

## Comparative morphology of threespine *Gasterosteus aculeatus* and ninespine *Pungitius pungitius* sticklebacks in lowland streams of southeastern England

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**Abstract.** We examined the relative growth of threespine sticklebacks collected from 11 lowland streams in southeastern England during late autumn to test for between-catchment variations as well as development-related shifts in morphology. And, to understand better the sympatric relationship between threespine *Gasterosteus aculeatus* and ninespine *Pungitius pungitius* sticklebacks in small lowland streams, we compared growth variability in 35 mensural characters and morphological indices related to functional morphology in adults from one of these sites. Threespine demonstrated great plasticity, with some morphological differences observed between stream catchments, mainly in ventral spine and dorsal spine length, pre-anus and preanal distances, fin depth, and ventral body width (represented in the basipterygium medial plate width). Developmentally, three groups of mensural characters were found: 18 characters best explained by a linear regression (isometric growth), 9 characters best explained by a quadratic equation (gradual allometry), and 8 characters best explained by a split-linear regression (mainly isometric growth, with an abrupt shift in proportional growth – between 40 and 58 mm SL). However, these shifts did not appear associated with common ecological, physiological and/or behavioural functions. Compared with ninespine stickleback, threespine has a more robust body with stronger and more prominent spines, shorter tail, and eyes situated more backwards. Functional morphological indices suggest threespine to be a slower but more manoeuvrable swimmer than ninespine, which acquires a relatively more cruiser-form shape with reduced drag and more lift.

**Key words:** fish ecomorphology, growth variability, mensural characters, principal components analysis, split-linear regression, functional morphology indices, resources use

### Introduction

Threespine *Gasterosteus aculeatus* and ninespine *Pungitius pungitius* sticklebacks are sympatric in lowland streams, fresh, brackish and sea waters (W o o t t o n 1976), though ninespine is said to be more associated with aquatic vegetation (W h e e l e r 1969), in particular *Myriophyllum* sp., *Elodea* sp. and mats of filamentous algae (C o p p 1992). Small lowland streams of eastern England are of particular interest as their upper reaches are generally inhabited by one or two fish species only (C o p p et al. 1998), often the threespine and sometimes the ninespine as well, representing a so-called 'stickleback zone'

(Maitland 1994). These streams have become partially intermittent, i.e. part of the stream (usually the lower reaches) run dry for some or much of the year as a result of nearby groundwater extraction. This intermittence results in the isolation of upstream fish populations, which can be accentuated by the presence of retention structures such as natural and human-made weirs. Thus, the potential for migration of these stickleback populations between streams is limited if not non-existent, and the potential for between-stream variations in morphological adaptation enhanced.

Threespine stickleback have often been used as a model for studies of behaviour, physiology, reproduction, morphology and evolution (Wootton 1976, 1984, McPhail 1984, Lavin & McPhail 1985, 1986, 1993, Campbell 1991, Hart & Ison 1991, Hart & Gill 1992, Bell & Foster 1994), but ninespine have received relatively little attention (e.g. Hynes 1950, Nelson 1968, Craig & Fitzgerald 1982). The study of external morphology (body shape and body proportions) is a useful tool for both comparative (Norton et al. 1995) and developmental investigations (e.g. Kováč et al. 1999). Within threespine stickleback populations, studies of morphological variation have been common (Banbura et al. 1989, Bowne 1994, Reimchen 1994), in particular with respect to the phenomenon of the 'limnetic' morph found in certain lakes of coastal western North America of the threespine (McPhail 1984, Lavin & McPhail 1985, 1986, Schluter 1993). Morphologically, genetically and trophically distinct from the 'original' benthic threespine, the limnetic form remains categorised as a species pair rather than a new species, and some evidence of digression has been observed (Kraak et al. 2001). Adapted to life in the open waters of these lakes, the limnetic morph has larger eyes, longer gill rakers, a smaller mouth and a lower body depth than the benthic form. Stream populations of sticklebacks, whilst still essentially benthic, are confronted with elevated water velocities and thus should demonstrate some morphological adaptation to these lotic conditions, which can vary considerably between stream catchments. Thus, one of the aims of the present investigation was to compare the morphology of threespine sticklebacks in different stream catchments so as to determine whether any local adaptation exists and, if so, how this develops during the threespine's ontogeny.

Comparative studies of threespine and ninespine sticklebacks are rare (Lewis et al. 1972, Delbeek & Williams 1987, Copp et al. 1998), and an ecomorphological perspective could aid in explaining their life histories (Motta et al. 1995). Morphological differences tend to be great in sympatric forms (Schluter 1993), therefore the second aim of this study was to compare the morphology of threespine and ninespine sticklebacks to understand better the basis of their sympatry in small streams; this includes attributes related to swimming or manoeuvrability, which may help to explain interspecific differences in habitat use (Webb & Weis 1986).

## **Study Area, Material and Methods**

For the between-catchment comparison of threespine morphology, specimens were collected in November 1992 at one location in each of eleven streams: Essendon Brook (National Grid Reference: TL 271 074), River Purwell (NGR: TL 197 303), River Hiz (NGR: TL 190 302), Ashwell Spring (NGR: TL 398 270), River Chess (NGR: TL 006 964), River Beane (NGR: TL 278 207), River Rib (NGR: TL 386 199), River Colne (NGR: TL 200 059), River Gade (NGR: TL 053 074), Hadley Brook (NGR: TL 273 974) and St-Ippollitts Brook (NGR: TL

194 281). These streams feed two different river catchments. The rivers Chess, Gade and Colne feed the River Thames west of London, whereas Essendon Brook, River Beane, River Rib, and Hadley Brook are tributaries of the River Lee, which joins the Thames east of London. St-Ippollitts Brook and the River Purwell are tributaries of the River Hiz, which leads into the River Great Ouse catchment via the River Ivel, whereas Ashwell Spring is a source stream of the River Cam, which joins the Great Ouse north of Cambridge (for a map of the Great Ouse basin, see Copp 1992). These streams can have an intermittent character (e.g. Essendon and Hadley brooks) or their continuity is interrupted by man-made barriers such as weirs, so movement between them is incidental at best. Threespine sticklebacks were the most abundant species at all the study sites, with bullhead *Cottus gobio* the only other species observed in high abundance at some sites. Other species encountered in decreasing order of frequency and abundance were: stone loach *Barbatula barbatula*, minnow *Phoxinus phoxinus*, brown trout *Salmo trutta*, chub *Leuciscus cephalus*, pike *Esox lucius*, and gudgeon *Gobio gobio*.

For the interspecific comparison of three and ninespine sticklebacks, adult specimens were collected between November 1993 and March 1994 from St-Ippollitts Brook, as described above (see also Copp et al. 1998). Water depth at the St-Ippollitts site varied, but was not observed to exceed 0.5 m during 2 years of field sampling on the brook. The shape of the stream bed was natural, riffles, runs and pools, but had steep riparian banks leading up from the water's edge on either side. The stream bottom was composed mainly of gravel, pebbles and sand, with small areas of silt deposits found among the beds of *Callitriche* sp., *Apium* sp., and *Ranunculus* sp.

All fish were collected by electrofishing (DC at 120 pulses per sec., 500 V, 3 Amp) using a portable apparatus (Deka 3000) modified by Copp (1989), immediately killed with an overdose of benzocaine, then preserved in 4 % formaldehyde. In the laboratory, 37 mensural characters (see list in Table 1) including total length (TL) and Standard length (SL) were measured as described by Penzance (1962) using vernier callipers to the nearest 0.05 mm on 65 specimens each of threespine and ninespine from St-Ippollitts Brook for the interspecific comparison, and on 250 specimens of threespine stickleback for the between-catchment comparisons. The dorsal (D), anal (A), pectoral (P), ventral (V) and caudal (C) fins are denoted by their respective abbreviations. Deviations in sample size from 250 in the between-catchment comparison were due to missing data for one or more characters. To allow inter-population comparisons with past and future studies, all mensural characters except SL and TL were expressed in percent SL and evaluated subsequently using variation analysis (Snedecor 1946). Between-stream comparisons of each character were undertaken using the coefficient of difference ( $C_{dif}$ ) to test for morphological differences in 0+ juveniles from the eight regions:

$$C_{dif} = \frac{x_2 - x_1}{s_1 + s_2}$$

where  $x_2$  and  $x_1$  are the arithmetic means, and  $s_1$  and  $s_2$  are the standard deviations of the mensural characters at the sites compared. Real differences in a mensural character between 90 % of the population from two given sites exists when  $C_{dif} > 1.28$  (Mayr et al. 1953).

The patterns of relative growth were determined using regression analyses of the raw data for mensural characters (SL and TL excepted) and plotted against SL. From

a developmental point of view, there are three possible patterns in the development of mensural fish characters: 1) growth proportional to that of standard length (SL) throughout the ontogeny (isometric growth); 2) growth proportionally faster or slower than SL (positive or negative allometric growth); and 3) growth proportional (isometric) up to a certain SL followed by a shift to a different proportional rate (either higher or lower). Thus, isometric growth is linear, whereas gradual allometry can be best explained by a quadratic regression, either concave upwards (character grows proportionally faster than SL) or concave downwards (character grows proportionally slower than SL). Shifts in isometric growth, which separate two intervals of isometric growth, are revealed by a split, or estimated break point, between two linear regressions (e.g. N i c k e r s o n et al. 1989).

For each relationship between a character and SL of threespine stickleback, we tested for linearity (isometry), gradual allometry and split linearity as described in K o v á ě et al. (1999). Our null hypothesis was that growth in threespine stickleback was isometric, and therefore best described by a simple linear regression. The first alternative hypothesis was that growth in threespine stickleback was gradual allometric, and best described by a quadratic equation. The second alternative hypothesis was that growth in threespine stickleback occurred in two different isometric intervals and was best described by a split linear regression. We fitted simple linear, quadratic and split linear regression models to plots of the dependent variables against SL, and tested the quadratic and split linear models for significant improvements in fit over the simple linear model using F-tests (S o k a l & R o h l f 1981, K o v á ě et al. 1999). The second alternative hypothesis was only accepted if the split-linear fit was significantly better than both the simple linear and quadratic fits.

Before the data from 11 stream populations were combined together, normality of each sample and each character was evaluated using the Kolmogorov-Smirnov test of goodness of fit (B i c k e l & D o k s u m 2001). Since the populations studied were isolated from each other, and their normality was not rejected in 384 from 385 cases tested, they represented mathematically independent entities, and thus, it was possible to combine the data for the overall growth variability analysis (B i c k e l & D o k s u m 2001).

To assess integrated, multi-character transformations in body morphology, we natural-log transformed the data to reduce the effect of size, retaining the data of specimens for which all 35 characters were available (244 specimens for the between-basin comparison, 130 specimens for the interspecific comparison), and subjected the reduced data sets to double-centred principal components analysis (PCA) as per S a g n e s et al. (1997) using the ADE software package (C h e s s e l & T h i o u l o u s e 1998). E l l i p s e s (90%) were generated for specimens from each stream in the between-catchment comparison of threespine sticklebacks to reveal patterns related to stream origin, and for each species in the interspecific comparison of three and ninespine sticklebacks.

## **Results**

### **Development of external morphology in threespine**

The SL of the 250 threespine stickleback specimens from all eleven streams ranged from 26.1 to 72.9 mm, and TL ranged from 29.85 to 80.25 mm (Table 1). Of the characters examined in threespine, 18 demonstrated isometric growth, i.e. they were best described by simple linear regressions; nine characters were allometric, i.e. they were best described by

**Table 1.** Mean, standard deviation, standard error, minimum, maximum, coefficient of variance (C.var) and number of specimens for mensural characters of threespine stickleback from eleven streams in Hertfordshire (England).

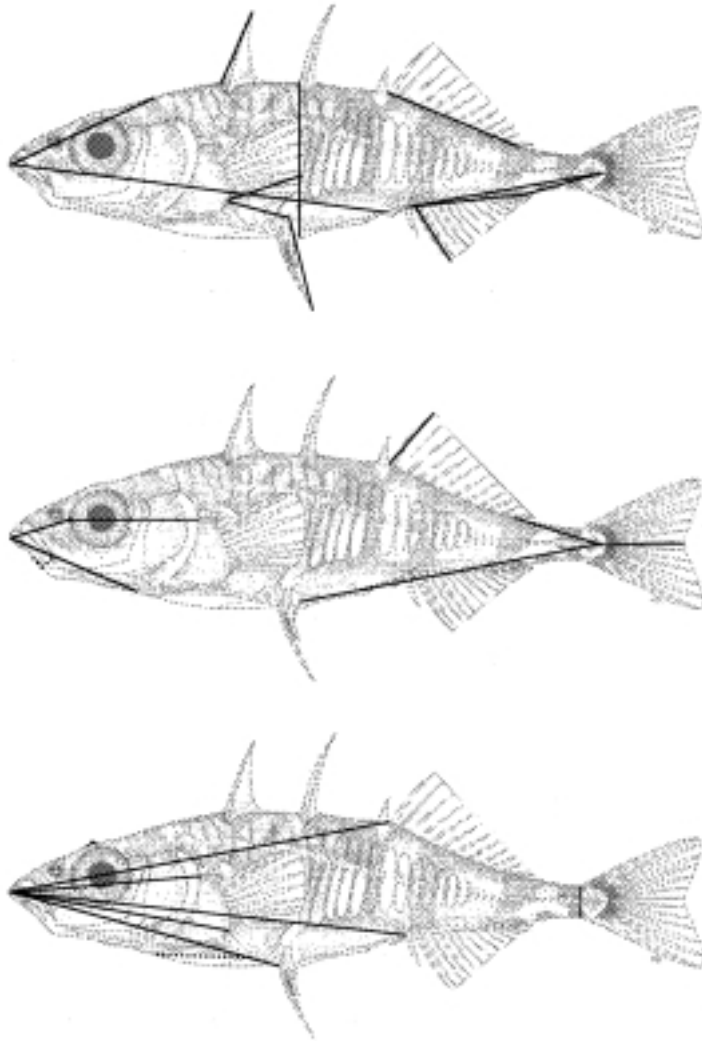
Character	mean	SD	SE	min.	max.	C.var.	n
Total length (mm)	50.5	10.14	3.21	29.9	80.3	20.09	250
Standard length (mm)	44.9	9.17	3.05	26.1	72.9	20.42	250
in % of Standard length							
1 dorsal head length	26.1	0.90	0.95	23.7	27.9	3.44	250
2 lateral head length	31.0	1.36	1.17	28.1	33.7	4.38	250
3 ventral head length	19.4	1.77	1.34	16.1	23.2	9.09	250
4 interorbital distance	6.7	0.49	0.71	5.6	8.0	7.28	250
5 preorbital distance	10.3	0.83	0.92	8.4	12.2	8.11	250
6 eye diameter	8.0	0.55	0.75	6.9	9.3	6.88	250
7 postorbital distance	14.2	0.62	0.79	12.6	15.3	4.37	250
8 predorsal distance	61.2	1.51	1.24	57.4	65.6	2.47	250
9 preanal distance	69.8	3.04	1.76	62.0	76.8	4.36	250
10 pre-anus distance	67.5	3.01	1.75	59.7	74.2	4.46	249
11 preventral distance	46.1	1.73	1.33	41.2	50.2	3.76	250
12 prepectoral distance	37.6	1.40	1.19	33.8	42.2	3.73	250
13 caudal fin length	13.1	1.14	1.08	10.6	16.0	8.70	250
14 dorsal spine length	7.4	1.16	1.08	5.3	10.8	15.62	250
15 dorsal fin length	25.2	1.33	1.16	21.3	28.6	5.28	250
16 dorsal fin depth	12.0	1.33	1.16	9.8	15.5	11.05	250
17 postdorsal distance	15.6	1.15	1.08	13.4	18.5	7.38	250
18 postanal distance	14.9	1.65	1.29	12.2	19.5	11.08	250
19 anal fin length	18.1	1.63	1.29	14.3	21.6	9.04	248
20 post-anus distance	35.0	2.34	1.54	30.4	40.4	6.69	250
21 ventral spine length	11.8	1.30	1.15	9.5	15.7	11.01	250
22 pectoral fin length	15.4	1.04	1.03	13.1	18.1	6.76	250
23 P - V distance	10.0	1.48	1.23	6.3	13.6	14.78	250
24 body depth	26.3	1.63	1.29	22.3	30.5	6.19	250
25 minimum body depth	4.6	0.35	0.59	3.9	5.5	7.52	250
26 anal fin depth	11.6	1.26	1.13	9.1	14.6	10.85	250
27 postventral distance	56.7	2.09	1.46	51.5	62.6	3.69	250
28 internasal distance	4.4	0.39	0.63	3.4	5.2	8.93	249
29 head width	13.4	1.16	1.09	10.9	16.4	8.71	250
30 mouth width	7.7	0.58	0.77	6.0	9.0	7.50	249
31 ectocoracoid length	17.8	1.20	1.11	15.2	20.4	6.74	249
32 basipterygium length	18.7	1.26	1.13	16.2	21.9	6.75	250
33 basipterygium - anus dist.	8.1	2.19	1.49	3.4	12.4	26.90	250
34 basipt. medial plate width	6.2	1.21	1.11	3.4	8.6	19.55	250
35 basipterygium width	12.5	1.05	1.03	9.9	14.5	8.40	250

quadratic regressions, and the remaining eight characters (ventral head length, interocular distance, predorsal distance, preanal distance, preventral distance, prepectoral distance, minimum body depth and ectocoracoid length) were best described by split linear regressions, being isometric with an abrupt change in their growth rate relative to SL (F-tests, Table 2, Fig. 1).

The eight mensural characters with abrupt isometric growth can be divided into two groups, based on non-overlapping standard error bars (Fig. 2). Group 1 consisted of three characters (interorbital distance, minimum body depth and ectocoracoid length) in which all the breakpoints fell within the range between 37–45 mm of SL; group 2 was composed of five characters (ventral head length, predorsal distance, preanal distance, preventral distance and prepectoral distance) with breakpoints in the range from 47 to 60 mm of SL (Fig. 2).

**Table 2.** Statistics for linear (L), quadratic (Q) and split linear (S) regressions for mensural characters of threespine stickleback from streams in Hertfordshire, England, with probabilities, standard error and number. NS = not significant.

Character	r <sup>2</sup> linear	r <sup>2</sup> quadratic	F-test Q/L	P	r <sup>2</sup> split lin.	F-test S/Q	P	F-test S/L	P	best model	break- point	SE	n
1 dorsal head length	0.9702	0.9703	1.09	NS	0.9707	2.88	NS	1.99	NS	L	-	-	250
2 lateral head length	0.9523	0.9544	11.45	<0.01	0.9555	6.19	<0.05	8.94	<0.01	S	57.8	2.68	250
3 ventral head length	0.8875	0.8922	10.67	<0.01	0.893	1.89	NS	6.30	<0.05	Q	-	-	250
4 interorbital distance	0.8906	0.9024	29.66	<0.01	0.9051	6.88	<0.01	18.62	<0.01	S	43.6	1.49	250
5 preorbital distance	0.9354	0.9366	4.57	<0.05	0.9374	3.31	NS	3.96	<0.05	Q	-	-	250
6 eye diameter	0.8778	0.9581	471.43	<0.01	0.8877	-153.54	NS	10.86	<0.01	Q	-	-	250
7 postorbital distance	0.9527	0.9883	750.55	<0.01	0.9583	-176.35	NS	16.55	<0.01	Q	-	-	250
8 predorsal distance	0.9877	0.9797	-97.25	NS	0.9886	190.81	<0.01	9.27	<0.01	S	50.1	2.10	250
9 preanal distance	0.9795	0.9765	-31.30	NS	0.9804	49.20	<0.01	5.89	<0.05	S	49.9	2.63	250
10 pre-anus distance	0.9764	0.8145	-214.76	NS	0.9771	1737.97	<0.01	3.43	NS	L	-	-	249
11 pre-ventral distance	0.9643	0.9656	9.13	<0.01	0.9664	5.75	<0.05	7.53	<0.01	S	54.1	2.48	250
12 prepectoral distance	0.9623	0.9644	15.11	<0.01	0.9661	12.11	<0.01	13.95	<0.01	S	57.7	2.02	250
13 caudal fin length	0.8079	0.8145	8.79	<0.01	0.8160	1.93	NS	5.37	<0.05	Q	-	-	250
14 dorsal spine length	0.5111	0.5114	0.16	NS	0.5169	2.81	NS	1.49	NS	L	-	-	250
15 dorsal fin length	0.9486	0.9492	3.10	NS	0.9497	2.38	NS	2.75	NS	L	-	-	250
16 dorsal fin depth	0.7344	0.7406	5.86	<0.05	0.7440	3.25	NS	4.58	<0.05	Q	-	-	250
17 postdorsal distance	0.9152	0.9180	8.28	<0.01	0.9179	-0.16	NS	4.04	<0.05	Q	-	-	250
18 postanal distance	0.7804	0.7819	1.63	NS	0.7835	1.85	NS	1.74	NS	L	-	-	250
19 anal fin length	0.8073	0.8082	1.15	NS	0.8074	-1.03	NS	0.06	NS	L	-	-	248
20 post-anus distance	0.8998	0.9002	0.84	NS	0.9017	3.76	NS	2.31	NS	L	-	-	250
21 ventral spine length	0.8695	0.8695	0.02	NS	0.8698	0.56	NS	0.29	NS	L	-	-	250
22 pectoral fin length	0.8914	0.8915	0.37	NS	0.8925	2.19	NS	1.28	NS	L	-	-	250
23 P - V distance	0.8294	0.8301	0.93	NS	0.8294	-0.89	NS	0.02	NS	L	-	-	250
24 body depth	0.9212	0.9213	0.30	NS	0.9213	3.96	<0.05	0.18	<0.05	L	40.3	3.16	250
25 minimum body depth	0.8661	0.8692	5.96	<0.05	0.8713	3.96	<0.05	5.00	<0.05	S	-	-	250
26 anal fin depth	0.7573	0.7577	0.46	NS	0.7608	3.13	NS	1.80	NS	L	-	-	250
27 postventral distance	0.9706	0.9712	4.76	<0.05	0.9711	-0.85	NS	1.94	NS	Q	-	-	250
28 internasal distance	0.8206	0.8206	0	NS	0.8211	0.70	NS	0.35	NS	L	-	-	249
29 head width	0.8704	0.8705	0.18	NS	0.8709	0.76	NS	0.47	NS	L	-	-	250
30 mouth width	0.9284	0.9314	10.83	<0.01	0.9319	2.00	NS	6.44	<0.05	Q	-	-	249
31 ectocoracoid length	0.9010	0.9043	8.54	<0.01	0.9085	11.16	<0.01	10.03	<0.01	S	42.5	2.12	249
32 basipterygium length	0.8962	0.8962	0.15	NS	0.8970	1.93	NS	1.04	NS	L	-	-	250
33 basipterygium - anus distance	0.7466	0.7468	0.15	NS	0.7473	0.57	NS	0.36	NS	L	-	-	250
34 basipterygium medial plate width	0.9414	0.9415	0.69	NS	0.9415	-0.31	NS	0.19	NS	L	-	-	250
35 basipterygium width	0.9566	0.9567	0.51	NS	0.9571	2.51	NS	1.51	NS	L	-	-	250

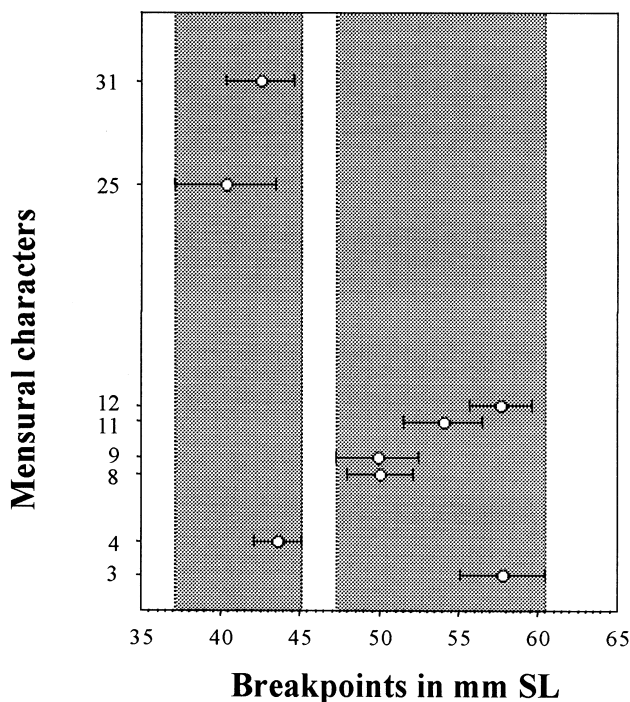


**Fig. 1.** Illustration of the three groups of mensural characters in threespine stickleback from eleven streams in Hertfordshire (England) demonstrating the three types of growth: a) isometric, b) positive or negative allometric, and c) split-isometric (i.e. growth up to a certain SL followed by a shift to a different proportional rate).

### Between-catchment comparisons of threespine

Most of the 35 characters expressed in % SL showed limited variation, the most variable characters being basipterygium-anus distance and basipterygium medial plate width (Table 1). This variability, however, was enhanced by different morphology of the specimens from Colne and Hadley Brook compared to specimens from most of the other sites (Tables 3 and 4, Fig. 3).

In accounting for size in the analyses, it cannot be ignored that Colne and Hadley specimens were significantly smaller (Scheffe F-test at 95% for both) than the other populations (Table 5; ANOVA,  $F = 51.332$ ,  $df = 233$ ,  $P < 0.0002$ ). However, significant differences were also found in those characters, which are characterised by linear relative



**Fig. 2.** Estimates of the breakpoints and their standard errors (see Table 2) for the morphometric characters demonstrating significant abrupt changes in slope when plotted against SL in threespine sticklebacks from eleven streams in Hertfordshire (England). Two groups of characters are identified based on non-overlapping standard error intervals. The sequence of variables is the same as in Table 2.

growth, and thus are not affected by size (Table 2). These differences were mainly associated with spine length (dorsal, ventral), A-fin depth, pre-anus, postanal and basipterygium-anus distances, and ventral body width (basipterygium width and basipterygium medial plate width, Tables 3 and 4). The PCA of composite threespine morphology (Fig. 3) corroborates the differences in morphology of the specimens from the River Colne and Hadley Brook, as compared to all other sites (Tables 3 and 4), their ellipses having limited overlap with (i.e. morphological similarity to) other stream populations except the River Gade, which is within the Colne basin.

#### Comparison of threespine and ninespine stickleback morphology

The 65 specimens of threespine stickleback (Ga) examined here (low and partially-plated forms representing 81.46 and 19.64 %, respectively) were significantly longer (Student's  $t = 10.933$  and  $9.989$ , respectively; both:  $P = 0.0001$ ) than the ninespine (Pu): Ga mean TL = 64.6 mm, SE = 0.086,  $n = 65$ , Pu mean TL = 51.9 mm, SE = 0.078,  $n = 65$ ; Ga mean SL = 57.0 mm, SE = 0.077,  $n = 65$ ; Pu mean SL = 46.4 mm, SE = 0.074,  $n = 65$ . The relationships between TL and SL were: threespine,  $TL = 1.109SL + 0.139$  ( $F = 4724.843$ ,  $df = 64$ ,  $P = 0.0001$ ,  $r^2 = 0.987$ ); ninespine,  $TL = 1.05SL + 0.321$  ( $F = 4755.121$ ,  $df = 64$ ,  $P = 0.0001$ ,  $r^2 =$



**Table 3.** A comparison of mensural characters of threespine stickleback from the River Colne with those from streams in Hertfordshire (England), using the Coefficient of Difference. Values underlined and in bold indicate statistically valid differences ( $P = 0.05$ ) between the specimens from that site and other sites, characters underlined and in bold are characterised with linear relative growth, and are not affected by size of specimens (SL).

No	Character/sites compared	Hiz	St-Ipps	Bean	Chess	Ashwell	Rib	Purwell	Essendon
1	dorsal head length	0.52	0.39	0.34	0.12	0.51	0.35	0.36	0.25
2	lateral head length	1.15	0.85	0.65	0.18	0.60	0.55	0.76	0.32
3	ventral head length	0.93	1.14	<b><u>1.39</u></b>	1.25	<b><u>1.70</u></b>	1.22	0.71	0.77
4	interorbital distance	<b><u>2.05</u></b>	<b><u>1.63</u></b>	<b><u>1.60</u></b>	0.66	0.97	0.87	<b><u>1.39</u></b>	0.65
5	preorbital distance	1.16	1.42	1.01	0.87	0.38	0.36	0.84	0.75
6	eye diameter	0.09	0.13	0.44	0.40	0.13	0.22	0.44	0.03
7	postorbital distance	0.87	1.00	0.96	0.04	0.34	0.43	0.57	0.39
8	predorsal distance	0.80	0.80	0.48	0.80	0.47	0.65	1.03	0.45
9	preanal distance	<b><u>1.86</u></b>	<b><u>1.30</u></b>	<b><u>1.46</u></b>	<b><u>1.35</u></b>	1.01	0.65	<b><u>1.42</u></b>	0.83
10	<b><u>pre-anus distance</u></b>	<b><u>2.07</u></b>	<b><u>1.36</u></b>	<b><u>1.49</u></b>	<b><u>1.49</u></b>	0.91	0.91	<b><u>1.79</u></b>	0.69
11	preventral distance	0.18	0.37	0.69	0.87	0.06	0.35	0.59	0.40
12	prepectoral distance	0.96	0.52	0.36	0.08	0.60	0.37	0.56	0.43
13	caudal fin length	0.46	0.65	0.53	0.25	0.59	0.66	0.02	0.07
14	<b><u>dorsal spine length</u></b>	<b><u>2.39</u></b>	<b><u>1.71</u></b>	<b><u>2.02</u></b>	<b><u>1.40</u></b>	<b><u>1.36</u></b>	<b><u>1.61</u></b>	<b><u>1.36</u></b>	1.23
15	dorsal fin length	0.54	0.13	0.06	0.13	0.14	0.00	0.13	0.01
16	dorsal fin depth	<b><u>1.50</u></b>	1.11	<b><u>1.35</u></b>	<b><u>1.48</u></b>	0.78	0.99	0.92	0.58
17	postdorsal distance	1.26	<b><u>1.30</u></b>	1.13	1.24	0.84	1.10	1.04	1.14
18	<b><u>postanal distance</u></b>	<b><u>1.51</u></b>	<b><u>1.46</u></b>	1.17	1.50	1.02	1.04	<b><u>1.36</u></b>	1.14
19	anal fin length	0.80	0.43	0.39	0.06	0.43	0.44	0.52	0.21
20	post-anus distance	1.22	1.05	0.78	0.94	1.21	1.09	1.37	0.74
21	<b><u>ventral spine length</u></b>	<b><u>2.21</u></b>	<b><u>2.15</u></b>	<b><u>2.00</u></b>	<b><u>1.49</u></b>	<b><u>1.68</u></b>	<b><u>1.68</u></b>	<b><u>1.47</u></b>	<b><u>1.85</u></b>
22	pectoral fin length	0.03	0.04	0.28	0.11	0.26	0.28	0.31	0.34
23	P - V distance	1.08	0.87	0.92	1.23	0.82	1.20	0.49	0.31
24	body depth	0.26	0.16	0.41	0.54	0.37	0.13	0.32	0.05
25	minimum body depth	0.66	0.01	0.21	0.01	0.30	0.22	0.08	0.12
26	<b><u>anal fin depth</u></b>	<b><u>1.94</u></b>	<b><u>2.07</u></b>	<b><u>1.98</u></b>	1.19	<b><u>1.48</u></b>	<b><u>1.47</u></b>	<b><u>1.47</u></b>	1.01
27	postventral distance	0.74	0.27	0.34	0.08	0.29	0.23	0.04	0.27
28	internasal distance	1.09	0.47	0.39	0.37	0.89	0.65	0.20	0.25
29	head width	0.73	1.00	1.00	<b><u>1.43</u></b>	0.43	0.69	0.39	0.04
30	mouth width	0.77	1.12	1.07	0.95	0.28	0.36	0.34	0.61
31	ectocoracoid length	0.25	0.29	0.21	1.06	1.27	0.30	0.27	0.79
32	basipterygium length	0.04	0.36	0.07	0.93	0.25	0.31	0.26	0.73
33	<b><u>basipteryg. - anus distance</u></b>	<b><u>2.14</u></b>	1.27	<b><u>1.55</u></b>	<b><u>1.29</u></b>	<b><u>1.34</u></b>	0.96	1.01	0.57
34	<b><u>basipter. med. plate width</u></b>	<b><u>2.64</u></b>	<b><u>1.98</u></b>	<b><u>2.06</u></b>	<b><u>2.15</u></b>	<b><u>1.78</u></b>	<b><u>1.51</u></b>	<b><u>1.78</u></b>	<b><u>1.65</u></b>
35	<b><u>basipterygium width</u></b>	<b><u>1.50</u></b>	<b><u>1.33</u></b>	<b><u>1.58</u></b>	1.23	0.76	0.97	0.87	0.70

0.987). No sexual dimorphism was found in ninespine, and in threespine only basipterygium length rendered significantly different slopes (no overlap of 95% confidence limits).

In terms of composite morphology, the two species are clearly distinguishable (Fig. 4a), mainly by a greater anal-fin length and post-anus distance in ninespine and greater dorsal and ventral spine lengths in threespine (Fig. 4b). In functional terms, the ninespine has higher body shape factor scores, which indicates it is less manoeuvrable than the threespine (Webb & Weihs 1986), though the relative position of maximum width suggests the opposite (Fig. 5). From the caudal peduncle depth factor, the two species appear to begin with similar abilities to reduce drag and generate lift, but the ninespine's values decrease with increased size, suggesting that it acquires a more cruiser-form shape, reducing drag and generating more lift at the expense of manoeuvrability (Fig. 5). The relative position of the shoulder is closer to the head in ninespine.

**Table 4.** A comparison of mensural characters of threespine stickleback from Hadley Brook with those from streams in Hertfordshire, using the Coefficient of Difference. Values underlined and in bold indicate statistically valid differences ( $P = 0.05$ ) between the specimens from that site and other sites, characters underlined and in bold are characterised with linear relative growth, and are not affected by specimen size (SL).

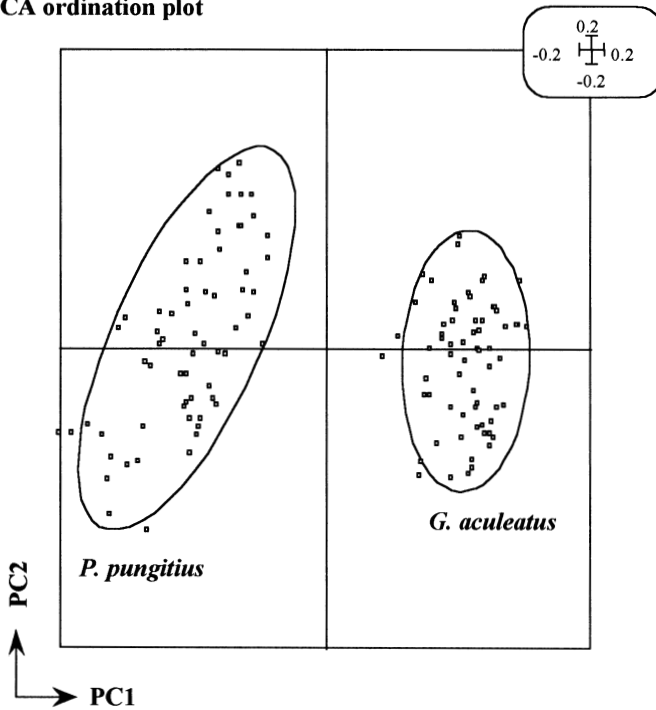
No	Character/sites compared	Hiz	St-Ipps	Bean	Chess	Ashwell	Rib	Purwell
1	dorsal head length	0.45	0.32	0.27	0.04	0.45	0.28	0.29
2	lateral head length	0.88	0.61	0.44	0.01	0.42	0.35	0.49
3	ventral head length	0.58	0.85	1.10	0.96	<b><u>1.34</u></b>	0.94	0.40
4	interorbital distance	<b><u>1.45</u></b>	1.12	1.09	0.23	0.57	0.48	0.87
5	preorbital distance	1.17	<b><u>1.48</u></b>	1.00	0.83	0.26	0.22	0.79
6	eye diameter	0.46	0.74	1.03	0.21	0.76	0.41	0.20
7	postorbital distance	0.68	0.79	0.74	0.25	0.18	0.25	0.38
8	predorsal distance	0.77	0.77	0.44	0.77	0.42	0.61	1.01
9	preanal distance	<b><u>1.76</u></b>	1.22	<b><u>1.39</u></b>	1.27	0.94	0.60	<b><u>1.35</u></b>
<b>10</b>	<b><u>pre-anus distance</u></b>	<b><u>1.87</u></b>	1.22	<b><u>1.35</u></b>	<b><u>1.33</u></b>	0.82	0.81	<b><u>1.63</u></b>
11	preventral distance	0.37	0.19	0.51	0.66	0.24	0.14	0.38
12	prepectoral distance	1.06	0.59	0.44	0.17	0.70	0.45	0.65
13	caudal fin length	0.29	0.44	0.35	0.07	0.41	0.46	0.17
<b>14</b>	<b><u>dorsal spine length</u></b>	<b><u>1.70</u></b>	1.26	<b><u>1.48</u></b>	1.03	1.02	1.14	0.97
15	dorsal fin length	0.83	0.47	0.24	0.13	0.45	0.35	0.20
16	dorsal fin depth	0.89	0.61	0.80	0.90	0.40	0.52	0.43
17	postdorsal distance	1.19	1.23	1.08	1.17	0.82	1.05	1.00
<b>18</b>	<b><u>postanal distance</u></b>	<b><u>1.49</u></b>	<b><u>1.45</u></b>	1.17	<b><u>1.48</u></b>	1.03	1.05	<b><u>1.35</u></b>
19	anal fin length	0.56	0.18	0.16	0.18	0.24	0.21	0.25
20	post-anus distance	1.05	0.87	0.58	0.76	1.02	0.88	1.20
<b>21</b>	<b><u>ventral spine length</u></b>	<b><u>1.76</u></b>	<b><u>1.70</u></b>	<b><u>1.57</u></b>	1.12	1.26	1.27	1.10
22	pectoral fin length	0.43	0.32	0.63	0.44	0.60	0.63	0.14
23	P - V distance	1.26	1.04	1.08	1.40	0.99	<b><u>1.36</u></b>	0.74
24	body depth	0.24	0.14	0.38	0.49	0.35	0.11	0.29
25	minimum body depth	0.65	0.07	0.25	0.07	0.33	0.27	0.01
<b>26</b>	<b><u>anal fin depth</u></b>	<b><u>1.30</u></b>	<b><u>1.31</u></b>	<b><u>1.29</u></b>	0.72	0.93	0.88	0.81
27	postventral distance	0.63	0.15	0.23	0.08	0.18	0.09	0.10
28	internasal distance	0.67	0.21	0.14	0.15	0.60	0.39	0.07
29	head width	0.13	0.42	0.52	0.79	0.78	0.12	0.10
30	mouth width	0.57	0.84	0.82	0.74	0.07	0.08	0.11
31	ectocoracoid length	0.12	0.07	0.16	0.51	0.68	0.06	0.10
32	basipterygium length	0.39	0.03	0.33	0.36	0.16	0.14	0.63
<b>33</b>	<b><u>basipteryg. - anus distance</u></b>	<b><u>1.57</u></b>	0.85	1.11	0.90	0.97	0.61	0.63
<b>34</b>	<b><u>basipter. med. plate width</u></b>	<b><u>1.90</u></b>	<b><u>1.46</u></b>	<b><u>1.51</u></b>	<b><u>1.50</u></b>	1.25	1.01	1.28
<b>35</b>	<b><u>basipterygium width</u></b>	<b><u>1.42</u></b>	1.27	<b><u>1.48</u></b>	1.18	0.77	0.95	0.87

## Discussion

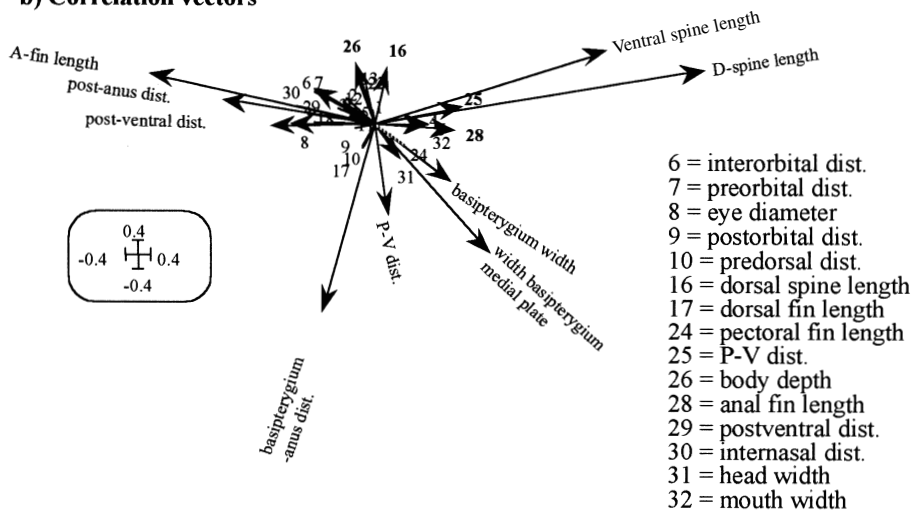
### Development of external morphology in threespine

Eight mensural characters demonstrated significant breaks in relative growth in the specimens from 26.1 to 72.9 mm SL (Fig. 1c). However, not all of the breakpoints in these characters appeared at the same SL, and three of them (interocular distance, ventral head length, preanal distance) were amongst the characters showing difference between sites (Fig. 3, Tables 3 and 4). The range of SL at which the slopes changed significantly was quite wide (37 mm to 60 mm), and the characters in which these changes occurred did not seem to be associated with some common ecological, physiological and/or behavioural functions (see also Kováč et al. 1999). Perhaps the only exception could be the relatively sudden increase in the rate of growth of the anterior part of stickleback's body, as indicated by the breakpoints

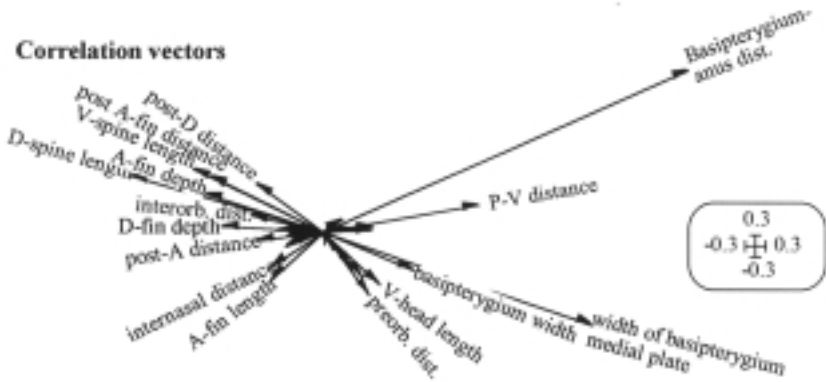
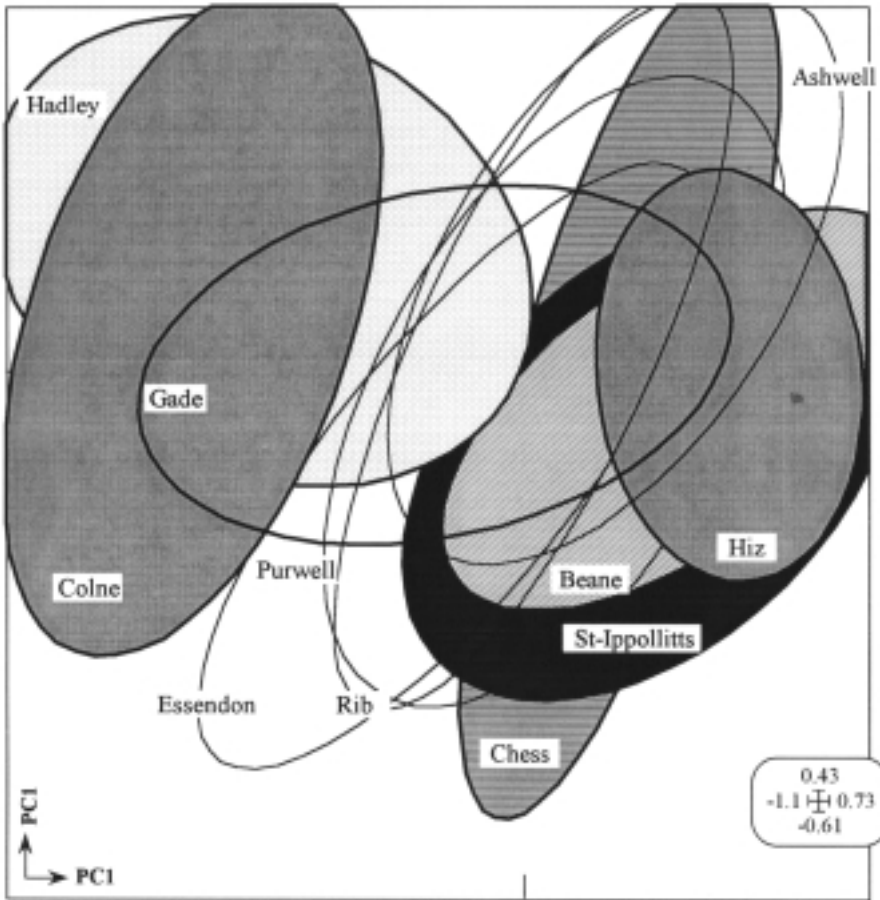
**a) PCA ordination plot**



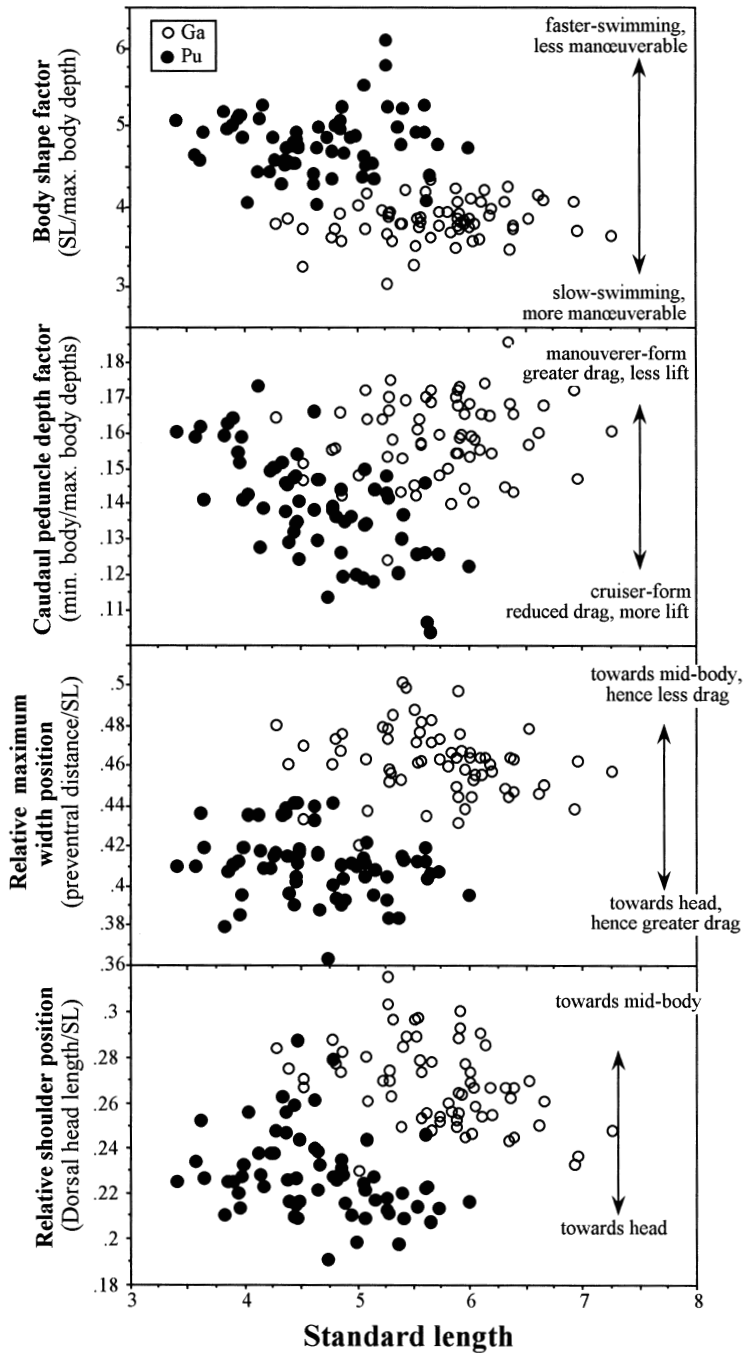
**b) Correlation vectors**



**Fig. 4.** a) Ordination of first two components from double-centred principle components analysis (S a g n e s et al. 1997) of the natural-log transformed data set (130 specimens  $\times$  35 characters) for threespine and ninespine sticklebacks from St-Ippollitts Brook, Hertfordshire (England). b) Correlation vectors, the length of which indicates a character's relative importance to the trace.



**Fig. 3.** Ordination of first two components from double-centred principle components analysis (S a g n e s et al. 1997) of the natural-log transformed data set (244 specimens x 35 characters), with correlation vectors (the length of which indicates a character's relative importance to the trace) for threespine sticklebacks from various streams in Hertfordshire (England).



**Fig. 5.** Body shape, caudal peduncle depth, relative maximum width and relative shoulder position factor values plotted against SL for three-spine (Ga) and nine-spine (Pu) sticklebacks from St-Ippollits Brook, Hertfordshire (England).

in predorsal and preanal distances, which occurred at the same SL (50.1 and 49.9 mm, respectively). This may be associated with the preparation of the body to accommodate the increasing volume of the body cavity occupied by gonads, and the shifts in proportional growth of the preventral and prepectoral distances, as well as the ventral head length, may reflect the completion of this process, as maximum gonadal growth (gonadosomatic index) is achieved within the 45–60 mm SL size range (Copp et al. 2002).

The anterior edge of C-fin is only very slightly forked in threespine stickleback, and the proportional length of this fin decreased with SL ( $y = -0.001068x^2 + 0.212878x - 1.490274$ ). A long C-fin with straight rear edge is a subject of turbulent flow, which increases resistance of water (Aleyev 1963). Such a shape of C-fin does not allow the fish to swim fast, and represents a disadvantage in case of longer migrations; on the other hand, it increases their manoeuvrability. Therefore, if the C-fin in threespine stickleback becomes shorter as the fish grow, then their ability to swim for longer distances improves. This might be associated with movements that threespine undertake to establish territories and attract mates during the spawning period (Berg 1949, Svetovidov 1964, Paepke 1983). Although such movement may not be typical for stream sticklebacks, it is likely that they share this morphological pattern with their relatives living in different environments. On the other hand, relative maximum body depth in our stream threespine sticklebacks (isometric growth, being 3.6-times shorter than SL) was far from that found in sticklebacks from Baltic Sea (4.4-times shorter than SL) but close to sticklebacks from inland waters of Germany (3.3-times shorter than SL; Paepke 1983). A relatively deeper body reduces resistance during axial rotation of body (Aleyev 1963), and thus enhances manoeuvrability of sticklebacks living in small streams.

In some species of fish, such as stone loach and perch, a coincidence between developmental patterns in morphology and habitat use has been found (Kováč et al. 1999, Šedivá et al. 2000). Since we did not observe such a consistent group of morphometric characters with abrupt change (associated with some common ecological, physiological and/or behavioural functions), these developments in external morphology in the threespine probably do not correspond to any shifts in resource use. This may be because stickleback is not a species with typical indirect development (Flecker-Balon 1989), or it may simply reflect the limited lower range of sizes (none < 26.1 mm SL) available for examination, which probably was not sufficient to reveal all developmental patterns (V. Kováč, unpublished data).

#### Between-catchment comparison of threespine

Although there were some differences in body morphology between streams in southeastern England (Fig. 3, Tables 3 and 4), these do not appear to suggest a divergence in habitat-related phenotype such as observed in lakes of northwestern North America (e.g. McPhail 1984). The variations we observed could be size related (Table 5), however, size was accounted for in the analyses, and only characters with isometric growth (in which size effect is entirely eliminated) were considered in our analysis. Nonetheless, our results do suggest that the Colne and Hadley sticklebacks differ morphologically from other streams in the region.

Both the Colne and Hadley populations had longer dorsal and ventral spines, deeper anal fins, longer tail components (shorter pre-anus and basipterygium-anus distances but longer postanal distance) and narrower bodies (basipterygium medial plate width and basipterygium width) than most of the other populations. In most cases, these differences

were found to be statistically significant. However, the coefficient of difference is a statistical tool designed to test for inter-population differences but is not sensitive enough to evaluate nuances in morphology. Differences found with CD can even be used to substantiate the existence of valid subspecies (e.g. Holčík & Skořepa 1971, Kováč 1987). Therefore, the morphological distinction of Colne and Hadley sticklebacks is quite obvious. On the other hand, the inter-population differences found are relatively diverse, and there are only two characters (ventral spine length and basipterygium medial plate width) in which Colne sticklebacks differ from all other populations. Whereas, no such difference was observed in any character in the Hadley sticklebacks. In the absence of a divergence in habitat-related phenotype (e.g. McPhail 1984), our results substantiate further that the threespine stickleback is a species with great morphological plasticity, and its “variation can be considerable even within very restricted geographic areas” (Baker et al. 1998).

**Table 5.** Site code, mean Standard length (SL) in mm, standard error, minimum, maximum, coefficient of variation (C.var) and number of specimens of threespine stickleback from streams in Hertfordshire (England). Significant differences in mm SL (post-priori Scheffe F-test at 95%) between streams indicated with asterisks.

Stream	code	SL mm	SE	min.	max.	C.var	n					
Essendon	Ess	44	3.16	39	50.45	7.18	20					
St-Ippollitts	Ipps	54.79	8.1	40.3	72.9	14.78	35					
Purwell	Pur	46.91	4.49	33.35	53.6	9.58	25					
Hiz	Hiz	52.23	4.58	47.7	66.25	8.76	15					
Ashwell	Ash	45.58	4	39.4	57.4	8.78	21					
Chess	Che	45.2	2.84	41.7	53	6.29	17					
Beane	Bea	51.43	6.3	40.65	70.2	12.25	35					
Rib	Rib	42.37	3.93	36.55	50.6	9.26	14					
Colne	Col	31.69	3.74	26.1	39.6	11.79	26					
Gade	Gad	41.22	3.83	36.2	49.9	9.29	18					
Hadley	Had	32.72	6.09	26.8	54.7	18.61	24					
	Ips	Pur	Hiz	Ash	Che	Bea	Rib	Col	Gad	Had		
Essendon	*		*			*		*		*		
St-Ippollitts		*		*	*		*	*	*	*		
Purwell								*		*		
Hiz							*	*	*	*		
Ashwell								*		*		
Chess								*		*		
Beane							*	*	*	*		
Rib								*		*		
Colne									*			
Gade										*		

Other possible factors in the interpretation of morphological differences among the stickleback populations are predation pressure and related sources of mortality. Our specimens were sampled from streams inhabited mainly by small-bodied fish species, except in the River Rib at Barwick and the River Gade at Hemel Hempstead. At these sites, brown trout *Salmo trutta* were observed in low density (a few specimens sampled). Other potential predators were birds (mainly kingfisher *Alcedo atthis*). Bird predation could be accentuated at some sites (e.g. River Chess) where a small proportion (< 20 %) of sticklebacks were infested with the Cestoda parasite *Schistocephalus solidus*. The behaviour of sticklebacks heavily infected by this parasite is modified, and they swim slowly around

near the surface in lentic areas of the water body, which makes them more susceptible to predation (e.g. Giles 1983, 1987). However, predation pressure on sticklebacks in the Colne and Hadley brooks was probably limited to avian, and as such did not differ from the majority of other sites. Therefore, predation does not appear to be a consistent factor generating significant morphological differences among the populations studied.

### Comparison of threespine and ninespine sticklebacks

Keeping in mind the great morphological and ecological diversity in threespine stickleback (e.g. McPhail 1984, Lavin & McPhail 1985, 1986, 1993, Schluter 1993, Walker 1997, Baker et al. 1998, this paper), it is difficult to draw general conclusions about ecomorphological relationships between threespine and ninespine sticklebacks. From a morphological point of view, threespine stickleback differ from ninespine mainly by having a relatively longer snout (but not a longer head), preanal distance, pre-anus distance, preventral distance, both dorsal and ventral spines, a deeper body, greater minimum body depth, longer and wider basipterygium, and wider basipterygium medial plate, but relatively shorter A-fin, post-anus distance and postventral distance. Basically, the threespine has a more robust body with stronger and more prominent spines, shorter tail, and with the eyes situated more backwards than the ninespine stickleback. On the other hand, both species share some common patterns in external morphology related to swimming capability and locomotion, such as the longitudinal position of maximum body depth and the shape of caudal fin (Aleyev 1963). Nevertheless, some differences were found in the shape factors. So, how are these morphological differences associated with differences in habitat use?

Although the ninespine stickleback has been reported to prefer vegetated areas to open water (Wheeler 1969, Copp 1992, Copp et al. 1998), the body shape factor suggests that ninespine should be relatively better adapted for open waters. It possesses a more thunniform (i.e. cruiser-like) body, which is usually associated with preference of stronger currents, such as found in open water (Webb & Weihs 1986). In general, microhabitat use between threespine and ninespine stickleback differs mainly in the density of macrophyte cover, with ninespine preferring more densely vegetated areas than threespine, whereas the opposite has been observed with respect to ligneous debris (Copp 1992). Ninespine are generally indifferent to substratum character, whereas threespine demonstrate weak preferences (Copp 1992). From the data available, morphological differences between threespine and ninespine stickleback in streams of southeastern England do not appear to impede co-habitation of similar microhabitats. Distinctions in habitat use are probably related to the type of macrophyte bed preferred as well as the available and exploited food resources (e.g. Grenouillet & Pont 2001), and some evidence of this at the catchment scale exists with respect to macrophyte preferences (Copp 1992). As such, the scale of perception probably plays a role in understanding the sympatry of threespine and ninespine sticklebacks, and this will be addressed in a subsequent article.

### Acknowledgements

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