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Multi-tissue stable-isotope analyses can identify dietary specialization

Alexander L. Bond[1]*†, Timothy D. Jardine[2] and Keith A. Hobson[1]‡

[1]*Environment Canada, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, Canada; and* [2]*School of Environment and Sustainability, Toxicology Centre, University of Saskatchewan, Saskatoon, SK S7N 5B3, Canada*

Summary

1. Individual specialization along one or more niche axes is now recognized as an integral and ubiquitous aspect of populations. A major challenge, however, is quantifying the level of specialization using robust metrics that are applicable across species and ecosystems. Measuring stable-isotope values in multiple tissues with different isotopic turnover rates could be one mechanism for quantifying specialization.

2. We used simulation studies of stable-isotope values to investigate how the recently proposed relative index of specialization varies in relation to variance in prey isotope values, diet–tissue discrimination factors, specialist group size and tissue half-life, and applied specialization metrics to two systems – Australian freshwater fish and marine birds in the Canadian Arctic.

3. In all simulations, populations comprised entirely of generalists were easily separated from those with even small amounts (5%) of individual specialization. In some cases, however, specialization measured using isotope values with bimodal distributions may appear similar to those with univariate distributions, but this can be detected by examining the original data. All fish and bird species examined showed varying degrees of individual specialization.

4. Analysing stable isotopes in multiple tissues can provide a useful index of the degree of specialization within a population that can be compared to the same metric measured in other groups or species.

Key-words: Food Webs, individual specialization, Mitchell River, Northwater Polynya, simulation study, stable isotopes

Introduction

Individual specialization, or the residual variation in a population's niche after accounting for age, sex or morphotype, has consequences for species' survival and evolution (Bolnick *et al.* 2003; Araújo, Bolnick & Layman 2011). This concept was recognized by Darwin (1859) and first formalized as part of the niche variation hypothesis, which postulates that wider niches at the population level (as a consequence of individual specialization) would result in greater morphological variation (Van Valen 1965) and larger populations (Roughgarden 1972), a hypothesis that has generally been supported (Bolnick *et al.* 2007). Despite this, many ecological studies treat individuals within a population as homogenous and unvarying, in the face of increasing evidence to the contrary (Bolnick *et al.* 2002, 2003; Layman, Newsome & Crawford 2015). While measuring species' niches can be challenging (Colwell & Fuentes 1975; Futuyma & Moreno 1988; Devictor *et al.* 2010), the increased realization that individual specialization is widespread means it is an important attribute to try to understand and quantify.

Quantifying the degree of specialization, beyond a dichotomous specialist/generalist label using objective criteria, has only recently been operationalized (Bolnick *et al.* 2002; Matich, Heithaus & Layman 2011). Within any population, there will be some degree of specialization, where some individuals exploit different resources along an axis or axes of their niche space (Roughgarden 1974; Araújo *et al.* 2007; Newsome *et al.* 2007; Araújo, Bolnick & Layman 2011). To move beyond 'specialization in the eye of the beholder' (Futuyma & Moreno 1988) and classifications that are largely dependent on the niche axes that are studied (e.g. prey composition, prey size, habitat temperature; Bolnick *et al.* 2002), we need robust, transferable metrics to quantify this important ecological attribute.

Individual specialization is most often measured in the diets of individuals, but can reflect any sort of resource use (Bolnick *et al.* 2002), and typically concerns the realized niche (Chase & Leibold 2003). This is done by partitioning a population's niche into two components: a within-individual component (variance in resource use within individuals, WIC) and a between-individual component (variance in resource use among individuals, BIC). Together, WIC and BIC measures of variance are the total niche width (TNW) of a population

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*Correspondence author. E-mail: alex.bond@rspb.org.uk
Present addresses:
†RSPB Centre for Conservation Science, Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, UK
‡Department of Biology, Western University, Biological & Geological Sciences Building, London, ON N6A 5B7, Canada

(Roughgarden 1972, 1974). The degree of specialization can be measured as the proportion of TNW expressed by WIC (WIC/TNW; Bolnick *et al.* 2002), or as the ratio of BIC/WIC as a relative index of specialization (RIS; Matich, Heithaus & Layman 2011).

Variation in stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values in animal tissues has been used frequently to measure specialization (Fry, Joern & Parker 1978; Gu, Schelske & Hoyer 1997; Bearhop *et al.* 2004; Matthews & Mazumder 2004). Because stable-isotope values of body tissues reflect nutrients assimilated over many feeding events, they integrate morphological, physiological and behavioural specialization (Futuyma & Moreno 1988). There are, however, several caveats to the use of stable isotopes to measure individual specialization (Matthews & Mazumder 2004; Araújo, Bolnick & Layman 2011). First, variation in prey stable-isotope values can affect the classification of consumer populations as generalists or specialists because limited variance in isotope values across different prey groups may mask individual specialization (Matthews & Mazumder 2004), though simulation exercises can overcome this problem (Araújo *et al.* 2007). Secondly, the use of models like IndSpec (Bolnick *et al.* 2002) and RInSp (Zaccarelli, Bolnick & Mancinelli 2013) generally requires the diet of individuals to be measured as proportional contributions of different sources (e.g. per cent biomass or per cent frequency of occurrence from gut contents). To derive these proportions from stable-isotope data, all possible prey sources must also be analysed and the data included in mixing models (e.g. Parnell *et al.* 2010, 2013). These models require diet–tissue discrimination factors (the change in isotope values between prey and consumer) that can vary depending on the tissue, diet, age and species of the individual, and are only meaningful if possible prey sources are isotopically distinct (Caut, Angulo & Courchamp 2009; Bond & Diamond 2011). Thirdly, the relative number of individuals that exhibit specialist behaviour may disproportionately influence the overall specialization index of the population. If the majority of individuals in a population are generalists, detecting specialization may prove challenging (Poisot *et al.* 2012). Finally, the recent promising development of exploiting differential tissue isotopic turnover rates to determine RIS (Matich, Heithaus & Layman 2011) may also be sensitive to the relative difference in turnover rates among the tissues selected (Boecklen *et al.* 2011). It is also important to note the difference between dietary and isotopic specialization. Individuals feeding on the same prey resources may differ isotopically, while those with similar stable-isotope values may differ in their diet (Bond & Jones 2009; Karnovsky, Hobson & Iverson 2012). The link between the measured stable-isotope values and ecology of the species and individuals must be considered.

To date, the relative sensitivity of specialization metrics to the above confounding factors has never been evaluated (Matthews & Mazumder 2004). Our objective was to critically examine the role of isotopic variance among prey types, diet–tissue isotopic discrimination values, number of specialist individuals in a population, number of specialist subpopulations and tissue turnover rates in quantifying individual specialization. We did this by simulating data for generalists and specialists under two- and three-prey source scenarios to evaluate the utility and transferability of recently reported specialization indices using multi-tissue isotope models (Matich, Heithaus & Layman 2011). We then considered how the confounding factors listed above might influence the interpretation of WIC, BIC and RIS by calculating these indices in two case studies: freshwater fish from tropical Australia, and marine birds in the Canadian Arctic.

Materials and methods

SIMULATIONS

We defined 'generalists' as a population of individuals exploiting the same resources in similar proportions and 'specialists' as a population of individuals who differ from each other in their resource use. Under these definitions, a population that relies on one or a few resources, but where individuals do not differ in their resource use, is equivalent to a generalist population (Bolnick *et al.* 2003; Bearhop *et al.* 2004). We assumed that prey items were at isotopic equilibrium, a common assumption when using stable-isotope values to infer diet (Boecklen *et al.* 2011; Hopkins III & Ferguson 2012).

Our starting conditions consisted of a generalist population of 20 individuals that exploited two food sources equally (50%), and a specialist population comprised of two groups of 10 individuals: one that

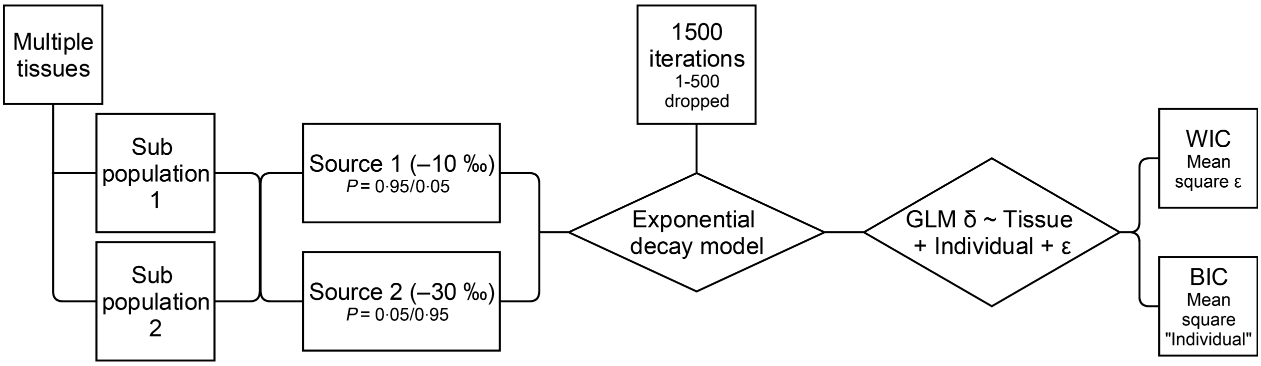

Fig. 1. A conceptual diagram showing the simulation of two-source specialization models for two subpopulations.

exploited one food source 95% of the time and another source 5% of the time (Subpop1, Fig. 1) and the other in the opposite proportions (5% and 95%, Subpop2, Fig. 1). The two food sources had $\delta^{13}C$ values of –10·0 ± 1·0‰ and –30·0 ± 1·0‰, and two tissues were sampled from each consumer, one with a half-life of 5 days typical of a metabolically active tissue such as liver and the other with a longer half-life of 100 days commonly observed in cartilaginous and calcareous tissues (Boecklen *et al.* 2011). We did not consider more complex aspects of physiology, such as isotopic routing or concentration-dependent models (Voigt *et al.* 2008; Boecklen *et al.* 2011).

We modelled BIC and WIC and calculated WIC/TNW and RIS (Matich, Heithaus & Layman 2011) for the generalists and specialists and determined the effect of (i) differences in the isotope values of food sources, (ii) differences in isotopic diet–tissue discrimination factors, (iii) differences in the size of specialist groups, (iv) the number of specialist groups and (v) differences in tissue elemental turnover rates. In all simulations, we used a dynamic exponential decay model where 20 individuals were iterated over 1500 time steps to simulate repeated sampling. Consumer populations' initial isotope value was set at the mean of the sources weighted for their proportional use, and at each time step, they consumed one of the resources with a predefined probability. We discarded the first 500 iterations, which represented the maximum time to obtain isotopic equilibrium [calculated as five times the longest tissue isotopic half-life ($t_{1/2}$)], and used means from the remaining 1000 iterations (Fig. 1).

We then extended this model to include three possible isotopic sources: –10·0 ± 1·0‰, –20·0 ± 1·0‰ and –30·0 ± 1·0‰ with varying dietary combinations. In these cases, generalists consumed 33·33% from each source, and specialists consumed either 90% of one source and 5% of each of the other two (Specialist A; Table 1) or 45% from two sources, with 10% from the remaining source (Specialist B; Table 1, Fig. S1, Supporting Information). We included this three-source system because individuals specializing on a prey of intermediate isotopic value (e.g. −20‰) would be similar to those consuming equal parts of prey with extreme isotopic values (e.g. −10‰ and −30‰) (Matthews & Mazumder 2004).

At each time step, we estimated WIC and BIC based on analysis of variance (anova) outputs where stable-isotope values were a function of 'tissue', and 'individual' was included as a random factor. BIC is the mean sum of squares of the 'individual' factor, and WIC is the residual mean sum of squares (Matich, Heithaus & Layman 2011). We then calculated WIC/TNW (where TNW = WIC + BIC) and RIS (where RIS = BIC/WIC). All analyses were conducted in r 3.1.3 (R Core Team 2015).

SIMULATION PARAMETERS

To test how the difference in prey isotope values affected specialization indices, we used the conditions described above and performed 80 iterations, adjusting source isotope values by ±0·25‰ at each step starting at –10·0‰ and –30·00‰ and ending at –15·0‰ and –15·25‰, respectively. This allowed us to examine how pure specialists and generalists vary in WIC, BIC and TNW across the range of source values commonly observed in natural systems (Matthews & Mazumder 2004; Fry 2006). We used a single-isotope approach, calculating specialization using one isotope (e.g. $\delta^{13}C$), following Matich, Heithaus & Layman (2011).

We modelled the effect of diet–tissue discrimination factors, again starting with the initial conditions detailed above, and performing 30 iterations, each one increasing the difference in discrimination factors between the two tissues in increments of 0·2‰ to a maximum of 6·0‰ that represents the natural range of values observed across species and tissues (Post 2002; Caut, Angulo & Courchamp 2009). Based on the results (see below), we only considered a two-source system.

We tested the effects of specialist group size by starting with the original conditions of the specialist population with an even split between the two groups [10 individuals specializing on one food source (Subpop1) and 10 specializing on another (Subpop2)]. We then varied the number of individuals belonging to Subpop1 and Subpop2 until we had every combination from 1 to 19 individuals in each group. We examined the effect of the number of subpopulations by simulating the effect of varying source differences on populations with 2, 4, 6, 8 and 10 specialist subpopulations using the two-source model, with subpopulations distributed evenly across the resource gradient (e.g. for four subpopulations, the proportions of one source were 5%, 33%, 66%, and 95%). We also performed similar analyses using simulated proportional data (i.e. output from stable-isotope mixing models) for populations with 2 and 10 specialist subpopulations and found identical results.

To assess the effect of differences in tissue half-lives on specialization indices, we simulated a similar data set by starting with two tissues where $t_{1/2}$ = 2 and 200 days. These represent extreme values observed in laboratory diet-switch studies for fast (avian blood plasma) and slow (adult fish blood cells) turnover tissues (Boecklen *et al.* 2011). We then adjusted the $t_{1/2}$ by ±0·5 days at each step, ending when $t_{1/2}$ = 100 and 101 days (199 iterations).

To model the responses of specialists using three sources, we used the same parameterization as in the two-source modelling, but ran three different groupings for Specialist A (A1, A2, A3) and three for Specialist B (B1, B2, B3; Table 1). When modelling the effect of the difference among sources, we calculated the source difference as the difference between the two extreme sources; the middle source remained unchanged.

We used two case studies to evaluate how specialization indices might vary among co-habiting species and some of the caveats associated with their interpretation. We chose these case studies because they differ considerably in taxonomy and climate, food sources in these systems are well-characterized (Hobson *et al.* 2002; Hobson & Bond

Table 1. Model inputs of dietary source proportions for two subpopulations of specialists consuming three isotopically distinct sources

Specialist group	Subpopulation 1			Subpopulation 2		
	Source 1 (–10‰)	Source 2 (–20‰)	Source 3 (–30‰)	Source 1 (–10‰)	Source 2 (–20‰)	Source 3 (–30‰)
A1	90	5	5	5	90	5
A2	90	5	5	5	5	90
A3	5	90	5	5	5	90
B1	10	45	45	45	10	45
B2	10	45	45	45	45	10
B3	45	10	45	45	45	10

2012; Jardine *et al.* 2012), and multiple tissues were sampled from each of three species, allowing for calculation of specialization indices according to Matich, Heithaus & Layman (2011). They are therefore illustrative of how these indices might be compared within and among systems.

CASE STUDY 1: AUSTRALIAN FRESHWATER FISH

The Mitchell River in northern Queensland, Australia, is a productive floodplain river with multiple habitats available for foraging by fishes. Fish in this system have access to both dry and wet season food source pathways, ranging from ^{13}C-depleted riverine insects that feed on plankton and periphyton to ^{13}C-enriched marine prey, with seasonally available floodplain resources (a mix of aquatic, semi-aquatic and terrestrial insects, crustaceans and molluscs) having intermediate δ^{13}C values (Jardine *et al.* 2012). Specialization in this instance is therefore largely driven by habitat specialization. We used stable-isotope data from muscle, and liver, from barramundi (*Lates calcarifer*, $n = 9$), catfish (*Neoarius graeffei*, $n = 9$) and gizzard shad (*Nematalosa come*, $n = 9$) to test for individual dietary specialization using both the original isotope data (δ^{13}C) and source-proportion data from a linear mixing model. For simplicity, this latter model used the two extreme end-members in this system, riverine prey ($\delta^{13}\text{C} = -27{\cdot}4$‰) and marine prey ($\delta^{13}\text{C} = -17{\cdot}1$‰), to calculate a proportion freshwater diet for each tissue in each individual.

CASE STUDY 2: ARCTIC MARINE BIRDS

The Northwater Polynya in Canada's Arctic supports millions of breeding seabirds and is the most productive polynya or seasonally ice-free body of water (Hobson *et al.* 2002). We studied specialization in three seabirds: planktivorous dovekies (*Alle alle*, $n = 9$), and piscivorous thick-billed murres (*Uria lomvia*, $n = 10$) and black-legged kittiwakes (*Rissa tridactyla*, $n = 11$) using isotope data from bone collagen, muscle and liver (Hobson & Bond 2012). Because we lacked comprehensive prey data from this system, we did not attempt to calculate source proportions using the isotope data and instead calculated metrics using the raw data only. For all species in both case studies, data from multiple tissues were sampled from the same individual using a multivariate approach (Matich, Heithaus & Layman 2011).

Results

SIMULATIONS

Differences in the δ^{13}C value of prey sources affected specialization indices of specialists but not generalists. The former had RIS values >50 while the latter remained *c.* 1 regardless of source differences, and WIC/TNW was essentially 0 in specialists and around 0·5 for generalists. Difficulty separating specialists and generalists using WIC/TNW and RIS arose only when δ^{13}C differences between sources were <1·0‰ (Fig. 2). Above source differences of 5·0‰, specialists approached an asymptote for WIC/TNW of ~0 and for RIS of 50–100, while TNW exhibited an exponential increase that was driven by increases in BIC. In most three-source simulations (A1, B1, B2, A3, B3), specialists and generalists could also be separated on the basis of WIC/TNW and RIS when δ^{13}C differences were >1·0‰. However, when extreme values had the greatest probability of selection (A2), there was no difference in the RIS of specialists and generalist (Fig. S2).

Changing the diet–tissue discrimination factors between the two tissues did not change the specialization parameters. Specialists' RIS ranged from 129 to 150 (Fig. 3), while that of generalists ranged from only 1·04 to 1·10.

The size of specialist groups had little effect on the RIS of the populations. With even one specialist in 20 individuals, the RIS was markedly higher than generalist populations in every

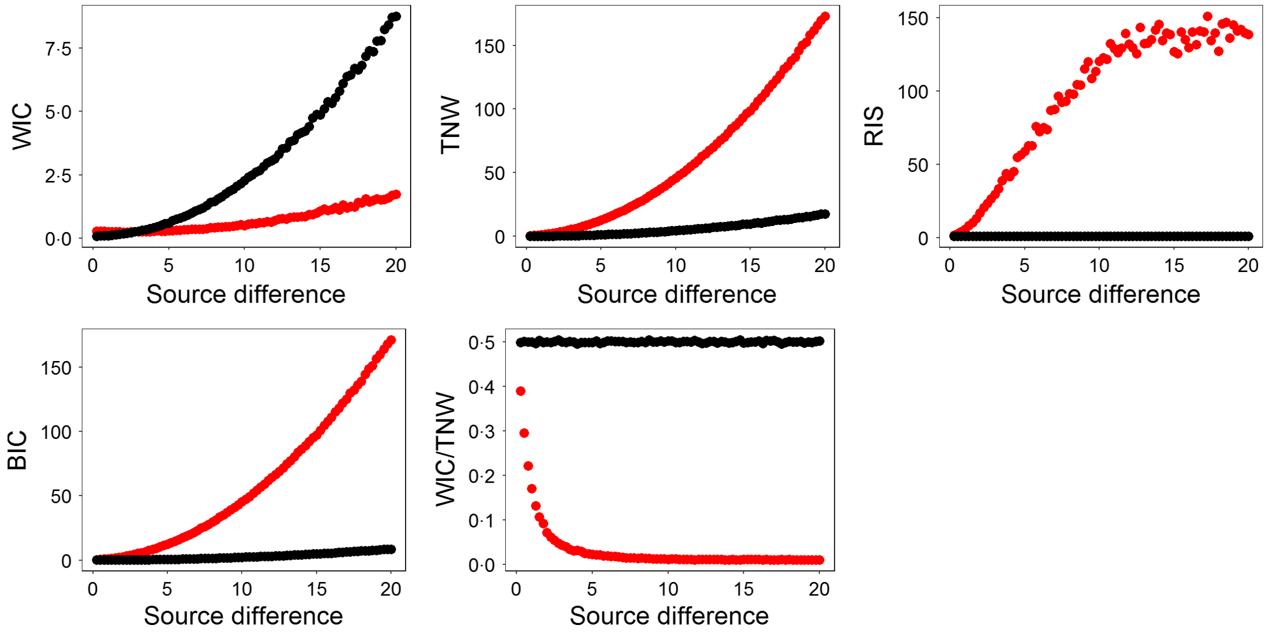

Fig. 2. Specialization indices for two-source simulations of specialist (red) and generalist (black) populations showing the effect of altering the difference between the source isotope values.

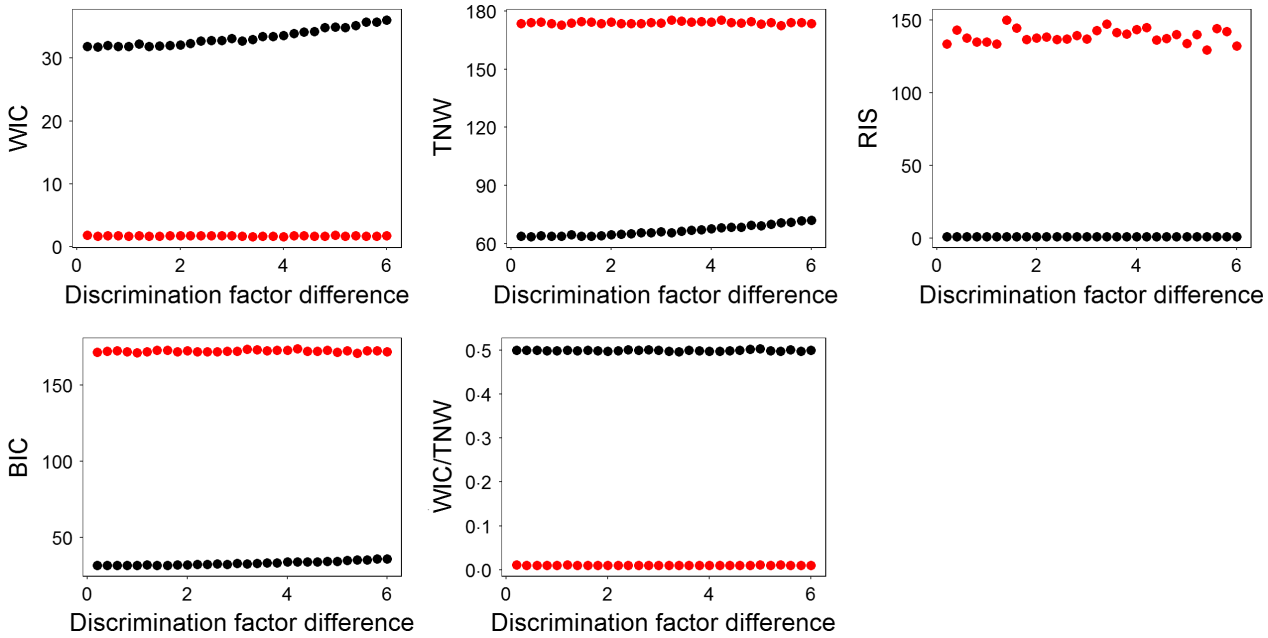

Fig. 3. Specialization indices for two-source simulations of specialist (red) and generalist (black) populations showing the effect of altering the difference in diet–tissue discrimination factors between two tissues.

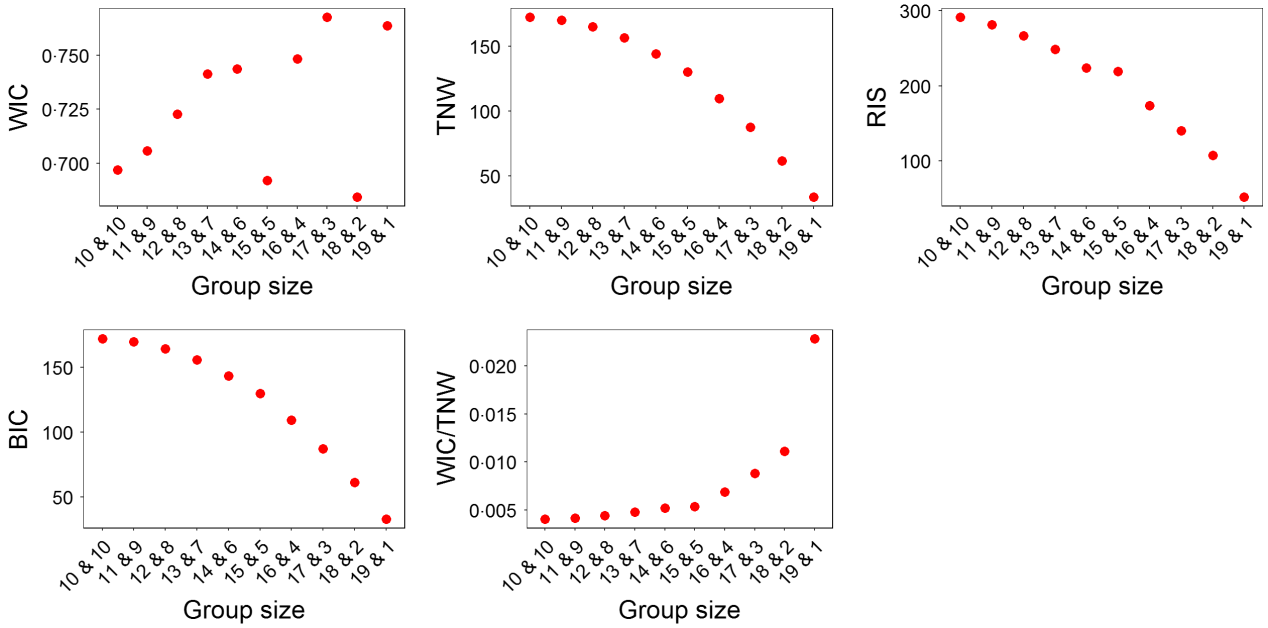

Fig. 4. Specialization indices for two-source simulations of specialist (red) populations showing the effect of altering the size of specialist subpopulations.

simulation, except A2 and B2 (Figs 4 and S3). In these cases where WIC/TNW and RIS could not distinguish between specialists and generalists (A2 and B2), a secondary analysis using frequency distributions of consumer isotope values was able to separate the two. Specialist populations exhibiting these source combinations have bimodal rather than unimodal distributions (Fig. S4).

Unlike the limited effect of the size of specialist groups, the number of subpopulations drastically affected the RIS at any given point along the scale of source differences. In general, the RIS decreased (a result of a small increase in WIC, but large decrease in BIC) with an increasing number of specialist subpopulations (Fig. 5). While the RIS remained high when there were two subpopulations (1·73–147·02), for four subpopulations and above, RIS declined to 1·35–23·06, approaching but never equalling values for generalists (Fig. 5).

Similar to results for the evaluation of source differences, varying the difference in half-lives of two tissues changed

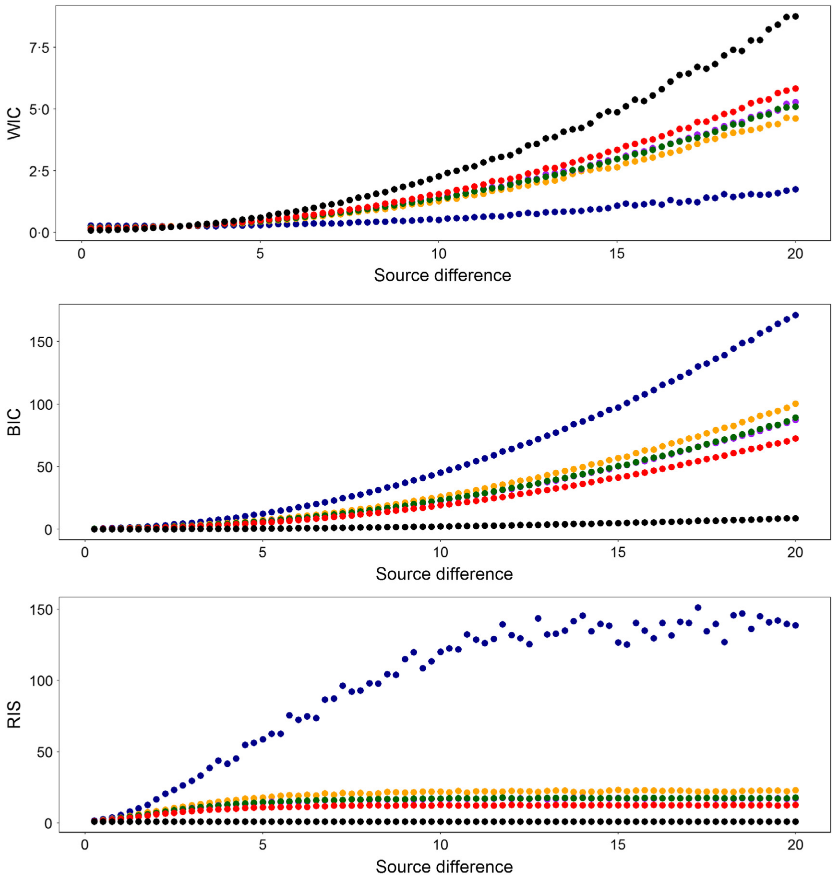

Fig. 5. The effect of the number of subpopulations (dark blue: 2, orange: 4, purple: 6, green: 8, red: 10, black: generalists) on RIS values from two-source simulations altering the difference between isotopic sources.

specialists' RIS, but not that of generalists. Populations of specialists were also always distinguishable from populations of generalists when using WIC/TNW and RIS (Fig. 6). The same pattern emerged for most three-source models (A1, B1, A3, B3), the exception being A2 and B2, in which the extreme endpoints had the greatest probability of being sampled (Table 1) resulting in a lower BIC and an RIS indistinguishable between specialists and generalists (Fig. S5), but these were also distinguishable based on histograms.

AUSTRALIAN FRESHWATER FISH

Within-individual components (WIC) for all three fish species were low (<0·005 using mixing model outputs), and, driven by low BIC, predatory barramundi had the lowest RIS of the three species (2·47). Herbivorous gizzard shad and omnivorous catfish had higher BIC and therefore higher RIS (6·46 and 11·60) than barramundi (Table 2). The estimates of RIS using δ^{13}C values (range 2·47–8·94) were very similar to those derived from source proportions (proportion of freshwater carbon) (Table 2).

ARCTIC CANADIAN MARINE BIRDS

The three bird species exhibited much higher levels of individual specialization (RIS range: 19·43–56·36 for raw data) compared with the Australian fish (Table 2). While the WIC was similar (range: 0·26–0·31), the birds had much higher BIC (6·06–14·93) compared with the Australian fish (Table 2). Dovekies, which eat mostly zooplankton and small fish, had the least amount of individual specialization (RIS: 19·43) and piscivorous black-legged kittiwakes the highest (RIS: 56·36) which rivalled that of our simulated specialists.

Discussion

In general, we found that dietary specialization indices (WIC/TNW and RIS) calculated from multi-tissue isotope data are robust to known confounding factors such as the range in source isotope ratios and differences in tissue turnover rates. In all cases, true generalists, those feeding equally on a suite of resources, always had a WIC/TNW around 0·5 and an RIS around 1·0. We note that our WIC/TNW values deviate somewhat from those conceptualized previously (Bolnick *et al.* 2003), where this metric ranges from 0 to 1 depending on the underlying distribution of values. Our simulated generalists had values comparable to a scenario with two populations having only partially overlapping resource use (scenario 'B' in Fig. 2 from Bolnick *et al.* 2003). In our simulations, any degree of individual specialization within the population (including as little as 1/20 individuals) resulted in a decrease in WIC/TNW and an increase in RIS, approximating two populations with vastly divergent diets (scenario 'C' in Fig. 2 from Bolnick *et al.* 2003). Differences in source isotope values and tissue half-lives also drove changes in these indices,

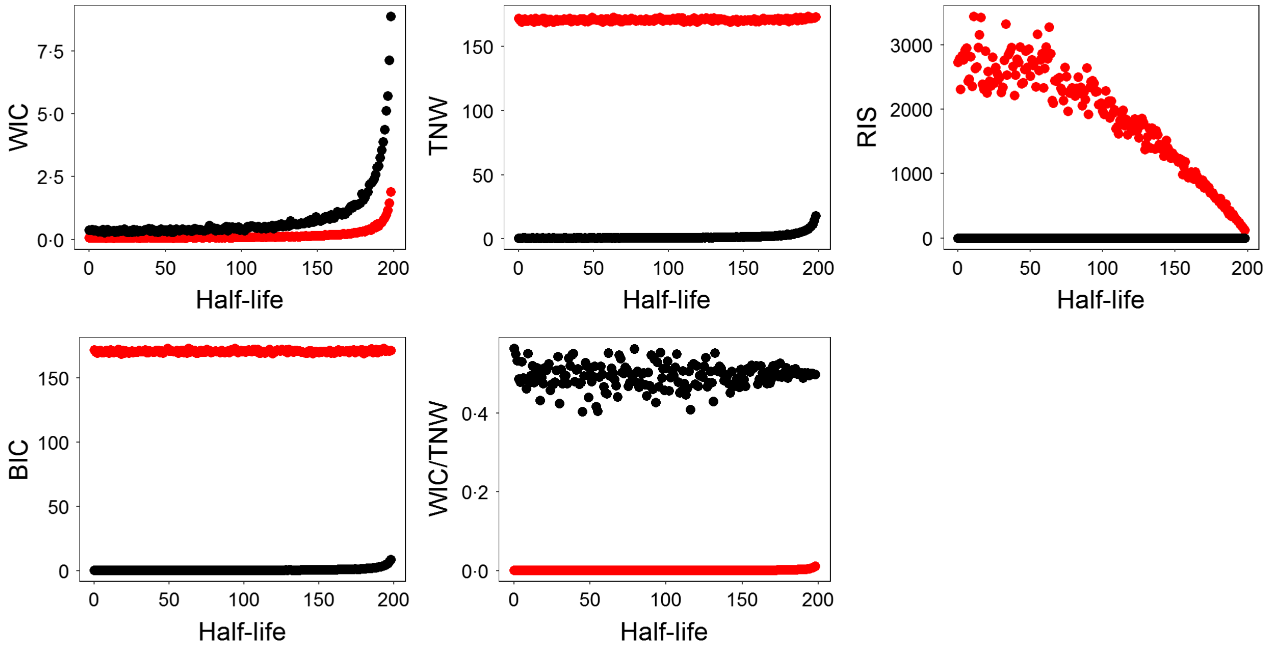

Fig. 6. Specialization indices for two-source simulations of specialist (red) and generalist (black) populations showing the effect of altering the tissue half-lives.

Table 2. Dietary specialization indices for Australian freshwater fish, and Canadian Arctic marine birds based on multiple tissue isotope values and on estimated source proportions (freshwater fish only)

	Isotope values			Proportion estimates		
Species	WIC	BIC	RIS	WIC	BIC	RIS
Lates calcarifer	0·20	0·50	2·47	0·002	0·005	2·47
Nematalosa come	0·45	2·84	6·31	0·004	0·027	6·46
Neoarius graeffei	0·27	2·37	8·94	0·002	0·021	11·60
Alle alle	0·31	6·06	19·43	–	–	–
Rissa tridactyla	0·26	14·93	56·36	–	–	–
Uria lomvia	0·28	12·23	43·62	–	–	–

WIC, within-individual component; BIC, between-individual component; RIS, relative index of specialization.

approaching asymptotes above a source difference of 5–10‰ and a half-life difference of 10–50 days. These thresholds therefore establish the minimum criteria under which one-time, multi-tissue sampling of consumers will allow meaningful quantification of dietary specialization (Bolnick *et al.* 2002, 2003; Matich, Heithaus & Layman 2011).

An important assumption is that prey are in isotopic equilibrium (i.e. the stable-isotope values in tissues of predators reflect the time-integrated average of the local environment) (Woodland *et al.* 2012). When this is not the case (e.g. for environments with seasonally or spatially variable source isotope values such as flowing waters; Woodland *et al.* 2012; Jardine *et al.* 2014), the degree of dietary specialization may be overestimated, as it will appear that individuals have a broader range of stable-isotope values in the fast-turnover tissues being compared. Complementing isotope analyses with stomach content analyses can help mitigate this challenge by providing additional evidence that organisms are consuming different prey types, rather than simply tracking isotopic variability in space and time

An important feature of the multi-isotope approach is that a generalist will never exhibit low WIC/TNW or high RIS. This means that 'false positives' of specialization are less of a concern. However, while we demonstrated that the WIC/TNW and RIS consistently separate generalists and specialists using the multi-tissue isotope approach, there were exceptions. Simulations where two specialist subpopulations converged on the same mid-point (groups A2 and B2) were indistinguishable from generalist populations using RIS therefore representing a 'false negative'. These two groups can be separated simply by examining the modality of the consumers' isotope values or source-proportion estimates. But the advantage of an indexing method is moving beyond describing the simple presence of specialization (which is likely ubiquitous to some extent), to describing the degree of specialization. As the number of specialist groups within a subpopulation increases, the distribution will tend towards unimodality, as in Matthews & Mazumder's (2004) 'random generalists, large range' and 'individual specialists, small range' example.

The number of specialist subpopulations affected the magnitude of RIS, and in all cases, it was greater than generalists' RIS over a wide range of isotopic source differences with the exception of very small differences (<1–2‰; Fig. 5). The asymptotic RIS differed depending on the number of subpopulations, decreasing with an increasing number of specialist groups. This is expected, and generalist populations (which could alternatively be described as a very large number of small specialist populations) reach an RIS asymptote near 1.

Using stable-isotope values to examine dietary specialization, as opposed to gut content analysis, is advantageous because the former indicate assimilated nutrients, and they integrate morphology, physiology and behaviour (Bolnick *et al.* 2007). Further, if isotope values vary spatially in a predictable way, they may capture habitat specialization that might not be evident based on gut contents because prey items may be common to multiple habitats. Previous applications of stable-isotope data to measure dietary specialization compared calculated WIC/TNW to null-model-derived estimates (Matthews & Mazumder 2004; Araújo *et al.* 2007) or used isotope values to calculate proportional contributions to diet (Matich, Heithaus & Layman 2011). The diet–tissue discrimination factors required in such calculations introduce both statistical error (through their inherent uncertainty and quantified as the SD) and possibly decrease accuracy of the resulting niche axis measurements (Bond & Diamond 2011).

Stable-isotope values can also indicate aspects of individual habitat specialization, as they vary spatially at several scales in marine, freshwater and terrestrial systems (Hobson, Piatt & Pitocchelli 1994; Hobson *et al.* 2012a, b; McMahon, Hamady & Thorrold 2013). Sampling multiple tissues could therefore provide some indication of habitat specialization at the individual level at a variety of spatial and temporal scales (Quillfeldt *et al.* 2008). Consumers foraging on the same resource over different habitats (e.g. littoral and pelagic zones in lakes) would show greater isotopic variance (Post 2002). Similarly, prey isotope values that vary considerably over time (e.g. plankton in freshwater lakes; Zohary *et al.* 1994) will increase the variance of isotope values in predators. By using relative measures, the RIS should be robust to temporal variation.

In our case studies, within ecosystems, the relative ranking of specialization of the three species was the same regardless of whether the raw isotope data were used to calculate WIC/TNW and RIS or whether mixing models were first used to calculate source proportions (Parnell *et al.* 2010). By either measure, barramundi in Australia and dovekies in the Northwater Polynya were the least specialized species relative to their conspecifics. In the case of barramundi, their low RIS of 2·47 was near that of our simulated generalists (RIS $\approx$ 1). The distribution of data for barramundi was normal (Shapiro–Wilk: $P = 0{\cdot}802$) so the low RIS was not due to a bimodal distribution that can artificially depress RIS (Fig. S4), suggesting that specialization is indeed low in this species. At the other extreme, both catfish and black-legged kittiwakes had high RIS values, the latter having values that rivalled those of our simulated specialists. We note, however, that even our most specialized fish species had a low RIS relative to bull sharks in a system with similar isotopic characteristics (Matich, Heithaus & Layman 2011), and all of our fishes had relatively low BIC, suggesting limited among-individual variation in diet. The euryhaline fish species gizzard shad had the highest BIC and highest WIC because their populations can disperse into distinct habitats where they likely forage before converging on common spawning grounds.

Other factors such as trophic position could explain variation in specialization. Higher trophic level organisms may exhibit reduced specialization because they can channel two dominant but distinct energy pathways such as algal and detrital sources or couple two different habitats such as pelagic and littoral (Rooney *et al.* 2006; McCann 2011). A very high WIC for tiger sharks (0·021), generated using source-proportion data, led to a low RIS for this predator (Matich, Heithaus & Layman 2011), comparable to our predatory barramundi. However, another predator, the bull shark had high BIC and low WIC, resulting in high RIS values suggesting specialization (Matich, Heithaus & Layman 2011). This disparity was also observed in our avian data. Planktivorous dovekies had the lowest RIS of the three bird species, possibly a function of the limited spatial variation in plankton δ^{13}C in oceanic environments, while piscivorous murres were highly specialized, with δ^{13}C ranging from –21·8 to –19·3‰, and very high BIC (range: 6·06–14·93), matching behavioural observations of murre foraging (Woo *et al.* 2008; Elliott, Woo & Gaston 2009). Black-legged Kittiwakes had the highest BIC of any species group (56·36), indicating a large degree of specialization among individuals, again matching behavioural observations (Irons 1998; McKnight *et al.* 2011; Ceia & Ramos 2015).

Our measures of dietary specialization are based on the stable-isotope values of consumers, which can vary by species, sex, tissue, diet, location and many other factors (Bearhop *et al.* 2002; Cherel & Hobson 2007; Codron *et al.* 2012). Furthermore, individuals with different stable-isotope values in the same tissue synthesized at the same time may have fundamentally different diets, and individuals with similar diets may have different stable-isotope values, which are mediated by the various factors listed above. It is therefore crucial to separate the difference between inferences of diet measured using conventional means and isotopic composition (Bond & Jones 2009; Karnovsky, Hobson & Iverson 2012).

Resource limitation could also promote specialization because trophic efficiencies may drive niche partitioning (Bolnick *et al.* 2003; Araújo *et al.* 2007). As such, we might expect productive tropical freshwaters (Davies, Bunn & Hamilton 2008) and productive Arctic oceans (Sakshuag 2004) to contain organisms with generalist diets (Hobson, Piatt & Pitocchelli 1994; Blanchette *et al.* 2014). However, both our tropical fishes and Arctic birds had broad variation in specialization, suggesting that variation among species within ecosystems may be greater than specialization of ecological communities across ecosystems. Regardless, the use of multi-tissue analysis opens up possibilities for the testing of numerous hypotheses proposed to control specialization (Araújo, Bolnick & Layman 2011). As with most applications of stable isotopes to trophic ecology, however, we urge caution when interpreting results in systems with poorly quantified variability in prey isotope values or population substructure.

Conclusions

Measuring individual dietary specialization in populations can be accomplished with stable-isotope values from two contrasting tissues that differ in their isotopic turnover rate. Care must be taken, however, when interpreting RIS values, as the distribution of prey isotope data may hide evidence of specialization (as in our cases A2 and B2). Further research should focus on comparisons of inert tissues (feathers, hair) or serial non-destructive sampling of the same tissue (e.g. blood) over time, and whether similar conclusions about dietary specialization can be drawn.

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Data accessibility

Data sets and R scripts deposited on figshare: http://dx.doi.org/10.6084/m9.figshare.2058834.v1 (Bond, Jardine & Hobson, 2016).

References

Araújo, M.S., Bolnick, D.I. & Layman, C.A. (2011) The ecological causes of individual specialisation. *Ecology Letters*, **14**, 948–958.

Araújo, M.S., Bolnick, D.I., Machado, G., Giaretta, A. & dos Reis, S.F. (2007) Using δ^{13}C stable isotopes to quantify individual-level diet variation. *Oecologia*, **142**, 643–654.

Bearhop, S., Waldron, S., Votier, S.C. & Furness, R.W. (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology*, **75**, 451–458.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & MacLeod, H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, **73**, 1007–1012.

Blanchette, M.L., Davis, A.M., Jardine, T.D. & Pearson, R.G. (2014) Omnivory and opportunism characterise food webs in a large dry-tropics river system. *Freshwater Science*, **33**, 142–158.

Boecklen, W.J., Yarnes, C.T., Cook, B.A. & James, A.C. (2011) On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 411–440.

Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M. & Svanbäck, R. (2002) Measuring individual-level resource specialization. *Ecology*, **83**, 2936–2941.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. & Forister, M.L. (2003) The ecology of individuals: incidence and implications of individual specialization. *American Naturalist*, **161**, 1–28.

Bolnick, D.I., Svanbäck, R., Araújo, M.S. & Persson, L. (2007) Comparative support for the niche variation hypothesis that more generalized populations are also more heterogeneous. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 10075–10079.

Bond, A.L. & Diamond, A.W. (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications*, **21**, 1017–1023.

Bond, A.L., Jardine, T.D. & Hobson, K.A. (2016) Data and code from "Multi-tissue stable-isotope analyses can identify dietary specialization". *Methods in Ecology and Evolution*, doi: https://dx.doi.org/10.6084/m9.figshare.2058834.v1

Bond, A.L. & Jones, I.L. (2009) A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions and caveats. *Marine Ornithology*, **37**, 183–188.

Caut, S., Angulo, E. & Courchamp, F. (2009) Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, **46**, 443–453.

Ceia, F.R. & Ramos, J.A. (2015) Individual specialization in the foraging and feeding strategies of seabirds: a review. *Marine Biology*, **162**, 1923–1938.

Chase, J.M. & Leibold, M.A. (2003) *Ecological Niches: Linking Classical and Contemporary Approaches*. University of Chicago Press, Chicago, IL, USA.

Cherel, Y. & Hobson, K.A. (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Marine Ecology Progress Series*, **329**, 281–287.

Codron, D., Sponheimer, M., Codron, J., Newton, I., Lanham, J.L. & Clauss, M. (2012) The confounding effects of source isotopic heterogeneity on consumer–diet and tissue–tissue stable isotope relationships. *Oecologia*, **169**, 939–953.

Colwell, R.K. & Fuentes, E.R. (1975) Experimental studies of the niche. *Annual Review of Ecology and Systematics*, **6**, 281–310.

Darwin, C.R. (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. John Murray, London, UK.

Davies, P.M., Bunn, S.E. & Hamilton, S.K. (2008) Primary production in tropical streams and rivers. *Tropical Stream Ecology*, 1st edn (ed. D. Dudgeon), pp. 23–42. Academic press, London, UK.

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., Venail, P., Villéger, S. & Mouquet, N. (2010) Defining and measuring ecological specialization. *Journal of Applied Ecology*, **47**, 15–25.

Elliott, K.H., Woo, K.J. & Gaston, A.J. (2009) Specialization in murres: the story of eight specialists. *Waterbirds*, **32**, 491–506.

Fry, B. (2006) *Stable Isotope Ecology*. Springer, New York, NY, USA.

Fry, B., Joern, A. & Parker, P.L. (1978) Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. *Ecology*, **59**, 498–506.

Futuyma, D.J. & Moreno, G. (1988) The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, **19**, 207–233.

Gu, B., Schelske, C.L. & Hoyer, M.V. (1997) Intrapopulation feeding diversity in blue tilapia: evidence from stable-isotope analyses. *Ecology*, **78**, 2263–2266.

Hobson, K.A. & Bond, A.L. (2012) Extending an indicator: year-round information on seabird trophic ecology using multiple-tissue stable-isotope analyses. *Marine Ecology Progress Series*, **461**, 233–243.

Hobson, K.A., Piatt, J.F. & Pitocchelli, J. (1994) Using stable isotopes to determine seabird trophic relationships. *Journal of Animal Ecology*, **63**, 786–798.

Hobson, K.A., Fisk, A.T., Karnovsky, N.J., Holst, M., Gagnon, J.-M. & Fortier, M. (2002) A stable isotope (δ^{13}C, δ^{15}N) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. *Deep-Sea Research Part II Topical Studies in Oceanography*, **49**, 5131–5150.

Hobson, K.A., Ofukany, A.F.A., Soto, D.X. & Wassenaar, L.I. (2012a) An isotopic baseline (δ^{13}C, δ^{15}N) for fishes of Lake Winnipeg: implications for investigating impacts of eutrophication and invasive species. *Journal of Great Lakes Research*, **38**, 58–65.

Hobson, K.A., Van Wilgenburg, S.L., Wassenaar, L.I., Powell, R.L., Still, C.J. & Craine, J.M. (2012b) A multi-isotope (δ^{13}C, δ^{15}N, δ^{2}H) feather isoscape to assign Afrotropical migrant birds to origins. *Ecosphere*, **3**, art. 44.

Hopkins III J.B. & Ferguson, J.M. (2012) Estimating the diets of animals using stable isotopes and a comprehensive Bayesian mixing model. *PLoS One*, **7**, e28478.

Irons, D.B. (1998) Foraging area fidelity of individual seabirds in relation to tidal cycles and flock feeding. *Ecology*, **79**, 647–655.

Jardine, T.D., Pusey, B.J., Hamilton, S.K., Pettit, N.E., Davies, P.M., Douglas, M.M., Sinnamon, V., Halliday, I.A. & Bunn, S.E. (2012) Fish mediate high food web connectivity in the lower reaches of a tropical floodplain river. *Oecologia*, **168**, 829–838.

Jardine, T.D., Hadwen, W.L., Hamilton, S.K., Hladyz, S., Mitrovic, S.M., Kidd, K.A. *et al.* (2014) Understanding and overcoming baseline isotopic variability in running waters. *River Research and Applications*, **30**, 155–165.

Karnovsky, N.J., Hobson, K.A. & Iverson, S.J. (2012) From lavage to lipids: estimating diets of seabirds. *Marine Ecology Progress Series*, **451**, 263–284.

Layman, C.A., Newsome, S.D. & Crawford, T.G. (2015) Individual-level niche specialization within populations: emerging areas of study. *Oecologia*, **178**, 1–4.

Matich, P., Heithaus, M.R. & Layman, C.A. (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. *Journal of Animal Ecology*, **80**, 294–305.

Matthews, B. & Mazumder, A. (2004) A critical evaluation of intrapopulation variation of δ^{13}C and isotopic evidence of individual specialization. *Oecologia*, **140**, 361–371.

McCann, K.S. (2011) *Food Webs*. Princeton University Press, Princeton, NJ, USA.

McKnight, A., Irons, D.B., Allyn, A.J., Sullivan, K.M. & Suryan, R.M. (2011) Winter dispersal and activity patterns of postbreeding black-legged kittiwakes *Rissa tridactyla* from Prince William Sound, Alaska. *Marine Ecology Progress Series*, **442**, 241–253.

McMahon, K.W., Hamady, L.L. & Thorrold, S.R. (2013) A review of ecogeochemistry approaches to estimating movements of marine animals. *Limnology and Oceanography*, **58**, 697–714.

Newsome, S.D., Martínez del Rio, C., Bearhop, S. & Phillips, D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, **5**, 429–436.

Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L. (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS One*, **5**, e9672.

Parnell, A., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W. *et al.* (2013) Bayesian stable isotope mixing models. *Environmetrics*, **24**, 387–399.

Poisot, T., Canard, E., Mouquet, N. & Hochberg, M.E. (2012) A comparative study of ecological specialization estimators. *Methods in Ecology and Evolution*, **3**, 537–544.

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

Quillfeldt, P., McGill, R.A.R., Masello, J.F., Weiss, F., Strange, I.J., Brickle, P. & Furness, R.W. (2008) Stable isotope analysis reveals sexual and environmental variability and individual consistency in foraging of Thin-billed Prions. *Marine Ecology Progress Series*, **373**, 137–148.

R Core Team (2015) *R: A Language and Environment for Statistical Computing*, Version 3.1.3 [computer program]. R Foundation for Statistical Computing, Vienna, Austria.

Rooney, N., McCann, K.S., Gellner, G. & Moore, J.C. (2006) Structural asymmetry and the stability of diverse food webs. *Nature*, **442**, 265–269.

Roughgarden, J. (1972) Evolution of niche width. *American Naturalist*, **106**, 683–718.

Roughgarden, J. (1974) Niche width: biogeographic patterns among *Anolis* lizards. *American Naturalist*, **108**, 429–442.

Sakshuag, E. (2004) Primary and secondary production in the Arctic seas. *The Organic Carbon Cycle in the Arctic Ocean* (eds R. Stein & R.W. MacDonald), pp. 57–81. Springer, Berlin, Germany.

Van Valen, L. (1965) Morphological variation and width of ecological niche. *American Naturalist*, **99**, 377–390.

Voigt, C.C., Rex, K., Michener, R.H. & Speakman, J.R. (2008) Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissue and exhaled breath. *Oecologia*, **157**, 31–40.

Woo, K.J., Elliott, K.H., Davidson, M., Gaston, A.J. & Davoren, G.K. (2008) Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. *Journal of Animal Ecology*, **77**, 1082–1091.

Woodland, R.J., Rodríguez, M.A., Magnan, P., Glémet, H. & Cabana, G. (2012) Incorporating temporally dynamic baselines in isotopic mixing models. *Ecology*, **93**, 131–144.

Zaccarelli, N., Bolnick, D.I. & Mancinelli, G. (2013) RInSp: an R package for the analysis of individual specialization in resource use. *Methods in Ecology and Evolution*, **4**, 1018–1023.

Zohary, T., Erez, J., Gophen, M., Berman-Frank, I. & Stiller, M. (1994) Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. *Limnology and Oceanography*, **39**, 1030–1043.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Conceptual diagrams of three-source simulations.

Fig. S2. Specialization indices for three-source simulations. Comparisons of specialization indices for specialists and generalists when altering the difference in stable isotope endpoints.

Fig. S3. Specialization indices for three-source simulations. Comparisons of specialization indices for specialists and generalists when altering the size of specialist groups.

Fig. S4. Consumer stable isotope values of two specialist groups (red) and a generalist (black) from scenario A2 simulated for 100 individuals.

Fig. S5. Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference in tissue half-lives.

Multi-tissue stable-isotope analyses can identify dietary specialization

Alexander L. Bond, Timothy D. Jardine, and Keith A. Hobson

Supporting Information

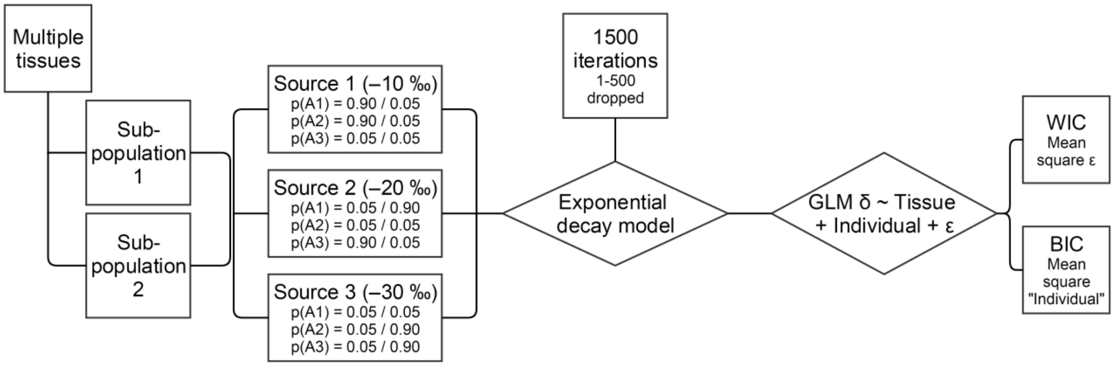

Figure S1a. A conceptual diagram showing the simulation of three-source specialization models for two subpopulations for scenarios A1, A2, and A3 (see Table 1)

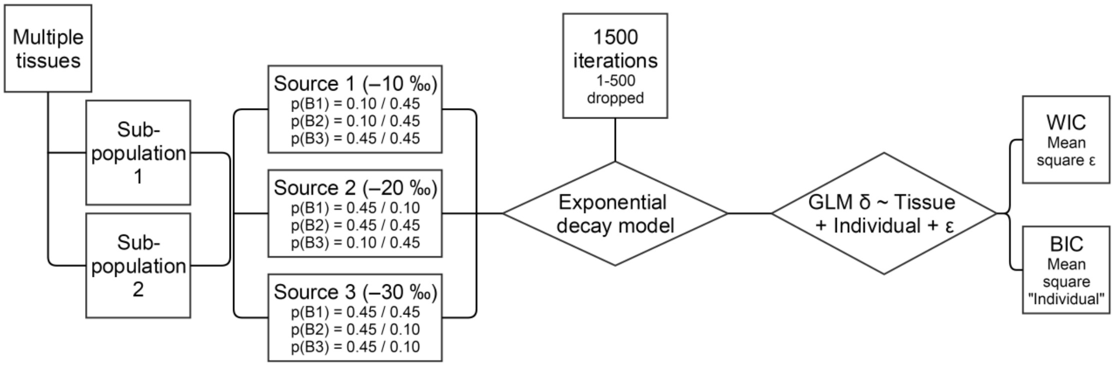

Figure S1b. A conceptual diagram showing the simulation of three-source specialization models for two subpopulations for scenarios B1, B2, and B3 (see Table 1).

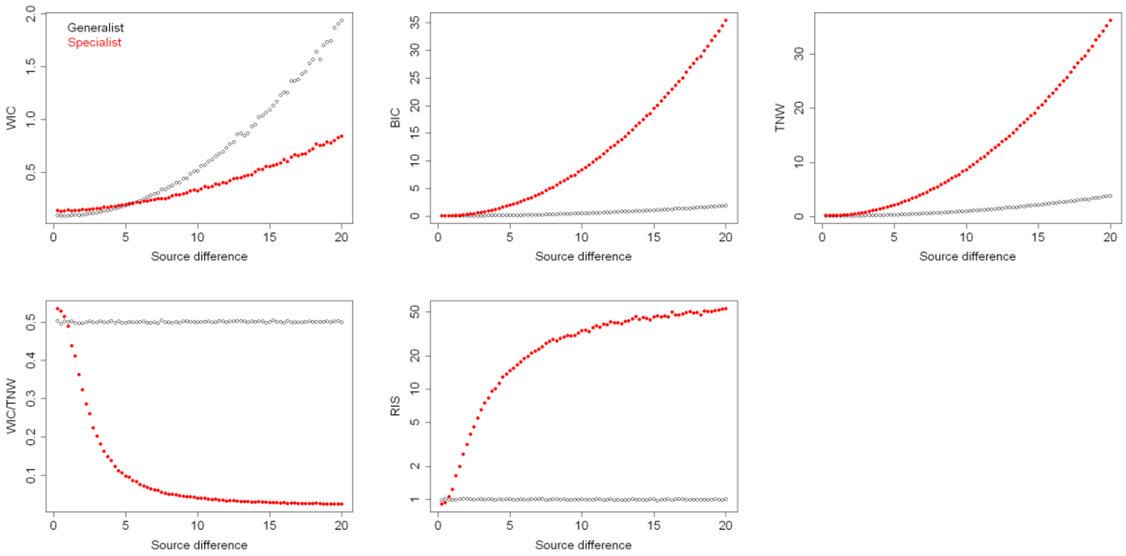

Figure S2a. Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference between the source isotope values in scenario A1 (Table 1).

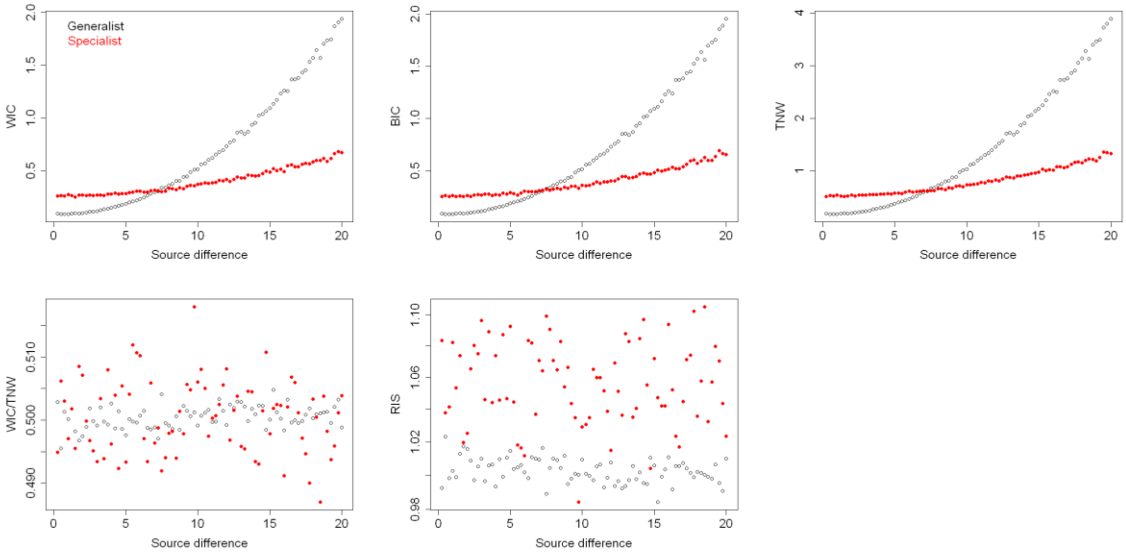

Figure S2a. Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference between the source isotope values in scenario A2 (Table 1).

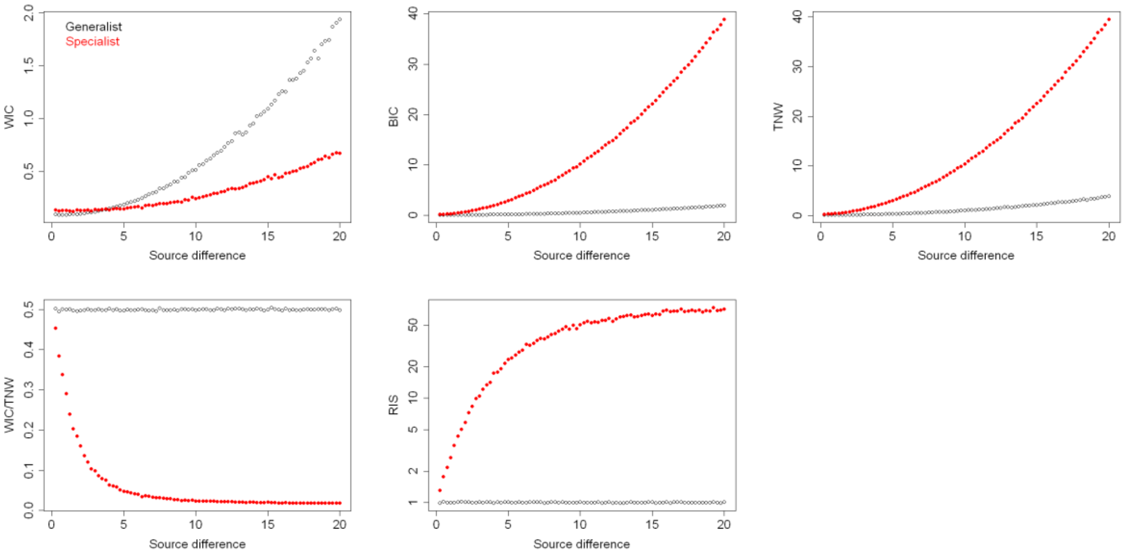

Figure S2c – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference between the source isotope values in scenario A3 (Table 1).

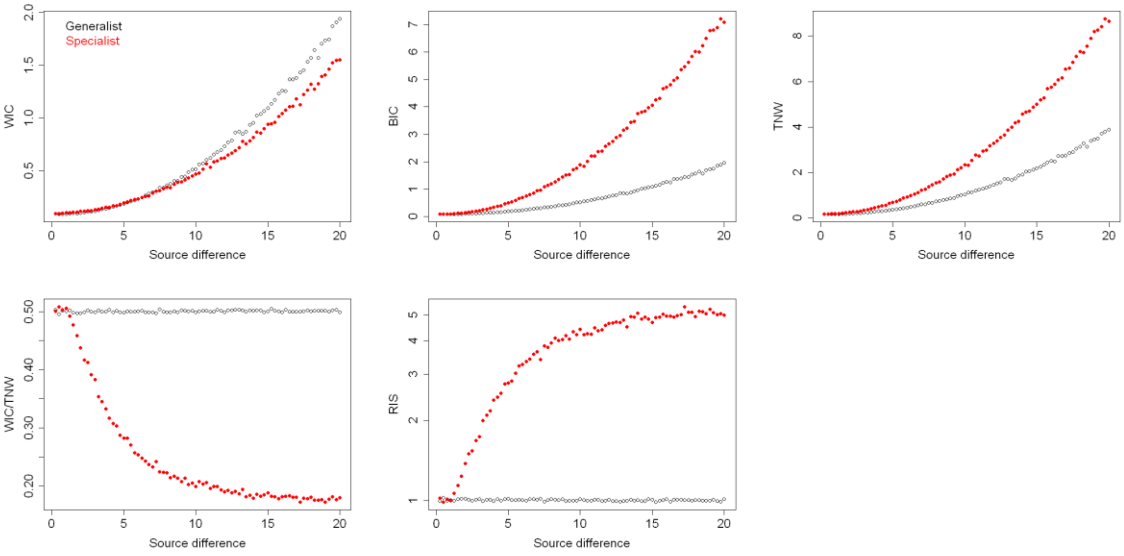

Figure S2d – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference between the source isotope values in scenario B1 (Table 1).

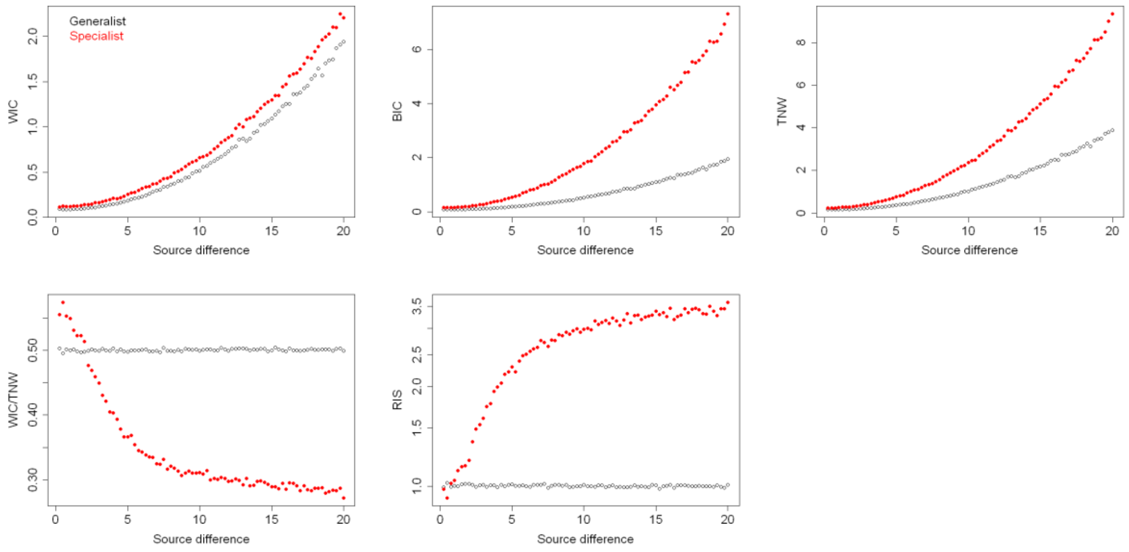

Figure S2e – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference between the source isotope values in scenario B2 (Table 1).

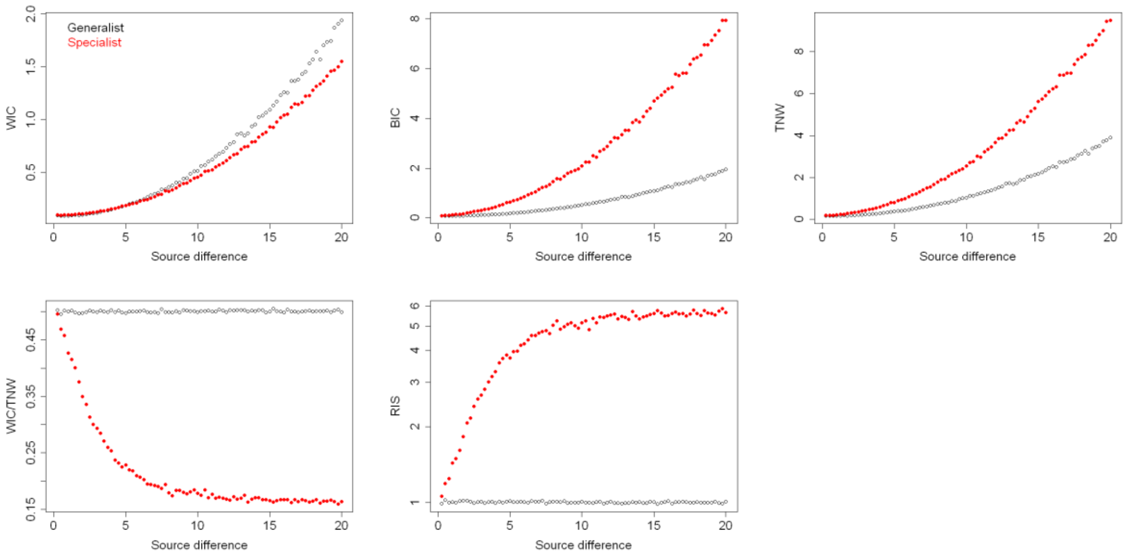

Figure S2f – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference between the source isotope values in scenario B3 (Table 1).

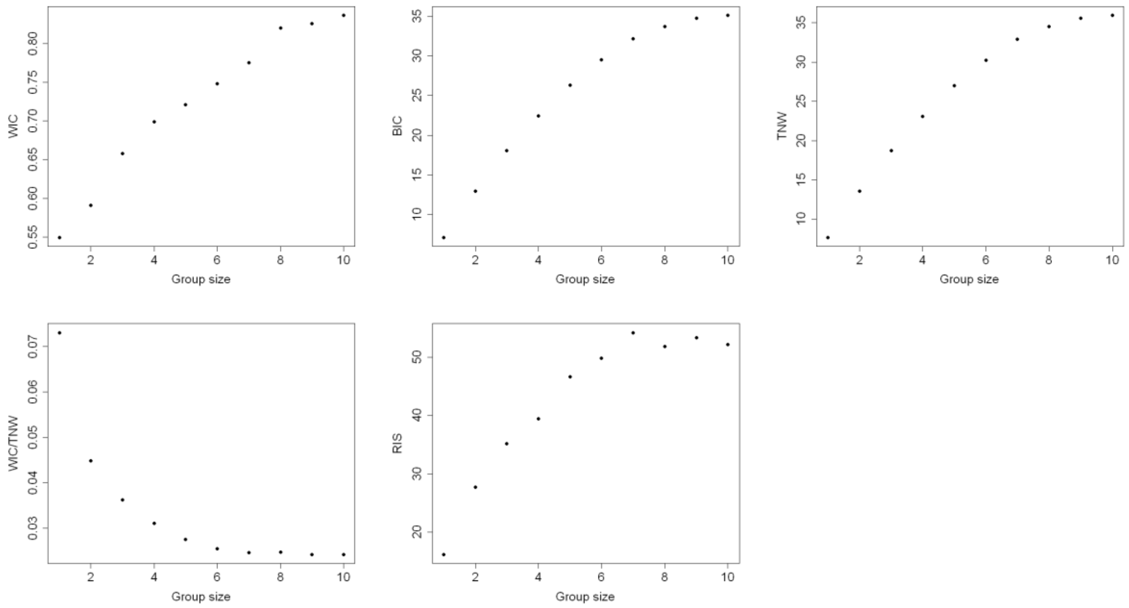

Figure S3a – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the size of specialist groups in scenario A1 (Table 1).

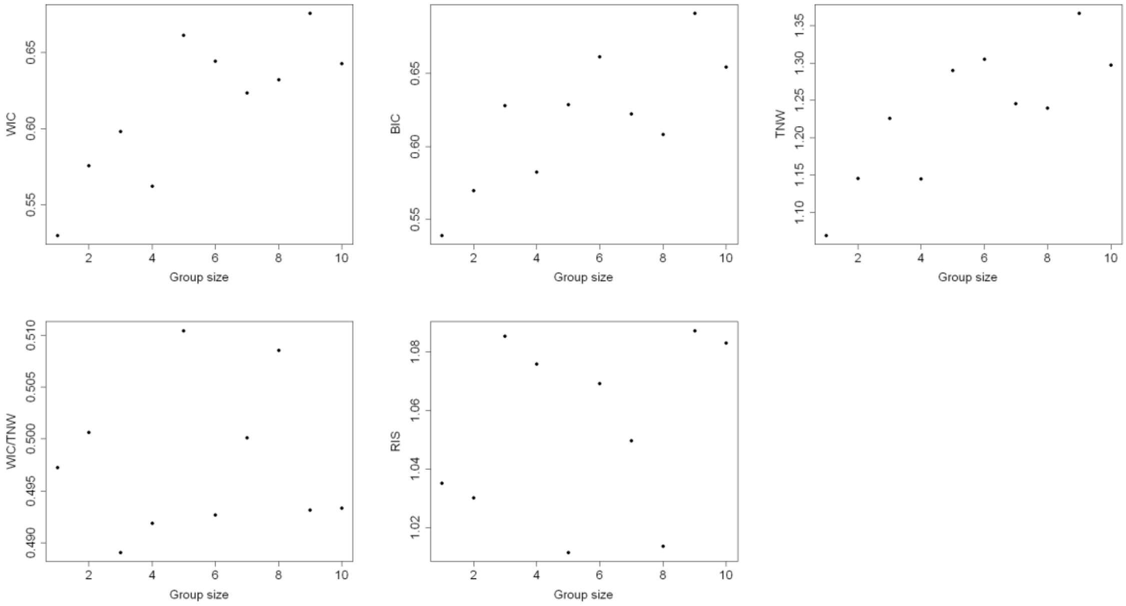

Figure S3b – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the size of specialist groups in scenario A2 (Table 1).

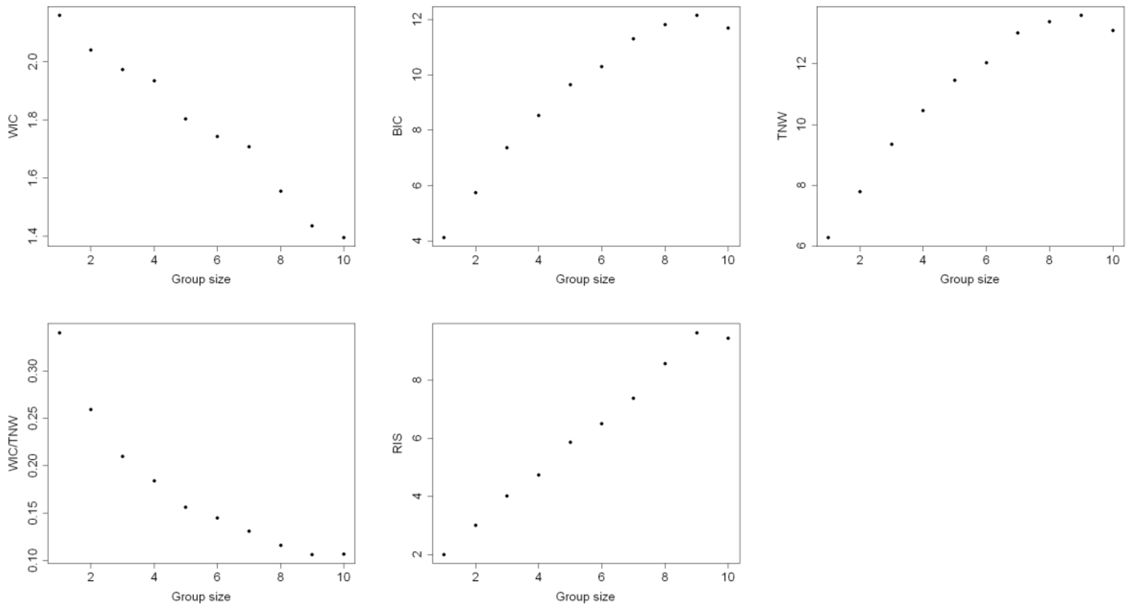

Figure S3c – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the size of specialist groups in scenario A3 (Table 1).

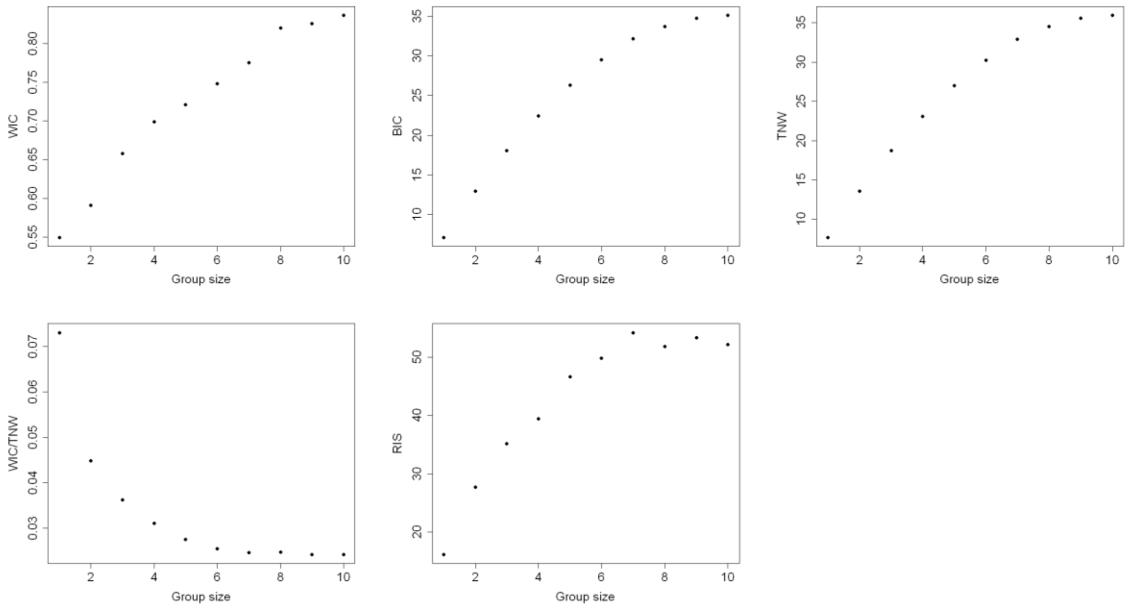

Figure S3d – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the size of specialist groups in scenario B1 (Table 1).

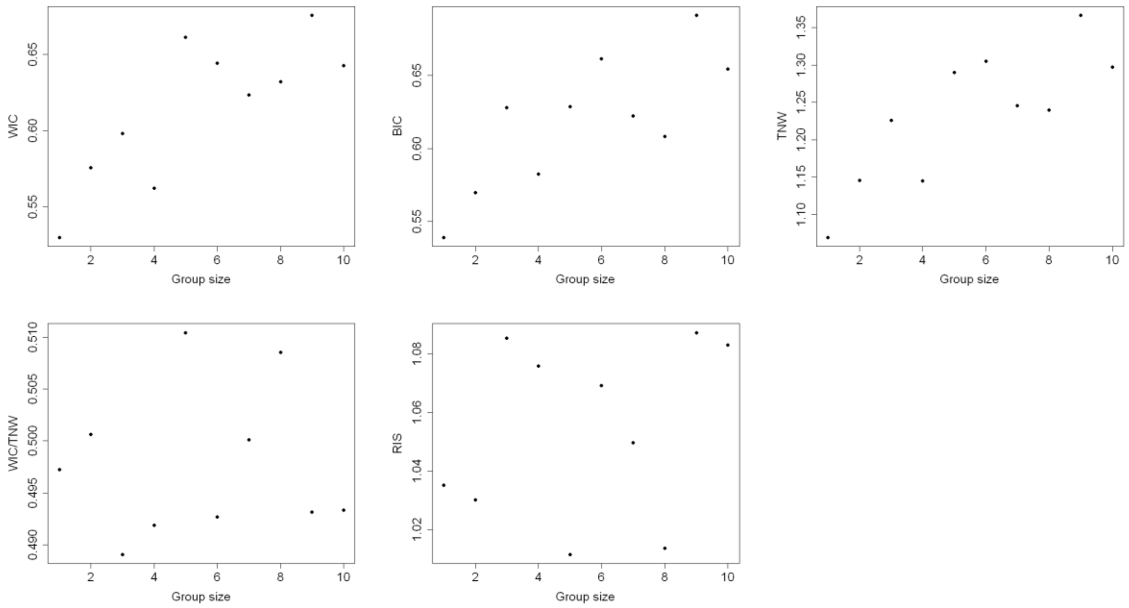

Figure S3e – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the size of specialist groups in scenario B2 (Table 1).

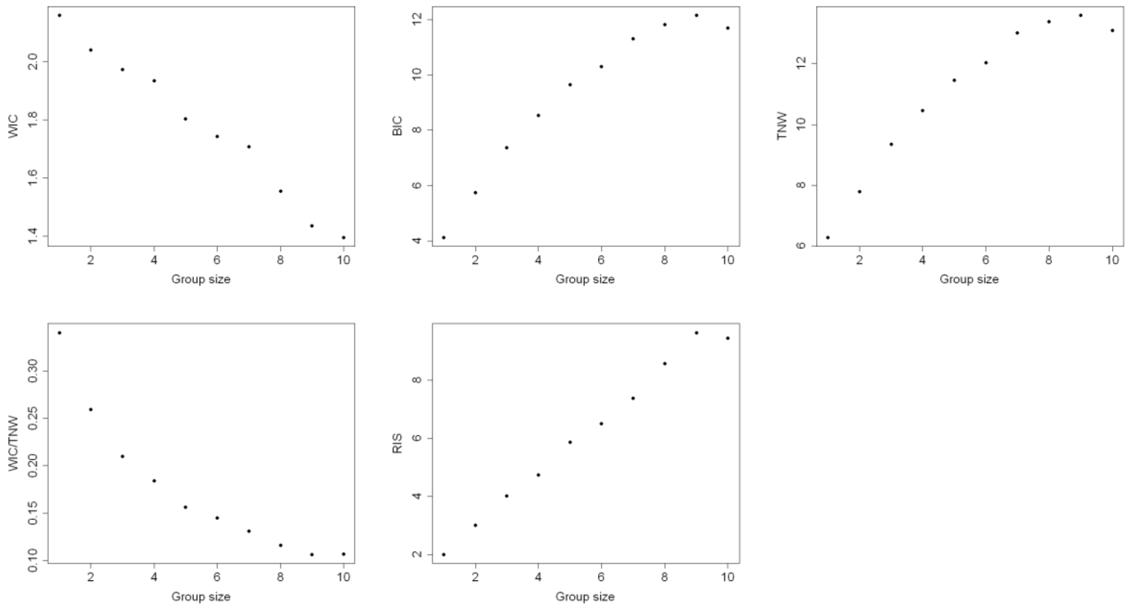

Figure S3f – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the size of specialist groups in scenario B3 (Table 1).

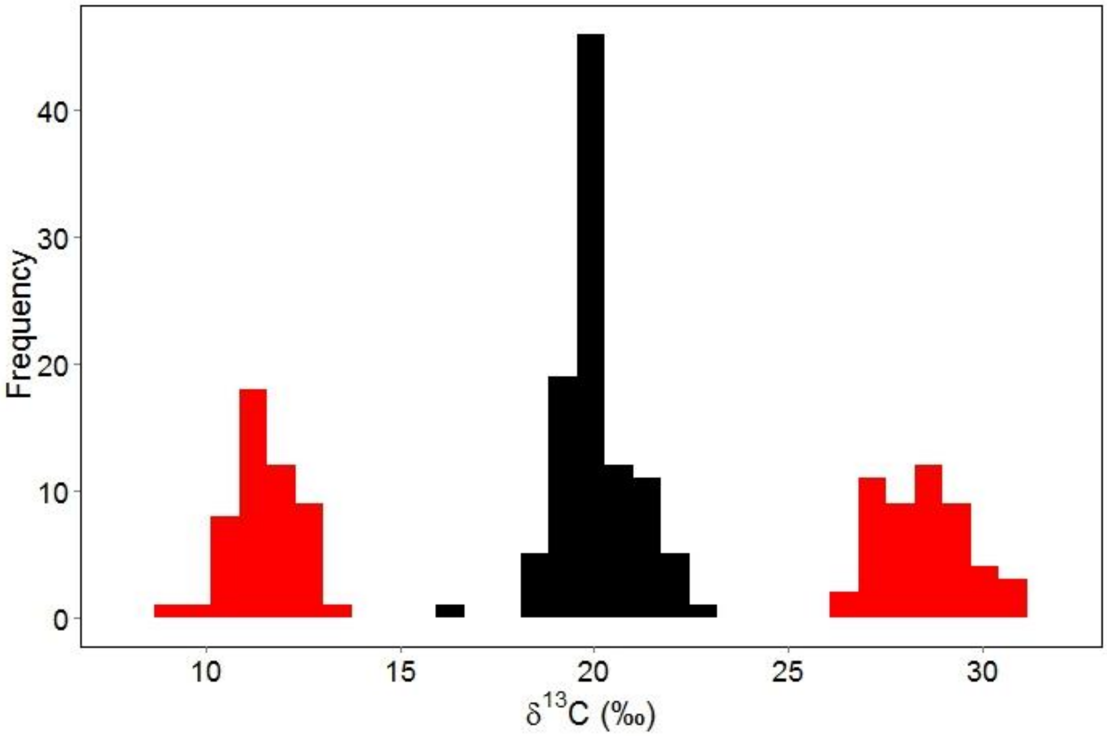

Figure S4 – Consumer stable isotope values of two specialist groups (red) and a generalist (black) from scenario A2 simulated for 100 individuals. These two groups have similar WIC/TNW and RIS but can be distinguished by comparing frequency distributions of isotope values.

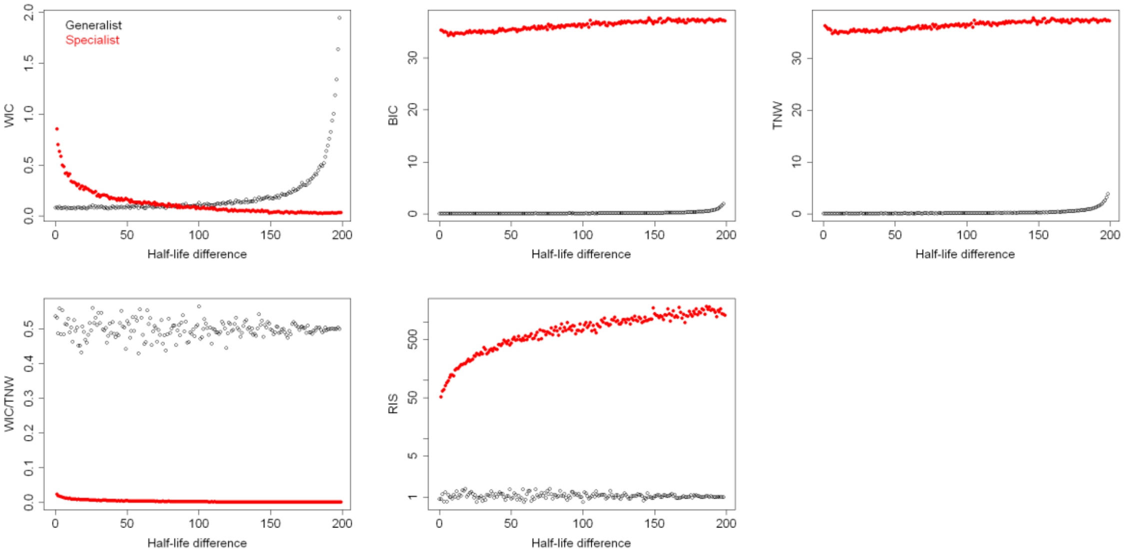

Figure S5a – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference in tissue half-lives in scenario A1 (Table 1).

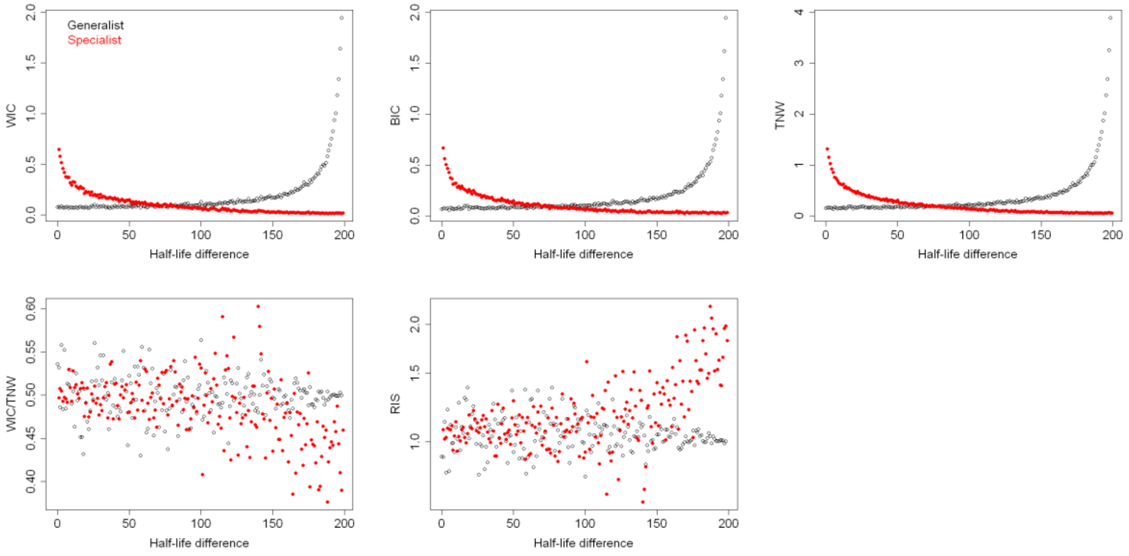

Figure S5b – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference in tissue half-lives in scenario A2 (Table 1).

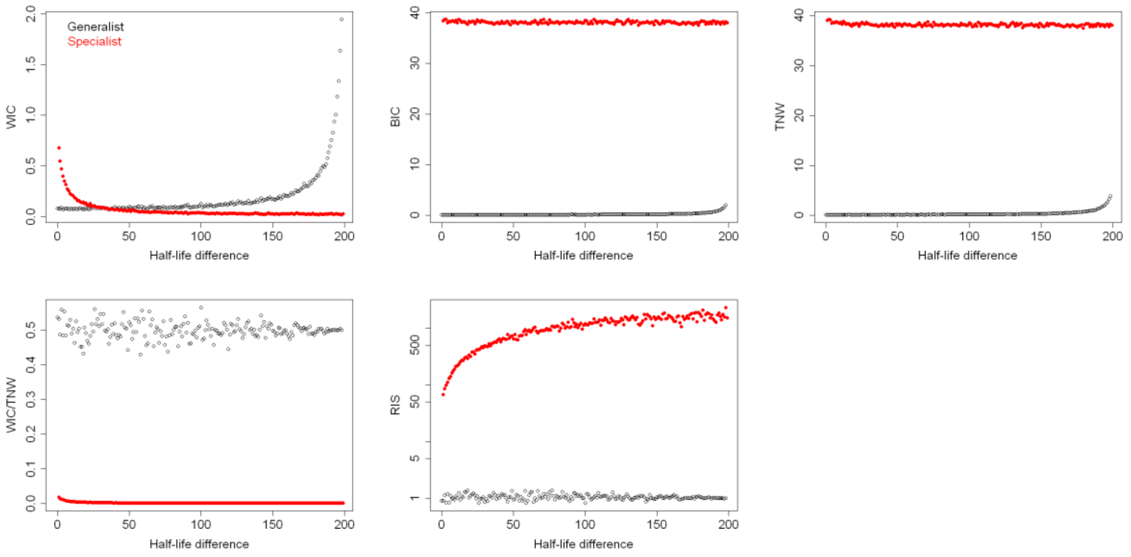

Figure S5c – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference in tissue half-lives in scenario A3 (Table 1).

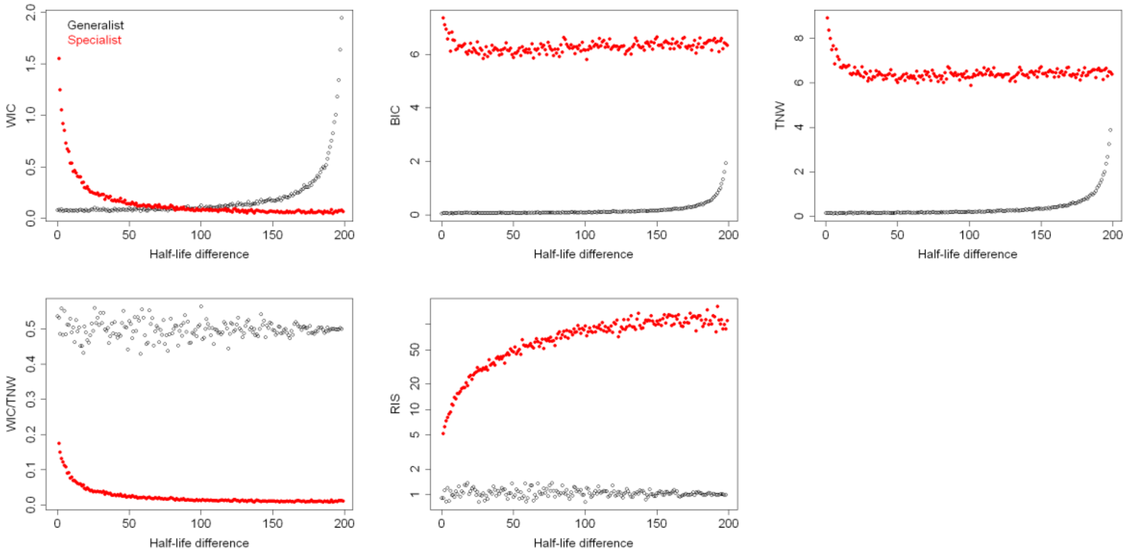

Figure S5d – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference in tissue half-lives in scenario B1 (Table 1).

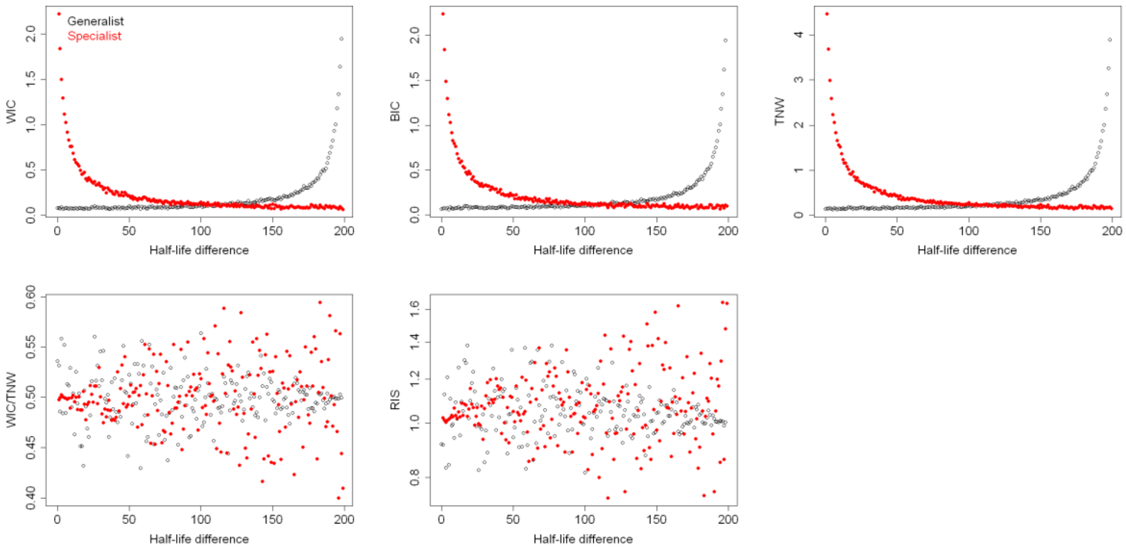

Figure S5e – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference in tissue half-lives in scenario B2 (Table 1).

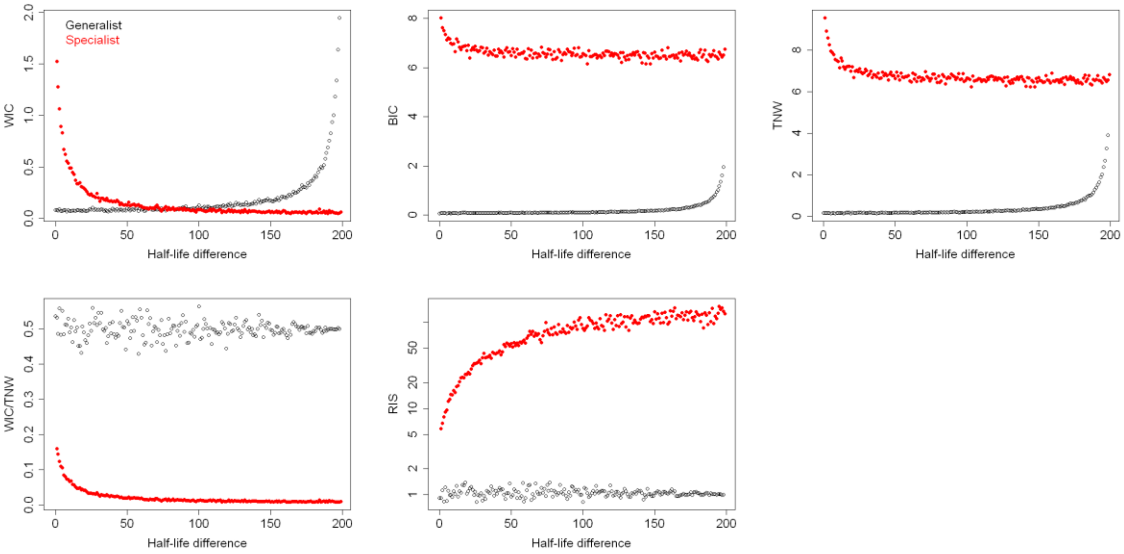

Figure S5f – Specialization indices for three-source simulations of specialist and generalist populations showing the effect of altering the difference in tissue half-lives in scenario B3 (Table 1).