

The evolution of habitat specialisation in a group of marine triplefin fishes

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Abstract There has been considerable interest in the directionality of resource specialisation during the diversification of lineages. We developed a quantitative method to investigate habitat specialisation in a radiation of New Zealand triplefin fishes, as habitat use appears to be an important axis of diversification in this marine group. The degree of specialisation in 15 species was calculated by comparing each individual to all other individuals of a species, thus allowing for quantitative distinction between species. Species differed in habitat specialisation, but Bayesian comparative methods found no directional trend in the evolution of resource use. Further analyses showed that specialisation had evolved gradually and was phylogenetically constrained, with most differences between species arising toward the tips of the tree. No correlation between the degree of specialisation and body size was detected in this group, suggesting that habitat specialisation evolved independently of body size. Habitat specialisation does not appear to have been an impediment to ecological diversification in this group. Rather, diversification in these fishes appears to have followed different evolutionary trajectories in habitat specialisation, one in which species have sub-partitioned available resources, and another in which species have expanded their use of resources. These findings support recent studies suggesting that diversification does not necessarily proceed from generalised ancestors to specialised descendants.

Keywords Ecological specialisation · Phylogenetic comparative method · Habitat use · Coexistence · Tripterygiidae

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Introduction

It has been commonly hypothesised that adaptive radiations are characterised by a directional trend from generalist to specialist species (Futuyma and Moreno 1988; see Schluter 2000 for a list of theoretical reasons, Simpson 1953; Mayr 1942; Schluter et al. 1997), leading to a recognisable progression towards greater resource specialisation. However, recent studies suggest that exceptions are common in nature (e.g. Nosil and Mooers 2005; Nosil 2002; Elliott et al. 1999; Morse and Farrell 2005). Schluter (2000) reviewed the phylogenetic evidence for resource specialisation in a broad range of taxonomic groups, and found little support for a trend towards increasing resource specialisation. Instead, the founders of radiations were frequently specialist species that gave rise to both specialist and generalist descendants (Schluter 2000).

Testing the generalist-to-specialist hypothesis in a phylogenetic context requires the following criteria: a speciose taxon of interest, detailed species-level ecological data, and diversification along a resource axis. One limitation of earlier studies that used phylogenetic methods to examine progression during the course of lineage divergence is that they carried the assumption that the phylogeny itself was error-free (Schluter 2000). Recently developed Bayesian Markov Chain Monte Carlo analyses directly incorporate phylogenetic uncertainty (Pagel and Meade 2004; Pagel et al. 2004), enabling this deficiency to be overcome. With this in mind, we used the New Zealand triplefin fauna (*F. Tripterygiidae*) to investigate the evolution of habitat breadth on a continuous scale. This system meets all of the criteria listed above, allowing us to answer Schluter's plea for work that could corroborate the patterns identified by earlier studies.

Triplefin fishes (Tripterygiidae) display their highest regional diversity in New Zealand, with 26 endemic species (Clements 2003). The majority of these blennioid species are sympatric throughout coastal New Zealand, and occur from the North Cape (37°68'S, 178°55'E) to Stewart Island (47°32'S, 167°50'E), showing no obvious latitudinal trends in abundance (Fricke 1994). Previous work has indicated that there has been little specialisation in diet, jaw morphology and male breeding colouration (Feary 2001; Wellenreuther and Clements 2007), but considerable diversification in physiology (Brix et al. 1999; Hickey and Clements 2003) and habitat (Feary and Clements 2006; Wellenreuther et al. 2007, *in press*). Furthermore, species-specific habitat associations appear to be the results of active choice at settlement (Wellenreuther and Clements *in press*), and thus may be adaptive. New Zealand triplefin fishes are extremely philopatric, and individuals occupy the same small patch of habitat (2–4 m²) for their entire demersal life following a pelagic larval phase (Clements 2003). Given this extreme philopatry and temporal stability in habitat use, habitat use can be effectively estimated in a single encounter with an adult individual. Although New Zealand triplefin species are fairly similar in morphological traits, closely related species show considerable interspecific variation in body size (Wellenreuther and Clements 2007). As body size and habitat use are known to be related in other taxa (Nagel and Schluter 1998), it is possible that body size is related to habitat use in this group.

We used a combination of ecological and phylogenetic information to analyse the evolution of habitat specialisation in the New Zealand triplefin fauna. First, we tested whether species differ in the degree of habitat specialisation. Estimates of habitat specialisation were obtained with a novel statistical method that was developed to calculate the overlap between individuals in the quantitative use of habitat resources. This method has important advantages over the commonly used binary or discrete coding method (specialist versus generalist), as it allows the fine-scale differentiation in the degree of specialisation between species. Second, the evolution of habitat specialisation was

investigated in detail using comparative Bayesian phylogenetic methods. Specifically, we tested whether (i) the evolution of habitat specialisation displays a directional trend (i.e. from generalised to more specialised species), (ii) the evolutionary mode, tempo and phylogenetic association of habitat specialisation are consistent with a Brownian-motion model of trait evolution, and (iii) whether there is a correlation between body size and the degree of specialisation.

Materials and methods

Sampling and ecological data

Habitat use of triplefin species was recorded quantitatively in the Hauraki Gulf (36°36'S, 174°50'E) in northeastern New Zealand from January 2002 to May 2005 using underwater visual census. The Hauraki Gulf is large and has a wide variety of accessible habitats, enabling the quantification of habitat characteristics of a number of triplefin species over a range of exposures and depths at coastal and offshore sites. The habitat measures ranged from large-scale differences between-sites (exposure) to moderate-scale differences within-sites (depth), and fine-scale microhabitat differences (substratum types). Substratum types were measured as percentage coverage of the habitat (0–100%) and included rock, cobble, gravel, sand/mud, coralline algae and macroalgae. Species habitat use was quantified at a total of 33 sites (155 visual censuses) following the same methodology as described in Wellenreuther et al. (2007). Species for which sufficient habitat data (>10 observations of adults) could be recorded included *Bellapiscis lesleyae*, *Cryptichthys jojettae*, *Forsterygion flavonigrum*, *F. lapillum*, *F. malcolmi*, *F. varium*, *Grahamina capito*, *G. nigripenne*, *Karalepis stewarti*, *Obliquichthys maryannae*, *Notoclinops caerulepunctus*, *N. segmentatus*, *N. yaldwyni*, *Ruanoho decemdigitatus* and *R. whereo*. All of these species belong to endemic genera (Fricke 1994; Clements et al. 2000), and thus their nearest relatives are all present in New Zealand waters.

Analysis of habitat specialisation

The first goal was to assess the hypothesis that habitat specialisation differs between species. We calculated the degree of habitat specialisation for each species using the depth and exposure of the habitat (2 variables), the substratum use (7 structural variables), and lastly all habitat variables together (9 variables). We derived the specialisation coefficient for each species by comparing the similarity of habitat use between all individuals of a species (Table 1) using the double-scaled Euclidean distance measure (described in Wellenreuther et al. 2007). Briefly, this method works by calculating the squared discrepancy between two individuals on a variable and then dividing this value by the maximum possible squared discrepancy for that variable. Summing and taking the square root of these 'scaled' discrepancies across habitat variables yields a scaled Euclidean distance that varies between 0 and some value >1. Therefore, we carried out a further scaling operation to convert this coefficient into a unit metric (0–1) by dividing the Euclidean distance by the square-root of the number of variables comprising the distance computation, producing a coefficient between 0 (no distance) and 1 (maximum distance between variables given the maximum and minimum bounds for each variable). This dual scaling ensured that the coefficient were comparable as different variable magnitudes might otherwise distort a conventional Euclidean distance. Finally, in order to complete the process, we expressed the double-scaled

Table 1 Number of triplefins observed and maximum body size (standard length, SL) of species

Species	Number of individuals	Maximum length (SL)
<i>F. lapillum</i> ¹	2865	71
<i>G. nigripenne</i> ¹	152	88
<i>F. malcolmi</i> ²	80	123
<i>O. maryannae</i> ²	596	60
<i>R. decemdigitatus</i> ³	79	120
<i>R. whereo</i> ³	1371	81
<i>B. lesleyae</i>	99	65
<i>C. jojettae</i>	66	55
<i>F. flavonigrum</i>	247	53
<i>F. varium</i>	830	125
<i>G. capito</i>	178	91
<i>K. stewarti</i>	35	127
<i>N. caerulepunctus</i>	158	38
<i>N. segmentatus</i>	1894	49
<i>N. yaldwyni</i>	334	53

Superscripts denote sister-species pairs (Hickey and Clements 2005)

Euclidean distance as a similarity coefficient by subtracting it from 1, thus yielding the double-scaled Euclidean similarity (DSE-S) coefficient, which we used as a proxy of habitat specialisation. For each habitat variable, all individuals of a species were compared with each other and all resulting pairwise DSE-S coefficients were subsequently averaged to obtain the mean degree of species-wide specialisation of habitat use for that variable. To investigate whether habitat specialisation has mainly occurred in the physical or structural habitat the DSE-S values for (i) depth and exposure and (ii) structural habitat variables were combined. To investigate the overall evolutionary trends the DSE-S specialisation index for all 9 habitat variables was used. A DSE-S value of 0 indicates that all individuals within a species use completely different habitats (i.e. generalist), and 1 indicates that all individuals within a species use exactly the same habitats (i.e. specialist). The computer program “Habitat Diversity Analysis” (available from <http://www.pbmetrix.com>) was used to generate the calculations, and to construct the coefficient distribution percentiles to provide the mean. It should be noted that the DSE-S values cannot be used for statistical significance testing, because they do not present a vector of independent observations.

While the first two habitat specialisation analyses were conducted to examine separately the degree of specialisation in depth and exposure, and in substratum use, we conducted the phylogenetic analysis (see below) using the combined specialisation coefficients for all habitat variables, as the aim was to investigate the evolution of the overall degree of habitat specialisation in the fauna.

Evolution of habitat specialisation

The second goal was to assess the hypothesis that habitat specialisation shows a directional trend in the evolution of the New Zealand triplefin fauna. Common ancestry is the most likely reason why closely related species share similar ecological features, therefore data for related species cannot be considered as independent points in comparative studies (Harvey and Pagel 1991). For this reason, we employed a phylogenetic comparative method to investigate the evolution of habitat specialisation. The phylogeny and branch lengths for the 15 triplefin species were obtained using MrBayes (Ronquist and

Huelsenbeck 2003; Huelsenbeck and Ronquist 2001) based on fragments from three mitochondrial genes (12S, 16S and control region) and a nuclear gene (ETS2) (details published in Hickey and Clements 2005). To calculate the posterior probabilities distribution of trees, we conducted a Bayesian Markov Chain Monte Carlo analysis using the program BayesPhylogenies (Pagel et al. 2004, Pagel and Meade 2004). We used the mixture model in BayesPhylogenies, which allows different sites in the aligned data to evolve in qualitatively distinct ways, but does not require a priori assumptions about these patterns or the partitioning of the sequence data (Pagel and Meade 2004). We specified the sister-species pair *R. whero* and *R. decemdigitatus* as the outgroup following the topology of Hickey and Clements (2005). Trees were generated for 1 million generations, with sampling every 20,000 generations, and the first 20% were discarded as ‘burn-in’. Figure 1 shows a consensus network tree with posterior probabilities for the species relationships (Holland and Moulton 2003).

The ecological habitat dataset consisted of the mean specialisation DSE-S coefficients of the overall habitat use (see above) and maximum body length data (standard length) for each of the 15 triplefin species. We obtained triplefin body length data from specimen records at the Museum of New Zealand, Te Papa Tongarewa (Table 1). We analysed the evolution of habitat specialisation using the generalised least squares model implemented in BayesContinuous (Pagel and Meade 2004; Pagel et al. 2004), which takes phylogenetic uncertainty into account. Non-independence among taxa is controlled for by specifying a variance–covariance matrix based upon the assumed phylogeny. We used likelihood-ratio tests to test specific hypotheses. The log-likelihood of the model corresponding to a null hypothesis (H_0) is compared with the model for an alternative hypothesis (H_1), where the

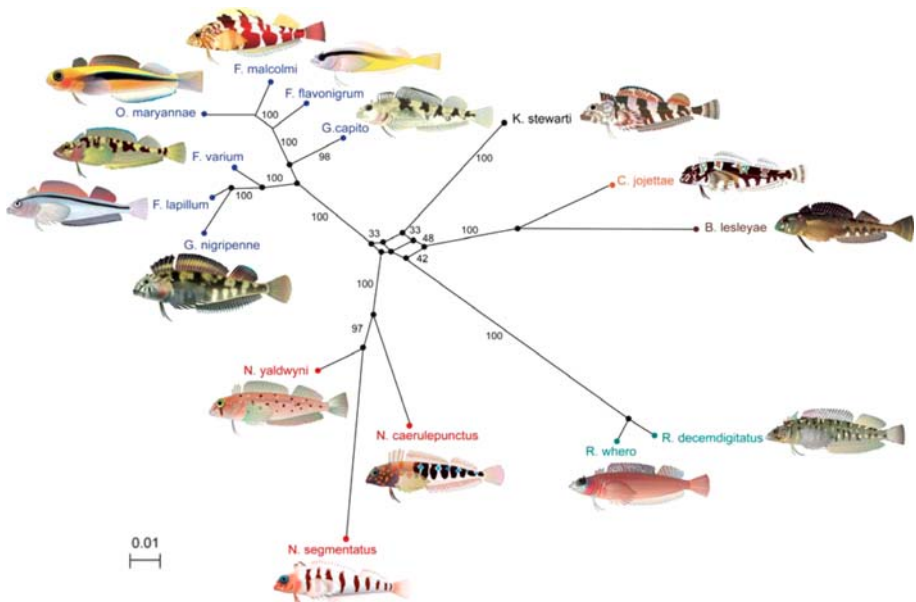


Fig. 1 Consensus network tree (Holland and Moulton 2003) with posterior probabilities for three mitochondrial genes (12S, 16S and control region) and a nuclear gene (ETS2) (sequencing details published in Hickey and Clements 2005) of 15 triplefin species ($x = 0.2$). The *Ruanoho* sister species pair was used as the outgroup for the analysis following the topology of Hickey and Clements (2005)

likelihood-ratio = $-2 \log [H_0/H_1]$. The likelihood-ratio statistic is asymptotically distributed as a chi-square variate with degrees of freedom equal to the difference in the number of parameters between the two models.

BayesContinuous incorporates the constant-variance random walk (Brownian-motion) model of character evolution, but it can also accommodate a directional component for trait evolution (Pagel 1997, 1999). We performed likelihood-ratio tests to determine whether a random walk model described the data significantly better than a directional random walk model. The random walk model has a single parameter, the instantaneous variance of evolution. This model would suggest that trait evolution had involved changes in both directions (i.e. simultaneous increase and decrease) across different paths in the phylogenetic tree. In contrast, the directional random walk assumes a model with a steady increase or decrease in trait value over time. The directional model accounts for the same variance of evolution as the non-directional model, but in addition has a parameter describing the tendency for directional change. It should be noted that under the random model the reconstructed ancestral state estimate is predicted to fall somewhere within the range of observed values in the species data. In contrast, the ancestral character state estimate under a directional model can lie outside of the range of observed values in the data (Pagel 1999). In addition, we also estimated the likelihood of directional evolution in habitat specialisation with kappa set to zero (for a definition of kappa see below), to test for bias introduced by differences in lineage-specific speciation rate. Setting kappa to zero forces all internode distances between branch tips and the root to be equal to 1, and thus branch lengths would be directly proportional to the number of speciation events on each branch. The log likelihood of this model was then compared with the likelihood of the model where habitat specialisation evolves randomly.

The third goal was to test whether the phylogenetic association, mode and tempo of trait evolution fit the model of Brownian motion. The program BayesContinuous allows estimation of three scaling parameters, denoted by lambda (λ), kappa (κ) and delta (δ), which describe phylogenetic associations, mode, and tempo of trait evolution, respectively. Lambda determines whether characters evolve independently of phylogeny. A lambda value of 1 indicates that phylogeny constrains the evolution of the trait, whereas a lambda value of 0 suggests that trait evolution has proceeded independently of phylogeny. Kappa measures punctuational versus gradual evolution of characters on a phylogeny. A kappa value of 0 suggests a punctuated mode of evolution in which changes in the degree of specialisation occurred rapidly, whereas a kappa value of 1 suggests that the evolution has proceeded in a gradual manner. Finally, delta determines whether character change is concentrated at the root or toward the tips of a phylogeny. A delta value of <1.0 suggests species-specific adaptation—that is, longer paths (i.e. paths from the root to the tips that contain greater numbers of nodes) contribute more to trait evolution than shorter ones. In contrast, a delta of >1.0 indicates a greater rate of evolution in the earlier states followed by slower rates of evolution among related species. These parameters can be estimated by comparing the log-likelihood of the H_0 model containing default values for the scaling parameters with the log-likelihood of the alternative H_1 model, in which one parameter is permitted to take its maximum likelihood value.

Lastly, the fourth goal was to assess the hypothesis that body size and habitat specialisation are phylogenetically related. In addition, body size divergence between sister-species pairs was calculated as the absolute value of the percentage size difference between the species. This was done to test whether divergence in body size between sister-species pairs has been associated with habitat specialisation.

Results

Triplefin species varied in the range of depths and exposures used, and this was reflected in high interspecific variation in the degree of specialisation (0.77–1.00, Fig. 2a). The high habitat specialisation of *G. nigripenne*, *B. lesleyae*, *G. capito* and *R. decemdigitatus* (0.94–1.00) reflected the fact that these four species had pronounced preferences for particular depths and exposures. *Cryptichthys jojettae* also showed small variation in depth and exposure, and had the fifth highest specialisation coefficient (0.91, Fig. 2a). All other species had specialisation coefficients of under 0.9, with three species having a coefficient of under 0.8 (i.e. *F. malcolmi*, *O. maryannae* and *F. flavonigrum*), indicating that these species are relatively generalised in terms of depth and exposure use (Fig. 2a). The degree of specialisation in substratum variables did not differ as much between species as for depth and exposure (Fig. 2b). *Bellapiscis lesleyae* had the highest specialisation coefficient (0.96), indicating the use of a narrow range of substratum variables (Fig. 2b). The specialisation coefficients of the remaining 14 triplefin species ranged from 0.72 to 0.83 (Fig. 2b). This shows that New Zealand triplefins have specialised predominately along the depth and exposure axis, while specialisation for substratum types appears to be less pronounced. Lastly, all variables (depth, exposure and substratum use) were analysed together to calculate the overall specialisation for each species (Fig. 2c). The specialisation coefficients for all habitat variables ranged from 0.72 to 0.96. Overall, *B. lesleyae* was by far the most specialised species (0.96), while *F. lapillum* and *G. capito* were the least specialised (<0.8) of the triplefin species examined (Fig. 2c). The value of specialisation coefficients was not attributable to the sample size of species ($r^2 = 0.19$, $P = 0.10$).

The directional model did not perform significantly better than the random walk model in describing phylogenetic differences in specialisation (Likelihood-ratio test = 1.83, $P = 0.18$). This test cannot unequivocally rule out a directional trend, but if evolution in the trait is proportional to genetic divergence then there is no evidence for directional effects. Similarly, there was no evidence that speciation rate on particular lineages affected our results, as the likelihood of directional evolution in habitat specialisation with kappa set to zero was also statistically not significant (Likelihood-ratio test = 1.98, $P = 0.16$). Thus the hypothesis that habitat specialisation in New Zealand triplefin fishes proceeds as a directional walk in time was rejected.

The model in which lambda was allowed to assume its maximum likelihood value did not perform significantly better than the model in which the default settings (=1) were used, indicating that phylogeny to some extent constrains the evolution of habitat specialisation observed (Likelihood-ratio test = 2.7, $P = 0.10$). Similarly, the maximum likelihood parameter of kappa did not perform significantly better than when the default parameters were used, rejecting punctuated evolution of habitat specialisation (Likelihood-ratio test = 0.17, $P = 0.68$). The maximum likelihood estimate for delta was 6.8 and differed significantly from the default settings of 1 (Likelihood-ratio test = 18, $P < 0.001$), suggestive of increased trait evolution towards the tips of a phylogeny.

Lastly, we tested whether the specialisation coefficients correlate phylogenetically with triplefin body size. The results showed no evidence for a trait correlation (Likelihood-ratio test = 0.017, $P = 0.90$), suggesting that the evolution of the two traits has proceeded independently. However, body size comparisons of sister-species pairs indicated high divergence in maximum body length (*F. lapillum* and *G. nigripenne* 21%, *F. malcolmi* and *O. maryannae* 69%, and *R. decemdigitatus* and *R. whereo* 39%, see Table 1), and in all cases the larger of the pair was the more specialised species (Fig. 2c).

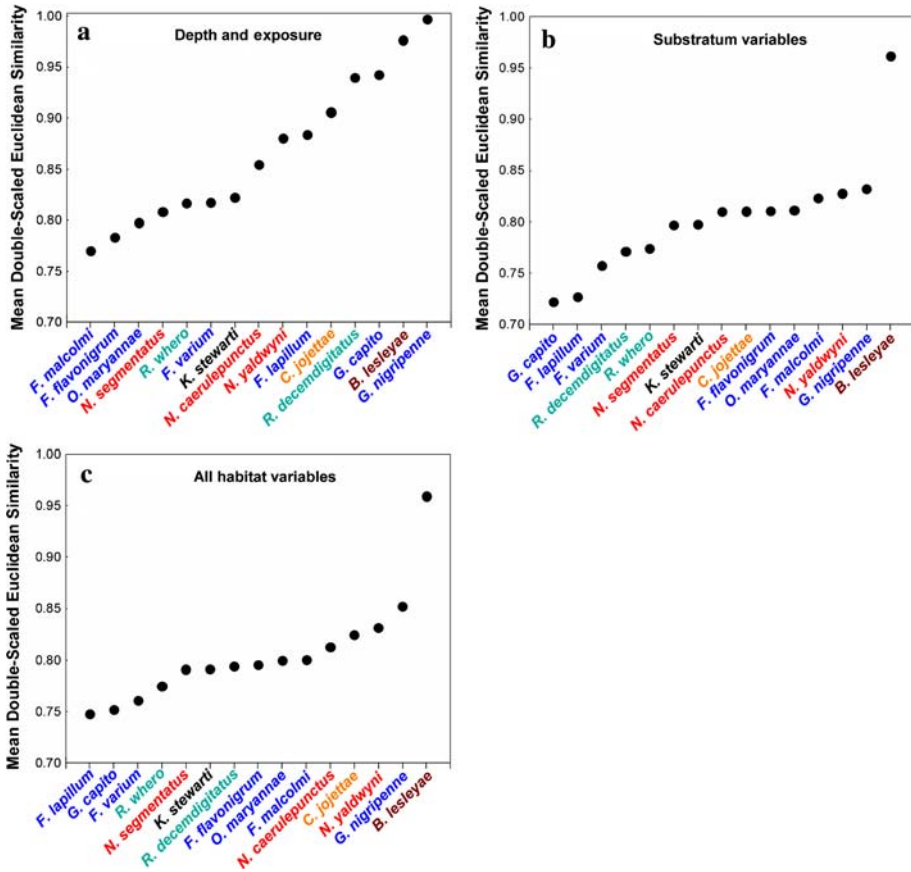


Fig. 2 Habitat specialisation coefficients of 15 triplefin species in (a) depth and exposure (b) substratum types and (c) both combined. The black circles show the mean double-scaled Euclidean similarity coefficient for each species. The colour codes present different genera

Discussion

It was traditionally thought that ecological diversification proceeds from generalised ancestors to more specialised descendants, and that ecological specialisation is largely irreversible once achieved (Mayr 1942; Futuyma and Moreno 1988; Simpson 1953). Here we show evidence that the evolution of habitat specialisation in the New Zealand triplefin radiation is phylogenetically constrained, but the data are not consistent with the traditional view that radiations are typically founded by resource generalists that give rise to descendants that become more specialised through time as species diversity increases.

Our study employed a method that allowed us to describe accurately the degree of habitat specialisation between species, and thus has important advantages over the commonly used discrete coding method (i.e. specialist species versus generalist species). First, our method is able to detect fine-scale differences in specialisation between species. Second, all specialisation coefficients are scaled between 0 and 1, and are thus comparable within and between studies. Lastly, our method is suitable to estimate specialisation for all

quantitatively measured resources (e.g. prey types ingested, temperature of the habitat), and thus has wide application. The vast majority of studies to date have coded character traits as binary, discrete entities when investigating the evolution of habitat specialisation (e.g. Nosil 2002; Desdevises et al. 2002; Rubinoff and Sperling 2002) and only few have considered the potential impact of the method by which species have been assigned a degree of specialisation. Stephens and Wiens (2003) used discrete and continuous coding methods to analyse emydid turtle evolution, and found that the use of different coding methods can have profound effects on the outcome of phylogenetic tests. Specifically, they suggested that the use of continuous coding methods may have produced the recent exceptions to the generalist-to-specialist hypothesis (Stephens and Wiens 2003). This highlights that classification of species into discrete groups can be problematic, as ecological traits typically vary continuously within and between species, thus, a discrete coding method is likely to fail to detect fine scale differences between species.

Our first aim was to examine whether New Zealand triplefin species differ in the degree of habitat specialisation. Interspecific comparisons demonstrated that species differ in the degree of specialisation for both (i) the depth and exposure and (ii) the substratum types of the habitat. Species generally showed a higher degree of resource specialisation in the depth and exposure of the habitat, thus indicating that the use of depth and exposure is less flexible than the use of substratum types. This is consistent with previous work on habitat partitioning in this group in that interspecific differences are mainly structured through partitioning in the exposure and depth of the habitat (Wellenreuther et al. 2007, *in press*; Feary and Clements 2006; Syms 1995). The combined analysis of all habitat variables demonstrated that *B. lesleyae* yielded the highest habitat specialisation coefficient in this group, as it was exclusively found in very shallow and rocky areas. We note that the variation in depth and exposure of this species may have been underestimated, as highly exposed intertidal habitats could not be sampled subtidally. However, given that not only the use of depth and exposure but also the use of substratum variables was indicative of specialisation, it is unlikely that the result was simply a sampling artefact. In comparison, *F. lapillum* and *G. capito* exhibited the widest habitat breadth, and can thus be described as the least specialised of the species studied. The remainder of the triplefin species surveyed may be considered as moderately specialised in terms of habitat use given the spectrum of habitat use between *B. lesleyae* and *F. lapillum*/*G. capito*.

Questions concerning species coexistence have been related to specialisation in resource utilisation. Specialists, by definition, have highest fitness in a narrow set of habitats and the trade-off is typically exhibited across habitat types, whereas generalists do not exhibit trade-offs across habitat types (McPeck 1996; Caley and Munday 2003). Many studies have demonstrated that such trade-offs facilitate the coexistence of habitat specialist and generalist species (e.g. Munday 2004; Kneitel and Chase 2004; Brown 1996). For example, Brown (1996) suggested that habitat generalists might coexist with competing habitat specialists if they exploit the shared environment at a larger spatial scale, because this would allow them to exploit whichever habitats are unused or underused by the more specialised species. The results of the present study suggest that habitat shifts of triplefin fishes are most likely to occur in the use of substratum types, as the selection of depth and exposure was generally more specialised in the vast majority of species. For example, the ability to use a wide range of habitats may allow *F. lapillum* and *G. capito* to use whichever substratum type is unused or underutilised by other species, as long as it is within the species' preferred depth and exposure range. Thus, coexistence in New Zealand triplefin species may be achieved by slight shifts in substratum use by the more generalised species, thereby allowing the survival of both species in the same area.

Our second aim was to use comparative phylogenetic methods to investigate whether the evolution of habitat specialisation in the New Zealand triplefin radiation shows a progressive sequence towards increasing specialisation. Bayesian analyses of triplefin habitat specialisation showed that the directional model did not fit the data significantly better than a random walk model, rejecting the notion that there is a general trend towards increasing or decreasing habitat specialisation. This indicates that the evolution of habitat specialisation has proceeded in both directions, towards one that favours specialisation and another that favours generalisation in habitat use. Furthermore, this also indicates that the degree of habitat specialisation in the common ancestor of these fishes (i.e. at the root of the phylogeny) most probably falls somewhere within the range of the observed values (Pagel 1999), and thus lies somewhere between the least and most specialised species. This finding is inconsistent with traditional models of adaptive radiation (e.g. Mayr 1942; Simpson 1953), in which a generalist ancestor is assumed to yield specialist descendants (reviewed in: Schluter 2000). This traditional model has commonly been explained by the greater probability of generalist species to access novel environments and resource types (Futuyma and Moreno 1988). Diversification of the founding species is thought to result in more and more specialised species, and this process is presumed to continue until new resources become scarce or until specialisation leads to an ecological ‘dead-end’ (Futuyma and Moreno 1988; Kelley and Farrell 1998). The results from the present study indicate that both expansion to utilise new resources and finer partitioning of existing resources have been involved in the diversification of New Zealand triplefin fishes.

The third aim of the study was to use phylogenetic analyses to investigate whether the mode and phylogenetic association of triplefin habitat specialisation conform to a Brownian-motion model of trait evolution and whether body size is correlated with the degree of specialisation. Maximum likelihood estimates showed that lambda (phylogenetic association) and kappa (phylogenetic mode) were not significantly different from the null hypothesis. These results demonstrate that the evolution of habitat specialisation in New Zealand triplefins has a phylogenetic component, and that habitat specialisation has occurred gradually over time. Lastly, the maximum likelihood value for delta was estimated to be 6.8 and fitted the data significantly better than 1. This indicates that differences between species in habitat specialisation have evolved more rapidly in later phases of evolution. Phylogenetic signal has been reported for the majority of comparative datasets (e.g. Freckleton et al. 2002), supporting the general assumption of a Brownian-motion model of trait evolution that more closely related species are more similar to each other than less closely related species. Further analysis of our data set demonstrated that the evolution of habitat specialisation has proceeded independently of body size. The absence of a significant trait correlation between body size and habitat specialisation suggests that body size evolution in New Zealand triplefin species is not clearly linked with specialisation in habitat use, however, it was interesting to note that the more specialised species of a sister-species pair generally had the greater size. This was particularly pronounced for the sister-species pairs *F. malcolmi*/*O. maryannae* and *R. decemdigitatus*/*R. whero*, and indicates that differences in body size may have been involved in species divergences.

In summary, habitat specialisation does not appear to have been an evolutionary impediment to ecological diversification in the New Zealand triplefin fauna. Instead, species appear to have followed different evolutionary trajectories in habitat specialisation, one in which species appear to have sub-partitioned available resources, while the other one led to an expansion in the use of resources. Fossil triplefin otoliths (Schwarzhan and Grenfell 2002) and a calibrated phylogeny of the group (Clements et al. unpublished) suggest that much of the extant diversity in this group arose in the Nukumaruan

(1.63–2.4 ma) and Mangapanian (2.4–3.0 ma) stages of the Pliocene. This was a period of cooling when the Miocene warm water mollusc fauna was replaced by the recent cool water mollusc fauna (Beu 2004; Beu et al. 2004), and thus New Zealand triplefins may have radiated under low levels of competition into habitats previously occupied by warm water species. These findings support the notion that continuous expansion into new resources and novel environments is one of the most widespread feature of many adaptive radiations (Schluter 2000). This finding stands in contrast to the long standing hypothesis that ecological generalists give rise to specialists more often than the reverse (e.g. Simpson 1953; Mayr 1942), but is in accord with recent studies on the evolution of resource specialisation in fishes (Elliott et al. 1999) and turtles (Stephens and Wiens 2003) and the host range evolution of phytophagous insects (Nosil 2002; Nosil and Mooers 2005; Crespi and Sandoval 2000; Sandoval and Nosil 2005).

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