NRC Research Press

ARTICLE

Differential temperature preferences of vertebral phenotypes in *Gasterosteus*

T.E. Reimchen and K.D. Cox

Abstract: Across a broad diversity of freshwater and marine fish that have wide latitudinal distributions, 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 that are associated 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 15 °C among trials. Results show that six trials yielded statistically significant or near-significant results, of which five trials were in the predicted direction of more vertebrae in cooler waters. The effects were more expressed in fish with small bodies (35–55 mm) than in fish with larger bodies (60–85 mm) and in longer duration trials. We believe that these data are novel and are consistent with recent studies on swimming efficacy among vertebral phenotypes; the data also hint at much greater ecological functionality than is currently assumed by intrapopulation variation in this meristic trait.

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

Résumé : Pour une grande variété de poissons marins et d'eau douce répartis sur de grandes fourchettes de latitudes, la variabilité intraspécifique du nombre de vertèbres définit une tendance robuste vers un plus grand nombre de vertèbres aux latitudes plus froides (la règle de Jordan). La variabilité de ce caractère méristique est déterminée durant le développement précoce des larves par une combinaison d'influences héréditaires et modulées par la température. Nous avons évalué expérimentalement, pour la première fois, s'il y a ségrégation active le long d'un gradient de température de différents phénotypes vertébraux d'une même population et, le cas échéant, si cette ségrégation concorde avec de grandes tendances géographiques chez différents taxons vers un nombre accru de vertèbres associé à des eaux plus froides. En utilisant des épinoches à trois épines (*Gasterosteus aculeatus* L., 1758) de deux populations, nous avons mené 22 essais avec chacun environ 50 poissons et un gradient de température allant de 2 à 15 °C selon l'essai. Six des essais ont donné des résultats statistiquement significatifs ou quasi significatifs, dont cinq vont dans le sens prédit d'un plus grand nombre de vertèbres en eau plus froide. L'expression des effets est plus nette chez les classes de plus petite taille (35–55 mm) que chez les poissons plus grands (60–85 mm) et dans les essais de plus longue durée. Nous croyons que ces données sont originales et qu'elles concordent avec les résultats d'études récentes sur l'efficacité de la nage pour différents phénotypes vertébraux; les données indiquent également une fonctionnalité écologique beaucoup plus grande que ce qui est supposé actuellement sur la base des variations de ce caractère méristique au sein de différentes populations. [Traduit par la Rédaction]

Mots-clés : préférences comportementales, épinoche à trois épines, *Gasterosteus aculeatus*, règle de Jordan, plasticité, nombre de vertèbres, gradient de température.

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 McDowall 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 (Tåning 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 multiple studies

Received 11 September 2015. Accepted 23 October 2015.

T.E. Reimchen and K.D. Cox. Department of Biology, University of Victoria, P.O. Box 3020, Victoria, BC V8W 3N5, Canada.
Corresponding author: T.E. Reimchen (e-mail: reimchen@uvic.ca).

Can. J. Zool. **94**: 1–5 (2016) dx.doi.org/10.1139/cjz-2015-0193

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.

Materials and methods

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 long, 10 cm wide, 8 cm deep) 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 1 h, a temperature gradient from 2 to 10 °C developed between the coolest and the warmest chambers, the gradient varying among trials due to daily differences in wind and air temperatures. After 3 h, 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 2-week period, half of the trials using mainly subadults (35–55 mm 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 subadults and 166 adults were used (Table 1).

The second experiment (eight trials) was carried out in the University of Alberta aquatic facilities. A 2 m 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, which 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 9–14 °C 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 h (three trials), 48 h (one trial), 72 h (two trials), 120 h (one trial), and 192 h (one 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: 72–151 in the short-duration trials (3 h trials) and 42–68 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 to 35 (predominantly 33 and 34) in the Drizzle Lake population and from 30 to 33 (predominantly 31 and 32) in the Hasse Lake population (Table 1). Statistical associations between vertebral phenotype and chamber were examined using nonparametric 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 log-linear 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 trail (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 and >40 mm SL) but found no significant difference in mean vertebral counts ($F_{[1,321]} = 1.1$, $P = 0.3$). We also partitioned the subadult Drizzle Lake fish into two classes (≤40 and >40 mm SL) but found no differences between the groups ($F_{[1,547]} = 1.2$, $P = 0.2$).

Table 1. Summary of experimental protocol for temperature-preference experiments of vertebral phenotypes in threespine stickleback (*Gasterosteus aculeatus*).

Trail	Duration (h)	Temperature range (°C)	Vertebral range	N_{SA}	N_A
Drizzle Lake 1	3	14.0–24.0	33–34	0	11/5
Drizzle Lake 2	3	15.5–17.5	32–34	0	7/3
Drizzle Lake 3	3	11.0–15.0	32–34	0	11/3
Drizzle Lake 4	3	8.5–12.5	32–34	59/23	0
Drizzle Lake 5	3	13.0–19.0	32–34	53/20	0
Drizzle Lake 6	3	13.5–19.5	32–34	14/3	16/7
Drizzle Lake 7	3	8.0–14.5	32–35	74/26	0
Drizzle Lake 8	3	9.0–14.5	32–34	27/7	0
Drizzle Lake 9	3	10.5–15.0	32–34	41/14	0
Drizzle Lake 10	3	8.5–12.0	32–34	19/13	7/2
Drizzle Lake 11	3	8.0–14.0	32–34	0	6/11
Drizzle Lake 12	3	9.0–16.0	32–34	0	39/16
Drizzle Lake 13	3	9.0–20.5	32–35	103/44	0
Drizzle Lake 14	3	9.0–16.0	32–35	0	9/12
Hasse Lake 1	3	5.5–9.8	30–32	5/36	9/20
Hasse Lake 2	3	7.0–13.8	30–33	18/37	19/40
Hasse Lake 3	3	10.5–15	30–33	18/53	32/56
Hasse Lake 4	72	4.0–9.1	30–33	11	28
Hasse Lake 5	120	5.0–10.0	31–33	5	38
Hasse Lake 6	192	5.0–10	30–33	6	38
Hasse Lake 7	72	7.0–13.8	30–33	9	57
Hasse Lake 8	48	10.5–14.5	30–33	1	48

Note: N_{SA} and N_A indicate sample sizes of subadult and adult stickleback, respectively, for each sex (male/female) if available.

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 showed a marginal effect ($P = 0.08$), each in the predicted direction of greater vertebrae in colder waters. We restricted the analyses to males, which is the most abundant in the samples, and the statistical trends were similar to the combined sexes ($P < 0.01$ in two cases and $P = 0.09$ in the third case). 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. Log-linear 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 (Fig. 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 10 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 (vertebra × 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 ($P > 0.4$).

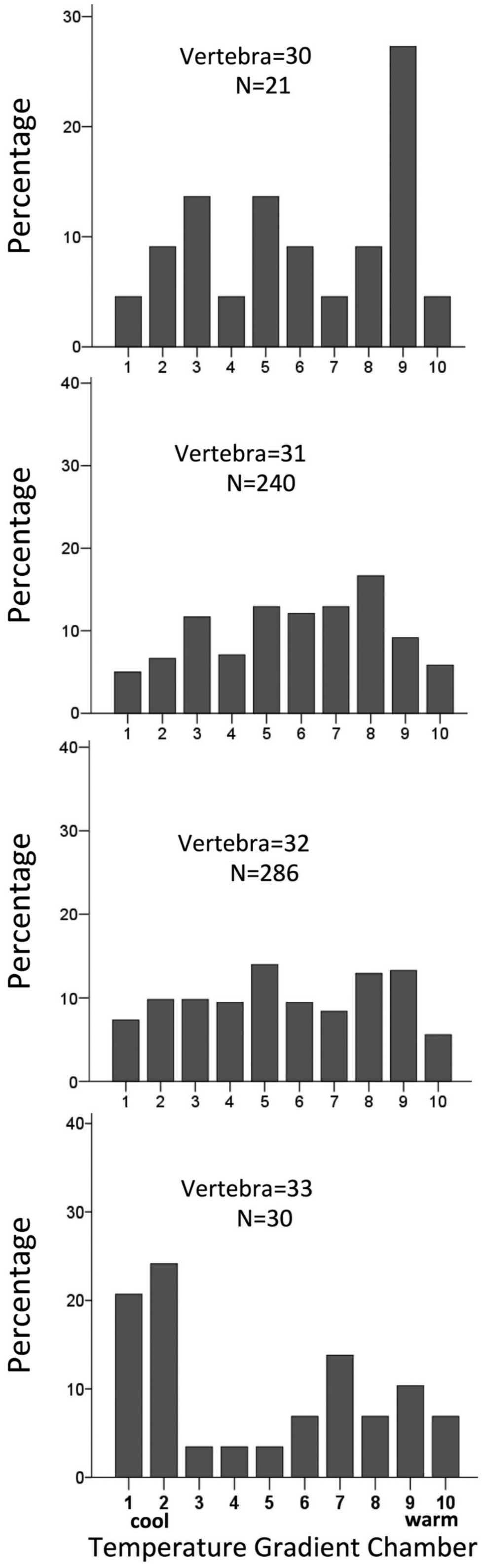
Discussion

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 6 trials with significant or near significant effects ($P < 0.1$), 5 trials 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 (~35 mm 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 microhabitats? 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 and 10 °C. Hydrodynamic studies of larval Atlantic herring (*Clupea harengus* L., 1758) 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 subadult

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



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. Løken et al. (1994), working with Norwegian Arctic cod (*Gadus morhua* L., 1758), 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 behavioural 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 (25–175 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 1962). 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, then 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, then a behavioural preference could emerge from variable swimming efficacy in waters differing in temperature or 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, then several practical and conceptual applications emerge. First, if fish are segregating over a temperature gradient according to vertebral phenotype, then 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.

Acknowledgements

T.E.R. thanks S. Douglas and multiple volunteers for overland transfer of ice to the field site at Drizzle Lake. J. Westly assisted in the field and laboratory during the Hasse Lake trials. This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) operating grant to T.E.R. (NRC2354).

References

Aguirre, W.E., Walker, K., and Gideon, S. 2014. Tinkering with the axial skeleton: vertebral number variation in ecologically divergent threespine stickleback populations. Biol. J. Linn. Soc. **113**: 204–219. doi:10.1111/bij.12316.

Alho, J.S., Leinonen, T., and Merilä, J. 2011. Inheritance of vertebral number in the three-spined stickleback (*Gasterosteus aculeatus*). PLoS ONE, **6**(5): e19579. doi:10.1371/journal.pone.0019579.

Al-Shemmeri, 2012. Engineering fluid mechanics. BoBoCoAe, TAS and Ventus Publishing ApS, Frederiksberg, Denmark.

Ando, D., Shinriki, Y., Miyakoshi, Y., Urabe, H., Yasutomi, R., Aoyama, T., Sasaki, Y., and Nakajima, M. 2011. Seasonal variations in and effect of incubation water temperature on vertebral number in naturally spawning chum salmon *Oncorhynchus keta*. Fish. Sci. **77**: 799–807. doi:10.1007/s12562-011-0390-3.

Baumann, H., Rosales, Casián, J.A., and Conover, D.O. 2012. Contrasting latitudinal variations in vertebral number and sex determination in Pacific versus Atlantic silverside fishes. Copeia, **2012**: 341–350. doi:10.1643/CG-10-189.

Billerbeck, J.M., Ortí, G., and Conover, D.O. 1997. Latitudinal variation in vertebral number has a genetic basis in the Atlantic silverside, *Menidia menidia*. Can. J. Fish. Aquat. Sci. **54**(8): 1796–1801. doi:10.1139/f97-084.

Brainerd, E.L., and Patek, S.N. 1998. Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in tetraodontiform fishes. Copeia, **1998**: 971–984. doi:10.2307/1447344.

Brooks, S., and Johnston, I.A. 1994. Temperature and somitogenesis in embryos of the plaice (*Pleuronectes platessa*). J. Fish Biol. **45**: 699–702. doi:10.1111/j.1095-8649.1994.tb00936.x.

Fowler, J.A. 1970. Control of vertebral number in teleosts—an embryological problem. Q. Rev. Biol. **45**: 148–167. doi:10.1086/406492.

Fuiman, L.A., and Batty, R.S. 1997. What a drag it is getting cold: partitioning the physical and physiological effects of temperature on fish swimming. J. Exp. Biol. **200**: 1745–1755. PMID:9319652.

Gosline, W.A. 1971. Functional morphology and classification of teleostean fishes. University Press of Hawaii, Honolulu.

Hubbs, C.L. 1922. Variations in the number of vertebrae and other meristic characters of fishes correlated with the temperature of water during development. Am. Nat. **56**: 360–372. doi:10.1086/279875.

Lindsey, C.C. 1962. Experimental study of meristic variation in a population of threespine sticklebacks, *Gasterosteus aculeatus*. Can. J. Zool. **40**(2): 271–312. doi:10.1139/z62-028.

Lindsey, C.C. 1975. Pleomerism, the widespread tendency among related fish species for vertebral number to be correlated with maximum body length. J. Fish. Res. Bd. Can. **32**(12): 2453–2469. doi:10.1139/f75-283.

Lindsey, C.C. 1978. Form, function, and locomotory habits in fish. *In* Fish physiology. Vol. II. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 1–100.

Lindsey, C.C. 1988. Factors controlling meristic variation. *In* Fish physiology. *Edited by* D.J. Hoar and D.J. Randall. Academic Press, New York. pp. 197–274.

Lindsey, C.C., and Arnason, A.N. 1981. A model for responses of vertebral numbers in fish to environmental influences during development. Can. J. Fish. Aquat. Sci. **38**(3): 334–347. doi:10.1139/f81-044.

Lindsey, C.C., and Harrington, R.W., Jr. 1972. Extreme vertebral variation induced by temperature in a homozygous clone of the self-fertilizing cyprinodontid fish *Rivulus marmoratus*. Can. J. Zool. **50**(6): 733–744. doi:10.1139/z72-100.

Løken, S., Pedersen, T., and Berg, E. 1994. Vertebrae numbers as an indicator for the recruitment mechanism of coastal cod of northern Norway. ICES Mar. Sci. Symp. **198**: 510–519.

Long, J.H., and Nipper, K.S. 1996. The importance of body stiffness in undulatory propulsion. Am. Zool. **36**: 678–694. doi:10.1093/icb/36.6.678.

McDowall, R.M. 2004. Variation in vertebral number in galaxiid fishes, how fishes swim and a possible reason for pleomerism. Rev. Fish Biol. Fish. **13**: 247–263. doi:10.1023/B:RFBF.0000033121.97066.c1.

Reid, G.K. 1961. Ecology of inland waters and estuaries. Chapman and Hall Ltd., London.

Reimchen, T.E. 1988. Inefficient predators and prey injuries in a population of giant stickleback. Can. J. Zool. **66**(9): 2036–2044. doi:10.1139/z88-299.

Reimchen, T.E. 1994. Predators and evolution in threespine stickleback. *In* Evolution of the threespine stickleback. *Edited by* M.A. Bell and S.A. Foster. Oxford University Press, Oxford. pp. 240–273.

Reimchen, T.E., and Nelson, J.S. 1987. Habitat and morphological correlates to vertebral number as shown in a teleost, *Gasterosteus aculeatus*. Copeia, **1987**: 868–874. doi:10.2307/1445549.

Spouge, J.L., and Larkin, P.A. 1979. A reason for pleomerism. J. Fish. Res. Bd. Can. **36**(3): 255–269. doi:10.1139/f79-041.

SPSS Inc. 2008. SPSS statistics for Windows. Version 22.0 [computer program]. SPSS Inc., Chicago.

Swain, D.P. 1992. Selective predation for vertebral phenotype in *Gasterosteus* *aculeatus*: reversal in the direction of selection at different larval sizes. Evolution, **46**: 998–1013. doi:10.2307/2409752.

Swain, D.P., and Lindsey, C.C. 1984. Selective predation for vertebral number of young sticklebacks, *Gasterosteus aculeatus*. Can. J. Fish. Aquat. Sci. **41**(8): 1231–1233. doi:10.1139/f84-146.

Swain, D.P., and Frank, K.T. 2000. Depth-dependent vertebral number of Atlantic cod (*Gadus morhua*) on the Magdalen Shallows and Scotian Shelf: stock mixing or microgeographic trends within populations? Can. J. Fish. Aquat. Sci. **57**(12): 2393–2401. doi:10.1139/f00-218.

Tåning, A.V. 1952. Experimental study of meristic characters in fishes. Biol. Rev. **27**: 169–193. doi:10.1111/j.1469-185X.1952.tb01392.x.

Webb, P.W. 1982. Locomotor patterns in the evolution of actinopterygian fishes. Am. Zool. **22**: 329–342. doi:10.1093/icb/22.2.329.