Dietary ecology of terapontid grunters (Pisces: Terapontidae) with particular reference to ontogeny and phylogeny

PhD thesis submitted by

Aaron Marshall Davis

BSc, MAppSci, James Cook University

in August 2012

for the degree of Doctor of Philosophy
in the School of Marine and Tropical Biology
James Cook University

Statement on the contribution of others

Supervision was provided by Professor Richard Pearson (James Cook University) and Dr Brad Pusey (Griffith University). This thesis also includes some collaborative work. While undertaking this collaboration I was responsible for project conceptualisation, laboratory and data analysis and synthesis of results into a publishable format. Dr Peter Unmack provided the raw phylogenetic trees analysed in Chapters 6 and 7. Peter Unmack, Tim Jardine, David Morgan, Damien Burrows, Colton Perna, Melanie Blanchette and Dean Thorburn all provided a range of editorial advice, specimen provision, technical instruction and contributed to publications associated with this thesis. Greg Nelson-White, Pia Harkness and Adella Edwards helped compile maps.

The project was funded by Internal Research Allocation and Graduate Research Scheme grants from the School of Marine and Tropical Biology, James Cook University (JCU). In-kind field collection was also funded in part by the Australian Government's Natural Heritage Trust National Competitive Component and Land and Water Australia. In-kind support was also provided by TropWater (formerly Australian Centre for Tropical Freshwater Research) at JCU in the form of field equipment and sample collection.

Acknowledgments

I would initially like to thank my supervisors Brad Pusey and Richard Pearson for their invaluable guidance, patience and support over the course of this project. Your capacity for insights and fixes to seemingly intractable challenges was always much appreciated. I am also immensely grateful to David Morgan and Colton Perna for providing many of the fish specimens so integral to this study, as well as useful discussion. Several other collaborators on various thesis topics provided tremendous and prompt support, encouragement and advice: Melanie Blanchette for her work ethic, organizational ability and eye for detail in both the field and laboratory; Tim Jardine for his expertise and enthusiasm about all things stable isotope-related; and Peter Unmack, without whose phylogenetic contributions this thesis would undoubtedly have suffered considerably.

An array of people, including Damien Burrows, Michael Pusey, Mark Kennard, Dean Thorburn, Adam Kerezsy, Matthew Knott, Jason Shaffer, Ian Dixon, Mark Adams, Gerry Allen, Jon Armbruster, Michael Baltzly, Cindy Bessey, Joshua Brown, Chris Burridge, Stephen Caldwell, Adam Fletcher, David Galeotti, Chris Hallett, Michael Hammer, Jeff Johnson, Alfred Ko'ou, Andrew McDougall, Masaki Miya, Sue Morrison, Tim Page, Ikising Petasi, Ross Smith and the Hydrobiology team, and the staff from ERISS and Northern Territory Fisheries, are also all thanked for their efforts in helping to collect and/or providing specimens. Kent Hortle and Andrew Storey are thanked for provision of data and advice regarding Papuan species. Additional samples for genetic work were provided by the Australian, Northern Territory, Queensland, South Australian, Western Australian, and University of Kansas museums and by the Smithsonian National Museum of Natural History; thanks to their staff and donors for providing samples. I am grateful to Stephen Lewis, a great sounding board regarding manuscript submission, for his endless patience and effort in helping me compensate for my poor graphical abilities.

I owe a debt of gratitude to both the pioneering and current fish and evolutionary biologists whose work has been a constant source of inspiration, especially Richard Vari, whose seminal work on the terapontids was an invaluable resource throughout my thesis. Thanks are also warranted to the numerous journal editors, anonymous reviewers and thesis examiners who revised the various data chapters/manuscripts making up this thesis. Their advice and critiques

greatly improved the quality of my research, and provided an invaluable learning experience with regard to better analyzing, interpreting and presenting of my data.

Finally, I am forever indebted to my parents Steve and Kris at so many levels: for always encouraging and fostering an interest in the natural world; for their unwavering sacrifice and support towards my education; and for their invaluable help and company (as well as sustenance) in the field. Who would have thought all those childhood days spent in the backyard creek catching spangled perch (*L. unicolor*) with grasshoppers on bent pins would lead to this? Finally thank you so much to my own little family – Birony, Brooke, Sophie and Hayley. Thank you for the support, forbearance and comedic relief over many years, without which I could never have completed this work.

Abstract

Ecological processes, such as major habitat and dietary diversification, are considered to play a major role in the adaptive radiation of many vertebrates. While the Australian continent's long-term biogeographic isolation provides an ideal and relatively independent testing ground for associated hypotheses, ecological processes have received little attention in the context of the radiation of Australia's freshwater fishes. This thesis therefore examines the role of dietary habits, habitat affiliation and ontogeny in one of Australia's largest families of freshwater fishes, the terapontid grunters (Terapontidae), in the context of evolutionary theory.

Stomach-content analyses (SCA) of 22 north-Australian terapontid species identified distinct ontogenetic dietary shifts in all species for which sufficient data were available, with many species passing through several discrete trophic categories during their life histories. Carnivory was prevalent in juvenile terapontids, with diets dominated by aquatic insect larvae and microcrustacea, followed by divergence into a broad spectrum of feeding groups comprising carnivory (including piscivory and lepidophagy), omnivory (including frugivory and consumption of allochthonous prey), specialized herbivory and detritivory in larger size classes.

Stable isotope analyses (SIA) of fish tissues largely corroborated the size-related dietary shifts identified by SCA. The combination of SIA and SCA identified the important role of ontogenetic dietary shifts in the trophic ecology of terapontids in the Burdekin River. SIA was particularly useful in indicating that, for fish species with pronounced size-related dietary shifts, the basal carbon sources supporting those species can also change markedly with ontogeny. The ontogenetic dietary shifts revealed by both SIA and SCA were so profound that different size classes of certain species occupied different trophic as well as isotopic niches.

Body size, and its relationship to allometric development of several morphological features, appears to be a significant constraint dictating the trophic habits of many terapontids at different life-history stages. Preliminary analyses identified the role of allometric growth (both positive and negative allometry) – in characters such as intestinal length, maxilla length and mouth width – in driving considerable ontogenetic divergence in interspecific morphological trajectories. Despite these complex associations between body size and growth of morphological variables, multivariate analyses showed that morphology has a significant relationship to diet, both within and between terapontid species, explaining ~50% of dietary

variation in the 22 studied species and their constituent ontogenetic trophic units. Many of the diet-morphology relationships evident in the terapontids parallel those documented in other fish assemblages around the globe: intestinal length and sub-terminal mouth orientation positively correlated with detritivory and consumption of aquatic algae; intestinal length negatively correlated with carnivory; and conical tooth shape, maxilla length, mouth width, head length and eye diameter all positively correlated with piscivory and prey size.

The potential role of historical habitat transitions in the marked trophic diversification within the terapontids was investigated using a new molecular phylogeny (using mitochondrial and nuclear genes) incorporating 36 species. Ancestral habitat reconstruction indicated that ancestral terapontids were euryhaline, with a single transition to freshwater environments being ancestral to all contemporary Australasian freshwater species. Mapping of adult terapontid feeding modes on to the molecular phylogeny indicated that carnivorous dietary habits were displayed by ancestral terapontids, which subsequently diversified into a range of additional carnivorous, omnivorous, herbivorous and detritivorous diets following the invasion of fresh waters. The evolution of herbivorous-detritivorous diets – a rare evolutionary occurrence in most other fish lineages – is especially prevalent in Australia's freshwater terapontids, with plant and/or detrital material being predominant in around two-thirds of the species included in this study. Comparative analyses suggested that following the freshwater invasion, the single clade exhibited an increased rate of diversification, radiating at more than twice the background rate of the rest of the family. The marine-freshwater transition within the Terapontidae therefore appears to have resulted in much greater dietary radiation and lineage diversification in fresh waters than in euryhaline-marine habitats.

Ontogeny has also apparently played a major role in the evolutionary ecology and phylogenetic diversification of the Terapontidae, specifically in the role of intestinal length in dietary habits. Several different patterns of ontogenetic increase in intestinal length were evident in terapontid species, with increasing intestinal complexity during ontogeny driving this variability. Phylogenetically independent contrasts indicated that the interspecific differences in intestinal length resulting from these ontogenetic mechanisms explained ~60% of the variability in the proportion of plant-detrital material in terapontid diets. The ontogenetic development of intestinal complexity therefore appears to represent an important functional innovation driving the adaptive radiation of Australia's freshwater terapontids, facilitating the adoption of omnivorous, herbivorous and detritivorous diets.

Australia is notable for the lack of dietary diversification among its freshwater fishes. These results identify the terapontids as Australia's most trophically diverse fish family, with size-related dietary shifts a fundamental feature of individual species' dietary habits. Trophic studies of fish and food webs that ignore the possibility of size-related shifts in prey and basal sources are, therefore, simplistic and potentially flawed. This study has also demonstrated that selective pressures have driven terapontid morphology to converge with other ecologically comparable fishes across the globe. The capacity to modify intestinal morphology during ontogeny appears to have been an important facilitator of trophic diversification during the terapontid freshwater radiation. Australia's biogeographic history, specifically its lack of herbivorous-detritivorus primary freshwater fishes, may have provided the necessary 'ecological opportunity' for the adaptive radiation of the freshwater terapontids.

Contents

List of tables

Statistically significant allometric scaling relationship (i.e., where the 95% confidence interval for slope does not overlap with an isometric slope of 1.0) are highlighted in bold. n signifies the number of intestinal length measurements per species. _____ 195

List of figures

Publications arising from thesis

Publication arising from Chapter 2 of this thesis:

Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2011. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78**: 265-286.

Publication arising from Chapter 3 of this thesis:

Davis, A.M., Pearson, R.G. and Pusey, B.J. 2011. Contrasting intraspecific dietary shifts in two terapontid assemblages from Australia's wet-dry tropics. *Ecol. Freshw. Fish* **21**: 42-56.

Publication arising from Chapter 4 of this thesis:

Davis, A.M., Pusey, B.J. and Pearson, R.G. 2012. Trophic ecology of terapontid fishes (Pisces: Terapontidae): the role of morphology and ontogeny. *Mar. Freshwater Res.* **63**: 128-141.

Publication arising from Chapter 5 of this thesis:

Davis, A.M., Blanchette, M.L., Pusey, B.J., Pearson, R.G. and Jardine, T.D. (in press). Gut-content and stable-isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. *Freshwater Biol*.

Publication arising from Chapter 6 of this thesis:

Davis, A.M., Unmack, P.J., Pusey, B.J. and Pearson, R.G. 2012. Marine-freshwater transitions are associated with the evolution of dietary diversification in terapontid grunters (Teleostei: Terapontidae). *J. Evol. Biol.* **25**:1163-1179.

Publications arising from thesis-related research:

Davis, A.M. and Pusey, B.J. 2010. Trophic polymorphism and water clarity in northern Australian *Scortum* (Pisces: Terapontidae). *Ecol. Freshw. Fish* **19**: 638-643.

Davis, A.M., Pusey, B.J., Thorburn, D.C., Dowe, J.L., Morgan, D.L. and Burrows, D. 2010. Riparian contributions to the diet of terapontid grunters in wet-dry tropical rivers. *J. Fish Biol.* **76**: 862-879.

Davis, A.M. and Perna, C.N. 2009. Evidence in predation on terrestrial cane toads, *Bufo marinus*, by the sooty grunter, *Hephaestus fuliginosus*, in northern Australia. *Nthn Terr. Nat.* **21**: 45-48.

Chapter 1: General Introduction

1.1 Fish dietary diversity, ecomorphology, and ontogeny

Dietary diversity

A major goal in evolutionary biology is to understand the forces that generate and maintain biological diversity. Adaptive radiation – the diversification of species and associated adaptations in response to natural selection and ecological opportunity – is recognized as one of the most important processes responsible for the origin of biological diversity. The benchmark synthesis by Schluter (2000) on the phenomenon of adaptive radiation has prompted increasing appreciation that understanding of species' ecology is fundamental to understanding evolution. Careful study of the interface between ecology and evolution within and among populations has yielded a wealth of information about how natural selection and ecological divergence drives evolutionary diversification (Schluter, 2000; 2003). Current evolutionary theory coupled with numerous empirical studies indicate that, even if not prerequisites, adaptive radiations frequently rely on some form of 'ecological opportunity' (Schluter, 2000; Gavrilets and Vose, 2005; Losos, 2010). Ecological opportunity may result from processes such as extinction of competitors, diversification in resource base, acquisition of a key 'functional innovation' that allows the organism to use resources in a different or more effective manner, or from colonization of a new adaptive zone with underutilized niches (Schluter, 2000; Losos, 2010; Losos and Mahler 2010).

The Teleostei, with more than 24,000 currently recognized species, is the most diverse group of vertebrate animals, comprising almost half of all currently known vertebrates (Helfman *et al*., 1997; Lundberg *et al*., 2000). Teleost fish occupy virtually every available aquatic habitat on earth, and consume a wide diversity of food items from both aquatic and terrestrial ecosystems. They include some of the most well-documented and celebrated adaptive radiations, such as those of the African rift-lake cichlids and the northern hemisphere's three-spined sticklebacks, (Schluter, 1995; Barlow, 2000; Streelman and Danley, 2003). Despite the wide array of feeding modes amongst fishes, the development of certain trophic habits, such as herbivory and detritivory, have been infrequent evolutionary phenomena (Horn and Ojeda, 1999; Nelson, 2006; Lujan *et al*., 2011). While plant-based diets have a broad taxonomic distribution among

mammals (~25%)), the occurrence of herbivory is much more restricted (2-5% of species) amongst other vertebrate groups (Choat and Clements, 1998; Espinoza *et al*., 2004). The morphological and physiological specializations that facilitate access to the nutrients held within plant cells have accordingly attracted considerable interest from fish ecologists and evolutionary biologists (Horn, 1989; German, 2011; Lujan *et al*., 2011).

Ecomorphology

The broad interaction between fish form and function (particularly feeding ecology) is a subject that has also long intrigued fish biologists (e.g., Al-Hussaini, 1949). The field of ecomorphology, with its underlying tenet of ecology being related to morphology, provides a framework for addressing adaptation and the extent of morphological and ecological co-evolution (Karr and James, 1975; Motta *et al*., 1995b). Ecomorphology aims to integrate anatomical, ecological, behavioural and evolutionary studies to describe the interrelationship between the functional morphology of organisms and their environment (Wainwright and Richard, 1995). Behavioural, ecological, physiological, historical and ontogenetic factors can all interact to influence the strength of the linkage between morphological and ecological characters (Motta *et al*., 1995b). Nevertheless, some morphological characters have widely accepted and repeatedly demonstrated functional relevance to diet. For example, intestinal length is commonly correlated with degree of herbivory (Elliott and Bellwood, 2003); mouth gape tends to be strongly correlated with prey size and degree of piscivory (Wainwright and Richard, 1995; Mittelbach and Persson, 1998); relative orientation of the mouth indicates location of feeding (e.g., water surface, benthic) or of predator position in relation to prey (Gatz, 1979; Wikramanayake, 1990); and dentition typically provides a reasonable indication of diet, with conical holding teeth corresponding to carnivory, and flattened cutting teeth to herbivory (Gatz, 1979; Stoner and Livingston, 1984).

Ontogeny, ecology and phylogeny

The ecological importance of body size has long been recognised, with differences in body size a major mechanism by which various species avoid competition for resources (Werner and Gilliam, 1984), and in some situations it is an important reflection of community structure (Schoener, 1974). One of the most pervasive and influential concepts in community ecology is the guild structure of assemblages (Simberloff and Dayan, 1991). Guilds have always been regarded as consisting of “complete” species, with little recognition of possible ontogenetic changes in resource use by the guild members as suggested by the original defnition of a guild

(Root, 1967). In size-structured populations, it is common for individuals to exploit several niches sequentially in the course of their life history (Werner and Gilliam, 1984). The change during life history from one niche to another is referred to as an ontogenetic niche shift.

Ontogenetic diet shifts (size-related patterns of feeding) are a particularly common feature of fish ecology (e.g., Stoner and Livingston, 1984; Winemiller, 1989; Gill and Morgan, 2003). These changes arise partly because fishes have indeterminate growth that results in body sizes ranging over orders of magnitude within a species, and also because size is directly related to risk of predation and foraging ability (Werner, 1984; Ross, 1986). For some populations, ontogenetic resource changes can be so pronounced that the population can be divided into discrete size classes or stages, with each stage fulfilling a different role in the ecosystem (Osenberg *et al*., 1994). Ontogenetic resource shifts can greatly complicate study of community interactions such as competition and food web structure (Werner and Gilliam, 1984), and the usefulness of the taxonomic species concept in investigating guild structure of fishes can be accordingly undermined (Munoz and Ojeda, 1998).

Until recently, documentation of size-related dietary shifts in fish has been based on stomach content analysis, but increasingly, stable isotope analysis (SIA) – utilising the differential enrichment of naturally occurring stable isotope ratios (typically carbon and nitrogen) of tissues (DeNiro and Epstein, 1978; Petersen and Fry, 1987) – is being used to clarify ontogenetic dietary shifts in fishes. The majority of demonstrated isotopic shifts have been reported from simple, often plankton-driven marine or lacustrine food chains where size-structured feeding is expected to be pronounced (Post, 2002; Galván *et al*., 2010). In more trophically complex river ecosystems, with a greater diversity of food sources and weaker size-structuring, isotopic evidence of size-related diet shifts may be difficult to identify (Bunn *et al*., 1999; Jepsen and Winemiller, 2002; Douglas *et al.,* 2005). Isotopic studies related to size-based feeding are particularly rare for Australia's highly variable tropical freshwater ecosystems.

The potential implications of the role of ontogenetic phenomena in phyletic evolution of contemporary species have a long and controversial history (Haeckel, 1876). Understanding the interplay between selective forces, developmental pathways and morphological change in shaping phenotypic diversity remains a fundamental goal of evolutionary biology (Hall, 1998). Variations in the timing and rate of change of developmental events are considered among the most common mechanisms through which morphological change and novelties originate during

phyletic evolution (Gould, 1977; Alberch *et al*., 1979; Marroig and Cheverud, 2005). The evolution of fish diversity has provided fertile ground for biologists assessing the role of ontogeny in evolutionary modification. The phylogenetic relationships between the beloniform fish has long been a staple of the recapitulation versus paedomorphosis debate (Gould, 1977; Boughton *et al*., 1991; Lovejoy, 2000). Alterations of ontogenetic pathways in tooth attachment modes, patterns of skull morphology and intestinal looping patterns have been touted as the cause of the major evolutionary diversity across a range of other teleost fish lineages (Fink, 1981; Zihler, 1982; Yamaoka, 1985). These studies indicate that the relationships between developmental biology and phylogeny hold great potential in the study of the evolutionary biology of fishes. However, the interaction between ecological and developmental genetic mechanisms in the development of morphological and phenotypic variability remains unknown for the majority of traits and organisms.

The Comparative Method.

One of the issues presenting both constraints and opportunities to ecomorphological studies is the phylogenetic relatedness among species. Species sharing a common ancestor are non-independent, with phylogenetic proximity voiding the assumption of sample independence underpinning many conventional statistical tests; if overlooked, this issue may cause the relationship between ecology and morphology to be overstated (see Felsenstein, 1985). Most researchers now accept that studies of the evolution of ecological features should be framed within the context of a phylogeny (Douglas and Matthews, 1992; Motta and Kotrschal, 1992). Recently developed methods in phylogenetic systematics that integrate diverse data from the phylogenetic relationships, functional morphology, comparative anatomy and ecology of a number of related species can provide powerful phylogenetically informed hypothesis-testing capacity for questions of evolutionary biology (Harvey and Pagel, 1991). Comparative approaches utilising phylogenetic relationships have been utilized to great effect in illustrating the evolution of feeding biology in a number of fish groups (Wainwright and Lauder, 1992; Winterbottom and McLennan, 1993; Westneat, 1995; Correa *et al*., 2007).

1.2 The Australian freshwater fish fauna

The long-term biogeographic isolation resulting from the split of Australia and Antarctica from Gondwanaland approximately 100-110 MYA has produced several distinctive evolutionary

characteristics in the Australian freshwater fish fauna. They include a markedly depauperate fauna by global standards and an evolution largely independent of other continental assemblages (Coates, 1993; Lundberg *et al*., 2000; Unmack, 2001; Allen *et al*., 2002). Australia's freshwater fish fauna is particularly unusual for its prevalence of acanthopterygian fishes which typically dominate marine environments, and a corresponding lack of primary division ostariophysian fishes which usually dominate freshwater habitats (Williams and Allen, 1987; Allen *et al*., 2002). While there is a widespread presumption in the literature that many Australian freshwater fishes are derived from marine ancestors, this supposition is not always supported by biogeographic and phylogenetic evidence (Lundberg *et al*., 2000).

A major tenet of modern evolutionary biology is that colonization of a new habitat often opens up new ecological opportunities and thus promotes lineage diversification (Schluter, 2000; Gavrilets and Losos, 2009). While numerous successful examples are documented from terrestrial systems, particularly in oceanic archipelagos (Carson and Kaneshiro, 1976; Grant and Grant, 2008), the effects of marine-to-freshwater habitat shifts on lineage diversification have rarely been tested. Marine and fresh waters are very different ecosystems: in addition to a significant difference in salinity, locally adapted coinhabitants provide different resources and competitions (Lee and Bell, 1999). Transitions between marine and freshwater habitats in either direction are therefore a particularly interesting aspect of the evolutionary biology of many Australasian 'freshwater' fish groups, although the evolutionary implications of such major habitat shifts have received little research focus in an explicitly phylogenetic context.

In addition to Australia's low species diversity of freshwater fishes, the limited dietary variation in the Australian freshwater ichthyofauna contrasts with the marked trophic diversity even within individual families in some parts of the world. Australia's freshwater fishes are characterised by a predominance of carnivores (particularly aquatic invertivores) and omnivores (Coates, 1993; Kennard *et al*., 2003; Pusey *et al*., 2004; Douglas *et al*., 2005). The generalized or average diets of many Australian fish have been quite well documented, but understanding of ontogenetic and ecomorphological relationships is not well developed. Some studies have documented ontogenetic diet transitions (Gill and Morgan, 1998; 2003; King, 2005; Pusey *et al*., 2004), but such knowledge is lacking for the majority of species. Insights into ecomorphological associations between dietary ontogeny and the morphology of Australia's freshwater fish are even less developed. Some limited aspects of ecomorphological ontogeny in body size, mouth gape and associations with diet are addressed in a small number

of Australian studies (see Pusey *et al*., 1995; Pusey *et al*., 2000; King, 2005, Tibbetts and Carseldine, 2005), but the dearth of ecomorphological studies from the Australasian region has been long recognised as a significant information gap for the field (Norton *et al*., 1995).

1.3 Study organisms

The Terapontidae, commonly referred to as the terapon perches, trumpeters or grunters, has already been noted as novel trophic group in a continent more notable for a pronounced lack of fish dietary diversity (Kennard *et al*., 2003). The Terapontidae is a small family of 50-60 species that have a conservative percimorph body plan (Mees and Kailola, 1977). The distributional range of the family is Indo-Pacific, extending from the Red Sea and eastern African coast, east to Tonga, north to Japan and south to marine waters around southern Australia (Vari, 1978). A number of species are marine or estuarine (although several of these species freely enter fresh waters), but the majority of species are restricted to the fresh waters of Australia and New Guinea. The Terapontidae is also among Australia's most species rich freshwater fish families, ranking only behind the Gobiidae and Eleotridae in terms of diversity, and reaching its highest radiation at both species and generic levels in northern Australia (Vari, 1978; Allen *et al*., 2002).

Resolving the taxonomy of the terapontids, both within and above the family level, has proved a long-standing challenge (Cuvier and Valenciennes, 1829; Whitley, 1943; Mees and Kailola, 1978; Vari, 1978). The most comprehensive treatment of the family is that of Vari (1978), whose genus-level phylogeny, based predominantly on comparative morphology, provided a hypothesis for within-family relationships. Vari (1978) utilised 33 synapomorphies among a series of morphological, osteological and meristic characters in a Hennigan analysis to define the cladistic sequence of 15 genera within the family (Figure 1.1): (1) *Leiopotherapon*, (2) *Amniataba*, (3) *Lagusia*, (4) *Hannia*, (5) "*Terapon*" (differs from *Terapon* in the strict sense), (6) *Pelates*, (7) *Terapon*, (8) *Pelsartia*, (9) *Rhyncopelates*, (10) *Mesopristes*, (11) *Hephaestus*, (12) *Bidyanus*, (13) *Scortum*, (14) *Pingalla* and (15) *Syncomistes*. The new genus "*Terapon*" was provisionally recognized in this initial revision but was not described due to uncertainty surrounding its relationships. After some initial invalid nomenclature issues were recognized and resolved, Allen (1993) proposed the replacement generic name *Variichthys* for the two

currently identified species placed within the genus (Figure 1.1). The synonymized genus *Helotes* which was previously confused with species from the genus *Pelates* has also been recently recognized (Sun, 1991; Johnson, 1999; 2010).

Vari's (1978) character analysis revealed patterns in a number of the features used to differentiate the evolution of the family that are notable from the perspective of dietary ecology. He suggested that a sequence of four intestinal patterns occurs within the Terapontidae, beginning at the basal (plesiomorphic) condition of a simple two-loop intestinal pattern as an adult for genera 1 – 10. Genera 11 – 13 exhibit an intermediate pattern of six loops in adult stages. Juveniles of genera 11-13 exhibit the two-loop pattern seen in the adults of genera 1 – 10 before undergoing an ontogenetic elongation and folding to produce the more complex adult pattern. Vari (1978) noted that this pattern appeared to have been secondarily lost in a distinctive subunit of *Hephaestus* species including *H. adamsoni, H. trimaculatus, H. suavis* and *H. carbo*. The adult life stages of genera 14 – 15 (*Pingalla* and *Syncomistes*) purportedly undergo a further ontogenetic shift to produce a highly convoluted and elaborate intestinal pattern, with the final and most complex intestinal pattern unique (autoapomorphic) to *Syncomistes*.

Terapontid dentition and jaw structure follows a similar evolution across the family from the plesiomorphic condition of simple, non-depressible conical teeth (genera 1 - 11) through to highly complex, flattened, depressible teeth and laterally directed dentary seen in other more derived genera (*Scortum*, *Pingalla*, *Syncomiste*s). Vari (1978) speculated that these intestinal and dentitional changes might reflect evolution toward increased herbivory, although this hypothesis remains untested. The sparse dietary data available for the Australian species does support this contention (Bishop *et al*., 2001; Pusey *et al*., 2004), although the subject is yet to be addressed in a systematic or comprehensive manner.

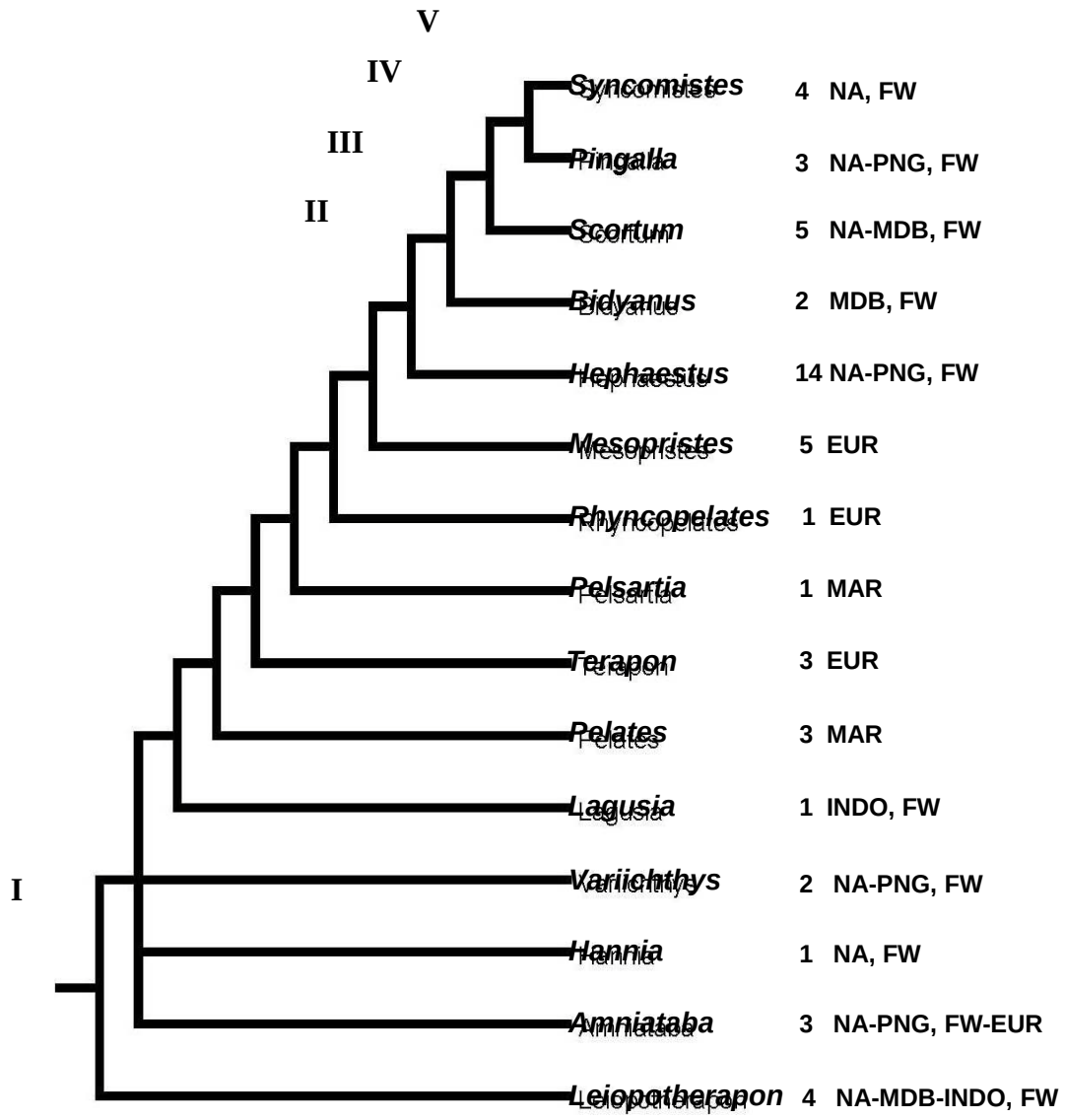

Figure 1.1 Cladogram depicting terapontid generic relationships derived from comparative morphology (adapted from Vari, 1978). For each genus, the number of species per genus recognised today, present distribution and habitat associations are indicated. NA, Northern Australia; MDB, Murray-Darling basin; PNG, Papua New Guinea; INDO, Indonesia. FW, exclusively freshwater; MAR, marine; and EUR, euryhaline. Node numbers: I, plesiomorphic condition of conical dentition and "s" shaped intestinal convolution; II, "six loop" intestinal configuration; III, depressible dentition; IV, moderately flattened dentition; V, highly complex intestinal configuration, highly flattened dentition and dentary modification. Note that *Amniataba, Hannia* and *Variichthys* form an unresolved trichotomy. Vari (1978) also identified two distinct sub-clades within the *Hephaestus* genus ("genus a" develops "6-loop" intestinal pattern; and "genus b" retains plesiomorphic "s-shaped" intestine).

The Vari (1978) phylogeny is uncorroborated by molecular approaches that are increasingly providing insights into patterns of evolutionary change beyond those possible with classical morphology-based approaches (Streelman *et al*., 2002; Lovejoy and Collette, 2001). Whether the Terapontidae has a marine or freshwater origin is uncertain, as is the case with several other prominent Australian freshwater fish families (Lundberg *et al*., 2000; Sparks and Smith, 2004). The dietary ecology of northern Australia's terapontids as a group has been discussed only summarily. Previous assessment of the degree of phylogenetic variation in diet of Australia's freshwater fishes has suggested that the terapontids rank with the Percichthyidae and Ariidae as the most trophically diverse of the Australian freshwater fish families (Kennard *et al*., 2001). However, while there is some indication of dietary diversification in Australia's terapontids, quantitative studies of the diets of many species and genera are largely absent.

1.4 Project aims and thesis outline

The Terapontidae represents one of the more intriguing components of the Australian freshwater fish fauna, and one that is amenable to comparative ecomorphological and evolutionary methods. The family, while relatively small, exhibits a range of natural diets (including apparently specialized detritivory and herbivory) as well as considerable variability in morphological characters related to feeding modes (dentition and intestinal complexity). A proposed (although not independently corroborated) phylogeny provides an ideal opportunity to frame questions regarding the evolution of feeding biology within the terapontid family in a historical phylogenetic context. Terapontids therefore provide an opportunity to test current theories regarding a range of ecological and evolutionary phenomena such as the role of size-related dietary shifts in trophic ecology, dietary-ecomorphological relationships, and the role of major habitat transitions on lineage radiation. With continental Australia relatively removed, both geographically and phylogenetically, from previously studied assemblages, its *tabula rasa* (blank slate) status provides a unique testing ground for studying the evolution of freshwater fish families.

The research objectives of this thesis are addressed in six data chapters, outlined below. These chapters are formatted in a publication manuscript style (see Appendix 4), and are followed by a concluding discussion chapter, which synthesizes the results of the data chapters.

Chapter 2 quantifies the diets of northern Australian terapontids, describing the extent of ontogenetic dietary shifts within the context of a broader classification of species' trophic diversity.

Chapter 3 examines the relationships between changes in body size and dietary resource utilization by terapontid assemblages in two catchments that exhibit contrasting flow regimes.

Chapter 4 describes the ontogeny of terapontid morphological characters and their associations with diet, investigating whether morphology can be used to predict the dietary habits of terapontid species throughout their life history.

Chapter 5 describes the diets of the terapontids inhabiting the Burdekin River and examines the congruence between stomach content analysis and stable isotope signatures (carbon and nitrogen) of terapontids.

Chapter 6 explores the phylogenetic evolution of terapontid habitat and trophic ecology using a new species-level molecular phylogeny, investigating the number and timing of marine-freshwater transitions, and the association between freshwater incursions and dietary diversification.

Chapter 7 examines the process of ontogenetic development of intestinal length in the Terapontidae within the context of molecular phylogenetic relationships.
Chapter 8 assesses the contribution of this thesis to our understanding of the ecology and evolution of the Australian freshwater Terapontidae, and to evolutionary theory.

Chapter 2: Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification

Published in the Journal of Fish Biology ***78****: 265-286 (2011)*

2.1 Introduction

Diversification in trophic ecology is regarded as one of the primary axes of vertebrate evolutionary radiation (Streelman and Danley, 2003), and most diverse taxa display substantial divergence in dietary habits (Greenwood, 1981). Trophic variation into a wide and occasionally novel array of feeding modes is regarded as central to the spectacular phylogenetic diversification of a number of major fish lineages such as the labrids, characiforms and cichlids (Fryer and Iles, 1972; Greenwood, 1981; Winemiller *et al*., 1995; Westneat and Alfaro, 2005; Correa *et al*., 2007). This marked trophic diversity contrasts with the limited dietary variation displayed by the Australian freshwater ichthyofauna, which is characterised by an abundance of carnivores (particularly aquatic invertivores) and omnivores (Merrick and Schmida, 1984; Kennard *et al*., 2001). Specialised dietary modes such as detritivory and herbivory, common feeding modes of fishes elsewhere (Knoppel, 1970; Lowe-McConnell, 1975; Choat and Clements, 1998; Matthews, 1998), are rare in Australia's fresh waters (Pusey *et al*., 2000).

Many studies of fish feeding habits, within both community and phylogenetic contexts, have neglected potential ontogenetic shifts in trophic ecology, instead focusing on the biological species as the functional entity of interest. Fish populations tend to be strongly size structured due to a combination of small hatching size and indeterminate growth, and resultant changes in body size that often span several orders of magnitude (Werner, 1986). Consequently, ontogenetic changes in diet are particularly pervasive amongst fishes in both marine (Stoner and Livingston, 1984; Clements and Choat, 1990; Muñoz and Ojeda, 1998) and freshwater environments (Werner and Gilliam, 1984; Winemiller, 1989; Gill and Morgan, 1998; Piet, 1998; Pusey *et al*., 1995, 2000; Rayner *et al*., 2009). An array of terminologies such as 'feeding stanzas' (Tyler, 1972), 'ontogenetic trophic units' (Stoner and Livingston, 1984), 'ontogenetic niches' (Werner and Gilliam, 1984) and 'ecological species' (Polis, 1984) have been proposed in recognition that the different size classes of many species use different resources. Failure to

adequately address ontogenetic dietary shifts has been demonstrated to mask important underlying dietary segregation in fish feeding guild classifications (Muñoz and Ojeda, 1998).

In a continent notable for low freshwater fish diversity and a lack of fish dietary variation, the Australian terapontid fishes represent an unusual group. The Terapontidae, commonly referred to as the terapon perches, trumpeters or grunters, is a small family (*ca*. 52 species recognised) exhibiting a conservative percomorph body plan (Mees and Kailola, 1977; Vari, 1978). The distribution of the family is Indo-Pacific, extending from the Red Sea and eastern African coast, east to Tonga, north to Japan and south to marine waters around southern Australia (Vari, 1978). Although a number of species are primarily marine or estuarine, most species are restricted to the freshwater environments of Australia and New Guinea. The Terapontidae is one of Australia's most diverse freshwater fish families at both species and generic levels, reaching its highest species richness in northern Australia (Allen *et al*., 2002). By virtue of their numerical and biomass dominance in many northern Australian aquatic ecosystems (see Pusey *et al.*, 2004), terapontids are likely to play influential roles in community trophodynamics. The dietary ecology of several northern Australian terapontid species has been described by a number of studies (Bishop *et al.* 2001; Morgan *et al.*, 2004; Pusey *et al*., 2004; Davis *et al.*, 2010). Collective data from a limited suite of species has indicated considerable trophic diversity within the family, including carnivory, omnivory, herbivory and detritivory. Similarly, previous assessment of the degree of phylogenetic variation in diet of Australia's freshwater fishes has suggested that the Terapontidae and the Percichthyidae rank as the most trophically diverse of the major Australian freshwater fish families (Kennard *et al*., 2001). The occurrence of substantial ontogenetic dietary shifts has also been documented as a prominent feature of the ecology of several terapontid species (Pusey *et al*., 2004; Davis *et al*., 2010), although the influence of body size on terapontid trophic habits has only been addressed occasionally.

The full extent of dietary diversification within Australia's terapontids is yet to be established, and the dietary ecology of northern Australia's terapontids as a group has been discussed only summarily. There is, in general, an absence of quantitative data concerning the diet of many terapontid species, and in some cases, entire genera. This chapter describes the diets of 21 of the approximately 24 terapontids known to occur across northern Australia, including the first trophic data for 11 species whose dietary habits were previously undescribed. Specific chapter aims involved: (1) determining the extent of ontogenetic dietary changes across northern Australia's terapontids; and (2) investigating the consequences of these ontogenetic shifts

within the context of a broader classification of the diversity of northern terapontid feeding habits.

2.2 Materials and Methods

2.2.1 Study area and specimen collection

Terapontids were collected between 2004 and 2009, during several projects examining the diversity and ecology of northern Australian riverine fishes (B. J. Pusey unpubl. data; D. L. Morgan unpubl. data). Thirty-seven catchments were sampled from north-eastern Queensland's wet-dry tropics through to the Pilbara region of Western Australia (Figure 2.1). Collection was undertaken in the dry season (primarily May to November) each year due to difficulties with access during the monsoonal wet season. Electro-fishing (boat mounted and backpack) and seine netting were the primary collection techniques, with efforts made to sample as wide a range of size classes of fish for each species as possible at all survey sites. Specimens were anaesthetised in clove oil and pithed immediately after capture prior to preservation/fixation in either 10% buffered formalin or absolute ethanol. Specimens greater than 100 mm standard length (SL) were injected with preservative using a hypodermic syringe, or the visceral cavity was opened by incision to ensure optimal fixation of viscera and gut contents. Species nomenclature follows that used in Allen *et al*. (2002).

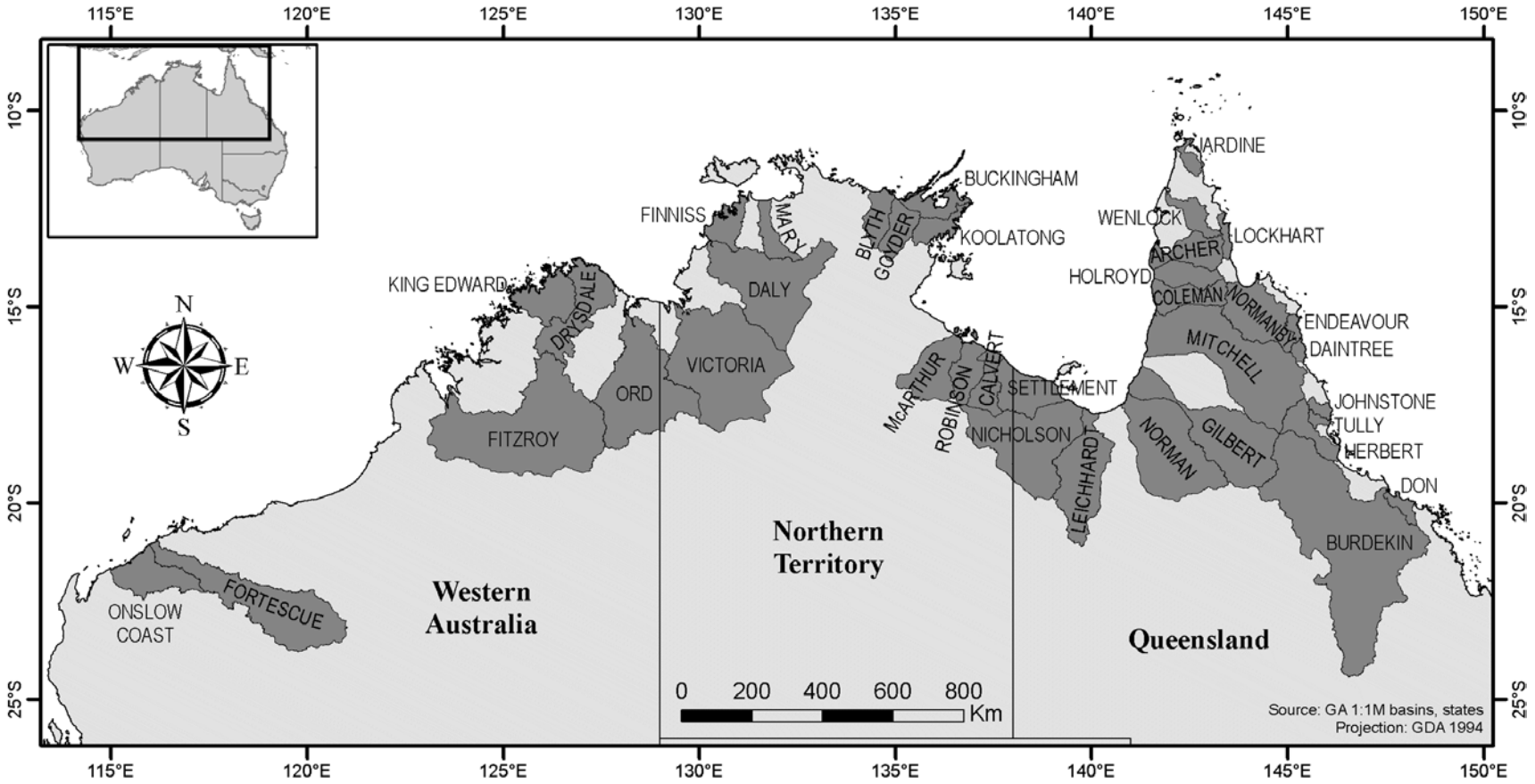

Figure 2.1 Map of northern Australia. Highlighted catchments indicate areas from which specimens were obtained. The catchment boundaries for the broader northern Australian drainage divisions defined by the Australian Water Resources Council (1976) are outlined in bold.

2.2.2 Quantification of diet

In the laboratory, standard length (SL) was measured for each specimen prior to excision of the viscera from the body cavity. Stomachs qualitatively assessed to be more than 20% full were transferred to a watch glass and the contribution of prey/food items was estimated by the indirect volumetric method of Hyslop (1980). Food items were identified to the lowest practical taxonomic level and grouped within 41 prey classes (Table 2.1).

Table 2.1 Dietary categories used in stomach content analysis. The fine category outlines dietary items used in ontogenetic dietary assessments; the broad category outlines pooled dietary items used in broader feeding group classification.

Fine category	Broad category
Chironomidae (larvae or pupa)	Aquatic Diptera larvae
Simuliidae	
Ceratopogonidae	
Diptera larvae (misc.)	
Ephemeroptera larvae	Ephemeroptera larvae
Trichoptera larvae	Trichoptera larvae
Odonata larvae	Odonata larvae
Lepidoptera larvae	Other aquatic invertebrates
Corixidae	
Notonectidae	
Naucoridae	
Aquatic Coleoptera (adults and larvae)	
Water surface invertebrates (Mesoveliidae, Gerridae)	Water surface invertebrates
Parastacidae	Macrocrustacea
Palaemonidae	
Atyidae	
Crustacea (misc.)	
Copepoda	Zooplankton
Cladocera	
Acarina	
Ostracoda	Ostracoda
Gastropoda, Mollusca	Gastropoda, Mollusca
Orthoptera	Terrestrial invertebrates
Formicidae	
Aerial-terrestrial invertebrates (misc.)	
Terrestrial vertebrates	Terrestrial vertebrates
Fish	Fish
Fish scales	Fish scales
Fish eggs	Eggs
Arthropoda eggs	
Inorganic fraction	Inorganic fraction
Detritus	Detritus
Biofilm-auwfuchs	
Porifera	
Filamentous algae	Filamentous algae
Aquatic macrophytes	Aquatic macrophytes
Terrestrial fruits	Terrestrial vegetation
Terrestrial seeds, flowers and leaves	
Other terrestrial plant parts (roots, bark etc.)	Misc. plant material
Unidentified Arthropoda fragments	Unidentified Arthropoda fragments
Unidentified fraction	Unidentified fraction

2.2.3 Data analysis – ontogenetic dietary variation

Dietary data from individual species with sufficient specimen numbers were grouped (averaged) into either sequential 10 or 20 mm SL size classes, depending upon the total size range and specimen numbers available for each species. The diet category 'Unidentified' was excluded from all analyses, with remaining dietary data arcsine square-root transformed, a transformation recommended for proportional data to improve normality (Sokal and Rohlf, 1995). Agglomerative hierarchical cluster analysis employing the Sorensen (Bray-Curtis) distance measure (Clarke, 1993; McCune and Grace, 2002) in combination with flexible-beta linkage (ß = -0.25) was applied to each individual species' dataset to group intraspecific size classes into distinct 'ontogenetic trophic units' (OTUs) (*sensu* Stoner and Livingston, 1984). Flexible-beta was the preferred linkage method due to its lower propensity for chaining compared to many other commonly used linkage techniques (McCune and Grace, 2002), a feature considered beneficial in an ontogenetic grouping assessment where dietary shifts are potentially gradual, rather than discrete.

Resultant dendrograms were pruned subjectively at a level that identified appropriate groupings of size classes. The validity of these groups (OTUs) within each species was then tested independently using multi-response permutation procedures (MRPP). MRPP is a non-parametric procedure for testing the hypothesis of no difference between two or more groups of entities, and is similar to ANOSIM in concept, but utilises a different test statistic: the probability of achieving the result (*P*) as well as a description of within-group homogeneity (*A*) is reported (Biondini *et al.*, 1985; McCune and Grace, 2002). Each individual fish in a species was assigned an *a priori* categorical group based upon the clustering outputs and previous dendrogram pruning. Pairwise MRPP (using Sorensen (Bray-Curtis) distance measures; n/sum(n) weighting) were then applied to provide a non-parametric multivariate test of differences at $P < 0.05$ between intra-specific trophic units (groups not involved in testing were simply excluded from analyses).

Considerable variation exists in the geographic distribution of the examined terapontid species. Several species have a very restricted range, limited in some cases to just one or two catchments, while others are among Australia's most widespread freshwater fish species (see Allen *et al.*, 2002). Datasets for three of the widespread species were subjected to additional analysis to provide some indication of the degree of spatial variation in diet. Individual species' datasets were divided according to distribution across northern Australia's broad drainage divisions

(Australian Water Resources Council, 1976) (see Figure 2.1). Species data within each drainage division was divided according to the OTU size intervals identified previously for each species. A dissimilarity matrix (Bray-Curtis distance measure) was generated comparing each species' OTU across drainage divisions. Resultant dissimilarity values for each pair-wise comparison between equivalent size class OTUs across different drainage divisions were averaged to provide a measure of spatial variability in diet between similar-sized fishes of the same species.

2.2.4 Data analysis – Determination of feeding group membership

The incorporation of ontogenetic dietary shifts into definition of broader terapontid feeding habits involved a combination of agglomerative clustering and ordination approaches. Average diets for each individual species' OTUs were calculated by pooling the dietary data from all individuals in the relevant size categories identified from cluster analysis. Each available species' OTUs, as well as mean diets from species with datasets too small to enable assessment of ontogenetic variation, were then collectively assessed to define broader dietary groups across the family. In order to reduce data noise in this broader classification scheme, the original 40 food categories used in individual species ontogenetic dietary assessments were reduced to 20 (Table 2.1). These pooled dietary categories were developed to highlight distinctiveness of food items and reflect differences in food origin (autochthonous versus allochthonous; benthos versus water column versus water surface) and size.

Hierarchical agglomerative clustering, as above, was used to classify the previously identified OTUs into similar feeding groups. Distinct groups were subjectively identified and differences between groups were tested by pairwise MRPP (as above). Given the underlying subjectivity inherent in any clustering algorithm (as well as the forced nature of clustering analyses *per se*), non-metric multidimensional scaling (NMS, Kruskal, 1964; Mather, 1976), based on the OTU by OTU similarity matrix as used above, was also performed. Preliminary NMS analyses in the PC-ORD 'autopilot' mode were used to identify the optimal number of axes for an ordination solution. A subsequent final ordination also included a Monte-Carlo simulation (100 runs of randomised data) to assess the probability of the final ordination configuration occurring by chance. All multivariate analyses (hierarchical clustering and ordination) were carried out in the PC-ORD® Ver. 5.01 software package (McCune and Mefford, 1999).

2.3 Results

The diets of a total of 3705 fish from 22 terapontid species were examined. Three species (*Terapon jarbua* (Forsskål), *Mesopristes argenteus* (Cuvier) and *Amniataba caudavittatus* (Richardson)) typically associate with estuarine-marine environments, but occasionally penetrate the lower freshwater reaches of many rivers and it is from such habitats that they were collected for this study. The remainder were strictly freshwater fishes.

2.3.1 Ontogenetic diet shifts

Discrete ontogenetic trophic units (OTUs) were identified for all 13 species that had sufficient sample size for ontogenetic analysis (Figure 2.2). The number of OTUs ranged from two to four feeding units per species. Dendrograms were scaled by Wishart's objective function (Wishart, 1969), converted to percentage of information retained (McCune and Grace, 2002). All of the separate ontogenetic trophic unit designations presented on each dendrogram were confirmed with subsequent MRPP analyses ($P < 0.05$), with the exception of *Pingalla gilberti* Whitley. Only a single specimen of *P. gilberti* less than 40 mm SL was collected, which negated the minimum group size required for MRPP analysis. The diet of this single juvenile was sufficiently different from that of other conspecifics, as well as consistent with trends observed in other species (i.e. invertivory in juveniles), that its diet was designated as a separate ontogenetic trophic unit.

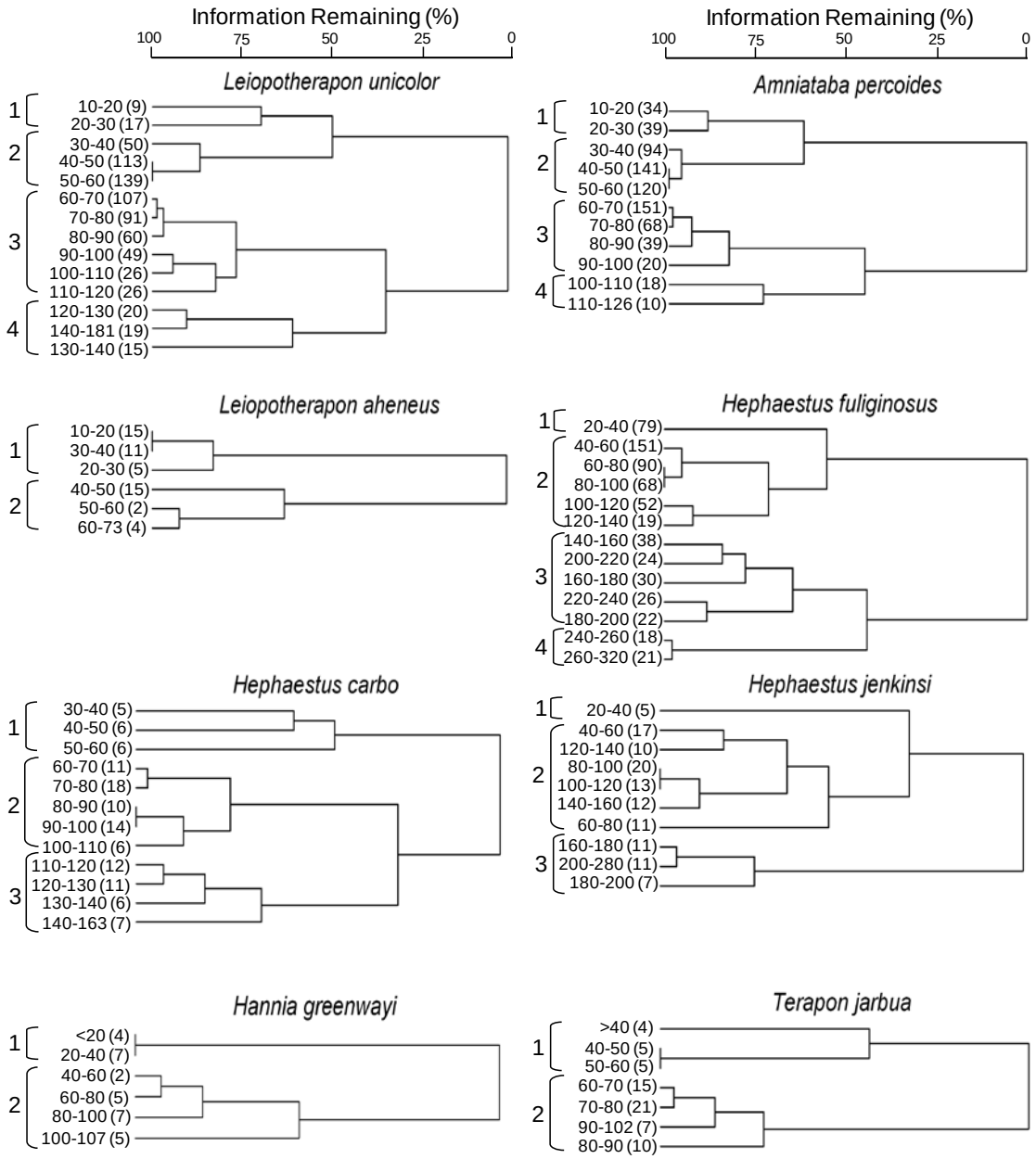

Figure 2.2 Classification of size classes of 13 terapontid species by diet, using the Bray-Curtis dissimilarity measure and flexible-beta linkage. Numbered groupings indicate sequential 'ontogenetic trophic units.' Numbers in brackets signify specimen numbers in each size class.

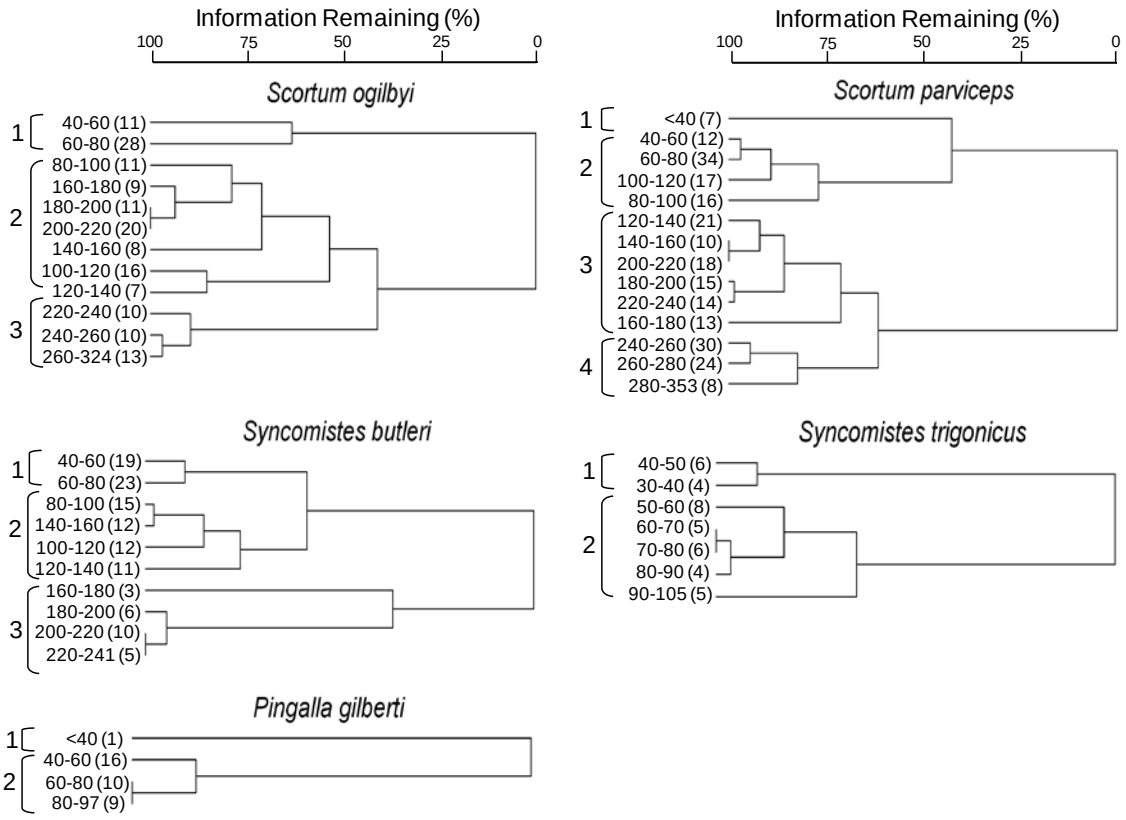

Figure 2.2 (cont.) Classification of size classes of 13 terapontid species by diet, using the Bray-Curtis dissimilarity measure and flexible-beta linkage. Numbered groupings indicate sequential 'ontogenetic trophic units.' Numbers in brackets signify specimen numbers in each size class.

The specific nature of ontogenetic dietary shifts varied substantially among species (Table 2.2). The diets of *Leiopotherapon unicolor* (Günther) and *Hephaestus carbo* (Ogilbyi and McCulloch) were dominated by shifts from aquatic invertebrates in smaller fish toward increasing consumption of fish, macrocrustacea and terrestrial-aerial invertebrates by larger size classes. The diets of *Amniataba percoides* (Günther), *Leiopotherapon aheneus* (Mees) and *Hannia greenwayi* Vari were marked by a transition from aquatic invertivory to omnivory, particularly consumption of filamentous algae. *Hephaestus fuliginosus* (Macleay) and *Hephaestus jenkinsi* (Whitley) demonstrated very similar ontogenetic dietary transitions away from aquatic invertebrates in smaller fishes to omnivory in larger fishes. Both species exhibited increasing consumption of aquatic plant material (filamentous algae and aquatic macrophytes), fish, macrocrustacea and allochthonous food, particularly terrestrial vegetation in larger size classes. *Scortum ogilbyi*

Whitley and *Scortum parviceps* (Macleay) changed from carnivorous-omnivorous diets in smaller size classes to diets almost entirely dominated by aquatic plant material (filamentous algae and aquatic macrophytes) in larger fish. The diets of *Syncomistes trigonicus* Vari and *P. gilberti* were marked by substantial increases in consumption of filamentous algae and detritus as size increased. *Syncomistes butleri* Vari underwent a similar shift from omnivorous dietary habits in smaller specimens to a diet dominated by detritus and filamentous algae in the largest fishes, but also included substantial consumption of encrusting sponges, accounting for ~20% of diet in the largest size class. The diet of *T. jarbua* involved increasing consumption of fish scales and terrestrial invertebrates (particularly orthopterans) with increasing size.

Sample sizes were insufficient for the assessment of dietary shifts in eight terapontid species. Diet for these species, averaged across all sizes, is outlined in Table 2.3. Size distributions in all eight species, as indicated by mean sizes, were biased towards larger specimens and diets accordingly reflected that of sub-adult and adult size classes.

Table 2.2 Volumetric dietary data for terapontid species' ontogenetic trophic units. Only dietary categories that totaled more than 5% within any individual species' ontogenetic trophic units are indicated.

	Terapon jarbua		***Amniataba percoides***				***Leiopotherapon unicolor***				***L. aheneus***		***Hephaestus fuliginosus***				***H. carbo***		
Ontogenetic Trophic Unit	**1**	**2**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**1**	**2**	**3**	**4**	**1**	**2**	**3**
Size range (mm)	**>60**	**60-102**	**>30**	**30-60**	**60-100**	**100-126**	**>30**	**30-60**	**60-120**	**120-181**	**>40**	**40-73**	**>40**	**40-140**	**140-240**	**240-320**	**30-60**	**60-110**	**110-163**
Broad dietary category																			
Diptera larvae	52.2	18.2	32.3	33.9	20.4	12.4	66.0	23.9	10.6	0.6	34.1	5.9	33.8	25.3	0.8	-	33.6	17.1	5.9
Ephemeroptera	1.7	14.1	11.6	17.1	9.5	6.8	16.0	19.8	7.7	0.2	18.3	5.5	18.5	8.7	1.1	0.1	16.0	9.3	5.9
Trichoptera			6.1	13.0	12.3	4.6	-	15.6	8.1	1.3	6.5	6.2	28.6	15.1	1.5	0.3	8.3	32.1	22.3
Odonata larvae			2.7	2.5	3.7	5.4	-	5.0	7.7	1.5	-	6.8					6.7	9.5	11.2
Other aquatic invertebrates			0.4	6.5	9.3	5.4	2.0	9.1	9.5	4.2			-	7.8	5.9	1.1	21.9	6.5	4.0
Surface Invertebrates																			
Macrocrustacea	13.3	12.3					-	3.2	19.2	23.0			-	7.5	13.7	9.9	1.7	13.1	15.7
Zooplankton			35.9	7.1	1.6	0.1	16.0	6.4	1.4	-	25.6	1.3	5.3	3.7	-	-	6.6	-	-
Ostracoda																			
Mollusca, Gastropoda																			
Terrestrial invertebrates	2.7	11.0					-	1.0	6.2	14.6			0.1	1.5	6.0	3.1	0.8	3.8	15.8
Fish	12.3	13.1					-	1.1	8.5	37.4			-	2.0	10.8	11.9	-	1.6	12.5
Fish scales	13.3	30.0																	
Eggs																			
Inorganic																			
Detritus											7.3	11.1	1.0	4.1	3.5	1.9			
Filamentous algae			0.3	8.8	24.2	15.1	-	6.1	9.5	4.8	2.4	56.9	0.5	15.2	15.9	3.7			
Aquatic macrophytes			-	0.2	1.7	35.2							-	0.5	7.0	22.0			
Terrestrial vegetation													0.1	1.0	26.8	38.4			
Misc. plant parts																			
Terrestrial vertebrates														0.2	4.5	6.5			

Table 2.2 (cont.)

	H. jenkinsi			*Scortum ogilbyi*			*S. parviceps*				*Syncomistes butleri*			*S. trigonicus*		*Hannia greenwayi*		*Pingalla gilberti*	
Ontogenetic Trophic Unit	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**1**	**2**	**1**	**2**	**1**	**2**
Size range (mm)	**>40**	**40-160**	**160-280**	**>80**	**80-220**	**220-324**	**>40**	**40-120**	**120-240**	**240-353**	**>80**	**80-160**	**160-241**	**>50**	**50-105**	**>40**	**40-107**	**>40**	**40-97**
Broad dietary category																			
Diptera larvae	44.3	9.6	2.5	9.4	1.4	0.1	58.8	39.4	2.6	1.7	38.9	12.6	0.2	18.0	1.0	35.4	13.6	72.0	15.2
Ephemeroptera	3.8	16.7	2.5	8.9	0.6	-								9.0	0.2	45.5	32.6	10.0	0.7
Trichoptera	6.3	15.5	2.6				28.0	0.1	1.4	0.2	6.2	0.6	-	9.1	1.0			10.0	0.5
Odonata larvae																-	6.1		
Other aquatic invertebrates	38.8	9.5	3.6	10.0	0.5	0.6										8.6	5.0		
Surface Invertebrates																			
Macrocrustacea	-	3.8	7.8					5.2	0.0	2.6									
Zooplankton	5.8	2.9	0.1	9.6	-	-	6.0	0.2	1.2	1.0				14.3	0.4	10.4	-	8.0	0.2
Ostracoda																			
Mollusca, Gastropoda	-	0.1	6.2																
Terrestrial inverts																			
Fish	-	4.9	6.8													-	9.3		
Fish scales																			
Eggs																			
Inorganic																		-	12.0
Detritus	-	2.1	8.0	7.1	18.0	7.1	0.5	4.0	9.2	12.8	32.2	47.0	41.0	32.3	67.0			-	34.0
Filamentous algae	-	15.4	12.2	38.4	56.4	52.0	-	42.0	53.7	50.0	12.2	31.4	46.6	13.1	26.0	-	19.7	-	36.5
Aquatic macrophytes	-	3.6	8.2	-	12.0	33.1		6.8	23.3	18.3									
Terrestrial vegetation	-	1.8	19.1					0.5	1.8	5.5									
Misc. plant parts	-	0.6	7.1								5.0	5.4	11.6						
Terrestrial vertebrates	-	-	5.3																

Table 2.3 Average diets for eight northern Australian terapontid species. The original 40 dietary categories used in dietary definition have been pooled and coded according to the broad dietary categories outlined in Table 2.1.

Species	***H. tulliensis***	***H. epirrhinos***	***P.midgleyi***	***S. rastellus***	***V. lacustris***	***P. lorentzi***	***M.argenteus***	***A. caudavittatus***
Size Range (mean)	**81-217mm (156.4)**	**193-275mm (223.7mm)**	**43-71mm (57.3)**	**68-165mm (115.2)**	**112-181mm (150mm)**	**48-116mm (65.6)**	**85-226mm (147.4mm)**	**67-105mm (80mm)**
Broad diet category								
Unid. Arthropoda fragments	2.2		3.3	0.5	1.5	2.7	0.8	2.5
Diptera larvae	1.9		5.3	1.7	5.2	9.8	0.3	38.2
Ephemeroptera larvae	0.9		0.7	1.7		4.8	7.4	
Trichoptera larvae	5.0	4.3	1.3	1.3		2.9	8.3	
Odonata larvae	1.3	5.0			1.3		1.8	
Other aquatic inverts.	2.9				19.7		3.1	
Terrestrial-aerial inverts.	1.1				12.4			1.7
Macrocrustacea	3.4	46.7				9.5	42.8	
Zooplankton	0.1		3.7		0.1	3.8		
Ostracoda	0.6							5.7
Mollusca/Gastropoda					5.2		10.4	13.5
Fish	3.6	24.3					6.2	9.8
Eggs	0.3			0.1			2.4	
Inorganic	0.7		29.3	6.3		2.9	2.7	0.7
Detritus	6.8		47.0	38.3		22.0	1.1	
Filamentous algae	48.4	19.7	6.3	47.7	18.8	30.8		25.5
Aquatic macrophytes	0.8				33.2	1.3		2.5
Terrestrial vegetation	20.1				0.8	0.8		
Misc. plant parts			3.0			8.8		

The average dissimilarity in diet of OTUs within the three widespread species (*L. unicolor*, *A. percoide*s and *H. fuliginosus*) was <30% for all three species (Table 2.4), indicating that the diets of similar-sized fish within each species were comparable, regardless of geographic location.

Table 2.4 Mean ± S.D. Bray-Curtis dissimilarity values for pairwise between-drainage-division dietary comparison of three terapontid species' OTUs.

Species	Average OTU diet dissimilarity (%)	Drainage divisions analysed
Amniataba percoides	25.3 ± 11.5	Timor Sea, Gulf of Carpentaria, North-East Coast
Leiopotherapon unicolor	25.4 ± 13.3	Timor Sea, Gulf of Carpentaria, North-East Coast
Hephaestus fuliginosus	27.2 ± 9.9	Timor Sea, Gulf of Carpentaria, North-East Coast

2.3.2 Terapontid feeding group classification

Hierarchical Clustering.

Seven broad trophic groups were identified from hierarchical clustering of species' ontogenetic trophic units (Table 2.2), with the average species diets outlined in Table 2.3 and Figure 2.3. The scale-eating habits of *T. jarbua* separated the two trophic units of this species from other species into their own lepidophagous carnivore group (Group 1). The larger size classes of four species (*L. unicolor*, *M. argenteus*, *H. carbo* and *Hephaestus epirrhinos* Vari and Hutchins) formed a macrophagous carnivore group (Group 2), the diet of which was dominated by larger, mobile aquatic animal prey such as fish and macrocrustaceans.

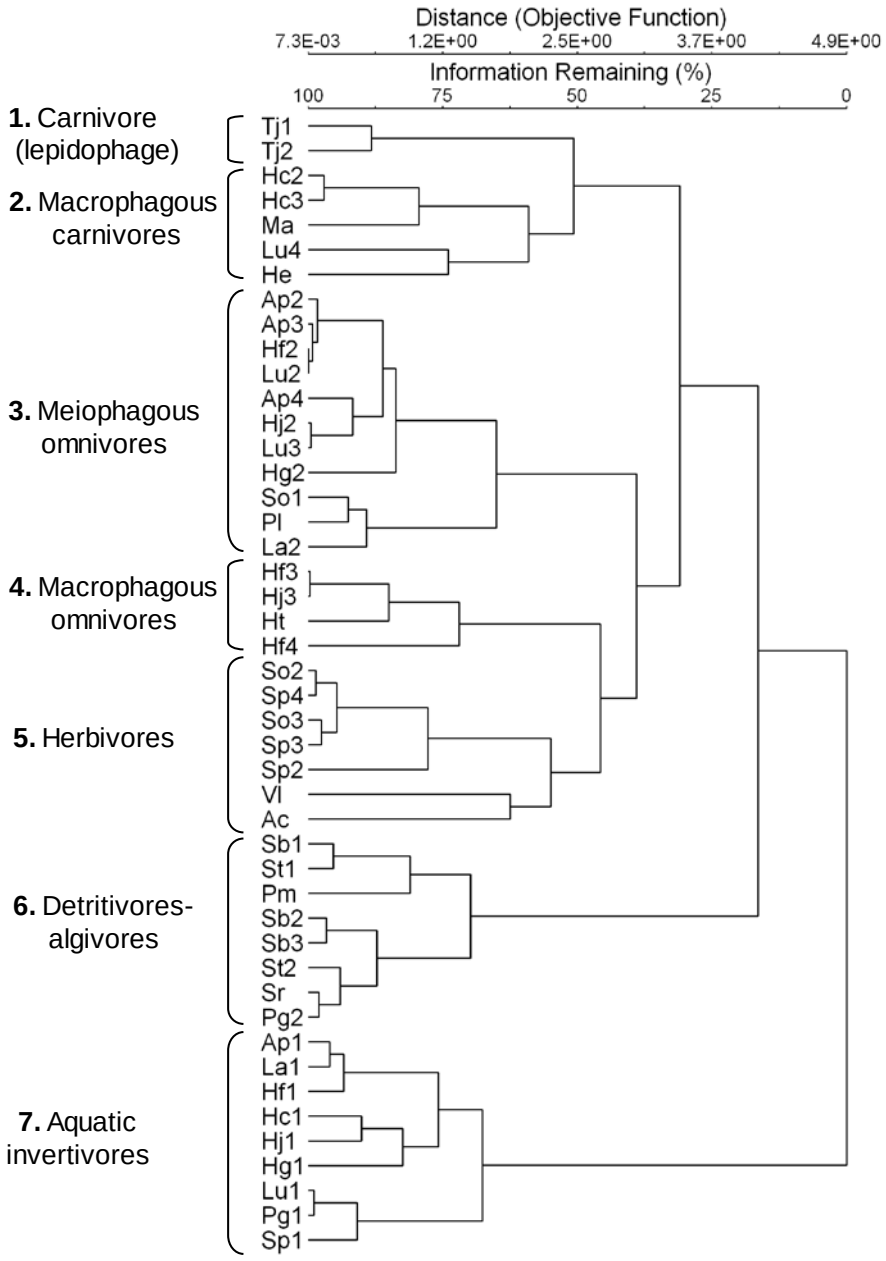

Figure 2.3 Classification of terapontid diets. Fish epithets are formed from initial letters in genus and species names; numeric suffixes refer to the sequential ontogenetic trophic units identified within individual species by previous hierarchical clustering procedures. Lack of a numeric identifier refers to average diets for species outlined in Table 2.2.

A meiophagous omnivore group (Group 3), characterised by diets of aquatic invertebrates (particularly insects) and plant material comprised the largest OTUs of several smaller-bodied species (*A. percoides*, *H. greenwayi*, *L. aheneus* and *Pingalla lorentzi* (Weber)), as well as intermediate size classes of a number of larger species (e.g., *H. fuliginosus*, *H. jenkinsi* and *L. unicolor*). There was considerable variation in the extent of herbivory in this group, as some species consumed only small amounts of plant material, while others such as *S. ogilbyi*, *P. lorentzi* and *L. aheneus* consumed significant amounts of plant material (primarily filamentous algae) (Tables 2.2 and 2.3).

The larger size classes of three *Hephaestus* species were all included in a macrophagous omnivore group (Group 4). The diet of this group was characterised by a high diversity of food types that included larger prey items such as fish, macrocrustacea, plant material (aquatic macrophytes), terrestrial-riparian vegetation (fruits, flowers, seeds) and, in the case of *H. fuliginosus* and *H. jenkinsi*, a range of terrestrial vertebrates such as small frogs, reptiles and birds. The larger size classes of the two *Scortum* species as well as *Variichthys lacustris* (Mees and Kailola) and *A. caudavittatus* formed a group of herbivores (Group 5). The diets of larger-size classes of the two *Scortum* species in particular were dominated by filamentous algae and aquatic macrophytes. While clustering suggested the diets of *V. lacustris* and *A. caudavittatus* aligned more closely with *Scortum* species than other fishes, the diets of these two species contained larger amounts of animal prey. Five species in the *Syncomistes* and *Pingalla* genera formed a group with diets characterised by consumption of detritus and filamentous algae (Group 6).

The most distinctive trophic grouping (Group 7) was composed of the smaller size classes of a wide range of terapontid species whose diets were very similar regardless of taxonomy. All OTUs in this feeding group were < 60 mm SL and had diets dominated by a limited suite of aquatic invertebrate taxa, namely dipteran larvae, ephemeropteran nymphs, trichopteran larvae and zooplankton (microcrustaceans and mites). All dietary groups identified from hierarchical clustering were shown to be significantly different by MRPP analyses across groups (Table 2.5).

Table 2.5 Significance values of pairwise MRPP comparisons of terapontid feeding groups identified from hierarchical cluster analysis. Probability values (*P*) are unshaded; within-group homogeneity values (*A*) are shaded.

Groups	1	2	3	4	5	6	7
1		<0.05	<0.01	<0.05	<0.01	<0.01	<0.01
2	0.164		<0.001	<0.01	<0.001	<0.001	<0.001
3	0.159	0.141		<0.001	<0.001	<0.001	<0.001
4	0.325	0.194	0.173		<0.01	<0.001	<0.001
5	0.248	0.26	0.157	0.162		<0.001	<0.001
6	0.287	0.322	0.214	0.289	0.2		<0.001
7	0.167	0.253	0.177	0.331	0.318	0.329	

NMS Ordination.

The final 3-dimensional analysis had a stress of 8.58, indicative of a 'good ordination' with no real risk of drawing false inferences (McCune and Grace, 2002), a final instability of 0.00009, and took 65 iterations for the final solution. The ordination was statistically significant, with a Monte-Carlo *P* value of <0.01. While a 3-dimensional solution was optimal, the majority of variance was explained by axis 3 (0.562 of variance) and axis 1 (0.274 of variance), with axis 2 representing 0.106 of variance (cumulative r^2 of 0.942).

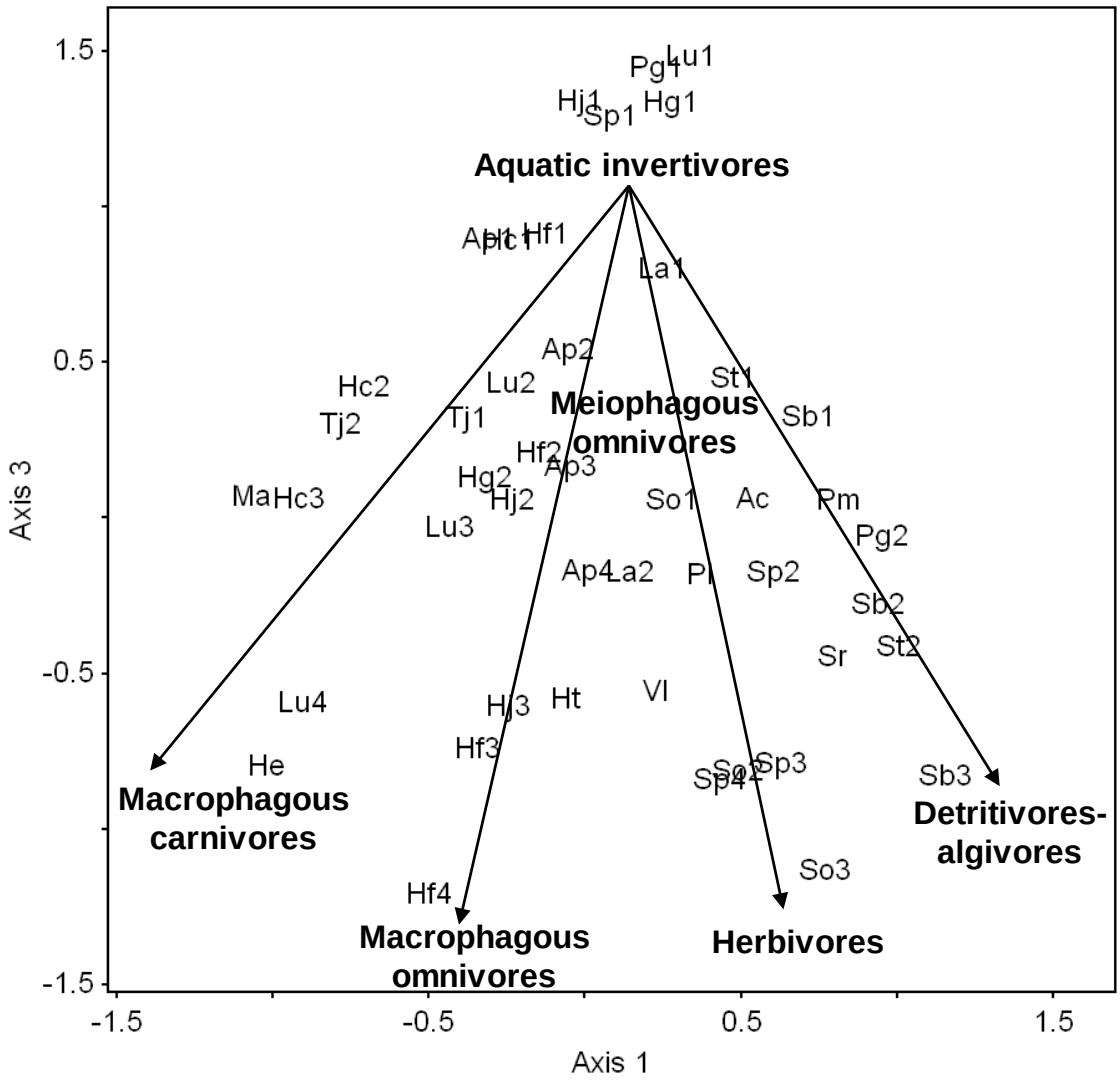

Figure 2.4 NMS ordination (axes 1 and 3) of terapontid feeding groups. Arrows represent general direction of ontogenetic dietary trajectories for different feeding classifications. Fish epithets relate to initial letters in genus and species names. Numeric suffixes refer to the ontogenetic trophic units identified within individual species by previous hierarchical clustering procedures. Lack of a numeric identifier refers to average species diets for species outlined in Table 2.3.

The ordination plot illustrates a range of clear and increasingly divergent shifts in dietary composition with increasing size in a wide range of terapontids (Figure 2.4). In a similar outcome to the clustering results, the diets of smaller size classes of many terapontids were very

similar before diverging with size along a number of distinctive ontogenetic trajectories. However, NMS ordination indicated that the diet of smaller size classes of *S. ogilbyi*, *S. trigonicus* and *S. butleri* was not as dissimilar to smaller size classes of other species as suggested by cluster analysis. The early OTUs of these species aligned relatively closely in ordination space to the highly carnivorous dietary habits displayed by other small terapontid size classes. Ordination outputs also showed that the diets of many species that ultimately assume diverse feeding habits in larger size classes pass through a similar intermingled omnivorous feeding group in intermediate size classes (equating to the meiophagous omnivore feeding group identified through hierarchical clustering).

Table 2.6 outlines the correlations between food items and distributions of ontogenetic trophic units in ordination space. Dietary items strongly correlated with axis 3 included aquatic dipteran larvae, ephemeropteran nymphs, zooplankton (positive) as well as miscellaneous plant material, aquatic macrophytes, terrestrial vegetation and filamentous algae (negative correlations). The dietary items that correlated most strongly with dispersion of species along axis 1 were detritus and filamentous algae (positive correlation) and macrocrustacea and fish (negatively correlated).

Table 2.6 Pearson and Kendall correlations with NMS ordination axes. Axes are ordered according to decreasing proportion of ordination variance explained. Dietary items are ordered according to descending strength of correlation value with axis 3. Only those items with a highly significant correlation are listed (critical value for *r* is 0.460, *P* <0.001).

	Axis 3	**Axis 1**	**Axis 2**
Diet category	***r***	***r***	***r***
Aquatic dipteran larvae	0.881		
Ephemeroptera	0.71		
Zooplankton	0.668		
Miscellaneous plant material	-0.468		
Terrestrial vegetation	-0.547		
Aquatic macrophytes	-0.585		
Filamentous algae	-0.706	0.57	
Detritus		0.711	-0.65
Terrestrial-aerial invertebrates		-0.53	0.619
Odonata larvae		-0.609	
Fish		-0.718	
Macrocrustacea		-0.803	
Inorganic			-0.576

2.4 Discussion

The diets of the smallest size classes of most terapontid species are very similar regardless of the eventual feeding habits displayed by larger size classes. Carnivory is prevalent in juveniles, with diets dominated by aquatic insects (particularly dipteran larvae) and microcrustacea. While a number of species in this study were identified as omnivores in the smallest collected size classes (*S. ogilbyi*, *S. trigonicus* and *S. butleri*), availability of juvenile specimens was limited. Collection of smaller individuals in these species would likely reveal higher levels of carnivory. The smallest *S. ogilbyi* collected in this study (<60 mm SL), for example, were essentially carnivorous, with aquatic invertebrate prey accounting for over 80% of average diet. The dietary ontogeny of fish can be influenced by interaction between external factors (e.g. predation risk, food supply, habitat changes) and internal ones (changes relating to digestive physiology, oral and intestinal morphology, ecological relationships with internal symbionts and feeding behaviour) (see Montgomery, 1977; Stoner and Livingston, 1984; Werner and Gilliam, 1984; Rimmer, 1986; Drewe *et al*., 2004). The general similarity in dietary habits of small terapontids is likely due to a number of these constraints linked to body size (mouth gape, digestive anatomy) and the requirement for readily available nitrogen needed for growth (see White, 1985).

Terapontid species that remained predominantly carnivorous throughout their life history nevertheless also exhibited ontogenetic dietary shifts. Distinctive transitions from invertebrate feeding in small juveniles to consumption of larger, more mobile and evasive prey such as fishes and macrocrustacea in sub-adult and adult size classes is a recurrent theme among many carnivorous fishes (Winemiller, 1989; Muñoz and Ojeda, 1998; Mittelbach and Persson, 1998). Keast (1985) referred to species that assume piscivorous habits late in life history as ‘secondary piscivores’, as opposed to ‘specialist piscivores’ which begin feeding on fish soon after birth. While piscivory is clearly an important aspect of the dietary ecology of several species, this definition of later-onset piscivory is appropriate to the adoption of piscivorous habits in most terapontids. With the exception of the specialised lepidophagous carnivore *T. jarbua*, substantial piscivory apparently does not emerge until relatively late in the life history of terapontids. Even in the most piscivorous fish examined here (*L. unicolor*), significant predation on other fishes was not evident until fish were relatively large (>120 mm SL and approximately two-three years of age according to Pusey *et al*., 2004). Previous research has documented significant variation in levels of piscivory by *L. unicolor* (see Pusey *et al*., 2004),

as well as an important community structuring role in some northern Australian ecosystems through predation on other species (Kennard, 1995). The results of this study suggest that ontogeny plays a substantial role in mediating observed levels of piscivory by this widespread and often highly abundant species and may therefore influence associated ecosystem functions also. The role of any size-related ecological constraints such as prey handling capacity (Mittelbach and Persson, 1998) or predation-risk–habitat-use associations (Mittelbach, 1981) on expression of piscivory in terapontids is yet to be investigated.

More pronounced ontogenetic dietary change involving transition from an essentially carnivorous habit to omnivorous, herbivorous or detritivorous habits was detected for many species. That most herbivorous fish begin life as carnivores or omnivores before adopting herbivory later in life has been long established (Montgomery, 1977; Rimmer, 1987; Drewe *et al*., 2004; Tibbetts and Carseldine, 2005). Several terapontids thus conform to the dietary patterns displayed by ecologically comparable fish in other families, displaying shifts from carnivory in juveniles, through omnivory in intermediate size classes, to various forms of herbivory or detritivory in large sizes.

Northern Australia's terapontids have clearly assumed a variety of feeding and dietary strategies, with considerable trophic diversification evident in larger size classes of many species. The dietary diversity displayed by these terapontids is likely comparable to that displayed by the entire continental Australian freshwater fish fauna (see Kennard *et al*., 2001). Particularly noteworthy is the prevalence of herbivorous-detritivorous dietary habits within the northern Australian terapontids. A number of other Australian fish families such as the Clupeidae (herrings), Mugilidae (mullets), Melanotaeniidae (rainbowfishes) and Plotosidae (eel-tailed catfishes) have freshwater representatives that utilize substantial amounts of plant material (see Pusey *et al*., 2004), but these species are probably more correctly defined as omnivores or detritivores. Genuine Australian freshwater herbivores are likely restricted to a single euryhaline species within the Hemiramphidae (garfishes) (Tibbetts and Carseldine, 2005) and the Terapontidae. It is within the terapontids that herbivorous dietary habits are expressed most frequently and to the greatest degree out of all of Australia's freshwater fishes. Whatever pre-adaptations to herbivorous-detritivorous lifestyles were possessed by earlier terapontids, they have allowed the family to exploit a number of ecological niches that other Australian freshwater fishes have either been excluded from, or have simply failed to utilise significantly.

As well as the significant dietary diversification (by Australian standards) exhibited by terapontids, several species exhibit feeding habits that are unusual at a global scale. Although consumption of terrestrial vegetation (fruit, flowers, foliage, seeds) has been documented in over 30 families of freshwater fish (Correa *et al*., 2007), it still represents an unusual dietary mode for fishes. The importance of terrestrial vegetation to the diet of Australia's freshwater fishes has been previously regarded as inconsequential (Kennard *et al*., 2001, Douglas *et al*., 2005). This study, as well as other recent research (Rayner *et al*., 2009; Davis *et al*., 2010) has, however, highlighted the significant frugivorous habits in larger size classes of a number of northern Australia's large-bodied *Hephaestus* species. The significant consumption of Porifera (sponges) evident in the diet of *S. butleri* is another novel dietary inclusion. Sponges typically possess a number of deterrents to predation such as sharp spicules, a fibrous collagenous structure and chemical defenses. While spongivory is rare in fish, it has been reported in a number of freshwater and marine families such as the Atherinidae, Cichlidae, Pomacentridae and Sparidae (Randall and Hartman, 1968; Wulff, 1997; Barlow, 2000; Allen *et al*., 2005). What nutritional benefit the otherwise exclusively herbivorous-detritivorous larger size classes of *S. butleri* may derive from freshwater sponge consumption is currently unknown, but opportunistic feeding on sponges has been posited as a solution to the problem of meeting dietary nitrogen requirements in marine herbivores (Wulff, 1997). Scale-eating (lepidophagy) is regarded as a derived, highly specialised dietary habit (Sazima, 1983). The lepidophagous habits, along with ontogenetic shifts towards increasing scale-eating, and an associated suite of behavioural and morphological feeding adaptations to this specialised feeding mode have been documented previously in *T. jarbua* (Whitfield and Blaber, 1978).

Despite the existence of these specialised feeding habits, this data chapter has revealed that the development of highly specialised feeding strategies, where consumption is limited to just one or two dietary items, is mostly absent. Versatility in feeding habits is clearly a feature of dietary ecology of most terapontids. Aquatic invertivory and/or some degree of omnivory typify the dietary habits of the majority of species. Few terapontids, with the possible exception of some *Syncomistes* or *Scortum* species, demonstrate highly canalised feeding strategies, and most possess some capacity to forage on a diversity of prey.

The degree of dietary diversification by northern Australia's terapontids raises questions from several evolutionary perspectives. The trophic spectrum documented in this study is interesting given the relatively small number of fishes (10 genera, 21 species) compared to other

phylogenetically and ecologically diverse fish groups such as the cichlids or labrids which comprise hundreds or thousands of species. Also of interest is that the substantial trophic variation evident amongst the terapontids appears to have been achieved within the constraints of a conservative percomorph body plan, with modest variation in overall body form. Adoption of diverse dietary modes has occurred without the marked divergence in body size, osteology (skull form, mouth shape, pharyngeal morphology) and body shape seen in many other fish families that demonstrate significant trophic variation (see Westneat and Alfaro, 2005). Similarly, the spectacular diversification of feeding modes seen within families of the Labroidei (including the closely related Cichlidae and Labridae) is widely accepted as occurring due to the evolutionary advantage of a unique pharyngeal jaw apparatus (Liem, 1973; Rice and Lobel, 2003). Functional novelties that may have facilitated the trophic radiation of the Terapontidae are yet to be described.

Several of the species examined here have a very restricted range, limited in some cases to just one or two catchments in northern Australia (e.g. *L. aheneus*, *H. greenwayi*, *Scortum parviceps*, *Syncomistes rastellus* Vari and Hutchins, *V. lacustris*, *H. epirrhinos*, *Pingalla midgleyi* Allen and Merrick and *P. lorentzi*). The potential for broad spatial variability in diet for these species would accordingly be limited. A number of other species, particularly *L. unicolor* and *A. percoides*, have very broad distributions across much of Australia (see Allen *et al*., 2002). That the diets for equivalent-sized fish of these species were on average much more similar than not, regardless of geography, suggests the identified ontogenetic shifts are a consistent feature of dietary ecology, even in these widespread fishes.

This data chapter further emphasises the dietary diversity of Australian terapontids, with no other Australian freshwater fish family exhibiting a comparable trophic spectrum, particularly with regard to adoption of herbivorous-detritivorous feeding modes. The dietary variation exhibited by other trophically diverse Australian families such as the Percichthyidae is essentially restricted to carnivorous dietary modes (see Kennard *et al*., 2001; Pusey *et al*., 2004). The terapontids can be regarded as constituting an Australian freshwater counterpart, albeit on a much smaller scale, to the well-known cichlid and characiform families that characterise the ichthyofauna of South America and Africa. The Terapontidae have undergone substantial speciation (at least from an Australian perspective) in the freshwater environments of northern Australia. Current evolutionary paradigms regarding diversification of vertebrate lineages emphasise trophic divergence as being a central component of many phylogenetic

radiations (see Streelman and Danley, 2003). Assessment of the relationship between the phylogenetic and ecological radiation occurring in an evolutionarily distant Australian freshwater fish family such as the terapontids will be an interesting test of current models (see Chapter 6 and 7).

Chapter 3: Contrasting intraspecific dietary shifts in two terapontid assemblages from Australia's wet-dry tropics.

*Published in Ecology of Freshwater Fish **21**: 42-56 (2011).*

3.1 Introduction

Studies from tropical environments demonstrate little agreement on the trophic organization of fish assemblages, particularly in relation to seasonal hydrology. Several studies have documented low temporal variability in community trophic structure (Rayner *et al.*, 2009; Pusey *et al.*, 2010), whereas others have identified higher dietary specialization and lower dietary overlap during high-water periods as a feature of neotropical fish assemblages (Lowe-McConnell, 1964; Goulding, 1980). A contrasting tendency toward more pronounced trophic resource differentiation under dry-season or drought conditions has also been found (Zaret and Rand, 1971; Winemiller, 1989; Jepsen *et al.*, 1997). A potentially confounding factor is the role of evolutionary history: much apparent differentiation in dietary habits can be attributed to family-level evolutionary differences in body form or behavior, rather than an active process of resource partitioning to reduce competitive interaction (Rayner *et al.*, 2009; Pusey *et al.*, 2010). The co-existence of closely related and morphologically similar species can therefore provide substantial insights into the role of differential resource use in assemblage trophic structure.

Another factor that complicates the study of the intra- and interspecific feeding interactions in many aquatic food webs is the strong size-structuring evident in fish populations. The body sizes of conspecific individuals in some species can span several orders of magnitude (Werner, 1986), with concomitant changes in feeding and habitat use occurring throughout life histories. In some cases these shifts are so profound that an individual species can assume several functional ecological roles during its life span. The concept of the 'ontogenetic niche' (*sensu* Werner and Gilliam, 1984) is accordingly well-established in fish ecology (Chapter 2; Stoner and Livingston, 1984; Post, 2003; Davis *et al.*, 2011b).

Wet-dry tropical rivers account for a considerable proportion of global river regimes (Latrubesse *et al.*, 2005), but have received little ecological attention in comparison to tropical and temperate systems. This situation is particularly prevalent in Australia, where studies on wet-dry tropical rivers are relatively sparse (although see Bishop *et al.*, 2001; Pusey *et al.*,

2000). Australia's wet-dry monsoonal rivers account for almost 40% of total continental discharge (Lake, 1971; Bishop and Forbes, 1991), as well as supporting a large proportion of Australia's freshwater fish diversity (Allen *et al.,* 2002). Australia's wet-dry tropical rivers are characterized by highly seasonal flows, sharing a climatic-hydrological regime with tropical savanna environments across much of the world (Haines *et al.,* 1988).

There has been growing advocacy for aquatic ecology to move beyond assessment of localized within–catchment processes to adopt an increased spatio-temporal or 'macro-ecological' domain of investigation (Hugueny *et al.,* 2010). The basis of this approach is comparative 'natural experiment' studies where a limited number of potentially important attributes differentiate communities. Recent eco-hydrological classification of continental flow regimes (Kennard *et al.,* 2010) provides a useful starting point for more focused investigations of relationships between flow regime and ecology in an Australian context. Several distinctive flow regime classes have been documented across the tropics (Kennard *et al.,* 2010). Here I examine the functional organization of two northern assemblages of Australian Terapontidae (a family of generalized perciform fishes) inhabiting different catchments from both intra- and interspecific perspectives. The two catchments share many bio-climatic and catchment land-use similarities, but exhibit contrasting long-term flow regimes. I aimed to determine 1) the relationships between changes in body size and dietary resource utilization in the two assemblages; 2) whether the patterns of resource use are consistent between systems; and 3) whether there is any evidence of seasonal effects on resource use.

3.2 Materials and Methods

3.2.1 Study area and specimen collection

Both study catchments were located within northern Australia's wet-dry tropics. The region has a sub-humid to humid tropical (monsoonal) climate, characterized by pronounced seasonality in rainfall patterns and discharge regimes. Highest river flows typically occur from December to April (the wet season) with lowest flows occurring between August and October.

Burdekin River

The Burdekin River catchment is the fifth largest in Australia (130,000 km²), with a sub-humid monsoonal climate, located in the wet-dry tropics of north-eastern Australia (Figure 3.1). Regional vegetation is dominated by sclerophylous *Eucalyptus* and *Acacia* woodlands, with the predominant catchment land use being low-intensity cattle grazing. The riparian zone is dominated by *Melaleuca* and occasional open vine thickets (Pearson 1991). More than 80% of annual rainfall occurs during the summer wet season between November and April. Average annual rainfall through much of the study area is 750 mm/year, although rainfall in some upper catchment reaches in the wet-tropics bioregion can be substantially higher (Rogers *et al.*, 1999). The flow regime of the Burdekin River is amongst the most variable in the world for rivers of comparable size (Puckridge *et al.*, 1998).

The upper Burdekin River system is low gradient, with a significantly under-fit channel characterized by steep banks, and minimal off-channel, floodplain habitat. Despite its large size, the Burdekin River has a very low diversity of instream habitats. The river is largely characterized by long shallow reaches dominated by a sand and fine gravel substratum (Pearson, 1991). Flow regimes in the Upper Burdekin catchment are classified as 'unpredictable intermittent' (Kennard *et al.*, 2010). This flow regime class was notable for extreme levels of both intra- and inter-annual flow variability and variable timing of maximum flows. Periods of no flow occur in approximately 9% of years of data record (Gauging Station 120002C- Burdekin River at Sellheim, 1947-2005), although permanent long, shallow pools persist along the river.

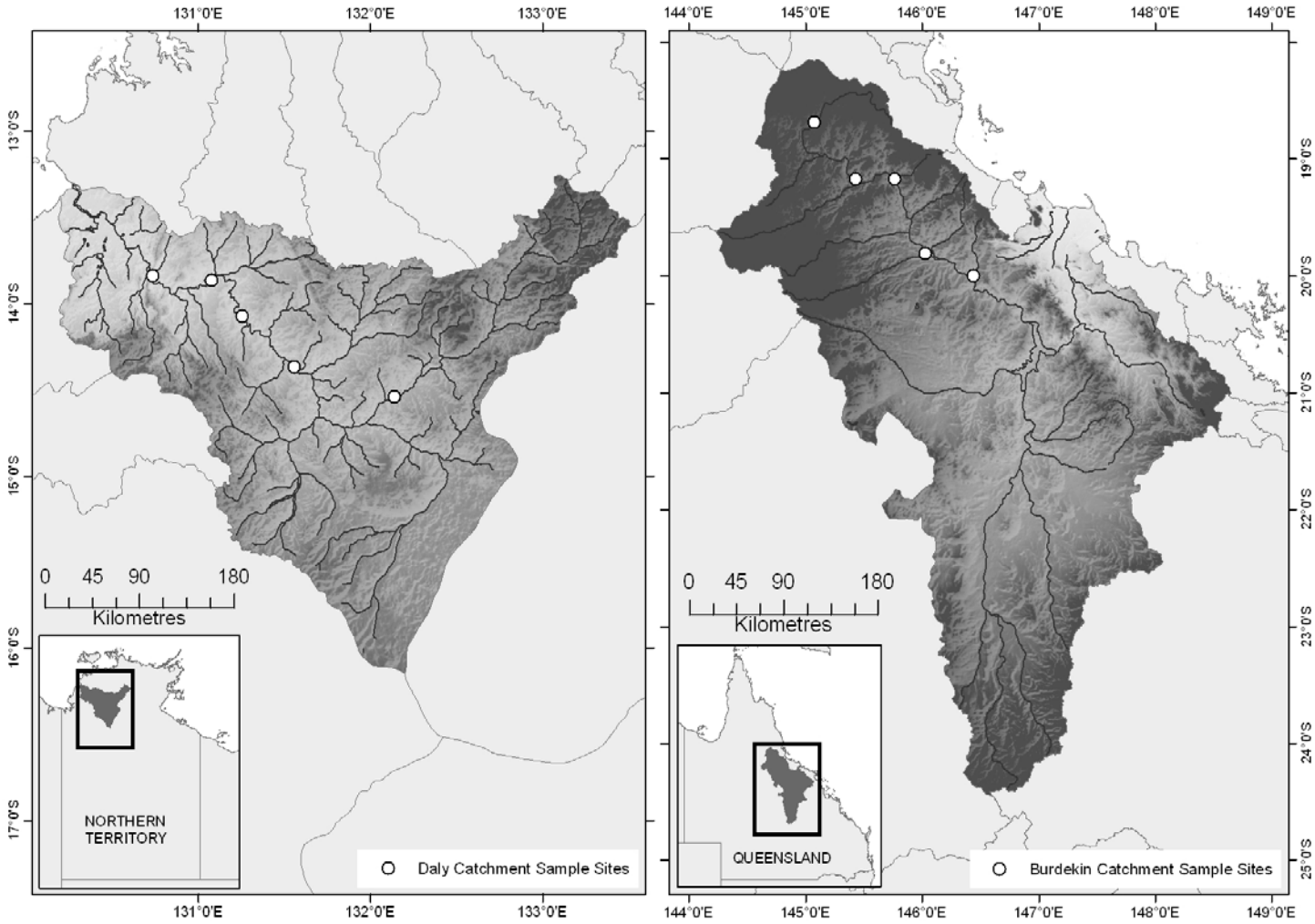

Figure 3.1 Location of the two study catchments and sampling locations within each river system (left, Daly River; right, Burdekin River).

Daly River

The Daly River catchment is 52,600 km², located in the Northern Territory (Figure 3.1). Catchment vegetation is dominated by *Eucalyptus* savanna woodlands and open forest, with the predominant land use being low-intensity cattle grazing. More than 90% of the Daly catchment's approximate 1000 mm annual rainfall occurs during the summer wet season between November and March (Jolly, 2001). Dry-season rainfall (May – October) is negligible, with long periods of slowly declining flows. While approximately a quarter of global tropical savanna rivers regularly cease to flow (Dodds, 1997), the Daly River is perennial, with dry-season baseflow maintained by groundwater input from extensive limestone and dolomite formations in the middle catchment. The long-term flow regime in perennial reaches of the Daly River is classified as 'stable summer baseflow' (Kennard *et al.*, 2010). While a strong seasonal run-off signal is evident in the Daly (majority of run-off in summer), discharge is typically very stable within and among years (low variability in daily and annual flows). The river is typically 40-60m in width, with steep banks ranging from 10-20m in height, rising in a series of terraces (Faulks, 1998). Lower terraces are vegetated by strips of *Melaleuca* trees and lower terraces are often dominated by dense closed forest communities, with many trees typical of monsoon closed forests (Lamontagne *et al.*, 2005). River substratum in the studied middle reaches is consistently dominated by coarse sand and fine gravel (Townsend and Padovan, 2005).

Fish Assemblages

The Terapontidae (grunters) are among the most diverse families occurring in the Burdekin and Daly River catchments, and amongst the most numeric and biomass dominant fishes in many habitats across both systems (Pusey *et al.*, 1998; Pusey B.J. *unpublished data*). The two catchments share very similar terapontid species assemblages in a taxonomic or functional sense. Three of Australia's more widespread fish species, spangled perch *Leiopotherapon unicolor* (Günther), barred grunter *Amniataba percoides* (Günther) and sooty grunter *Hephaestus fuliginosus* (Macleay) are common to both catchments. The small-headed grunter *Scortum parviceps* (Macleay) is an endemic herbivore widespread across the Burdekin catchment, whereas the closely related algivore-detritivore Butler's grunter *Syncomistes butleri* Vari is found in the Daly catchment.

3.2.2 Quantification of diet

Fish assemblages were collected at five sites on the main channel of the upper Burdekin River over two sampling periods (1989-1992 and 2005-2008). These were the 'upstream' Burdekin River sites outlined in Pusey *et al.* (1998). Fish were collected using a combination of backpack electrofishing, gill netting and beach seine netting (see Pusey *et al.*, 1998 for a full description). The three sampling methods sampled different macrohabitats within each sample reach (i.e. electrofishing: riffles and glides with in-stream cover; seine netting: open run and glide habitats; gill-netting: deepwater pools). Sampling was conducted in May-June and October-November of each year.

In the Daly River, fish were collected at five main channel perennial sites on five separate occasions. Seasonal timing of collection in the Daly catchment was similar to that of the Burdekin, commencing in June 2006 and ending in July 2008, with regular June-July and September-October sample occasions occurring over that time period. Fish were collected via backpack and boat electrofishing, a combination which covered all major macrohabitats within a sample reach.

Standard length (SL) was measured for each specimen prior to excision of the stomach and viscera from the body cavity. Stomachs that were estimated to be more than 20% full were transferred to a watch glass and the contribution of each prey/food item was estimated by the indirect volumetric method of Hyslop (1980). Near-empty stomachs were excluded to prevent bias in the calculation of prey diversity or proportional contribution (Pusey *et al.*, 1995). All stomachs that were >20% full were treated similarly in subsequent analyses. No association between diet composition and stomach fullness was therefore assumed, which may impart some error into analyses, although effects were expected to be minimal. Food items were identified to the lowest practical taxonomic level and grouped within the following 22 food classes: 1, unidentified fraction (UI); 2, unidentified Arthropoda fragments (UAF); 3, Chironomidae larva or pupa (Chi); 4, Ephemeroptera larva (Eph); 5, Trichoptera larva (Tri); 6, Simuliidae larva (Sim); 7, Odonata larva (Odo); 8, water surface invertebrates (Gerridae/Mesoveliidae/Veliidae: SIn); 9, other aquatic invertebrates (OAI); 10, terrestrial/aerial invertebrates (Hymenoptera; Odonata adults: TIn); 11, Palaemonidae (Pal); 12, Atyidae (Aty); 13, Zooplankton (Copepoda; Cladocera; Acarina: Zoo); 14, Ostracoda (Ost); 15, Mollusca-Gastropoda (Mol); 16, fish (Fis);

17, detritus (Det); 18, filamentous algae (FA); 19, aquatic macrophytes (AM); 20 terrestrial vegetation (TVg); 21, miscellaneous plant parts (MPP); and 22, terrestrial vertebrates (TVt).

3.2.3 Data analysis – ontogenetic dietary variation

Fish were allocated to one of four length classes, corresponding to standard lengths of < 40 mm, 40-80 mm, 80-160 mm and > 160 mm. These size classes correspond closely to the species' ontogenetic trophic unit (OTU) intervals identified in Chapter 2 and Davis *et al.* (2011b). The 'unidentified' diet category was excluded from all analyses. Dietary data for any subsequent analyses was square-root transformed (Platell and Potter, 2001).

3.2.4 Data analysis – Spatial and temporal variation in diet

While the focus of this study was a comparison of assemblage resource partitioning between two separate catchments, initial analyses were conducted to document both within-catchment (between-site) and temporal (between-years) variation in diets, with the intent to justify pooling of data in subsequent analyses. To explore between-site variability in diet, mean volumetric dietary data for each species' OTU size interval at each site within a drainage division was calculated. A Bray-Curtis similarity matrix was generated to compare all site-by-species OTU comparisons within each catchment. Resultant similarity values for each pair-wise comparison between equivalent size class OTUs across different sites within a catchment were averaged to provide a measure of spatial variability in diet between similar-sized fishes of the same species within the same catchment. To quantify temporal variability in diets between years, average dietary data for all species' OTUs were separated according to year and season (early dry and late dry) in each catchment. A Bray-Curtis similarity matrix was generated comparing all season-by-species OTU comparisons within each catchment between years. Resultant similarity values for each pair-wise comparison between equivalent size class OTUs across the same season between years within each catchment were averaged to provide a measure of yearly variability in diet between similar-sized fishes of the same species during the same season. The Bray-Curtis similarity measure ranges between 0 - 100, representing zero to total similarity in diet composition respectively. Although there are no critical levels with which similarity values

can be compared, this study considered a Bray-Curtis similarity value higher than 60 to indicate no biologically significant difference in dietary habits (Grossman, 1986).

Average (±S.E) OTU Bray-Curtis diet similarity between sites was 64.1 (±2.48) in the Upper Burdekin and 63.5 (±2.2) in the Daly, indicating that there was little within-catchment spatial variation in diet among similar-sized fishes of the same species in either catchment. Average (±S.E) OTU Bray-Curtis diet similarity between equivalent OTUs during the same season, but compared between different years, was 70.1 (±2.9) in the Upper Burdekin and 73.5 (±3.4) in the Daly. These results indicate that there was minimal spatial (between-site) or between-year variation in average diet among similar-sized fishes of the same species and in the same season in either catchment. It was therefore assumed that pooling data between sites and across years in each catchment would not confound subsequent analyses.

3.2.5 Data analysis – Multivariate Analyses

Non-metric multidimensional scaling ordination (NMDS: Kruskal, 1964) was carried out to provide an unconstrained visual representation of terapontid community trophic structure in ordination space. The non-parametric nature of this analysis circumvents the underlying distributional assumptions associated with many other ordination methods and is widely regarded as the preferred technique when underlying ecological relationships are unknown (Clarke, 1993). Samples collected from each catchment in May-July were pooled to represent the early dry season; similarly, all September-November samples from each catchment were pooled to represent a late dry season (see Winemiller, 2003; Pusey *et al.*, 2010). Seasonal datasets from each catchment were then separated into the designated OTU size classes. The analysis was conducted on a combined OTU by OTU similarity matrix for both catchments across both seasons, constructed using the Bray-Curtis dissimilarity coefficient. To augment the NMDS ordination, the relative dispersion of species' diets in each catchment on the ordination plot was quantified using multivariate dispersion indices from the MVDISP routine in PRIMER (Somerfield and Clarke, 1997). Multivariate dispersion (spread of variance in diet similarities) can provide an indication of divergence in niche space (i.e., decreased dietary overlap) within communities. All multivariate analysis of dietary data was conducted using PRIMER® v.7 software (Clarke and Gorley, 2006).

The collective trophic guild structure of the two catchment assemblages, as well as evidence for seasonal guild switching, was investigated via hierarchical cluster analysis, employing the Bray-Curtis similarity matrix and group average linkage. The similarity profile analysis routine in PRIMER (SIMPROF, with a $P < 0.01$ and number of permutations = 999) was applied to clustering outputs to objectively identify valid clusters. SIMPROF performs permutation tests at every node of a completed dendrogram, to identify statistically significant clusters in samples that are *a priori* unstructured (Clarke and Gorley, 2006; Clarke *et al.,* 2008). The SIMPER (similarity of percentages) sub-routine was used to determine dietary categories characteristic of each identified guild. SIMPER decomposes average Bray-Curtis similarities between all pairs of samples in groups (or between groups of samples), into percentage contributions from each dietary item (Clarke, 1993).

3.2.6 Data analysis – Dietary overlap

The comparative level of dietary overlap evident between catchment assemblages, as well as within catchments between seasons, was compared using the Bray-Curtis similarity matrix. Bray-Curtis similarity values range from 0% (no shared species) to 100% (all prey species are shared and consumed in the same proportion), and the index can be used to provide a measure of dietary overlap (Marshall and Elliott, 1997). Prey groups that are mutually absent from two diets (double zeros in proportion) are excluded from the similarity calculation as joint absences may not indicate a common negative feeding preference (Legendre and Legendre, 1998).

To contrast the average levels of resource overlap occurring between catchment assemblages, all Bray-Curtis pairwise similarity values over both seasons within each catchment were summed and compared using an unequal variance *t*-test (see Ruxton, 2006) (SPSS version 16.0, SPSS Inc., Chicago, IL). Seasonal differences in the average intensity of resource overlap occurring within each catchment were compared similarly by means of an unequal variance *t*-test of the average Bray-Curtis diet similarity from all pairwise OTU x OTU comparisons in the early dry season compared to the late dry season. The intensity of overlap was assessed against the following levels: high (>60), intermediate (40 – 60) or low (<40), following Grossman (1986) and Ross (1986).

3.2.7 Data analysis – Niche breadth

Levin's standardised measure of niche breadth, B_A (Hulbert, 1978), was used to compare the levels of dietary specialization between each catchment's collective OTU assemblage, and to assess seasonal differences in niche breadth within catchments, as follows:

$B_L = 1/(\sum p_i^2)$ and $B_A = (B_L - 1)/(n - 1)$

Where: B_L = Levin's measure of niche breadth; p_i = proportional contribution of resource i to the total diet ($\sum p_i = 1.0$); B_A = Levin's standardised niche breadth; n = number of possible resource (diet) categories. It is useful to standardize B_L to a scale of 0 (minimum niche breadth and maximum specialisation) to 1 (maximum niche breadth and minimum specialisation) to allow comparisons among species (Krebs, 1999). The significance of any difference in the average levels of dietary specialization (niche breadth) evident across both catchments (both seasons combined) was tested using a two-tailed, unequal variance *t*-test. Seasonal differences in the average niche breadth occurring within catchment assemblages were tested in the same manner. All niche breadth values were calculated on the average species OTU diets outlined in Tables 3.1 and 3.2.

3.3 Results

Stomach contents were identified for 1170 fish from the Burdekin catchment and 850 fish from the Daly River. Four food items (filamentous algae, chironomid larvae, trichopteran larvae and ephemeropteran nymphs) accounted for almost 70% of total diet in Burdekin terapontids when averaged across all species-OTUs (Table 3.1). These four items similarly accounted for a substantial proportion of average diet (45%) across Daly River terapontid OTUs, with detritus (10.3%) also constituting an important food category in the Daly River (Table 3.2). The unidentified dietary component was relatively minor for species-OTUs in both catchment, with a maximum of 3.3% for any Daly River size class and a maximum for 5.2% for any Burdekin River species-OTU in any season.

The Bray-Curtis similarity value for comparison of average assemblage diets (Table 3.1 and 3.2) was 80.65, highlighting considerable similarity in average OTU diet between catchments. It was therefore assumed that within-catchment variability was unlikely to exert any confounding effects on between-catchment comparisons of resource partitioning.

3.3.1 NMDS ordination

Comparison of diet trajectories in ordination space indicated pronounced size-related dietary shifts in species from the Daly River, regardless of season (Figure 3.2). The diets of small juveniles in the Daly (<40 mm SL) all grouped closely, suggesting considerable similarity in diet. Size-related diet shifts, while evident in Burdekin River terapontids, were more constrained. This interpretation was supported by the outcomes of the MVDISP test, with the multivariate dispersion value for the entire Daly River assemblage (1.102) being substantially greater than that of the Burdekin (0.871).

Table 3.1 Mean contribution (%) and standard deviation of prey items in the average diet of Burdekin River terapontid OTUs according to season. ED refers to early dry season, LD refers to late dry season. Abbreviations for dietary items are listed in the text, N is the number of stomachs analysed per OTU.

Species	Size class - season	Diet	Chi	Eph	Tri	Sim	Odo	Sln	OAl	Tln	Pal	Aty	Zoo	Ost	Mol	Fis	Det	FA	AM	TVg	MPP	TVt	N
A.percoides	<40 ED	Av.	20.2	19.3	14.8	6.2	0.9	-	-	1.8	-	-	2.8	-	0.5	-	2.3	27.3	-	-	-	-	13
		S.D.	35.7	36.2	25.6	20.7	3.0	-	-	6.0	-	-	6.6	-	-	-	4.1	43.1	-	-	-	-	
	40-80 ED	Av.	30.2	27.9	9.9	2.0	1.4	0.1	2.9	1.9	-	-	0.1	0.5	0.2	0.1	0.2	14.8	-	0.2	-	-	54
		S.D.	36.1	34.9	19.7	13.7	6.1	3.7	7.7	8.9	-	-	0.7	2.0	-	0.9	1.0	26.6	-	1.3		-	
	>80 ED	Av.	26.9	21.3	4.0	4.9	-	-	-	2.3	-	-	-	1.4	-	-	-	37.7	-	-	-	-	17
		S.D.	36.0	37.5	9.9	19.4	-	-	-	8.8	-	-	-	4.3	-	-	-	42.3	-	-	-	-	
	<40 LD	Av.	16.4	48.6	10.7	-	-	-	0.7	-	-	-	20.7	-	-	-	-	-	-	-	-	-	9
		S.D.	22.9	50.1	28.3	-	-	-	1.9	-	-	-	38.8	-	-	-	-	-	-	-	-	-	
	40-80 LD	Av.	29.3	28.4	16.8	4.5	0.1	0.1	1.9	0.4	-	-	0.7	0.2	0.3	0.5	-	13.8	-	-	-	-	171
		S.D.	38.6	37.7	29.1	18.7	1.9	4.0	8.6	2.8	-	-	6.3	1.5	2.7	5.8	0.2	29.7	-	-	-	-	
	>80 LD	Av.	25.5	22.5	10.3	22.1	0.9	-	-	-	-	-	-	-	-	-	-	5.6	1.7	-	-	-	12
		S.D.	30.5	35.9	13.8	36.3	2.6	-	-	-	-	-	-	-	-	-	-	16.7	5.2	-	-	-	
S. parviceps	40-80 ED	Av.	-	-	-	10.0	-	-	-	-	14.3	-	-	-	-	-	1.4	58.0	14.3	1.4	-	-	8
		S.D.	-	-	-	32.0	-	-	-	-	37.8	-	-	-	-	-	3.8	48.8	37.8	3.8	-	-	
	80-160 ED	Av.	-	-	-	-	-	-	-	-	11.8	-	-	-	-	0.4	-	61.2	26.5	0.1	-	-	18
		S.D.	-	-	-	-	-	-	-	-	33.2	-	-	-	-	1.7	-	48.1	43.7	0.6	-	-	
	>160 ED	Av.	-	-	0.1	1.5	-	-	-	2.6	-	-	-	-	-	-	2.7	68.4	22.7	-	-	-	56
		S.D.	-	-	0.6	9.5	-	-	-	13.5	-	-	-	-	-	-	9.8	43.4	39.4	-	-	-	
	40-80 LD	Av.	2.0	0.1	0.3	-	-	-	0.6	3.0	-	5.0	0.1	0.2	-	-	5.0	70.0	13.0	-	-	-	21
		S.D.	2.7	0.2	1.0	-	-	-	2.1	16.5	-	22.7	0.2	0.7	-	-	8.4	33.9	22.4	-	-	-	
	80-160 LD	Av.	2.0	0.2	4.7	7.1	5.5	-	0.1	2.0	-	-	-	-	-	0.8	-	62.5	9.5	-	-	-	22
		S.D.	1.0	0.7	16.7	22.9	21.1	-	0.4	1.0	-	-	-	-	-	3.9	-	45.2	30.1	-	-	-	
	>160 LD	Av.	-	-	-	8.8	-	-	-	-	2.4	-	-	-	-	-	2.4	58.4	25.5	2.4	-	-	42
		S.D.	-	-	-	27.6	-	-	-	-	15.6	-	-	-	-	-	15.6	41.9	35.7	15.6	-	-	

Table 3.1 (cont.)

Species	Size class - season	Diet	Chi	Eph	Tri	Sim	Odo	Sln	OAl	TIn	Pal	Aty	Zoo	Ost	Mol	Fis	Det	FA	AM	TVg	MPP	TVt	N
H. fuliginosus	<40 ED	Av.	18.8	17.3	26.8	15.8	-	-	1.4	1.8	-	-	-	-	-	-	-	5.7	-	-	-	-	17
		S.D.	32.3	31.2	37.2	33.6	-	-	5.6	7.0	-	-	-	-	-	-	-	22.7	-	-	-	-	
	40-80 ED	Av.	16.4	12.0	44.9	2.5	0.4	0.1	0.9	0.4	-	-	0.4	0.3	-	3.7	-	10.9	0.4	-	-	-	28
		S.D.	26.0	22.2	39.5	7.0	1.9	5.6	1.7	2.1	-	-	2.0	1.1	-	19.2	-	24.4	1.9	-	-	-	
	80-160 ED	Av.	6.7	3.3	28.1	12.4	2.8	-	2.0	6.4	4.7	0.7	0.4	0.2	-	2.0	0.8	21.0	0.2	1.5	-	-	56
		S.D.	22.9	14.5	33.9	27.9	13.2	-	4.2	22.4	15.7	5.3	2.7	0.8	-	9.8	3.2	30.3	1.2	9.1	-	-	
	>160 ED	Av.	6.8	2.9	14.3	8.2	0.5	2.4	0.6	16.0	7.9	-	-	-	-	21.6	-	13.1	1.8	-	-	-	20
		S.D.	15.5	10.4	31.7	25.5	2.3	10.4	3.3	31.2	25.1	-	-	-	-	39.2	-	31.6	6.1	-	-	-	
	<40 LD	Av.	15.8	12.0	25.0	-	-	-	44.3	-	-	-	2.5	-	-	-	-	-	-	-	-	-	6
		S.D.	12.6	13.4	50.0	-	-	-	35.1	-	-	-	5.0	-	-	-	-	-	-	-	-	-	
	40-80 LD	Av.	18.9	16.9	33.1	15.0	1.1	-	2.0	1.1	-	-	2.1	-	1.7	-	0.2	2.8	-	0.2	-	-	59
		S.D.	31.1	30.3	38.2	32.0	7.3	-	9.2	8.5	-	-	7.4	-	13.1	-	1.3	15.2	-	1.6	-	-	
	80-160 LD	Av.	15.2	16.0	34.6	6.7	0.6	-	2.3	1.9	2.6	-	-	-	0.5	-	1.2	10.3	3.0	0.1	-	-	38
		S.D.	25.7	30.1	35.7	17.8	3.3	-	12.3	5.7	14.3	-	-	-	3.3	-	3.8	23.5	9.4	0.8	-	-	
	>160 LD	Av.	14.8	3.0	41.3	3.8	0.2	3.0	1.0	-	3.3	0.5	0.3	-	-	-	0.4	0.5	15.7	5.1	-	-	14
		S.D.	29.4	8.1	46.7	13.9	0.8	10.9	2.8	-	7.2	1.3	1.2	-	-	-	1.4	1.5	30.4	13.8	-	-	
L. unicolor	<40 ED	Av.	32.1	21.0	18.3	0.3	3.3	-	1.4	1.3	2.2	2.2	0.1	1.8	-	0.3	0.1	7.3	4.4	-	-	-	46
		S.D.	41.4	34.0	33.7	2.2	16.4	-	6.7	8.5	14.9	14.9	0.4	9.1	-	1.8	0.7	21.9	20.8	-	-	-	
	40-80 ED	Av.	19.9	18.3	21.3	1.4	7.6	1.8	4.2	2.9	-	3.4	2.0	1.4	1.5	0.1	-	7.7	2.2	-	-	-	106
		S.D.	31.7	27.9	30.0	9.6	22.1	8.8	14.4	14.4	-	14.4	13.3	5.1	1.2	0.7	-	22.3	13.4	-	-	-	
	>80 ED	Av.	14.6	25.9	19.1	-	3.0	-	1.0	12.2	2.0	0.9	-	0.6	-	4.9	1.1	4.6	6.1	-	-	-	34
		S.D.	28.7	34.1	27.2	-	17.4	-	3.4	31.3	11.6	5.2	-	2.7	-	17.2	6.1	18.1	24.2	-	-	-	
	40-80 LD	Av.	35.2	27.2	9.6	1.3	3.7	-	3.1	0.5	0.6	0.6	2.3	1.0	0.5	1.8	0.1	4.1	1.4	-	-	-	167
		S.D.	40.8	36.7	22.2	10.4	16.1	-	13.6	6.6	7.8	7.8	13.8	5.8	2.6	11.5	0.7	16.2	9.5	-	-	-	
	>80 LD	Av.	27.7	20.7	14.3	-	-	1.0	7.0	-	3.3	1.7	1.2	0.2	-	6.6	0.2	8.7	0.3	1.3	-	-	61
		S.D.	36.4	33.2	29.7	-	-	7.3	24.6	-	18.0	10.5	7.1	1.4	-	21.1	1.3	21.5	2.0	9.8	-	-	
	Average diet		15.8	14.6	16.1	5.4	1.3	0.3	3.4	2.3	2.2	0.6	1.4	0.3	0.3	1.7	0.7	23.0	6.0	0.5	0.0	0.0	

Table 3.2 Mean contribution (%) and standard deviation of prey items in the average diet of Daly River terapontid OTUs according to season. ED refers to early dry season, LD refers to late dry season. Abbreviations for dietary items are listed in the text, N is the number of stomachs analysed per OTU.

Species	Size class - season	Diet	Chi	Eph	Tri	Sim	Odo	Sln	OAl	Tln	Pal	Aty	Zoo	Ost	Mol	Fis	Det	FA	AM	TVg	MPP	TVt	N
S. butleri	40-80 ED	Av.	13.8	-	3.0	30.8	-	-	2.5	-	-	-	0.7	-	-	-	37.7	9.9	-	-	0.4	-	26
		S.D.	25.5	-	6.9	41.9	-	-	6.3	-	-	-	2.9	-	-	-	38.8	20.0	-	-	1.4	-	
	80-160 ED	Av.	0.7	0.1	1.1	19.2	-	-	-	-	-	-	-	-	-	-	44.6	27.4	-	-	6.2	-	17
		S.D.	2.4	0.2	1.8	36.3	-	-	-	-	-	-	-	-	-	-	32.7	28.1	-	-	16.9	-	
	>160 ED	Av.	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	46.0	39.8	-	-	13.3	-	13
		S.D.	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	28.2	27.7	-	-	18.2	-	
	40-80 LD	Av.	1.0	8.2	13.3	26.0	-	-	0.6	-	-	-	-	-	-	-	27.6	4.3	-	-	17.7	-	11
		S.D.	2.2	27.1	22.0	37.7	-	-	2.1	-	-	-	-	-	-	-	35.7	7.7	-	-	36.2	-	
	80-160 LD	Av.	2.9	0.4	0.6	36.9	-	-	0.4	-	-	-	-	-	-	1.4	39.9	12.7	-	-	4.0	-	7
		S.D.	7.6	1.1	1.5	41.0	-	-	1.1	-	-	-	-	-	-	3.8	35.4	22.0	-	-	7.7	-	
	>160 LD	Av.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34.0	47.5	-	-	17.5	-	3
		S.D.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.5	31.8	-	-	24.7	-	
L. unicolor	<40 ED	Av.	28.7	33.2	6.5	1.8	7.8	-	5.2	-	-	-	9.9	-	-	-	1.1	3.9	-	-	0.4	-	19
		S.D.	22.8	31.5	10.0	6.9	23.6	-	13.5	-	-	-	23.8	-	-	-	2.7	17.2	-	-	1.8	-	
	40-80 ED	Av.	15.6	13.8	18.0	4.9	6.9	-	12.6	2.1	8.0	0.9	1.7	0.6	0.6	1.4	1.4	6.0	0.1	0.3	0.2	-	154
		S.D.	21.8	19.3	24.7	13.9	18.3	-	23.7	11.4	23.2	6.5	6.7	3.5	4.9	8.0	3.0	15.6	1.0	2.9	2.1	-	
	>80 ED	Av.	2.4	2.9	5.3	2.1	3.7	0.4	8.8	5.6	31.4	4.1	-	0.4	-	3.2	0.9	8.0	13.6	2.0	0.3	-	45
		S.D.	9.2	8.8	12.6	8.6	16.8	-	18.5	18.9	41.3	19.3	-	2.7	-	15.3	3.1	21.5	26.5	10.8	1.6	-	
	<40 LD	Av.	13.0	51.5	29.5	-	-	-	1.5	-	-	-	0.5	-	-	-	1.5	-	-	-	-	-	6
		S.D.	9.9	37.5	36.1	-	-	-	2.1	-	-	-	0.7	-	-	-	2.1	-	-	-	-	-	
	40-80 LD	Av.	10.7	10.9	14.8	4.9	11.2	-	16.3	2.0	5.2	-	2.7	0.9	-	3.5	1.3	12.9	-	0.2	-	-	50
		S.D.	17.7	16.0	25.8	18.4	22.3	-	23.2	12.9	17.8	-	8.1	4.4	-	12.1	3.6	24.7	-	1.2	-	-	
	>80 LD	Av.	0.8	-	0.4	0.2	8.6	-	8.2	21.2	41.4	-	-	-	-	5.0	0.6	13.6	-	-	-	-	8
		S.D.	1.8	-	0.9	0.4	19.2	-	17.2	44.1	42.8	-	-	-	-	11.2	1.3	23.8	-	-	-	-	

Table 3.2 (cont.)

Species	Size class - season	Diet	Chi	Eph	Tri	Sim	Odo	Sln	OAl	Tln	Pal	Aty	Zoo	Ost	Mol	Fis	Det	FA	AM	TVg	MPP	TVt	N
H. fulig.	<40 ED	Av.	25.7	17.1	35.1	8.4	2.5	-	2.3	-	-	-	3.3	-	-	-	-	-	-	-	-	-	17
		S.D.	33.5	29.4	34.5	14.5	10.0	-	3.7	-	-	-	4.9	-	-	-	-	-	-	-	-	-	
	40-80 ED	Av.	11.0	8.0	18.6	26.3	4.5	0.1	13.3	0.2	5.7	0.2	1.4	-	0.2	0.4	2.9	3.6	-	1.1	0.1	-	90
		S.D.	20.9	15.6	22.3	35.8	14.0	0.6	22.5	2.1	18.9	2.1	5.2	-	1.6	2.8	11.7	11.0	-	9.9	0.5	-	
	80-160 ED	Av.	7.4	6.0	18.6	13.5	2.3	-	6.5	0.9	16.6	2.3	-	0.2	-	-	3.6	6.3	4.8	4.4	-	-	30
		S.D.	15.6	12.1	25.4	29.1	5.5	-	12.1	2.9	33.3	12.8	-	0.6	-	-	7.1	18.6	17.8	15.7	-	-	
	160 ED	Av.	-	0.2	0.1	0.3	0.2	-	7.0	0.9	20.2	0.4	-	-	-	14.4	0.8	10.1	13.9	28.7	-	2.7	26
		S.D.	-	1.0	0.4	1.4	1.0	-	23.2	3.1	32.6	1.2	-	-	-	34.3	3.9	19.6	30.4	39.6	-	11.9	
	<40 LD	Av.	21.9	24.1	35.9	0.6	0.2	-	1.3	-	-	-	3.7	0.9	-	1.4	3.7	3.8	-	-	-	-	20
		S.D.	17.8	22.8	32.3	1.7	0.9	-	2.3	-	-	-	7.4	1.8	-	3.7	12.2	11.7	-	-	-	-	
	40-80 LD	Av.	10.3	19.6	16.5	15.3	5.4	-	18.4	0.3	1.5	-	5.0	0.5	0.5	0.4	2.0	1.8	-	-	0.2	-	24
		S.D.	16.8	17.8	20.0	28.0	17.2	-	26.9	1.2	5.1	-	11.0	1.2	2.2	1.4	4.4	6.3	-	-	1.0	-	
	80-160 LD	Av.	1.0	7.3	14.1	30.2	2.5	-	14.7	4.1	5.8	1.3	-	-	-	-	0.3	4.4	-	13.5	0.5	-	12
		S.D.	2.9	22.1	20.4	37.0	7.2	-	23.2	10.0	12.6	2.9	-	-	-	-	0.9	9.9	-	27.9	1.7	-	
	>160 LD	Av.	-	-	-	-	-	-	0.6	-	49.0	-	-	-	-	16.2	-	5.6	-	8.0	0.6	19.8	7
		S.D.	-	-	-	-	-	-	1.3	-	35.9	-	-	-	-	23.0	-	8.8	-	17.9	1.3	44.3	
A.percoides	<40 ED	Av.	48.9	18.8	7.6	0.2	1.9	-	4.2	-	2.1	1.0	5.8	0.3	-	0.2	4.2	2.0	-	-	-	-	29
		S.D.	32.8	25.3	11.2	0.9	10.2	-	6.9	-	11.3	5.3	12.5	0.9	-	0.9	13.5	7.0	-	-	-	-	
	40-80 ED	Av.	31.7	9.8	15.7	3.1	4.2	0.1	14.3	-	0.4	0.5	0.7	0.2	0.9	-	3.2	11.1	1.2	-	-	-	101
		S.D.	28.8	18.6	21.9	9.8	16.0	0.5	20.7	-	2.0	3.3	2.4	1.2	3.9	-	10.1	19.5	6.3	-	-	-	
	>80 ED	Av.	9.5	2.5	2.9	0.7	4.9	-	10.1	0.3	5.0	-	0.1	0.1	4.8	-	6.5	10.4	40.6	-	-	-	32
		S.D.	15.2	4.0	8.7	1.7	19.1	-	21.2	1.2	16.5	-	0.5	0.5	15.7	-	15.3	24.4	46.4	-	-	-	
	<40 LD	Av.	38.1	17.3	1.1	-	0.9	-	3.7	-	-	2.4	30.1	0.1	-	4.3	0.3	0.2	-	-	-	-	28
		S.D.	30.2	24.2	5.8	-	4.8	-	8.7	-	-	9.9	29.5	0.6	-	13.3	1.3	1.0	-	-	-	-	
	40-80 LD	Av.	30.5	17.2	10.6	2.7	0.6	-	20.2	-	-	0.4	0.6	1.6	1.6	5.1	0.2	7.1	-	0.1	0.1	-	15
		S.D.	28.3	24.4	19.7	6.9	2.1	-	33.0	-	-	1.3	1.3	4.1	4.4	15.9	0.8	13.4	-	0.5	0.5	-	
	>80 LD	Av.	35.0	5.0	-	18.0	-	-	7.0	-	-	-	-	-	-	-	5.0	15.0	5.0	-	-	-	3
		S.D.	37.0	4.0	-	24.4	-	-	8.0	-	-	-	-	-	-	-	4.0	24.3	4.0	-	-	-	
	Average diet		13.9	10.5	10.4	9.5	2.6	0.1	6.9	1.4	7.4	0.5	2.6	0.2	0.3	2.2	10.3	10.3	3.1	2.2	2.4	0.9	

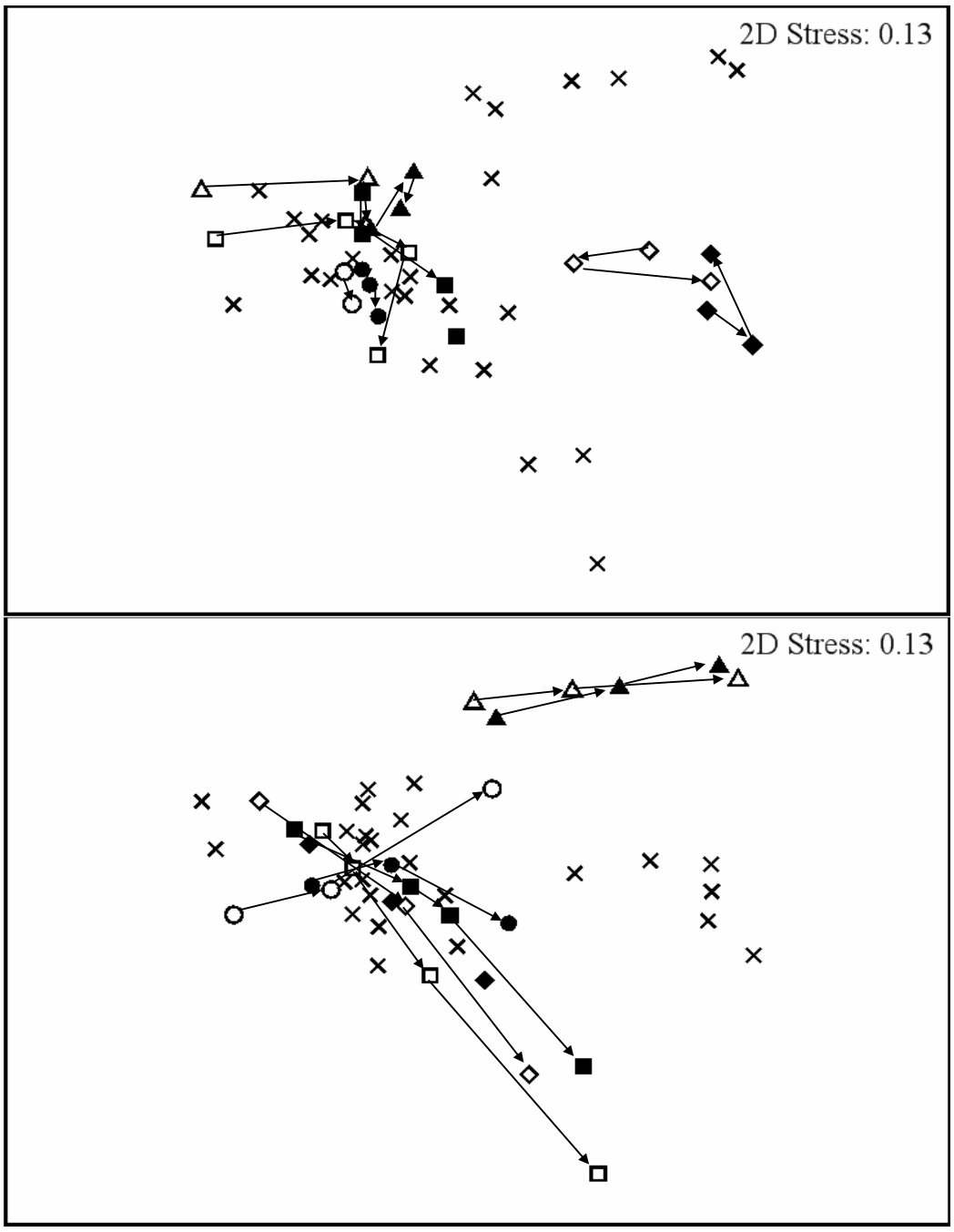

Figure 3.2 Non-metric multidimensional scaling ordination of combined Daly and Burdekin River terapontid OTU diets. Ontogenetic trajectories for species in each catchment are presented separately for clarity; A: Burdekin River OTUs depicted by symbols as follows: ▲ - *A. percoides* (early dry), △ - *A. percoides* (late-dry), ■ - *H. fuliginosus* (early-dry), □ - *H. fuliginosus* (late-dry), ● - *L. unicolor* (early-dry), ○ - *L. unicolor* (late-dry), ◆ - *S. parviceps* (early-dry), ◇ *S. parviceps* (late-dry); × - all Daly OTUs B: Daly River OTUs depicted by by symbols as follows: ▲ - *S. butleri* (early dry), △ - *S. butleri* (late-dry), ■ - *H. fuliginosus* (early-dry), □ - *H. fuliginosus* (late-dry), ● - *A. percoides* (early-dry), ○ - *A. percoides* (late-dry), ◆ - *L. unicolor* (early-dry), ◇ *L. unicolor* (late-dry); × - all Burdekin OTUs.

3.3.2 Hierarchical clustering

Cluster analysis and SIMPROF tests for significant guild structure identified seven coherent trophic groups ($P < 0.01$) encompassing a diversity of trophic levels across the two assemblages (Figure 3.3). Each group was allocated to a functional feeding guild on the basis of dominant items in diet identified through SIMPER analysis (Table 3.3).

Dietary items such as chironomid larvae, ephemeropteran nymphs, trichopteran larvae and filamentous algae were particularly important for several guilds, although the relative contributions or total range of dietary items differentiated several specific guilds that consumed these prey items. One guild (herbivores) was exclusive to the Burdekin catchment, with three guilds (macrophagous consumers, detritivores-algivores and meiophagous omnivores) unique to the Daly River assemblage. The remaining four guilds comprised OTUs from both catchments.

Size-related and seasonal guild shifts for each species, catchment and season are summarized in Table 3.4. Single species occupying multiple trophic guilds occurred in both catchments. Ontogenetic niche shifts were particularly evident in *H. fuliginosus* in both catchments. This species simultaneously occupied three distinct trophic guilds in both the early and late dry season in the Daly River, while it was the only Burdekin species to occupy more than two simultaneous trophic guilds in any season (late dry season). The total number of intraspecific trophic guild shifts in the Daly was more than double that of the Burdekin (although no *L. unicolor* <40 mm SL were collected in the Burdekin dry-season samples). Species such as *L. unicolor* and *A. percoides*, which exhibited consistent trophic niche shifts in the Daly, regardless of season, were relatively constrained in their occupation of multiple trophic guilds in the Burdekin, particularly in the early dry season.

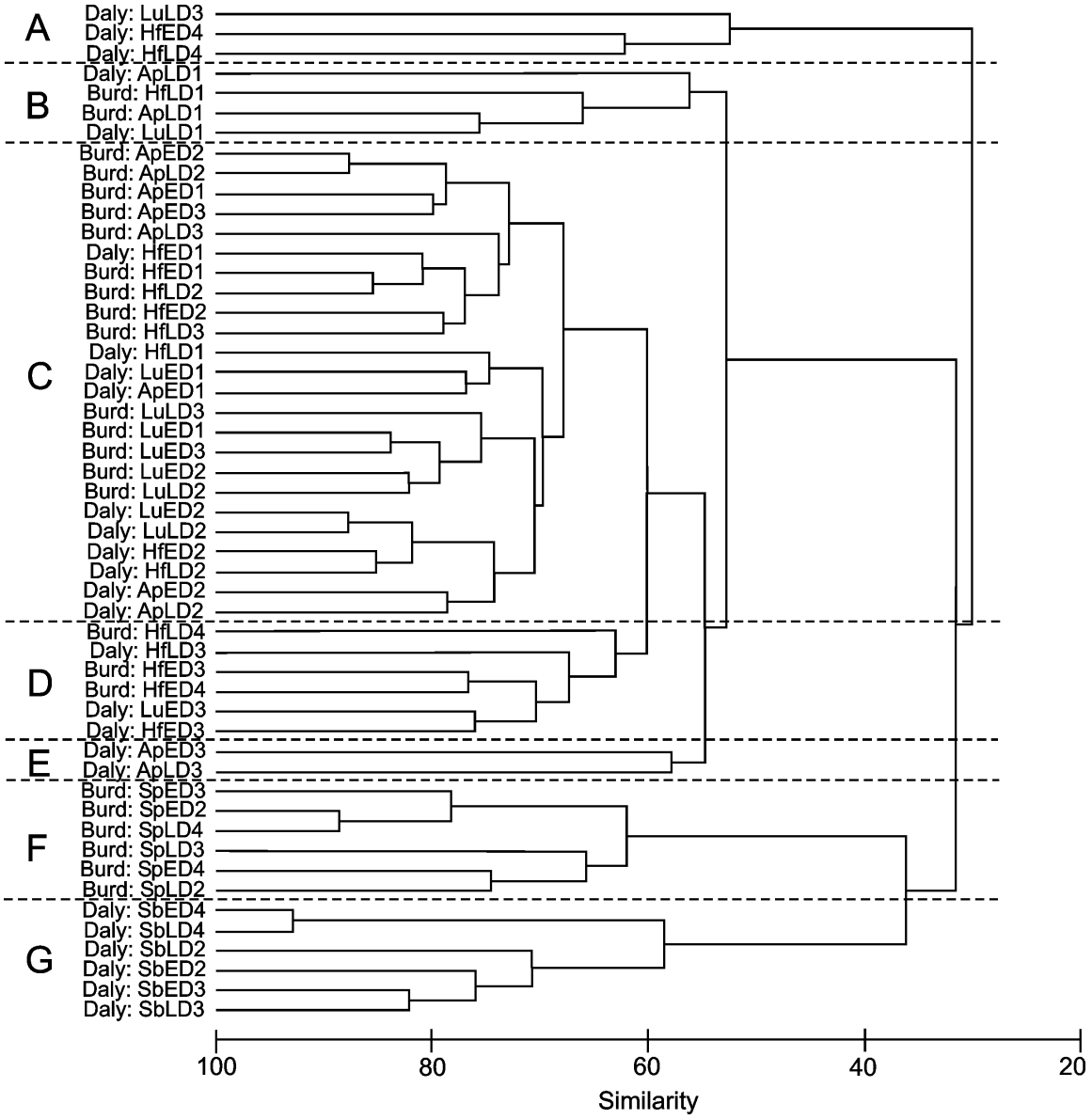

Figure 3.3 Cluster analysis outlining combined dietary guild structure of the Daly and Burdekin River terapontid assemblages. Trophic guilds are identified as: A - macrophagous consumers; B – invertivore-planktivores; C – invertivores-algivores; D – generalized omnivores; E – meiophagous omnivores; F – herbivores and G – detritivores-algivores. Catchment codes precede each species name: Burd; Burdekin; Daly; Daly. Species names are coded according to genus and species initials. Seasonal codes follow species name: ED, early-dry; LD, late-dry. Numeric suffixes denote OTU size classes: 1, <40 mm; 2, 40-80 mm; 3, 80 – 160 mm; 4, > 160 mm.

Table 3.3 Average Bray-Curtis diet similarity within each trophic guild and the average diet proportion for the major food groups characterising each guild.

Guild	Descriptor	Average similarity	Major dietary items	Average Proportion	% Contribution to guild similarity
A	Macrophagous consumers	55.76	Palaemonidae	36.03	36.03
			Fish	19.09	55.12
			Fil. algae	18.18	73.3
			Other aq. insects	9.5	82.74
			Terr. Vegetation	6.48	92.97
B	Invertivore-planktivores	62.83	Ephemeroptera	31.74	31.74
			Chironomidae	28	57.94
			Trichoptera	18.6	78.36
			Zooplankton	12	90.26
C	Invertivore-algivores	70.68	Chironomidae	20.29	20.29
			Ephemeroptera	18.9	39.16
			Trichoptera	16.7	55.87
			Fil. algae	9.59	65.45
			Simuliidae	6.31	71.77
			UI Arthr. Frag	6.2	77.97
			Other aq. insects	5.7	83.67
D	Generalised omnivores	67.97	Trichoptera	15.6	15.6
			Palaemonidae	10.31	25.9
			Simuliidae	10.31	36.2
			Fil. algae	8.55	44.7
			Other aq. insects	8.38	53.1
			Chironomidae	8.07	61.2
			Ephemeroptera	7.97	74.93
			Terr. invert.	5.03	79.96
E	Meiophagous omnivores	57.92	Fil. algae	20.34	20.34
			Chironomidae	19.5	39.78
			Other aq. insects	16.8	56.5
			Detritus	14.14	70.64
			Aq. Macrophyte	14.14	84.78
			Ephemeroptera	9.9	94.67
F	Herbivores	67.32	Fil. algae	54.96	54.96
			Aq. Macrophyte	26.77	81.62
			Simuliidae	5.4	87.1
			Detritus	3.8	91.1
G	Detritivore-algivores	63.14	Detritus	41.17	41.17
			Fil. algae	24.48	65.66
			Misc. plant parts	14.45	80.11
			Simuliidae	12.19	92.29

Table 3.4 Summary of species' seasonal ontogenetic guild shifts for the Burdekin and Daly River terapontid assemblages.

Catchment	Species	Season	Ontogenetic trophic guild sequence	Ontogenetic shifts
Daly	*Amniataba percoides*	Early Dry	C, C, E	1
	Hephaestus fuliginosus		C, C, D, A	2
	Leiopotherapon unicolor		C, C, D	1
	Syncomistes butleri		G, G, G	0
		Late Dry		
	Amniataba percoides		B, C, E	2
	Hephaestus fuliginosus		C, C, D, A	2
	Leiopotherapon unicolor		B, C, A	2
	Syncomistes butleri		G, G, G	0
			Total	10
Catchment	**Species**	**Season**	**Ontogenetic trophic guild sequence**	**Ontogenetic shifts**
Burdekin		Early Dry		
	Amniataba percoides		C, C, C	0
	Hephaestus fuliginosus		C, C, D, D	1
	Leiopotherapon unicolor		C, C, C	0
	Scortum parviceps		F, F, F	0
		Late Dry		
	Amniataba percoides		B, C, C	1
	Hephaestus fuliginosus		B, C, C, D	2
	Leiopotherapon unicolor		*NA*, C, C	0
	Scortum parviceps		F, F, F	0
			Total	4

Trophic guild shifts by within-species OTUs between seasons were uncommon for both assemblages. Guild D (invertivores-planktivores) was the only trophic guild unique to a season in either catchment, with several small juvenile OTUs from both catchments assuming this dietary mode in the late dry season. The shift by the largest OTU of *L. unicolor* (80-160 mm SL) from generalized omnivory in the early dry season to macrophagy in the late dry season was the only additional seasonal niche shift.

3.3.3 Dietary overlap

The OTU by OTU Bray-Curtis similarity matrices for each catchment and season are presented in Tables A1.1-A1.2. Average (± S.E.) Bray-Curtis dietary overlap for both seasons combined was significantly lower in the Daly River (45.59±1.63, N=156) than in the Burdekin River (52.85±1.71, N=144) (two-

tailed, unequal variance *t*-test, d.f. = 296, $t = 3.07$, $P < 0.01$). Much of this difference could be attributed to seasonal differences in degree of collective dietary overlap in the Daly River. The average level of dietary overlap was significantly lower (two-tailed, unequal variance *t*-test, d.f. = 153, $t = 2.447$, $P < 0.05$) in the Daly late dry assemblage (41.6±2.19, N=78) compared to the early dry (49.57±2.35, N=78). There was no significant difference in average dietary overlap between the early dry (54.35±2.38, N=78) and late dry (51.077±2.44, N=66) seasons in the Burdekin River terapontid assemblage (two-tailed, unequal variance *t*-test, d.f. = 140, $t = 0.958$, $P > 0.05$). These levels of dietary overlap were both within the range of 40-60, indicating what is generally regarded as a moderate level of dietary overlap (see Grossman 1986; Ross 1986).

Tables A1.1 and Table A1.2 highlight the degree of dietary overlap occurring between the smallest and largest OTUs of each species, according to catchment and season. Intraspecific pairwise dietary overlap between several Burdekin River OTUs was of high intensity (>80) for species such as *A. percoides* and *L. unicolor*, particularly in the early dry season, indicative of minimal size-related dietary divergence. Intraspecific dietary overlap between the smallest and largest individuals of species such as *H. fuliginosus* and *L. unicolor* in the Daly River was very low in comparison (<20), particularly in the late dry season (see also Table 3.1). This outcome demonstrated pronounced size-related dietary shifts with low potential for competition between size-range extremes in these species.

3.3.4 Niche breadth

Few consistent patterns were evident in terapontid OTU dietary specialization in relation to size, season or catchment effects (Table 3.5). The OTUs of the herbivorous *S. parviceps* (Burdekin River) and the detritivorous-algivorous *S. butleri* (Daly River) demonstrated the highest levels of dietary specialization of all species, particularly in larger size classes. There were no significant differences detected ($P > 0.05$) in overall levels of dietary specialization (both seasons combined) between catchments, or within catchments between seasons (two-tailed, unequal variance *t*-tests).

Table 3.5 Levin's niche breadth (B_L) values for terapontid OTUs according to catchment and season.

Burdekin		Levin's niche breadth		Daly		Levin's niche breadth	
Species	Size class	Early-dry	Late-dry	Species	Size class	Early-dry	Late-dry
A.percoides	>40	0.12	0.06	*A.percoides*	>40	0.07	0.06
	40-80	0.10	0.09		40-80	0.11	0.12
	>80	0.07	0.12		>80	0.11	0.10
H. fuliginosus	>40	0.13	0.06	*H. fuliginosus*	>40	0.08	0.09
	40-80	0.07	0.10		40-80	0.16	0.15
	80-160	0.14	0.11		80-160	0.13	0.22
	>160	0.17	0.09		>160	0.06	0.12
L. unicolor	>40	0.11	na	*L. unicolor*	>40	0.04	0.09
	40-80	0.16	0.10		40-80	0.21	0.20
	>80	0.15	0.13		>80	0.08	0.15
S. parviceps	40-80	0.04	0.02	*S. butleri*	40-80	0.10	0.07
	80-160	0.03	0.04		80-160	0.06	0.06
	>160	0.02	0.04		>160	0.04	0.04
Average		0.10	0.08			0.12	0.10

3.4 Discussion

There is considerable plasticity in the magnitude of size-related dietary shifts in the two terapontid assemblages inhabiting the two different environments. Diets of small juveniles (<40 mm SL) within both catchments were invertivorous, regardless of season. While several Burdekin River species were largely invertivorous across all size classes, the diet of larger Daly River terapontids diverged significantly from the initial period of high juvenile dietary overlap. Similarity in resource use early in life history between closely related species, prior to niche divergence with increasing size, has been documented in a range of fish (Mittelbach, 1984; Mark *et al.*, 1987; Garner, 1996; Olson and Young, 2003) and other aquatic vertebrates (Mushinsky *et al.*, 1982).

Werner and Gilliam (1984) noted that patterns of intraspecific resource use may vary on a continuum from minimal resource partitioning among size classes, extending through to discrete ontogenetic diet/habitat shifts with no direct interaction between different size classes. The intraspecific trophic trajectories of Burdekin River and Daly River terapontid assemblages clearly align closely with either of these two extremes, at least for some species. The low intraspecific dietary overlap evident between the smallest and largest OTUs of many Daly terapontids coupled with greater numbers of size-related feeding

guilds indicates different size classes acting as ecologically different species. Differential expression in the magnitude of size-related dietary shifts, more than any other factor, appeared to play the major role in the greater trophic guild differentiation in the Daly compared to the Burdekin River assemblage.

Allochthonous food such as riparian fruits and terrestrial vertebrates (frogs and carrion) were conspicuously absent from the diets of Burdekin terapontids. In the Daly, these items were major contributors to the diet of larger individuals of species such as *H. fuliginosus*. This result likely reflects the differences in riparian vegetation between the Burdekin and Daly. The upper Burdekin riparian zone has low floristic diversity, dominated by *Melaleuca leucadendron* (Pearson, 1991) whereas the Daly riparian zone is dominated by a floristically complex, and highly baseflow-dependent (pheatophytic) closed-monsoon forest (Lamontagne *et al.*, 2005; O'Grady *et al.*, 2006). Flow regime and access to groundwater have been shown to be important determinants of vegetation assemblage structure and lateral zonation in northern Australian riparian ecosystems (O'Grady *et al.*, 2006; Pettit *et al.*, 2001). A diverse monsoon-forest riparian community is a feature of perennial systems across northern Australia, whereas systems with less reliable dry-season flow are dominated by *Melaleuca*. Thus, the contrasting flow regimes of the two rivers appear to have an important influence on their respective food webs.

While detritivorous or herbivorous dietary strategies are rare within Australia's entire freshwater ichythyofauna (Kennard *et al.*, 2001), the Terapontidae is one of the few Australian families to include these diets to a significant extent (Davis *et al.*, 2011b). The evolution of these dietary strategies has very probably reduced much interspecific competition for food, even within assemblages of closely related species. Adoption of a specialized diet may still constitute a viable life history trait in these variable river systems if the niche is unoccupied and/or the targeted resource (detritus or aquatic vegetation in this case) is continuously available despite environmental variability.

Both the Daly and Burdekin River terapontid assemblages exhibited minor spatial and between-year variability in diets, as has been observed in other tropical Australian river systems (Rayner *et al.*, 2009; Pusey *et al.*, 2010). The decreased dry-season dietary overlap in the Daly is similar to that in several other tropical systems (Zaret and Rand, 1971; Winemiller, 1989; Jepsen *et al.*, 1997). Baseflow stability and predictability of daily flow (key variables differentiating the Daly and Burdekin) have been identified as particularly influential hydrological factors in filtering the suite of ecological traits likely to be favoured in a region (Poff and Allan, 1995). The maintenance of relatively stable and predictable baseflow conditions in the Daly during the dry season may facilitate niche complementarity as a mechanism to avoid intense competitive interactions both between and within species. However, the exact mechanism

producing the reduced dry-season dietary overlap in the Daly is somewhat unclear. While there were some guild shifts associated with the transition to the dry season in the Daly, a similar number also occurred in the Burdekin, and it is unlikely that the significantly lower overlap evident in the late dry season can be solely attributed to these few species' shifts. Given that guild structure remained relatively stable, it is also possible that OTUs showed greater patterns of intra-guild resource sub-division during the late dry season (see Winemiller and Pianka, 1990).

This chapter shows that size-related dietary shifts within terapontids can exhibit considerable plasticity. While fish dietary information needs coupling with resource availability data to rigorously test relationships, differences in long-term flow regime, particularly baseflow perenniality, may have played a substantial role in the observed differences in diet shifts between the studied assemblages. Broader relationships between interspecific interactions, species' traits and long-term hydrological variability have received considerable attention (Grossman *et al.*, 1982; Winemiller and Rose, 1992; Townsend and Hildrew, 1994; Poff and Allan, 1995, Poff, 1997; Blanck *et al.*, 2007), the implications of environmental variability on ontogenetic resource use, however, remain largely untested.

Chapter 4: Trophic ecology of terapontid fishes (Pisces: Terapontidae): the role of morphology and ontogeny

Published in Marine and Freshwater Research ***63****:128-141 (2012).*

4.1 Introduction

Ecomorphology describes the relationships between an organism's ecology and its functional morphology, and provides a framework for addressing the extent of morphological and ecological co-evolution (Winemiller *et al.,* 1995; Motta *et al.,* 1995*b*). The interface between fish morphology and patterns of prey use (diet) has been the focus of considerable study, although there is substantial divergence in research outcomes. A number of studies have identified a strong association between fish morphology and diet (Winemiller *et al.,* 1995; Piet, 1998; Hugueny and Pouilly, 1999), whereas others have suggested that any relationships are weak or inconsistent at best (Grossman, 1986; Douglas and Matthews, 1992; Motta *et al.,* 1995a). Several morphological characters with functional linkages to dietary ecology have been repeatedly identified, however. For example, intestinal length is commonly correlated with degree of herbivory (Elliott and Bellwood, 2003); mouth gape tends to be strongly correlated with prey size and degree of piscivory (Wainwright and Richard, 1995; Mittelbach and Persson, 1998); relative orientation of the mouth indicates the depth at which feeding typically occurs or of predator position in relation to prey (Gatz, 1979; Wikramanayake, 1990); and dentition typically provides a reasonable approximation of diet, with conical holding teeth corresponding to carnivory, and flattened cutting teeth prevalent in herbivorous fishes (Gatz, 1979; Stoner and Livingston, 1984).

Most ecomorphological studies of fish have focused upon comparisons of the adult morphologies of different species, in many cases deliberately avoiding any appraisal of the role of ontogeny (the developmental history of an organism) on co-variation between diet and morphology (exceptions include Wainwright and Richard, 1995; Piet, 1998). This emphasis on terminal morphologies is surprising considering the otherwise long-standing appreciation of ontogenetic differences in resource use, particularly diet, within fish species (Werner and Gilliam, 1984; Stoner and Livingston, 1984). Considerable support for the ontogeny-morphology-diet interaction emerged from the work of Piet (1998), who demonstrated that the majority of morphological variation in some fish assemblages can be attributed to intra- rather than inter-specific differences in body size. As an added complicating factor, attributing observed ontogenetic changes in diet directly to changes in body size alone is potentially

misleading, as many key morphological characters that determine feeding performance often grow non-proportionately (i.e. allometrically) as fish increase in size (Wainwright and Richard, 1995). Allometric changes in morphology are commonplace in many fishes, and often a key correlate with ontogenetic diet shifts (Kramer and Bryant, 1995; Wainwright and Richard, 1995; Piet, 1998; Cassemiro *et al.,* 2008).

The Australian freshwater fish fauna offers some interesting features from an evolutionary perspective, such as a predominance of 'secondary' freshwater species (derived from marine ancestors) and their evolution independent of other continental faunas (see Allen *et al.,* 2002). Detailed ecomorphological research is largely lacking for Australia's freshwater fish species, although a few studies have assessed dietary shifts in relation to ontogenetic changes in attributes such as body size, mouth gape and intestinal length (see Pusey *et al.,* 1995; Pusey *et al.,* 2000; Tibbetts and Carseldine, 2005). This dearth of ecomorphological studies from the Australasian region has been long-recognised as a significant information gap for the field (Norton *et al.,* 1995). In a continent notable for its pronounced lack of fish dietary variation (Coates, 1993; Kennard *et al.,* 2001), the terapontid grunters offer a promising study subject for examining the relationships between fish form and function, both within and between species. The Terapontidae is one of the most trophically diverse of Australia's freshwater fish families, having feeding habits that span carnivorous, omnivorous, herbivorous and detritivorous modes (Chapter 2; Kennard *et al.,* 2001; Bishop *et al.,* 2001; Davis *et al.,* 2011b). Pronounced ontogenetic diet shifts are also a prominent feature of terapontid dietary ecology. Diets of juvenile terapontids appear similar among species prior to pronounced transitions through multiple, distinct dietary stages (carnivory, omnivory, herbivory), with the separate intra-specific size classes of many terapontids exhibiting dietary habits more closely allied with other species than conspecifics of different size classes (Davis *et al.,* 2011b). Considerable variations in morphological characters with potential linkages to diet, such as dentition, mouth gape and intestinal complexity/length have also been documented across the Terapontidae (Mees and Kailola, 1977; Vari, 1978; Davis *et al.,* 2010).

Terapontids therefore provide an opportunity to test previous ecomorphological outcomes against a fish family relatively removed, both geographically and phylogenetically, from previously studied assemblages. This chapter addresses the following questions: (1) What role does ontogeny play in the development of terapontid dietary morphology? (2) Is there a correlation between terapontid morphology and diet? (3) If significant relationships exist between terapontid morphological variables and dietary habits, how do these diet-morphology associations compare with other fish groups at a global level? The fundamental premise of ecomorphology lies in predictable convergences (i.e. morphological similarities) occurring in distantly related fish groups sharing similar ecological niches (Winemiller, 1991). Thus, it is

expected that the diet-morphology relationships repeatedly documented in other fish groups (intestinal length correlating positively with herbivory-detritivory and negatively with carnivory, mouth gape, head length and eye diameter positively correlated with piscivory and prey size) should be paralleled by those in the Terapontidae.

4.2 Materials and Methods

4.2.1 Study area and specimen collection

The ecomorphological analyses described in this study are based on the same 21 Australian terapontid species' diets and intra-specific ontogenetic trophic units (OTUs; *sensu* Stoner and Livingston, 1984) documented in Chapter 2 (Davis *et al.*, 2011b). 'Ontogenetic trophic units' refers to significantly different, size-specific dietary habits identified from hierarchical clustering procedures conducted on individual species' diets over their entire size range (Chapter 2; Davis *et al.*, 2011b). Fish were collected from freshwater habitats across northern Australia through the period 2004-2009. The 21 collected species represent the dominant proportion (ca. 87%) of terapontid species diversity in northern Australia. Full descriptions of fish collection methodology, dietary quantification and OTU definition are outlined in Chapter 2. Table 4.1 provides a summary of each species' OTUs as well as broader feeding group classifications. Dietary habits characterizing each feeding category were as follows: invertivores – diets dominated by small invertebrate prey (zooplankton, chironomid, ephemeropteran and trichopteran larve); meiophagous omnivores – diets dominated by small invertebrate prey and filamentous algae; macrophagous omnivores – diets dominated by an array of large plant (aquatic macrophytes, riparian fruit) and animal prey (fish, macrocrustacea, terrestrial vertebrates); macrophagous carnivores – diets dominated by large animal prey (fish, macrocrustacea, terrestrial invertebrates); herbivores – diets dominated by filamentous algae and aquatic macrophytes; detrivivores – diets dominated by detritus and filamentous algae; and lepidophages – diets dominated by fish scales and fish. It should be noted that the same feeding group categorization is evident through multiple OTUs of some species. In these cases, several significantly different feeding OTUs, which were identified in intra-specific analyses, grouped together in broader interspecific classification of terapontid dietary habits.

Table 4.1 Summary of terapontid species 'ontogenetic trophic unit' (OTU) size ranges (mm SL) and feeding group categorisation derived from hierarchical clustering in Davis *et al*. (2011b).

Species	OTU	Size range	Dietary Category
Amniataba caudovittatus	Ac	67-105	Herbivore
Amniataba percoides	Ap1	14-29	Invertivore
	Ap2	30-59	Meiophagous omnivore
	Ap3	60-99	Meiophagous omnivore
	Ap4	100-126	Meiophagous omnivore
Hannia greenwayi	Hg1	17-39	Invertivore
	Hg2	40-107	Meiophagous omnivore
Hephaestus fuliginosus	Hb1	18-40	Invertivore
	Hb2	40-139	Meiophagous omnivore
	Hb3	140-239	Macrophagous omnivore
	Hb4	240-320	Macrophagous omnivore
Hephaestus carbo	Hc1	37-59	Invertivore
	Hc2	60-109	Macrophagous carnivore
	Hc3	110-163	Macrophagous carnivore
Hephaestus epirrhinos	He	193-275	Macrophagous carnivore
Hephaestus jenkinsi	Hj1	27-39	Invertivore
	Hj2	40-159	Macrophagous omnivore
	Hj3	160-280	Macrophagous omnivore
Hephaestus tulliensis	Ht	81-217	Macrophagous omnivore
Leiopotherapon aheneus	La1	13-39	Invertivore
	La2	40-73	Meiophagous omnivore
Leiopotherapon unicolor	Lu1	18-29	Invertivore
	Lu2	30-59	Meiophagous omnivore
	Lu3	60-119	Meiophagous omnivore
	Lu4	120-181	Macrophagous carnivore
Mesopristes argenteus	Ma	85-226	Macrophagous carnivore
Pingalla gilberti	Pg1	34-39	Invertivore
	Pg2	40-97	Detritivore-algivore
Pingalla lorentzi	Pl	48-116	Meiophagous omnivore
Pingalla midgleyi	Pm	43-71	Detritivore-algivore
Scortum ogilbyi	So1	53-79	Meiophagous omnivore
	So2	80-219	Herbivore
	So3	220-324	Herbivore

Table 4.1 (contd)

Species	OTU	Size range	Dietary Category
Scortum parviceps	Sp1	15-39	Invertivore
	Sp2	40-119	Herbivore
	Sp3	120-239	Herbivore
	Sp4	240-353	Herbivore
Syncomistes butleri	Sb1	43-79	Detritivore-algivore
	Sb2	80-159	Detritivore-algivore
	Sb3	160-241	Detritivore-algivore
Syncomistes rastellus	Sr	68-165	Detritivore-algivore
Syncomistes trigonicus	St1	32-49	Detritivore-algivore
	St2	50-105	Detritivore-algivore
Terapon jarbua	Tj1	37-59	Lepidophage
	Tj2	60-102	Lepidophage
Varrichthys lacustris	Vl	112-181	Herbivore

4.2.2 Morphological variables

Ten linear morphological variables were used to describe species' morphology (Figure 4.1), with selection of morphological variables emphasizing characters that have been previously demonstrated to be relevant to the feeding habits investigated in this study (Gatz, 1979; Winemiller, 1991, Piet, 1998; Hugueny and Pouilly, 1999). Measurements were made to the nearest 0.01mm for measures <150 mm and to the nearest 1 mm for measures > 150 mm. In addition to the linear, mensural variables, two coded variables were scored with integer values for each species: mouth orientation (MO) - coded according to the inclination of a plane perpendicular to the longitudinal axis of the body and tangential to upper and lower jaws when the fish mouth is open (1 = supra-terminal, 2 = terminal and 3 = sub-terminal), and tooth shape (TS) - coded as 1 = conical, 2 = slightly flattened and 3 = highly flattened dentition (following Vari (1978)).

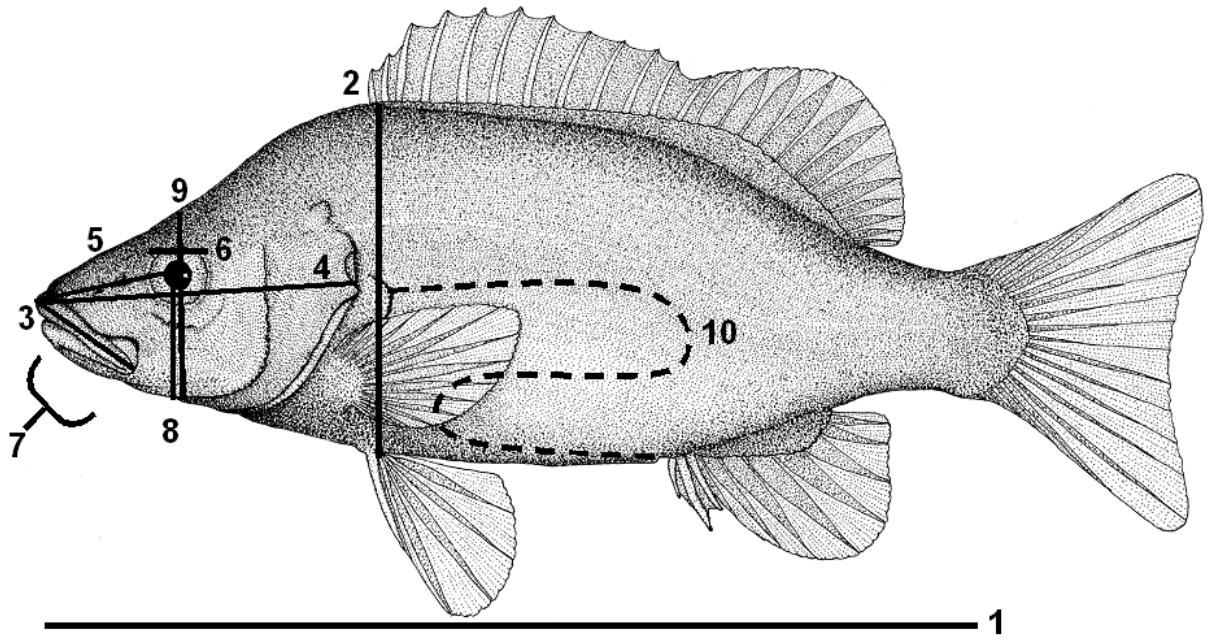

Figure 4.1 A representative terapontid species, *Hephaestus fuliginosus*, with ten mensural morphometric variables indicated: 1 = standard length (SL) - length of the fish from anterior-most section of jaws to the caudal flexure; 2 = body depth (BD) - maximum vertical distance from dorsum to ventrum; 3 = maxilla length (ML) - distance from the tip of the snout to the posterior edge of the upper maxilla; 4 = mouth width (MW) - maximum horizontal mouth gape; 5 = head length (HL) - distance from the tip of snout to the most caudal extension of the operculum; 6 = snout length (SNL) - distance from the pupil of the eye to the tip of the snout; 7 = eye diameter (ED) - horizontal distance from eye margin to eye margin; 8 = eye height (EH) - vertical distance from centre of pupil to ventrum; 9 = head depth (HD) - vertical distance from dorsum to ventrum passing through the centre of the pupil, and; 10 = intestinal length (IL) - length of the digestive tract, measured from pyloric caeca to anus, fully extended without stretching.

4.2.3 Ontogenetic changes in terapontid morphology

Allometric growth

With the study focus on the association between both intra- and inter-specific morphology and diet, initial analyses focused on defining the extent of non-proportional growth in morphological variables occurring during the ontogeny of individual terapontid species. Quantification of the nature and magnitude of allometric growth within and between species is necessary to provide context to subsequent analyses

specifically exploring the direct association between terapontid ontogeny, morphology and diet. The ontogenetic scaling relationship of each morphological variable versus body size was assessed using reduced major axis regression (Sokal and Rohlf, 1981). Intestinal length, maxilla length, mouth width, head length, snout length, eye diameter and body depth were regressed against the independent variable (standard length) on a logarithmic scale (Log_{10}). A single morphological shape variable relating to relative eye position in the dorso-ventral plane was also created by standardizing eye height against head depth in all individuals. This produced simple linear regressions described by the equation:

$$\log (Y) = \log (a) + b \log (X).$$

A scaling relationship was considered allometric if the 95% confidence interval for its slope failed to overlap the slope predicted for isometry (a slope of 1.0 for linear variables and 0 for relative eye position). If slope did not significantly differ from 1 or 0 for linear measures and relative eye position respectively, variables showed isometric growth. Significant values of $b > 1$ indicated positive allometric growth, whereas values of $b < 1$ indicated negative allometry. All regression procedures were conducted using RMA software (Bohanak and van der Linde, 2004).

4.2.4 Multivariate Analyses

Several multivariate approaches were used to explore the relationship firstly between between terapontid ontogeny and morphology, and subsequently between ontogeny, morphology and diet. All multivariate analyses were based around the morphologies and diets of the 'species-OTUs' identified in Chapter 2. Allometric growth of morphological variables during ontogeny can introduce significant computational biases into multivariate analyses, and appropriate statistical approaches to address the covariation between body size and the size/shape of morphological characters have been extensively debated (Atchley and Anderson, 1978; Reist, 1985; Winemiller, 1991). Use of ratio values or size-standardized measures is a common approach to address this problem, although even minor allometric relationships have been demonstrated as inadequate as a size-standardisation procedure (Atchley and Anderson, 1978; Reist, 1985). Following Reist (1985), removal of body-size effects for each linear morphological variable was achieved in this study by extraction of size residuals from linear regression of each variable against standard length. Log_{10}-transformed values for each morphological character for all measured individuals in all species were combined in a single multispecies dataset for each morphological character and regressed against log_{10}-transformed standard length. Residual values for each individual in a species'

OTU size class from this 'global regression' were then averaged to provide a size-free quantification of each morphometric variable for that OTU. This correction for body size and allometric effects was only carried out on variables where significant allometry was evident from previous regression analyses. If a variable displayed isometric (proportional) growth, no body-size correction was necessary prior to subsequent multivariate analyses.

Eight species analysed in Chapter 2 had insufficient specimen numbers for definition of intraspecific OTUs, with dietary data presented as average species' diets. Similarly, ontogenetic changes in diet or morphology for these eight species could not be considered in this study. Morphological variables for all individuals were included in the multi-species 'global regression', with resultant residual value for each variables averaged over the available size range for these eight species and expressed as a species mean. Size distributions in these eight species were biased towards larger specimens (Chapter 2), so diets and morphologies reflected those of sub-adult and adult size classes. From this point forward the collective 46 average species and species' OTUs will be referred to as 'OTUs', unless otherwise specified. This variable number of OTUs across different species could introduce computational biases toward species represented by multiple OTUs into subsequent analyses, but are difficult to avoid given available data. Table A2.1 (appendix) summarises the raw values for morphological variables (prior to body size-allometric corrections) (46 OTUs x 11 morphological variables).

Principal Components Analysis (PCA) was used as a preliminary analysis to identify the dominant patterns in morphological variation occurring both within and between species. PCA summarizes any similarities in allometric growth between species evident from previous regression analyses as well as highlighting any interspecific divergences in morphology mediated by allometric effects. PCA partitions a resemblance matrix into a set of independent orthogonal axes, the first few of which model the largest proportion of variance among the original variables that can be explained (McCune and Grace, 2002). PCA (using the correlation coefficient cross-products matrix) was performed on a data matrix comprising the average residual values for each linear morphological variable, relative eye position (a raw ratio variable for which no allometric effects were detected in regression analyses), coded integer values for mouth orientation and tooth shape, and average $\log_{10}$-transformed standard length (SL) for each terapontid OTU. Standard length ($\log_{10}$-transformed) was included as a variable because body size, more than any other morphological feature, appears to play a dominant role in determining fish diet (see Wainwright and Richard, 1995; Motta *et al.*, 1995*a*). There has been considerable argument mounted against completely removing all body size effects in ecomorphological studies due to their pervasive ecological and evolutionary significance (Douglas, 1987; Winemiller, 1991). With the ontogenetic

emphasis of this study, inclusion of SL as a morphological variable also allowed the relationship between body size and growth of other morphological characters to be explored. With logarithmic transformation, SL scaled closely to all other average residual-based and integer-coded variables, and being just one of 11 morphological attributes was unlikely to unduly dominate results (see Winemiller, 1991; Winemiller *et al.,* 1995). A Monte-Carlo randomization test (5000 iterations) was conducted to assess the significance of extracted axes.

Canonical correspondence analysis (CCA) (ter Braak, 1986) was used to examine the multivariate relationship between terapontid dietary habits and morphology. CCA selects the linear combinations (canonical variables) from two datasets (in this case with diet as the independent variable and morphology as the dependent), with the constraint that the two canonical variables are maximally correlated. The second and any subsequent CCA axes also select linear combinations of morphological variables that maximize dispersion of diet scores, but with the stipulation of being uncorrelated to previous CCA axes. CCA is a powerful tool for the direct assessment of the association between diet and morphology, and has seen extensive application in studies of fish ecomorphological relationships (Winemiller *et al.*, 1995; Piet, 1998; Pouilly *et al.,* 2003). The morphological dataset used in CCA was the same 46 OTU x 11 morphological variable matrix as that used in PCA. The dietary matrix (46 OTUs x 21 dietary categories) was based on arcsine square-root transformed volumetric data of the dietary proportions for each OTU derived from Chapter 2 and Davis *et al*. 2011b (also presented in Table A2.2). The statistical significance of the diet-morphology axes extracted by the CCA was validated through a Monte-Carlo test (5000 iterations). All ordination analyses were carried out in the PC-ORD® Ver. 5.01 software package (McCune and Mefford, 1999).

Felsenstein (1985) noted that the shared evolutionary history of closely related species (as in this study) will void the underlying assumptions of data independence inherent to many statistical approaches. No corroborated species-level phylogeny currently exists for the Terapontidae, although Vari (1978) outlined a broad generic-level phylogeny for the family. This study also has the additional confounding potential of multiple intra-specific 'pseudo-species' (i.e., OTUs), which will share many morphological and ecological traits. To assess the effect of 'phylogenetic proximity' on diet-morphology interactions a data matrix based on an ordinal taxonomic distance was used to approximate phylogenetic distance (see Winemiller, 1991; Hugueny and Pouilly, 1999; Pouilly *et al.*, 2003 for similar approaches). A value of 1.5 was set for the relationship between con-specific OTUs, 2 for congenerics, 3 for species separated by three or fewer genera in the generic phylogenetic sequence of Vari (1978), 4 for genera within five, and 5 for a generic separation of > 5. The non-parametric Mantel test (Mantel, 1967) was used to assess the

correlation between this phylogenetic proximity matrix and both a diet and a morphological similarity matrix (based on the same dietary and morphological data matrices used in the preceding CCA). A partial Mantel statistic (Smouse *et al.*, 1986) was also calculated between the diet and morphology matrices, while using taxonomy as a co-variate (essentially controlling for the effect of phylogenetic relatedness). Statistical significance was estimated by a permutation test (10,000 permutations), and due to multiple comparisons, a Bonferroni correction (0.05/n) was used to assign significance ($P = 0.05$, divided by 3 = 0.017). Thus, the significance of the diet-morphology relationship was assessed when removing the potentially confounding effect of taxonomic proximity.

Classification and regression tree analysis (CART – Breiman *et al.*, 1984) was used to develop a complementary predictive model for the relationship between terapontid ontogeny, diet and morphology. CART is a powerful and flexible non-parametric method analogous to discriminant function analysis that can be used to assess complex relationships between explanatory (predictor) and response variables (De'ath and Fabricius, 2000). While yet to see application in fish ecomorphological studies CART methodologies have been successfully applied in a range of ecological studies of complex relationships between explanatory and response variables where generalized linear modeling approaches have failed (De'ath and Fabricius, 2000; Vayssières *et al.*, 2000). The CART methodology explains variation in the response variable by using a binary recursive partitioning algorithm to repeatedly partition the dataset into a series of homogeneous, mutually exclusive groups based on the best available predictor variable.

CART analysis offers a number of advantages over traditional statistical methods including: use of a constraint paradigm rather than a correlation-based model; the capacity for modeling of non-linear, hierarchical relationships among mixed variable datasets (interval, continuous, categorical etc.); an invariance to monotonic transformations of data (thereby eliminating the need for data transformation); minimal sensitivity to outlier effects in the final model; and relatively simple interpretation (Bell, 1999; De'ath and Fabricius, 2000). CART models also possess a range of features that have considerable potential advantage over standard correlation-based multivariate approaches in ecomorphological studies, particularly one involving ontogeny. While CART models are unable to account for phylogenetic relationships in the same manner as explicit comparative methods (Harvey and Pagel, 1991), the problems of statistical independence and shared evolutionary history that challenge standard correlation-based models are not applicable to development of CART constraint-based predictive models (see Jones *et al.*, 2006). Similarly, CART models are also insensitive to strong correlations among explanatory variables: instead, collinear variables are identified as surrogates and accordingly strengthen the analysis by maximizing the amount of available information (De'ath and Fabricius, 2000). The capacity to identify

context dependency or utilize 'conditional' information is another defining advantage of CART models (Vayssières *et al.*, 2000). In CART analyses, all variables are considered at each split regardless of their use in previous splits, the CART model thereby selecting the variable containing the most information in the multivariate space it is analyzing, identifying complex, non-additive interactions among explanatory variables. The CART methodology should therefore be well-suited to analyzing patterns among inherently correlated biological traits identified both within and between species.

The CART model was developed using the seven feeding groups identified from hierarchical clustering in Chapter 2 as the categorical response variable (see Table 4.1 for summary). Given that CART models require no data transformation, predictor variables were based on raw values for SL, IL, ML, MW, SNL, HL, ED, EP, BD, mouth orientation and tooth shape for each of the 46 terapontid OTUs. Classification tree models were developed using the STATISTICA v. 7.0 (StatSoft Inc. USA) Classification Trees module, employing a "CandRT" split selection method and the Gini measure goodness-of-fit criterion to determine variable splits. The optimal size of the decision tree (tree pruning) was determined by "V-fold cross validation" (50 sets) based on the one-standard error rule (De'ath and Fabricius, 2000). V-fold cross validation in STATISTICA v. 7.0 divides the original dataset into ten equal, mutually exclusive subsets. Each sub-set is dropped out in turn, with a tree built from remaining subsets used to predict the responses from the omitted subset, with the final optimal tree producing the smallest estimated error rate. In addition to identifying the best predictor variable and its value at each split, the CART analysis also identifies the overall importance of all predictor variables (scaled from 0 to 100) at each split. This is analogous to assessing the importance of a variable in a multiple regression analysis by its overall contribution to all possible regression models, rather than for its importance to the ultimate (optimal) 'best model'.

4.3 Results

4.3.1 Allometric analyses

A variety of significant allometric relationships were evident in the growth of terapontid morphological characters. Summary results of regression analyses are outlined in Table 4.2, with detailed statistical results for each species' morphology shown in Table A2.3 (appendix). Intestinal length was the morphological variable displaying the most pronounced ontogenetic allometry. Positive allometric growth in intestinal length (IL) was observed in all species with the exception of *Amniataba caudovittatus* and *Pingalla midgleyi*, where slopes did not significantly differ from isometry (Table 4.2). Patterns of

allometric increase did, however, differ considerably across species: *Leiopotherapon unicolor*, *Amniataba percoides*, *Hannia greenwayi*, *Terapon jarbua* and *Mesopristes argenteus* demonstrated the lowest relative increases in positive IL allometry; *Hephaestus carbo, Hephaestus epirrhinos, Hephaestus jenkinsi, Hephaestus fuliginosus* and *Varrichthys lacustris* were characterized by moderate positive allometric increases in IL; and *Leiopotherapon aheneus, Syncomistes butleri, Syncomistes trigonicus, Syncomistes rastellus, Scortum parviceps* and *Scortum ogilbyi* demonstrated the highest positive gradients for allometric development of IL. To provide additional context to these results, juvenile terapontids (<50 mm SL), regardless of species, all share relative intestinal lengths (RIL=IL/SL) <= 1.0 prior to significant growth. *Scortum* and *Syncomistes* species develop intestinal lengths between 5 to 8 times standard length in larger size classes. *Hephaestus fuliginosus, Hephaestus jenkinsi* and *Leiopotherapon aheneus* develop moderate relative intestinal lengths (RIL 2-3) in largest specimens. Species such as *Leiopotherapon unicolor*, *Hephaestus carbo, Amniataba percoides* and *Hannia greenwayi*, while demonstrating positive allometric growth in intestinal length, retain relative intestinal lengths ca. 1.0 throughout their life history.

The ontogenetic development of maxilla length (ML) exhibited several scaling patterns (Table 4.2). *Amniataba percoides, Syncomistes trigonicus, Hephaestus fuliginosus, Hephaestus jenkinsi, Hephaestus carbo, Hannia greenwayi* and *Leiopotherapon unicolor* all exhibited significant positive allometric growth in maxilla length during ontogeny. Rates of allometric increase were highest in these last five species indicating that maxilla length became significantly larger in relationship to standard length in these species as they grew. No significant allometric relationships were detected for ML in *Amniataba caudovittatus, Hephaestus epirrhinos, Hephaestus tullensis, Leiopotherapon aheneus, Mesopristes argenteus, Pingalla midgleyi, Syncomistes butleri, Syncomistes rastellus* and *Varrichthys lacustris. Pingalla gilberti* and both *Scortum* species demonstrated significant negative allometric growth during ontogeny, indicating that maxilla length became proportionately smaller as fish size increased. A number of species exhibited positive allometric increases in mouth width (*Amniataba percoides, Syncomistes rastellus, Terapon jarbua, Hannia greenwayi, Hephaestus jenkinsi, Leiopotherapon aheneus, Pingalla lorentzi, Varrichthys lacustris*), with highest positive rates evident in species such as *Hephaestus carbo, Hephaestus fuliginosus* and *Leiopotherapon unicolor*. *Scortum ogilbyi* was the only species demonstrating significant negative allometry in mouth width as body size increased.

Table 4.2 Summary table for allometric relationships between standard length (SL) and intestinal length, (IL), maxilla length (ML), mouth width (MW), head length (HL), snout length (SNL), eye diameter (ED), body depth (BD) and relative eye position (EP). +ve = positive allometric growth, -ve = negative allometric growth; blank cells = no significant allometric relationship detected.

	Morphological variables							
Species	**IL**	**ML**	**MW**	**HL**	**SNL**	**ED**	**EP**	**BD**
Amniataba caudovittatus						-ve		
Amnitatba percoides	+ve	+ve	+ve	-ve	-ve	-ve		+ve
Hannia greenwayi	+ve	+ve	+ve			-ve		+ve
Hephaestus carbo	+ve	+ve	+ve	-ve	-ve	-ve		+ve
Hephaestus epirrhinos	+ve							
Hephaestus fuliginosus	+ve	+ve	+ve	-ve	-ve	-ve		+ve
Hephaestus jenkinsi	+ve	+ve	+ve	-ve	-ve	-ve		+ve
Hephaestus tulliensis	+ve			-ve		-ve		
Leiopotherapon aheneus	+ve		+ve		+ve	-ve		
Leiopotherapon unicolor	+ve	+ve	+ve	-ve	-ve	-ve		-ve
Mesopristes argenteus	+ve					-ve		
Pingalla gilberti	+ve	-ve		-ve		-ve		
Pingalla lorentzi	+ve		+ve	-ve		-ve		+ve
Pingalla midgleyi				-ve		-ve		
Scortum ogilbyi	+ve	-ve	-ve	-ve	-ve	-ve		+ve
Scortum parviceps	+ve	-ve		-ve	-ve	-ve		+ve
Syncomistes butleri	+ve			-ve	-ve	-ve		+ve
Syncomistes rastellus	+ve		+ve	-ve	-ve	-ve		+ve
Syncomistes trigonicus	+ve	+ve	+ve		+ve	-ve		+ve
Terapon jarbua	+ve	-ve	+ve	-ve		-ve		
Varrichthys lacustris	+ve		+ve	-ve	-ve	-ve		

Negatively allometric growth in head length was identified for all species with the exception of *Amniataba caudovittatus, Hannia greenwati, Hephaestus epirrhinos, Leiopotherapon aheneus, Mesopristes argenteus* and *Syncomistes trigonicus*, where no allometric relationship was detected. *Leiopotherapon aheneus* and *Syncomistes trigonicus* were the only species to demonstrate positive allometric increases in snout length, with several species exhibiting negative allometry (*Amniataba percoides, Hephaestus carbo, Hephaestus fuliginosus, Hephaestus jenkinsi, Leiopotherapon unicolor, Scortum ogilbyi, Scortum parviceps, Syncomistes butleri, Syncomistes rastellus* and *Varrichthys lacustris*). Significant negative allometric scaling in eye diameter was identified in all species with the exception of *Hephaestus epirrhinos*, indicating that eye diameter became disproportionately smaller in

most species as they increased in size. Significant positive allometric increases in body depth were evident in a large number of species, with only *Leiopotherapon unicolor* demonstrating significant negative allometry in body depth. No significant allometric effects were detected for relative eye position, indicating relative stability of this measure through all species' growth.

4.3.2 Multivariate Analyses

Principal components analysis

The first two PC axes of the morphological dataset explained a cumulative 63.7% of total variance in morphological space (Figure 4.2*a*, Table 4.3). Only the eigenvalues of the first two principal components were considered meaningful according to the Rnd-Lambda stopping rule (Peres-Neto *et al.*, 2005), with Monte-Carlo simulations similarly highlighting statistical significance for these two axes at $P < 0.05$. PC1 accounted for 46.95% of the variance, PC 2 accounted for 17%. Size-adjusted residual values for head length, maxilla length, and eye diameter were the dominant variables driving positive OTU distribution along PC1. OTUs with the highest positive scores on PC1 included *Hephaestus epirrhinos*, *Mesopristes argenteus*, and the OTUs of *Leiopotherapon unicolor*, *Hephaestus fuliginosus*, *Hephaestus carbo*, *Hephaestus jenkinsi* and *Terapon jarbua*. Mouth orientation, tooth shape and size adjusted residual values for intestinal length had significant negative loadings along PC 1. Negative scores on PC1 were demonstrated by the OTUs of *Scortum*, *Syncomistes* and *Pingalla* species (all with varying degrees of flattened tooth shape and sub-terminal mouth orientations). Size-adjusted residual values for body depth and eye diameter had significant positive loadings on PC2, while eye position and maxilla length had the highest negative loadings.

Table 4.3 Axis eigenvalues, proportion of variance modeled, cumulative variance modeled and variable loadings from the first two principal components of PCA of terapontid morphological characters.

	PCA	
	Axis 1	**Axis 2**
Eigenvalue	5.17	1.87
Proportion (%) of variance explained	46.96	17.0
Monte-Carlo test (*P*-value, 5000 permutations)	<0.001	0.01
Morphological variable loadings		
Standard length	-0.016	0.307
Intestinal length	-0.354	-0.136
Maxilla length	0.352	-0.302
Head length	0.364	-0.027
Snout length	0.252	0.012
Mouth width	0.295	-0.265
Eye diameter	0.317	0.363
Body depth	0.0244	0.655
Eye position	0.293	-0.341
Tooth shape	-0.372	-0.151
Mouth orientation	-0.379	-0.160

Figure 4.2*b* indicates ontogenetic trajectories for several species that had three or more OTUs defined through their life history. These trajectories emphasize the complex role of allometric changes in morphological characters in driving the morphological divergence within and among species. The early OTUs of most species cluster relatively closely in morphological space, indicating the similarity in morphology of juvenile terapontids before substantial growth occurs. As species increase in size, allometric growth results in increasing morphological divergence and progressively diminished overlap in morphological space. The increasingly larger OTUs of species such as *Syncomistes butleri*, *Scortum ogilbyi* and *Scortum parviceps* which exhibit the most pronounced positive allometry in intestinal length (as well as significant negative allometry in maxilla length in the last two species; Table 4.2) progressively diverged negatively along PC 1. In contrast, species such as *Leiopotherapon unicolor*, *Hephaestus fuliginosus*, *Hephaestus carbo* and *Hannia greenwayi*, which exhibited pronounced positive allometry in characters such as maxilla length and width, showed substantial positive divergence along PC 1. Body depth was the variable with the strongest loading on PC 2 (positive). With many species displaying significant positive allometry in body depth (Table 4.2), there was also considerable corresponding positive divergence along PC 2 as these species increased in size.

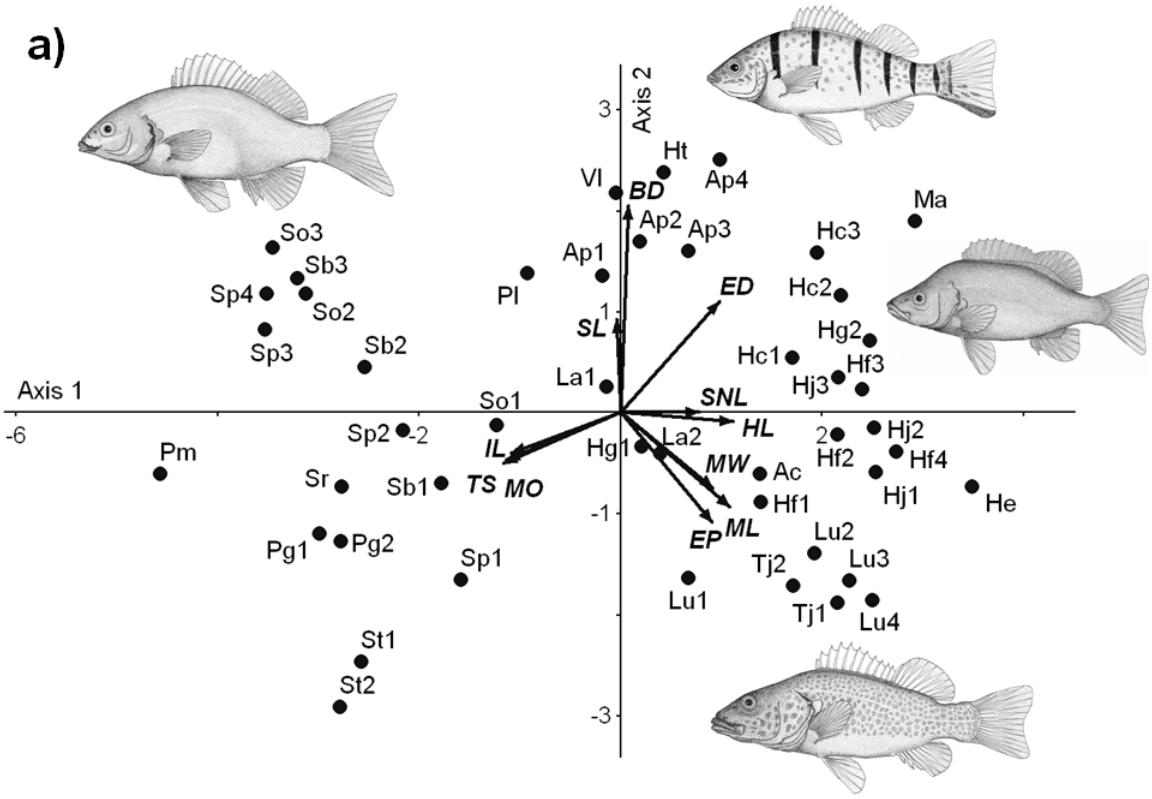

Figure 4.2*a*. Principal components analysis of nine mensural and two coded morphometric variables for 46 terapontid OTUs. Eigenvectors (italicized) for morphological variables coded as follows: SL-standard length; IL-intestinal length; ML; maxilla length; MW-mouth width; HL-head length; SNL-snout length; ED-eye diameter; EP-eye position; BD-body depth; MO-mouth orientation; TS-tooth shape.

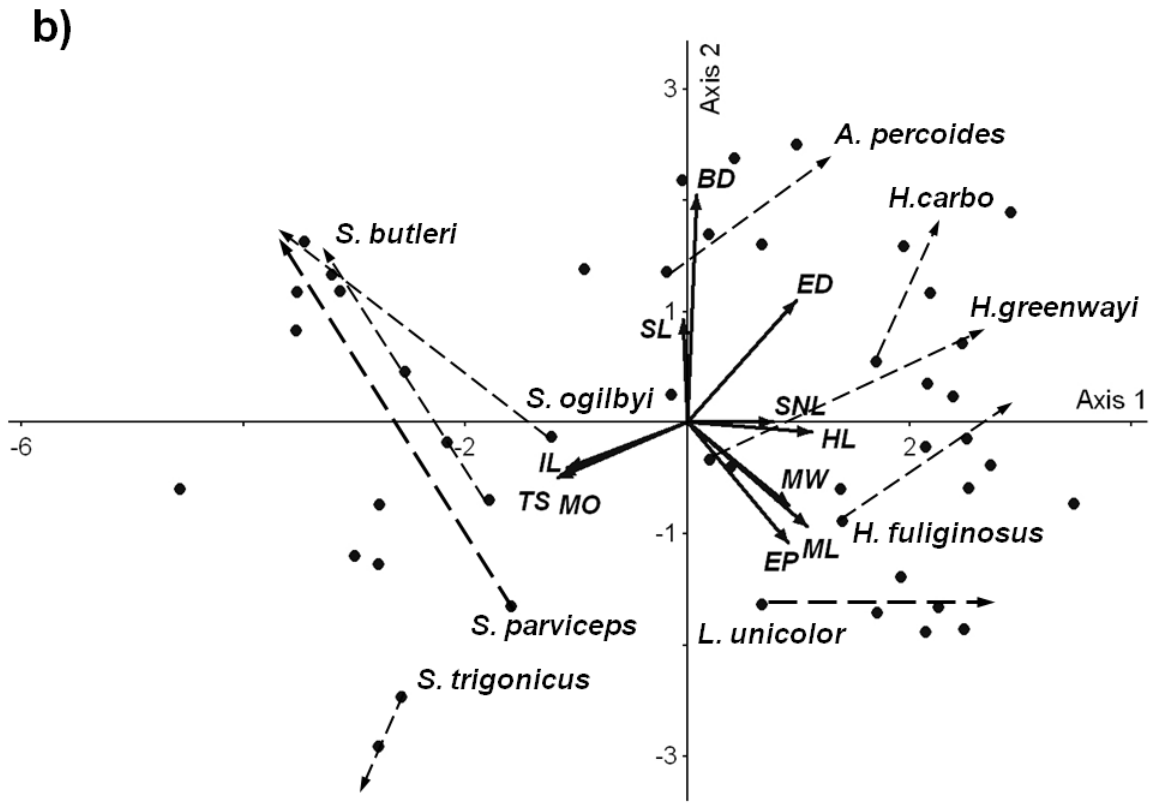

Figure 4.2*b* Same PCA with approximate morphological trajectories (dashed) occurring through ontogeny for species OTUs depicted by arrows.

4.3.3 Canonical correspondence analysis

Canonical correspondence analysis revealed a significant relationship between diet and morphology (Monte-Carlo test: $P = 0.001$) for the first three axes, accounting for 50% of the total variation in fish dietary composition (Table 4.4). Interpretation was limited to axis 1 (23%) and axis 2 (21%), as these two axes had the largest r^2 values. The first CCA axis was very strongly positively correlated with standard length, reflecting a pronounced body-size gradient. The first morphological axis was paired with a first dietary axis that was positively influenced by feeding on aquatic macrophytes, terrestrial vegetation, terrestrial vertebrates, miscellaneous plant material, filamentous algae and fish, and decreasing consumption of zooplankton, aquatic dipteran larvae and ephemeropteran larvae. High positive species scores along the first axis were exhibited by a range of large-bodied OTUs, regardless of feeding group designation (Figure 4.3*b*). The lowest (negative) scores on axis 1 were demonstrated by the early, small-sized OTUs of a wide range of species (all invertivores). In species with multiple OTUs this highlights a

common shift away from invertebrate-dominated diets in smaller size classes, regardless of eventual dietary habits in adult size classes.

Table 4.4 Summary of the results from canonical correspondence analysis (CCA) relating OTU diets to morphological variables. Dietary items with the scores >0.5 on each CCA axis are also listed.

Total variance ("inertia") in the dietary data = 1.3024		**Axis 1**	**Axis 2**	**Axis 3**
Eigenvalue		0.299	0.262	0.09
% of variance explained		23.0	20.1	6.9
Monte-Carlo test (*P*-value, 5000 permutations)		0.001	0.001	0.001
Pearson Correlation: Specis-Environment		0.974	0.956	0.806
Diet/morphology intraset correlations*	Variable			
	SL	0.953	0.194	0.041
	IL	0.068	-0.890	-0.093
	ML	-0.264	0.659	-0.623
	HL	-0.141	0.630	-0.064
	SNL	-0.071	0.248	-0.251
	MW	-0.111	0.682	-0.517
	ED	-0.053	0.611	0.135
	BD	0.071	0.059	0.454
	EP	-0.046	0.526	-0.294
	Tooth shape	0.162	-0.865	-0.294
	Mouth orientation	0.128	-0.750	-0.079
Dietary Composition	Aquatic Diptera	-0.875		
	Ephemeroptera	-0.846		
	Zooplankton	-1.218		
	Macrocrustacea		0.990	-0.578
	Fish	0.527	1.271	-0.840
	Aquatic macrophytes	1.284		1.394
	Terrestrial vegetation	1.428	0.500	
	Terrestrial vertebrates	1.995	1.314	-0.806
	Terrestrial invertebrates		1.040	
	Scales		1.314	-2.528
	Detritus		-1.294	-0.606
	Fil. algae	0.664	-0.606	
	Misc. plant	0.912	-0.922	
	Inorganic		-0.966	
	Ostracoda	-0.711		0.937
	Molluscs		0.926	0.792
	Other aq. Invert.		0.580	
	Odonata larvae		0.701	

* Correlations are "intraset correlations" of ter Braak (1986)

The second axis was strongly positively correlated with size adjusted residual values for mouth width, maxilla length, head length and eye diameter, and negatively correlated with size adjusted residual intestinal length, tooth shape and mouth orientation. The second morphological axis was positively influenced by feeding on macrocrustacea, fish, fish scales and terrestrial vertebrates and negatively influenced by consumption of detritus, inorganic material, miscellaneous plant material and filamentous algae. These outcomes indicate that high residual values for morphological variables, such as maxilla length, mouth width, head length and eye diameter, were positively correlated with carnivorous dietary habits in terapontids, particularly consumption of larger prey items as fish increased in size. In contrast, high residual values for intestinal length, as well as flattened tooth shape and sub-terminal mouth orientation, were positively correlated with detritivory and consumption of inorganic material, miscellaneous plant material and filamentous algae. High residual values for intestinal length were negatively correlated with carnivory, indicating that OTUs with long intestines consumed minimal animal material. Similarly, flattened tooth shape was negatively correlated with consumption of animal prey, highlighting a significant relationship between conical dentition and carnivory.

There was considerable separation of OTUs in geometric space with regard to feeding group classifications along this axis (Figure 4.3*b*). The macrophagous carnivores and macrophagous omnivores, associated with conical dentition, oblique mouth orientation, large body size and high values for mouth width, maxilla length and eye diameter were predominantly positively distributed along axis 2 in the upper right quadrant of the biplot. The shorter intestinal lengths of the macrophagous carnivores in comparison to the macrophagous omnivores arranged them more positively along axis 2. Conversely, the herbivorous and detritivorous-algivorous OTUs, characterized by high RILs, flattened tooth shape and terminal or sub-terminal mouths, dominated the lower right quadrant of the biplot. Several species were also positioned with morphologies apparently discordant with their observed diet. *Varrichthys lacustris* and *Amniataba caudovittatus*, both classified as herbivores, were positively distributed along axis 2, morphologically aligning with omnivorous species. *Pingalla lorentzi*, a nominal meiophagous omnivore, was strongly negatively positioned along axis 2, aligning most closely with detritivorous-algivorous species on the basis of morphology.

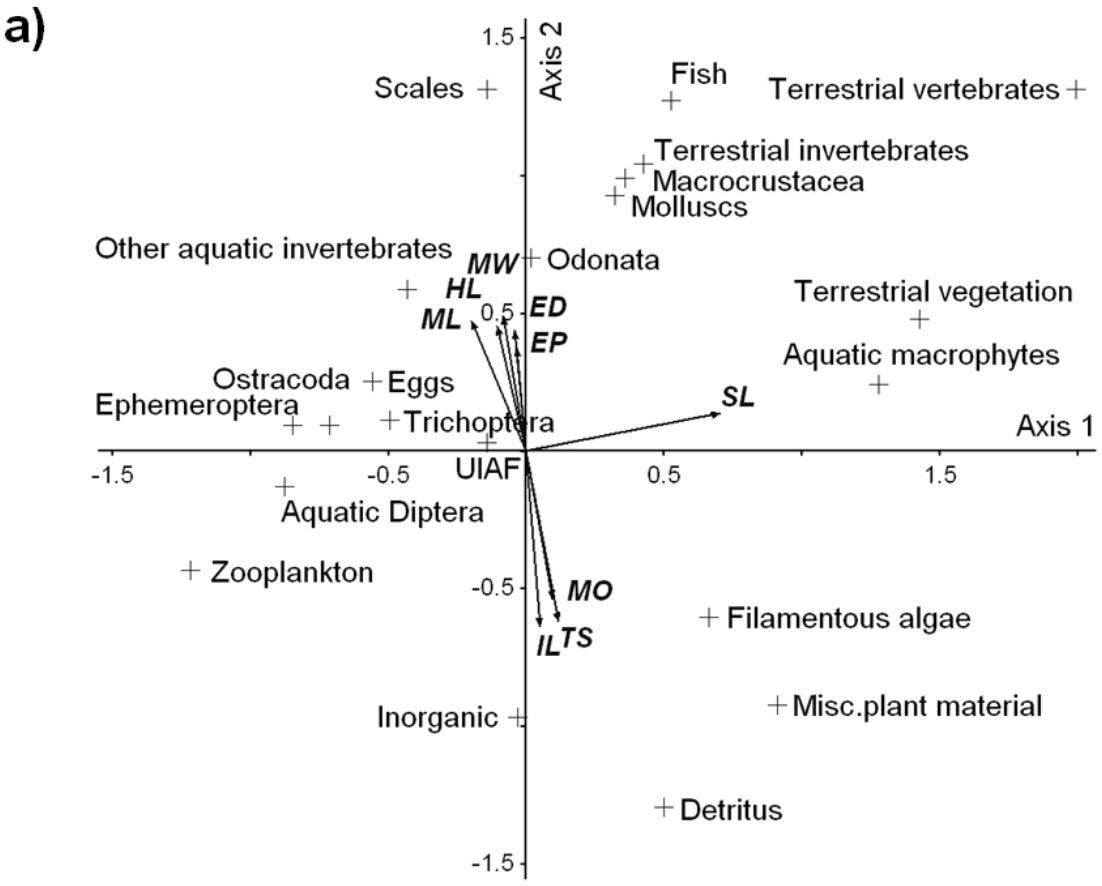

Figure 4.3 Canonical Correspondence Analysis ordination biplot with terapontid dietary composition related to eleven morphological variables. Overlays for morphological attributes and dietary items (*a*) and fish (*b*) are presented separately for clarity. Fish OTUs are categorised according into seven feeding groups: open triangles represent lepidophages, solid triangles represent macrophagous carnivores, inverted open triangles represent meiophagous omnivores, inverted solid triangles represent macrophagous omnivores, open diamonds represent herbivores, closed solid diamonds represent detritivores, open circles represent invertivores (Davis *et al.,* 2011b).

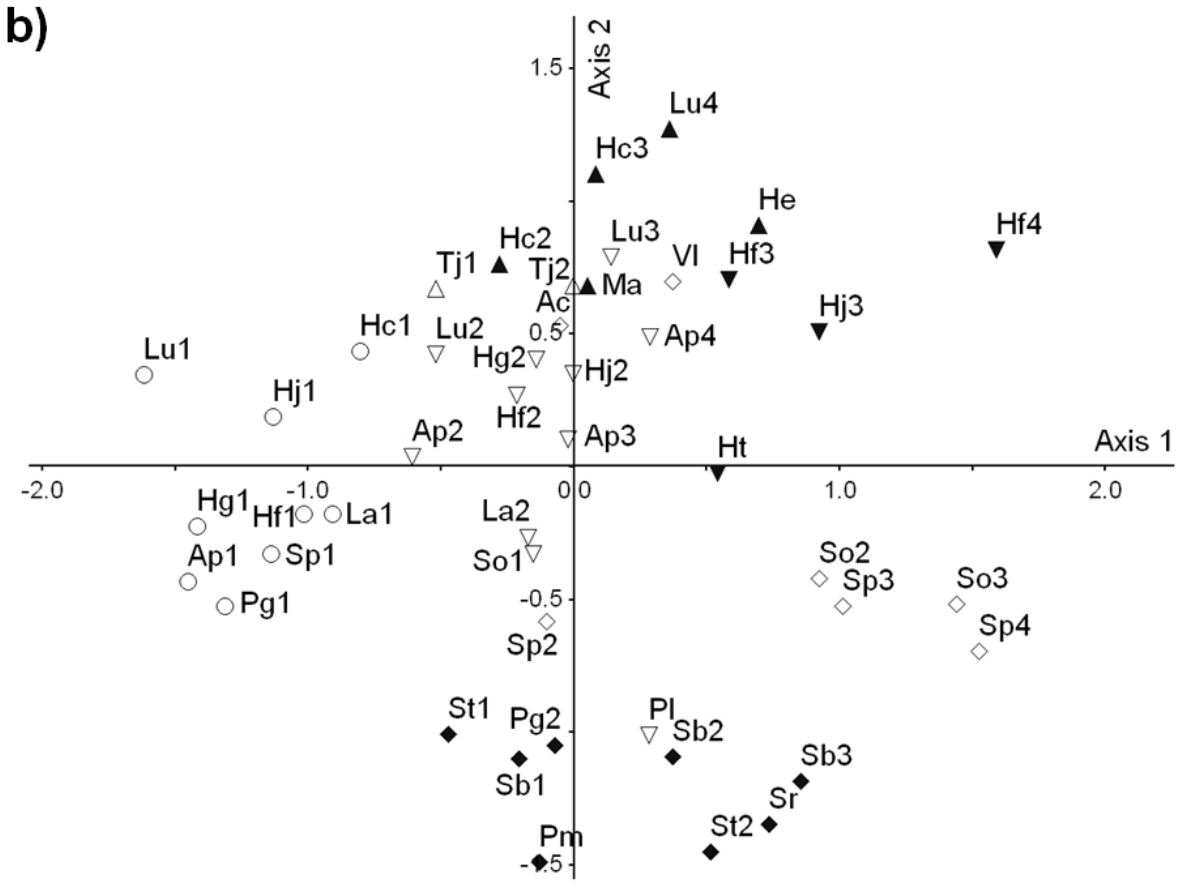

Figure 4.3b Canonical Correspondence Analysis ordination biplot with terapontid dietary composition related to eleven morphological variables. Overlays for morphological attributes and dietary items (*a*) and fish (*b*) are presented separately for clarity. Fish OTUs are categorised according into seven feeding groups: open triangles represent lepidophages, solid triangles represent macrophagous carnivores, inverted open triangles represent meiophagous omnivores, inverted solid triangles represent macrophagous omnivores, open diamonds represent herbivores, closed solid diamonds represent detritivores, open circles represent invertivores (Davis *et al.,* 2011b).

The complex effects of increases in body size (standard length) and allometric growth evident in regression and PCA analyses are also reflected in CCA outputs. The smallest invertivorous OTUs of many species clustered closely indicating general similarity in morphology and diet at low standard lengths. The strong body size gradient evident in CCA axis 1 is coupled with increasing divergence (both positive and negative) along CCA axis 2 in many species that demonstrated some of the most pronounced allometry in variables with the strongest correlations to diet. The OTUs of species with the most pronounced positive allometry in intestinal length (*Scortum* and *Syncomistes* species) increasingly diverge negatively along CCA axis 2 highlighting increasing consumption of plant and detrital material as fish

(and particularly intestinal length) increase in size. Several species (*L. unicolor* and *Hephaestus* species) that exhibit pronounced positive allometric increases in maxilla length and mouth width increasingly diverge positively along CCA axis 2 as consumption of prey items such as fish and macrocrustacea similarly increase.

4.3.4 Mantel tests

The result of the Mantel tests showed pairwise correlations between dietary, morphological and phylogenetic distance matrices all to be statistically significant (Table 4.5). The partial Mantel test, using taxonomic proximity as a covariable, demonstrated that the diet-morphology relationship was still significant when controlling for the effect of taxonomy. Thus, a phylogenetic effect is apparent in diet-morphology relationships within the studied terapontids, but relationships are still significant when this effect is removed.

Table 4.5 Correlation coefficient and statistical significance values for Mantel test and partial Mantel tests on diet, morphology and taxonomy distance matrices.

Comparisons	Covariable	*r* (Mantel)	*P**
Diet-morphology		0.495	<0.0001
Diet-taxonomy		0.149	<0.0001
Morphology-taxonomy		0.277	<0.0001
Diet-morphology	Taxonomy	0.478	<0.0001

* Bonferroni-corrected probability = 0.017 (0.05/3)

4.3.5 CART model

Classification and regression tree analysis produced a simple tree separating six terapontid feeding groups (five splitting nodes and six terminal nodes) (Figure 4.4). The final decision tree used five of the eleven morphological variables to classify the six feeding groups. The overall importance value of predictor variables to the development of the final classification tree (scaled from 100 = most important to 0 = least important) were as follows: intestinal length 100; standard length 89; mouth width 75; maxilla length 71; snout length 70; tooth shape 65; eye diameter 64; head length 61; mouth orientation 59; body depth 58; and eye position 53. Therefore all variables played a role in construction of the tree, although the last three made relatively minor contributions to splitting criteria across all nodes of the tree. The CART

model correctly classified feeding group membership on the basis of morphology for 38 of the 46 OTUs used in the analysis (82.6% accuracy), with a total misclassification rate of 17.4% (Table 4.6).

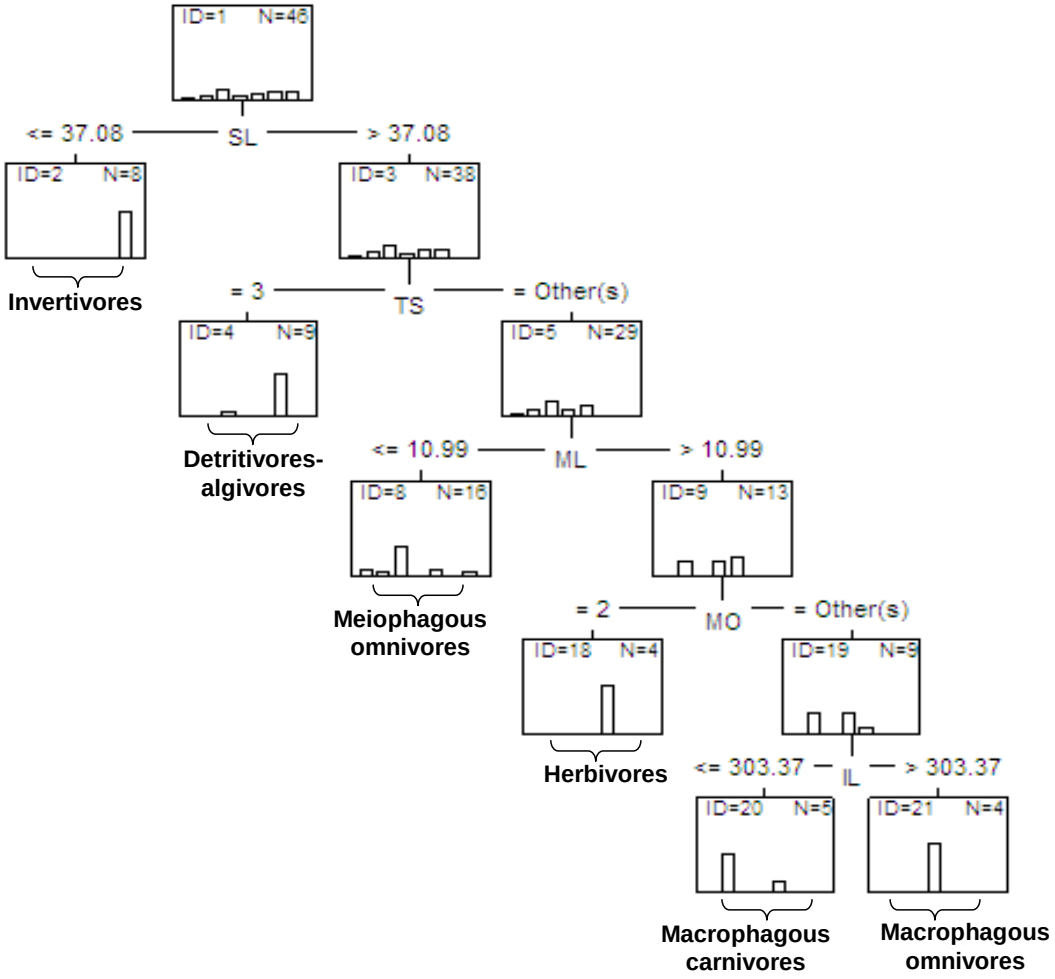

Figure 4.4 Classification tree for seven terapontid feeding groups based on eleven morphological variables. Each of the five splits (non-terminal nodes) is labelled with the variables determining each split as follows: SL-standard length; TS-tooth shape; ML-maxilla length; MO-mouth orientation; IL-intestinal length. Each node is labeled with an identifier (ID) designating node number and the number of observations (OTUs) within each node (N). Histograms outline the distribution of OTUs within each node.

Two feeding groups were correctly classified in their entirety (Table 4.6): they were the detritivores-algivores (node 18) and the macrophagous omnivores (node 21). One feeding group, the lepidophagous OTUs of *Terapon jarbua*, was entirely misclassified, aligning with macrophagous carnivores according to their morphology. Other misclassified OTUs included Hc1 and Hc2, which were designated as macrophagous carnivores and invertivores respectively, but were classified as meiophagous omnivores on the basis of morphology. *Pingalla lorentzi* (a meiophagous omnivore on the basis of dietary data) was

predicted to align with other congenerics as a detritivore-algivore on the basis of morphology. Three herbivores, *Amniataba caudovittatus*, *Varrichthys lacustris* and the second OTU of *Scortum parviceps* (40-139 mm SL) were classified as meiophagous omnivores.

Table 4.6 Misclassification table for the seven terapontid feeding groups based on a CART model with seven nodes. Rows are *a priori* feeding groups (Chapter 2), columns are feeding groups predicted from morphology by CART analysis. Row totals are indexed as number correct/number misclassified.

	Lepidophages	Macrophagous carnivores	Meiophagous omnivores	Macrophagous omnivores	Herbivores	Detritivores-algivores	Invertivores	Totals	Misclassified OTUs
Lepidophages	-	2	-	-	-	-	-	0/0	-
Macrophagous carnivores	2	4	1	-	-	-	-	4/3	Tj1, Tj2,Hc2
Meiophagous omnivores	-	-	10	-	-	1	-	10/1	Pl
Macrophagous omnivores	-	-	-	4	-	-	-	4	-
Herbivores	-	-	3	-	5	-	-	5/3	Ac, So2, Vl
Detritivores-algivores	-	-	-	-	-	7	-	7	-
Invertivores	-	-	1	-	-	-	8	8/1	Hc1
	Total misclassification rate:							8/46	

4.4 Discussion

The role of body size and allometry in terapontid trophic habits

Ontogeny and its interaction with morphology are dominant influences on the dietary ecology of terapontids, and divergence in morphology during ontogeny is paralleled by a divergence in dietary habits for most species. Results of CCA highlighted body size *per se* as one of the most, if not the most, important variables influencing the feeding habits of terapontids during their life history. Body size has been long appreciated as an important determinant of species' diets (Motta *et al.*, 1995*a*; Wainwright and Richard, 1995; Pusey *et al.*, 2000). However, relationships between body size and diet should be treated with caution from an ecomorphological perspective (see Wainwright and Richard, 1995). Attributing the ontogenetic changes in diet directly to body size is misleading, as it is typically changes in key morphological characters that themselves change with size (often allometrically), and that determine feeding performance. Ontogenetic dietary changes are typically induced by changes associated with growth of morphological structures correlated with body size (Wainwright and Richard, 1995), and

allometric changes in morphology are commonplace in fishes (Kramer and Bryant, 1995; Wainwright and Richard, 1995; Cassemiro *et al.*, 2008).

This chapter indicates that the interactions between body size and allometric growth of particular morphological characters play an important role in the dietary habits of terapontids. Differences in the patterns of allometric growth of characters such as intestinal length, maxilla length and mouth width were largely responsible for driving a considerable component of the morphological and dietary divergence evident across terapontid ontogenies. Variations in the development of intestinal length were a particularly influential factor in the differentiation in terapontid morphology. Positive allometric growth in intestinal length, regardless of dietary predilections, has been documented in numerous fish species (Kramer and Bryant, 1995). Terapontids display a similar pattern, with even fishes that assume entirely carnivorous dietary habits throughout their life history (*Hephaestus carbo*, *Mesopristes argenteus*) exhibiting significant positive allometric growth in intestinal length. Intestinal length was strongly positively associated with detritivory and to a lesser extent consumption of filamentous algae, and negatively associated with carnivory in CCA, as has been documented in other studies (Piet, 1998; Hugueny and Pouilly, 1999). However, tooth shape and mouth orientation had stronger correlations to the same diet-morphology relationship that emerged from the CCA analysis. Several species that exhibited the highest rates of allometric increase in intestinal length (*Scortum* and *Syncomistes* species) also demonstrated some of the most profound ontogenetic dietary shifts, transitioning from carnivory-omnivory in small size classes to specialized detritivory and herbivory in largests OTUs.

Morphometrics relating to mouth gape have been repeatedly demonstrated as correlating positively with prey size (Gatz, 1979; Wainwright and Richard, 1995; Piet, 1998) and piscivorous feeding habits (Winemiller *et al.*, 1995). Similar results were obtained in this study, with both maxilla length and mouth width the two variables most positively correlated with consumption of large aquatic prey (fish, macrocrustacea). This chapter also highlighted the positive correlation of maxilla length and mouth width with consumption of large allochthonous prey such as terrestrial fruits and terrestrial vertebrates. A large gape has been previously identified as the morphological character most strongly associated with consumption of terrestrial food resources within the Terapontidae (Davis *et al.*, 2010). For several of these macrophagous species (*Leiopotherapon unicolor*, *Hephaestus fuliginosus*), ontogenetic increases in gape size were also associated with significant positive allometry, indicating that the gape gets proportionately larger as fish grow (see also Piet, 1998; Wainwright and Richard, 1995). Interestingly, the significant negative allometry evident for mouth gape observed in the herbivorous *Scortum* species studied here parallels that documented in other herbivorous fishes (see Piet, 1998).

The relationship between terapontid diet and morphology

Despite complex associations between body size and growth of other morphological variables, the results show that morphology has a significant relationship to diet, both within and between terapontid species. Canonical correspondence analysis focusing specifically on the diet-morphology interaction identified a significant relationship between morphology and variation in diet. However, the amount of variation explained, while statistically significant, accounted for only 50% of data variance, with changes in standard length alone accounting for a large part of explained variability. Clearly, behavioural, ecological, physiological, historical and ontogenetic factors may all interact to influence the relationship between morphological and ecological characters (Motta *et al.*, 1995*b*). The number of morphological variables (11 in total) used in this study was lower than in some previous studies (see Gatz, 1979; Winemiller *et al.*, 1995). Additionally, the diversity of dietary categories (21 in total) used in this study was relatively broad, which may have masked some associations between morphology and diet.

The foraging ecology of the terapontids may also have contributed to study results. While trophically diverse by Australian standards, few terapontids, except perhaps some herbivores and detritivore-algivores, exhibit highly specialized dietary habits (Chapters 1 and 2; Davis *et al.*, 2011a; 2011b). Invertivory and various forms of omnivory – trophic habits that seem to require a lower level of morphological specialization – are prevalent across the terapontid OTUs described here. As noted by Pouilly *et al.* (2003), studies of assemblages dominated by invertivores and omnivores frequently conclude relatively weak relationships between diet and morphology (see Douglas and Matthews, 1992; Motta *et al.*, 1995a).

The Terapontidae have a relatively conservative percomorph body plan (Mees and Kailola, 1977). Results of this study, however, highlight significant morphological variability within the constraints of this broader morphotype, with much of this variability correlating with dietary habits. Appreciation of the dominant role of body size within a species' ecomorphology is also clearly important in understanding the often pronounced ontogenetic dietary changes exhibited by many fish species. Body size and its relationship to ontogenetic development of many morphological parameters appear to constrain the potential trophic habits of many terapontids.

Classification and regression tree analysis provided a useful complement to the more traditional CCA approach, producing a predictive decision tree model that indicated a strong coupling of morphology to dietary habits, while accounting for ontogeny. The first major split of the CART model separated all small

juveniles, regardless of species, from larger size classes, and it was this division that perhaps underlines the value of CART approaches. This split has considerable justification from an ecological perspective as ontogeny would be expected to constrain morphology and diet to the greatest degree in these small fishes. The inter-specific differences in morphology evident in larger size classes due to changes in body size and/or allometric divergence in parameters such as IL and ML are not expressed in juveniles. Additionally, the dietary associations of variables such as dentition would be expected to be linked to other variables such as intestinal length to manifest significant relationships to diet. Subsequent splits in the CART model used variables other than standard length to differentiate feeding groups. This initial split makes intuitive ecological sense, but represents an issue that standard 'generalised linear models' would have minimal capacity to address adequately.

Some CART misclassifications (*Pingalla lorentzi*, *Amniataba caudovittatus, Varrichthys lacustris*) were possibly due to erroneous initial feeding classifications based on small sample sizes and/or single collection occasions (Chapter 2), which provided only a limited representation of diets. Similarly, the CART model misclassified the lepidophagous OTUs of *Terapon jarbua*, including this species with macrophagous carnivores on the basis of morphology. Apart from the significant consumption of scales that distinguished the species, the diet of *Terapon jarbua* was otherwise very similar to that of macrophagous carnivores, including large proportions of fish, macrocrustacea and terrestrial invertebrates (Chapter 2). Some misclassifications are therefore possibly more an artifact of the initial *a priori* dietary designations resulting from dietary datasets and cluster procedures in Chapter 2 than inadequacies of the CART model.

CART models have been used primarily to this point in species-community distribution and habitat modeling applications (see De'ath and Fabricius, 2000; Vayssières *et al*., 2000; Olden *et al.*, 2008). This study suggests that CART models may also have considerable utility in ecomorphological analyses, particularly ontogenetic assessments, where complex hierarchical or non-linear interactions may exist between characters such as body size and other variables (see also Karels *et al.* 2004). The capacity to handle mixed datasets is particularly germane to organisms such as fish, where many morphological characters are either discrete and/or categorical in nature (e.g., presence/absence of sensory barbels, gill filament or fin shape), or are difficult to quantify with a simple continuous measure (e.g., tooth form). While CART models circumvent some of the data non-independence issues that confront standard statistical approaches, they can provide none of the evolutionary insight into ecomorphological relationships that genuine phylogenetically based comparative methods confer. CART models may be particularly useful in circumstances such as this study where robust phylogenetic information is

unavailable, and may also have considerable scope for identifying specific variables for inclusion in explicit phylogenetically based comparative methods.

The role of phylogeny

The Mantel tests demonstrated the significant relationship of phylogeny with both morphology and diet. While ecomorphological relationships were significant when accounting for taxonomic proximity, phylogenetic relationships may still have exerted an effect on results of multivariate analyses assessing diet-morphological relationships. A robust species-level phylogenetic framework and explicit comparative approach (Felsenstein, 1985; Harvey and Pagel, 1991) may provide a more powerful insight into the morphology, ecology and evolutionary relationships of the Terapontidae. Comparative approaches utilizing morphological characters very similar to those used in this chapter have been used to great effect in recent phylogenetically informed assessments of dietary ecomorphology (see Higham *et al.,* 2006; Wagner *et al.,* 2009). However, it has been suggested that if an analysis has high taxonomic diversity (i.e. high ratio of clades to species), any integration of phylogenetic information is unlikely to greatly affect the significance of any identified relationships (see Weathers and Siegel, 1995; Ricklefs and Starck, 1996). The analysis here was confined to a phylogenetically diverse dataset from a single family (21 species across 10 genera), so it is likely that inclusion of phylogenetic information would not greatly alter outcomes.

Terapontid dietary ecomorphology versus global examples.

Morphology explains a significant component of both the interspecific and intraspecific dietary variability across terapontid diets. Many of the diet-morphology relationships evident in the terapontids (relative intestinal length and sub-terminal mouth orientation positively correlated with detritivory; intestinal length negatively correlated with carnivory, conical tooth shape, maxilla length, mouth width, head length and eye diameter positively correlated with piscivory and prey size) parallel those documented in other fish assemblages (Winemiller *et al.,* 1995; Piet, 1998; Hugueny and Pouilly, 1999; Pouilly *et al.,* 2003). A criticism leveled at correlative ecomorphological research (such as this study) is that it does not provide an explicit functional link between observed patterns of prey use and morphology (Norton *et al.,* 1995). However, the repeated demonstration of re-occurring ecomorphological relationships among distantly related fish groups provides compelling evidence for convergence shaped by evolutionary forces, reducing the likelihood that the ecomorphological pattern is a random event. This chapter indicates that the ecomorphological pattern observed across the Terapontidae is not a chance phenomenon, but represents a common parallel with many other distantly related fishes, fashioned by similar selective pressures and adaptive processes.

Chapter 5: Gut-content and stable-isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river

In press in Freshwater Biology (2012).

5.1 Introduction

Body size is a fundamental determinant of fish trophodynamics, with size-related dietary shifts prevalent in the life histories of many fish species (Mittelbach and Persson, 1998; Jennings *et al.*, 2002; Davis *et al.*, 2011b). The resulting 'ontogenetic niches' (*sensu* Werner and Gilliam, 1984) can greatly complicate our understanding of feeding interactions in aquatic food webs. Dietary studies have traditionally relied upon stomach content analysis (SCA) to identify carbon sources or food web structure (Hyslop, 1980). SCA has several inherent limitations such as providing only a short-term (hours to days) dietary 'snapshot' of recently ingested items (Hyslop, 1980), the requirement of high sampling frequency to obtain a reliable time-integrated overview of dietary habits, and minimal indication of the degree of assimilation of dietary items (Parkyn *et al.*, 2001). These limitations have led to increasing emphasis on stable isotope analysis (SIA) as a tool to assess aquatic food web structure and function (Vander Zanden *et al.*, 1997; Post, 2002).

SIA uses the differential fractionation of naturally occurring stable isotopes (typically carbon and nitrogen) of tissues as dietary tracers through food chains (Petersen and Fry, 1987). Carbon isotope ($\delta^{13}C$) signatures of consumers directly reflect that of their prey items, changing <1‰ per trophic level, thereby providing information on the sources of organic material supporting a food chain. In contrast, nitrogen isotopes ($\delta^{15}N$) are useful in identifying the trophic level at which an animal typically feeds (Peterson and Fry, 1987; Post, 2002). $\delta^{15}N$ increases at an average of 3.4 ‰ per trophic level (Minagawa and Wada, 1984; Peterson and Fry, 1987; Post, 2002) although fractionation rates can vary significantly in relation to species' phylogenetic position or dietary habits (Vanderklift and Ponsard, 2003; Mill *et al*., 2007). The combined measurement of both isotopes can therefore provide insights into both source materials and species' trophic levels. A major advantage of isotopic approaches is that they provide temporally integrated information (weeks to months) on dietary habits, reflecting foods that are actually assimilated by the consumer. Stomach content data can, however, provide information on taxonomic and size composition of diets that cannot be inferred from stable isotope analysis alone. The specifics of spatio-

temporal feeding patterns or predator-prey interactions in complex systems where species consume a diversity of prey items may also be difficult to elucidate from stable isotope ratios used in isolation (see Layman *et al*., 2005). A combination of isotopic approaches and traditional stomach content analysis techniques is increasingly being used to improve interpretation of feeding studies in various aquatic food webs (Parkyn *et al*., 2001; Mantel *et al*., 2004; Layman *et al*., 2005a).

SIA is also being increasingly used to clarify ontogenetic dietary shifts in fishes. The use of δ^{15}N as an indicator of trophic position suggests that size-related dietary transitions are common, although the magnitude of effects can vary (Jennings *et al.*, 2002; Galván *et al.*, 2010). The majority of demonstrated isotopic shifts have been from simple, often plankton-driven marine or freshwater food chains where size-structured feeding is expected to be pronounced (Post, 2003; Galván *et al.*, 2010). Food webs in tropical rivers, however, tend to be characterised by omnivory, and by being short, diffuse and highly interconnected (Jepsen and Winemiller, 2002; Winemiller, 2004; Douglas *et al.*, 2005; Layman *et al*., 2005a; 2005b; Pusey *et al.*, 2010; Rayner *et al.*, 2010). Trophic enrichment of nitrogen isotopes in tropical rivers is often less than the predicted 3-4‰ (Kilham *et al*., 2009), with many large predatory fish in these rivers occupying similar trophic positions to smaller-bodied fishes (Layman *et al.*, 2005b), suggesting that many species feed across multiple trophic levels (Jepsen and Winemiller, 2002; Douglas *et al.*, 2005). In these more trophically complex systems, with a greater diversity of production sources and weaker size-structuring, isotopic evidence of size-related diet shifts may be difficult to document. Simultaneous studies of SCA and SIA of different size classes of species from tropical fresh waters are rare, with many studies limiting isotopic assessments to adult fish to avoid the confounding effects of ontogeny.

Northern Australia's terapontid grunters are an ideal group to examine the utility of SIA in discerning ontogenetic dietary shifts in tropical freshwater fishes. The Terapontidae is one of the most trophically diverse of Australia's freshwater fish families, with pronounced ontogenetic dietary shifts (determined by SCA) a prominent aspect of species' dietary ecology (Pusey *et al.*, 2004; Davis *et al.*, 2011a; 2011b). This study examines the diets of the terapontid grunters inhabiting the Burdekin River, one of Australia's largest wet-dry tropical catchments. The aims of the study were to: (1) compare the correspondence between SCA and SIA in determining diet; (2) examine changes in trophic position with ontogeny, and (3) assess whether proportions of food exploited by terapontids vary in relation to body size.

5.2 Materials and Methods

5.2.1 Study area and specimen collection

Fish were collected from the upper Burdekin catchment in the wet-dry tropics of north-eastern Australia (Figure 5.1). The Burdekin catchment is the fifth largest in Australia (130,000 km²). Study sites were located upstream of the Burdekin Falls Dam in the Basalt, Cape/Campaspe, and Burdekin Rivers, and Keelbottom Creek. The region experiences a sub-humid tropical (monsoonal) climate, characterised by pronounced seasonality in rainfall and discharge. Highest annual river flows typically occur from December to April (the 'wet season'), with lowest flows between August and October (the 'dry season'). The flow regime of the Burdekin River is amongst the most variable in the world for rivers of comparable size (Puckridge *et al.*, 1998), and flows in the upper Burdekin study area have been classified as 'unpredictable intermittent' (Kennard *et al.*, 2010). This flow-regime class is notable for extreme intra- and inter-annual flow variability and variable timing of maximum flows. Regional vegetation is dominated by sclerophylous *Eucalyptus* and *Acacia* woodlands, and the riparian zone typically composed of *Melaleuca* and occasional open vine thickets (Pearson, 1991). The upper catchment is sparsely populated, with the predominant land use being low-intensity cattle grazing. The upper Burdekin River system is of low gradient, with an under-fit channel, steep banks and minimal off-channel, floodplain habitat. Despite its size, the Burdekin River has low diversity of instream habitats. The river is largely characterised by long shallow reaches dominated by a sand and fine gravel substratum (Pearson, 1991).

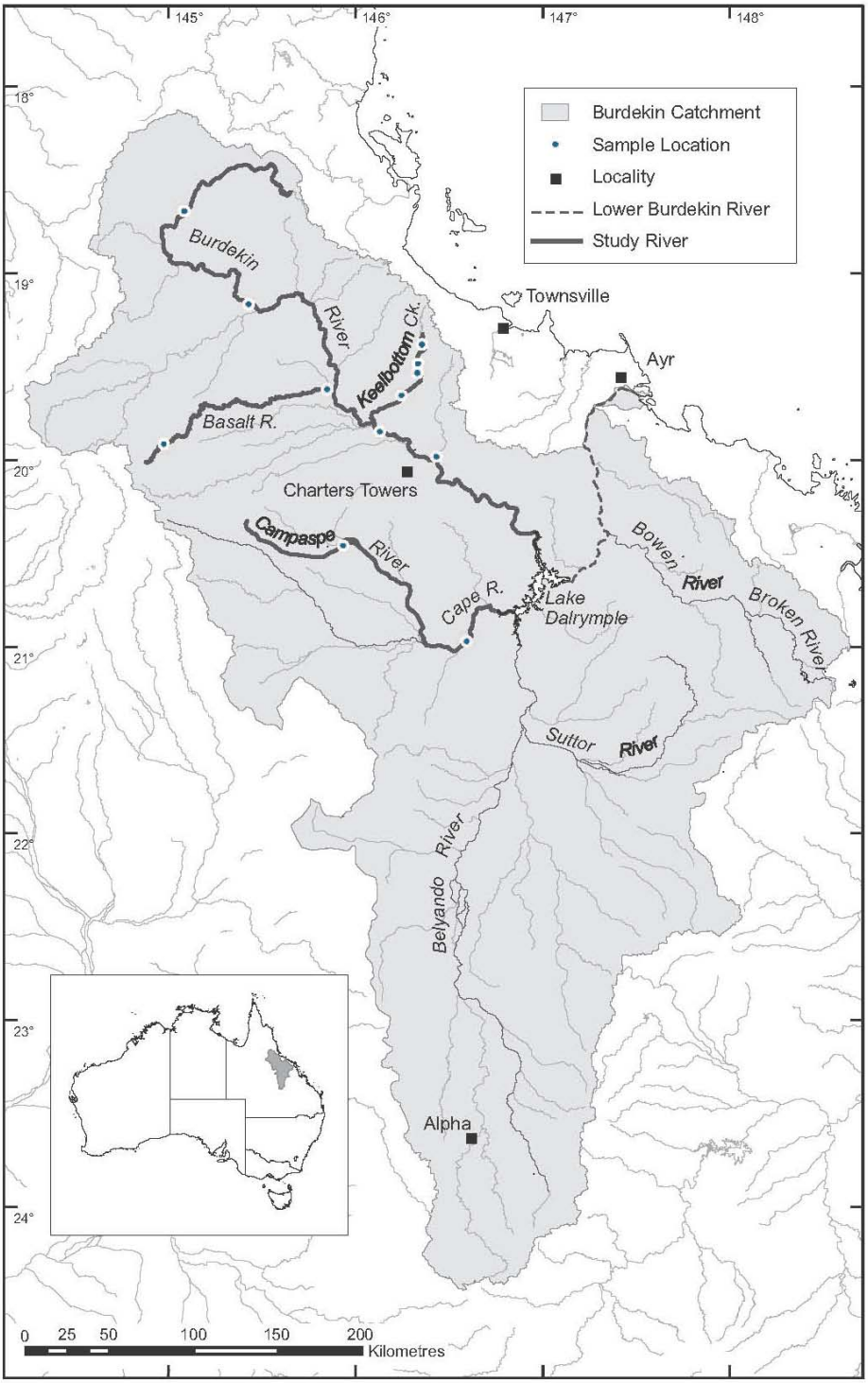

Figure 5.1 Study site locations in the Upper Burdekin River catchment. Data © Commonwealth of Australia (Geoscience Australia) 1990.

Study species

The four species of Terapontidae (grunters) that occur in the Burdekin River are collectively the most abundant fishes, by numbers and biomass, in this system (Pusey *et al.,* 1998). They comprise three of Australia's more widespread fish species – spangled perch *Leiopotherapon unicolor* (Günther), barred grunter *Amniataba percoides* (Günther) and sooty grunter *Hephaestus fuliginosus* (Macleay) – which are common elements of the fish assemblage, and the small-headed grunter *Scortum parviceps* (Macleay), which is endemic to the Burdekin catchment.

Sampling and sample processing

Fish were collected at twelve sites across major tributaries of the upper Burdekin River from September to November 2009 (late dry season), using electro-fishing (boat mounted and backpack) and gill netting, with efforts made to sample as wide a range of size classes for each species as possible at all sites. Macroinvertebrates were collected using a 2 m hand-held 250 µm mesh dip net from all major habitat types (edge, bed, macrophytes, runs) at each site. Samples were pooled to represent site-level abundance and diversity. Animals were sorted live on-site, and placed into distilled water for a minimum of 3 hours to facilitate purging of gut contents. Zooplankton samples were collected at each site via 20 vertical hauls of a 63-µm net through the water column.

Potential basal food sources collected at each site (when present) were: aquatic and terrestrial vascular plants, submerged leaves, filamentous algae, seston (a surrogate for phytoplankton, also including traces of suspended particulate matter), and biofilm (epilithon). Multiple leaves of the dominant terrestrial and aquatic vascular plants occurring at each site were clipped directly from the plant. Submerged leaf packs were collected from the substratum at each site and stored in plastic bags for transport. Samples of **f**ilamentous algae were collected with forceps from the substratum and stored in the same manner. Seston was collected using 20 vertical hauls of a 20 µm plankton net at each site. For biofilm collection, three stones at each site were scrubbed with a toothbrush and the collected material was washed through 800 µm and 250 µm sieves to remove coarse detritus and macroinvertebrates, respectively, with the resultant slurry from each stone being collected in an individual tube. With the exception of plankton and biofilm samples (see below), all samples were stored on ice in the field and immediately frozen upon return to the laboratory prior to processing.

Fish specimens were measured (standard length in mm; SL) and weighed prior to excision of the stomach and viscera from the body cavity. Stomachs estimated to be more than 20% full were transferred to a glass dish and the contribution of each food item was determined by the indirect volumetric method of

Hyslop (1980). Food items were identified to the lowest practical taxonomic level within 46 categories, including species level for fish, family level for macroinvertebrates and functional groups for items such as filamentous algae and detritus (Table 5.1). For fish > 40 mm SL, a fillet of dorsal white muscle was collected for SIA, as this tissue typically has lower lipid and inorganic carbonate content than other tissues, and yields lower variability in δ^{15}N and δ^{13}C values (Pinnegar and Polunin, 1999). For fish < 40 mm SL, whole fish excluding head, tail and viscera were used for analysis. Different tissues tend to follow similar patterns of isotopic change, so should not have unduly influenced resulting trends (Doucett *et al.,* 1999). Samples of abdominal muscle were excised from larger decapods crustaceans. All aquatic macroinvertebrates were pooled into functional feeding groups according to taxonomy first (e.g. family) and, if greater sample size was needed, combined according to feeding mode based on Hawking and Smith (1997), but always at least within the same taxonomic order, and processed whole as a composite sample. All animal tissue samples were oven-dried at 55°C to a constant mass before being ground to a fine homogeneous powder using a pestle and mortar.

Leaves of all aquatic and terrestrial vascular plants and filamentous algae were washed with distilled water, brushed, and visually inspected to ensure removal of contaminants prior to oven-drying. Zooplankton and phytoplankton samples underwent initial processing prior to refrigerated storage (0°C). Each zooplankton sample was poured through 250 µm and 60 µm sieves and rinsed with distilled water, with the 60 µm size fraction retained for storage. Each phytoplankton sample was poured through 60 µm and 20 µm sieves to remove contaminants larger than 60 µm, rinsed thoroughly with distilled water and the 20 µm size fraction was retained. Biofilm samples were centrifuged in an Eppendorf 5702 centrifuge at ~1000 rpm for 10 minutes to concentrate material. The chlorophyll-rich top fraction was collected from each 60 mL tube, then filtered through pre-combusted Whatman GF/C glass fibre filter papers (0.7 µm), and stored frozen in aluminum foil prior to oven-drying.

Stable Isotope Analysis

Isotopic analysis for δ^{15}N, δ^{13}C, %C, %N, and C/N was conducted by the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. Samples were analysed in continuous-flow mode using a Thermo-Finnigan Delta plus Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer. Data were normalised using four internationally accepted isotope reference standards (International Atomic Energy Agency standards CH6, CH7, N1, and N2). External precision on these standards was ± 0.10‰ or better for δ^{13}C and ± 0.20‰ or better for δ^{15}N. δ^{13}C and δ^{15}N data are expressed relative to standard reference material: Vienna Pee Dee Belemnite for carbon, and atmospheric N for nitrogen.

Lipid correction

Following Logan *et al.,* (2008), a subset of samples from each fish species was analyzed in duplicate (one lipid extracted, one non-extracted) to develop an appropriate lipid correction equation. Lipid extraction was done using the modified Folch *et al.,* (1957) technique at the Colorado Plateau Stable Isotope Laboratory. Approximately 10–20 mg of ground tissue was soaked in a 2:1 chloroform: methanol (by volume) solvent mixture and the material suspended by stirring. After 15 min, the sample was centrifuged (3000 rpm for 5 min), the supernatant discarded (i.e., the analysis was not quantitative for lipids), and the pellet re-suspended in the solvent mixture. These steps were repeated at least three times or until the solvent ran clear. Finally, the pellet was dried (60 °C) and ground. About 1000 μg of the sample was analyzed for $\delta^{13}C$ and $\delta^{15}N$ using the isotopic analysis techniques outlined above.

$\delta^{13}C$ values of all terapontids were corrected for the potential effects of lipid content prior to any analyses. Based on the subset of methanol-chloroform extracted samples, the relationship between bulk tissue $\delta^{13}C$, lipid extracted $\delta^{13}C$ and bulk tissue C:N was used to develop the following lipid correction equation for terapontids:

$$\delta^{13}C_{corrected} = \delta^{13}C_{untreated} + 3.33 \log(C{:}N_{bulk}) - 3.62 \quad (r^2 = 0.64).$$

This equation fell between the correction predictions of Post *et al.,* (2007) and Logan *et al.,* (2008) (Figure 5.2).

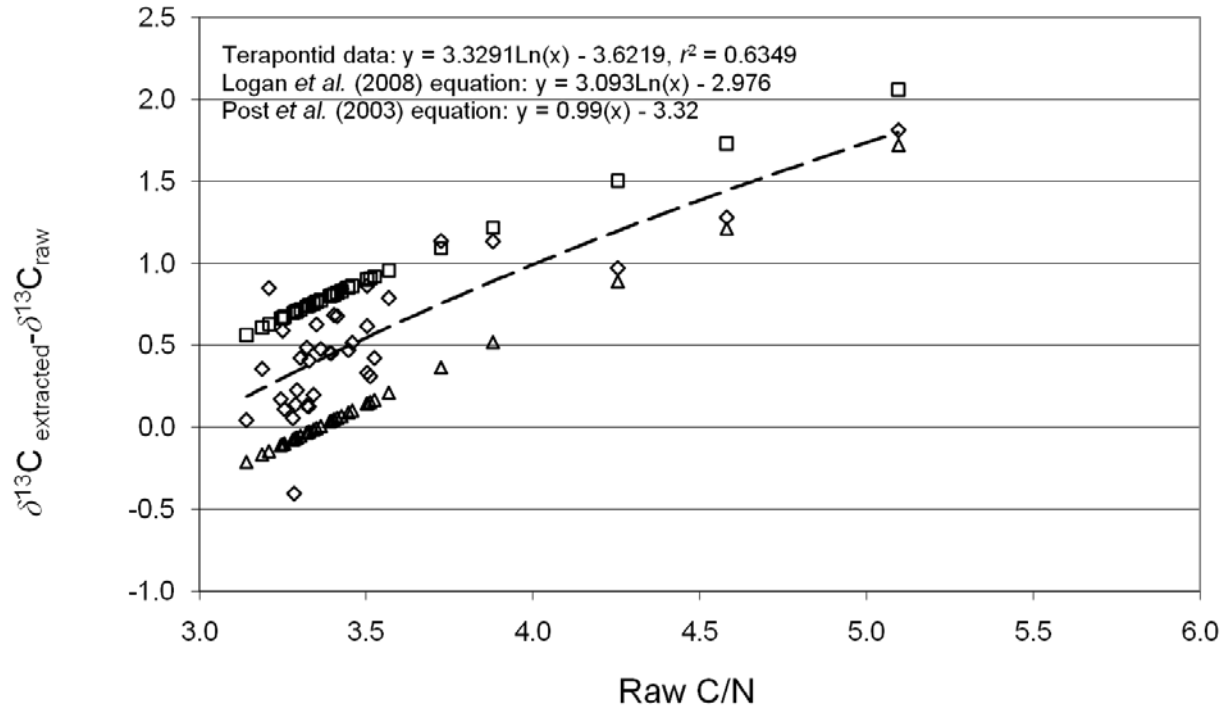

Figure 5.2 Lipid correction model fits to differences in terapontid species' tissue bulk $\delta^{13}C$ and lipid-free $\delta^{13}C$ versus bulk tissue C:N following 2 : 1 chloroform : methanol lipid extractions (diamonds). Equation shows model estimate for Terapontidae (dashed regression line) in relation to those of Post *et al.*, (2007) (triangles) and Logan *et al*., (2008) (squares).

5.2.2 Data analysis – Dietary shifts

Due to the questionable applicability of the taxonomic species as a valid functional ecological unit in fish ecology, I again employed the concept of the 'ontogenetic trophic unit' (OTU; *sensu* Stoner and Livingston, 1984), where an individual terapontid species was subdivided into size classes (standard length; < 40 mm, 40–80 mm, 80–160 mm and > 160 mm) with different known ecological (trophic) roles (Chapter 2). Dietary data was square-root transformed, and the level of dietary overlap between largest and smallest OTU's was compared using the Bray-Curtis similarity index, which provides values ranging from 0% (no shared species) to 100% (all prey species shared and consumed in the same proportion), and can be as a measure of dietary overlap (Marshall and Elliott, 1997). Although there are no defined critical levels with which similarity values can be compared, the intensity of overlap was assessed as high (> 60), intermediate (40 – 60) or low (< 40), following Ross (1986).

5.2.3 Data analysis – Size-related shifts in trophic position (SCA)

Stomach content data was used to estimate a unique trophic position for individual fish following the formula:

$$TP_{SCA} = \sum (V_i \,.\, T_i) + 1$$

where TP_{SCA} = the trophic position of a species size-class weighted by the volumetric contribution of the i^{th} prey item (V_i), and T_i = trophic position of the i^{th} prey item (*sensu* Winemiller, 1990; Vander Zanden *et al.,* 1997). Trophic positions of prey items were allocated by major taxonomic groups ranging from primary producers (algae, aquatic macrophytes, terrestrial vegetation) and detritus-inorganic material (trophic position 1.0) to predaceous invertebrates and fish (trophic position 3.0). Estimated trophic positions of prey items were assigned according to the concept of the dominant functional feeding group (*sensu* Merritt and Cummins, 1996) of the majority of members of the group (Table 5.1). Trophic categories for macroinvertebrate groups were derived from Hawkings and Smith (1997). Feeding designations and trophic positions for the common prey fish species were based on the trophic habits outlined in Pusey *et al.,* (2010). Trends in trophic position according to size (standard length) at a species level, as well as for all individuals of each species at each site, were analysed using linear regression.

Table 5.1 Estimated trophic position values for prey categories used in calculation of trophic position for Burdekin River terapontids. Functional feeding group classifications for invertebrates were sourced from Hawking and Smith (1997), fish from Pusey *et al*., (2004).

Prey Category		Functional feeding group	Estimated trophic position
Unidentified Arthropoda		Omnivores	2.5
Diptera	Chironomidae	Omnivores	2.5
	Simuliidae	Omnivores	2.5
	Ceratopogonidae	Carnivores	3
	Tabanidae	Carnivores	3
Ephemeroptera	Ephemeroptera	Detritivores-herbivores	2
Trichoptera	Trichoptera	Omnivores	2.5
Odonata	Odonata	Carnivores	3
Hemiptera	Corixidae	Omnivores	2.5
	Naucoridae	Carnivores	3
	Gerridae	Carnivores	3
	Mesoveliidae	Carnivores	3
Coleoptera	Dytiscidae	Carnivores	3
	Hydrophilidae	Herbivores	2
	Elmidae	Herbivores	2
	Unidentified aquatic Coleoptera	Omnivores	2.5
Lepidoptera	Pyralidae	Herbivores	2
Terrestrial-aerial invertebrates	Orthoptera	Herbivores	2
	Formicidae	Omnivores	2.5
	Unidentied terrestrial invertebrates	Omnivores	2.5
Macrocrustacea	Palaemonidae	Omnivores	2.5
	Atyidae	Omnivores	2.5
Zooplankton	Copepoda	Omnivores	2.5
	Cladocera	Detritivores-herbivores	2
	Ostracoda	Omnivores	2.5
	Acarina	Carnivores	3
Mollusca	Bivalvia	Filter feeders	2
	Gastropoda	Herbivores	2
Terrestrial vertebrates		Omnivores	2.5
Fish	*Mel. splendida*	Algivore-terrestrial invertivore	2.5
	Crat. stercusmuscarum	Aquatic invertivore-planktivore	3.0
	Ambassis agassizii	Aquatic invertivore-planktivore	3.0
	Oxyeleotris lineolatus	Invertivore-carnivore	3.5
	Nematalosa erebi	Detritivore-herbivore	2
	Oreochromis mossambicus	Detritivore-herbivore	2
	Unidentified fish	Omnivores	2.5
	Fish Eggs	Omnivores	2.5
Inorganic	Inorganic		1
Detritus	Detritus		1
Microalgae			1
Filamentous Algae			1
Aquatic macrophytes			1
Terrestrial vegetation			1

5.2.4 Data analysis – Size related shifts in trophic position (SIA)

It is widely recognised that estimation of trophic position from SIA is sensitive to the assumed nitrogen fractionation value used in calculations (DeNiro and Epstein, 1981; Post, 2002; Vanderklift and Ponsard, 2003). Following the method described in Winemiller *et al.,* (2007), trophic position based on isotopic data was calculated using the formula:

$$TP_{SIA} = [(\delta^{15}N_{consumer} - \delta^{15}N_{source})/3.3] + 1$$

where $\delta^{15}N_{source}$ was the mean of all basal sources occurring at each site, and the denominator 3.3 was an estimated mean trophic enrichment (fractionation) between consumers and their diet. The 3.3‰ estimate was calculated using the isotopic data from all terapontid species at < 40 mm SL. All terapontids in this size range have similar invertivorous diets (Chapter 2 and Chapter 3), dominated by larvae of three orders of aquatic insect (Ephemeroptera, Diptera and Trichoptera) that are lower-level consumers. The mean $\delta^{15}N_{source}$ was subtracted from the $\delta^{15}N$ of all terapontids < 40 mm SL at each site and divided by 2 (to account for the secondary consumer status of fish in this size range). This metric is analogous to the calculation of average fractionation rate throughout the food web proposed by Kilham *et al.,* (2009).The resultant 3.3‰ fractionation estimate was slightly above the average fractionation rate of 3.0‰ derived for white muscle tissue from the meta-analysis of Vanderklift and Ponsard (2003), and aligned closely with average fractionation rates (3.4‰) derived from the meta-analysis of Post (2002). Trends in trophic position according to standard length for individuals of each species at each site, as well as at a broader species level, were analysed using linear regression.

$\delta^{15}N$ fractionation rates of primary invertebrate consumers can be substantially lower than those of secondary consumers such as fish (Vander Zanden and Rasmussen, 2001; Vanderklift and Ponsard, 2003). Therefore, to provide context to changes in $\delta^{15}N$ values of terapontids during ontogeny, mean $\delta^{15}N$ values for different prey items were also plotted to compare $\delta^{15}N$ values and fractionation rates of invertebrate and fish prey items occupying the same nominal trophic positions (Table 5.1). Differences in the mean $\delta^{15}N$ values between fish and invertebrate consumers in each trophic category (i.e. herbivores-detritivores, omnivores and carnivores) were assessed using one-way ANOVA.

5.2.5 Data analysis-Estimation of size-related assimilated basal source material from SIA

Changes in $\delta^{13}C$ according to standard length for individuals of each species were analysed using linear regression. The relative contribution of different basal carbon sources to diet of different OTU's was assessed using SIAR (Stable Isotope Analysis in R; Parnell *et al.*, 2010), a Bayesian mixing model that runs on the R platform (R Development Core Team, 2009). Bayesian mixing models have the advantage of allowing the variation and uncertainties associated with isotopic estimates and trophic enrichment to be propagated through the model, with outputs being more reflective of the natural variability within a system. The SIAR model is fit via Markov Chain Monte Carlo methods producing simulations of values of dietary proportions of sources consistent with the data using a Dirichlet prior distribution (Parnell *et al.*, 2010). Prior to SIAR analyses, related basal sources with similar isotopic compositions were grouped to minimise the number of sources and simplify the range of possible solutions (Phillips, Newsome and Gregg, 2005). Biofilm typically has a large component of attached filamentous algae (Rasmussen, 2010), and exploratory analyses identified $\delta^{13}C$ values for filamentous algae as having a significant correlation with biofilm across sites ($r^2 = 0.51$, $P < 0.05$), so the two sources were combined at site level, and termed 'benthic algae'. Five food sources – terrestrial C4 grasses, terrestrial C3 vegetation (all other terrestrial vegetation and submerged leaf packs), seston, aquatic macrophytes (submerged) and benthic algae – were used in the mixture modeling.

The SIAR model was run at both the catchment level (using the overall mean and standard deviation for each of the five basal sources and the overall mean for each OTU), and at the individual site level (using the values for available basal sources at each site and the site mean for each OTU), to assess broad- and fine-scale trends in species' size-related feeding. Each terapontid species was coded into its size-class groups (OTUs). Trophic enrichment factors (TEFs) for nitrogen were corrected using the value of 3.3±0.5‰ (mean ±1S.D. as above). A $\delta^{13}C$ fractionation rate of 0.4±1.3‰ was assumed following Post (2002). The SIAR mixing model was run for 500,000 iterations, discarding the first 50,000 samples. The resulting distributions of probability-density functions of the feasible foraging solutions produced by SIAR allowed direct identification of the most probable solution for basal carbon sources supporting each species' OTUs (Parnell *et al.*, 2010). The upper and lower 95% credibility intervals were used to describe the contribution for each diet item (Phillips and Gregg, 2003).

5.3 Results

5.3.1 Stomach content analysis

A total of 337 stomachs from the four terapontid species were examined. Ontogenetic shifts were prominent features of all species' diets (Figure 5.3). All species were invertivorous in their smaller size classes, with diets dominated by Diptera larvae (particularly Chironomidae and Simuliidae), as well as Trichoptera and Ephemeroptera. As they grew, fish shifted away from this reliance on invertebrates, although the nature of these shifts varied among species. *Amniataba percoides* continued to consume large numbers of invertebrates in all size classes, but filamentous algae and aquatic macrophytes became increasingly important with size. *Hephaestus fuliginosus* displayed pronounced size-related dietary change, with smaller invertebrates diminishing in importance, and larger animals such as fish (primarily *Melanotaenia splendida*), shrimp (Palaemonidae), and plants (aquatic macrophytes, filamentous algae) dominating the diet of larger individuals. With increased size, *L. unicolor* shifted from invertivory to generalised carnivory, consuming an increasingly broader array of predominantly animal prey (aquatic insects, terrestrial insects, prawns and fish). *Scortum parviceps* transitioned from a largely invertivorous diet to an almost exclusive diet of aquatic and terrestrial plant material and detritus in larger specimens. These diets and ontogenetic shifts are largely consistent with previous reports of the trophic ecology of these species (Pusey *et al*., 2010; Davis *et al*., 2011a; 2011b). Bray-Curtis analysis demonstrated high dietary similarity within species' OTUs across sites (unpublished data), concurring with previous reports (Pusey *et al*., 2010; Davis *et al*., 2011a; 2011b).

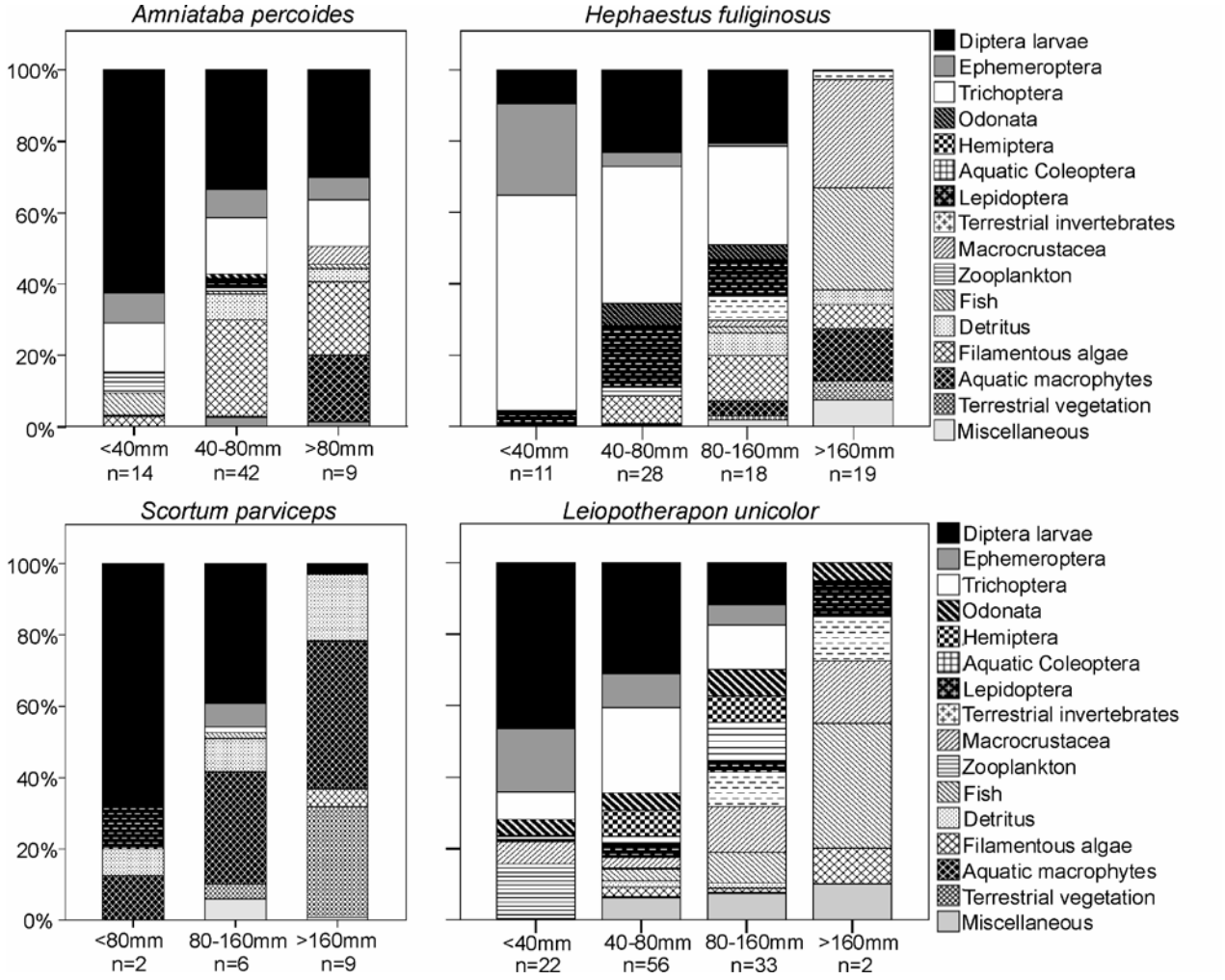

Figure 5.3 Volumetric proportions (%) of major prey items across Burdekin terapontid size classes. Prey categories are based on those presented in Table 5.1. The number of individuals (*n*) examined in each size class is indicated.

Within-species Bray-Curtis dietary overlap values based on SCA between the smallest and largest OTU's of *A. percoides*, *H. fuliginosus*, *L. unicolor* and *S. parviceps* were 45.6, 2.27, 11.53 and 28.83 respectively. With the exception of *A. percoides*, where overlap between size classes was moderate (i.e. Bray-Curtis dietary overlap between 40 – 60), size-related diets shifts in all other species resulted in low (> 40) overlap. The extent of the ontogenetic dietary shift in *H. fuliginosus* was particularly pronounced, with essentially no similarity in diet between the < 40 mm SL and > 160 mm SL size classes.

5.3.2 Size-related shifts in trophic position (SCA)

Linear regression of trophic position estimates calculated from SCA showed that, with the exception of *S. parviceps*, significant size-related shifts in trophic position were not apparent (Figure 5.4). *Scortum parviceps* demonstrated a significant negative trend in trophic position as it shifted from a diet dominated by invertebrates to herbivory in larger size classes. The remaining terapontids fed across trophic positions through much of their life histories, often ranging between primary consumers (trophic position 2) through to tertiary predators (trophic position ~ 4) within a similar size range.

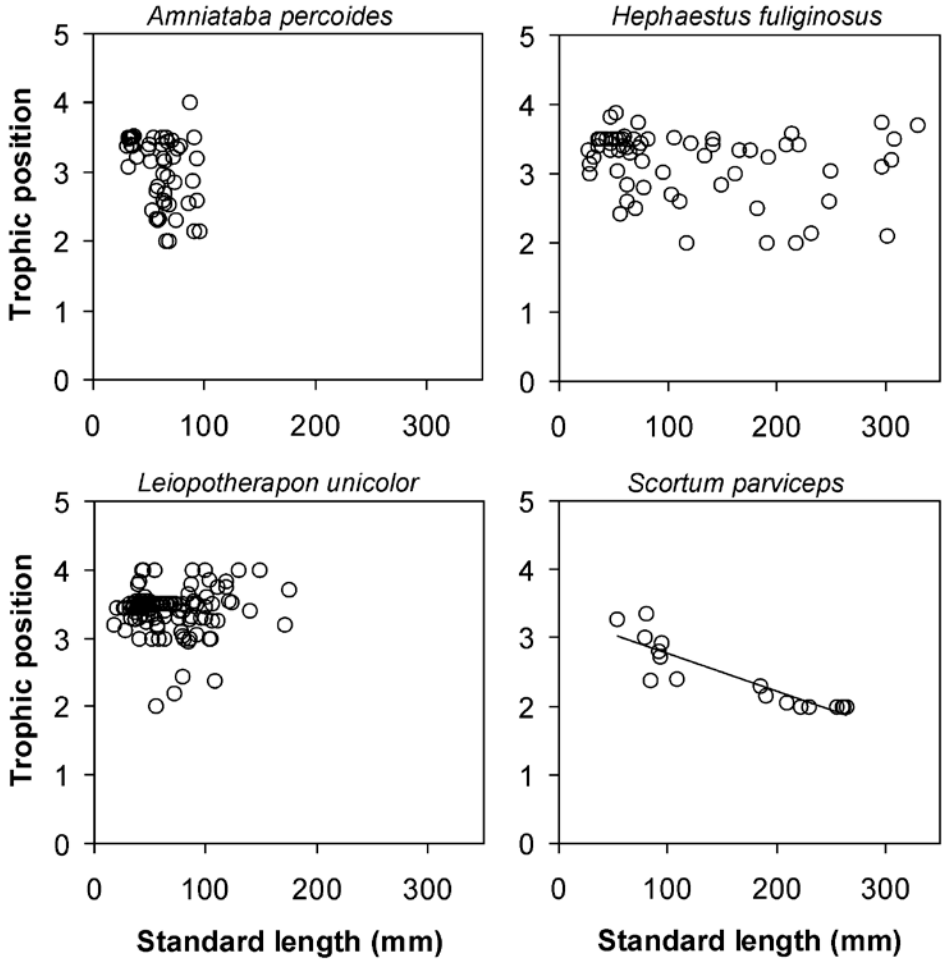

Figure 5.4 Size-related trophic position estimates for Burdekin River terapontids based on SCA. The regression line represents a significant relationship between standard length and trophic position for *S. parviceps* (r^2 = 0.796, P <0.001).

5.3.3 Stable Isotope Analyses

Size-related shifts in trophic position.

At the species level there was a small positive relationship between trophic level, indicated by δ^{15}N, and standard length for *H. fuliginosus* and *L. unicolor*, but not for *A. percoides* or *S. parviceps* (Figure 5.5). The weak positive relationships (< 0.5 trophic position) occurred between individuals < 40 mm SL and >160 mm SL in both species. These relationships between trophic position and standard length were also largely reflected at a site level, with significant positive relationships between δ^{15}N and standard length evident at several sites for both *H. fuliginosus* and *L. unicolor*, with positive but non-significant trends evident at most remaining sites (Table 5.2).

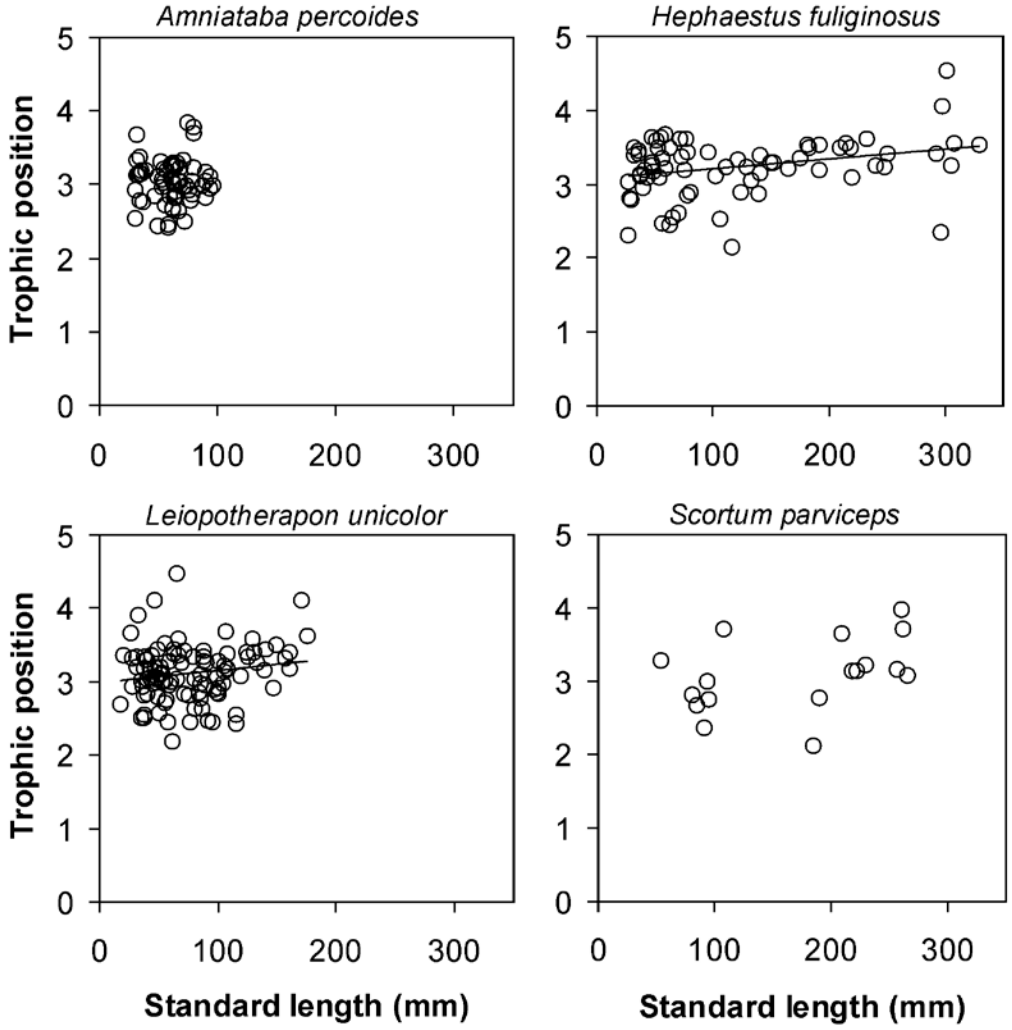

Figure 5.5 Size-related trophic position estimates for Burdekin River terapontids based on SIA. Solid lines represent significant regression relationships between standard length and trophic position (for *H. fuliginosus* r^2 = 0.164, P < 0.01; for *L. unicolor* r^2 = 0.06, P < 0.05).

There were no significant relationships between diet- and isotope-based estimates of trophic position for individual fishes in any of the species (Figure 5.6), indicating low correspondence between the two techniques. Isotope-based trophic positions for *S. parviceps* tended to be higher than those derived from SCA.

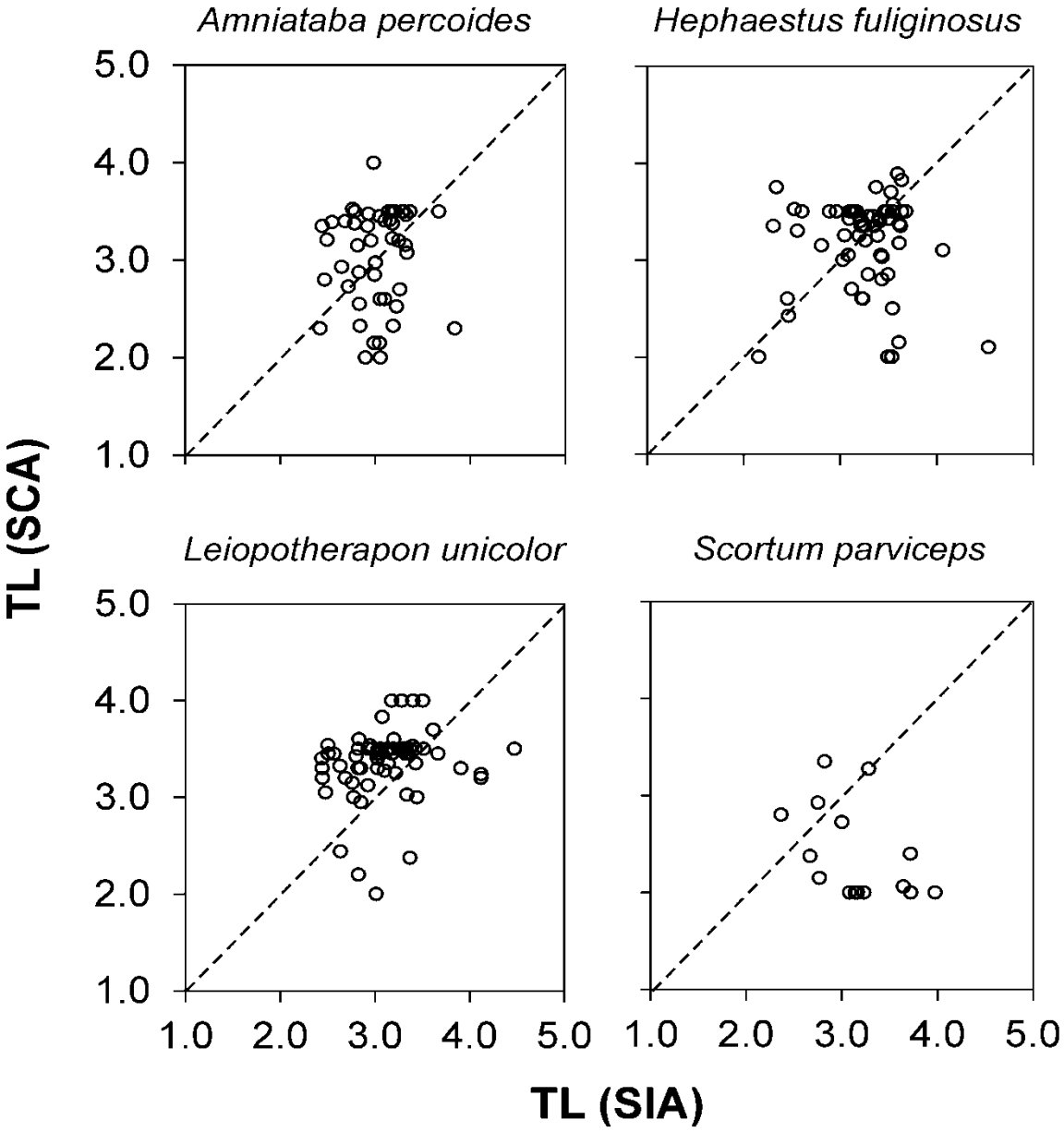

Figure 5.6 Comparison of trophic position values derived from stomach content analysis (SCA) and stable isotope analysis (SIA) for four Burdekin River terapontid species. The diagonal line represents total correspondence between the two methodologies. For *A. percoides* r^2 =0.004, $P > 0.05$; *H. fuliginosus* r^2 = 0.001, $P > 0.05$; *L. unicolor* r^2 = 0.04, $P > 0.05$; and *S. parviceps* r^2 = 0.20, $P > 0.05$.

Comparison of δ^{15}N values for fish and invertebrate prey in terapontid diets indicated that fish from all trophic positions (herbivore-detritivore, omnivore and carnivore) consistently had elevated δ^{15}N values compared to invertebrate consumers ostensibly occupying similar trophic niches (Figure 5.7). There were significant differences between mean δ^{15}N values of fish and invertebrates at all positions of the food web (herbivores: $F_1 = 60.8$, $P < 0.001$; omnivores: $F_1 = 45.6$, $P < 0.001$; carnivores: $F_1 = 163.8$, $P < 0.001$).

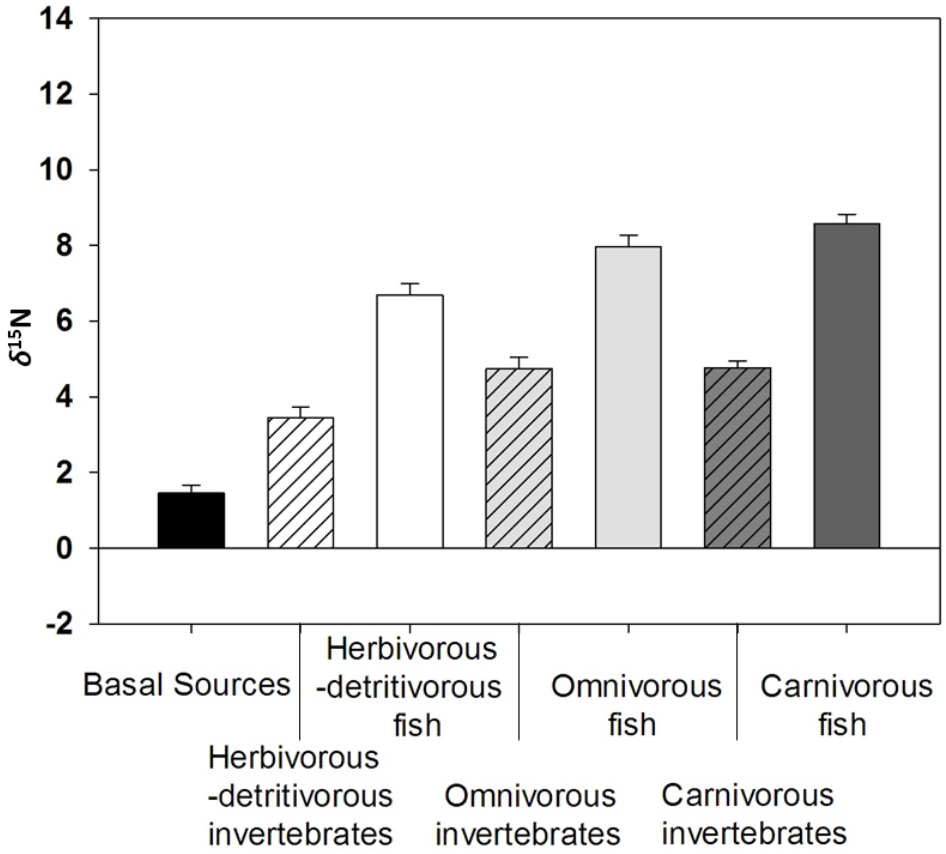

Figure 5.7 Mean (±S.E.) δ^{15}N values for basal sources, with invertebrates and fish grouped according to trophic position classifications outlined in Table 5.1.

Size-related shifts in δ^{13}C.

Regression analysis indicated that there was no significant rate of increase of δ^{13}C with standard length in *A. percoides* or *S. parviceps* at the species level (Figure 5.8). A significant positive relationship between δ^{13}C and standard length was evident in *L. unicolor* with individuals becoming progressively enriched in δ^{13}C as length increased ($r^2 = 0.413$, $P < 0.001$). A positive size-related shift in δ^{13}C enrichment was also evident in *H. fuliginosus* ($r^2 = 0.392$, $P < 0.001$) with increased size. These relationships between overall

species' δ^{13}C values and standard length were largely reflected at the site level, with significant positive relationships evident at several sites for both *H. fuliginosus* and particularly *L. unicolor* (Table 5.2), and similar but non-significant trends in regressions at most remaining sites.

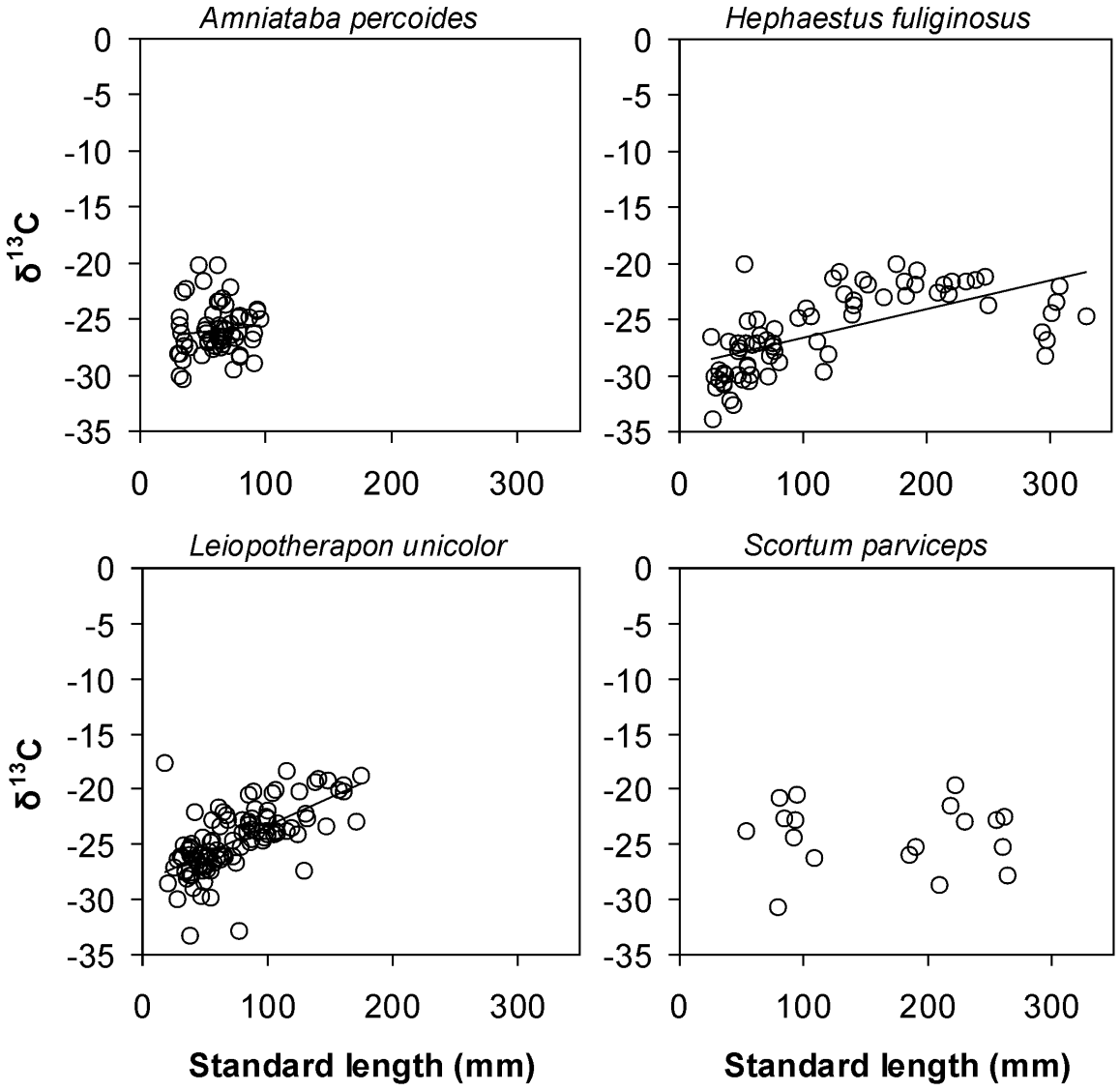

Figure 5.8 Trends in size-related δ^{13}C for Burdekin River terapontids. Solid lines represent significant regression relationships between standard length and δ^{13}C.

Table 5.2 Numbers (and size range, mm SL) of each terapontid species collected at each site in the Burdekin catchment.

Site name	*A. percoides*	*H. fulginosus*	*S. parviceps*	*L. unicolor*
BU1	-	9 (26-301)$^{\#\#,*}$	-	5 (75-147)*
BU1a	11 (31-63)^^	28 (29 - 303)**	4 (80-108)	15 (26-159)
BU2	10 (52-94)	8 (103-308)$^{\#\#}$	2 (262-265)	11 (34-115)**
BU3	-	11 (39-209)$^{\#\#,**}$	4 (53-94)	11 (18-130)**
BA1	12 (53-90)	1 (296)	-	2 (66-106)
BA2	12 (61-93)	5 (49-141)	2 (185-190)	17 (37-175)$^{\#\#,**}$
C1	-	2 (183-305)	6 (209-260)	13 (33-171)**
C2	-	-	-	2 (40-65)
K1	8 (30-66)$^{\#}$	2 (124-192)	-	3 (35-126)
K2	2 (49- 58)	-	-	3 (38-116)$^{\#,**}$
K3	5 (56-67)	8 (32-330)**	-	17 (36-133)**
K4	1 (72)	-	-	3 (38-96)

*, ** indicates significant positive δ^{13}C-body size relationship at $P < 0.10$, $P < 0.05$ respectively
$^{\#}$, $^{\#\#}$indicates significant positive δ^{15}N-body size relationship at $P < 0.10$, $P < 0.05$ respectively
^^ indicates significant -negative δ^{15}N-body size relationship at $P < 0.05$

5.3.4 Estimation of size-related assimilated basal source material from SIA

Mean δ^{15}N and δ^{13}C values of basal sources across the 12 sites revealed low variability within categories, with source isotopic ratios generally consistently positioned relative to each other (Figure 5.9, Table 5.3): terrestrial C3 vegetation was the most ^{13}C-depleted source at all sites, aquatic macrophytes were typically the most ^{13}C-enriched aquatic plant at most sites, while C4 grasses were the most distinct basal sources, exhibiting the most enriched δ^{13}C values. Mean δ^{13}C values of the autochthonous basal sources (benthic algae, aquatic macrophytes, and seston) were not well differentiated, although there was some divergence in δ^{15}N across these sources. The dominant animal and fish prey in terapontid diets exhibited intermediate δ^{15}N between terapontids and basal sources.

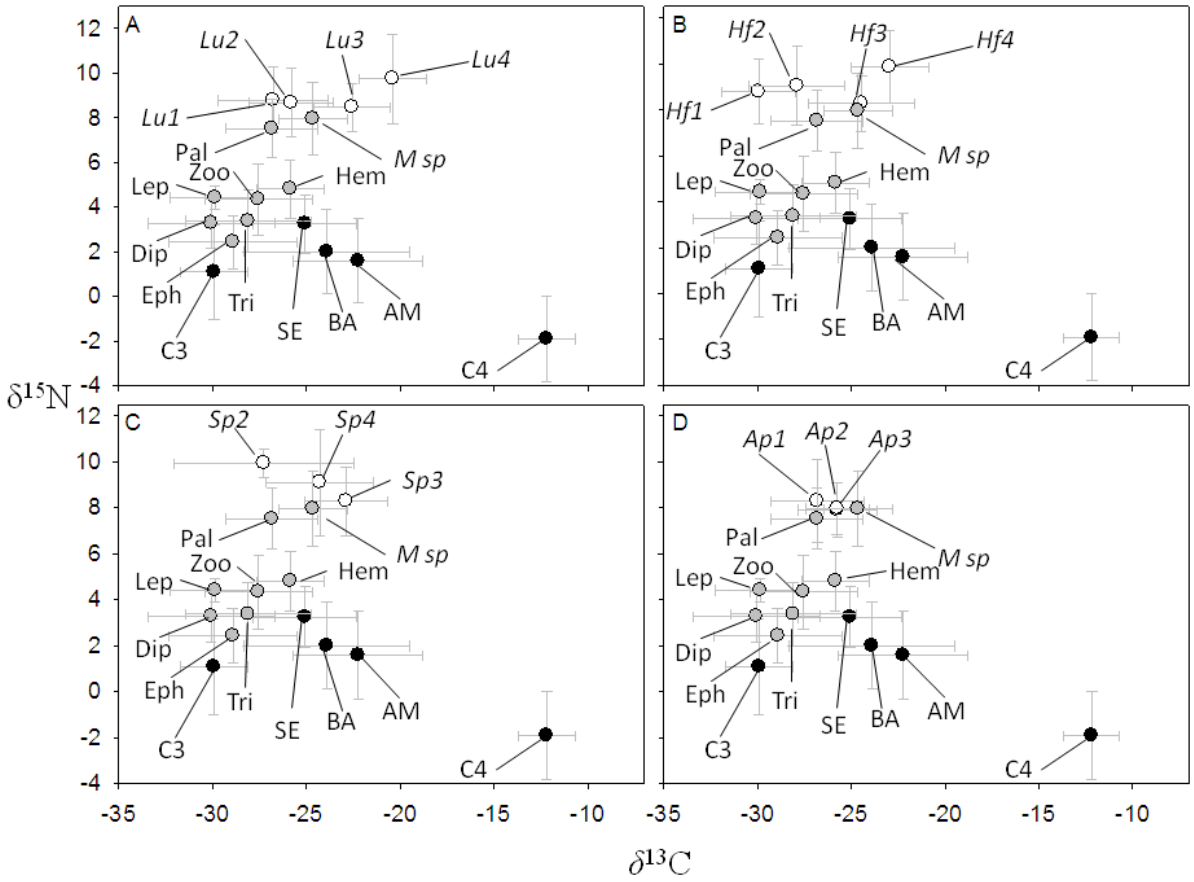

Figure 5.9 Stable isotope values (mean ±1 S.D. for δ^{13}C versus δ^{15}N) of potential primary sources (solid circles), prey items (grey circles) and terapontid fishes (open circles) collected from Upper Burdekin aquatic habitats. Figures A-D represent the four respective species: *L. unicolor*; *H. fuliginosus*; *A. percoides*; and *S. parviceps*. Terapontid data are presented as intraspecific OTUs, epithets are formed from initial letters in genus and species names; numeric suffixes refer to ontogenetic trophic unit size classes (1 – < 40 mm, 2 – 40-80 mm, 3 – 80-160 mm and 4 – > 160 mm). Intermediate prey items are labeled: Dip – Diptera larvae; Tri – Trichoptera larvae; Eph – Ephemeroptera larvae; Lep – Lepidoptera larvae; Zoo – Zooplankton; Pal – Palaemonidae (shrimp); Hem – Aquatic Hemiptera; and *Msp* – *Melanotaenia splendida* (fish).

There was considerable overlap in the δ^{13}C and δ^{15}N ratios of many terapontid OTUs, particularly smaller (< 80 mm SL) sizes (Figure 5.9). The ontogenetic shifts demonstrated in the regression analyses of individual species' isotopic values are also evident in the δ^{13}C- δ^{15}N biplots. Progressive enrichment in both δ^{13}C and δ^{15}N was particularly evident in the sequential OTUs of *H. fuliginosus* and *L. unicolor* as their isotopic values diverged from their high overlap as juveniles. There was some variation in isotopic values of several of the significant prey items in terapontid diet. The invertebrate larvae (Diptera, Ephemeroptera, Lepidoptera and Trichoptera) which predominated in the diets of smaller terapontids (<

80 mm SL) were relatively depleted in $\delta^{13}C$. Prey items that increased in importance as species such as *H. fuliginosus* and *L. unicolor* grew (e.g., palaemonid shrimps, the fish *M. splendida* and aquatic macrophytes) were relatively enriched in $\delta^{13}C$.

Table 5.3 Average $\delta^{13}C$ and $\delta^{15}N$ isotope values for basal sources at 12 upper Burdekin River sampling sites. Lack of a source at a site due to absence during field collection indicated by -.

	C3 terrestrial vegetation		Seston		Benthic algae		Aquatic macrophytes		C4 grasses	
Site name	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
BU1	-27.9	1.9	-20.4	2.6	-23.5	0.8	-18.8	-0.3	-12.0	-0.8
BU1a	-30.5	1.2	-27.1	3.3	-25.4	1.6	-23.9	4.1	-12.3	-3.0
BU2	-30.8	0.3	-27.1	4.5	-21.7	3.2	-19.3	2.9	-	-
BU3	-30.6	1.2	-24.4	0.9	-25.0	2.7	-22.4	1.2	-	-
BA1	-29.6	-1.4	-23.9	2.3	-26.8	0.7	-21.9	1.8	-	-
BA2	-29.8	0.3	-21.6	1.2	-24.0	-1.7	-	-	-	-
C1	-29.1	1.3	-28.5	4.6	-28.0	3.5	-	-	-	-
C2	-29.4	4.1	-	-	-24.9	2.9	-	-	-	-
K1	-29.5	-0.4	-22.3	3.8	-15.6	-0.2	-16.5	1.3	-	-
K2	-31.2	0.6	-28.4	4.2	-30.8	3.3	-	-	-	-
K3	-31.5	-0.7	-26.5	4.7	-17.3	4.7	-	-	-	-
K4	-30.4	1.8	-25.4	3.6	-	-	-	-	-14.8	0.8

The ability of the SIAR mixing model to resolve proportions of different food sources in terapontid diets varied between size classes at both site and catchment levels. Results were similar at both scales so are presented here for the catchment scale only. The carbon sources supporting the diet of *H. fuliginosus* and *L. unicolor* became less well resolved as fish increased in size (Figure 5.10). Terrestrial C3 vegetation made the dominant contribution in both species for size classes < 80 mm SL; however, the C3 pathway progressively diminished in importance for fish > 80 mm in both species, with a range of basal carbon sources making similar feasible contributions to the diet. A similar (though less pronounced) trend in relative importance of C sources was evident in *A. percoides*. C3 vegetation was the major contributor to diet in specimens < 40 mm SL (95% credibility interval: 45% to 62%), remaining the dominant C source in all size classes, although as fish grew its importance diminished. No obvious size-related patterns in relative contributions of different carbon sources were evident for *S. parviceps*, with seston, C3 vegetation, aquatic macrophytes and biofilm making similar contributions to diet across all size classes.

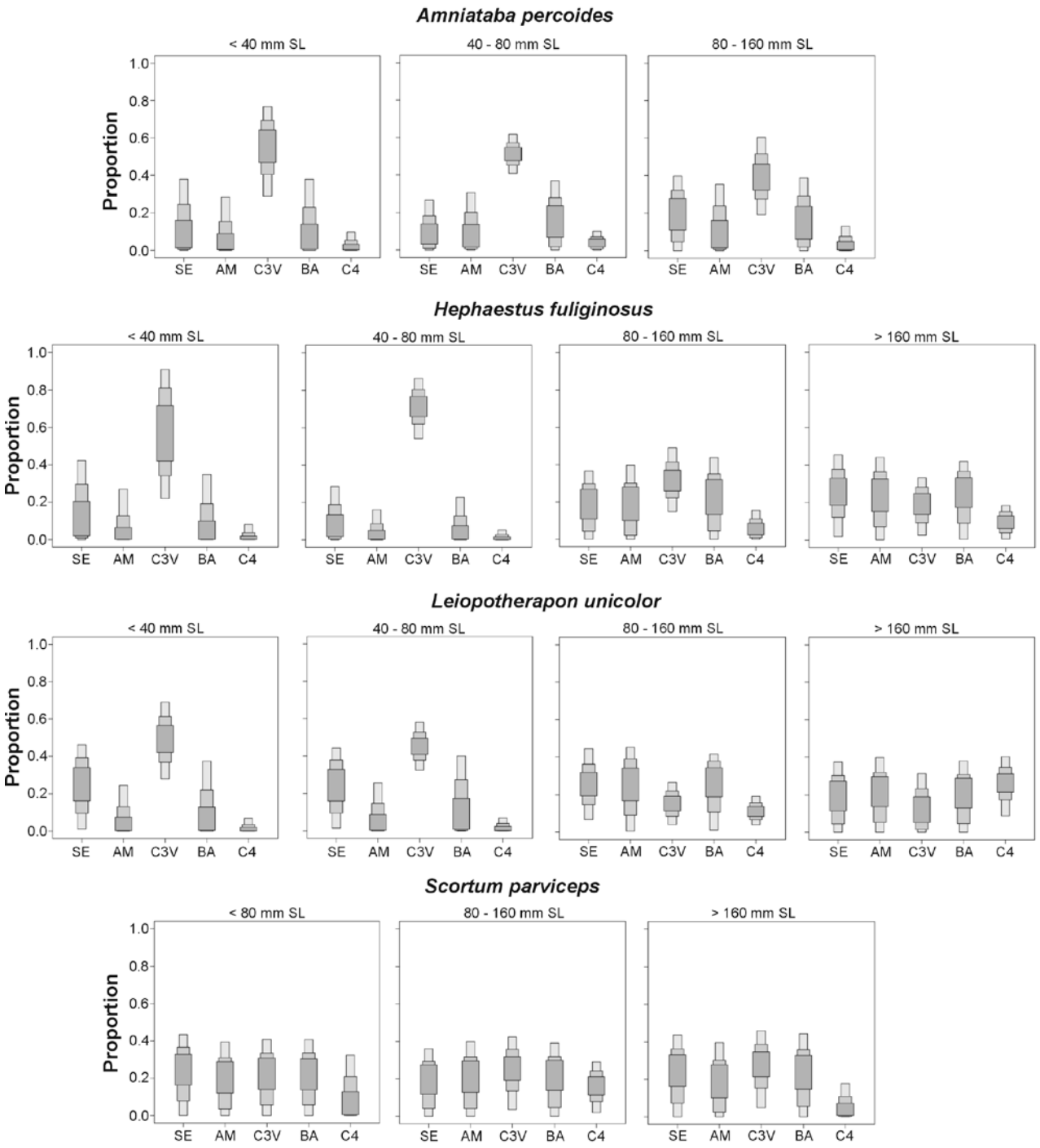

Figure 5.10 Boxplots derived from the SIAR mixing model showing the contribution of different primary carbon sources to the diets of Burdekin terapontid size classes using $\delta^{13}C$ and $\delta^{15}N$ isotopes. The proportions show credibility intervals plotted at 95, 75 and 50% credibility intervals. Carbon sources are labeled: SE – seston; AM – aquatic macrophytes; C3V – C3 terrestrial vegetation; BA – benthic algae and C4 – C4 terrestrial grasses.

These catchment-scale trends were also largely reflected in SIAR results conducted at a site scale. C3 vegetation was typically the primary carbon source supporting *A. percoides*, *L. unicolor* and *H. fuliginosus* in OTUs < 80 mm SL at most sites (Table 5.4). C3 vegetation diminished markedly in significance for the larger OTUs of *L. unicolor* and *H. fuliginosus* at most sites, where different basal sources (benthic algae, seston and aquatic macrophytes) made the dominant contribution to diet, except where the model could not discriminate any dominant source. Instances where an individual carbon source was clearly dominant for a species' OTU at a site (i.e. minimum contribution to model outputs > 0.4 for the lower 95% probability value) were rare but, when evident, generally took the form of C3 vegetation as the major source for fish OTUs < 80 mm SL. Multiple carbon sources making likely contributions to OTUs (> 0.2 for the lower 95% probability value) were commonplace across species (particularly in larger size classes) and sites.

Table 5.4 SIAR modeling summaries for terapontid OTUs according to site. Basal sources are: C3 – C3 terrestrial vegetation; BA – benthic algae; SE – seston; AM – aquatic macrophytes; C4 – C4 terrestrial grasses. Sources for each OTU are ordered according to their probability of contribution from SIAR modeling; sources with a minimum contribution to model outputs of > 0.2 and > 0.4 for the lower 95% probability value are indicated by * and ** respectively. Bold font indicates a source with a lower 95% probability value > 0. [1] signifies OTUs with only 1 individual available, necessitating use of the "SIARSOLO" command in SIAR.

	Amniataba percoides			***Hephaestus fuliginosus***			
Site	**<40 mm SL**	**41-80 mm SL**	**81-160 mm SL**	**<40 mm SL**	**41-80 mm SL**	**81-160 mm SL**	**>160 mm SL**
BU1a	**C3*, SE*, AM, BA,** C4	**C3*, BA, SE, AM, C4**	-	**C3*, SE*, AM, BA**, C4	**SE*, C3*, AM,** BA, C4	**C3, BA, SE, AM, C4**	**AM, SE, BA, C4, C3**
BU1	-	-	-	**BA*, C3, AM,** SE, C4	**C3*, BA*, AM,** SE, C4	**C3, BA, SE, AM, C4**	**SE, C3, BA, AM, C4**
BU2	-	-	-	-	-	**BA, AM, SE, C3**	**BA*, AM*, SE, C3**
BU3	-	-	-	**C3*, BA, SE, AM**	**C3*, BA*, SE, AM**	**BA*, AM, SE, C3**	**BA, AM, SE, C3**
K1	**C3*, BA, SE, AM**	**C3*, BA, SE, AM**	-	-	-	**C3*, SE, BA, AM**	**C3*, SE, BA, AM**
K2	-	-	-	**C3**, SE,** BA	**SE*, BA*, C3**	**BA*, SE*, C3**	**SE*, BA*, C3***
K3	-	**SE*, BA*, C3***	-	-	-	-	-
K4	-	[1] **C4*, C3*, SE**	-	-	-	-	-
BA1	-	**C3*, BA*, SE, AM**	**BA*, C3, SE, AM**	-	-	-	**BA*, C3*, SE, AM**
BA2	-	**C3**, SE,** BA	**C3*, SE*, BA**	-	**SE*, C3*, BA***	**SE*, C3*, BA***	-
C1	-	-	-	-	-	-	SE, BA, C3
C2	-	-	-	-	-	-	-

Table 5.4 (cont.)

	Scortum parviceps			*Leiopotherapon unicolor*			
Site	**41-80 mm SL**	**81-160 mm SL**	**>160 mm SL**	**<40 mm SL**	**41-80 mm SL**	**81-160 mm SL**	**>160 mm SL**
BU1a	**SE, C3, AM, BA,** C4	**C3, BA, SE, AM, C4**	-	**C3*, SE*, AM, BA,** C4	**SE*, C3, AM, BA,** C4	**AM*, SE, BA, C3, C4**	-
BU1	-	-	-	-	**BA*, C3*,** AM, SE, C4	**C3*, BA, SE, AM,** C4	-
BU2	-	-	**C3, BA, SE, AM**	**C3*, SE*, BA, AM**	**C3*, BA*, SE,** AM	**BA*, AM*, SE,** C3	-
BU3	**BA, AM, SE, C3**	**BA*, AM, SE, C3**	-	**BA*, C3, AM, SE**	**C3*, BA*, SE, AM**	[1] **BA*, AM, SE, C3**	-
K1	-	-	-	[1] **C3****, SE, BA, AM	**C3*, SE, BA, AM**	**C3, SE, BA, AM**	-
K2	-	-	-	**C3*, BA*, SE**	**SE*, C3*, BA**	**SE**, BA*,** C3	-
K3	-	-	-	[1] **SE*, BA*, C3***	**SE*, BA*, C3**	**BA*, SE*,** C3	-
K4	-	-	-	[1] **C3****, SE, C4	[1] **C3****, SE, C4	[1] **C3*, SE*, C4**	-
BA1	-	-	-	-	**AM*, SE, BA, C3**	**AM, SE, BA, C3**	-
BA2	-	-	**C3*, BA*, SE**	**C3*,SE*, BA**	**C3**,SE*,** BA	**SE*, BA*,** C3	**SE*, BA*, C3**
C1	-	-	BA*, SE*, C3	**SE*, BA*, C3***	**SE*, BA*, C3**	**SE*, BA*, C3**	**BA*, SE*, C3**
C2	-	-	-	[1] **C3**, BA**	[1] **C3**, BA**	-	-

These SIA results align well with dietary shifts documented from SCA. SIAR modeling of source contributions to diet of the key invertebrates in juvenile stomach contents showed a major reliance on C3 vegetation (unpublished data), highlighting their role in transfer of energy to these secondary consumers. The combined volumes of these ^{13}C-depleted invertebrates decreased markedly as standard length increased for *L. unicolor*, *H. fuliginosus* and *S. parviceps* (Figure 5.11). In contrast, volumetric contribution of these insect taxa to the diet of *A. percoides* remained relatively constant as fish size increased, with this species also remaining heavily reliant on C3 vegetation across all size classes. Other prey items contributing to the diet of larger size classes of *H. fuliginosus* and *L. unicolor* (i.e. Palaemonidae, *M. splendida*) demonstrated reliance on a broad range of relatively ^{13}C-enriched basal sources (unpublished data). These two terapontids exhibited a corresponding enrichment in ^{13}C with increased size, and reliance on a diversity of ^{13}C-enriched basal sources.

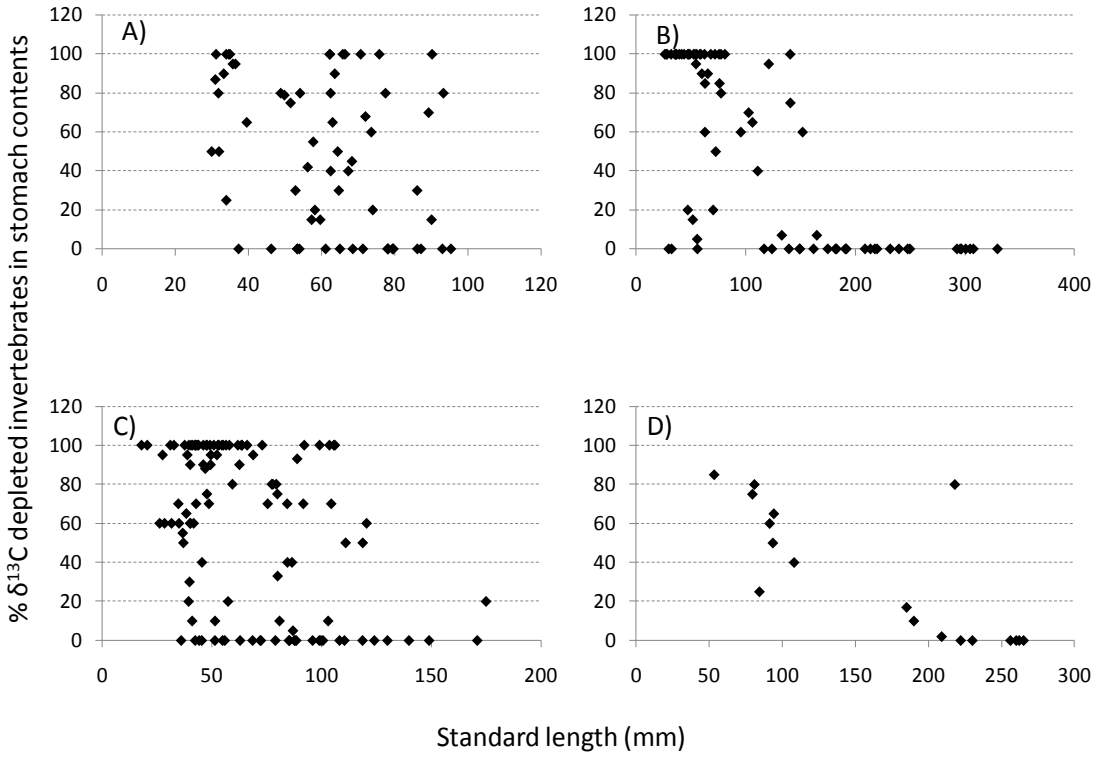

Figure 5.11 Percentage of stomach contents contributed by highly ^{13}C depleted invertebrate families (Diptera, Ephemeroptera, Trichoptera and Lepidoptera larvae) plotted against standard length for four Burdekin River terapontids: A) *Amniataba percoides*; B) *Hephaestus fuliginosus*; C) *Leiopotherapon unicolor* and D) *Scortum parviceps*.

5.4 Discussion

SIA and SCA both indicated that feeding across several trophic positions with several basal sources (i.e., omnivory) is important in the diets of Burdekin River terapontids. This finding supports the view that omnivory is an adaptive response to seasonal variations in water level and trophic resources that characterise hydrologically variable tropical river systems (Lowe-McConnell, 1987; Jepsen and Winemiller, 2002). In a system as variable as the Burdekin River, species traits are expected to revolve around resource generalism and opportunistic foraging (Townsend and Hildrew, 1994; Poff and Allan, 1995; Pusey *et al.*, 2010). However, the contributions of individual sources varied considerably over the life history of fish. Both SIA and SCA approaches suggested that the early life-history stages of all terapontid species were heavily reliant on a limited set of prey (insect larvae) and C3 terrestrially- derived plants. The diets of two of species in particular (*H. fulginosus* and *L. unicolor*) became more generalised with size, and as a result were less well resolved with SIAR. Therefore, while large terapontids may be generalists, smaller individuals are more specialised. Identifying these stages is critical to understanding the trophic ecology of these species, and ultimately, the broader food web.

Correspondence and mismatches between SCA and SIA.

The two methods for assessing size-related dietary shifts produced both congruent and conflicting results. There was broad agreement between SCA and carbon mixing-model data. Shifts away from insect prey evident from SCA were reflected in increasingly enriched ^{13}C isotope data as both *L. unicolor* and *H. fuliginosus* increased in size, with corresponding mixing model outputs demonstrating progressively reduced importance of C3 riparian vegetation (with low $\delta^{13}C$). The ontogenetic dietary shifts revealed by both SIA and SCA were so profound in *H. fuliginosus* and *L. unicolor* that different life stages essentially acted as different trophic species. In contrast, *A. percoides*, which exhibited small size-related shifts from its insect diet, exhibited relatively minor shifts in its organic carbon sources through its life history. However, there was little agreement between the SCA and SIA estimates of trophic position. *Hephaestus fuliginosus* and *L. unicolor*, which exhibited no significant increases in trophic position on the basis of SCA, demonstrated significant, although minor, increases in trophic position according to SIA. *Scortum parviceps*, the only species to demonstrate a significant decrease in trophic position through SCA, exhibited no correlation between body size and trophic position according to SIA.

Caut *et al.* (2009) noted that disparity between stomach content and isotopic estimates of trophic position can be due to several factors such as insufficient sample sizes, biases in the contributions of basal sources used to derive trophic position estimates from δ^{15}N data, and errors in assumed δ^{15}N fractionation rates. While the sample of only 18 individuals of *S. parviceps* in this study may have been insufficient for accurate appraisal of diet, SCA for this species was similar to that in much larger studies in the same catchment (Pusey *et al.*, 2010; Davis *et al.*, 2011a), indicating that this was not the case. Sample sizes for the other species were relatively high and also concurred with previous research, indicating that SCA-derived estimates of trophic position were robust with regard to sample size.

The issue of assumed fractionation rates is particularly pertinent to trophic position estimates in isotopic studies. *Scortum parviceps* demonstrated the greatest disparity between the analytical methods, at least in relation to trophic position, with SIA significantly over-estimating trophic position in larger size classes compared to SCA. The dietary shift from carnivory (invertivory) in juveniles to herbivory in the largest size classes should have been accompanied by a relative reduction in δ^{15}N as fish grew. Apparent over-estimation of the trophic position of nominally herbivorous species using SIA is not uncommon (Carseldine and Tibbetts, 2005; Layman *et al.*, 2005b; Mill *et al.*, 2007; Winemiller *et al.*, 2011). Differences in food quality and feeding and excretion rates can cause herbivorous fishes to deviate significantly from the frequently cited 3-4% trophic-step enrichment rate applicable to more carnivorous species (Mill *et al.*, 2007), an effect that could account for the anomalies in the trophic position estimates for *S. parviceps*. *Scortum parviceps* differs from other sympatric terapontids not only in diet (Davis *et al.*, 2011*b*; this Chapter) but also in exhibiting many of the ecomorphological features of a specialised herbivore, such as flattened dentition and a long intestine (Vari 1978; Davis *et al.*, 2012a). Changes in trophic enrichment in relation to ontogeny constitutes an information gap in isotopic ecology, and represents an issue of considerable significance in herbivores, many of which, like *S. parviceps*, switch from carnivory to herbivory (see also Carseldine and Tibbetts, 2005).

The variable allocation of assimilated elements to different physiological processes via 'isotopic routing' has been suggested as another explanation for discrepancies between ingested foods and isotopic values of different tissues (Gannes *et al.*, 1997; Perga and Gerdeaux, 2005). While many studies have documented ontogenetic relationships between body size and δ^{13}C and δ^{15}N isotopes, these effects could result from changing physiological allocation of isotopes,

or changes in tissue turnover rates during ontogeny (Overman and Parrish, 2001), although these phenomena are poorly described (Fry and Arnold, 1982; Doucett *et al.*, 1999). The fact that stomach-content data aligned closely with $\delta^{13}C$ isotopic changes for three of the four species in this study provides more credence to isotopic changes in $\delta^{13}C$ being directly attributable to dietary shifts. Isotopic change in ectotherms (such as fish), is dominated by the effects of growth, which slows as fish age (Hesslein *et al.*, 1993, Suzuki *et al.*, 2005). Therefore, old slow-growing fish such as the large size classes reported here may not respond isotopically despite a recent change in diet, especially considering that elimination of ^{15}N-enriched nitrogen may occur slowly in muscle tissue of fish (MacNeil *et al.*, 2006). This latter point may be particularly relevant for species such as *S. parviceps* that become more herbivorous as they age, switching from a diet with a high N content and high $\delta^{15}N$ (animal protein) to one with low N content and low $\delta^{15}N$ (plant protein).

Another potential source of error in isotope-derived trophic position estimates is the change in the relative fractionation rates of key prey species that drive size-related diet shifts. Several studies have indicated that $\delta^{15}N$ fractionation rates of vertebrates can be higher than those for invertebrates (Vander Zanden and Rasmussen, 2001; Vanderklift and Ponsard, 2003). A similar outcome was apparent in this study, with invertebrate prey across all trophic guilds demonstrating much lower $\delta^{15}N$ values than corresponding fish species in the same trophic guilds. While it could be argued that these results underline the value of SIA in revealing the true trophic position of consumers, it is also possible that the ontogenetic increases in $\delta^{15}N$ for species such as *H. fuliginosus* and *L. unicolor* simply reflect higher relative $\delta^{15}N$ fractionation rates of larger-bodied and longer-lived prey (fish, macrocrustacea) that underpin ontogenetic changes in diet. Using $\delta^{15}N$ as a proxy for trophic position may be valid for specialised predators in relatively simple, linear food chains with significant size-based feeding (see Post, 2003), but the habits of more generalised carnivores and omnivores that feed among multiple trophic levels and include taxonomic groups with varying fractionation rates will likely introduce considerable uncertainty into assignment of trophic levels.

Shifts in basal production sources and the effects of omnivory

There is long-standing debate as to the dominant basal carbon sources supporting tropical aquatic food webs (Lewis *et al.*, 2001; Thorp and Delong, 2002; Douglas *et al.*, 2005; Jepsen and Winemiller, 2007; Zeug and Winemiller, 2008; Reid *et al.*, 2008; Lau *et al.*, 2009). Previous SIA studies have suggested that algal carbon is the dominant basal source supporting

tropical aquatic food webs (Lewis *et al.*, 2001; Thorp and Delong, 2002; Jepsen and Winemiller, 2007; Lau *et al.*, 2009), including freshwater ecosystems in northern Australia (Douglas *et al.*, 2005). In contrast, this study identified a range of autochthonous and allochthonous carbon sources supporting terapontid diets over different stages of their life history, although C3 vegetation was particularly important in the smaller size classes. Species shifted away from these relatively specialised juvenile diets to feeding across multiple carbon sources as they grew. An increasing number of studies are, similarly, highlighting the role of allochthonous plant matter in riverine and stream ecosystem function (Zeug and Winemiller, 2008; Lau *et al.*, 2009), including Australia (Reid *et al.*, 2008). The role of allochthonous detritus may be particularly relevant to the upper Burdekin River due to several aspects of its habitat structure. Pearson (1991) noted that although the upper Burdekin river bed is wide (~250m), for much of the dry season the wetted channel is relatively narrow (< 20m) and typically flows close to either bank, so that the influence of riparian vegetation may be greater than expected for such a large river. It is increasingly recognised that single conceptual models of carbon dynamics are not broadly applicable to different river systems, habitat units or individual species (Hoeinghaus *et al.*, 2007; Zeug and Winemiller, 2008). Similarly, with ontogenetic dietary shifts being such a fundamental component of fish dietary ecology, a single conceptual entity is unlikely to adequately represent a given species at all times.

Many of the size-related dietary shifts observed here occurred with only minor changes in trophic position. Even in species such as *L. unicolor* and *H. fuliginosus* where isotope-based increases in tropic position were evident, these changes were small (< 0.5 trophic position) and may well have been due to changes in fractionation rates of prey rather than changes in trophic position *per se*. Highly variable tropical systems characterised by short food chains, omnivory and little size-based feeding (Douglas *et al.*, 2005; Layman *et al.*, 2005*b*) may be conducive to major size-related trophic shifts coupled with minimal apparent change in trophic position. This study demonstrates the use of SIA and δ^{15}N-derived estimates of trophic position may yield limited, and potentially erroneous, insights into the nature and extent of ontogenetic diet shifts in many species. It is important to note that due to resourcing constraints this study was based on samples from one year's late dry season. Additional research is required to make robust inferences about basal production sources supporting terapontid diets, as there may be substantial hydrology-mediated shifts in the primary production sources (Zeug and Winemiller, 2008, Jardine *et al.*, 2012).

Limitations of Mixing-Model Approaches.

While isotopic mixing models have emerged as a popular tool in isotopic studies, some caution is warranted in interpretation of outputs. Mixing-model outputs do not provide a definitive solution for the organic matter sources supporting particular species, instead outlining a distribution of mass-balanced solutions from a nominated set of possible contributions (Phillips and Gregg, 2003). The ultimate resolution of mixing-model outputs is therefore dependent on the isotopic distinctiveness of basal sources. When multiple carbon sources demonstrate considerable overlap in isotopic value, as in this study, resolution of model inputs can be limited. The apparent role of particular carbon sources may simply reflect their similarity in isotopic value to other contributing sources, rather than their real contributions to higher level consumers. There is also increasing theoretical and empirical evidence that generalist populations, which broadly utilise a wide diversity of resources, are often composed of a heterogeneous assemblage of relatively specialised individuals of the same species (Bolnick *et al*., 2007). While beyond the scope of this study, populations composed of a collection of specialised individuals reliant on a range of different carbon sources will certainly pose challenges for mixing models applied at a broader site or population level (as in this study). Another of the most cited caveats for mixing model analyses is their susceptibility to assumed TEFs that have not been validated at species or tissue levels (Caut *et al.,* 2009; Bond and Diamond, 2011). However, one of the major strengths of the SIAR platform is the capacity to incorporate variation in TEFs into the model; the current study used large standard deviations of 0.5 and 1.4‰ for nitrogen and carbon respectively, likely leading to larger error estimates around source proportions.

Conclusions

Stable isotope analysis and stomach content analysis both demonstrated the important role of ontogenetic dietary shifts in the trophic ecology of Burdekin River terapontid species. However, each method had inherent limitations in defining trophic shifts and elucidating food web structure. It has been long appreciated that stable isotope ratios are most informative when used in conjunction with stomach content analyses (Mantel *et al.,* 2004; Layman *et al.,* 2005), but this dual approach may be particularly relevant to tropical aquatic ecosystems. With the widespread omnivory and minor increases in trophic position through life history, even in species with marked ontogenetic dietary shifts, the results of SIA considered in isolation will likely provide minimal insight into food web structure and the functional role of fish. Additionally, the meaningful resolution of SIA is contingent upon primary food sources that

exhibit distinct isotopic values. Because of the diversity of potential basal sources in riverine ecosystems, often with considerable isotopic overlap, diets and functional roles of consumers can be difficult to resolve solely on the basis of SIA. A range of associated uncertainties regarding fractionation rates through trophic levels, and the confounding role of ontogeny, further challenge meaningful interpretation of SIA mixing model outputs. Due to the cosmopolitan feeding habits of tropical species and wide-ranging solutions emerging from mixing model predictions (particularly in larger size classes of several species), concurrent use of SCA was valuable in disentangling the trophic complexity and specific structure of predator-prey interactions. Results of this study also indicate that for certain fish species, particularly those that exhibit pronounced size-related dietary shifts, the basal sources may change markedly with size. Ignoring the possibility of size-related shifts in organic matter supporting fish species is a simplistic and potentially flawed approach to definition of aquatic food web function.

Chapter 6: Marine-freshwater transitions are associated with the evolution of dietary diversification in terapontid grunters (Teleostei: Terapontidae).

*Published in the Journal of Evolutionary Biology **25**:1163-1179 (2012).*

6.1 Introduction

The interface between marine and fresh waters represents an imposing physiological challenge for aquatic species, and constitutes a barrier that a number of entire phyla (e.g., Echinodermata, Brachiopoda) and clades within other groups have failed to overcome (Lee and Bell, 1999). The suite of osmoregulatory mechanisms that facilitate permanent freshwater residency are therefore thought to represent a significant evolutionary adaptation for biota (Lee and Bell, 1999). The successful invasion of a novel habitat can also have significant evolutionary ramifications. The ecological opportunities associated with such profound habitat shifts are regarded as an important catalyst of diversification in a range of aquatic taxa, providing new scope for morphological, ecological and, ultimately, species radiation (Lee and Bell, 1999; Schluter, 2000; Streelman and Danley, 2003). Rapidly developing comparative approaches (Harvey and Pagel, 1991) that integrate diverse data from the geneaology, ecology and morphology of related species are increasingly being used to elucidate the significant role of marine-freshwater incursions in phyletic radiations of a range of biota (Cook *et al*., 2006; Betancur-R, 2010; Yamanoue *et al*., 2011).

Marine-freshwater transitions are a particularly interesting aspect of the evolutionary biology of many fish groups. Some fish lineages have apparently achieved the feat on multiple occasions (Lovejoy *et al*., 2006; Bentancur-R, 2010; Yamanoue *et al*., 2011), while radiations in several otherwise ecologically diverse fish lineages (Cypriniformes, Characiformes, Labridae, Acanthuridae) has been limited to either marine or freshwater environments (Lee and Bell, 1999; Nelson, 2006). The evolutionary outcome of successfully crossing the marine-freshwater ecotone is particularly relevant to the Australasian freshwater fish fauna. Due to its long biogeographic isolation the Australian freshwater fish fauna possesses several distinct evolutionary features such as being depauperate by global standards and having evolved largely independently of other continental faunas (Coates, 1993; Lundberg *et al*., 2000; Unmack, 2001;

Allen *et al*., 2002). It is particularly unusual for its prevalence of acanthopterygian fishes, which typically dominate marine environments, and for an almost complete lack of ostariophysan fishes, which usually dominate freshwater habitats on all other continents. The majority of Australia's fish fauna is purportedly composed of 'secondary' freshwater acanthopterygian species (i.e., freshwater species derived from marine ancestors), many of which have strong affinities with tropical Indo-Pacific marine fishes (Williams and Allen, 1987; Allen *et al*., 2002).

Fishes that successfully colonize freshwater habitats are usually confronted with a variety of new trophic niches (Bell and Andrews, 1997; Robinson and Schluter, 2000). Adaptation to these niches could lead to evolutionary diversification, including speciation. Diversification in trophic ecology is regarded as central to the spectacular phylogenetic diversification of a number of major fish lineages, particularly in the southern hemisphere (Fryer and Iles, 1972; Schaefer and Lauder, 1986; Winemiller *et al*., 1995; Streelman and Danley, 2003; Westneat and Alfaro, 2005; Correa *et al*., 2007). Despite the diversity of feeding modes evident amongst fishes, some trophic habits have evolved infrequently. Herbivores and detritivores, despite their numerical dominance in many communities (Knoppel, 1970; Lowe-McConnell, 1975), are confined to just a few families of teleost fishes (Choat and Clements, 1998; Horn, 1998; Horn and Ojeda, 1999). Moreover, the evolutionary patterns emerging from numerous phylogenetic studies have led to the general view that carnivory is plesiomorphic and herbivorous dietary habits derived in ray-finned fish evolution (Winterbottom and McClennan, 1993; Horn and Ojeda, 1999; Vermeij and Lindberg, 2000).

Australian fishes in the family Terapontidae provide a good model to evaluate the link between freshwater colonization and phylogenetic diversification. The Terapontidae contains 54 valid species in 16 genera, which occupy a broad range of environments. The family is unusual in that while it has such a broad marine distribution spanning much of the Indo-West Pacific, the majority of species (~40) are entirely restricted to the freshwater environments of Australia, Papua New Guinea and Indonesia (Vari, 1978; Allen *et al*., 2002). The remaining species are either exclusively marine or euryhaline in habit. While most Australian freshwater fishes show a lack of dietary diversification (see Merrick and Schmida, 1984; Kennard *et al*., 2001), the Terapontidae possess a remarkable diversity of feeding strategies, including specialized herbivorous and detritivorous trophic habits (Davis *et al*., 2011a, 2011b). While there is an abundance of ecological information recently emerging for the family, particularly freshwater

species, a comprehensive assessment of the dietary habits, including marine forms, is lacking. The diversification of terapontid feeding traits could be linked to the freshwater invasion of this group from ancestral marine species. However, to formally test this idea requires a well-resolved phylogeny of the family upon which foraging traits and habitat affiliations can be mapped. The most comprehensive treatment of the family is that of Vari (1978), whose genus-level phylogeny, based on comparative morphology, provided a hypothesis for within-family relationships. This phylogeny, however, has been uncorroborated by molecular phylogenetic approaches that are increasingly providing additional insights into patterns of evolutionary change to those possible with classical morphology-based phylogenetic approaches (Streelman *et al*., 2002; Lovejoy and Collette, 2001).

If terapontid fishes had a marine origin, then their colonization of fresh water in Australia would have provided scope for both ecological and phyletic diversification. However, whether the Terapontidae has a marine or freshwater origin has been somewhat uncertain, as is the case with several other prominent Australian freshwater fish families (Lundberg *et al*., 2000; Sparks and Smith, 2004). Fossil evidence suggests that the Terapontidae has had a long evolutionary history, with fossil terapontid material collected from a freshwater oil-shale formation dated to ~40-45 Ma (Turner, 1982; Henstridge and Missen, 1982). Vari (1978) speculated on an ancient origin for the family, suggesting that ancestral terapontids may have occupied the rivers, estuaries or shorelines of parts of Gondwana. Allen *et al*. (2002) suggested that freshwater grunters evolved from marine ancestors (most likely during the late Cretaceous or early Tertiary period). The basal (plesiomorphic) genera on Vari's (1978) terapontid phylogeny, however, are largely dominated by exclusively freshwater species, with marine-euryhaline genera occupying intermediate positions in the phylogeny (Figure 6.1). With freshwater genera restricted to Australia, New Guinea and other Indo-Australian Gondwanaland fragments, Lundberg *et al*. (2000) suggested that terapontids could be ancestrally freshwater fishes, with several more recently evolved marine species. The assumption of a marine derivation and number of potential marine and freshwater transitions occurring within the family is yet to be assessed, as are the phyletic and ecological outcomes of these transitions.

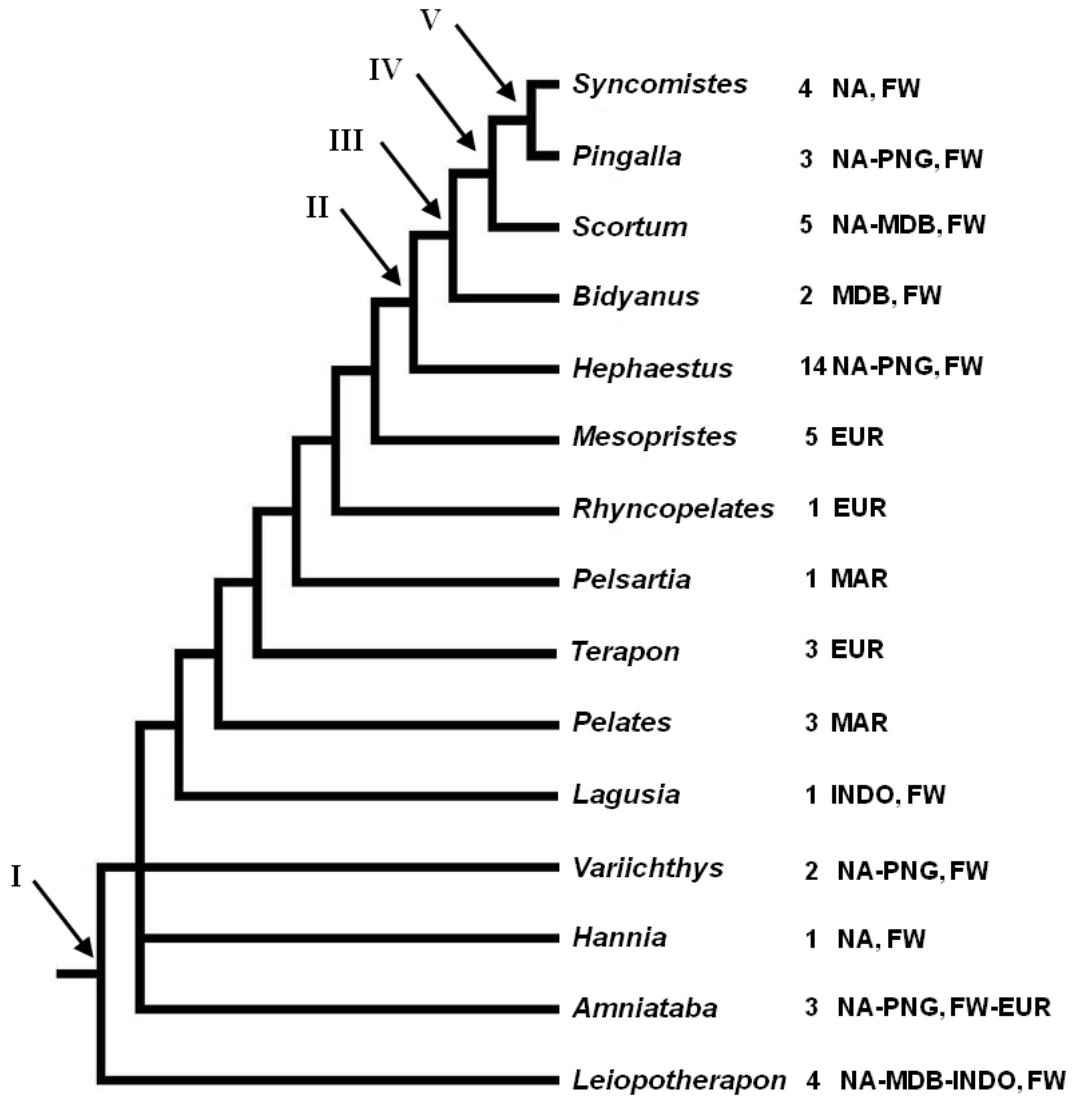

Figure 6.1 Cladogram depicting terapontid generic relationships derived from comparative morphology (adapted from Vari, 1978). For each genus, the number of species per genus recognised today, present distribution and habitat associations are indicated. NA, Northern Australia; MDB, Murray-Darling basin; PNG, Papua New Guinea; INDO, Indonesia. FW, exclusively freshwater; MAR, marine; and EUR, euryhaline. Node numbers: I plesiomorphic condition of conical dentition and "s" shaped intestinal convolution; II "six loop" intestinal configuration; III depressible dentition; IV moderately flattened dentition; V highly complex intestinal configuration, highly flattened dentition and dentary modification. Note that *Amniataba, Hannia* and *Variichthys* form an unresolved trichotomy. Vari (1978) also identified two distinct sub-clades within the *Hephaestus* genus ("genus a"; develops "6-loop" intestinal pattern, and "genus b"; retains plesiomorphic "s-shaped" intestine).

While there is some recent research highlighting the role of marine invasions in shaping Australia's freshwater fish fauna (Betancur-R, 2010; Unmack and Dowling, 2010), the macro-ecological processes following a marine-freshwater incursion are less well-studied. With the uncertainty over their habitat origins, and as exceptions to the limited dietary diversity of Australia's freshwater fishes, terapontids raise questions as to their habitat origins and phylogenetic derivation of trophic modes. The broad goal of this data chapter is to examine the phylogenetic evolution of terapontid habitat affiliations and dietary diversification in a new species-level molecular phylogeny. I test three hypotheses: (1) that similar to many prominent Australasian fish families, freshwater terapontids are derived from marine ancestors; (2) trophic radiation in the Terapontidae is consistent with that seen in other fishes, with carnivorous dietary habits plesiomorphic within the family; and (3) transitions across the marine-freshwater interface have underpinned the rapid diversification of freshwater lineages within the family. Results are discussed in the context of the biogeographic history of Australia's freshwater fishes.

6.2 Materials and Methods

6.2.1 Molecular study taxa and sampling

For the phylogenetic analysis I obtained 37 of the 54 species of Terapontidae (Supplementary online Table. 1). This included all but one genus (*Lagusia*), most marine species and most Australian freshwater species. Two species from the synonymized genus *Helotes,* which have previously been confused with species from the genus *Pelates,* were included (Sun, 1991; Johnson, 1999, 2010). Also included were two individuals of *Hannia greenwayi* Vari 1978 as the samples examined showed different phylogenetic relationships for all genes examined, thus making their phylogenetic placement ambiguous. Of the species missing from analyses, six are in the genus *Hephaestus* from New Guinea, while three species are in *Mesopristes*. The remainder are single missing species from seven genera.

6.2.2 DNA isolation, amplification and sequencing

Genomic DNA was extracted from muscle tissue from each specimen using the DNeasy Tissue Kit (QIAGEN Inc., Chatsworth CA). The mtDNA gene cytochrome *b* (cyt*b*) gene was initially amplified using two primers that flanked this gene: Glu31 GTGACTTGAAAAACCACCGTT and Tera.Thr.33 TAACCTTCAGCCTCCTGCTTACA. In many cases amplification of the gene in two overlapping regions using Glu31 and either Hd.GP GGRTTGTTTGAGCCTGTYTCGT or Hd.Alt GGRTTGTTGGAGCCTGTTTCAT was needed. For species in the *Leiopotherapon-Amniataba* clade Glu18 5'-TAACCAGGACTAATGRCTTGAA-3' was used instead of Glu31. For the second half of the gene eight different forward primers in conjunction with Tera.Thr.33 were used. Final concentrations for polymerase chain reaction (PCR) components per 25 µL reaction were as follows: 25 ng template DNA, 0.25 µM of each primer, 0.625 units of Taq DNA polymerase, 0.1 mM of each dNTP, 2.5 µL of 10X reaction buffer and 2.5mM $MgCl_2$. Amplification parameters were as follows: 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 48°C for 30 s, and 72°C for 60 s, and 72°C for 7 min. Two nuclear genes were also amplified, the recombination activating gene 1 and gene 2 (RAG1 and RAG2, which are jointly referred to as RAG) which are adjacent to each other. In fishes RAG1 consists of three exons and two introns (each of which are typically quite conservative) while RAG2 contains only one exon. All nuclear sequence was obtained by nested PCR (Supplementary online Table. 2). The first reaction size of 10 µL used PCR conditions listed above except extension was for 90 s. This first PCR reaction was then diluted to 1:49, and 1 µL of this product was added to a second 25 µL reaction. PCR products were examined on a 1% agarose gel using SYBR safe DNA gel stain (Invitrogen, Eugene, OR, USA). PCR products were purified using a Montage PCR 96 plate (Millipore, Billerica, MA, USA). Sequences were obtained via cycle sequencing with Big Dye 3.0 dye terminator ready reaction kits using 1/16th reaction size (Applied Biosystems, Foster City, CA). Sequencing reactions were run with an annealing temperature of 52°C following the ABI manufacturer's protocol. Sequenced products were purified using sephadex columns. Sequences were obtained using an Applied Biosystems 3730 XL automated sequencer at the Brigham Young University DNA Sequencing Center.

6.2.3 Analysis of sequence data

DNA sequences were edited using Chromas Lite 2.0 (Technelysium, Tewantin, Queensland, Australia) and imported into BioEdit 7.0.5.2 (Hall, 1999). Cyt*b* was aligned by eye while RAG sequences were aligned using the online version of MAFFT 6.822 (Katoh and Toh, 2010) using the accurate G-INS-i algorithm with the scoring matrix for nucleotide sequences set to 1PAM / K=2, a gap opening penalty of 1.53 and an offset value of 0.1. All coding sequences were checked for unexpected frame shift errors or stop codons in Mega 4.1 (Tamura *et al.*, 2007). Editing resulted in a 1141 base pair (bp) fragment of cyt*b* and a 3903 and 905 bp fragment of RAG1 and RAG2 respectively for a total of 5949 bp for each individual included in this study. Combined phylogenetic analyses were performed with both likelihood and parsimony approaches using GARLI 2.0 (Zwickl, 2006) and TNT 1.1 (Goloboff *et al.*, 2008). For maximum likelihood (ML) analysis the best-fitting model of molecular evolution was identified using the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada and Crandall, 1998) using PAUP* 4.0b10 (Swofford, 2003). For cyt*b* Modeltest identified TrN+I+G as the best model and for RAG GTR+I+G was the best model. For maximum likelihood (ML) analysis, GARLI with four search replicates using the default settings with two partitions representing cyt*b* and RAG with their respectrive models was ran. For bootstrapping, 1000 replicates were run with the previous settings except that the options genthreshfortopoterm was reduced to 10,000 and treerejectionthreshold was reduced to 20 as suggested in the GARLI manual to speed up bootstrapping. For maximum parsimony (MP) analysis, robustness of nodes was only estimated using TNT. Standard bootstrapping was conducted with a traditional tree search starting with Wagner trees with 10 random additions of taxa and TBR branch swapping for 10,000 replicates.

Trees were rooted with representatives from several related families based on Yagishita *et al*. (2009) including *Girella punctata* Gray, 1835 (cyt*b* GenBank AP011060.1) combined with RAG from *G. tricuspidata* (Quoy and Gaimard 1824), *Kuhlia mugil* (Forster, 1801), *Kuhlia rupestris* (Lacepède 1802), *Kyphosus cinerascens* (Forsskål, 1775) (cyt*b* GenBank AP011061.1) combined with RAG from *K. incisor* (Cuvier 1831), *Microcanthus strigatus* (Cuvier 1831) (cyt*b* GenBank AP006009.1), *Oplegnathus punctatus* (Temminck and Schlegel 1844) (cyt*b* GenBank AP011066.1) and *Scorpis lineolata* Kner 1865 (cyt*b* GenBank AP011063.1). Cyt*b* sequences obtained from GenBank are from the same species, but not the same individuals that were sequenced for RAG.

6.2.4 Ecological character classification

Dietary classification

Categorizing dietary data into discrete character states for the Terapontidae is difficult for several reasons. Specialised (or simple) dietary habits, where feeding is limited to a small number of dietary items, are rare within the Terapontidae. Most species consume a diversity of prey types, and significantly different variations of broad trophic modes such as omnivory or carnivory are common (Chapter 2). Ontogenetic dietary shifts are also common within the Terapontidae, with many species exhibiting several significantly different 'ontogenetic niches' during their life history (Davis *et al*., 2011b). Quantitative natural diet data for terapontid species was collated from a number of sources including published primary literature, unpublished governmental, consultancy and technical reports, as well as personal communications. The full list of species, their naming authority and references for dietary data used in this study is provided in Appendix 3. For those species with multiple dietary datasets available, data was discriminated on the basis of several factors with preference given to larger datasets and/or those assessing larger, preferably adult, size classes. For those datasets presenting dietary habits over a range of size classes, the largest size class information subset was preferentially used with juvenile data excluded from analyses. Volumetric or proportional data, one of the most frequently used indices of dietary contributions (Hyslop, 1980), was also the most commonly presented data type across data sources (~75% of studies), and was accordingly used as the standard method of data representation. Datasets presenting data in gravimetric units (dry or wet mass) were treated as directly comparable to volumetric techniques. Use of frequency of incidence or occurrence data was avoided where possible, but when used through necessity, it was transformed so as to approach proportional representation, following Kennard *et al*. (2001). For species with multiple datasets available, I averaged diet across populations among seasons and/or among studies.

A hierarchical clustering procedure was used to classify terapontid diets into similar feeding categories for character state reconstruction. Trophic data from collated studies were grouped into 12 broad dietary categories: unidentified component, zooplankton, macrocrustacea; other aquatic invertebrates; terrestrial invertebrates; fish; algae; aquatic vascular plants; terrestrial plant material; detritus-sediment; unidentified plant material and terrestrial vertebrates. The 'unidentified' diet category was excluded from all analyses and resultant dataset for each species normalized to 100% and then arcsine transformed to improve normality (Sokal and

Rohlf, 1995). Agglomerative hierarchical cluster analysis employing the Sorensen (Bray-Curtis) distance measure (McCune and Grace, 2002) in combination with flexible-beta linkage (ß = -0.25) were used to group species' diets. Resultant dendrograms were pruned subjectively, with the validity of these groupings then tested independently using non-parametric multi-response permutation procedures (MRPP; McCune and Grace, 2002).

Macro-habitat classification

Three broad macro-habitat types were differentiated for each terapontid species' habitat affiliation: marine, euryhaline (recorded occurrences across marine, estuarine and freshwater environments), and freshwater (exclusively freshwater). Habitat associations were sourced predominantly from published literature (Vari, 1978; Mees and Kaiolola, 1978; Allen, 1991; Pusey *et al*., 2004) and web-based distributional records (Froese and Pauly, 2011).

6.2.5 Analysis of character evolution

The most commonly used phylogenetic approach in studies of ecological diversification is ancestral character state reconstruction (Schluter, 2000). The choices for trait definition and coding (i.e., continuous vs. discrete characters) in ancestral reconstructions and their influence on results remains a matter of some debate (Stephens and Wiens, 2003). Coding ecological habits such as diet and habitat affiliations, which likely vary on a continuum rather than as discrete categories, is simplistic (Pianka, 2000), although representing complex characters such as diet with a simple continuous trait can be similarly problematic. Considering that available habitat data for most terapontids is qualitative in nature, and the macro-habitat scale of study questions (i.e., marine-freshwater transitions), character state reconstructions for habitat association were limited to discrete (categorical) characters. A combination of discrete and continuous approaches was used to reconstruct the evolution of dietary habits in Terapontidae.

Historical patterns of terapontid habitat association and dietary evolution were hypothesized by optimising discrete (categorical) and continuous characters onto the molecular phylogeny and branch lengths obtained from ML analysis, utilising the character reconstruction techniques in Mesquite 2.74 (Maddison and Maddison, 2006). Habitat and dietary data were available for all 37 species assessed in the genetic phylogeny except for *Helotes sexlineatus* (Quoy and Gaimard 1825), which most ichthyologists have failed to recognise as a valid species (Johnson, 1999,

2010). Dietary data for *H. sexlineatus* were accordingly coded as missing data ("?") in dietary character state reconstruction. Because alternative methods of categorical character state reconstruction can produce conflicting results (Ekman *et al*., 2008), both maximum parsimony (MP) and maximum likelihood (ML) methods of ancestral state reconstruction were employed (Schluter *et al*., 1997; Pagel, 1999). Parsimony ancestral state reconstruction, which minimizes the amount of character change given a tree topology and character state distribution, has been widely used, but may over-represent confidence in ancestral character states (Schluter *et al*., 1997). For the MP analysis, character transitions were considered to be unordered (changes between any character state are equally costly). One character or the other was assigned to a node if it created fewer steps, otherwise the node was considered equivocal.

Character states were also reconstructed using a ML approach, which finds the ancestral states that maximize the probability that the observed states would evolve under a stochastic model of evolution (Schluter *et al*., 1997; Pagel, 1999). A symmetrical Mk1 model (Lewis, 2001), which assumes equal forward and backward character transition rates, was used as the evolutionary model. A major advantage of ML is that the analysis takes branch lengths into account, allows uncertainty associated with each reconstructed ancestral state to be quantified and is preferable for medium-sized trees (Mooers and Schluter, 1999; Pagel, 1999). Likelihood ratios at internal nodes are compared by pairs, and were reported as proportional likelihoods. While likelihoods do not necessarily translate into levels of statistical significance, a difference of 2 log units for a character (i.e., ~7.4 times more probable than any other alternative state) was employed to assign states at a node, otherwise the node was considered equivocal (defined as 'the rule of thumb'; Pagel, 1999).

As a complement to categorical ancestral character state reconstruction to evaluating evolutionary changes in diet, we also reconstructed ancestral states of arcsine-transformed percent animal prey in diet using the ML phylogeny and squared-change parsimony in Mesquite 2.74 (Maddison and Maddison, 2006). For clarity and ease of interpretation, these variables were then untransformed into raw percentages.

6.2.6 Diversification analyses

To identify periods of potential diversification rate shifts within terapontid history, we used MEDUSA, a recently developed phylogenetic method which allows diversification rate shifts to be to be identified on an incompletely sampled phylogeny (Alfaro *et al*., 2009). MEDUSA utilizes a diversity tree as its basis, which corresponds to a time-calibrated or ultrametricised phylogeny, with a species richness value assigned to each tip of the tree based on taxonomic diversity. The MEDUSA algorithm is a stepwise procedure that first fits a single birth-death model (one birth rate and one death rate) to the entire diversity tree using a joint phylogenetic (edge-length) and taxonomic (richness) likelihood function developed by Rabosky *et al*. (2007). Then a series of increasingly complex models (e.g., 2-rate, 3-rate, etc.) are fitted to the tree with rate shifts occurring at internals nodes giving the highest likelihood. Medusa produces corrected Akaike criterion (AICc) scores, which are a bias correction for small sample sizes. Models are then compared using AICc scores to choose the best fit model. All MEDUSA analyses were implemented in GEIGER, a package developed for the statistical application R (http://www.r-project.org). I used the "runMedusa" and "summaryMedusa" commands in GEIGER, and chose the model with the lowest AICc value.

BEAST 1.6.1 (Drummond and Rambaut, 2007) was used to produce the ultrametric tree required for the MEDUSA analysis. BEAUti 1.6.1 was used to generate the input file for BEAST. An uncorrelated lognormal relaxed molecular clock was used with rate variation following a tree prior using the speciation birth death process. The same models of molecular evolution as per the ML analysis were employed. The tree from the ML analysis was used to fix the topology so that only branch lengths were estimated. Analyses were run for 50 million generations, with parameters logged every 1,000 generations. TreeAnotater 1.6.1 was used to create the final tree for input to MEDUSA. To create the terapontid diversity tree, the 37 taxon topology was pruned down to 25 tips. To preserve the maximum amount of phylogenetic resolution, tips were assigned a diversity of one (a single tip species) where possible. Following Alfaro *et al*. (2009), the diversity tree was resolved subject to two constraints; firstly tips had to represent monophyletic and non-nested taxonomic groups, and secondly, existing terapontid species richness was completely partitioned across the represented taxonomic groups. To account for missing species it was necessary to collapse several clades (Figure 6.7) and assign additional tip richness values to groups such as *Pelates* (assigned a tip richness of three), *Mesopristes-Rhyncopelates* (tip richness of six), *Scortum* (tip richness of five), *Pingalla* (tip

richness of three), *Syncomistes* (tip richness of four), *Variichthys* (tip richness of two), *Leiopotherapon* (tip richness of two), *Hephaestus* "genus a" (tip richness of six) and *Hephaestus* "genus b" (tip richness of seven). Assignment of unsampled species richness was based on previous phylogenetic and taxonomic descriptions and currently recognized terapontid species diversity (Vari, 1978; Froese and Pauly, 2011; Nelson, 2006).

6.3 Results

6.3.1 Genetic phylogeny

The genetic dataset consisted of sequences from 38 ingroup and 7 outgroup samples. The species *Terapon puta* Cuvier, 1829 had an insertion in cyt*b* of a single base pair present in the last codon which created a premature stop codon (which is not uncommon in cyt*b*). Of the 5949 bp sequenced, 3859 characters were invariant, 722 characters were variable but parsimony uninformative, and 1368 characters were parsimony informative. ML recovered one tree with a likelihood score of -36324.681391 (Figure 6.2). Overall, most nodes in the tree were well resolved with strong support (Hillis and Bull, 1993) evidenced by bootstrap values > 80 for the ML analyses. In contrast, MP provided no support at deeper nodes, although most shallow nodes had similar support from ML and MP (Figure 6.2). One supported node differed between analyses, MP recovered the three lineages of (*Hephaestus* (*Scortum*, *Syncomistes*/*H. tulliensis*) as (*Scortum* (*Hephaestus*, *Syncomistes*/*H. tulliensis*). Support for deeper nodes between the various marine species was either low, or <50; the marine species were typically separated by longer branches than those between the freshwater species, which may make resolution / obtaining bootstrap support more difficult from a single gene. However, the ML topology obtained for marine species was similar to MP except for placement of *Terapon theraps* Cuvier 1829 and *Pelsartia humeralis* (Ogilby, 1899) (TNT: data not shown).

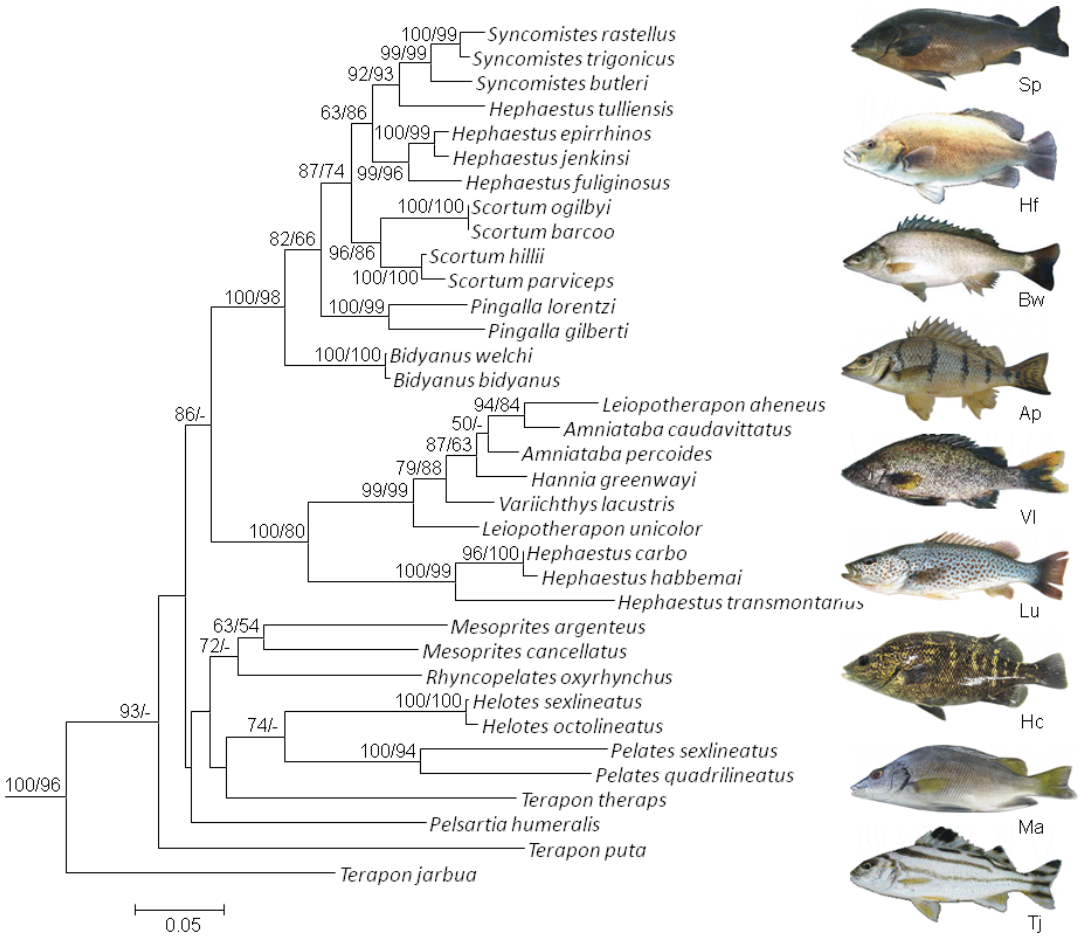

Figure 6.2 Maximum likelihood tree (-ln = -36324.681391) for Terapontidae species based on a combined analysis of cytochrome *b* and the recombination activation 1 and 2 gene sequences (5952 bp). Topology and branch lengths were estimated using maximum likelihood in GARLI with 1000 bootstrap replicates. Robustness of nodes was also estimated using TNT for maximum parsimony for 10,000 replicates. Bootstrap values are presented as ML/MP, with an # representing nodes with support from both methods > 99. One supported node differed between analyses, MP recovered the three lineages of (*Hephaestus* (*Scortum*, *Syncomistes*/*H. tulliensis*) as (*Scortum* (*Hephaestus*, *Syncomistes*/*H. tulliensis*), only the ML bootstrap value is shown for this incongruent node. Outgroup species were pruned from the tree. Images are identified by initials of genus and species nearby in the tree. Species labelled by abbreviation of generic and species names, identifiable from full names in tree.

6.3.2 Diet classification

Six broad feeding categories emerged from cluster analysis (Figure 6.3): zooplanktivore-invertivore – diets dominated by zooplankton and other invertebrate prey; invertivores – diets characterized by small aquatic insects and invertebrates; omnivores – diets consisting of a variety of both animal (invertebrates, fish) and plant material (aquatic and terrestrial vascular

plants, filamentous algae); generalist carnivores – diets dominated by large animal prey (fishes, macrocrustacea); herbivores- diets dominated aquatic plant material; detrivivores-algivores – diets dominated by detritus and filamentous algae. All of the six feeding category designations were significantly different in MRPP analyses, with significance values for all pairwise comparisons ranging between $P < 0.019$ to $P < 0.00001$. These categories were coded as categorical traits (0-5) and optimised onto the terapontid molecular phylogeny.

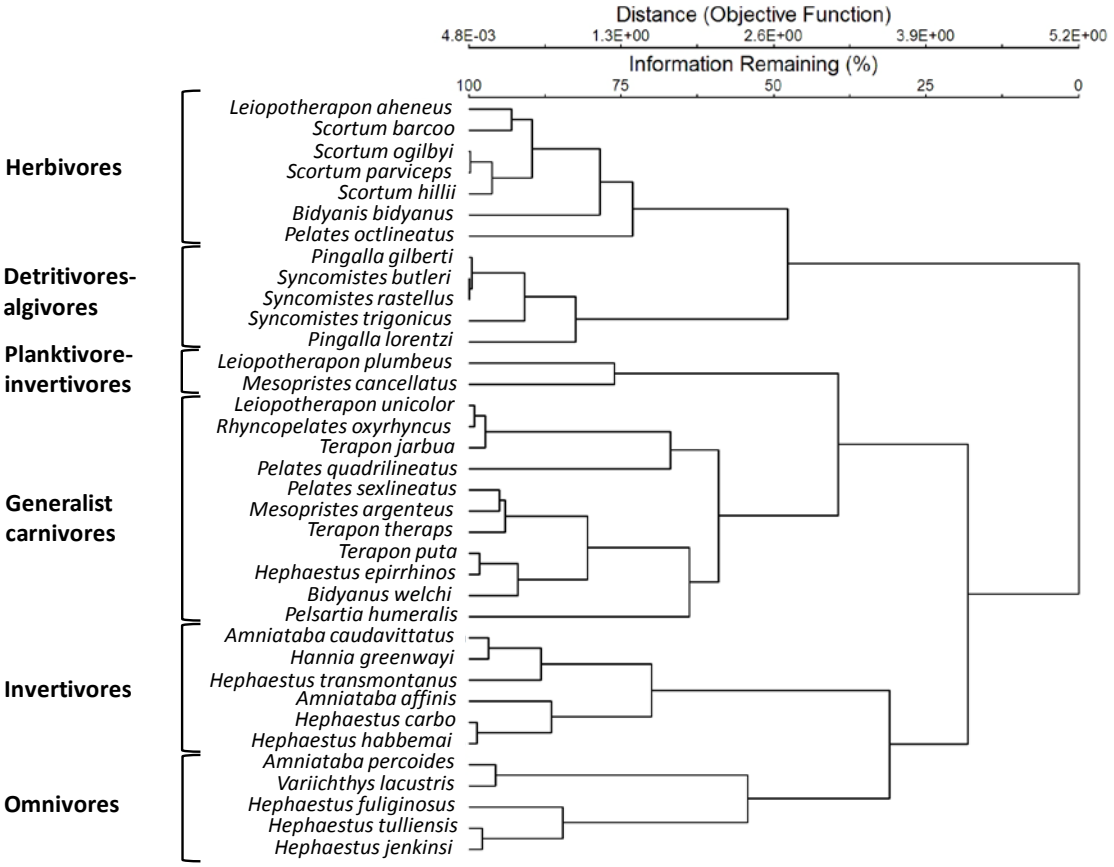

Figure 6.3 Classification of terapontid species' diets, using Sorensen (Bray-Curtis) distance measures with flexible beta linkage method.

6.3.3 Reconstruction of ancestral character states

Both MP and ML ancestral character state reconstruction produced similar outcomes regarding the evolutionary development of terapontid macro-habitat affiliations. Reconstruction of habitat association in the molecular tree unambiguously indicated the ancestral terapontid habitat type as euryhaline (Figure 6.4). Reconstruction of habitat preferences suggested a number of entirely marine-associated species likely evolved from this basal euryhaline species, as well as all freshwater species following a single transition to freshwater environments. Both approaches also suggested there has also been a single reversion from freshwater habits back to the plesiomorphic euryhaline habitat condition in *Amniataba caudavittatus* (Richardson, 1845).

MP dietary reconstruction identified a "generalist carnivore" as the unambiguous character state for the basal terapontid, a feeding habit that has been largely retained in marine and euryhaline terapontids (Figure 6.5). Zooplanktivore-invertivore and herbivorous feeding habits both evolved on single occasions in marine-euryhaline terapontids (*Mesopristes cancellatus* (Cuvier, 1829) and *Helotes octolineatus* Jenyns, 1840 respectively). MP reconstruction also indicated that the dietary habit of the initial terapontid invading freshwater environments was also a generalist carnivore. ML reconstruction could only resolve unequivocal character states at 10 internal nodes of the phylogeny, all of which were close to tips (Figure 6.5). Character state reconstruction suggested that following a single invasion of fresh waters, a rapid cladogenesis occurred, producing a range of dietary habits. This included habits that had rarely or never evolved in marine or euryhaline terapontids (detritivory-algivory, meiophagous and macrophagous omnivory and to some extent herbivory).

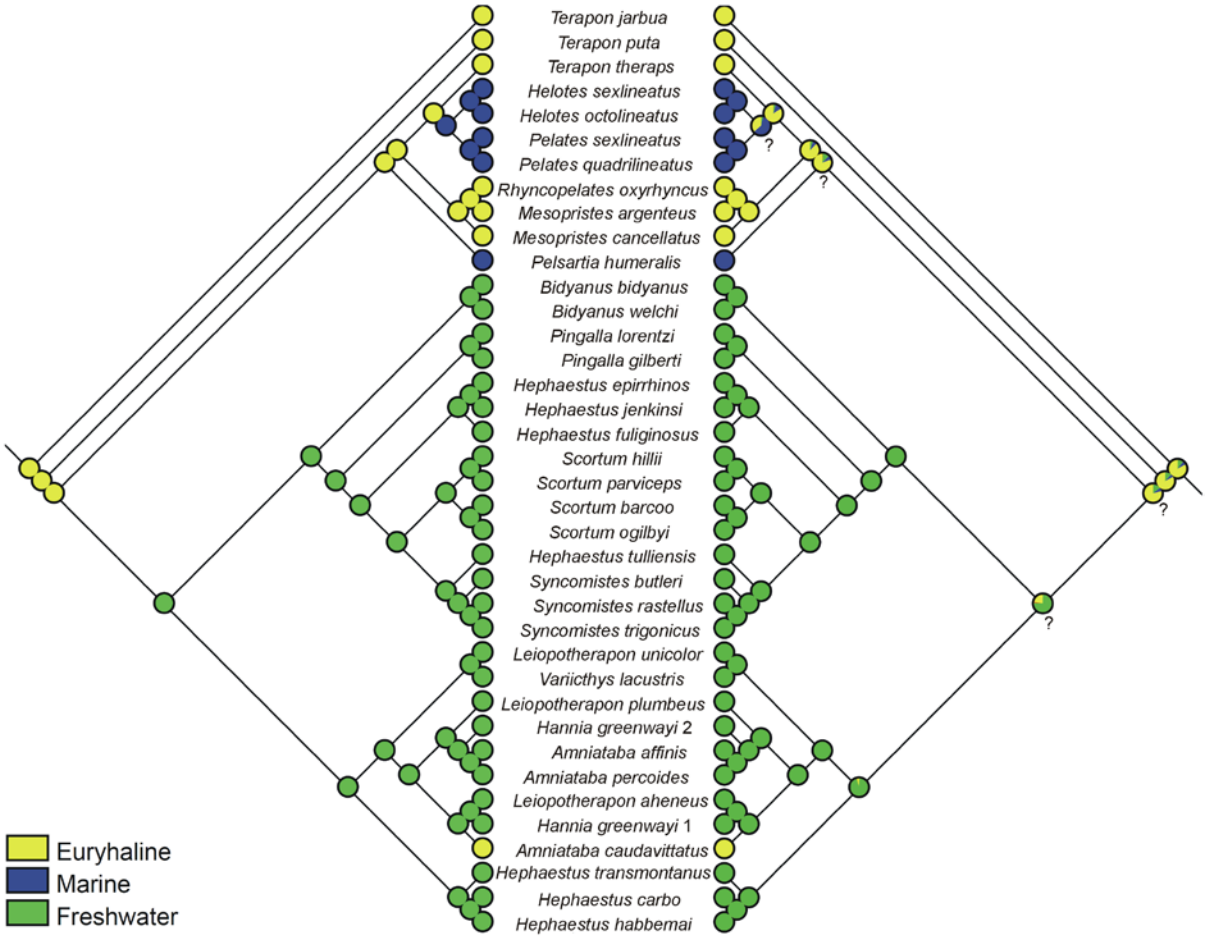

Figure 6.4 Maximum parsimony (left cladogram) and maximum likelihood (right cladogram) ancestral character reconstruction for the evolution of habitat association in the terapontid molecular phylogeny. Circles at terminal nodes represent the observed character state for extant species. Pie charts for ancestral nodes show estimated probabilities for reconstructed character states at that internal node. Significant support (more than 2 log units difference in ML) for an unequivocal character state at a node exists unless otherwise indicated with "?", in which case the node is equivocal.

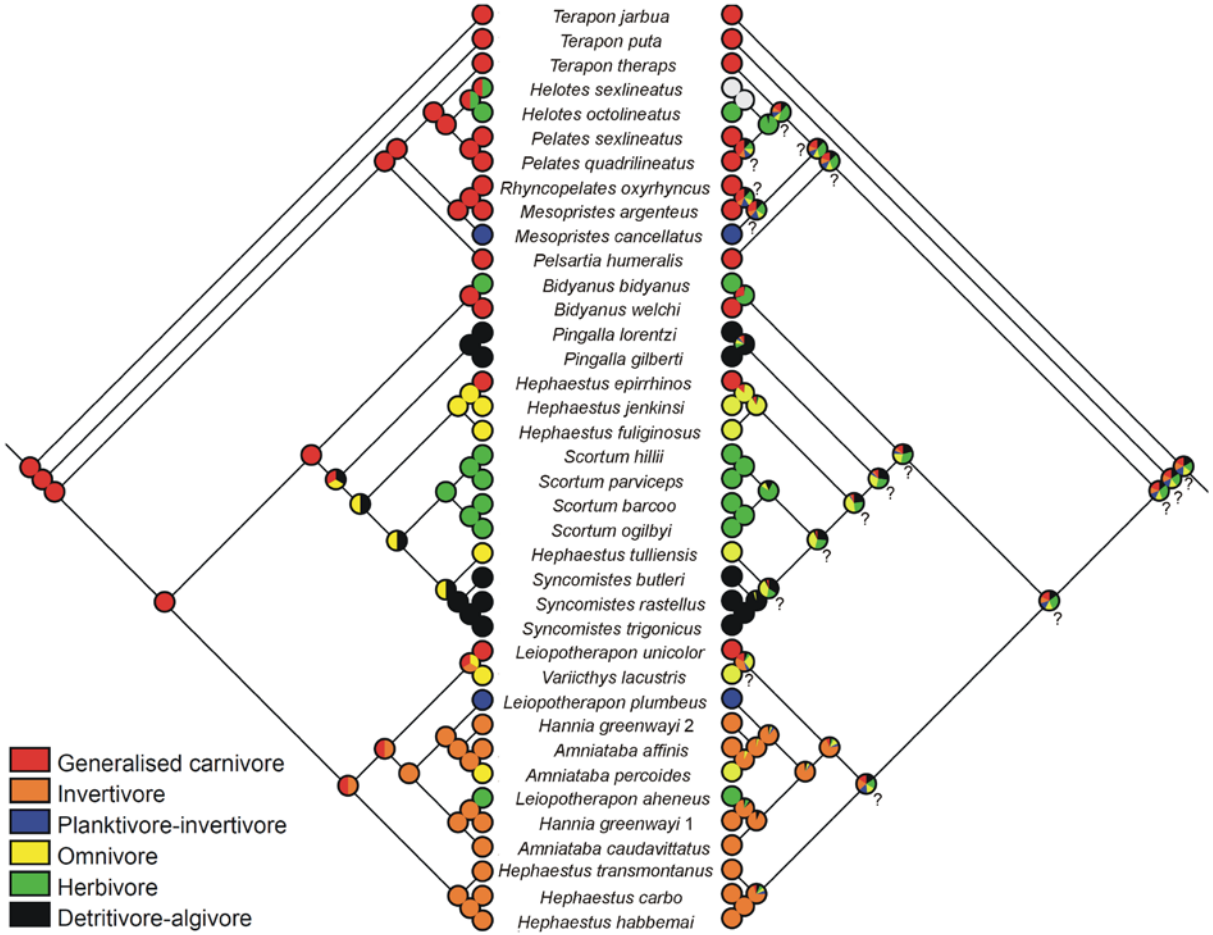

Figure 6.5 Maximum parsimony (left cladogram) and maximum likelihood (right cladogram) ancestral character reconstruction for the evolution of dietary ecology in the terapontid molecular phylogeny. Circles at terminal nodes represent the observed character state for extant species. Pie charts for ancestral nodes show estimated probabilities for reconstructed character states at that internal node. Significant support (more than 2 log units difference in ML) for an unequivocal character state at a node exists unless otherwise indicated with "?", in which case the node is equivocal.

The two major clades of freshwater terapontids each evolved a range of entirely new feeding modes compared to their euryhaline-marine predecessors. Invertivory, detritivory-algivory and meiophagous omnivory were new dietary modes to have evolved in the *Amniataba*, *Hannia*, *Leiopotherapon*, *Variichthys* and *Hephaestus* "genus b" clade, with *Leiopotherapon unicolor* (Günther 1859) retaining the ancestral macrofaunal predator feeding habit. Several new feeding habits such meiophagous and macrophagous omnivory, herbivory and detritivory-algivory evolved in the clade containing *Bidyanus*, *Scortum*, *Pingalla*, *Syncomistes* and *Hephaestus* "genus a". Both reconstruction approaches suggested the loss of macro-omnivorous feeding habits and reversion to the ancestral macrofaunal predator dietary mode in *Hephaestus epirrhinos* Vari and Hutchins, 1978.

Mapping of consumption of animal prey on the molecular phylogeny showed reliance on animal prey to be extremely labile in terapontids, particularly freshwater species (Figure 6.6). With the exception of *H. octolineatus*, animal prey dominated the diet of remaining marine and euryhaline terapontids, with hypercarnivory (>80% proportion of animal prey in diet) commonplace. In contrast, transitions away from carnivory to diets dominated by non-animal prey have evolved on multiple occasions in freshwater terapontids. Squared change parsimony reconstructions of ancestral diets suggested the basal terapontid was essentially carnivorous (~82% animal prey in diet). Likewise, the most recent common ancestor of all freshwater species was predominantly carnivorous (~75% animal prey in diet), before multiple transitions to non-animal prey diets on multiple occasions following the invasion of Australasian freshwaters.

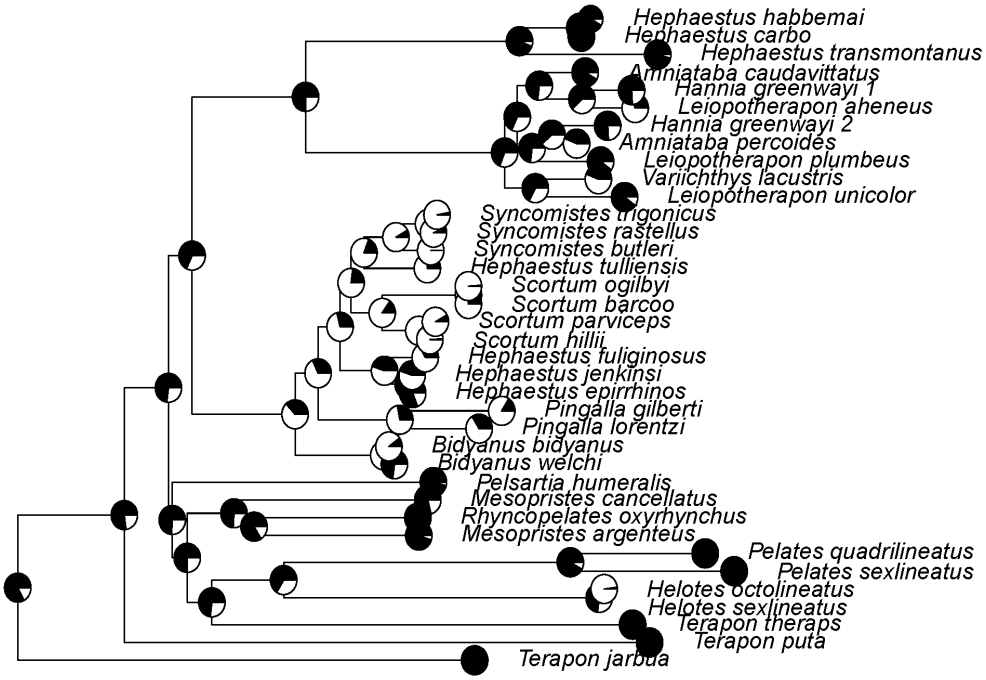

Figure 6.6 Squared-change parsimony reconstructions of percent animal prey mapped onto the maximum-likelihood phylogram of terapontid species used in this study. Pie charts of terminal taxa represent the percent animal prey in that species' diet according to the literature. Pie charts in of internal nodes indicate squared-change parsimony reconstructions of percent animal prey in the diet for that ancestor.

6.3.4 Diversification analyses

The diversification analysis found the net rate of diversification (*r*) of terapontids in a whole-tree birth model to be 4.79 (Table 6.1), with a log-likelihood value of –0.35. MEDUSA identified support for a birth-death model with one shift in diversification rate, occurring on the branch leading to the node uniting all freshwater species, as the best-fit model (log-likelihood value of 4.73) explaining the current diversity of terapontids (Figure 6.7). The net diversification rate for this clade (10.8) was ~2.7 higher than the background rate of diversification (3.95) occurring in other terapontid lineages, namely the marine-euryhaline clade. MEDUSA also inferred that the freshwater clade subtended by this rate shift exhibited a

strikingly higher species turnover (ε, extinction fraction d/b) compared to the background species turnover in other terapontid lineages. This outcome must be interpreted with some caution, as extinction rates are widely recognized as difficult to estimate with great precision in molecular phylogenies, particularly in the absence of fossil data (Rabosky, 2010). Results do provide a strong indication for an increased rate of diversification in the clade containing freshwater terapontids compared to other marine-euryhaline lineages.

Table 6.1 Table 6.1 Estimates of diversification rates for clades as noted in Figure 6.7. (*r*, net rate of diversification, *ε*, extinction fraction (d/b), AICc, corrected AIC scores for each model that added a turnover in diversification rate in the respective clade).

# of shifts	**clade**	***r***	***ε***	**AICc**
0 (whole-tree birth-death model)	whole tree	4.79	0.60	4.95
	1 (marine-euryhaline clade)	3.95	3.47×10^{-11}	
1	2 (freshwater clade)	10.84	0.135	1.94

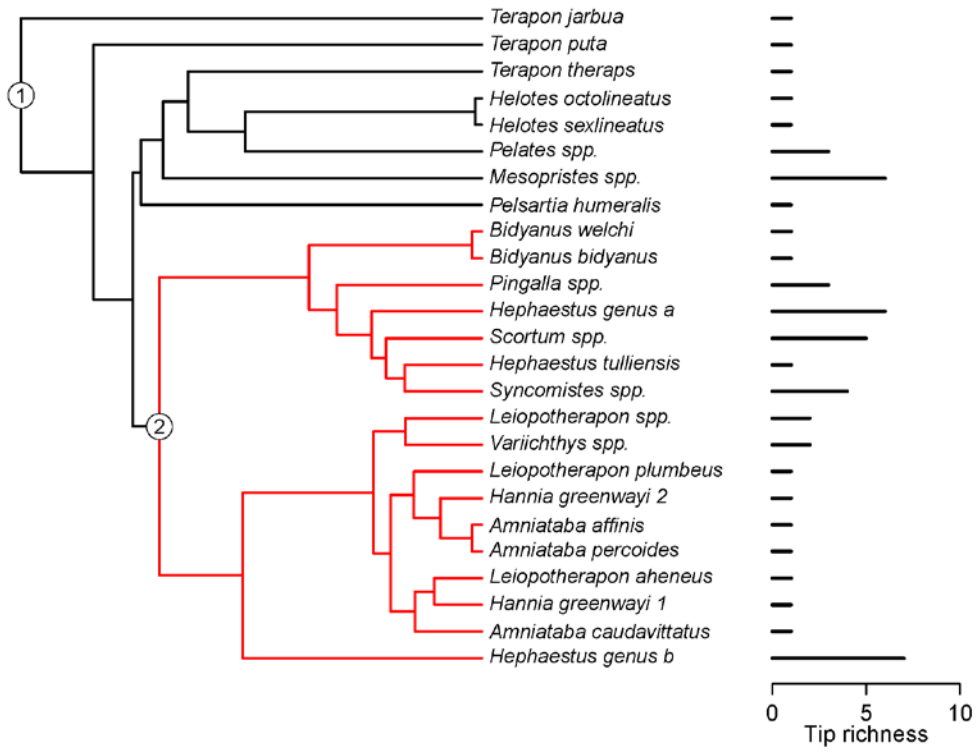

Figure 6.7 Phylogenetic placement of diversification rate shifts in the Terapontidae. Tip richness values to the right of each taxon name give the estimated lineage diversity (extant species richness) for the given taxon. Changes in rates of diversification (*r*) detected using MEDUSA are denoted by numbers at the appropriate node.

6.4 Discussion

The combined mitochondrial and nuclear gene analysis yielded a well supported tree with most nodes resolved. Mapping of terapontid habitat onto the molecular phylogeny predicted that a single transition to freshwater environments by euryhaline terapontids and subsequent cladogenesis produced all Australasian freshwater species within the family. Analysis of feeding modes predicted carnivorous habits were present in ancestral terapontids, with carnivorous modes remaining prevalent in extant marine and euryhaline species. The transition to freshwater habitats was followed by dietary diversification into a range of novel trophic habits largely restricted to freshwater species. In addition to this substantial ecological (dietary) diversification in fresh waters, comparative analyses suggested higher net diversification rates in the freshwater clade compared to marine-euryhaline lineages.

Terapontidae systematics

The topology of the molecular tree is quite different from Vari's morphological tree, in that the relationships between most of the genera differ and several genera are clearly not monophyletic. Freshwater terapontids were represented by two major clades. One clade included *Leiopotherapon*, *Amniataba*, *Hannia* and *Variichthys* as well as the available species representatives from *Hephaestus* "genus b" (as per Vari, 1978). The other major freshwater clade contained the following genera: *Hephaestus* "genus a", *Bidyanus*, *Scortum*, *Pingalla* and *Syncomistes*. At the broadest level there is some congruence between groupings within the two freshwater lineages and the marine species. Vari's tree has the same genera present in these groups, but the order of their relationships is different, as well as the within group relationships (Vari, 1978). Molecular evidence suggests that four genera are not monophyletic (Figure 6.2). *Hephaestus* was recovered in three different lineages, and could even be more diverse given that six species from New Guinea were not included. Neither *Amniataba* or *Leiopotherapon* were monophyletic and again, one additional *Leiopotherapon* species was not sampled. Within the marine species, *Terapon* was not recovered as monophyletic, lending support to the placement of *T. theraps* into the genus *Pseudoterapon* as proposed by Lee and Tsai (1999), although bootstrap support in that portion of the tree was lacking. Support was found for separation of the genera *Pelates* and *Helotes* as per Sun (1991) and Johnson (2010). A number of species have either experienced introgression or have incorrect taxonomy. The two *Bidyanus* species were almost identical for cyt*b*, but had a number of differences for RAG, suggesting mitochondrial introgression. Several species pairs had essentially none, or only minor variation between them despite apparent morphological differences: *Hephaestus carbo* (Ogilby and McCulloch, 1916) and *Hephaestus habbermai* (Weber 1910); *H. octolineatus* and *Helotes sexlineatus*; *Scortum barcoo* (McCulloch and Waite, 1917) and *Scortum ogilbyi* Whitley, 1951; *Syncomistes rastellus* Vari and Hutchins, 1978 and *Sycomistes trigonicus* Vari, 1978; and *Hephaestus epirrhinos* and *Hephaestus jenkinsi* (Whitley 1945). Individuals of these species that were examined at cyt*b* from additional geographic locations confirmed these results (data not shown). Further work focused on additional nuclear genes and morphological re-evaluation is necessary to resolve these taxonomic issues.

Marine-freshwater transitions

The pattern of freshwater invasion by terapontids presents a number of interesting evolutionary outcomes. Ancestral habitat reconstruction refuted the first study hypothesis, and a widely held

view of the family's phylogenetic history, that Australasian freshwater terapontids are derived from marine ancestors. Instead, the most likely hypothesis of terapontid evolutionary history is that both marine and obligate freshwater terapontids evolved from a common euryhaline ancestor, with a single invasion of freshwater environments. The osmotic adaptations that facilitate permanent freshwater residency are thought to represent significant evolutionary transitions for invading fauna (Lee and Bell, 1999). Coates (1993) noted that the colonisation of Australian continental fresh waters by 'marine' species would not have been a simple one-step process, but that secondary Australian freshwater fishes would almost certainly have emerged after a requisite transitional step through brackish-estuarine adapted species. Transitions from hyperosmotic euryhaline habits to exclusively freshwater life histories are important steps in the diversification of a number of fishes and other lineages over a range of time scales, from ancient to very recent (Lee and Bell, 1999; Waters and Wallis, 2001; Raeymaekers *et al*., 2005; Lovejoy *et al*., 2006; Whitehead, 2010). The inherent physiological resilience of euryhaline species is likely a major contributor to successful invasions of novel osmotic habitats.

While several fish clades such as the Gasterosteiformes and Fundulidae have successfully invaded freshwater habitats on multiple occasions (Schluter, 2000; Whitehead, 2010), terapontids parallel a number of other notable fish families with just a single invasion of freshwaters. South American freshwater stingrays, needlefishes and anchovies (Lovejoy *et al.*, 1998; Bloom and Lovejoy, 2012), sculpins (Yokoyama and Goto, 2005), African herrings (Wilson *et al.*, 2008), freshwater pufferfishes (Yamanoue *et al.*, 2011) and the Terapontidae all share a very similar pattern of a single or very few invasions of fresh water by a marine lineage into a specific geographic area. Regardless of the number of marine to freshwater invasions within a lineage, most studies further suggest directionality from marine to fresh water appears to be the rule not only in fishes but other marine taxa (Lee and Bell, 1999). Transitions from freshwater to marine habitats, particularly successful re-invasions of ancestral marine habitats, are relatively rare (Betancur-R, 2009; Whitehead, 2010; Bloom and Lovejoy, 2012). While comparative osmotolerance data is lacking for most freshwater terapontids, extensive habitat and distribution data for available for many species (Pusey *et al*., 2004) make it clear they are limited to freshwater habitats. A similar habitat limitation also exists in several other freshwater invading lineages such as Fundulidae (Whitehead, 2010) and Engraulidae (Bloom and Lovejoy, 2012). The evolution of an exclusively freshwater physiology within Terapontidae appears to be largely coupled with contraction of the physiological plasticity seen in euryhaline ancestors. This suggests the freshwater form in terapontids is a derived physiological specialised state,

although, interestingly, there has been an apparent single reversion back to the ancestral euryhaline phenotype in *A. caudavittatus*. This phenomenon of phylogenetic niche/biome conservatism is a common theme of many marine to freshwater habitat transitions (Lee and Bell, 1999; Whitehead, 2010; Bloom and Lovejoy, 2012). Two hypotheses have been proposed to explain the asymmetry in evolutionary directionality of osmotic tolerance shifts: the genetic and mechanistic difficulties in evolving a hyperosmotic and euryhalinic phenotype from a hyposmotic/stenohalinic phenotype; and competitive exclusion by incumbent faunas (Whitehead, 2010; Bloom and Lovejoy, 2012).

Evolution of terapontid diet

Mapping of feeding modes onto the molecular terapontid phylogeny, largely confirmed the second study hypothesis, suggesting carnivory is plesiomorphic within the family, with all other terapontid trophic habits evolving from this ancestral diet state (Figure 6.5). The successful invasion of fresh water by these ancestral euryhaline carnivores was followed by significant dietary diversification (Figures 6.4 and 6.5). Feeding habits diverged from generalist carnivory to a range of trophic modes including omnivory, herbivory and detritivory. However, invertivorous and omnivorous feeding habits account for a substantial proportion of dietary habits among freshwater terapontids, paralleling the trophic characteristics of the broader Australiasian ichthyofauna (Coates, 1993; Kennard *et al*., 2001). The main trophic opportunities available to fishes probably greatly differ between marine, estuarine and freshwater environments (Coates, 1993), with submerged aquatic macrophytes, allochthonous materials (e.g., terrestrial leaves, fruits, seeds, insects and vertebrates) and detritus being less important sources of food in estuarine-marine environments than in fresh waters. Differential consumption of these 'novel' prey items accounts for much of the trophic diversification evident in freshwater species compared to euryhaline-marine counterparts.

In addition to the evolutionary significance of the terapontid marine-freshwater invasion, the subsequent evolution of herbivorous and detritivorous feeding habits primarily within a number of obligate freshwater terapontid genera also represents a novel evolutionary event. Herbivory has evolved just once in marine-euryhaline terapontids, in *H. octolineatus*, a species found in south-western Australian marine waters. In contrast, herbivory and detritivory have evolved independently in multiple freshwater species across several genera. Despite the diversity of feeding modes amongst fishes, herbivory and detritivory are relatively rare, being confined to restricted sets of families within teleosts (Choat and Clements, 1998; Horn, 1998). Fishes that

consume macroalgae, diatoms or angiosperms as the major part of their diet make up less than 5% of the 426 recognised families of teleostean fishes, with herbivorous representation even less pronounced at species level (Horn, 1998; Horn and Ojeda, 1999). Diets with plant and/or detrital material making up the dominant proportion of diet accounted for ~2/3 of the freshwater terapontids included in this study.

A central question in the evolutionary biology of terapontid fishes is the relationship between morphology and dietary habits across the family. It is expected that, following the invasion of a new habitat, species will show rapid cladogenesis and associated ecomorphological (often diet-related) diversification (Schluter, 2000; Streelman and Danley, 2003). Considerable morphological divergence certainly exists in characters like intestinal convolution, oral anatomy and dentition across the Terapontidae, particularly amongst freshwater species (Figure 6.1, Vari, 1978). Previous ecomorphological research conducted without a comparative phylogenetic framework suggests that terapontid characters such as intestinal length and dentition have significant correlations to dietary habits such as herbivory and detritivory (Davis *et al*., 2010). The molecular phylogeny presented in this study can provide a basis for phylogenetically informed assessment of diet-morphology correlations and 'character releases' (*sensu* Schluter, 2000) associated with the terapontid freshwater invasion.

Implications of freshwater invasion on family diversification

Comparative analyses identified the invasion of fresh waters, in addition to ecological (trophic) diversification, also produced an increased diversification rate in the radiating freshwater clade relative to background rates. While freshwater terapontids have diversified at approximately twice the rate of marine-euryhaline lineages, the scale of the freshwater radiation has been relative modest (< 40 recognised species) compared to radiations seen in other fishes (i.e., Barlow, 2000; Westneat and Alfaro, 2005). With fossil evidence suggesting that the Terapontidae have had a long evolutionary history in fresh waters (~40–45 Ma; Turner, 1982), freshwater terapontids have had ample apparent opportunity time for substantial species radiation. However, it should be noted that terapontids are just one of many ancestrally euryhaline or marine lineages that have invaded Australian fresh waters (others include Ariidae, Plotosidae, Atherinidae, Ambassidae, Apogonidae and Eleotridae), all of which have exhibited similarly modest subsequent species radiations (i.e., < 50 species at most and much lower diversity in many cases) in freshwater habitats (see Allen *et al*., 2002). Taxonomic examination of many Australian species is lacking and current estimates of species richness are clearly

underestimates (Lundberg *et al*., 2000), which may affect understanding of the implications of freshwater invasions. Australian continental fresh waters are nevertheless characterized by a range of attributes (broad-scale aridity, highly variable hydrology, lack of habitat diversity) that may restrict opportunities for substantial radiation compared to other continents (Allen *et al*., 2002).

Biogeography and the dietary habits of Australia's freshwater fishes

While species-level diversification following the freshwater invasion in terapontids has been relatively limited, this major habitat transition has triggered substantial diversification in trophic ecology. Ecological opportunity theory proposes that organisms freed from competitive pressure, such as through the invasion of novel and/or unoccupied habitats, will experience an 'ecological release' typified by increased cladogenesis and/or phenotypic and morphological evolution (Schluter, 2000). Habitats with depauperate fish communities and a lack of pre-adapted incumbent fauna are similarly regarded as a macroecological condition that facilitates colonisation and subsequent radiation by invading species (Lee and Bell, 1999; Robinson and Schluter, 2000). For example, Winemiller *et al*. (1995) speculated that the relative paucity of herbivorous and detritivorous trophic habits in the South American cichlid fauna was due to competitive exclusion from this niche by characiform and siluriform fishes. A contrasting situation may have existed on the Australian continent because the timing of its isolation from Gondwana precluded the presence of groups such as cichlids, characiforms, cypriniformes and most siluriformes. Together these lineages, which are absent from Australia, have evolved the dominant proportion of herbivorous and detritivorous feeding modes in freshwater fishes (see Schaefer and Lauder, 1986; Barlow, 2000). Australia and New Guinea do possess an indigenous siluriform fauna (ariid and plotosid catfishes), although these groups, like the Terapontidae, are thought to be secondarily derived from marine ancestors (Allen *et al*., 2002; Bentancur-R, 2010).

Australia's known freshwater-evolved fish families such as Neoceratodontidae and Osteoglossidae, as well as groups with likely ancient freshwater origins such as Lepidogalaxiidae, Galaxiidae, Retropinnidae, Melanotaeniidae, Pseudomugilidae and Percichthyidae, are all predominantly carnivorous or omnivorous (Pusey *et al*., 2004). Species relying on plant material or detritus as their dominant food are rare across Australia's freshwater fish fauna (Kennard *et al*., 2001). It is notable that the few non-terapontid herbivorous-detritivorous species in Australian fresh waters, such as species of Clupeidae,

Engraulidae, Mugiliidae and Hemirhamphidae, often occur in euryhaline habitats and are probably similarly derived from euryhaline-marine ancestors (Pusey *et al*., 2004). Herbivorous-detritivorous trophic habits may have constituted a vacant niche for early freshwater-invading fish families such as the Terapontidae. Similar mechanisms have been recently proposed as facilitating the phylogenetic diversification of ariid catfishes, which have undergone their greatest freshwater radiations in the same biogeographical realm as the Terapontidae (Bentancur-R, 2010).

The dietary diversity displayed by freshwater terapontids raises the question of why there are few herbivorous-detritivorous or omnivorous marine-euryhaline species. Recent molecular studies of the suborder Percoidei suggest that terapontids are closely related to families such as the Kyphosidae, Kuhliidae and Oplegnathidae (Yagishita *et al*., 2009; Near *et al*., 2012). In particular, Kyphosidae are a prominent family of marine herbivores (Horn, 1989), likely exhibiting herbivorous habits since the Eocene (Bellwood, 2003). The long-term existence of these related families, and other herbivorous marine fish lineages (see Bellwood, 2003) may have played a role in constraining evolution of herbivory or detritivory in marine terapontids. While marine-euryhaline terapontids have largely retained the ancestral condition of carnivory, they have evolved some relatively novel feeding habits within the constraints of this broader carnivorous lifestyle – for example, lepidophagy (scale-eating) in *Terapon jarbua* (Forsskål, 1775) (Whitfield and Blaber, 1978; Sazima, 1983) and parasite-cleaning by juvenile *Rhyncopelates oxyrhyncus* (Temminck and Schlegel, 1842) (Shigeta *et al*., 2001). The degree of terapontid dietary diversification documented in this study is interesting given the relatively small number of species (37) compared to phylogenetically and ecologically diverse fish groups such as cichlids or labrids with hundreds or thousands of species. While freshwater terapontids display the greatest variation in feeding habits in the family, the Terapontidae as a whole clearly has considerable scope for dietary diversification, regardless of habitat affiliation.

Conclusions

This chapter indicates that Australasian freshwater and marine terapontids are derived from euryhaline ancestors. The successful invasion of fresh waters triggered a substantial diversification in trophic habits, in addition to significant increases in diversification rates in freshwater lineages compared to euryhaline-marine clades. The absence of most primary freshwater fishes in continental Australia, particularly herbivores-detritivores, may have facilitated the colonisation and subsequent diversification in the under-utilised trophic niche

space present on the Australian continent. While the current molecular phylogeny contains the majority of putative terapontid genera, it lacks several taxa including one genus (*Lagusia*) from Sulawesi and several poorly known freshwater species from New Guinea. The availability of additional phylogenetic and ecological information from these species will no doubt refine understanding of the evolution of this distinctive family. Future comparative assessment of the correlations between morphological and ecological characters will also provide interesting tests of the interdependence between diet and the morphological diversification evident in terapontids. The results of this research provide compelling evidence that crossing the marine-freshwater boundary is an important trigger for fish ecological diversification.

Chapter 7: Ontogenetic development of intestinal length and evolution of diet in the trophic radiation of an Australasian fish family (Terapontidae).

In review at BMC Evolutionary Biology

7.1 Introduction

Morphological divergence associated with dietary shifts has played a major role in the phyletic radiation of many vertebrates (e.g., Grant, 1986; Albertson *et al*., 1999; Streelman and Danley, 2003; Vitt *et al*., 2003; Clements and Raubenheimer, 2006). These evolutionary changes in diet and trophic morphology can occur rapidly (Espinoza *et al*., 2004; Burbink and Pryon, 2009), even within ecological timescales (Herrel *et al*., 2008). However, the frequency with which particular dietary modes have evolved varies considerably across different vertebrate lineages. While plant-based diets have a broad taxonomic distribution among mammals (> 25%) (Price *et al*., 2012), the occurrence of herbivory is much more restricted (2–5% of species) amongst other vertebrate groups (Choat and Clements, 1998; Espinoza *et al*., 2004). Despite the wide array of feeding modes amongst fishes and the biomass dominance of herbivorous and detritivorous fishes in many communities (Knoppel, 1970: Lowe-McConnell, 1975), the development of herbivorous-detritivorous trophic habits has been an infrequent evolutionary phenomenon, being largely confined to a few families of teleosts (Choat and Clements, 1998; Horn, 1998; Horn and Ojeda, 1999; Nelson, 2006; Lujan *et al*., 2011). The morphological and physiological specializations that facilitate access to the nutrients held within plant cells have accordingly attracted considerable interest from ecologists and evolutionary biologists (Horn, 1989; Choat and Clements, 1998; Karasov and Martinez del Rio, 2007; German, 2011; Lujan *et al*., 2011).

One of the most widely identified ecomorphological relationships between organismal morphology and ecology, and one particularly relevant to dietary radiations involving plant-detrital diets, is intestinal length. The vertebrate digestive tract represents a functional link between foraging (energy intake) and energy management and allocation, but is energetically costly to maintain, and may account for 20–25% of the whole animals metabolic rate (Karasov *et al*., 2011). A core prediction of digestive theory (*sensu* Silby, 1981; Karasov and Martinez del Rio, 2007) is that the consumption of food with a high content of indigestible material

results in an increase in gut dimensions. Numerous studies have shown that digestive tracts tend to be shortest in carnivorous species, intermediate in omnivores and longest in herbivorous and detritivorous species, a general relationship evident across all vertebrate classes (Stevens and Hume, 1995; Ricklefs, 1996; Karasov *et al*., 2011). The functional significance of this association lies in the need for species on low protein – high roughage diets to have longer guts in order to ingest larger volumes of lower-quality food, increase absorptive surface area and maximise digestive efficiency (German, 2011). However, most previous research conducted on diet-intestinal length relationships has made little acknowledgment of the evolutionary history of the studied species (Karasov and Martinez del Rio, 2007). Species sharing a common ancestor are not evolutionarily independent, and phylogenetic proximity voids the assumption of sample independence underpinning many conventional statistical tests, thereby creating difficulties in attributing morphological-ecological relationships to adaptive causes rather than phylogenetic artefacts (Felsenstein, 1985). Applying caution to inferences drawn from phylogenetically naive diet-intestinal length studies is being increasingly advocated (Elliott and Bellwood, 2003, German and Horn, 2006; Karasov and Martinez del Rio, 2007; German *et al*., 2010). While an abundance of comparative ecomorphological studies of oral kinematics, food procurement and dietary habits in vertebrates has recently emerged (Herrel, 2001; 2009; Westneat, 2004; Higham *et al*., 2006), the association between diet and intestinal length has received surprisingly little phylogenetically informed attention; recent exceptions include Wagner *et al*. (2009) and German *et al*., (2010).

While developmental plasticity has long been posited to play a key role in the origin and diversification of novel traits (Pfennig *et al*., 2010), the developmental processes underpinning interspecific differences in intestinal length are a largely neglected aspect of evolutionary study. Interspecific variations in intestinal length between closely related species are largely driven by variations in allometric intestinal growth during ontogeny (Kramer and Bryant, 1995; Davis *et al*., 2012a). Substantive allometric increases in intestinal length typically involve additional intestinal looping or convolution that must be accommodated in the body cavity (Zihler, 1982). Previous research has suggested that looping patterns are not random, with an underlying phylogenetic component, so that patterns of development of intestinal looping have been used to reconstruct the phylogenetic systematics of a number of fish lineages (Vari, 1978; Zihler, 1982; Yamaoka, 1985). Yamaoka (1985) noted that use of intestinal complexity as a tool in systematic research involves a ‘two-storey’ structure, with the first storey comprising a qualitative aspect (coiling pattern), and the second storey composed of the quantitative

(functional) component of intestinal length. It appears that there are no studies that integrate the description of ontogenetic development patterns with recent techniques in molecular phylogenetic reconstruction and comparative approaches. Concurrent appraisal of the ontogenetic processes producing variation in intestinal lengths, and the ultimate functional significance of these processes (i.e., associations with diet) in a phylogenetic context are similarly lacking.

Northern Australia's Terapontidae (grunters) offer a promising candidate for examining the relationship between intestinal length and diet, and the phylogenetic context for ontogenetic development of intestinal length. The Terapontidae is one of the most species rich and trophically diverse of Australia's freshwater fish families, exhibiting feeding habits that span carnivorous, omnivorous, herbivorous and detritivorous modes (Davis *et al*., 2011b). A genus-level phylogeny of the family by Vari (1978) relied heavily on differences in ontogenetic development of intestinal configuration as a diagnostic character. Vari's (1978) morphological character analysis suggested that a sequence of four intestinal patterns of increasing complexity in the Terapontidae, beginning at the ancestral (plesiomorphic) condition of a simple two-loop intestine throughout life history in the genera *Leiopotherapon, Amniataba, Hannia, Variichthys, Lagusia, Terapon, Pelates, Pelsartia, Rhyncopelates* and *Mesopristes* (Figure 7.1). The genera *Hephaestus, Bidyanus* and *Scortum* have an intermediate "six-loop" pattern. Juveniles of these genera have the two-loop pattern seen in ancestral genera before undergoing an ontogenetic elongation and folding to produce more complex patterns as adults. Vari noted that this pattern appears to have been secondarily lost in a subunit of *Hephaestus* referred to as *Hephaestus* "genus b". The adult life stages of the genera *Pingalla* and *Syncomistes* purportedly undergo a further ontogenetic shift to produce a highly convoluted and elaborate intestinal pattern, with the final and most complex intestinal pattern unique (autoapomorphic) to *Syncomistes*. Juveniles of the species in *Pingalla* and *Syncomistes* possess a similar intestinal convolution to adults of genera exhibiting the adult "six-loop" pattern, with Vari presuming these species pass through the simple "two-loop" pattern earlier in ontogeny.

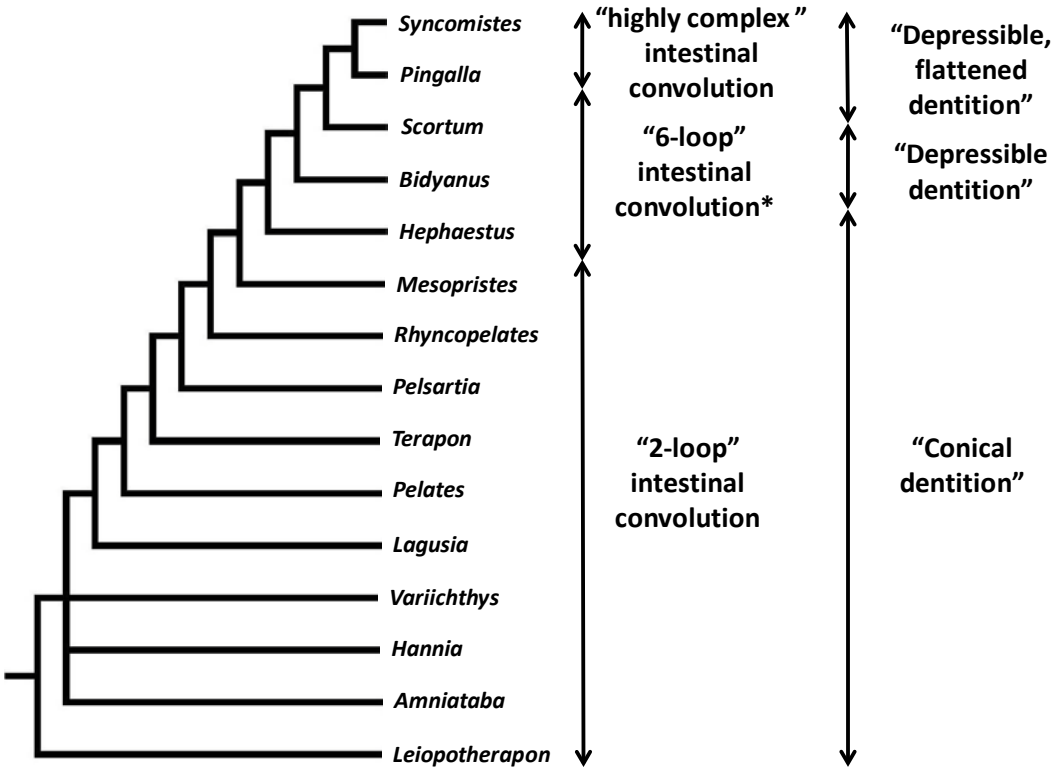

Figure 7.1 Cladogram depicting terapontid generic relationships derived from comparative morphology (adapted from Vari, 1978), showing intestinal convolution and dentition characters used to differentiate genera. Note that *Amniataba, Hannia* and *Variichthys* form an unresolved trichotomy. Vari (1978) also identified two distinct sub-clades within the genus *Hephaestus* ("genus a" which develops a "6-loop" intestinal pattern, and "genus b" which retains the plesiomorphic "2-loop" intestine).

The molecular-based phylogeny presented in Chapter 6 suggests a different topology for this phylogeny, as well as substantial lineage and dietary diversification, particularly the adoption of plant and detritus-based diets, upon a single invasion of freshwater environments by ancestral euryhaline-marine terapontids. There is now compelling theoretical and empirical evidence that ecological processes can play a significant role in the early stages of speciation (Schluter, 2001). High rates of evolution often occur following colonization of a novel environment as a result of shifts in selection pressures driven by differences in climate, vegetation, resource base, competitors, or predators (Blondel, 2000). The ecological opportunity hypothesis (Schluter, 2000) suggests that lineages invading a novel adaptive zone often undergo a 'release'

characterised by cladogenesis and associated ecomorphological diversification. Since increases in intestinal complexity within terapontids are apparently limited to freshwater forms (Vari, 1978), as are the majority of herbivorous-detritivorous diets in the family (Davis *et al*. 2011b), modifications of intestinal complexity could represent such an ecomorphological character release. Previous ecomorphological research conducted on the Terapontidae without an explicit comparative approach has suggested that characters like intestinal length have significant correlations to dietary habits such as herbivory and detritivory that characterise freshwater terapontids (Davis *et al*. 2012a). Further research within a phylogenetic framework is required to test the correspondence between dietary habits and the functionality of variations in terapontid intestinal morphology.

Here I utilise a suite of phylogenetic comparative methods to address two study aims: firstly I re-examine the process of ontogenetic development of intestinal length in the Terapontidae within the context of a molecular phylogeny. Patterns of ontogenetic intestinal configuration are described and then combined with ancestral character state reconstruction to examine the evolutionary history of intestinal complexity within terapontids, including the number of gains/losses of particular intestinal patterns within the family. Secondly, in line with predictions of digestive theory, I predict greater intestinal length in species that consume higher proportions of plant and detrital food items than those consuming animal prey. If this hypothesized relationship exists, it will provide evidence for dietary ecomorphological diversification, based around modification of intestinal length, which is likely to be a significant driver of the phyletic and trophic radiation evident in Australia's freshwater terapontids.

7.2 Materials and Methods

7.2.1 Taxon sampling, molecular markers, and phylogeny reconstruction

To construct a framework for comparative study a phylogenetic analysis of 28 terapontid species was performed based upon combined nuclear and mitochondrial DNA (mtDNA) sequences from Chapter 6. The ingroup consisted of 28 species and included nine Australian marine-euryhaline species, all genera and 18 of 24 species of Australian freshwater terapontids, and one species present only in New Guinea. Two representative sequences of one species (*Hannia greenwayi*) were included due to their different placement in the topology. These

species exhibit the six major trophic habits displayed by Australia's freshwater terapontids: invertivores, macrofaunal predators, meiophagous omnivores, macrophagous omnivores, herbivores and detritivores-algivores documented in Chapters 2 and 6 (Davis *et al.*, 2011a; 2012b).

Sequence data consisted of an 1141 base pair (bp) fragment of the mtDNA gene cytochrome *b* (cyt*b*) and a 3896 and 905 bp fragment of the nuclear recombination activation genes RAG1 and RAG2 (hereafter referred to as RAG) respectively for a total of 5942 bp for each individual included in our study. The previous Chapter 6 dataset (Davis *et al*., 2012b; Dryad Digital Repository doi:10.5061/dryad.4r7b7hg1) was used, with taxa lacking IL data trimmed out, and the dataset realigned. Cyt*b* was aligned by eye while RAG sequences were aligned using the online version of MAFFT 6.822 (Katoh and Toh, 2010) using the accurate G-INS-i algorithm with the scoring matrix for nucleotide sequences set to 1PAM / K=2, a gap opening penalty of 1.53 and an offset value of 0.1. Combined partitioned phylogenetic analyses were performed with maximum likelihood (ML) using GARLI 2.0 (Zwickl, 2006). The best-fitting model of molecular evolution was identified using the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada and Crandall, 1998) using PAUP* 4.0b10 (Swofford, 2003). For cyt*b* Modeltest identified TrN+I+G as the best model and for RAG GTR+I+G was the best model. GARLI was run with 10 search replicates using the default settings with two partitions representing cyt*b* and RAG with their respective models. For bootstrapping, 1000 replicates were run with the previous settings except that the options genthreshfortopoterm was reduced to 10,000 and treerejectionthreshold was reduced to 20 as suggested in the GARLI manual to speed up bootstrapping. Trees were rooted with representatives from several related families based on Yagishita *et al.* (2009), see Davis *et al.* (2012b) for details.

7.2.2 Specimen collection

Fish for dietary and morphometric quantification were collected from a number of fish surveys of freshwater and marine habitats across Australia (Davis *et al*., 2011b) and Papua New Guinea, as well as being sourced from museum collections. Fish were primarily preserved in either buffered formalin or ethanol. Incisions were made into the body wall of larger specimens or fixative was injected by hypodermic syringe into the body cavity to aid fixation of internal organs.

7.2.3 Intestinal coiling pattern description and intestinal length measurement

After weighing fish and measuring standard length (SL), specimens were dissected and the entire digestive system and viscera were removed from the body cavity. All terapontids possess a Y-shaped stomach with a straight descending limb from the oesophagus, followed by a blind sac formed at the bend of the stomach, which leads anteriorly to the pyloric limb on the left side of the body (Vari, 1978). Intestinal convolution patterns posterior to the pyloric outlet were observed using a dissecting microscope and sketched and photographed from dorsal, ventral, left and right aspects. While Vari (1978) described intestinal patterns from the left side of the body, this study followed Yamaoka (1985) by defining intestinal patterns from the ventral aspect, which facilitates definition of the bilaterally symmetrical body structure of fishes. After description of intestinal coiling structure, the intestine was carefully uncoiled to avoid stretching and intestinal length (IL) was measured as the distance from the pyloric outlet to the rectum. Species' means for standard length and intestinal length were $\log_{10}$ transformed to homogenise variance prior to analysis and to increase data independence.

7.2.4 Reconstructing the evolutionary history of terapontid intestinal length development

The historical patterns of terapontid intestinal development were hypothesized utilising ancestral character reconstruction techniques in Mesquite 2.75 (Maddison and Maddison, 2011). The "Trace Over Trees" function in Mesquite, which reconstructs ancestral history on multiple phylogenies, was used to incorporate phylogenetic uncertainty in ancestral reconstructions of character states. In order to generate a collection of trees the Bayesian method BEAST 1.7.1 was used (Drummond and Rambaut, 2007), with input files generated using BEAUti 1.7.1. The analysis used an uncorrelated lognormal relaxed molecular clock with rate variation following a tree prior using the speciation birth-death process, and the same models of sequence evolution for the nuclear and mtDNA partitions as per the ML analysis above. BEAST analyses were run for 50 million generations, with parameters logged every

100,000 generations. Multiple runs were conducted to check for stationarity and that independent runs were converging on a similar result. The treefile was summarized using TreeAnnotator 1.7.1 with the mean values placed on the maximum clade credibility tree. The first 10% of trees were removed as burn-in, providing 450 trees for reconstructing ancestral states, with ancestral states summarized onto the maximum clade credibility tree. States were summarized for each node by counting all trees with uniquely best states. If no state was more parsimonious than the other, the reconstruction at that node was classed as equivocal. The frequency of each state was reported for all trees containing that ancestral node, with the variability of inferred states among trees providing a measure of the degree to which ancestral state reconstructions for the node concerned are affected by uncertainty in tree topology and branch lengths. Adult intestinal configurations were coded as discrete (categorical) character states and optimised onto the molecular phylogeny. Because alternative methods of character state reconstruction can produce conflicting results, both maximum parsimony (MP) and maximum likelihood ML methods of ancestral state reconstruction were employed (Schluter *et al*., 1997; Pagel, 1999). Parsimony ancestral state reconstruction, which minimizes the amount of character change given a tree topology and character state distribution, has been widely utilised but may over-represent confidence in ancestral character states (Schluter et al. 1997). For the MP analysis, character transitions were considered to be unordered (changes between any character state are equally costly). A character was assigned to a node if it created fewer steps, otherwise the node was considered equivocal.

ML ancestral character state reconstruction finds the ancestral states that maximize the probability that the observed states would evolve under a stochastic model of evolution (Schluter *et al*., 1997; Pagel, 1999). A symmetrical Mk1 model (Lewis, 2001), which assumes equal forward and backward character transition rates (i.e., all changes equally probable), was used as the evolutionary model. A major advantage of ML is that the analysis takes branch lengths into account, allows the uncertainty associated with each reconstructed ancestral state to be quantified, and is preferable for medium-sized trees (Mooers and Schluter, 1999; Pagel, 1999). Likelihood ratios at internal nodes were compared by pairs, and were reported as proportional likelihoods. While likelihoods do not necessarily translate into levels of statistical significance, a difference of 2 log units for a character (i.e., ~7.4 times more probable than any other alternative state) was employed to assign states at a node, otherwise the node was considered equivocal (defined as ‘the rule of thumb’) (Pagel, 1999).

7.2.5 Dietary data

Pronounced ontogenetic diet shifts in association with significant allometric growth in many diet-ecomorphological characters are a prominent feature of terapontid ontogenetic biology (Davis *et al*., 2011a; 2011b). To limit any confounding effects of ontogeny on comparative analyses in the present study, assessment focused on the morphologies and dietary habits of the largest size classes only (i.e., when intestinal length was most fully developed). Although the full range of items contributing to the diet of the examined terapontids have been quantified (22 different food classes; Davis *et al*., 2011b), in this study, gut contents were simply categorised as the percent contribution of plant-detrital material to species' diet (i.e., the combined contribution of detritus, filamentous algae, aquatic macrophytes, terrestrial vegetation and miscellaneous plant parts). Arcsine transformations of dietary percentages were conducted prior to further analysis to improve normality (Sokal and Rohlf, 1995).

7.2.6 Body-size–intestinal length correction

Appropriately correcting for body size effects and allometric scaling of morphological traits, while simultaneously taking phylogeny into account, poses an ongoing challenge for comparative studies (Revell, 2009). To remove effects of body size and allometric scaling of intestinal length between terapontid species, the "phyl_resid" function outlined in Revell (2009) was used to regress mean species' intestinal lengths against mean standard lengths to produce phylogenetic size-corrected residuals in the R package "phytools" (http://www.r-project.org) (Revell, 2011). Hereafter, reference to intestinal length refers to the phylogenetically size-corrected estimate.

7.2.7 Testing for phylogenetic signal

To test whether the traits considered in this study (intestinal length and volumetric plant-detrital proportions in diet) individually showed evidence of phylogenetic signal two alternative

metrics were utilised – the *K* statistic (Blomberg *et al*., 2003) and Pagel's λ (Pagel, 1999). These statistics compare the observed fit of the data to the phylogeny with the analytical expectation based on the topology and branch lengths of the phylogeny, assuming a Brownian (random walk) model of character evolution. Blomberg's *K* quantifies the amount of phylogenetic signal in the tip data relative to the expectation ($K = 1$) for a trait that evolved by Brownian motion along the specified topology and branch lengths (Blomberg *et al*., 2003). Values of *K* close to 0 indicate random evolution of traits, values close to 1 correspond to a Brownian motion-type evolution, and values < 1 indicate strong phylogenetic signal and trait conservatism. Following Blomberg *et al*. (2003), *K*'s significance was assessed using a data randomization test conducted by randomly permutating the tips of the phylogeny 1000 times. A significant phylogenetic signal was indicated if the observed *K* value was greater than across 95% of the randomizations.

Pagel's λ provides the best fit of the Brownian motion model to the tip data by means of a maximum likelihood approach (Freckleton *et al*., 2002). Thus, if $\lambda = 1$, the trait evolved according to the Brownian motion, and λ can take any value from 0 (i.e., a star phylogeny, where the trait shows no phylogenetic signal) to >1 (more phylogenetic signal than expected under the Brownian motion). The significance of λ can be assessed by a likelihood ratio comparison of nested models with particular values (i.e., 0 or 1).Tests for phylogenetic signal were implemented using the "phylosignal" and "*Kcalc"* functions in "phytools" (Revell, 2011). Both statistics were calculated for traits based on the maximum clade credibility tree.

7.2.8 Phylogenetic comparative analysis

Correlations between intestinal length and dietary composition were examined both with and without phylogenetic correction. To remove the possible correlation associated with phylogenetic relatedness I calculated phylogenetically independent contrasts (PIC; Felsenstein, 1985) of intestinal length and proportion of plant-detrital material in species' diets. For PIC analysis, the molecular topology with branch lengths was imported into Mesquite 2.75 (Maddison and Maddison, 2011). The PDAP (Phenotypic Diversity Analysis Package) module (Garland *et al*., 1992; Midford *et al*., 2010) implemented in Mesquite was used to calculate standardised independent contrasts for the correlation between size-corrected intestinal length

and arcsine-transformed proportion of plant-detrital material in diet at 28 internal nodes on the terapontid phylogeny. The Pearson product-moment correlation coefficient *r* (computed through the origin) and its associated *P* value are reported. The relationship between the phylogenetically independent contrasts was then determined by using a RMA as there is considerable variation in calculation of both morphological and dietary variables.

Initial diagnostic tests found that the estimated branch lengths were adequate for the assumptions of independent contrasts (Garland *et al*., 1992). While PIC is reasonably robust to violations of branch length assumptions (Garland *et al*., 1999), additional PICs were calculated using topologies with several arbitrary branch lengths as a sensitivity analysis for any potential uncertainty associated with branch lengths derived in the molecular phylogeny: branch lengths set to unity (1.0; similar to a speciation model of character evolution), contemporaneous tips with internodes set to one (Pagel, 1992), contemporaneous tips with internodes set to one less that the number of descendant tip species (Grafen, 1989), and contemporaneous tips with internodes set to the log of number of descendant tip species (Nee in Purvis, 1995). All tree manipulation was done using Mesquite (version 2.75).

To assess the effects of failing to control for phylogenetic relatedness, a phylogenetically naive RMA regression (i.e., assuming a star phylogeny) was also conducted to assess the relationship between intestinal length residuals (calculated from an ordinary least squares regression of standard length versus intestinal length) versus arcsine-transformed proportion of plant-detrital material in diet.

7.3 Results

7.3.1 Phylogenetic analysis

Maximum likelihood recovered one tree with a likelihood score of -34413.698284 (Figure 7.2). Overall, most nodes within the freshwater radiation in the tree were well resolved with strong support (Hillis and Bull, 1993) evidenced by bootstrap values mostly >80. Marine-euryhaline species relationships mostly had no bootstrap support.

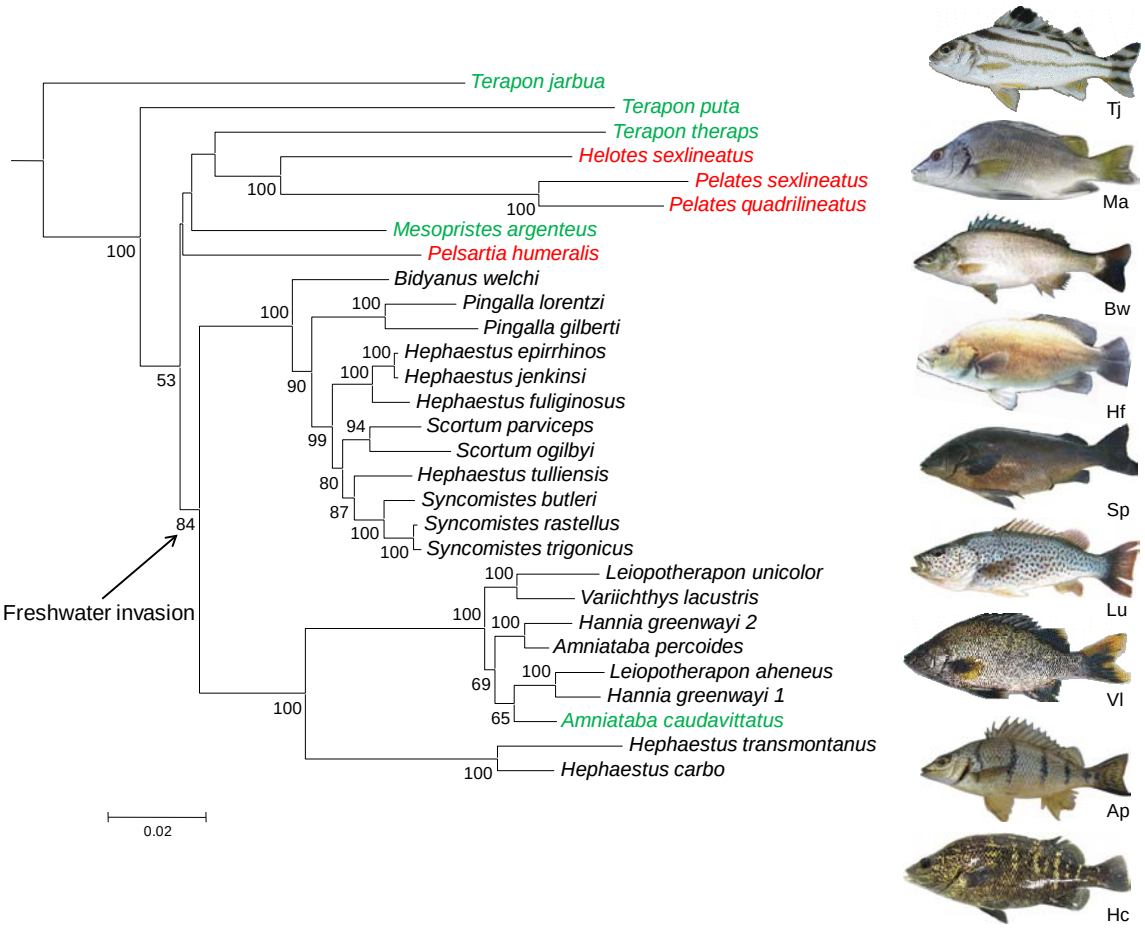

Figure 7.2 Maximum likelihood (ML) tree for 28 terapontid species based on analysis of combined nuclear and mitochondrial DNA. All bootstrap values are based on 1000 pseudoreplicates. Outgroup species were pruned from the tree. Images are identified by initials of genus and species nearby in the tree. Taxon names are colour-coded according to macrohabitat associations identified in Davis *et al.* (2012b): red = marine, green = euryhaline, and black = freshwater. The node signifying invasion of Australasian freshwater habitat is indicated. Species labelled by abbreviation of generic and species names, identifiable from full names in tree.

7.3.2 Dietary and morphological quantification

Specimen numbers, dietary data, standard length and intestinal length averages of each species and trophic classifications from existing literature are presented in Table 7.1. There was broad variability evident in terapontid diets, with some species' diets dominated by plant-detrital material, through to others that consumed virtually only animal prey. Relative intestinal length (IL/SL) is the most commonly used descriptor in diet-morphology assessments (Horn 1989), so RIL ranges are provided for comparison with published data (Table 7.1).

Table 7.1 Study species, specimen numbers (*n*), mean values (±S.D.) for each species' morphological measurements, percentage plant-detrital material in diet, trophic classifications and RIL range. *n* signifies the number of intestinal length measurements per species, with the sample numbers used to derive dietary data in parentheses. Trophic classifications sourced from Davis *et al*. (2011b) and Davis *et al*. (2012b).

Species	*n*	SL (mm)	IL (mm)	RIL Range	% Plant-detritus	Trophic classification*
Amniataba caudavittatus	12 (14)	93.9 ± 27.8	85.4 ± 30.6	0.7–0.9	7	Invertivore
Amniataba percoides	28 (48)	110.5 ± 6.8	112.8 ± 15.0	0.8–1.2	55.8	Meiophagous omnivore
Bidyanus welchi	9 (10)	204.1 ± 25.8	347.2 ± 36.8	1.6–2.2	30	Generalist carnivore
Hannia greenwayi	10 (19)	81.2 ± 25.7	74.0 ± 39.8	0.6–1.2	23.2	Invertivore
Helotes sexlineatus	36 (36)	123.4 ± 26.6	177.3 ± 23.2	1.3–1.7	78	Herbivore
Hephaestus fuliginosus	20 (42)	266.9 ± 23.1	556.3 ± 143.5	1.6–3.5	67.2	Macrophagous omnivore
Hephaestus carbo	25 (27)	129.2 ± 13.6	126.7 ± 23.0	0.8–1.1	1.4	Invertivore
Hephaestus epirrhinos	3 (3)	223.7 ± 44.7	303.0 ± 93.3	1.2–1.5	19.6	Generalist carnivore
Hephaestus jenkinsi	22 (33)	195.9 ± 30.3	415.7 ± 83.2	1.5–2.8	54.9	Macrophagous omnivore
Hephaestus transmontanus	20 (20)	76.8 ± 4.17	51.65 ± 5.18	0.6–0.8	0.4	Invertivore
Hephaestus tulliensis	14 (15)	171.5 ± 24.6	439.6 ± 90.5	1.7–3.0	76.7	Macrophagous omnivore
Leiopotherapon aheneus	18 (25)	50.7 ± 9.7	96.3 ± 39.0	1.2–3.1	68.1	Herbivore
Leiopotherapon unicolor	30 (70)	136.8 ± 15.1	122.6 ± 20.1	0.8–1.2	8.9	Generalist carnivore
Mesopristes argenteus	13 (13)	156.7 ± 33.6	188.2 ± 63.8	0.9–1.4	3.8	Generalist carnivore
Pelates quadrilineatus	7 (7)	112.7 ± 17.0	106.6 ± 15.7	0.9–1.02	0.8	Generalist carnivore
Pelates sexlineatus	16 (16)	94.9 ± 15.2	85.1 ± 17.5	0.8–1.0	1.9	Generalist carnivore
Pelsartia humeralis	2 (2)	153.5 ± 7.78	142 ± 9.9	0.9–0.9	4	Generalist carnivore
Pingalla gilberti	29 (35)	67.5 ± 16.2	117.0 ± 30.2	1.2–2.3	82.6	Detritivore-algivore
Pingalla lorentzi	12 (12)	67.1 ± 18.7	122.5 ± 42.6	1.5–2.0	66	Detritivore-algivore
Scortum ogilbyi	17 (25)	275.0 ± 32.8	1297.6 ± 296.4	3.7–7.1	92	Herbivore
Scortum parviceps	28 (31)	264.0 ± 13.8	1427.8 ± 248.1	3.6–7.6	97	Herbivore
Syncomistes butleri	18 (21)	200.0 ± 18.9	786.5 ± 191.0	3.1–5.6	99.8	Detritivore-algivore
Syncomistes rastellus	12 (13)	108.4 ± 38.6	415.4 ± 294.1	3.0–6.4	92.3	Detritivore-algivore
Syncomistes trigonicus	23 (26)	71.9 ± 16.3	232 ± 123.9	3.0–5.3	97.3	Detritivore-algivore
Terapon jarbua	26 (20)	106.0 ± 29.4	111.4 ± 22.7	0.9–1.2	1	Generalist carnivore
Terapon puta	6 (6)	131.2 ± 35.5	125.7 ± 37.6	0.9–1.0	3.6	Generalist carnivore
Terapon theraps	8 (8)	148.9 ± 14.1	144.8 ± 20.2	0.9–1.1	0.1	Generalist carnivore
Variichthys lacustris	11 (11)	150.4 ± 34.8	141.1 ± 82.0	0.7–1.1	53	Meiophagous omnivore

Reduced major axis regressions of $\log_{10}$–transformed standard length versus $\log_{10}$–transformed intestinal length for each species over the available studied size are also outlined in supporting information (Table 7.2).

Table 7.2 Results for scaling analyses of reduced major axis regressions of Log_{10}–transformed standard length versus Log_{10}–transformed intestinal length for 27 terapontid species. Statistically significant allometric scaling relationship (i.e., where the 95% confidence interval for slope does not overlap with an isometric slope of 1.0) are highlighted in bold. *n* signifies the number of intestinal length measurements per species.

Species	Intestinal length category	Slope (a)	a (confidence limits)	Y-intercept	r^2	*n*	Size range (mm SL)
Amniataba caudovittatus	"Two-loop"	1.299	0.946-1.439	-0.635	0.964	11	67-163
Amniataba percoides	"Two-loop"	**1.385**	1.339-1.431	-0.761	0.929	432	15-126
Hannia greenwayi	"Two-loop"	**1.194**	1.041-1.347	-0.423	0.954	15	16-124
Hephaestus carbo	"Two-loop"	**1.274**	1.216-1.333	-0.581	0.954	87	37-160
Hephaestus epirrhinos	"Two-loop"	**1.516**	1.26-1.772	-1.085	1.000	3	193-275
Hephaestus transmontanus	"Two-loop"	1.767	0.838-5.480	-1.619	0.112	20	68-84
Leiopotherapon unicolor	"Two-loop"	**1.252**	1.223-1.280	-0.572	0.936	479	11-170
Mesopristes argenteus	"Two-loop"	**1.257**	1.177-1.346	-1.183	0.952	13	86-223
Pelates quadrilineatus	"Two-loop"	0.999	0.602-1.270	-0.022	0.891	7	93-137
Pelates sexlineatus	"Two-loop"	**1.317**	1.190-1.455	-0.675	0.952	16	70-117
Terapon jarbua	"Two-loop"	0.943	0.818-1.02	0.129	0.894	32	39-156
Terapon puta	"Two-loop"	1.095	0.611-1.300	-0.221	0.971	6	79-167
Terapon theraps	"Two-loop"	1.496	0.706-1.835	-1.092	0.822	8	120-170
Varrichthys lacustris	"Two-loop"	1.150	0.427-1.246	-0.358	0.941	12	39-181
Bidyanus welchi	"Six-loop"	**1.594**	1.282-4.464	-1.146	0.769	8	131-235
Hephaestus fuliginosus	"Six-loop"	**1.440**	1.408-1.471	-0.769	0.965	292	24-320
Hephaestus jenkinsi	"Six-loop"	**1.574**	1.478-1.671	-0.988	0.926	80	32-280
Hephaestus tulliensis	"Six-loop"	**1.766**	1.291-2.241	-1.295	0.780	16	81-217
Pingalla gilberti	*"Pingalla"*	**1.470**	1.146-1.794	-0.625	0.676	30	34-97
Pingalla lorentzi	*"Pingalla"*	**1.316**	1.042-1.591	-0.324	0.913	12	48-116
Scortum ogilbyi	*"Scortum"*	**1.626**	1.501-1.752	-0.817	0.884	80	53-324
Scortum parviceps	*"Scortum"*	**1.681**	1.599-1.763	-0.931	0.976	74	15-297
Syncomistes butleri	*"Syncomistes"*	**1.820**	1.694-1.987	-1.214	0.956	100	38-241
Syncomistes rastellus	*"Syncomistes"*	**1.938**	1.249-2.628	-1.373	0.777	13	68-166
Syncomistes trigonicus	*"Syncomistes"*	**2.356**	2.155-2.557	-2.042	0.948	32	32-105
Helotes sexlineatus	*"Helotes"*	**1.289**	1.174-1.387	-0.441	0.934	32	87-159
Leiopotherapon aheneus	*"L. aheneus"*	**1.806**	1.650-1.962	-1.123	0.943	34	13-73

7.3.3 Ontogenetic development of intestinal morphology

The simplest intestinal pattern consisted of two loops and was evident immediately after post-larval metamorphosis in all species examined (Figure 7.3A-7.3B). The first loop occurred posterior of the pylorus near the rear of the body cavity, after a slight dextral curve immediately posterior to the pylorus. The intestine continued anteriorly until a second loop occurred ventral to the stomach, where after the intestine continued posteriorly to the anus producing an "s-shaped or two-loop" layout. This simple configuration was evident throughout the life history of *Leiopotherapon unicolor*, *Amniataba percoides*, *A. caudivittata*, *Hannia greenwayi*, *Hephaestus carbo*, *Hep. epirrhinos*, *Hep. transmontanus*, *Pelates sexlineatus*, *P. quadrilineatus*, *Terapon theraps*, *T. puta*, *T. jarbua* and *Varichthys lacustris* (Figure A4.1, A4.8, A4.9, A4.10); however, significant allometric increases in intestinal length were achieved in several species by increasing the length of each loop in both anterior and posterior directions (Figure 7.3B). RILs of <1.2 typify terapontid species that retained the two-loop intestinal configuration throughout life history (Table 7.1). The "two-loop" intestinal configuration was the juvenile morphology of all remaining species.

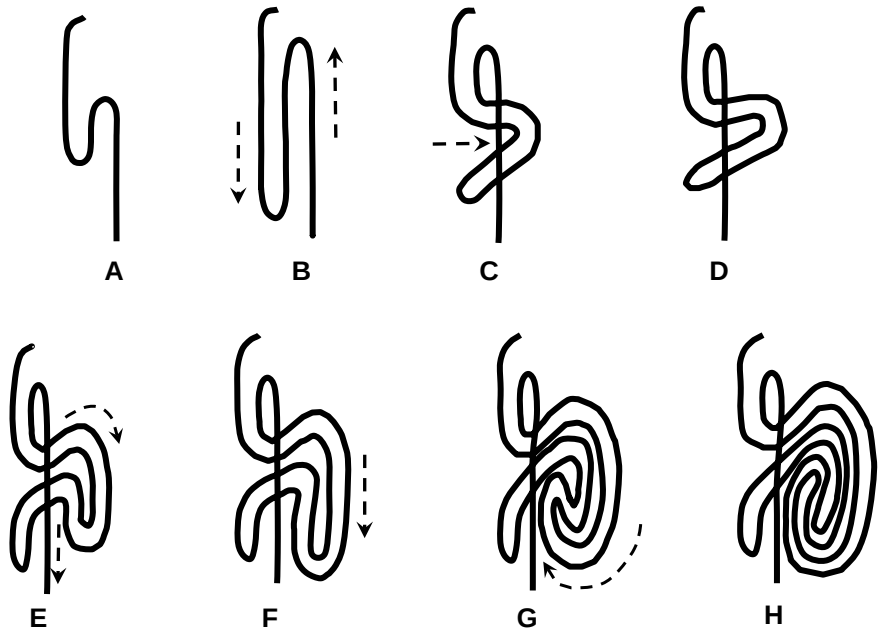

Figure 7.3 Patterns of ontogenetic development of intestinal layout in *Leiopotherapon unicolor* (A to B), *Amniataba (*A to B), *Hannia* (A to B), *Variichthys* (A to B), *Bidyanus* (A to D), *Hephaestus* (A to D), *Pingalla* (A to F) and *Scortum* species (A to H). Intestinal tracts are viewed ventrally, the anterior most portion of the intestine (outlet of the pylorus) is always located to the top of each figure. Arrows indicate major directions of intestinal lengthening or looping characterising each stage.

In all remaining species except *L. aheneus* and *Hel. sexlineatus*, a transverse folding and elongation of the middle portion of the two-loop intestinal pattern occurred, directing the elongated section to the left of the body cavity (Figure 7.3C and 7.3D) and ventrally beneath the posteriorly directed section of the two-loop pattern. This produced the "six-loop" configuration described by Vari (1978). This pattern remained the intestinal layout throughout the remaining life history of *Bidyanus welchi*, *Hep. fuliginosus*, *Hep. jenkinsi* and *Hep. tulliensis* (Figure A4.2, A4.3). Adult RILs of ~2 to 2.5 characterized these species (Table 7.1). In *Pingalla* and *Scortum* species, the loops on the right-hand side of the body cavity continued to proceed dorso-anteriorly before turning to lengthen in a posterior direction (Figure 7.3E-7.3F). In *Pingalla* species this remained the intestinal layout of adults. A further increase in intestinal complexity occurred in *Scortum* species characterised by additional convolution added in a spiral configuration (Figure 7.3G-7.3H). In all of these species the majority of convolution occurred on the left-hand side of the body cavity. The RILs of *Scortum* species averaged ~4.5, and reached over 7 in some specimens (Table 7.1; Figure A4.5).

A different development of intestinal configuration was evident in *Syncomistes* species. Similarly to early ontogenetic intestinal pattern in *Hephaestus*, *Pingalla* and *Scortum* species, *Syncomistes* species develop the "six-loop" pattern. Rather than then proceeding ventrally and to the right of the body cavity as in other species, the looping in *Syncomistes* species proceeds anteriorly before folding and lengthening to the right-hand side of the body cavity. At the same time, posterior looping from the "six-loop" configuration proceeded to the left hand side of the body cavity behind the stomach (Figure 7.4C-7.4E). This was followed by a reversal of looping directions in both the anterior and posterior sections, looping back to the left- and right-hand side of the body cavity respectively (Figure 7.4F- 7.4I). This complex intestinal configuration resulted in RILs of *Syncomistes* reaching over 6 in some specimens (Table 7.1; Figure A4.8).

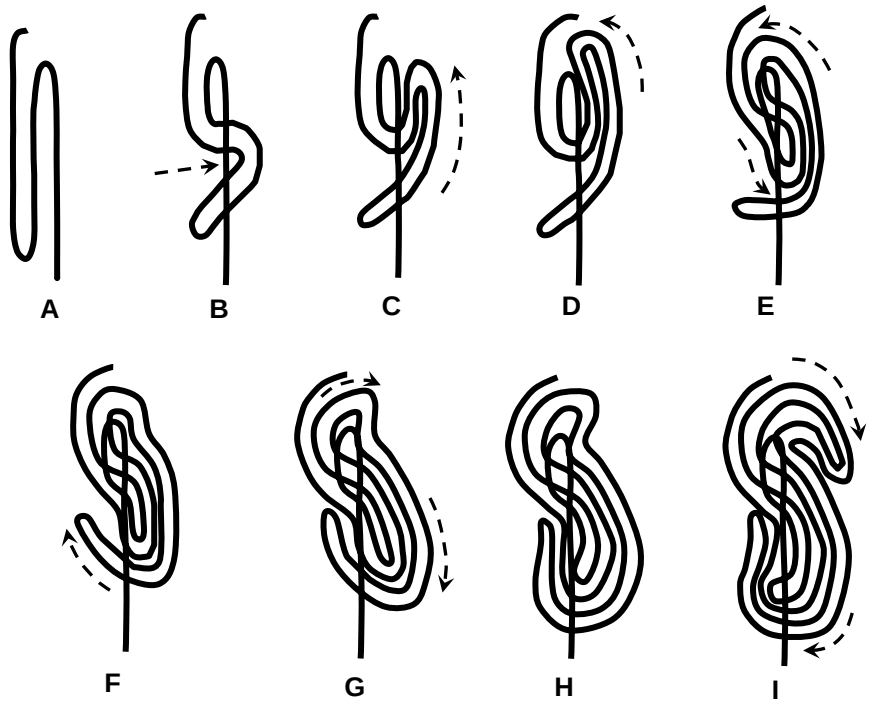

Figure 7.4 Ontogenetic development of intestinal layout in *Syncomistes* species.

Another distinct pattern of ontogenetic intestinal looping was evident in *Leiopotherapon aheneus*. From the initial two-loop pattern the anterior loop lengthened in an anterior direction along the ventral surface of the stomach close to the pyloric outlet (Figure 7.5A-7.5B). This was followed by a folding in the middle section of the intestine (Figure 7.5C-7.5E). This folding initially proceeded anteriorly along the dorso-ventral plane of the body before turning to the right-hand side of the body cavity (Figure 7.5F-7.5G). The majority of folding in this pattern occurred on the right-hand side of the body. Intestinal lengths of *L. aheneus* typically reached between 2-3 times standard length in larger specimens (Table 7.1; Figure A4.6).

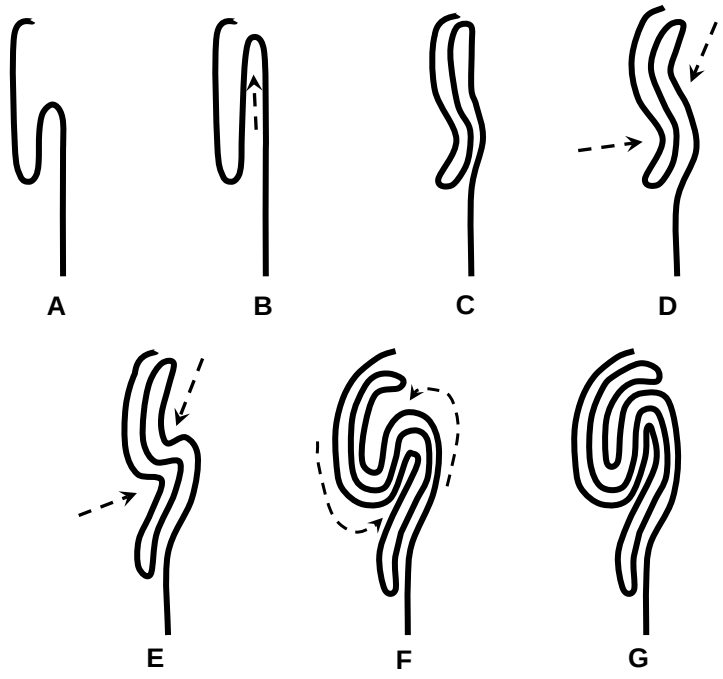

Figure 7.5 Ontogenetic development of intestinal layout in *Leiopotherapon aheneus*.

A final distinct pattern of ontogenetic intestinal looping was also evident in *Helotes sexlineatus*. From the initial two-loop pattern, both the posterior and anterior loops extended in both directions during ontogeny. The anterior loop then extended past the pyloric outlet, before looping around the anterior aspect of the stomach, crossing the dorso-ventral plane to lengthen into the anterior, right-hand side of the body cavity (Figure 7.6D-7.6E). While only a comparatively minor increase in complexity, this configuration produced higher RILs compared to the standard "two-loop" intestinal layout (Table 7.1; Figure A4.9).

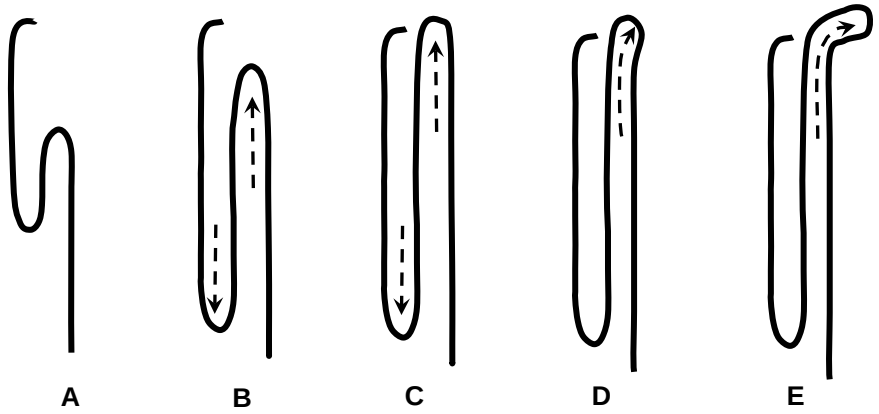

Figure 7.6 Ontogenetic development of intestinal layout in *Helotes sexlineatus*.

7.3.4 Character optimisations and reconstruction of ancestral states

Optimising adult intestinal configuration patterns onto the maximum clade credibility phylogeny indicated that the ontogenetic development of increased intestinal complexity has evolved independently on three occasions in terapontid fishes. While a range of patterns of ontogenetic increase in intestinal complexity have evolved in the clade containing *Hephaestus*, *Scortum*, *Bidyanus*, *Syncomistes* and *Pingalla* species, ontogenetic increases in intestinal convolution were limited to just a single species (*L. aheneus*) in the other major freshwater clade, as well as on a single occasion in the euryhaline/marine clade (*Hel. sexlineatus*). An examination of ancestral state reconstructions across the 450 trees from the BEAST analysis yielded very similar predictions between parsimony and likelihood analyses (Figure 7.7) and the inferred ancestral states for terapontid intestinal length configuration were not substantially affected by uncertainty in tree topology, branch lengths, or character state reconstruction methods. Both MP and ML analysis suggested that the "two-loop" pattern is unequivocally plesiomorphic within the Terapontidae, and the "two-loop" intestinal pattern was also exhibited by the most recent common ancestor of all freshwater species (i.e., at the time of freshwater invasion). Both reconstruction approaches also indicated that the evolution of adult intestinal complexity followed a complex pattern of multiple independent gains and one loss within both major freshwater clades. Both approaches indicated that the "six-loop" intestinal configuration was a precursor to the range of more complex intestinal patterns evident in *Pingalla*, *Scortum*

and *Syncomistes* species. Character state reconstruction also suggested that the two similar patterns of increase evident in *Pingalla* and *Scortum* species evolved independently. An apparent reversion to the plesiomorphic state of an adult "two-loop" intestinal pattern was also evident in *Hep. epirrhinos*, the only species within this clade to retain this intestinal configuration as an adult.

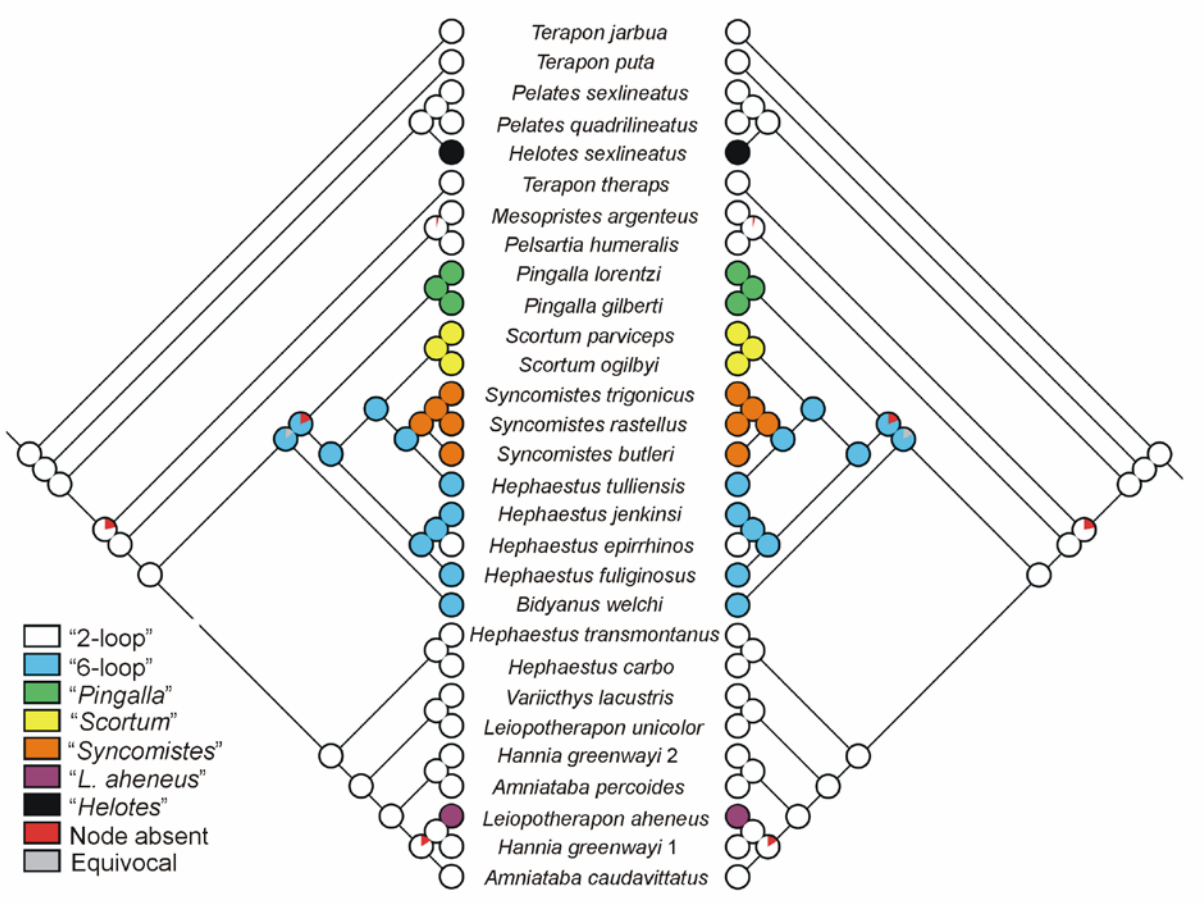

Figure 7.7 Summary of maximum likelihood (left graph) and maximum parsimony (right) ancestral character reconstruction of adult intestinal configuration for 450 terapontid trees displayed on the maximum clade credibility tree. Circles at terminal nodes represent the observed character state for extant species. Pie charts for ancestral nodes show estimated proportions for reconstructed character states at that internal node.

7.3.5 Phylogenetic signal

Blomberg's K and Pagel's λ for proportion of plant-detrital material and intestinal length both demonstrated significant levels of phylogenetic signal, indicating that neither variable was independent and, therefore, phylogenetic comparative methods were justified in further analyses. While the estimates of phylogenetic signal for the two variables were both significant, the patterns of phylogenetic signal were not convergent. Phylogeny was a significant predictor of variation in plant-detrital material in terapontid diet ($K = 0.63$, observed PIC variance = 0.84, $P = 0.003$, Pagel's $\lambda = 0.86$, $P < 0.001$). However, both K *and* λ were estimated to be considerably less than 1, suggesting a phylogenetic signal lower than the one expected under Brownian motion and, accordingly, substantial evolutionary lability in terapontid diet, even between closely related species. Phylogeny accounted for a larger component of variability in intestinal length in the terapontids ($K = 1.05$, observed PIC variance = 0.294, $P < 0.001$, Pagel's $\lambda = 0.94$, $P < 0.001$), suggesting a phylogenetic signal close to what would be expected under Brownian motion in both statistics.

7.3.6 Comparative analyses

After correcting for phylogenetic proximity, the independent contrasts of intestinal length versus diet were significantly, and positively, correlated with the percentage of plant-detrital material in terapontid diet , explaining 63% of variation in diet composition (r^2= 0.63, RMA slope = 1.36, $P < 0.001$). Twenty three of the 28 independent contrasts were positive, and occurred across both deep and shallow nodes of the phylogeny (Figure 7.8). Several of the most positive contrasts occurred at nodes within the phylogeny (nodes 38, 55, 24, 29 and 19) that were precursors to gains/losses in intestinal complexity identified in the character mapping and ancestral character reconstruction (Figure 7.7). PICs with branch lengths set to unity (r^2 = 0.55, RMA slope = 1.57, $P < 0.001$), (Nee in Purvis 1995) branch lengths (r^2 = 0.61, RMA slope = 1.50, $P < 0.001$), Grafen (1989) branch lengths (r^2 = 0.53, RMA slope = 1.57, $P < 0.001$) and Pagel's (1999) length (r^2 = 0.56, RMA slope = 1.51, $P < 0.001$) all produced similar results to the molecular phylogeny. A phylogenetically naive RMA regression also identified a significant positive relationship between intestinal length residuals and arcsine-transformed proportion of plant-detrital material in diet, although this analysis did explain a greater proportion of data variation than any of the phylogenetic comparative analyses (r^2 = 0.80, RMA slope = 1.35, $P < 0.001$).

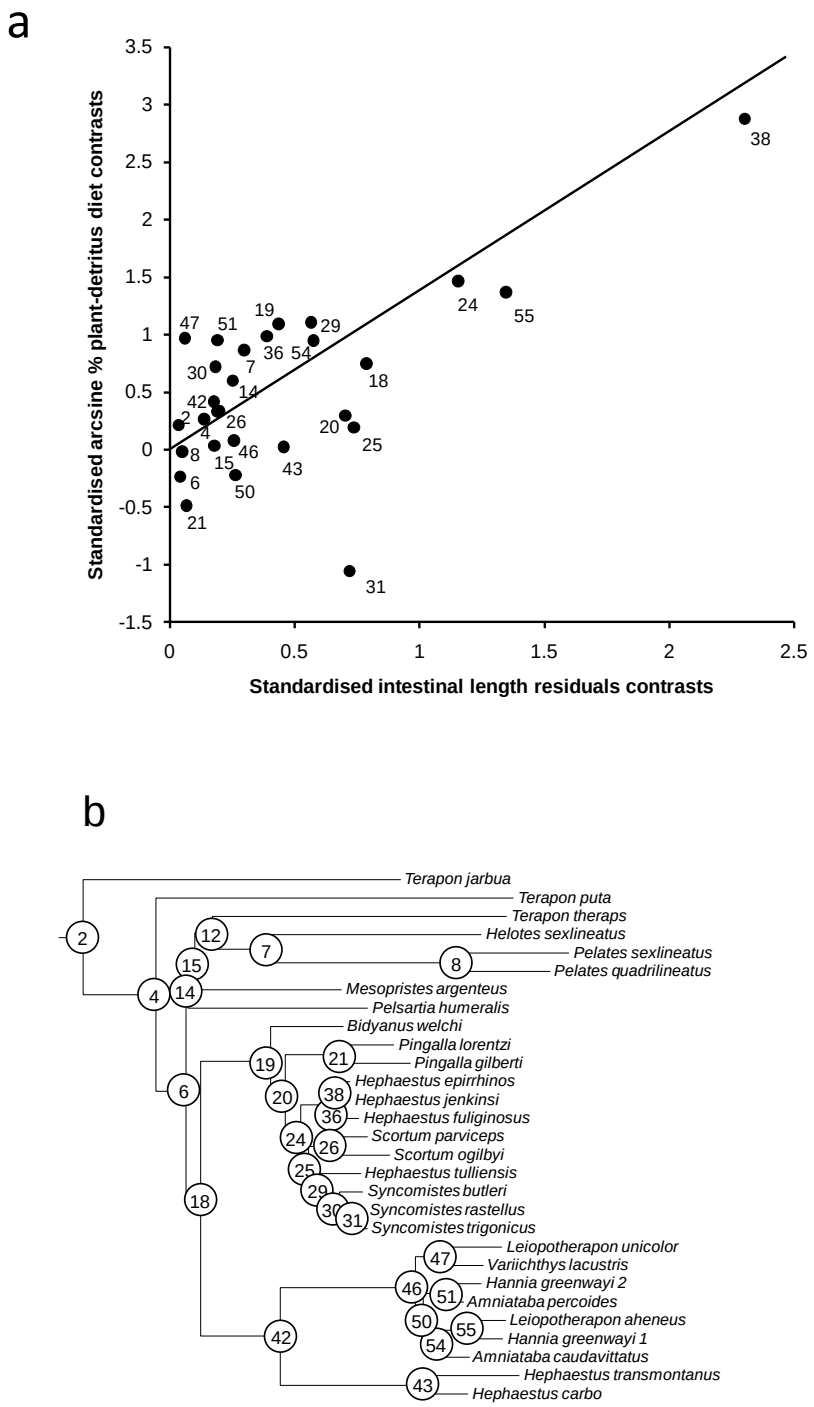

Figure 7.8 (a) Relationship between phylogenetically independent contrasts of intestinal length residuals and contrasts of arcsine transformed proportion of plant-detrital material in diet. Numbers represent the nodes (contrasts) indicated in the phylogeny in (b).

7.4 Discussion

Evolution of intestinal length and dietary radiation in terapontids

Several patterns of ontogenetic development of increased intestinal length were evident in the terapontid species examined. The interspecific differences in intestinal length resulting from these ontogenetic developmental mechanisms explained a substantial amount of the variability in the volume of plant-detrital material in terapontid diets. Results indicate that the widely held ecomorphological maxim of increasing digestive tract length equating with increasing consumption of plants and/or detritus, holds true for terapontids, even when accounting for phylogenetic relationships between species. The capacity to increase intestinal length, and associated herbivorous-detritivorous dietary habits, have evolved independently across multiple marine-euryhaline and freshwater genera within the Terapontidae, but are especially pronounced in freshwater species. Diets with plant and/or detrital material being the dominant proportion accounted for ~2/3 of the freshwater terapontids in this study, a pattern reflected at a broader family level (Davis *et al*., 2012b). Species that consume macroalgae, diatoms or angiosperms as the major part of their diet make up less than 5% of the 426 recognised families of the Telostei, with herbivorous representation even less pronounced at the species level (Horn, 1998; Horn and Ojeda, 1999). The evolution of herbivory in numerous lineages of terrestrial vertebrates is frequently associated with considerable evolutionary diversification (Sues, 2000). The evolution of herbivory and plant-detrital diets are similarly prominent in many of the more species rich and ecologically diverse marine and freshwater fish lineages, often marking a profound shift in the phylogenetic trajectories, species diversity and ecological impact of certain clades (Bellwood, 2003; Barlow, 2000). The significant correlation between intestinal length and plant-detrital material in the diet of approximately 55% of extant terapontid species suggests the capacity to develop long intestines during ontogeny has facilitated the widespread adoption of herbivorous and detritivorous diets across the family.

This study produced a number of commonalities as well as contrasts to the previous work on the family outlined in Vari (1978). Both studies identified the “two-loop” intestinal configuration as being the plesiomorphic adult pattern within the Terapontidae. This study suggested a number of different contrasts to the patterns of intestinal development evident across the family, at both species and family levels. The secondary loss of the “six-loop” intestinal layout Vari (1978) proposed in “*Hephaestus* genus b” instead appears due to the polyphyly of *Hephaestus* and phylogenetic location of this “*Hephaestus* genus b” in a separate

clade of species with a "two-loop" intestinal layout. Vari (1978) suggested that *Scortum* species shared the same adult "six-loop" intestinal pattern as *Bidyanus* and *Hephaestus* species (Figure 7.1). The current study instead highlighted *Scortum* and *Syncomistes* species as developing the most complex intestinal patterns of any terapontid species. This study also identified previously undescribed pattern of ontogenetic intestinal length increase in *L. aheneus* and *Hel. octolineatus.* The different topology emerging from molecular relationships compared to Vari's (1978) phylogeny also suggested a different sequence of intestinal length complexity across the family. Rather than the progressive increase in complexity as genera become more derived, proposed by Vari (1978) (Figure 7.1), a more complex historical process of development was predicted from molecular relationships. Character-state reconstruction inferred that the relatively complex intestinal configurations of adult *Pingalla, Scortum* and *Syncomistes* species all evolved from the six-loop pattern on three separate occasions. The novel ontogenetic development documented in both *L. aheneus* and *Hel. octolineatus* also demonstrated that the capacity for significantly increasing intestinal length during ontogeny has evolved independently in both major clades of freshwater terapontids as well as euryhaline-marine species. These multiple independent origin of increased intestinal complexity across several clades suggests convergent evolution toward increased intestinal length in herbivorous-detritivorous terapontids.

While the role of ontogenetic phenomena in phyletic evolution remains strongly debated (Gould, 1977, Alberch *et al*., 1979; Webster and Zelditch, 2005), modification of ontogenetic development is proposed as one of the most common mechanisms through which morphological change and novelties originate during phyletic evolution. While not explicitly assessed as part of this study, the development of intestinal complexity in terapontids exhibits several elements of heterochronic processes (Gould, 1977; Alberch *et al*., 1979), where ontogeny is modified to produce morphological novelty. Several possible peramorphic (recapitulatory) processes, for example, could explain the apparent repetition of adult intestinal layouts (two-loop and six-loop patterns) of ancestral forms during the ontogeny of many descendent terapontid taxa, before additional intestinal complexity is added to ancestral configurations. A range of associated heterochronic processes (acceleration, hypermorphosis and pre-displacement) can all produce descendent phenotypes that transcend the ancestral form (Gould, 1977; Alberch *et al*., 1979). Similarly, paedomorphic phenomena, where adults retain the juvenile morphology of putative ancestral taxa, could similarly explain the apparent retention of two-loop intestinal layout throughout the life history of *Hep. epirrhinos*, within a

clade of other closely related *Hephaestus* species demonstrating a six-loop adult intestinal configuration (Figure 7.6). Without a range of additional size/age and possibly shape-based data on terapontid ontogenetic trajectories (*sensu* Alberch *et al*., 1978; Webster and Zelditch, 2005), the exact role of heterochronic processes can only be speculated upon. Recapitulation does, however, also appear to be a recurrent theme in the development of intestinal length complexity in a number of fish lineages (Yamaoka, 1985). With additional genetic and morphological data, terapontids may provide a valuable model lineage for elucidating the role of modification of ontogeny as a driver of evolutionary diversification.

The utility of intestinal length as a predictor of diet

While standard regression and PIC approaches both highlighted significant relationships between intestinal length and plant-detrital material in the diet, the amount of variability explained was lower in the PIC analysis. This underlines the importance of comparative methods in not overstating the strength of the association between morphology and ecology. Although intestinal length emerged from the phylogenetically informed analysis as a useful predictor of diet, a substantial amount of unexplained variability was also evident in the relationship. Behavioural, ecological, physiological and historical factors can all interact to influence the strength of the congruence between morphological and ecological characters (Motta *et al*., 1995b). Issues associated with age, phenotypic plasticity, antecedent food availability (i.e., periods of starvation) as well as the relative levels of different dietary substrates have emerged from both field and controlled laboratory studies as possibly inducing changes in intestinal length (Horn, 1989, Sturmbauer *et al*., 1992; German and Horn, 2006; Davis and Pusey, 2010). Recent research has also highlighted a capacity in certain terapontid species for considerable intraspecific intestinal length plasticity in response to environmental and ecological stimuli (Davis and Pusey, 2010). This capacity for phenotypic plasticity in response to different trophic opportunities could promote initial divergence in dietary habits, and potentially provide scope for natural selection to extend and consolidate the phenotypic response. Research into the specific physiological mechanisms used by terapontids to access plant-detrital nutrients (*sensu* Horn, 1989), are yet to be assessed.

Fishes are notoriously opportunistic in feeding behaviours, and whether plant or detrital material ingested by many species in this study is actually assimilated or simply refractory in nature is unknown. Use of stable isotope data, specifically employing $\delta^{15}N$ values to identify trophic level, has been used to identify dietary assimilation in recent ecomorphological studies

(see Wagner *et al*., 2009), although aspects of herbivore-detritivore digestive physiologies can also confound the utility of isotopic approaches in deriving trophic position (see Mill *et al*., 2009). Intestinal length considered in isolation is also in many ways a simplistic indicator of the functional morphology of fish intestinal tracts. Other aspects of digestive morphology and physiology such as intestinal diameter, digesta passage rates, ultra-structural surface area and digestive enzyme profiles can also have significant associations with diet (Al- Hussaini, 1949; Hofer, 1988; Frierson and Foltz, 1992; Tibbetts, 1997; Elliott and Bellwood, 2003; German *et al*., 2010). Potential linkages between dietary habits and other aspects of terapontid morphology such as oral, pharyngeal and dentition structure (see Figure 7.1) are yet to be assessed.

Terapontids as a model system for studying dietary diversification

Horn (1989) suggested that studies of trophically diverse lineages using cladistics and assessment of digestive tract characters could be useful in elucidating the process of evolution of herbivorous-detritivorous trophic habits. Terapontids provide such a model to demonstrate the process of evolution of herbivory and detritivory from an ancestrally carnivorous lineage. With carnivory the likely ancestral habit of the euryhaline-marine ancestors of Australia's freshwater terapontids, the invasion of fresh waters saw adoption of a variety of omnivorous, herbivorous and detritivorous dietary habits during the terapontid freshwater radiation (Davis *et al*., 2012b). Paleoecological conditions that may have facilitated the dietary diversification of early freshwater invading terapontids, particularly adoption of plant and detrital-based diets, include a probable lack of an incumbent herbivorous-detritivorous fish fauna and a range of vacant niches (Davis *et al*., 2012b). Similar processes relating to ecological opportunity and release from competitive constraints have also been recently proposed to explain the significant morphological disparification and lineage diversification evident in Australasian ariid catfishes following a similar freshwater invasion (Betancur-R *et al*., 2012). It is anticipated that following invasion of a new habitat, species will show a rapid burst of cladogenesis and associated ecomorphological (often diet-related) diversification (Schluter, 2000; Streelman and Danley, 2003). The majority of morphological divergence in characters like intestinal convolution and dentition appear to have occurred independently on several occasions in freshwater terapontids (Vari, 1978; this study). The significant relationship between intestinal length and proportion of plant-detrital material in the diet of terapontids suggests that the evolution of longer intestines, in particular, facilitated much of the dietary diversification evident in Australian freshwater environments.

Conclusions

Intestinal length emerged as a significant correlate to interspecific dietary variation in terapontids, even after accounting for phylogeny. The ontogenetic development of intestinal complexity appears to represent an important functional innovation driving much of the ecological (trophic) radiation evident within the Terapontidae. The significant correlation between trophic morphology (intestinal length) and proportion of recalcitrant material in terapontid diet suggests resource-based divergent selection as an important diversifying force in the adaptive radiation of Australia's freshwater terapontids, particularly adoption of omnivorous, herbivorous and detritivorous dietary habits. Moreover, the ontogenetic development of a range of intestinal convolutions being limited to freshwater terapontids is suggestive of ecomorphological character release within the family following invasion of fresh waters by ancestral euryhaline-marine species. Much previous research has suggested that modifications of oral anatomy and functional associations with initial food procurement are one of the primary drivers of fish lineage diversification (Schaefer and Rosen, 1961; Lauder, 1982; Westneat, 2004; Higham *et al*., 2006). The capacity to modify intestinal morphology-physiology in light of new digestive challenges may also be an important facilitator of trophic diversification during phyletic radiations (see also Wagner *et al*., 2009; Herrel *et al*., 2008; Konow et *al*., 2011).

Chapter 8: General discussion

8.1 Overview

The results dealing with the contemporary ecology of the Terapontidae identify size-related dietary shifts as a complex and apparently ubiquitous aspect of terapontid trophic habits. While there was considerable scope evident for spatial dynamism in intra-specific dietary shifts, even in a system such as the Burdekin River, where ontogenetic effects were relatively constrained, major size-related transitions in both the prey items consumed and primary supporting carbon sources were evident across all species. Body size, growth and associations with key morphological variables such as mouth gape and intestinal length appear to place significant constraints on the size and nature of dietary resources available to terapontids at different stages of life history. Small body size, mouth gapes and short intestines limit food choice for terapontid juveniles to small invertebrate prey. Increases in body size (in combination with dietary-ecomorphological variables) open up a broader range of trophic opportunities to terapontids as they mature. Size-based feeding is widely known to generate complex interactions and feedback dynamics with regard to a range of ecological issues such as competitive partitioning, recruitment bottlenecks, and subsequent population and broader community structure in fish assemblages (see Werner and Gilliam, 1984; Olson *et al*., 1995 Post, 2003). Terapontids are clearly not exceptions to these effects.

The Australian freshwater fish fauna offers some interesting features from an evolutionary perspective, such as a predominance of 'secondary' freshwater species (derived from marine ancestors) and their evolution independent of other continental faunas (see Allen *et al.*, 2002). Despite its isolation and age, Australia's freshwater fish fauna displays comparatively depauperate species diversity by global standards. Continental Australia therefore provides an ideal opportunity to test contemporary ecological and evolutionary theory within a unique fauna and environment relatively removed, both geographically and phylogenetically, from previously studied assemblages. At a global level, rapidly developing methods in phylogenetic systematics that integrate diverse data from the functional morphology, comparative anatomy and ecology of a number of related species are providing powerful hypothesis-testing capacity for questions of evolutionary biology (Harvey and Pagel, 1991). However, while approaches such as molecular systematics are increasingly providing valuable insights into the taxonomic relationships and hitherto unrealized levels of species diversity of the Australian freshwater

fauna (Sparks and Smith, 2004; Cook *et al*., 2006; Unmack and Dowling, 2010), comparative phylogenetic techniques focusing on evolutionary and ecological processes have seen minimal application in the Australian freshwater context.

In this thesis I have undertaken a combined ecological and phylogenetic approach to investigate the role of trophic ecology in shaping the evolutionary ecology of the Terapontidae fishes, a family that has undergone most of its evolutionary radiation in Australia and Papua New Guinea. Dietary diversity, both within and between species, is the overriding hallmark of terapontid feeding ecology. A range of historical factors (habitat transitions) and developmental processes (ontogenetic modification of morphology) appear to have driven much of the family's diversification in trophic ecology. The terapontid grunters represent one of the most successful radiations of an Australian freshwater fish family, in terms of species richness (see Allen *et al*., 2002), and especially with respect to dietary ecology. The range of dietary items consumed by the family is comparable to the trophic spectrum exhibited by the entire Australian freshwater icthyofauna (see Kennard *et al*., 2001; Davis *et al*., 2011b). Results of this study also revealed several notable evolutionary features to the family's phylogenetic history, with comparative analyses suggesting that terapontids represent a significant secondary radiation of an actinopterygian fish lineage in freshwater environments (Davis *et al*., 2012b). Even at a global level, few other secondary fish radiations (Schluter, 2000; Lovejoy *et al*., 2006; Collette and Lovejoy, 2012) exhibit a comparable combination of species and ecological (trophic) diversification.

The results demonstrated that much of the terapontid trophic radiation has revolved around increasing consumption of a variety of plant and/or detrital materials. With a large component of the earth's organic carbon sequestered in both living and dead plant material, the capacity for herbivory and/or detritivory opens up an abundant resource for organisms to exploit (Cebrian, 1999; Lujan *et al*., 2011). Although abundant, this resource is trophically inaccessible to many vertebrates because of low nutritional quality and abundance of indigestible structural polymers (Choat and Clements, 1998; Cebrian, 1999). Investigation of dietary overlap of terapontid assemblages underlined how the increasing adoption of plant-detrital diets during ontogeny can greatly alleviate potential competitive interactions with closely related sympatric species. The evolution of herbivory in numerous lineages of terrestrial vertebrates has been frequently associated with considerable evolutionary diversification in those lineages (Sues, 2000). Like the Terapontidae, evolution of herbivory and plant-detrital diets is also prominent in many of

the more species rich and ecologically diverse marine and freshwater fish lineages, often marking a profound shift in the phylogenetic trajectories, species diversity and ecological impact of certain clades (Bellwood, 2003; Barlow, 2000).

Australian freshwater environments do not immediately spring to mind when considering the phenomenon of fish adaption radiation, partly because of the relatively low species diversity of freshwater fish in Australia. Nevertheless, the results of this thesis indicate a high degree of ecological radiation within the terapontids relative to their species diversity. Accordingly, the results raise interesting questions as to whether the phylogenetic diversification in the Terapontidae constitutes an adaptive radiation in the same sense as more celebrated and larger-scale examples such as the paradigmatic Darwin's finches, Caribbean anoles and African cichlids (see Losos and Mahler, 2010). Adaptive radiation has been defined as the process of diversification from a single ancestral form into a variety of ecological or geographic niches to produce new morphologically and ecologically differentiated taxa (Gavrilets and Losos, 2009). However, considerable debate still surrounds what constitutes an adaptive radiation, how it can be diagnosed in nature, and whether the phenomenon is a common mode of biological diversification or merely a rare occurrence evident in a few conspicuous and well-studied clades (Glor 2010; Losos and Mahler, 2010).

While popular and evolutionary literature has largely emphasized species richness in adaptive radiations, more recent syntheses suggest that an evolutionary radiation needs to incorporate two distinct aspects of diversity – species richness and phenotypic diversity (frequently termed 'adaptive disparity' to avoid confusion with 'species diversity') (Losos and Mahler, 2010). The term 'adaptive radiation' should therefore refer to clades exhibiting an exceptional extent of adaptive disparity (Losos and Mahler, 2010). Accordingly, some species-rich, but ecologically monotonous lineages should not be considered adaptive radiations, whereas some clades having unexceptional species diversity, but demonstrating exceptional phenotypic diversity, constitute adaptive radiations.

While the Terapontidae is only moderately species-rich by global standards, it does rank as one of Australia's most species-diverse families (Allen *et al*., 2002). The family also displays marked ecological (trophic) diversity, in relation to the constrained trophic diversity displayed by the rest of Australia constituent fish fauna (see Kennard *et al*., 2001 for comparison). It is also worth noting that in addition to the morphological variability documented within

terapontids (Vari, 1978; this study), diversity at higher levels of taxonomy (i.e., genus and higher) usually equates to diversity of morphological forms (Foote, 1997). The terapontids are, accordingly, notable for their high diversity at a generic level, only exceeded by the Eleotridae and Gobiidae in Australia (Figure 8.1). Much of the ecological diversity in the Australian eleotrids and gobies appears to be associated with reproductive mode and a reduction in dependence on estuarine and marine habitats for larvae rather than dietary ecology (see Pusey *et al*., 2004). With their coupling of ecological (trophic diversity) and associated ecomorphological disparity (intestinal length and oral anatomy), the terapontids thus meet the definition of an adaptive radiation.

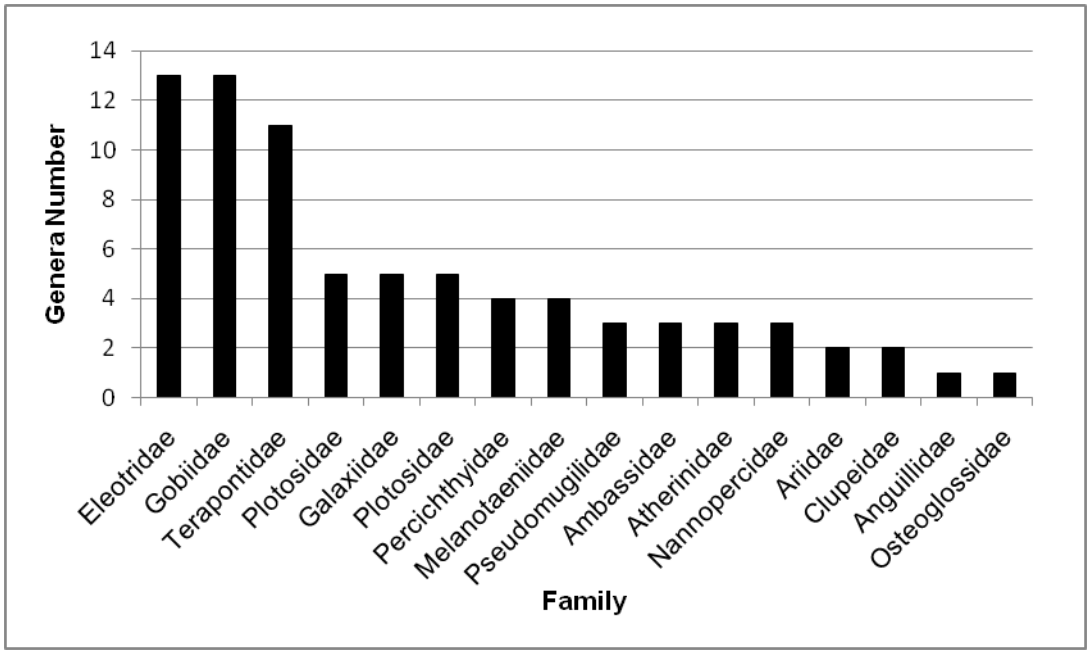

Figure 8.1 Genus-level diversity of major Australian freshwater fish families (data from Allen *et al*., 2002).

8.2. Future research directions

In this thesis I have addressed several key issues relating to the phylogeny, ecology and morphology of terapontid grunters. My findings have also highlighted a number of key questions relating to the evolutionary history of the family that could be explored further.

Resolving the 'taxonomic impediment' of Australasian fish taxonomy

A fundamental requirement in deciphering the underlying processes driving lineage diversification is an accurate inventory of species diversity (Donnellan *et al*., 1993). The issue of 'taxonomic impediment' is particularly relevant to Australian freshwater fishes, where taxonomic effort compared to other developed countries has been poor, and a considerable proportion of species diversity likely remains scientifically undocumented (Lundberg *et al*., 2000; Pusey *et al*., 2004). The genetic analyses in this study highlighted the very real possibility of cryptic species (e.g., *Hannia greenwayi*) in the Terapontidae. Similarly, Bostock *et al*. (2006) almost inadvertently identified two highly divergent haplotypes of *Leiopotherapon aheneus*, strongly suggestive of further cryptic speciation existing within the family. As well as this evidence emerging from two relatively geographically restricted 'species', the Terapontidae contains several of Australia's more widespread freshwater species, and would undoubtedly benefit from a more thorough taxonomic re-evaluation. It is likely that careful study of many widespread species will lead to an increase in the number of recognized species. Taxonomic inventory of the Indo-Papuan freshwater fish fauna, the other 'hotspot' of terapontid freshwater diversity (see Vari, 1978; Allen, 1991), has been even more limited. A more comprehensive assessment of terapontid species diversity and ecology would greatly facilitate the study of macroevolutionary patterns within the family.

Better definition of fish nutritional ecology

In contrast to the abundant research and long-established field of 'nutritional ecology' (Karasov and Martinez del Rio, 2007) that has been so beneficial to the study of terrestrial herbivory, the nutritional targets, food composition and associated digestive functioning of herbivorous-detritivorous fish remain much less well defined (see Choat and Clements, 1998; Clements *et al*., 2009). As noted by Logothetis *et al*. (2001) 'what it takes to be a herbivore remains a largely unanswered question in fish digestive physiology'. Accordingly, there has been considerable recent effort on the part of predominantly marine fish researchers to redress these gaps and develop a more unified and robust nutritional ecology approach to investigating fish herbivory (see Choat and Clements, 1998, Crossman *et al*., 2005, Clements and Raubenheimer, 2006). While these gaps are being addressed in the marine environment (incrementally in some areas; Clements *et al*., 2009), they are at least equally pronounced in freshwater species, and pose a considerable impediment to understanding the trophic ecology and food web function of herbivorous-detritivorous freshwater fishes (Mill *et al.*, 2009; Lujan *et al*., 2011).

The nutritional targets of the many nominal herbivores, detritivores and omnivores within the terapontids are currently unclear. SIA results from this study, for example, were largely uninformative for terapontid species such as *Scortum parviceps*, which was designated as highly herbivorous on the basis of SCA (Chapter 5). A better understanding of the nutritional ecology and digestive physiology of terapontids, particularly specialised herbivores and detritivores, is fundamental to fully comprehending their ecological roles (see Mill *et al*., 2009). Many marine 'herbivores' once commonly perceived to be algivores have been revealed by detailed dietary analyses to be highly dependent on amorphic detritus scraped from the epilithic algal complex – a loose assemblage of detritus, microbes, algae and diatoms that commonly collects on submerged surfaces in aquatic systems (van Dam *et al*. 2002; Crossman *et al*., 2005, Lujan *et al*., 2011). Similarly, recent studies have indicated that freshwater 'detritivorous' fishes assimilate carbon from biofilm and seston, and nitrogen from intermediate microbial decomposers in the environment, and are not actually capable of direct assimilation of vascular plant carbon (i.e. morphic detritus) (Lujan *et al*., 2011). Whether nominal terapontid detritivores such as *Syncomistes* and *Pingalla* species are genuine detritivores, or are actually targeting amorphic detritus, with its associated algal and microbial complexes, is also unknown. A nutritional ecological approach, however, incorporating knowledge of diet, functional morphology, intake, digestive physiology and dietary assimilation (*sensu* Karasov and Martinez del Rio 2007; Clements *et al*., 2009) would provide a more robust foundation with which to resolve the trophic habits of terapontids.

Initial food procurement

While this thesis focused primarily on the role of intestinal length in the terapontid trophic radiation (i.e. post ingestion), assessment of the biomechanics of food capture or procurement and buccal handling/processing of ingested material is likely to be another fruitful avenue of research in terapontid evolutionary history. Morphological and functional changes to the biomechanics and musculoskeletal functional morphology that underpins prey capture and handling are considered critical components in the impressive evolutionary diversification and ecological success of teleosts (Liem, 1980; Wainwright and Bellwood, 2002; Cooper and Westneat, 2009; Cooper *et al*., 2010). Fishes capture a diversity of prey using a wide range of movements of the skull, jaws and associated structures (e.g., Lauder, 1985; Lauder and Shaffer, 1993). Prey capture behaviors have been classified as suction feeding, biting, filter feeding, and ram feeding: non-exclusive categories that designate the central strategy for obtaining food (e.g., Lauder and Shaffer, 1985). Morphological and biomechanical studies of a range of fish

groups (e.g., Labridae, Pomacentridae) suggest that changes to aspects of the feeding apparatus have been of great importance during the extensive trophic radiation of several taxonomically and ecologically diverse lineages (Liem, 1980; Cooper *et al*., 2010) . There are marked changes in oral anatomy (mouth gape, dentition, dentary rotation etc.) across several genera within the Terapontidae (Figure 1.1; Vari, 1978). Quantification of any changes to the morphology and feeding kinematics of terapontids in relation to trophic diversification would be a valuable complement to the role of intestinal modification documented in this thesis. Gerhke (1988), for example, reported considerable plasticity in the feeding mechanics and behaviour of *L. unicolor* when offered different prey types. How this sort of behavioural and kinematic flexibility translates more broadly across the Terapontidae, particularly to more trophically derived forms, remains unknown.

The role of biogeography in terapontid trophic diversification.

A detailed assessment of the biogeographic processes that have shaped present-day terapontid species' distributional patterns and ecology is beyond the scope of this study. Biogeography, however, has potentially played a major role in a number of the phylogenetic patterns evident in current terapontid trophic ecology. Mapping of diet onto phylogeny indicated that many Australian species within genera such as *Syncomistes, Pingalla, Hephaestus* and *Scortum* share very similar dietary habits (Figure 6.5), although instances of species sympatry within these genera are rare (Allen *et al*., 2002). Several examples also exist of terapontid species that exhibit either disjunct Australian and New Guinean populations (*V. lacustris, P. lorentzi*), or closely allied 'sister species' that occur separately in each country (*H. carbo – H. raymondi*, *A. percoides – A. affinis, H. fuliginosus – H. roemeri*), separated by only minor morphological or genetic differences (Vari, 1978; this study). Several Australian genera (*Hephaestus, Scortum, Bidyanus*) similarly have multiple species occupying contiguous catchments. Diets remain very similar in these geographically separated species and sister species, suggesting species diversification, but minimal ecological divergence. Vicariant events producing these disjunct species and sister-species complexes likely played a major role in driving the subsequent speciation within freshwater terapontids.

While continental Australia has been essentially geologically quiescent, with contemporary drainage divisions established by the Paleocene (Veevers, 1991), a range of vicariance events have been proposed to explain the complex distributions of many Australian freshwater fishes, including the Terapontidae. Northern Australia and Papua New Guinea (the hot spot of

terapontid species diversity) share a complex history of inter-fluvial connections associated with major sea level shifts occurring during Quaternary glacial cycles (Chivas *et al*., 2001). The existence of a freshwater lake (Lake Carpentaria) between northern Australia and southern PNG between 12,000 and 14,000 years BP is proposed to have played a particularly important role in the phylogeography of aquatic taxa (including terapontids) during the late Pleistocene (Bowman *et al.*, 2010). Several terapontid genera are distributed across both northern and southern Papuan zoogeographic provinces, which probably separated 5-6 million years ago (Allen, 1991), suggesting the presence of freshwater terapontids in PNG since at least the Miocene/Pliocene. Similarly, genetic research for a broad range of freshwater fish species across northern Australia suggests that Lake Carpentaria played little role in determining the present day distribution of species or genetic divergence (see discussion in Pusey *et al*., 2011). The biogeographic dispersal histories of the freshwater terapontids are likely complex, and would undoubtedly benefit from further assessment.

Specific biogeographic aspects surrounding the evolution of terapontid diet within the context of broader Australian fish feeding habits may also warrant investigation, particularly examination of the geographical distribution and underlying evolutionary processes of Australia's herbivorous freshwater fishes. The relationships between latitude, and the purported diversity and abundance of tropical marine herbivorous fishes in comparison to temperate regions, has provoked considerable debate (Floeter *et al*., 2005). While hypotheses of latitudinal temperature thresholds on the digestive processes of herbivorous fishes have gained considerable traction (Floeter *et al*., 2005), the issue remains contentious and unresolved (Clements *et al*., 2009). Dietary information suggests that significantly more plant and detrital material is consumed by northern than southern Australian fishes (Kennard *et al*., 2001). Ecological and evolutionary syntheses capturing the underlying phylogenetic and foraging constraints producing these patterns would be informative tests of contemporary theories regarding herbivory in fish.

8.2. Conclusions

Australia is notable for the lack of dietary diversification among its freshwater fishes. This thesis identifies the terapontids as Australia's most trophically diverse fish family, with size-related (ontogenetic) dietary shifts a fundamental aspect of individual species' trophic habits. Trophic studies of fish and food webs that ignore these often pronounced size-related shifts in the specific dietary items and range of basal carbon sources utilized by fish throughout their history are, therefore, simplistic and potentially flawed. As well its significance to the contemporary ecology of terapontids, ontogeny has apparently also played a major role in shaping the evolutionary ecology and phylogenetic diversification of the Terapontidae. The ontogenetic development of intestinal complexity in particular appears to represent an important functional innovation driving the adaptive radiation seen in Australia's freshwater terapontids, specifically enabling the widespread adoption of omnivorous, herbivorous and detritivorous diets across the family. Australia's biogeographic history, specifically its lack of herbivorous-detritivorus primary freshwater fishes, may have provided the necessary 'ecological opportunity' for the adaptive radiation of the freshwater terapontids.

References

Alberch, P., Gould, S.J., Oster, G.F. and Wake, D.B. 1979. Size and shape in ontogeny and phylogeny. *Paleobiology* 5: 296 – 317.

Albertson, R.C., Markert, J.A., Danley, P.D. and Kocher, T.D. 1999. Phylogeny of a rapidly evolving clade: the cichlid fishes of Lake Malawi, East Africa. *Proc. Natl. Acad. Sci. USA* **96**: 5107–5110.

Alfaro, M.E., Santini, F., Brock, C., Alamillo, H., Dornburg, A., Rabosky, D.L., Carnevale, G. and Harmon, L.J. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proc. Natl. Acad. Sci. USA* **106**: 13410-13414.

Al-Hussaini, A.H. 1949. On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: anatomy and histology. *Q. J. Microscop. Sci.* **90**: 109 – 139.

Allen, G.R. 1991. *Field Guide to the Freshwater Fishes of New Guinea*. Christensen Research Institute, Madang, Papua New Guinea.

Allen, G.R. 1993. *Variichthys*, a replacement name for the terapontid fish genus *Varia* and first record of *V. lacustris* from Australia. *Rec. Western Aust. Mus.* **16**: 459–460.

Allen, G.R., Midgley, S.H. and Allen, M. 2002. *Field Guide to the Freshwater Fishes of Australia*. Western Australian Museum, Perth, Western Australia.

Allen, M., Morgan, D.L. and Gill, H.S. 2005. Distribution, zoogeography and biology of the Murchison River hardyhead (*Craterocephalus cuneiceps* Whitley, 1944), an atherinid endemic to the Indian Ocean (Pilbara) Drainage Division of Western Australia. *Ecol. Freshw. Fish* 14: 209-224.

Atchley, W.R., and Anderson, D.E. 1978. Ratios and the statistical analysis of biological data. *Syst. Zool.* 27: 71-78.

Australian Water Resources Council. 1976. *Review of Australia's water resources 1975*. Australian Water Resources Council, Australian Government Department of Natural Resources, Canberra, Australia.

Barlow, G.W. 2000. *The Cichlid Fishes.* Perseus Publishing, Cambridge, Massachusetts.

Bell, J.F. 1999. Tree-based methods. In: *Machine Learning Methods for Ecological Applications*. (A. Fielding, ed), pp. 89-105. Kluwer, Dordrecht.

Bell, M.A. and Andrews, C.A. 1997. Evolutionary consequences of colonization of fresh water by primitively anadromous fishes. In: *Evolutionary Ecology of Freshwater Animals*,

Current Trends and Case Studies (B. Streit, T. Städler and C.M. Lively, eds), pp. 323–363. Birkhäuser, Basel.

Bellwood, D.R. 2003. Origins and escalation of herbivory in fishes: a functional perspective. *Paleobiology* **29**: 71–83.

Benavides, A.G., Cancino, J.M. and Ojeda, F.P. 1994. Ontogenetic change in the diet of *Aplodactylus punctatus* (Pisces: Aplodactylidae): an ecophysiological explanation. *Mar. Biol.* **118**:1–5.

Betancur-R, R. 2010. Molecular phylogenetics support multiple evolutionary transitions from marine to freshwater habitats in ariid catfishes. *Mol. Phylogenet. Evol*. **55**: 249–258.

Betancur-R,R., Ortí, G., Stein, A.M., Marceniuk, A.P. and Pyron, R.A. 2012. Apparent signal of competition limiting diversification after ecological transitions from marine to freshwater habitats. *Ecol. Lett*. DOI: 10.1111/j.1461-0248.2012.01802.x

Bishop, K.A., Allen, S.A., Pollard, D.A. and Cook, M.G. 2001. *Ecological Studies on the Fishes of the Alligator Rivers Region, Northern Territory: Autecology*. Office of the Supervising Scientist Report 145, Supervising Scientist, Canberra, Australia.

Bishop, K.A. and Forbes, M.A. 1991. The freshwater fishes of northern Australia. In: *Monsoonal Australia: Landscape, Ecology and Man in the Northern Lowlands* (C.D. Haynes, M.G. Ridpath, and M.A.J. Williams, eds), pp. 79-107. A.A. Balkema, Rotterdam.

Biondini, M.E., Bonham, D.C. and Redente, E.F. 1985. Secondary successional patterns in sagebrush (*Artemisia tridentate*) community as they relate to soil disturbance and soil biological activity. *Vegetatio* **60:** 25-36.

Blanck, A., Tedesco, P.A. and Lamouroux, N. 2007. Relationships between life-history strategies of European freshwater fish species and their habitat preferences. *Freshwater Biol*. **52**: 843-859.

Blomberg, S.P., Garland, *T. and* Ives, A.R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717–745.

Blondel, J. 2000. Evolution and ecology of birds on islands: trends and prospects. *Vie et Milieu* **50**: 205–220.

Bloom, D. D. and Lovejoy, N.R. 2012. Molecular phylogenetics reveals a pattern of biome conservatism in New World anchovies (family Engraulidae). *J. Evol. Biol.* 25: 701–715.

Bohanak, A.J. and van der Linde, K. 2004. *RMA: Software for Reduced Major Axis regression, Java Version (Version 1.21)*. Available at http://www.kimvdlinde.com/professional/rma.html.. [Accessed 01 March 2011].

Bolnick D. I., Svanback R., Araujo M. S. and Persson, L. 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proc. Natl. Acad. Sci. USA* **104**: 10075-10079.

Bond, A. and Diamond, A. 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol. Appl.* **21**: 1017-1023.

Bostock, B.M., Adams, M., Laurenson, L.J.B. and Austin, C.M. 2006. The molecular systematics of *Leiopotherapon unicolor* (Gunther, 1859): testing for cryptic speciation in Australia's most widespread freshwater fish. *Biol. J. Linn. Soc*. **87**:537–552.

Boughton, D.A., Collette, B.B. and McCune, A.R. 1991. Heterochrony in jaw morphology of needlefishes (Teleostei: Belonidae). *Syst. Zool*. **40**: 329 – 54.

Breiman, L., Freidman, J., Olshen, R.A., and Stone, C.J. 1984. *Classification and Regression Trees*. Wadsworth International Group, Belmont, California.

Burbrink, F.T. and Pyron, R.A. 2010. How does ecological opportunity influence rates of speciation, extinction and morphological diversification in New World ratsnakes (Tribe Lampropeltini)? *Evolution* **64**:934-943.

Carseldine, L. and Tibbetts, I.R. 2005. Dietary analysis of the herbivorous hemiramphid *Hyporhamphus regularis ardelio*: an isotopic approach. *J. Fish Biol.* **66**: 1589–1600.

Carson, H.L. and Kaneshiro, K.Y. 1976. Drosophila of Hawaii: Systematics and ecological genetics. *Annu. Rev. Ecol. Syst.* **7**:311–345.

Cassemiro, F.A.S., Rangel, T.F.L.V.B., Pelicice, F.M. and Hahn, N.S. 2008. Allometric and ontogenetic patterns related to feeding of a neotropical fish, *Satanoperca pappaterra* (Perciformes, Cichlidae). *Ecol. Freshw. Fish* **17**: 155-164.

Caut, S., Angulo, E. and Courchamp, F. 2009. Variation in discrimination factors (δ^{15}N and δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. *J. Appl. Ecol*. **46**: 443-453.

Cebrian, J. 1999. Patterns in the fate of production in plant communities. *Am. Nat*.: 154, 449–468.

Chow, S. and Hazama K. 1998. Universal PCR primers for S7 ribosomal protein gene introns in fish. *Mol. Ecol*. **7**: 1255–1256.

Choat, J.H. and Clements, K.D. 1998. Vertebrate herbivores in marine and terrestrial environments: a nutritional ecology perspective. *Annu. Rev. Ecol. Syst.* **29**: 375-403.

Chivas, A.R., Garcia, A., van der Kaars, S., Couapel, M.J.J., Holt, S., Reeves, J.M., Wheeler, D.J., Switzer, A.D., Murray-Wallace, C.V., Banerjee, D., Price, D.M., Wang, S.X., Pearson, G., Edgar, N.T., Beaufort, L., de Deckker, P., Lawson, E. and Cecil, C.B. 2001. Sea-level and environmental changes since the last interglacial in the Gulf of Carpentaria, Australia: an overview. *Quatern. Int.* **83–85**: 19–46.

Clarke, K.R. and Gorley, R.N. 2006. *PRIMER v6: User Manual/Tutorial*. Primer-E, Plymouth.

Clarke, K.R., Somerfield, P.J. and Gorley, R.N. 2008. Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. *J. Exp. Mar. Biol. Ecol.* **366**: 56-69.

Clements, K.D. and Choat, J.H. 1990. Influence of season, ontogeny and tide on the diet of the marine herbivorous fish *Odax pullus* (Odacidae). *Mar. Biol.* **117**: 213-220.

Clements, K.D. and Raubenheimer, D. 2006. Feeding and nutrition. In: *The Physiology of Fishes, 3rd ed*. (D.H. Evans and J.B. Claiborne eds), pp. 47–82. CRC Press, Gainesville.

Clements, K.D., Raubenheimer, D. and Choat, J.H. 2009. Nutritional ecology of marine herbivorous fishes: ten years on. *Funct. Ecol*. **23**: 79–92.

Coates, D. 1993. Fish ecology and management of the Sepik-Ramu, New Guinea, a large contemporary tropical river basin. *Environ. Biol. Fishes* **38**: 345-368.

Cook, B.D., Baker, A.M., Page, T.J., Grant, S.C., Fawcett, J.H., Hurwood, D.A. *et al*. 2006. Biogeographic history of an Australian freshwater shrimp, *Paratya australiensis* (Atyidae): the role life history transition in phylogeographic diversification. *Mol. Ecol*. **15**: 1083–1093.

Cooper W. J. and Westneat M.W. 2009. Form and function of damselfish skulls: rapid and repeated evolution into a limited number of trophic niches. *BMC Evol. Biol*. **17**: 1–17.

Cooper, W.J., Parsons, K., McIntyre, A., Kern, B., McGee-Moore, A., *et al*. 2010. Bentho-Pelagic Divergence of Cichlid Feeding Architecture Was Prodigious and Consistent during Multiple Adaptive Radiations within African Rift-Lakes. *PLOS ONE* **5**: e9551. doi:10.1371/journal.pone.0009551

Connell, J.H. and Orias, E. 1964. The ecological regulation of species diversity. *Am. Nat*. **98**: 399-314.

Correa, S.B., Winemiller, K.O., Lopez-Fernandez, H. and Galetti, M. 2007. Evolutionary perspectives on seed consumption and dispersal by fishes. *Bioscience* **57**: 748-756.

Crossman, D.J., Choat, J.H. and Clements, K.D. 2005. Nutritional ecology of nominally herbivorous fishes on coral reefs. *Mar. Ecol. Prog. Ser.* **296**: 129–142.

Cuvier, G. and Valenciennes, M. 1829. *Histoire Naturelle des Poissons* **58**. Strasbourg, Paris.

Davis, A.M., Pearson, R.G. and Pusey, B.J. 2011a. Contrasting intraspecific dietary shifts in two terapontid assemblages from Australia's wet-dry tropics. *Ecol. Freshw. Fish* **21**: 42-56.

Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2011b. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78**: 265-286.

Davis, A.M., Pusey, B.J., Thorburn, D.C., Dowe, J.L., Morgan, D.L. and Burrows, D. 2010. Riparian contributions to the diet of terapontid grunters in wet-dry tropical rivers. *J. Fish Biol.* **76**: 862-879.

Davis, A.M. and Pusey, B.J. 2010. Trophic polymorphism and water clarity in northern Australian *Scortum* (Pisces: Terapontidae). *Ecol. Freshw. Fish* 19:638-643.

Davis, A.M., Pusey, B.J. and Pearson, R.G. 2012a. Trophic ecology of terapontid fishes (Pisces: Terapontidae): the role of morphology and ontogeny. *Mar. Freshwater Res.* **63**: 128-141.

Davis, A.M., Unmack, P.J., Pusey, B.J. and Pearson, R.G. 2012b. Marine-freshwater transitions are associated with the evolution of dietary diversification in terapontid grunters (Teleostei: Terapontidae). *J. Evol. Biol.* **25**:1163-1179.

De'ath, G. and Fabricius, K.E. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* **81**: 3178-3192.

DeNiro, M. J. and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Ac.* **42**: 495-506.

Dodds, W. K. 1997. Distribution of runoff and rivers related to vegetative characteristics, latitude, and slope: A global perspective. *J. N. Am. Benthol. Soc.* **16**:162–168.

Doucett, R.R., Booth, R.K., Power, G. and McKinley, R.S. 1999. Effects of spawning migration on the nutritional status of anadromous Atlantic salmon (*Salmo salar*): insights from stable isotope analysis. *Can. J. Fish. Aquat. Sci.* **56**: 2172-2180.

Donnellan S., Adams, M., Hutchinson, M. and Baverstock. P.R. 1993. The identification of cryptic species in the Australian herpetofauna: a high research priority. In: *Herpetology*

in Australia: a diverse discipline (D. Lunney and D. Ayres eds), pp. 121–126. Surrey Beatty and Sons, Sydney, Australia.

Douglas, M.E. 1987. An ecomorphological analysis of niche packing and niche dispersion in stream fish clades. In: *Community and evolutionary ecology of North American stream fishes* (W.J. Matthews and D.C. Heins, eds), pp. 144-149. University of Oklahoma Press, Norman, Oklahoma.

Douglas, M.E. and Matthews, W.J. 1992. Does morphology predict ecology? Hypothesis testing with a freshwater stream fish assemblage. *Oikos* **65**: 213 – 224.

Douglas, M.M., Bunn, S.E. and Davies, P.M. 2005. River and wetland food webs in Australia's wet-dry tropics: general principles and implications for management. *Mar. Freshwater Res.* **56**: 329 – 342.

Dowling, T.E., Tibbets, C.A., Minckley, W.L. and Smith, G.R. 2002. Evolutionary relationships of the plagopterins (Teleostei: Cyprinidae) from cytochrome *b* sequences. *Copeia* **2002**: 665–678.

Drewe, K.E., Horn, M.H., Dickson, K.A. and Gawlicka, A. 2004. Insectivore to frugivore: ontogenetic changes in gut morphology and digestive enzyme activity in the characid fish *Brycon guatemalensis* from Costa Rican rainforest streams. *J. Fish Biol.* **64**: 890 – 902.

Drummond, A.J. and Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**: 214.

Ekman, S., Andersen, H.L. and Wedin, M. 2008. The limitations of ancestral state reconstruction and the evolution of the ascus in the Lecanorales (lichenized Ascomycota). *Syst. Biol*. **57**: 141–156.

Elliott, J.P. and Bellwood, D.R. 2003. Alimentary tract morphology and diet in three coral reef fish families. *J. Fish Biol.* **63**: 1598–1609.

Espinoza, R.E., Wiens, J.J. and Tracy, C.R. 2004. Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. *Proc. Natl. Acad. Sci. USA* **101**: 16819–16824.

Faulks, J.J. 1998. *Daly River Catchment, An assessment of the physical and ecological condition of the Daly River and its major tributaries. Top End Waterways project. Technical report TR99/10.* Northern Territory Department of Lands, Planning and Environment, Darwin, Northern Territory, Australia.

Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1 – 15.

Fink, W.L. 1981. Ontogeny and Phylogeny of Tooth Attachment Modes in Actinopterygian Fishes. *J. Morph.* **167**: 167-184.

Floeter S.R, Behrens M.D, Ferreira C.E.L, Paddack M.J and Horn M.H. 2005. Geographical gradients of marine herbivorous fishes: patterns and processes. *Mar. Biol.* **147**: 1435–1447.

Folch, J., Lees, M. and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues, *J. Biol. Chem.* **226:** 497-509.

Foote, M. 1997. The evolution of morphological diversity. *Annu. Rev. Ecol. Syst.* **28**: 129–152.

Freckleton, R.P., Harvey, *P.H. and* Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* 160:712–726.

Frierson, E.W. and Foltz, J.W. 1992. Comparison and estimation of Absorptive Intestinal Surface areas in two species of Cichlid Fish. *Trans. Amer. Fish. Soc.* **121**: 517 – 523.

Froese, R. and Pauly, D. 2011. *FishBase*. World Wide Web electronic publication. www.fishbase.org, version (08/2011).

Fryer, G. and Iles, T.D. 1972. *The Cichlid Fishes of the Great Lakes of Africa: their Biology and Evolution*. Oliver and Boyd, Edinburgh.

Fry, B. and Arnold, C.R.E. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* **54**: 200-204.

Galván, D.E., Sweeting, C.J. and Reid, W.D.K. 2010. Power of stable isotope techniques to detect size-based feeding in marine fishes. *Mar. Ecol. Prog. Ser.* **407**: 271-278.

Gannes, L.Z., O'Brien, D.M. and Martinez del Rio, C. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* **78**: 1271-1276.

Garland, T., Jr., Bennet, A.F. and Rezende, E.L. 2005. Phylogenetic approaches in comparative physiology. *J. Exp. Biol*. **208**:3015–3035.

Garland, T., Jr., Harvey, P.H. and Ives, A. R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol*. **41**:18–32.

Garland, T., Midford, P.E. and Ives, A.R. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. *Am. Zool*. **39**:374–388.

Garner, P. 1996. Microhabitat use and diet of 0+ cyprinid fishes in a lentic, regulated reach of the River Great Ouse, England. *J. Fish Biol*. **48**: 367-382.

Gatz, A.J. 1979. Ecological morphology of freshwater stream fishes. *Tulane Stud. Zool. Bot.* **21**: 91-124.

Gavrilets, S. and Losos, J.B. 2009. Adaptive radiation: Contrasting theory with data. *Science* **323**:732–737.

Gavrilets, S. and Vose, A. 2005. Dynamic patterns of adaptive radiation. *Proc. Natl Acad. Sci. USA* **102**:18040–18 045.

Gehrke, P.C. 1988. Influence of gut morphology, sensory cues and hunger on feeding behavior of spangled perch, *Leiopotherapon unicolor* (Guenther, 1859), (Percoidei, Teraponidae). *J. Fish Biol.* **33**: 189–201.

German, D.P., Neuberger, D.T., Callahan, M.N., Lizardo, N.R. and Evans, D.H. 2010. Feast to famine: the effects of food quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae). *Comp. Biochem. Phys. A* **155**: 281-293.

German, D.P. 2011. Digestive efficiency. In: *Encyclopedia of Fish Physiology, From Genome to Environment* (A.P. Farrell, J.J. Cech, J.G. Richards and E. D. Stevens, eds), pp. 1596-1607. Elsevier, San Diego, California.

German D.P. and Horn, M.H. 2006. Gut length and mass in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. *Mar. Biol.* **148**:1123–1134.

German D.P., Nagle, B.C., Villeda, J.M, Ruiz, A.M., Thompson, A.W., Bald, S.C. and Evans, D.H. 2010. Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): effects on gut size and digestive physiology. *Physiol. Biochem. Zool.* **83**:1–18.

Gill, H.S. and Morgan, D.L. 2003. Ontogenetic changes in the diet of the black-stripe minnow *Galaxiella nigrostriata* (Shipway, 1953) (Galaxiidae) and the salamanderfish *Lepidogalaxias salamandroides* (Mee, 1961) (Lepidogalaxiidae). *Ecol. Freshw. Fish* **12**: 151-158.

Gill, H.S. and Morgan, D.L. 1998. Larval development of *Nannatherina balstoni* Regan (Nannopercidae), with a description of ontogenetic changes in diet. *Ecol. Freshw. Fish* **7**: 132-139.

Glor, R. E. 2010. Phylogenetic insights on adaptive radiation. *Annu. Rev. Ecol. Evol. Syst.* **41**: 251-270.

Goloboff, P.A., Farris, J.S. and Nixon, K.C. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* **24**: 774–786.

Grafen, A. 1989. The phylogenetic regression. *Philos. Trans. R. Soc. Lond. B* **326**: 119–157.

Grant, P.R. 1986. Ecology and Evolution of Darwin's Finches. Princeton University Press, Princeton, New Jersey.

Grant, P.R. and Grant, B.R. 2008. *How and Why Species Multiply: The Radiation of Darwin's Finches*. Princeton University Press, Princeton, New Jersey.

Greenwood, P.H. 1981. *The Haplochromine Fishes of the East African Lakes*. Cornell University Press, Ithaca, New York.

Grossman, G.D., Moyle, P.B. and Whitaker Jr., J.O. 1982. Stochasticity in structural and functional characteristics of an Indian stream fish assemblage: a test of community theory. *Am. Nat*. **120**: 423-454.

Grossman, G.D. 1986. Food resource partitioning in a rocky intertidal fish assemblage. *J. Zool*. **1**: 317-355.

Goloboff, P.A., Farris, J.S. and Nixon, K.C. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* **24:** 774–786.

Gould, S.J. 1977. *Ontogeny and Phylogeny*. Harvard University Press, Cambridge, Masschusetts.

Goulding, M. 1980. *Fishes and the Forest: Explorations in Amazonian Natural History*. University of California Press, Berkeley, California.

Haeckel, E. 1876. *The History of Creation* (translated by E. R. Lankester). H. S. King and Co., London.

Hall, B. K. 1998. *Evolutionary Developmental Biology*. Chapman and Hall, New York.

Hall, T.A. 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser*. **41**: 95–98.

Haines, A.T., Finlayson, B.T. and McMahon, T.A. 1988. A global classification of river regimes. *Appl. Geochem*. **8**: 252-272.

Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E. and Challenger, W. 2008. GEIGER: Investigating evolutionary radiations. *Bioinformatics* **24**:129–131.

Harvey, P.H. and Pagel, M.D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Hall, T.A. 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser*. **41**: 95–98.

Hawking, J.H. and Smith, F.J. (1997) *Colour guide to the invertebrates of Australian inland waters*. Cooperative Research centre for Freshwater Ecology. Albury, NSW, 2460.

Helfman, G.S., Collette, B.B. and Facey, D.E. 1997. *The Diversity of Fishes*. Blackwell Science, Malden, Massachusetts.

Herrel A., Meyers, J.J., Nishikawa, K.C. and De Vree, F. 2001. The evolution of feeding motor patterns in lizards: modulatory complexity and possible constraints. *Am. Zool.* **41**:1311–1320.

Herrel, A., Podos, *J.*, Vanhooydonck, *B. and* Hendry, A.P. 2009. Force-velocity trade-off in Darwin's finch jaw function: a biomechanical basis for ecological speciation? *Funct. Ecol.* 23*:* 119–125.

Herrel, A., Huyghe K., Vanhooydonck, B., Backeljau, T., Breugelmans, K., Grbac, I., Van Damme, R. and Irschick, D.J. 2008. Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *Proc. Natl. Acad. Sci. USA* **105**:4792–4795.

Hesslein, R.H., Hallard, K.A. and Ramlal, P. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by δ^{34}S, δ^{13}C, and δ^{15}N. *Can. J. Fish. Aquat. Sci.* **50**: 2071-2076.

Henstridge, D.A. and Missen, D.D. 1982. Geology of oil-shale deposits with The Narrows graben, Queensland, Australia. *Am. Assoc. Pet. Geol. Bull*. **66**: 719–731.

Hillis, D.M. and Bull, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol*. **42**: 182–192.

Hoeinghaus, D.J., Winemiller, K.O. and Agostinho, A. A. 2007. Landscape-scale hydrologic characteristics differentiate patterns of carbon flow in large river food webs. *Ecosystems* **10**: 1019-1033.

Hofer, R. 1988. Morphological adaptations of the digestive tract of tropical cyprinids and cichlids to diet. *J. Fish Biol.* **33**: 399 – 408.

Horn, M. H. 1989. Biology of marine herbivorous fishes. *Ocean. Mar. Biol. Annu. Rev.* **27**: 167–272.

Horn, M.H. 1998. Feeding and digestion. In: *The Physiology of Fishes* (D.H. Evans, ed), pp. 43–63. CRC Press, Boca Raton, Florida.

Horn, M.H. and Ojeda, F.P. 1999. Herbivory. In: *Intertidal Fishes: Life in Two Worlds* (M.H. Horn, K.L. Martin and M.A. Chotkowski, eds), pp. 197–222. Academic Press, San Diego, California.

Higham, T.E., Hulsey, C.D., Rican, O. *and* Carroll, A.M. 2007. Feeding with speed: prey capture evolution in cichlids. *J. Evol. Biol.* 20*:* 70–78.

Hugueny, B. and Pouilly, M. 1999. Morphological correlates of diet in an assemblage of West African freshwater fishes. *J. Fish Biol.* **54**: 1310 – 1325.

Hugueny, B., Oberdoff, T. and Tedesco, P.A. 2010. Community ecology of river fishes: a large scale perspective. In: *Community ecology of stream fishes: concepts, approaches and techniques* (D. Jackson and K. Gido, eds). American Fisheries Society Symposium 73. *In press*.

Hurlbert, S.H. 1978. The measurement of niche overlap and some relative. *Ecology* **59**: 66-77.

Hyslop, E.J. 1980. Stomach content analysis – a review of methods and their application. *J. Fish Biol.* **17**: 411 - 429.

Jardine, T.D., Pusey, B.J., Hamilton, S.K., Pettit, N.E., Davies, P.M., Douglas, M.M., Sinnamon, V., Halliday, I.A. and Bunn, S.E. 2012. Fish mediate high food web connectivity in the lower reaches of a wet-dry tropical floodplain river. *Oecologia* **168**: 829-38.

Jennings, S., Greenstreet, S. P. R., Hill, L., Piet, G. J., Pinnegar, J. K. and Warr, K. J. 2002. Long term trends in the trophic structure of the North Sea fish community: evidence from stable isotope analysis, size-spectra and community metrics. *Mar. Biol.* **141**: 1085-1097.

Jepsen, D.B. and Winemiller, K.O. 2002. Structure of tropical river food webs revealed by stable isotope ratios. *Oikos* **96**: 46-55.

Jepsen, D.B., Winemiller, K.O. and Taphorn, D.C. 1997. Temporal patterns of resource partitioning among *Cichla* species in a Venezuelan blackwater river. *J. Fish Biol.* **51**: 1085-1108.

Johnson, J.W. 1999. Annotated checklist of the fishes of Moreton Bay, Queensland, Australia. *Mem. Qld Mus.* **43**: 709–762.

Johnson, J.W. 2010. Fishes of the Moreton Bay Marine Park and adjacent continental shelf waters, Queensland, Australia. *Mem. Qld Mus*. **54**: 299–353.

Jolly, P. 2001. *Daly River Catchment Water Balance*. Northern Territory Department of Infrastructure, Planning and Environment, Darwin, Northern Territory.

Jones, M.J., Fielding, A. and Sullivan, M. 2006. Analysing extinction risk in parrots using decision trees. *Biodivers. Conserv.* **15**: 1993-2007.

Karels, T.J., Bryant, A.A. and Hik, D.S. 2004. Comparison of discriminant function and classification tree analyses for age classification of marmots. *Oikos* **105**: 575-587.

Katoh, K. and Toh, H. 2010. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* **26**: 1899–1900.

Karr, J.R. and James, F.C. 1975. Eco-morphological configurations and convergent evolution in species and communities. In: *Ecology and Evolution of Communities* (M.L. Cody and J.M. Diamond, eds), pp. 258 – 291. Harvard University Press, Cambridge, Massachusetts.

Kapoor, B.G., Smit, H. and Verighina, A.I. 1975. The alimentary canal and digestion in teleosts. *Adv. Mar. Biol.* **13**: 109-239.

Karasov, W.H. and Hume, I.D. 1997. Vertebrate gastrointestinal system. In: *Handbook of Comparative Physiology* (W.H. Dantzler, ed), pp. 407–480. American Physiological Society, Bethesda, Maryland.

Karasov, W.H., and Martínez del Rio, C. 2007. *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins.* Princeton University Press, Princeton, New Jersey.

Karasov, W. H., Martínez del Rio, C. and Caviedes-Vidal, E. 2011. Ecological physiology of diet and digestive systems. *Ann. Rev. of Phys*. **73**: 69–93.

Katoh, K. and Toh, H. 2010. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* **26**: 1899–1900.

Keast, A. 1985. The piscivore feeding guild of fishes in small freshwater ecosystems. *Environ. Biol. Fishes* **12**: 119-129.

Kennard, M.J. 1995. *Factors Influencing Freshwater Fish Assemblages in Floodplain Lagoons of the Normanby River, Cape York Peninsula: a Large Tropical Australian River*. MSc Thesis, Griffith University, Brisbane, Australia.

Kennard, M., Pusey, B. and Arthington, A. 2001. *Trophic Ecology of Freshwater Fishes in Australia. Summary Report CRC for Freshwater Ecology Scoping Study SCD6.* CRC for Freshwater Ecology, Griffith University, Brisbane, Australia.

Kennard, M., Pusey, B., Olden, J., Mackay, S., Stein, J. and Marsh, N. 2010. Ecohydrological classification of natural flow regimes to support environmental flow assessments: an Australian case study. *Freshwater Biol*. **55**: 171-193.

Kilham, S. S., Hunte-Brown, M., Verburg, P., Pringle, C. M., Whiles, M. R., Lips, K. R. and Zandona, E. 2009. Challenges for interpreting stable isotope fractionation of carbon and nitrogen in tropical aquatic ecosystems. *Verh. Internat. Verein. Theoret. Angew. Limnol.* **30**: 749-753.

King, A.J. 2005. Ontogenetic dietary shifts of fishes in an Australian floodplain river. *Mar. Freshwater Res.* **56**: 215-225.

Knoppel, H.A. 1970. Food of central Amazonian fishes. *Amazonia* **2**: 257 – 352.

Konow, N. and Bellwood, D. R. 2011. Functional Disparity and Ecological Diversification in Marine Angelfishes, f. Pomacanthidae. *PLOS One* **6**: e24113.

Kramer, D.L. and Bryant, M.J. 1995. Intestine length in the fishes of a tropical stream: 1. Ontogenetic allometry. *Environ. Biol. Fishes* **42**: 115-127.

Krebs, C.J. 1999. *Ecological Methodology*, 2nd *edn*. Addison Wesley Longman, New York.

Kruskal, J.B. 1964. Nonmetric multidimensional scaling: a numerical method. *Pyschometrika* **29**: 1-27.

Lake, J.S. 1971. *Freshwater Fishes of Australia.* Nelson, Sydney, Australia.

Lamontagne, S., Cook, P.G., O'Grady, A. and Eamus, D. 2005. Groundwater use by vegetation in a tropical savanna riparian zone (Daly River: Australia). *J. Hydrol*. **310**: 280-293.

Lau, D.C.P., Leung, K.M.Y. and Dudgeon, D. 2009. What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong. *Freshwater Biol*. **54**: 127-141.

Lauder, G.V. 1982. Patterns of evcolution in the feeding mechanism of actinopterygian fishes. *Am. Zool*. **20**: 275-285.

Layman, C.A., Winemiller, K.O. and Arrington, D.A. 2005a. Describing the structure and function of a Neotropical river food web using stable isotopes, stomach contents, and functional experiments. In: *Dynamic Food Webs: Multispecies Assemblages, Ecosystem Development and Environmental Change* (P.C. de Ruiter, V. Wolters, and J.C. Moore, eds), pp. 395-406. Elsevier, Amsterdam.

Layman, C. A., Winemiller, K. O., Arrington, D. A. and Jepsen, D. B. 2005b. Body size and trophic position in a diverse tropical food web. *Ecology* **86:** 2530-2535.

Lee, C.E. and Bell, M.A. 1999. Causes and consequences of recent freshwater invasions by saltwater animals. *Trends Ecol. Evol.* **14**: 284–288.

Lee, S.C. and Tsai, M.P. 1999. Molecular systematics of the thornfishes genera *Terapon* and *Pelates* (Perciformes: Teraponidae) with reference to the new genus *Pseudoterapon*. *Zool. Stud.* **38**: 279–286.

Legendre, P. and Legendre, L. 1998. *Numerical Ecology*. Elsevier Scientific, Amsterdam.

Lewis, W.R., Hamilton Jr, S.K., Rodriquez, M.A., Saunders, J.F. III and Lasi M.A. 2001. Food web analysis of the Orinoco floodplain based on production estimates and stable isotope data. *J. N. Am. Benthol. Soc.* **20**: 241-254.

Lewis, P.O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol*. **50**: 913–925.

Liem, K.F. 1973. Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Syst. Zool.* **22**: 425-441.

Liem, K.F. 1980. Adaptive Significance of Intraspecific and Interspecific Differences in the Feeding Repertoires of Cichlid Fishes. *Am. Zool*. **20**: 295–314.

Lobel, P.S. 1981. Trophic biology of herbivorous reef fishes, alimentary pH and digestive capabilities. *J. Fish Biol.* **19**: 365-397.

Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A. and Lutcavage, M.E. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modeling methods. *J. Anim. Ecol.* **77**: 838-846.

Logothetis, E.A., Horn, M.H. and Dickson, K.A. 2001. Gut morphology and function in *Atherinops affinis* (Teleostei: Atherinopsidae), a stomachless omnivore feeding on macroalgae. J. Fish Biol. **59**:1298–1312.

Losos, J.B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. *Am. Nat.* **175**: 623–639.

Losos, J.B. and Mahler D.L. 2010. The interaction of ecological opportunity, adaptation, and speciation. In: *Evolution Since Darwin: The First 150 Years, Adaptive radiation* (M. Bell, D.J. Futuyma, W.F. Eanes, J.S. Levinton, eds), pp 381–420. Sinauer, Sunderland, Massachusetts.

Lovejoy, N.R., Albert, J.S. and Crampton, W.G.R. 2006. Miocene marine incursions and0 marine/freshwater transitions: evidence from Neotropical fishes. *Sth Am. Earth Sci*. **21**: 5–13.

Lovejoy, N.R. and Collette, B.B. 2001. Phylogenetic relationships of New World needlefishes (Teleostei: Belonidae) and the biogeography of transitions between marine and freshwater habitats. *Copeia* **2001**: 324–338.

Lowe-McConnell, R.H. 1964. The fishes of the Rupunini savanna district of British Guiana, South America. Part 1. Ecological groupings of fish species and effects on the seasonal cycle of fish. *Zool. J. Linn. Soc*. **45**: 103-144.

Lowe-McConnell, R.H. 1975. *Fish Communities in Tropical Freshwaters*. Longman Press, London.

Lundberg, J.G., Kottelat, M., Smith, G.R., Stiassny, M.L.J. and Gill, A.C. 2000. So many fishes, so little time: an overview of recent ichthyological discovery in continental waters. *Ann. Missour. Botan. Gard.* **87**: 26–62.

Lujan, N.K., German, D.P. and Winemiller K.O. 2011. Do wood grazing fishes partition their niche? Morphological and isotopic evidence for trophic segregation in neotropical Loricariidae. *Funct. Ecol.* **25**: 1327–1338.

MacNeil, M.A., Drouillard, K.G. and Fisk, A.T. 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Can. J. Fish Aquat. Sci.* **63**: 345-353.

Maddison, W.P. and Maddison, D.R. 2006. *Mesquite: a modular system for evolutionary analysis.* Available at: http://mesquiteproject.org.

Maddison, W.P., *and* Maddison, D.R. 2011. *Mesquite: a modular system for evolutionary analysis, ver. 2:75,* http://mesquiteproject.org.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Res.* **27**: 209-220.

Mantel, S.K., Salas, M. and Dudgeon, D. 2004. Food web structure in a tropical forest stream. *J. N. Am. Benthol. Soc.* **23**: 728-755.

Mark, W., Hofer, R. and Weiser, W. 1987. Diet spectra and resource partitioning in the larvae and juveniles of three species and six cohorts of cyprinids from a subalpine lake. *Oecologia* **78**: 330-337.

Marshall, S. and Elliott, M. 1997. A comparison of univariate and multivariate numerical and graphical techniques for determining inter- and intraspecific feeding relationships in estuarine fish. *J. Fish Biol.* **51**: 526-545.

Mather, P.M. 1976. *Computational Methods of Multivariate Analysis in Physical Geography.* J. Wiley and Sons, London.

Matthews, W.J. 1998. *Patterns in Freshwater Fish Ecology.* Chapman and Hall, New York.

McCune, B., and Grace, J.B. 2002. *Analysis of Ecological Communities*. MjM Software Design, Glenden Beach, Oregon.

McCune, B., and Mefford, M.J. 1999. *PC-ORD. Multivariate Analysis of Ecological Data*. MjM Software Design, Glenden Beach, Oregon.

Marroig, G. and Cheverud, J.M. 2005. Size as a line of least evolutionary resistance: diet and adaptive morphological radiation in New World monkeys. *Evolution* **59**:1128-1142.

Mees, G.F. and Kailola, P.J. 1977. The freshwater Therapontidae of New Guinea. *Zool. Verhand.* **153**: 3-88.

Merrick, J.R and Schmida, G.E. 1984. *Australian Freshwater Fishes: Biology and Management*. Griffin Press, Netley, Australia.

Merritt R.W. and Cummins K.W. 1996. *An Introduction to the Aquatic lnsects of North America* (Third Edition). Kendall Hunt Publishing, Iowa, USA.

Midford, P. E., Garland, Jr., T., *and* Maddison, W.P. 2010. *PDAP Package, version 1.15. Available at* http://mesquiteproject.org/pdap_mesquite/

Mill, A.C., Pinnegar, J.K. and Polunin, N.V.C. 2007. Explaining isotope trophic-step fractionation: why herbivorous fish are different. *Funct. Ecol.* **21**: 1137-1145.

Minagawa, M., and Wada, E. 1984. Stepwise enrichment of 15N along food chains: further evidence and the relation between 15N and animal age. *Geochim. Cosmochim. Ac.* **48**: 1135–1140.

Mittelbach, G.G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. *Ecology* **62**: 1370-1386.

Mittlebach, G.G. 1984. Predation and resource partitioning in two sunfishes (Centrarchidae). *Ecology* **65**: 499-513.

Mittelbach, G.G. and Persson, L. 1998. The ontogeny of piscivory and its ecological consequences. *Can. J. Fish. Aquat. Sci.* **55**: 1454-1465.

Montgomery, W.L. 1977. Diet and gut morphology in fishes, with special reference to the monkeyface prickleback, *Cebidichthys violaceus* (Stichaeidae: Blennioidei). *Copeia* **1977**: 178-182.

Mooers, A.Ø. and Schluter, D. 1999. Reconstructing ancestral states with maximum likelihood: support for one- and two-rate models. *Syst. Biol.* **48**: 623–633.

Morgan, D.L., Rowland, D., Gill, H.S and Doupé, R.G. 2004. Implications of introducing a large piscivore (*Lates calcarifer*) into a regulated northern Australian river (Lake Kununurra, Western Australia). *Lakes Reserv. Res. Man.* **9**: 181-193.

Motta, P.J., Clifton, K.B., Hernandez, P., and Engold, B.T. 1995a. Ecomorphological correlates in ten species of subtropical seagrass fishes: diet and microhabitat utilization. *Environ. Biol. Fishes* **44**: 37 – 60.

Motta, P.J., Norton, S.F., and Luczkovich, J.L. 1995b. Perspectives on the ecomorphology of bony fishes. *Environ. Biol. Fishes* **44**: 11 – 20.

Muñoz, A.A. and Ojeda, F.P. 1998. Guild structure of carnivorous intertidal fishes of the Chilean coast: implication of ontogenetic diet shifts. *Oecologia* **114**: 563-573.

Mushinsky, H.R., Hebrard, J.J. and Vodopich, D.S. 1982. Ontogeny of water snake foraging ecology. *Ecology* **63**: 1624-1629.

Near, T.J., Sandel, M., Kuhn, K.L., Unmack, P.J., Wainwright, P.C. and Smith, W.M. 2012. Nuclear gene inferred phylogenies resolve the relationships of the enigmatic pygmy sunfishes, *Elassoma* (Teleostei: Percomorpha). *Mol. Phylogenet. Evol*. doi: 10.1016/j.ympev.2012.01.011

Nelson, J.S. 2006. *Fishes of the World*. John Wiley and Sons, Hoboken.

Norton, S.F. 1995. A functional approach to ecomorphological patterns of feeding in cottid fishes. *Environ. Biol. Fishes* **44**: 61-78.

Norton, S.F., Luczkovich, J.L., and Motta, P.J. 1995. The role of ecomorphological studies in the comparative biology of fishes. *Environ. Biol. Fishes* **44**: 287-304.

O'Grady, A. P., Eamus, D., Cook, P. G. and Lamontagne, S. 2006. Groundwater use by riparian vegetation in the wet-dry tropics of northern Australia. *Aust. J. Bot*. **54**: 145 - 154.

Olden, J.D., Lawler, J.J., and Poff, N. L. 2008. Machine learning methods without tears: a primer for ecologists. *Q. Rev. Biol.* **83,** 171-193.

Olson, M. H., Mittelbach, G.G. and Osenberg, C.W. 1995. Competition between predator and prey: resource-based mechanisms and implications for stage-structured dynamics. *Ecology* **76**:1758-1771.

Olson, M.H. and Young, B.P. 2003. Patterns of diet and growth in co-occurring populations of largemouth bass and smallmouth bass. *Trans. Am. Fish. Soc*. 1207-1213.

Osenberg, C. W., Olson, M. H. and Mittelbach, G. G. 1994. Stage structure in fishes: resource productivity and competition gradients. In: *Theory and Application of Fish Feeding Ecology* (D. J. Stouder, K. L. Fresh and R. J. Feller, eds), pp. 51–70. University of South Carolina Press, Columbia, South Carolina.

Overman, N.C. and Parrish, D.L. 2001. Stable isotope composition of walleye: ^{15}N accumulation with age and area-specific differences in $\delta^{13}C$. *Can. J. Fish Aquat. Sci.* **58**: 1253-1260.

Pagel, M.D. 1992. A method for the analysis of comparative data. *J. Theor. Biol.* **156**:431–442.

Pagel, M. 1999a. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Syst. Biol.* **48**:612–622.

Pagel, M. 1999b. Inferring the historical patterns of biological evolution. *Nature* **401**:877–884.

Parkyn, S.M., Collier, K.J. and Hicks, B.J. 2001. New Zealand stream crayfish: functional omnivores but trophic predators? *Freshwater Biol.* **46**: 641-652.

Parnell, A., Inger, R., Bearhop, S. and Jackson, A.L. 2010. Source partitioning using stable isotopes: coping with too much variation. *PLOS ONE* 5: e9672. doi: 10.1371/ journal. pone.0009672

Pearson, R.G. 1991. *Ecology of the Burdekin River*. Australian Centre for Tropical Freshwater Research Report No. 91/01, James Cook University, Townsville, Queensland, Australia.

Peres-Neto, P.R., Jackson, D.A., and Somers, K.M. 2005. How many principal components? Stopping rules for determining the number of non-trivial axes revisited. *Comput. Stat. Data Anal.* **49**: 974-997.

Perga, M.E. and Gerdeaux, D. 2005. 'Are fish what they eat' all year round? *Oecologia* **144:** 598-606.

Peterson, B.J. and Fry, B. 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* **18**: 293-320.

Pettit, N. E., Froend, R. H. and Davies, P. M. 2001. Identifying the natural flow regime and the relationship with riparian vegetation for two contrasting western Australian rivers. *Reg. Rivers Res. Man.* **17**: 201-215.

Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D. and Moczek, A.P. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* **25**:459–467.

Phillips, D.L. and Gregg, J.W. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136: 261-269.

Phillips, D.L., Newsome ,S.D. and Gregg J.W. 2005. Combining sources in stable isotope mixing models: alternative methods. *Oecologia* **144**: 520-527.

Pianka, E.R. 2000. *Evolutionary Ecology, 6th edn.* Addison Wesley Longman, New York.

Piet, G.J. 1998. Ecomorphology of a size-structured tropical freshwater fish community. *Environ. Biol. Fishes* **51**: 67 – 86.

Piet, G.J., Pet, J.S., Guruge, W.A.H.P., Vijverberg, J. and Van Densen, W.L.T. 1999. Resource partitioning along three niche dimensions in a size-structured tropical fish assemblage. *Can. J. Fish. Aquat. Sci.***56**: 1241-1254.

Pinnegar, J.K. and Polunin, N.V.C. 1999. Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: implications for the study of trophic interactions. *Funct. Ecol.* **13**: 225-231.

Platell, M.E. and Potter, I.C. 2001. Partitioning of food resources among 18 abundant benthic carnivorous fish species in marine waters on the lower west coast of Australia. *J. Exp. Mar. Biol. Ecol.* **261**: 31-54.

Poff, N.L. and Allan, J.D. 1995. Functional organisation of stream fish assemblages in relation to hydrological variability. *Ecology* **76**: 606-627.

Polis, G.A. 1984. Age structure component of niche width and intraspecific resource partitioning: can age groups function as ecological species? *Am. Nat.* **123**: 541-564.

Posada, D. and Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703-718.

Post, D.M. 2003. Individual variation in the timing of ontogenetic niche shifts of largemouth bass. *Ecology* **84**: 1298-1310.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J. and Montaña, C.G. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152: 179-189.

Pouilly, M., Lino, F., Bretenoux, J.G. and Rosales, C. 2003. Dietary-morphological relationships in a fish assemblage of the Bolivian Amazonian floodplain. *J. Fish Biol.* **62**: 1137-1158.

Price, S.A., Hopkins, S.S.B, Smith K.K., and Roth, V.L. 2012. Tempo of trophic evolution and its impact on mammalian diversification. *Proc. Natl. Acad. Sci. USA* **109**: 7008-7012.

Puckridge, J.T., Sheldon, F., Walker, K.F. and Boulton, A.J. 1998. Flow variability and the ecology of large rivers. *Mar. Freshwater Res.***49**: 55-72.

Purvis, A. 1995. A composite estimate of primate phylogeny. *Philos. Trans. R. Soc. Lond. B* **348**:405–421.

Pusey, B.J., Arthington, A.H. and Read, M.G. 1998. Freshwater fishes of the Burdekin River, Australia: biogeography, history and spatial variation in community structure. *Environ. Biol. Fishes* **53**: 303-318.

Pusey, B.J., Arthington, A.H. and Read, M.G. 2000. The dry-season diet of freshwater fishes in monsoonal tropical rivers of Cape York Peninsula, Australia. *Ecol. Freshw. Fish* **9**: 177-190.

Pusey, B.J. Arthington, A.H., Stewart-Koster, B. and Kennard, M.J. 2010. Trophic generalism in freshwater fish assemblages of a hydrologically variable northern Australian river. *J. Fish Biol.* **77**: 731-753.

Pusey, B.J., Kennard, M. and Arthington, A. 2004. *Freshwater Fishes of North-Eastern Australia.* CSIRO Publishing, Melbourne, Australia.

Pusey, B., Kennard, M., Burrows, D., Perna, C., Kyne, P., Cook, B. and Hughes, J. 2011. Freshwater fish. Chapter 6. In: *Aquatic biodiversity of the Wet-Dry Topics of Northern Australia: patterns, threats and future* (B.J. Pusey ed), pp. 71-92. . Charles Darwin University Press, Darwin, Australia.

Pusey, B.J., Read, M.G. and Arthington, A.H. 1995. The feeding ecology of freshwater fishes in two rivers of the Australian Wet Tropics. *Environ. Biol. Fishes* **43**: 85-103.

Pybus, O.G. and Harvey, P.H. 2000.Testing macro-evolutionary models using incomplete molecular phylogenies. *Proc. Royal Soc. Lond. Part B.* **267**: 2267-2272.

R Development Core Team. 2009. *R: A Language and Environment for Statistical Computing.* (R Foundation for Statistical Computing, Vienna). Available at: http://www.r-project.org/ (accessed 20 September 2009).

R Development Core Team. 2011. *R: a language and environment for statistical computing. (R Foundation for Statistical Computing, Vienna, Austria). ISBN 3-900051-07-0, available at URL* http://www.R-project.org

Revell, L.J. 2009. Size-correction and principal components for interspecific comparative studies. *Evolution* **63**:3258–3268.

Revell, L.J. 2011. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol. doi:* 10.1111/j.2041-210X.2011.00169.x

Rabosky, D.L. 2010. Extinction rates should not be estimated from molecular phylogenies. *Evolution* **64**: 1816–1824.

Rabosky, D. L., Donnellan, S. C., Talaba, A. L. and Lovette, I. J. 2007. Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proc. R. Soc. B* **274**: 2915–2923.

Raeymaekers, J.A.M., Maes, G.E., Audenaert, E. *and* Volckaert, F.A.M. 2005. Detecting Holocene divergence in the anadromous-freshwater three-spined stickleback (*Gasterosteus aculeatus*) system. *Mol. Ecol*. 14*:* 1001–1014.

Rasmussen, J.B. 2010. Estimating terrestrial contribution to stream invertebrates and periphyton using a gradient-based mixing model for delta ^{13}C. *J. Anim. Ecol.* **79**: 393-402.

Randall, J.E. and Hartman, W.D. 1968. Sponge-feeding fishes of the West Indies. *Mar. Biol.* **1**: 216-225.

Rayner, T., Pusey, B.J. and Pearson, R.G. 2009. Spatio-temporal dynamics of fish feeding in the lower Mulgrave River, northeastern Queensland; the influence of seasonal flooding, instream productivity and invertebrate abundance. *Mar. Freshwater Res.* **60**: 97-111.

Rayner, T.S., Pusey, B.J., Pearson, R.G. and Godfrey, P.C. 2010. Food web dynamics in an Australian Wet Tropics River. *Mar. Freshwater Res.* **61**: 909-917.

Reid D.J., Quinn G.P., Lake P.S. and Reich P. 2008.Terrestrial detritus supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study. *Freshwater Biol.* **53:** 2036-2050.

Reist, J.D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. *Can. J. Zool.* **63**: 1429-1439.

Robinson, B.W. and Schluter, D. 2000. Natural selection and the evolution of adaptive genetic variation in northern freshwater fishes. In: *Adaptive genetic variation in the wild* (T.A. Mousseau, B. Sinervo and J.A. Endler, eds), pp. 65-94. Oxford University Press, Oxford.

Rogers, L.G., Cannon, M.G. and Barry, E.V. 1999. *Land Resources of the Dalrymple Shire*. Queensland Department of Natural Resources, Brisbane, Australia.

Rice, A.N. and Lobel, P.S. 2003. The pharyngeal jaw apparatus of the Cichlidae and Pomacentridae: Function in feeding and sound production. *Rev. Fish Biol. Fish.* **15**: 433-444.

Ricklefs, R.E. and Starck, J.M. 1996. Applications of phylogenetically independent contrasts: a mixed progress report. *Oikos* **77**: 167-172.

Rimmer, D.W. 1986. Changes in diet and the development of microbial digestion in juvenile buffalo bream, *Kyphosus cornelii*. *Mar. Biol.* **92**: 443 – 448.

Robinson, B.W. and Schluter, D. 2000. Natural selection and the evolution of adaptive genetic variation in northern freshwater fishes. In: *Adaptive genetic variation in the wild* (T.A. Mousseau, B. Sinervo and J.A. Endler, eds), pp. 65-94. Oxford University Press, Oxford.

Root, R. B. 1967. The niche exploitation pattern of the Bluegray Gnatcatcher. *Ecol.Monog.* **37**:317–350.

Ross, S.T. 1986. Resource partitioning in fish assemblages: a review of field studies. *Copeia* **1986:** 352-388.

Ruxton, G.D. 2006. The unequal variance *t*-test is an underused alternative to Student's *t*-test and the Mann-Whitney *U* test. *Behav. Ecol.* **17**: 688-690.

Sazima, I. 1983. Scale-eating in characoids and other fishes. *Environ. Biol. Fishes* **9**: 87-101.

Schaefer, S.A. and Lauder, G.V. 1986. Historical transformation of functional design: evolutionary morphology of feeding mechanisms in Loricarioid catfishes. *Syst. Zool*. **35**: 498–508.

Schaefer, B. and Rosen, D.E. 1961. Major adaptive levels in the Actinopterygian feeding mechanism. *Am. Zool.* **1**: 187-204.

Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* **76**:82–90.

Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, New York.

Schluter, D. 2001. The ecology and origin of species. *Trends Ecol. Evol*. **16**:372–380.

Schluter, D., Price, T., Mooers, A.Ø. and Ludwig, D. 1997. Likelihood of ancestor states in adaptive radiation. *Evolution* **51:** 1699–1711.

Schoener, T. W. 1974. Resource partitioning in ecological communities. *Science* **185**: 27-39.

Shigeta, T., Usuki, H. and Gushima, K. 2001. Interaction between cleaner and host: the black porgy cleaning behaviour of juvenile sharpnose tigerfish, *Rhyncopelates oxyrhyncus* in the Seto Inland Sea, Western Japan. In: *Proceedings of the 30th U.S. – Japan meeting on Aquaculture* (Y. Nakamura, J. P. McVey, K. Leber, C. Neidig, S. Fox, and K. Churchill, eds.), pp 139–147. Sarasota, Florida, December, 3–4. Mote Marine Laboratory, Sarasota, Florida.

Sibly, R.M. 1981. Strategies of digestion and defecation. In: *Physiological Ecology: an evolutionary approach to resource use (*C.R. Townsend and P. Calow, eds), pp.109-139. Blackwell Scientific Publications, Oxford.

Simberloff, D. and Dayan, T. 1991. The guild concept and the structure of ecological communities. *Annu. Rev. Ecol. Syst.* **22**:115–143.

Smouse, P.E., Long, J.C. and Sokal, R. 1986. Multiple regression and correlation extensions of the Mantel test of matrice correspondence. *Syst. Zool*. **35**: 627-632.

Sokal, R.R. and Rohlf, F.J. 1995. *Biometry: the Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman and Co., New York.

Somerfield, P.J. and Clarke, K.R. 1997. A comparison of some methods commonly used for the collection of sublittoral sediments and their associated fauna. *Mar. Environ. Res*. **43**: 145-156.

Sparks, J.S. and Smith, W.L. 2004. Phylogeny and biogeography of the Malagasy and Australasian rainbowfishes (Teleostei : Melanotaenioidei): Gondwanan vicariance and evolution in freshwater. *Mol. Phylogenet. Evol.* **33**: 719–734.

Stevens, C.E., and Hume, I.D. 1995. *Comparative Physiology of the Vertebrate Digestive System. 2nd Edition.* Press Syndicate of the University of Cambridge, Melbourne.

Sturmbauer C., Mark, W. and Dallinger, R. 1992. Ecophysiology of aufwuchs-eating cichlids in Lake Tanganyika: niche separation by trophic specialization. *Environ. Biol. Fishes* **35**:283–290.

Stephens, P.R. and Wiens, J.J. 2003. Ecological diversification and phylogeny of emydid turtles. *Biol. J. Linn. Soc*. **79**: 577 – 610.

Stoner, A.W. and Livingston, R.D. 1984. Ontogenetic patterns in diet and feeding morphology in sympatric sparid fishes from seagrass meadows. *Copeia* **1984**: 174 – 187.

Streelman, J.T., Alfaro, M., Westneat, M.W., Bellwood, D.R. and Karl, S.A. 2002. Evolutionary history of the parrotfishes: biogeography, ecomorphology, and comparative diversity. *Evolution* 56*:* 961–971.

Streelman, J.T. and Danley, P.D. 2003. The stages of vertebrate evolutionary radiation. *Trends Ecol. Evol.* **18**: 126-131.

Sues, H.-D., ed. 2000. Evolution of Herbivory in Terrestrial Vertebrates: Perspectives from the Fossil Record. Cambridge University Press, Cambridge.

Sun, B.L. 1991. A new species of Teraponidae from China. *Acta Zool. Sin.* **37**: 254–257.

Swofford, D.L. 2003. *PAUP*. Phylogenetic analysis using parsimony (* and other methods), version 4.0b10*. Sinauer, Sunderland.

Suzuki, K.W., Kasai, A., Nakayama, K. and Tanaka, M. 2005. Differential isotopic enrichment and half life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. *Can. J. Fish Aquat. Sci.* **62**: 671-678.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**: 1596–1599.

ter Braak, C.J.F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* **67**: 1167-1179.

Thorp, J.H. and Delong, A.D. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. *Oikos* **96**: 543-550.

Tibbetts, I.R. 1997. The distribution and function of mucous cells and their secretions in the alimentary tract of *Arrhamphus sclerolepis krefftii. J. Fish Biol.* **50**: 809-820.

Tibbetts, I.R. and Carseldine, L. 2005. Trophic shifts in three subtropical Australian halfbeaks (Teleostei: Hemiramphidae). *Mar. Freshwater Res.***56**: 925 – 932.

Townsend, C.R. and Hildrew, A.G. 1994. Species traits in relation to a habitat templet for river systems. *Freshwater Biol.* **31**: 265-275.

Turner, S. 1982. A catalogue of fossil fish in Queensland. *Mem. Qld Mus*. **20**: 599–611.

Tyler, A.V. 1972. Food resource division among northern, marine, demersal fishes. *J. Fish. Res. B. Can.* **29**: 997-1003.

Unmack, P.J. 2001. Biogeography of Australian freshwater fishes. *J. Biogeogr.* **28**: 1053–1089.

Unmack, P. J. and Dowling, T. E. 2010. Biogeography of the genus *Craterocephalus* (Teleostei: Atherinidae) in Australia. *Mol. Phylogenet. Evol*. **55**: 968–984.

van Dam, A.A., Beveridge, M.C.M., Azim, M.E. and Verdegem, M.C.J. 2002. The potential of fish production based on periphyton. *Rev. Fish. Biol. Fish*. **12**:1–31.

Vanderklift, M.A. and Ponsard, S. 2003. Sources of isotopic variation in consumer-diet δ^{15}N: a meta-analysis. *Oecologia* **74**: 231-235.

Vander Zanden, M.J., Cabana, G. and Rasmussen, J.B. 1997. Comparing the trophic position of freshwater fish calculated using stable nitrogen isotopes (δ^{15}N) and literature dietary data. *Can. J. Fish Aquat. Sci.* **54**: 1142-1158.

Vander Zanden, M.J. and Rasmussen, J.B. 2001. Variation in δ^{15}N and δ^{13}C trophic fractionation: implications for aquatic food web studies. *Limnol. Oceanog.* **46**: 2061-2066.

Vari, R.P. 1978. The terapon perches (Percoidei: Teraponidae): a cladistic analysis and taxonomic revision. *B. Am. Mus. Nat. Hist.* **159**: 175 – 340.

Vayssièrres, M.P., Plant, R.E., and Allen-Diaz, B.H. 2000. Classification trees: An alternative non-parametric approach for predicting species distributions. *J. Veg. Sci.***11**: 679-694.

Veevers, J.J. 1991. Phanerozoic Australia in the changing configuration of Proto-Pangea through Gondwanaland and Pangea to the present dispersed continents. In: *Austral Biogeography* (P.Y. Ladiges, C.J. Humphries and L.W.Martinelli eds), pp. 1–12. CSIRO, Melbourne.

Vermeij, G.J. and Lindberg, D.R. 2000. Delayed herbivory and the assembly of marine benthic ecosystems. *Paleobiology* **26**: 419 - 430.

Vitt, L.J., Pianka, E.R., Cooper, W.E. and Schwenk, K. 2003. History and global ecology of squamate reptiles. *Am. Nat.* 162:44–60.

Wagner, C.E., McIntyre, P.B., Buels, K.S., Gilbert, D.M. and Michel, E. 2009. Diet predicts intestine length in Lake Tanganyika's cichlid fishes. *Funct. Ecol.* **23**: 1122-1131.

Wainwright, P.C. and Lauder, G.V. 1992. The evolution of feeding biology in sunfishes (Centrarchidae). In: *Systematics, Historical Ecology, and North American Freshwater Fishes* (R.W. Mayden, ed), pp. 472 – 491. Stanford University Press, Stanford.

Wake, D. B. 2009. What salamanders have taught us about evolution. *Annu. Rev. Ecol. Evol. Syst*. **40**: 333–352.

Waters, J.M. and Wallis, G.P. 2001. Cladogenesis and loss of the marine life-history phase in freshwater galaxiid fishes (Osmeriformes: Galaxiidae). *Evolution* **55**: 587–597.

Westneat, M.W. 1995. Feeding, function and phylogeny: analysis of historical biomechanics in labrid fishes using comparative methods. *Syst. Biol.* **44**: 361 – 383.

Westneat, M.W. 2004. Evolution of levers and linkages in the feeding mechanisms of fishes. *Integr. Comp. Biol.* **44**: 378–389.

Weathers, W.W. and Siegel, R.B. 1995. Body size establishes the scaling of avian postnatal metabolic rate: an interspecific analysis using phylogenetically independent contrasts. *Ibis* **137**: 532-542.

Welcomme, R.L. 1979. *Fisheries Ecology of Floodplain Rivers*. Longman, London.

Werner, E.E. 1986. The mechanisms of species interactions and community organisation in fish. In: *Community Ecology* (J. Diamond and T.J. Case, eds), pp.344-358. Harper and Row, New York.

Werner, E.E. and Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size structure populations. *Annu. Rev. Ecol. Syst.* **15**: 393 – 415.

Westneat, M.W. 1995. Feeding, function and phylogeny: analysis of historical biomechanics in Labrid fishes using comparative methods. *Syst. Biol.* **44**: 361 – 383.

Westneat, M.W. and Alfaro, M.E. 2005. Phylogenetic relationships and evolutionary history of the reef fish family Labridae. *Mol. Phylogenet. Evol*. **36**: 370–390.

Westneat, M.W. and Alfaro, M.E. 2005. Phylogenetic relationships and evolutionary history of the reef fish family Labridae. *Mol. Phylogenet. Evol.* **36**: 370-390.

White, T.C.R. 1985. When is a herbivore not a herbivore? *Oecologia* **67**: 596 – 597.

Whitehead, A. 2010. The evolutionary radiation of diverse osmotolerant physiologies in killifish (Fundulus Sp.). *Evolution* **64**: 2070–2085.

Whitfield, A.K. and Blaber, J.M. 1978. Scale-eating habits of the marine teleost *Terapon jarbua* (Forsskål). *J. Fish Biol.* **12**: 61-70.

Whitley, G.P. 1943. Ichthyological notes and illustrations (Part 2). *Aust. Zool*. **10**: 167–187.

Wikramanayake, E.D. 1990. Ecomorphology and biogeography of a tropical stream fish assemblage: evolution of assemblage structure. *Ecology* **71**: 1756-1764.

Williams, W.D. and Allen, G. R. 1987. Origins and adaptations of the fauna of inland waters. In: *Fauna of Australia, Volume 1A, General Articles* (G.R. Hyne and D.W. Walton, eds), pp. 184–201. Government Printing Service, Canberra, Australia.

Wilson, A.B., Teugels, G.G. and Meyer, A. 2008. Marine incursion: the freshwater herring of Lake Tanganyika are the product of a marine invasion into West Africa. *PLOS ONE* **3**: e1979.

Winemiller, K.O. 1989. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan llanos. *Environ. Biol. Fishes* **26**: 177-199.

Winemiller, K.O. and Pianka, E.R. 1990. Organization in natural assemblages of desert lizards and tropical fishes. *Ecol. Monog.* **60**: 27-55.

Winemiller, K.O., Kelso-Winemiller, L.C. and Brenkert, A.L. 1995. Ecomorphological diversification and convergence in fluvial cichlid fishes. *Environ. Biol. Fishes* **44**: 235-261.

Winemiller, K.O. 2003. Floodplain river foodwebs: generalizations and implications for fisheries management. In: *Proceedings of the Second International Symposium on Management of Large Rivers for Fisheries* (R. Welcomme and T. Pter, eds), pp. 285-309. FAO, Bangkok.

Winemiller, K.O., Akin S. and Zeug S.C. 2007. Production sources and food web structure of a temperate tidal estuary: integration of dietary and stable isotope data. *Mar. Ecol. Prog. Ser*. **343:** 63–76.

Winemiller, K.O., Zeug, S.C., Robertson, C.R., Winemiller, B.K. and Honeycutt, R.L. 2011. Food web structure of coastal streams in Costa Rica revealed by dietary and stable isotope analyses. *J. Trop. Ecol.* **27:** 463-476.

Winterbottom, R. and McLennan, D.A. 1993. Cladogram versatility: evolution and biogeography of acanthuroid Fishes. *Evolution* **47**: 1557–1571.

Wishart, D. 1969. An algorithm for hierarchical classifications. *Biometrics* **25**: 165–170.

Wulff, J.L. 1997. Parrotfish predation on cryptic sponges of Caribbean coral reefs. *Mar. Biol.* **129**: 41-52.

Yagishita, N., Miya, M., Yamanoue, Y., Shirai, S.M., Nakayama, K., Suzuki, N. *et al.* 2009. Mitogenomic evaluation of the unique facial nerve pattern as a phylogenetic marker within the perciform fishes (Teleostei: Percomorpha*). Mol. Phylogenet. Evol.* **53**: 258–266.

Yamanoue, Y., Miya, M., Doi, H., Mabuchi, K., Sakai, H. and Nishida, M. 2011. Multiple invasions into freshwater by pufferfishes (Teleostei: Tetraodontidae): a mitogenomic perspective. *PLOS ONE* **6**: e17410. doi:10.1371/journal.pone.0017410.

Yamaoka, K. 1985. Intestinal coiling pattern in epilithic algal-feeding cichlids (Pisces, Teleostei) of Lake Tanganyika, and its phylogenetic significance. *Zool. J. Linn. Soc.* **84**: 235 – 261.

Yokoyama, R. and Goto, A. 2005. Evolutionary history of freshwater sculpins, genus *Cottus* (Teleostei: Cottidae) and related taxa, as inferred from mitochondrial DNA phylogeny. *Mol. Phylogenet. Evol.* **36**: 654–668.

Zeug, S. C. and Winemiller, K. O. 2008. Evidence supporting the importance of terrestrial carbon in a large-river food web. *Ecology* **89**: 1733–1743.

Zihler, F. 1982. Gross morphology and configuration of digestive tracts of cichlidae (Teleostei, Perciformes): phylogenetic and functional significance. *Netherlands J. Zool.* **32**: 544-571.

Zwickl, D. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. dissertation, The University of Texas at Austin.

Appendix 1: Burdekin and Daly River dietary overlap data

Table A1.1 Bray-Curtis dietary overlap indices for Burdekin River terapontid assemblage in the early-dry and late-dry season. Species names are coded according to genus and species initials. Seasonal codes follow species name: ED, early-dry; LD, late-dry. Numeric suffixes denote OTU size classes: 1<40mm; 2 40-80mm; 3 81–160mm; 4 > 160mm. Shaded cells highlight level of dietary overlap between the largest and smallest OTU of each individual species.

	ApED1	**ApED2**	**ApED3**	**HfED1**	**HfED2**	**HfED3**	**HfED4**	**LuED1**	**LuED2**	**LuED3**	**SpED2**	**SpED3**
ApED1												
ApED2	78.97											
ApED3	80.08	77.48										
HfED1	75.05	75.63	69.77									
HfED2	71.55	75.30	64.42	77.60								
HfED3	68.53	66.59	57.12	66.80	70.71							
HfED4	60.08	60.96	52.88	61.27	66.13	76.79						
LuED1	68.52	77.84	66.55	69.39	71.66	67.93	62.19					
LuED2	74.27	76.99	65.23	71.20	73.28	67.45	60.15	83.45				
LuED3	63.95	69.08	57.16	64.31	70.28	71.13	69.94	84.03	74.75			
SpED2	36.15	24.57	36.99	23.73	22.73	45.60	40.66	27.73	20.85	27.36		
SpED3	22.72	19.73	29.39	10.98	20.21	31.97	34.33	28.03	17.66	26.71	79.69	
SpED4	43.95	33.19	44.80	24.70	27.58	36.51	33.28	29.82	34.80	31.57	68.64	68.254

Table A1.1 (cont.)

	ApLD1	ApLD2	ApLD3	HfLD1	HfLD2	HfLD3	HfLD4	LuLD2	LuLD3	SpLD2	SpLD3
ApLD1											
ApLD2	60.41										
ApLD3	52.94	74.08									
HfLD1	65.08	56.89	45.93								
HfLD2	56.35	77.57	74.29	61.54							
HfLD3	47.29	76.64	71.54	53.62	82.44						
HfLD4	41.22	59.65	56.84	51.76	67.62	75.37					
LuLD2	56.96	80.19	71.85	52.42	72.72	71.31	62.35				
LuLD3	54.34	75.21	64.10	58.41	65.20	69.27	64.51	77.67			
SpLD2	14.82	37.39	28.78	14.49	28.71	42.64	37.73	35.92	37.00		
SpLD3	19.45	53.17	53.45	19.04	47.87	58.17	46.45	51.49	40.54	67.44	
SpLD4	0.85	26.16	29.53	0.83	22.85	41.60	41.67	21.44	26.95	59.65	59.47

Table A1.2 Bray-Curtis dietary overlap indices for Daly River terapontid assemblage in the early-dry and late-dry season. Species names are coded according to genus and species initials. Seasonal codes follow species name: ED, early-dry; LD, late-dry. Numeric suffixes denote OTU size classes: 1<40mm; 2 40-80mm; 3 81–160mm; 4 > 160mm. Shaded cells highlight level of dietary overlap between the largest and smallest OTU of each individual species.

	SbED2	**SbED3**	**SbED4**	**LuED1**	**LuED2**	**LuED3**	**HfED1**	**HfED2**	**HfED3**	**HfED4**	**ApED1**	**ApED2**
SbED2												
SbED3	72.94											
SbED4	49.84	76.92										
LuED1	51.90	30.17	17.65									
LuED2	48.96	30.19	16.36	68.93								
LuED3	37.70	28.76	17.57	48.48	70.27							
HfED1	46.07	22.87	1.32	71.43	65.38	39.03						
HfED2	59.57	39.03	16.45	64.19	84.01	62.25	64.82					
HfED3	50.27	37.58	18.23	53.49	76.34	76.13	56.75	79.16				
HfED4	25.28	21.04	17.40	25.15	44.89	65.69	13.14	40.49	56.61			
ApED1	46.70	25.01	16.32	77.02	70.90	51.61	69.25	67.14	61.96	30.31		
ApED2	54.83	35.35	22.44	72.43	80.17	58.92	67.27	76.76	70.49	37.32	74.31	
ApED3	49.88	34.22	25.04	53.45	64.42	66.19	41.00	64.03	65.60	50.96	55.56	69.27

Table A1.2 (cont.)

	SbLD2	SbLD3	SbLD4	LuLD1	LuLD2	LuLD3	HfLD1	HfLD2	HfLD3	HfLD4	ApLD1	ApLD2
SbLD2												
SbLD3	73.52											
SbLD4	53.53	57.26										
LuLD1	43.22	22.71	6.47									
LuLD2	49.25	41.46	18.40	52.19								
LuLD3	23.78	33.73	21.04	15.18	59.10							
HfLD1	53.18	38.47	17.69	75.37	67.21	28.57						
HfLD2	56.52	41.51	12.92	59.83	80.89	40.15	70.92					
HfLD3	58.81	43.59	13.66	37.45	68.35	47.17	44.17	66.95				
HfLD4	18.73	21.91	15.87	5.60	28.83	49.04	17.58	17.71	34.21			
ApLD1	30.39	24.42	4.45	51.55	53.50	29.21	63.37	55.73	36.37	15.52		
ApLD2	47.17	38.23	14.95	52.72	72.86	39.51	69.74	71.33	55.70	23.38	65.52	
ApLD3	50.85	56.36	30.32	37.40	52.30	34.56	50.47	52.72	46.33	13.64	45.22	56.93

Appendix 2: Terapontid dietary and morphological data

Table A2.1 Summary data of raw terapontid OTU morphologies used in study analyses. OTU codes are outlined in Table 4.1 (main text). All linear variables (standard length, intestinal length, maxilla length, mouth width, head length, snout length, eye diameter, body depth) are presented as average lengths (mm). Relative eye position is presented as a ratio variable (eye height/head depth) and tooth shape and mouth position as coded integer values.

OTU	Standard length	Intestinal length	Head length	Snout length	Maxilla length	Mouth width	Eye diameter	Eye position	Body depth	Tooth Shape	Mouth orientation
Ap1	24.8	15.9	8.3	3.9	2.1	1.7	2.9	0.67	8.0	1	1
Ap2	46.2	34.9	14.6	7.1	3.5	3.9	5.2	0.68	18.0	1	1
Ap3	76.0	65.7	22.0	10.5	5.6	6.7	6.9	0.69	29.2	1	1
Ap4	113.5	112.8	33.3	16.5	8.6	12.1	9.4	0.67	48.1	1	1
Ac	75.3	67.7	21.8	10.9	7.5	8.3	6.9	0.71	27.0	1	1
Hg1	23.5	16.8	8.0	3.7	2.2	2.2	2.9	0.65	8.5	1	1
Hg2	78.7	74.0	25.1	13.3	8.9	8.3	7.5	0.71	27.0	1	1
Hf1	32.9	28.6	11.6	5.6	3.2	2.7	3.5	0.72	11.7	1	1
Hf2	71.6	78.3	27.1	13.3	7.2	8.3	6.6	0.71	26.3	1	1
Hf3	166.7	303.8	54.0	26.0	21.3	19.7	11.1	0.70	73.2	1	1
Hf4	288.8	556.3	87.6	42.3	31.2	40.2	15.1	0.78	106.8	1	1
Hc1	47.3	37.5	15.7	7.2	5.0	4.8	4.8	0.68	19.2	1	1
Hc2	81.3	71.3	26.3	12.1	9.0	9.1	7.2	0.67	34.5	1	1
Hc3	138.1	126.7	42.0	19.6	14.4	18.0	10.1	0.63	55.7	1	1
Hj1	33.1	26.5	10.8	5.9	3.7	3.1	3.4	0.70	12.2	1	1
Hj2	91.2	153.3	31.2	15.8	10.7	10.2	7.4	0.70	36.2	1	1
Hj3	194.7	415.7	62.9	30.7	20.9	23.2	12.8	0.70	74.5	1	1
He	226.7	303.0	74.5	36.2	32.1	25.3	14.4	0.74	78.3	1	1
Lu1	23.1	14.5	9.2	4.0	2.3	2.7	2.6	0.66	7.8	1	1
Lu2	42.7	34.0	14.1	6.2	4.9	3.7	4.5	0.77	15.9	1	1
Lu3	87.8	66.8	28.1	12.2	9.1	9.7	7.1	0.77	26.9	1	1
Lu4	139.2	122.6	48.4	20.6	17.0	20.0	9.5	0.74	47.6	1	1

Table A2.2 (cont.) Summary data of raw terapontid OTU morphologies used in study analyses. OTU codes are outlined in Table 4.1 (main text). All linear variables (standard length, intestinal length, maxilla length, mouth width, head length, snout length, eye diameter, body depth) are presented as average lengths (mm). Relative eye position is presented as a ratio variable (eye height/head depth) and tooth shape and mouth position as coded integer values.

OTU	Standard length	Intestinal length	Head length	Snout length	Maxilla length	Mouth width	Eye diameter	Eye position	Body depth	Tooth Shape	Mouth orientation
Ht	151.9	375.3	46.9	23.7	14.0	16.2	12.0	0.61	63.0	1	1
La1	35.2	33.2	11.1	4.9	2.5	3.2	3.8	0.63	8.2	1	1
La2	51.4	96.3	16.3	7.4	4.9	5.1	4.8	0.69	18.4	1	1
Ma	132.8	169.1	43.1	22.8	15.8	14.3	11.9	0.65	58.1	1	1
Pg1	33.1	24.0	11.2	5.6	2.6	2.4	3.3	0.65	11.3	3	3
Pg2	65.1	117.0	19.4	10.3	4.7	5.3	5.5	0.70	22.0	3	3
Pm	57.1	155.3	15.8	8.1	3.8	4.7	4.5	0.64	20.4	3	3
Pl	65.6	118.3	18.8	9.0	5.1	6.0	5.8	0.66	27.9	3	1
Sp1	31.9	37.3	10.1	4.6	3.0	3.0	3.1	0.63	10.2	2	2
Sp2	73.9	188.7	22.2	10.5	6.1	7.1	5.9	0.60	26.7	2	2
Sp3	212.8	773.9	58.0	25.2	11.2	20.0	9.8	0.62	60.0	2	2
Sp4	263.1	1431.7	70.4	32.0	17.6	25.8	12.3	0.62	103.6	2	2
So1	66.5	157.0	20.8	9.2	6.4	7.1	5.3	0.63	26.1	2	2
So2	176.4	521.4	47.1	20.5	12.3	18.0	10.0	0.60	65.6	2	2
So3	246.6	1297.6	63.5	28.0	18.6	24.5	12.5	0.63	159.4	2	2
St1	39.0	57.4	11.2	5.3	3.4	3.5	3.6	0.68	12.5	3	2
St2	76.5	232.2	21.3	11.3	6.7	7.3	4.9	0.71	23.2	3	2
Sb1	63.4	126.1	19.6	10.3	5.6	5.9	4.9	0.65	23.3	3	2
Sb2	120.4	320.4	34.3	17.9	10.3	10.9	7.7	0.61	45.3	3	2
Sb3	187.3	786.5	50.1	26.1	17.9	16.7	10.3	0.60	84.8	3	2
Sr	119.3	415.4	32.8	17.9	9.7	11.6	7.1	0.65	42.4	3	2
Tj1	49.5	44.9	16.1	7.2	6.0	6.5	4.5	0.70	16.4	1	1
Tj2	77.3	82.5	22.0	10.8	9.8	10.5	6.2	0.71	26.8	1	1
Vl	149.7	141.1	40.8	18.5	12.2	15.3	10.3	0.65	62.5	1	1

Table A2.2 Volumetric dietary data for terapontid species' ontogenetic trophic units. Only dietary categories that totaled more than 5% within any individual species' ontogenetic trophic units are outlined.

	Terapon jarbua		***Amniataba percoides***				***Leiopotherapon unicolor***				***L. aheneus***		***Hephaestus fuliginosus***				***H. carbo***		
Ontogenetic Trophic Unit	**1**	**2**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**1**	**2**	**3**	**4**	**1**	**2**	**3**
Size range (mm)	**>60**	**60-102**	**>30**	**30-60**	**60-100**	**100-126**	**>30**	**30-60**	**60-120**	**120-181**	**>40**	**40-73**	**>40**	**40-140**	**140-240**	**240-320**	**30-60**	**60-110**	**110-163**
Broad dietary category																			
Diptera larvae	52.2	18.2	32.3	33.9	20.4	12.4	66.0	23.9	10.6	0.6	34.1	5.9	33.8	25.3	0.8	-	33.6	17.1	5.9
Ephemeroptera	1.7	14.1	11.6	17.1	9.5	6.8	16.0	19.8	7.7	0.2	18.3	5.5	18.5	8.7	1.1	0.1	16.0	9.3	5.9
Trichoptera			6.1	13.0	12.3	4.6	-	15.6	8.1	1.3	6.5	6.2	28.6	15.1	1.5	0.3	8.3	32.1	22.3
Odonata larvae			2.7	2.5	3.7	5.4	-	5.0	7.7	1.5	-	6.8					6.7	9.5	11.2
Other aquatic inverts			0.4	6.5	9.3	5.4	2.0	9.1	9.5	4.2			-	7.8	5.9	1.1	21.9	6.5	4.0
Surface Invertebrates																			
Macrocrustacea	13.3	12.3					-	3.2	19.2	23.0			-	7.5	13.7	9.9	1.7	13.1	15.7
Zooplankton			35.9	7.1	1.6	0.1	16.0	6.4	1.4	-	25.6	1.3	5.3	3.7	-	-	6.6	-	-
Ostracoda																			
Mollusca, Gastropoda																			
Terrestrial invertebrates	2.7	11.0					-	1.0	6.2	14.6			0.1	1.5	6.0	3.1	0.8	3.8	15.8
Fish	12.3	13.1					-	1.1	8.5	37.4			-	2.0	10.8	11.9	-	1.6	12.5
Fish scales	13.3	30.0																	
Eggs																			
Inorganic																			
Detritus											7.3	11.1	1.0	4.1	3.5	1.9			
Filamentous algae			0.3	8.8	24.2	15.1	-	6.1	9.5	4.8	2.4	56.9	0.5	15.2	15.9	3.7			
Aquatic macrophytes			-	0.2	1.7	35.2							-	0.5	7.0	22.0			
Terrestrial vegetation													0.1	1.0	26.8	38.4			
Misc. plant parts																			
Terrestrial vertebrates														0.2	4.5	6.5			

Table A2.1 (cont.)

	H. jenkinsi			*Scortum ogilbyi*			*S. parviceps*				*Syncomistes butleri*			*S. trigonicus*		*Hannia greenwayi*		*Pingalla gilberti*	
Ontogenetic Trophic Unit	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**1**	**2**	**1**	**2**	**1**	**2**
Size range (mm)	**>40**	**40-160**	**160-280**	**>80**	**80-220**	**220-324**	**>40**	**40-120**	**120-240**	**240-353**	**>80**	**80-160**	**160-241**	**>50**	**50-105**	**>40**	**40-107**	**>40**	**40-97**
Broad dietary category																			
Diptera larvae	44.3	9.6	2.5	9.4	1.4	0.1	58.8	39.4	2.6	1.7	38.9	12.6	0.2	18.0	1.0	35.4	13.6	72.0	15.2
Ephemeroptera	3.8	16.7	2.5	8.9	0.6	-								9.0	0.2	45.5	32.6	10.0	0.7
Trichoptera	6.3	15.5	2.6				28.0	0.1	1.4	0.2	6.2	0.6	-	9.1	1.0			10.0	0.5
Odonata larvae																-	6.1		
Other aquatic inverts	38.8	9.5	3.6	10.0	0.5	0.6										8.6	5.0		
Surface Invertebrates																			
Macrocrustacea	-	3.8	7.8					5.2		2.6									
Zooplankton	5.8	2.9	0.1	9.6	-	-	6.0	0.2	1.2	1.0				14.3	0.4	10.4	-	8.0	0.2
Ostracoda																			
Mollusca, Gastropoda	-	0.1	6.2																
Terrestrial inverts																			
Fish	-	4.9	6.8													-	9.3		
Fish scales																			
Eggs																			
Inorganic																		-	12.0
Detritus	-	2.1	8.0	7.1	18.0	7.1	0.5	4.0	9.2	12.8	32.2	47.0	41.0	32.3	67.0			-	34.0
Filamentous algae	-	15.4	12.2	38.4	56.4	52.0	-	42.0	53.7	50.0	12.2	31.4	46.6	13.1	26.0	-	19.7	-	36.5
Aquatic macrophytes	-	3.6	8.2	-	12.0	33.1		6.8	23.3	18.3									
Terrestrial vegetation	-	1.8	19.1					0.5	1.8	5.5									
Misc. plant parts	-	0.6	7.1								5.0	5.4	11.6						
Terrestrial vertebrates	-	-	5.3																

Table A2.1 (cont.)

Species **Size Range (mean)**	***H. tulliensis*** **81-217mm (156.4)**	***H. epirrhinos*** **193-275mm (223.7mm)**	***P.midgleyi*** **43-71mm (57.3)**	***S. rastellus*** **68-165mm (115.2)**	***Varrichthys lacustris*** **112-181mm (150mm)**	***P. lorentzi*** **48-116mm (65.6)**	***Mesopristes argenteus*** **85-226mm (147.4mm)**	***A. caudavittatus*** **67-105mm (80mm)**
Broad diet category								
Diptera larvae	1.9		5.3	1.7	5.2	9.8	0.3	38.2
Ephemeroptera larvae	0.9		0.7	1.7		4.8	7.4	
Trichoptera larvae	5.0	4.3	1.3	1.3		2.9	8.3	
Odonata larvae	1.3	5.0			1.3		1.8	
Other aquatic inverts	2.9				19.7		3.1	
Surface Invertebrates								
Terrestrial-aerial inverts	1.1				12.4			1.7
Macrocrustacea	3.4	46.7				9.5	42.8	
Zooplankton	0.1		3.7		0.1	3.8		
Ostracoda	0.6							5.7
Mollusca/Gastropoda					5.2		10.4	13.5
Fish	3.6	24.3					6.2	9.8
Fish scales								
Eggs	0.3			0.1			2.4	
Inorganic	0.7		29.3	6.3		2.9	2.7	0.7
Detritus	6.8		47.0	38.3		22.0	1.1	
Filamentous algae	48.4	19.7	6.3	47.7	18.8	30.8		25.5
Aquatic macrophytes	0.8				33.2	1.3		2.5
Terrestrial vegetation	20.1				0.8	0.8		
Misc. plant parts			3.0			8.8		
Terrestrial vertebrates								

Table A2.3 Results for scaling analyses of reduced major axis regressions of Log_{10} – transformed standard length versus eight Log_{10} –transformed morphological variables for terapontids. Morphological variables coded as follows: IL-intestinal length; ML; maxilla length; MW-mouth width; HL-head length; SNL-snout length; ED-eye diameter; EP-eye position; and BD-body depth. Regression equation slope (b), isometric slope, constant (y-axis intercept), r^2 (square of the correlation coefficient), 95% confidence interval for slope and number of sample points (n) are outlined for each species and variable. Statistically significant allometric relationships (b≠1) are highlighted in bold.

Amniataba caudovittatus

Variable	b	Isometry	Constant	r^2	Confidence limits	n
Intestinal length	1.363	1.0	-0.766	0.926	0.849-1.878	6
Maxilla length	0.876	1.0	-0.793	0.984	0.723-1.029	6
Maxilla width	0.964	1.0	-0.890	0.890	0.33-1.597	6
Head length	0.704	1.0	0.017	0.770	0.003-1.405	6
Snout length	1.187	1.0	-1.189	0.785	0.175-2.20	6
Eye Diameter	**0.301**	1.0	0.270	0.730	-0.03-0.634	6
Eye position	-0.640	0.0	1.920	0.590	-1.12-0.023	6
Body depth	0.857	1.0	-0.200	0.886	0.456-1.262	6

Amniataba percoides

Variable	b	Isometry	Const	r^2	Confidence limits	n
IL	**1.385**	1.0	-0.761	0.929	1.339-1.431	432
ML	**1.094**	1.0	-1.284	0.900	1.067-1.121	623
MW	**1.239**	1.0	-1.480	0.967	1.169-1.308	72
HDL	**0.903**	1.0	-0.343	0.992	0.877-0.928	72
SNL	**0.933**	1.0	-7.139	0.981	0.894-0.973	72
ED	**0.724**	1.0	-0.513	0.973	0.687-0.761	72
EP	0.008	0.0	0.662	0.002	-0.057-0.073	72
BD	**1.057**	1.0	-0.496	0.966	1.042-1.073	72

Hannia greenwayi

Variable	b	Isometry	Const	r^2	Confidence limits	n
IL	**1.194**	1.0	-0.423	0.954	1.041-1.347	15
ML	**1.049**	1.0	-1.010	0.992	1.012-1.086	28
MW	**1.175**	1.0	-1.316	0.987	1.113-1.237	28
HDL	0.996	1.0	-0.489	0.988	0.994-1.048	28
SNL	1.071	1.0	-0.907	0.974	0.990-1.151	28
ED	**0.803**	1.0	-0.647	0.957	0.723-0.882	28
EP	0.231	0.0	0.437	0.786	-0.175-0.287	28
BD	**1.068**	1.0	-0.542	0.991	1.023-1.114	28

Table A2.3 (cont.) Results for scaling analyses of reduced major axis regressions of Log_{10} – transformed standard length versus eight Log_{10} –transformed morphological variables for terapontids. Morphological are variables coded as follows: IL-intestinal length; ML; maxilla length; MW-mouth width; HL-head length; SNL-snout length; ED-eye diameter; EP-eye position; and BD-body depth. Regression equation slope (b), isometric slope, constant (y-axis intercept), r^2 (square of the correlation coefficient), 95% confidence interval for slope and number of sample points (n) are outlined for each species and variable. Statistically significant allometric relationships (b≠1) are highlighted in bold.

Hephaestus carbo						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.274**	1.0	-0.581	0.954	1.216-1.333	87
ML	**1.059**	1.0	-1.075	0.981	1.031-1.087	105
MW	**1.270**	1.0	-1.463	0.982	1.216-1.323	60
HDL	**0.916**	1.0	-0.333	0.990	0.888-0.944	60
SNL	**0.937**	1.0	-0.709	0.981	0.896-0.977	60
ED	**0.665**	1.0	-0.415	0.952	0.619-0.711	60
EP	-0.091	0.0	0.841	0.073	-0.19-0.01	60
BD	**1.068**	1.0	-0.507	0.986	1.043-1.093	103
Hephaestus epirrhinos						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.516**	1.0	-1.085	1.000	1.26-1.772	3
ML	1.154	1.0	-1.206	0.986	-0.591-2.900	3
MW	1.396	1.0	-1.886	0.998	0.501-2.291	3
HDL	1.180	1.0	-0.909	0.985	-0.725-2.9	3
SNL	0.999	1.0	-1.361	0.950	-1.829-3.837	3
ED	0.493	1.0	-0.001	0.860	-0.33-1.307	3
EP	-0.007	0.0	0.755	0.003	-1.61-1.66	3
BD	1.204	1.0	-0.936	0.995	0.15-2.255	3
Hephaestus fuliginosus						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.440**	1.0	-0.769	0.965	1.408-1.471	292
ML	**1.120**	1.0	-1.222	0.985	1.107-1.132	461
MW	**1.249**	1.0	-1.472	0.992	1.213-1.284	75
HDL	**0.935**	1.0	-0.347	0.997	0.918-0.952	75
SNL	**0.927**	1.0	-0.643	0.927	0.904-0.950	75
ED	**0.676**	1.0	-0.460	0.983	0.648-0.703	75
EP	-0.001	0.0	0.721	0.000	-0.05-0.05	75
BD	**1.069**	1.0	-0.565	0.993	1.056-1.077	455

Table A2.3 (cont.) Results for scaling analyses of reduced major axis regressions of Log_{10} – transformed standard length versus eight Log_{10} –transformed morphological variables for terapontids. Morphological are variables coded as follows: IL-intestinal length; ML; maxilla length; MW-mouth width; HL-head length; SNL-snout length; ED-eye diameter; EP-eye position; and BD-body depth. Regression equation slope (b), isometric slope, constant (y-axis intercept), r^2 (square of the correlation coefficient), 95% confidence interval for slope and number of sample points (n) are outlined for each species and variable. Statistically significant allometric relationships (b≠1) are highlighted in bold.

Hephaestus jenkinsi						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.574**	1.0	-0.988	0.926	1.478-1.671	80
ML	**1.054**	1.0	-1.075	0.975	1.022-1.086	119
MW	**1.150**	1.0	-1.261	0.985	1.107-1.192	72
HDL	**0.945**	1.0	-0.363	0.992	0.919-0.970	72
SNL	**0.919**	1.0	-0.610	0.985	0.89-0.952	72
EyeD	**0.701**	1.0	-0.500	0.969	0.644-0.738	72
EyePos	-0.022	0.0	0.750	0.010	-0.08-0.04	72
BD	**1.046**	1.0	-0.519	0.993	1.029-1.064	102
Hephaestus tulliensis						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.766**	1.0	-1.295	0.780	1.291-2.241	16
ML	0.940	1.0	-0.906	0.903	0.772-1.108	19
MW	1.104	1.0	-1.225	0.909	0.883-1.325	19
HDL	**0.856**	1.0	-0.216	0.957	0.735-0.978	19
SNL	0.921	1.0	-0.725	0.946	0.779-1.062	19
ED	**0.580**	1.0	-0.198	0.806	0.391-0.769	19
EP	-0.285	0.0	1.242	0.341	-0.549-0.02	19
BD	0.953	1.0	-0.280	0.965	0.851-1.056	19
Leiopotherapon aheneus						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.806**	1.0	-1.123	0.943	1.650-1.962	34
ML	0.996	1.0	-1.007	0.975	0.945-1.047	41
MW	**1.221**	1.0	-1.384	0.942	1.116-1.326	41
HDL	1.042	1.0	-0.569	0.977	0.985-1.099	41
SNL	**1.093**	1.0	-1.003	0.975	1.032-1.155	41
ED	**0.696**	1.0	-0.503	0.918	0.623-0.770	41
EP	0.192	0.0	0.347	0.210	-0.06-0.324	41
BD	1.033	1.0	-0.481	0.985	0.995-1.074	48

Table A2.3 (cont.) Results for scaling analyses of reduced major axis regressions of Log_{10} – transformed standard length versus eight Log_{10} –transformed morphological variables for terapontids. Morphological are variables coded as follows: IL-intestinal length; ML; maxilla length; MW-mouth width; HL-head length; SNL-snout length; ED-eye diameter; EP-eye position; and BD-body depth. Regression equation slope (b), isometric slope, constant (y-axis intercept), r^2 (square of the correlation coefficient), 95% confidence interval for slope and number of sample points (n) are outlined for each species and variable. Statistically significant allometric relationships (b≠1) are highlighted in bold.

Leiopotherapon unicolor						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.252**	1.0	-0.572	0.936	1.223-1.280	479
ML	**1.187**	1.0	-1.315	0.960	1.169-1.204	709
MW	**1.283**	1.0	-1.511	0.977	1.228-1.339	75
HDL	**0.955**	1.0	-0.405	0.996	0.938-0.972	75
SNL	**0.934**	1.0	-0.728	0.991	0.909-0.958	75
ED	**0.637**	1.0	-0.402	0.958	0.599-0.676	75
EP	0.078	0.0	0.590	0.158	-0.026-0.129	75
BD	**0.970**	1.0	-0.423	0.958	0.955-0.986	511
Mesopristes argenteus						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.257**	1.0	-1.183	0.952	1.177-1.346	13
ML	0.991	1.0	-0.937	0.969	0.890-1.092	17
MW	1.164	1.0	-1.320	0.961	0.992-1.337	17
HDL	1.021	1.0	-0.533	0.993	0.958-1.08	17
SNL	0.988	1.0	-0.740	0.993	0.926-1.050	17
ED	**0.743**	1.0	-0.501	0.951	0.616-0.871	17
EP	-0.040	0.0	0.740	0.047	-0.176-0.096	17
BD	1.014	1.0	-0.419	0.994	0.968-1.060	17
Pingalla gilberti						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.470**	1.0	-0.625	0.676	1.146-1.794	30
ML	**0.819**	1.0	-0.825	0.932	0.744-0.893	36
MW	1.120	1.0	-1.309	0.920	0.999-1.242	36
HDL	**0.910**	1.0	-0.363	0.984	0.886-0.954	36
SNL	1.050	1.0	-0.892	0.973	0.985-1.114	36
ED	**0.744**	1.0	-0.608	0.958	0.686-0.802	36
EP	0.050	0.0	0.607	0.029	-0.058-0.159	36
BD	1.008	1.0	-0.496	0.970	0.946-1.071	36

Table A2.3 (cont.) Results for scaling analyses of reduced major axis regressions of Log_{10} – transformed standard length versus eight Log_{10} –transformed morphological variables for terapontids. Morphological are variables coded as follows: IL-intestinal length; ML; maxilla length; MW-mouth width; HL-head length; SNL-snout length; ED-eye diameter; EP-eye position; and BD-body depth. Regression equation slope (b), isometric slope, constant (y-axis intercept), r^2 (square of the correlation coefficient), 95% confidence interval for slope and number of sample points (n) are outlined for each species and variable. Statistically significant allometric relationships (b≠1) are highlighted in bold.

Pingalla lorentzi						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.316**	1.0	-0.324	0.913	1.042-1.591	12
ML	0.921	1.0	-0.966	0.983	0.837-1.005	12
MW	**1.236**	1.0	-1.442	0.973	1.035-1.437	12
HDL	**0.919**	1.0	-0.367	0.994	0.85-0.987	12
SNL	1.019	1.0	-0.867	0.989	0.912-1.126	12
ED	**0.530**	1.0	-0.182	0.910	0.363-0.696	12
EP	0.106	0.0	0.464	0.402	-0.02-0.234	12
BD	**1.148**	1.0	-0.642	0.986	1.052-1.244	12
Pingalla midgleyi						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	2.256	1.0	-1.797	0.924	-5.634-10.147	3
ML	0.891	1.0	-0.990	0.995	0.068-1.714	3
MW	1.457	1.0	-1.865	0.995	0.153-2.761	3
HDL	**0.804**	1.0	-0.212	0.999	0.64-0.968	3
SNL	0.631	1.0	-0.271	0.974	-0.654-1.916	3
EyeD	**0.680**	1.0	-0.542	0.999	0.397-0.962	3
EyePos	-0.831	0.0	2.107	0.962	-2.92-1.263	3
BD	0.873	1.0	-0.225	1.000	0.723-1.021	3
Scortum ogilbyi						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.626**	1.0	-0.817	0.884	1.501-1.752	80
ML	**0.775**	1.0	-0.613	0.975	0.753-0.798	120
MW	**0.942**	1.0	-0.862	0.982	0.901-0.987	70
HDL	**0.848**	1.0	-0.229	0.988	0.835-0.861	70
SNL	**0.843**	1.0	-0.577	0.994	0.822-0.865	70
ED	**0.656**	1.0	-0.471	0.760	0.623-0.689	70
EP	-0.024	0.0	0.665	0.014	-0.089-0.041	70
BD	**1.038**	1.0	-0.478	0.987	1.016-1.059	70

Table A2.3 (cont.) Results for scaling analyses of reduced major axis regressions of Log_{10} – transformed standard length versus eight Log_{10} –transformed morphological variables for terapontids. Morphological are variables coded as follows: IL-intestinal length; ML; maxilla length; MW-mouth width; HL-head length; SNL-snout length; ED-eye diameter; EP-eye position; and BD-body depth. Regression equation slope (b), isometric slope, constant (y-axis intercept), r^2 (square of the correlation coefficient), 95% confidence interval for slope and number of sample points (n) are outlined for each species and variable. Statistically significant allometric relationships (b≠1) are highlighted in bold.

Scortum parviceps						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.681**	1.0	-0.931	0.976	1.599-1.763	74
ML	**0.876**	1.0	-0.872	0.984	0.850-0.903	73
MW	1.022	1.0	-1.065	0.993	0.993-1.052	70
HDL	**0.915**	1.0	-0.366	0.998	0.902-0.928	70
SNL	**0.897**	1.0	-0.665	0.992	0.869-0.925	70
ED	**0.593**	1.0	-0.353	0.949	0.545-0.641	70
EP	0.008	0.0	0.597	0.003	-0.04-0.056	70
BD	**1.104**	1.0	-0.651	0.992	1.081-1.128	73
Syncomistes butleri						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.560**	1.0	-0.714	0.956	1.494-1.627	97
ML	0.995	1.0	-1.038	0.983	0.969-1.02	109
MW	0.998	1.0	-1.039	0.966	0.943-1.054	70
HDL	**0.873**	1.0	-0.281	0.993	0.851-0.895	70
SNL	**0.869**	1.0	-0.556	0.988	0.841-0.899	70
ED	**0.674**	1.0	-0.519	0.957	0.63-0.717	70
EP	-0.079	0.0	0.781	0.082	-0.16-0.01	70
BD	**1.096**	1.0	-0.604	0.987	1.072-1.121	109
Syncomistes rastellus						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.938**	1.0	-1.373	0.777	1.249-2.628	13
ML	1.007	1.0	-1.095	0.942	0.846-1.167	13
MW	**1.126**	1.0	-1.277	0.987	1.012-1.241	13
HDL	**0.914**	1.0	-0.380	0.996	0.863-0.965	13
SNL	**0.884**	1.0	-0.581	0.997	0.840-0.928	13
ED	**0.648**	1.0	-0.491	0.971	0.547-0.748	13
EP	0.086	0.0	0.475	0.200	-0.07-0.24	13
BD	**1.100**	1.0	-0.639	0.990	1.027-1.172	13

Table A2.3 (cont.) Results for scaling analyses of reduced major axis regressions of Log_{10} – transformed standard length versus eight Log_{10} –transformed morphological variables for terapontids. Morphological are variables coded as follows: IL-intestinal length; ML; maxilla length; MW-mouth width; HL-head length; SNL-snout length; ED-eye diameter; EP-eye position; and BD-body depth. Regression equation slope (b), isometric slope, constant (y-axis intercept), r^2 (square of the correlation coefficient), 95% confidence interval for slope and number of sample points (n) are outlined for each species and variable. Statistically significant allometric relationships (b≠1) are highlighted in bold.

Syncomistes trigonicus						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**2.356**	1.0	-2.042	0.948	2.155-2.557	32
ML	**1.160**	1.0	-1.338	0.979	1.099-1.221	35
MW	**1.086**	1.0	-1.187	0.986	1.002-1.171	30
HDL	0.962	1.0	-0.483	0.996	0.921-1.002	30
SNL	**1.120**	1.0	-1.058	0.991	1.051-1.189	30
ED	**0.464**	1.0	-0.181	0.949	0.393-0.535	30
EP	0.108	0.0	0.508	0.186	-0.04-0.258	30
BD	**1.078**	1.0	-0.645	0.979	1.021-1.135	34
Terapon jarbua						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.201**	1.0	-0.348	0.905	1.008-1.394	31
ML	**0.932**	1.0	-0.786	0.950	0.872-0.992	51
MW	**1.089**	1.0	-1.033	0.994	1.026-1.152	35
HDL	**0.702**	1.0	0.018	0.995	0.663-0.74	35
SNL	0.912	1.0	-0.682	0.978	0.810-1.013	35
ED	**0.717**	1.0	-0.563	0.956	0.600-0.834	35
EP	0.047	0.0	0.621	0.099	-0.06-0.154	35
BD	1.007	1.0	-0.470	0.966	0.955-1.059	53
Varrichthys lacustris						
Variable	**b**	**Isometry**	**Const**	**r^2**	**Confidence limits**	**n**
IL	**1.409**	1.0	-1.359	0.939	1.301-1.917	11
ML	1.099	1.0	-1.308	0.980	0.982-1.216	11
MW	**1.367**	1.0	-1.789	0.901	1.043-1.69	11
HDL	**0.846**	1.0	-0.228	0.980	0.755-0.936	11
SNL	**0.860**	1.0	-0.603	0.959	0.729-0.990	11
ED	**0.577**	1.0	-0.242	0.688	0.284-0.869	11
EP	-0.039	0.0	0.736	0.008	-0.372-0.299	11
BD	1.024	1.0	-0.433	0.977	0.906-1.141	11

Appendix 3: Dietary studies used to derive terapontid species' dietary habits.

References

Amniataba caudavittatus (Richardson, 1845): Wise, B.S., Potter, I. C. and Wallace, J. H. 1994. Growth, movements and diet of the terapontid *Amniataba caudavittata* in an Australian estuary. *J. Fish Biol.* **45**: 917–931.

*Amniataba percoide*s (Günther, 1864): Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Bidyanus bidyanus (Mitchell, 1838): J.Koehn, (personal communication).

Bidyanus welchi (McCulloch and Waite, 1917): A. M. Davis (unpublished data).

Hannia greenwayi Vari, 1978: Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Hephaestus carbo (Ogilby and McCulloch, 1916): Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Hephaestus epirrhinos Vari and Hutchins, 1978: Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Hephaestus fuliginosus (Macleay, 1883): Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Hephaestus habbemai (Weber, 1910): Storey, A.W. 1998. *A review of dietary data from fish of the Fly River System: A precursor to constructing a food web. A report prepared for Ok Tedi Mining Ltd.* Wetland Research and Management. Glen Forrest, Western Australia.

Hephaestus jenkinsi (Whitley, 1945): Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Hephaestus transmontanus (Mees and Kailola, 1977): Coates, D. and Ulaiwi, W.K. 1995. A simple model for predicting ecological impacts of introduced aquatic organisms: a case study of common carp, *Cyprinus carpio* L., in the Sepik-Ramu River Basin, Papua New guinea. *Fish. Manag. Ecol.* **2**: 227–242.

Hephaestus tulliensis De Vis, 1884: Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Leiopotherapon aheneus (Mees, 1963) Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Leiopotherapon unicolor (Günther, 1859): Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Mesopristes argenteus (Cuvier, 1829): Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Mesopristes cancellatus (Cuvier, 1829): Macabalang, S.D., Gani, A.A. and Kamad, I.C. 1984. Biological investigation and survey of pigek. *BFAR XII Res. J.* **17**: 1–17.

Helotes octolineatus Jenyns, 1840: Guenther, J. 2002. *Influence of habitat configuration on the diet of Pelates octolineatus (Terapontidae), Haletta semifasciata, Siphonognathus radiatus and*

Neodax balteatus (Odacidae). Fisheries Action Program NHT Project No. 974219. Flinders University, Adelaide, South Australia.

Pelates quadrilineatus (Bloch, 1790): Warburton, K. and Blaber, S.J.M. 1992. Patterns of recruitment and resource use in a shallow-water fish assemblage in Moreton Bay, Queensland. *Mar. Ecol. Prog. Series* **90**: 113–126.

Pelates sexlineatus (Quoy and Gaimard, 1825): Sanchez-Jerez, P., Gillanders, B.M. and Kingsford, M.J. (2002). Spatial variation in abundance of prey and diet of trumpeter (*Pelates sexlineatus*: Teraponidae) associated with *Zostera capricorni* seagrass meadows. *Austral Ecol.* **27**: 200–210.

Pelsartia humeralis (Ogilbyi, 1899): Robertson, A.I. and Lenanton, R.C.J. 1984. Fish Community Structure and Food Chain Dynamics in the Surf Zone of Sandy Beaches: The role of detached Macrophyte Detritus. *J. Exp.Mar.Biol.Ecol.* **84**: 265–283.

Pingalla gilberti Whitley, 1955: Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Pingalla lorentzi (Weber, 1910): Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Rhynchopelates oxyrhynchus (Temminck and Schlegel, 1842): Froese, R. and Pauly, D. 2000. FishBase 2000: concepts, design and data sources. ICLARM, Los Baños, Laguna, Philippines.

Scortum barcoo (McCulloch and Waite, 1917): Balcombe, S. R., Bunn, S. E., Davies, P. M. and McKenzie-Smith, F. J. 2005. Variability of fish diets between dry and flood periods in an arid zone floodplain river. *J. Fish Biol.* **67**: 1552-1567.

Scortum hillii (Castelnau, 1878)*:* A.M. Davis (unpublished data)

Scortum ogilbyi Whitley, 1951: Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Scortum parviceps (Macleay, 1883): Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Syncomistes butleri Vari, 1978: Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Syncomistes rastellus Vari and Hutchins, 1978: Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

*Syncomistes trigonicu*s Vari, 1978: Davis, A.M., Pearson, R.G., Pusey, B.J., Perna, C., Morgan, D.L. and Burrows, D. 2010. Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *J. Fish Biol.* **78:** 265–286.

Terapon jarbua (Forsskål, 1775): Whitfield, A.K. and Blaber, S.J.M. 1978. Scale-eating habits of the marine teleost *Terapon jarbua* (Forskal). *J. Fish Biol.***12**: 61–70.

Terapon puta Cuvier, 1829: Haywood, M.D.E, Heales, D.S., Kenyon, R.A., Loneragan, N.R. and Vance, D.J. 1998. Predation on juvenile tiger prawns in a tropical Australian estuary. *Mar. Ecol. Prog. Series* **162**: 201–214.

Terapon theraps Cuvier, 1829: Senta, T., Kimura, M. and Kanbara, T. 1993. Predation of fishes on open-ocean species of sea-skaters (*Halobates* spp.). *Jap. J. Ichthyol*. **40**: 193–198.

Variichthys lacustris (Mees and Kailola, 1977): Storey, A.W. 1998. *A review of dietary data from fish of the Fly River System: A precursor to constructing a food web. A report prepared for Ok Tedi Mining Ltd.* Wetland Research and Management. Glen Forrest, Western Australia.

Appendix 4: Ontogenetic development of terapontid intestinal convolution.

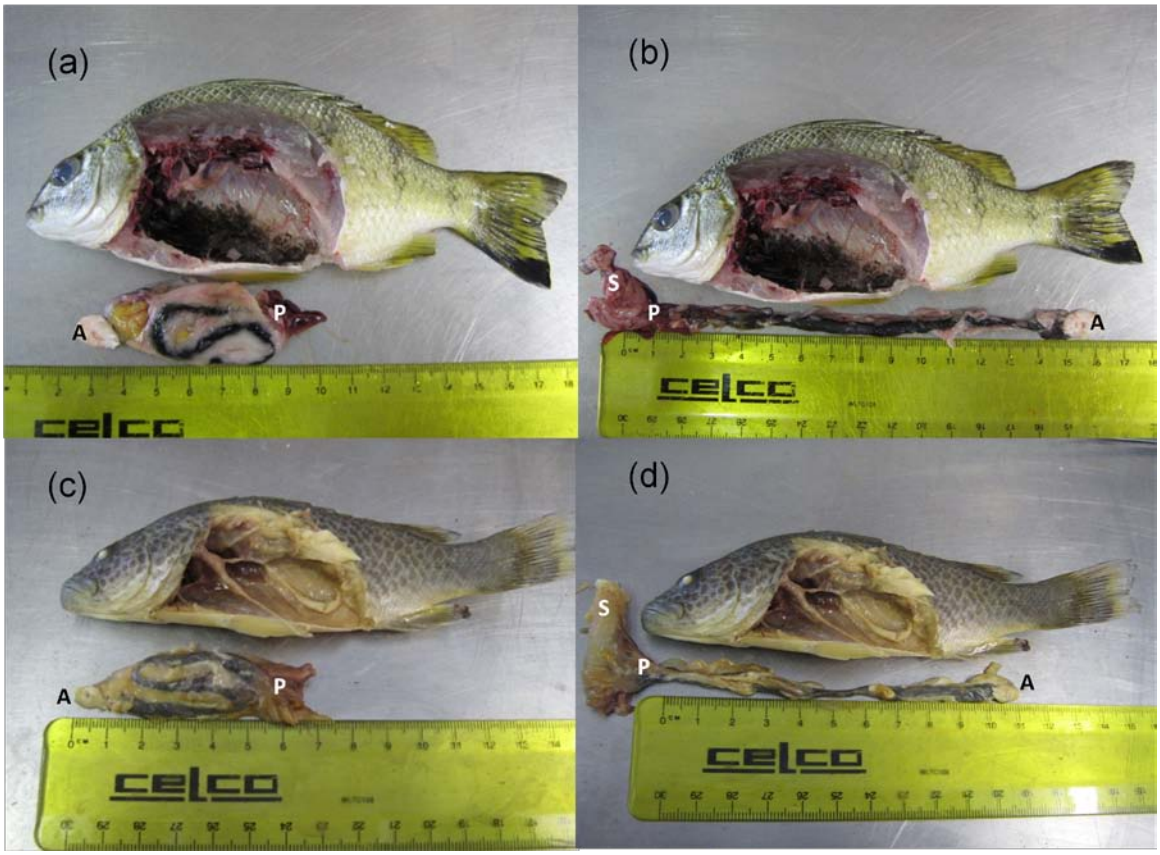

Figure A4.1 Terapontid intestinal morphology. (a) *Amniataba percoides* adult, "two-loop" intestine, excised and rotated; (b) *Amniataba percoides* intestine fully extended. (c) *Leiopotherapon unicolor* adult, "two-loop" intestine, excised and rotated; (d) *Leiopotherapon unicolor* intestine fully extended. S, stomach; P, pylorus; A, anus.

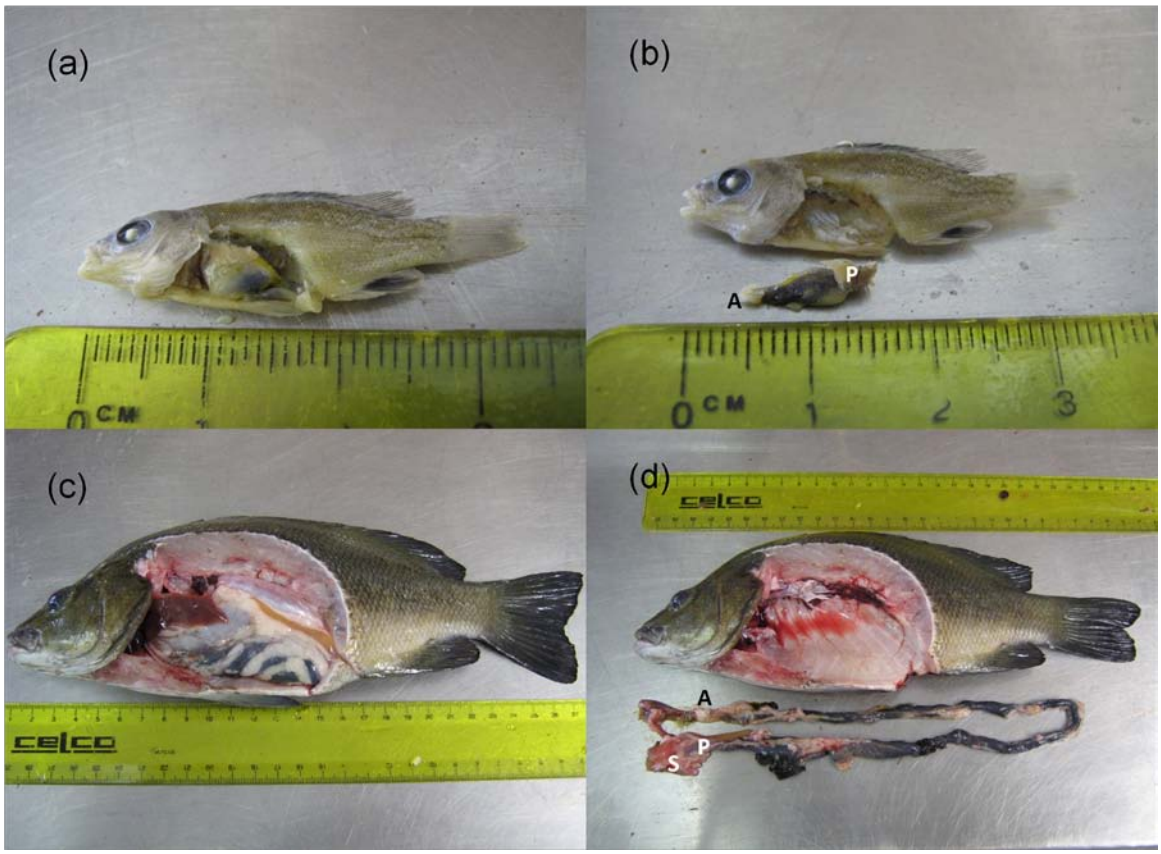

Figure A4.2 Terapontid intestinal morphology. (a) *Hephaestus fuliginosus* juvenile, “two-loop” intestine in situ; (b) *Hephaestus fuliginosus* juvenile intestine, excised and rotated; (c) *Hephaestus fuliginosus* adult, “six-loop” intestine in situ; (d) *Hephaestus fuliginosus* intestine extended. S, stomach; P, pylorus; A, anus.

Figure A4.3 Terapontid intestinal morphology. (a) *Bidyanus welchi* adult, “six-loop” intestine, excised; (b) *Pingalla gilberti* sub-adult “six-loop” intestine in situ; (c) *Pingalla gilberti* adult, “*Pingalla*” intestine in situ; (d) *Pingalla gilberti* adult intestine extended. S, stomach; P, pylorus; A, anus.

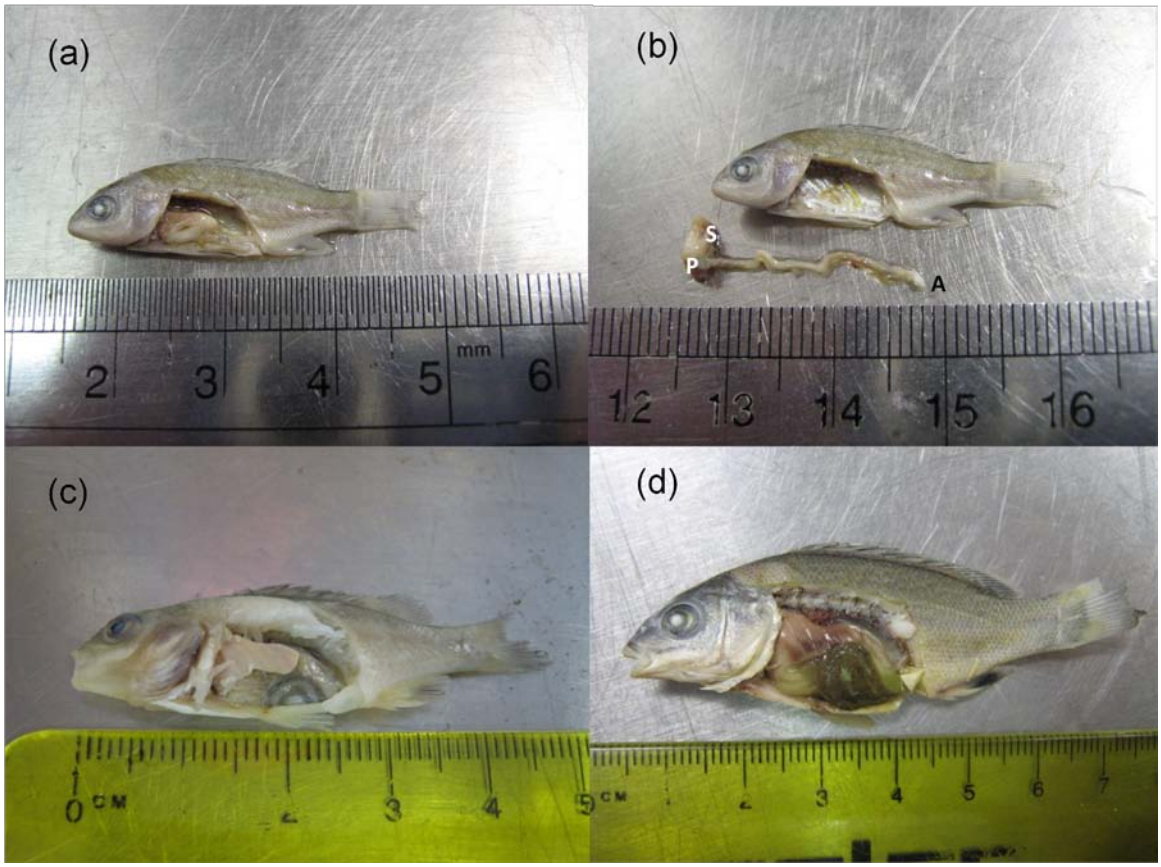

Figure A4.4 Terapontid intestinal morphology. (a) *Scortum parviceps* juvenile, “two-loop” intestine in situ; (b) *Scortum parviceps* juvenile, “six-loop” intestine fully extended (c) *Scortum parviceps* juvenile, early “six-loop” intestine in situ; (d) *Scortum parviceps* sub-adult, late “six-loop” intestine in situ . S, stomach; P, pylorus; A, anus.

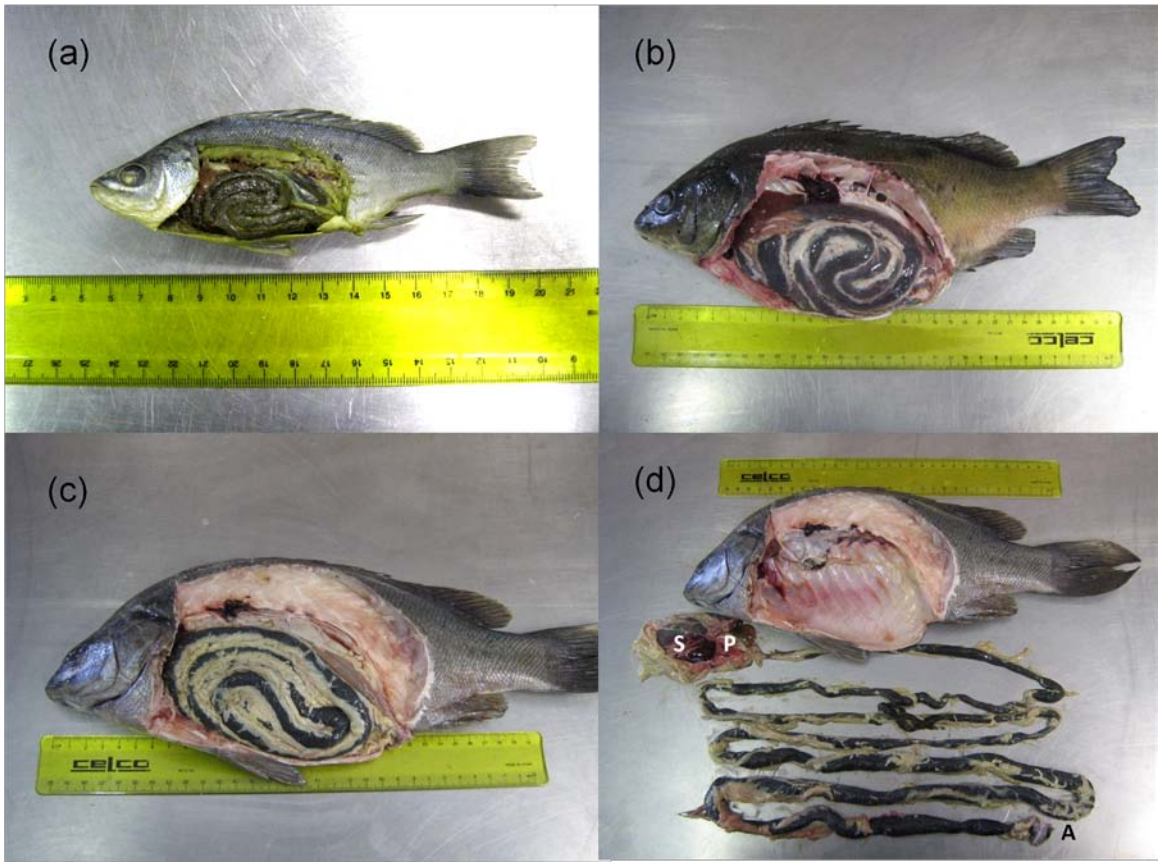

Figure A4.5 Terapontid intestinal morphology. (a) *Scortum parviceps* sub-adult, intestine in situ; (b) *Scortum parviceps* adult, "*Scortum*" intestine in situ (c) *Scortum parviceps* adult, "*Scortum*" intestine fully developed in situ; (d) *Scortum parviceps* adult intestine extended. S, stomach; P, pylorus; A, anus.

Figure A4.6 Terapontid intestinal morphology. (a) *Leiopotherapon aheneus* juvenile, “two-loop” intestine excised; (b) *Leiopotherapon aheneus* adult, “*L. aheneus*” intestine in situ (c) *Leiopotherapon aheneus* adult, “*L. aheneus*” intestine, excised and rotated; (d) *Leiopotherapon aheneus* adult intestine extended. S, stomach; P, pylorus; A, anus.

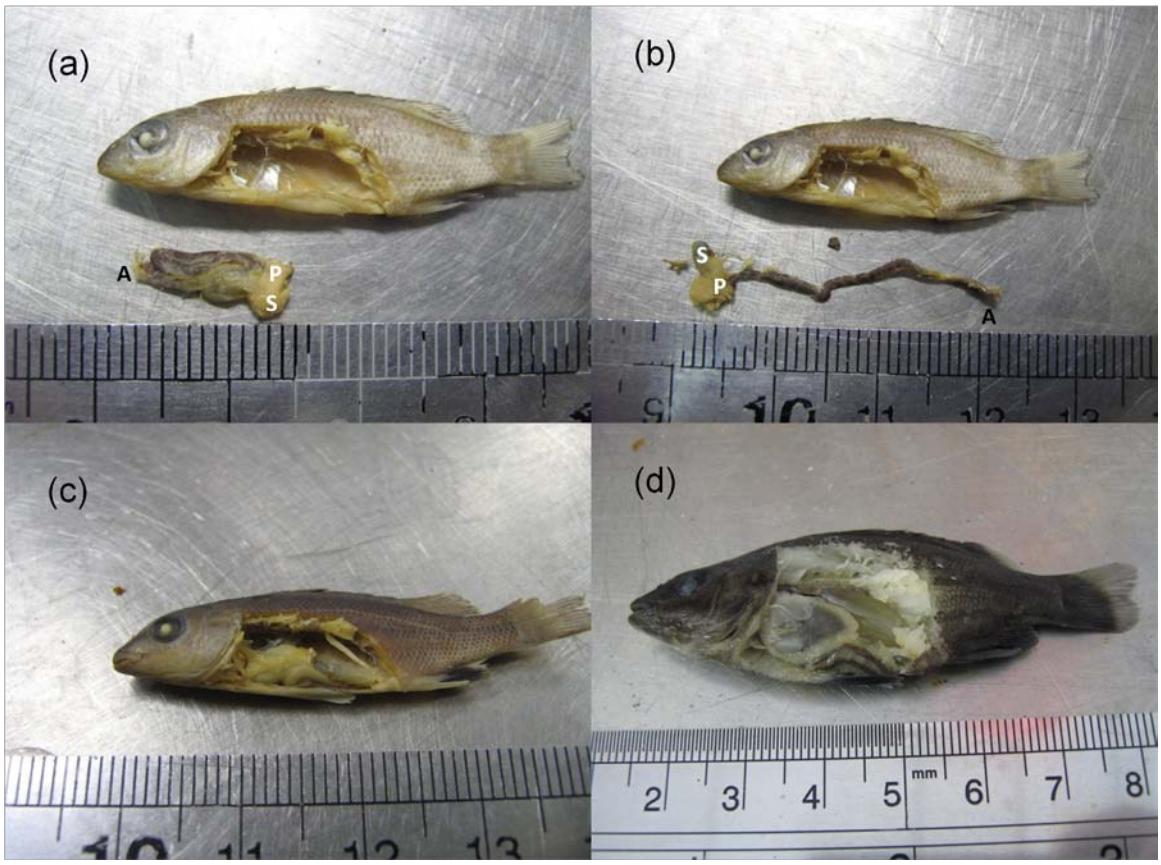

Figure A4.7 Terapontid intestinal morphology. (a) *Syncomistes butleri* juvenile, “two-loop” intestine, excised and rotated; (b) *Syncomistes butleri* juvenile, “two-loop” intestine fully extended; (c) *Syncomistes butleri* juvenile, early “six-loop” intestine in situ; (d) *Syncomistes butleri* juvenile, “six-loop” intestine in situ. S, stomach; P, pylorus; A, anus.

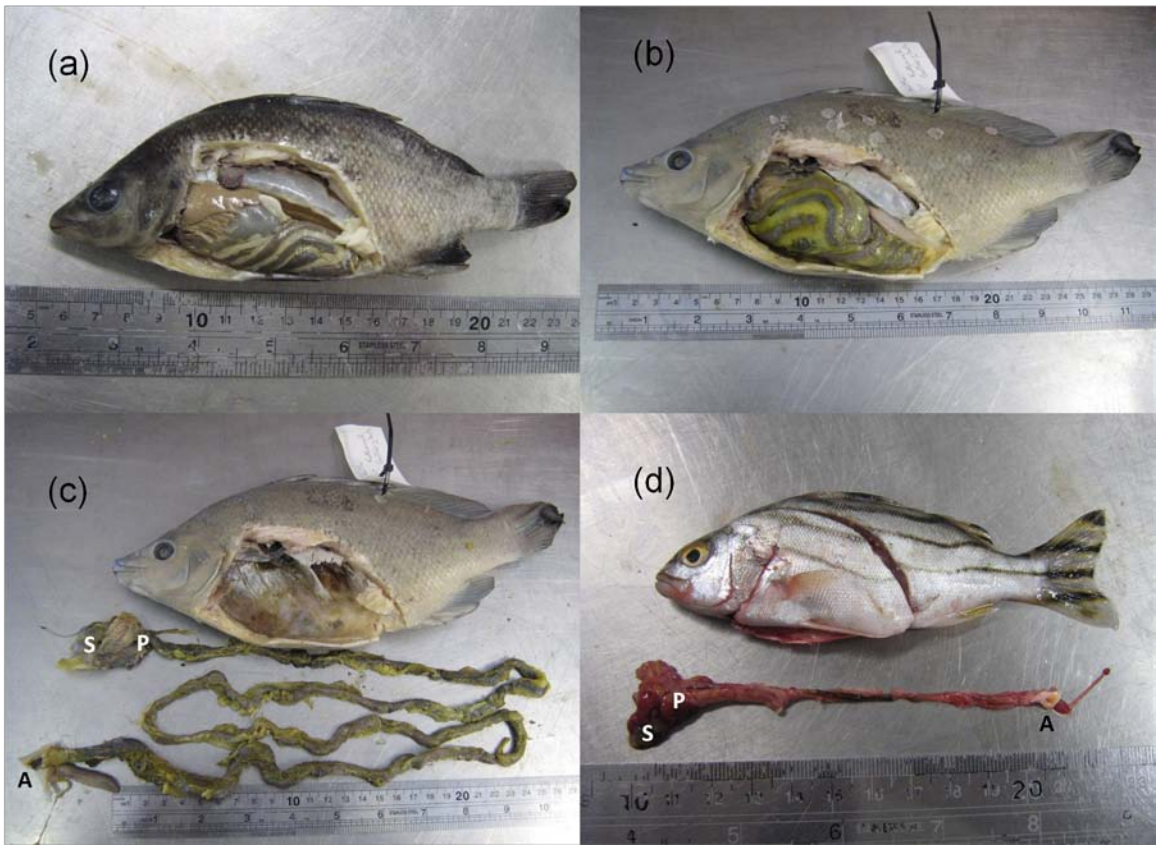

Figure A4.8 Terapontid intestinal morphology. (a) *Syncomistes butleri sub-adult*, intestine in situ; (b) *Syncomistes butleri* adult, "*Syncomistes*" intestine in situ; (c) *Syncomistes butleri* adult, "*Syncomistes*" intestine fully extended; (d) *Terapon jarbua* adult, "two-loop" intestine fully extended. S, stomach; P, pylorus; A, anus.

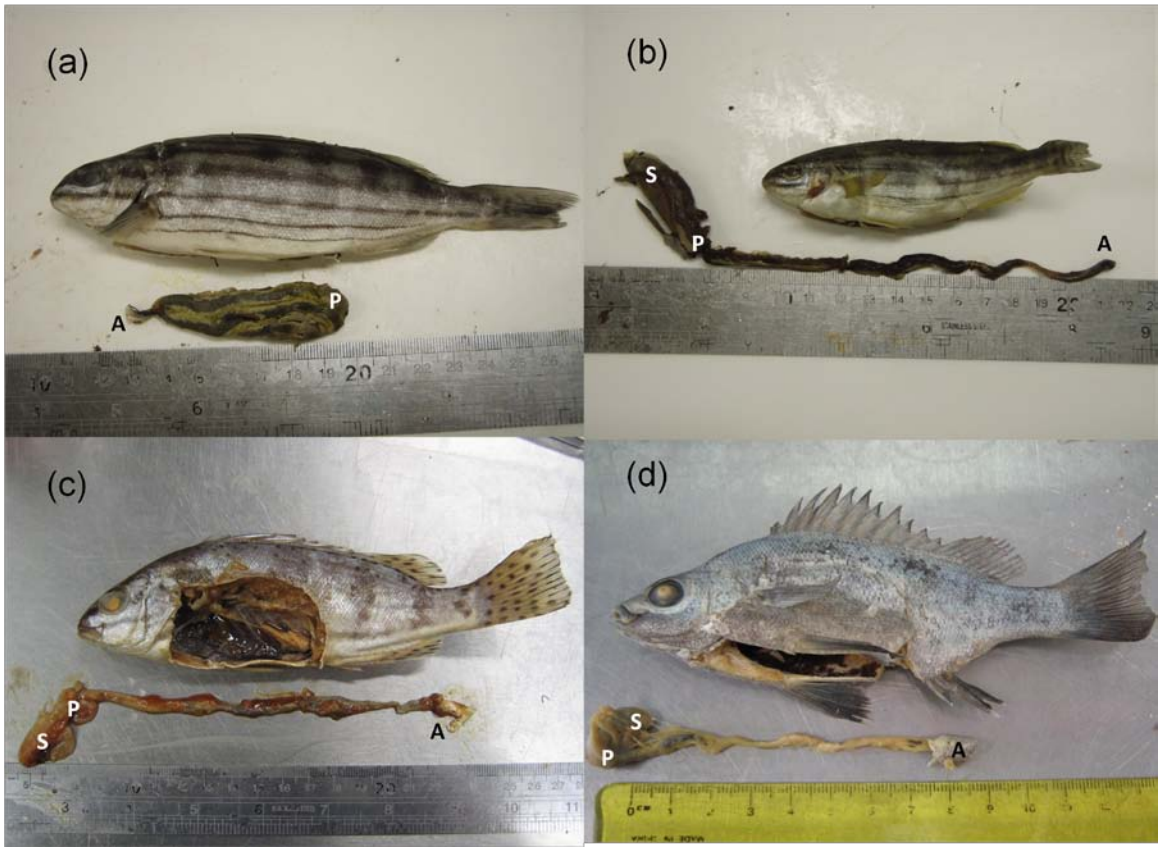

Figure A4.9. Terapontid intestinal morphology. (a) *Helotes sexlineatus* adult, "*Helotes*" intestine, excised and rotated; (b) *Helotes sexlineatus* adult, "*Helotes*" intestine fully extended; (c) *Pelsartia humeralis* adult, "two-loop" intestine fully extended; (d) *Variichthys lacustris* adult, "two-loop" intestine fully extended. S, stomach; P, pylorus; A, anus.

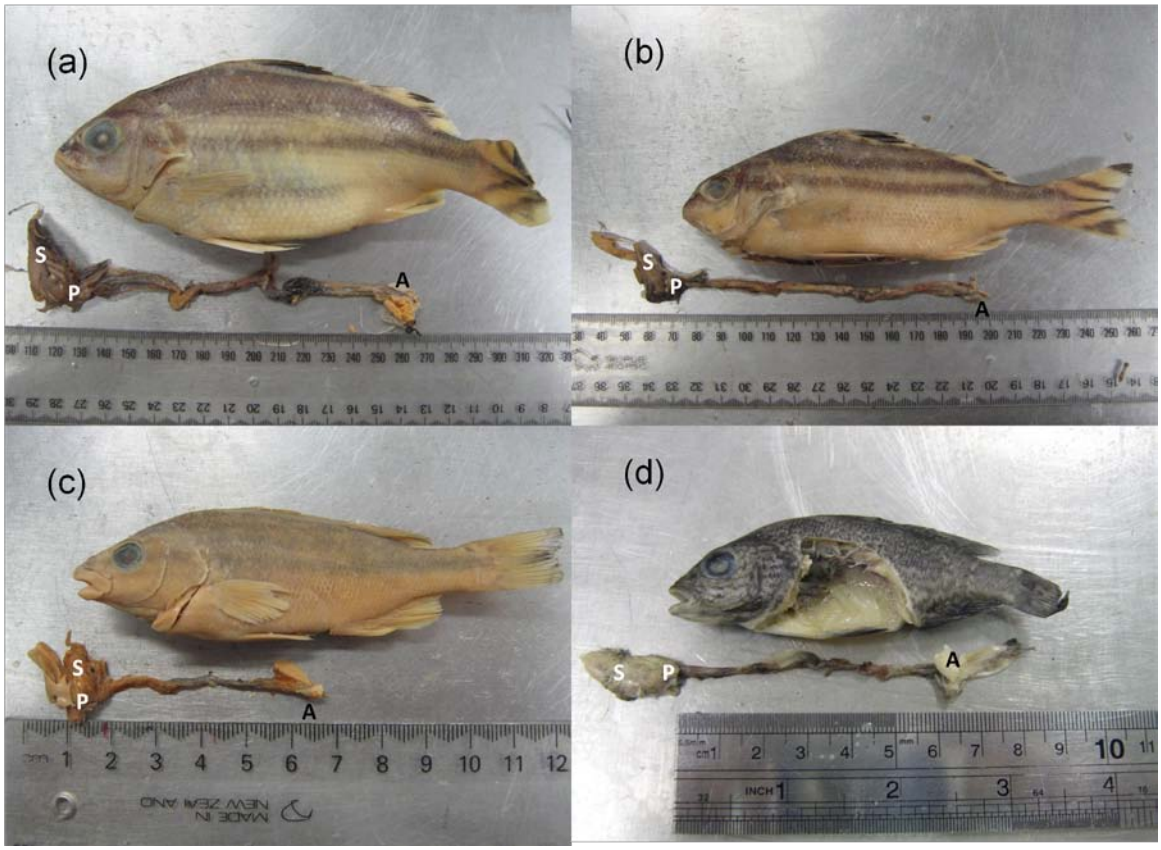

Figure A4.10 Terapontid intestinal morphology. (a) *Terapon theraps* adult, “two-loop” intestine fully extended; (b) *Terapon puta* adult, “two-loop” intestine fully extended; (c) *Hephaestus transmontanus* adult, “two-loop” intestine fully extended; (d) *Hephaestus carbo* adult, “two-loop” intestine fully extended. S, stomach; P, pylorus; A, anus.