.*, 2012). Sex-specific reproductive roles are also considered to contribute to morphological trait variation between males and females (*e.g.*, Bakker & Mundwiler, 1999; Casselman & Schulte-Hostedde, 2004). Despite parental care playing a significant role in individual fitness and reproductive success, sexual dimorphic trait variation related to parental care is rarely considered in functional ecological studies that have correlated morphological traits with ecomorphological functions in fishes (*e.g.*, Luiz, Olden, *et al.*, 2019; Toussaint *et al.*, 2016; Villéger *et al.*, 2017).

Mouth-brooding is a type of parental care in fishes where parents incubate eggs and/or larva in the buccal cavity to provide a safe environment for improved offspring growth and survival (*e.g.*, Corrie *et al.*, 2008; Keenleyside, 1991; Oppenheimer, 1970). Mouth-brooding can be uniparental (*i.e.*, performed exclusively by the male or the female) or biparental (performed by both sexes) (Blumer, 1982; Keenleyside, 1991) and may impose costs to the caring parent, requiring behavioural (Oliveira & Almada, 1998), physiological (Keller *et al.*, 2017; Reardon & Chapman, 2010) and/or morphological trait adaptations (Barnett & Bellwood, 2005; Ronco *et al.*, 2019; Tkint *et al.*, 2012). The head region of fishes performs multiple key functions, including feeding, respiration and visual perception for feeding and predator avoidance. Head shape also plays a role in locomotory performance, as it serves as a bow for streamlined swimming to decrease drag in the water (Tkint *et al.*, 2012; Van Wassenbergh *et al.*, 2015). Due to the postulated constructional limitations in the head region, optimising mouth-brooding in combination with other functions may impose performance costs on feeding, locomotion and/or respiration potentially leading to functional trade-offs (Barel, 1983; Hulsey *et al.*, 2007; Tkint *et al.*, 2012). Therefore, morphological adaptations related to feeding, respiratory and locomotion performance could be expected to occur in mouthbrooders, or if mouth-brooding is uniparental, morphological adaptations could be sexually dimorphic and present only in the caring sex (*e.g.*, Herler *et al.*, 2010; Hoey *et al.*, 2012; Ronco *et al.*, 2019).

In some uniparental mouth-brooding fishes, the sexes demonstrate sexual dimorphism in morphometry and osteology of the head-jaw region (*e.g.*, Herler *et al.*, 2010; Hoey *et al.*, 2012; Oliveira & Almada, 1995). Nonetheless, whether mouth-brooding leads to other morphological differences (*e.g.*, morphological or functional traits) as

well as performance trade-offs in the adult carer is still largely speculative (but see Tkint *et al.*, 2012; Van Wassenbergh *et al.*, 2015). Some studies have also suggested that the adult mouth-brooding carer can undergo temporary internal and external trait changes only while incubating eggs (*e.g.*, Butler *et al.*, 2017; Okuda *et al.*, 2002; Van Wassenbergh *et al.*, 2015). In the paternal mouth-brooding cardinalfish *Apogon doederleini* (Jordan and Snyder 1901), males differ from females in the morphometric measures of the head-jaw region, including the buccal cavity, during the breeding period but not in the non-breeding season (Okuda *et al.*, 2002). The female mouth-brooding cichlid *Astatotilapia burtoni* (Günther 1894) displays compartmentalised swimbladders compared to non-brooders of the same sex at the same time, presumably to compensate for the weight of the eggs in the buccal cavity (Butler *et al.*, 2017). Nonetheless, morphological trait flexibility and the temporal longevity of these traits are poorly understood and are important to develop the understanding of the fitness costs to adults of mouth-brooding in fishes.

In this study, the authors explore sexual dimorphism and morphological trait adaptations related to feeding, locomotion, respiration and breeding functions in two paternal mouth-brooding fishes, the mouth almighty *Glossamia aprion* (Richardson 1792) and blue catfish *Neoarius graeffei* (Kner and Steindachner 1867). They explicitly test in both species for (a) morphological variation between sexes, *i.e.*, sexual dimorphism between females and males that were not brooding eggs (hereafter termed "non-brooder males"), and (b) morphological variation between mouth-brooding males (hereafter termed "brooder males") and non-brooder males. They predict that (a) due to the added mouth-brooding function in only males, non-brooder males will exhibit morphological trait differences when compared to females as an adaptation when mouth-brooding is performed, and (b) because the suggested mechanism for mouth-brooding is temporal flexibility (expansibility) of the buccal cavity region during brooding (Barnett & Bellwood, 2005; Okuda *et al.*, 2002), brooder males will exhibit morphological trait differences when compared to non-brooder males (see Table 1 for specific hypotheses predictions).

2 | MATERIALS AND METHODS

2.1 | Study species

G. aprion (Perciformes: Apogonidae) is a small predatory, nocturnal, freshwater fish. The average length at maturity is *c.* 60 mm for males and *c.* 70 mm for females (Bishop *et al.*, 2001). It is widely distributed in northern Australia and southern New Guinea (Pusey *et al.*, 2017). This species is a paternal mouthbrooder, where males incubate eggs and larvae in their buccal cavity for 2–3 weeks (Fauth, 2017; Pusey *et al.*, 2004). *G. aprion* is reported to show sexual dichromatism during the breeding period (Fauth, 2017; Pusey *et al.*, 2004) and exhibit intraspecific variation in morphological traits as a response to habitat variability and predominant flow conditions (Abecia *et al.*, 2018).

N. graeffei (Siluriformes: Ariidae) is a moderate- to large-sized omnivorous fish with an average length at maturity of *c.* 270 mm for

TABLE 1 List of morphological traits with their formula, function, functional performance implications and trait predictions for sexual dimorphism and male breeding state

Trait	Formula	Trait function	Implications for mouth-brooding, feeding, locomotory and respiratory performance	Trait predictions
Body lateral shape	$\frac{Hd}{Bd}$	Determines head size, hydrodynamism and swimming performance ability (*e.g.*, fast-start movements, steady swimming and manoeuvrability)	Mouth-brooding may require an increase in head size for a larger buccal cavity space to facilitate brooding and efficient respiration (*e.g.*, Herler *et al.*, 2010; Hoey *et al.*, 2012; Ostlund-Nilsson & Nilsson, 2004) Variation in this trait may impact swimming ability (*e.g.*, streamlining/drag reduction: Van Wassenbergh *et al.*, 2015)	Non-brooders compared to females will have higher values due to larger buccal volume and head size for mouth-brooding[a] Brooders compared to non-brooders will have higher values due to an enlarged buccal cavity when mouth-brooding[b]
Relative gape size	$\frac{Mh \times Mw}{SL^2}$	Determines the maximum prey size that can be swallowed and therefore indicative of the size range of prey that can be swallowed	Mouth-brooding may require higher mouth height, longer relative maxillary length and bigger buccal cavity relative to standard length (*e.g.*, Hess, 1993; Almada and Oliveira, 1995; Barnett & Bellwood, 2005; Herler *et al.*, 2010; Hoey *et al.*, 2012) Variation in these traits can affect the size range and types of prey that can be eaten	Non-brooders compared to females will have larger gape size, longer maxillary length and higher oral shape index[a] Brooders will have a larger relative gape size and vertically oriented oral gape shape and larger relative maxillary length due an expanded buccal cavity when compared to non-brooders[b]
Relative maxillary length	$\frac{Jl}{SL}$	Estimates the strength of the jaw and the size of the mouth in lateral view, therefore determines the maximum size or size range of prey that can be swallowed		
Oral gape shape	$\frac{Mh}{Mw}$	Determines the shape of prey eaten		
Relative eye size	$\frac{Ed}{SL}$	Determines light sensitivity or visual acuity when finding prey and may indicate the time of foraging (diurnal *vs.* nocturnal)	Mouth-brooding requires a dorsal shift in eye position and reduced eye size to accommodate for a larger buccal cavity (*e.g.*, Barel, 1983; Herler *et al.*, 2010)	Non-brooders will have smaller relative eye size relative to standard length and a more dorsal eye and oral gape position relative to body depth when compared to females due to a bigger buccal cavity[a]
Eye vertical position	$\frac{Eh}{Bd}$	Indicates fish position in the water column (*e.g.*, surface *vs.* benthos) when feeding in the water column therefore may indicate the position of prey items; *e.g.*, more dorsal eye position is associated with surface feeding	Variation in these traits can affect the size range and types of prey that can be eaten. In addition, variation in relative eye size can affect the ability to detect prey and avoid predators (Fernald, 1990)	Brooders will have higher values (more dorsal) for eye vertical and oral gape position but lower values for relative eye size when compared to non-brooders due to an expanded buccal cavity during mouth-brooding[b]
Oral gape position	$\frac{Mo}{Bd}$	Indicates fish position in the water column (surface *vs.* benthos) when feeding in the water column, therefore may indicate the position of prey items; *e.g.*, more dorsal gape position is associated with surface feeding		
Buccal volume	$\frac{Buccal\ volume}{SL^3}$	Determines the number of eggs individuals can brood in their mouth (reproductive potential)	Larger buccal volume increases male reproductive potential and is advantageous for egg churning and respiration during mouth-brooding (*e.g.*, Barnett & Bellwood, 2005; Herler *et al.*, 2010; Hess, 1993; Okuda *et al.*, 1998)	Non-brooders will have larger buccal volumes for mouth-brooding when compared to females[a] Brooders compared to non-brooders will have bigger buccal volumes due to an enlarged buccal cavity[b]

Notes: Adapted from Luiz, Crook, *et al.* (2019), Toussaint *et al.* (2016) and Villéger *et al.* (2017)

Morphological measurements: standard length (SL), mouth height (Mh), mouth width (Mw), body depth (Bd), eye height (Eh), eye diameter (Ed), head depth (Hd), maxillary jaw length (Jl) and oral gape position (Mo).

[a]Denotes predictions in table for sexual dimorphism.

[b]Denotes predictions in table for breeding state.

males and c. 285 mm for females (Pusey et al., 2004). This species is widespread across northern Australia and in New Guinea (Pusey et al., 2017). N. graeffei is a paternal mouthbrooder that incubates its eggs and larvae in its buccal cavity for 6–8 weeks (Pusey et al., 2004; Rimmer, 1985a). The pelvic-fin shape is sexually dimorphic, with males having a narrower pelvic fin and females having a rounded fin base (Pusey et al., 2004; Rimmer, 1985b). The rounded pelvic fins of females are considered to act as claspers for holding the partner closely during mating or as a holding site during the transfer of eggs to the male's buccal cavity. These fins are observed to thicken and enlarge before the spawning season (Pusey et al., 2004; Rimmer & Merrick, 1983).

2.2 | Sample collection and processing

Samples of G. aprion and N. graeffei were collected from 14 coastal draining rivers in the Northern Territory (see Appendix S1 for the list of rivers and numbers of individual fish collected). This region is in a wet-dry tropical savannah climate, driven by the equatorial southern monsoonal weather systems, generating highly seasonal and summer-dominated rainfall occurring mostly during November to April (Warfe et al., 2011).

Individuals were collected by electrofishing (boat and backpack) on four sampling occasions and seasons during 2016–2019 (early dry August 2016, post wet May–June 2016, late dry October–November

2016/2017/2019 season). A total of 385 G. aprion and 126 N. graeffei individuals were collected and measured. Fish were euthanised in an overdose solution of AQUI-S (>175 mg l^{-1} for 20 min), labelled and bagged individually; kept in an ice slurry in the field; and then frozen until further analysis. In the field, brooder males were differentiated by the presence of eggs or larvae in their mouth. If a mouth-brooding male was collected, the eggs were carefully removed from the buccal cavity and placed in a small bag, which was then stored with the brooding adult in an individual bag for further analysis.

In the laboratory, the samples were thawed, allowed to soften and dried with a paper towel. All samples were then photographed using a digital Nikon Camera model D500, with each individual fish laid flat on a board with its left side up and fins arranged and pinned in a way displaying its natural position and form. A number of morphological measurements were performed (Figure 1) to calculate the morphological traits based on previous studies (Table 1; Toussaint et al., 2016; Villéger et al., 2017; Luiz, Crook, et al., 2019). The authors used these measurements to derive the most used morphological traits in freshwater fish functional studies (Brosse et al., 2021; Su et al., 2021). Some measurements were first made directly on specimens: weight (W) to the nearest 0.01 g using a precision balance, standard length (SL), mouth height (Mh) and mouth width (Mw) using vernier callipers to the nearest 0.1 mm. Additional morphological measurements such as body depth (Bd), eye height (Eh), eye diameter (Ed), head depth (Hd), maxillary jaw length (Jl) and oral gape position (Mo) were extracted from each
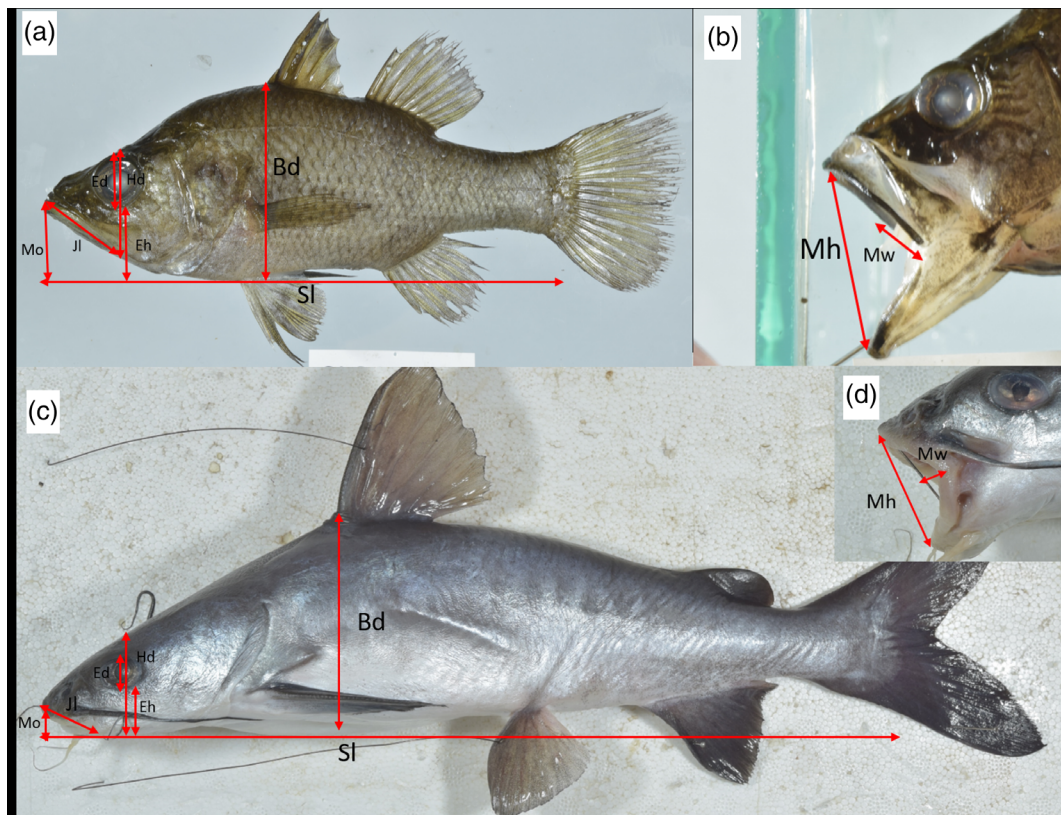
FIGURE 1 Body measurements taken in each fish sample and used in Table 1: standard length (SL), mouth height (Mh), mouth width (Mw), body depth (Bd), eye height (Eh), eye diameter (Ed), head depth (Hd), maxillary jaw length (Jl) and oral gape position (Mo) on (a, b) Glossamia aprion and (c, d) Neoarius graeffei

individual's photograph using the image editor ImageJ (Rueden et al., 2017).

The volume of the buccal cavity was determined using a volumetric bead method adapted from Hess (1993). Small plastic beads, with a diameter of 5 mm for *G. aprion* and 8 mm for *N. graeffei*, were used to completely fill and measure the size of the buccal cavity. Buccal cavity was filled until the lips can still normally close over to ensure that no beads were forced into the oesophagus (adapted from Hess, 1993). Bead weight was recorded, and the volume of the buccal cavity was estimated by multiplying the weight of the beads used to fill the buccal cavity with a conversion factor. The conversion factor was calculated by measuring the weight of the beads required to fill a 5 ml graduated cylinder, allowing the total volume occupied by the beads (beads and interstitial space) to be estimated (Hess, 1993). To account for the effect of body size differences among individuals, buccal cavity volume was standardised to SL^3, following Barnett and Bellwood (2005).

The samples were then dissected, sexed and categorised as females, non-brooder males or brooder males, resulting in the following sample numbers: *G. aprion* 190 females, 25 brooders and 170 non-brooders; *N. graeffei* 63 females, 15 brooders and 48 non-brooders.

2.3 | Statistical analysis

Linear mixed effect models were used to test for differences in morphological traits across the three groups: (a) females, (b) brooder males and (c) non-brooder males. Because female individuals do not mouth brood, using these level combinations allows us to test the effects of sex (female *vs.* non-brooder male) and breeding state (brooder male *vs.* non-brooder male) in a single model, improving the power of our analysis. The authors then validated every model by inspecting (a) the scatterplot of residuals *vs.* fitted values to verify homogeneity (see Appendices S2 and S3) and (b) the histogram of the residuals for normality (Zuur et al., 2009). Site of collection was included as a random factor. The analyses were performed using the lmerTest package (Kuznetsova et al., 2018) in R 3.3.3 software (R Development Core Team, 2019).

2.4 | Ethical statement

This research project was approved by the Charles Darwin University Animal Ethics Committee (permit numbers: A16032 and A19022).

3 | RESULTS

3.1 | Prediction 1 – morphological trait variation between sexes, *i.e.*, sexual dimorphism between females and non-brooder males

The relationship of each morphological (body) measure to SL and body depth showed no evidence of strong allometry for *G. aprion* and *N. graeffei* non-brooder males and females (see Supporting Information

Appendices S4 and S5). Nonetheless, head depth was significantly different between non-brooders and females in *G. aprion* but not in *N. graeffei*. SL was also significantly different between non-brooder males and females in both *G. aprion* and *N. graeffei* (see Appendix S7).

The sexual dimorphic traits exhibited by both study species were species specific. *G. aprion* was sexually dimorphic in two of the eight traits examined (Figure 2; Table 2; Supporting Information Appendices S8 and S10). Non-brooder males had significantly larger buccal volumes and bigger eye diameters relative to SL compared to females. All the other morphological traits, namely body lateral shape, relative gape size, oral gape position, eye vertical position and relative maxillary length, showed no difference between non-brooders and females.

Sexual dimorphism in traits was also detected in *N. graeffei* but only in relative gape size (Figure 3; Table 3; Supporting Information Appendices S9 and S11). Non-brooder males had higher relative gape size values (*i.e.*, wider mouth dimensions relative to SL) compared to females. Contrary to authors' prediction, *N. graeffei* non-brooders
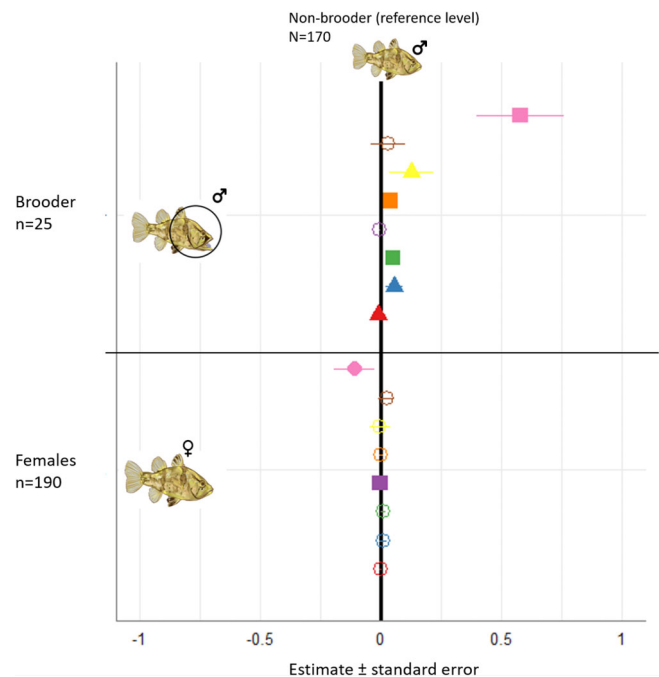
FIGURE 2 Estimate coefficients ± s.e. of the linear mixed effect models of *Glossamia aprion* with site as random factor, combined sex–breeding state category as fixed factor (levels = non-brooder males, brooder males and females) and morphological traits as dependent variables: buccal volume, relative gape size, oral gape shape, body lateral shape, relative eye size, eye vertical position, oral gape position and relative maxillary length. The plot shows the non-brooder male level as the reference level in comparison to brooder males (upper panel) and females (lower panel). *P*-values for each trait are indicated as (○) not significant, (●) <0.05, (▲) <0.01 and (■) <0.001. Circle indicates the presence of the eggs in the mouth. Morphological traits: ─●─, bucal_volume; ─●─, relative_gape_size; ─●─, oral_gape_shape; ─●─, body_lateral_shape; ─●─, relative_eye_size; ─●─, eye_vertical_position; ─●─, oral_gape_position; ─●─, relative_maxillary_length. *P*-level: ○, n.s.; ●, *; ▲, **; ■, ***

TABLE 2 Morphological trait coefficients in *Glossamia aprion* using a linear mixed effect model with site as random factor and combined sex–breeding state category (levels = non-brooder males, brooder males and females) as fixed factor

Trait	Estimate	s.e.	df	t-Value	P-value	Response (prediction)
Buccal volume						
Non-brooders *vs.* **females**	−0.110	0.043	376.781	−2.530	0.012 (<0.05*)	−(−)
Non-brooders *vs.* **brooders**	0.579	0.092	381.610	6.291	<0.001*	+(+)
Relative gape size						
Non-brooders *vs.* **females**	0.001	0.001	377.700	1.133	0.285	NS(−)
Non-brooders *vs.* **brooders**	0.001	0.002	374.300	0.720	0.472	NS(+)
Oral gape shape						
Non-brooders *vs.* **females**	−0.009	0.032	374.263	−0.291	0.771	NS(−)
Non-brooders *vs.* **brooders**	0.169	0.069	380.131	2.438	0.015 (<0.05*)	+(+)
Body lateral shape						
Non-brooders *vs.* **females**	0.002	0.004	378.500	0.429	0.668	NS(−)
Non-brooders *vs.* **brooders**	0.040	0.008	366.400	4.905	<0.001*	+(+)
Relative eye size						
Non-brooders *vs.* **females**	−0.004	0.001	373.239	−3.533	<0.001*	−(+)
Non-brooders *vs.* **brooders**	−0.004	0.002	377.387	−1.699	0.090	NS(−)
Eye vertical position						
Non-brooders *vs.* **females**	0.008	0.006	379.900	1.394	0.164	NS(−)
Non-brooders *vs.* **brooders**	0.051	0.012	351.800	4.337	<0.001*	+(+)
Oral gape position						
Non-brooders *vs.* **females**	0.009	0.008	379.700	1.137	0.256	NS(−)
Non-brooders *vs.* **brooders**	0.055	0.017	358.600	3.240	<0.001*	+(+)
Relative maxillary length						
Non-brooders *vs.* **females**	−0.004	0.008	377.981	−0.587	0.558	NS(−)
Non-brooders *vs.* **brooders**	−0.045	0.016	380.553	−2.793	<0.001*	−(+)

Notes: Estimate coefficient values apply to levels in bold font. Asterisk (*) indicates significant differences at *P* < 0.05, (NS) not significant, (+) increase or (−) decrease in trait values relative to non-brooders.

were not sexually dimorphic in buccal volume. All the other morphological traits were comparable between the two groups.

3.2 | Prediction 2 – morphological trait variation between brooder males and non-brooder males

The relationship of each morphological (body) measure to SL and body depth showed no evidence of strong allometry for *G. aprion* brooder and non-brooder males (see Appendix S4). Nonetheless, *N. graeffei* brooders showed negative allometry for mouth width, mouth height and head depth relative to SL compared to non-brooder males (see Appendix S5). Twelve of the 15 *N. graeffei* brooders were sampled at one collection date (November 2019) in the Mary River, whereas the rest of the *N. graeffei* samples were collected in 2016–2017 across different rivers. Moreover, head depth and SL were significantly different between brooder and non-brooder males in both *G. aprion* and *N. graeffei* (see Appendix S7).

Brooder and non-brooder males differed significantly in several morphological traits in both species (Figures 2 and 3; Tables 2 and 3). *G. aprion* brooders were significantly different from non-brooder males in six of the eight traits examined except for relative gape size and relative eye size (Figure 2; Table 2; Supporting Information Appendices S8 and S10). Brooder males had significantly larger buccal volumes than non-brooder males. The body lateral shape of brooders was also greater than non-brooders where brooders had larger heads relative to body depth. Oral gape shape, eye vertical position and oral gape position were significantly higher in brooders than non-brooders, whereas the relative maxillary length was higher in non-brooders. Overall, *G. aprion* brooders had larger heads, higher eye and mouth positions relative to body depth, shorter jaws relative to SL and bigger buccal cavities compared to non-brooder males (see Appendix S8).

Brooder and non-brooder males of *N. graeffei* significantly differed in four of the eight morphological traits examined (Figure 3; Table 3; Appendices S9 and S11). Similar to *G. aprion*, *N. graeffei* brooders had significantly larger buccal volumes and higher body
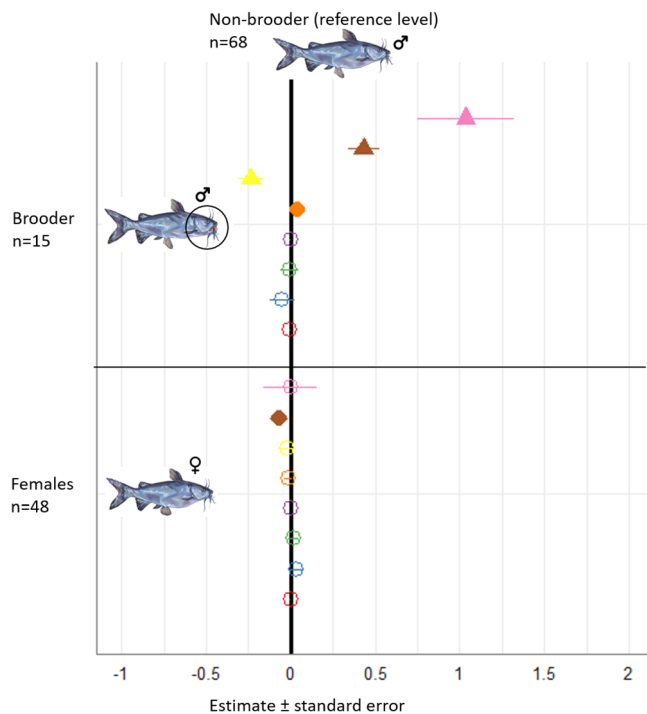
FIGURE 3 Estimate coefficients ± s.e. of the linear mixed effect models of *Neoarius graeffei* with site as random factor, combined sex–breeding state category as fixed factor (levels = non-brooder males, brooder males and females) and morphological traits as dependent variables: buccal volume, relative gape size, oral gape shape, body lateral shape, relative eye size, eye vertical position, oral gape position and relative maxillary length. The plot shows the non-brooder male level as the reference level in comparison to brooder males (upper panel) and females (lower panel). *P*-values for each trait are indicated as (○) not significant, (●) <0.05 and (▲) <0.001. Circle indicates the presence of the eggs in the mouth. Morphological traits: —●—, bucal_volume; —●—, relative_gape_size; —●—, oral_gape_shape; —●—, body_lateral_shape; —●—, relative_eye_size; —●—, eye_vertical_position; —●—, oral_gape_position; —●—, relative_maxillary_length. *P*-level: ○, n.s.; ●, *; ▲, ***

lateral shape trait values relative to non-brooder males. *N. graeffei* brooders showed an increase in relative gape size and a decrease in oral gape shape trait values relative to non-brooders. *N. graeffei* brooders had bigger and horizontally wider mouth gapes relative to body size, larger heads relative to body depth and bigger buccal cavities compared to non-brooders (see Appendix S9).

4 | DISCUSSION

The two paternal mouth-brooding species examined in this study demonstrated evidence of both sexually dimorphic and breeding-specific morphological adaptations in traits associated with feeding, locomotion and breeding. Brooder males of both species differed in several traits compared to non-brooder males, indicating that breeding state was an important driver of morphological intraspecific trait variation. Trait differences between brooder and non-brooder males of

both species also suggest that there are temporary changes in morphological traits occurring during parental care. *G. aprion* was sexually dimorphic in relative eye size and buccal volume, with non-brooding males having increased relative eye sizes and buccal volumes compared to females. *N. graeffei* was sexually dimorphic only in relative gape size, with males having larger relative gape sizes than females. Sexual dimorphism in traits is likely adaptations to minimise the trade-off between mouth-brooding and feeding performance, but specific trait variations may be a response to the species' inherent feeding requirements (*e.g.*, Hoey *et al*., 2012; Ronco *et al*., 2019; Tkint *et al*., 2012). In past studies, sexual dimorphism in the morphology of uniparental mouthbrooders has been reported to potentially compromise the brooder's feeding ability (*e.g.*, bite force reduction, Tkint *et al*., 2012) as well as swimming performance (*e.g.*, streamlining, Van Wassenbergh *et al*., 2015) due to the added function of mouth-brooding.

The two studied species showed species-specific differences in sexual dimorphic traits, despite sharing the same specialised mouth-brooding strategy. These differences may be a reflection of their contrasting feeding behaviour (*e.g.*, Tkint *et al*., 2012) and dietary preferences (*e.g.*, Hoey *et al*., 2012). *N. graeffei* is a generalist omnivorous species; male brooders may take advantage of greater gapes allowing them to brood more and/or larger fertilised eggs while keeping their ability to utilise a wider range of prey sizes or food items in their diet (Hoey *et al*., 2012; Luiz, Crook, *et al*., 2019; Villéger *et al*., 2017). *G. aprion*, on the contrary, is a specialised nocturnal ambush carnivore (Pusey *et al*., 2004). A larger buccal volume may enhance the male brooders' capacity to hold a large number of eggs, while the larger relative eye size may improve their visual ability to find and capture prey (Bellwood *et al*., 2014; Hess, 1993; Schmitz & Wainwright, 2011) as mouth-brooding can be reproductively costly to brooding *G. aprion* males (Abecia *et al*., 2021).

Two potential mechanisms could explain the morphological trait variations between brooder and non-brooder males recorded in the species of this study: (a) that non-brooders had not bred before and consequently are not demonstrating traits required for breeding or (b) that the altered traits associated with the brooding state are temporary changes during breeding. The first mechanism is plausible because the apparent differences in traits associated with breeding may not appear until maturity or only once breeding has commenced (*e.g.*, Rimmer, 1985b). In the current study, the authors are unable to determine whether males classified as non-brooding have bred previously. Nonetheless, although a number of non-brooder males in this study were immature (68 of 170 for *G. aprion* and 13 of 63 for *N. graeffei*), when these individuals are removed from the analysis, brooders still differed significantly in several morphological traits compared to non-brooders (see Appendices S6, S12 and S13). Based on this, the authors suggest that temporary trait change is the more likely explanation.

Previous studies have reported temporary changes in buccal morphology, including greater soft tissue flexibility and lower jaw expansibility, during mouth-brooding (Barnett & Bellwood, 2005; Okuda *et al*., 2002). In the current study, the buccal cavity volumes of both

TABLE 3   Morphological trait coefficients in *Neoarius graeffei* using a linear mixed effect model with site as random factor and combined sex–breeding state category (levels = non-brooder males, brooder males and females) as fixed factor

Trait	Estimate	S.E.	df	t-Value	P-value	Response (prediction)
Buccal volume						
Non-brooders *vs.* **females**	−0.002	0.082	119.821	−0.019	0.985	NS(−)
Non-brooders *vs.* **brooders**	1.034	0.146	122.999	−7.067	<0.001*	+(+)
Relative gape						
Non-brooders *vs.* **females**	−0.066	0.028	121.430	−2.357	0.02 (<0.05*)	−(−)
Non-brooders *vs.* **brooders**	0.431	0.048	93.692	9.030	<0.001*	+(+)
Oral gape shape						
Non-brooders *vs.* **females**	−0.024	0.022	122.116	−1.071	0.286	NS(−)
Non-brooders *vs.* **brooders**	−0.235	0.037	83.738	−6.361	<0.001*	−(+)
Body lateral shape						
Non-brooders *vs.* **females**	−0.012	0.012	122.413	−0.990	0.324	NS(−)
Non-brooders *vs.* **brooders**	0.040	0.019	28.526	2.135	0.042 (<0.05*)	+(+)
Relative eye size						
Non-brooders *vs.* **females**	−0.0004	0.001	123.000	−0.676	0.500	NS(+)
Non-brooders *vs.* **brooders**	−0.001	0.001	123.000	−1.368	0.174	NS(−)
Eye vertical position						
Non-brooders *vs.* **females**	0.018	0.016	119.541	1.135	0.258	NS(−)
Non-brooders *vs.* **brooders**	−0.004	0.028	121.877	−0.156	0.876	NS(+)
Oral gape position						
Non-brooders *vs.* **females**	0.033	0.021	119.711	1.567	0.120	NS(−)
Non-brooders *vs.* **brooders**	−0.049	0.037	120.863	−1.315	0.191	NS(+)
Relative maxillary length						
Non-brooders *vs.* **females**	−0.002	0.002	123.000	−1.401	0.164	NS(−)
Non-brooders *vs.* **brooders**	−0.004	0.002	123.000	−1.517	0.132	NS(+)

Notes: Estimate coefficient values apply to levels in bold font. Asterisk (*) indicates significant differences at $P < 0.05$, (NS) not significant, (+) increase or (−) decrease in trait values relative to non-brooders.

G. aprion and *N. graeffei* brooders were significantly larger than those of non-brooder males as well as females, suggesting that a larger buccal volume is required for parental caring obligations in these species. The caring sex of some uniparental mouth-brooding species has comparable buccal volumes to the non-caring sex when not brooding eggs (Barnett & Bellwood, 2005; Okuda *et al.*, 2002). Males of *A. doederleini*, a paternal mouthbrooder confamilial to *G. aprion*, exhibited larger buccal volumes and differed in several buccal cavity morphometric measurements during the breeding period compared to females, suggesting plasticity (or expansibility) in buccal cavity morphometry (Okuda *et al.*, 2002). The results for *A. doederleini* are quite similar to the present study where several morphological traits of *G. aprion* and *N. graeffei* brooders differed significantly from females, whereas a comparison between non-brooder males and females showed only minimal trait differences (only one to two traits). As with the current study, the measurements made on *A. doederleini* during the breeding and non-breeding periods were taken on different individuals, so whether these traits change through time is still not proven for this species (Okuda *et al.*, 2002) or the species of the present

study. Theoretically, temporary alteration in morphological traits during mouth-brooding would be more advantageous than a permanent change in morphology as the traits required for successful mouth-brooding may not necessarily result in optimum feeding (*e.g.*, Barnett & Bellwood, 2005; Hoey *et al.*, 2012; Tkint *et al.*, 2012).

An enlarged buccal cavity volume provides several advantages to the adult carer when brooding, such as increasing the reproductive potential or the number of eggs that they can brood (Hess, 1993), improving churning (mixing) and aeration of brooded eggs (Hess, 1993; Okuda *et al.*, 1998; Van Wassenbergh *et al.*, 2016) and promoting efficient respiration of the brooding adult (Ostlund-Nilsson & Nilsson, 2004). Nonetheless, the increase in the dorso-ventral height of the head (represented by higher values for body lateral shape) due to buccal cavity enlargement in both *G. aprion* and *N. graeffei* during parental care may affect the brooder's increased drag in the water, *i.e.*, streamlining (Van Wassenbergh *et al.*, 2015). Several studies have suggested that locomotory performance (*e.g.*, burst swimming, fast-start movements and sustained swimming) and aerobic swimming capacity could be compromised while mouth-

brooding (Ostlund-Nilsson & Nilsson, 2004; Schurch and Taborsky, 2005; Van Wassenbergh *et al.*, 2015).

Overall, the authors showed that mouth-brooding involves not only species-specific sexual dimorphism but also morphological trait differences when breeding. They suggest that the most likely mechanism for the trait variation between brooders and non-brooders in their study is temporary alteration in morphology, most obviously the enlargement of the buccal cavity during mouth-brooding to facilitate successful brooding. Given that the male head performs multiple critical functions such as feeding, brooding, respiration and visual acuity for predators, they suggest it is more likely and advantageous that any traits required for breeding are temporary during the breeding period only. Nonetheless, further research is needed to evaluate whether these potential temporary morphological trait differences also occur in other uniparental mouth-brooding species and if the results of past studies that reported on sexual dimorphism were influenced by a mixture of breeding and non-breeding individuals combined in one category. Investigation of the mechanism, as well as possible ecological selection pressures driving trait differences between brooders and non-brooders, is also required. Authors' results also supported the *a priori* prediction that uniparental mouth-brooding is associated with sexual dimorphism in morphological traits. The sexually dimorphic traits between non-brooder males and females for both species seemingly confer both feeding and breeding advantages to the caring sex. Nonetheless, one must interpret the results of *N. graeffei* with caution because of its small sample size. Moreover, 12 out of the 15 *N. graeffei* brooders were collected on a single day and in one site (Mary River), whereas the *N. graeffei* females and non-brooding males were collected during four sampling trips over 1 year (2016–2017) and across different rivers. Further investigation of brooding males sampled over a broader time and spatial scale would help elucidate whether the negative allometric relationship of some of the body measures to SL in this species occurs in other brooder males. Behavioural studies relating morphology to feeding performance in uniparental mouthbrooders would help decipher whether such a link exists. The authors' findings support the growing importance of considering intraspecific variation, including information on sex-specific reproductive roles, in functional ecology research.

AUTHOR CONTRIBUTIONS

J.E.A. was involved in the generation of the concept, laboratory work, data analysis, writing and manuscript preparation.

A.J.K. assisted with the concept, funding, ideas, writing and manuscript preparation.

O.J.L. helped with ideas, statistical analysis and manuscript preparation.

D.A.C. assisted with ideas, writing and manuscript preparation.

S.C.B. helped with ideas, writing and manuscript preparation.

D.W. was involved in field work and ideas.

ACKNOWLEDGEMENTS

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