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Differential temperature preferences of vertebral phenotypes in *Gasterosteus*

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Abstract

Across a broad diversity of freshwater and marine fish that have a wide latitudinal distribution, intraspecific variability in vertebral number shows a robust trend for higher vertebral counts in colder latitudes (Jordan's Rule). Variability in this meristic trait is determined during early larval development by a combination of heritable and temperature-mediated influences. We experimentally evaluate for the first time whether different vertebral phenotypes from a population actively segregate across a temperature gradient and if so, whether the segregation is consistent with broad geographical trends across taxa of greater vertebral counts associating with cooler waters. Using threespine stickleback (*Gasterosteus aculeatus* L., 1758) from two populations, we conducted 22 trials with ~50 fish per trial and a temperature gradient ranging from 2 to 15⁰C degrees among trials. Results show that 6 trials yielded statistically significant or near-significant results of which 5 trials were in the predicted direction of more vertebrae in cooler waters. The effects were more expressed in small body-size classes (35-55 mm) than in larger fish (60-85 mm) and in longer duration trials. We feel these data are novel and consistent with recent studies on swimming efficacy among vertebral phenotypes but also hint at much great ecological functionality than currently assumed to intrapopulation variation in this meristic trait.

Key words:

behavioural preferences, *Gasterosteus aculeatus*, Jordan's Rule, plasticity, vertebral number, temperature gradient, threespine stickleback

INTRODUCTION

Intraspecific variation in vertebral number is widespread in fishes. In species distributed across a north-south gradient, those in colder climates tend to have greater vertebral counts (Hubbs 1922; Fowler 1970; Lindsey and Arnason 1981; Baumann et al. 2012, exceptions in McDowell 2004). This geographical trend, generally known as Jordon's Rule, originates from a combination of heritable effects and temperature-sensitive phenotypic plasticity with vertebral number typically fixed during embryo development prior to hatching (Taning 1952; Lindsey 1962, 1975, 1988; Lindsey and Harrington 1972; Brooks and Johnston 1994; Alho et al. 2011; Ando et al. 2011). While subject to multiple approaches and interpretations, the significance of this variation remains poorly resolved. That more vertebrae tend to occur in colder latitudes could be associated with the benefits of increased axial flexibility and burst acceleration in the cooler and more viscous waters (Spouge and Larkin 1979; Conover et al. 1997; McDowall 2004).

Is this extensive vertebral variability, initially identified over large geographical regions, also evident at a small spatial scale within populations? In one of the first evaluations of microspatial patterns of vertebral phenotypes, Reimchen and Nelson (1987), studying threespine stickleback (*Gasterosteus aculeatus* L., 1758) from a small lake, observed differences in vertebral frequencies between littoral and offshore habitats and between surface and benthic layers in the water column. Although differential survival was possible, one of the alternate interpretations to these results could be different habitat preferences of vertebral phenotypes. This possibility has not yet been examined in the multiples studies of vertebral variation and is

surprising as vertical temperature gradients in the water column are one of the most widespread physical attributes available to fish in marine and freshwater habitats.

In the present paper, we examine whether different vertebral phenotypes of stickleback prefer different temperatures. Extending from Jordon's Rule and the assumption that vertebral variability has ecological function, we hypothesize that within a population, fish with more vertebrae will prefer colder water temperatures than fish with fewer vertebrae.

METHODS AND MATERIALS

Our first experiment was carried out in the field in June 1985 with the giant black threespine stickleback (*G. aculeatus*) from Drizzle Lake, Haida Gwaii, off the west coast of Canada (details of study area in Reimchen 1994). Four linear metal troughs (each 100 cm length, width 10 cm, depth 8 cm) were assembled into a square and eight flap doors placed in a horizontal position around the apparatus chamber which when released produced eight chambers of equal size. A heating coil was inserted into one side of the apparatus and an external ice bath attached to the opposite side and the unit filled with water from the littoral zone at Drizzle Lake. Stickleback were collected from the same littoral site where the water was obtained and transferred directly to the apparatus. To minimize potential stress on the fish, these were not handled during the transfer. An opaque lid was placed over the unit to exclude direct sunlight and the apparatus left undisturbed. Over approximately one hour, a temperature gradient from 2° C to 10° C developed between the coolest and warmest chambers, the gradient varying among trials due to daily differences in wind and air temperature. After three hours, flap doors were simultaneously released remotely and this confined fish into separate chambers. Water temperatures were recorded and the fish removed from each chamber and euthanized. Fourteen

trials were carried out over a two week period, half of the trials using mainly sub-adults (35-55mm standard length- SL) and half using adults (60-85 mm SL), the numbers of each varying among trials (11-146) with lower numbers for the larger fish. In total, 544 sub-adults and 166 adults were used (Table 1).

The second experiment (8 trials) was carried out in University of Alberta aquatic facilities. A two meter long linear trough was constructed (10 cm deep * 10 cm wide) and a temperature gradient achieved using a thermocooler and a heating coil on opposite ends of the trough. Flap doors were added along the length of the unit that when closed would create 10 chambers of equal size along the temperature gradient. Threespine stickleback (35-50 mm SL) were collected from Hasse Lake, Alberta, transferred to university facilities and kept at 9-14⁰C for 1-2 weeks prior to the experiment. The fish were added in the middle of the unit and allowed to disperse as the temperature gradient developed (Table 1). We altered the duration of the trials (3 hours- 3 trials, 48 hours - 1 trial, 72 hours - 2 trials, 5 days-1 trial and 8 days-1 trial) to examine potential influence of acclimation time on behaviour. After each trial, the flap doors were released remotely, trapping stickleback in chambers. In total, 577 fish were used, 72 to 151 in the short duration (3 hour trials) and 42 to 68 fish in the extended duration trials.

Following each trial, fish were removed and euthanized in MS222 and preserved in 10% formalin. Drizzle Lake fish and the short duration trials for the Hasse Lake fish were scored for sex. Fish were x-rayed and radiographs scored under a dissecting scope for SL and total vertebra. Hasse Lake stickleback were also scored for numbers of abdominal and caudal vertebrae. Number of vertebrae ranged from 31-35 (predominantly 33 and 34) in the Drizzle Lake population and 30-33 (predominantly 31 and 32) in the Hasse Lake population (Table 1). Statistical associations between vertebral phenotype and chamber were examined using non-

parametric tests (SPSS Inc. 2008). Because the temperature mean and range differed substantially among trials (2 - 10°C) for the Drizzle Lake field experiments (Table 1), we partitioned the gradient into three categories (low, medium, high) for each trial and separated the data for the two body size classes. We did multiple analyses and examined each trial separately (sexes combined, sexes separate), initially with univariate contingency tests and secondly using loglinear tests (sexes combined, sexes separate). The temperature range for the linear chamber trials in the laboratory with the Hasse Lake stickleback was about 5°C in each trial and we did a log-linear analysis using temperature (df=9), vertebral phenotype (df=4) and trial (df=7). Although the Hasse Lake fish were unimodal in body size (range 30-55 mm SL), any association between body length and vertebral number within each size class could confound any interpretations of potential temperature preferences. As such, we partitioned body size into two size classes (≤ 40 , > 40 mm SL) but found no significant difference in mean vertebral counts ($F_{1,321}=1.1$, $P=0.3$). We also partitioned the sub-adult Drizzle Lake fish into two classes (≤ 40 , > 40 mm SL) but found no differences between the groups ($F_{1,547}=1.2$, $P=0.2$).

Results

Field experiments with the Drizzle Lake fish using the square chamber yielded a weak inverse association between vertebral number and temperature. Of the seven trials using small fish (30-55 mm SL), two showed significant effects ($P<0.01$) and a third a marginal effect ($P=0.08$), each in the predicted direction of greater vertebrae in colder waters. We restricted the analyses to males, the most abundant in the samples, and the statistical trends were similar to the combined sexes. Loglinear analysis (males only) showed a marginal effect (partial chi-square= $P=0.06$) with greater vertebral number in the cooler temperature. Among seven trials with large

fish (65-85 mm SL), none reached significance (all with $P>0.2$). Male-only analysis did not alter this association.

Laboratory experiments with a linear chamber with Hasse Lake stickleback were tested over different exposure durations to the temperature gradient. Loglinear analysis showed no significant vertebra * temperature * trial interaction (partial $\chi^2_{189}=125.4$, $P>0.9$) but a significant vertebra * temperature interaction (partial $\chi^2_{27}=43.2$, $P<0.03$) with greater vertebral counts in cooler waters (Figure 1). We collapsed the 10 chambers into three temperature groups (low, medium, high) independent of the number of fish in each group. Among the eight trials, one of the three short duration trials showed a significant ($P<0.02$) effect with more vertebrae in warmer waters, the opposite of the predicted direction, while three of the five extended duration trials showed a trend ($P<0.03$, $P<0.08$, $P<0.2$) towards more vertebrae in cooler waters. We also collapsed the temperature gradient over the ten chambers into three categories (low, medium and high) such that there were similar numbers of fish in each category. Analyses of these data were consistent and showed a significant trend for higher vertebral counts in cooler waters (verts*temperature: partial $\chi^2_6=17.5$, $P<0.01$). We also examined whether there was any association with the ratio of abdominal versus caudal vertebrae, with low ratios reflecting proportionately more caudal vertebrae. One of the eight trials showed a significant ($P<0.01$) trend for lower ratios in cooler temperatures and with no trends in the remaining trials ($P>0.4$).

Discussion

Based on an earlier study showing spatial differences in vertebral number in a lake population of *Gasterosteus* (Reimchen and Nelson 1987), we tested whether vertebral phenotypes of wild-captured fish differed in their temperature preference over a thermal

gradient. More specifically, and extending from the taxonomically and geographically robust trends characterized in Jordon's Rule and the assumption that vertebral variability was ecologically relevant, we predicted that within a population, fish with more vertebrae, would prefer cooler temperatures relative to those with fewer vertebrae. We detected no such effect in 16 of 22 trials but of the remaining six trials with significant or near significant effects ($P < 0.1$), five were in the predicted direction and were consistent with the hypothesis of ecologically-mediated variability in vertebral number. It is possible that our results are conservative. The minimum body size of fish we used (~35mm SL) was much larger than what Swain (1992) used (~15 mm SL) in detecting differences in swimming mode among vertebral phenotypes in stickleback.

With our experiments, we are not able to identify the proximal mechanisms for the trends. Individual fish may be seeking out temperatures encountered during early ontogeny or during recent foraging periods and as such the experimental results could be a correlated by-product of previous behaviour and habitat. Use of laboratory-hatched fish would address this possibility.

How might different vertebral phenotypes benefit from choosing different thermal micro-habitats? Greater number of vertebrae increases axial flexibility (Long and Nipper 1996; Brainerd and Patek 1998) and this can influence maneuverability, burst acceleration and cruising speed (Gosline 1971; Lindsey 1978; Webb 1982; Swain 1992; Long and Nipper 1996; Brainerd and Patek 1998; McDowall 2004). Each of these swimming parameters is influenced by water viscosity which varies predictably with temperature. Based on standard equations for fluid motion (Al-Shemmeri 2012), viscosity would increase 30% between 20°C and 10°C. Hydrodynamic studies of larval Atlantic herring (*Clupea harengus*) indicate that 48% of the variation in swimming speed is explained by a combination of temperature and viscosity

(Fuiman and Batty 1997). As such, it is reasonable that any correlation between vertebral phenotype and behavioural temperature preference could be favoured if this leads to more efficient swimming, for example during migration or foraging. It might also be particularly useful during evasive responses to predators as individuals differing by a single vertebra are known to have altered susceptibility to predators (Swain and Lindsey 1984; Swain 1992). Juvenile and sub-adult stickleback tend to have high levels of predator-mediated mortality (Reimchen 1988, 1994) increasing the potential for differential responses of vertebral phenotypes.

One of the most common physical parameters in marine and freshwater habitats is the vertical temperature gradient and a prediction emerging from our study is that this gradient might be differentially exploited by vertebral phenotypes within a population of fish. Temperature differences of 10°C can occur over a distance of 10 m in a temperate lake thermocline and can persist throughout summer months (Reid 1961). Despite the extensive interest in meristic variation in fish, vertebral phenotype frequencies along a depth transect have rarely been documented. We have found only several studies with such data and results are broadly consistent with our prediction. Löken *et al.* (1994), working with Norwegian Arctic cod (*Gadus morhua*), found that within age classes, there was increased vertebral numbers with water depth. However, this was thought to reflect mixtures of different stocks varying in vertebral counts rather than behavioral preferences within a stock. Swain and Frank (2000), sampled cod (*G. morhua*) in a vertical transect in the western Atlantic and identified a linear increase in vertebral counts with water depth (25 m to 175 m). Among the multiple interpretations they considered for this trend, including the potential for vertebrae-specific temperature preferences, they were

not able to resolve alternate hypotheses. Our study on stickleback suggests that temperature stratification could contribute to these vertical clines in vertebral phenotypes.

It is well established that the extent of axial segmentation is influenced by both genetic background and temperature-dependent development plasticity (Lindsey 1982). Direct estimates of heritability of vertebral number average 66% across diverse taxa and are 36% in *Gasterosteus* (Alho et al. 2011). If this heritability also applies to our populations, it suggest the potential for additional genetic covariation between this meristic variation and behavioural temperature preferences. Alternatively, if vertebral variability results completely from developmental plasticity, a behavioural preference could emerge from variable swimming efficacy in waters differing in temperature/viscosity. In *Gasterosteus*, one of the best intrapopulation predictors of vertebral number in a lake population of stickleback was relative body depth, with increased vertebrae occurring with reduced body depth, a trend occurring in both sexes and across multiple size classes (Reimchen and Nelson 1987; see also Aguirre et al. 2014). This covariation between a meristic trait (vertebrae) and a metric trait (body shape) within populations suggests further developmental complexity structuring vertebral number.

If our results in these initial experiments are confirmed, several practical and conceptual applications emerge. First, if fish are segregating over a temperature gradient according to vertebral phenotype, sampling natural populations would require increased attention to vertical and horizontal thermal gradients and these could be expected to change between daytime and nighttime or across seasons. Second, it seems probable that foraging activity periods and fitness of vertebral phenotypes would vary as a function of temperature regime particularly across a thermocline where large differences in temperature and viscosity can occur. Third, we suspect our evidence for different temperature preferences are part of a more integrated meristic and

behavioural phenotype and may contribute to the interpretation of the geographical and taxonomically robust trends in Jordan's Rule.

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Figure caption:

Figure 1. Temperature preference experiments using Hasse Lake threespine stickleback differing in vertebral number. For each vertebral phenotype (30 to 33), bars show the percentage of fish along the temperature gradient for the combined 8 trials. N=total number of fish. Three-way log linear analyses: verts* temperature* trial: partial $\chi^2_{189}=125.4$, $P>0.9$; verts* temperature: partial $\chi^2_{27}=43.2$, $P<0.03$.

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Table 1. Summary of experimental protocol for temperature preference experiments of vertebral phenotypes in threespine stickleback.

Trail	Duration	Temp Range °C	Vert. Range	N _{SA}	N _A
Drizzle Lake 1	3hrs	14.0-24.0	33-34	0	11/5
Drizzle Lake 2	3hrs	15.5-17.5	32-34	0	7/3
Drizzle Lake 3	3hrs	11.0-15.0	32-34	0	11/3
Drizzle Lake 4	3hrs	8.5-12.5	32-34	59/23	0
Drizzle Lake 5	3hrs	13.0-19.0	32-34	53/20	0
Drizzle Lake 6	3hrs	13.5-19.5	32-34	14/3	16/7
Drizzle Lake 7	3hrs	8.0-14.5	32-35	74/26	0
Drizzle Lake 8	3hrs	9.0-14.5	32-34	27/7	0
Drizzle Lake 9	3hrs	10.5-15.0	32-34	41/14	0
Drizzle Lake 10	3hrs	8.5-12.0	32-34	19/13	7/2
Drizzle Lake 11	3hrs	8.0-14.0	32-34	0	6/11
Drizzle Lake 12	3hrs	9.0-16.0	32-34	0	39/16
Drizzle Lake 13	3hrs	9.0-20.5	32-35	103/44	0
Drizzle Lake 14	3hrs	9.0-16.0	32-35	0	9/12
Hasse Lake 1	3hrs	5.5-9.8	30-32	5/36	9/20
Hasse Lake 2	3hrs	7.0-13.8	30-33	18/37	19/40
Hasse Lake 3	3hrs	10.5-15	30-33	18/53	32/56
Hasse Lake 4	3 days	4.0-9.1	30-33	11	28
Hasse Lake 5	5 days	5.0-10.0	31-33	5	38
Hasse Lake 6	8 days	5.0-10	30-33	6	38
Hasse Lake 7	3 days	7.0-13.8	30-33	9	57
Hasse Lake 8	2 days	10.5-14.5	30-33	1	48

Notes: N_{SA}, N_A indicates sample sizes of subadult and adult stickleback for each sex (m/f) if available.