To feed or to breed: morphological constraints of mouthbrooding in coral reef cardinalfishes

Andrew S. Hoey1,2,*, David R. Bellwood2,3 and Adam Barnett2,4

1Red Sea Research Center, King Abdullah University of Science and Technology, Thuwal 23955-6900, Kingdom of Saudi Arabia
2ARC Centre of Excellence for Coral Reef Studies, and 3School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia
4Fisheries, Aquaculture and Coasts Centre, Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania 7001, Australia

Functionally coupled biomechanical systems are widespread in nature and are viewed as major constraints on evolutionary diversification, yet there have been few attempts to explore the implications of performing multiple functions within a single anatomical structure. Paternally mouthbrooding cardinalfishes present an ideal system to investigate the constraints of functional coupling as the oral jaws of male fishes are directly responsible for both feeding and reproductive functions. To test the effects of (i) mouthbrooding on feeding and (ii) feeding on reproductive potential we compared the feeding apparatus between sexes of nine species of cardinalfish and compared brood characteristics among species from different trophic groups, respectively. Mouthbrooding was strongly associated with the morphology of the feeding apparatus in males. Male cardinalfishes possessed longer heads, snouts and jaws than female conspecifics irrespective of body size, trophic group or evolutionary history. Conversely, reproductive potential also appeared to be related to trophic morphology. Piscivorous cardinalfishes produced larger, but fewer eggs, and had smaller brood volumes than species from the two invertebrate feeding groups. These interrelationships suggest that feeding and reproduction in the mouth of cardinalfishes may be tightly coupled. If so this may, in part, have contributed to the limited morphological diversification exhibited by cardinalfishes.

Keywords: functional coupling; mouthbrooding; ecomorphology; cardinalfish; coral reef

1. INTRODUCTION

Organismal form is often related to its function and may be assumed to reflect the adaptive significance of the structure [1]. Over the past few decades, integrative approaches to organismal form and function have greatly enhanced our understanding of ecological and evolutionary relationships among species and assemblages [2–5]. Morphological variation has been directly related to evolutionary histories, ecological parameters, diet, habitat use, movement and survival across a broad range of taxa [4]. For example, wing shape in bats, birds, insects and pterosaurs [6–9], fin shape in fishes [10], and trunk morphology in snakes [11] have been related to locomotion; and variation in oral jaw morphology in mammals and fishes [12,13] and beak shape in birds [14] have been related to diet. While many of these associations are based on one-to-one mapping of morphology to function, the coupling (i.e. one-to-many) or decoupling (i.e. many-to-one) of structural components may directly influence these relationships [1,15].

Functional decoupling—the duplication and redundancy of individual design elements—has contributed to increased diversity in many groups through the release of functional constraints [1,3,16]. Perhaps the best-documented example of functional decoupling is the development of a pharyngeal jaw apparatus in several fish families [2,17,18]. The evolution of a second set of jaws that could specialize on prey processing freed the oral jaws from potential constraints involved with food processing. This key structural innovation has, in turn, facilitated increased diversity in the oral jaws. Such delineation of tasks is exemplified in the Great Lake cichlids and coral reef labrids, where it has allowed the oral jaws to evolve numerous innovations, facilitating remarkable trophic diversification through the collection of an expanded range of food items [3,19,20].

In contrast to functional decoupling, the implications of functional coupling (i.e. a single anatomical system performing multiple functions) are less well understood. Although it is assumed that the constraints of performing multiple functions will limit evolutionary diversification, few studies have directly examined the effects of functional coupling on organismal design. This is surprising given the relatively widespread occurrence of functionally coupled systems; body regions that are involved in multiple functions are a basic feature of organisms [15]. The few studies that have examined the implications of functional coupling, although largely restricted to examining muscle activity patterns associated with feeding in fishes, have provided little support for the notion that functional coupling constrains diversification [21,22]. Wainwright & Turingan [21] reported that the coupling of prey capture and processing in the oral jaws did not...
constrain the behavioural versatility of the queen triggerfish *Balistes vetula*. Meanwhile, comparisons of muscle activity patterns associated with the coupled tongue-bite and oral jaw apparatus in a salmonid and an osteoglossomorphs suggested that coupled systems have not posed a limitation on diversification [22].

Mouthbrooding in fishes, while restricted to approximately 10 families [23,24], presents an ideal system to investigate the constraints of functional coupling as the oral jaws perform both reproductive and feeding functions. This combination of major processes in the one structure may represent one of the clearest examples of functional coupling in aquatic environments. The objective of this study, therefore, was to examine the potential functional implications of mouthbrooding in coral reef cardinalfishes (Family Apogonidae). Cardinalfishes are a species-rich and abundant group of primarily marine fishes, in which all species are paternal mouthbrooders that display moderate trophic and morphological diversity [25]. Unlike the mouthbroodingAppledicichthys ciliids in which the functional decoupling of the oral and pharyngeal jaws may mask the functional constraints of mouthbrooding, cardinalfishes have a generalized percomorph branchial basket and do not possess well-developed pharyngeal jaws or dentition. Specifically, the aims of this study were to compare the morphology of the feeding apparatus among species and sexes of cardinalfish, and to compare brood characteristics among species with different trophic morphologies. Differences in feeding structures were investigated between sexes to determine if sex (and, by association, brooding) was related to the morphology of the feeding apparatus. If mouthbrooding influenced feeding we would expect to find consistent variation in the morphology of the feeding apparatus among sexes. Conversely, if feeding influenced mouthbrooding, we would expect brood characteristics to be related to trophic group.

2. METHODS
This study was conducted on Lizard Island (14°40′S, 145°28′E), northern Great Barrier Reef (GBR), Australia during October–December. Nine species of cardinalfish (*Archamia fucata*, *Apoigon doederleinii*, *Chelidiodipterus artus*, *Chelidiodipterus macrodon*, *Chelidiodipterus quinquelineatus*, *Nectamia fusca*, *Ostorhinchus cyanosoma*, *Pristaipogon exostigma* and *Zoramia fragilis*) were selected as they were locally abundant at the study site, and encompassed broad taxonomic, phylogenetic, morphological and trophic variation [24–29]. These species also spanned the maximum range of body sizes of species recorded from the GBR [30].

(a) Effect of mouthbrooding on feeding
To explore the effects of mouthbrooding on feeding mode we compared the morphology of the feeding apparatus between sexes for the nine cardinalfish species. Ten individuals of each sex from each of the nine species were collected from reefs in the Lizard Island lagoon using the fish anaesthetic clove oil [31] and hand nets. Fish were euthanized in an ice–sea water slurry immediately following collection. Within approximately 2 h of capture (i.e. while fresh and relaxed), each specimen was weighed and a series of external measurements recorded; standard length, snout length, head length and width, horizontal gape, vertical gape, upper jaw protrusion and lower jaw protrusion (see Barnett et al. [25] for detailed descriptions). The visceral cavity was dissected open and the sex of each specimen determined by macroscopic examination of the gonads. All specimens were then fixed in 10 per cent sea water-buffered formalin for approximately three weeks before being transferred to 70 per cent ethanol for storage.

To allow examination of internal morphological characters, fixed specimens were cleared and stained (following Konow & Bellwood [32]). Briefly, specimens were immersed in an enzymatic pre-soak detergent, followed by potassium hydroxide (1.5%) digestion, and stained with Alizarin red to highlight osteological elements. Subsequently, the dimensions of the lower jaw and tooth height were measured using a dissecting microscope with a graduated eyepiece. Lower jaw length, or jaw out-lever, was measured as the distance from the lower jaw articulation (LJA) to the anteriormost tooth. The closing and opening in-levers were measured as the distance from the LJA to the point of attachment of the adductor mandibulae and the interopercular-mandibular ligament, respectively. Subsequently, jaw closing and opening lever ratios were calculated as the ratio of the respective in-lever to the out-lever, or jaw length [13,33]. To account for the variation in body size among individuals all linear morphological variables were divided by the cube root of body mass. Analysis of residuals from least-squares regressions of the morphological variables with the cube root of body mass indicated that the relationships were isometric. Consequently, residuals offered no advantage over mass-standardized ratios for removing the effect of body size. Standardizing by body length produced similar results.

To compare morphological variation in the feeding apparatus among species and sexes a two-factor multivariate analysis of variation (MANOVA) was used. Assumptions of the MANOVA were examined using residual analysis and no transformation was necessary. A principal component analysis (PCA) was then used to determine how the feeding apparatus differed among sexes and species of cardinalfish. The PCA was based on a covariance matrix of the mean of the mass-standardized morphological variables for each sex and species combination (*n* = 10 individuals).

(b) Effect of feeding morphology on mouthbrooding
The influence of feeding morphology on mouthbrooding was investigated by comparing the buccal cavity volume, brood volume and size, and number of eggs among cardinalfish species from different feeding morphologies. Between 6 and 20 brooding males (*mean* = 13 individuals) from seven cardinalfish species were collected, as described above. These species encompassed three broad trophic morphologies: a largely piscivorous group with elongate heads and snout (*C. artus*, *C. macrodon* and *C. quinquelineatus*); a generalist invertebrate feeding group with large gapes (*A. fucata*, *O. cyanosoma* and *Z. fragilis*); and a generalist invertebrate feeding group with wide heads (*A. doederleinii* [25]). An absence of brooding male *P. exostigma* and *N. fusca* at the time of the study precluded analyses for these species. Immediately following capture, the egg masses were removed from the mouths of brooding males and preserved in formaldehyde–acetic acid–calcium chloride. Specimens were measured and weighed and the mouths filled with neutral cure silicone to provide a cast of the buccal cavity (modified from Okuda et al. [34]). After the silicone had set, the buccal cast was removed and the volume determined by water displacement (following Barnett & Bellwood [24]).
The volume of individual broods, or egg clutches, was measured by placing the entire egg mass into a graduated cylinder filled with water, lightly packing down and recording the volume occupied by the egg mass. This method quantifies the overall brood volume (i.e., eggs, chorionic filaments and space between eggs). The number of eggs in each brood was estimated by comparing the weight of the entire brood (to the nearest milligram) with the weight of a subsample of each brood (approx. 5% of the egg mass) for which the number of eggs had been counted. Individual egg volume was estimated by haphazardly selecting 20 eggs from each brood and measuring the diameter of each egg under a microscope. All eggs appeared spherical and subsequently the volume was calculated as \(4/3\pi r^3\). To account for variation in body size among individuals, buccal cavity volume was divided by body mass, and brood volume and the number of eggs were divided by buccal cavity volume. Egg volume was not standardized as it was poorly related to both body mass (\(r^2 = 0.09\)) and buccal cavity volume (\(r^2 = 0.07\)).

Buccal cavity volume, brood volume, number of eggs and egg size were compared among trophic groups and species using a nested MANOVA. The assumptions of the MANOVA were examined using residual analysis. Consequently, the number of eggs and egg size were log-transformed to meet the assumptions of the MANOVA (i.e., normality and homoscedasticity). A PCA was then used to determine how these brooding characteristics differed among trophic groups.

(c) Interrelationships between feeding and mouthbrooding

To provide a quantitative examination of the interrelationships between feeding morphology and mouthbrooding characteristics a series of correlations were used. The factor scores for each species on the first two principal components (i.e., PC1 and PC2) from the morphological and mouthbrooding PCAs were correlated with the mouthbrooding and feeding morphology variables, respectively. The correlations were based on mean values for males of the seven cardinalfish species.

(d) Morphological diversity

Existing morphological data for 130 labrid species from the GBR [33] were used for comparative purposes to assess the relative morphological diversity of the Apogonidae. A PCA based on a correlation matrix of four shared morphological characters (i.e., jaw opening and closing lever ratios, horizontal gape and upper jaw protrusion) was used to explore morphospace occupation among the 9 cardinalfish species from the present study and the 130 labrids. To account for the variation in body size among the 139 species, residuals were calculated for horizontal gape and protrusion from least-squares regressions with cube-root of body mass (following Wainwright et al. [33]). Morphological diversity of the cardinalfishes and seven putatively monophyletic labrid groups (i.e., scarids, hypsigynelines, cheilines, pseudochelines, novaculines, julidines and labrichthyines) was calculated as the variance of factor scores on each of the four principal components for the members of each group, scaled by the variance explained by each component, and summed across all principal components (following [35,36]). This measure of morphological diversity does not scale with sample size, thereby facilitating comparisons among groups of varying size.

3. RESULTS

(a) Effect of mouthbrooding on feeding

The MANOVA indicated that feeding morphology differed significantly among the nine species (Wilks’s \(\lambda = 0.004, p < 0.001\)) and sexes (Wilks’s \(\lambda = 0.657, p < 0.001\)) of cardinalfish. There was no significant interaction between species and sex (Wilks’s \(\lambda = 0.527, p = 0.150\)). The PCA showed clear morphological variation among species and sexes, with the first two components collectively explaining 81.8 per cent of the total variation. The ordination identified three distinct groups, with species within trophic groups clustering together. The first group (A. fucata, N. fusca, O. cyanosoma and Z. fragilis) was characterized by larger gapes and greater protrusion of the lower jaw; the second group (C. artus, C. macrodon and C. quinquelineatus) by longer heads and larger teeth; and the third group (A. doederleini and P. exostigma) by wider heads and greater jaw opening lever ratios. Sexual dimorphism was evident for all species. Although the dimorphism was not as pronounced as interspecific variation in morphological characteristics, the differences in males were highly consistent (Wilks’s \(\lambda = 0.657, p < 0.001\); figure 1a). Male cardinalfishes all had higher loadings on the second principal component than female conspecifics. These differences were driven by males having longer heads, snouts and jaws than females of the same size for all species (figure 1b).

(b) Effect of feeding morphology on mouthbrooding

Feeding morphology appeared to be associated with mouthbrooding characteristics in male cardinalfishes (figure 2). The mouthbrooding characteristics differed significantly among the three trophic groups (Wilks’s \(\lambda = 0.124, p < 0.001\)) and among species within each trophic group (Wilks’s \(\lambda = 0.168, p < 0.001\)). The PCA showed marked variation in mouthbrooding characters among species, with species within trophic groups clustering together. The first group (C. artus, C. macrodon and C. quinquelineatus) was separated from the two remaining groups along PC1, which explained 76.6 per cent of the total variation. This group was characterized by smaller standardized buccal cavities, smaller brood volumes (relative to buccal volume) and larger, but relatively fewer, eggs than species from the other trophic groups (figure 2b). The two remaining groups were separated along PC2, with A. fucata, O. cyanosoma, Z. fragilis having larger buccal cavities relative to body size and smaller brood volumes relative to buccal volume than A. doederleini (figure 2b).

(c) Interrelationships between feeding and mouthbrooding

Overall, brood characteristics, by proxy of the first axis of the mouthbrooding PCA, were negatively correlated with relative head length \((r = -0.778, p = 0.040)\), snout length \((r = -0.787, p = 0.036)\) and tooth height \((r = -0.852, p = 0.015)\), indicating that species with longer heads, snouts and teeth had larger eggs and reduced brood volumes compared with the other species. There was also a negative, but non-significant relationship between the second axis of the mouthbrooding PCA and vertical gape \((r = -0.727, p = 0.064)\). Conversely, the morphology of the feeding apparatus, by proxy of the
first axis of the morphology PCA, displayed a positive relationship with buccal volume \( (r = 0.887, p = 0.008) \) and a marginally non-significant negative relationship with egg size \( (r = -0.762, p = 0.062) \). None of the other variables examined were related to the overall brood characteristics or morphology of the feeding apparatus (electronic supplementary material, tables S1 and S2).

(d) Morphological diversity

Cardinalfishes displayed limited morphospace occupation and morphological diversity compared with the labrids (figure 3). The first two components of the PCA explained 77.7 per cent of the total variation, with the cardinalfishes occupying a markedly smaller and completely separate region of morphospace than the labrids (figure 3a). This separation was primarily associated with the larger horizontal gapes of the cardinalfishes (figure 3b). The Apogonidae showed the lowest morphological diversity of the 10 groups compared, representing only 11 and 16 per cent of the diversity seen with the parrotfishes and wrasses, respectively (figure 3c). Even the morphological diversity within the six species of Labrichthyines was over double that of the nine cardinalfish species.

4. DISCUSSION

Functional coupling is widespread in natural systems and is often viewed as a major constraint on evolutionary diversification [3,15,18], yet there have been few attempts to explore the implications of performing multiple functions within a single anatomical structure (see Wainwright & Turingan [21] and Konow & Sandford [22] for exceptions). Using paternal mouthbrooding cardinalfishes as a model system, we found strong interrelationships between feeding and reproduction, supporting the view that functional coupling may constrain organismal design. Mouthbrooding was correlated with the morphology of the feeding apparatus, with male cardinalfishes having consistently larger morphological elements than female conspecifics. This sexual dimorphism was evident across all species, irrespective of body size, functional group or evolutionary lineage, suggesting that it is not only widespread but may also be attributable to mouthbrooding. Conversely, trophic morphology was correlated with reproductive potential. Piscivorous cardinalfishes produced larger but fewer eggs and, despite having smaller buccal cavities (relative to body size), their egg masses occupied a smaller proportion of their buccal cavities than species from the two invertebrate feeding groups. Reproductive potential also varied among the two invertebrate feeding groups, with those species characterized by larger gapes...
having greater buccal volumes but reduced buccal cavity occupation than species characterized by wide heads and greater jaw opening lever ratios. These relationships between trophic morphology and reproductive potential may be influenced by phylogenetic constraints. However, this effect is likely to be limited as, with the exception of the three piscivorous species, all cardinalfish species examined were from distinctly different clades [27–29].

Sexual dimorphism of the buccal cavity appears to be widespread among mouthbrooding fishes. Males of the paternally mouthbrooding cardinalfishes [24,34] and jawfishes [37], and females of the maternally mouthbrooding cichlids [38,39], have been shown to have larger buccal cavities than the respective non-brooding sexes. This expanded buccal volume has been attributed to larger osteological elements and/or increased flexibility of soft tissues in the brooding sex [24,34,39,37–39]. The significance of the larger buccal volume has been related to increased reproductive potential [40], and increased respiratory efficiency of both the eggs [37] and the brooding parent [41]. Despite these perceived advantages, causality between mouthbrooding and buccal volume cannot be assumed without further examination. The observed sexual dimorphisms may be related to alternate functions or auto-correlated with other morphological elements and not represent active phenotypes. While several studies have examined the relationship between mouthbrooding and morphology [24,34,37–39], this is the first study to investigate the associations between trophic morphology and reproductive potential in mouthbrooding fishes. Consequently, our current understanding of the functional basis of this relationship is limited.

Together with feeding and brooding, the mouth is an integral component of the respiratory pump of fishes, forcing water over the eggs and the gills of the parent. While larger eggs have been related to increased juvenile growth and survival [42], the lower surface area-to-volume ratio of large eggs reduces the relative rate of diffusion and their ability to acquire sufficient oxygen to meet their metabolic demands [43]. For example, van den Berghe & Gross [44] reported that large coho salmon (Oncorhynchus kisutch) eggs had lower survival than smaller eggs when laid in gravel nests with low oxygen. Therefore, the reproductive strategy of the piscivorous cardinalfishes in the present study may represent a trade-off between egg size and egg quantity, with the smaller relative egg mass allowing for greater churning, oxygen diffusion and, in turn, increased survival for their larger eggs.

The egg mass may also impede the respiratory function of the mouthbrooding parent by reducing the irrigation of the gills. Indeed, variation in brood size between two species of cardinalfish (Zoramia leptocantha and Z. fragilis) has been related to their tolerance of oxygen depletion [41]. Mouthbrooding reduced the respiratory scope and sustained aerobic swimming capacity of both species; however, the species with the smaller brood (Z. leptocantha) displayed a greater tolerance to hypoxic conditions. Effectively, males are forced into a trade-off between potential reproductive success, or brood size, and the capacity for oxygen intake [41]. This in turn, may be influenced by the ecology of individual species, particularly those factors that influence oxygen availability and demand (e.g. habitat use, swimming ability, fish size and diet).

Our results, while only correlative, provide one of the first examples of the constructional constraints of performing two functions within one structure. Just as the decoupling of biomechanical systems may lead to greater morphological diversification of associated structures...
[1,3], functional coupling may impede diversification through opposing trade-offs in the function and performance of structures involved in multiple processes. Cardinalfishes have an evolutionary history that mirrors that of the Labridae in terms of the age of origination and timing of diversification [29]. Interestingly, cardinalfishes appear to show none of the morphological diversification seen in the Labridae, a family with functional decoupling. Comparisons of morphological diversity among the nine cardinalfish species (present study) and 130 GBR labrids [33] revealed limited morphospace occupation and diversity among the cardinalfish species. Even the highly specialized Labrichthyines showed over twice the diversity of the nine cardinalfish species. While this preliminary comparison only includes 9 of the 62 cardinalfishes recorded from the GBR [30], these species span the maximum size range among cardinalfishes, represent 6 of the 14 major cardinalfish groups in the the most recent cardinalfish phylogeny and represent all major trophic categories in the family. It thus appears that the limited morphological diversity reported herein is unlikely to increase substantially with greater sampling.

Although more morphological and evolutionary studies of cardinalfishes are needed to establish any causality between trophic morphology and reproductive potential, and to establish the role of functional coupling in influencing diversification, the results of the present study highlight the potential for such relationships. The nature of the coupling may determine the possibility of optimizing or compromising adaptations, the direction of the evolutionary change and the variability of key traits. Ultimately, to understand the implications of functionally coupled systems, investigations should examine the nature of these interrelationships across a broader range of taxa within a well-resolved phylogenetic framework. In such a framework, the Apogonidae present an interesting opportunity to explore the evolution of functional and developmental determinants of coupled biomechanical systems.

We thank M. Depczynski and F. Merida for field assistance; the staff of the Lizard Island Research Station for logistical support; and C. Fulton, J. Hoey, M. Hoogenboom and N. Konow for helpful discussions. Comments from two anonymous reviewers greatly improved the manuscript. Financial support was provided by the Australian Research Council. James Cook University animal experimentation ethics approval no. A650.

REFERENCES

3 Lauder, G. V. 1981 Form and function: structural analysis in evolutionary morphology. Paleobiology 7, 430–442.


