ADAPTIVE SIGNIFICANCE OF VARIATION IN VERTEBRAL NUMBERIN FISHES: EVIDENCE IN Gasterosteus aculeatus ANDMylocheilus caurinus
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Variation in vertebral number is widespread in fishes, and is partly genetic in origin. Its adaptive significance was tested in young threespine sticklebacks Gasterosteus aculeatus by predation experiments and by direct measurements of swimming performance, and in young peamouth chub Mylocheilus caurinus by predation experiments. Counts of wild fry grouped by length or age were examined for evidence of selection for vertebral number in the wild.

Survival of sticklebacks exposed to predation by sunfish Lepomis gibbosus was greater for fish with 31 TV (total vertebrae) than for those with 32 TV at lengths of 8.1-8.3 mm (mean at end of experiments), but not at smaller ( 7.6 mm ) or larger (8.9-11.2 mm) lengths, using fish from either Holden or Kennedy Lake, B.C. Among Holden Lake sticklebacks, greater survival of fish with 31 TV at small lengths (8.1-8.3 mm) was due to fish with a high ratio of $14 \mathrm{AV} / 17 \mathrm{CV}$ (abdominal/caudal vertebrae). At intermediate (9.8-10.0 mm) or large (11.2 mm) lengths, survival was greatest for fish with intermediate (14/18, 13/17) or low (13/18) ratios, respectively. Among Kennedy Lake sticklebacks, the ratios at advantage during predation also decreased as prey size increased, but higher ratios were favoured at a given size. Selective predation for vertebral number or ratio was not attributable to size selection or selection for body proportions (precaudal/caudal or abdominal/caudal lengths), and did not vary between cover (presence/absence) or temperature (15/20 or $25 C$ ) treatments.

Burst swimming performance of Holden Lake sticklebacks at 15C was superior among fry with a high ratio of $14 \mathrm{AV} / 17 \mathrm{CV}$ at small lengths ( $7.4-7.8 \mathrm{~mm}$ ), among those with an intermediate ratio (14/18) at intermediate lengths (7.8-8.3 mm), and among those with a low ratio (13/18) at large lengths (8.3-9.0 mm). At even larger lengths (9.0-11.5 mm), performance was unrelated to $A V / C V$. Effects of $A V / C V$ were similar at $25 C$, except that fry with high or intermediate ratios were superior at slightly shorter lengths at this higher temperature. The effect of $A V / C V$ on performance differed greatly between water and an $0.1 \%$ solution of methylcellulose at 15 C . Effects of $A V / C V$ could not be attributed to effects of precaudal/caudal length ratio. Differences between predation and swimming performance experiments in the lengths at which particular ratios were optimal probably reflected growth and the concentration of mortality at smaller lengths during predation experiments.

Vertebral counts of wild stickleback fry in Holden Lake indicated selection favouring a high ratio ( $14 \mathrm{AV} / 17 \mathrm{CV}$ ) and a low total count (31) at small lengths (7.3-7.8 mm), and intermediate ratios (14/18, 13/17) and a high total count (32) at slightly larger lengths (7.8-8.3 mm).

Survival of peamouth chub during predation by sunfish or smallmouth bass Micropterus dolomieui was greater for fish with 44 TV than for those with 45 TV at lengths of $10.8-11.1 \mathrm{~mm}$ (mean at end of experiments), but not at smaller lengths (when vertebrae of most fry were undeveloped). Selection for vertebral number could not be attributed to size selection by predators.

Selection in the wild favoured fry with 44 TV at small lengths (9.3-9.9 mm), those with 45 TV at slightly larger lengths (9.9-10.6 mm), and neither number at even larger lengths (10.6-14.0 mm).

Effects of vertebral number on performance may involve optimal anteroposterior gradients in flexibility. Explanations are suggested for pleomerism and Jordan's rule, in terms of selection operating on the larvae or fry soon after hatching. Result's are related to the maintenance of variation in vertebral number within populations, and to the possible adaptive significance of environmentally induced variation in vertebral number.

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## Introduction

The adaptive significance of variation within species is a central problem in evolutionary biology. Present interest in this problem has been stimulated largely by the discovery of vast amounts of allozyme variation in populations (e.g., Harris 1966; Lewontin and Hubby 1966). This variability, greater than had been expected by population geneticists, can be interpreted in two ways. It may be selectively neutral, and persists simply because no selective forces act on it. Or, selective mechanisms capable of maintaining polymorphism may be more widespread than had been supposed. This latter possibility has led to much theoretical work on the conditions under which diversifying selection in space or time can maintain genetic variation within populations (Levene 1953; Dempster 1955; Haldane and Jayaker 1963; Bryant 1976; Hedrick et al. 1976; Spieth 1979; Ewing 1979).

An analogous and older problem exists regarding the maintenance of morphological and behavioural variation in populations. This problem is complicated by the possibility that such variation may reflect environmentally induced rather than genetic variation, or may result from indirect selection acting on genetically correlated traits.

The mechanisms maintaining morphological variation have received much attention in the gasterosteid fishes. These fishes are conspicuously variable for characters relating to the extent of body armature (number of lateral plates, number and size of
dorsal and pelvic spines). Predation has usually been implicated as an important factor in selection operating on these characters (McPhail 1969; Hagen and Gilbertson 1972, 1973; Moodie 1972; Moodie et al. 1973; Moodie and Reimchen 1976; Bell and Haglund 1978; Reimchen 1980, 1983; Reist 1980a,b; Blouw and Hagen 1984a,b). Some workers have emphasized a direct functional significance of the various character states in avoiding a variety of predators in a variety of circumstances (e.g., Reimchen 1980, 1983). Others have suggested that the character of direct selective significance may not be the extent of body armature itself, but rather some correlated behavioural character (e.g., Moodie et al. 1973; Reist 1980a,b; Blouw and Hagen 1984a).

Variation in the number of vertebrae is widespread within and among fish populations. This variation is partly environmental in origin (e.g., Lindsey and Arnason 1981; Swain and Lindsey 1986a,b), but also has a genetic component (Lindsey 1962; Ali and Lindsey 1974; Leary et al. 1985a). The 'neutralist' view has been that such variation persists because, within narrow limits, the precise vertebral count is without selective significance (Fowler 1970). Wide malleability of vertebral count in response to environmental influences during early development has been considered consistent with a lack of strong selective advantage to particular counts. Fowler (1970) has argued that, within limits, it may be more adaptive to 'let' the number of vertebrae vary due to random environmental changes, than to fix the number genetically, since to do so would limit a
fish's ability to respond to stresses exerted by mutation or environmental change on other developmental processes of greater selective significance (Bateson 1963).

A contrasting 'selectionist' view is supported by the widespread tendency among related forms for higher vertebral number to be associated with longer maximum body length (pleomerism) and with higher latitude or colder waters (Jordan's rule) (Lindsey 1975). It has been argued that these trends might reflect selection, not for vertebral number per se, but rather for characters such as egg size or developmental rate, which incidently influence the number of vertebrae formed in embryos (Hubbs 1926, 1928). Alternatively, a functional advantage, perhaps involving locomotory ability, may be associated with some optimum number of vertebrae (Lindsey 1975). This might involve flexibility, myomere number or some other feature of the axial segmentation. If so, pleomerism suggests that the functionally optimum number of segments might depend on body size, and Jordan's rule suggests that the optimum number at a given size might depend on water temperature or viscosity.

In this thesis, $I$ test the hypotheses that some selective advantage is associated with particular numbers of vertebrae, that the number at advantage varies with body size, and that the optimum number at a given size depends on water temperature or viscosity. I assume that vertebral number is adapted not to the maximum body lengths in a population, but rather to the fry lengths, when mortality is high and selection related to locomotory ability (to escape predators) is likely to be most
stringent. These hypotheses are tested in two species, the threespine stickleback Gasterosteus aculeatus (Linnaeus) and peamouth chub Mylocheilus caurinus (Richardson), using predation experiments in which vertebral counts of survivors of predation are compared to those of unexposed controls. I also look for evidence of selection in the wild, by comparing vertebral counts of wild fry of various sizes and ages. In addition, I test the hypothesis that locomotory performance depends on vertebral number, by direct measurements of burst swimming performance of G. aculeatus fry of various sizes and at various water temperatures and viscosities. Finally, results of these tests are related to the maintenance of variation in vertebral number within and among populations, and to the possible adaptive significance of environmental influences on vertebral number.

Part I. Predation experiments with threespine sticklebacks

Material and Methods

Breeding sticklebacks were collected from either Kennedy or Holden Lakes on Vancouver Island. Kennedy Lake is a large oligotrophic lake containing all three lateral plate morphs (Hagen and Gilbertson 1972) of G. aculeatus; Holden Lake is a small eutrophic lake containing only the low plated morph. Each male was artificially crossed to two females, and their offspring were reared in the laboratory at about 17C. Fry were held at densities of about $100 / \mathrm{L}$ for the first few days after hatching and about $30 / L$ thereafter. Fry were fed natural plankton, Artemia nauplii and nematodes.

For each of three experiments conducted in 1982 using Kennedy Lake fish, the offspring of a single collection of parents (about 50 crosses) were mixed and distributed among six 190-L tanks (of green fiberglass, approximate water dimensions $110 \times 50 \times 34 \mathrm{~cm}) \quad 15-20 \mathrm{~d}$ after hatching. Two tanks were designated as control tanks and four as experimental tanks. One control and two experimental tanks were maintained at 15C; the remaining tanks were maintained at either 17 or 20C. In experiment K 1 , fry were first distributed in lots of $10-30$ fish alternately among the three tanks to be held at 15 C until each had received its full complement of fish, and then alternately among the three tanks to be held at 20C. In experiments $K 2$ and K3, fry were distributed in small lots alternately among all six tanks. Tanks were illuminated by overhead fluorescent lights on
a photoperiod of 16.5 h light: 7.5 h dark. Water flow was 1-2L/min in each tank. Cover was usually provided by shredded plastic suspended near each end of experimental tanks, but was removed while assessing the extent of predation and was usually not replaced when the estimated number of fry eaten approached the desired number. After about 15 h of acclimation, fry in experimental tanks were exposed to predation by 10-30 pumpkinseed sunfish (Lepomis gibbosus) for $10-50 \mathrm{~h}$ until 50-60 \% of the initial number had been eaten. Sunfish were replaced every 2-5 $h$, and removed overnight in experiments lasting more than 1 d. The sunfish used were collected from Holden Lake, and averaged 24 mm in standard length (range $15-30 \mathrm{~mm}$ ). Fry were fed each morning 1-2 $h$ before the introduction of sunfish. Further details are given in Table 1.

Three collections of parents were made from Holden Lake in 1983 (80-110 crosses each), and offspring of each used in two experiments at different sizes. Experiments resembled those conducted in 1982 except that (1) temperatures were either 15 or 25 C , (2) cover was provided in only one of the two experimental tanks at each temperature, and (3) sunfish were replaced only every day. When cover was provided it remained in place for the entire experiment. Details are given in Table 2.

At the end of each experiment, fry were killed in anaesthetic (MS222), preserved in $10 \%$ buffered formalin, stained with alcian blue, cleared with trypsin and $0.5 \% \mathrm{KOH}$, and stored in glycerine (following Dingerkus and Uhler (1977), except that 2 mg of alcian blue per ml of stain solution were used here).

Table 1. Description of 1982 predation experiments using Kennedy Lake sticklebacks as prey. Percent cover is percent of predator-hours (pred. $h$ ) with cover. Pred. $h=$ no. predators $x h$ present. Duration in $h$ includes night periods without predators. Predators are sunfish collected from Holden Lake.


Table 2. Description of 1983 predation experiments using Holden Lake sticklebacks as prey. Cover is present (Y) or absent ( $N$ ) during all predator-hours (pred.-h). Duration in $h$ includes night periods without predators Predators are sunfish collected from Holden Lake. Prey in experiments with the same parent code are offspring of the same collection of parents.

| Exp. | Parents | Treatment | Temp. ( C) | Cover | Duration |  | standard length of predators (mm) |  | $\begin{gathered} \% \\ \text { eaten } \end{gathered}$ | No. eaten per pred.-h | No. surviving | standard <br> length of survivors (mm) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | h | Pred.-h | Mean | SD |  |  |  | Mean | SD |
| H1 | A | Contral | 15 | N | 46 | 0 |  |  | 0 |  | 119 | 7.62 | 0.53 |
|  |  |  | 25 | N | 23 | 0 |  |  | 0 |  | 121 | 7.63 | 0.57 |
|  |  | Expt 1. | 15 | $Y$ | 52 | 298 | 23.13 | 0.74 | 55 | 0.55 | 135 | 7.97 | 0.52 |
|  |  |  | 15 | N | 46 | 298 | 23.47 | 1. 19 | 63 | 0.63 | 112 | 7.98 | 0.54 |
|  |  |  | 25 | Y | 22 | 119 | 23.15 | 1.00 | 55 | 1.39 | 133 | 7.83 | 0.54 |
|  |  |  | 25 | N | 22 | 122 | 23.60 | 0.81 | 59 | 1.44 | 124 | 7.78 | 0.49 |
| H 2 | $\therefore \mathrm{B}$ | Control | 15 | $N$ | 52 | 0 |  |  | 0 |  | 124 | 8.27 | 0.49 |
|  |  |  | 25 | $N$ | 33 | 0 |  |  | 0 |  | 125 | 8.35 | 0.48 |
|  |  | Expt 1. | 15 | $Y$ | 54 | 496 | 24.30 | 0.76 | 56 | 0.34 | 132 | 8.65 | 0.55 |
|  |  |  | 15 | N | 50 | 419 | 24.28 | 0.79 | 62 | 0.45 | 113 | 8.79 | 0.54 |
|  |  |  | 25 | Y | 29 | 165 | 24.10 | 0.74 | 58 | 1.05 | 127 | 8.56 | 0.52 |
|  |  |  | 25 | N | 32 | 195 | 24.10 | 0.74 | 64 | 0.98 | 108 | 8.69 | 0.53 |
| H3 | C | Control | 15 | N | 25 | 0 |  |  | 0 |  | 124 | 8.56 | 0.51 |
|  |  |  | 25 | N | 13 | 0 |  |  | 0 |  | 125 | 8.50 | 0.63 |
|  |  | Expt 1. | 15 | Y | 26 | 136 | 28.07 | 0.80 | 62 | 1.36 | 115 | 8.90 | 0.56 |
|  |  |  | 15 | N | 24 | 92 | 27.93 | 0.80 | 58 | 1.90 | 126 | 8.92 | 0.60 |
|  |  |  | 25 | Y | 12 | 97 | 28.00 | 0.82 | 65 | 2.03 | 104 | 8.97 | 0.52 |
|  |  |  | 25 | $N$ | 12 | 109 | 28.00 | 0.82 | 70 | 1.94 | 89 | 8.97 | 0.50 |

Table 2. contd.


Counts were made of centra, excluding the urostyle. The last centrum frequently bore two neural or haemal arches. Such complex vertebrae were counted as one. Vertebrae were designated as 'abdominal' or 'caudal' on the basis of haemal spine length. In over $80 \%$ of $f i s h$ a sharp discontinuity in haemal spine length occurred in the vicinity of the first anal basal (Fig. 1). The first vertebra bearing a long haemal spine was designated the first caudal vertebra; normally this was the first vertebra whose haemal spine projected posterior to the first anal basal. In those fish whose haemal spine length increased gradually near the first anal basal, the following criteria were used. In the 1983 (Holden Lake) experiments, the first vertebra bearing a haemal spine 1.5 or more times as long as its centrum, or at least $85 \%$ as long as the longest haemal spine in the vicinity of the anal basals, was designated the first caudal vertebra. In the 1982 (Kennedy Lake) experiments (recounted in 1986), the first vertebra whose haemal spine length was $80 \%$ or more of the maximum spine length in the vicinity of the anal basals was designated the first caudal vertebra. Of 332 fish counted using both methods, the designation of the first caudal vertebra differed between methods in only 5 fish; in 3 fish the first method designated one fewer caudal vertebrae, and in 2 fish one more.

Total length was measured from the tip of the snout to the end of the hypural plate. In Kennedy Lake fish, length of the vertebral column was also measured, from the anterior edge of the first vertebra to the posterior edge of the hypural plate. In

Figure 1. Axial skeleton of threespine stickleback fry. A. Entire skeleton. B-D. Division between abdominal and caudal vertebrae marked by arrow (B. most common division, D. most rare division). Scale 1 mm . Fish lengths $8.0(A, B)$ or 8.1 ( $\mathrm{C}, \mathrm{D}$ ) mm. CL is caudal length.

fish from both lakes, caudal length was measured from the anterior edge of the first vertebra whose haemal spine opposed or projected posterior to the midline of the first anal basal, to the posterior edge of the hypural plate (Fig. 1). The vertebrae included in this length normally corresponded to those designated as caudal vertebrae according to the criteria described above. However, in some instances, the first caudal vertebra was excluded from this length, or rarely, the last abdominal vertebra was included in it (Fig. 1 C and 1 D , respectively). In these cases, in fish from Holden Lake, an adjusted caudal length was calculated by adding or subtracting the average length of a vertebra in the caudal length of the fish in question. Adjusted caudal lengths could not be calculated in Kennedy Lake fish, since the counts of caudal vertebrae made when caudal lengths were measured did not employ those criteria described above.

Independence of count distribution and treatment was assessed by chi-square tests calculated using BMDP4F (Dixon 1981). Yates' correction for continuity was used in all $2 x 2$ comparisons with sample sizes of 200 or less. Probabilities calculated using this correction may be conservative (Sokal and Rohlf 1981; Dixon 1981). Linear regressions were calculated and equality of slopes tested using $B M D P 1 R$ and $B M D P 1 V$, respectively (Dixon 1981). Analyses of variance and covariance were performed using BMDP7D, BMDP1V or BMDP2V.

Results

## Total Vertebrae

1. Kennedy Lake experiments

Vertebral count distributions were apparently homogeneous among tanks before exposure to predators. In each of the first two experiments, distributions in the two control tanks were virtually identical (Table 3). Differences between the control distributions in the third experiment were not statistically significant ( $\mathrm{p}=0.14$, with data grouped as fish with or without 31 vertebrae).

The first two experiments provided striking evidence of selective predation with respect to vertebral number (Table 3). The frequency of fish with 31 vertebrae was consistently greater in experimental than in control tanks after predation. This difference is highly significant ( $\mathrm{p}=0.0014$, grouping over both experiments; count distributions are homogeneous between experiments within predation treatments, $p>0.20$ ). Judging from the difference in counts between control and experimental groups, the survival rate of fish with 31 vertebrae was about 1.7 and 1.3 times that of fish with 32 vertebrae in the first and second experiments, respectively.

The third experiment differed from the previous two in average prey size. Judging from the size distributions of control and experimental groups, the majority of the fish eaten were about 8.5-10.5 mm long, whereas most of those eaten in the

Table 3. Vertebral counts of Kennedy Lake sticklebacks exposed (experimental) or unexposed (control) to predation by sunfish. Probabilities are from chi-square tests of independence between vertebral count and predation treatment, with fish grouped as those with or without 31 vertebrae.

first two experiments were about $7.0-8.5 \mathrm{~mm}$ long. Predation on these longer fish was not significantly selective for vertebral number; the frequency of fish with 31 vertebrae was only slightly greater in experimental than in control groups.

Predation was also selective with respect to prey size. The average length of fish surviving predation in experimental groups was usually slightly greater than that of fish unexposed to predation in the corresponding control groups (Table 3). Vertebral number and length were positively correlated among fish in all groups, but this relationship was not significant in any control group (Table 4). Moreover, this correlation was in the direction that would cause size selective predation to counteract rather than contribute to the observed differences in vertebral numbers. Therefore, selectivity of predation with respect to vertebral number cannot have been due to size selection in these experiments.

Although significant in no control group, the correlation between vertebral number and length was significant in experimental groups in one experiment (K2 in Table 4). This increase in significance in experimental relative to control groups could result from selection in favour of fish with 31 vertebrae only at smaller sizes within the range exposed to predators in this experiment, or may simply reflect increased sample size in experimental groups. Slopes of regressions between vertebral number and length did not differ significantly between experimental and control groups in any of the three experiments (Table 4).

Table 4. Regressions between vertebral count and length in 1982 experiments using offspring of Kennedy Lake sticklebacks. Data are grouped over replications within predation treatments since slopes are equal among replications ( $p>0.35$ ).

| Exp. | Treatment | No. | Slope | Probability of zero slope | Probability of equal slopes between treatments |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $k 1$ | Control | 176 | 0.091 | 0. 12 | 0.28 |
|  | Expt 1. | 463 | 0.012 | 0.78 |  |
| K2 | Control | 243 | 0.031 | 0.59 | 0.31 |
|  | Expt 1 . | 608 | 0.099 | 0.006 |  |
| K3 | Control | 284 | 0.056 | 0.22 | 0.69 |
|  | Expt 1 . | 653 | 0.032 | 0.43 |  |

No significant effect of temperature on the extent of selective predation was detected. Vertebral counts of survivors in experimental groups were virtually identical at the two temperatures in experiment $K 1$. In experiments $K 2$ and $K 3$, $f i s h$ with 32 vertebrae were slightly more frequent in experimental groups at the higher temperature, but differences between temperatures were not statistically significant ( $\mathrm{p}=0.16$ and 0.49 , respectively), and may simply reflect the lower percent mortality at the higher temperatures.
2. Holden Lake experiments

Selective predation for vertebral number of Holden Lake sticklebacks in 1983 resembled that seen using Kennedy Lake fish in 1982. At average prey lengths of about 8.1-8.2 mm (i.e., among fish 8.5 mm or less in length in experiments H 2 and H 3 ), survival during exposure to predation was again greater for fish with 31 vertebrae than for those with other counts (Table 5). This increase in the frequency of fish with 31 vertebrae in experimental compared to control groups was highly significant ( $\mathrm{p}=0.0025$, combining both experiments since the frequency in control groups was homogeneous between experiments ( $p=0.82$ )). The extent of selective predation in these experiments was unrelated both to temperature and to the availability of cover ( $p>0.65$ ). Predation was also selective with respect to prey size in these experiments. Vertebral number and length were positively correlated among fish in $H 2$ and H3 control groups (Table 6). Since survival during predation was greater for larger fish, this correlation was again in the direction that

Table 5. Vertebral counts of Holden Lake sticklebacks exposed (experimental) or unexposed (control) to predation by sunfish. Probabilities are from chi-square tests of independence between count and predation treatment. with fish grouped as those with or without the favoured count (3i in experiments at sizes under 8.5 or over 11.0 mm ; 32 in experiments at other sizes). Results of experiments H2 and H3 are shown separately for fish 8.5 mm or less in length, or over 8.5 mm in length.


Table 5. contd.


Table 5. conta.

| Exp. | Treatment | Temp. ( C ) | Cover | Mean length ( mm) | No. surviving | Vertebral count (\%) |  |  |  | Probability |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  | $\leqslant 30$ | 31 | 32 | $\geqslant 33$ |  |
| H4 | Contral | 15 | N | 9.83 |  |  |  |  |  |  |
|  |  | 25 | N | 9.76 | 122 | $8.2$ | $68.9$ | $21.3$ | 1.6 |  |
|  |  |  |  |  | 245 | 6.9 | 66.5 | 25.7 | 0.8 |  |
|  |  |  |  |  |  |  |  |  |  | 0.36 |
|  | Expt 1. | 15 | Y | 10.26 | 128 | 9.4 | 67.2 | 22.7 | 0.8 |  |
|  |  | 15 | N | 10.31 | 128 | 7.8 | 63.3 | 28.9 |  |  |
|  |  | 25 | Y | 10.03 | 121 | 8.3 | 66.1 | 25.6 |  |  |
|  |  | 25 | $N$ | 10.22 | 125 | 6.4 | 54.4 | 38.4 | 0.8 |  |
|  |  |  |  |  | 502 | 8.0 | 62.7 | 28.9 | 0.4 |  |
| H5 | Control | $15$ |  |  | $120$ | $10.8$ | $69.2$ | $20.0$ |  |  |
|  |  | $25$ | N | $10.12$ | $119$ | $11.8$ | $70.6$ | $16.8$ | 0.8 |  |
|  |  |  |  |  | 239 | 11.3 | 69.9 | 18.4 | 0.4 |  |
|  |  |  |  |  |  |  |  |  |  | 0.39 |
|  | Expt 1. | $15$ |  | $10.21$ | 133 | $10.5$ | 70.7 |  | 0.8 |  |
|  |  | $15$ | $N$ | $10.05$ | 126 | $12.7$ | 68.3 | $19.0$ |  |  |
|  |  | 25 | Y | 10.05 | 124 | 8.9 | 64.5 | 25.8 | 0.8 |  |
|  |  | 25 | N | 9.66 | 128 | 13.3 | 64.1 | 21.9 | 0.8 |  |
|  |  |  |  |  | 511 | 11.4 | 66.9 | 21.1 | 0.6 |  |
| H6 | Control | 15 | $N$ | 11.13 | 125 | 8.8 | 69.6 | 20.8 | 0.8 |  |
|  |  | 25 | N | 11.19 | 113 | 9.7 | 64.6 | 23.9 | 1.8 |  |
|  |  |  |  |  | 238 | 9.2 | 67.2 | 22.3 | 1.3 | 0.39 |
|  | Exptl. | $15$ |  |  | 110 | 8.2 | 72.7 | 18.2 | 0.9 |  |
|  |  | 15 | $N$ | $11.67$ | 130 | 6.9 | 67.7 | 23.1 | $2.3$ |  |
|  |  | 25 | Y | 11.54 | 113 | 4.4 | 66.4 | 27.4 | - 1.8 |  |
|  |  | 25 | $N$ | 11.66 | 116 | 6.9 | 75.0 | 18.1 |  |  |
|  |  |  |  |  | 469 | 6.6 | 70.4 | 21.7 | 1.3 |  |

Table 6. Regressions between vertebral number and length in experiments using offispring of Holden Lake sticklebacks. Only results grouped over replications within predation treatments are shown. since slopes are equal among replications ( $p>0.08$ ).

| Exp. | Treatment | No. | Slope | Probability of zero slope | Probability of equal slopes between treatments |
| :---: | :---: | :---: | :---: | :---: | :---: |
| H1 | Control | 240 | -0.020 | 0.79 | 0.39 |
|  | Expt 1 . | 504 | 0.057 | 0.27 |  |
| H2 | Control | 172 | 0.230 | 0.11 | 0.35 |
| $\leqslant 8.5 \mathrm{~mm}$ | Expt 1 . | 205 | 0.056 | 0.65 |  |
| $\begin{aligned} & \mathrm{H} 3 \\ & \leqslant 8.5 \mathrm{~mm} \end{aligned}$ | Control | 137 | 0.724 | $<0.0001$ | 0.001 |
|  | Expt 1 . | 105 | -0.119 | 0.55 |  |
| $\begin{aligned} & \mathrm{H} 2 \\ & >8.5 \mathrm{~mm} \end{aligned}$ | Control <br> Expt 1 . | 77 | 0.237 | 0.37 | 0.24 |
|  |  | 275 | -0.115 | 0.31 |  |
| $\begin{aligned} & \mathrm{H} 3 \\ & >8.5 \mathrm{~mm} \end{aligned}$ | Control <br> Expt 1 . | 112 | 0.348 | 0.11 | 0.46 |
|  |  | 329 | 0. 194 | 0.032 |  |
| H4 | $\begin{aligned} & \text { Control } \\ & \text { Expt1. } \end{aligned}$ | 245 | 0.088 | 0.12 | 0.18 |
|  |  | 502 | 0.183 | $<0.0001$ |  |
| H5 | Control <br> Expt1. | 239 | 0.111 | 0.046 | 0.47 |
|  |  | 511 | 0.061 | 0.13 |  |
| H6 | $\begin{aligned} & \text { Control } \\ & \text { Expt1. } \end{aligned}$ | 238 | -0.004 | 0.94 | 0.71 |
|  |  | 469 | 0.019 | 0.59 |  |

would cause size selective predation to counteract rather than contribute to the observed differences in vertebral numbers. Therefore, as in the Kennedy Lake experiments, the selectivity of predation with respect to vertebral number in these experiments cannot have been due to size selection.

Among fish 8.5 mm or less in length in experiment H 3 , the correlation between vertebral number and length was significant in control but not in experimental groups (Table 6). Slopes differed significantly between the two treatments ( $\mathrm{p}=0.001$ ). This difference between control and experimental groups could be explained by stronger selection in favour of fish with 31 vertebrae at longer lengths below 8.5 mm .

At average prey lengths below 8.0 or above 8.5 mm , predation was not significantly selective for total vertebral number (Table 5). At the smaller size, fish with 31 vertebrae were slightly more frequent in experimental than in control groups; at the larger sizes, fish with 32 vertebrae were usually more frequent in experimental than in control groups. None of these differences were significant when temperature and cover treatments were grouped together ( $p>0.35$ ). However, the effect of predation appeared to differ between cover treatments in experiment H 4 ( $\mathrm{p}=0.019$ ). In this experiment, fish with 32 vertebrae appeared to be at an advantage over fish with other vertebral counts in the absence, but not in the presence of cover ( $\mathrm{p}=0.054$ and 0.68 , respectively). In no other experiment did the selectivity of predation appear to depend upon the availability of cover ( $\mathrm{p}>0.50$ ). Vertebral numbers in experimental groups did
not differ significantly between temperatures in any experiment ( $p>0.10$ in all cases, $p>0.35$ in all but two cases). Predation was selective for prey size in all experiments except H5. Among experiments at prey lengths below 8.0 or above 8.5 mm , the correlation between vertebral number and length was significant in control groups only in H5 (Table 6). In other experiments at these sizes, this correlation, though not significant, was in the direction that would cause size selective predation to contribute to the slight differences in vertebral number observed between experimental and control groups.
3. Summary

The selectivity of predation for total vertebral number of sticklebacks depends upon prey length and is consistent between populations (Fig. 2). In both the Kennedy and the Holden Lake populations, fish with 31 vertebrae are at a significant advantage over fish with other vertebral counts when exposed to predation at average lengths of $8.0-8.5 \mathrm{~mm}$, but not when exposed at smaller ( 7.6 mm ) or greater ( $8.9-11.2 \mathrm{~mm}$ ) lengths. When selective predation was significant, its extent was unrelated to either temperature or the availability of cover. Although predation was also usually selective with respect to prey size, selective predation for vertebral number could not be attributed to size selection in any experiment at average lengths of $8.0-8.5$ mm .

Figure 2. Change in percent frequency of vertebral counts in sticklebacks exposed to predation at various mean sizes (experimental minus control fish). Points below the horizontal axis are experiments in which fish with 31 vertebrae are more frequent in exposed than in unexposed groups; those above are experiments in which fish with 32 vertebrae are more frequent in exposed groups.


## Abdominal and Caudal Vertebrae

1. Holden Lake experiments

Count classes based on numbers of abdominal and caudal vertebrae are shown in Table 7 for offspring of Holden Lake fish. Three classes are common: fish with 14 abdominal and 17 caudal vertebrae (31 total vertebrae (TV)), those with 14 abdominal and 18 caudal vertebrae ( 32 TV ), and those with 13 abdominal and 18 caudal vertebrae (31 TV).

Results of the predation experiments in terms of count classes based on the ratio of abdominal to caudal vertebrae (AV/CV) are shown in Table 8 and Fig. 3. Data are grouped over replications within predation treatments, since the frequency of fish with the favoured ratio(s) did not differ between temperatures nor between cover treatments among experimental groups in any experiment (p>0.35). At the smallest prey size (7.6 mm), no count class was favoured during exposure to predation. However, as prey size was increased above this value, fish with progressively lower ratios $A V / C V$ were favoured during exposure to predation. At average prey lengths of about 8.1-8.2 mm , fish with a high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ were significantly more frequent after predation in experimental groups than in unexposed control groups ( $p=0.006$ grouping experiments $H 2 S$ and $H 3 S$, since the frequency of fish with this ratio did not differ between experiments ( $\mathrm{p}=0.91$ )). At average prey lengths of about 9.8-10.0 mm , fish with intermediate to low ratios of $0.78-0.72 \mathrm{AV} / \mathrm{CV}$ were more frequent after exposure to predation than in unexposed

Table 7. Vertebral count classes in control groups of Holden Lake predation experiments, based on numbers of abdominal and caudal vertebrae.

Vertebral number

| Total | Abdominal | Caudal | No. | Ratio <br> Abd./Caud. |
| :---: | :---: | :---: | :---: | :---: |
| 30 | 15 | 15 | 1 | 1.00 |
|  | 14 | 16 | 68 | 0.88 |
|  | 13 | 17 | 68 | 0.76 |
|  | 12 | 18 | 5 | 0.67 |
| 31 | 15 | 16 | 35 | 0.94 |
|  | 14 | 17 | 701 | 0.82 |
|  | 13 | 18 | 196 | 0.72 |
| 32 | 15 | 17 | 52 | 0.88 |
|  | 14 | 18 | 290 | 0.78 |
|  | 13 | 19 | 24 | 0.68 |

Table 8. Ratios of abdominal to caudal vertebrae in Holden Lake sticklebacks exposed (exptl.) or unexposed (control) to predation by sunfish. Results of experiments H 2 and H3 are shown separately for fish 8.5 mm or less (S) or over 8.5 mm (L) in length. Probabilities are from chi-square tests of independence between count class and predation treatment, with fish grouped as those with or without the ratio(s) favoured during exposure to predation. (In experiment H1, a ratio of 0.82 is tested). Abbreviations are total vertebral number (TV), number of abdominal vertebrae (AV), and number of caudal vertebrae (CV).

| Vertebral count class (\%) |  |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| E×p. | Treatment | Mean length (mm) | $\begin{array}{r} \mathrm{AV}: \\ \mathrm{TV}: \\ \mathrm{AV} / \mathrm{CV}: \end{array}$ | $\geqslant 0.88$ | $\begin{gathered} 14 \\ 31 \\ 0.82 \end{gathered}$ | $\begin{gathered} 14 \\ 32 \\ 0.78 \end{gathered}$ | $\begin{gathered} 13 \\ 30 \\ 0.76 \end{gathered}$ | $\begin{gathered} 13 \\ 31 \\ 0.72 \end{gathered}$ | other | No. surviving | Favoured ratio(s) | Probability |
| H1 | Control | 7.63 |  | 11.3 | 45.4 | 19.6 | 4.6 | 13.8 | 5.5 | 240 | (0.82) | 0.87 |
|  | Expt1. | 7.89 |  | 11.9 | 46.0 | 17.7 | 5.4 | 16.5 | 2.6 | 504 |  |  |
| H2S | Control | 8.06 |  | 14.6 | 45.3 | 29.7 | 1.2 | 6.4 | 2.9 | 172 | 0.82 | 0.038 |
|  | Exptl. | 8.15 |  | 15.2 | 56.1 | 19.5 | 2.0 | 6.3 | 1.0 | 205 |  |  |
| H3S | Control | 8.12 |  | 13.2 | 46.0 | 19.7 | 10.2 | 10.9 |  | 137 | 0.82 | 0.062 |
|  | Expt 1 . | 8.20 |  | 11.5 | 58.1 | 14.3 | 4.8 | 10.5 | 1.0 | 105 |  |  |
| H2L | Control | 8.87 |  | 9.1 | 57.1 | 20.8 | 2.6 | 9.1 | 1.3 | 77 | 0.78 | 0.43 |
|  | Expt 1 . | 9.05 |  | 13.4 | 50.5 | 25.1 | 1.5 | 7.3 | 2.2 | 275 |  |  |
| H3L | Control | 9.03 |  | 10.7 | 44.6 | 24.1 | 4.5 | 14.3 | 1.8 | 112 | 0.78 | 0.46 |
|  | Expt 1 . | 9.17 |  | 10.6 | 41.9 | 27.7 | 5.2 | 13.4 | 1.2 | 329 |  |  |
| H4 | Control | 9.79 |  | 10.7 | 49.8 | 20.4 | 2.9 | 13.9 | 2.4 | 245 | 0.78-0.72 | 0.066 |
|  | Expt 1 . | 10.21 |  | 8.8 | 45.2 | 24.3 | 4.6 | 15.3 | 1.8 | 502 |  |  |
| H5 | Control | 10.01 |  | 9.2 | 46.0 | 13.0 | 5.9 | 21.8 | 4.2 | 239 | 0.78-0.72 | 0.011 |
|  | Expt 1 . | 9.99 |  | 4.9 | 41.5 | 17.8 | 7.8 | 24.9 | 3.1 | 511 |  |  |
| H6 | Control | 11.16 |  | 8.4 | 52.5 | 17.2 | 5.5 | 11.8 | 4.7 | 238 | 0.72 | 0.006 |
|  | Expt1. | 11.59 |  | 5.4 | 49.0 | 17.9 | 3.4 | 20.0 | 4.3 | 469 |  |  |

Figure 3. Change in percent frequency during predation of vertebral count classes based on the ratio of abdominal to caudal vertebrae (experimental minus control frequencies), in 1983 experiments with Holden Lake sticklebacks. Solid bars above horizontal axis are vertebral count classes increasing in frequency during exposure to predation; open bars below axis are those decreasing during predation. Mean length in control group at end of experiment is given in each panel. Probabilities are as in Table 8. Abbreviations TV, AV and CV are numbers of total, abdominal and caudal vertebrae, respectively.

control groups ( $\mathrm{p}=0.002$ grouping experiments H 4 and H 5 , since the frequency of fish with these ratios did not differ between these experiments ( $\mathrm{p}=0.44$ )). Finally, fish with a low ratio of 0.72 $A V / C V$ were significantly more frequent after predation at the largest prey size tested (11.2 mm).

This closer examination of the Holden Lake experiments reveals that selective predation for total vertebral number in these experiments is evidently the result of selection for the ratio $A V / C V$. The increased frequency of fish with 31 vertebrae after predation at lengths of about $8.1-8.2 \mathrm{~mm}$ is accounted for by the increase in frequency of fish with a high ratio of 0.82 AV/CV; fish with 31 vertebrae and a low ratio of $0.72 \mathrm{AV} / \mathrm{CV}$ did not increase in frequency after predation at these sizes.

Selective predation with respect to the ratio AV/CV cannot be attributed to size selection in these experiments. In experiment $H 5$, no size selection occurred. In all other experiments, longer fish tended to have greater survival during exposure to predation. In experiments H 2 S and H 3 S , fish with the favoured ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ were slightly (though not significantly) less frequent at greater lengths in control groups (Table 9). Hence, size selection could not have contributed to the observed increase in the frequency of fish with this ratio during predation in these two experiments. In experiments H2L, H4 and H6, fish with the favoured ratio(s) were slightly more frequent at greater lengths in control groups (Table 9). Hence, size selection may have contributed slightly to the observed increase in the frequency of fish with these ratios during

Table 9. Relationship between vertebral count class and length in control groups in Holden Lake experiments. Frequencies of fish with the favoured ratio(s) are shown for lengths less than or equal to the mean (S) or greater than the mean (L). Probabilities are chi-square tests of independence between vertebral count and size classes, with fish grouped as those with or without the favoured ratio.

| Exp. | Favoured ratio AV/CV | $\begin{aligned} & \text { Size } \\ & \text { class } \end{aligned}$ | Mean length (mm) | Frequency of favoured ratio (\%) | No. | Probability |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| H1 | (0.82) | S | 7. 16 | 43.0 | 114 | 0.47 |
|  |  | 1 | 8.05 | 47.6 | 126 |  |
| H2S | 0.82 | S | 7.74 | 51.4 | 72 | 0.23 |
|  |  | L | 8.29 | 41.0 | 100 |  |
| H3S | 0.82 | 5 | 7.80 | 46.8 | 62 | 1.00 |
|  |  | L | 8.38 | 45.3 | 75 |  |
| H2L | 0.78 | 5 | 8.70 | 14.6 | 41 | 0.26 |
|  |  | L | 9.07 | 27.8 | 36 |  |
| H3L | 0.78 | S | 8.77 | 24.2 | 62 | 1.00 |
|  |  | L | 9.35 | 24.0 | 50 |  |
| H4 | 0.78-0.72 | S | 9.29 | 36.9 | 122 | 0.93 |
|  |  | L | 10.30 | 37.4 | 123 |  |
| H5 | 0.78-0.72 | 5 | 9.51 | 35.5 | 124 | 0.10 |
|  |  | L | 10.54 | 46.1 | 115 |  |
| H6 | 0.72 | S | 10.53 | 10.3 | 116 | 0.51 |
|  |  | L | 11.76 | 13.1 | 122 |  |

predation in these other experiments. However, in no case did the frequency of the favoured ratio(s) differ significantly between fish below or above the mean length in control groups (Table 9). Since this comparison involves a much greater difference in mean size than that between control and experimental groups, it is unlikely that selective predation with respect to the ratio $A V / C V$ can be explained by size selection in any of these experiments. To further rule out this possibility, I calculated the counts expected in experimental groups as a result of size selection alone. The expected frequency $E$ of $f$ ish with the favoured ratio(s) was calculated as

$$
E=(\Sigma j \operatorname{Pj} N j) / N t
$$

where $P j$ is the frequency of fish with the favoured ratio(s) in the jth size class in control groups, $N j$ is the number of fish in the jth size class in experimental groups, and Nt is the total number of fish in experimental groups. Four size classes were distinguished: (1) L $\leqslant \overline{\mathrm{X}}-0.68 \mathrm{~s},(2) \overline{\mathrm{X}}-0.68 \mathrm{~s}<\mathrm{L} \leqslant \overline{\mathrm{X}},(3) \overline{\mathrm{X}}<\mathrm{L}$ $\leqslant \overline{\mathrm{X}}+0.68 \mathrm{~s}$, and (4) $L>\overline{\mathrm{X}}+0.68 \mathrm{~s}$, where $\overline{\mathrm{X}}$ and s are the mean length and its standard deviation in control groups. In all experiments in which the frequency of fish with the favoured ratio $A V / C V$ differed significantly between experimental and control groups, it also differed significantly between experimental groups and those expected as a result of size selection alone (Table 10).

Table 10. Ratio of abdominal to caudal vertebrae (AV/CV) in Holden Lake sticklebacks exposed (experimental) or unexposed (control) to predation by sunfish, and the ratio expected in exposed fish if predation is selective for prey size but not for the ratio $\mathrm{AV} / \mathrm{CV}$. Frequencies of fish with the favoured ratio are shown. Probabilities are chi-square tests comparing the observed frequency in experimental groups and that expected due to size selection alone. Probabilities are calculated assuming that the expected frequency due to size selection alone is a sampled value, with sample size equal to that in control groups.

Frequency of favoured ratio (\%)

| Exp. | Frequency of favoured ratio (\%) |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Favoured ratio AV/CV | Experimental |  |  |  |
|  |  | Control | Expected due to size selection | Observed | Probability |
| H1 | (0.82) | 45.4 | 46.0 | 46.0 | >0.90 |
| H2S | 0.82 | 45.3 | 44.6 | 56.1 | 0.025-0.05 |
| H3S | 0.82 | 46.0 | 46.1 | 58.1 | 0.05-0.10 |
| H2L | 0.78 | 20.8 | 16.8 | 25.1 | 0.10-0.25 |
| H3L | 0.78 | 24.1 | 24.8 | 27.7 | 0.50-0.75 |
| H4 | 0.78-0.72 | 37.2 | 37.4 | 44.2 | $0.05-0.10$ |
| H5 | 0.78-0.72 | 40.7 | 40.5 | 50.5 | 0.01-0.025 |
| H6 | 0.72 | 11.8 | 12.9 | 20.0 | 0.01-0.025 |

2. Kennedy Lake experiments

The division of the vertebral column into abdominal and caudal regions in Kennedy Lake fish differed markedly from that seen in Holden Lake fish (Table 11). Kennedy Lake fish tended to have one more abdominal vertebra than Holden Lake fish with the same total vertebral count. Thus, ratios of abdominal to caudal vertebrae tended to be higher in Kennedy than in Holden Lake fish.

In experiments $K 2$ and $K 3$, results resembled those seen using Holden Lake fish, in that the ratios of abdominal to caudal vertebrae at advantage during predation appeared to decrease as prey size increased (Table 12 and Fig. 4). At the smaller prey size in experiment K 2 , fish with high ratios of $0.88-0.94 \mathrm{AV} / \mathrm{CV}$ were more frequent after predation in experimental groups than in unexposed control groups ( $p=0.027$ ). Conversely, at the larger prey size in experiment K 3 , fish with low ratios of 0.78 - 0.82 $A V / C V$ were more frequent after predation ( $p=0.024$ ). However, the actual ratios at advantage during predation at a given size differed between the two populations. At average prey sizes of about $8.1-8.2 \mathrm{~mm}$, ratios of $0.88-0.94$ were favoured in Kennedy Lake fish, while one of 0.82 was favoured in Holden Lake fish.

Results in experiment $K 1$ departed somewhat from the pattern described above. In this experiment, fish with total vertebral counts of 31 appeared to be at advantage during predation regardless of the proportion of vertebrae that were abdominal. However, results did conform to the expected pattern to the

Table 11. Vertebral count classes in control groups of Kennedy Lake predation experiments, based on numbers of abdominal and caudal vertebrae.

Vertebral number

| Total | Abdominal | Caudal | No. | Abd./Caud. |
| :---: | :---: | :---: | :---: | :---: |
| 30 | $\begin{array}{r} 15 \\ 14 \end{array}$ | $\begin{aligned} & 15 \\ & 16 \end{aligned}$ | $\begin{array}{r} 6 \\ 22 \end{array}$ | $\begin{aligned} & 1.00 \\ & 0.88 \end{aligned}$ |
| 31 | $\begin{aligned} & 16 \\ & 15 \\ & 14 \end{aligned}$ | $\begin{aligned} & 15 \\ & 16 \\ & 17 \end{aligned}$ | $\begin{array}{r} 1 \\ 198 \\ 138 \end{array}$ | $\begin{aligned} & 1.07 \\ & 0.94 \\ & 0.82 \end{aligned}$ |
| 32 | $\begin{aligned} & 16 \\ & 15 \\ & 14 \end{aligned}$ | $\begin{aligned} & 16 \\ & 17 \\ & 18 \end{aligned}$ | $\begin{array}{r} 12 \\ 250 \\ 70 \end{array}$ | $\begin{aligned} & 1.00 \\ & 0.88 \\ & 0.78 \end{aligned}$ |
| 33 | 15 | 18 | 6 | 0.83 |

Table 12. Ratios of abdominal to caudal vertebrae in Kennedy Lake sticklebacks exposed (experimental) or unexposed (control) to predation by sunfish. Probabilities are from chi-square tests of independence between vertebral count classes and predation treatments, with fish grouped as those with or without the ratios favoured during exposure to predators. In experiment $K 1$, two ratios ( 0.94 or 0.82 ) are tested. Abbreviations are as in Table 8 .

| Vertebral count class (\%) |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Exp. | Treatment | Mean <br> length ( mm ) | $\begin{array}{r} \mathrm{AV}: \\ \text { TV: } \\ \mathrm{AV} / \mathrm{CV}: \end{array}$ | $\begin{gathered} 15 \\ 31 \\ 0.94 \end{gathered}$ | $\begin{gathered} 15 \\ 32 \\ 0.88 \end{gathered}$ | $\begin{gathered} 14 \\ 31 \\ 0.82 \end{gathered}$ | $\begin{gathered} 14 \\ 32 \\ 0.78 \end{gathered}$ | Other | No. surviving | Favoured ratios | Probability |
| K1 | Control | 8.31 |  | 21.0 | 40.3 | 19.9 | 17.6 | 1.1 | 176 | 0.94 | 0.038 |
|  | Expt 1. | 8.43 |  | 29.2 | 32.2 | 24.6 | 10.8 | 3.2 | 463 | 0.82 | 0.21 |
| K2 | Control | 8. 18 |  | 24.7 | 30.5 | 22.2 | 12.3 | 10.3 | 243 | 0.88-0.94 | 0.027 |
|  | Expt 1 . | 8.50 |  | 31.3 | 32.1 | 21.5 | 5.8 | 9.4 | 608 |  |  |
| $K 3$ | Control | 8.92 |  | 35.6 | 37.0 | 17.3 | 3.2 | 7.0 | 284 | 0.78-0.82 | 0.024 |
|  | Expt 1. | 9. 10 |  | 35.7 | 30.5 | 21.3 | 6.1 | 6.4 | 653 |  |  |

Figure 4. Change in percent frequency during predation of vertebral count classes based on the ratio AV/CV, in 1982 experiments using Kennedy Lake sticklebacks. Symbols and abbreviations are as in Fig. 3.


AV/CV |  | 1 | 1 | 1 | 1 |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | $\begin{array}{lllll}T V & 31 & 32 & 31 & 32\end{array}$ VERTEBRAL COUNT

extent that this increase in frequency of fish with 31 vertebrae after predation was significant among fish with a high ratio of $0.94 \mathrm{AV} / \mathrm{CV}(\mathrm{p}=0.038)$, but not among those with a lower ratio of $0.82 \mathrm{AV} / \mathrm{CV}(\mathrm{p}=0.21)$.

Selective predation with respect to the ratio of abdominal to caudal vertebrae cannot be attributed to size selection in these experiments. In all three experiments, larger fish tended to have higher survival during predation, but the frequency of fish with the favoured ratios was lower in larger than in smaller fish before predation in control groups (Table 13).
3. Summary

Selective predation for total vertebral number in these experiments apparently resulted from selection for the ratio of abdominal to caudal vertebrae. In 1983 experiments with Holden Lake sticklebacks, survival at small prey sizes (8.1-8.3 mm) was greatest among fry with a high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$. At intermediate (9.8-10.0 mm) or large ( 11.2 mm ) prey sizes, survival was greatest for fry with intermediate to low (0.78-0.72) or low (0.72) ratios, respectively. In 1982 experiments with Kennedy Lake sticklebacks, the ratios at advantage during exposure to predation also tended to decrease as prey size increased. However, ratios tended to be higher in Kennedy than in Holden Lake fish, and higher ratios were favoured at a given prey size in the Kennedy lake fish. Selective predation for vertebral count ratio was not attributable to size selection.

Table 13. Ratios of abdominal to caudal vertebrae in control groups in Kennedy Lake experiments, at lengths less than or equal to (S) or greater than (L) the mean length. Probabilities are from chisquare tests of independence between vertebral count and length classes, with fish grouped as those with or without the ratios favoured during predation. In experiment K1, two ratios (0.94 or 0.82 ) are tested. Abbreviations are as in Table 8.


## Body Proportions

1. Holden Lake experiments

Selection with respect to the ratio of abdominal to caudal vertebrae could result from selection with respect to body proportions. To test this possibility, I considered the precaudal/caudal length ratio, where precaudal length is the total minus the caudal length. As might be expected, the ratio of abdominal to caudal vertebrae and this length ratio were directly and highly significantly ( $p<0.0001$ ) related (Table 14). However, only about $30-65 \%$ of the overall variation in vertebral count ratio (VR) is explained by variation in length ratio (LR). (I use the adjusted (see Material and Methods) caudal length in these analyses since length ratios calculated with this adjustment explain over twice as much of the variation in $V R$ as do those calculated without it). Within a vertebral count class, variation in LR is considerable (Fig. 5). This variation within vertebral count classes may result partly from variation in relative head lengths, and partly from variation in the spacing of vertebrae (i.e., in the number of abdominal or caudal vertebrae per unit of abdominal or caudal body length).

In most experiments, the regression of $V R$ on $L R$ differs significantly between control and experimental groups in slope or intercept, indicating selective predation for VR, LR or both. LR decreased during exposure to predation in experiments $H 1$, H2S and H5, and increased during predation in experiment H6 (Table 15). LR and total length were inversely related ( $p<0.05$ ) in all four

Table 14. Regression of the ratio of abdominal to caudal vertebrae (VR) on the ratio of precaudal to caudal length (LR) in control and experimental groups of Holden Lake experiments.

| Exp. | Treatment |  |  |  | Probability |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | VR Vs LR |  |  |  | equality of |  |  |
|  |  | No. | Intercept | Slope | zero slope | slopes and intercepts | equality of slopes | $r^{2}$ |
| H1 | Control | 240 | 0. 1514 | 0.4683 | $<0.0001$ | 0.0005 | 0.009 | $\begin{aligned} & 0.40 \\ & 0.32 \end{aligned}$ |
|  | Expt 1 . | 504 | 0.3215 | 0.3518 | $<0.0001$ |  |  |  |
| H2 | Control | 249 | 0.1806 | 0.4572 | $<0.0001$ | 0.0001 | 0.038 | $\begin{aligned} & 0.44 \\ & 0.47 \end{aligned}$ |
|  | Expt 1 . | 480 | 0.0710 | 0.5446 | $<0.0001$ |  |  |  |
| H3 | Control | 249 | 0.1136 | 0.4971 | $<0.0001$ | 0.60 | 0.78 | $\begin{aligned} & 0.53 \\ & 0.48 \end{aligned}$ |
|  | Expt 1 . | 434 | 0.1310 | 0.4863 | $<0.0001$ |  |  |  |
| H4 | Control | 245 | 0.0746 | 0.5341 | $<0.0001$ | 0.026 | 0.007 | 0.480.61 |
|  | Expt 1 . | 502 | -0.0740 | 0.6430 | $<0.0001$ |  |  |  |
| H5 | Control | 239 | -0.0398 | 0.5965 | $<0.0001$ | 0.0006 | 0.13 | $\begin{aligned} & 0.66 \\ & 0.63 \end{aligned}$ |
|  | Expt 1 . | 511 | -0. 1042 | 0.6494 | $<0.0001$ |  |  |  |
| H6 | Control | 238 | 0.0273 | 0.5657 | $<0.0001$ | $<0.0001$ | 0.085 | 0.54 |
|  | Exptl. | 469 | -0.0800 | 0.6308 | <0.0001 |  |  | 0.66 |

Figure 5. Ratios of precaudal/caudal length (LR) in vertebral count classes VR of Holden Lake sticklebacks exposed (experimental, open bars) or unexposed (control, black bars) to predation by sunfish. VR is the ratio of abdominal to caudal vertebrae. Results are shown for three cases: A. H1, predation selective for LR but not VR; B. H2S, selection favouring low LR but high VR; and C. H6, selection favouring high LR but low VR.


| Table | 15. Body proportions of Holden Lake sticklebacks exposed (exptl.) or unexposed (control) to predation by sunfish. Body proportions are the pre-caudal length divided by the caudal length. When length ratios are related (p<0.05) to total length, adjusted ratios are also shown. When slopes of this relationship differ (p<0.O5) between predation treatments, slopes within treatments are also shown. Methods of adjustment are : (A) no adjustment made, (B) ratios adjusted to grand mean length (standard analysis of covariance), (C) ratios in control group adjusted to mean length in experimental group, or (D) ratios in experimental group adjusted to mean length in control group. |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Probab | ity of |  |  |  |  |  | cauda 1 / | audal le | th ratio |  |
| Exp. | Slope | zero slope | equal <br> slopes | Treatment | Slope | Probability of zero slope | No. | Mean | SE | Adjusted mean | Adjustment method | Probability of equal means |
| H1 | -0.00229 | $<0.001$ | 0.42 | Control <br> Exptl. | -- |  | $\begin{aligned} & 240 \\ & 504 \end{aligned}$ | $\begin{aligned} & 1.3832 \\ & 1.3642 \end{aligned}$ | $\begin{aligned} & 0.00433 \\ & 0.00297 \end{aligned}$ | $\begin{aligned} & 1.3782 \\ & 1.3666 \end{aligned}$ | B | 0.029 |
| H2S | -0.00176 | 0.039 | 0.023 | Control Exptl. | $\begin{array}{r} -0.00367 \\ 0.00181 \end{array}$ | $\begin{aligned} & 0.004 \\ & 0.87 \end{aligned}$ | $\begin{aligned} & 172 \\ & 205 \end{aligned}$ | $\begin{aligned} & 1.3830 \\ & 1.3671 \end{aligned}$ | $\begin{aligned} & 0.00497 \\ & 0.00455 \end{aligned}$ | $\begin{aligned} & 1.3788 \\ & 1.3671 \end{aligned}$ | C | 0.079 |
| H3S | -0.00487 | $<0.001$ | 0.56 | Control <br> Expt 1 . | -- | -- | $\begin{aligned} & 137 \\ & 105 \end{aligned}$ | $\begin{aligned} & 1.3928 \\ & 1.3946 \end{aligned}$ | $\begin{aligned} & 0.00526 \\ & 0.00601 \end{aligned}$ | $\begin{aligned} & 1.3907 \\ & 1.3973 \end{aligned}$ | B | 0.41 |
| H2L | 0.00113 | 0.20 | 0.41 | $\begin{aligned} & \text { Control } \\ & \text { Exptl } \end{aligned}$ | -- | -- | 77 275 | $\begin{aligned} & 1.3673 \\ & 1.3639 \end{aligned}$ | $\begin{aligned} & 0.00719 \\ & 0.00374 \end{aligned}$ | -- | A | 0.66 |
| H3L | -0.00188 | 0.007 | 0.015 | $\begin{aligned} & \text { Control } \\ & \text { Expt } . \end{aligned}$ | $\begin{array}{r} 0.00136 \\ -0.00278 \end{array}$ | $\begin{aligned} & 0.41 \\ & 0.0003 \end{aligned}$ | $\begin{aligned} & 112 \\ & 329 \end{aligned}$ | $\begin{aligned} & 1.3830 \\ & 1.3753 \end{aligned}$ | $\begin{aligned} & 0.00646 \\ & 0.00374 \end{aligned}$ | $\begin{aligned} & 1.3829 \\ & 1.3802 \end{aligned}$ | D | 0.71 |
| H4 | -0.00090 | 0.002 | 0.54 | ```Control Exptl.``` | -- | -- | $\begin{aligned} & 245 \\ & 502 \end{aligned}$ | $\begin{aligned} & 1.3635 \\ & 1.3566 \end{aligned}$ | $\begin{aligned} & 0.00402 \\ & 0.00276 \end{aligned}$ | $\begin{aligned} & 1.3604 \\ & 1.3581 \end{aligned}$ | B | 0.64 |
| H5 | -0.00117 | $<0.001$ | 0.66 | Control Expt 1 . | -- | -- | $\begin{aligned} & 239 \\ & 511 \end{aligned}$ | $\begin{aligned} & 1.3951 \\ & 1.3687 \end{aligned}$ | $\begin{aligned} & 0.00417 \\ & 0.00285 \end{aligned}$ | $\begin{aligned} & 1.3952 \\ & 1.3686 \end{aligned}$ | B | $<0.001$ |
| H6 | -0.00055 | 0.033 | 0.56 | $\begin{aligned} & \text { Control } \\ & \text { Expt1. } \end{aligned}$ | -- | -- | $\begin{aligned} & 238 \\ & 469 \end{aligned}$ | $\begin{aligned} & 1.3695 \\ & 1.3817 \end{aligned}$ | $\begin{aligned} & 0.00432 \\ & 0.00304 \end{aligned}$ | $\begin{aligned} & 1.3676 \\ & 1.3827 \end{aligned}$ | B | 0.005 |

[^0]experiments, but selective predation with respect to $L R$ cannot be attributed to size selection alone. In experiment $H 1$, a significant decrease in $L R$ during predation persists after adjustment of control and experimental groups to a common total length. In experiment $H 2 S$, the mean $L R$ of experimental groups remains lower than that of control groups after adjustment, although the difference between groups only approaches significance $(p=0.08)$. In experiment $H 5$, the mean $L R$ of experimental groups was lower than that of control groups even though no size selection occurred. In experiment H6, size selection counteracted selection with respect to LR. In summary, low LR was sometimes favoured during predation at small (H1, H2S)or medium (H5) prey sizes, while high LR was favoured at large prey size (H6).

Results are shown separately for each vertebral count class in Table 16 and Fig. 5. In all experiments with significant overall selection for $L R$, it is in the same direction in all count classes. However, significant interaction does occur between vertebral count class and predation treatment in experiment H 5 , and possibly in experiments H 3 L and H 4 . In experiment $H 5$, the decrease in $L R$ during predation is greatest in the count class with the highest mean LR (VR $\geqslant 0.88$ ). In experiment H2S, selection for low $L R$ was apparently partly obscured by selection for high (0.82) VR. After adjusting to a common total length in this experiment, the decrease in LR during predation only approached significance combining vertebral count classes (Table 15), but was highly significant within vertebral

Table 16. Change in precaudal/caudal length ratio (LR) within vertebral count classes in Holden Lake sticklebacks after exposure to predation in 1983. Data are the mean in experimental groups minus that in control groups. Unadjusted data and data adjusted to a common total length (TL) are shown. Adjustment A employs standard analysis of covariance; tests of zero slope and equality of slopes among groups are shown for the regression of LR on TL. Groups with slopes differing ( $p<0.05$ ) from the slope of other groups combined are noted. A second adjustment $B$ is shown when slopes are heterogeneous among groups. PT is the probability that mean LR does not differ between predation treatments, and PT $\times C T$ the probability of no interaction between predation treatment and count class. Abbreviations: $C=$ control. $E=$ experimental, $C 72=$ control with 0.72 abd./caud. vertebrae.


Note: Adjustment B:
(1) H1: slopes homogeneous among control groups; LR in control groups adjusted to mean TL in experimental groups.
(2) H2S, 3S, 3L, and 6: slopes homogeneous among groups omitting the group noted in table; LR is adjusted to the mean TL of all groups combined, using either the slope in the differing group or that in all other groups combined.
(3) H4: slope is significant in C77 only: LR in C77 adjusted to mean TL in E77
count classes (Table 16).

Selection with respect to $L R$ cannot account for selection for the ratio of abdominal to caudal vertebrae (VR). In experiment $H 1$, selection was significant for LR but not for VR. In experiment $H 2 S$, selection favoured high ( 0.82 ) VR but low LR. In experiments $H 3 S$ and $H 4$, selection approached significance for $\operatorname{VR}(p=0.06-0.07)$ but not for $L R(p=0.40-0.60)$. In experiment $H 6$, selection favoured low (0.72) VR but high LR. Thus, in some experiments ( $\mathrm{H} 1, \mathrm{H} 3 \mathrm{~S}$, and H 4 ) selection was significant for one but not the other prey attribute, while in other experiments (H2S and H6) selection for one attribute counteracted selection for the other.
2. Kennedy Lake experiments

Body proportion measurements were made in experiments $K 1$ and K2 only. Two length ratios were considered: abdominal/caudal length (LR1) and precaudal/caudal length (LR2) (abdominal length $=$ total - head - caudal length; precaudal length $=$ head length + abdominal length). The two length ratios were highly correlated ( $\mathrm{p}<0.0001$ ); 80 and $86 \%$ of the variation in the precaudal/caudal ratio was explained by variation in the abdominal/caudal ratio in experiments $K 1$ and $K 2$, respectively.

These length ratios could not be compared among classes of the usual vertebral count ratio $V R$, since the criteria finally employed to distinguish abdominal from caudal vertebrae were not those used when these length measurements were made. Instead, a ratio VR', based on the numbers of vertebrae anterior to or
opposed and posterior to the first anal basal, will be used here. VR and VR' were equivalent in $89 \%$ of $f i s h$ in experiments $K 1$ and K2. In the remainder, the division used in VR' differed by one vertebra from that used in VR.

Both length ratios showed a highly significant correlation with $V R^{\prime}$ (Table 17). However, variation in length ratio is considerable within any one vertebral count class, even for the abdominal/caudal ratio (Fig. 6). In the case of this latter length ratio, most variation within count classes must be due to variation in the spacing of vertebrae.

Relationships between $L R$ and total length or $V R$ (or VR') differed somewhat between Kennedy and Holden Lake fish. LR was inversely related to total length in Holden Lake fish (Table 15), but directly related to total length in Kennedy Lake fish (Table 18). More of the variation in $V R$ was explained by variation in LR in Holden than in Kennedy Lake fish (Tables 14 and 17). This occurs despite the inclusion of head length in the $L R$ used in the Holden Lake comparison, but not in LR1 in the Kennedy Lake comparison. Finally, regressions of VR on LR usually differed between control and experimental groups in the Holden Lake experiments (Table 14), but not in the Kennedy Lake experiments (Table 17). Some of these differences might result from the use of more appropriate criteria to distinguish abdominal from caudal vertebrae in the $L R$ analysis of Holden Lake experiments.

Table 17. Regression of vertebral count ratio VR' on length ratios LR in Kennedy Lake fish. VR' is the number of vertebrae anterior to the first anal basal divided by the number opposing or posterior to it. LR1 is the length from the first vertebra to the first opposing or posterior to it. LRi is the length from the first vertebra to the firs latter point to the end of the hypural plate. LR2 resembles LR $t$, but includes the head length in the numerator. Regressions are shown separately for control and experimental groups, and equality of slopes and intercepts tested between the two.


Figure 6. Ratios of abdominal/caudal length in vertebral count classes VR' of Kennedy Lake sticklebacks exposed (experimental, open bars) or unexposed (control, black bars) to predation by sunfish. VR' is the number of vertebrae anterior to the first anal basal divided by the number opposing and posterior to it.


LR2=ABDOMINAL / CAUDAL LENGTH

Table 18. Body proportions of Kennedy Lake sticklebacks exposed (exptl.) or unexposed (control) to predation by sunfish. Body proportions LR1 and LR2 are as defined in Table 17. When LR is significantly (p<0.05) related to total length TL, adjusted LR is also shown. When slopes of regressions of LR on TL differ significantly between predation treatments, slopes within treatments are also shown. Adjustment methods are as in Table 15, except that in $D$ control and experimental groups are adjusted to the grand mean TL using the separate slopes within each group.

| Probability |  |  |  |  |  |  | Probability of zero slope | Mean SE |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Exp. | LR | Slope | zero <br> slope | equal <br> slopes | Treatment | Slope |  |  |  | Adjusted mean | Adjustment method | Probability of equal means |
| K1 | 1 | -0.00001 | 0.96 | 0.53 | $\begin{aligned} & \text { Control } \\ & \text { Expt1. } \end{aligned}$ |  |  | $\begin{aligned} & 0.9827 \\ & 0.9946 \end{aligned}$ | $\begin{aligned} & 0.00430 \\ & 0.00265 \end{aligned}$ | -- | A | 0.018 |
|  | 2 | 0.00121 | 0.008 | 0.28 | $\begin{aligned} & \text { Control } \\ & \text { Expt1. } \end{aligned}$ | - | -- | $\begin{aligned} & 1.7039 \\ & 1.7246 \end{aligned}$ | $\begin{aligned} & 0.00655 \\ & 0.00404 \end{aligned}$ | $\begin{aligned} & 1.7052 \\ & 1.7241 \end{aligned}$ | B | 0.015 |
| K2 | 1 | 0.00034 | 0.10 | 0.004 | ```Control Exptl.``` | $\begin{array}{r} 0.00129 \\ -0.00004 \end{array}$ | $\begin{aligned} & 0.003 \\ & 0.85 \end{aligned}$ | $\begin{aligned} & 0.9870 \\ & 0.9797 \end{aligned}$ | $\begin{aligned} & 0.00332 \\ & 0.00207 \end{aligned}$ | $\begin{aligned} & 0.9919 \\ & 0.9797 \end{aligned}$ | C | 0.002 |
|  | 2 | 0.00133 | $<0.001$ | 0.015 | $\begin{aligned} & \text { Control } \\ & \text { Expt1. } \end{aligned}$ | $\begin{aligned} & 0.00250 \\ & 0.00086 \end{aligned}$ | $\begin{aligned} & 0.0001 \\ & 0.012 \end{aligned}$ | $\begin{aligned} & 1.6920 \\ & 1.6900 \end{aligned}$ | $\begin{aligned} & 0.00486 \\ & 0.00304 \end{aligned}$ | $\begin{aligned} & 1.6967 \\ & 1.6879 \end{aligned}$ | D | 0. 12 |

Selective predation with respect to LR differed between the two experiments (Table 18). During exposure to predation, mean LR increased in experiment K 1 , but decreased in experiment K 2 (after adjustment of control and experimental groups to a common total length). Thus, selection with respect to $L R$ may have contributed to selection for the ratio of abdominal to caudal vertebrae in experiment K 1 , but counteracted selection for this ratio in experiment K 2 . Therefore, in these experiments as in the Holden Lake experiments, selection with respect to vertebral count ratios cannot in general be attributed solely to selection with respect to length ratios.
3. Summary

In 1983 experiments using Holden Lake fish, low LR was sometimes favoured during predation at small or medium fry sizes, while high LR was favoured at large fry sizes. Similarly, in 1982 experiments with Kennedy Lake fish, low LR was favoured at the smaller fry size in experiment K 2 , and high LR at the larger fry size in experiment K 1 . Selective predation for vertebral count ratio was not attributable to this selection for LR. In some experiments, selection was significant for one but not for the other prey attribute, while in other experiments selection for one attribute counteracted selection for the other.

Part II. Burst swimming performance of threespine sticklebacks

Materials and Methods

Fish used were laboratory-reared offspring of breeding sticklebacks collected from Holden Lake in August 1983, June August 1984, and June 1985. In 1983, 113 crosses were made from a single collection of parents, and the embryos produced reared together at about 17C in the same 75 L tank. In 1984, between 1 and 15 crosses were made from each of 8 collections of parents, and embryos reared at temperatures between 14 and 23C. After hatching, fry from a single rearing temperature and collection of parents were mixed together, and held at 15C until use. In 1985, 5 crosses were made from a single collection of parents, and embryos reared at. 14 C , or at 22 C until 46 h after fertilization and 14C thereafter. After hatching in all years, fry were fed Artemia nauplii twice daily. Illumination was artificial, but simulated the natural photoperiod.

Burst swimming performance was measured of fry about 7-11.5 mm in length in 1983 and 1984, and of fry 9.4-11.5 mm in length in 1985. Performance was measured in a tank 1 m square in 1983, or 0.6 m square in 1984 and 1985. Water depth was 3 cm . Single fry were introduced into a $3 x 3 \mathrm{~cm}$ container in this tank. A 250 W floodight was switched on, a sliding door in the container opened, and fry allowed to emerge from the container. Burst swimming was initiated using an a.c. electric shock stimulus applied through two stainless steel grids 15 cm apart. In 1983,
swimming was in a $2 \mathrm{~V} / \mathrm{cm}$ electric field; in 1984 and 1985, swimming followed a 32 msec stimulus of $8 \mathrm{~V} / \mathrm{cm}$ (with the exception noted below). Swimming was filmed through a mirror mounted overhead at a 45 degree angle, on 16 mm cine-film at 64 frames/sec. In 1983, water temperature varied between 15 and 17C; in 1984 and 1985, the filming arena was immersed in a water bath which maintained temperatures usually within 0.5 C of either 14.5 or 24.5 C in 1984 , or 15.0 C in 1985 . Temperature treatments will be referred to as 15 C or 25 C . In 1984 , some fry were also filmed at 15 C in a viscous solution of $0.1 \%$ methylcellulose (viscosity about 0.021 poise, measured with an Ostwald viscometer). Some fry filmed in this solution experienced a stimulus of 48 msec duration; performance was similar whether stimulus duration was 32 or 48 msec . In summary, performance was measured under two temperature treatments (15 or 25C) and three viscosity treatments: about 0.009 poise (in 25C water), 0.011 poise (in 15 C water) or 0.021 poise (in 15 C methylcellulose solution). Fry were acclimated to the experimental temperature and viscosity for at least 8 h , and fasted for $8-24 \mathrm{~h}$ before use.

After filming, fry were killed in anaesthetic, preserved in $10 \%$ buffered formalin in individually numbered vials, and cleared and stained as described above. Counts were made of centra excluding the urostyle. The last vertebra was often complex; such vertebrae were counted as one. Fish with other vertebral irregularities were omitted from analyses. Abdominal and caudal vertebrae were distinguished on the basis of haemal spine length. The first vertebra whose haemal spine length was $80 \%$ or more of
the maximum spine length in the vicinity of the anal basals was designated the first caudal vertebra. Total length was measured to the posterior edge of the hypural plate. Caudal length was measured from the anterior end of the first caudal vertebra.

Film records were analysed frame by frame. The greatest distance travelled in 4 frame intervals (0.0625 s) at 15 C , or in 3 frame intervals (0.047 s) at 25 C , was calculated for each fish. The shorter time interval was used at 25 C because bursts of swimming tended to be of shorter duration at this higher temperature. The recorded bursts of swimming were subjectively scored for response quality before vertebral counts were known. Three criteria were considered: (1) an indication of convulsion or paralysis before, during or after bursts, (2) a failure to respond with a sustained burst of swimming, and (3) a.change in depth during bursts. The first factor was the most frequent problem in responses to the extreme stimulus used in 1983; the frequency of this problem increased as fry size increased. This problem was avoided in 1984 and 1985 by using a brief electric pulse as a stimulus. However, fish more often failed to respond with a sustained burst of swimming to this more mild stimulus. Failure to respond enthusiastically was most frequent in the 15 C , 0.011 poise treatment, and was apparently unrelated to size. I attempted to objectively select responses for analysis as follows. In each size class in each experiment, I calculated the percentage of responses subjectively scored as good. Using a slightly lower percentage $X, I$ then selected the fastest $X \%$ of responses within each count class of a given size class. The
percentages used were:
(1) 15C, 1983: $70 \%$ for the length class $7.4-7.8 \mathrm{~mm}, 60 \%$ for lengths of $7.8-8.3 \mathrm{~mm}, 55 \%$ for lengths of $8.3-9.0 \mathrm{~mm}$, and $40 \%$ for length classes 9.0-9.8 and 9.8-11.4 mm;
(2) 15C, 1984: 70\% for all length classes;
(3) 15C, 1985: 60\% for all length classes;
(4) 25C, 1984: 80\% for all length classes; and,
(5) 15C, 0.1\% methylcellulose: $80 \%$ for all length classes. Results were also examined using responses selected on the basis of subjective scores of response quality. Both selection procedures revealed the same pattern in results, so only those results obtained using the objective procedure are described here.

In most analyses described below, fish are grouped in length classes. Length classes were defined in ocular micrometer units (1 unit $=0.0816667 \mathrm{~mm}$ ). The length classes usually used were: 81-90, 91-95, 96-101, 102-110, 111-120, and 121 or more. These classes will be referred to as: $6.6-7.4 \mathrm{~mm}, 7.4-7.8 \mathrm{~mm}, 7.8-8.3$ $\mathrm{mm}, 8.3-9.0 \mathrm{~mm}, 9.0-9.8 \mathrm{~mm}$, and $>9.8 \mathrm{~mm}$.

Effects of vertebral count on swimming performance, and interactions between length and the effect of count, were tested by one- or two-way analyses of variance (ANOVA), and by regression analyses. ANOVA was computed using BMDP7D (Dixon 1981). Homogeneity of variances among groups was tested using

Levene's test (Brown and Forsythe 1974a). Variances differed significantly among groups in only two tests: (1) the effect of count at sizes over 9.8 mm at 15 C in 1983 , and (2) this effect in the 7.4-7.8 mm length class at 15 C in $0.1 \%$ methylcellulose. In these two cases, equality of means was tested using the Welch statistic (Brown and Forsythe 1974b). Regression lines were calculated using BMDP1R, and equality of slopes tested using BMDPIV. The significance of regressions of slope on vertebral count ratio was tested as described by Sokal and Rohlf (1981, p.503-505). The third order interaction between viscosity treatment, length class and count class was tested using BMDP4V.

Results

Abdominal and caudal vertebrae

1. Performance at 15C

The same pattern in swimming performance was seen both in 1983 and 1984 among fish filmed at 15 C at lengths of $7.4-9.0 \mathrm{~mm}$ (Fig. 7). Performance depended not on the absolute number of vertebrae, but on the ratio of abdominal to caudal vertebrae. Performance was best among fish with a high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ (abdominal/caudal vertebrae) at small sizes (7.4-7.8 mm), among those with an intermediate ratio (0.78) at intermediate sizes (7.8-8.3 mm), and among those with a low ratio (0.72) at large sizes (8.3-9.0 mm). This interaction between length and optimum vertebral count ratio was significant in both years (Table 19). Within length classes, the effect of vertebral count ratio on performance was significant according to ANOVA at small and large

Figure 7. Burst swimming performance at 15C of stickleback fry 6.6-9.0 mm in length in 1983 (A) and 1984 (B). Fry are 'grouped by length and by the ratio of abdominal to caudal vertebrae ( $A V / C V$ ), and plotted at the mean length in each group. Symbol areas are proportional to sample sizes.


Table 19. Signiftcance of effects of vertebral count class on burst swimming performance of stickleback fry within length classes at 15c. Vertebral count classes are based on the ratio of abdominal to caudal vertebrae. To avoid small sample sizes, fish with ratios above 0.82 are omitted, and those with intermediate ratios of 0.76 and 0.78 grouped together. Probabilities are for the effect of count class in one- or two-way analyses of variance. Variances are homogeneous among groups in all comparisons. The maximum and minimum sample sizes are given in parentheses for each comparison. Data are shown in Fig. 7 .

| Year | Length class (mm) |  |  |  | Interaction Count $\times$ Length |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | 6.6-7.4 | 7.4-7.8 | 7.8-8.3 | 8.3-9.0 |  |
| 1983 | -- | $\begin{aligned} & 0.098 \\ & (6-24) \end{aligned}$ | $\begin{gathered} 0.62 \\ (5-15) \end{gathered}$ | $\begin{gathered} 0.13 \\ (7-24) \end{gathered}$ | $\begin{gathered} 0.042 \\ (5-24) \end{gathered}$ |
| 1984 | $\begin{gathered} 0.095 \\ (10-31) \end{gathered}$ | $\begin{gathered} 0.020 \\ (10-40) \end{gathered}$ | $\begin{gathered} 0.27 \\ (17-45) \end{gathered}$ | $\begin{gathered} 0.017 \\ (28-43) \end{gathered}$ | $\begin{gathered} 0.001 \\ (10-45) \end{gathered}$ |
| Both | -- | 0.003 | 0.23 | 0.006 |  |

sizes in 1984, but not in 1983 (when sample sizes tended to be small, Table 19). Combining both years in a two-way analysis, effects of vertebral count ratio were highly significant at small and large sizes (Table 19). At these small and large sizes, performance tended to show a linear relationship to vertebral count ratio between 0.72 and 0.82 . At the small size, this relationship was positive and significant in both years; at the large size, it was negative and significant in both years (Table 20).

Regressions of swimming performance on length within vertebral count classes are shown in Table 21 . At 15C, slopes differ significantly among vertebral count classes in both years. In both years, slopes increased as vertebral count ratio decreased between 0.82 and 0.72 . This linear regression of slope on vertebral count ratio was significant in 1984 ( $p<0.025$ ), but not in 1983 ( $p>0.25$; however, degrees of freedom were minimal $(1,1)$ in the 1983 calculation).

Results are available only in 1984 for lengths less than 7.4 mm . The trend seen between lengths of 7.4 and 9.0 mm did not extend to these smaller sizes at 15C; performance was not best among fish with a high vertebral count ratio of 0.82 or 0.88 (Fig. 7B). Fish with an intermediate ratio appeared to perform best, but effects of vertebral count ratio on performance were not significant at these sizes under 7.4 mm (Table 19).

Table 20. Regressions of swimming performance of stickleback fry on the ratio of abdominal to caudal vertebrae (VR) and total length (TL), within length classes at 45 C . Only ratios between 0.82 and 0.72 inciusive are included. Abbreviations are b (partial regression coefficient), $n$ (sample size) and $P(b)$ (probability that the coefficient is zero).

|  |  |  | Length class (mm) |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Year | Variable |  | 6.6-7.4 | 7.4-7.8 | 7.8-8.3 | 8.3-9.0 |
| 1983 |  | $n$ | -- | 38 | 32 | 47 |
|  | VR | b $P(b)$ | --- | $\begin{gathered} 15.5628 \\ 0.032 \end{gathered}$ | $\begin{aligned} & -4.5185 \\ & 0.53 \end{aligned}$ | $\begin{array}{r} -13.4940 \\ 0.038 \end{array}$ |
|  | TL | b $P(b)$ | -- | $\begin{gathered} -0.6021 \\ 0.81 \end{gathered}$ | $\begin{gathered} -1.6979 \\ 0.39 \end{gathered}$ | $\begin{aligned} & 1.0022 \\ & 0.33 \end{aligned}$ |
| 1984 |  | n | 50 | 61 | 87 | 104 |
|  | VR | $\begin{aligned} & b \\ & P(b) \end{aligned}$ | $\begin{aligned} & 0.3896 \\ & 0.92 \end{aligned}$ | $\begin{gathered} 12.9016 \\ 0.006 \end{gathered}$ | $\begin{gathered} -2.3170 \\ 0.50 \end{gathered}$ | $\begin{array}{r} -12.1149 \\ 0.0007 \end{array}$ |
| - | TL | b $P(b)$ | $\begin{aligned} & 1.8036 \\ & 0.019 \end{aligned}$ | $\begin{aligned} & 2.0705 \\ & 0.16 \end{aligned}$ | $\begin{aligned} & 0.2000 \\ & 0.83 \end{aligned}$ | $\begin{aligned} & 2.0114 \\ & 0.003 \end{aligned}$ |

Table 21. Coefficients of regressions of swimming performance of stickleback fry on length, within vertebral count classes VR. VR is the ratio of abdominal to caudal vertebrae. Regressions are over the length interval $6.9-9.0 \mathrm{~mm}$ in the 25 C treatment, and 7.4-9.0 in the 15C treatments. Abbrevations are MC, $0.1 \%$ methylcellulose; $P(b)$, probability that slope is zero; $P($ equal $b)$. probability that slopes are equal among vertebral count classes; $n$. sample size.

| Treatment | VR | n | intercept | slope | P(b) | $P($ equal b) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 15C 1983 | 0.82 | 63 | 7.4261 | 1.4271 | 0.0008 | 0.014 |
|  | 0.78 | 36 | 5. 1687 | 1.7478 | 0.0027 |  |
|  | 0.72 | 18 | -14.6921 | 4.1631 | 0.0003 |  |
| 15C 1984 | 0.88 | 14 | 0.6901 | 2.2721 | 0.018 | 0.001 |
|  | 0.82 | 128 | 12.3298 | 0.8845 | 0.0027 |  |
|  | 0.78 | 26 | 10.5446 | 1. 1402 | 0.10 |  |
|  | 0.76 | 43 | 0.7743 | 2.3191 | <0.0001 |  |
|  | 0.72 | 55 | -4.6274 | 2.9851 | <0.0001 |  |
| 25C | 0.88 | 34 | 1.9724 | 2.2709 | - 0.0010 | 0.086 |
|  | 0.82 | 183 | 9. 1220 | 1.3815 | $<0.0001$ |  |
|  | 0.76-0.78 | 30 | 5.7740 | 1.8084 | 0.022 |  |
|  | 0.72 | 47 | -4.0622 | 3.0621 | $<0.0001$ |  |
| 15C MC | 0.88-0.94 | 26 | -12.1075 | 3.7128 | 0.0001 | 0.097 |
|  | 0.82 | 110 | -7.9750 | 3.2144 | $<0.0001$ |  |
|  | 0.76-0.78 | 26 | 5.0943 | 1.6003 | 0.035 |  |
|  | 0.72 | 52 | -6.7205 | 3.0609 | $<0.0001$ |  |

Swimming performance was measured at 15 C at lengths over 9.0 mm in three years (Fig. 8). The ranking of vertebral count classes with respect to performance differed among years. At lengths of $9.0-9.8 \mathrm{~mm}$, fish with an intermediate ratio of 0.78 appeared to perform poorly and those with other ratios to perform equally well in 1983 and 1984. However, in 1985, performance in this length class appeared to be inversely related to vertebral count ratio, as had been seen at slightly smaller sizes (8.3-9.0 mm ) in both previous years. At lengths of 9.8-11.5 mm, fish with a ratio of 0.78 again appeared to perform relatively poorly in 1983, while performance appeared to be similar among all vertebral count classes in 1985. None of these differences in performance among vertebral count classes was significant ( $p=0.10-0.63$ ), either within years or grouping over years in two-way analyses of variance. Nor was the interaction between year and the effect of count class significant at either size ( $p=0.19$ at lengths of $9.0-9.8 \mathrm{~mm}, \mathrm{p}=0.28$ at lengths of $9.8-11.5$ mm). Swimming performance is apparently unrelated to vertebral count ratio at 15 C at lengths between 9.0 and 11.5 mm (or, the optimum ratio at these large sizes is not among those tested, i.e. not between 0.72 and $0.88 \mathrm{AV} / \mathrm{CV}$ ).
2. Performance at 25 C

Effects of vertebral count ratio on swimming performance between lengths of 6.9 and 9.0 mm at 25 C resembled those seen between lengths of 7.4 and 9.0 mm at 15 C . Performance was best among fish with a high ratio at small sizes, among those with an intermediate ratio at an intermediate size, and among those with

Figure 8. Burst swimming performance at 15 C of stickleback fry 9.0-11.5 mm in length in 1983 (A), 1984 (B) and 1985 (C). Symbols and sample sizes are as in Fig. 7 legends, except as noted.

a low ratio at large sizes (Fig. 9). However, the sizes at which performance was best among fish with high or intermediate ratios appeared to be shifted to slightly shorter lengths at 25C compared to 15C. Performance was best among fish with a high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ at lengths of $7.4-7.8 \mathrm{~mm}$ at 15 C , but at lengths of 6.9-7.4 mm at 25C. Similarly, performance appeared to be best among fish with intermediate ratios of 0.76-0.78 at 7.8-8.3 mm at 15 C , but at $7.4-7.8 \mathrm{~mm}$ at 25C. This interaction between temperature treatment and the effect of vertebral count ratio on performance was significant in the $7.4-7.8 \mathrm{~mm}$ length class ( $p=0.051$ ), but not in other length classes ( $p>0.20$, but sample sizes are small in the smaller length class). (Probabilities are from two-way ANOVAs within length classes with temperature (1984 15C or 25 C ) and vertebral count ratios (0.82, 0.72 and in all but the smallest length class 0.76-0.78) as treatments). Effects of vertebral count ratio on performance were not significant at 25 C within any length class ( $\mathrm{p}>0.20$ ), but sample sizes tended to be small for all ratios but 0.82 (usually less than 10 , sometimes less than 5 within a length class). However, the regression of performance on vertebral count ratio was significant over the range $0.72-0.82$ in the $8.3-9.0 \mathrm{~mm}$ length class at 25C ( $b=-12.1080, \mathrm{p}=0.054$ ).

Slopes of regressions of swimming performance on length showed the same pattern among vertebral count classes at 25C as was seen at 15C (Table 21). At both temperatures, slopes increased as vertebral count ratio decreased from 0.82 to 0.72 . At 25C, differences in slope among vertebral count classes were

Figure 9. Burst swimming performance at 25 C of stickleback fry 6.9-9.0 mm in length. Symbols and sample sizes are as in Fig. 7 legends, except as noted.

significant over this range of ratios ( $p=0.053$ ), but only approached significance including the more extreme ratio of 0.88 ( $p=0.086$ ). Differences in slope between the ratios that performed best at small or large sizes (0.82 and 0.72, respectively) were highly significant ( $p=0.015$ ).

Regressions of swimming performance on length are shown in Fig. 10 for fish with vertebral count ratios of 0.72 or 0.82 at each of the two temperatures. The similarities and differences between effects of vertebral count ratio on performance at the two temperatures are clearly seen in this figure. At both temperatures, slopes of this regression are greater among fish with the low ratio than among those with the high ratio. At both temperatures, fish with the high ratio are superior at small sizes, while those with the low ratio are superior at large sizes. In both replications at 15 C , the two regression lines intersect at the same length ( $8.07-8.08 \mathrm{~mm}$ ). However, at 25C, the lines intersect at a smaller size ( 7.85 mm ). Regression lines were calculated over the length ranges $7.4-9.0 \mathrm{~mm}$ at 15 C , and $6.9-9.0 \mathrm{~mm}$ at 25 C . However, the shift in intersection point between temperatures is not a result of this change in range. Regression lines calculated over the range $7.4-9.0 \mathrm{~mm}$ at 25 C also intersect at 7.85 mm .
3. Performance at 15 C in $0.1 \%$ methylcellulose

Swimming performance in an $0.1 \%$ solution of methylcellulose at 15 C is shown in Fig. 11 for lengths between 6.6 and 9.0 mm . Overall performance was not greatly affected by the high

Figure 10. Regressions of swimming performance on length among stickleback fry with vertebral count ratios of 0.72 or $0.82 \mathrm{AV} / \mathrm{CV}$, at 15 C in 1983 (A), at 15 C in 1984 ( B$)$, and at 25C in 1984 (C). The probability of equal slopes between vertebral count ratios is given by ' $p$ '. The intersection point between the two lines is shown on the length axis in each panel.


Figure 11. Burst swimming performance of stickleback fry $6.6-9.0 \mathrm{~mm}$ in length in an $0.1 \%$ solution of methylcellulose at 15 C . Symbols and sample sizes are as in Fig. 7 legends, except as noted.

viscosity of this solution. Within a length and count class, performance in this solution averaged $92 \%$ (range 80-101\%) of that in water at 15 C . (These comparisons are with the best $80 \%$ of responses within a length and count class in the 1984 15C treatment; that below is with the best $70 \%$ of responses as shown in Fig. 7B). However, the effect of vertebral count ratio on performance differed greatly between this and the previous treatments. Performances of fish with high or low ratios of 0.82 or $0.72 \mathrm{AV} / \mathrm{CV}$ were similar in this viscous solution within all length classes between 6.6 and 9.0 mm . Effects of vertebral count ratio on performance were not significant within any length class according to ANOVA $(p=0.16-0.98)$. But the interaction between length and the effect of vertebral count ratio on performance was significant over the range $7.4-9.0 \mathrm{~mm}$ ( $p=0.028$ ). This interaction presumably reflects the superior performance of fish with intermediate ratios (0.76-0.78) at small sizes and their inferior performance at large sizes (Fig. 11). Comparing performance in water or this viscous solution at 15 C , the interaction between length and the effect of vertebral count ratio differed significantly between viscosity treatments ( $p=0.004$, for the third order interaction in the ANOVA testing effects of count ratio ( $0.72,0.76-0.78,0.82$ ), length class (as grouped in Fig. 11) and viscosity (1984 15C: water or $0.1 \%$ methylcellulose)).

Similar conclusions are reached comparing the slopes of regressions of performance on length among vertebral count classes (Table 21). The inverse relationship between slope and
vertebral count ratio seen in the previous treatments for ratios between 0.72 and 0.82 is absent in the methylcellulose treatment. Instead, slopes in this treatment are similarly high among fish with high or low ratios, and low among those with intermediate ratios. Differences in slope are not significant overall ( $p=0.097$ ), but are highly significant if fish are grouped as those with intermediate (0.76-0.78) or with other ratios ( $p=0.017$ ) .

In summary, the effect of vertebral count ratio on swimming performance differed greatly between water and $0.1 \%$ methylcellulose at 15C. The significance of this difference is difficult to assess. It does not seem to be due to a toxic effect of methylcellulose, since performance was not greatly diminished in the $0.1 \%$ solution of it. (Even long term exposure to $0.1 \%$ methylcellulose is relatively innocuous. Fish were reared for a month in water or this solution; survival in methylcellulose was slightly better than in water). A small decrease in viscosity (about 0.002 poise) from 15C to 25C appeared to shift the sizes at which fish with high or intermediate vertebral count ratios were superior to slightly smaller sizes. Perhaps the large increase in viscosity (about 0.010 poise) from water to $0.1 \%$ methylcellulose at 15 C produced a large shift in the other direction, so that the effects seen in water at 15 C would be seen in $0.1 \%$ methylcellulose at sizes larger than those examined. Alternatively, the effects of such large changes in viscosity may differ qualitatively from those of the small changes normally experienced in nature.

Body Proportions

Effects of the ratio of abdominal to caudal vertebrae on swimming performance could result from effects of body proportions on performance. To test this possibility, I considered the precaudal/caudal length ratio (where precaudal length is total minus caudal length). The correlation between vertebral count ratio (VR) and this length ratio (LR) was highly significant ( $p<0.0001$ ) among fish filmed at 15C both in 1983 and 1984. Variation in LR explained $57 \%$ of the variation in VR among 1984 fish, but only $25 \%$ of this variation among 1983 fish.

Effects of $V R$ and $L R$ on performance were compared by examining the simple linear regressions of performance on each and the partial regression coefficients of each in multiple linear regressions (Table 22). Regressions were calculated separately within the length classes $7.4-7.8 \mathrm{~mm}$ and $8.3-9.0 \mathrm{~mm}$, among fish filmed at 15 C in either 1983 or 1984 . Simple linear regressions of performance on $V R$ were significant ( $p<0.05$ ) in all four cases, while those on $L$ were significant in only one case and approached significance $(p=0.063)$ in only one other. Partial regression coefficients on $V R$ were significant in all but one case, while those on $L R$ were significant in no cases. In all but one case, signs of the partial coefficients on $L R$ were opposite to those on VR and to those of the simple coefficients on both VR and LR. Thus, effects of $V R$ on swimming performance are clearly not attributable to effects of LR on performance.

Table 22. Regressions of swimming performance on the ratio of abdominal to caudal vertebrae (VR), on the precaudal/caudal length ratio (LR). and on VR, LR and TL (total length), Within small or large length classes at 15C. Only fish with VR between 0.72 and 0.82 inclusive are included. Abbreviations are as in Table 20.


Swimming performance depended not on the absolute number of vertebrae, but on the ratio of abdominal to caudal vertebrae. At 15C, performance was superior among fry with a high ratio of 0.82 $A V / C V$ at small sizes (7.4-7.8 mm), among those with an intermediate ratio (0.78) at intermediate sizes (7.8-8.3 mm), and among those with a low ratio (0.72) at large sizes (8.3-9.0 mm). Performance at even smaller or larger sizes did not differ significantly among the ratios tested. A similar effect of vertebral count ratio on performance was seen at 25 C , except that performance of fry with high or intermediate ratios was superior at slightly smaller sizes at this higher temperature. The effect of vertebral count ratio on performance differed greatly between water and $0.1 \%$ methylcellulose. Fry with high (0.82) or low (0.72) ratios performed equally well in the viscous solution within all length classes between 6.6 and 9.0 mm. Effects of vertebral count ratio on swimming performance were not attributable to an effect of precaudal/caudal length on performance.

Part III. Changes in vertebral count with length in wild stickleback fry.

Material and Methods

Stickleback fry were captured by dip net ( 25 x 17 cm gape) from Holden Lake, B.C. Collections were made at four sites, from a canoe floating near shore (Fig. 12). Fry were collected from sites A1, A3 and A4 between May 7 and June 11, 1984, and from site $B$ between June 4 and 14,1984 . Sites were between $5 \times 5 \mathrm{~m}$ and 8 x 12 m in area. Sites $A 3$ and $A 4$ were adjoining; site $A 1$ was separated from A3 by about 20 m , and site B from A1 by about 200 m.

After collection, fry were killed in anaesthetic, preserved in $10 \%$ buffered formalin, and cleared and stained as described in Part I. Centra were counted, total lengths measured and abdominal and caudal vertebrae distinguished as described in Part II. The last vertebra often bore two neural and/or haemal arches; such vertebrae were counted as one. Counts were equivocal in about $1.5 \%$ of fish due to other irregularities; these equivocal counts were excluded from analyses. Vertebral development was insufficient to obtain counts from $12 \%$ of fish 6.1-6.6 mm in length, $0.5 \%$ of fish $6.7-7.2 \mathrm{~mm}$ in length, and $0.1 \%$ of fish $7.3-7.7 \mathrm{~mm}$ in length, among fish collected from $A$ sites. Development was sufficient to obtain counts from all fish collected from site B.

Figure 12. Map of Holden Lake, B.C., showing sampling sites.


Fry were grouped for analysis according to the ratio of abdominal to caudal vertebrae (AV/CV). Four groups were distinguished: (1) 0.82 AV/CV (14 AV/ 17 CV ), (2) 0.78-0.76 $\mathrm{AV} / \mathrm{CV}(14 / 18,13 / 17),(3) \quad 0.72 \mathrm{AV} / \mathrm{CV}$ (13/18), and (4) other ratios (mostly 0.88 or more). Probabilities are from chi-square tests, calculated using BMDP4F (Dixon 1981).

## Results

Differences in vertebral counts of recruits were tested among sites by comparing counts of fry under 7.4 mm in length. Frequencies of the four vertebral count classes did not differ among sites $A 1, A 3$ and $A 4(p=0.18)$, but did differ between these sites and site $B(p=0.0085)$. Hence, fish from sites $A$ and $B$ are analysed separately below.

Vertebral count ratios of fry grouped by length are shown in Fig. 13, pooling collections from different dates. At site B, fry with a high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ increased in frequency, and those with intermediate ratios of $0.76-0.78$ decreased in frequency, as mean length increased from about 6.9 to 7.5 mm ( $\mathrm{p}=0.020$ ). As mean length increased further to about 8.0 mm , the reverse change in frequency occurred ( $p=0.024$ ). No significant changes in frequency occurred with further increases in fry length ( $p=0.80$ ).

Among fry collected from site $A$, no significant changes in frequency occurred between mean lengths of 6.9 and 7.5 mm ( $p=0.13$ ). However, the same decrease in frequency of fish with the high ratio and increase in frequency of those with the

Figure 13. Frequencies of vertebral count classes in wild stickleback fry grouped by length. Count classes are based on the ratio of abdominal to caudal vertebrae.
A. Fry collected from site A; B. Fry collected
from site B. Symbols as in Fig. 14.



intermediate ratios was seen as mean length increased further from 7.5 to $8.0 \mathrm{~mm}(\mathrm{p}=0.040)$. Again, no significant changes in frequency occurred with even further increases in fry length ( $\mathrm{p}=0.51$ ) .

These changes in frequency could reflect selection favouring fry with a high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ at lengths of about 7.3-7.7 mm at site B , and those with intermediate ratios of $0.76-0.78$ at lengths of about 7.8-8.3 mm at both sites A and B. Alternate explanations would be that one (though probably not both) of the changes in frequency between successive length classes might reflect changes in the vertebral counts of recruits to the population over time, or an initial correlation between count and length in recruits. Vertebral count frequencies of recruits (i.e. fry under 7.4 mm in length) to the two sites are shown over time in Fig. 14. With data grouped as shown in the figure, frequencies of the four vertebral count classes in recruits did not differ significantly among days, either at site $B(p=0.46)$ or at site $A(p=0.18)$. No trend in the frequency of the most common vertebral count ratio ( 0.82 ) is seen over the extended sampling period at site A (Fig. 14A). However, frequencies of the intermediate and 'other' classes do tend to differ between recruits collected before or after day 23. Differences in count frequencies between samples grouped as those collected at site A before or after this date are significant ( $p=0.006$ ). However, the increase in frequency of fry with intermediate ratios between mean lengths of 7.5 and 8.0 mm at site A is not explained by this increase in frequency between early and late recruits. The two

Figure 14. Frequencies of vertebral count classes in fry less than 7.4 mm in length, grouped by collection date. Count classes are the ratio of abdominal to caudal vertebrae ( $A V / C V$ ). Samples from successive days grouped to give minimum sample sizes of 50 for site $A$ (panel A) and 35 for site $B$ (panel B).

length classes contained the same proportion of fry collected late in the sampling period $(47.6$ and $47.5 \%$ of fry in the length classes 7.3-7.7 and $7.8-8.3 \mathrm{~mm}$, respectively, were collected after day 25 (the later date is used to allow for the growth of fry into these length classes)). Furthermore, the same changes in vertebral count are seen as mean fry length increases from 7.5 to 8.0 mm in both early and late collections (Table 23), though these changes in frequency are significant in neither subsample alone ( $p=0.086$ and 0.21 , respectively).

The alternate explanation invoking an initial correlation between count and length in recruits can be similarly discredited. Most fry were apparently recruited at lengths below 7.8 mm . Thus, the greatest effect of a correlation between count and recruitment length should be seen between the two smallest length classes, i.e. between mean lengths of 6.9 and 7.5 mm . However, no significant difference in vertebral count occurred between these lengths at site A. A correlation between vertebral number and hatching length might be expected in view of the developmental processes thought to be involved in determining vertebral number (Lindsey and Arnason 1981). If so, a positive correlation is expected, and an increase in the frequency of fry with an intermediate ratio of $0.78 \mathrm{AV} / \mathrm{CV}$ (i.e., 32 total vertebrae) is predicted between mean lengths of 6.9 and 7.5 mm . When changes in vertebral count are seen between these lengths, they are opposite to these predicted changes. Thus, an initial correlation between vertebral count and length in recruits may counteract rather than contribute to the changes in vertebral

Table 23. Vertebral count class frequencies (\%) of sticklebacks 8.3 mm or less in length, collected from site $A$ between May 7 and 31 , or between June 4 and 11. 1984. Vertebral count classes based on the ratio of abdominal to caudal vertebrae.

| Date | ```Length class (mm)``` | Mean length (mm) | Vertebral count class (\%) |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | 0.82 | 0.76-0.78 | 0.72 | Other | Sample size |
| May 7-31 | 6.1-7.2 | 6.86 | 58.3 | 13.0 | 10.0 | 18.6 | 408 |
|  | 7.3-7.7 | 7.46 | 59.9 | 14.8 | 7.4 | 17.9 | 364 |
|  | 7.8-8.3 | 7.99 | 55.2 | 21.2 | 4.9 | 18.7 | 364 |
| June 4-11 | 6.1-7.2 | 6.94 | 60.5 | 16.7 | 10.5 | 12.2 | 294 |
|  | 7.3-7.7 | 7.46 | 63.1 | 19.6 | 7.6 | 9.7 | 331 |
|  | 7.8-8.3 | 8.00 | 55.0 | 24.3 | 9.1 | 11.6 | 329 |

count observed between mean lengths of 6.9 and 7.5 mm .

Fry were collected from site $A$ over an extended period, and were grouped over time in comparisons among length classes. Effects of any change in the counts of recruits over time, or of any correlation between count and length among newly hatched fry, would be mostly cancelled by this grouping over time. However, fry from site $B$ were collected over only a brief period, so comparisons among length classes of these fry are more likely to be confounded by such effects. These problems can be eliminated by restricting comparisons to a single cohort of fry. Such an analysis can be attempted for site $B$ if it is assumed that fry grow from one length class to the next between successive collection dates. Judging from the peaks in length distributions of fry in successive collections, growth rates of fry were at least about $0.2 \mathrm{~mm} / \mathrm{d}$. Successive mean lengths differ by 0.6 and 0.5 mm respectively for the first three length classes, so this assumption may be valid for collections 3d apart. Large numbers of fry were collected from site $B$ on days 36 and 39 only. Counts of fry in one length class on day 36 are compared with those of fry in the next length class on day 39 in Fig. 15. The same changes in frequency are seen in these comparisons as were seen in the comparisons grouped over all collection dates, though levels of significance are reduced. Thus, it seems likely that the changes in vertebral count seen among length classes reflect selection for vertebral count in those length classes.

Figure 15. Vertebral count ratios of stickleback fry in two 'cohorts' at site B, comparing counts in length class $i$ on day 36 and length class $i+1$ on day 39. Symbols as in Fig. 14. A. $i=6.1-7.2 \mathrm{~mm}$; B. $i=7.3-7.7 \mathrm{~mm}$.
(


Total vertebral counts of fry in the various length classes are shown in fig 16. Changes in frequency are those expected given the apparent selection for the ratio of abdominal to caudal vertebrae seen in Fig. 13. The only significant change seen at site $A$ was an increase in frequency of fry with 32 vertebrae, and a corresponding decrease in frequency of those with 31 , as mean length increased to 8 mm . Most of this change occurred between mean lengths of 7.5 and 8 mm . On the other hand, the most significant change seen at site $B$ was an increase in frequency of fry with 31 vertebrae and decrease in frequency of those with 32 , as mean length increased from 6.9 to 7.5 mm . As at site $A$, the reverse change in frequencies occurred as mean length increased further to 8 mm , but this second change only approached significance at site $B$. At neither site did significant changes in frequency occur as mean length increased above 8 mm .

In summary, changes in the relative frequencies of vertebral counts with length among wild stickleback fry in Holden Lake are consistent with selection favouring a high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ and a low total count of 31 at small sizes of $7.3-7.7 \mathrm{~mm}$, and intermediate ratios of $0.76-0.78 \mathrm{AV} / \mathrm{CV}$ and a high total count of 32 at slightly larger sizes of $7.8-8.3 \mathrm{~mm}$. Effects of changes in the vertebral counts of recruits over time, or of an initial correlation between count and length in recruits, cannot be completely eliminated, but are probably not the main causes of these changes in relative frequency with length. These changes persist when collections from different dates are pooled (so that length and recruitment date or size will not be strongly

Figure 16. Percent frequencies of total vertebral counts, in fry grouped by length at site A (panel A) or B (panel B). Only, counts between 30 and 32 ( $>98 \%$ of all counts) are shown.

confounded), and when fry in one length class of a given collection are compared with those in the next length class of the next collection.

Part IV. Predation experiments with peamouth chub.

Material and Methods

Ripe peamouth chub Mylocheilus caurinus were collected from Holden Lake on April 28 and May 11, 1983, and 7 (PM1) or 27 (PM8) crosses respectively made in the laboratory (each between a single male and female). Embryos from each set of crosses were incubated in separate 20 L tanks at 14C. After hatching, fry were fed Artemia nauplii twice daily, with occasional supplements of nematodes and natural plankton. Conditions were crowded, and growth and development slow compared to those of fry in the wild (see below).

Between 22 and 56 d after hatching, fry were distributed among two control and four experimental tanks, and those in experimental tanks exposed to predation by sunfish or smallmouth bass Micropterus dolomieui until $50-60 \%$ of the initial number had been eaten. In each of five experiments, fry were exposed at two temperatures ( 15 or $25 \mathrm{C},+/-1 \mathrm{C}$ ) and two cover treatments (cover present or absent). Experimental design and procedure were the same as those described in detail above for the 1983 experiments with sticklebacks, except that predators were not removed overnight in these experiments. Details of each experiment are given in Table 24.

In all but experiment 5, some fry were below the size at which vertebrae are sufficiently formed to be countable. Experiments were done at these small sizes since selection with respect to vertebral number could reflect selection for myomere

Table 24. Description of predation experiments using peamouth chub as prey. Predators are sunfish (S) or bass (B). Predator-hours (pred-h) are calculated using lighted periods only.

| Exp. | Par. | Tmt. | Temp. (C) | Cover | Duration |  |  | Length | (mm) |  | No. <br> eaten <br> per pred-h | No. surviving |  | Rearing mortality (\%) | Length (mm) of preserved survivors |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | n | Pred-h | Type | Mean | SD | eaten |  | Pres. | Reared |  | Mean | SD |
| 1 | PM8 | Con | 15 | N | 28 | 0 | - | - | - | 0 | - | 60 | 155 | 5.8 | 8.84 | 0.37 |
|  |  |  | 25 | N | 27 | 0 | - | - | - | 0 | - | 49 | 161 | 23.0 | 9.02 | 0.40 |
|  |  | Exp | 15 | Y | 26 | 203 | S | 31.2 | 2.6 | 55 | 1.35 | 70 | 155 | 10.3 | 8.95 | 0.32 |
|  |  |  | 15 | N | 22 | 154 | S | 30.4 | 2.1 | 52 | 1.7 .1 | 62 | 186 | 37.1 | 8.86 | 0.30 |
|  |  |  | 25 | $Y$ | 23 | 140 | 5 | 29.4 | 2.4 | 61 | 2.34 | 37 | 168 | 19.6 | 9.05 | 0.29 |
|  |  |  | 25 | N | 24 | 148 | S | 30.0 | 2.6 | 66 | 2.42 | 54 | 131 | 12.2 | 9.06 | 0.33 |
| 2 | PM 1 | con | $15$ |  | $78$ |  | - | - | - |  | - |  |  | 20.8 | $9.89$ |  |
|  |  |  | $25$ | $N$ | $54$ | $0$ | - | - | - | $0$ | - | $53$ | $149$ | $0.0$ | $10.13$ | $0.58$ |
|  |  | Exp | 15 | Y | 98 | 2208 | B | 14.8 | 1.7 | 48 | 0. 12 | 75 | 201 | 1.0 | 10.03 | 0.49 |
|  |  |  | 15 | N | 96 | 2082 | B | 14.5 | 2.1 | 51 | 0.13 | 61 | 196 | 17.9 | 9.85 | $0.55$ |
|  |  |  | 25 | Y | 52 | 830 | B | 16.7 | 2.3 | 64 | 0.40 | 34 | 153 | 0.0 | 10.23 | 0.57 |
|  |  |  | 25 | N | 54 | 868 | B | 16.6 | 2.1 | 64 | 0.38 | 41 | 146 | 0.0 | 10.18 | 0.59 |
| 3 | PM8 | Con | 15 | N | 49 | 0 | - | - | - | 0 | - | 58 | 142 | 7.0 | 10.14 | 0.53 |
|  |  |  | 25 | N | 29 | 0 | - | - | - | 0 | - | 48 | 152 | 5.3 | 10.38 | 0.47 |
|  |  | Exp | 15 | Y | 48 | 614 | B | 22.8 | 4.3 | 61 | 0.50 | 52 | 142 | 3.5 | 10.42 | 0.48 |
|  |  |  | 15 | N | 46 | 589 | B | 21.8 | 4.0 | 68 | 0.58 | 45 | 116 | 15.5 | 10.67 | 0.48 |
|  |  |  | 25 | Y | 28 | 192 | B | 23.8 | 4.8 | 60 | 1.56 | 57 | 143 | 1.4 | 10.56 | 0.46 |
|  |  |  | 25 | N | 30 | 214 | B | 23.3 | 5.5 | 57 | 1.33 | 50 | 163 | 7.0 | 10.47 | 0.41 |

Table 24 . contd.

| Exp. | Par. | Tmt. | Temp. (C) | Cover | Duration |  |  | Length | (mm) | \% | No. eaten per pred-h | No. surviving |  | Rearing mortality (\%) | Length (mm) of preserved survivors |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | h | Pred-h | Type | Mean | SD | eaten |  | Pres. | Reared |  | Mean | SD |
| 4 | PM 1 | Con | 15 | $N$ | 73 | 0 | - | - | - | 0 | - | 48 | 215 | $0.0$ | $10.81$ | $0.74$ |
|  |  |  | 25 | N | 49 | 0 | - | - | - | 0 | - | 43 | 162 | $0.0$ | $10.86$ | $0.67$ |
|  |  | Exp | 15 | Y | 70 | 1599 | B | 17.8 | 2.8 | 49 | 0.15 | 86 | 167 | 0.0 | 10.77 | 0.71 |
|  |  |  | 15 | $N$ | 74 | 1751 | B | 17.5 | 2.0 | 49 | 0.14 | 74 | 187 | 0.0 | 10.91 | 0.74 |
|  |  |  | 25 | Y | 47 | 610 | B | 19.0 | 0.8 | 55 | 0.44 | 54 | 167 | 0.0 | 11.12 | 0.72 |
|  |  |  | 25 | $N$ | 48 | 623 | B | 19.0 | 2.8 | 57 | 0.46 | 47 | 163 | 0.0 | 11.14 | 0.85 |
| 5 | PM8 | Con | 15 | $N$ | 31 | 0 | - | - | - | 0 | - | 109 | 0 | - | 11.60 | 0.62 |
|  |  |  | 25 | N | 27 | 0 | - | - | - | 0 | - | 110 | 0 | - | 11.84 | 0.73 |
|  |  | Exp | 15 | $Y$ | 30 | 377 | B | 23.5 | 4.8 | 58 | 0.43 | 114 | 0 | - | 12.25 | 0.89 |
|  |  |  | 15 | $N$ | 30 | 359 | B | 23.6 | 5.2 | 58 | 0.44 | 116 | 0 | - | 12.11 | 0.85 |
|  |  |  | 25 | Y | 26 | 150 | B | 25.7 | 5.5 | 60 | 1. 10 | 111 | 0 | - | 12.19 | 0.81 |
|  |  |  | 25 | N | 26 | 152 | B | 25.7 | 6.0 | 60 | 1.09 | 110 | 0 | - | 12.26 | 0.82 |

Abbreviations: Par $=$ parental crosses
Tmt $=$ predation treatment: control (con) or experimental (exp) Pres = preserved at end of experiment without further rearing Other abbreviations as in Table 2.
number or some other feature of the axial segmentation, and might thus occur before vertebral development is complete. At the end of all but experiment 5 , fry in each tank were divided into quarters using a 'plankton splitter'. One-quarter of the fry were preserved for length measurements, and the remainder reared to a larger size. All fry were preserved at the end of experiment 5.

Fry were killed in anaesthetic, preserved in $10 \%$ buffered formalin, and cleared and stained as described above. Length was measured from the tip of the snout to the posterior edge of the hypural 'plate'. Counts were made of centra, excluding the urostyle and including the members of the Weberian complex. The last or second last vertebra frequently bore two neural and occasionally two haemal arches. Such complex vertebrae were counted as one. Fish with other vertebral irregularities were rare (Table 25) and were excluded from analyses. Counts reported for experiments 1 and 2 are of reared fry only, since vertebrae of all or most fry preserved without further rearing were undeveloped in these experiments. In experiments 3 and 4, most fry preserved without post-experimental rearing had countable vertebrae. In both experiments, counts of reared fry and of fry without post-experimental rearing but with developed vertebrae differed by less than $1 \%$ over all treatments. Accordingly, counts reported for experiments 3 and 4 are of both reared and non-reared fry. Counts reported for experiment 5 are entirely of fry without post-experimental rearing.

Table 25. Vertebral development and abnormalities of peamouth chub surviving predation expertments. Abnormalities not including complex vertebrae in the last or second last positions. Length is the mean in controls at the end of experiments.

| Exp. | Length (mm) | Undeveloped vertebrae (\%) |  | Abnormalities (\%) |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Without rearing | After rearing | Nonreared | Reared |
| 1 | 8.9 | 100.0 | 0.0 | - | 2.1 |
| 2 | 10.0 | 57.5 | 0.7 | 6.9 | 2.0 |
| 3 | 10.2 | 25.8 | 1.3 | 0.0 | 0.6 |
| 4 | 10.8 | 13.6 | 1.2 | 1.7 | 1.3 |
| 5 | 11.7 ${ }^{\text {\% }}$ | 0.0 | - | 0.4 | - |

Abdominal and caudal vertebrae could not be distinguished. At the smaller sizes examined, haemal arches and spines were undeveloped or rudimentary. The designation of the abdominal/caudal division was strongly size dependent, using either closure of the haemal arch (when developed) or position relative to other structures such as the anal fin basals as criteria. The use of relative haemal spine length as a criterion was not practical, since changes in length near the abdominal/caudal division were not discontinuous as described above for Gasterosteus.

In the analysis of experiments 3 and 5 , vertebral counts are compared between predation treatments among fish above or below a certain length at the end of experiments $(10.35 \mathrm{~mm}$ in experiment 3, 11.5 mm in experiment 5). In experiment 3 , reared.fish were assigned to the small or large size class as follows. First, the percentage of fry below 10.35 mm at the end of the experiment (say $\mathrm{x} \%$ ) was determined for each of the six groups using the samples preserved without post-experimental rearing. Then, the smallest $X \%$ of fry in the corresponding reared group were assigned to the small size class, and the remainder to the large size class.

Lengths were compared by ANOVA, calculated using BMDP7D. Variances were tested for heterogeneity using Levene's test (Brown and Forsythe 1974a), and means compared using the Brown-Forsythe test (Brown and Forsythe 1974b) when variances were significantly ( $p<0.05$ ) heterogeneous. Count distributions were compared by chi-square tests, calculated using BMDP4F. To
avoid classes with small expected values, counts were grouped as 44, 45 and 'other' (Table 29), or as 44 and 'other' when sample sizes were small (Tables 26 and 30). Yates correction for continuity was used in $2 x 2$ comparisons with sample sizes under 200. Linear regressions were calculated using BMDP1R, and equality of slopes tested using BMDP1V.

Results

Rearing mortalities were high in some groups in experiments 1, 2 and 3 (Table 24). Mortality was largely due to infection by fungi, bacteria or parasitic flukes, and is not expected to be strongly selective for vertebral number at the stages of development reared here (Lindsey 1962). Vertebral counts of fry dying during post-experimental rearing were obtained for two groups in experiment 2. Counts of these fry (Table 26) did not differ significantly from those of survivors of post-experimental rearing in these groups ( $p=0.38$ combining the two groups since counts do not differ between groups within mortality treatments, $\mathrm{p}>0.40$ ) . Sample size was, however, small for fish dying during rearing. If rearing mortality was selective, the data suggest that it was lower for fish with 44 vertebrae. Given such selective mortality, fish with 44 vertebrae would be expected to be more frequent in replications with high mortality than in those with low mortality. This relationship was not seen. In experiments 1 and 2, rearing mortality was high in one control group and low in the other (Table 24). In both experiments, fry with 44 vertebrae were actually less frequent among survivors in the group with high mortality. Thus, the available evidence

| e 26. Vertebral counts of fry surviving (Surv) or not surviving (Mort) post-experimental rearing in two groups from experiment 2. |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Group |  | $\leqslant 43$ | 44 | 45 | $\geqslant 46$ | No. |
| 1. Control 15C | Mort <br> Surv | $0.9$ | $\begin{aligned} & 50.0 \\ & 56.6 \end{aligned}$ | $\begin{aligned} & 50.0 \\ & 41.6 \end{aligned}$ | $0.9$ | $\begin{array}{r} 20 \\ 113 \end{array}$ |
| 2. Exptl 15 C No cover | Mort Surv | $\begin{aligned} & 5.0 \\ & 1.9 \end{aligned}$ | $\begin{aligned} & 55.0 \\ & 62.3 \end{aligned}$ | $\begin{aligned} & 40.0 \\ & 35.1 \end{aligned}$ | $0.6$ | $\begin{array}{r} 20 \\ 154 \end{array}$ |

suggests that rearing mortality was not strongly selective for vertebral number in these experiments.

The mean length of survivors of predation in experimental groups tended to be slightly greater than that of fry in control groups at the end of all experiments (Table 27). However, size selection was statistically significant in only experiments 3 and 5. Rates of predation in these two experiments were intermediate to those in the experiments without significant size selection (Table 24).

Regressions between vertebral number and length are shown in Table 28. Data are grouped over replications within predation treatments since slopes did not differ among such replications in any experiment (p>0.18). The signs of slopes in control groups differed between the two sets of crosses: slopes were negative before predation among offspring of PM1 parents (experiments 2 and 4), but positive among those of PM8 parents (experiments 1, 3 and 5). In experiments 1 and 2, slopes differed significantly from zero in neither control nor experimental groups. Among fry with post-experimental rearing in experiments 3 and 4, slopes differed significantly from zero in control but not in experimental groups. In experiment 5, slope differed significantly from zero in the experimental but not in the control group. Differences in significance between control and experimental groups might be explained by the difference in sample size between the two groups in experiment 5, but not in experiments 3 and 4. In the latter experiments, regressions were significant in the group with the smaller sample size. Mean

Table 27. Mean lengths of peamouth chub fry exposed (exptl.) or unexposed (control) to predation. Means are the unweighted averages of the means within temperature treatments. Means within temperature treatments are also shown when the interaction (INT) between predation (PT) and temperature (TMP) treatments is significant.

|  | Mean length (mm) |  |  |  | Probability |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Exp. | Temp. | Control | Expt 1. | E-C | PT | TMP | INT |
| 1 | Both | 8.93 | 8.98 | 0.05 | 0.26* | 0.0001 | 0.72 |
| 2 | Both ${ }^{\text {a }}$ | 10.01 | 10.08 | 0.07 | 0.31 | 0.0001* | 0.88 |
| 3 | Both | 10.26 | 10.53 | 0.27 | $<0.0001$ | 0.052 | 0.026 |
|  | 15C | 10.14 | 10.54 | 0.40 | $<0.0001$ |  |  |
|  | 25C | 10.38 | 10.52 | 0. 14 | 0.081 |  |  |
| 4 | Both | 10.84 | 10.98 | 0. 14 | 0.11 | 0.061 | 0.17 |
| 5 | Both | 11.72 | 12.20 | 0.48 | <0.0001* | 0.028 | 0.11 |

* Variances heterogeneous ( $p<0.05$ ). Brown-Forsythe test used.

| Exp. | Type | Predation treatment | No. | Intercept | Slope | Probability of zero slope |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | Rear | Control $\text { Expt } 1 \text {. }$ | $\begin{aligned} & 266 \\ & 494 \end{aligned}$ | $\begin{aligned} & 43.8435 \\ & 44.3808 \end{aligned}$ | $\begin{array}{r} 0.0405 \\ -0.0015 \end{array}$ | $\begin{aligned} & 0.13 \\ & 0.93 \end{aligned}$ |
| 2 | , Rear | Control <br> Exptl. | $\begin{aligned} & 258 \\ & 634 \end{aligned}$ | $\begin{aligned} & 44.8603 \\ & 44.3083 \end{aligned}$ | $\begin{array}{r} -0.0439 \\ 0.0015 \end{array}$ | $\begin{aligned} & 0.73 \\ & 0.92 \end{aligned}$ |
| 3 | Pres | Control <br> Expt1. | $\begin{array}{r} 60 \\ 170 \end{array}$ | $\begin{aligned} & 42.1349 \\ & 45.4714 \end{aligned}$ | $\begin{array}{r} 0.2083 \\ -0.0953 \end{array}$ | $\begin{aligned} & 0.24 \\ & 0.41 \end{aligned}$ |
|  | Rear | Control <br> Expt 1 . | $\begin{aligned} & 268 \\ & 500 \end{aligned}$ | $\begin{aligned} & 43.1562 \\ & 43.9449 \end{aligned}$ | $\begin{aligned} & 0.1147 \\ & 0.0398 \end{aligned}$ | $\begin{aligned} & 0.002 \\ & 0.20 \end{aligned}$ |
| 4 | Pres | Control Exptl. | $\begin{array}{r} 82 \\ 216 \end{array}$ | $\begin{aligned} & 45.1025 \\ & 43.9354 \end{aligned}$ | $\begin{array}{r} -0.0708 \\ 0.0330 \end{array}$ | $\begin{aligned} & 0.41 \\ & 0.48 \end{aligned}$ |
|  | Rear | Control <br> Expti. | $\begin{aligned} & 358 \\ & 675 \end{aligned}$ | $\begin{aligned} & 45.0395 \\ & 44.5598 \end{aligned}$ | $\begin{aligned} & -0.0605 \\ & -0.0217 \end{aligned}$ | $\begin{aligned} & 0.035 \\ & 0.27 \end{aligned}$ |
| 5 | Pres | Control <br> Expt 1 . | $\begin{aligned} & 219 \\ & 451 \end{aligned}$ | $\begin{aligned} & 44.2292 \\ & 43.6556 \end{aligned}$ | $\begin{aligned} & 0.0194 \\ & 0.0635 \end{aligned}$ | $\begin{aligned} & 0.72 \\ & 0.043 \end{aligned}$ |

lengths varied more among replications of reared experimental groups than between replications of reared control groups in experiments 3 and 4. But regressions were also significant within replications of control groups but not within those of experimental groups in these experiments. Thus, the differences in significance between control and experimental groups are not explained by grouping over a wider range of mean lengths in the latter. Differences in the significance of regressions between control and experimental groups could result from selective predation for vertebral number. These observed changes in significance between groups exposed or unexposed to predation are consistent with selection favouring fry with 44 vertebrae at larger sizes in the range $9.2-11.2 \mathrm{~mm}$ (mean $+/-2 \mathrm{SD}$ ) in experiment 3, and at smaller sizes in the ranges 9.4-12.3 mm in experiment 4 and $10.4-13.1 \mathrm{~mm}$ in experiment 5 (Fig. 17).

Vertebral counts of fry exposed or unexposed to predation are shown in Table 29. Data are grouped over temperature and cover treatments, since counts did not differ significantly between levels of these treatments in experimental groups in any experiment ( $p>0.13$ ) and since the non-significant variations in count between levels did not show any pattern consistent over experiments. Counts of fry exposed to predation did not differ from those of unexposed controls in any experiment. In experiment 1 , a decrease in the frequency of fry with extreme vertebral counts after exposure to predation approached significance ( $p=0.065$ ). The greatest change in frequency seen in the five experiments was an increase in the frequency of fry with

Figure 17. Regressions between vertebral number and length in control (C) and experimental (E) groups, peamouth chub predation experiments 3-5. Length shown on lower horizontal axis is at the end of experiments (for exp. 3 and 4, regressions are among fish with post-experimental rearing, but lines are transposed to the estimated lengths before rearing; scales of reared lengths are also shown). Asterisks indicate slopes significantly different from zero ( $\mathrm{p}<0.05$ ). Lines drawn over the range $\overline{\mathrm{X}}+/-1.5 \mathrm{SD}$.




| Exp. | Predation treatment | Vertebral number (\%) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | $\leqslant 43$ | 44 | 45 | $\geqslant 46$ | No. | P |
| 1 | Control | 4.1 | 59.9 | 33.3 | 2.6 | 267 | 0.065 |
|  | Expt 1 . | 2.4 | 60.4 | 36.4 | 0.8 | 503 |  |
| 2 | Control | 0.7 | 64.4 | 34.5 | 0.4 | 278 | 0.19 |
|  | Exptl. | 2.9 | 61.6 | 35.3 | 0.2 | 654 |  |
| 3 | Control | 1.5 | 57.7 | 38.7 | 2.1 | 333 | 0.31 |
|  | Expt 1 . | 2.9 | 55.8 | 38.4 | 2.9 | 683 |  |
| 4 | Control | 2.7 | 62.3 | 34.1 | 0.9 | 440 | 0.18 |
|  | Expt 1 . | 1.8 | 67.2 | 30.1 | 0.9 | 891 |  |
| 5 | Control | 0.9 | 54.3 | 42.9 | 1.8 | 219 | 0.76 |
|  | Exptl. | 1.6 | 55.9 | 40.6 | 2.0 | 451 |  |

44 vertebrae after exposure to predation in experiment 4 (by about $5 \%, p=0.18$ ). In experiments 3 and 5 , selection in favour of fry with 44 vertebrae could have been obscured by size selection. Counts are compared within small or large size classes in these two experiments in Table 30 . In experiment 3 , fry with 44 vertebrae are sligthly more common after than before exposure to predation at lengths over 10.35 mm , but the increase in frequency (5.5\%) is not significant ( $p=0.23$ ). Conversely, at the smaller lengths in this experiment, fry with 44 vertebrae are less frequent after exposure to predation, but this difference again lacks significance $(p=0.10)$ and could in any event be attributed at least partly to size selection. In experiment 5, fry with 44 vertebrae are significantly more common after than before exposure to predation at lengths under $11.5 \mathrm{~mm}(p=0.031)$, but not at greater lengths. This difference at the shorter lengths cannot be attributed to size selection.

In summary, these experiments provide some evidence of selection in favour of fry with 44 vertebrae at lengths between about 10.3 and 11.5 mm , but not at smaller lengths. Evidence for selective predation with respect to vertebral number is strong in the experiment at the largest prey size (5), but only weak and circumstantial in the experiments at intermediate prey sizes (3 and 4): In experiments at smaller sizes (1 and 2), predation was selective with respect to neither vertebral number nor size, and correlations between vertebral number and length were significant neither before nor after predation. One possibility, suggested by the absence of size selection in these experiments, is that

Table 30. Vertebral counts of peamouth chub fry exposed or unexposed to predation at small or large lengths in experiments 3 and 5. Abbreviations as in Table 29.

| Exp. | Length class (mm) | Mean length ( mm ) | Predation treatment | Vertebral number (\%) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | $\leqslant 43$ | 44 | 45 | $\geqslant 46$ | No. | P |
| 3 | <10.35 | 9.94 | Control | 1.7 | 64.4 | 33.3 | 0.6 | 174 | 0. 10 |
|  |  | 10.06 | Expt 1. | 4.2 | 56.1 | 36.9 | 2.8 | 214 |  |
|  | > 10.35 | 10.76 | Control | 1.3 | 50.0 | 44.9 | 3.8 | 158 | 0.23 |
|  |  | 10.80 | Expti. | 2.4 | 55.5 | 39.4 | 2.8 | 465 |  |
| 5 | <11.5 | 11.13 | Control | 1.0 | 53.6 | 43.3 | 2.1 | 97 | 0.031 |
|  |  | 11.16 | Exptl. | . | 69.9 | 29.0 | 1.1 | 93 |  |
|  | >11.5 | 12.18 | Control | 0.8 | 54.9 | 42.6 | 1.6 | 122 | 0.61 |
|  |  | 12.47 | ExptI. | 2.0 | 52.2 | 43.6 | 2.2 | 358 |  |

high predator efficiency overshadowed differences in performance related to vertebral number or size of prey at these small sizes. This possibility is discounted by a very low predation rate in experiment 2. Apparently, fry which will develop 44 or 45 vertebrae perform equally well at these small sizes.

Part V. Changes in vertebral number with length in wild peamouth chub fry

Material and Methods

Fry were collected from Holden Lake and from its main inlet stream, Hemer Creek, in 1984. Spawning of peamou'th chub appeared to be confined to the creek, and occurred on several nights between late April and early June. After hatching, fry migrated into the lake at night, beginning at dusk. In 1984, the first fry migration into the lake occurred on the evening of May 12 or 13. Fry migrating into the lake were collected between May 14 and July 1, using a Surber sampler set about 15 m upstream of the lake. Sets were of 5 or rarely 15 min duration between 2200 and 2300 h , and sampled the entire water column. Additional sets were made at earlier or later times on two occasions. Collections were made every day or two. Fry were also collected from the lake between May 14 and June 27 at A sites, and between May 31 and June 27 at site B (Fig. 12). Lake collections were made by dip net ( $25 \times 17 \mathrm{~cm}$ gape), from a canoe floating near shore.

Vertebrae were undeveloped in fry collected from the creek, in most fry collected from the lake in May, and in smaller fry collected from the lake in June. Fry collections from the creek were divided into halves or quarters using a plankton splitter. One-half or -quarter were reared in the laboratory to a larger size, and the remainder killed in anaesthetic and preserved in 10\% buffered formalin. Fry collections from the lake were
treated in one of three ways:
(1) All fry collected on May 14 and 18 were reared to a larger size, since sample sizes were relatively small.
(2) Fry collected between May 20 and June 18, and on June 27, were divided into two groups using a plankton splitter. One group was killed in anaesthetic and preserved in $10 \%$ buffered formalin. Before June 11 or 14 (depending on collection site), the second group was reared to a larger size. After these dates, fry in the second group were anaesthetised and divided into size groups. Fry in the smaller groups were reared to a larger size, and those in the larger group preserved in $10 \%$ buffered formalin.
(3) Fry collected on June 22 were anaesthetised and divided into small ( $<9.5 \mathrm{~mm}$ ) or large ( $>9.5 \mathrm{~mm}$ ) size classes. Those in the small size class were reared to a larger size, and those in the large class preserved in formalin.

Fry reared to a larger size and those preserved upon collection from the lake were cleared and stained as described in Part I. Total length was measured, and centra counted as described in Part IV. Vertebral abnormalities (other than accessory arches on the last or second last vertebra) occurred in 1.3\% of fry reared after collection from the creek, in $1.7 \%$ of those reared after collection from the lake, and in $0.8 \%$ of those preserved upon collection from the lake. These abnormal fry were excluded from analyses. Fry preserved upon collection from the creek were not cleared and stained, but were measured. Lengths of these fry cannot be compared directly to those of cleared and
stained fry. Clearing and staining appeared to reduce fry length by about $0.5-0.7 \mathrm{~mm}$.

Linear regression was calculated using BMDP1R. Mean lengths were compared by ANOVA, calculated using BMDP7D. Other probabilities are from chi-square tests, calculated using BMDP4F. Vertebral counts were usually grouped as 44, 45 or 'other' in order to avoid small expected values in chi-square tests.

Results

Fry migration into the lake was greatest before May 21, and usually slight thereafter (Fig. 18). Peak migrations occurred on the evenings of May 15 and May 18 or 19. Lesser peaks occurred on June 1 and 19. About $95 \%$ of fry entering the lake between 2200 and 2300 h had 44 or 45 vertebrae (Table 31). Vertebral counts differed markedly between fry entering the lake before or after May 17 ( $\mathrm{p}<0.0001$ ). Counts of 44 vertebrae occurred in only about $45 \%$ of fry entering before this date, but in over $70 \%$ of those entering thereafter. Average length of fry entering the lake also varied over the migration period, decreasing from early to late migration dates ( $p<0.0001$ according to linear regression analysis, Fig. 19). Within an evening, fry migrations were apparently homogeneous over time with respect to vertebral number, but not with respect to length (Table 32). Hence, vertebral counts of fry entering the lake between 2200-2300 $h$ on a given evening (Table 31) are apparently representative of those entering the lake over the entire

Figure 18. Number of peamouth chub fry captured per 5 min Surber sampler set, at 2200-2300 h in Hemer Creek, 1984.



Figure 19. Lengths of peamouth chub fry captured in Hemer Creek between 2200 and 2300 h . Vertical bars are standard errors. Regression line between length and collection day is shown. $P$ is the probability that the slope of this line is zero.


Table 32. Vertebral counts and lengths of peamouth chub fry collected from Hemer Creek at different times on the same night. $P(1)$ and $P(v)$ are the probabilities that lengths and counts, respectively, do not differ among collection times

| Date | Time | Length ( mm ) |  |  |  | No. per 5 min | $\begin{gathered} \text { Rearing } \\ \text { mortality } \\ (\%) \end{gathered}$ | Vertebral number (\%) |  |  |  | No. counted | P(v) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Mean | SD | No. | P(1) |  |  | 43 | 44 | 45 | 46 |  |  |
| May 1 | 2215-2230 | 8.87 | 0.33 | 48 | 0.0064 | 807 | 21.3 | 1.8 | 46.2 | 49.9 | 2.0 | 941 | 0.81 |
|  | 0115-0130 | 9.05 | 0.29 | 48 |  | 58 | 27.3 | 5.6 | 44.2 | 50.0 | 0.0 | 54 |  |
| May 16 | 2115-2120 | 9.15 | 0.21 | 11 |  | 59 | 16.7 | 5.0 | 47.5 | 47.5 | 0.0 | 40 |  |
|  | 2145-2150 | 9.02 | 0.27 | 48 | 0.39 | 338 | 18.3 | 2.8 | 51.1 | 45.4 | 0.7 | 141 | 0.72 |
|  | 2215-2200 | 9.02 | 0.31 | 48 |  | 378 | 15.5 | 4.3 | 44.3 | 49.3 | 2.1 | 142 |  |

evening.

Length distributions of fry collected from the lake between May 21 and June 27 are shown in Fig. 20. Little growth of fry was apparent in the lake before about May 28. Average fry length actually decreased from May 21 to 23. Vertebrae were undeveloped in all fry collected before May 28 (Table 33). Between May 25 and June 8, the proportion of fry with developed vertebrae increased at all lengths. By June 4, vertebrae were developed in most fry 9.3 mm or more in length.

Vertebral counts of fry collected from the lake between May 14 and 31 are shown in Table 34. Because vertebrae were undeveloped in most fry collected on these dates, counts are of fry reared in the laboratory after collection. Fry are grouped over all four collection sites, since vertebral counts differed neither among $A$ sites ( $p=0.85$ ) nor between sites $A$ and $B$ ( $\mathrm{p}=0.59$ ). Vertebral counts differed among collection dates ( $\mathrm{p}<0.0001$ ). The frequency of fry with 44 vertebrae increased between May 14-18 and May 21 ( $p<0.0001$ ), and decreased between May 21 and later collections $(p=0.013)$. The increase in frequency of fry with 44 vertebrae between May 18 and 21 in the lake presumably results from the increased frequency of this vertebral count among fry migrating into the lake on May 19 (Table 31). The decreased frequency of this vertebral count between fry collected from the lake on May 21 and 23 might reflect greater mortality among newly arrived fry in the lake. At any rate, vertebral count distributions were relatively homogeneous among collections made after May 21. In particular,

Figure 20. Length distributions of peamouth chub fry collected from Holden Lake between May(M) 21 and June(J) 27, 1984. The broken and solid lines connect the estimated median and smallest lengths, respectively, of fry in cohort $A$ (see text). Only fry over about 9 mm in length are shown for June 22.


Table 33. Percent of peamouth chub fry with developed vertebrae, among those collected from Holden Lake at the size and on the date shown. Empty cells indicate fewer than 5 fry preserved without laboratory rearing.

omu=ocular micrometer unit; $1 \mathrm{omu}=0.1641667 \mathrm{~mm}$.
$M=$ May, $J=$ June.

the frequency of fry with 44 vertebrae varied little over the period May 23-31 ( $\mathrm{p}=0.83$ ).

In the following analyses, three 'cohorts' of fry are distinguished. Fry entering the lake before May 22 comprise cohort A; those entering the lake about June 1, cohort $B$; and those entering June 19-21, cohort C. An attempt to delimit cohort A is shown in Fig. 20. In this figure, the broken line connects the estimated median length of fry of this cohort in each collection from the lake, and the solid line shows the approximate lower size limit of this cohort in each collection. The solid line is drawn assuming that fry of the smallest and median lengths have similar growth rates. Since smaller fry may have lower growth rates, this line may be drawn conservatively high and exclude a small proportion of fry actually in cohort A. However, this conservative procedure should also ensure that those fry assigned to cohort $A$ do not include a significant proportion of fry entering the lake after May 21. No attempt is made to delimit cohorts $B$ and $C$ in Fig. 20. Cohort $B$ can be first distinguished as the mode at small sizes in the June 4 sample, and cohort $C$ as that in the June 27 sample. Cohort $C$ was also present in the June 22 sample, but is not shown in Fig. 20 on this date because most fry below 9 mm in length in this sample were reared to a larger size after collection (see Material and Methods) .

A positive correlation between vertebral number and length might be expected in cohort $A$, since fry entering the lake late in the period May 14-21 had fewer vertebrae than did those entering early in this period. However, such an association was not seen in any collection between May 23 and 31 (Table 35). The absence of such an association can be explained by the apparent lack of growth among fry in the lake before May 24.

Vertebral counts of fry preserved upon collection in June are shown in Fig. 21 , grouped by length class and collection date. Fry below 9.3 mm in length are omitted from the figure, due to a high frequency of undeveloped vertebrae at these sizes (Table 33). In most collections, the frequency of fry with 44 vertebrae tended to decrease as length increased. Within collections obtained later than about June 8 , this tendency could be at least partly explained by confounding between length and cohort, since fry with 44 vertebrae were more common in cohorts entering the lake late in the season than in those entering early. However, the significant difference among length classes on June 4 cannot be so explained. Nor is it likely that such confounding can explain the significant difference seen among length classes when fry collected between June 4 and 22 are grouped together. In the former case, all fry compared are of the same cohort; in the latter case, most confounding between length and cohort has been eliminated by grouping over time, at least for lengths under about 12 mm . These changes in length could reflect selection in favour of fry with 45 vertebrae at lengths of about $9.9-10.6 \mathrm{~mm}$.

Table 35. Vertebral counts of small or large fry in cohort $A$, in samples collected between May 23 and 31 .

| Date | Length class (mm) |  | Vertebral count (\%) |  |  |  | No. | P |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\begin{aligned} & \text { After } \\ & \text { rearing } \end{aligned}$ | Before rearing (estimated) |  |  |  |  |  |  |
|  |  |  | $\leqslant 43$ | 44 | 45 | 46 |  |  |
| May 23 | $<10.6$ | $<8.4$ | 7.6 | 60.6 | 31.8 | 0.0 | 132 | 0.86 |
|  | $>10.6$ | >8.4 | 4.0 | 60.0 | 34.0 | 2.0 | 100 |  |
| May 25 | 9.6-10.9 | 7.8-8.4 | 1.9 | 59.6 | 37.9 | 0.6 | 161 | 0. 17 |
|  | > 10.9 | >8.4 | 0.0 | 61.7 | 38.3 | 0.0 | 141 |  |
| May 28 | 9.6-10.6 | 8.1-8.7 | 2.9 | 64.5 | 30.2 | 2.3 | 172 | 0.41 |
|  | > 10.6 | >8.7 | 2.2 | 61.0 | 35.7 | 1.1 | 182 |  |
| May 31 | 10.1-10.9 | 8.6-9.3 | 4.0 | 55.6 | 39.9 | 0.5 | 198 | 0.55 |
|  | $>10.9$ | >9.3 | 2.0 | 60.2 | 36.6 | 1.2 | 246 |  |

Figure 21. Frequencies of fry with 44 vertebrae, in length classes of collections between June 4 and 27. Probabilities are from chi-square tests with counts grouped as 44 or other.


No sample appropriate for comparison with fry grouped over cohorts is available to test for selection at smaller lengths. Such samples are available only for cohort $A$ alone. Vertebral counts of fry in cohort $A$ are shown in Fig. 22 , both for fry collected on May 31 when vertebrae were mostly undeveloped and for those collected in June when vertebrae were mostly developed. Counts are of reared fish for the May sample, and of fish preserved upon collection for the June samples. Fry below 9.3 mm in length are again omitted from the June samples. The same relationship between vertebral number and length is seen in the June samples of cohort $A$ alone as was seen in these samples grouped over cohorts: Fry with 44 vertebrae decreased in frequency, and those with 45 vertebrae increased in frequency, between the 9.3-9.9 and 9.9-10.6 mm length classes ( $p=0.022$ ). Since vertebral number and length were unrelated in May samples (Table 35), this change in frequency indicates selection favouring fry with 45 vertebrae at the larger size. A similar (though non-significant) result can be seen comparing only the larger fry in the May 31 and June 4 samples (Table 36). On the other hand, fry with 44 vertebrae were more frequent, and those with 45 vertebrae less frequent, in June samples at lengths of 9.3-9.9 mm than in the May sample. This comparison is valid since vertebral number and length were unrelated in May samples, and suggests selection favouring fry with 44 vertebrae at lengths below 9.9 mm . This result is confirmed comparing counts of smaller fry in the May 31 and June 4 samples (Table 36 ). Finally, vertebral counts vary little with length between 10.6 and 14.0 mm , suggesting that selection favours neither fry with

Figure 22. Vertebral counts of fry in cohort $A$, among fish reared after collection on May 31 and among those preserved upon collection between June 4 and 22. Fry collected in June grouped by length.


Table 36. Vertebral counts of small or large fry in cohort $A$, on May 31 or June 4. Counts are of fry preserved upon collection on June 4, or reared after collection on May 31. Lengths of reared fry are the estimated lengths before rearing.

| Vertebral number (\%) |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Group |  | $\leqslant 43$ | 44 | 45 | 46 | No. | P |
| May 31 | $8.8-9.3 \mathrm{~mm}$ | 3.0 | 55.4 | 41.1 | 0.6 | 168 | 0.022 |
| June 4 | $9.3-9.9 \mathrm{~mm}$ | 2.5 | 69.3 | 28.1 | 0.0 | 199 |  |
| May 31 | $>9.3 \mathrm{~mm}$ | 2.0 | 60.2 | 36.6 | 1.2 | 246 | 0.56 |
| June 4 | $>9.9 \mathrm{~mm}$ | 3.0 | 54.8 | 40.7 | 1.5 | 135 |  |

44 nor those with 45 vertebrae at these greater lengths.

Since counts are compared here of reared and non-reared fry, and since rearing mortality varies substantially among groups, the possibility that rearing mortality may be selective with respect to vertebral number must be considered. In the previous section (Part IV), it appeared that rearing mortality was not significantly selective for vertebral number. Results in this section likewise show no indication of selective rearing mortality. No consistent pattern is seen between vertebral number and extent of rearing mortality in Tables 31 and 34 . In Table 37, replications with substantially different rearing mortalities are shown for four groups. The first group consists of fry collected from Hemer Creek between 2250 and 2315 on June 13. The second and third replications of this group are subsamples from the same Surber sampler set, separated using a plankton splitter. Each of the remaining three groups consist of samples from different a sites, collected on the same date. In no case do vertebral counts differ significantly among replications, despite wide variation in the extent of rearing mortality. Nor is any consistent pattern seen among replications between vertebral number and the extent of rearing mortality. It is unlikely that the significant differences seen above among reared groups, or between reared and non-reared groups, result from varying extents of rearing mortality.

Table 37. Vertebral counts in replications of groups with widely varying extents of rearing mortality.

| Group |  | Repl. | Rearing | Vertebral number (\%) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | (\%) | $\leqslant 43$ | 44 | 45 | 46 | No. | P |
| Hemer Cr., | June 13 | 1 | 15.7 | 7.0 | 69.8 | 23.3 | 0.0 | 43 |  |
|  |  | 2 | 24.1 | 6.3 | 77.8 | 15.9 | 0.0 | 63 | 0.81 |
|  |  | 3 | 2.9 | 5.3 | 72:1 | 22.6 | 0.0 | 190 |  |
| Holden L.. | May 14 | A1 | 21.2 | 3.2 | 52.4 | 41.3 | 3.2 | 63 | 0.70 |
|  |  | A3 | 12.5 | 2.9 | 50.0 | 46.1 | 1.0 | 102 |  |
|  | May 18 | A 1 | 34.7 | 3.1 | 50.0 | 46.9 | 0.0 | 32 |  |
|  |  | A3 | 24.1 | 3.3 | 45.8 | 51.0 | 0.0 | 153 | 0.24 |
|  |  | A4 | 14.6 | 2.6 | 26.3 | 68.4 | 2.6 | 38 |  |
| May 21 |  | A 1 | 25.1 | 2.1 | 68.4 | 28.4 | 1.1 | 190 |  |
|  |  | A3 | 58.0 | 2.1 | 70.2 | 27.7 | 0.0 | 47 | 0.49 |
|  |  | A4 | 18.0 | 5.4 | 67.0 | 25.9 | 0.9 | 112 |  |

Vertebrae were undeveloped in some of the smaller non-reared fry but in few of the reared or larger non-reared fry. However, this difference can be discounted as a major cause of differences in vertebral number among groups. Among fry used in predation experiments (Part IV), vertebral number and degree of development were apparently unrelated. Reared and non-reared subsamples, differing by $12-24 \%$ in the numbers of fry with undeveloped (uncountable) vertebrae, differed by less than $1 \%$ in vertebral numbers. Even if the degree of vertebral development did differ among vertebral numbers, the small differences between groups in the proportions of fry with countable vertebrae could not produce the large differences in vertebral number seen in some comparisons. For example, in cohort $A$, vertebrae were undeveloped in about $4 \%$ of non-reared fry in the $9.3-9.9 \mathrm{~mm}$ length class in June samples, and in no reared fry of the May samples or non-reared fry in the $9.9-10.6 \mathrm{~mm}$ length class of June samples. However, the number of fry with 44 vertebrae in the first sample differed by $10 \%$ or more from the numbers in the latter two samples.

In summary, changes in the vertebral counts of fry in Holden Lake among May samples, in which vertebrae of most fry were undeveloped and uncountable, probably reflect changes in the vertebral counts of recruits into the lake, or greater mortality of new recruits, rather than selection for vertebral number per se. On the other hand, changes in the vertebral counts of fry in the lake between May and June samples, and between length classes of June samples, indicate selection favouring fry with 44
vertebrae at lengths below 9.9 mm , and those with 45 vertebrae at lengths between 9.9 and 10.6 mm . At greater lengths (between 10.6 and 14.0 mm ), selection appears to favour neither number. Selection for vertebral number at the smaller sizes appears to occur only after vertebrae are sufficiently developed to be countable.

Experiments with Gasterosteus

The experiments with Gasterosteus have demonstrated differences in swimming performance of fry with different vertebral counts, selective predation with respect to vertebral number of fry and changes in mean vertebral number of wild fry with length. The same pattern in relative performance was seen both in predation and in swimming performance experiments using sticklebacks from Holden Lake. Swimming performance was best, and survival during exposure to predation greatest, among fry with a high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ at small sizes, among those with intermediate ratios at intermediate sizes, and among those with a low ratio at large sizes. Changes in vertebral count with length among wild fry also conformed to this pattern, in that fry with the high ratio sometimes increased in frequency at small sizes, as did those with the intermediate ratios at slightly larger sizes.

This similarity in pattern among experiments suggests that the selective mortality seen in predation experiments in the laboratory, and apparently seen among fry in the wild, may result from differences in the swimming performance of fry with different vertebral numbers. The changes in vertebral count seen among wild fry are precisely those expected if this were so. Fry with the high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ increased in frequency in the wild at just those sizes when burst swimming performance is best among such fry, as did fry with the intermediate ratios of
0.78-0.76. However, results of the predation experiments differed from those of the swimming performance experiments in two important respects.

First, the two experiments appeared to differ in the effect of temperature. Results appeared similar between temperatures in predation experiments, but the sizes at which relative swimming performance was best shifted slightly between temperatures for fry with high or intermediate vertebral count ratios. This apparent difference may simply reflect the relatively wide range of sizes exposed to predation in any one experiment, and the relatively slight shift between temperatures in the size at which fry with particular vertebral count ratios perform the best.

Second, particular vertebral count ratios appeared to be favoured at somewhat larger sizes in predation than in swimming performance experiments. Discrepancies between mean lengths in the two types of experiments were about $0.5-0.8 \mathrm{~mm}$ at the smaller sizes, $1.5-2 \mathrm{~mm}$ at the intermediate sizes, and 2.5 mm at the large sizes. Since lengths in predation experiments are those at the end of experiments, these discrepancies could be due to growth during predation experiments. This explanation would require growth rates of $0.2-0.5 \mathrm{~mm} / \mathrm{d}$ at 15 C and $0.6-1.5 \mathrm{~mm} / \mathrm{d}$ at 25 C in predation experiments at small sizes (experiments $\mathrm{H} 1-3$ ), and 0.5-1.1 mm/d at 15 C and $0.9-1.4 \mathrm{~mm} / \mathrm{d}$ at 25 C in those at larger sizes (experiments $\mathrm{H}^{-6}$ ). These rates are mostly within the range reported for larvae and fry of other species, especially at the higher temperature (Table 38). They are, however, high in this range, and are in most cases considerably

Table 38. Growth rates reported for larvae and fry of several fish species, in the laboratory (L) or wild (W).

greater than rough estimates of growth rates of wild fry in Holden Lake ( 0.2 or $0.4 \mathrm{~mm} / \mathrm{d}$ for wild fry under or over 8.2 mm in length, respectively). But the estimated growth rates of wild fry are minimum estimates, judged from the distance between adjacent peaks in length distributions of fry in successive collections. In any case, growth rates during predation experiments may have been greater than those of similarly sized fry in the wild, due either to high food densities in the laboratory or to the change from crowded to uncrowded conditions during experiments. Growth is typically slow at the smallest fry sizes when feeding is first initiated, and increases rapidly at larger sizes when feeding is well established (e.g., Kramer and Smith 1960; Dey 1981; West 1966; Griswold and Smith 1972). Feeding was well established in fry used in predation experiments, though sizes were small due to crowded holding conditions. Perhaps growth rates of these fry when transferred to the uncrowded experimental conditions were similar to those of larger fry with well established feeding. Also, growth rates of the survivors of predation in these experiments may have been greater than the average rates in the population, if selection favoured the more vigorous and faster growing individuals. In any case, the length discrepancies between the two types of experiments can also be explained even given slower growth rates, if mortality in predation experiments was concentrated at the smaller sizes. Such was the case in most experiments, since predation was usually size selective, favouring larger prey.

In the above view, as fry grew during predation experiments (except H6), they experienced selection for first one and then a second vertebral count. The result observed at the end of each experiment is thus the net result of selection over these various intervals. Hence, selection within any one interval may have been much more intense than that judged on the basis of net changes in frequency between control and experimental groups. Selection at the larger sizes near the end of experiments would not be expected to completely cancel effects of selection at the small sizes near the start of experiments, because predation rates were probably greatest early in experiments when predators were most hungry, nil during night periods later in experiments when predators were absent and low during day periods later in experiments when predator numbers were generally much reduced.

An alternate view is that the selective predation seen in the laboratory is not the result of differences in the burst swimming performance of fry with different vertebral numbers. Fast-start performance may be more important in avoiding predators than burst swimming performance, but could not be measured at the available framing rates. Perhaps it too depends on vertebral count, but particular counts perform the best at somewhat larger sizes in fast-starts than in burst swimming. Or the observed selective predation might have resulted from a correlation between vertebral number and some other morphological or behavioural character of importance in avoiding predators. At the sizes used here; few other morphological characters likely to be of importance in avoiding predators are yet expressed:
lateral plates are not yet formed, spines are rudimentary or undeveloped, and fin rays are not fully formed. Behavioural differences related to vulnerability to predation have been found between different lateral plate phenotypes in sticklebacks (Moodie et al. 1973) but there the different phenotypes were from different populations. The sticklebacks used here were from a single population, so such associations between traits are less likely.

However, the changes in vertebral count frequencies seen in wild fry argue against this alternate view. Fry with high or intermediate vertebral count ratios increased in frequency in the wild at just those sizes when their burst swimming performance was the best. At the sizes recorded at the end of predation experiments, changes in vertebral count frequencies either were absent in the wild (experiments $44-6$ ) or were not those predicted from experiment (H1-3). Thus, it seems likely that the selective predation seen in the laboratory resulted from an effect of vertebral number on swimming performance, and that the length discrepancies seen between predation and swimming performance experiments resulted from growth during predation experiments and the concentration of mortality at the smaller sizes in these experiments.

Changes in vertebral count frequencies of wild fry differed from those predicted from swimming performance experiments in one respect. Fry with the low ratio of 0.72 AV/CV did not significantly increase in frequency at any size in the wild. However, fry with this ratio were rare in the wild, so selection
in their favour over a brief period may not have produced detectable changes in frequency. In laboratory experiments, these fry were favoured at larger sizes than were those with high or intermediate ratios. Growth rates at these larger sizes appeared to be greater in the wild than those at the smaller sizes, so fry with the low ratios may have been favoured during even briefer periods in the wild than were those with high or intermediate ratios.

Results also differed somewhat between sites. Fry with the high ratio of $0.82 \mathrm{AV} / \mathrm{CV}$ increased in frequency at small sizes at site B but not at site A. This difference could reflect temporal rather than spatial variation. Fry from site A were mostly collected on earlier dates and may have experienced lower temperatures as embryos, than fry from site B. Size at hatching often increases as incubation temperature decreases (see below). The apparent difference in selection between sites $A$ and $B$ could be explained by recruitment of larger fry to site A. Or it might reflect temporal or spatial differences in the size or type of predators.

Predation experiments were replicated using fish from two populations, from ecologically dissimilar lakes with a wide geographic separation. Fish from the two populations differed considerably in morphology (e.g., in lateral plate number). Yet, results were similar between populations, in that fry with high ratios of abdominal to caudal vertebrae were favoured at small sizes, and those with low ratios at larger sizes. However, vertebral count ratios differed markedly between populations, and
different actual ratios were favoured at the same fry size in the two populations. Perhaps the optimum vertebral count ratio depends not just on fry length, but also on other dimensions such as width or depth (Lindsey 1975; Lindsey and Lavin 1986) which may have varied between the two populations.

In these experiments, the character of functional importance was apparently not total vertebral number, but rather the ratio of abdominal to caudal vertebrae. However, the two characters were related. Fry with low total numbers tended to have high ratios. Accordingly, selection for the ratio resulted in selection for total number, both in laboratory experiments (K1, K2, H2, H3) and in the wild. Fry with low numbers (31) were favoured at small sizes, those with high numbers (32) at slightly larger sizes.

Experiments with Mylocheilus

Results using Mylocheilus resembled those using Gasterosteus. Selection acting on wild fry appeared to favour low vertebral numbers (44) at small lengths (9.3-9.9 mm), high vertebral numbers (45) at slightly greater lengths (9.9-10.6 mm), and neither number at even greater lengths (10.6-14.0 mm). As with Gasterosteus, selection appeared to occur at slightly larger sizes in predation experiments than in the wild. Again, lengths reported for predation experiments were those at the end of experiments, so this discrepancy could be due to growth during experiments, or to the concentration of mortality at the smaller sizes in experiments. Of course, differences between results in
the laboratory and in the wild could also be due to physiological, morphological or behavioural differences between laboratory-reared and wild fry, in the case of Mylocheilus. However, this possible explanation does not hold for Gasterosteus, since results were similar between wild fry and laboratory-reared fry in swimming performance experiments.

Evidence from predation experiments was less conclusive using Mylocheilus than using Gasterosteus. Most of the experiments using Mylocheilus were apparently conducted at least partly at sizes below those to which vertebral numbers were adapted, or at sizes where selection is absent because vertebrae are undeveloped. (Note that vertebral development listed in Table 25 is among fry at the end of experiments; development may have been less advanced at the start of experiments). In all experiments but 5, sampling error may have been increased relative to that in experiments with Gasterosteus, due to subsampling for fry to be reared to a larger size. The necessity of rearing fry after all but experiment 5 also probably introduced error to the partitioning of results by fry size. Finally, as in Gasterosteus, the character of functional importance in Mylocheilus may have actually been the ratio of abdominal to caudal vertebrae, even though this ratio could not be practically distinguished. Variation in this ratio among laboratory-reared fry with the same total number of vertebrae may have reduced selection for total number in the laboratory.

Both in laboratory experiments and in the wild, selection for fry with low vertebral numbers (44) first occurred at just those sizes when vertebrae were first developed. This coincidence suggests that the structures directly involved in this selection may be the vertebral centra themselves, rather than some related feature of the axial segmentation such as myomeres or nerves. Also because of this coincidence, it is not possible to determine from these results the lower limit of the size range over which fry with 44 vertebrae are favoured. If vertebrae were developed at smaller sizes, as may occur at higher temperatures, selection might favour fry with 44 vertebrae at yet smaller sizes than those seen here, or it might favour fry with yet lower vertebral numbers at these smaller sizes.

Effect of vertebral number on burst swimming performance

Burst swimming was initiated in this study using an electric shock stimulus. While this is not a natural stimulus, responses to electric shocks are apparently comparable to those to more typical stimuli (cf. Webb and Corolla 1981). For example, mean burst speeds of northern anchovy larvae, Engraulis mordax, are similar whether stimulated by an electric shock (Webb and Corolla 1981) or by pursuit by a predator (Webb 1981).

Maximum velocities obtained in this study were about 38 and $54 \mathrm{BL} / \mathrm{s}$ (body lengths/sec) at 15 and 25 C , respectively (for fry with a mean length of 8.5 mm ). These values are comparable to those obtained by Fuiman (1986) for zebra danio Danio rerio larvae (50-65 BL/s at. $21-30 \mathrm{C}$ ), but higher than those obtained by

Webb and Corolla (1981) for anchovy larvae (about $25 \mathrm{BL} / \mathrm{s}$ at 17C) and by Ryland (1963) for plaice Pleuronectes platessa larvae (11-16 $\mathrm{BL} / \mathrm{s}$ at 6.5C). (Values were obtained by Ryland (1963) using a stopwatch and measurement grid, and so were probably underestimates (Fuiman 1986)).

The $Q(10)$ for maximum velocity in this study was 1.4 between 15 and 25C. Fuiman (1986) obtained the same value for maximum velocity of zebra danio larvae between 21 and 30C. Webb (1978) obtained a value of 1.7 for subadult rainbow trout Salmo gairdneri between 5 and 15C, but found that maximum velocity was independent of temperature in this species at higher temperatures (15-25C).

Spouge and Larkin (1979) developed a model relating thrust to vertebral number based on hydrodynamic considerations. Their model indicated an effect of the absolute number of locomotor vertebrae on performance, rather than of the ratio of abdominal to caudal vertebrae as demonstrated here. A relationship between optimum vertebral number and length was predicted by their model, but this relationship conformed to the observed increase in vertebral number with length (Lindsey 1975) only at lengths over about 10 cm . Thus, pleomerism is explained by their model only if selection is concentrated at large lengths near the maximum recorded for a population. If selection for vertebral number is most intense at larval and fry lengths (and these are positively correlated with adult length), their model predicts a decrease in vertebral number with maximum length, a relationship opposite to the observed trend. Spouge and Larkin's model also contained
factors dependent on temperature (viscosity and power per unit of muscle volume), but tended to indicate that vertebral number would decrease in colder waters. This is in disagreement with Jordan's rule, though Spouge and Larkin suggested that this rule might still be explained by systematic variation with temperature of other variables in their model (hydrodynamic efficiency, body proportions or vertebral morphology).

Predictions of the Spouge and Larkin model thus conform neither to the effects of vertebral number on performance observed in this study, nor to the trends between vertebral number and length (pleomerism) or water temperature (Jordan's rule) observed among fish populations (if selection is assumed to be concentrated at small sizes). The model was derived using Lighthill's (1970) 'elongated-body' theory for locomotion with undulations of small amplitude. Since amplitude is not small during burst swimming, the use of Lighthill's (1971) large-amplitude theory might have been more appropriate. Lighthill (1970) assumed that the wave of bending passed along a swimming fish's body was a sine-wave of constant amplitude and velocity. This assumption is certainly not valid over a large change in position along a fish's body (e.g., Batty 1981), but may be reasonable when the change in position is small. In deriving some equations in their model, Spouge and Larkin assumed the length of the caudalmost vertebra to be infinitesimally small, an assumption of questionable validity. A critical assumption made by Spouge and Larkin was that the lateral velocity of the caudalmost vertebra relative to the preceding one
depends only upon the rate of contraction of the preceding myotome. This assumption ignores the effects of bending moments transmitted from more anterior segments and of water resistance acting on a body of variable flexibility. It is based on the view that the waves of bending seen on a swimming fish result from coincident waves of contraction, a view recently discredited by Blight (1976, 1977).

The idea that the waves of bending seen passing down the body of a swimming fish are caused by coincident waves of muscular contraction was suggested in early studies of fish locomotion (Breder 1926; Gray 1933a), and has persisted to the present (e.g., Wardle and Videler 1980). However, Blight (1977) questioned this view, suggesting that waves of bending will be generated even when all muscle segments contract simultaneously on a side, so long as the fish body is of variable flexibility, relatively stiff at the anterior end and flexible at the caudal end. The importance of this gradient in flexibility in generating waves of bending was first demonstrated by Gray (1933b), who showed that these waves were transmitted along the body of a swimming whiting Gadus merlangus only when the caudal fin was intact. Electromyograms of swimming activity in amphibians and fish also support Blight's alternate view. All myotomes on a side are seen to contract simultaneously during burst swimming, both in larvae of the newt Triturus helveticus (Blight 1976) and in adult carp Cyprinus carpio (Kashin et al. 1979). During slower steady swimming, a longitudinal delay in myotomal contraction is seen, but the waves of muscular
activation do not coincide with the waves of bending (Blight 1976; Kashin et al. 1979). For example, in steady swimming of the adult tench Tinca tinca, the onset of muscular activity is delayed and the bursts of activity become shorter, toward the caudal end of the animal (Blight 1976). This results in a period of simultaneous activity of most of the myotomes on a side, coinciding with the 'S' position of the body (when such simultaneous activity is least expected according to conventional theory). Blight ( 1976,1977 ) suggests that the function of this longitudinal delay in muscle activity is not the propagation of waves of bending, but rather the reduction of lateral oscillation of the head and the adjustment of tail flexibility for wave conduction at different frequencies. Finally, Blight's theory is also supported by kinematic analysis of fast swimming in the saithe Pollachius virens (Hess and Videler 1984). This analysis indicates that the bending moment does not travel as a running wave from head to tail as the lateral body curvature does, but rather behaves as a standing wave. That is, left and right sides produce alternate contractions simultaneously over the whole body length.

The importance of a gradient in flexibility in generating the running waves of bending characteristic of fish locomotion suggests an explanation for the effect of vertebral count ratio on swimming performance. Much of the difference in flexibility between abdominal and caudal regions of a fish results from differences in girth. However, flexibility should also be influenced by the number of segments (i.e., hinges) per unit
length, especially in larval fish whose abdominal flexibility is not so restricted by wide girth as is that of adults. Fish with a high ratio of abdominal to caudal vertebrae will have a relatively low gradient in flexibility between abdominal and caudal regions; those with a low ratio, a relatively high gradient. Thus, selection for the ratio of abdominal to caudal vertebrae may reflect selection for some optimum gradient in flexibility between abdominal and caudal regions. The selection for body proportions seen in predation experiments using Holden Lake sticklebacks is also consistent with this suggestion. Selection favoured a high ratio of abdominal to caudal vertebrae at small sizes, and a low ratio at large sizes. Conversely, selection favoured a low ratio of precaudal to caudal length at small sizes, and a high ratio at large sizes. Thus, selection for both characters favoured a low gradient in flexibility at small sizes, and a high gradient at large sizes.

Changes in the optimum gradient in flexibility with length might result from kinematic changes with length. Webb (1976) observed that fast-start kinematic pattern was size dependent in rainbow trout 10 to 40 cm in length. Webb et al. (1984) found that during steady swimming the wavelength and amplitude of body waves were relatively smaller in larger rainbow trout between lengths of 5 and 56 cm . However, these relationships, observed in relatively large fish, may not extend to fry sizes. Batty (1981) found that wavelength and amplitude were relatively similar in 7 and 10 mm plaice larvae at cruising speed. But Batty (1981) did find that maximum tail beat frequency decreased
greatly as size increased, a change that might favour relatively stiffer caudal regions at smaller sizes (Blight 1977). Hunter (1972) reported relatively larger tail beat amplitudes in larger anchovy larvae, and Batty (1984) observed a change in swimming style between 11 and 22 mm herring larvae. The amplitude of lateral oscillation and maximum angle of the body to the direction of motion increased linearly towards the tail in 11 mm larvae, and more rapidly than linearly towards the tail in 22 mm larvae. These latter changes with length might favour more flexible caudal regions at larger sizes.

Batty (1984) suggested that the changes in swimming style seen as herring larvae grow may reflect an increase in Reynolds number ( $R$ ) during growth. At low $R(<20)$, resistive (viscous) forces are of predominant importance in generating thrust, while at high $R$ ( $>200$ ), reactive (inertial) forces dominate contributions to thrust (Webb and Weihs 1986). Batty (1984) proposed that the swimming style of small larvae, in which amplitude is large over most of the body, relies mainly on resistive forces for propulsion, while that of large larvae, in which amplitude increases rapidly towards the tail, relies mainly on reactive forces for propulsion. While these arguments may apply to the routine activities of fish larvae, they are not likely to be relevent to burst swimming activities, since even the smallest larvae can accelerate to speeds high enough to generate values of $R \gg 200$ (Webb and Weihs 1986). The rapid changes in morphology seen during larval development (e.g., Webb and Weihs 1986) may be more important causes of the concommitant
changes in swimming style and optimum vertebral count ratio with length during these stages.

The optimum vertebral count ratio at a given size appeared to depend on water temperature, at least at small sizes. This dependency might be explained by changes in water viscosity (as suggested by the experiments with methylcellulose). As water temperature increases, viscosity decreases. The resultant decrease in water resistance might favour a relatively greater gradient in flexibility at a given size in order to maintain the same kinematic form at higher temperature. Just such a result was seen in these experiments at smaller sizes (compare results in the $7.4-7.8 \mathrm{~mm}$ length class at 15 and 25C). Alternatively, the arguments of the previous paragraph notwithstanding, the effect of temperature might reflect an effect on Reynolds number. As water temperature increases, viscosity decreases and velocity at a given size increases. Both changes increase Reynolds number. If the change in optimum vertebral count ratio between small and large fry lengths results from an increase in Reynolds number and a shift in the relative importance of resistive and reactive forces in propulsion, this shift would occur at a smaller size at higher temperature. However, as noted above, the high Reynolds number associated with burst speeds of even the smallest larvae argues against this alternative explanation.

Vertebral count ratio appeared to affect performance only over a narrow range of fry lengths. Perhaps the changes in anteroposterior flexibility associated with changes in vertebral count ratio are important only over a narrow range of Reynolds
number. Or, the ratios optimal at smaller or larger sizes may have been absent from the populations studied (because selection is never concentrated at these more extreme sizes in these populations). Alternatively, factors other than vertebral count ratio (e.g., longitudinal variation in girth) may be of predominant importance in determining longitudinal gradients in flexibility at the more extreme (especially larger) sizes.

The results obtained in this study indicate that the ratio of abdominal to caudal vertebrae is of fundamental importance in determining swimming performance over a narrow range of fry sizes. At these sizes, swimming occurs at Reynolds numbers near, though somewhat higher than, those associated with a transition between the predominance of resistive or reactive forces in propulsion. The explanations suggested above for this effect of vertebral count ratio on performance, and for the dependency of the optimum ratio on fry length and water temperature, are obviously highly speculative. Kinematic analyses at burst speeds of fry of various lengths and vertebral numbers are needed before more definitive explanations can be suggested.

Genetic control of the ratio of abdominal to caudal vertebrae

The vertebral character of selective importance appears to be not the absolute number of vertebrae, but rather the ratio of abdominal to caudal vertebrae. Two simple models can be suggested for the genetic control of this character. The number of abdominal and the number of caudal vertebrae may each be
controlled by separate genes. Assuming no pleiotropy or linkage disequilibrium, the expected frequency of any vertebral count class in a randomly mating population is then simply the product of the frequencies of the corresponding numbers of abdominal and caudal vertebrae:

$$
p(a+c)=p(a) p(c)(\operatorname{model} 1) .
$$

Alternatively, the total number of vertebrae and the ratio of abdominal to caudal vertebrae may be determined by separate genes. Then, the expected frequency of a given count class (ratio) is the product of the frequency of the corresponding total number and the frequency of the count class given the total number:

$$
p(a+c)=p(t) p(a+c \mid t) \text { where } t=a+c(\text { model } 2) .
$$

The two models are fitted to the counts in control groups of the Holden Lake predation experiments in Table 39. In model 2, the ratio of abdominal to caudal vertebrae is assumed to be a threshold character. Liability is assumed to be normally distributed with the same mean and standard deviation in all total vertebral count classes. Thresholds of liability are assumed to be the mid-points between the observed ratios of abdominal to caudal vertebrae. For example, among fish with 31 total vertebrae, three classes are seen: 15/16=0.9375 AV/CV, $14 / 17=0.8235 \mathrm{AV} / \mathrm{CV}$, and $13 / 18=0.7222 \mathrm{AV} / \mathrm{CV}$. Thresholds between these classes are assumed to be 0.8805 and 0.7729. Assumed thresholds thus differ among total vertebral count classes. Model 2 is fitted only to total count classes with three or more

Table 39. Fits of two models for the genetic control of the ratio of abdominal to caudal vertebrae to counts observed in control groups of Holden Lake predation experiments. Contributions to chi-square are shown including only classes to which both models are fitted. All chi-square probabilities are less than 0.05 .


Abbreviations: M1=Model 1, M2=Model 2, TV=Total vertebrae.
Abd/Caud=abdominal to caudal ratio in continuous units of liability, estimated separately for each TV.

[^1]ratios $A V / C V$. Weighted averages of the means and standard deviations in liability calculated for each total count class are used in model 2.

Predictions of both models depart significantly from the observed count frequencies in all control groups. In all cases, predictions of model 1 depart much more widely from the observed counts than do those of model 2. Most departures of model 1 from observation are explained by an observed excess of fish with 31 vertebrae and deficit of those with more extreme counts. These departures cannot be explained by any simple hypothesis involving pleiotropy or linkage disequilibrium. On the other hand, most departures of model 2 from observation can be so explained. These departures indicate a negative correlation between total vertebral number and mean abdominal/caudal ratio (in continuous units of liability) (Table 39). Among fish with 31 or 32 vertebrae, this correlation results in an increase in the frequencies of abdominal /caudal ratios of $14 / 17$ and/or $14 / 18$, compared to those expected if the two traits were independent. These ratios are the two that are sometimes selected for in the wild in this population (Part III). Thus, selection would be expected to favour the observed correlation between total vertebral number and abdominal /caudal ratio. Since the fish used in this analysis were reared in a common laboratory environment, this correlation presumably reflects a genetic correlation (i.e., pleiotropy or linkage disequilibrium) rather than an environmental one.

Variation also appears to occur among parental groups in which count class (14/17 or $14 / 18$ ) is more frequent than expected if total vertebral number and abdominal /caudal ratio were independent. This variation may represent seasonal variation (A parents were collected July 24 , $B$ parents August 4-5, and $C$ parents August 10). Seasonal variation could reflect genetic variation among parental groups, or an effect of seasonal variation in the prefertilization environment or age of parents (Swain and Lindsey $1986 \mathrm{a}, \mathrm{b}$ ). Since there may also be seasonal variation in which count is most favoured by selection, this variation could be adaptive.

Most work on genetic correlations among traits has been directed towards an examination of correlated responses to selection (Falconer 1981; Atchley et al. 1982). However, genetic correlations may themselves be responses to selection. Cheverud (1982) suggested that developmentally and functionally related traits should be relatively highly integrated with respect to genetic factors, but failed to find such an association among macaque cranial traits. He suggested that this failure might reflect the evolution of genetic correlations among developmentally and functionally independent traits through stochastic processes. Although the results reported here are only preliminary, they are consistent with a genetic integration of functionally related vertebral traits in response to selection. A quantitative genetic analysis is needed to estimate the genetic correlation between these traits, and to look for an environmental correlation between them. Further work is also
needed to examine the existence, basis and possible adaptive significance of any seasonal variation in the correlation between these traits.

Variation among populations

1. Pleomerism

Results described here suggest an explanation for pleomerism. In both G. aculeatus and M. caurinus, selection appeared to favour fry with a low vertebral count at small sizes, and those with a higher vertebral count at slightly larger sizes. In fishes, large females tend to produce large eggs (Fowler 1972; Gall 1974; Kazakov 1981), and large eggs tend to produce large fry, both at hatching and at complete yolk absorption (Fowler 1972; Gall 1974; Wallace and Aasjord 1984; Beacham et al. 1985). Thus, offspring of large females might face less exposure to selection at sizes when low counts are favoured than would those of small females (Fig. 23). If so, higher counts would tend to be favoured in offspring of large females, lower counts in those of small females.

This explanation requires overlap between the sizes at which selection operates on vertebral number and those at which offspring of large and small females are first potentially exposed to selection (referred to here as 'recruited'). For example, it cannot explain pleomerism if all offspring of both large and small females are recruited at sizes below those at which selection favours lower vertebral numbers. However, the required overlap appears to occur in both the species studied

Figure 23. A model explaining pleomerism. Recruitment size is the size when fry are first potentially exposed to selective predation, i.e. when fry are free-swimming and vertebrae are developed. $V_{N}$ and $V_{N+1}$ are fry with $N$ or $N+1$ vertebrae.

here. The smallest modes in size distributions of stickleback fry collected from Holden Lake overlapped with the sizes at which selection favoured low vertebral numbers (31) in these fry. Similarly, selection appeared to favour a low vertebral count (44) in peamouth chub fry at just those sizes when vertebrae were first developed in these fry.

Selection operating on the fry might result in pleomerism even if offspring of both large and small females are recruited at sizes below those at which selection favours low vertebral numbers. Large eggs may produce more rapidly growing fry than small eggs (Wallace and Aasjord 1984). If differences in length-specific growth between fry produced from small or large eggs decrease as length increases, higher vertebral numbers will tend to be favoured in fry produced from large eggs than in those produced from small eggs.

If pleomerism does result from selection operating on the fry soon after hatching, the evidence in $G$. aculeatus is that it is related not to selection operating directly on the total number of vertebrae, but rather to selection operating on the ratio of abdominal to caudal vertebrae. In $G$. aculeatus, the optimum ratio decreased as fry length increased. If this result is a general one, pleomerism is expected only when the ratio and the total number of vertebrae are inversely correlated. Such a correlation was seen in both the stickleback populations studied here, but few other data are available to test its generality. An inverse correlation between the ratio and the total number will occur if most variation in the total number is produced by
variation in the caudal number. This pattern of variation has been observed in the paradise fish Macropodus opercularis (Lindsey 1954), in the medaka Oryzias latipes (Ali and Lindsey 1974), and in Rivulus marmoratus (Swain 1979). In the population of G. aculeatus studied by Lindsey (1962), most environmentally induced variation occurred in the abdominal region (i.e., anterior to the anal spine), while inherited variation appeared most markedly in the caudal region. Thus, the pattern of variation expected to produce pleomerism is in fact seen in most of the few cases examined. If pleomerism does result from selection for the ratio of abdominal to caudal vertebrae, the correlation with maximum adult size should be stronger for this ratio than for the total number, and the degree to which a group exhibits pleomerism should depend partly on the strength of the inverse correlation between the ratio and the total number. Perhaps some groups that fail to exhibit pleomerism do so because the total number does not show a strong inverse correlation with the ratio $A V / C V$ in these groups.

Lindsey (1975) noted that in a few instances pleomerism occurs between the sexes. He cited three examples. In blennies of the genus Ecsenius, males reach larger lengths and have more vertebrae than do females. In the shark Etmopterus spinax, females are larger and have more vertebrae than males. Finally, males of the capelin Mallotus villosus tend to be larger than females; males tend to have more vertebrae than females in British Columbia and Murmansk, but fewer than females in Newfoundland. I can offer two explanations for differences in
vertebral number between the sexes. First, selection for greater egg-carrying capacity might favour a high proportion of abdominal to caudal vertebrae in females. In the stickleback populations studied here, such selection would favour a lower vertebral count in females (since the proportion and the total number are inversely correlated). Second, in some fishes, sex determination is temperature-dependent (Harrington 1968,1971; Conover 1984; Sullivan and Schultz 1986). When spawning is over a protracted period of rising or falling temperatures, this temperature dependency may produce a difference both in size and in vertebral number between the sexes. For example, in the Atlantic silverside Menidia menidia, a higher proportion of females are produced among larvae incubated at lower temperatures; females tend to be larger than males because most are produced earlier in the spawning season and thus experience a longer growing season (Conover 1984). Since the number of vertebrae produced in embryos is also affected by incubation temperature, differences in vertebral number will also be expected between the sexes. If low incubation temperature tends to produce high vertebral counts in embryos, the larger sex will tend to have the higher mean vertebral number. This tendency might be reinforced by seasonal variation in selection for vertebral number (see below).

In summary, selection operating on larvae or fry soon after hatching may provide a sufficient explanation for most instances of pleomerism. Additional factors, such as temperature-dependent sex determination or selection for a high proportion of abdominal to caudal vertebrae in females, may be required to explain
instances of pleomerism between the sexes. Selection may also operate on vertebral number at adult sizes, but such selection need not be hypothesized to explain pleomerism. In fact, the failure of some groups to exhibit pleomerism might reflect a poor correlation between larval and maximum adult sizes.

## 2. Jordan's rule

A functional explanation may also be sought for Jordan's rule. Body size tends to be larger in colder waters, both within species (Ray 1960) and within families (Lindsey 1966) of poikilotherm vertebrates. Since higher mean vertebral numbers are associated with greater maximum body lengths (pleomerism), latitudinal clines in vertebral number might be purely a reflection of these latitudinal clines in body size. However, Lindsey (1975) showed that Jordan's rule cannot be attributed solely to latitudinal clines in maximum body length. The most striking demonstration of this was provided by the wrasse family Labridae. These fishes showed a marked latitudinal cline in vertebral number, but no latitudinal cline in maximum body length.

However, Jordan's rule might be attributable to latitudinal clines in egg and larval sizes, rather than to those in maximum adult sizes. Like adult sizes, egg and larval sizes tend to be larger in colder waters (e.g., Marshall 1953). These clines are presumably at least partly genetic, but may also be partly attributable to a direct modification of egg and larval sizes by water temperatures during development. For example, in the
pupfish Cyprinodon n. nevadensis, larger eggs are produced by females held in colder water (Shrode and Gerking 1977). Similarly, colder incubation temperatures produce larger larvae at hatching in a variety of species (Salmo trutta, Gray 1929; Gadus macrocephalus, Alderdice and Forrester 1971; Clupea pallasi, Alderdice and Velsen 1971; Belone belone, Fonds et al. 1974; Salmo salar, Hamor and Garside 1977, Peterson et al. 1977; Oncorhynchus tshawytscha, Heming 1982; but see Beacham and Murray (1986) for an exception to this trend). Thus, higher vertebral numbers may be favoured by selection in colder waters because the larger larvae produced in these waters face less exposure to selection at sizes when low vertebral numbers are favoured, than do the smaller larvae produced in warmer waters. Again this explanation requires an overlap between the sizes at which selection operates on vertebral number and those at which larvae produced in warm or cold waters are recruited to the population potentially exposed to selection. Examination of latitudinal variation in larval sizes in the wrasses would provide a test of this hypothesis.

Jordan's rule might also be partly attributable to latitudinal clines in the body size of predators. The extent to which predation is selective depends both on prey and predator sizes. Predators that are very large compared to prey will be relatively unselective with respect to the escape performance of prey. Thus, the prey size upon which selection operates most stringently may be larger in colder waters, due to the larger size of predators in these waters.

Water temperature also influences a variety of other factors which may affect the extent or direction of selection for vertebral number. For example, both growth rates and predation rates are expected to increase with increasing water temperature. Changes in these rates, if uniform over all sizes, will not affect the net direction of selection for vertebral number, but they will influence the extent of this selection. Acceleration of growth or predation rates would be expected to reduce or increase selection for vertebral number, respectively. Water temperature or viscosity also apparently affects the size range over which a particular vertebral number is favoured by selection. As temperature increases (or viscosity decreases), particular counts appear to be favoured at slightly smaller sizes (at least for high and intermediate vertebral count ratios). The effect of this shift on the extent and net direction of selection depends on: (1) whether the shift is greater for higher ratios, as is suggested in the results reported here; (2) how overall mortality rates and the proportion of mortality that is potentially selective vary with size and temperature; and, (3) recruitment size, and how much it decreases as temperature increases. Depending on how these factors interact, a decrease in the sizes at which particular counts are favoured due to an increase in temperature (or decrease in viscosity) could favour either higher or lower vertebral numbers, or whichever number was otherwise at net advantage.

In summary, latitudinal clines in vertebral number might be attributable to latitudinal clines in egg and larval sizes, and in the size distributions of predators. Water temperature and viscosity also affect a variety of other factors that may influence selection for vertebral number, but the net effect of these various factors is uncertain.

Variation within populations

Populations of gasterosteid fishes are often conspicuously polymorphic for a variety of morphological characters in addition to vertebral number (e.g., numbers of lateral plates or dorsal spines, presence or absence of pelvic spines). The factors important in maintaining variation in vertebral number within populations differ from those suggested for these other polymorphisms in some respects. Particular vertebral numbers appear to be optimum at particular fry sizes because of a functional advantage involving swimming performance. In contrast, no obvious functional advantage is associated with variation in such characters as the number of lateral plates in G. aculeatus (Moodie 1972; Hagen and Gilbertson 1973) or the number of dorsal spines in Apeltes quadracus (Blouw and Hagen 1984a,b). Selection is often presumed to act indirectly on these other characters, via selection on genetically correlated behavioural traits (Moodie et al. 1973; Kynard 1979; Huntingford 1981; Blouw and Hagen 1984a,b). Secondly, in these other polymorphisms, each phenotype is generally supposed to be most fit in a different circumstance, or selection in favour of one phenotype is supposed to be opposed by indirect selection in
favour of the other. For example, the presence of pelvic spines may be of advantage in avoiding vertebrate predation, while their absence may be advantageous in avoiding invertebrate predation (Reimchen 1980; Reist 1980,a,b). Or, the morphological advantage of pelvic spines in avoiding vertebrate predation may be opposed by a behavioural advantage correlated with the absence of spines (Reist 1980a). In the case of vertebral number, opposing selective forces result, not from extrinsic differences in the circumstances of selection (e.g., type of predator) or from a balance between direct and indirect selection, but rather from an intrinsic effect of fry size on the optimum number.

These opposing selection pressures cannot of themselves maintain a stable polymorphism, except in the unlikely event that selection at one size is exactly balanced by selection at another. More probably, selection will operate most stringently at one size, and overall fitness will be greatest for the vertebral number that is optimal at that size. Even so, variation might still be maintained by spatial, seasonal or annual variation in the size on which selection operates most stringently. Such variation could result from variation in the size distribution of predators, or from variation in water temperature (which will influence hatching size, the size at which a particular number is optimal, growth rates and predation rates). That such spatial or seasonal variation in selection coefficients occurs is suggested by the apparent differences in selection for vertebral number of stickleback fry between sites in Holden Lake. Selection in favour of fry with high counts of

32 was most significant among fry collected early in the season from site $A$, while selection in favour of those with low counts of 31 was most significant among those collected later in the season from site B. Conditions under which such spatial or temporal heterogeneity in selection coefficients can maintain genetic variation in populations have received much theoretical consideration (e.g., Levene 1953; Dempster 1955; Haldane and Jayaker 1963; Hedrick et al. 1976; Ewing 1979).

About $14 \%$ of fry collected from Holden Lake had only 30 vertebrae. About a third of these had an intermediate ratio of abdominal to caudal vertebrae (0.76), and are expected to be favoured by selection at about the same 'large' sizes as are most fry with 32 vertebrae. However, the majority of fry with 30 vertebrae had an exceptionally high ratio of abdominal to caudal vertebrae (0.88). An advantage to this high ratio might be expected at very small sizes (smaller than those when a ratio of 0.82 (31 total vertebrae) was optimal), but such an advantage was not seen in the admittedly sparse data at these small sizes. Perhaps development of these small fry was insufficient for a potential advantage to be expressed. On the other hand, selection may favour fry with this high ratio, not because of greater survival at some size, but rather because of greater fecundity due to a high abdominal to caudal ratio in body proportions. Thus, additional variation in vertebral number may be maintained in populations because of spatial and temporal variation in the net effect of differential survival and fecundity among the various phenotypes.

A single genotype developing in a single external environment may produce two or more different vertebral numbers in embryos. This is clearly demonstrated within clones of the self-fertilizing cyprinodont fish Rivulus marmoratus (Lindsey and Harrington 1972; Harrington and Crossman 1976; Swain and Lindsey 1986a,b), and presumably also occurs in outbreeding species. This variation must result from random 'accidents' of development or developmental noise (Waddington 1957).

Genotypes whose phenotypic expression is least affected by such developmental noise (i.e., those with greatest developmental stability) are often considered to be the most fit. This is the rationale behind the many recent studies of effects of levels of heterozygosity or genomic coadaptation on developmental stability (e.g., Angus and Schultz 1983; Leary et al. 1984, 1985a,b; Graham and Felley 1985). However, when there is environmental heterogeneity in selection coefficients, the opposite may be true: genotypes producing two or more phenotypes in a given environment may be more fit than those producing a single phenotype.

Consider the following simple (haploid) model. Genotypes A, $B$ and $C$ produce phenotypes $P$ and $Q$ in proportions (1,0), (p, 1-p) and ( 0,1 ), respectively. Genotypes $A$ and $C$ represent 'pure' strategies, each producing a single phenotype, while B represents a 'mixed' strategy. Environments 1 and 2 occur with frequencies $v$ and $1-v$. In environment 1 , the fitnesses of phenotypes $P$ and $Q$
are $F$ and 1, respectively; in environment 2, they are 1 and $G$, respectively ( $F>1, G>1$ ). The respective fitnesses of genotypes $A, B$ and $C$ are $F, p F+1-p$, and 1 in environment 1 , and $1, p+G-p G$, and $G$ in environment 2. If environmental heterogeneity is temporal, the most fit genotype is the one with greatest geometric mean fitness (Haldane and Jayaker 1963, Gillespie 1977). Geometric mean fitnesses of the three genotypes are:

A: $F^{\vee}$
B: $\quad(\mathrm{pF}+1-\mathrm{p})^{V}(\mathrm{p}+\mathrm{G}-\mathrm{pG})^{1-V}$
C: $G^{1-V}$

The conditions under which each of the genotypes is the most fit are shown in fig. 24, for various levels of $v$ and $p$. The mixed strategist tends to be the most fit when differences in fitness between the phenotypes are great in both environments, especially when environmental unpredictability is high (i.e., v is near 0.5). Levins (1962) reached a similar conclusion with respect to the fitness of populations (as opposed to the fitness of genotypes considered here), using a qualitative geometric analysis.

Random variation may also occur between clutches. Kaplan and Cooper (1982) have shown that in this case also the mixed strategist is most fit (in a haploid model) when fitness differs greatly between phenotypes in a given environment and environmental unpredictability is high. (They considered only this one case, analogous to the case $F=G=6, v=0.5$ in the model

Figure 24. Conditions under which mixed or pure strategies are optimal when there is temporal variation in selection. The mixed strategist is the most fit under conditions between the curves for a given $p$, the pure strategist $A$ below the lower curve, and the pure strategist $C$ left of the upper curve. Details in text.



described here). There is no evidence for this sort of random variation in the phenotypic expression of vertebral number, though data to test for its existence are sparse (but see Ali and Lindsey 1974, p.961).

In a population of interbreeding individuals, the relevant question is which strategies will be maintained in the population by selection. The conditions under which a strategy will be maintained in an interbreeding population are likely to be less stringent than those under which it is optimal in a haploid one, but it is beyond the scope of this discussion to calculate these conditions. The important point here is that under some conditions of environmental heterogeneity, genotypes with lower developmental stability will be favoured by selection over those with higher stability. Thus, instances of relatively low developmental stability may not indicate developmental constraints on adaptation, but rather represent an optimal adaptation to environmental unpredictability. Spatial and temporal heterogeneity in selection coefficients for vertebral number has been suggested above. The developmental noise observed in the expression of vertebral number may be an adaptation to this heterogeneity.

Finally, distinction should be made with respect to level of phenotypic expression. Vertebral number is usually thought of as a threshold trait. Such traits have an underlying continuity with thresholds that impose discontinuities on their visible expression. The underlying continuous variable, termed the liability, is both genetic and environmental in origin, and could
in principle be studied as a metric character (Falconer 1981). In the case of vertebral number, liability could refer to rates of embryonic growth and differentiation, or numbers of cells available for incorporation into somites (Lindsey and Arnason 1981). Developmental noise at the level of observed vertebral number is relevant to the discussion above, while noise at the level of underlying liability is the more relevant to questions of effects of heterozygosity or genomic coadaptation on developmental stability. Differences in noise at the level of observed vertebral number do not necessarily indicate differences in the noisiness of the underlying liability; instead they may simply reflect shifts in the mean liability between thresholds of liability (i.e., shifts in the mean vertebral number between whole values) (Swain 1987).

Phenotypic plasticity

Phenotypic plasticity may be defined as the extent to which the phenotype produced by a genotype depends on the developmental environment. Phenotypic plasticity may be favoured by natural selection when (1) the relative fitness of phenotypes depends strongly on environmental conditions, (2) the environment experienced by individuals cannot be chosen, and (3) the developmental environment is correlated with those environmental factors that determine which phenotype is the most fit (Levins 1963, 1968). The possible adaptive significance of phenotypic plasticity has long been emphasized in plants (Bradshaw 1965), and is now receiving considerable attention in animals. Recent examples include studies of phenotypic plasticity of life history
traits in the snail Lymnaea elodes (Brown 1985), temperature-dependent sex determination in the fish Menidia menidia (Conover 1984), and the effect of temperature on egg size in the fishes Cyprinodon n. nevadensis (Shrode and Gerking 1977) and Etheostoma spectabile (Marsh 1984).

The number of vertebrae produced in fish embryos depends on water temperature. Patterns of response of vertebral number to developmental temperature are usually either declivous or U-shaped (Fowler 1970). This plasticity may be adaptive, since the relative fitness of different vertebral numbers also depends on water temperatures during embryo development and the early free-swimming larval stages. Females held in relatively cold water may produce relatively large eggs (Shrode and Gerking 1977), and embryos developing in relatively cold water usually produce relatively large larvae at hatching (see references above). As a result of these effects, selection should tend to favour genotypes that produce a declivous response of vertebral number to developmental temperature. However, water temperature (or viscosity) also apparently affects the size range over which a particular vertebral number is optimal. This latter effect could favour either higher or lower vertebral numbers at colder temperatures, depending on a variety of factors (see above). The pattern of response of vertebral number to developmental temperature actually favoured by selection would depend on these factors and on the relative magnitude of this latter effect and the effect of water temperature on hatching size. The ubiquity of declivous and $U$-shaped responses suggests that selection
normally favours one of these two patterns.

The variable pattern of response to incubation temperature within populations (declivous or U-shaped) is in contrast to the typically inverse relation between vertebral number and water temperature among populations (Jordan's rule). Perhaps differences among populations in hatching size due to a combination of genetic and environmental influences typically overshadow differences in the size ranges when particular vertebral numbers are optimum due to environmental influences alone. Alternatively, U-shaped patterns of response within populations may be artifacts of experimental conditions. Parental holding temperature before fertilization affects the number of vertebrae produced in offspring (Dentry and Lindsey 1978; Swain and Lindsey 1986a). When parents are held at one temperature and their offspring reared at a variety of temperatures, an apparent $U$-shaped response to rearing temperature might be produced even though a declivous response would result if parents were held at the rearing temperature of their offspring (C.C. Lindsey, pers. comm.).

Just as Jordan's rule has an analogue in the effect of developmental temperature on vertebral number, pleomerism may have an analogue in an effect of parental size or age on the number of vertebrae produced in offspring. In R. marmoratus, offspring produced long after the onset of breeding tend to have more vertebrae than do those produced soon after the onset of breeding (Swain and Lindsey 1986b). Similarly, in a population of $G$. aculeatus, large females tend to produce offspring with
more vertebrae than do small females (unpublished data). This effect is presumably non-genetic, since large females do not themselves appear to have more vertebrae in this population. Large females often tend to produce large eggs in fishes (see above), but relationships between female age or size and offspring vertebral number are not likely to be proximate effects of differences in egg size (Lindsey and Ali 1971). Instead, they probably result from biochemical differences between the eggs of small or large (or young or old) females. However, they may be ultimate effects of differences in egg size. Since large females tend to produce large eggs and thus large offspring, higher vertebral counts should be favoured by selection in offspring of large (or old) females. Thus, the observed effect of female age or size on offspring vertebral number may be an adaptation to differing selection pressures operating on offspring of parents of different ages or sizes.

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[^0]:    Note: Regressions are calculated with length in ocular micrometer units: 1 unit $=0.082083 \mathrm{~mm}$.
    $S E=$ standard error.

[^1]:    $+N<50$

