Stable isotope niche differentiation in sticklebacks with symmetric and asymmetric pectoral fins

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Fluctuating asymmetry (FA) is often, but controversially, viewed as an indicator of fitness and a target of selection. In the brook stickleback, *Culaea inconstans* (Kirtland), FA of the pectoral fins, which are the main source of propulsion, is inversely correlated with fecundity. We examined the hypothesis that asymmetry of the pectoral fins could affect locomotion in such a way as to influence foraging and niche use in prereproductive brook stickleback. Nitrogen and carbon stable isotope analysis showed the diet of symmetric and asymmetric males diverged with increasing body size. Larger symmetric males fed at higher trophic levels and had a diet based on carbon emanating from a more pelagic source than their asymmetric counterparts. Such effects were not observed in females or smaller males. The number of chironomid larvae found in the gut was greater on average in asymmetric than symmetric fish. The results from this study strongly suggest FA of pectoral fins affects the foraging behaviour of *C. inconstans* and that stable isotope analyses of individual phenotypes provides a useful tool for assessing the ecological consequences of FA. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, **92**, 617–623.

ADDITIONAL KEYWORDS: brook stickleback – diet – fluctuating asymmetry – SIA – symmetry.

INTRODUCTION

Fluctuating asymmetry (FA) has generated interest as a potential indicator of fitness and a focus of natural and sexual selection (Møller & Swaddle, 1997). Fluctuating asymmetry of a dimensional or meristic trait is characterized by the left minus right difference and has a normal distribution with a mean equal to zero (Van Valen, 1962; Palmer, 1994; Palmer & Strobeck, 2003). The developmental and genetic basis of FA is not well understood and its importance is arguable (Houle, 1998) but FA is perceived as a simple means of recognizing optimum phenotypes or phenotypic quality, with symmetric individuals possessing superior developmental stability, quality, or health in the form of a reduced parasite load (Reimchen, 1997; Reimchen & Nosil, 2001). Although the validity of using FA as an index of fitness and developmental stability is controversial (Lens *et al.*, 2002),

the fact that numerous studies suggest FA is negatively correlated with fitness and mating success requires explanation. Studies on a range of organisms from fishes to humans show the trait in question is often sexually dimorphic and may be subject to direct assessment by potential mates. In other instances, it may be that mates are responding instead to some other more readily recognizable trait associated with symmetry (Moodie & Moodie, 1996).

Few studies have considered the functional and ecological implications of FA when investigating why a symmetric trait confers superiority (for exceptions, see Moodie & Moodie, 1996; Bergstrom & Reimchen, 2000; Nosil & Reimchen, 2001; Reimchen & Nosil, 2001). Previously, Hechter, Moodie & Moodie (2000) showed that FA in the pectoral fin rays of the brook stickleback, *Culaea inconstans* (Kirtland), was inversely correlated with female fecundity. The pectoral fins in this and other sticklebacks are important propulsive organs, and their symmetry may affect locomotion, foraging, and male reproductive success.

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Culaea, *Gasterosteus*, and *Pungitius* males use the pectoral fins to direct a forward flow of water over the eggs during incubation. Populations of *Culaea* occupying rivers and streams are characterized by lower levels of pectoral fin ray FA than those inhabiting the standing water of ponds and lakes, which may relax demands on their swimming performance (Moodie, 1978). The pectoral fins of males also have a key role in courtship and parental behaviour. In *Culaea*, males have larger pectoral fins throughout life (Hechter *et al.*, 2000). Likewise, in *Gasterosteus aculeatus*, males engaged in parental care have larger pectoral fins than nonparental males and parents with relatively larger fins have more food in their stomachs and are in better condition (Bakker & Mundwiler, 1999). These observations lead us to explore the possibility of a functional relationship between an asymmetric structure (the pectoral fins) and specific fitness traits. Fin asymmetry could be influencing the swimming efficiency of *Culaea* and thus its foraging performance and ability to accumulate energy stores that affect fecundity. We therefore examined the possibility of differential resource use or niche space through assessment of stable isotopes signatures of muscle tissues for symmetric and asymmetric fish. Stable isotopes provide insight on trophic position as well as the spatial foraging habitat (Minagawa & Wada, 1984; France, 1995). An extensive literature shows that elevated δ^{15}N values indicate feeding at a higher trophic level. Depleted δ^{13}C (i.e. more negative) values suggest a dietary emphasis on pelagic algal species because, in freshwater systems, pelagic algae show less ^{13}C fractionation during carbon fixation than do benthic algae. This difference can carry through to higher trophic levels (Hecky & Hesslein, 1995) and be used to determine the relative importance of benthic and pelagic algal sources in a fish's diet. Variation in ^{13}C fractionation has been observed in different fish species with primarily pelagic or benthic diets (Fry *et al.*, 1999).

MATERIAL AND METHODS

The study population is semi-isolated and is the only fish species present in a small (10 ha) lake in southern Manitoba. Length frequency and mark–recapture data showed that these fish rarely live beyond 1 year and spawn over a period of a few weeks beginning in late May (Moodie, 1986). A sample of 551 young-of-the-year fish (10–16 weeks in age) were captured in unbaited minnow traps in late September. Upon removal from the traps, the fish were placed on frozen CO_2. Fish were subsequently thawed, each fish was given an identifying number, sex, total body length (TL), and weight were recorded, and the sagittal otoliths removed. Fish were then fixed for 24 h in 5%

formalin and then rinsed and stored in 50% isopropyl alcohol. Relative ages of the two classes of fish (i.e. symmetric and asymmetric) were determined by measuring the otolith area of digital images with WinFolia software (Boehlert, 1985).

Stable isotopes of carbon and nitrogen (^{13}C and ^{15}N) were determined for a randomly (random numbers table applied to fish identity numbers) selected subsample of 82 symmetric and all 81 asymmetric fish. Tissue used for isotope analysis was taken from the flank musculature 2 years after the fish were placed in alcohol. Analysis was performed at the University of Saskatchewan Isotope Laboratory. Tissues were dried at 60 °C for 2 weeks and powdered with a Wig-L-Bug grinder (Crescent Dental Co.). For each fish, approximately 1 mg dry mass was weighed and sealed in a 3.5×5 mm tin capsule. All samples were analysed for continuous-flow isotope ratio mass spectrometry of nitrogen and carbon in a Robo prep elemental analyser interfaced with a Europa 20 : 20 isotope ratio mass spectrometer at the stable isotope facility, University of Saskatchewan. Isotopic ratios (heavy isotope/light isotope) are expressed in δ notation and reflect deviation in parts per mil (‰) from international standards (PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen). Measurement error was approximately ±0.1‰ for ^{13}C and ±0.3‰ for ^{15}N. Although formalin fixation can deplete δ^{15}N by approximately 1.65‰ (Sarakinos, Johnson & Vander Zanden, 2002), all fish were stored in the same jar of formalin and processed as a group. After recording these variables, pectoral fin ray counts were obtained during which the previously measured parameters were concealed. An earlier study of this population (Hechter *et al.*, 2000) showed that fin ray counts (thus symmetry) can be measured without error. Individuals were considered symmetric when the number of fin rays on each side was equal. Ray counts of asymmetric individuals typically differed by one ray or rarely by two. The length and overall size of the fin rays was relatively uniform. The rays at both ends of the fin are only slightly shorter than those in the middle of the series. Thus, the form of measurement error involving the questionable presence of a structure described for meristic traits by Palmer (1994) did not occur in the present study.

Gut contents were assessed with previously measured parameters hidden. Ninety-seven percent of fish with food in their gut had consumed chironomid larvae. A few fish had eaten one or two ostracods, gammarids, or parts of insects. Total mass and size of food items other than head capsules of chironomid larvae could not be reliably evaluated due to varying degrees of digestion and mastication. We therefore compared the gut contents of symmetric and asymmetric fish for the number of chironomid larvae as their heads resist digestion.

Linear relationships between an isotope outcome variable (δ^{15}N or δ^{13}C) and body length were examined in separate regressions on symmetric males ($N = 45$), asymmetric males ($N = 44$), symmetric females ($N = 37$), and asymmetric females ($N = 36$). The differences in resulting slopes for symmetric and asymmetric fish within each sex were tested for statistical significance using beta-hat analysis (Milliken & Johnson, 2002) to determine whether an equal slopes or an unequal slopes covariance analysis should be applied with the isotope signature as the outcome variable, asymmetry (no, yes) as the study variable, and length as the covariate. Levene's test was used to evaluate the assumption of homogeneity of variances in the analyses. Statistically significant outliers to the regression models were identified by a test for outliers (Kleinbaum *et al.*, 1998). Analyses were conducted with and without statistically significant outliers included.

A multiple linear regression model was used to test whether mean number of chironomid larvae on a square root scale (NOC) differed between symmetric and asymmetric fish adjusted for body length and sex. Linear relationships between an isotope outcome variable (δ^{15}N or δ^{13}C) and NOC were examined in symmetric males, asymmetric males, symmetric females, and asymmetric females by applying multiple regressions to these four subgroups with the isotope signature as the response variable and NOC and body length as explanatory variables.

All statistical analyses were carried out using Statistical Analysis System (SAS), release 8.2.

RESULTS

The slope of the linear regression of δ^{15}N on body length in symmetric male fish (slope estimate = 0.063, SE = 0.011) differed significantly ($P = 0.046$) from the slope for that regression in asymmetric ones (slope estimate = 0.030, SE = 0.012; Fig. 1), indicating the mean difference in δ^{15}N for symmetric and asymmetric males depended on the length of fish (i.e. the regression lines of body length and δ^{15}N for asymmetric and symmetric males were not parallel). For this reason, the mean δ^{15}N for asymmetric and symmetric males were compared at selected body lengths using an unequal slopes covariance model in accordance with the suggestion of Milliken & Johnson (2002) that percentiles can be used as objective criteria for selecting covariate values at which comparisons may be made when regression lines are not parallel. Thus, applying the unequal slopes covariance model, the mean difference in δ^{15}N for asymmetric and symmetric males was evaluated at the 90th (61.1 mm), 75th

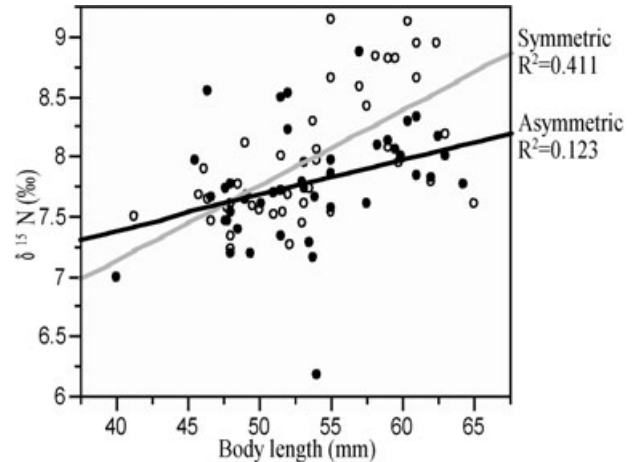**Figure 1.** Regression of δ^{15}N(‰) on body length (mm TL) of symmetric (○) and asymmetric (●) males.

(58.2 mm), 50th (53.2 mm), 25th (48.6 mm), and 10th (46.7 mm) percentiles of total body lengths where the length percentiles were obtained from the combined group of symmetric and asymmetric males. The mean δ^{15}N was significantly greater in symmetric than asymmetric males in the 90th, 75th, and 50th length percentiles ($P < 0.048$) and a similar trend ($P < 0.086$) was observed at the 25th percentile (Table 1). As the observation on an asymmetric male with δ^{15}N = 6.2 and length = 54.1 was a statistically significant outlier, the preceding analyses were repeated with this individual excluded. The conclusion that the difference in mean δ^{15}N for asymmetric and symmetric males occurred at the larger body lengths remained the same when the outlier was excluded. The slope for δ^{15}N on body length with this outlier excluded (slope = 0.031, SE = 0.010) was virtually identical to that obtained in the analysis with the outlier included (slope = 0.030, SE = 0.012). With the outlier excluded, the difference in slopes between asymmetric and symmetric male fish remained statistically significant as did the mean differences in δ^{15}N for asymmetric and symmetric males evaluated at the 90th (61.1 mm) and 75th (58.2 mm) percentiles of total body lengths. However, the *P*-value (= 0.087) for the difference between the mean δ^{15}N for asymmetric (7.815) and symmetric males (7.963) evaluated at the 50th (53.2 mm) percentile is greater than 0.05, when the significant outlier was excluded.

The slope of the regression of δ^{13}C on body length for symmetric males (estimated slope = 0.046, SE = 0.013) was significantly different ($P = 0.028$) from the slope for asymmetric males (estimated slope = 0.083, SE = 0.010; Fig. 2), thus indicating the difference in mean δ^{13}C for asymmetric and symmetric males was not the same at different fish lengths. Therefore, the

Table 1. Comparison of length-adjusted mean $\delta^{15}N$ [standard errors (SE) in parentheses] for asymmetric ($N = 44$) and symmetric males ($N = 45$) obtained from an unequal slopes covariance analysis evaluated at the 90th, 75th, 50th, 25th, and 10th percentiles of body length (mm in parentheses)

| Body length (mm) | Mean $\delta^{15}N‰$ | | t-test statistic for difference between adjusted means | P-value (two-sided) |
	Asymmetric (SE)	Symmetric (SE)		
90th percentile (61.1)	8.012 (0.114)	8.460 (0.111)	2.82	0.006
75th percentile (58.2)	7.926 (0.088)	8.278 (0.086)	2.86	0.005
50th percentile (53.2)	7.778 (0.066)	7.963 (0.065)	2	0.048
25th percentile (48.6)	7.642 (0.087)	7.674 (0.086)	0.27	0.790
10th percentile (46.7)	7.585 (0.103)	7.555 (0.102)	−0.21	0.834

Table 2. Comparison of length-adjusted mean $\delta^{13}C$ [standard errors (SE) in parentheses] for asymmetric ($N = 44$) and symmetric males ($N = 45$) obtained from an unequal slopes covariance analysis evaluated at the 90th, 75th, 50th, 25th, and 10th percentiles of body length (mm in parentheses)

| Body length (mm) | Mean $\delta^{13}C‰$ | | t-test statistic for difference between adjusted means | P-value (two-sided) |
	Asymmetric (SE)	Symmetric (SE)		
90th percentile (61.1)	−26.445 (0.114)	−26.895 (0.110)	−2.84	0.006
75th percentile (58.2)	−26.687 (0.088)	−27.028 (0.085)	−2.79	0.006
50th percentile (53.2)	−27.104 (0.066)	−27.258 (0.066)	−1.67	0.099
25th percentile (48.6)	−27.487 (0.087)	−27.469 (0.086)	−0.15	0.880
10th percentile (46.7)	−27.258 (0.064)	−27.104 (0.066)	−1.67	0.611
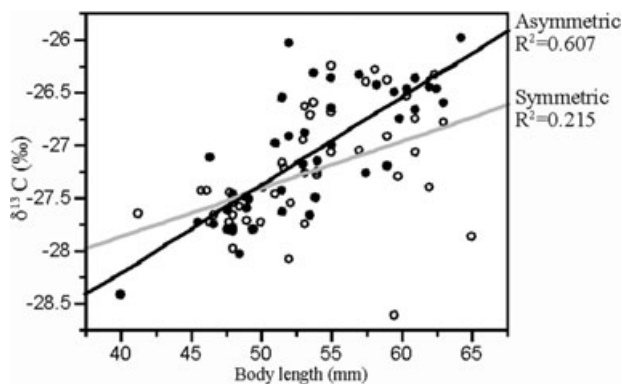
Figure 2. Regression of $\delta^{13}C(‰)$ on body length (mm TL) of symmetric (○) and asymmetric (●) males.

difference in mean $\delta^{13}C$ for asymmetric and symmetric males was evaluated in an unequal slopes covariance model at the 90th (61.1 mm), 75th (58.2 mm), 50th (53.2 mm), 25th (48.6 mm), and 10th (46.7 mm) percentiles for lengths of males considered together. Mean $\delta^{13}C$ was significantly more negative in symmetric males at the 90th and 75th length percentiles ($P < 0.006$). There was a similar trend ($P < 0.09$) at the 50th length percentile (Table 2). The above analyses were repeated excluding a symmetric male with $\delta^{13}C = -28.6$ and length = 59.6 that was a statistically significant outlier. With this outlier excluded, the difference in slopes for $\delta^{13}C$ on body length between symmetric and asymmetric male fish remained significant at the 5% level. With the outlier excluded, the slope for symmetric fish was 0.053 (SE = 0.012) compared to 0.046 (SE = 0.013) with the outlier included. The mean differences in $\delta^{13}C$ for asymmetric and symmetric males evaluated at the 90th (61.1 mm) and 75th (58.2 mm) percentiles of total body lengths remained statistically significant when the outlier was excluded from the analysis.

In females, mean $\delta^{15}N$ adjusted for length did not significantly differ ($P = 0.52$; equal slopes covariance) between symmetrics (mean $\delta^{15}N = 8.240$, SE = 0.096) and asymmetrics (mean $\delta^{15}N = 8.150$, SE = 0.096). Likewise, mean $\delta^{13}C$ adjusted for length did not significantly differ ($P = 0.47$; equal slopes covariance) between symmetric (mean $\delta^{13}C = -26.990$, SE = 0.085) and asymmetric (−26.922, SE = 0.085) females. Equal slopes univariate covariance analyses were applied because beta-hat analyses indicated that, in females (unlike in males), the mean difference between symmetrics and asymmetrics in $\delta^{15}N$ or in $\delta^{13}C$ did not vary with body length.

Table 3. Means and standard errors of body length and otolith area (arbitrary units) in sample cross-classified by sex and symmetry

	Mean length (mm)	Mean otolith area (mm²)
Symmetric ♀	55.22 ± 0.830	418.91 ± 7.478
Asymmetric ♀	55.28 ± 0.822	424.39 ± 8.141
Symmetric ♂	53.54 ± 0.836	398.65 ± 9.096
Asymmetric ♂	53.35 ± 0.840	400.42 ± 8.659

There was a significant positive linear association between $\delta^{15}N$ and length ($P < 0.02$), and also between $\delta^{13}C$ and length ($P < 0.01$), in all four subgroups (symmetric females, asymmetric females, symmetric males, asymmetric males), suggesting the niche space of fish shifted with increasing body size.

The difference in mean NOC between symmetric and asymmetric fish adjusted for length was consistent over sex. The mean NOC adjusted for length was 1.71 for symmetric males, 2.76 for asymmetric males, 1.62 for symmetric females, and 2.77 for asymmetric females. Multiple regression analysis revealed symmetric fish averaged significantly ($P = 0.019$) fewer chironomid larvae in their guts (mean = 1.66, SE = 0.012) than asymmetric fish (mean = 2.76, SE = 0.012) after adjustment for body length and sex. Levenes' test ($P = 0.442$) indicated the variance for NOC within symmetric and asymmetric fish did not significantly differ.

A statistically significant positive linear relationship between $\delta^{13}C$ and NOC in the gut was found for symmetric females in the multiple linear regression with $\delta^{13}C$ as the response variable and NOC and body length as explanatory variables (slope estimate for NOC = 0.241, SE = 0.101, $P = 0.024$). A positive linear relationship between $\delta^{13}C$ and NOC was also observed for asymmetric females but was not statistically significant (estimate of slope for NOC = 0.078, SE = 0.056, $P = 0.168$). Although symmetric and asymmetric females may have a different relationship between $\delta^{13}C$ and NOC, as revealed by their slope estimates for NOC in the multiple regressions (0.241 for symmetric females versus 0.078 for asymmetric females), we did not detect a significant difference in these slopes for NOC for symmetric and asymmetric females ($P = 0.14$). When the data on symmetric and asymmetric females were considered together in the multiple regression analysis, there was a significant linear relationship between $\delta^{13}C$ and NOC (slope estimate for NOC = 0.144, SE = 0.053, $P = 0.008$). The slopes for NOC in the separate regressions for symmetric and asymmetric males were, respectively, 0.032 and –0.002 ($P > 0.62$), indicating no linear relationship between $\delta^{13}C$ and NOC in males.

In the four separate multiple regression analyses (symmetric females, asymmetric females, symmetric males, asymmetric males) with $\delta^{15}N$ as the response variable and NOC and body length as explanatory variables, the slope estimates for NOC were all close to zero ($P > 0.69$).

No significant differences in the relative lengths and ages of symmetric and asymmetric fish were detected (Table 3).

DISCUSSION

Gasterosteids such as *Culaea* depend on their pectoral fins for locomotion and reproduction and there is evidence for correlations between fin asymmetry and these functions (Moodie, 1978; Hechter *et al.*, 2000). This morphological imbalance may have a functional consequence that affects the fitness of an asymmetric phenotype. Foraging behaviour could be altered and efficiency lowered if fin asymmetry negatively affects swimming performance. Differences in the stable isotope content of the tissues of symmetric and asymmetric males implies foraging behaviour was affected. The existence of a correlation between $\delta^{13}C$ and a measure of the gut contents (NOC), controlling for length, suggests that the differences we observed in $\delta^{13}C$ indeed reflect dietary differences. The fact the guts of asymmetric females contained more chironomid larvae than those of symmetric females cannot be interpreted as an indication of superior fitness of asymmetric fish. Fish choose the range of prey items that maximizes energetic gain (Werner & Hall, 1974). Whether that will lead to a choice of numerous and therefore probably small items, or large and therefore probably fewer items, interacts with other variables such as handling time, searching time, caloric value, and digestibility of the items. A large number of consumed food items could indicate inability to capture bigger, more energy efficient items (Bozek, DeBrey & Lockwood, 1994). The differences that we observed in isotope values and the number of chironomid larvae in the guts suggest differences in foraging behaviour exist between symmetric and asymmetric

fishes, although it is not possible to assign fitness levels to these differences.

Caution is necessary in interpreting results of stable isotope analysis as evidence of trophic relationships when age is a confounding factor (Overman & Parrish, 2001). Although we cannot exclude the possibility, it is unlikely that the differences observed in the present study are due to age-related factors as the fish compared were born within a few weeks of each other and were of almost identical body length. As a further precaution, we statistically controlled for length (a surrogate for age) by always including it as a covariate in the analyses testing for the effect of asymmetry on the isotope and gut data. Finally, otolith analysis did not reveal significant differences in age of symmetric and asymmetric fish. If these isotope differences do reflect trophic differences (Vander Zanden & Rasmussen, 1999) they suggest that the diet of symmetric males in the larger size percentiles is more dominated by carbon emanating from a pelagic source than the diet of asymmetric males. Both symmetric and asymmetric males and females appear to feed at higher trophic levels as their body length increases. The pelagic component of their diet is reduced as body length increases. The fact isotope ratio differences between symmetric and asymmetric fish appeared only in males could be related to the dimorphism in relative pectoral fin size which exists in this species (Hechter *et al.*, 2000). Males have relatively larger pectoral fins than females and fin asymmetry may degrade their swimming performance more than that of females. The significantly lower number of chironomid larvae present in the guts of symmetric fish also points to the existence of trophic differences between symmetric and asymmetric fish.

Other than Hechter *et al.* (2000), we found only one previous study demonstrating a relationship between FA of pectoral fins and function. In *Pomatoschistus microps*, a species in which the male fans the fertilized eggs with the pectoral fins, there was a significant positive relationship between male fin surface area asymmetry and both the nest area and proportion of the nest surface occupied by eggs. There was, however, no significant relationship between asymmetry and the number of eggs in the nest, nor egg density (Sasal & Pampoulie, 2000). Isotope analysis in the present study yielded persuasive evidence of trophic differences related to asymmetry in male*s*. In both females and males, gut content analysis revealed significantly fewer chironomid larvae on average in symmetric fish compared to asymmetric ones. In females, the number of chironomid larvae in the gut (a snapshot of diet at a point in time) was significantly positively correlated with δ^{13}C, a time-integrated measure of the source of algal components

of the diet. Dietary differences also occur between symmetric and asymmetric phenotypes of the pelvis and of the lateral plate series in the threespine stickleback, *G. aculeatus* (Reimchen & Nosil, 2001). In the water boatman, *Callicorixa vulnerata*, individuals with asymmetric feeding structures show reduced survival relative to symmetric ones under conditions of food competition (Nosil & Reimchen, 2001). These results, combined with our data on *Culaea*, suggest a potentially important role for ecological factors in structuring fitness differences between symmetric and asymmetric individuals.

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