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1 Phylogenetic effects on functional traits and life history
2 strategies of Australian freshwater fish.

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10

11 **Abstract**

12 Understanding the biogeographic and phylogenetic basis to interspecific
13 differences in species' functional traits is a central goal of evolutionary biology and
14 community ecology. We quantify the extent of phylogenetic influence on functional traits
15 and life-history strategies of Australian freshwater fish to highlight intercontinental
16 differences as a result of Australia's unique biogeographic and evolutionary history. We
17 assembled data on life history, morphological and ecological traits from published
18 sources for 194 Australian freshwater species. Interspecific variation among species could
19 be described by a specialist-generalist gradient of variation in life-history strategies
20 associated with spawning frequency, fecundity and spawning migration. In general,
21 Australian fish showed an affinity for life-history strategies that maximise fitness in
22 hydrologically unpredictable environments. We also observed differences in trait lability
23 between and within life history, morphological and ecological traits where in general
24 morphological and ecological traits were more labile. Our results showed that life-history
25 strategies are relatively evolutionarily labile and species have potentially evolved or
26 colonised in freshwaters frequently and independently allowing them to maximise
27 population performance in a range of environments. In addition, reproductive guild
28 membership showed strong phylogenetic constraint indicating that evolutionary history is
29 an important component influencing the range and distribution of reproductive strategies
30 in extant species assemblages. For Australian freshwater fish, biogeographic and
31 phylogenetic history contribute to broad taxonomic differences in species functional
32 traits, while finer scale ecological processes contribute to interspecific differences in
33 smaller taxonomic units. These results suggest that the lability or phylogenetic relatedness
34 of different functional traits affects their suitability for testing hypothesis surrounding
35 community level responses to environmental change.

36

37 Introduction

38 Understanding the biogeographic and phylogenetic basis to interspecific
39 differences in species' functional traits is a central goal of evolutionary biology and
40 community ecology. Organisms express different functional trait characteristics which
41 allow them to persist in a variety of environments, however this expression is
42 influenced or constrained by environmental and phylogenetic history (Peres-Neto et
43 al. 2012). On an evolutionary timescale, the levels of spatial and temporal variation
44 inherent in an environment act to select particular combinations of morphological,
45 behavioural and reproductive traits (strategies) which confer the ability of a particular
46 species to persist and reproduce in that environment. In time, this habitat templet
47 (sensu Southwood 1977) acts to filter out unsuccessful strategists from the potential
48 pool of colonists thereby controlling broad-scale species distributions and local
49 community composition (Townsend and Hildrew 1994; Poff 1997). Life history
50 strategies are often considered important for species fitness and long term survival
51 because they relate to reproductive success and therefore population performance
52 (Winemiller and Rose 1992).

53 Identifying the major axes of life-history strategy variation in freshwater fish
54 assemblages has had a long history in the literature beginning with the r - K model
55 first introduced by MacArthur and Wilson (1967). More recently, based on
56 mechanistic life history trade-offs between reproduction, growth and survival,
57 Winemiller and Rose (1992) identified three reproductive strategies as endpoints of a
58 triangular continuum resulting from adaptive responses to environmental conditions:
59 periodic, opportunistic and equilibrium. These strategies optimize fitness within
60 environmentally predictable, unpredictable and stable systems, respectively. A
61 number of studies have since supported the association of reproductive traits into

62 these life history strategies across various continents (Olden et al. 2006; Tedesco and
63 Hugueny 2006; Mims et al. 2010; Olden and Kennard 2010), however it remains to be
64 seen if these predictions hold true for Australian freshwater species. Given Australia's
65 generally arid climate, relatively high hydrologic variability and strong seasonality
66 (White 1994; Unmack 2001; Kennard et al. 2010) it might be expected that the
67 Australian fish fauna will have relatively few equilibrium strategists and be dominated
68 by opportunistic and periodic strategists. Mims et al. (2010) found a high proportion
69 of opportunistic strategists in south eastern United States which has experienced
70 similar extremes in aridity and hydrologic variability to much of the Australian
71 continent.

72 Adaptation alone cannot account for the syndromes of tightly linked traits
73 repeatedly observed among taxa (Poff et al. 2006). Intuitively, species which share a
74 common ancestry are more likely to show similar trait expressions than species from
75 disparate phylogenetic backgrounds, and indeed, trait states of phylogenetically
76 related species should be correlated simply because they are descendant from a
77 common ancestor (Cheverud et al. 1985). Deep phylogenetic origins in some trait
78 characteristics implies that changes in state would require a suite of co-evolved traits
79 to adapt concurrently, and therefore changes in these characteristics may be relatively
80 infrequent. Alternatively, trait characteristics with seemingly more recent origins
81 should be less dependent on co-occurring traits and therefore may respond to local
82 environmental conditions with frequent independent adaptations (Kellermann et al.
83 2012). If phylogenetic dependence is a dominant force constraining functional trait
84 composition within a species assemblage we might expect one or a number of
85 dependant traits to be constrained to change state given an associated change in its
86 paired trait. Alternatively, if adaptation to local environmental conditions is important

87 for shaping trait composition we might expect functional trait states to evolve
88 independently numerous times across a phylogeny (homoplasy) (Wake 1991;
89 Blomberg et al. 2003; Poff et al. 2006). Thus, some traits may appear more
90 evolutionarily labile than others, based on the dominant process controlling their rates
91 of evolution. It has been suggested that life-history and morphological traits are more
92 constrained by phylogeny whereas ecological and behavioural traits tend to be more
93 evolutionarily labile (Usseglio-Polatera et al. 2000; Blomberg et al. 2003; Poff et al.
94 2006), however these patterns are yet to be fully investigated for continental
95 freshwater fish faunas. Understanding how strongly trait expression is influenced by
96 phylogeny remains a key objective for aquatic ecologists due to its potential
97 importance in understanding mechanisms of community assembly, trait-environment
98 interactions and determinants of species distributions (Webb et al. 2002; Diniz-Filho
99 et al. 2011).

100 Historically, evaluating the links between the functional trait composition of
101 biotic communities and the patterns of phylogenetic structure was unintuitive and the
102 results somewhat inconclusive (Webb et al. 2002; Graham et al. 2012). However,
103 recent analytical advancements quantifying trait lability, character evolution and
104 phylogenetic relatedness have the potential for gaining much deeper insights into
105 ecological/evolutionary patterns and processes (Diniz-Filho et al. 2012). Integrating
106 community assembly theory with phylogenetics has wide ranging implications for
107 biogeographic, taxonomic and ecological studies that seek to understand the processes
108 that generate variation in the diversity, identity and abundance of co-occurring species
109 and conserve them accordingly (Kraft et al. 2007).

110 Our primary objective in this paper is to quantify functional trait diversity in
111 Australian freshwater fish and evaluate the degree to which trait expression is

constrained by phylogeny. We hypothesise that the Australian freshwater fish fauna will be dominated by species that mature earlier in life and at a small size and will show an affinity for the ‘opportunistic’ endpoint strategy (sensu Winemiller and Rose 1992). Our analysis of functional trait diversity within Australian species is used to demonstrate how suites of co-evolved traits drive multivariate differences among species. To explore the role of phylogenetic constraint and the importance of adaptive responses to environmental variation in functional traits we quantify trait lability and co-evolution across a phylogeny of Australian freshwater fish. We expect that differences in trait lability will exist within and among life-history, morphology and ecological trait groups. Specifically, we hypothesise that life-history traits will show higher phylogenetic constraint compared with ecological and morphological trait groups and that trait characters with deeper phylogenetic origins will be more evolutionary constrained than trait characters with relatively recent origins. By gaining a broad understanding of the variation in functional traits and the degree of phylogenetic constraint on these traits, this study aims to provide new insight for future trait based studies linking trait assemblages to environmental gradients.

Materials and methods

Trait database

We assembled functional trait information for freshwater fish occurring in six primary Australian drainage divisions: North-East Coast, South-East Coast, Murray-Darling Basin, Lake Eyre Basin, Timor Sea, and Gulf of Carpentaria (AWRC 1976). The region is characterised by a diversity of landforms, climate and aquatic habitat types and contains over 90% of the total freshwater fish species found in Australia

137 (the majority of the remainder belonging to the family Galaxiidae and occurring in the
 138 Tasmanian drainage division) (Allen et al. 2003). Following Allen et al. (2003), we
 139 define a freshwater fish as one that can reproduce in freshwater and those diadromous
 140 species that spend the majority of their life cycle in fresh waters. For 194 native
 141 freshwater fish species (35 families and 81 genera), we collected information on
 142 seventeen traits (life history, morphology, ecology) that could be justified on the basis
 143 of our current state of knowledge and information available for the majority of focal
 144 species (Table 1). Life history traits describe longevity, age at maturation (female),
 145 length at maturation (female), movement associated with reproduction, spawning
 146 substrate, spawning frequency, reproductive guild (following Balon 1975), total
 147 fecundity, egg size, and degree of parental care (following Winemiller 1989). Parental
 148 care was quantified as the $\sum x_i$ for $i = 1-3$, where $x_1 = 0$ if no special placement of
 149 zygotes, $x_1 = 1$ if special placement of zygotes, $x_1 = 2$ if both zygotes and larvae
 150 maintained in nest, $x_2 = 0$ if no parental protection of zygotes or larvae, $x_2 = 1$ if brief
 151 period of protection by one sex (<1 month), $x_2 = 2$ if long period of protection by one
 152 sex (>1 month) or brief care by both sexes, $x_2 = 4$ or lengthy protection by both sexes
 153 (>1 month), $x_3 = 0$ if no nutritional contribution to larvae, $x_3 = 2$ if brief period of
 154 nutritional contribution to larvae (<1 month), $x_3 = 4$ if long period of nutritional
 155 contribution to larvae (1–2 months), and $x_3 = 8$ if extremely long period of nutritional
 156 contribution to larvae (>2 months). Morphological traits described maximum body
 157 Length (related to habitat and food availability), shape factor (related to
 158 maneuverability and feeding mode), swim factor (related to swimming performance
 159 and flow preference), eye size (related to trophic preference and predation success)
 160 and maxilla size (related to prey size and feeding mode). Ecological traits described
 161 vertical position and trophic guild according to adult feeding mode based on

162 published diet analyses.

163 Trait assignments were based on multiple sources of information including
164 species accounts in comprehensive texts (i.e. Merrick and Schmida 1984; McDowall
165 1996; Allen et al. 2003; Pusey et al. 2004; Lintermans 2007), species descriptions
166 from the primary literature, state agency reports, university reports, graduate theses
167 and electronic databases available on the World Wide Web (e.g. FishBase). All trait
168 information was assigned based on a majority of evidence rule with preference given
169 to adult female measurements where possible (see Olden and Kennard 2010 for more
170 details on trait assignments). Our database consisted of ordinal and continuous data
171 types. Ordinal data were assigned a single trait state and median values were recorded
172 when ranges were presented for continuous data. Existing information for species
173 traits can be confounded by imprecise measurement (e.g. total fecundity),
174 inconsistency among measurements and studies (e.g. single/batch/protracted spawning
175 season), missing data, intraspecific variation in trait expression and ontogeny (e.g.
176 trophic preference). Where such issues arose we employed our expert knowledge to
177 assign trait values (as per Olden et al. 2006; Tedesco and Hugueny 2006).

178

179 Interspecific variation in functional traits of Australian freshwater fish

180 We summarised interspecific variation in Australian fish functional traits using
181 principle coordinate analysis (PcoA), an ordination method which optimally
182 represents the variation of a multidimensional data matrix with reduced
183 dimensionality (Legendre and Legendre 1998). PCoA was performed on a species-by-
184 trait dissimilarity matrix calculated using Gower's coefficient, an appropriate metric
185 given our data set contained mixed data types.

186 We tested for multivariate differences in traits between various levels of

taxonomic resolution (e.g. order, family, genus) using permutational multivariate analysis of variance (PERMANOVA). We also tested for multivariate homogeneity of group dispersions using PERMDISP2, a multivariate analogue of Levene's test for homogeneity of variances. PERMDISP2 tests if the dispersions (variances) of one or more groups (e.g. order) are different by calculating the distances of group members (species) to the group centroid and subjecting them to a permutation test for homogeneity of multivariate dispersions (PERMUTEST). PERMUTEST performs an ANOVA-like permutation test on the group dispersions and produces pairwise comparisons between groups as a means of Post-Hoc testing. PERMANOVA and PERMDISP2 were performed on those groups with two or more species (i.e. 11 Orders, 22 Families, 34 Genera). Pairwise comparisons among species order, family and genus from the test of multivariate homogeneity of variances were considered significant at $\alpha=0.01$. All data analyses were performed in R (v2.13.1; The R Foundation for Statistical Computing 2011) using the 'vegan' package.

To summarise variation in life history strategies among species we plotted the life history attributes of juvenile survival [equal to $\ln(\text{egg size} + 1) + \ln(\text{parental care} + 1)$], fecundity and onset of reproduction (length at maturity) in three dimensional space to produce a tri-lateral life-history continuum similar to that of Winemiller and Rose (1992).

Evolutionary lability and phylogenetic relatedness among traits.

To quantify the phylogenetic basis to trait lability, co-evolution among traits and evolutionary history we first we constructed a phylogenetic tree of Australian freshwater fish based on morphological relationships among species [sourced from

212 Tree of Life (<http://tolweb.org/tree/>)] because molecular phylogenies were not
213 available for many of the taxa used in this study (Supplementary material appendix1).
214 We used a taxonomic framework to infer phylogenetic relationships at lower
215 taxonomic levels (i.e. family and genus) and treated all genera in a family as a
216 polytomy. This tree was then converted into a triangular distance matrix based on the
217 number of nodes between extant taxa. We used PAUP (v4.0b10; Swafford 2003) to
218 reconstruct our distance matrix in the form of a rooted, neighbour-joining tree with
219 unity branch lengths. The inclusion of polytomies and the use of unity branch lengths
220 is a suitable alternative when branch length information is missing (Halsey et al.
221 2006; Schweiger et al. 2008).

222 All phylogenetic analyses were based on a discretised version of the trait
223 database. Quartiles were used to delineate between trait states after inspection of the
224 frequency distributions revealed this to be an appropriate basis for discretising the
225 data. Each of the seventeen functional traits was traced onto our tree using the
226 parsimony criterion (reconstruction based on the minimum number of trait state
227 changes given our tree and the observed distribution of trait states) using Mesquite
228 (V2.75; Maddison and Maddison 2011). We treated continuous variables (prior to
229 discretising) as ordered (i.e. the number of steps from state i to state j is $|i-j|$) and
230 categorical variables as unordered (i.e. one step between each change of state).

231 To examine if a shift in one trait state corresponds to a shift in its paired trait
232 state (which would indicate evolutionary relatedness) we employed paired-
233 comparison analysis in Mesquite V2.75 (Maddison and Maddison 2003). We used the
234 ‘most pairs’ algorithm (see Maddison 2000) to generate pairs of taxa used in the trait
235 comparisons. The most pairs algorithm looks to maximise the degrees of freedom by
236 maximising the number of trait pairings under the condition that no two pairings can

237 be connected along the same branch of the phylogeny (Maddison 2000). Given the
238 number of possible combinations of pairings we considered pairwise comparisons
239 significant at $\alpha=0.01$. p-values did not change when dependant and independent
240 variables were reversed.

241 Evolutionary trait lability was estimated by calculating: 1) the minimum
242 number of parsimony steps in a trait state; and 2) the consistency index (CI; Kluge
243 and Farris 1969). The CI is a measure of convergent evolution (homoplasy) by
244 quantifying the ratio of the minimum number of steps to the actual number of steps
245 observed for each trait (Klassen et al. 1991). The CI ranges from near 0 to 1 where
246 high CI values indicate low levels of homoplasy and low CI values indicate high
247 levels of homoplasy (Klassen et al. 1991; Poff et al. 2006). Intuitively, high levels of
248 homoplasy would indicate high evolutionary lability in a given trait. We also
249 calculated Blomberg's K statistic (Blomberg et al. 2003) using the 'picante' package
250 in R (v2.13.1; The R Foundation for Statistical Computing 2011). All branch lengths
251 were set to 1 and we resolved all multichotomies. The K statistic is a measure of
252 phylogenetic signal that compares the observed signal in a trait to the signal under a
253 Brownian motion model of trait evolution on a phylogeny. Values range between
254 close to 0 and >1 where close to 0 corresponds to a random or convergent pattern of
255 evolution, values around 1 correspond to a Brownian motion process, and values >1
256 indicate strong phylogenetic signal and conservatism of traits. In general, the higher
257 the K statistic, the more phylogenetic signal in a trait.

258 We demonstrated the extent of trait lability and phylogenetic history by
259 reconstructing the ancestral states of reproductive guild membership and life-history
260 strategy in freshwater fish at the family level. We assigned trait states based on a

majority of evidence rule for species within each family and retained all assumptions as per previous analysis.

Results

Interspecific variation in functional traits of Australian freshwater fish

Ordination of 194 Australian freshwater fish species according to seventeen functional traits revealed two major gradients of trait variation represented by the first two PCoA axes that collectively explain 51.7% of the total variation. The first PC (PC1; 31.3% of total variation) shows two prominent groups of species in the ordination space with a clustering of Atheriniformes, Salmoniformes, Clupeiformes, and larger, non-benthic Perciformes in the negative space from smaller, benthic Perciformes (i.e. Gobiidae and Eleotridae) and plotosid catfishes in the positive space (Fig. 1a). PC1 separates species with high parental care and egg guarding reproductive strategies, amphidromous spawning migrations and fusiform body shapes (positive values on PC1) from non-benthic, omnivorous species with low parental investment in brood survivorship (non-guarding reproductive behaviour and low parental care) (negative values on PC1) (Fig. 1b, c). The second PC (PC2; 25.8% of total variation) represents a ‘periodic’ - ‘opportunistic’ gradient (sensu Winemiller and Rose 1992) and can be seen in ordination space by the clustering of the Atheriniformes and Salmoniformes in positive space from the Anguiliformes, Clupeiformes, Ariid catfish and larger Perciformes in the negative ordination space (Fig. 1a). PC2 clearly separates ‘periodic’ species, which tend to be highly fecund, late maturing, long lived, large growing pelagic spawners, in the negative space from ‘opportunistic’ species, which tend to be non-migratory, batch spawning herbivore-

286 detritivores, in the positive space (Fig. 1b, c).

287 The taxonomic basis to the variation in traits observed in the ordination
288 analysis was supported by the results of the PERMANOVA analysis which confirmed
289 significant multivariate differences in functional traits between species grouped at
290 taxonomic levels of order ($p= 0.012$), family ($p= <0.001$) and genus ($p= <0.001$)
291 (Table 2). The test for within group dispersion also showed significant differences
292 between taxonomic order ($p= <0.001$), family ($p= 0.008$) and genus ($p= <0.001$)
293 (Table 2). Post-hoc testing with PERMUTEST highlighted significant pairwise
294 differences between the Perciformes, which were highly dispersed in ordination
295 space, from the Atheriniformes, Salmoniformes and Siluriformes which were less
296 dispersed in ordination space (Supplementary material Appendix 2, Table A2) (Fig.
297 1a).

298 Australian freshwater fish showed an affinity for the life-history continuum
299 model proposed by Winemiller and Rose (1992). We found strong evidence for the
300 triangular adaptive surface bound by the opportunistic, periodic and equilibrium
301 endpoint strategies (Fig. 2). There was an obvious clustering of species towards the
302 opportunistic strategy endpoint. This space was dominated by fish of the order
303 Atheriniformes and Salmoniformes, with some Perciformes representatives such as
304 the smaller bodied Gobiidae, Eleotridae and Chandidae families (Fig. 2). There were
305 relatively few species occupying the ‘equilibrium’ endpoint space with only some
306 members of the Ariid catfishes (Order: Siluriformes) and the Osteoglossids present in
307 this space. The Anguilliformes and some of the larger Perciformes (e.g.
308 Centropomidae) occupied the ‘periodic’ endpoint; however the majority of species
309 occupied an intermediate position in the life-history space.

310

311 Evolutionary lability and phylogenetic relatedness among traits.

312 Pairwise comparison analysis showed a low degree of relatedness among trait
313 parings, however some did indicate an evolutionary linkage (i.e. a change in one trait
314 state constrained by a change in another trait state) (Supplementary material Appendix
315 3, Table A3). Significant positive pairwise comparisons were observed between the
316 life-history traits of total fecundity and length at maturity, and movement
317 classification. Significant positive correlations were also observed between shape
318 factor and swim factor, and between maxilla size and trophic guild (Appendix 3, Table
319 A3).

320 Estimation of trait lability and phylogenetic signal after reconstruction of
321 ancestral states and evaluation of trait variation among species revealed broad
322 differences in lability among and within life-history, morphological and ecological
323 traits (Table 3). There was broad agreement among the three measures of trait lability
324 where morphological traits tended to be more evolutionarily labile than life history
325 traits. Life history traits such as the frequency of reproductive bouts and parental
326 investment in brood survivorship (reproductive guild and parental care) appeared to
327 show the greatest phylogenetic signal, whereas, total fecundity, egg size, longevity,
328 and the onset of reproductive maturity (age and length at maturity) were the most
329 labile. Morphological traits showed only minor variation in lability with the exception
330 of maximum length which showed a strong phylogenetic signal for Blomberg's K
331 statistic (Table 3). The ecological trait describing vertical position showed a stronger
332 phylogenetic signal than trophic guild membership (Table 3).

333 Reconstruction of ancestral reproductive guild membership and life-history
334 strategy for freshwater fish at the family level using the most parsimonious resolution
335 showed differences in the degree of phylogenetic signal between the two measures

(Fig. 3). For reproductive guild, reconstruction unambiguously indicated an ancestral ‘broadcast’ (open substrate spawning, non egg guarding) spawning strategy from which a number of alternative reproductive guilds likely evolved. Greater investment in parental care (e.g. nest building, egg guarding, live bearing) appeared to evolve from this basal state in the Osteoglossidae, Ariidae and a number of Perciform families. Internal nodes were also unambiguously assigned to open substrate spawning indicating a high degree of phylogenetic signal to reproductive guild membership. For life-history strategies, ancestral state reconstruction was only able to unambiguously resolve seven internal nodes (all of which were close to the tips) indicating a high degree of lability among life-history strategies. Reconstruction was unable to distinguish the basal life-history strategy between periodic and equilibrium strategists and showed that opportunistic life-history strategies likely evolved (or colonised) more recently in the Australian freshwater fish fauna (Fig. 3). This was particularly evident of families in the orders Salmoniformes, Osmeriformes, Atheriniformes and Perciformes.

Discussion

There has been a long history of using functional traits to link species distributions and assemblages to gradients of environmental variation (e.g. Blanck et al. 2007; Tedesco et al. 2008; Erős et al. 2009; Olden and Kennard 2010; Logez et al. 2012). However, relatively few studies have combined this type of analysis with a quantitative assessment of evolutionary lability and phylogenetic constraint for freshwater organisms in order to gain a mechanistic understanding of community assembly processes (but see Poff et al. 2006; Peres-Neto et al. 2012). Our study is the

361 first to use a phylogenetic framework to explore the patterns of interspecific variation
362 in functional trait characteristics, life-history strategy differentiation, trait lability and
363 evolutionary history for freshwater fishes. We show that the majority of Australian
364 fish species examined have a strong affinity for the ‘opportunistic’ strategy (sensu
365 Winemiller and Rose 1992) with fewer periodic and equilibrium species. We showed
366 that biogeographic and phylogenetic history contribute to broad taxonomic differences
367 in species functional traits and that suites of co-occurring traits differentiate taxa on
368 multiple gradients of variation in life-history strategies. We also found strong
369 evidence for differences in evolutionary lability and phylogenetic history within and
370 between life-history, morphological and ecological traits which underpin the observed
371 interspecific differences in functional traits within Australian fish fauna.

372 The Australian freshwater fish fauna has a long history of isolation from other
373 continental species pools and as such is typified by high levels of endemism
374 (Unmack, 2001), and unique associations of life-history, morphological and
375 ecological traits that contrast with freshwater fish faunas from disparate phylogenetic
376 and biogeographic backgrounds. Australian species tend to reach maturity earlier (1.5
377 ± 0.3 years) on average than North American (2.5 ± 0.2 years; Olden and Kennard,
378 2010) and European species (3.2 ± 0.2 years; Blank et al. 2007), a trend which may be
379 partly explained by Australia’s increasing aridity in the last 500,000 years (White
380 1994; Unmack 2001). This would select for trait states better suited to less predictable
381 rainfall and unstable environmental conditions where early maturation would
382 maximise reproductive potential following disturbances or high rates of adult
383 mortality (Winemiller and Rose 1992). Australian species also tended to have larger
384 egg sizes (2.1 ± 0.3 mm) on average than North American (1.6 ± 0.1 mm; Olden and
385 Kennard 2010), South American (1.5 ± 0.2 mm; Winemiller, 1989), African (1.6 ± 0.1

386 mm; Tedesco et al. 2008) and European (1.6 ± 0.2 mm; Blanck et al. 2007) species, a
387 trait expression which is reportedly an adaptive response to poor (i.e. resource
388 limited) environmental conditions (Pianka 1970; Sibly and Calow 1986; Roff 1992).
389 This would suggest that relative to global fish faunas, Australian species tend to
390 maximize reproductive success in unpredictable and resource limited environments by
391 producing larger eggs at an earlier age. Typically however, larger egg sizes are
392 associated with stable environmental conditions and an association with the
393 'equilibrium' strategy (Winemiller and Rose 1992). We found that Australian
394 'equilibrium' species were uncommon which suggests that this strategy is likely not
395 advantageous given that it is favoured in stable environments, however attributes
396 related with this strategy, such as large egg size, may be beneficial for some species
397 occurring in unpredictable environments such as those found throughout much of
398 Australia (i.e. those occupying an intermediate endpoint strategy; Olden and Kennard
399 2010). Thus, it appears that biogeographic history and multiple environmental factors may
400 drive patterns of interspecific differences in functional trait expression between and
401 within continental species pools.

402 Our study shows that interspecific variation in functional traits of Australian
403 freshwater fish can be explained by two major gradients of variation in life-history
404 strategies. On the primary gradient, the Australian fish fauna were arranged between
405 benthic invertivores with relatively high parental care, egg guarding reproductive
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407
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409
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411 strategies and amphidromous spawning migrations, and non-benthic omnivores,
412 which offer little parental investment in brood survivorship (non-guarding
413 reproductive behaviour and low parental care). For example, broad differences in
414 functional traits exist between the Gobiid and Eleotrid families, and the Percichthyid,
415 Terapontid, and Chandid families within the order Perciformes, and the Plotosid and
416 Ariid catfish families within the order Siluriformes. We postulate that these
417 differences may be the result of a complex history of colonisation and radiation events
418 over a long temporal scale. This theory is evidenced by Australia's long history of
419 isolation from other continents (Unmack 2001) and the hypothesised strong marine
420 origins of many Australian fish families (Allen et al. 2003). The second gradient
421 represented a life history strategy gradient from small, non migratory, repeat spawners
422 associated with the 'opportunistic' strategy (Winemiller and Rose 1992) to large,
423 catadromous, highly fecund species associated with the 'periodic' strategy. This
424 gradient was also supported by the life history classification which positioned the
425 majority of Australian species in the 'opportunist' or 'periodic' space. These results
426 are not unexpected given that opportunistic and periodic species tend to be associated
427 with unpredictable and/or strongly seasonal flow regimes such as those that dominate
428 the Australian continent (Kennard et al. 2010). Interspecific differences in the
429 functional traits of Australian fish therefore appear to be the result of the interaction
430 between multiple colonisation and radiation events, disturbance history and
431 environmental filters (e.g. Huey et al. 2010; Davis et al. 2012; Sternberg and Kennard
432 in press)

433 It is widely accepted that phylogenetic history is an important component of
434 determining which traits or various combinations of traits are present in extant species
435 pools (Webb et al. 2002; Diniz-Filho et al. 2011). We found a strong taxonomic basis

436 to the interspecific variation in functional traits which suggests that species with
437 recent divergences tend to be more similar in their functional characteristics, as
438 compared with more distantly related lineages (Cheverud et al. 1985). However, our
439 test for within group dispersion highlights that variation also exists within the
440 speciose orders such as the Perciformes, Siluriformes and Atheriniformes (Appendix
441 2, table A2). These results suggest that at higher taxonomic resolutions, phylogenetic
442 origins are important for structuring freshwater fish trait composition (i.e. family level
443 clustering in ordination space), while at finer taxonomic scales, ecological processes
444 acting in response to environmental conditions may be responsible for interspecific
445 variation in functional traits (i.e. within family dispersion in ordination space)
446 (Schluter 2000; Davis et al. 2012).

447 Our analysis showed significant pairwise correlations between some functional
448 traits suggesting that a change in the state of one trait may be constrained by changes
449 in another trait (Maddinson 2000). This phylogenetic relatedness may highlight a
450 number of evolutionary trade-offs among life-history, morphological and ecological
451 traits and suggests there are physiological limitations on the adaptation of traits (Wake
452 1991; Klingenberg 2005). For life-history characters, we found a correlation between
453 age at maturity and total fecundity that suggests a potential life-history trade-off
454 between colonisation success and long-term survival/persistence. For morphological
455 characters, we found a significant correlation between shape and swim factor which
456 may indicate a potential physiological trade-off between manoeuvrability and
457 swimming performance. Finally, pairwise correlations between maxilla size and
458 trophic guild suggest an energetic trade-off between prey availability and predation
459 success. In general, there was a low degree of correlation among paired traits,
460 however, this result is not unexpected given that Grafen and Ridley (1996) argue that

pairwise comparison analysis may have a low power to detect correlations. Nonetheless, these results highlight the potential phylogenetic relatedness between functional traits and the degree of evolutionary lability within life-history, morphological and evolutionary traits.

It has been suggested that evolutionarily labile traits are more responsive to local environmental selection than are traits heavily constrained by phylogeny and that traits free from phylogenetic effects are predicted to converge overtime in response to local selection (e.g. Usseglio-Polatera et al. 2000; Blomberg et al. 2003; Poff et al. 2006). We found evidence for differences in trait lability within and between life-history, morphological and ecological functional traits and showed that, in general, morphological traits were more labile than life-history traits. We also showed differences in lability between freshwater fish reproductive guild and life-history strategy as defined by Balon (1975) and Winemiller and Rose (1992), respectively. For reproductive guild, broadcast spawning appears to be the ancestral reproductive strategy from which higher parental investment later evolved. This evolutionary history is consistent with a continental fish fauna that has strong marine origins where broadcast spawning and diadromous migrations are common life-history characteristics (Winemiller and Rose 1992). The deep evolutionary origin of this reproductive strategy and the high degree of phylogenetic relatedness among freshwater families indicates that changes in reproductive strategy may be relatively infrequent and require a suite of co-occurring traits (such as diadromy) to change state simultaneously. In contrast, life-history strategy tended to be more evolutionarily labile suggesting that fish life-histories, which incorporate measures of reproductive output, generation time, and parental investment in progeny, frequently and independently evolve or colonise in freshwaters in order to maximise population

486 performance in a variety of environments, habitat types and flow regimes. Thus, life-
487 history strategy may be an important metric for elucidating trait-environment
488 interactions as it is likely more responsive to local environmental conditions than
489 other, more constrained measures such as reproductive guild.

490 Our study represents the first time that the evolutionary lability of functional
491 traits for freshwater fish has been quantified in a phylogenetic framework and
492 provides important information for future studies that seek to relate phylogenetically
493 unconstrained functional traits with environmental gradients to test hypothesis
494 surrounding community level responses to environmental variation. However, we
495 highlight the following challenges. Firstly, we recognise that our estimation of trait
496 characters does not incorporate intraspecific variation in species traits and stress the
497 importance of encompassing this variation in order to confidently observe and predict
498 interspecific differences in species traits (Bolnick et al. 2011; Morrongiello et al.
499 2012). Depending on the direction (i.e. trait values higher or lower than reported), the
500 degree of error in classification (minor versus significant error in trait estimation) and
501 the prevalence of intraspecific variation among taxa in the phylogeny, actual values of
502 trait lability may be higher or lower than those reported. However, reconstructing
503 ancestral states using a discretised dataset of trait values does allow for some degree
504 of intraspecific trait variation because trait states are expected to vary more between
505 species than within species (Albert et al. 2011). Secondly, our phylogenetic analysis
506 was conducted with a tree based on taxonomic relationships among species. This
507 analysis would undoubtedly benefit from quantitative genetic information in order to
508 better tease out phylogenetic relationships between species and evolutionary lability
509 in functional traits (Pianka 2000). Incorporating genetic information into tests of
510 phylogenetic dependence generally reduces the risk of Type I error rates and

misclassifying the evolutionary origins of monophyletic groups, however, in the case where genetic information is not available, taxonomic information has been shown to be an adequate substitute (Kelly and Woodward 1996; Freckleton et al. 2002).

This study has shown that for Australian freshwater fish, biogeographic and phylogenetic history contribute to broad taxonomic differences in species functional traits, while finer scale ecological processes contribute to interspecific differences in smaller taxonomic units. We have also demonstrated that the lability or phylogenetic relatedness of different functional traits affects their suitability for testing hypothesis surrounding community level responses to environmental change. These results present future trait based studies with a framework for intuitively selecting taxonomic resolution and functional traits a priori as a means of answering specific hypotheses in relation to phylogenetic relatedness and/or trait-environment relationships.

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535 References

- 536 Albert, C. H. et al. 2011. When and how should intraspecific variability be considered
537 in trait-based plant ecology?—Perspect. Plant Ecol. 13: 217-225.
- 538 Allen, G. R. et al. 2003. Field Guide to the Freshwater Fishes of Australia. - Western
539 Australian Museum, Perth, WA.
- 540 AWRC. 1976. Review of Australian Water Resources 1975. Department of Natural
541 Resources. Australian Water Resources Council. - Australian Government
542 Publishing Service, Canberra.
- 543 Balon, E. K. 1975. Reproductive Guilds of Fishes: A Proposal and Definition. - J.
544 Fish. Res. Board. Can. 32: 821-864.
- 545 Blanck, A. et al. 2007. Relationships between life-history strategies of European
546 freshwater fish species and their habitat preferences. - Freshwater Biol. 52:
547 843-859.
- 548 Blomberg, S. P. et al. 2003. Testing for phylogenetic signal in comparative data:
549 behavioral traits are more labile. - Evolution 57: 717-745.
- 550 Bolnick, D. I. et al. 2011. Why intraspecific trait variation matters in community
551 ecology. - Trends Ecol. Evol. 26: 183-192.
- 552 Cheverud, J. M. et al. 1985. The quantitative assessment of phylogenetic constraints
553 in comparative analyses: sexual dimorphism in body weight among
554 primates. - Evolution 39: 1335-1351.
- 555 Davis, A. M. et al. 2012. Marine–freshwater transitions are associated with the
556 evolution of dietary diversification in terapontid grunters (Teleostei:
557 Terapontidae). - J. Evol. Biol. 25: 1163-1179.
- 558 Diniz-Filho, J. A. F. et al. 2011. Eigenvector estimation of phylogenetic and
559 functional diversity. - Funct. Ecol. 25: 735-744.

560 Diniz-Filho, J. A. F. et al. 2012. On the selection of phylogenetic eigenvectors for
 561 ecological analyses. - *Ecography* 35: 239-249.

562 Freckleton, R. P. et al. 2002. Phylogenetic analysis and comparative data: A test and
 563 review of evidence. — *Am. Nat.* 160: 712-726.

564 Graham, C. H. et al. 2012. Untangling the influence of ecological and evolutionary
 565 factors on trait variation across hummingbird assemblages. - *Ecology* 93:
 566 S99-S111 (Supplement).

567 Halsey, L. G. et al. 2006. A comparative analysis of the diving behaviour of birds and
 568 mammals. – *Funct. Ecol.* 20: 889-899.

569 Huey, J. A. et al. 2010. High levels of genetic structure in the Australian freshwater
 570 fish, *Ambassis macleayi*. – *J. N. Am. Benthol. Soc.* 29: 1148-1160.

571 Kellermann, V. et al. 2012. Phylogenetic constraints in key functional traits behind
 572 species' climate niches: patterns of desiccation and cold resistance across 95
 573 *Drosophila* species. - *Evolution* 66: 3377-3389.

574 Kelly, C. K. and Woodward, F. I. 1996. Ecological correlates of plant range size:
 575 Taxonomies and phylogenies in the study of plant commonness and rarity
 576 in Great Britain. - *Philos. Trans. R. Soc. B-Biol. Sci.* 351: 1261-1269.

577 Kennard, M. J. et al. 2010. Classification of natural flow regimes in Australia to
 578 support environmental flow management. - *Freshwater Biol.* 55: 171-193.

579 Klassen, G. et al. 1991. Consistency indices and random data. – *Syst. Biol.* 40: 446-
 580 457.

581 Klingenberg, C. P. 2005. Developmental constraints, modules and evolvability. - In:
 582 Hallgrímsson B, Hall BK (eds), *Variation: A central concept in biology*.
 583 Elsevier Academic Press, pp 219–247.

584 Kluge, A. G. and Farris, J. S. 1969. Quantitative phyletics and the evolution of
 585 anurans. - Syst. Biology. 18: 1-32.

586 Kraft, N. J. B. et al. 2007. Trait evolution, community assembly, and the phylogenetic
 587 structure of ecological communities. - Am. Nat. 170: 271-283.

588 Legendre, P. and Legendre, L. 1998. Numerical ecology, 2 edn. Elsevier Science.

589 Lintermans, M. 2007. Fishes of the Murray-Darling Basin: An introductory guide. -
 590 Murray Darling Basin Commission, Canberra.

591 Logez, M. et al. 2012. A continental-scale analysis of fish assemblage functional
 592 structure in European rivers. – Ecography 35: 1-12.

593 MacArthur, R. H. and Wilson, E. O. 1967. The theory of island biogeography. -
 594 Princeton Univ. Press.

595 Maddison, W. P. 2000. Testing character correlation using pairwise comparisons on a
 596 phylogeny. – J. Theor. Biol. 202: 195-204.

597 Maddison, W. P. and Maddison, D. R. 2011. Mesquite: a modular system for
 598 evolutionary analysis (Version 2.75). - <http://mesquiteproject.org>.

599 McDowall, R. M. 1996. Freshwater Fishes of South-Eastern Australia, 2 edn. - Reed
 600 Books.

601 Merrick, J. R. and Schmida, G. E. 1984. Australian freshwater fishes: biology and
 602 management. - Griffin Press Ltd.

603 Mims, M. C. et al. 2010. Life history trait diversity of native freshwater fishes in
 604 North America. – Ecol. Freshw. Fish. 19: 390-400.

605 Morrongiello, J. R. et al. 2012. Spatial variation in egg size and egg number reflects
 606 trade-offs and bet-hedging in a freshwater fish. – J. Anim. Ecol. 81: 806–
 607 817.

608 Olden, J. D. and Kennard, M. J. 2010. Intercontinental comparison of fish life history
609 strategies along a gradient of hydrologic variability. - In: Gido KB, Jackson
610 DA (eds), Community ecology of stream fishes: concepts, approaches, and
611 techniques. Am. Fish. Soc., pp. 83-107.

612 Olden, J. D. et al. 2006. Life-history strategies predict fish invasions and extirpations
613 in the Colorado River Basin. – Ecol. Monogr. 76: 25-40.

614 Peres-Neto, P. R. et al. 2012. Assessing the effects of spatial contingency and
615 environmental filtering on metacommunity phylogenetics. - Ecology 93:
616 S14-S30 (Supplement).

617 Pianka, E. R. 1970. On r-and K-Selection. – Am. Nat. 104: 592-597.

618 Pianka, E. R. 2000. Evolutionary Ecology, 6th edn. - Addison Wesley.

619 Poff, N. L. 1997. Landscape filters and species traits: Towards mechanistic
620 understanding and prediction in stream ecology. – J. N. Am. Benthol. Soc.
621 16: 391-409.

622 Poff, N. L. R. et al. 2006. Functional trait niches of North American lotic insects:
623 traits-based ecological applications in light of phylogenetic relationships. –
624 J. N. Am. Benthol. Soc. 25: 730-755.

625 Pusey, B. J. et al. 2004. Freshwater Fishes of North-Eastern Australia. - CSIRO Pub.

626 Roff, D. A. 1992. The evolution of life histories: theory and analysis. - Chapman and
627 Hall.

628 Schluter, D. 2000. The ecology of Adaptive Radiation. - Oxford Univ. Press.

629 Schweiger, O. et al. 2008. A comparative test of phylogenetic diversity indices. -
630 Oecologia 157: 485-495.

631 Sibly, R. and Calow, P. 1986. Why breeding earlier is always worthwhile. – J. Theor
632 Biol. 123: 311-319.

633 Southwood, T. R. E. 1977. Habitat, templet for ecological strategies – Presidential
634 Address to British Ecological Society, 5 January 1977. – J. Anim. Ecol. 46:
635 337-365.

636 Sternberg, D. and Kennard, M.J. (in press). Environmental, spatial and phylogenetic
637 determinants of fish life-history traits and functional composition of
638 Australian rivers. Freshwater Biol. doi:10.1111/fwb.12166

639 Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other
640 Methods). Version 4. - Sinauer Ass.

641 Tedesco, P. and Hugueny, B. 2006. Life history strategies affect climate based spatial
642 synchrony in population dynamics of West African freshwater fishes. -
643 OIKOS 115: 117-127.

644 Tedesco, P. A. et al. 2008. River hydrological seasonality influences life history
645 strategies of tropical riverine fishes. - Oecologia 156: 691-702.

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

648 Unmack, P. J. 2001. Biogeography of Australian freshwater fishes. – J. Biogeogr. 28:
649 1053-1089.

650 Usseglio-Polatera, P. et al. 2000. Biological and ecological traits of benthic freshwater
651 macroinvertebrates: relationships and definition of groups with similar
652 traits. - Freshwater Biol. 43: 175-205.

653 Wake, D. B. 1991. Homoplasy - the result of natural-selection, or evidence of design
654 limitations. - Am Nat 138: 543-567.

655 Webb, C. O. et al. 2002. Phylogenies and Community Ecology. – Annu. Rev. Ecol.
656 Syst. 33: 475-505.

657 White, M. E. 1994. After the greening: the browning of Australia. - Kangaroo Press.

658 Winemiller, K. O. 1989. Patterns of variation in life history among South American
659 fishes in seasonal environments. - *Oecologia* 81: 225-241.

660 Winemiller, K. O. and Rose, K. A. 1992. Patterns of life-history diversification in
661 north-american fishes - implications for population regulation. – *Can. J.*
662 *Fish. Aquat. Sci* 49: 2196-2218.

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665

666 Supplementary material (Appendix EXXXXXX at
667 <www.oikosoffice.lu.se/appendix>). Appendix 1–3.

668 Tables

669 Table 1. Description of 17 life history, morphological and ecological traits collated for
670 194 species (from 35 families) of Australian freshwater fish.

Trait	Description	Abbreviation
Life History		
Longevity	Maximum potential life span (years)	Long
Age at maturation	Mean age at maturation (years)	AgeMat
Length at maturation	Mean total length at maturation (cm)	LenMat
Movement Classification	No movement associated with spawning	MVT_None
	Potadromous	MVT_Pota
	Amphidromous	MVT_Amph
	Anadromous	MVT_Anad
	Catadromous	MVT_Cata
Spawning substrate	Mineral (e.g. gravel, rocks)	SPSUB_Min
	Organic (e.g. plants, wood)	SPSUB_Org
	Various (mineral and organic)	SPSUB_Var
	Pelagic	SPSUB_Pel
	Other (e.g. buccal)	SPSUB_Oth
Spawning frequency	Single spawning per season	SPFRQ_SinS
	Batch/repeat/protracted spawner per season	SPFRQ_MulS
	Single spawner per lifetime	SPFRQ_SinL
Reproductive guild	Nonguarders (open substratum spawners)	RG_NgOS

	Nonguarders (brood hiders)	RG_NgBH
	Guarders (substratum choosers)	RG_GSC
	Guarders (nest spawners)	RG_GNS
	Bearers (internal)	RG_BI
	Bearers (external)	RG_BE
Total Fecundity	Total number of eggs or offspring per breeding season	TFec
Egg size	Mean diameter of mature (fully yolked) ovarian oocytes (mm)	EggS
Parental care	Metric representing the total energetic contribution of parents to their offspring sensu Winemiller (1989)	PC
Morphology		
Maximum body length	Maximum total body length (cm)	MaxL
Shape factor	Ratio of total body length to maximum body depth	ShapeF
Swim factor	Ratio of minimum depth of the caudal peduncle to the maximum body depth	SwimF
Eye size	Ratio of eye diameter to total body length	ES
Maxilla size	Ratio of maxilla length to total body length	MS
Ecology		
Vertical position	Benthic	VP_Ben
	Nonbenthic	VP_NonBen

Trophic guild	Herbivore-detritivore (ca. > 25% plant matter)	TG_HeDe
	Omnivore (ca. 5 - 25% plant matter)	TG_Omni
	Invertivore	TG_Inve
	Invertivore-piscivore (>10% Fish)	TG_InvePisc

671

672

673 Table 2. Multivariate differences between (PERMANOVA) and group dispersions
674 within (BETADISPER) species traits at various levels of taxonomic resolution.
675 Pairwise differences in group dispersions between orders, families and genera are
676 presented in Supplementary material Appendix 2, Table A2.

Group	Between Group F	Group p	Within Group F	Group P	Pairwise differences
Order	1.828	0.012	10.788	<0.001	Appendix 2, Table A2
Family	2.857	<0.001	2.016	0.008	Appendix 2, Table A2
Genus	26.348	<0.001	2.654	<0.001	Appendix 2, Table A2

677

678

679 Table 3. Evolutionary lability of traits grouped into 3 categories (see Table 1 for trait
680 abbreviations) for a phylogenetic tree of Australian freshwater fish (196 taxa). Mean
681 ($\pm$ SE) number of steps, consistency index (CI), and Blomberg's K statistic across all
682 traits in a group are also presented.
683

	Parsimony	Consistency	Blomberg's
Trait	Steps	Index	K
Life-History			
Long	64	0.047	0.521
AgeMat	62	0.048	1.279
LenMat	68	0.044	1.193
MVT	50	0.080	0.358
SPWSUB	44	0.091	0.441
SPWFRQ	27	0.074	1.152
RG	18	0.222	0.570
Tfec	74	0.041	0.397
EggS	66	0.045	0.399
PC	29	0.069	0.392
Mean $\pm$ SE	50.2 $\pm$ 6.3	0.076 $\pm$ 0.017	0.670 $\pm$ 0.119
Morphology			
MaxL	74	0.041	1.300
ShapeF	72	0.042	0.719
SwimF	78	0.051	0.717
ES	78	0.038	0.858
MS	79	0.038	0.539

Mean $\pm$ SE	76.2 $\pm$ 1.4	0.042 $\pm$ 0.002	0.827 $\pm$ 0.127
Ecology			
VP	19	0.053	0.642
TG	62	0.048	0.367
Mean $\pm$ SE	40.5 $\pm$ 21.5	0.051 $\pm$ 0.003	0.504 $\pm$ 0.137

684

685

686 **Figures**

687 Figure 1. (a) Two-dimensional ordination resulting from the Principle Coordinate
688 Analysis (PCoA) of the 17 functional traits for 194 Australian freshwater fish species.
689 Species are grouped by 'order'. The first two axis of the PCoA explained 57.1% of the
690 total variation in species traits. (b) Eigenvector plot of continuous trait vectors with
691 significant loadings ($p < 0.001$) on the first two principle coordinates. (c) Eigenvector
692 plot of nominal trait centroids with significant loadings ($p < 0.001$) on the first two
693 principle coordinates.

694

695 Figure 2. Diversity of life-history strategies for 194 Australian freshwater species in
696 three dimensional space defined by fecundity, length at maturity and juvenile
697 survivorship. Species are located within a triangular space with endpoints defined by
698 opportunistic, periodic, and equilibrium life history strategies (sensu Winemiller and
699 Rose 1992).

700

701 Figure 3. Maximum parsimony ancestral character reconstruction for the evolution of
702 reproductive guild (after Balon 1975) and life-history strategy (after Winemiller and
703 Rose 1992) in the Australian freshwater fish fauna presented at the family level.
704 Circles at terminal nodes represent the majority of observed character states for that
705 family. Pie charts at internal nodes show estimated probabilities for reconstructed
706 character states at that ancestral node. Reproductive guild characters are: RG_NgOS =
707 Nonguarders (open substratum spawners); RG_NgBH = Nonguarders (brood hidens);
708 RG_GSC = Guardians (substratum choosers); RG_GNS = Guardians (nest spawners);
709 RG_BE = Bearers (external). Numbers in parentheses refer to family level richness.

Figure 1. (a) Two-dimensional ordination resulting from the Principle Coordinate Analysis (PCoA) of the 17 functional traits for 194 Australian freshwater fish species. Species are grouped by 'order'. The first two axis of the PCoA explained 57.1% of the total variation in species traits. (b) Eigenvector plot of continuous trait vectors with significant loadings ($p < 0.001$) on the first two principle coordinates. (c) Eigenvector plot of nominal trait centroids with significant loadings ($p < 0.001$) on the first two principle coordinates.

255x289mm (150 x 150 DPI)

Figure 2. Diversity of life-history strategies for 194 Australian freshwater species in three dimensional space defined by fecundity, length at maturity and juvenile survivorship. Species are located within a triangular space with endpoints defined by opportunistic, periodic, and equilibrium life history strategies (sensu Winemiller and Rose 1992).
245x165mm (150 x 150 DPI)

Figure 3. Maximum parsimony ancestral character reconstruction for the evolution of reproductive guild (after Balon 1975) and life-history strategy (after Winemiller and Rose 1992) in the Australian freshwater fish fauna presented at the family level. Circles at terminal nodes represent the majority of observed character states for that family. Pie charts at internal nodes show estimated probabilities for reconstructed character states at that ancestral node. Reproductive guild characters are: RG_NgOS = Nonguarders (open substratum spawners); RG_NgBH = Nonguarders (brood hiders); RG_GSC = Guardians (substratum choosers); RG_GNS = Guardians (nest spawners); RG_BE = Bearers (external). Numbers in parentheses refer to family level richness.

324x317mm (150 x 150 DPI)

Table A2. Pair-wise comparisons among species Order, Family and Genus from test of multivariate homogeneity of group dispersions. Significant correlations at $\alpha=0.01$ are highlighted in bold.

[illegible]

FAMILY

[illegible]

[illegible]

GENUS Cont.

[illegible]

Metadata for Table A2.

Order	ID	Family	ID	Genus	ID
Anguilliformes	1	Anguillidae	1	Ambassis	1
Atheriniformes	2	Ariidae	2	Anguilla	2
Beloniformes	3	Atherinidae	3	Bidyanus	3
Clupeiformes	4	Chandidae	4	Chlamydogobius	4
Mugiliformes	5	Clupeidae	5	Craterocephalus	5
Osteoglossiformes	6	Eleotridae	6	Eleotris	6
Perciformes	7	Gadopsidae	7	Gadopsis Galaxias	7
Petromyzontiformes	8	Galaxiidae	8	Glossogobius	8
Pleuronectiformes	9	Gobiidae	9	Gobiomorphus	9
Salmoniformes	10	Kuhliidae	10	Hephaestus	10
Siluriformes	11	Melanotaeniidae	11	Hypseleotris	11
Synbranchiformes	12	Mordaciidae	12	Kimberleyeleotris	12
		Mugilidae	13	Kuhlia	13
		Osteoglossidae	14	Leiopotherapon	14
		Percichthyidae	15	Macquaria	15
		Plotosidae	16	Maccullochella	16
		Pseudomugilidae	17	Melanotaenia	17
		Soleidae	18	Mogurnda	18
		Synbranchidae	19	Mordacia	19
		Terapontidae	20	Nannoperca	20
		Toxotidae	21	Neosilurus	21
				Neoarius	22
				Ophisternon	23
				Oxyeleotris	24
				Philypnodon	25
				Pingalla	26
				Porochilus	27
				Pseudomugil	28
				Scleropages	29
				Scortum	30
				Syncomistes	31
				Toxotes	32
					33

Table A3. Pair-wise comparisons between traits (see Table 1 for list of traits) across a phylogenetic tree of Australian freshwater fish (194 taxa). Significant pair-wise comparisons ($\alpha=0.01$) are highlighted in bold. “+” indicates direction of relationship.

TRAIT	Long	AgeMat	LenMat	MVT	SPWSUB	SPWFRQ	RG	Tfec	EggS	PC	MaxL	ShapeF	SwimF	ES	MS	VP
Long																
AgeMat	0.313															
LenMat	0.343	0.032														
MVT	0.188	0.125	0.032													
SPWSUB	0.032	0.250	0.125	0.344												
SPWFRQ	0.313	0.063	0.125	0.188	0.125											
RG	0.250	0.500	0.250	0.250	0.500	0.250										
Tfec	0.344	0.063	0.008 (+)	0.004 (+)	0.344	0.188	0.250									
EggS	0.188	0.125	0.125	0.188	0.063	0.125	0.500	0.188								
PC	0.063	0.313	0.188	0.063	0.313	0.125	0.250	0.125	0.500							
MaxL	0.344	0.125	0.032	0.188	0.250	0.063	0.500	0.032	0.250	0.125						
ShapeF	0.032	0.063	0.125	0.125	0.063	0.250	0.250	0.188	0.250	0.313	0.125					
SwimF	0.188	0.125	0.313	0.188	0.313	0.250	0.250	0.032	0.063	0.500	0.063	0.008 (+)				
ES	0.500	0.125	0.500	0.500	0.250	0.125	0.500	0.250	0.250	0.500	0.125	0.250	0.250			
MS	0.188	0.125	0.125	0.313	0.250	0.250	0.250	0.032	0.250	0.250	0.125	0.344	0.313	0.500		
VP	0.250	0.250	0.250	0.125	0.125	0.125	0.500	0.250	0.250	0.500	0.250	0.063	0.125	0.500	0.500	
TG	0.125	0.250	0.125	0.188	0.500	0.500	0.500	0.063	0.250	0.125	0.125	0.125	0.032	0.250	0.010 (+)	0.250