Feeding ecomorphology in angelfishes, f. Pomacanthidae: the implications of functional innovations on prey-dislodgement in biting reef fishes

PhD thesis submitted by

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STATEMENT OF CONTRIBUTION OF OTHERS

This thesis includes some collaborative work with my supervisor, Prof. David R. Bellwood (Chapter 2-4), with Prof. Peter C. Wainwright (Chapter 4) and with Dr. Wayne Mallett (Appendix 1). While undertaking these collaborations, I was responsible for conceptualising the project, execution of experiments, data analysis and synthesis of results into a publishable format. My co-authors assisted financially, with editorial advice and with technical instruction for programs and experimental equipment.

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Thesis Abstract

On coral reefs, biting teleosts form a major component of reef fish assemblages. Nevertheless, they have been largely overlooked in functional research, while their ram-suction feeding counterparts have received considerable attention over the past few decades. This thesis therefore examines the functional basis of biting in coral reef fishes, with a focus on the marine angelfishes (f. Pomacanthidae), and other deep-bodied squamipinnid fishes.

To evaluate the magnitude and role of functional specialisation associated with prey-capture in angelfishes, a basal species, *Pomacanthus semicirculatus* (Cuvier, 1931) was selected as a model taxon for comprehensive functional analysis. The feeding apparatus of *Pomacanthus* contains two biomechanical mechanisms of particular interest: an intramandibular joint, and a suspensorial linkage with two novel points of flexion. Prey-capture kinematics were quantified using motion analysis of high-speed video, generating performance profiles to illustrate timing of onset, duration and magnitude of movement in the novel mechanisms. Mandible depression and suspensorial rotation coincide during jaw protrusion, and augment mandible protrusion to increase head length typically by 30%. Jaw closure at peak jaw protrusion appears to result from contraction of the *adductor mandibulae* segment A2, the only segment with insertions facilitating rotation of the dentary by approx. 30º relative to the articular. Feeding events are concluded by a high-velocity jaw retraction typically lasting 20-50 ms, and completed in 450-750 msec. *Pomacanthus* feeding morphology and kinematics differ from other biting teleosts, and

more closely resemble some long-jawed ram-suction feeders, with the novel feeding kinematics matching an unusual diet of structurally resilient and firmly attached prey.

Ten angelfish species representing all phylogenetic lineages were chosen from the GBR fauna, in order to analyse morphological and kinematic disparity in the angelfish feeding apparatus. Angelfish cranial architecture exhibits remarkable evolutionary stability with constructional changes restricted to key suspensorial specialisations governing increased jaw protrusibility, differential jaw protrusion angles and variations in alimentary tract morphology. Whilst it was previously suggested that intramandibular joints increase mechanical complexity and expand jaw-gape, in angelfishes the joint is a synapomorphy with novel gape-restricting kinematics. Individual means of the 32 most informative kinematics variables in *Pomacanthus* were extracted from high-speed video of feeding events. Concordant with phylogenetic evidence, the derived pygmy-angel subgenera, *Centropyge [Centropyge]* and *C. [Xiphypops]* differ significantly in several traits, whereas the basal *Pomacanthus* subgenera are largely indistinguishable. The monotypic *Pygoplites* exhibits the most pronounced flexion and *Genicanthus* consistently demonstrate the most restricted flexion in most variables measured.

Mapping of informative alimentary traits to a phylogeny delineated divergent angelfish feeding guilds. Grab-and-tearing omnivory on sponges and other sturdy prey is utilised by several large and robust taxa and constitutes the basal trophic guild. More gracile, biting omnivory is commonly utilised in derived pygmy-angel taxa, while dislodging herbivory arose both in the basal large-bodied *P. [Euxiphipops]* and in the derived *C. [Xiphypops]*; planktivory in *Genicanthus* is atavistic. Gape-restricting intramandibular flexion, suspensorial rotation augmenting lower jaw protrusion and a high-

velocity jaw retraction are important functional innovations with major implications for angelfish feeding morphology and kinematics. Coupled with distinct size differences amongst taxa, these traits form the functional basis for a considerable ecological diversification in angelfishes.

The functional basis of biting in reef fishes was investigated in 11 deep-bodied families, to examine the relationships between novel intramandibular joints and associated trophic ecology. The results suggest convergent intramandibular joint evolution leading to biting strategies in at least five families. Restricted flexion repeatedly coincides with functional reversion to zoo-planktivory while basal ram-suction feeders generally lack flexion. In angelfishes, intramandibular joints are symplesiomorphic and evolutionarily stable, exhibiting limited kinematic divergence, averaging flexion of 27±11.1º and causing jaw occlusion at peak protrusion. Angelfish kinematics contrast with all other intramandibular joint bearers, in which gape-expanding flexion concludes prior to jaw-closure. Intramandibular flexion and transition from ram-suction to biting in butterflyfishes coincide, with flexion magnitude, culminating in the crown-group of *Corallochaetodon* (16±6.6º) and *Citharoedus* (49±2.7º).

Character mapping and optimisation revealed that up to seven intramandibular flexion transitions/reversals consistently correspond with trophic transitions from free-living to attached prey. Whilst functional patterns reflect convergence of this joint, the evolutionary origin of intramandibular flexion in the squamipinnid fishes remains ambiguous. Nevertheless, a complex evolutionary history appears to have led to widespread intramandibular joint occurrence in extant biting groups, suggesting that this is a major functional innovation, and a functional prerequisite to biting in many reef fish taxa.

Thesis abstract

In summary, the functional innovations of the angelfish feeding apparatus allow these fishes to pass ecological thresholds and exploit novel trophic strategies, using grab-and-tearing for herbivory and spongivory. Intramandibular joints appear to have been an important functional innovation, playing a similar role in driving the ecological diversification of the squamipinnes as the pharyngeal jaw apparatus in the Labroidei. However, an emerging trend of reduced feeding apparatus disparity in biters, when compared to ram-suction feeding taxa, supports the theory that novel traits can pose constraints on functional diversification. The results herein illustrate the utility of direct performance testing in quantifying disparity patterns at the organismal and assemblage-level and emphasise the potential for combining ecomorphological and biomechanical techniques in elucidating the functional basis of the biting feeding mode.

Chapter 1: General Introduction

1.1 Principles of ecomorphological research

Over the past half-century, a vast research effort has focussed on tropical marine reef ecosystems, which are high-complexity habitats supporting fish faunas of unequalled diversity. The rapid evolution from 65 myBP of scleratinian coral reefs prompted an adaptive radiation of perciform teleost fishes. Contemporary teleost assemblages are now characterised by many taxa that forage by actively biting prey from the substratum. The taxonomic and numerical abundance of biting marine reef teleosts appear to exceed those in any other aquatic ecosystem, yet the functional basis of biting teleosts and their interactions with the living and calcified reef matrix remain largely unexplored (Wainwright & Bellwood, *in Sale*, 2002). Consequently, the primary aim of this thesis is to investigate the ecomorphology and functional basis of biting in reef fishes.

Ecomorphological analyses invariably involve a multidisciplinary approach, with several distinct components. Constructional morphology examines the specific traits in the teleost feeding apparatus, or the *'tools'* available to an organism. Functional analysis quantifies the biomechanics of discrete compartments, such as the oral jaws. Behaviour invariably modulates the kinematics or *'movements'* such as jaw protrusion or mandible depression within compartments. Consequently a quantification of behavioural performance is important to determine the range of organismal *'choices'* of how to deploy their apparatus. Phylogenetic information provides a *'historical perspective'* where convergence, divergence or shared ancestry of ecomorphological traits can be

evaluated (Motta & Kotrchal, 1992; Wainwright & Reilly, 1994, Norton *et al*., 1995). Finally, ecology places the organism in the environment where morphology and behavioural traits, which define the potential niche, are expressed in relation to available resources to define the realized niche. This causal chain of relationships linking morphology, function and behaviour to the ecology of organisms and assemblages in the context of their evolutionary history is a powerful combined approach that can be used to examine many central questions in biology: How does the appearance of novel traits in the functional morphology of organisms influence their ecology? How does disparity in such functional morphology traits in closely related organisms influence the ecological diversification of assemblages? Can certain traits be identified that are of exceptional ecological significance, and does the evolution of these traits precede or succeed ecological diversification?

Ecomorphology, as outlined above has been applied in recent years to studies of feeding and locomotion in reptiles (Garland & Losos, 1994; Dwyer & Kaiser, 1997; Zaaf *et al*., 1999; Aerts *et al*., 2000), birds (Herrel *et al*., 2005) and bats (Norberg, 1994; Freeman, 2000). In parallel, the approach has been applied in studies of teleost functional systems governing essential survival and maintenance tasks, including locomotion, foraging and anti-predator behaviours (Walker, 1997; Fulton & Bellwood, 2002; Wainwright *et al*., 2002) and vision (Van der Meer *et al*., 1995). Over the past three decades, the number of teleost feeding ecomorphology analyses have grown, reflecting an unparalleled complexity of their feeding apparatus, as well as their monumental ecological diversity and high taxonomical and numerical abundances in all major aquatic ecosystems (Hulsey & Wainwright, 2002; Wainwright & Bellwood, 2002; Streelman & Danley, 2003; Wainwright *et al*., 2004; Hulsey & García de León, 2005).

1.2 Additions to ecomorphology

In addition to traditional ecomorphology, recent approaches have accentuated our understanding of relationships between traits in the complex teleost feeding apparatus by quantifying functional disparity by–proxy. Analyses of geometric morphometrics apply multiple ordination techniques to morphometric datasets and differentiate taxa in accordance with constructional differences (Walker, 1997; Loy *et al*., 1998; Linde *et al*., 2004). Resultant morphological groupings have subsequently been linked to observed ecological guilds (Adams & Rohlf, 2000; Kassam *et al*., 2003a, b) or been used to reflect phenotypic plasticity amongst populations (Langehans *et al*., 2003; Svanbäck & Eklöv, 2004). Morphometrics data have also been projected into biomechanical models of discrete functional compartments, such as the anterior-jaw, hyoid and opercular mechanisms (Hulsey & Wainwright, 2002; Bellwood, 2003; Wainwright *et al*., 2004; Westneat, 2004). These models are based on engineering concepts and examine velocity and force-transmission trade-offs in single levers (Aerts *et al*., 1987; Westneat, 2003) and complex four-bar linkage systems (Barel *et al*., 1975; Westneat, 1990; Muller, 1996).

The biomechanical approach was used in examination of feeding apparatus trait disparity in a phylogenetic context amongst marine wrasses and parrotfishes (f. Labridae). A major result was the identification of multiple convergences in the evolution of fast jaws associated with capture of elusive prey (Wainwright *et al*., 2004; Westneat *et al*., 2005). Evidence merging from these biomechanical analyses has indicated that a high degree of structural redundancy exists in many teleost feeding systems. Morphology maps to functional disparity patterns in a ‘many-to-one’ fashion. This implies that the ecological diversification resulting from a range of morphological forms is likely to be

less than the biomechanical predictions of functional disparity (Alfaro *et al*., 2004, 2005; Bellwood *et al*., 2005; Wainwright *et al*., 2005). These findings have highlighted direct performance testing techniques in quantifying functional disparity amongst organisms as a logical priority in ecomorphological research.

1.3 Functional studies of fish feeding

The relative ease with which freshwater fish taxa can be kept and reared in captivity has allowed comprehensive performance testing of prey-capture in these taxa. In both cichlids (f. Cichlidae) and sunfishes (f. Centrarchidae), feeding kinematics were experimentally quantified using high-speed video and sonomicrometry (Aerts *et al*., 1987; Aerts, 1990; Richard & Wainwright, 1995; Gillis & Lauder, 1995; Wainwright & Shaw, 1999; Wainwright *et al*., 2001; Sanford & Wainwright, 2002; Sass & Motta, 2002; Waltzek & Wainwright, 2003). Electromyography studies have identified trends of relatively generalised motor patterns, and few derived exceptions amongst taxa using divergent prey-capture strategies (Galis & Drucker, 1996; Grubich & Wainwright, 1997; Wainwright, 2002; Carroll, 2004). Finally, the combined musculoskeletal output was brought together in synthesis (Svanbäck *et al*., 2002; Carroll *et al*., 2004). Whilst direct performance testing in the freshwater studies led to conclusions on a different experimental basis to the labrid studies, similar evolutionary trends of fast jaws for elusive prey-capture amongst new-world cichlids were established (Hulsey & García de León, 2005). In contrast with the freshwater studies, marine analyses incorporating experimental performance testing of behavioural modulation remain relatively scarce, and restricted to analyses of unusual taxa (Westneat & Wainwright, 1989, Bergert &

Wainwright, 1997; Gibb, 1997), durophages (Friel & Wainwright, 1997; 1998; 1999; Wainwright & Friel, 2000; Grubich, 2003) small species flocks of chaetodontids (Ferry-Graham *et al*., 2001a; 2001b) or labrids (Sanderson, 1988; 1990; Westneat, 1990; 1994; Ferry-Graham *et al*., 2001c; 2002).

Existing analyses have significantly advanced our understanding of the functional basis underlying prey-capture in modern teleosts by quantifying three major feeding modes; ram-feeding, suction-feeding and biting. Teleost taxa can be segregated amongst these major functional groupings according to the trophic strategies utilised (Liem, 1980a; Norton & Brainerd, 1993; Ferry-Graham *et al*., 2002). Functional analyses of modern teleosts similarly provided evidence of a putative polarisation in the evolutionary progression from generalized ram-suction feeding to more derived biting strategies (Liem, 1980a; Ferry-Graham & Lauder, 2001). In order to procure elusive, delicate or loosely attached prey, an aquatic predator will typically deploy a strategy along an axis ranging from 100% suction to 100% ram-feeding, now understood to comprise a complex continuum of enveloping strategies (Norton & Brainerd; 1993; Wainwright *et al*., 2001). Digital particle image velocimetry has facilitated detailed exploration of how this functional continuum can be modulated during centrarchid prey-capture (Ferry-Graham & Lauder, 2001; Day *et al*., 2005; Hingham *et al*., 2005). While ram-suction feeders occasionally may occlude their oral jaws onto prey, they generally do not utilise active biting strategies during prey-capture (Ferry-Graham *et al*., 2002).

Dislodgment of structurally resilient and/or sturdily attached benthic prey is achieved by applying the jaws to the feeding substratum in a grazing, scraping or tearing fashion. Biter foraging strategies thus appear at least as complex as the ram-suction

register, and the prey utilised may pose drastically differing biomechanical challenges to a biting predator (Choat, 1991; Purcell & Bellwood, 1993; Bellwood *et al*., 2003). Whilst biting has been argued of more infrequent occurrence amongst teleosts in general (Liem, 1980a) this strategy is particularly prominent in high-diversity reef ecosystems, such as African cichlids on lacustrine boulder reefs (Takamura, 1983; De Visser & Barel, 1996), and particularly on tropical marine reefs (Choat & Bellwood, 1991; Wainwright & Bellwood, 2002). In both ecosystems, biting assemblages have become taxonomically and numerically abundant after adaptive radiations from the recent origins of these reefal ecosystems (Bellwood & Wainwright, 2002; Salzburger *et al*., 2002; Bellwood, 2003; Ruber & Sardoya, 2005).

Biting coral reef teleosts may utilise all biota forming the reef living matrix and representing novel trophic guilds, including algae (Acanthuridae, Kyphosidae & Scaridae; Choat & Bellwood, 1985; Bellwood & Choat, 1991; Choat *et al*., 2004), sponges (Pomacanthidae and Ostrachiidae; Allen, 1981; Hourigan *et al*., 1989; Dunlap & Pawlik, 1996; Hill, 1998; Swearingen & Pawlik, 1998), coral (Labridae & Chaetodontidae; Motta, 1987, 1988, 1989; Wainwright *et al*., 2002; Berumen *et al*., 2005; Pratchett, 2005) and other modular invertebrate organisms, such as tunicates, ascidians and bryozoans. Accordingly, bio-erosion caused by biter foraging may result in considerable habitat modification (Bellwood, 1995a; b; Wulff, 1997; Hill & Hill, 2002), and the importance of herbivores in maintaining coral reef ecosystem resilience is well established (Miller & Hay, 1998; Bellwood *et al*., 2004a). Meanwhile, it can be argued that the only direct influence of ram-suction feeders on the living reef matrix is via their faecal nutrients (Marnane, 2000).

The current state of functional knowledge concerning feeding in marine reef teleost contrasts available ecological knowledge: Ram-suction is, due to its dominance amongst coral reef labroids, and the more intensely studied freshwater taxa, by far the better understood feeding mode Ferry-Graham & Lauder, 2001). Meanwhile, the morphological, functional and behavioural characteristics forming the basis of the biting feeding mode amongst reef fishes in particular, remains largely unexplored (Wainwright & Bellwood, 2002). As researchers are faced with the increasingly urgent task of selecting functional groups for conservation in order to maintain ecosystem resilience, a more detailed understanding of the functional basis governing a wide range of biting strategies on coral reefs is of immediate priority.

1.4 Functional innovations and decoupling

Derived traits with an overriding influence on functional evolution and/ or ecological diversification are interchangeably termed 'key' or 'major' innovations in the literature (Liem, 1973; Galis & Drucker, 1996; Wainwright, 2002) and will in this thesis simply be referred to as 'functional innovations'. The principle is well exemplified by labriform propulsion, where high aspect ratio pectoral fins augment a characteristic 'aquatic flying' almost exclusively utilising pectoral fin motion during sustained swimming. The labriform swimming mode has greatly influenced the water-column distribution and shaped the assemblage composition of in particular labrid fishes on reefs across broad spatial scales (Webb, 1984; Wainwright *et al*., 2002; Fulton *et al*., 2005).

In fish feeding, by far the most intensely studied and debated functional innovation is an extra set of pharyngeal jaws. This pharyngeal jaw apparatus (PJA)

provides a functional decoupling of the oral jaws from mastication tasks, permitting them to proliferate in shape and size to facilitate novel niche utilisation (Lauder, 1983; Galis & Drucker, 1996; Grubich, 2003; Wainwright, *in press*). Similar functional innovation is seen in the convergence of novel suspensorial flexion points augmenting lower jaw protrusion in extreme jaw-protruding chaetodontids (Motta, 1984; Ferry-Graham *et al*., 2001a; 2001b), cichlids (Waltzek & Wainwright, 2002; Hulsey & García de León, 2005) and in the labrid *Epibulus* (Westneat & Wainwright, 1989).

The majority of functional innovations identified were linked to an extensive diversification of ram-suction feeders within the Labroidei, a major perciform radiation on coral reefs (Rice & Lobel, 2004). Noticeable exceptions are the robust feeding apparatus modifications, involving muscular duplication and/or jaw element fusion for increased biting strength in durophagous tetraodontiform taxa (Turingan *et al*., 1995; Friel & Wainwright, 1997; 1998; 1999) and in the excavating scarines (Bellwood, 1994; Streelman *et al*., 2002; Bellwood *et al*., 2003). Whilst quantitative evidence of functional decoupling as well as functional innovations is lacking amongst biters, there has been the occasional description of an extra joint in the lower jaw of biters, including three scarine genera (Gobalet, 1980; Bellwood, 1994), two acanthurid (Purcell & Bellwood, 1993), a girellid (Vial & Ojeda, 1990) and a pomacanthid species (Gregory, 1933). Except for the scarines, such intramandibular joints (IMJ) appear restricted to members of an alternative marine perciform radiation, collectively termed the squamipinnes and comprising the acanthuroid, chaetodontoid and a few other deep-bodied taxa (Tyler *et al*., 1989). The IMJ is an obvious candidate for functional innovation status amongst biters; however kinematics, as well as the general prevalence and ecological role of such joints in teleost feeding biology remain to be quantified.

1.5 Study taxa, research aims and objectives

The marine angelfishes (f. Pomacanthidae) and the squamipinnid teleosts were chosen for analyses to provide insight into the functional basis of biting in teleosts, given the observed prevalence and inferred importance of biting teleosts in reef ecosystems.

Angelfishes were deemed of particular interest for initial biting functional analyses for a number of reasons. Angelfish comprise a relatively speciose and iconic reef fish assemblage almost exclusively comprising biting taxa with a few, readily identifiable planktivorous exceptions. Due to their popularity as marine ornamentals, extensive dietary knowledge has been generated and their ecological diversification was suggested to be extensive (Allen, 1981; Hourigan *et al*., 1989; Howe, 1993; Allen *et al*., 1998). Their utilisation of robust and sturdily attached prey, such as sponges and tunicates represents an unusual trophic guild, otherwise documented in few durophagous taxa only where alternative principles of muscular duplication and fusion of jaw elements provide the functional basis of forceful prey-procurement (e.g. Friel and Wainwright, 1997). Like most other biters, angelfishes were historically examined in a descriptive morphology context only (Gregory, 1933; Burgess, 1974; Blum, 1988). Confusion surrounded their evolutionary history (Burgess, 1974; Chung & Woo, 1998) until recently when robust phylogenetic evidence at the sub-generic level became available (Bellwood *et al*., 2004b). Phylogenetic evidence is an essential criterion in the selection of representative taxa for functional analyses including direct performance testing of feeding behaviour to facilitate a comprehensive ecomorphological analysis Westneat, 1995; Foote, 1997).

The majority of biting coral reef taxa belongs to the squamipinnes, deep-bodied fishes resulting from the perciform radiation into marine reef ecosystems during the early Tertiary (Tyler *et al*., 1989; Bellwood, 2003). The squamipinnes comprise the chaetodontoid fishes: angelfishes (f. Pomacanthidae), butterflyfishes (f. Chaetodontidae), and stripeys (f. Microcanthidae), the acanthuroid fishes: surgeonfishes (f. Acanthuridae), unicornfishes (subfamily Nasinae), the monotypic Moorish Idol (f. Zanclidae), rabbitfishes (f. Siganidae), scats (f. Scatophagidae), batfishes (f. Ephippidae), sicklefishes (f. Drepanidae) and sea-chubs (f. Girellidae).

Following the ecomorphological state of knowledge and the important gaps in available functional evidence outlined above, the specific aims of this thesis are to:

1) Quantify the structural, functional and behavioural traits characterising the biting feeding apparatus in the generalised angelfish genus *Pomacanthus*.

2) Quantify the disparity in feeding apparatus morphology and kinematics amongst marine angelfishes (f. Pomacanthidae).

3) Examine the role of intramandibular joints in biting, and investigate how widespread this trait is amongst squamipinnid teleosts on tropical marine reefs.

The specific research objectives, related to the aims listed above, are divided into data chapters. These chapters are formatted in a publication manuscript style, and are followed by a concluding discussion chapter, which examines the data chapters in unison to develop final conclusions.

Chapter 2 conducts a comprehensive ecomorphological analysis, including direct performance testing, of the feeding kinematics in a generalised marine angelfish, *Pomacanthus semicirculatus*. The findings are compared with available feeding kinematics evidence from ram-suction feeders and other biters.

Chapter 3 uses the ecomorphological approach to examine disparity in functional morphology and kinematics while phylogenetic evidence is used to optimise and ascertain a complete sub-generic representation at the family-level of marine angelfishes (f. Pomacanthidae). The discussion evaluates available evidence of functional constraints resulting from novel feeding apparatus traits.

Chapter 4 conducts a broad screening analysis of differential functional traits in squamipinnid biters. This examination investigates the functional basis, as well as the role of functional innovations where present in promoting the observed prevalence of these biting teleosts in tropical marine reef ecosystems.

Chapter 5 synthesises the results to discuss the major findings *in lieu* of existing functional and ecomorphological evidence, ultimately assessing the role of biters in reefal ecosystems and suggesting areas of future research focus.

Chapter 2: The functional biology of prey-capture in *Pomacanthus semicirculatus* (Teleostei, Pomacanthidae).

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2.1 Introduction

Numerous studies have examined the associations between feeding apparatus functional morphology, biomechanics and prey-capture kinematics in teleost fishes (see reviews by Liem, 1980a; Motta, 1984; Ferry-Graham & Lauder, 2001; Wainwright & Bellwood, 2002). These analyses have either tested or benefited directly from mathematical modelling of the biomechanical mechanisms in teleost skulls (Anker, 1974; Lauder & Liem, 1981; Muller, 1987; Westneat, 1990). In unison, such studies have provided the functional understanding necessary to encapsulate a seemingly monumental diversity of teleost feeding patterns into two distinct feeding modes (Liem, 1980a; Ferry-Graham *et al*., 2002): The ram-suction feeders, covering all enveloping feeding methods (Lauder, 1980; Motta, 1982; Westneat & Wainwright, 1989; Wainwright & Shaw, 1999; Ferry-Graham *et al.,* 2001b; Wainwright *et al*., 2001; Sanford & Wainwright, 2002; Svanbäck *et al*., 2002), and the biters, covering all dislodging strategies (Barel, 1983; Motta, 1985; 1988; Turingan *et al*., 1995; Ferry-Graham *et al*., 2001c; 2002).

On coral reefs, recent studies have successfully documented the ecomorphological relationships between morphology of the feeding apparatus, associated prey-capture kinematics, behavioural performance, and feeding ecology of both wrasses (f. Labridae) (Sanderson, 1990; Clifton & Motta, 1998; Ferry-Graham *et al*., 2001c; 2002; Hulsey & Wainwright, 2002; Wainwright *et al.,* 2004) and butterflyfishes (f. Chaetodontidae) (Motta, 1988; Ferry-Graham *et al*., 2001a; 2001b). Whilst insightful, these studies have concentrated predominantly on ram-suction feeding taxa, a continuum

of feeding modes that are primarily associated with capture of free-living, loosely attached and/or delicate prey (Motta, 1988; Sanderson, 1990; Wainwright *et al.,* 2004). Jaw closure kinematics associated with these feeding modes are generally considered inadequate for grabbing and dislodging firmly attached and/or structurally resilient prey (but see Ferry-Graham *et al.,* 2002).

While a number of studies have examined structural morphology in biting coral reef teleosts, these have focussed primarily on robust bioeroders and more gracile herbivorous or detritivorous taxa (Bellwood & Choat, 1990; Purcell & Bellwood, 1993; Bellwood, 1994; Alfaro *et al*., 2001; Ferry-Graham *et al.,* 2002; Streelman *et al*., 2002; Bellwood, 2003). Such grazing, scraping and excavating forms predominate amongst surgeonfishes (f. Acanthuridae) and parrotfishes (f. Scaridae), where structural attributes of the feeding apparatus, e.g. degree of jaw robustness or motility, reflect microhabitat use and differential patterns of food procurement (Bellwood & Choat, 1990; Purcell & Bellwood, 1993). However, with the exception of labrids (including some scarids) (Westneat, 1994; Alfaro *et al*., 2001; Ferry-Graham *et al.,* 2001c; 2002) and tetraodontiform fishes (Turingan *et al*., 1995) relatively little functional knowledge exists for biters, especially those that feed on structurally resilient and/or sturdily attached prey. Considering the prevalence of biting taxa on coral reefs, the paucity of information on both functional diversity and degree of complexity in morphology and kinematics underlying this assortment of feeding strategies stands out as a fundamental gap in our current understanding of feeding modes and their functional role in coral reef ecology (Wainwright & Bellwood, 2002).

The gracile and usually more derived biting taxa often possess an intramandibular joint (IMJ), a major innovation which increases morphological as well as functional

complexity by decoupling the mandible into two functional units and permitting rotation of the dentary on the articular. This may expand jaw gape, resulting in a larger area of substratum being contacted in each feeding event (Bellwood & Choat, 1990; Purcell & Bellwood, 1993; Bellwood, 1994; Streelman *et al*., 2002). Whilst IMJ kinematics remain unquantified, IMJ presence also appears to be associated with changes in the orientation of the body and the jaws to the substratum (Bellwood *et al*., 2004b), as well as the curvature of substratum utilised (Choat, 1991; Bellwood *et al*., 2003).

Of the coral reef teleosts putatively labelled as biters, the marine angelfishes (f. Pomacanthidae) form an interesting and hitherto neglected assemblage. Although taxonomically conservative (*c.* 80 spp.), they are iconic reef fishes with a circum-global distribution on tropical to warm-temperate reefs (Allen *et al*., 1998). Both pomacanthids and their well-studied sister family, the Chaetodontidae (Burgess 1974) possess bristle-shaped teeth arranged in multi-tier arrays, which may provide exceptional gripping ability during feeding (Motta, 1989). Chaetodontids are known to possess a wide range of biomechanical specialisations associated with several trophic guilds (Motta, 1985; 1988; Ferry-Graham *et al*., 2001a; 2001b) and a similarly wide range of trophic guilds has been inferred for pomacanthids (Allen, 1981; Allen *et al.,* 1998; Debelius *et al.,* 2003; Bellwood *et al*., 2004b). Whilst structural information exists (Gregory, 1933; Burgess, 1974), the functional aspects of pomacanthid feeding morphology and biomechanics have not been quantified (Wainwright & Bellwood, 2002). A recent molecular phylogeny has identified the large, robust omnivorous members of the genus *Pomacanthus* as the generalised pomacanthid taxon (Bellwood *et al*., 2004b). In contrast to the pygmy angelfishes, which primarily target delicate prey items, *Pomacanthus* species feed on firmly attached and structurally resilient invertebrate components of the

reef biota, including poriferans, tunicates, ascidians and soft corals (Allen, 1981; Allen *et al.,* 1998; Debelius *et al.,* 2003). These prey commonly favour confined and complex microhabitats (Richter *et al.,* 2001), which raises the question: how are structurally resilient prey items reached, seized and dislodged from confined habitats when the large body size in *Pomacanthus* (sometimes 50-60 cm in total length) would appear to hinder this foraging strategy? No previously described functional system readily explains the microhabitat utilisation and feeding patterns of *Pomacanthus*, and the present study aims to quantitatively analyse the functional morphology, kinematics and performance characteristics of the feeding apparatus in this basal pomacanthid taxon to investigate the structural and functional basis of pomacanthid prey procurement. We hypothesise that the pomacanthid feeding apparatus contains novel functional diversity, and that the associated feeding kinematics match the diverging pomacanthid feeding guilds. Specifically, we test if *Pomacanthus* has a functional IMJ, and if so, whether intramandibular kinematics facilitates an extended gape angle as previously suggested in other IMJ-bearing taxa.

2.2 Materials and methods

2.2.1 Study taxon and specimen collection

The Koran Angelfish *Pomacanthus semicirculatus* [Cuvier, 1831], is one of the largest of its genus, attaining over 50 cm standard length (SL), and has uniformly pale head coloration, making it particularly suitable for motion analyses. This species has a wide distribution throughout the Indo-west Pacific, and is typically found in high

complexity habitats with some vertical relief, where it feeds on structurally resilient attached prey, including sponges, tunicates, ascidians, soft corals and foliose calcareous or turf algae (Allen *et al.,* 1998, Konow unpublished data). A total of 11 specimens (164-330 mm SL; 42-85 mm head length, HL) were collected with a barrier net from mid-shelf reefs on the Great Barrier Reef.

2.2.2 Dissections, manipulations and clear staining

Specimens for dissections were euthanized by immersion in seawater with an overdose of clove oil (Eugenol; Munday & Wilson, 1997). While fresh, specimens were manipulated for identification of biomechanical linkages and frozen for dissection, or fixed in buffered 10% formaldehyde for clear-stain preparations and myology studies. Tissue clearing of fixed specimens (n=3) involved immersion in enzymatic pre-soak detergent (Gosztonyi, 1984) with subsequent KOH digestion and counter-staining for bone and cartilage, using a protocol modified from Dingerkus & Uhler (1977). Fixed specimens (n=7) were dissected to determine origin, insertion, fibre orientation and relative prominence of muscle complexes, as well as tendon, ligament and connective tissue morphology. Cleared and stained specimens, as well as dissections of fresh specimens were used for manipulative studies. These studies qualitatively examined biomechanical mechanisms adjoining the oral jaw, suspensorial and hyoid apparatus, with the neurocranium and pectoral girdle, during jaw protrusion, closure and retraction. During such manipulations, specimens were pinned to a reference grid background under a mounted digital camera and step-photographed while the following manipulations were carried out (see numerical labels for directions of manipulations in Fig. 2.1B): **1**,

posterior-directed force applied to the urohyal (isthmus), imitating contraction of the *m. sternohyoideus* and *m. hypaxialis*, with a demonstrated role in suction-feeder mandible depression. **2**, posterior-directed force applied to the supraoccipital crest, imitating contraction of the *m. epaxialis*, causing cranial elevation that has been demonstrated to facilitate mandible protrusion in ram-feeders. **3**, caudal rotation of the ventral opercular margin, imitating contraction of the *m. levator operculi*, causing displacement of the opercular linkage, tightening the opercular-mandibular ligament (LIM), and contributing to mandible depression. **4**, anterodorsal depression of the quadrate articular articulation, imitating contraction of the *m. levator arcus palatini,* causing anterior-directed suspensorial rotation, which has been demonstrated to augment mandible protrusion in some ram-feeders. **5**, dorsal rotation of the dentary with the articular fully depressed, imitating contraction of *m. adductor mandibulae* subsection 2 (A2), causing jaw closure. Anatomical and biomechanical diagrams were drawn directly from dissections using a camera lucida, or traced from digital stills of clear-stain preparations using Corel Draw v.10. (Corel Corp.). Osteology, myology and connective tissue nomenclature follows Winterbottom (1974) and Motta (1982).

2.2.3 Live specimen husbandry and experimental design

Specimens were held in individual experimental aquaria with shelter, at 26±2ºC with a 12:12 photoperiod and screened from external visual stimuli with an opaque nylon cloth. All fish were acclimated for 1-2 weeks prior to experimentation. For provisioning as well as feeding trials, rock oyster shells of uniform size (5-6 cm^2 surface area) and covered with sponge, turf algae, ascidian, tubeworm, and tunicate epifauna were

collected from local coastal marine pylons. During acclimation, specimens were trained to feed under floodlight illumination on epifauna from shells clipped into a stainless steel clip on a steel wire shaft mounted in a 300 g polymer base.

Prior to video recording, specimens were anaesthetised by immersion in seawater with 1% clove oil in ethanol (Munday & Wilson, 1997). While anaesthetised, reflective markers were attached with cyano-acrylic glue to the skin to provide external topographic landmarks for biomechanical linkages in the oral jaws, suspensorium, cranium and pectoral girdle (Fig. 2.1). This procedure was completed in less than 100 seconds and caused no apparent stress, as specimens typically fed vigorously shortly after recovery from anaesthesia.

2.2.4 Sampling and analysis of kinematics

High-speed videography was completed over a 2-5 day period for each specimen, with a total of three specimens (SL = 190, 245 & 330 mm; HL = 51, 63 & 85 mm) being observed. All aquaria were equipped with 2 cm^2 reference grid backgrounds and illuminated with two 500 W halogen floodlights during video recording. Specimens were presented with attached prey in the gap between the aquarium front and the reference grid background, to ensure the specimen was perpendicular to the lens axis, and recorded using a JVC GR-DVL9800u digital video camera at 200 images s^{-1} and a 1/250 s. shutter speed. Video sequences were captured to a PC hard drive via a Canopus DV Raptor capture board and converted to raw AVI format in Virtual Dub v.1.0. Five feeding events for each specimen were selected for comprehensive analysis of feeding kinematics and to generate a performance profile of key components of the feeding apparatus. Each frame

in selected sequences was separated to eight de-interlaced image fields, yielding stacks of 200 TIFF images s^{-1}, which were recompiled to AVI format in MatLab v.6.0 (Appendix 1) with resulting image stream resolution of 320 × 240 pixels. A further three specimens (SL = 197, 241, 261 mm; HL = 55, 61, 67 mm) were recorded using a 3Com single-CCD camera at 50 images s^{-1}. Sequences were captured real-time to hard drive using Pictureworks image recording software v. 2.0 and stored as AVI files for analysis. As this frame rate captured approx. 30 frames per feeding event, these sequences were only used for analysis of excursion maxima and velocity characteristics of feeding kinematics. All selected sequences were inspected in Virtual Dub and cropped from feeding event start (t_S) via protrusion onset (t_0) to maximum protrusion (t_{MAX}), bite (t_B), and feeding event conclusion (t_C). Onset of bite (t_{MAX}) coincided with maximum jaw gape and protrusion, with time of bite (t_B), being the frame showing jaw closure onto the prey. Sequences were submitted to analysis only if the full feeding event was completed in focus and in lateral profile. As performance maxima were the focus of this study, slow bites were rejected, as they appeared to result from predator hesitation. For the latter analyses, the high-speed sequences were sub-sampled at 50 images s^{-1} for standardisation and 10 feeding events for each of the six specimens filmed were analysed for maximum gape, maximum protrusion, and total feeding event duration (t_{TOT}). The contribution of body ram (R_B) and jaw ram (R_J, equalling R_B extracted from total ram, R_{TOT}) to prey approach were also recorded.

For the performance analysis, thirteen reference points (Fig. 2.1), a target point (t) on the prey where the strike landed, and an origin reference on the grid-background (used to normalise data for image flicker and in the event of slight, unnoticed prey movement) were tracked in Movias Pro v.1.0 (Pixoft-NAC, 2002). Here, x:y coordinates were

extracted for each reference point position in consecutive fields of the high-speed image stream. Visual inspection of video streams determined that protrusion duration varied more temporally than closure and retraction, and coordinate data columns from each bite were thus aligned to t_B, to minimise variation in feeding kinematics. Excel macros were used to calculate vector lengths (distances between paired coordinate points) and angles between paired vectors (i.e. three coordinate points). Means ± S.E. of resulting values were plotted as incremental displacements (image-by-image, in 5 ms increments) of angles (Fig. 2.4) and linear distance (Fig. 2.5) between digitised points in x:y coordinate space. Onset-timing, magnitude and duration is illustrated for the following kinematic variables: total ram movement relative to the prey (R_{TOT}), from which body-ram movement (R_B) was deducted to isolate jaw-ram movement (R_J), jaw gape expansion, premaxillary protrusion, mandibular rotation and protrusion, intramandibular rotation, preopercular rotation (as a proxy for suspensorial movement), opercular rotation (as a proxy for opercular linkage displacement), cranial elevation and isthmus movement (as a proxy for hyoid depression).

2.3 Results

Feeding apparatus kinematics in *Pomacanthus* displays an unusual timing pattern (Fig 2.3). After the preparatory and protrusion phases, a jaw closure phase precedes jaw retraction (Table 2.1). Specific kinematic profiles (Fig. 2.4 & 2.5) and associated morphological specialisations (Fig. 2.2) for the three significant phases in a *Pomacanthus* feeding event (protrusion, closure and retraction) are described in sequence below.

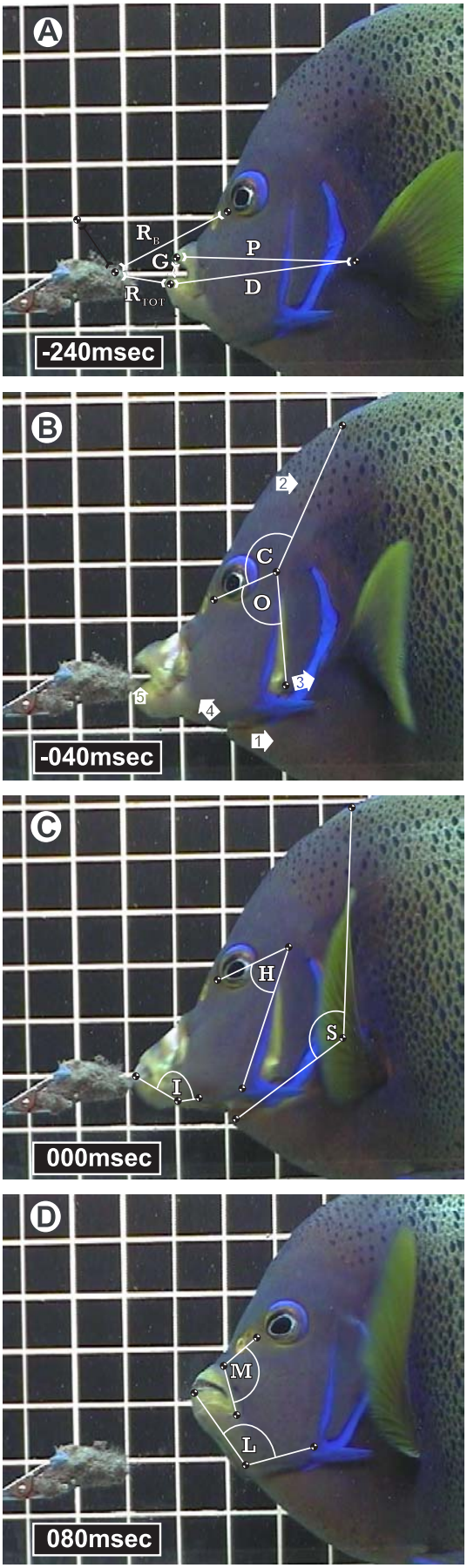

Figure 2.1 High-speed image frames from a 200 frames per second recording illustrating the feeding event in a *Pomacanthus semicirculatus* specimen (261 mm SL) feeding on sponge attached to a clip. Reference grid squares are 2 cm^2, and time in ms from bite (T_B) in bottom left corner of frames: A, protrusion onset; B, maximum protrusion; C, bite; D, prey-capture complete. Arrows with numerals (in B) refer to manipulations used on dissections (see text). Black and white dots indicate 15 landmarks on skull topography, prey and origin reference digitised in feeding sequences. Linear measurements (in A): R_{TOT}, Total ram; R_B, Body ram; G, gape distance; P, Premaxilla excursion; D, Dentary excursion. Black distance marker indicates origin-reference used to compensate for bite-related and unnoticed prey movements in analyses. Angular measurements (in B-D): C, cranial elevation; O, opercular rotation; H, suspensorial rotation; S, pectoral girdle rotation; I, intramandibular rotation; M, maxillary rotation; L, lower jaw depression.

2.3.1 Jaw protrusion

The hyomandibular bone and neurocranium have a synovial articulation on the ventral sphenotic margin (filled circle in Fig. 2.2A), which is associated with prominent *adductor arcus palatini* (AAP) and *levator arcus palatini* (LAP) musculature (Fig. 2.2B). Unusually, this permits anteroposterior movement of the hyomandibular, along with the closely associated elements of the suspensorium (Fig. 2.3A, 2.3B). Meanwhile, the latero-medial expansion capability of the suspensorium remains comparable to other teleosts. The pterygoid series is reduced anteriorly with the palatine loosely suspended by connective tissue between the pterygoids and a cartilaginous pad on the lateral ethmoid (open circle in Fig. 2.2A). Anteriorly directed manipulation of the hyoid-hyomandibular mechanism (**4** in Fig. 2.1B) results in a sliding of the palatopterygoid complex, and anterior movement of the suspensorium augmenting lower jaw protrusion (Fig. 2.3A, 2.3B). An interrupted pattern of suspensorial rotation is seen (Fig. 2.1C, angle H; Fig. 2.4A), with an early rotation of approx. 4º initiating at T_b–600-500 ms, preceding all other feeding kinematics, and designating the feeding event start, T_S.

The mandible (Fig. 2.2A) consists of a compact dentary with an elongated, curved ventral process, a crescent-shaped coronoid process, and an exceptionally elongate articular, which effectively lowers the mandible-quadrate articulation fossa, and a distinct angular (retro-articular) bone. The articular descending process connects to the hyoid apparatus via a stout mandibular-basihyal ligament and to the opercular series via a prominent interopercular-mandibular ligament (LIM in Fig. 2.2A); no preopercular-mandibular ligament is present. The alveolar and ascending premaxillary processes are similarly elongate, and the laterally flattened maxilla has a prominent internal premaxillary condyle articulating with ridges on the premaxilla,

Figure 2.2 Illustrations of the *Pomacanthus semicirculatus* skull (left lateral view) based on clear-stained preparations and dissections. A) Feeding apparatus when relaxed. B). Suspensorium and operculum rotated, and jaws protruded. C) Detail of protruded-closed oral jaws. Osteology labelling: an, Angular; art, Articular; d, Dentary; hyom, Hyomandibular; ihy, Interhyal; iop, Interoperculum; mpt, Metapterygoid; mx, Maxilla; op, Operculum; pal, Palatine; pmx, Premaxilla; pop, Preoperculum; ptr, Ectopterygoid; q, Quadrate; sop, Suboperculum; supcl, Supracleitrum; sym, Symplectic; urohy, Urohyal; lc, Lachrymal; Points of flexion are indicated in A between hyomandibular with nc, Neurocranium (filled circle) and between the palatopterygoid complex of the suspensorium with the lateral ethmoid (open circle). Open circle in C: IMJ, Intramandibular mechanism. Myology labelling: A1 (10% grey); A2 (50% grey, and medial to A1 and A3); A3 (30% grey), Adductor Mandibulae segments. LOP: Levator Operculi, DOP: Dilator Operculi, LAP: Levator Arcus Palatini, AAP: Adductor Arcus Palatini. Ligament labelling (all in 75% grey): EF, Naso-premaxilla elastic fibres; DLPM, Dorsal premaxilla-maxilla ligament; LIM, Interopercular-mandibular ligament; LIS, Interopercular-subopercular ligament; ILPM, Inner premaxilla-maxillary ligament; LPM, Palatine-maxillary ligament; LRDM, Articular-dentary-maxillary ligament; VLPM, Ventral premaxilla-maxilla ligament. Scale bars 10 mm.

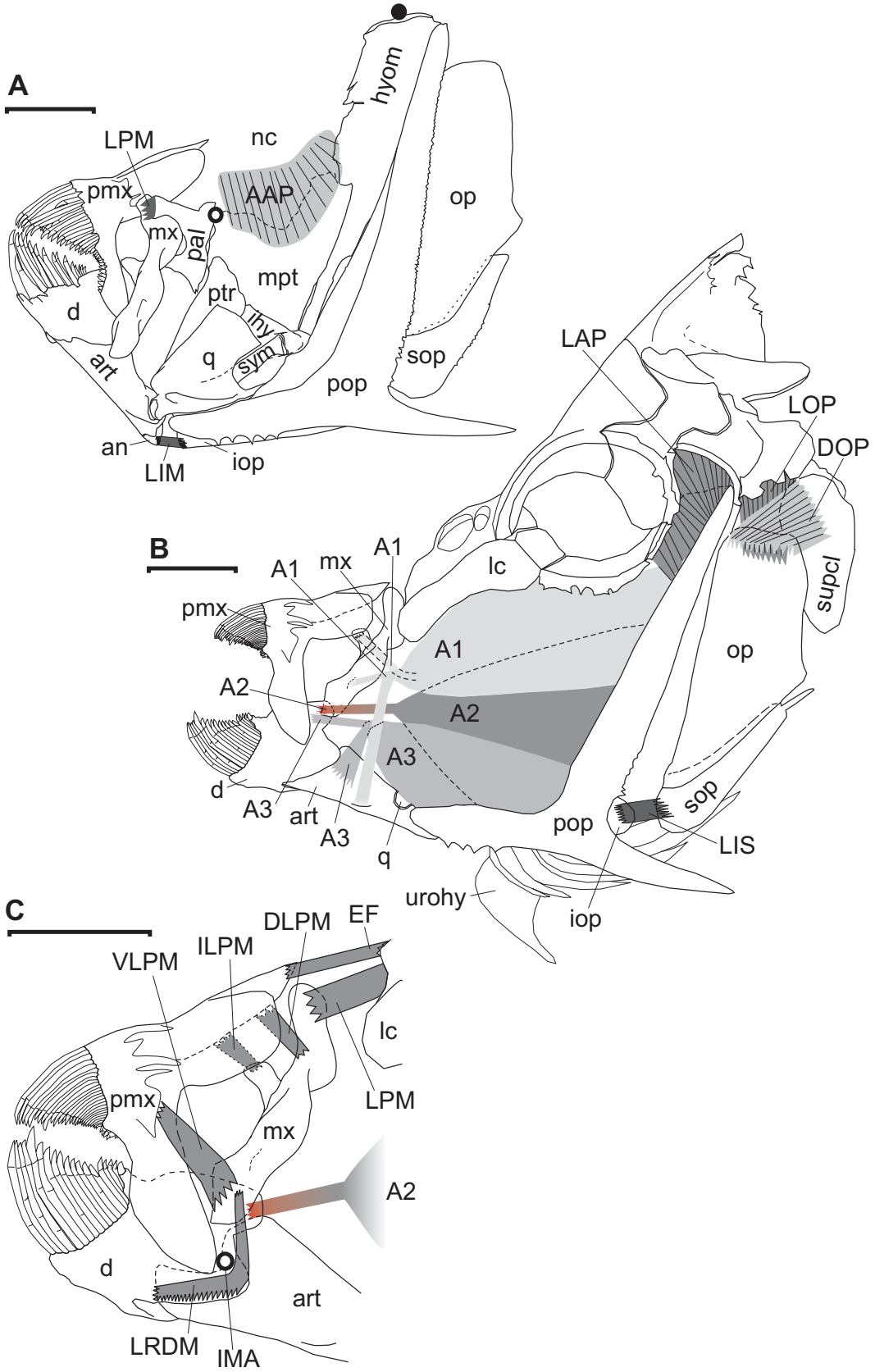
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Figure 2.2

and supported by a premaxillary-maxillary ligament (DLPM, in Fig. 2.2C). The antero-ventrally tapering maxillary arm (Fig. 2.2A) has a reduced cranial condyle (compared to e.g. chaetodontids, Motta, 1982). Initial suspensorial rotation is followed by suspensorial stasis during *c.* 300 ms, while the onset of mandible depression (Fig. 2.1D, angle L; Fig. 2.4B at T_B–150 ms) augments gape expansion by rotation of approx. 38º (T_B–350 ms). Gape expansion coincides with a rotation of the operculum by approx. 8º (Fig. 2.1B, angle O; Fig. 2.4C), reaching maximum rotation around T_b–20 ms.

The opercular series (Fig 2.2B) is formed by a vertical component, the fused operculum and suboperculum, which are connected by a ligamentous sheet (LIS) to the horizontally rectangular interoperculum, with a resting angle between mandible and interoperculum (Fig. 2.2A) of around 60º. Prominent LOP musculature can rotate the operculum around a synovial articulation on the dorso-caudal margin of the hyomandibular bone (Fig 2.3A), mimicked by manipulating the ventral opercular margin, and the adjoined interoperculum in a dorso-caudal direction (**3** in Fig. 2.1B). This displacement tightens the LIM (Fig. 2.2A), thereby causing mandible depression (Fig. 2.3A; 2.3B). As the oral jaws have a dorsally inclined resting position (Fig. 2.2A), due to extensive architectural reorganisation of the skull, the opercular series kinematics also causes rotational protrusion of the mandible (Fig 2.3A; 2.3B). The hyoid apparatus is flexible, with modest *protractor hyoideus, sternohyoideus* and *genihyoideus* musculature. Pectoral girdle rotation (measured as a proxy for hyoid depression, Fig. 2.1C, angle S) attains approx. 6.5º, around T_b–65 ms, with a prolonged duration. Similarly, the cranial articulation with the vertebra is mobile, with a raised supraoccipital crest enlarging the insertion surface for epaxial musculature.

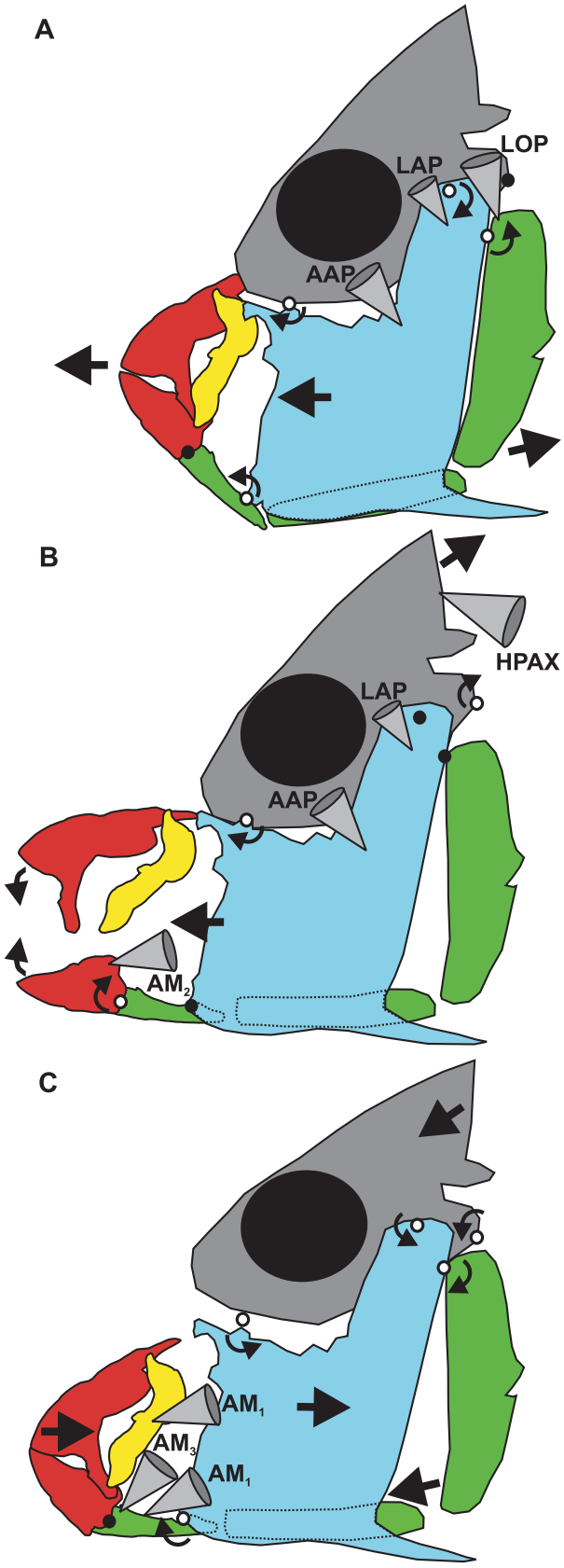

Figure 2.3 Schematic representation of skull kinematics in *Pomacanthus semicirculatus* illustrating the three significant phases of a grab-and-tearing feeding event; A. Jaw apparatus prior to protrusion onset, B. protruded, and C. protruded-closed state (upon jaw retraction, C. returns to A. after the recovery phase, in preparation for the next feeding event). Colour coding: Grey: neurocranium, Red: oral jaws, Yellow: maxilla, Blue: suspensorium, Green: opercular series and articular. Arrows indicate displacement, mediated by tendons and ligaments. Open circles indicate rotation and filled circles indicate passive linkage. Cones represent inferred contraction of a muscle group, with myology labelling as in Figure 2.2. HPAX: Hypaxialis musculature.

Figure 2.4 Mean kinematics profiles for three *Pomacanthus semicirculatus* (five bites per individual, all bites pooled ±SE), illustrating timing of onset, magnitude and duration of angular displacement (in degrees) in: A, hyomandibular; B, mandibular; C, opercular and D, intramandibular mechanisms. Note the alignment of kinematics around time of bite (T_B).

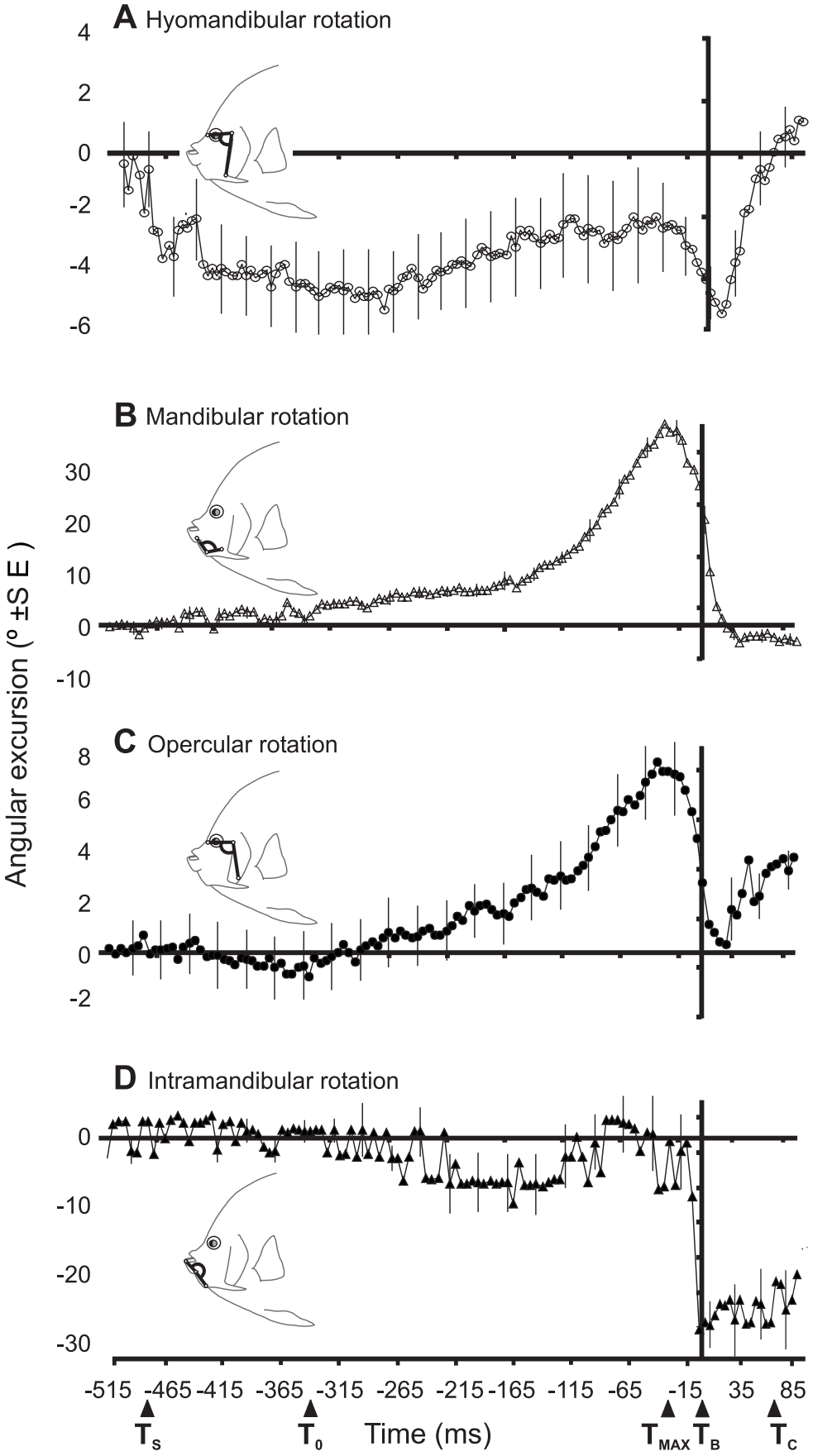
A Hyomandibular rotation
B Mandibular rotation
C Opercular rotation
D Intramandibular rotation
Angular excursion (° ±S E)
Time (ms)
-515 -465 -415 -365 -315 -265 -215 -165 -115 -65 -15 35 85
T_S
T_0
T_{MAX}
T_B
T_C

Figure 2.4

Cranial elevation (Fig. 2.1B, angle C) exhibits a slow and gradual increase to approx. 11º, with a peak around T_b–15 ms. In kinematic analyses, rotation in the suspensorial and cranial mechanisms are minimal around protrusion onset, only accelerating during the latter part. Despite the pronounced mobility in these mechanisms, neither isolated nor simultaneous manipulation (1 and 2 in Fig. 2.1B) resulted in mandible depression. The second stage of suspensorial rotation of approx. 4º (Fig. 2.4A) further augments mandible and premaxillary protrusion (|P| & |D| in Fig. 2.1A; Fig. 2.5A) and reaches maximum rotation around T_b.

2.3.2 Jaw closure

An intramandibular joint (IMJ) is present (Fig. 2.2C), with the lateral and medial walls of the dentary forming an articulating socket for the distal articular ascending process. Connective tissue restrains the dentary whilst allowing it to rotate on the articular, causing elevation of the tooth-bearing dentary surface. A single tendon from the medial A2 inserts into a deep medial fossa on the coronoid process of the dentary. No articular insertion of the A2 is present. The laterally convex, tooth-bearing surfaces of both the premaxilla and dentary contain tightly packed arrays of bristle-shaped teeth arranged in 5-7 tiers with tooth lengths decreasing posteriorly. A ventral premaxillary-maxillary ligament (VLPM in Fig. 2.2C), originating from the lateral premaxilla, inserts lateroventrally on the maxillary arm, while a prominent and modified articular-dentary-maxillary ligament (LRDM) connects the maxillary arm to almost the entire lateroventral surface of the dentary, but notably, not to the articular. Dentary manipulation (5 in Fig. 2.1B) causes tightening of this ligamentous array, forcing the tooth-bearing face

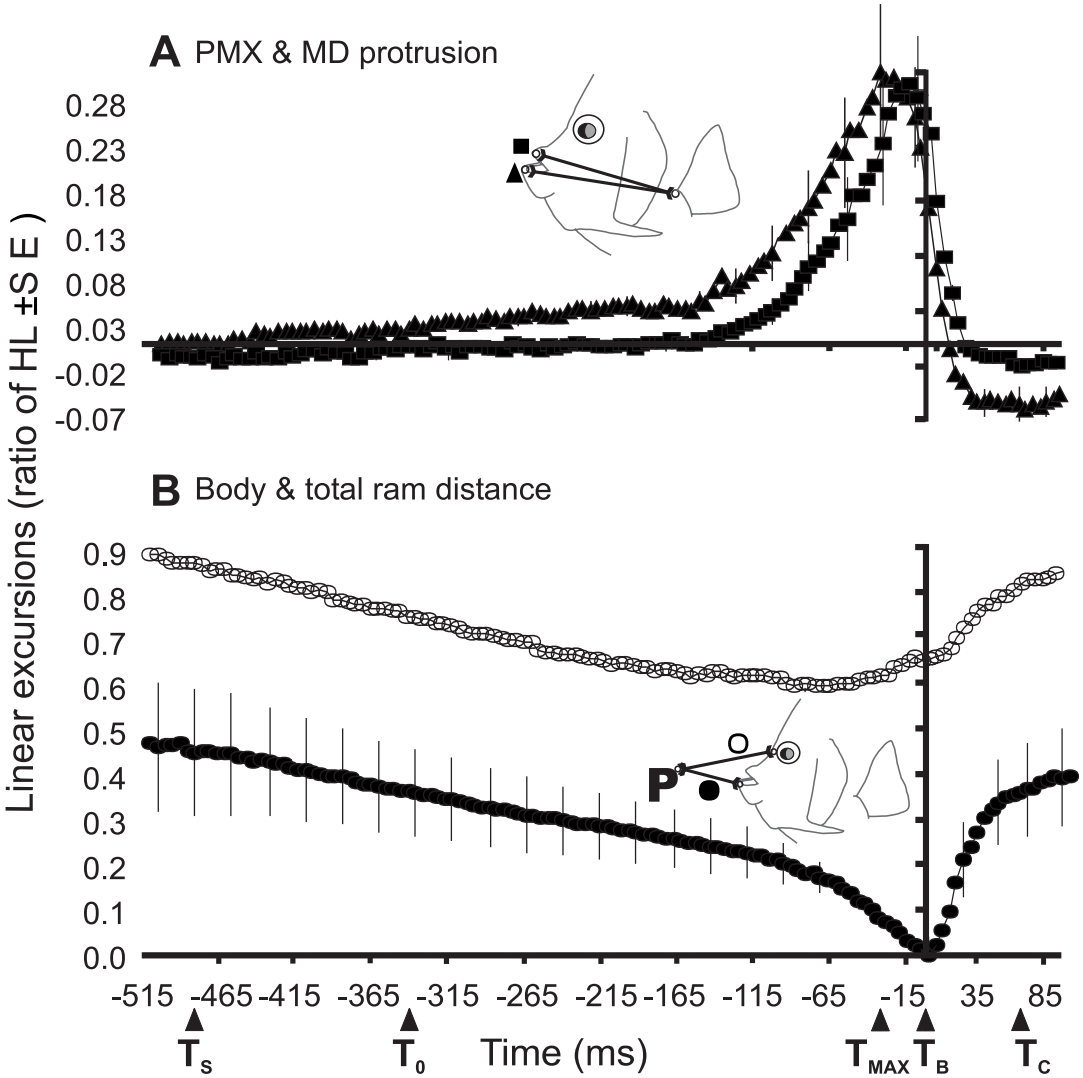

Figure 2.5 Mean kinematic profiles for three *Pomacanthus semicirculatus* (five bites per individuals, all bites pooled, vertical axis shows ratio of head length (HL) ±SE), illustrating timing of onset, magnitude and duration of linear displacement (as ratio of HL, head length) of: A, the premaxilla (square) and the mandible (triangle); and B, body-ram (open circle) and total-ram (closed circle).

Table 2.1 Performance characteristics of prey-capture kinematics in *Pomacanthus semicirculatus* with linear distances, angles and durations derived from high-speed video sequences of 30 separate feeding events (n=6 individuals with 10 bites each; all bites pooled for analysis). Linear, angular and ram variables follow Figure 2.1. *A.* Ram excursions and velocities are given for jaw protrusion, bite and retraction as maximum values (mean values indicated in parentheses). *B.* Jaw gape and protrusion with excursion maxima, timing of onset and maximum excursion relative to T_B and total duration. *C.* Angular excursions, with excursion means, timing of onset and maximum excursion relative to T_B and total duration.

A				Velocity maxima ($cm\ s^{-1}$)		
	Ram Variable	Distance	Maximum Excursion (cm)	Protrusion	Bite	Retraction
	R_{TOT}	Total ram	7.2 (5.6)	11.6 (6.4)	2.3 (1.6)	99.6 (52.4)
	R_J	Jaw ram	4.5 (2.9)	7.9 (4.8)	2.3 (1.6)	81.9 (45.0)
	R_P	Body ram	2.0 (0.6)	5.4 (1.5)	0.01 (0.005)	31.7 (7.4)

B							
	Linear Variable	Distance	Maximum excursion (%HL)	Onset (ms from T_B)	Maximum Excursion (ms from T_B)	Duration (ms)	Prot (ms) Ret (ms)
	G	Gape	11	-345	-45	345	300 45
	P	PMX protrusion	22	-150	-15	175	135 40
	D	MD protrusion	29	-155	-30	185	125 60

C						
	Angular Variable	Mechanism	Mean excursion (degrees)	Onset (ms from T_B)	Maximum Excursion (ms from T_B)	Duration (ms)
	C	Cranial	-11.2º	-455	-10	540
	O	Opercular	7.4º	-290	-30	310
	H	Hyomandibular	-6.5º	-575	10	755
	S	Sternohyoid	3.1º	-515	-5	600
	I	Intramandibular	-30.0º	-10	0	125
	L	Mandibular	37.9º	-340	-25	450
	M	Maxillary	17.5º	-250	-40	335

of the premaxilla onto the dentary tooth face, resulting in mouth closure (Fig. 2.1C, 2.3B-C), with the upper and lower jaw teeth occluding without superior or inferior overlap (Fig. 2.2C). Jaw closure kinematics (Fig. 2.3B) involve rotation of the intramandibular joint over approx. 5 ms, attaining approx. 30º (Fig. 2.1C, angle I, Fig. 2.4D), and occluding the protruded jaws at T_B.

Table 2.2 Prey-capture performance characteristics in *Pomacanthus* compared with previously studied acanthurid (A) and labroid (L) taxa, with designation of feeding modes (B, biting; S, suction; R, ram). (HL, Head length; IMJ, Intramandibular joint). Note that all taxa except *Pomacanthus* lack a dedicated mechanism for protruded jaw closure, and negative values represent speeds attained during jaw retraction. While total bite duration in *Pomacanthus* bears most resemblance to other biters, the inverse intramandibular joint kinematics, magnitude of jaw protrusibility and velocity maxima distinguishes *Pomacanthus* from other biters; *Pomacanthus* kinematics values bear a stronger resemblance to ram-feeders.

Taxa	Super-order	Feeding mode	Jaw protrusion (%HL)	Maximum jaw velocity (cm/s)	Protrusion duration (ms)	Retraction duration (ms)	IMJ kinematics	Author(s)
Pomacanthus	A	B	30	-82	550	60	closing	Present study
Ctenochaetus	A	B	6	-12	120	110	opening	Purcell & Bellwood, 1993
Astatotilapia	L	S	13	47	15	30	flexion	Aerts, 1985; Aerts *et al*., 1987
Chaetodon	A	S	7	8	24	24	-	Motta, 1985; 88
Forcipiger	A	R	30	13	30	40	-	Ferry-Graham *et al*., 2001a
Epibulus	L	R	65	230	35	76	-	Westneat & Wainwright, 1989
Petenia	L	R	55	65	24	-	-	Waltzek & Wainwright, 2003

2.3.3 Jaw retraction

The *m. adductor mandibulae* (Fig. 2.2B), whilst displaying the typical four divisions seen in teleosts, differs in some important respects. As noted above, a single tendon from the A2 inserts wholly on the dorsal surface of the dentary coronoid process. The A3 insertions are displaced posteriorly, away from the dentary, with one tendon from the ventrolateral A3 $_{\alpha}$ inserting in a shallow lateral fossa on the dentary, while the medial A3 $_{\beta}$ inserts on the sesamoid-articular, which is posteriorly displaced on the medial articular. The dorso-laterally situated A1 has two subsections: the A1$_{\alpha}$ inserts onto the primordial

ligament (the outer articular-maxillary ligament, or OLRM in Fig 2.2B); the $A1_{\beta}$ inserts in a medial fossa on the premaxillary condyle of the maxilla. Jaw retraction (Fig. 2.3C) occurs with a slight lag (5 ms) after T_B (Fig. 2.1D, angle L; Fig. 2.5A), and is associated with a pronounced lateral head jerk. Reverse body movement at this time, caused by a reverse pectoral fin stroke, yields an additional retraction of 20% HL from the prey (Fig. 2.5B). Jaw retraction kinematics is of high-velocity, encompassing 35º of mandible rotation and a linear excursion of approx. 30% HL. over 20-60 ms., to complete the feeding event at t_C.

2.3.4 Feeding event velocity regimes and performance

Linear excursions of gape, whole-jaw protrusion, -ram and body ram are summarised in Table 2.1. Mandible protrusion (Fig. 2.1A, $\Delta|D|$) attains about 30% HL, with subsequent retraction of the mandible beyond the resting point accounting for the negative protrusion values (Fig 2.4E). Premaxillary protrusion (Fig. 2.1A, $\Delta|P|$) attains approx. 27% HL, and occurs with an approximately 30 ms lag after mandible protrusion. During a feeding event, body ram, measured as the change in distance from prey to the nape (Fig. 2.1A, $\Delta|R_B|$) accounts for a 20% HL movement (Fig. 2.5B). Jaw protrusion is initiated outside a distance of 60% HL from the prey, and jaw-ram (Fig. 2.1A, $\Delta|R_{TOT}–R_B|$) typically covers approx. 30% HL. Body-ram velocities exhibit little change throughout the feeding event (Table 2.1); while the changes in jaw-ram velocity are notable (as visualised by varying curve slopes in Fig. 2.5B), with a slow protrusion (mean 6.4 cm s^{-1}), fast closure (mean 16.0 cm s^{-1}), and high-velocity retraction (mean 52.4 cm s^{-1}) during the feeding event phases (Table 2.1a).

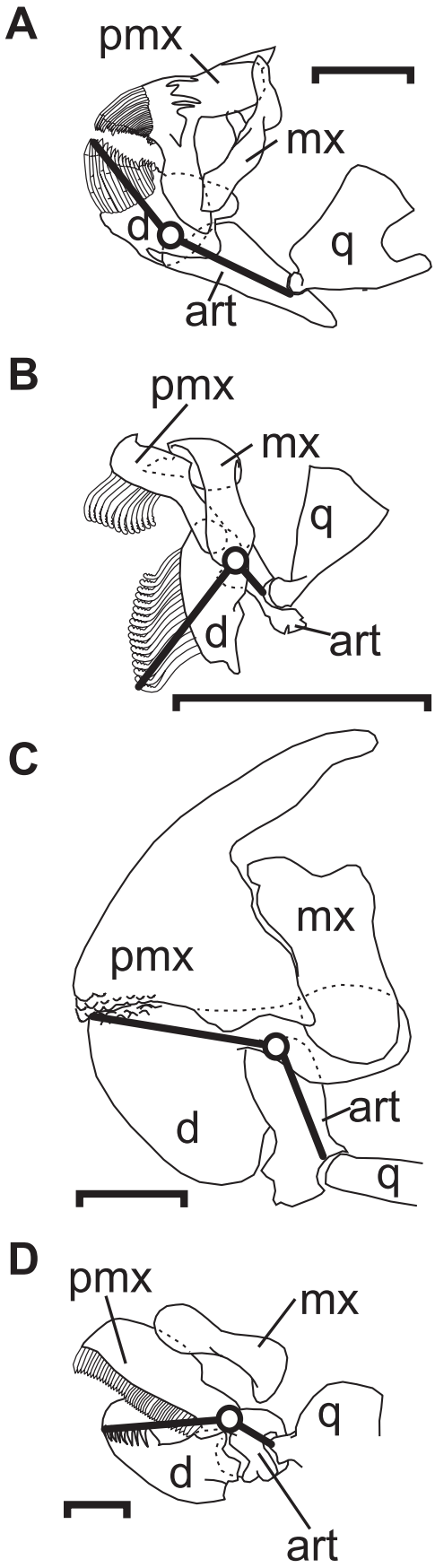

Figure 2.6 Camera lucida drawings of the feeding apparatus in four biting coral reef teleosts with intramandibular joints marked by open circles. Proximal (articular) and distal (dentary) components of the joints are marked by solid black bars (see text for explanation). A-B, joints rotated, C-D, joints relaxed. A. *Pomacanthus* (f. Pomacanthidae); B. *Ctenochaetus*, (f. Acanthuridae); C. *Scarus,* (f. Scaridae); D. *Escenius,* (f. Blennidae). Scale bars 10 mm, except D. 0.1 mm. Labelling follows Figure 2.2.

The conventional measurement of total bite duration (T_C–T_0) averages 450 ms, measured using jaw protrusion as proxy (Fig. 2.5A). However, when accounting for the early excursion of the suspensorium (Fig. 2.4A), mean bite duration (T_C–T_S) increases to about 600 ms, and sometimes approaches 750 ms (Table 2.2).

2.4. Discussion

The feeding apparatus morphology and associated feeding kinematics in *Pomacanthus* differ markedly from a generalised perciform suction or ram feeder. In suction feeding taxa, onset of cranial elevation commonly coincides with onset of oral gape expansion and jaw protrusion, followed by hyoid retraction and/or depression, while jaw occlusion occurs at completion of jaw retraction (Wainwright & Shaw 1999; Alfaro *et al*., 2001; Grubich, 2001; Ferry-Graham *et al*., 2001c; 2002; Sanford & Wainwright, 2002). In *Pomacanthus*, however, i) an intramandibular joint yields a novel sequence of onset timing in the retraction phase of the feeding event by facilitating protruded jaw closure; ii) steep resting angles enable the opercular mechanism to at least partially relieve cranial elevation and hyoid depression in the initiation of mandible depression; iii) antero-posterior rotation in the hyomandibular-cranial articulation, combined with pronounced palatoethmoid and palatopterygoid flexion facilitates suspensorial rotation which precedes other mechanisms and augments mandible protrusion, iv) suspensorial and opercular linkage stasis upon jaw closure appears to stabilise the feeding apparatus for optimised bite-force tenacity; and v) novel velocity regimes during the feeding event have great influence on feeding ecology.

2.4.1 The intramandibular joint

In an early descriptive account, Gregory (1933) noted "an incipient articulation of the dentary in the lower jaw of *Holacanthus [Angelichthys] ciliaris"*, but did not elaborate on functional implications, or the presence of intramandibular joints in other pomacanthids. In fact, intramandibular articulation may be the most significant morphological specialisation in the feeding apparatus of pomacanthids, with drastic consequences on feeding kinematics. Whilst bearing strong anatomical resemblance to IMJs described in other biting taxa (Fig. 2.6), the IMJ kinematics of *Pomacanthus* appear to be unique. In at least two acanthurid genera (*Acanthurus* and *Ctenochaetus;* Purcell & Bellwood, 1993) and three scarid genera (*Chlorurus*, *Hipposcarus* and *Scarus;* Bellwood, 1994; Streelman *et al*., 2002), IMJ kinematics, although unquantified, have been suggested to increase gape expansion and function while the jaws are retracted. In *Pomacanthus*, however, IMJ kinematics produce jaw closure with the mandible maximally depressed and the jaws at peak protrusion (Fig. 2.3). As a result, a distinct occlusion stage is added to the third phase of the feeding event, contrasting with the feeding kinematics in other IMJ bearers, as well as in perciform teleosts as a whole (Table 2.2; Ferry-Graham & Lauder, 2001).

2.4.2 Alternative mechanisms of mandible depression and jaw protrusion

Mandible depression kinematics in *Pomacanthus* appears to be driven by opercular rotation, thus differing from many other teleosts, and especially suction and ram-feeders, in which cranial and/or hyoid kinematics have an early onset (Muller, 1987; Aerts *et al.,* 1987; Alfaro *et al.,* 2001). In more basal fishes these mechanisms are

considered functional alternatives to the opercular linkage for initiation of mandible depression (Lauder, 1980; Carroll & Wainwright, 2003). However, our kinematic results as well as morphological properties of the *Pomacanthus* feeding apparatus suggest an reduced importance of these mechanisms in angelfishes. The onset timing of cranial elevation is delayed and during gape expansion and mandible depression the hyoid apparatus does not protrude ventrally (anterior to the isthmus in Fig. 2.1) as is typically the case in suction-feeders utilising this linkage (Motta, 1982; Aerts *et al.,* 1987).

Hyoid myology appears to be reduced compared to chaetodontids (Motta, 1982), yet the hyoid apparatus appears to be more flexible (Burgess, 1974; Motta, 1982). Our manipulation studies of the *Pomacanthus* feeding apparatus demonstrate that the oral jaws, suspensorium and opercular series constitute a functionally discrete unit, with component parts being capable of generating mandible depression, gape expansion, and oral jaw protrusion/retraction. The resultant displacements are of magnitudes comparable to those obtained in video kinematics yet exclude input from the hyoid linkage and the cranial lever mechanism. The observed lag in premaxillary protrusion, suggests that premaxillary kinematics is driven by that of the mandible, corresponding with 'type-B protrusion mechanism' (*sensu* Winterbottom, 1974; see also Motta, 1984) where the protruding mandible pulls the premaxilla anteriorly *via* ligamentous connections. Pomacanthids are unusual in having the oral jaws resting with a dorsal inclination relative to the interoperculum, which rests at a steep angle to the operculum (Gregory, 1933). Articular elongation increases the mandible out-lever, while anterior displacement of the quadrate articulation leaves the proximal articular as a hypertrophied opening in-lever (Fig. 2.6). Combined, these traits may provide the biomechanical leverage to make

opercular rotation the primary mechanism responsible for mandible depression and premaxillary protrusion (Anker, 1974).

Several lines of evidence support this interpretation, including the synchrony observed in opercular rotation and mandible depression kinematics (Fig. 2.4), and the well developed LOP musculature. Most labroids (including the extreme jaw-protruders) have an opercular-mandibular resting angle around 0º, and less developed opercular musculature (Wainwright *et al.,* 2004, Konow unpublished data). It is perhaps for this reason that opercular rotation has been considered of inferior importance when compared to the role of cranial elevation for initiation of mandible depression in teleosts (Westneat, 1990). Still, both Anker (1974) and Motta (1982) suggested that the opercular mechanism provided significant input to mandible depression initiation in several suction-feeding taxa. More recent experimental studies on suction-feeding cichlids and centrarchids have shown drastically reduced mandible depression performance after surgical severance of the interopercular-subopercular ligament (LIS: in Fig. 2.2A) while leaving the LIM, with the opercular-hyoid connection intact (Durie & Turingan, 2004). While the opercular mechanism may well represent a functional reversal to a basal teleost mechanism, dominant in Halecostome fishes and retained in some extant larval teleosts (Lauder & Liem, 1981; Adriaens *et al.,* 2001), it is noteworthy that similar opercular-mandibular angles are observed in both closely related (*Acanthurus* and *Ctenochaetus*) and more distantly related (*Scarus*) biting taxa. Given the paucity in kinematics data, it remains unclear if a functional opercular mechanism is a shared biter trait.

2.4.3 Within and between-mode performance variations

Mandible protrusion of 30% HL, as observed in *Pomacanthus,* may be considered extreme, and is a rare trait in teleosts. Such protrusion magnitude was previously only described in the cichlid genera *Petenia* and *Caquetaia* (Waltzek & Wainwright, 2003), the chaetodontid *Forcipiger* (Motta, 1984; Ferry-Graham *et al*., 2001a; 2001b), and the labrid *Epibulus* (Westneat & Wainwright, 1989). These taxa are all ram-suction feeders, possess extreme axial elongation of several jaw elements, and complex suspensorial mechanisms, either based on pivoting linkages (*Epibulus, Petenia* and *Caquetaia*) or suspensorial rotation around multiple points of flexion (*Forcipiger*). In comparison, *Pomacanthus* has suspensorial rotation around two novel points of flexion, contributing approximately 40% of the observed mandibular protrusion while depression of the dorsally inclined mandible contributes the remaining 60%. Axial bone elongation in *Pomacanthus*, albeit less pronounced than in other extreme jaw-protruders, is considerable in chaetodontoid terms (Motta, 1985; 1988). The resultant protrusion is of comparable magnitude to *Forcipiger*, for example, which displays the most extensive axial elongation of jaw osteology known in teleosts and three novel points of suspensorial flexion (Table 2.2). In contrast, the hyomandibular-cranial articulation of scarid and acanthurid IMJ-bearers lack antero-posterior rotation, and the palatoethmoid region shows little flexion and no reduction. Indeed little or no mandibular protrusion has been documented in these taxa (Motta, 1982; Purcell & Bellwood, 1993; Bellwood, 1994), whilst in *Ctenochaetus*, modest suspensorial rotation (Purcell & Bellwood, 1993) appears to augment gape angle and expansion increase rather than mandible protrusion.

The differences in axial bone elongation and incidence of derived mechanisms in the feeding apparatus of *Pomacanthus* and other extreme jaw-protruders may reflect

diverging structural requirements of ram-suction and biting kinematics during feeding (Table 2.2). In long-jawed ram-suction feeders, the prioritising of protrusion speed over jaw closure force (Barel, 1983) makes a latero-medially elongated jaw apparatus a logical prerequisite, providing stability in order to maintain precision during the dramatic, high-velocity protrusion kinematics (Westneat & Wainwright, 1989; Waltzek & Wainwright, 2003). Conversely, in *Pomacanthus*, peak-protruded jaw closure and jaw retraction appear to be critical feeding kinematics. The initial suspensorial rotation stage is followed by a prolonged stage (350 ms) of partially rotated, static posture. The second rotation stage, occurs immediately prior to jaw closure (T_B –15 ms), and coincides with maximal rotation of the opercular-, cranial-, and hyoid linkages. This late-protrusion constriction of the feeding apparatus presumably results from contraction of opercular, suspensorial, epaxialis and hypaxialis musculature and may serve to stabilise the oral jaw apparatus, thereby optimising the input from A2 contraction to dentary rotation, with a resultant direct force transmission for jaw closure. The close apposition of the hyomandibular bars, resulting from latero-medial skull compression, is an additional trait likely to govern bite forcefulness (Aerts, 1991).

Interestingly, while *Pomacanthus* jaw protrusion velocity is very slow (Table 2.1), mandible retraction velocity (approaching 100 cm s^{-1}) surpasses the high-velocity jaw movements of many ram feeders (Table 2.2). High retraction velocity corresponds well with the caudal displacement of A1 and A3 insertions. This displacement also leaves the A2 as the sole muscle rotating the dentary around the IMJ. Currently, anterior four-bar linkage models (Westneat, 1990; Hulsey & Wainwright, 2002; Wainwright *et al*., 2004) as well as models for mandibular mechanical advantage (Turingan *et al*., 1995; Wainwright & Shaw, 1999; Wainwright & Bellwood, 2002; Bellwood, 2003) do not

allow for IMJ presence (Wainwright *et al.,* 2004). The transmission coefficients of jaws with an IMJ are therefore unknown at present. However, it is noteworthy that *Pomacanthus* appears to be unique among IMJ-bearing teleosts in having the distal (dentary) portion of the IMJ equal to or longer than the proximal (articular) portion (Fig. 2.6). Whether this trait is causally related to pomacanthids being the only taxa with a closing IMJ remains to be determined.

Prey dislodgement force requirements could be met via alternative pathways, as mechanical output is not always linearly coupled with muscle contraction (Aerts *et al.,* 1987). At jaw occlusion the prey is clenched between tiered rows of bristle-shaped teeth in the protruded oral jaws, potentially yielding considerable gripping qualities. The protruded oral jaws appear to be stabilised in protruded-closed configuration by a rigid frame formed by the suspensorial and opercular rotation. A slight lag (5-7 ms) is observed prior to mandible retraction. It remains to be tested if this lag represents a stage of strain-energy storage in the *m. adductor mandibulae* sections involved with mandible retraction. Such an 'elastic recoil mechanism' was described in the mandible kinematics of *Astatotilapia*, where the power requirement for kinematics at the observed velocity exceeded the physical capability of mechanical output calculated from available muscle mass (Aerts *et al.,* 1987). In *Pomacanthus*, cranial stabilisation during the pre-retraction lag may be preventing jaw retraction initiation, thereby augmenting strain-energy build-up in the A1 and A3 musculature, which is mobilised upon skull musculature relaxation (bar the A2). Trade-offs between forcefulness and rapidity during *Pomacanthus* mandible retraction, along with the functional properties of tiered bristle tooth rows, require further investigation. Further biomechanical modelling and tensiometry combined with EMG appear to be the most promising avenues for future research.

2.4.4 Ecological implications of intramandibular joints

While the IMJ of *Pomacanthus* morphologically resembles that found in other biters, available evidence suggests that the IMJ kinematics differ and the feeding ecology certainly differs markedly. Only IMJs with inferred gape-expanding kinematics have previously been inferred in coral reef fishes (Fig. 2.6), such as the Acanthuridae (Purcell & Bellwood, 1993), the Scaridae (Gobalet, 1980; Bellwood 1994; Streelman *et al*., 2002) and IMJs also exist in the blennid genus *Escenius* (Konow, unpublished data). These taxa predominately graze or scrape planar or convex substrata (Choat & Bellwood, 1985; Choat, 1991; Bellwood *et al*., 2003; Depczynski & Bellwood, 2003). Hence, IMJ presence in *Pomacanthus* corresponds well with previous notions of biters exhibiting increased structural complexity in feeding apparatus morphology in accordance with the biomechanical challenges imposed by the substratum utilised (Wainwright & Bellwood 2002; Bellwood *et al*., 2003). However, the putatively unique IMJ kinematics of pomacanthids apparently relates to distinct ecological patterns of grab-and-tearing prey-capture, and may reflect a novel, yet unquantified, pattern of microhabitat utilisation.

The unusual IMJ kinematics may be particularly important in the larger, spongivorous taxa, such as *Pomacanthus,* known to prey on a wide range of invertebrate taxa, including sponges (Burns *et al*., 2003), gorgonians (Fenical & Pawlik 1991) and soft corals (Wylie & Paul, 1989)**.** Such prey typically possess potent predator-deterring toxins (Wylie & Paul, 1989), leading previous workers to the assumption that chemical defence may be the primary basis for predation deterrence in these important components of the non-coralline benthic reef community (Dunlap & Pawlik, 1996). Sponge toxin concentrations correlate well with the degree of within-habitat exposure to predation (Swearingen & Pawlik 1998). Chaetodontoid fishes appear to utilise toxic prey through

presumed tolerance of toxins (Wylie & Paul, 1989; Dunlap & Pawlik, 1996; Thacker *et al.,* 1998; Gleibs & Mebs, 1999), but a complementary explanation may exist: many of the less exposed (and less toxic) attached invertebrate taxa also exhibit less structural resilience, and while it is likely that chemical and structural defences function in concert to reduce predation, as commonly seen in algae (Hay, 1991), trade-offs are likely to exist between toughness and crypsis for many of the taxa consumed by pomacanthids (Wulff, 1997). The result may be that the least structurally defended species exhibit the most cryptic lifestyles, and the distribution and abundance of these invertebrate taxa may be shaped by the abundance of predators with jaw protrusibility, coupled with a grab and tearing force sufficient enough to utilise such crypto-benthic resources. Other predators robust enough to dislodge these taxa may simply be unable to reach them due to large body size. This opens an interesting avenue of ecological research into the relative importance of large angelfish taxa in shaping the distribution and abundance of toxic and/or structurally resilient, crypto-benthic reef taxa.

Microhabitat utilisation in *Pomacanthus* contrasts markedly with most other coral reef fishes that either ram-suction feed on free-living prey, or use gracile scraping or grazing strategies to procure delicate and/or loosely attached prey on convex or planar substratum surfaces. The unique microhabitat utilisation patterns in *Pomacanthus* are apparently facilitated by several unusual kinematic characteristics, all bearing more resemblance to jaw-ram feeders than to other biters (Table 2.2). As in long-jawed butterflyfishes, which are known to ram-feed on elusive non-attached prey in confined microhabitats, *Pomacanthus* exhibit extensive oral-jaw protrusion, enabling them to reach prey in complex and confined microhabitats. The unique IMJ kinematics, yielding peak-protruded jaw closure, combines with the tiered bristle tooth rows, which have

prehensile properties like 'Velcro-hooks', to reach in and obtain a high-tenacity grip on prey. Finally, the abrupt and high-velocity kinematics of jaw retraction, along with reverse body acceleration caused by pectoral fin and a lateral head jerk, generates sufficient tearing strength and/or momentum to dislodge prey with pronounced structural resilience. These distinct traits, coupled with the characteristic repetitive-bite foraging pattern observed in spongivorous angelfishes suggest these taxa represent a functionally, as well as ecologically distinct component of reef assemblages.

Overall, the prey-capture kinematics of *Pomacanthus* appears to differ markedly from biters described previously and, accordingly, the *Pomacanthus* feeding strategy could be considered as a new grab-and-tearing subcategory of the biting feeding mode. How widespread these novel feeding traits are, and their role in the evolutionary and ecological diversification of the Pomacanthidae will be evaluated in Chapter 3.

Chapter 3: Functional disparity and ecological diversification in marine angelfishes, f. Pomacanthidae

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3. 1 Introduction

Our current understanding of feeding ecomorphology in coral reef teleosts is founded on a limited amount of functional and behavioural evidence. In particular, there is a general lack of information about the feeding kinematics of taxa that use active biting to procure prey from the physically diverse reefal substrata. This paucity in biting data prevents an examination of critically important links between assemblage-level functional disparity and evolutionary diversification (Liem, 1980b; Streelman *et al*, 2002). It is currently not possible to compare kinematic disparity patterns amongst functionally distinct teleost trophic groupings, coined feeding modes (Norton & Brainerd, 1993), despite the potential for unique functional innovations evolving at different rates among these modes. Hence, examinations of the functional basis of biting in teleost assemblages are urgently needed. The importance of such examinations are equally reflected by our limited understanding of the evolutionary processes leading to a proliferation of biting taxa amongst Recent coral reef fishes (Wainwright & Bellwood, 2002).

A biting group particularly suitable for detailed functional analysis is the marine angelfishes, f. Pomacanthidae, which like other biters, predominately have been examined in a structural-descriptive context (e.g. Gregory, 1933; Burgess, 1974; Hourigan *et al*., 1989; Howe, 1993). A functional analysis of *Pomacanthus semicirculatus* revealed several feeding apparatus novelties, including suspensorial

rotation augmenting mandibular protrusion; intramandibular kinematics facilitating protruded jaw closure and high-velocity jaw retraction. These traits facilitate an unusual grab-and-tearing feeding strategy, permitting *Pomacanthus* to dislodge structurally resilient prey from confined attachment sites within the reef matrix (Chapter 2). Availability of a robust generic-level phylogenetic hypothesis (Bellwood *et al*., 2004b) reinforces the utility of pomacanthids as subjects for a comprehensive analysis of functional disparity (Westneat, 1995; Foote, 1997). This permits the distinction of convergent traits from traits resulting from shared ancestry in examining the influence of novel feeding traits on evolutionary ecology.

Qualitative dietary information has been generated for many popular aquarium species, which has prompted inferences of pomacanthids utilising divergent trophic guilds, including spongivory, herbivory and planktivory (Allen, 1981; Allen *et al*., 1998, Debelius *et al*., 2003). Alimentary traits could be useful in an examination of the validity of these purported trophic guilds as gut lengths commonly decrease from herbivores to omnivores, carnivores and planktivores (Al-Hussaini, 1947; Horn, 1989; Howe, 1993; Elliot & Bellwood, 2003). Different trophic guilds are commonly associated with distinct structural or functional specialisations, such as muscular stomachs (gizzards) or hindgut fermentation chambers (Horn, 1989; Choat *et al*., 2004; Clements *et al*., 2004). Pomacanthid gut morphology is relatively well understood (Howe, 1993) with hindgut fermentation being linked to herbivory in *Holacanthus* (Perez-España & Abitia-Cardenas, 1996; Martinez-Diaz & Perez-España, 1999).

In freshwater systems, functional analyses incorporating direct performance testing have comprehensively examined predatory ram-suction feeding assemblages, including sunfishes, the Centrarchidae (Wainwright, 1986, 1996; Wainwright &

Lauder, 1986; Wainwright & Shaw, 1999; Svanbäck *et al*., 2002; Carroll *et al*., 2004) and the Cichlidae (Wainwright *et al*., 2001; Hulsey & García de León, 2005). Comparable marine teleost analyses either involved case studies of extraordinary taxa (Westneat & Wainwright, 1989; Bergert & Wainwright, 1997), unusual lineages (Gibb, 1997; Carroll & Wainwright, 2003) and durophagous groups like the Tetraodontiformes (Turingan *et al*., 1995). Of the extensive perciform radiation on coral reefs, functional evidence predominately concerns ram-suction feeding species flocks of chaetodontid and labrid fishes (Sanderson, 1990, 1991; Westneat, 1994; Alfaro *et al*., 2001; Ferry-Graham *et al*., 2001a, 2002). Feeding kinematics remain unquantified in close biting relatives, such as corallivorous butterflyfishes and herbivorous parrotfishes (Motta, 1985; 1988; Bellwood, 1994). In parallel, extensive labrid analyses have projected morphological measurements into biomechanical models and linked the results to functional and ecological diversification (Hulsey & Wainwright, 2002; Wainwright *et al*., 2004; Westneat *et al*., 2005). However, limited labrid feeding kinematics testing restricts the availability of behavioural data to verify these links (Lauder, 1990).

It has been proposed that novel morphological and functional variation underpins divergent biting guilds (Bellwood *et al*., 2003) and that disparate functional systems govern biting and ram-suction feeding (Wainwright *et al*., 2004). Amongst the two dominating reef fish lineages, labroid and squamipinnid fishes (Tyler *et al*., 1989; Rice and Lobel, 2004), labroids are dominated by ram-suction feeders while biting strategies are particularly prominent amongst squamipinnids (Chapter 4). Whilst the assumed differences among feeding modes still require quantitative functional evaluations, evidence of functional innovations amongst biters is increasing (Chapter 2). Intramandibular joints (IMJs) appear to increase

mandible complexity in several biting coral reef teleost groups: the Acanthuridae (Purcell & Bellwood, 1993), Chaetodontidae (Motta, 1985; 1988; 1989), Girellidae (Vial & Ojeda, 1990; 1992) and Pomacanthidae (Gregory, 1933; Chapter 2). Despite existing in the labroid scarines (Gobalet, 1980; Bellwood & Choat, 1990, Bellwood, 1994, 2003), IMJs could be a comparable functional innovation in squamipinnid biters (Chapter 4) to the pharyngeal jaw apparatuses in labroid ram-suction feeders (Galis & Drucker, 1996; Streelman & Karl, 1997; Rice & Lobel, 2004).

The specific aims in this chapter are therefore threefold. Firstly, combining morphological examinations and experimental quantification of feeding kinematics, the constructional, functional and kinematic disparity underlying a putatively broad range of pomacanthid trophic guilds is examined. Secondly, the novel prey-capture system in pomacanthids is evaluated in the context of assemblage-level evolutionary ecology of angelfishes on the Great Barrier Reef, Australia. Lastly, pomacanthid functional disparity is compared with available disparity evidence across feeding modality and evolutionary history, from the Chaetodontidae and Labridae.

3.2 Materials and methods

3.2.1 Study taxa selection and collection

Taxon selection was guided by a phylogeny derived from 12 and 16S DNA (Fig. 3.1; Bellwood *et al*., 2004b). Specific study taxa (Table 3.1) were chosen for complete representation in the analysis of all lineages occurring on the Great Barrier Reef, with species selection optimised towards the most abundant and widespread taxa. For each of the eight study species (Fig. 3.2), a minimum of three specimens were collected on SCUBA (by the authors or by commercial collectors based in

Table 3.1 Summary of pomacanthid taxa examined in this study.

			Measurements		N		
Genus	Subgenus	Species	TL, mm max-min (mean)	HL, mm max-min (mean)	Total Examined	Kinematics	Dissections & clearstains
Centropyge	*Centropyge*	*bicolor*	115-109 (112)	25-22 (23)	6	3	3
Apolemichthys		*trimaculatus*	156-144 (151)	36-35 (37)	6	3	3
Genicanthus		*melanospilos*	147-113 (130)	27-22 (25)	6	3	3
Centropyge	*Xiphypops*	*bispinosa*	109-84 (103)	23-19 (22)	6	3	3
Paracentropyge		*multifasciata*	68-60 (65)	19-18 (18)	3	-	3
Pygoplites		*diacanthus*	151-145 (147)	39-37 (38)	6	3	3
Chaetodontoplus		*duboulayi*	234-210 (220)	47-42 (44)	6	3	3
Pomacanthus	*Euxiphipops*	*sexstriatus*	313-257 (291)	66-64 (65)	6	3	3
Pomacanthus	*Arusetta*	*semicirculatus*	408-227 (295)	85-51 (68)	11	6	5
Pomacanthus	*Acanthochaetodon*	*imperator*	204-126 (174)	47-31 (41)	3	-	3

Cairns, Australia) from the central and northern sectors of the Great Barrier Reef, Australia, using barrier nets, or hand nets and clove oil. As pomacanthids exhibit an order of magnitude difference in body size (Fig. 3.1), study specimens from the smaller species were selected to approach the maximum size recorded (Table 3.1).

The following species were included in this study (Fig. 3.2): A, *Centropyge [Centropyge] bicolor* and B, *Apolemichthys trimaculatus,* both gracile omnivores; C, *Genicanthus melanospilos,* a zooplanktivore that also procures attached invertebrate prey; D, *Centropyge [Xiphypops] bispinosa*, an omnivore that procures large rations of algae; E, *Pygoplites diacanthus,* a robust omnivore on attached invertebrates; F, *Chaetodontoplus duboulayi*, a robust omnivore on sponges and tunicates; G, *Pomacanthus [Euxiphipops] sexstriatus*, a purported herbivore on calcareous and turfing algae; H, *Pomacanthus [Arusetta] semicirculatus,* a spongivore that also utilises other sturdy modular invertebrate prey (Allen, 1981; Allen *et al*., 1998, Debelius *et al*., 2003).

Figure 3.1 Phylogeny of the GBR-pomacanthids (modified from Bellwood *et al*., 2004b) with clades corresponding with (sub) generic ranking (see Table 3.1). Thumbnails indicate the typical body-plan in each clade, and are drawn to scale with mean length of species within each taxon. For each clade, the horizontal scale bars indicate minimum, mean and maximum lengths (from left to right) for all species (except in the monotypic *Pygoplites*) relative to a maximum length in *Pomacanthus* of 60 cm. Block matrices indicate species numbers on the GBR with black squares, and species in other regional assemblages with grey squares (species numbers are according to Allen *et al*., 1998). Note the tendency of decreasing size and increased species richness in the 'pygmy angel clade'. The head length (HL) and total length (TL) measurements refer to Table 3.1.

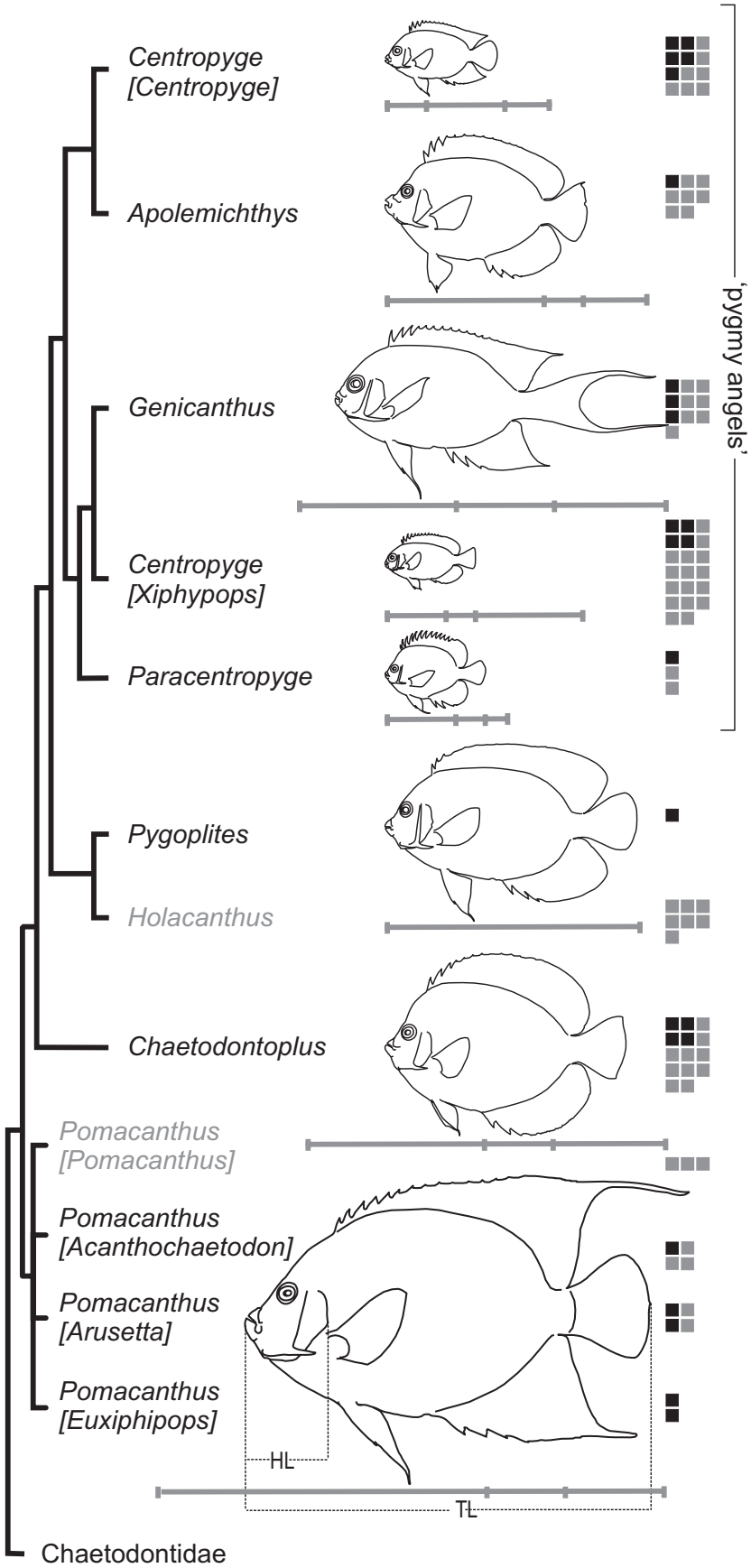
Centropyge
[Centropyge]
Apolemichthys
Genicanthus
Centropyge
[Xiphypops]
Paracentropyge
Pygoplites
Holacanthus
Chaetodontoplus
Pomacanthus
[Pomacanthus]
Pomacanthus
[Acanthochaetodon]
Pomacanthus
[Arusetta]
Pomacanthus
[Euxiphipops]
Chaetodontidae
'pygmy angels'
HL
TL

Figure 3.1

Whilst we primarily follow the taxonomy of Bellwood *et al*. (2004b), the subgenus *Pomacanthus [Pomacanthodes]* was rejected therein. Thus, we adopt suggestions made by Chung & Woo (1998) for a sub-generic classification of *Pomacanthus* (For alternative views, see R. Pyle, thesis). The confusion surrounding *Pomacanthus* is currently under investigation by C. Read and co-workers. Herein, morphological evidence was obtained for *P. [Acanthochaetodon] imperator*, the rare GBR monotype of *P. Acanthochaetodon* Bleeker 1876. However, this species appears morphologically similar to *P. [Arusetta] semicirculatus* with which it putatively shares trophic ecology (Allen *et al*., 1998). Additionally, the GBR monotype of *Paracentropyge* (*P. multifasciata*) proved to shy for video recording.

3.2.2 Live specimen husbandry and experimental design

Specimens were individually housed in experimental aquaria and maintained according to the protocol given in Chapter 2. Animals were encouraged to feed in a narrow passage between the aquarium front glass and a reference-grid background (Fig. 3.2). During acclimation specimens were trained to feed under floodlight illumination on experimental prey clipped into a stainless clip firmly mounted in the feeding passage. For provisioning and feeding trials, rock oyster shells of uniform size (5-6 cm^2 surface area) covered with sponge, turf algae, ascidian, tubeworm, and tunicate epifauna were collected from local coastal marine pylons. Ghost shrimp (*Acetes* sp.) were collected in adjacent waters.

Prior to experiments, 9 reflective markers were glued to the fish skin as reference markers for motion analysis (Chapter 2). Markers were placed over articulations in the oral jaws, suspensorium, pectoral girdle and the cranial-vertebral

Figure 3.2 The eight pomacanthid study taxa subjected to kinematics analysis. A, Centropyge [Centropyge] bicolor; B, Apolemichthys trimaculatus; C, Genicanthus melanospilos; D, Centropyge [Xiphypops] bispinosa; E, Pygoplites diacanthus; F, Chaetodontoplus duboulayi; G, Pomacanthus [Euxiphipops] sexstriatus; H, Pomacanthus [Arusetta] semicirculatus. The sequence of frames (A-H) illustrate the phases of a pomacanthid bite; A, preparatory; B-D, protrusion; E, bite (t_B); F-G, jaw retraction; and H, conclusion of the feeding event (t_C). Reference grids are 2cm, except in C, 1cm.

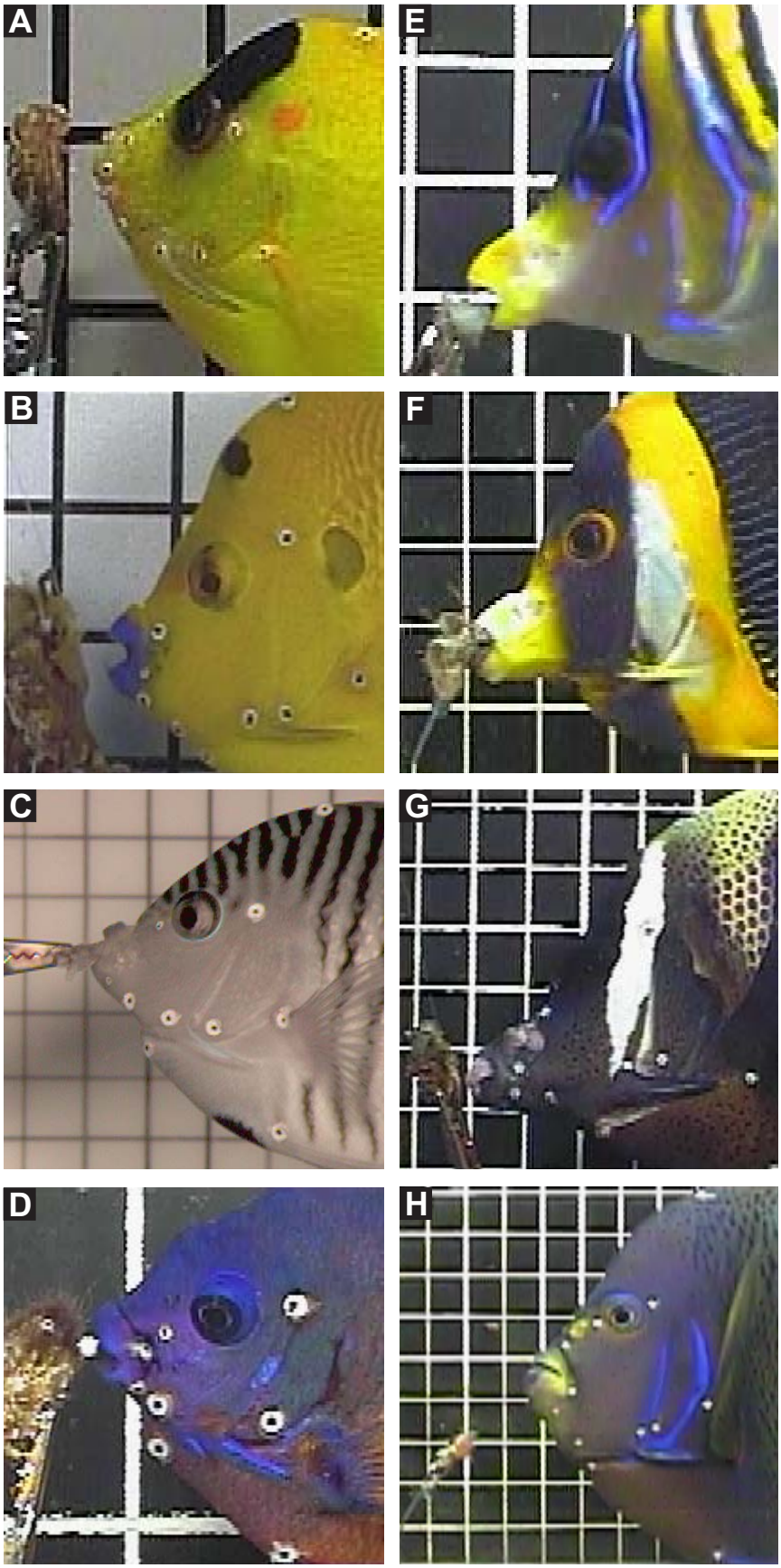

Figure 3.2

Figure 3.3 Skull preparation of *Pomacanthus [Euxiphipops] sexstriatus*, illustrating the generalised pomacanthid skull architecture, and the positions where reflective markers were glued to the skin of study animals, overlying joints and reference points prior to recording of high-speed video for kinematics analyses: 1, anterior-most dentary tooth tip; 2, base of pectoral fin; 3, anterior-most premaxillary tooth tip; 4, quadrate-mandibular articulation; 5, cranial-hyomandibular articulation; 6, anterior orbit; 7, base of first dorsal spine; 8, intramandibular joint; 9, sub-interopercular ligament. Scale bar, 15 mm.

connection, along with reference markers at the pelvic, pectoral and dorsal fin bases (Fig. 3.3).

3.2.3 Feeding performance and morphological sampling

To ensure a perpendicular orientation of the reflective markers to the lens axis, fish were presented with attached prey in the feeding passage. High-speed video was recorded using a JVC GR-DVL9800u digital video camera. *Genicanthus* specimens were recorded using a NAC Memrecam CI at 400 images s^{-1}. JVC video was converted to 200 images s^{-1} according to the protocol given in Appendix I (Chapter 2). No less than four feeding events for each specimen were analysed. Our use of large specimens increased temporal resolution of kinematics in high-speed video sequences, while also reducing the effects of allometry and ontogeny on kinematics. Since performance maxima were the focus of this study, prioritisation of rapid, aggressive bites posed the added benefit of excluding or at least reducing, the effects of predator satiation or hesitation (Sass & Motta, 2002). Fast bites also minimized the effects of a substantial variability in timing and duration of kinematics in the expansive phase (Chapter 2).

After completion of video recordings, specimens were euthanized in ice slurry and total length (TL) and head length (HL) measurements were taken. Alimentary data was obtained from fresh specimens following Elliott & Bellwood (2003). Three unpreserved specimens of even size from each study taxon were eviscerated and the viscera were carefully disentangled in order to measure the extended alimentary tract from the posterior-most point of the stomach to the anus, including the hindgut chambers where present. Gut length was standardised with TL and means of the resulting relative gut indices (±S.E.M) were mapped to the

pomacanthid phylogeny along with discrete gut morphology traits (fig. 3.6). Specimens were then either dissected fresh or fixed in formalin for tissue clearing and bone-cartilage counterstaining as in Chapter 2. Cleared and dissected specimens were manipulated to examine articulations of the jaws, suspensorium and hyoid compartments with the neurocranium and pectoral girdle. Anatomical diagrams were drawn directly from preparations using a dissector with Camera Lucida, and digitised in Corel Draw ver.10 (Fig. 3.4, 3.5) with osteology and connective tissue nomenclature following Chapter 2.

3.2.4 Morphological and kinematic data analyses

The correspondence between inferred trophic guilds (Allen, 1981; Allen *et al*., 1998; Debelius *et al*., 2003) and taxon-segregation based on their relative gut indices and presence/absence of hindgut fermenting chambers and gizzards was visualised by mapping of the traits to phylogeny. Character histories were traced using the parsimony optimisation criterion in Mesquite ver. 1.06 (Maddison & Maddison, 2005). Trophic transitions were optimised to the node of primary differentiation, while jaw morphology traits were optimised as character origin reconstructions on the cladogram branches. The statistical significance of resultant trophic guilds was examined using a Wards cluster analysis with Manhattan City Block as distance measure.

Video sequences were analysed only if the entire feeding event was completed in focus and in lateral profile. The total duration of feeding events (t_{TOT}) were cropped from protrusion onset (t_S), via time of bite (t_B) to completed jaw retraction (t_C) in Virtual Dub v.1.7.4. The nine reflective markers (positions indicated in Fig. 3.3) were tracked in Movias Pro v.1.5 and the extracted x:y

coordinates were used to calculate linear excursions (distances between coordinate pairs) and angular excursions (three coordinate pairs), as well as onset-timing, duration and velocity for seven joints and linkages: gape expansion and occlusion, dentary protrusion and retraction, mandibular depression, intramandibular rotation, preopercular rotation as proxy for suspensorial rotation, opercular rotation as proxy for opercular linkage displacement and neurocranial elevation (See Chapter 2).

A total of 32 kinematic variables were sampled from high-speed video in each species. As variation in individual skull kinesis was comparable to the variance found amongst individuals, kinematic means are based on all bites for each species. Multiple correlation analyses indicated that data transformations were required for the variables exhibiting significant auto-correlation ($r \geq 0.4$). Excursion magnitudes, total bite duration (t_{TOT}) and velocity variables were standardised with head length, while excursion durations and timing variables were standardised with total bite duration (t_{TOT}). Variables were omitted from analysis if significant auto-correlation amongst variables and/or with body-size persisted, resulting in a dataset of 16 informative variables. These predominately reflected timing (relative to time of bite; t_B), duration and velocity regimes of kinematics, and were all subjected to multivariate analysis. Multivariate analyses of variance (MANOVA) were used on the raw dataset, on size-corrected values and also on residual values from size correction, to evaluate the extent of variation in the dataset. The discriminate function analysis in Statistica (v.6.0) was used to examine the extent to which kinematics variables enable taxa to be distinguished in 2D-kinematic functional space. The canonical correspondence component in a DFA identifies and maximises variation among defined groups (Ferry-Graham *et al*., 2002). All three datasets gave similar plots and only the size-corrected data is presented in the following.

3.3 Results

3.3.1 Feeding apparatus and cranial functional morphology

The Great Barrier Reef angelfish assemblage includes representatives from 83% of all pomacanthid lineages (Fig. 3.1). All pomacanthid taxa share a laterally compressed head with an anteriorly reduced suspensorium and loosely suspended, protrusible jaws. An intramandibular joint (IMJ), pronounced antero-posterior flexion in the hyomandibular articulation with the sphenoid and a loose association of the palato-pterygoid region are also characteristic traits for all taxa (Fig. 3.4). The latter two flexion points permit excursion of the suspensorium over the lateral ethmoid to augment mandible protrusion (Fig. 3.2 E-G; Fig. 3.4 A, B). Manipulation studies showed that the anterior and posterior suspensorial elements in *Pygoplites*, *Genicanthus* and *C. [Xiphypops]* are more loosely associated than in other pomacanthids (Fig. 3.4C-D). In the 'pygmy angle clade' (Fig. 3.1), cartilaginous discs between the premaxillary condyles of the maxillae and the dorsal vomerine surface, form 'sliding-bearings' for the maxillary excursion over the dorsal vomerine surface during jaw kinesis (Fig. 3.4C-F). Pomacanthid mandibles generally rest at a characteristic dorsal incline (Fig. 3.4), whilst in *Centropyge [Xiphypops]* this angle is more horizontal, while the dentary rests at a steeper angle with the articular bone (Fig. 4D). *Centropyge [Xiphypops]* has sub-terminal protrusion (Fig. 3.5A), contrasting with anterior protrusion in all other pomacanthids (Fig. 3.5B). The tooth-bearing margin in *C. [Xiphypops]* and *Genicanthus* is planar (Fig 3.5C), contrasting with a convex margin in other pomacanthids (Fig. 3.5D).

Figure 3.4 Morphology of the feeding apparatus and cranium in the Pomacanthidae, drawn from cleared and stained, or dissected specimens (Table 3.1.) or photographs of cranial preparations. A, *Pomacanthus [Arusetta] semicirculatus* (protruded position)*;* B, *Chaetodontoplus duboulayi* (protrude)*;* C, *Pygoplites diacanthus* (resting); D, *Genicanthus melanospilos* (protruded)*;* E, *Centropyge [Xiphypops] bispinosa* (resting)*;* F, *C. [Centropyge] bicolor* (protruded). Osteology labelling in A, (B-F follows): art, articular; d, dentary; ect, ectopterygoid; hyom, hyomandibular; ihy, interhyal; iop, interoperculum; mpt, meta pterygoid; mx, maxilla; op, operculum; pal, palatine; pmx, premaxilla; pop, preoperculum (in fine stippling); q, quadrate; sop, suboperculum; supcl, supracleitrum; sym, symplectic; lc, lachrymal; v, vomer. Shading denotes space not occupied by bone; the maxillary-palatine, interopercular-articular and interopercular-subopercular ligaments are shown in black. Black shapes with white margin, as indicated with black arrowheads in C-F, are cartilaginous discs unique to the 'pygmy angel' clade (Fig. 3.1, 3.6). For clarity, drawings are discontinued medially, where the interhyal joins the urohyal. Scale bars, 5 mm.

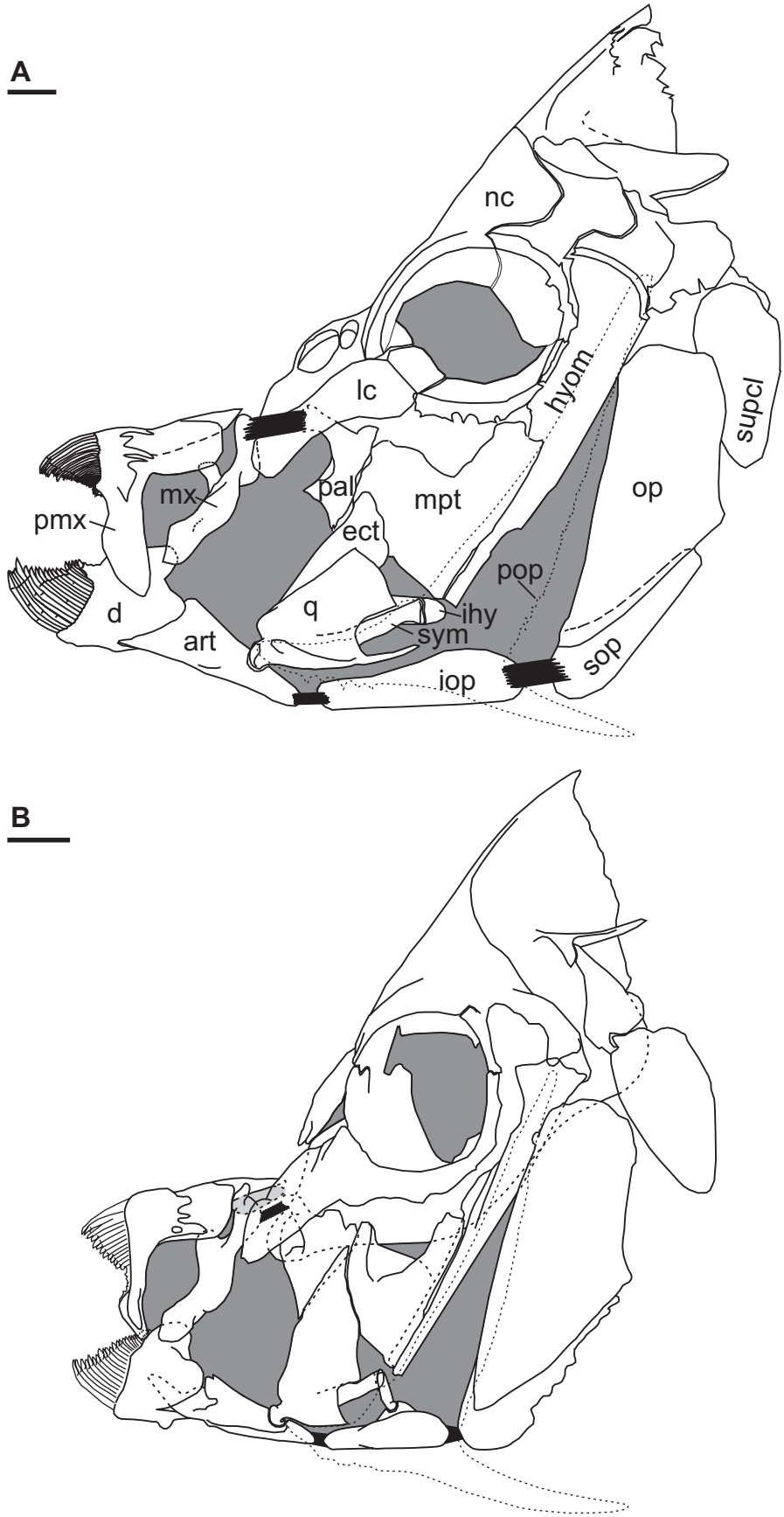
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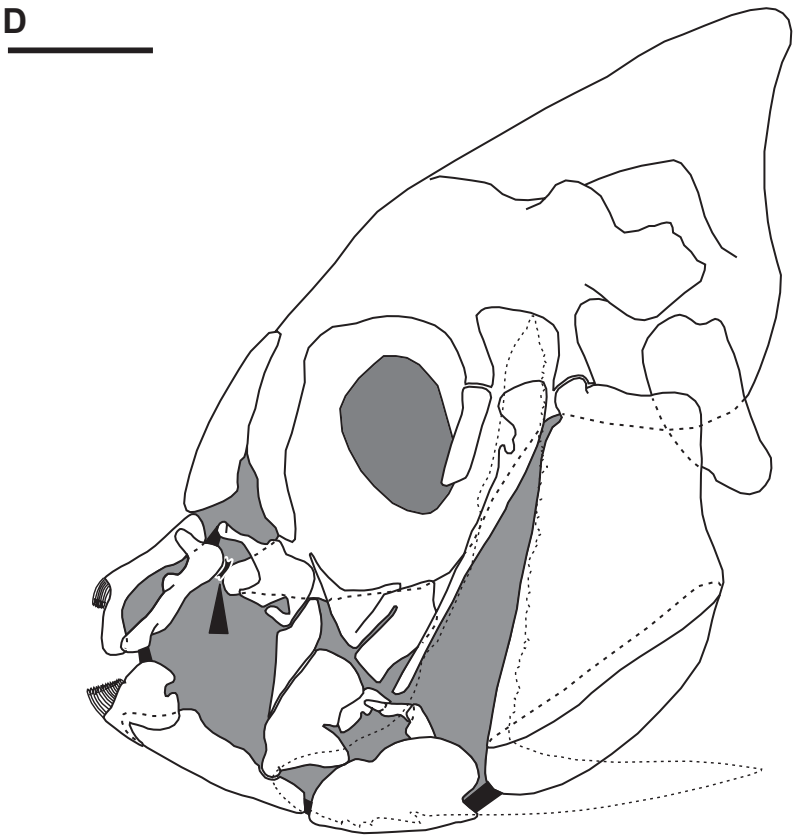
Figure 3.4



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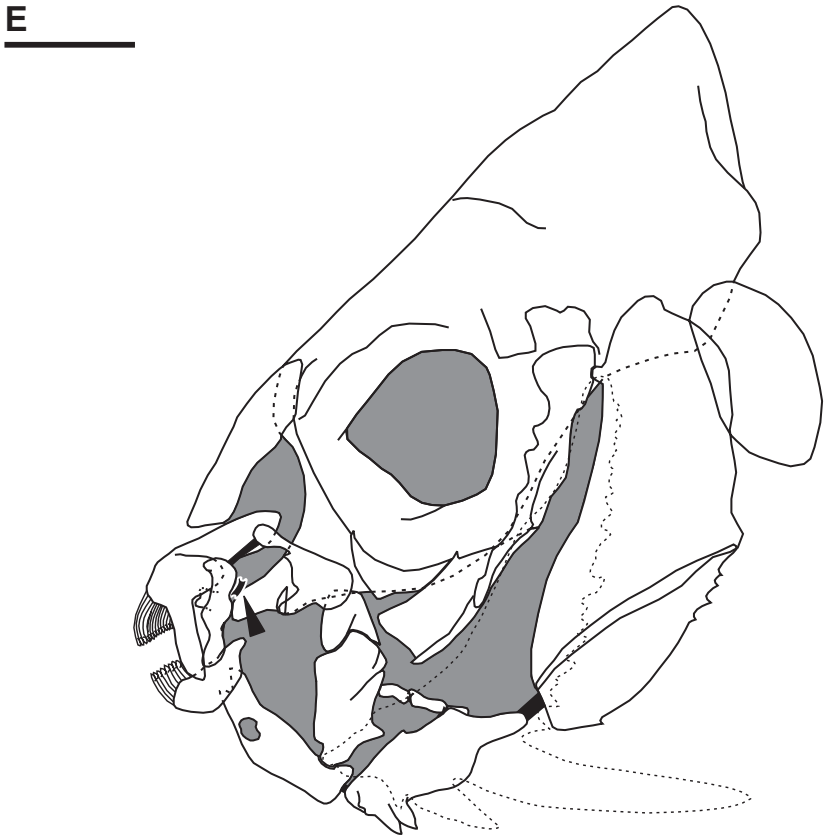

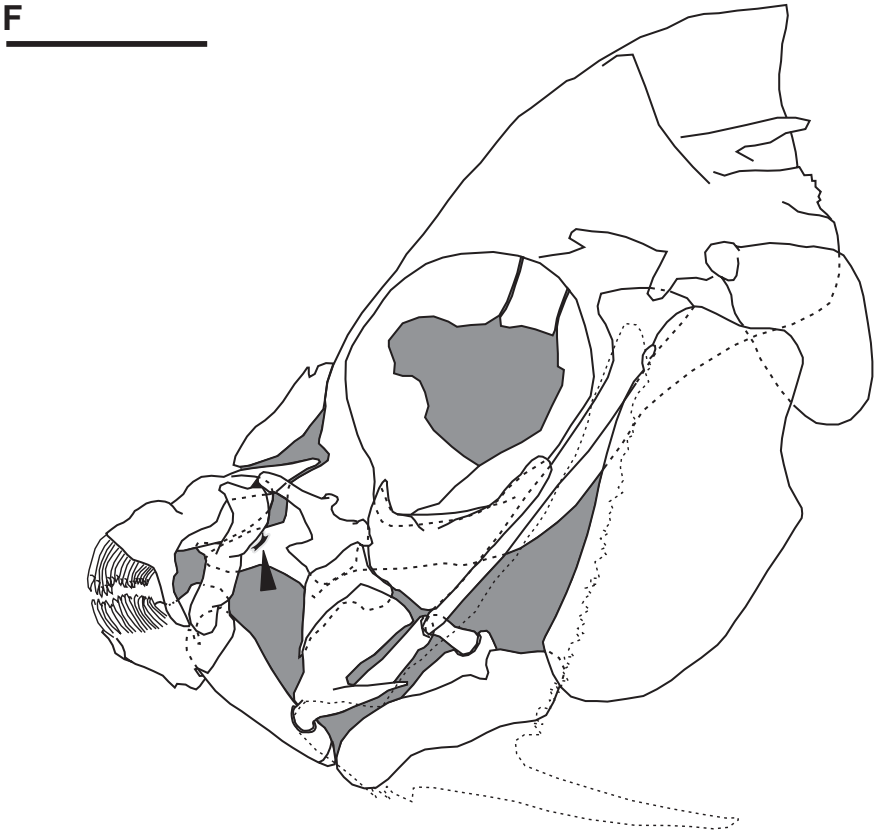

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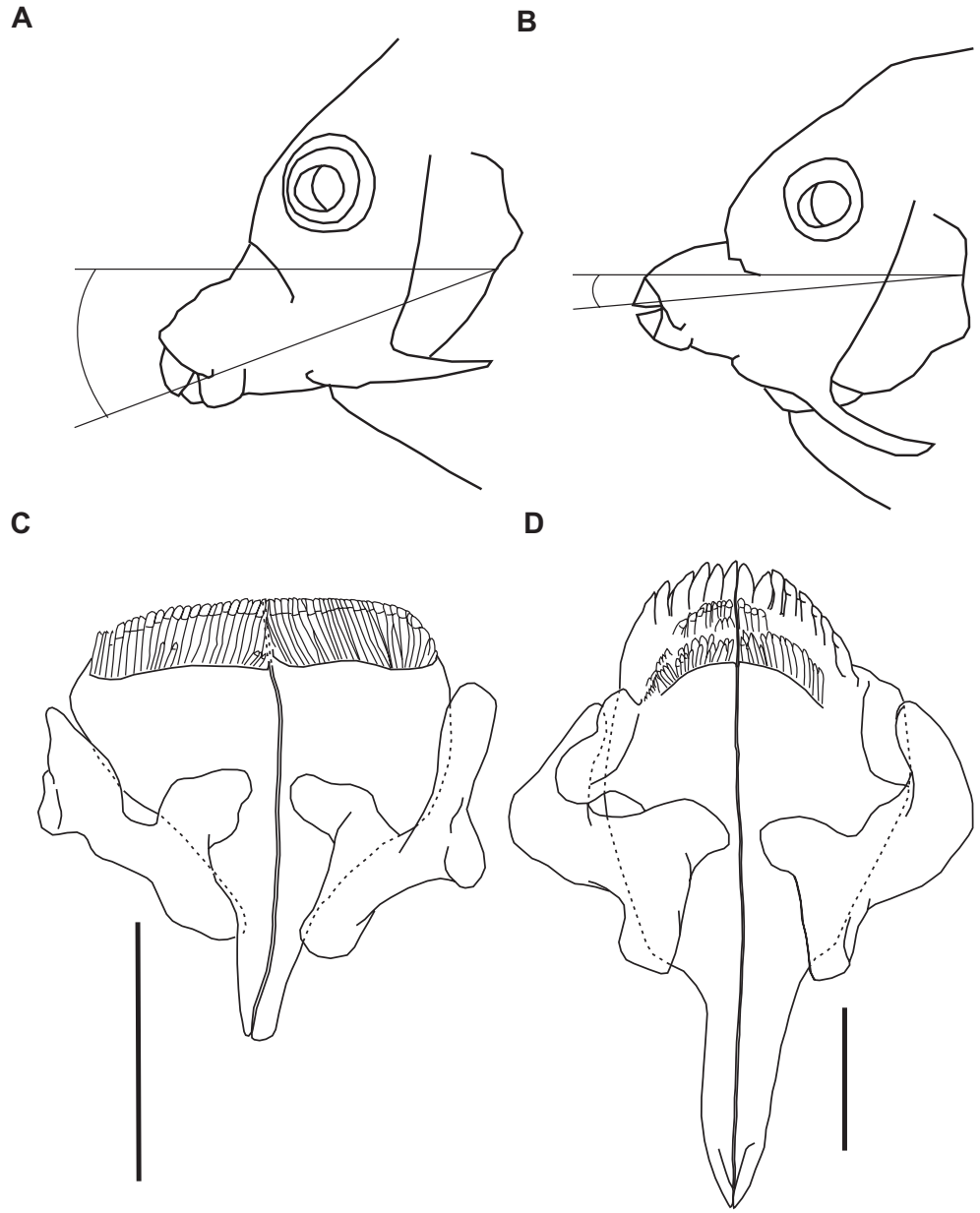

Figure 3.5 Functional and morphological differences among pomacanthid clades. A and B shows tracings from high-speed video images at maximum jaw protrusion. The diverging jaw protrusion pattern in A, *C. [Xiphypops]* (bottom leg of angle) from the position of the premaxilla in retracted state (top leg) is compared with B, *C. [Centropyge],* which is representative of the protrusion patter in all other pomacanthids. C and D show a ventral view of premaxillary and maxillary jawbones (scale bars, 5 mm.), illustrating the diverging tooth face profiles between C, the clade of *Centropyge [Xiphypops] – Genicanthus* (here exemplified by *C. [Xiphypops]*) and D, other pomacanthids (exemplified by *Pygoplites*).

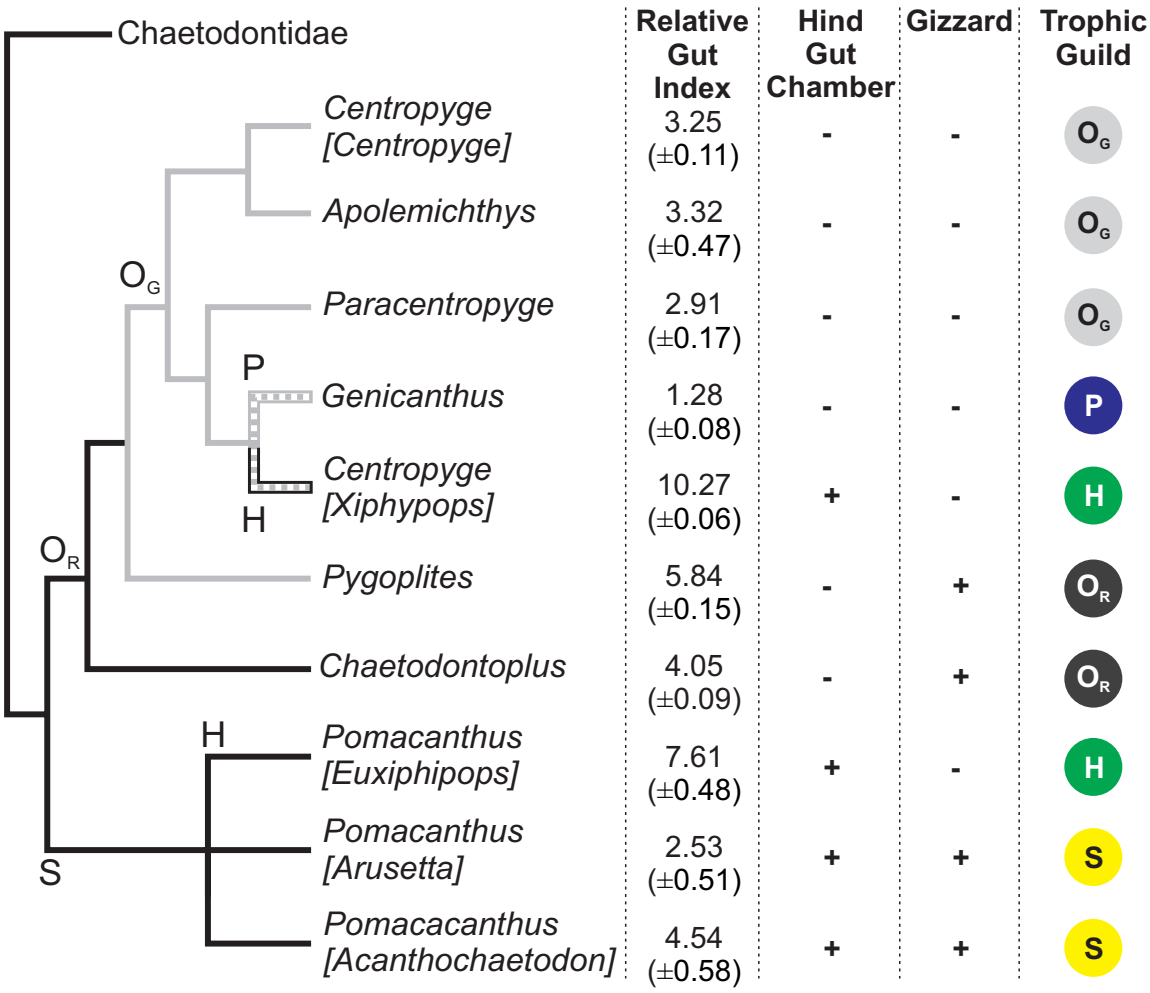

Figure 3.6 Mapping and optimising of character traits to the pomacanthid cladogram (Fig 3.1). Jaw morphology character-states (see text) are optimised to the phylogeny: shaded branches in the cladogram denote presence of cartilage discs (Fig. 3.4); stippled branches denote a planar frontal tooth margin in *Centropyge [Xiphypops]* and *Genicanthus,* while the black-outlined branch denotes the divergent protrusion pattern in *C. [Xiphypops]* (Fig. 3.5). In the character matrix the relative gut index (alimentary tract length*TL^{-1}; mean ±S.E.M; n=3) as well as hindgut chamber and gizzard presence (+) or absence (-) is indicated. Putative feeding modes (derived from Allen, 1981; Allen *et al*., 1998; Debelius *et al*., 2003) are indicated in the right hand character matrix column and optimised to the node of primary differentiation in the phylogeny: H, herbivorous; O_G, gracile omnivore; O_R, robust omnivore; P, planktivore, S, spongivore. Colour labels correspond with figure 3.8. Note the convergent herbivory origins in *P. [Euxiphipops]* & *C. [Xiphypops]*.

Optimisation of jaw morphology traits to the pomacanthid phylogeny shows that novelties in cranial morphology predominately are associated with the small-bodied and speciose 'pygmy angel clade' (Fig. 3.1, 3.6). Meanwhile, mapping of alimentary traits to the phylogeny separates taxa into five clusters corresponding with their putative trophic status. *C. [Xiphypops]* and *Pomacanthus [Euxiphipops]*, segregated by three times the relative gut indices ($GL*TL^{-1}$) of congeners, presence of a hindgut chamber, but absence of a gizzard. The large-bodied *P. [Arusetta]* and *P. [Acanthochaetodon]* segregated with short guts, a gizzard, and a short hindgut chamber, while *Pygoplites* shared a medium-length gizzard-bearing gut with *Chaetodontoplus*. *C. [Centropyge]*, *Apolemichthys* and *Paracentropyge* segregated by a short unspecialised gut, while *Genicanthus* approximated this cluster, having the shortest pomacanthid gut of only one body-length. A Wards cluster analysis on the morphological character matrix found identical groupings.

3.3.2 Feeding kinematics

All bites from the study species (Appendix II) were characterised by a relatively slow protrusion (0.07 - 0.21 $m*s^{-1}$) with highly variable duration (0.054 - 0.3 s) leading to maximum protrusion, averaging 23% HL, while only attaining 14% HL in *Genicanthus.* Maximum protrusion was followed by a distinct, rapid jaw closure (0.012 - 0.059 s), reaching time of bite (t_B). Half of the study taxa exhibited a slight lag in jaw kinesis (0.002 - 0.005 s) before a high-velocity jaw retraction (0.2 - 1.11 $m*s^{-1}$) concluded the bite at t_{TOT} (0.085 - 0.351 s). However, *Pygoplites* and *Chaetodontoplus* did not lag, while *Pomacanthus [Euxiphipops]* and *Centropyge [Xiphypops]* initiated jaw retraction 0.001 - 0.005 s prior to t_B.

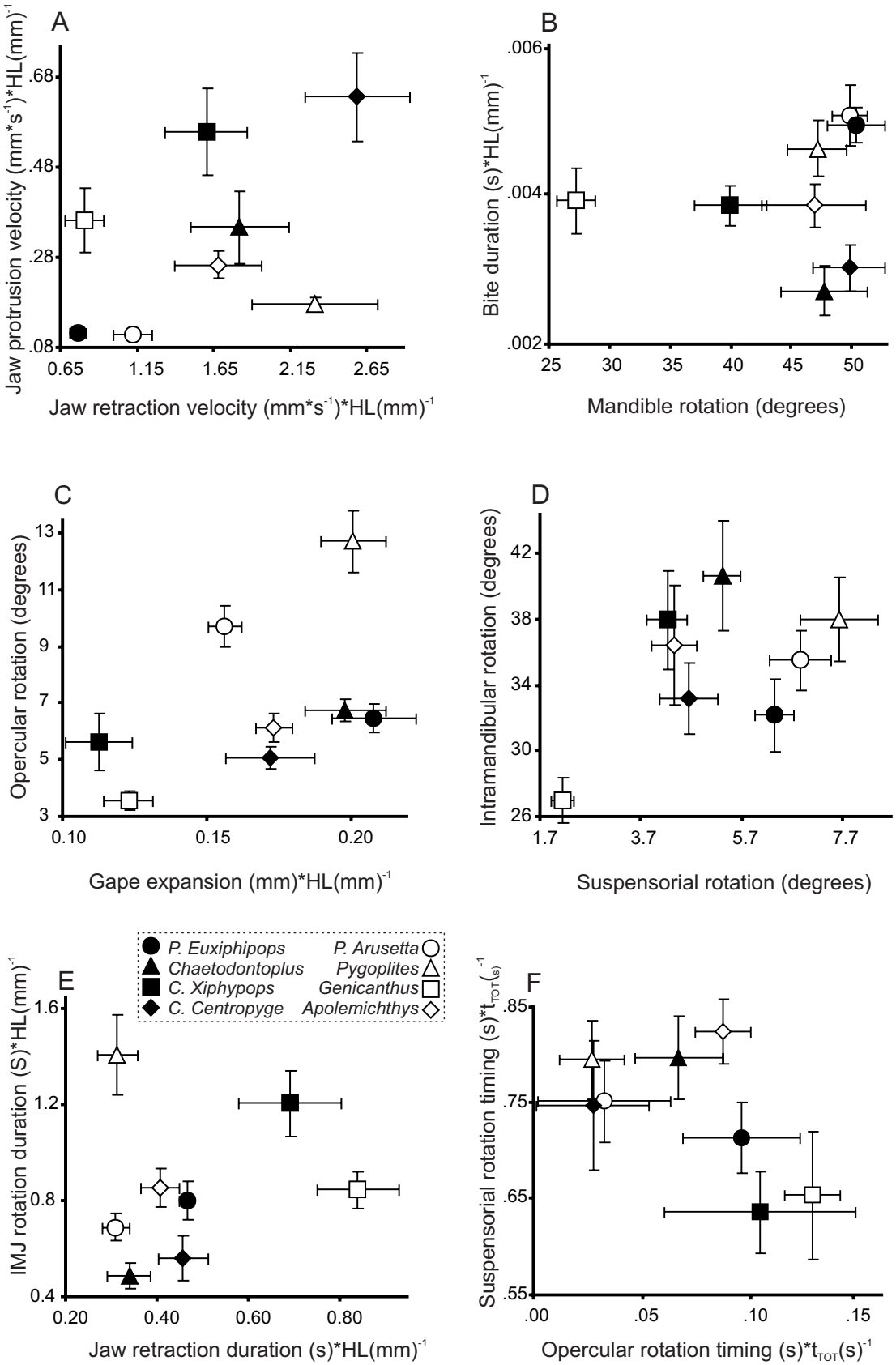

Figure 3.7 2D-kinematic functional space plots of representative kinematics in the eight study taxa (whiskers: ± S.E.M.).

Genicanthus showed the least difference in jaw protrusion and retraction durations (0.064 & 0.02 s ±0.01 S.E.M.) and a lower jaw retraction velocity (0.2 $m*s^{-1}$±0.03 S.E.M.) than other taxa. After standardisation of kinematics variables (with TL or t_{TOT}), all pygmy angels except *Genicanthus* exhibited proportionally faster jaw kinesis than the large-bodied *Pomacanthus* taxa (Fig. 3.7A). The putative herbivores, *C. [Xiphypops]* and *P. [Euxiphipops]* exhibited proportionally slower jaw retraction than their respective congeners, and only out-performed *Genicanthus* (Fig. 3.7A). These three taxa also segregated from other pomacanthids based on peak suspensorial and opercular rotation timing, relative to t_B (Fig. 3.7F).

Uncorrected values showed diminished mandible depression in *C. [Xiphypops]* and *P. [Euxiphipops]* compared to their respective congeners. After size-correction, this relationship persisted for the *Centropyge* taxa and was eliminated amongst the *Pomacanthus* taxa – illustrating a prevailing trend of more pronounced difference between *Centropyge* than among *Pomacanthus* taxa (Fig. 3.7B). In *C. [Xiphypops],* restricted mandible rotation contrasts with the sub-terminal protrusion pattern. The putative herbivores also segregated from their congeners based on reduced gape expansion (Fig. 3.7C) while increased jaw protrusion segregated *C. [Centropyge]* and *Pygoplites* from other taxa, and with pronounced difference from *C. [Xiphypops]*. Further differences between *P. [Euxiphipops]* and *P. [Arusetta]* included opercular (Fig. 3.7C) and intramandibular (Fig. 3.7D) flexion. *Pygoplites* exhibited more pronounced flexion than others (e.g. suspensorial, Fig. 3.7D and opercular rotation, Fig. 3.7C), while *Genicanthus* exhibited the least flexion in all magnitude variables (Fig. 3.7B, C, D). Suspensorial rotation and all of the four IMJ traits caused modest taxon segregation (Fig. 3.7D).

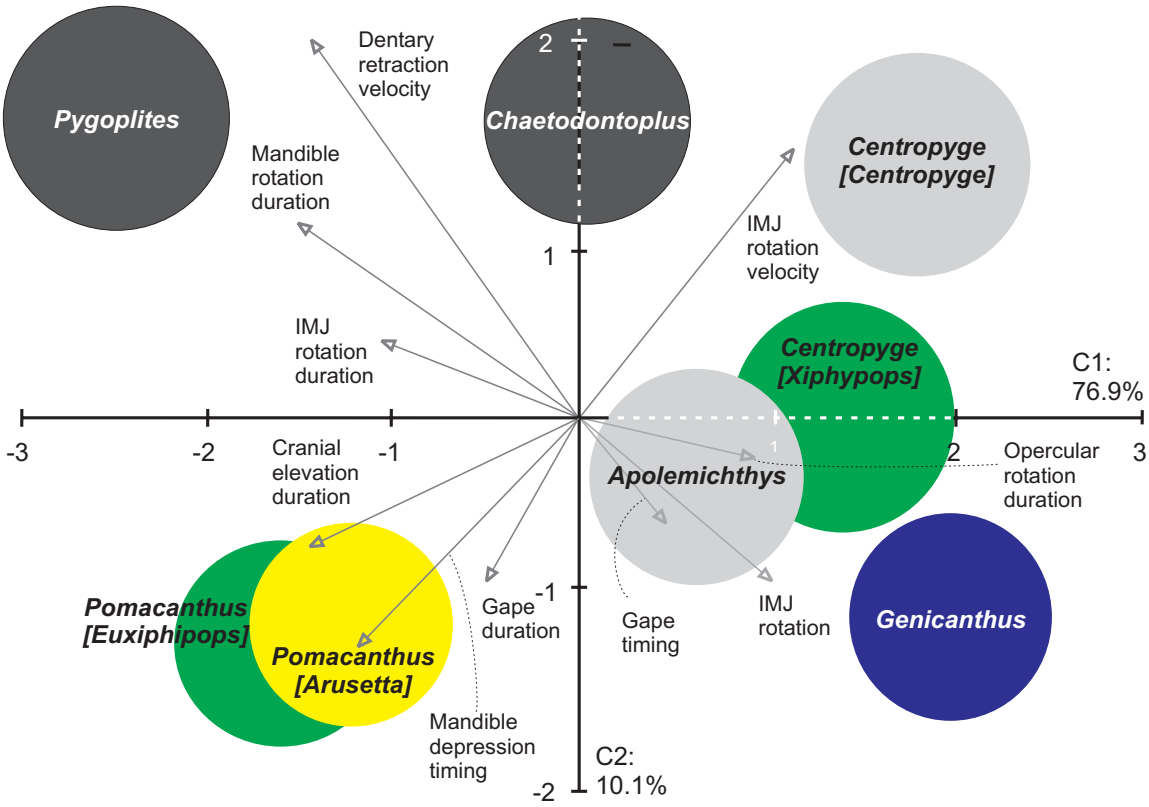

Figure 3.8 Scatter-plot of mean canonical scores for the first two canonical factors generated by the DFA analysis. The 10 most informative kinematics variables, which predominately reflect duration, velocity and timing regimes, are plotted as vectors to illustrate their combined effect in dispersing pomacanthid taxa across 2D-kinematic functional space. The 95% confidence eclipses for each taxon are coloured according to the trophic guilds in figure 3.6: blue, *Genicanthus*, zooplanktivore; yellow, *Pomacanthus [Arusetta]*, spongivore; green, *Centropyge [Xiphypops]* and *P. [Euxiphipops]*, herbivores; light grey, *C. [Centropyge]* and *Apolemichthys*, gracile omnivores; and dark grey, *Pygoplites* and *Chaetodontoplus*, robust omnivores.

However, *Chaetodontoplus* did exhibit more pronounced IM flexion and concluded this rotation proportionally faster (Fig. 3.7D), whilst *Pygoplites* was proportionally slower than other taxa (Fig. 3.7E). Finally, duration and timing variables effectively segregated the *Genicanthus – C. [Xiphypops]* clade (Fig. 3.7E) along with *P. [Euxiphipops]* (Fig 3.7F) from other taxa.

A MANOVA revealed significant variance amongst kinematic values (Wilk's lambda = 0.021, f_{105} = 3.36, p=0.00). Three canonical factors extracted from the discriminate function analysis (DFA) had eigenvalues of 0.68 or greater, with canonical factor loads of 76.9%, 10.1% and 3.2% respectively, together explaining 85.3% of the dataset variance. Canonical factor 1 (Fig. 3.8) was correlated with rotation duration in four of the five angular variables (opercular rotation, intramandibular flexion, cranial elevation and mandible depression), all showing weak correlations with body-size ($r \leq 0.4$). Of the 10 most informative characters, duration variables featured more prominently in taxon segregation (with 5) than timing (2), velocity (2) and magnitude variables (1). Some body size effects persisted in the data after standardisations, evident from the dispersal of taxa predominantly along C1 in accordance with differential body size (see also fig. 3.7A). This effect separated the *Pomacanthus* taxa with *Pygoplites* as an outlier from the pygmy clade with *Genicanthus* as an outlier. None of the canonical axes segregated *P. [Arusetta]* from *P. [Euxiphipops]*, which formed a tight cluster based on positive correlations with cranial rotation duration, mandible depression timing and gape expansion duration. Meanwhile, all three canonical axes displaced *C. [Centropyge]* from a cluster of *C. [Xiphypops], Genicanthus* and *Apolemichthys* based on a positive relationship with IMJ flexion velocity.

3.4 Discussion

3.4.1 Pomacanthid morphological disparity

The Great Barrier Reef assemblage of pomacanthids is a representative subset of pomacanthid evolutionary history. Amongst these GBR taxa, cranial architecture and feeding apparatus functional morphology exhibit evolutionary stability. A limited number of variations are observed from the generalised feeding apparatus in *Pomacanthus* described in detail in Chapter 2. Most structural novelties are symplesiomorphic for all pomacanthid clades, i.e. each is a pomacanthid synapomorphy compared with a generalised perciform teleost (Chapter 2). Structural divergence is predominately manifested in the 'pygmy angel clade', and primarily involves alterations in suspensorial morphology within the *Centropyge [Xiphypops] – Genicanthus* clade, as well as diminished size of oral jaw structures in *Genicanthus*.

The gape-restricting IMJ constitutes an important pomacanthid synapomorphy, the presence of which was previously only documented in *Pomacanthus* (Chapter 2), and alluded to by Gregory (1933) in the Caribbean subgenus *Holacanthus [Angelichthys]*. Novel suspensorial articulations and loose associations facilitating suspensorial rotation, and augmenting jaw protrusion are equally important derived traits among pomacanthid taxa. These suspensorial traits bear a closer resemblance with basal long-jawed butterflyfish ram-suction feeders (Motta, 1984; Ferry-Graham et al., 2001a, b) than with other IMJ-bearing biters. Amongst biters in general, associations between the suspensorium and

neurocranium are more rigid and jaw protrusion is negligible (Purcell & Bellwood, 1993; Bellwood, 1994). Whilst no immediate ancestors to the Pomacanthidae have been identified, *Drepane*, a close relative within the deep-bodied squamipinnes exhibits several feeding apparatus morphological similarities. These include some IM flexion and extensive jaw protrusibility (Jones, 1968; Tyler *et al*., 1989; Tyler & Sorbini, 1999; see also Chapter 4).

Segregation of the *Centropyge [Xiphypops]* and *Pomacanthus [Euxiphipops]* taxa from other pomacanthids based on alimentary morphology traits corresponds with available dietary evidence. Purported affinity towards herbivory in several species within these subgenera lends support to this evidence (Allen, 1981; Allen *et al*., 1998; Debelius *et al*., 2003). Whilst not quantified, a dominance of algal material did appear in the intestines of *Centropyge [Xiphypops]* and *Pomacanthus [Euxiphipops]* examined herein. When reflected onto phylogenetic evidence, these characteristics indicate that herbivory arose independently in the large-bodied *Pomacanthus* clade as well as in *Centropyge [Xiphypops]* within the pygmy angel clade. Short guts in *Genicanthus* correspond well with a functional reversal to zooplanktivory (Horn, 1989; Howe, 1993; Elliot & Bellwood, 2003). The unique planar tooth margin in the *Centropyge [Xiphypops]* – *Genicanthus* clade supports the clustering of these taxa in the phylogeny of Bellwood *et al*. (2004b). A flat anterior jaw shape allows *C. [Xiphypops]* to cover a greater area of grazing substratum or foliage per bite, and may thus be an alternative modification for herbivory to the gape-expanding IMJ kinematics and tricuspid tooth morphologies found in several squamipinnid grazers (Vial & Ojeda, 1992; Purcell & Bellwood, 1993; see also Chapter 4). Differences in relative gut length between *C. [Xiphypops]*

and *C. [Centropyge]* further support the priority placed on elevation of *C. [Xiphypops]* to full generic status in Bellwood *et al*. (2004b).

Sub-terminal jaw protrusion is an apomorphic trait in *C. [Xiphypops]* and cannot be explained by mandible depression kinematics, which is restricted compared with other pomacanthid taxa. Rather, slight alterations in suspensorial morphology and subtle architectural rearrangement of the mandibular resting angle to a more horizontal position appear to cause this protrusion pattern. Observations of pygmy angelfishes feeding both in the wild and in captivity indicate that *C. [Xiphypops]* adopts a position more parallel to the substratum than other pomacanthids (Konow, unpublished). A parallel body orientation to the substratum was previously suggested to facilitate an improved predator avoidance response (Alexander, 1967; Motta, 1984). The foraging strategy of *C. [Xiphypops]* corresponds with an ecological prerequisite of venturing further from shelter whilst foraging on photosynthetic algal prey (Eagle *et al*., 2001). Conversely, *C. [Centropyge]* predominately utilises attached invertebrate prey and tend to forage in closer association with shelter (see Eagle *et al*., 2001), theoretically making it less susceptible to predation.

3.4.2 Pomacanthid kinematics disparity

Herein, the first family-assemblage level data on intramandibular biting kinematics is presented, which appear to be conservative amongst pomacanthids. Whilst it has been presumed that intramandibular kinematics generally augment gape-expansion, functional evidence supporting this generalisation has only recently

become available (Chapter 4). According to current knowledge, no other teleost taxon possesses homologous IMJ kinematics that facilitates protruded mouth occlusion.

Significant differences were however present in other pomacanthid feeding kinematics, and many taxa could be segregated in 2D-kinematic functional space. Here, it should be noted that the variance-seeking nature of discriminate function analysis presumably has optimized the variance present amongst taxa. Interestingly, kinematic disparity was limited in functional traits previously suggested of particular biological significance in *Pomacanthus*, particularly protrusion magnitude and jaw retraction velocity (Chapter 2). Overall, subtle variations in duration, onset-timing and velocity variables, and not magnitude of flexion or linear excursion variables significantly influenced taxon segregation. The implications of these results are two-fold: as the influential kinematic traits are likely to be under more intrinsic behavioural and neuromuscular, and less pronounced osteological influence than magnitude variables (Ferry-Graham *et al*., 2001c; Nemeth, 1997a, b), it may be deduced that feeding kinematics, as well as morphology, remain fairly conservative amongst pomacanthid clades, with a notable exception of the *Centropyge [Xiphypops]* – *Genicanthus* clade. Accordingly, behavioural modulation is potentially a major factor driving the ecological diversification of angelfishes into novel trophic guilds.

Feeding kinematic traits generally segregated the putative herbivore *C. [Xiphypops]* from the gracile omnivorous congener *C. [Centropyge]*. However, the corresponding sister-taxa within the genus *Pomacanthus*, the herbivorous *P. [Euxiphipops]* and the spongivorous *P. [Arusetta]* rarely segregated in available 2D-

kinematic functional space. This can be taken as evidence of the overriding influence of body size on ecological diversification in most pomacanthid clades. Benefits of a large body appears most obvious amongst spongivores and robust omnivores, in providing an increase in absolute bite force (Clifton & Motta, 1989; Huber & Motta, 2004) as well as sufficient tearing force to rupture or dislodge structurally resilient prey such as sponges and tunicates. In the generalised taxon *Pomacanthus [Euxiphipops],* herbivory is also likely to be governed by the robust grab-and-tearing strategy, facilitating procurement of sturdy calcareous, encrusting and turf algae. Meanwhile, establishment of herbivory in *C. [Xiphypops]* appears to have driven the most pronounced species radiation seen in the family. Given the physical force-constraints of small body size, the limited gape expansion and divergent protrusion patterns in this clade suggest utilisation of more gracile ripping or shearing strategies to procure more delicate foliaceous algae.

Despite a close affinity to *C. [Xiphypops]*, *Genicanthus* separated from all other pomacanthid taxa by reduced magnitude of flexion in the majority of kinematic variables. Restricted mandible rotation and gape expansion combine with a diminutive protractible mouth to form the functional basis of inertial suction feeding (Motta, 1982; Howe, 1993), commonly interpreted as the generalized teleost feeding mode (Lauder, 1980; Liem, 1980a). Interestingly, while body size generally is seen to decline with phylogenetic differentiation of the pygmy angels, body size does not constrain planktivores, as illustrated by the large maximum, and pronounced variation in body sizes within *Genicanthus*. Interestingly, the trend of large body size persists in IMJ-bearing facultative planktivores from other genera, e.g. *Pomacanthus [Arusetta] rhomboides* (Randall, 1988) and members of the West

Tethyan genus *Holacanthus* (Perez-España & Abitia-Cardenas, 1996). This trend persists in the unicornfishes (Nasinae), an acanthuroid subfamily of planktivorous and benthic feeders (Klanten *et al*., 2004). The large body size in IMJ-bearing planktivorous taxa relative to confamiliars may in part be attributable to evolutionary interplay between their IMJ, the associated increase of niche-breath it may provide and resultant exaggerated growth capabilities. Increased body-size combined with intramandibular flexion may be facilitating a "Jack of all trades" strategy, by allowing procurement of sturdily attached prey when planktonic prey is unavailable.

3.4.3 Prey-capture disparity within and amongst feeding modes

Rather limited disparity in morphology and kinematics in the Pomacanthidae contrast with existing evidence from chaetodontid and labrid assemblages. In the following paragraphs it will be discussed how differences in morphology and functional traits among distinct evolutionary assemblages may have shaped diversification patterns in feeding kinematics and trophic strategies.

Amongst chaetodontids, labrids and pomacanthids, ram-suction and biting have evolved in different sequences. According to current phylogenetic evidence (Blum, 1981; Ferry-Graham *et al*., 2001b; but see Littlewood *et al*., 2004) generalised chaetodontid taxa utilise ram-suction feeding, while the crown-group (genus *Chaetodon*) comprise corallivorous biters. This differentiation pattern exemplifies the generalized evolutionary succession of feeding modes (Lauder, 1980; Liem, 1980a). Contrastingly in labrids, multiple convergences of fast ram-

suction feeder jaws appear and the biting parrotfishes are nested deep in the labrid phylogeny (Westneat & Alfaro, 2005).

Whilst IMJs appear to influence biting strategists in pomacanthid, chaetodontid, acanthuroid and scarine taxa alike (Chapter 4), other functional innovations have influenced the unevenness in functional divergence among these three assemblages. Labroids are united by their possession of a uniquely configured pharyngeal jaw apparatus that decouples the oral jaws from mastication tasks (Liem, 1973; Rice & Lobel, 2004; Wainwright, in press; but see Streelman & Karl, 1997). Novel points of suspensorial flexion augment mandible protrusion in all pomacanthids (resulting in a dentary protrusion mean of 23% HL) and in some basal long-jawed ram-suction feeding butterflyfishes (≤30%HL; Ferry-Graham *et al*., 2001b). A comparable system exists in one marine wrasse taxon only (*Epibulus,* 50%HL; Westneat & Wainwright, 1989), but is found in several freshwater labroids (Hulsey and García de León, 2005). Whilst a degree of suspensorial rotation is retained in biting butterflyfishes (Konow, unpublished), parrotfishes have rigid suspensoria, and non-protrusible oral jaws (Bellwood, 1994).

Correspondingly, the distribution of trophic strategies differs among the groups. Biting taxa appear to have adopted derived biting trophic guilds, such as herbivory and corallivory, governed on different patters of functional innovation. Corallivory, using picking and scraping strategies, dominates amongst butterflyfish biters, while herbivory has not been documented (Motta, 1985; 1987; 1988; 1989). In labrids, herbivory is restricted to scarids, and facilitated by scraping and excavating strategies (Bellwood, 1994; Streelman *et al*., 2002; Bellwood *et al*., 2003). Labrid corallivores commonly feed on mucous, which pose less

biomechanical challenges than other coralline tissue, and rarely use active biting (Clifton & Motta, 1998; Ferry-Graham *et al*., 2002). Pomacanthid trophic diversification extends over a broad range of sturdy to gracile attached invertebrate prey and herbivory both arose in taxa of large and diminutive body size. Overall, these divergent trends suggest that different combinations of functional innovations cause divergent evolutionary patterns of ecological diversification among these three assemblages. A similar pattern of different combinations of functional traits causing differential diversification patterns was previously only established at the family-level, amongst labrid fishes (Clifton & Motta, 1998; Wainwright *et al*., 2004).

Biting parrotfishes and angelfishes are characterised by different assortments of novel functional traits. However, similarities are evident in associated trends of niche transitions from generalised robust forms (excavating and grab-and-tearing) to derived gracile forms (scraping, ripping and shearing). The functional disparity amongst parrotfishes appeared restricted, relative to other monophyletic labrid assemblages (Wainwright *et al*., 2004). With the present pomacanthid evidence taken into consideration, a pattern is emerging – biting teleost assemblages appear to rely on conservative feeding mechanisms, shaped by symplesiomorphic functional innovations, including suspensorial flexion, pharyngeal jaws and, most prominently, intramandibular joints. In acquiring these novel functional systems, biters appear to achieve the functional potential to cross 'ecological thresholds' into novel niche utilisation. Consequently, it can be argued that divergence is preceding and shaping diversification amongst these biting teleosts (Liem, 1980b; Streelman *et al*., 2002).

While this evidence supports the status of the pomacanthid IMJ as an important functional innovation, it also contradicts a previous notion of IMJs

providing functional decoupling of the mandible (Vial & Ojeda, 1990). Vial & Ojeda (1990) suggested that IMJs divide mandibles into two mechanical units, the dentary and articular bones, thereby increasing its structural complexity. Given the disparate IMJ kinematic patterns reported herein (see also Chapter 4) the associated functional complexity is also increased. However, IMJs do not appear to decouple the function of associated structures with related functions, as traditionally implied by the term (*cf.* PJAs decoupling the oral jaws; Liem, 1973; Galis & Drucker, 1996). On the contrary, IMJs appear to pose constraints on functional versatility of the mandible. This is reflected in planktivorous IMJ-bearing taxa, where IM-flexion is restricted compared with IMJ-bearing sister taxa that exclusively bite from the substratum (see the pomacanthid genus *Genicanthus,* and the Nasinae, chapter 4). In fact, the ecomorphological comparisons of chaetodontid, labrid and pomacanthid taxa presented above provide several indications of functional innovations promoting evolution along certain ecological axes whilst simultaneously posing functional constraints on evolutionary diversification along alternative axes.

3.4.4 Implications for future studies

Despite a concerted effort in recent studies of labrid morphology and biomechanics, behavioural influence was rarely quantified (*cf.* Wainwright *et al*., 2004), and the resultant estimates of functional disparity may therefore be either inflated or conservative. Such inferences are particularly problematic *in lieu* of novel functional systems that are inadequately explained by existing biomechanical models, and this is the case with IMJ-bearing mandibles (Wainwright *et al*., 2004; but see Clifton & Motta, 1998). Behavioural modulation may pose drastic influences

on kinematics in response to intricate changes in predator-prey dynamics (Nemeth, 1997a&b; Ferry-Graham *et al*., 2001c; Sanford, 2001). Incongruent patterns of evolutionary change with conservative motor patterns influencing more labile osteological systems are also becoming apparent (Alfaro *et al*., 2001; Grubich, 2001; Wainwright, 2002). Whilst many-to-one mapping of highly redundant structural diversity to functional diversity is likely to greatly influence ecological diversification this theorem may altogether compromise ecomorphological predictions made by morphological-biomechanical proxies (Alfaro *et al*., 2005; Bellwood *et al*., 2005Wainwright *et al*., 2005).

Consequently, the similarities amongst angelfish and parrotfish biters of decreased disparity patterns cannot be directly compared as they were established on a widely differing experimental basis. Likewise, pattern similarities of multiple convergences in the evolution of fast jaws for evasive prey capture amongst cichlids and wrasses only involved direct performance testing of the cichlids (Hulsey & García de León, 2005; Westneat *et al*., 2005).

Experimental testing of feeding kinematics in scarid and labrid taxa will be an important step towards future comparisons of ecomorphological relationships between functionally distinct assemblages. This approach will provide a crucial link from morphology, via the by-proxy results obtained from biomechanical studies to the feeding ecology and field behaviour of fishes on marine reefs. As reflected by the labrid analyses, the by-proxy methods holds significant merit in ecomorphological analyses of large assemblages, provided biomechanical models of the functional system in question have been developed and tested against behavioural performance analyses. With robust phylogenetic hypotheses rapidly

becoming available for coral reef fishes a powerful tool is provided for selection of representative study taxa from all functionally distinct lineages. The current weighting of available evidence towards the ram-suction feeding mode of teleost prey-capture should prompt attention towards biting taxa in future functional and comparative analyses (Norton & Brainerd 1993; Alfaro *et al*., 2001; Ferry-Graham *et al*., 2002). Refreshing the functional research, including direct performance testing, into the cause and effects of IMJs in particular and functional innovations in general may help clarify how novel traits influence evolutionary patterns in functional ecology.

The status of the IMJ as an important functional innovation is becoming well established and the divergent kinematics of pomacanthid IMJs, compared with other IMJ-bearers, emphasise the increased potential for ecological diversification provided by such innovations at assemblage-level. The present functional analysis provides an unprecedented understanding of how novelties in functional apparatuses may prompt diversification along particular ecological axes, and pose functional constraints on evolutionary radiations along alternative axes.

Chapter 4: Intramandibular joints help reef fishes take diverse bites

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4. 1 Introduction

Reef fish assemblages may be distinguished from many other fish faunas by the dominance and diversity of taxa utilising biting strategies to either graze or scrape the substratum (Wainwright & Bellwood, 2002). Reef biter assemblages are dominated by the squamipinnes, comprising the chaetodontoid and acanthuroid fishes (Tyler, 1989; Tyler & Sorbini, 1999), but also the parrotfishes, and a few durophagous groups (Bellwood, 1994; Turingan *et al*., 1995; Grubich, 2003). Descriptions of intramandibular joints (IMJs) in reef fishes are likewise dominated by squamipinnid taxa (Vial & Ojeda, 1990; Purcell & Bellwood, 1993) and parrotfishes (Bellwood, 1994, Alfaro *et al*., 2001; Streelman *et al*., 2002). Such accounts led to suggestions that functional complexity of the biting feeding apparatus reflects novel biomechanical challenges associated with feeding on sturdily attached and/or structurally resilient prey (Bellwood, 2003; Bellwood *et al*., 2003). Despite a wide range of novel dislodging strategies in fishes utilising the biting feeding mode, the functional basis, and particularly prey-capture kinematics of biting remains unclear (Norton and Brainerd, 1993; Ferry-Graham *et al*., 2002; but see Alfaro *et al*., 2001).

In contrast, the role of functional innovations in reef fishes utilising the ram-suction feeding mode are far better understood (Ferry-Graham & Lauder, 2001). Examples include pharyngeal jaws, yielding functional decoupling of the oral jaws in labroids (Gobalet, 1980; Wainwright *et al*., 2004; Wainwright, in press) and suspensorial rotation, augmenting anterior jaw protrusion in generalised butterflyfishes (Ferry-

Graham *et al*., 2001a&b). Such functional innovations may accentuate ecological diversification by broadening the biomechanical basis, and associated behavioural capabilities for novel niche-exploitation (Streelman *et al*., 2002). Distinct associations of functional innovations with specialised trophic ecology have evolved in convergence amongst closely related cichlids (Winemiller *et al*., 1995; Roe *et al*., 1997), butterflyfishes (Motta, 1988); surgeonfishes (Purcell & Bellwood, 1993; Winterbottom & McLennan, 1993); more distant taxa (e.g. cichlids and centrarchids: Norton & Brainerd, 1993), and entirely unrelated taxa (ram-suction feeders: Chapter 2; pharyngeal biters: Grubich, 2003). An analysis of the functional traits underlying the evolution of biting in reef fishes stands out as a priority in order to fully understand the range of trophic strategies utilised by teleosts on coral reefs.

Functional evidence strengthening the link between IMJs and biting was established in Chapter 2 with the description of a novel gape-restricting IM-flexion in angelfishes, f. Pomacanthidae, coupled with their utilisation of an unusual grab-and-tearing feeding strategy. IM-flexion thus appears a likely key-element in promoting the trophic diversification, of both squamipinnid (pomacanthids (Chapter 3); acanthurids (Jones, 1968; Purcell & Bellwood, 1993)) and scarid fishes (Bellwood, 1994). Meanwhile, functional analyses of the putative pomacanthid sister-group, the Chaetodontidae, revealed unusual functional attributes and feeding ecomorphology in generalised ram-suction feeders (Ferry-Graham *et al*., 2001a&b). The butterflyfish crown-taxa (Ferry-Graham *et al*., 2001b; Smith *et al*., 2003) remain unexamined in a functional context, although their corallivorous feeding ecologies suggest the utilisation of biting strategies (Motta, 1985; 1988; 1989). The status of IM-flexion presence or absence in butterflyfishes is likely to further reflect evolutionary trends in closely related biting taxa, and they are thus a particularly important group for further examination.

The present study therefore addressed the following questions: is there a shared functional basis supporting the divergence of biters amongst squamipinnid reef fish lineages? What has the role of IMJs been in the evolution of biting in reef fishes? And, are IMJs present in biting butterflyfishes?

4.2 Materials and methods

4.2.1 Analysis of mandible morphology and kinematics

Study taxa were typically selected at the sub generic level (Table 4.1), which was previously determined to sufficiently reflect trends in ecological diversification (Bellwood *et al*., 2004b). As most available phylogenetic hypotheses examined genus, or family-level relationships, this analysis should detect evolutionary trends in morphology. The methodology used herein is described in-depth in chapter 2. Manipulations and dissections were conducted on n≥3 non-preserved specimens of all study taxa, and both dissected and intact specimens were fixed in 10% seawater-buffered formalin and clear-stained for bone and cartilage, for preparation of anatomical diagrams (Fig. 4.2).

Available live individuals of taxa found to exhibit some degree of intramandibular flexion were recorded to 200fps high-speed video during feeding for analysis and performance testing of intramandibular kinematics (Fig. 4.1A, 4.B). Video sequences of three feeding events were selected for each individual analysed.

Table 4.1 Taxa investigated in this study. *Kinematics from L. A. Ferry-Graham *et al*. (2001a). +Elusive/ attached prey treatments.

Family	Genus	Subgenus	Species	Video	Manipulation
Chaetodontidae (Butterflyfishes)	*Chaetodon*	*Citharoedus*	*Ornatissimus*	3	3
		Corallochaetodon	*Lunulatus*	3	3
		Discochaetodon	*Aureofasciatus*	0	3
		Gonochaetodon	*baronessa*	1	3
		Tetrachaetodon	*plebius*	1	3
		Megaprotodon	*trifascialis*	1	3
		Lepidochaetodon	*unimaculatus*	2	3
		Exornator	*xanthurus*	3*	3
		Radophorus	*lineolatus*	3	3
		Radophorus	*melannotus*	3	3
	Chelmon		*muelleri*	3	3
	Chelmonops		*curiosus*	1	3
	Coradion		*altivelis*	3	3
	Forcipiger		*longirostris*	3*	3
	Forcipiger		*flavissimus*	3*	3
	Hemitaurichthys		*polylepis*	3	3
	Heniochus		*varius*	1	3
	Amphichaetodon		*howensis*	1	2
Pomacanthidae (Angelfishes)	*Centropyge*	*Centropyge*	*bicolor*	3	3
	Centropyge	*Xiphypops*	*bispinosa*	3	3
	Apolemichthys		*trimaculatus*	3	3
	Genicanthus		*melanospilos*	3^{+}	3
	Paracentropyge		*multifasciata*	0	3
	Pygoplites		*diacanthus*	3	3
	Chaetodontoplus		*duboulayi*	3	3
	Pomacanthus	*Euxiphipops*	*sexstriatus*	3	3
	Pomacanthus	*Arusetta*	*semicirculatus*	3	3
Scatophagidae (Scats)	*Selenotoca*		*multifasciata*	3	3
Microcanthidae (Stripeys)	*Tilodon*		*sexfasciatus*	1	3
	Atypichthys		*latus*	0	3
Ephippidae (Batfishes)	*Platax*		*orbicularis*	2	3
Drepanidae (Sicklefishes)	*Drepane*		*punctata*	0	3
Siganidae (Rabbitfishes)	*Siganus*		*doliatus*	0	3
Zanclidae (Moorish Idol)	*Zanclus*		*cornutus*	2	3
Nasinae (Unicornfishes)	*Naso*		*liturathus*	0	3
	Naso		*tonganus*	0	2
Acanthuridae (Surgeonfishes)	*Paracanthurus*		*hepatus*	0	3
	Zebrasoma		*veliferum*	0	3
	Acanthurus		*olivaceus*	2	3
	Ctenochaetus		*striatus*	2	3
Scarinae (Parrotfishes)	*Scarus*		*flavipectoralis*	0	3
Blennidae (Blennies)	*Escenius*		*bicolor*	0	3

The sampling methodology is described in-depth in Chapter 2. Three landmarks were digitised in Movias v. 1.5 (Pixoft/ NAC, 2003): 1, the tip of the dentary; 2, the intramandibular joint (at the junction between the dentary and the articular bone); and 3, the quadrate-mandibular joint. From the resultant coordinate pair triplets, frame-by-frame angular measurements of intramandibular (IM) flexion throughout the feeding event were extracted, indicating the timing of onset, duration and magnitude of IM-flexion in performance plots (Fig. 4.1C, D). These performance plots were condensed to maximum values (±Std Dev.) for each clade (Fig. 4.3) where negative IM-flexion values indicate gape-restrictive and positive values indicate gape-expansive kinematic properties. IMJs of fresh-killed specimens previously used in video analyses were manipulated to evaluate the utility of manipulation techniques for predicting IMJ kinematics. Video and manipulation results matched closely, although video data variance exceeded manipulation variance (e.g. *Chaetodon [Corallochaetodon] lunulatus*, T-test, p=0.968; Std. Dev_{video}, 1.51; Std. $Dev_{manipulation}$, 0.50). Manipulation studies were consequently used to quantify IM-flexion in taxa where live individuals were unavailable (Table 4.1).

4.2.2 Phylogenetic mapping and optimising

We used a matrix-recombining algorithm with parsimony (Bininda-Emonds, 2004; Kerr, 2005) to combine existing consensus trees into a super-tree for the Girellidae, Pomacanthidae, Chaetodontidae, Scatophagidae, Microcanthidae, Ephippidae and the Acanthuroidei (Bellwood et al., 2004; Tyler et al, 1989; Smith et al., 2003; Tang et al., 1999, Clements et al., 2004). Character histories for both feeding mode (N. Konow unpublished data; FishBase, 2005) and IM-flexion and were traced using the parsimony optimisation criterion in Mesquite ver. 1.06 (Maddison & Maddison, 2005) to estimate trait transition points.

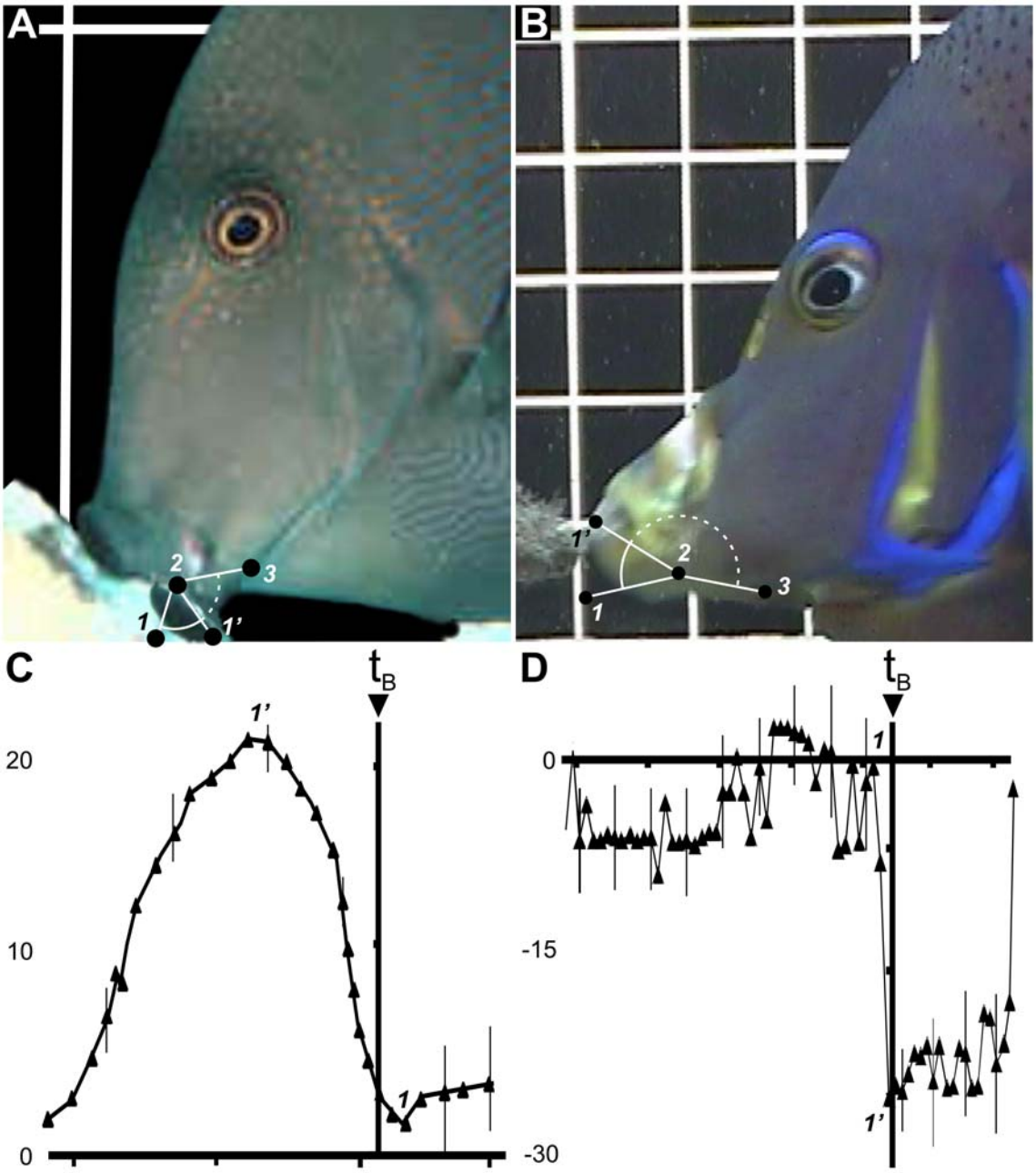

Figure 4.1 Sample images from high-speed video (A, B) and associated performance profile-plots of IMJ kinematics (C, D) for the common gape-expanding IMJ (A, C; *Ctenochaetus striatus*) and for the unique angelfish gape-restricting IMJ (B, D; *Pomacanthus semicirculatus*). 1, relaxed; 1', rotated dentary; 2 IMJ, 3, quadrate-articular joint. The X-axis of profile-plots indicates feeding-event duration, with time of jaw occlusion (t_B) at the Y-axis intercept. The Y-axis indicates degree of IM-flexion, with value-polarisation reflecting gape-expanding (positive) or restricting IMJ kinematics (negative values).

4.3 Results

4.3.1 Intramandibular morphology and kinematics

Intramandibular joints are present in the derived, biting butterflyfish belonging to the genera *Citharoedus* (Fig. 4.2 A) and *Corallochaetodon*. Kinematic onset occurs prior to maximum jaw protrusion and augments gape expansion by additional dentary tip rotation of ~49° and ~16° respectively (Fig. 4.3). Biting butterflyfish mandibles have a prominent lateral articular flange (Fig. 4.2A, 4.2B) with medially inserting ligaments connecting to the upper jawbones. Similarly, IMJs in siganids (Fig. 4.2F) and in *Girella* (Fig. 4.2H) augment gape expansion (Fig. 4.3), while more resembling the surgeonfish (Fig. 4.2E) and parrotfish IMJ by having steep resting angles between the dentary and articular bones (Fig. 4.2I). Contrasting with butterflyfishes, these articular bones lack lateral flanging, as do articular bones in angelfishes (Fig. 4.2C, 4.2D).

Angelfish IMJ kinematics are broadly comparable (Fig. 4.3), with a mean rotation of -35º (±4.8 Std Dev.) and rotation-onset lagging 5-6 ms after peak-jaw protrusion, eliciting a unique timing-sequencing of the feeding-event, with protruded jaw closure preceding a high-velocity jaw retraction. Divergence is principally seen in the zooplanktivorous genus *Genicanthus* (Fig. 4.3) where free-living prey elicits reduced IMJ kinematics, with mean rotation of -7±4.8º. Meanwhile, in correspondence with the grab-and-tearing mode of other angelfishes, attached prey elicits kinematic modulation, albeit still with reduced IM-flexion of -26.5±4.7º compared with the pomacanthid mean. Manipulations of IMJs in the predominantly zooplanktivorous Nasinae illustrated a similar trend of reduced IM-flexion (11.1±2.1º) from the acanthuroid mean of 18.7º. Intramandibular flexion was absent amongst the generalised butterflyfishes (Fig. 4.3), the

Figure 4.2 Camera Lucida drawings of jaw morphologies in representative study families: A, B: Chaetodontidae; C, D: Pomacanthidae; E: Acanthuridae; F: Siganidae; G: Scatophagidae; H: Girellidae (redrawn from Vial & Ojeda, 1990); I: Scaridae; J: Blennidae. Rostral cartilage in grey where present. Open circles indicate presence of intramandibular joints. Scale bars: 10 mm; except J, 0.1 mm.

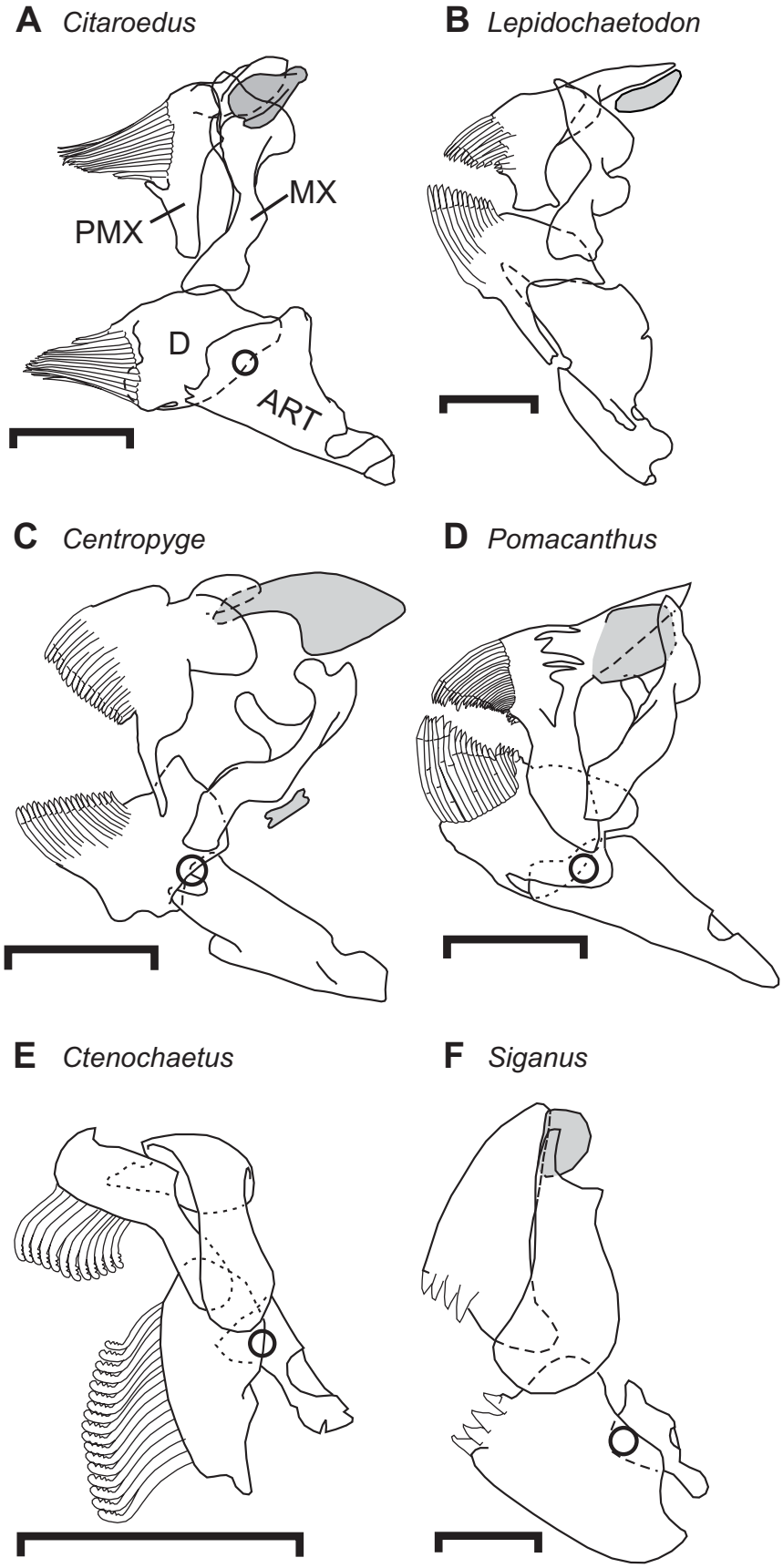
A Citaroedus
PMX
MX
D
ART
B Lepidochaetodon
C Centropyge
D Pomacanthus
E Ctenochaetus
F Siganus

Figure 4.2

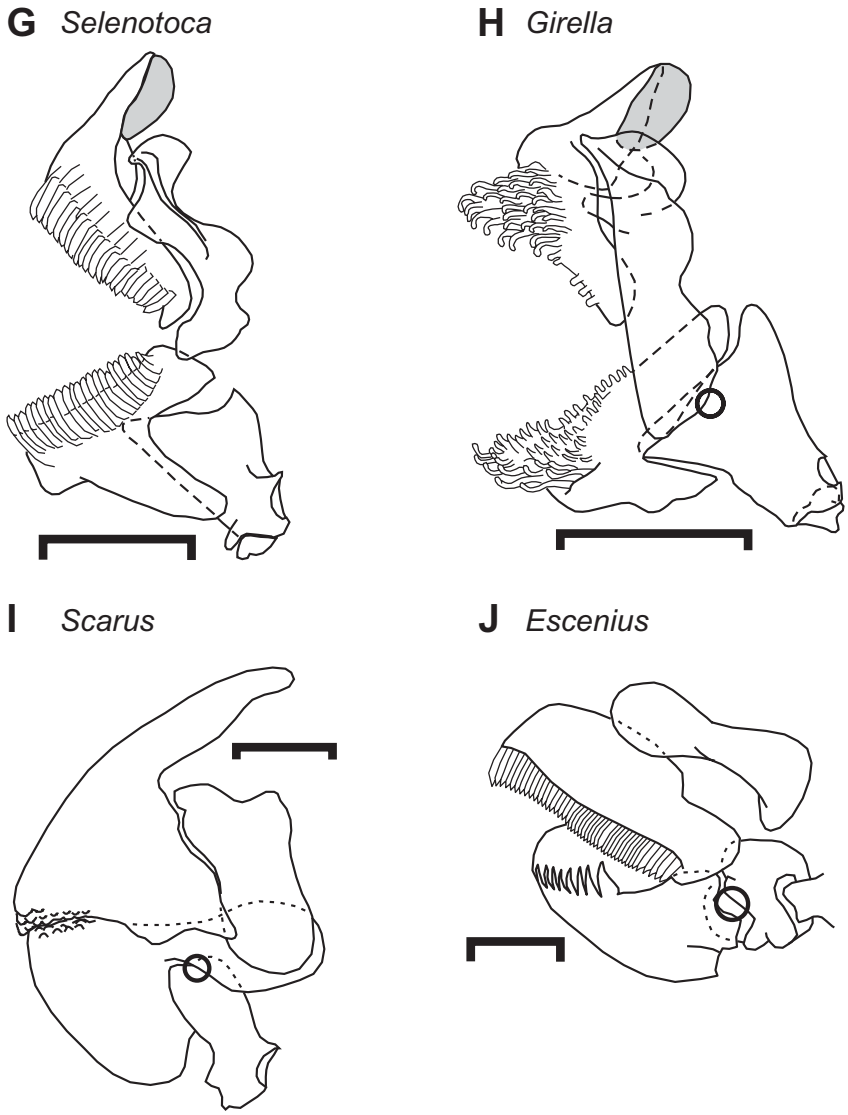

Figure 4.2, continued

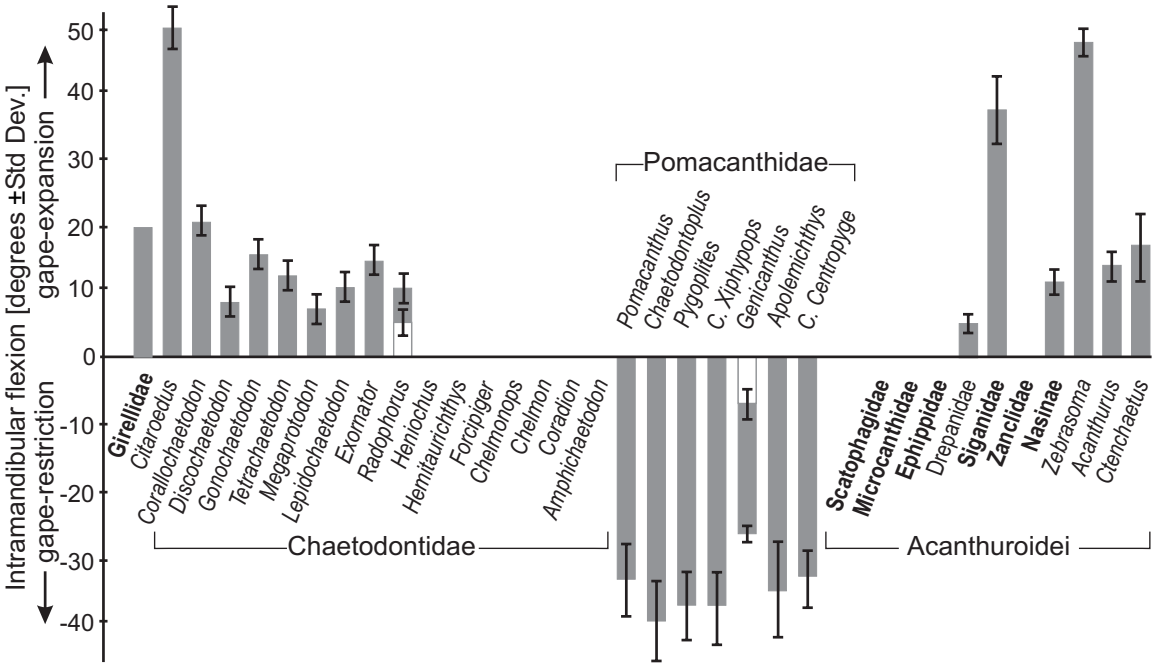

Figure 4.3 Intramandibular flexion clade-means based on kinematics and/or biomechanical analyses of n≤3 specimens (horizontal axis, see Table 4.1), with value-polarity (vertical axis) indicating jaw-closing (negative) or opening augmentation by IM-flexion (positive). In *C. Radophorus*, grey column represents *Chaetodon [Radophorus] melannotus* and white column the remaining taxa. In *Genicanthus*, grey column represents attached and white column free-living prey. *Girella* values were measured from mandible diagrams in Vial & Ojeda (1990).

Scatophagidae (Fig. 4.2G), Microcanthidae, and Ephippidae, while marginal flexion exists in *Drepane.* The lack of flexion in the monotypic invertebrate-picker *Zanclus* appears to be 'the exception to the rule' of intramandibular flexion in biting squamipinnid taxa.

4.3.2 Phylogenetic mapping of intramandibular flexion

Consensus of existing molecular and morphology-based phylogenies yielded a cladogram with the Girellidae forming a clade basal to an unresolved, 6-way polytomy of the Chaetodontidae, Pomacanthidae, Microcanthidae, Scatophagidae and Ephippidae (with *Drepane*), and the acanthuroid lineage: the Siganidae, *Zanclus*, Nasinae and Acanthuridae (Fig. 4.4). Optimisation of feeding mode to the cladogram shows ram-suction as the ancestral feeding mode in four radiations of this polytomy, while the biting status of derived butterflyfishes, angelfishes, most acanthurids and the kyphosids reveals ambiguity of the ancestral feeding-mode of the squamipinnes. Character mapping renders the gape-expanding IMJ type as the basal IMJ condition and an IMJ as a basal trait for the Pomacanthidae, Siganidae and Acanthuridae. In the Chaetodontidae, IM-flexion originates deep in the phylogeny, coinciding with transition from ram-suction to biting (Fig. 4.4) within *Chaetodon [Radophorus]*. Taxa from this subgenus are generally invertebrate pickers (Allen *et al*., 1998) with limited IM-flexion (approx. 4°), while a single corallivorous species, *C. [Radophorus] melannotus,* exhibits more pronounced flexion (*c.* 10°). IM-flexion increases with phylogenetic differentiation, culminating in the stout-jawed corallivorous crown-group, *C. [Corallochaetodon]* and *C. [Citharoedus]* (Fig. 4.2). Ambiguity also surrounds the basal state of IM-flexion in the study taxa, as IMJs only were identified in *Girella*, and other kyphosids that were unavailable for analysis.

Figure 4.4 Composite phylogeny for the squamipinnid families studied, based on available phylogenetic evidence. The polytomy reflects a lack of resolution, rather than conflict among alternate hypotheses. IMJ character-states are optimised to branches: filled branches, gape-expanding IMJ; open branches, gape-restricting IMJ; shaded branches, no IMJ; stippled branches, ambiguous. Feeding mode is mapped to clades using shading to delineate biting taxa. Representative study taxa; for the Chaetodontidae at sub-genus, the Pomacanthus and Acanthuridae at genus, and the remainder at family-level, are shown with thumbnails scaling to maximum total length (TL), to illustrate the evolution of body-plans (scale, 150 mm). Bracketed numbers indicate the number or species within clades, and asterisks indicate instances of reversal from biting to planktivory.

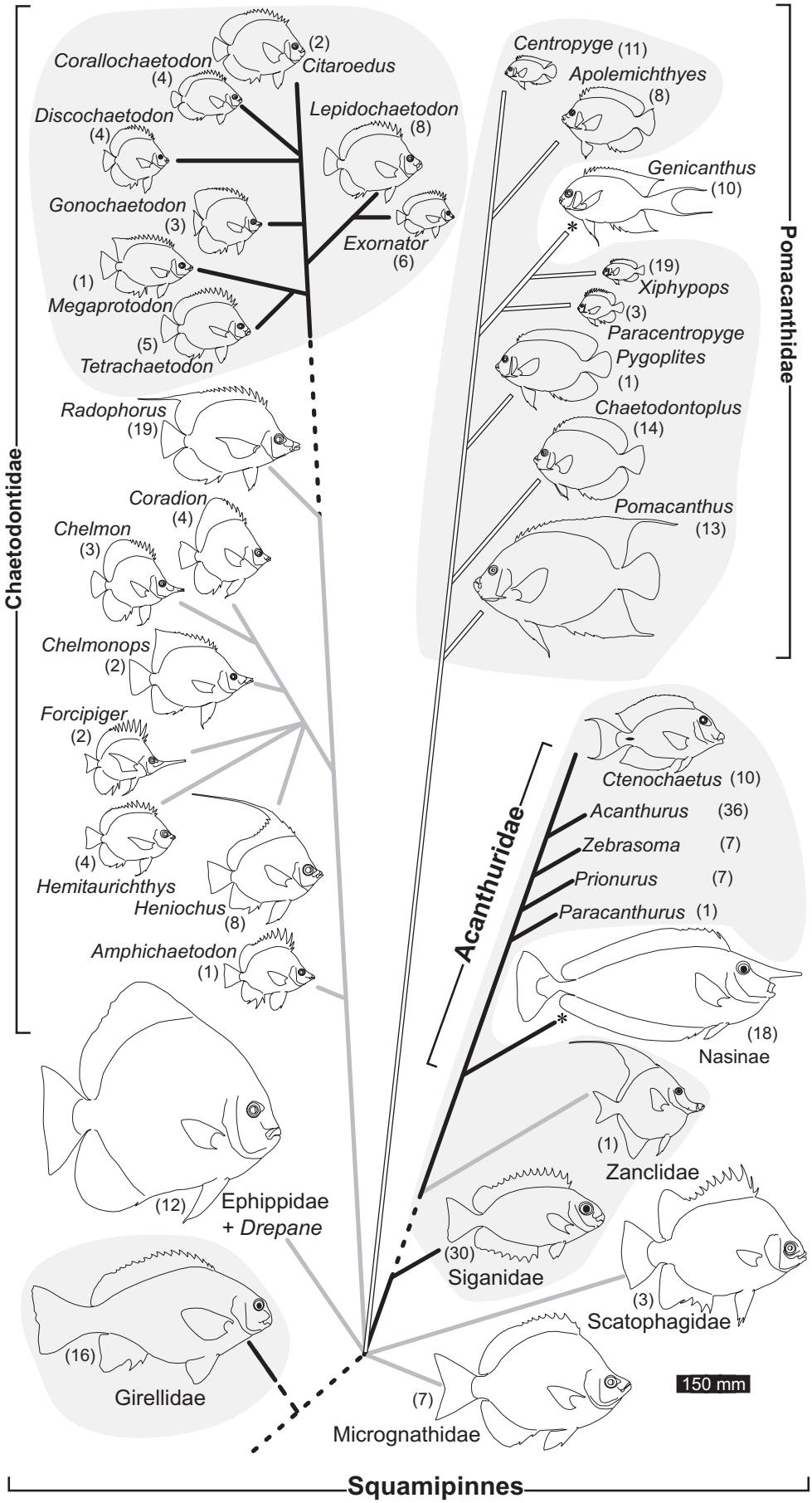
Chaetodontidae
Corallochaetodon
(4)
(2)
Citaroedus
Discochaetodon
(4)
Lepidochaetodon
(8)
Gonochaetodon
(3)
Exornator
(6)
(1)
Megaprotodon
(5)
Tetrachaetodon
Radophorus
(19)
Coradion
(4)
Chelmon
(3)
Chelmonops
(2)
Forcipiger
(2)
(4)
Hemitaurichthys
Heniochus
(8)
Amphichaetodon
(1)
(12)
Ephippidae
+ Drepane
(16)
Girellidae
Centropyge (11)
Apolemichthyes
(8)
Genicanthus
(10)
*
(19)
Xiphypops
(3)
Paracentropyge
Pygoplites
(1)
Chaetodontoplus
(14)
Pomacanthus
(13)
Pomacanthidae
Acanthuridae
Ctenochaetus (10)
Acanthurus (36)
Zebrasoma (7)
Prionurus (7)
Paracanthurus (1)
*
(18)
Nasinae
(1)
Zanclidae
(30)
Siganidae
(3)
Scatophagidae
(7)
Micrognathidae
150 mm
Squamipinnes

Figure 4.4

4.4 Discussion

Amongst squamipinnid reef fish taxa, a diverse range of biting strategies appear to dominate, and intramandibular joints with kinematics augmenting either gape expansion or occlusion appear to have arisen independently at least three times.

4.4.1 The prevalence of IMJs in biting reef fishes

The occurrence of IMJs is widespread in biting reef fishes amongst the derived chaetodontids, siganids, previously unstudied acanthurids, in addition to pomacanthids (Chapter 2; Chapter 3), girellids (Vial & Ojeda, 1990), acanthurids (Purcell & Bellwood, 1993), three scraping and grazing scarid genera (Bellwood, 1994; Bellwood *et al*., 2003; Streelman *et al*., 2002), and the blennid genus *Escenius* (Chapter 2; Fig. 4.2J). It appears that few major radiations of biting reef fishes lack IMJs, and these are either durophagous tetraodontiform fishes (Turingan *et al*., 1995) or robust, excavating parrotfishes (Bellwood 1994; Streelman *et al*., 2002; Bellwood *et al*., 2003). In robust taxa, duplication of musculature, or fusion of jaw elements yield increased feeding apparatus stability. Similarly, *Chaetodon [Lepidochaetodon] unimaculatus,* a known hard coral biter (Motta, 1985; 1987; 1988; Cox, 1994), possesses the most robust butterflyfish jaw apparatus (Fig. 4.2B) and exhibits fairly restricted IM-flexion (Fig. 4.3). In conjunction with functional reversal from biting to ram-suction feeding planktivory in IMJ-bearing taxa, IM-flexion appears to be restricted (e.g. in the Nasinae, *Genicanthus* and in *Pomacanthus rhomboides;* N. Konow, unpubl. data). Whilst IMJs exist in a cichlid (*Astatotilapia;* Aerts, 1985), and purportedly in poecillids (A. C. Gibb, pers.

comm. 2004), their role and prevalence amongst biting freshwater teleosts remains unclear.

4.4.2 Divergent IMJ kinematics and differential biting strategies

Pomacanthids occupy highly divergent feeding guilds (Allen *et al*., 1998), despite an evolutionary conservative IMJ morphology and kinematics. Generalised, robust genera grab-and-tear structurally resilient prey, and specialised, gracile taxa shear or tear turf algae, or delicate invertebrate prey. The unique gape-restrictive IMJ kinematics, coupled with considerable jaw protrusion and a high-velocity jaw retraction appear to provide a sufficiently versatile functional basis for ecological diversification in the Pomacanthidae (Chapter 3). The unusual capability of protruded jaw closure has allowed angelfishes to exceed the 'ecological thresholds' posed by robustness of attached prey and/or sturdiness of prey attachment, which otherwise are impassable to most reef fishes.

Gape-expanding IMJ kinematics remain the dominant form, present in all other squamipinnid, as well as some scarine and blennid biters, where differential magnitudes of IM-flexion contrasts the conservative degree of flexion in pomacanthid IMJs. This may either reflect differential optima in the musculoskeletal systems, or a more pronounced advantage of augmented gape-expansion, in allowing increased substratum-area coverage per bite, or in optimising the jaw and/or predator orientation to the substratum (Alexander, 1967; Motta, 1984; Purcell & Bellwood, 1993, Bellwood *et al*., 2004b). In the chaetodontid crown-group, gape-expanding IMJs facilitate coral-tissue scraping by taxa with robust jaw morphology and bristle-shaped teeth, and among generalised biting butterflyfishes, modest IM-flexion corresponds with altered

biomechanical requirements of rigid, pincer-like jaws with anterior-pointing bristle teeth for single-polyp picking (Motta, 1989).

4.4.3 Convergent, parallel or divergent IMJ evolution?

Biting strategies aided by an IMJ is a derived butterflyfish trait, but a basal trait in angelfishes, where no known ancestors display reduced IM-flexion. While ancestral taxa may remain as undiscovered fossils (Bellwood & Wainwright 2002), the status of *Drepane,* bearing highly protrusible jaws with IM-flexion, albeit modest, is particularly interesting. Disparate patterns of IMJ evolution in chaetodontoid fishes raise common-ancestry ambiguity, yet the observed kinematic divergence may result from minor reorganisations of jaw adductor muscle insertions and ligaments adjoining oral jaw elements (Chapter 2). *Citharoedus* and *Corallochaetodon* may comprise the chaetodontid crown-group (but see Littlewood *et al*., 2004) but they also bear the closest structural resemblance to angelfishes. Shared derived traits include a dorsal inclination of the resting mandible, pronounced rostro-lachrymal elongation, a frontal neurocranial cavity to accommodate the ascending premaxillary process and multi-tiered rows of bristle-shaped, hook-pointed teeth (Chapter 2). These characters provide too weak a basis for inferences to be made about chaetodontoid ancestry. However, the lack of differential IMJ character-states in angelfishes, ambiguous origin-separation of IM-flexion amongst chaetodontoid fishes, and uncertain ancestral hypotheses for putative sister families within the squamipinnes, all underscore a need for robust phylogenetic reconstructions. Re-examinations of existing hypotheses would benefit from calibrated clade age-estimates in order to resolve chaetodontoid common-ancestry and squamipinnid stem-group relationships (Bellwood *et al*., 2004b). The presence of IM-flexion in *Girella*

clearly illustrates this requirement. A deeper clade-resolution will help determine whether IM-flexion is a basal squamipinnid trait, and thus whether the present distribution of intramandibular joints results from multiple convergences, parallel radiations or reversals. Regardless, IMJs have been an equally important functional innovation in the evolution of the squamipinnes, as the pharyngeal jaw apparatus was in labroid evolution.

At the assemblage-level, it appears commonplace for biting reef fishes to utilise an intramandibular joint, sparking a remarkable presence of intramandibular flexion in at least five major extant squamipinnid groups. This supports the status of the IMJ as an important functional innovation, forming the basis for advanced grazing strategies in reef fishes. The widespread gape-increasing IMJ appears useful for grazing and scraping tasks posing limited biomechanical challenges (structural resilience and/or sturdy attachment). Gape-restricting IM-flexion is apparently unique to angelfishes, where it facilitates novel prey-utilisation involving differential biomechanical challenges including, but not limited to, the procurement of sturdily attached prey with a high structural integrity relative to predator size. Historically, this unique functional system is reflected by a remarkable trophic diversification of angelfishes in reef ecosystems.

Chapter 5: Concluding Discussion

In the past, extensive research in functional and evolutionary ecology of feeding in reef fishes has primarily focussed on dietary and foraging patterns, functional feeding morphology and, more recently, phylogenetic relationships. A detailed understanding has been generated for a broad range of trophic strategies, including the existence of an apparent emphasis amongst reef fishes on attached prey procurement. The primary missing link in order to conduct comprehensive ecomorphological analyses has been the feeding kinematics of biters, and consequently, this has been the central focus of this thesis.

5.1 Synthesis of thesis results

A requirement of obtaining feeding kinematics data for biting marine reef teleosts was pointed out in Chapter 1. Such data would complement the expanding ecological knowledge on these taxonomically and numerically abundant fishes in high-diversity marine reef ecosystems. Whilst the introduction only presented evidence from the teleost literature, an extensive insight has been obtained in a concurrent and prolific functional research into the feeding biology of chondrichthyans and generalised teleost lineages.

The functional analysis of *Pomacanthus* in Chapter 2 generated the first comprehensive evidence of feeding kinematic in an exclusively biting teleost. Three major trait novelties were revealed in the feeding apparatus of this generalised angelfish taxon with known sponge-feeding habits: suspensorial rotation was found to augment extreme jaw protrusion, intramandibular articulation causing occlusion of the jaws in a protruded state, followed by a high-velocity jaw retraction.

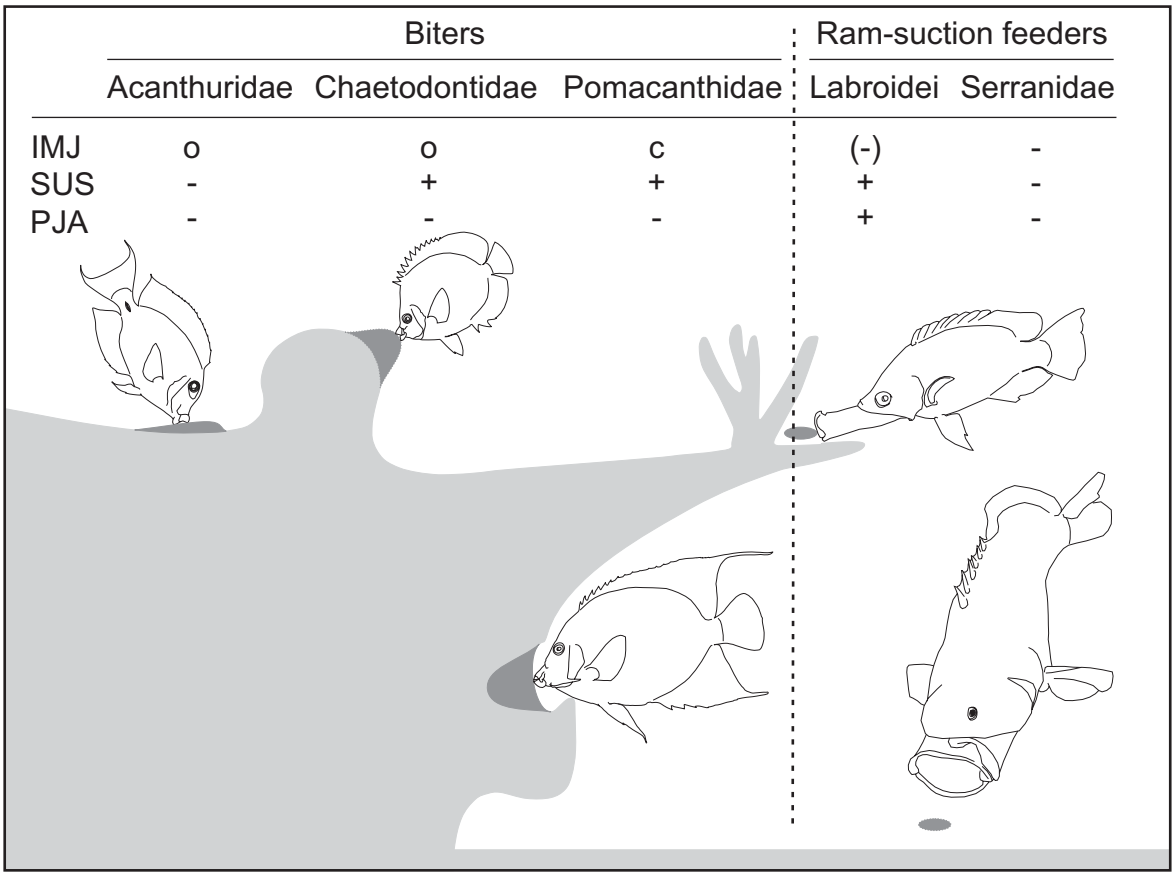

Figure 5.1 Relationships between prey posing differential biomechanical challenges and combinations of presence (+) and absence (-) of several functional innovations in biting and ram-suction feeding teleosts in marine reefs ecosystems. IMJ, intramandibular joint that facilitate C, gape occlusion (f. Pomacanthidae) or O, expansion (other squamipinnid teleosts); SUS, suspensorial rotation that augment jaw protrusion; PJA, a modified labroid pharyngeal jaw apparatus (also present in biting scarines) that provides functional decoupling of the oral jaws from mastication tasks. Whilst acanthurid and chaetodontid biters appear functionally suited to convex bite surfaces, pomacanthids appear unique amongst the squamipinnes in their functional prerequisites for convex surface utilisation. Intramandibular joints appear to drive biting strategies, including those found in the labroid scarines, while pharyngeal jaws drive the ram-suction feeding strategies that are restricted to procurement of elusive or loosely attached prey (Konow *et al*., *in prep*).

Suspensorial rotation was previously only identified in ram-suction feeders on highly elusive prey whilst in *Pomacanthus* it facilitates access to attached semi-cryptic benthos (fig 5.1). Previously, IMJ kinematics were unquantified, but inferred to expand jaw gape, whilst in *Pomacanthus* protruded jaw closure resulted from IM-flexion and facilitated grabbing of prey. Commonly, jaw protrusion velocity greatly exceeds retraction velocity in teleosts, yet in *Pomacanthus* a reverse velocity regime mediates a tearing dislodgement of sturdy sponge prey. The distinctive feeding apparatus in *Pomacanthus* appears functionally unique and well matched with their novel trophic guild, whilst it corresponds poorly with the teleost feeding modes previously quantified.

The evolutionary and ecological implications of the feeding apparatus characteristics described in a generalised *Pomacanthus* taxon were examined at the family-level of angelfishes in Chapter 3. Phylogenetic evidence enabled the selection of eight study species to represent all GBR lineages, and 83% of all monophyletic clades within the family. The phylogeny also reflected a generalised nature of pomacanthid spongivory and robust omnivory and convergent origins of herbivory in generalised robust taxa, as well as amongst the small, more gracile pygmy angelfishes. Overall, the broad trophic diversification of angelfishes into novel biting feeding guilds was well delineated by phylogenetic evidence. Differences in morphological traits amongst *Centropyge* taxa supported previous recommendation based on molecular evidence for taxonomical reclassification of the genus *Centropyge*.

The wide-ranging ecological diversification of angelfishes was well reflected by their highly divergent alimentary traits but contrasted with conservative evolutionary trends in the disparity of feeding apparatus traits and prey-capture kinematics. The nature of biting strategies was specialised, but remained conservative within the monophyletic

angelfish assemblage. Conservative specialised traits combined with a broad body size range in angelfishes to facilitate their adoption of novel reefal trophic guilds. This functional basis provided the capabilities required for angelfishes to overcome 'ecological thresholds' imposed by the different biomechanical challenges presented by robust or delicate attached prey. Quantitative experimental evidence illustrating the effects of novel, yet evolutionarily stable feeding kinematics on functional disparity at the family assemblage-level is unprecedented amongst teleosts. This is also the first comprehensive family-level functional analysis of ecomorphology in a monophyletic biting assemblage. Quantitative functional data has not previously been presented in support of the hypothesis of ecological disparity preceding assemblage-level species diversification (Liem, 1980b; Streelman *et al*., 2002). Whilst promoting diversification along the ecological axis of biting, angelfish feeding kinematics simultaneously appeared to functionally constrain angelfish proliferation along the alternative axis of ram-suction feeding.

An expanded functional analysis of representative squamipinnid reef fish taxa in Chapter 4 reflected the evolutionary history and kinematic characteristics of intramandibular flexion in biters. Disparity in IMJ kinematics provided evidence that suggested angelfishes are both functionally and ecologically distinct. Their force-driven grab-and-tearing feeding contrasts with more gracile grazing and scraping trophic guilds utilised by other IMJ-bearers, governed by gape-expanding IMJ kinematics, as previously hypothesised (Fig. 5.1). Application of a cut-and-paste super-tree technique to existing squamipinnid phylogenetic hypotheses provided insight into the evolutionary history of IMJs. The evolution of IM flexion in at least three, and possibly five squamipinnid lineages coincide with transition to biting and strongly suggest an overriding influence of IMJs on squamipinnid biter proliferation. This evidence supports a status of the IMJ as a functional

innovation in biters with an importance comparable to pharyngeal jaws (PJA) in the evolutionary radiation of reef ram-suction feeders (labroids and serranids in fig. 5.1).

Overall, this thesis constitutes a baseline of functional insight into the role of angelfishes and other squamipinnid biters in tropical marine reef ecosystems. With this functional knowledge in place, several avenues of ecological research can be explored, linking patterns of biter functional novelty and disparity to their astounding diversity in marine reef ecosystems.

5.2 Grab-and-tearing feeding kinematics and novel prey utilisation

The grab-and-tearing feeding kinematics of *Pomacanthus* matches a novel diet of sponges, tunicates and other structurally prey with structural resilience and/ or sturdy attachment that pose considerable biomechanical challenges to a predator. This raises questions concerning the physical force generated during prey dislodgement. How is sufficient tearing force generated by a predator that is unaided by gravity, to remove such prey from the reef? In *Pomacanthus*, protruded jaw closure, high-velocity jaw retraction and prehensile tooth rows appear to be the critical factors of prey dislodgement. Physical force generation during feeding is scarcely tested in lower vertebrates (Huber & Motta, 2004) and will be useful in examining the relationship between pomacanthid body size and the associated sponge-tearing capabilities. Direct tensiometry results can be compared with quantitative data from the bath sponge industry on structural integrity of sponge species preyed upon by angelfishes to reveal if a certain body size is required for spongivory.

The temporal lag between bite and jaw-retraction in *Pomacanthus* may represent a phase of strain-energy storage in the jaw adductors A1 and A3, analogous to the jaw

musculature strain-energy storage that facilitates rapid jaw depression in *Astatotilapia* (Aerts *et al*, 1987). Synchronisation of electromyography and high-speed video are potentially useful tools to examine the physiological factors of force-generation in angelfish grab-and-tearing.

Sponge feeding is unusual among trophic guilds because it involves a prey type with numerous anti-predatory mechanisms, including crypsis, noxious secondary metabolites, structural resilience and mechanical protection by spicules. Spongivory has so far received a biogeographically skewed attention, with field-based experimental studies mainly conducted on sponge-rich Caribbean reefs (Wulff, 1994; 1997; but see Burns *et al*., 2003). These studies generally involved comparisons of direct predation estimates on conspicuous sponge growth forms from reefal habitats or experiments where mangrove sponges, which in theory coexist with fewer potential predators, were transplanted to reefal habitats (Dunlap & Pawlik, 1996; Swearingen & Pawlik, 1998), or exposure of cryptic reefal sponges that actively take refuge from predators within the reef matrix (Wulff, 1997; Richter *et al*, 2001). Transplanted and/ or cryptic sponge taxa were consistently chosen over conspicuous reefal growth forms by sponge feeding taxa.

Traditionally, emphasis has been placed on the effect of sponge growth forms and abiotic differences in their wide habitat ranges on predation rates, rather than on the role of functional capabilities of sponge feeding taxa. In addition to many large body pomacanthid taxa (Hourigan *et al*., 1989; Hill & Hill, 2002; the present study), teleost sponge feeders include trunk, file and pufferfishes (Dunlap & Pawlik, 1996; Wulff, 1994). The functional basis facilitating durophagous trophic strategies, including sponge feeding in tetraodontiform fishes is well understood (Turingan & Wainwright, 1993; Turingan *et al*., 1995; Friel & Wainwright, 1997; 1998; 1999; Wainwright & Friel, 2000). However,

emerging evidence suggests that several other teleost groups may opportunistically feed on sponges. Parrotfishes are a good example, with members of the genera *Sparisoma* and *Scarus* preying on transplanted mangrove or exposed cryptic sponges (Dunlap & Pawlik, 1996; Wulff, 1997). *Sparisoma* species are robust excavators with limited intramandibular flexion and are likely to ingest interstitial sponges during their general foraging. In contrast, *Scarus* species are gracile scrapers and their intramandibular joints may pose a functional constraint on spongivory by preventing excavation of sturdy sponge forms.

The parrotfishes showed affinity towards cryptic sponge forms that may be less structurally and/or chemically defended (Wulff, 1997). This suggests that lack of suspensorial flexion and associated jaw protrusion, as seen in pomacanthids, functionally constrains non-excavating parrotfishes to feeding on the parts of cryptic sponges growing out of their cavities in the reef. Evidence is emerging of cryptic sponges constituting a much larger matrix component on photic zone reefs, than previously expected (Richter *et al*, 2001) and considerable growth-plasticity in sponge taxa (Hill & Hill, 2002). Combined with evidence of sponges being competitively superior to scleratinian corals in space acquisition, at least in the Caribbean (Hill, 1998), and evidence of spongivory being more common than hitherto expected, the need for further attention to the functional basis and ecological effects of spongivory becomes apparent. Sponge feeder removal for the marine ornamental trade could pose an unexpected threat of sponge-overgrowth to scleratinian corals, in addition to the numerous alternative coral reef ecosystem resilience threats already known (Bellwood *et al*., 2004a).

5.3 Functional disparity in biting teleosts

The functional characteristics of the feeding apparatus described in *Pomacanthus* (Chapter 2) was established in all other pomacanthid taxa studied (Chapter 3), and is thus symplesiomorphic for all pomacanthid clades, including the atavistic ram-suction feeding genus *Genicanthus.* Both morphological and functional disparity appears restricted in pomacanthids, and most taxa were largely inseparable in 2D kinematic functional space (Chapter 3). However, reduced disparity compared to the predominately ram-suction feeding labroids (Wainwright *et al*., 2004; Westneat *et al*., 2005) and long-jawed chaetodontids (Ferry-Graham *et al*., 2001a,b) is also seen in the biting IMJ-bearing scarines (Wainwright *et al*., 2004).

Despite restricted disparity, the novel functional systems in both angel and parrotfishes appear to have released sufficient evolutionary potential to facilitate a broad ecological diversification, allowing the crossing of an 'ecological threshold' otherwise preventing procurement of sturdily attached prey types. Limited disparity in biters may also reflect functional constraints resulting from novel traits. In unison, these evolutionary principles may permit diversification along certain axes (e.g. prey dislodgement) whilst restricting diversification along alternative axes (e.g. suction generation). Likely examples of such principles are the atavistic ram-suction feeding IMJ-bearers in the pomacanthid genus *Genicanthus*. This group of angelfishes utilise IMJ flexion for benthic prey procurement, while exhibiting a functional reversal to ram-suction feeding and reduced IM-flexion when presented with elusive prey (Konow *et al*., *in prep*). Similar behavioural modulation may occur in the planktivorous unicornfishes (Nasinae), where IM-flexion also is reduced compared with other acanthuroid IMJ bearers (Chapter 4). This evidence suggests that IMJs are of reduced utility in trophic strategies not relying on active biting.

Although functional data from angelfishes and structural data from parrotfishes lends reciprocal support, caution should be taken when comparing morphological and functional characteristics due to emerging evidence of extensive morphological redundancy in complex biomechanical apparatuses (Alfaro *et al*., 2005). This many-to-one mapping theory predicts a reduced ecological diversification potential from a set range of functional diversity, compared with estimates obtained in by-proxy analyses (Alfaro *et al*., 2005; Wainwright *et al*., 2005). Additionally, a functional system such as the angelfish feeding apparatus, which displays restricted disparity, whilst supporting a considerable ecological diversification, appears to contrast this scenario and may represent an alternative evolutionary path to high diversity. Eventual comparisons of functional disparity amongst assemblages should be based on equivalent data, and may require alternative measurements to those used in labrid studies (cumulated PC axes variances for monophyletic assemblages; see Wainwright *et al*., 2004).

Overall, the methodologies deployed herein demonstrated a potential for ecological diversity in taxa exhibiting restricted functional and morphological trait differences and holds promise of success in future comparisons of assemblage-level prey-capture disparity. Despite the limited kinematic disparity amongst pomacanthids the methodology showed definite utility in segregating taxa enough to detect divergence trends. This approach also takes into consideration the amplified variance in trait measurements invariably generated by behavioural modulation (Nemeth, 1997a, b; Ferry-Graham *et al*., 2001c; Sass & Motta, 2002).

5.4 The biting feeding mode

All angelfishes exhibit a unique sequencing of the feeding event, where jaw-closure preceded jaw retraction (Chapter 2 & 3), resulting in a novel 'grab-and-tearing' strategy that diverges from existing evidence from more gracile biters (Motta, 1985; 1988; 1989; Vial & Ojeda, 1990; Purcell & Bellwood, 1993; Bellwood, 1994) and other biters analysed herein (Chapter 4). Morphological and functional evidence herein reflects a complex array of biting strategies, ranging from generalised durophagous and excavating forms without IMJs, seen in tetraodontid and some scarine taxa, grab-and-tearing taxa with gape-restricting IMJ kinematics exclusive to the Pomacanthidae, and more gracile scraping and grazing strategies with gape-expanding IMJ kinematics dominating amongst other squamipinnid IMJ-bearers. Biting thus appears to be an equally complex continuum of feeding strategies as ram-suction feeding, illustrating the requirement for further examination of the biting feeding mode.

5.5 Divergent IMJ kinematics and differential microhabitat utilisation

Several studies have linked trends of altered microhabitat utilisation to morphology and the appearance of key innovations over evolutionary time (Streelman *et al*., 2002; Langerhans *et al*., 2003). Meanwhile, attempts to quantify specific microhabitat traits that reflect and promote morphological specialization, behavioural modulation and trophic diversification in fishes have been met with varying success (Green, 1996; Bean *et al*., 2002). Considering the divergence of IMJ kinematics in squamipinnid reef biters (Chapter 4), it appears of particular importance to test if relative surface curvature (planar, convex and concave) measured at the scale of jaw-gape and/or tooth bearing surface width (i.e. 1-

20 mm^2) where bites are taken constitute key microhabitat factors that segregate biters with differential IMJ kinematics (Fig. 5.1). Successful quantification of such relationships would provide valuable insights into these otherwise elusive interactions between differently equipped predators and prey posing differential biomechanical challenges.

Biting angel and butterflyfishes would be particularly interesting groups for examining relationships with microhabitats. Whilst both are conspicuous and relatively abundant components of fish faunas on most tropical marine reefs, the diversification of each of these putative sister-families appear to be driven by differential kinematics, functional traits and patterns of biting evolution (Chapter 4). Occlusive protrusible jaws and associated grab-and-tearing strategies are unique to angelfishes and appear to be specialisations towards concave microhabitat use. Angelfishes may thus be both functionally and ecologically unique in their ability to dislodge sturdy prey from protected attachment sites within the reef matrix. Butterflyfishes seem to conform better to the convex substratum preference of gape-expanding IMJ bearers (*cf.* Bellwood *et al*., 2003; Streelman *et al*., 2002). Acute resource selectivity has been argued in some corallivorous butterflyfishes (Berumen *et al*., 2005) whilst evidence of the opposite may reflect flexibility in feeding habits (Pratchett, 2005) or biogeography-related differences.

Chaetodon [Lepidochaetodon] unimaculatus has a robust mandible with restricted intramandibular flexion, and specialises on *Montipora* coral in Hawaii (Cox, 1994, but see Motta, 1988), whilst predominately utilising soft coral on the GBR (Anderson, *et al*., 1981; Pratchett, 2005). *Chaetodon [Citharoedus] ornatissimus* lunges at a range of scleratinian coral species in Hawaii (Motta, 1985), predominately at *Acropora hyacinthus* on the GBR (N. Konow, unpublished) and has more intramandibular flexion than any other butterflyfish (Chapter 4). Interestingly, Hawaiian *C. ornatissimus* attain high and divergent

relative gut lengths (RGI) of 20-30:1, while GBR specimens have more modest RGIs of 10-15:1 (N. Konow, unpublished). Despite arguments of flexibility in feeding habits driving variations in diet (Pratchett, 2005), it remains a possibility that butterflyfish resource-selectivity in some instances results from functional morphology constraints (Motta, 1988). One possible constraint could be the tooth-bearing surface anatomy closely matching the hard-tissue surface profiles of coral species preyed upon.

5.6 Prevalence of biters in tropical marine reef ecosystems

The evolutionary history and functional biology of biting reef fishes remains poorly understood despite the ubiquitous ecological status of many biting taxa in reefal ecosystems. Biting is a major feeding mode in seven squamipinnid families, and dominates one clade, the scarines, within the otherwise ram-suction dominated Labroidei. IMJs are utilised in all of these biting groups, except for the monotypic Zanclidae, strongly suggesting IMJs to be a functional innovation. Insufficient deep phylogenetic resolution of the squamipinnid fishes currently prevents further examination of evolutionary processes that promote biting on reefs (Chapter 4). Deep-node resolution will allow analyses to determine the ancestral character state of IM flexion. Despite a putative ancestral ram-suction feeding status in the ray-finned fishes (Lauder, 1980; Liem, 1980a) the cladogenesis of IMJ-bearing biters may have occurred earlier in the perciform evolution than revealed by existing evidence.

5.7 Biters with IMJs in other reefal ecosystems

The fact that both the role and prevalence of IMJs have largely been overlooked in marine reef ecosystems warrants a functional revisitation of African lacustrine cichlids, particularly the speciose biter assemblage inhabiting Lake Tanganyika. Are IMJs truly lacking in these notoriously diverse biting taxa? A diverging prominence of ram-suction and biting strategies appears evident amongst marine and freshwater assemblages. Does this reflect differential feeding challenges from associated microhabitat characteristics amongst marine and freshwater reef ecosystems? Extensive structural data from African lacustrine cichlids (Barel, 1983; Strauss, 1984; De Visser & Barel, 1996) only includes evidence of an IMJ in *Astatotilapia elegans* (Aerts, 1985). Interestingly, recent functional cichlid analyses have focussed almost exclusively on heroine taxa from New-World riverine ecosystems and established multiple convergences of suspensorial rotation augmenting jaw protrusion (Meyer, 1987; Norton & Brainerd, 1993; Wainwright *et al*., 2001; Hulsey & García de León, 2005). African lacustrine boulder reefs may contrast riverine habitats, in terms of relative microhabitat complexity, rendering a strong likelihood of at least gape-expanding IMJs being present in biting lacustrine cichlids.

Relationships between differential microhabitat complexity in marine and freshwater reef ecosystems and the relative abundance, functional composition and functional morphospace utilisation associated with biting taxa should be investigated at a broad spatial scale. Comparing biter ecomorphology and microhabitat utilisation patterns across high-complexity Indo-West Pacific coral reefs, intermediately complex Caribbean coral reefs and African lacustrine boulder reefs could aid our understanding of the relationships between patterns of functional disparity and the ecological diversification of species over evolutionary time (Liem, 1980b). The growing amount of phylogenetic

hypotheses for lacustrine cichlid (Salzburger *et al*., 2002), marine labroid (Westneat and Alfaro, 2005) and squamipinnid taxa (Read et al., *in prep*) will help ensure that such comparisons remain uncompromised by phylogenetic and biogeographical effects.

5.8 Thesis conclusions

In summary, a unique combination of functional innovations in the feeding apparatus of angelfishes allows them to overcome ecological thresholds and use grab-and-tearing to exploit novel trophic guilds including dislodging spongivory and herbivory. Intramandibular joints are an important biter functional innovation, playing a role in the ecological diversification of the squamipinnes comparable to pharyngeal jaws in labroid evolution. An emerging trend of reduced feeding apparatus disparity amongst biters, when compared with ram-suction feeding taxa, suggest that these novel traits may constrain diversification.

Results in this thesis demonstrated the utility of a functional approach to ecomorphology, involving experimental quantification of prey-capture kinematics: in detecting novel feeding kinematics (Chapter 2), in quantifying functional disparity of a monophyletic assemblage (Chapter 3) and in investigating the role of functional innovations in the ecological diversification of entire assemblages over evolutionary time (Chapter 4). The combination of this experimental method with methods projecting morphometrics into biomechanical models in a context of rapidly growing phylogenetic evidence will be integral to future investigations. Such a combined approach improves the chance of elucidating differences in the functional basis and the associated ecological role of divergent teleost feeding modes.

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APPENDIX I

Original code authored with Dr. Wayne A. Mallett. Sponsor: JCU, QPSF, APAC.

MatLab-code and instructions for realignment of the 8 quarter-fields per image, to produce 200 frames per second high-speed movie from a 25fps image stream recorded by the JVC GR-DVL9800 camera are given below.

Raw-AVI files are required for this procedure. VirtualDub (ver. 1.4.7 used) is one of several programs capable of re-formatting JVC AVI to raw-AVI.

With raw-AVI files named "*.avi" and placed in the directory "data" begin the video conversion procedure by typing:

```
cd data
avi2tif *.avi destination_directory
```

while substituting "*.avi" with the appropriate filename and "destination_directory" with a suitably chosen directory name.

This will create a series of *.tiff files in the chosen directory. These image files contain 8 quarter-fields each (on/off field of each quarter) arranged in the correct movie sequence.

The following may, or may not be necessary:

To extract the data from the tiff files (if needed) start MATLAB by typing:

```
matlab65 –nojvm
```

Once the MATLAB prompt is returned type

frames = tiffdata('frame',n);

where "n" is replaced with the number associated with the last tiff file in the movie sequence.

The expanded data can be saved in tiff files using the MATLAB command

tiffsave(frames);

This will produce a large amount of tiff files beginning with "frm" in the current working directory.

Images can be viewed on a correct aspect ratio by typing

tiffshow(frames{n})

where "n" is replaced with a frame number. The total number of frames available can be obtained by entering the MATLAB command

length(frames)

A MATLAB movie can be created/displayed from this data by typing

frms = tiffmovie(frames);

The resultant high-speed AVI file can then be produced of this MATLAB movie by typing

movie2avi(frms,'fish.avi','fps',25);

Name and number of frames displayed per second (from 25) of this AVI file can be changed as desired

MATLAB functions for video conversion procedure.

The following functions must be placed in separate *.M files, named according to the function, e.g. 'tiffdata' and be placed in the MATLAB directory

```
function  frms=tiffdata(prfx,nmax)
% TIFFDATA   Extract data from a series of tiff files.
%
% Example:   frames=tiffdata('mov',500)
%    This example extracts data from a series of 500 TIFF files
%    such as mov000.tif, mov001.tif, mov002.tif, etc into the
%    MATLAB variable frames.
file = sprintf('%s000.tif',prfx);
if (exist(file,'file'))

  frms = cell(1,8*(nmax+1));
  for i=0:nmax ,
    file = sprintf('%s%03d.tif',prfx,i);
    data = imread(file,'tif');
% ORIGINAL SEQUENCE
%    frms{8*i+1} = data(002:2:220,008:300,:);    %% Top-left  On
%    frms{8*i+2} = data(002:2:220,308:600,:);    %% Top-right On
%    frms{8*i+3} = data(226:2:444,008:300,:);    %% Bot-left  Off
%    frms{8*i+4} = data(226:2:444,308:600,:);    %% Bot-right Off
%    frms{8*i+5} = data(003:2:221,008:300,:);    %% Top-left  Off
%    frms{8*i+6} = data(003:2:221,308:600,:);    %% Top-right Off
%    frms{8*i+7} = data(227:2:445,008:300,:);    %% Bot-left  On
%    frms{8*i+8} = data(227:2:445,308:600,:);    %% Bot-right On
% MODIFIED SEQUENCE
    frms{8*i+1} = data(002:2:218,008:300,:);    %% Top-left  On
    frms{8*i+2} = data(002:2:218,308:600,:);    %% Top-right On
    frms{8*i+3} = data(226:2:442,008:300,:);    %% Bot-left  Off
    frms{8*i+4} = data(226:2:442,308:600,:);    %% Bot-right Off
    frms{8*i+5} = data(001:2:217,008:300,:);    %% Top-left  Off
    frms{8*i+6} = data(001:2:217,308:600,:);    %% Top-right Off
    frms{8*i+7} = data(225:2:441,008:300,:);    %% Bot-left  On
    frms{8*i+8} = data(225:2:441,308:600,:);    %% Bot-right On
  end

else

  frms = cell(1,8*nmax);
  for i=1:nmax ,
    file = sprintf('%s%03d.tif',prfx,i);
    data = imread(file,'tif');
```

```
    size(data)
    frms{8*i-7} = data(002:2:218,008:300,:);    %% Top-left  On
    frms{8*i-6} = data(002:2:218,308:600,:);    %% Top-right On
    frms{8*i-5} = data(226:2:442,008:300,:);    %% Bot-left  Off
    frms{8*i-4} = data(226:2:442,308:600,:);    %% Bot-right Off
    frms{8*i-3} = data(001:2:217,008:300,:);    %% Top-left  Off
    frms{8*i-2} = data(001:2:217,308:600,:);    %% Top-right Off
    frms{8*i-1} = data(225:2:441,008:300,:);    %% Bot-left  On
    frms{8*i  } = data(225:2:441,308:600,:);    %% Bot-right On
  end
```

function tiffsave(frms)

```
% TIFFSAVE   Store data in a series of tiff files.
%
% Example:   tiffsave(frms)
%    This example stores data in the MATLAB variable frms into
%    a series of TIFF files such as frm000.tif, frm001.tif,
%    frm002.tif, etc.
for i=1:length(frms) ,
  imwrite(frms{i},sprintf('frm%03d.tif',i),'tiff','Compression','none');
end
```

function tiffshow(file)

```
%
if (ischar(file))
  data = imread(file,'tiff');
  imsz = size(data);
  im_x = [1:imsz(2)];
  im_y = [1:2:imsz(1)]*2;
  image(im_x,im_y,data);
  axis image;
else
  imsz = size(file);
  im_x = [1:imsz(2)];
  im_y = [1:2:imsz(1)]*2;
  image(im_x,im_y,file);
  axis image;
end
```

function tiffshow(file)

```
%
if (ischar(file))
  image(imread(file,'tiff'));
else
  image(file);
end
```

```
function  frms=tiffread(prfx,n)
% TIFFREAD   Extract data from a series of tiff fields (frm*.tif).
%
% Example:   frms=tiffread('frm',432)
%    This example extracts data from a series of 432 TIFF
%    fields (frm*.tif) into the MATLAB variable frms.
% This function is helpful if conversion is halted
%    after TIFFSAVE
frms = cell(1,n);
for i=1:n ,
  file = sprintf('%s%03d.tif',prfx,i);
  frms{i} = imread(file,'tif');
end
```

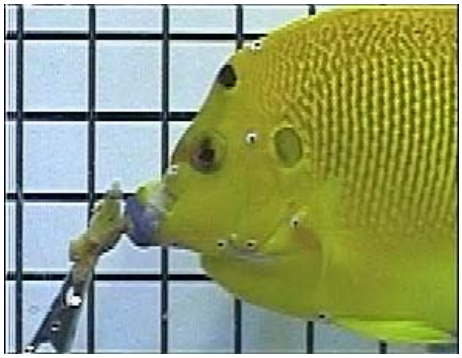
```
function  frms=tiffmovie(data)
%
if (iscell(data))
  imsz = size(data{1});
  im_x = [1:1:imsz(2)];
  im_y = [1:2:imsz(1)]*2;

  for i=1:length(data) ,
    image(im_x,im_y,data{i});
    axis image;    % Scale image.
    frms(i) = getframe;
  end
  movie(frms);
end
```

Please note: Attempts to remove a vertical skip in the resultant image stream were unsuccessful. This skip persists in commercial versions, i.e. DVtoAPAS (by Ariel Dynamics) and DVconvert (by BioMechanica). The use of origin-point tracking during motion-analyses allows computational removal of this artefact from kinematics data.

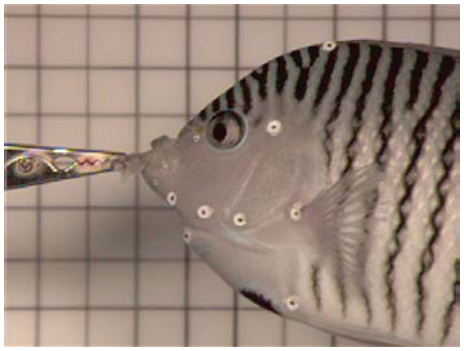
APPENDIX II

High-speed video sequences of feeding in representative pomacanthid study-taxa. Videos are 200 images per second, except *Genicanthus,* 400 images per second. Click thumbnails to play in Windows Media Player, QuickTime, Real Player or DivX. (CD-ROM provided).

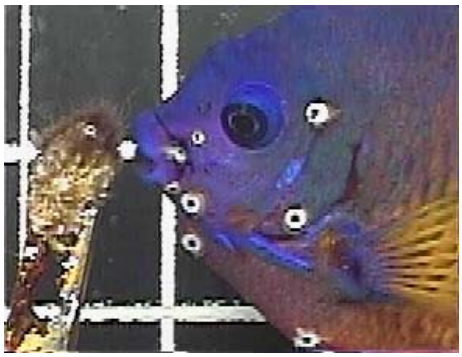
Centropyge [Centropyge] bicolor



Apolemichthys trimaculatus



Genicanthus melanospilos



Centropyge [Xiphypops] bispinosa

Pygoplites diacanthus

Chaetodontoplus duboulayi

Pomacanthus [Euxiphipops] sexstriatus

Pomacanthus [Arusetta] semicirculatus

APPENDIX III

Manuscripts arising from this thesis:

Chapter 2: **Konow, N. & Bellwood, D. R.** (2005). Prey-capture in *Pomacanthus semicirculatus* (Teleostei, Pomacanthidae): functional implications of intramandibular joints in marine angelfishes. *J. Exp. Biol.* 208:1421-1433.

Chapter 3: **Konow, N. & Bellwood, D. R.** (*submitted*). Ecomorphological disparity in the feeding apparatus of marine angelfishes. *The American Naturalist.*

Chapter 4: **Konow, N., Bellwood, D. R. & Wainwright, P. C.** (*in prep*). Intramandibular joints help reef fishes take diverse bites. *Proc. R.. Soc. Lond. Ser. B. Biology*

Manuscripts arising from thesis-related research:

Bellwood, D. R., van Herwerden, L. & Konow, N. (2004). Evolution and biogeography of marine angelfishes (Pisces: Pomacanthidae). *Mol. Phylogen. Evol. 33: 140-155*.

Konow, N., Fitzpatrick, R. & Barnett, A. (accepted). Adult Emperor angelfish (*Pomacanthus imperator*) clean Giant sunfishes (*Mola mola*) at Nusa Lembongan, Indonesia. *Coral Reefs*

APPENDIX IV

Publication arising from Chapter 2 of this thesis:

Konow, N. & Bellwood, D. R. (2005). Prey-capture in *Pomacanthus semicirculatus* (Teleostei, Pomacanthidae): functional implications of intramandibular joints in marine angelfishes. *J Exp Biol* 208:1421-1433.