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Differences in diet-induced flexibility in morphology and growth in a partially migratory species

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Abstract

Partial migration, in which some individuals of a population migrate while other individuals remain resident, is generally associated with ontogenetic shifts to better feeding or as a response to adversity, but its underlying mechanisms remain relatively unknown. Brown trout (*Salmo trutta*) exhibit partial migration, with some individuals remaining in fresh water (freshwater-resident) while others undertake an anadromous migration, gain most of their adult size at sea and then return to fresh water to spawn. The option adopted by an individual trout is thought to be partly determined by its growth performance in early life, which in the stochastic and dynamic environment of freshwater streams may be dependent on its flexibility. In order to examine potential effects of parent type on phenotypic flexibility we measured the metabolism, growth and morphology of full-sibling groups of offspring from freshwater-resident and anadromous parents both before and after a switch in diet. We found that fry had a higher growth rate and a more rounded head and body shape when reared on Chironomid larvae compared to when they were reared on *Daphnia*, but diet had no effect on standard metabolic rate. Interestingly, offspring of anadromous parents were less able to maintain their growth rate when fed on *Daphnia* than were those of freshwater-residents, and showed a correspondingly greater increase in growth following a switch from *Daphnia* to Chironomid larvae. Offspring of anadromous parents also showed less morphological flexibility in response to diet than did the offspring of freshwater-residents. We discuss how the migration history of the parents might interact with phenotypic flexibility in early life to influence the migration probability of the offspring.

67 **Key-words:** Alternative life history, ecotype, resident vs. migratory, *Salmo trutta*,
68 parental effects, salmonid metabolism, anadromous vs. non-anadromous, partial
69 migration
70
71

Draft

Introduction

Phenotypic flexibility, an organism's ability to match its morphology and physiology to current environmental conditions, is fundamental to adaptability and occurs when complimentary combinations of traits change in response to environmental conditions to maximize the efficiency of resource exploitation. For example, within fish there may be changes within the lifetime of the individual animal in gill raker spacing and mouth shape to suit shifts in prey type (Schluter, 1993), changes in body shape that are related to parallel changes in the velocity of water in which the fish is living (Peres-Neto and Magnan, 2004) and more recently differences in standard metabolic rate (the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state) in response to local food availability (Van Leeuwen et al. 2011; Auer et al. 2015). While much of the research surrounding phenotypic flexibility has been focussed on explaining patterns of resource polymorphisms within species in the context of adaptive radiation and speciation, it may also help explain other ecological patterns of intraspecific variation, one of which is the phenomenon of partial migration.

Partial migration, in which members of a population differ in whether or not they undertake migrations, occurs across a wide range of taxa including invertebrates (Hansson and Hylander 2009), fish (Dodson et al. 2013), birds (Newton 2008) and mammals (Ball et al. 2001). The commonest form is non-breeding partial migration (*sensu* Chapman et al. 2011), where migrants and residents breed sympatrically but overwinter apart. There have been many hypothesized explanations for this variation in migratory pattern, including competition for resources, differences in thermal tolerances and differences in arrival times/prior residence (see Chapman et al. 2011). In all cases

however, the migration can be viewed as a response to adversity (Taylor and Taylor 1977), but the degree of adversity will depend on the particular environmental conditions that are experienced at the time. For example, individuals that are of a larger body size or experiencing a higher food supply may generally have less to gain from migration (Chapman et al. 2011).

It is likely that both abiotic and biotic factors influence the decision to migrate or not, since it is potentially influenced by both genetic causes (i.e. determined by the parents through genetic or parental effects, so that offspring of migrants have a higher probability to migrate) and environmental factors (e.g. through condition-dependent migration; Brodersen et al. 2008). Berthold (1988) and Berthold and Pulido (1994) provide support for a genetic pre-disposition for migratory tendency and migration distance in the Blackcap *Sylvia atricapilla*. However, it has also been suggested that partial migration is driven by a complex interaction between the environment and genetics. In the “threshold model” the triggering of migration depends on whether or not a continuous character (“liability trait”) exceeds a genetically predetermined threshold value (Chapman et al. 2011; Dodson et al. 2013). In this scenario, individuals physiologically self-evaluate their performance against this threshold (e.g. of growth rate, body size or physiological condition), with migration being dependent on whether or not the threshold is exceeded (Fleming 1996; Pulido 2011; Dodson et al. 2013).

A well-documented example of a species exhibiting partial migration is the Brown trout *Salmo trutta*, a polymorphic species that adopts a continuum of life history strategies, with the two most common being freshwater-resident and anadromous migrant (which migrates to sea as a juvenile and returns to fresh water to spawn). The two

ecotypes can occur in sympatry, possibly derived from a single gene pool, with both anadromous and freshwater-resident adults having the ability to interbreed and produce offspring capable of adopting either life history (Wysujack et al. 2009; O'Neal and Stanford 2011). Freshwater-resident and anadromous trout appear indistinguishable during early life, and it is presumed that they only become separable when after one or more years the migrants turn silver in colour in preparation for entry to sea water ('smolting'; Jonsson 1985). Jonsson (1985) proposed that migrant brown trout are made up of the slower growing individuals in a population, which migrate in search of more productive habitats. It has also been suggested that metabolic constraints play an important role in determining physiological state and thus migration probability. The fish are often found in oligotrophic habitats in fresh water (e.g. upland temperate lakes and streams), and in this low food environment individuals with a lower growth efficiency, higher food requirement and/or higher metabolic rate (i.e. energy maximizers) will become energetically constrained earlier in life compared to those with higher growth efficiency, lower food requirement and/or lower metabolic rates (efficiency maximizers; Metcalfe et al. 1995; Forseth et al. 1999; Morinville and Rasmussen 2003; Rosenfeld et al. 2013). The individuals with the lower growth efficiencies and/or higher metabolic rates may therefore migrate in search of more productive habitats (lakes, oceans) to meet their outstanding metabolic needs.

It is likely that genetics interacts with growth history, current body size and physiological condition to determine whether or not the animal reaches the threshold that triggers migration. However, there may also be a role for the morphological and physiological flexibility of the organism (i.e. its phenotypic flexibility). For example

many species of fish adjust their body shape in response to diet type and water velocity to increase their efficiency of prey detection, capture and handling of prey items (Skúlason and Smith, 1995; Adams and Huntingford, 2002) and to reduce swimming costs (Peres-Neto and Magnan, 2004). Furthermore, flexibility in physiology may be equally important since individual brown trout that showed the biggest change in standard metabolic rate (SMR) when food availability was altered (either upwards or downwards) were recently found to have the fastest growth under the new food regime (Auer et al. 2015). Given that freshwater fluvial ecosystems are often regarded as being stochastic and that the decision to migrate is likely based on a cumulative assessment of performance over a range of environmental conditions experienced to date (i.e. a timespan of several years), it is possible that differences in the phenotypic flexibility of the individual may be more important in determining growth performance, and thus explaining patterns of partial migration, than whether it has a consistently “high” or “low” value for traits or conditions of interest. Therefore individuals who are more able to match morphology and physiology to current environmental conditions, and therefore to maximize growth (or minimize their energetic costs), may be more suited to freshwater fluvial habitats compared to less phenotypically flexible individuals who may be more suited to more homogenous habitats such as large lakes and oceans. If true, then offspring of freshwater-resident parents might be more likely to exhibit plasticity in early life than those of anadromous brown trout.

To explore these issues, we reared brown trout offspring from eggs of known parentage (i.e. freshwater-resident or anadromous) under two diets of equal energy content but potentially differing ease of digestion (*Daphnia* and Chironomid larvae),

which were then switched to determine if freshwater-resident brown trout fry are more able to match morphology and physiology to current environmental conditions compared to anadromous offspring.

Methods

BROODSTOCK COLLECTION

Twenty-four mature freshwater-resident (12 male and 12 female) and 14 anadromous (7 male and 7 female) brown trout were captured during the breeding season using electrofishing on 11 and 23 October 2013 from two neighbouring sub-tributaries of the River Tweed, Scotland. Freshwater-resident trout were collected from above an impassable dam on the Whiteadder River (55° 88'N, 2°57'W) while the anadromous trout were collected from the College Burn (55° 77'N, 2°18'W). Fish were classified as freshwater-resident or anadromous based on size and coloration (Eek and Bohlin 1997): freshwater-resident fish were smaller and dark brown in colour with red spots, while anadromous fish were larger and silvery-grey in colour with black spots. Both ecotypes were transported to the Belhaven Trout Company, Scotland, where they were held separately (keeping parental ecotypes discrete) in two round 1530 L aluminum tanks supplied with 8.1 ± 0.4 °C (mean $\pm$ SD) well water under ambient photoperiod and assessed every three days for ripeness.

Ripe fish were anaesthetized, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs were fertilized with sperm from a haphazardly-chosen male of the same life history origin to create 12 full sibling families from freshwater-resident parents and 7 full sibling families from anadromous parents. Freshwater-resident and

187 anadromous fish were spawned from 3 November - 29 November and 17 November - 4
188 December 2013 respectively.

189 EGG REARING, HATCHING AND EXPERIMENTAL PROCEDURES

190 Each family of eggs was housed separately in a plastic mesh egg basket, placed in one of
191 two (1m X 3m X 0.4m) rearing troughs supplied with well water and covered with dark
192 plastic sheeting to ensure eggs were in complete darkness. Water temperature during
193 incubation was 8.1 ± 0.4 °C and was recorded daily along with any dead eggs which were
194 carefully removed. Eggs were checked daily for hatching; those from freshwater-resident
195 and anadromous parents hatched from 19 December 2013 - 17 January 2014 and 30
196 December 2013 - 24 January 2014 respectively. Once eggs began to hatch, the newly
197 emerged offspring (alevins) were separated from the remaining eggs and gently placed
198 into a small mesh basket (one per family) located in the same two troughs as the egg
199 baskets.

200 On 31 January 2014 alevins were transported to the Scottish Centre for Ecology
201 and the Natural Environment, Scotland and housed in 15 L (50cm X 30cm X 15cm) clear
202 plastic aquaria on a partial recirculation system at a constant temperature of 9.2 ± 0.2 °C
203 (mean $\pm$ SD) and simulated ambient photoperiod. The aquaria each contained a single air
204 stone and were supplied with water pumped directly from Loch Lomond, which was first
205 treated with an ozone generator (Sander S1000, Germany) before being discharged into a
206 large sump. Water from the sump was pumped through an in-line 110W UV sterilizer
207 (Tropical Marine Center (TMC), Manchester, UK) before entering the aquaria. Return
208 water was gravity fed into a large free standing filter before being discharged back into
209 the main sump. Fish were monitored daily and any dead fish removed.

On 3 March 2014, at the time of first feeding, random selections of offspring from across families were haphazardly assigned into eight 15 L (50cm X 30cm X 15cm) clear plastic aquaria (keeping parental ecotypes discrete), with four aquaria per parental type and 10 fish per aquaria. The aquaria were placed inside a constant temperature room on a partial recirculation system at a temperature of 13.6 ± 1 °C (mean $\pm$ SD), with a simulated ambient photoperiod. Fish were fed *ad libitum* several times daily by pipetting food onto the surface of each aquaria, with excess food (which was clearly visible on the bottom of the aquaria after every feed) being removed by vacuum siphon at the end of each day. Diet treatments consisted of *Daphnia* (BCUK Aquatics, Lincolnshire, England; composition: protein 5%, fat 0.7%, fibre 1%, moisture 90%) or Chironomid larvae (BCUK Aquatics, Lincolnshire, England; composition: protein 5%, fat 0.5%, fibre 0.9%, moisture 89%); diet types were supplied frozen from the manufacturer and thawed daily before feeding. It was presumed that, although the two diets had an almost identical nutritional and water content, fish would grow more slowly on the *Daphnia* treatment due to the extra costs associated with digesting and processing the hard exoskeleton of the *Daphnia* (Swaffar and O'Brien 1996) in comparison with the soft body of the Chironomid larvae. Two replicate aquaria (i.e. 20 fish) per parental type were randomly allocated to each of the two diet treatments.

On 11 June 2014 fish were anaesthetized, measured (fork length ± 0.1 mm; body mass ± 0.0001 g), and tagged with a visible implant elastomer (Northwest Marine Technology, Inc.). They were then anaesthetized, re-measured and photographed on 2 July 2014, so that their growth rate (from 11 June to 2 July 2014) and morphological shape on their initial diets (3 March 2014 to 2 July 2014) could be measured (interval 1).

233 The SMR of all fish was then measured (see below) once over the period from 2 July
234 2014 to 12 July 2014. Once all fish had been subjected to metabolic measurements the
235 two diet types were switched (12 July 2014), so that all individuals previously fed
236 *Daphnia* were switched to a diet of Chironomid larvae and *vice versa*. The timing of this
237 switch (3 months since first feeding) was based on pilot trials showing that this was a
238 sufficient period to detect significant differences in diet-induced morphology. On 28
239 August 2014 all fish were again anaesthetized, re-measured and photographed, then their
240 SMR recorded (measurements over the next 10 days), for assessment of growth rate,
241 morphological shape change and metabolic rate following the diet switch (12 July to 28
242 August 2014; interval 2). Fish were maintained on their switched diets to further evaluate
243 the degree of shape change through later ontogeny (through to 30 September 2014, when
244 they were again anaesthetized and photographed; interval 3).

245 MEASURING STANDARD METABOLIC RATE

246 Aquaria were vacuum siphoned to remove food and debris the day before fish
247 were placed in respirometry chambers. This ensured that fish were unfed for at least 28 h
248 prior to oxygen uptake measurements, and had sufficient time to evacuate their guts; 28 h
249 post-feeding has been shown to be adequate for the specific dynamic action (SDA)
250 response to subside in salmonids (Cutts et al. 2002). SDA is an elevation in metabolic
251 rate due to the increased energy demands associated with digestion, immediately
252 following a meal (Rosenfeld et al., 2015), and is generally not considered part of SMR.

253 Oxygen uptake was measured over a 24 h period, from approximately 10.00 AM
254 onwards, using intermittent flow respirometry. Individual fish were placed into 1 of 8
255 separate (8.0cm length, 3.4cm diameter) glass respirometry chambers. Chambers were
256 submersed in a water bath housed inside a second constant temperature room kept at the

257 same temperature (13.6 ± 0.5 °C across all measurements) as the tanks in which growth
258 was measured. An air-stone in the water bath of the respirometer apparatus kept the water
259 fully saturated with oxygen. Chambers were wrapped in dark plastic to prevent visual
260 contact between individual fish during measurements, and all measurements were
261 conducted in the dark to further minimize fish activity (Cutts et al., 2002). Glass
262 respirometers and tygon tubing were used to minimize potential issues with use of
263 plastics and oxygen permeable materials (Stevens, 1992). Oxygen uptake was measured
264 for 20 min every 45 min on a continuous 25 min “on” and 20 min “off” cycle. During the
265 “on” cycle oxygenated water from the water bath was driven by a water pump (Eheim
266 300 universal, Deizisau, Germany) through each respirometer. Flow rate was regulated
267 by adjusting the tension of a hose clamp on the outflow side of the pump tubing to
268 prevent swimming and spontaneous behaviour during this period of flushing. After 25
269 minutes the pump creating the water turnover was automatically switched off (Superpro
270 MFRT-1 timer, Somerset, England) allowing for a decrease in oxygen concentration to be
271 measured during the 20 min “off” period, during which a peristaltic pump (Masterflex
272 L/S, London, England) was used to ensure adequate mixing within each respirometer.
273 Water oxygen concentration was measured every 1s for 20 min during this time period.
274 Oxygen concentration within the respirometer was measured using one of two oxygen
275 meters (FireStingO₂ oxygen meter; PyroScience) each fitted with 4 oxygen probes which
276 were placed in individual measurement chambers (Loligo systems, Tjele, Denmark)
277 connected inline between the outlet side of each respirometer and the peristaltic pump;
278 concentrations never dropped below 80% oxygen saturation in this experiment. Probes
279 were calibrated daily, and rates of background oxygen consumption were subtracted from

the observed values by measuring the oxygen concentration of water inside each of the respirometers in the absence of fish at the beginning and end of each measurement trial and assuming a linear decrease in oxygen concentration over the measurement period.

The rate of oxygen consumption was determined using the following equation (Ege and Krogh 1914):

$$MO_2 = V_w(\Delta C_w O_2) / \Delta t$$

where V_w is the volume of water in the respirometer and associated tubing minus the volume of the fish and $\Delta C_w O_2$ is the change in oxygen concentration of the water over time period Δt (Steffensen 1989). Oxygen concentration was calculated by correcting PO₂ (partial pressure oxygen) for barometric pressure and multiplying by αO_2 ($\mu\text{mol L}^{-1} \text{ torr}^{-1}$), the solubility coefficient at the observed temperature. Standard metabolic rate was estimated by using the average of the lowest 10% of values observed during the respirometry trial (Norin 2014). Following respirometry measures all fish were anaesthetized, blotted dry and weighed to the nearest 0.0001g.

MORPHOLOGICAL MEASURES

Lateral view photographs of all fish were taken using a Canon EOS 350D digital camera fixed to a camera stand and illuminated with two blue lights mounted on either side of the camera stand to ensure quality images for geometric morphometric analysis. For each photograph a scale reference was added to allow for the correction of shape change associated with changes in body size. Twenty consistently identifiable landmarks were digitised on each image (Figure 1) using tpsDig and tpsUtil software (Rohlf 2006 a,b). Landmark configurations for each specimen were aligned, translated, rotated and scaled to a unit of centroid size by using a Procrustes superimposition using the mean shape of all the images as the starting form (Rohlf and Slice, 1990). Shape change due to

differences in allometry and not a response to diet type were removed (size corrected) using a multivariate, pooled, within-group regression of the Procrustes coordinates on the log centroid size of the individual (Klingenberg and McIntyre 1998). The residuals of this regression were then used for all further analysis. Canonical variate analysis was undertaken in MorphoJ to assess the effect of diet on body shape, using the average Mahalanobis distance (D) between the two diet groups from a single parent type (freshwater-resident or anadromous) for each time interval. Comparison of the changes over time in the size of D for the offspring of freshwater-resident and anadromous fish indicates the relative degree of morphological flexibility of the two offspring types.

CALCULATIONS AND STATISTICAL ANALYSES

Specific growth rates of fish (percent per day) were calculated as $100[\log_e(\text{final mass}) - \log_e(\text{initial mass})]/\text{duration}$, where duration refers to the interval between measurements (Ricker 1975). Given the large variation in fish size and the confounding effect of size on metabolism and growth, we used residual SMR and residual growth in subsequent analysis. These residual values were calculated as residuals from the regression of absolute oxygen consumption or growth rate (SMR or Growth) on body mass (g); in order to standardise the results we used as a reference the combined data for offspring from freshwater-resident and anadromous fish habituated to the Chironomid larvae diet (i.e. during interval 1; $\log_{10}(\text{SMR}) = (1.02 * \log_{10} \text{mass}) + 0.7576$ (n= 38); $\log_{10}(\text{Growth}) = (0.0116 * \log_{10} \text{mass}) + 0.619$; n=38), plotted on double logarithmic axes. Prior to being log transformed a constant of one was added to the growth data to allow transformation of negative growth values (since some fish on the *Daphnia* food treatments lost weight).

We used linear mixed effects models (LME) to test for the effects of diet and parental life history on growth and SMR. All LME models initially included all possible two way interactions, with aquarium tank and individual included as random factors to control for potential tank effects and non-independence of measures for individuals (rSMR / rGrowth = Parental life history + Diet + Parental life history*Diet, random = Aquarium tank + Individual). Variance inflation factors (VIF's) for all explanatory variables were calculated prior to analysis; all VIF's were less than 3, indicating that collinearity among explanatory variables was unlikely to have affected our analyses (Zuur et al., 2009). Furthermore, visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Likelihood ratio tests comparing models with and without a given term were used to sequentially compare model fit; models were progressively simplified provided that any increase in the log-likelihood ratio statistic was non-significant ($p > 0.05$). Tukey and LS means tests were used to compare treatment groups. All analyses were conducted using R version 3.0.1 statistical software (R Core Team, 2013) and the lme4 function (Bates et al., 2011).

Results

STANDARD METABOLIC RATE

There was no significant effect of parental type on SMR, nor of initial diet (Fig. 2A) However overall there was a significant decrease in SMR when offspring were switched from their initial diets to their alternate diets (Fig.2B; Tukey, all less than $p < 0.001$), with fish switching from Chironomid larvae to *Daphnia* showing a greater

decrease in SMR compared to those individuals that switched from *Daphnia* to Chironomid larvae (Fig. 2C.; $\chi^2=5.51$, $df=1$, $p<0.02$).

GROWTH RATE

The effect of diet on growth rate depended on the parental type (Fig.2D; $\chi^2=28.08$, $df=3$, $p<0.001$), with offspring of freshwater-resident parents having a higher growth rate than those of anadromous parents, but only if on a diet of *Daphnia* during the first time interval (LSMEANS, $p=0.04$). There was no significant effect of parental type on growth when fry were feeding on Chironomid larvae (Tukey, $p=0.120$), or on *Daphnia* having previously been fed Chironomid larvae (Tukey, $p=0.598$). However fish grew faster on Chironomid larvae than on *Daphnia* ($\chi^2=293.1$, $df=3$, $p<0.001$), and the switch from *Daphnia* to Chironomid larvae produced a bigger increase in the growth of offspring from anadromous parents than those from freshwater-resident parents (Fig.2F.; $\chi^2=6.51$, $df=1$, $p=0.01$).

MORPHOMETRICS

There was a similar significant morphological response to diet in offspring of the two parental types (Fig. 3; Fig.4), with fish initially fed on Chironomid larvae developing a rounder body and head compared to those fed on *Daphnia*, which had a more slender body and head (Fig. 5). When the diet was switched, the fish responded by developing the appropriate morphology (i.e. those previously fed on *Daphnia* developed a rounder body and head when switched to Chironomid larvae, and vice versa). The extent of the diet-induced difference in morphology was similar for offspring of the two parental types during both interval one (anadromous: Mahalanobis distance = 3.91, $p<0.0001$,

freshwater-resident: Mahalanobis distance = 3.79, $p < 0.0001$) and interval two (anadromous: Mahalanobis distance = 2.64, $p < 0.0001$, freshwater-resident: Mahalanobis distance = 2.54, $p < 0.0007$; Fig 3; Fig. 4). However, during interval three the offspring of freshwater-resident parents diverged more in morphology in response to diet than did those of anadromous parents (anadromous: Mahalanobis distance = 2.54, $p < 0.004$, freshwater-resident: Mahalanobis distance = 3.67, $p < 0.0001$), even though all fish had been on the same diets since the beginning of interval two (Fig. 3; Fig. 4). This suggests a greater morphological flexibility in offspring of freshwater-residents than anadromous trout.

Discussion

The diet on which juvenile brown trout were reared had a significant effect on body (especially head) shape and growth, with fish fed on Chironomid larvae having a higher growth rate and developing a more rounded body shape compared to those fed on a *Daphnia* diet. There were initially no differences in SMR between fish on the two diets, nor between fish from different types of parent (i.e. freshwater-resident versus anadromous). However, given that SMR often differs by a factor of 2 or 3 between individual trout fry of the same age and size (Burton et al. 2011), this result may be due to low statistical power to detect differences among groups or potentially a more rapid compensation by metabolic rate to changes in diet. While brown trout show close coupling in the flexibility of their growth and metabolic rates (Auer et al. 2015), these traits may change at different rates. For example, while changes in growth may take several weeks to become apparent, Moran et al. (2013) demonstrated that brown trout exhibited genome-wide methylation changes (which may have altered the expression of

genes linked to metabolic rate) within 14 days of a dietary change. Individual differences in SMR within salmon and trout populations have been linked to variation in individual growth and life history strategies (e.g. timing of subsequent smolt migration; McCarthy 2000). Although we did not detect a difference in SMR between offspring type and diet type we did find a decrease in SMR when diets were switched (in either direction). Flexibility in SMR has been shown to occur in salmon and trout populations in relation to food availability, with individual SMR decreasing following a period of food restriction (Du Preez, 1987; Wieser et al., 1992) and increasing when food is supplied above baseline levels (O'Connor et al., 2000; Van Leeuwen et al., 2011; 2012); moreover, growth is fastest in those individuals that show the biggest change in SMR in response to changing food availability (Auer et al. 2015). However this doesn't explain the reduction in SMR in our study as it happened regardless of the direction of the diet switch and despite the fact that the fish were fed an equal ration (in terms of relative mass), calculated to be *ab libitum*, for each diet type. One possible explanation is an imbalance between new prey type, digestive tract performance and assimilation, producing a similar response to when food levels are changed. Vertebrate digestive tracts have been shown to respond over relatively short time scales to differences in prey consumption and food availability (Starck 1999; Armstrong and Bond 2013). For example, snakes can increase the capacity and activity of their digestive tract during a meal, and conversely decrease its capacity and activity during periods of food deprivation (Secor and Diamond 2000). Similarly, juvenile salmonids can dramatically increase the length of their intestine during sustained periods of increased food availability (Armstrong and Bond 2013). The switch in diet may have meant that the digestive system of the fish was initially

416 imperfectly matched to the type of food, which might produce a similar response to a
417 food shortage.

418 This idea of a difference in digestive requirements for the two food types is
419 supported by the analyses of growth rate. We found a difference in growth rate between
420 diet types – and this in turn was affected by the fish's parental type. *Daphnia*, although
421 relatively similar in proximate composition (and hence energy content per unit wet
422 weight) to Chironomid larvae (see methods section above), have a hard exoskeleton; this
423 is likely harder to digest (Swaffar and O'Brien 1996), compared to soft-bodied
424 Chironomid larvae, so it was not surprising that fish grew faster on a diet of Chironomid
425 larvae compared to those fed *Daphnia*. However, offspring of freshwater-resident brown
426 trout were more able to maintain their growth on the *Daphnia* diet than were those of
427 anadromous parents, so that the latter showed a greater fluctuation in growth rate
428 following a switch in diet, indicating potential differences between offspring from the
429 two types of parent in the ability to compensate for changes in food quality. Differences
430 in growth efficiency between freshwater-resident and anadromous individuals have been
431 demonstrated in previous studies. For example, Morinville and Rasmussen (2003)
432 demonstrated that individual migrant brook trout (*Salvelinus fontinalis*) had a lower
433 growth efficiency in the year prior to migration compared to sympatric resident brook
434 trout.

435 Lastly we found that the extent of the divergence in body shape induced by diet
436 (as measured by Mahalanobis distance between individuals fed Chironomid larvae and
437 *Daphnia*) was similar between offspring types for the first weeks of feeding (i.e. during
438 interval one, the first ~111 days since first feeding, and interval two, the next ~56 days

after the diet switch). However, while in offspring of anadromous trout the diet-induced change in shape was maintained at the same level (as indicated by a relatively constant Mahalanobis distance) for intervals two and three, the offspring of freshwater-resident trout fed on Chironomid larvae continued to diverge in shape over this time period from those fed on *Daphnia*. This suggests a greater plasticity in morphology in the offspring of freshwater-residents. Morphological flexibility in response to diet type is well documented and is generally related to an increase in efficiency of detection, capture and handling of prey items (Skúlason and Smith, 1995; Adams and Huntingford, 2002) and is a primary driver behind the expression of alternative trophic phenotypes. For example Walls et al. (1993) demonstrated that larval eastern long-toed salamanders (*Ambystoma macrodactylum columbianum*) fed tadpoles and brine shrimp nauplii developed significantly broader and deeper heads compared to those only fed brine shrimp nauplii. While we cannot be sure that the morphological differences induced by the two diets in this study were adaptive, the fact that the type of offspring with the greater morphological flexibility (i.e. the offspring of freshwater residents) also showed a greater ability to maintain growth on the poorer prey type is suggestive of an adaptive response. One potential explanation for the difference in morphological flexibility between offspring from alternative life histories is their contrasting requirements for niche shifts. Freshwater ecosystems are often regarded as being food-limited (Imre et al. 2005), so requiring adaptability in diet choice; moreover, freshwater-resident trout tend to move into deeper and slower-flowing habitats as they get older (e.g. deeper pools in rivers, and often eventually lakes; Klemetsen et al. 2003). These ontogenetic changes in diet and habitat likely both require changes to swimming capability and foraging mode (e.g. with the fish

becoming less active as they increase in size), so selecting for the ability to remain morphologically flexible throughout ontogeny (to minimize energetic costs and maximize prey capture efficiency). Freshwater-resident individuals may thus benefit from morphological flexibility, since this would help maintain growth in the unproductive and changeable freshwater environment. In contrast, fish migrating to sea will continue to be actively swimming against strong currents and obtaining prey by pursuit foraging, in a highly productive environment that allows narrow dietary specialisations, so possibly selecting against morphological flexibility.

One potential caveat to our study is that we were unable to determine whether the differences between offspring phenotypic flexibility were primarily due to genetic or maternal effects, but this would be difficult to establish given that the resident-anadromous dichotomy by its very nature prevents the use of the standard approach of rearing the parents in a common garden to rule out maternal effects.

In conclusion, the results of this study suggest that offspring from freshwater-resident and anadromous parental life history strategies show some differences in phenotypic flexibility that may be consistent with the future habitats individuals may encounter, with offspring of migratory fish being apparently morphologically less flexible and less able to maintain growth on a poor quality diet. Therefore we suggest that genetic and parental effects affecting phenotypic flexibility may contribute to the differences in performance observed in a common environment and may play a role in the perpetuation of non-breeding partial migration within populations of brown trout.

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Figure Captions.

Fig.1. Schematic diagram of the morphological landmarks used for analysis.

Fig.2. The effect of diet and parental type (squares = offspring of freshwater-resident parents (Res); circles = offspring of anadromous parents (Anad)) on standard metabolic rate (SMR) and growth rate. (a) SMR at the end of interval one of fish that had been fed since first feeding on *Daphnia* (D) and Chironomid larvae (B); (b) SMR at the end of interval two of fish that had been switched at the end of interval one from a diet of *Daphnia* to Chironomid larvae (DB; closed) or from Chironomid larvae to *Daphnia* (BD; open); note change in scale of ordinate compared to previous graph; (c) Change in SMR after the change in diet (negative values indicating a lower SMR after the switch). (d-f) Corresponding data for growth rates over (d) interval one and (e) interval two, and (f) change in growth rate after the change in diet (negative values indicating a slower growth rate after the switch). SMR and growth rates are expressed as residuals to correct for body mass. Error bars represent 95% confidence intervals. See text for statistical analysis.

Fig.3. Schematic diagram of the morphological shape response for offspring from anadromous (Anad)) and freshwater-resident (Res) parents during time periods when fish were reared on *Daphnia* (grey arrows) and Chironomid larvae (black arrows). Diets were switched at the start of interval two. Note the equivalent morphological responses to diet of the two offspring types during intervals one and two (i.e. a similar degree of initial morphological divergence between fry on the *Daphnia* and on the Chironomid larvae

diets, and a similar effect of a diet switch, as measured by Mahalanobis (D) distance). However, by interval three the offspring of freshwater-resident parents showed a greater dietary-induced morphological divergence ($D = 3.67$) compared to those of anadromous parents ($D = 2.54$).

Fig.4. The morphological difference in shape response for offspring from anadromous (open circles; dashed line) and freshwater-resident (closed circles; solid line) parents during time periods when fish were reared on *Daphnia* and Chironomid larvae. The Mahalanobis (D) distance quantifies the difference in body shape between fish of the same parentage that were reared on the two diets. See text for further description of the analyses.

Fig.5. Wire frame diagrams of the morphological shape change associated with canonical variance 1 (CV1) from the canonical variance analysis (CVA). CV1 split groups based on diet and accounted for 36.2% of the overall variation. Note the more rounded head and body shape in the fish fed Chironomid larvae compared to those fed *Daphnia*. Analysis is based on fish morphometrics averaged across all three intervals.

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