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Sympatry between threespine *Gasterosteus aculeatus* and ninespine *Pungitius pungitius* sticklebacks in English lowland streams

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The comparative biology of stream-dwelling threespine *Gasterosteus aculeatus* L. and ninespine *Pungitius pungitius* L. sticklebacks was examined in streams of eastern England. Threespine occurred throughout, but ninespine occurred < 50 km from stream source, varying from regular distribution to contagion. The species co-occurred more often than expected in macro and microhabitats, and dietary overlap occurred overall and within *Callitriche* beds. Threespine occurred infrequently and ninespine frequently in *Apium* beds, contrasting silt/detritus habitats. Threespine and ninespine microhabitat preferences differed in distance from bank, substratum composition, and amount of ligneous debris, and seems uninfluenced by bullhead *Cottus gobio*, which used faster flowing areas. Greater use of vegetation by ninespine is facilitated by a more stream-lined and small body, which imposes corporal space constraints on gonad size. To compensate, ninespine maintain a lower proportion of mature eggs, produce smaller batches more frequently than threespine and thus incur a lower annual investment to reproduction.

Introduction

Threespine *Gasterosteus aculeatus* and ninespine *Pungitius pungitius* sticklebacks are sympatric in lakes, estuaries and small streams throughout the holarctic region (Wootton 1976, 1984, Craig & FitzGerald 1982). Their sympatry in small streams is of particular interest in that available lentic habitat is relatively limited and could potentially lead to competition for microhabitat (Copp *et al*. 1998), particularly during reproduction. However competition between sticklebacks has not been clearly demonstrated (Wootton 1976, 1984), perhaps because the interactions between these species in streams have received limited study relative to still and brackish waters (Lewis *et al*. 1972, Delbeek & Williams 1987, Lavin & McPhail 1993, Prenda *et al*. 1997). This is particularly true of the ninespine regardless of the ecosystem type (Coad 1981), but especially for streams, with most studies undertaken in the UK and Canada (Hynes 1950, Nelson 1968, Coad & Power 1973, Craig & FitzGerald 1982). To address this gape in knowledge, a series of studies was undertaken on sticklebacks in the River Great Ouse basin, England, to elucidate the sympatric relationship between the two species in small lowland streams. The specific objectives of the present study were to: (1) test for interspecific associations in habitat use at different habitat scales (basin, stream reach, microhabitat), including interactions with co-occurring bullhead *Cottus gobio* at the microhabitat level; (2) compare diet and population densities in various macrohabitats (within stream reaches); and (3) provide a synthesis of this series of investigations, which included the behaviour of habitat use under experimental and field conditions (Copp *et al.* 1998), body morphology and its relevance to habitat use (Kováč *et al.* 2002), and reproductive allocation (Copp *et al.* 2002).

Study area, material and methods

The study was undertaken at four spatial scales (Fig. 1): catchment level (River Great Ouse), river reach level (River Hiz and St. Ippollitts Brook), macrohabitat level (*Callitriche* sp. beds, *Apium* sp., open water with silt/detritus), and microhabitat level (artificial stream, River Purwell, St. Ippollitts Brook). The River Great Ouse catchment drains an area of approximately 8585 km^2 in eastern England. The higher elevations in the south of the catchment drain chalk hills, descending north easterly towards Bedford through greensand alluvial deposits and then former peat/marsh lands around Ely before entering the North Sea at Kings Lynn. A total of 130 sites (stretches of stream, river, side-channel or backwater) throughout the catchment were visited once during the period early August to late September 1990 (for details, *see* Copp 1992). At each of the 130 sites, fish were sampled using point abundance sampling by electrofishing (PASE) adapted to small fishes (Copp & Garner 1995), with all 0+ and some 1+ fishes preserved in 4% formaldehyde. Sampling points were selected haphazardly (*see* Copp & Garner 1995), working in an upstream direction to avoid disturbance of the fish, with approximately 1 sampling point per 5 m distance.

At the stream reach level, sampling was undertaken during a three-week period in October 1992 at eleven sites of the River Hiz basin (Fig. 1), a sub-catchment of the River Ivel, which is a tributary of the Great Ouse. In the River Hiz basin, eleven sites were visited:

1. River Hiz near its source (Nat. Grid Ref. TL 202 278),
2. Ashbrook (TL 178 280),
3. St. Ippollitts Brook (TL 194 281),
4. River Hiz in Hitchin (TL 190 302),
5. River Purwell in Hitchin (TL 178 309),
6. confluence of the Rivers Hiz and Oughton (TL 188 313),
7. River Hiz at Ickleford (TL 186 318),
8. River Oughton upstream (TL 179 309),
9. River Hiz at New Ramerwick Farm (TL 186 338),
10. River Hiz at Arlesey (TL 189 364), and
11. River Hiz near Henlow (TL 189 378).

Channel width of the upstream sites 1, 2, 3, 4, 5, and 8 ranged from 1 to 3 m, with depths not exceeding 0.5 m; the other sites were 2–4 m wide and 0.5 to 1.5 m deep. Stream bottoms were composed mainly of gravel, pebbles and sand with patches of silt deposits amongst the beds of *Callitriche* sp., *Apium* sp. and *Ranunculus* sp. (sites 1, 3, 4, 5, 7, 8, 9, 10, 11), whereas some sites were heavily silted (sites 2 and 6) with a few patches of bare alluvia. The lower Hiz, the Ivel and the Great Ouse are regulated by sluice structures, which make the possibility of seasonal migrations between coast waters and the Hiz basin extremely difficult if not impossible. Fish were collected by continuous electrofishing (DC at 120 pulses per sec., 500 V, 3 Amp), using a portable apparatus (Deka 3000) modified as described by Copp and Garner (1995), over a known area (m^2) of river reach (measured with a tape measure). Fish were killed with an overdose of bensocaine, then preserved in 4% formaldehyde. In the laboratory, fixed specimens were preserved in a fresh solution of 30% industrial methylated spirits. The number of specimens of each species was counted. For sites at which at least 10 specimens of a species (as suggested by Mann *et al.* 1997) were captured per habitat type, diet was assessed, with a representative subsample of 10 selected randomly from those available (Delbeek & Williams 1987) when > 10 speci-

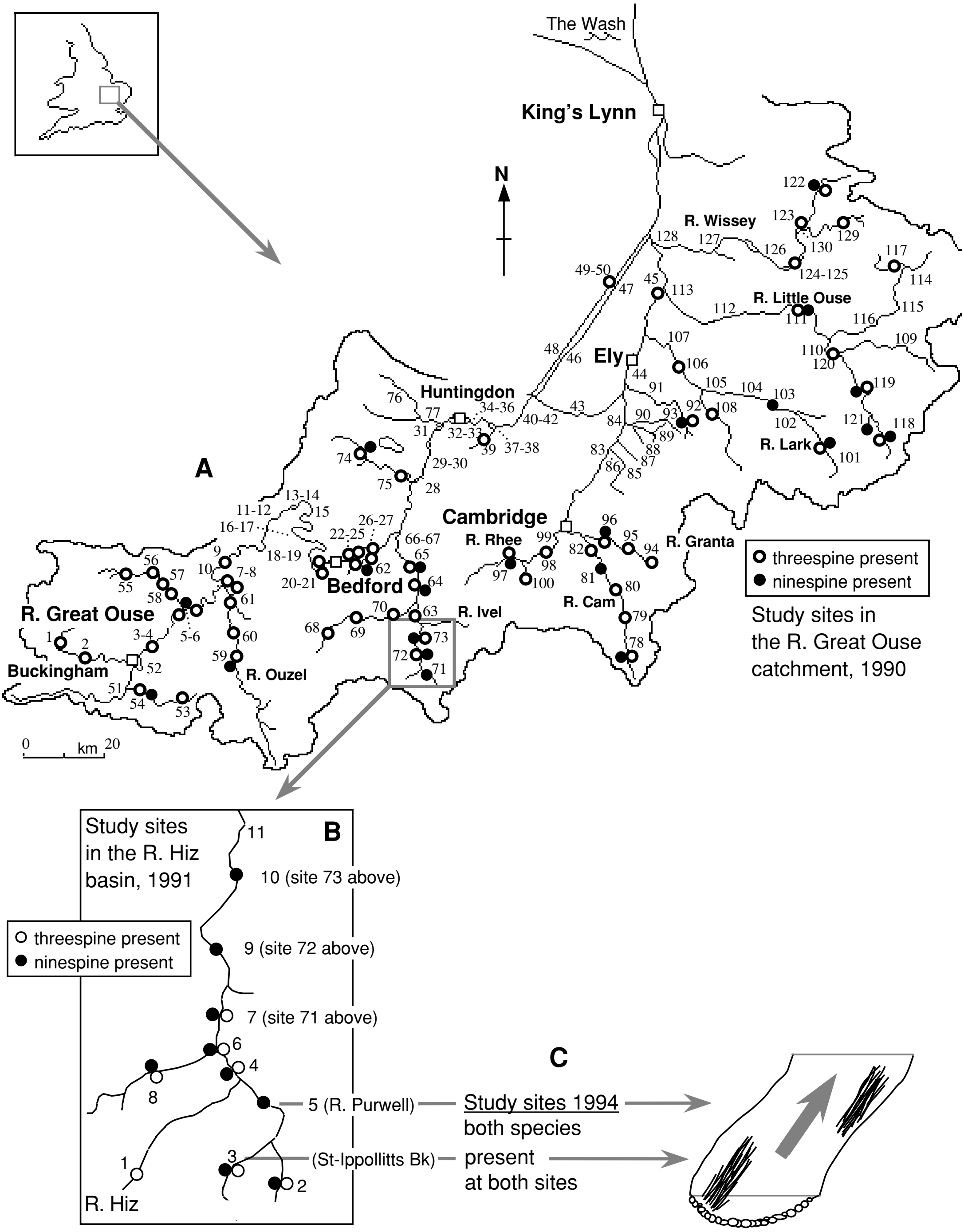

Fig. 1. Maps with distribution of threespine and ninespine sticklebacks at sites sampled in the River Great Ouse catchment (**A**) during August and September 1990 (redrawn from Copp 1992) and in the River Hiz basin (**B**) during October and November 1991, with an illustration of the channel character in the two reaches, St. Ippollitts Brook and River Purwell (**C**), studied in March 1994.

mens were captured. Sticklebacks were measured for standard length (SL), their entrails removed by dissection, the stomach contents identified under a microscope to a family level, and the abundance of individual food items in each stomach enumerated as per Hynes (1950). Dietary analysis of other species captured in the same samples is presented in Copp *et al*. (1994).

At the microhabitat level, we sampled within two stretches of the St. Ippollitts Brook and the River Purwell (sites 3 and 5 indicated above), the former running adjacent to cultivated fields and the latter within a local nature reserve near the town of Hitchin, Hertfordshire. Water depth in the two stretches varied but was not observed to exceed 0.5 m. Neither stream bed appeared to have been engineered, possessing a quasi-natural bed morphology, including riffles, runs and pools. However, the riparian banks leading up from St. Ippollitts Brook were steep due to bed incision and engineering of the ground leading down to the stream bank. 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.

Microhabitat sampling was undertaken during daytime over two days in March 1994 using PASE as described above. A total of 120 point samples were collected, 90 from St. Ippollitts Brook and 30 from the River Purwell. At each sample point, captured fishes were identified and measured for standard length (SL), then we measured 11 quantitative environmental variables: Distance from bank (≤ 0.39, 0.4–0.68, 0.69–0.97, > 0.97 m), Depth (≤ 0.10, 0.11–0.15, 0.16–0.20, > 0.20 m), Bottom: Mud & Silt (0%–25%, 26%–50%, > 50%), Bottom: Sand (0%–25%, 26%–50%, > 50%), Bottom: Pebbles & Cobbles (0%–25%, 26%–50%, > 50%), Ligneous debris (absent, 1–5, 6–10, > 10 items), submerged vegetation (0%, 1%–33%, 34%–66%, 67%–100%), number of overhanging bushes and trees (absent, 1, 2, > 3 items), Water velocity (0, 1–3.00, 3.01–6.00, > 6.00 cm s^{-1}), Oxygen concentration (≤ 9.9, 10.0–10.9, 11–11.9, ≥ 12 mg l^{-1}, measured at every tenth point).

Data analyses

Data from Copp (1992) on stickleback occurrences within the Great Ouse catchment were re-analysed using chi-square (χ^2) analysis to test for deviations from expected frequency of co-occurrence in the two species at the catchment level by study sites (macrohabitat level) and by point sample (microhabitat scale). To compare aggregation patterns, we used Green's (1966) index of dispersion, $[(s^2/x) - 1/nx - 1]$, where s^2 is the variance, x is the mean and n is the number of samples. Green's index, which is considered to be little influenced by differences in sample number (Elliot 1977), was plotted against distance from source to determine any spatial patterns and against sample number to control for potential bias due to sample number.

At the reach level, the densities (numbers per m^2) of threespine and ninespine sticklebacks in the three habitat types (*Callitriche* sp., *Apium* sp., silt/detritus) at eleven sites in the River Hiz basin were compared using Wilcoxon's signed-rank test, and Mann-Whitney *U*-test was used to compare the numbers of prey per SL found in the stomachs of the two species. Differences in SL within species between sites were tested for using analysis of variance (ANOVA) and the Fisher least significant difference (LSD) test (Sokal & Rohlf 1981). Body condition (plumpness) was assessed monthly for males and females using Fulton's condition factor as per Mills and Eloranta (1985b): $K = W10^5 \times SL^{-3}$, where W is the wet weight in g and SL is in mm. The contribution of individual prey selectivity (i.e. variations in resource use by each individual within a species) to diet composition and overlap was assessed using covariance matrix principle components analysis as described by de Crespin de Billy *et al*. (2000). The original diet data matrix was converted to proportions of the total number of items found in each gut, which removes the unequal weight among individuals and provides a more appropriate basis for analyses at the individual level (de Crespin de Billy *et al*. 2000). In the analysis, each prey taxon is linked to the population centroid by an arrow whose length is proportional to the relative abundance of that taxon. Additionally, the length of the arrow also depends on the variation of use of the corresponding prey among individual guts. Thus, dominant prey taxa are ordinated along the principal components (resource gradients), whereas rare prey types are concentrated around the origin. For each fish species from each habitat type, 90% contour ellipses (Green 1971) were generated to aid the interpretation of dietary overlap, which was calculated using the index of Zaret and Rand (1971) for which values ≥ 0.6 are suggested by them to indicate significant overlap, though no test statistics were provided.

At the microhabitat level in the pre-spawning period, data matrices containing fish abundance (samples-by-species) and microhabitat variables (samples-by-variables) were cross tabulated (with the former converted to absence/presence) to: determine the frequencies of occurrence, test for deviations from expected using the Fisher Exact test, and generate microhabitat profiles for all species of fish present in the samples, which included both species of stickleback as well as the bullhead *Cottus gobio*. The microhabitat electivities were calculated, as per Copp (1992) and Watkins *et al*. (1997), as the difference between the frequency of a species in the group of samples having a given category of microhabitat variable and the frequency of that species in all the samples. Electivities approaching –0.5 indicate avoidance and those approaching 0.5 indicate preference. The samples-by-species matrix in (absence/presence) was cross tabulated with itself to test (Fisher Exact) for deviations from expected co-occurrence of the species. To assess composite microhabitat use (e.g. Copp 1992, ter Braak & Verdonschot 1995), the two matrices were then subjected to canonical correspondence analysis (CCA, ter Braak 1986), using software by Chessel and Thioulouse (1998) and Thioulouse (1990). From the analyses, a triplot is produced, diagrammatically illustrating the main pattern of variation in assemblage composition as accounted for by the microhabitat variables (vectors) and the species distribution along each variable. Variable vectors can be extended in either direction to identify the position of a species relative to other species along that gradient (ter Braak 1986), thus providing an approximation of each species' microhabitat breadth and its associations with other species (Watkins *et al*. 1996).

Results

Threespine and ninespine sticklebacks occurred together more often than expected both at sites (reach level) and at sampling points (microhabitat level) within the Great Ouse catchment (Table 1). Threespine were observed throughout the basin, whereas ninespine were restricted to upstream reaches within 50 km of stream source (Fig. 2a). Their aggregation patterns were variable (regular, random, contagion), though most were randomly distributed; these clumping patterns do not appear to be influenced by distance from source (Fig. 2a) nor by the number of samples collected (Fig. 2b).

At the reach level in the River Hiz basin (lower map of Fig. 1), the two species did not occur together more often than expected (Fisher Exact test), contrasting the results for the Great Ouse catchment overall (Table 1). The frequencies of occurrence the species in the three habitat types (Table 2) did not differ significantly from expected (Fisher Exact test, $P > 0.05$), however the mean density of ninespine in *Callitriche* sp. beds was significantly higher than that of threespine (Fig. 3a). The mean densities of the two species were similar in *Apium* sp. beds, but threespine occurred only once in an *Apium* bed but in high density (Table 2). Contrastingly,

Table 1. Co-occurrence of three- and ninespine sticklebacks at 130 sites and in 2800 point samples in the River Great Ouse basin (data from Copp 1992), with expected values given in brackets and χ^2 statistics.

		Ninespine			
	Threespine	Absent	Present	χ^2	*P*
By site	absent	66 (58.2)	4 (11.9)	11.88	0.0006
	present	42 (49.9)	18 (10.2)		
By point	absent	900 (883.2)	31 (47.8)	27.29	0.0010
	present	226 (242.8)	30 (13.2)		

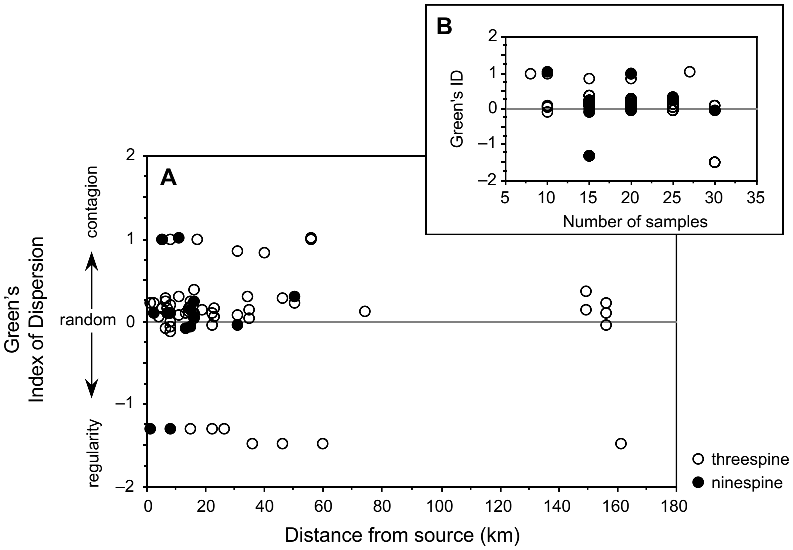

Fig. 2. — **A**: Green's (1966) index of dispersion for three and ninespine sticklebacks in the River Great Ouse catchment (Fig. 1), illustrating generally random distribution, with some clumping and regular distribution closer to stream source (reanalysed data from Copp 1992). — **B**: Green's index for the two species plotted against sample number.

threespine were in higher density and occurred more frequently in silt/detritus habitats (Fig. 3a), where ninespine occurred only once and in high numbers (Table 2). On a habitat-by-habitat basis, the two species were not associated with each other statistically (Fisher Exact, $P > 0.05$) except in *Callitriche* sp. beds ($P = 0.05$). The significantly smaller SL of ninespine in *Apium* sp. habitats, combined with the infrequent occurrence of threespine in *Apium* beds (Table 2), suggests that

Table 2. Relative densities (ind × 100 m^2) of three (Ga) and ninespine (Pu) stickleback in 3 types of macrohabitat (*Apium* sp., *Callitriche* sp., silt/detritus) at 11 sites on the River Hiz and its tributaries, during October and November 1991, by means of continuous electrofishing per unit area (m^2). Also, mean and standard error (SE) of standard length (SL) for sub-sample of specimens examined for diet.

		Callitriche sp.[1]		*Apium* sp.		silt/detritus	
Site	Area	Ga	Pu	Ga	Pu	Ga	Pu
1	75	0	0	0	0	33.33	0
2	60	0	0	0	0	111.67	46.67
3	138	0	0	38.41	5.07	0	0
4	560	1.07	3.21	0	0	0	0
5	182	0	0	0	15.38	0	0
6	510	3.53	5.49	0	0	0	0
7	704	0.43	3.55	0	0.28	0	0
8	1120	0	0.18	0	0	0.36	0
9	280	0	6.07	0	5.71	0	0
10	522	0	0	0	2.68	0	0
11	558	0	0	0	0	0	0
Total number:		27	90	53	67	96	28
Specimens							
Mean SL =		37.40	43.73	48.60	35.60[2]	43.75	45.10
SE of SL =		2.35	1.38	5.63	1.89	2.56	3.04
Number =		10	40	10	20	20	10

[1] significant differences (Wilcoxon's, $P < 0.05$) in density between species.

[2] significantly shorter standard length (ANOVA, $F = 6.638$, df = 67, $P = 0.0023$) within species between habitats (Fisher LSD at 95%).

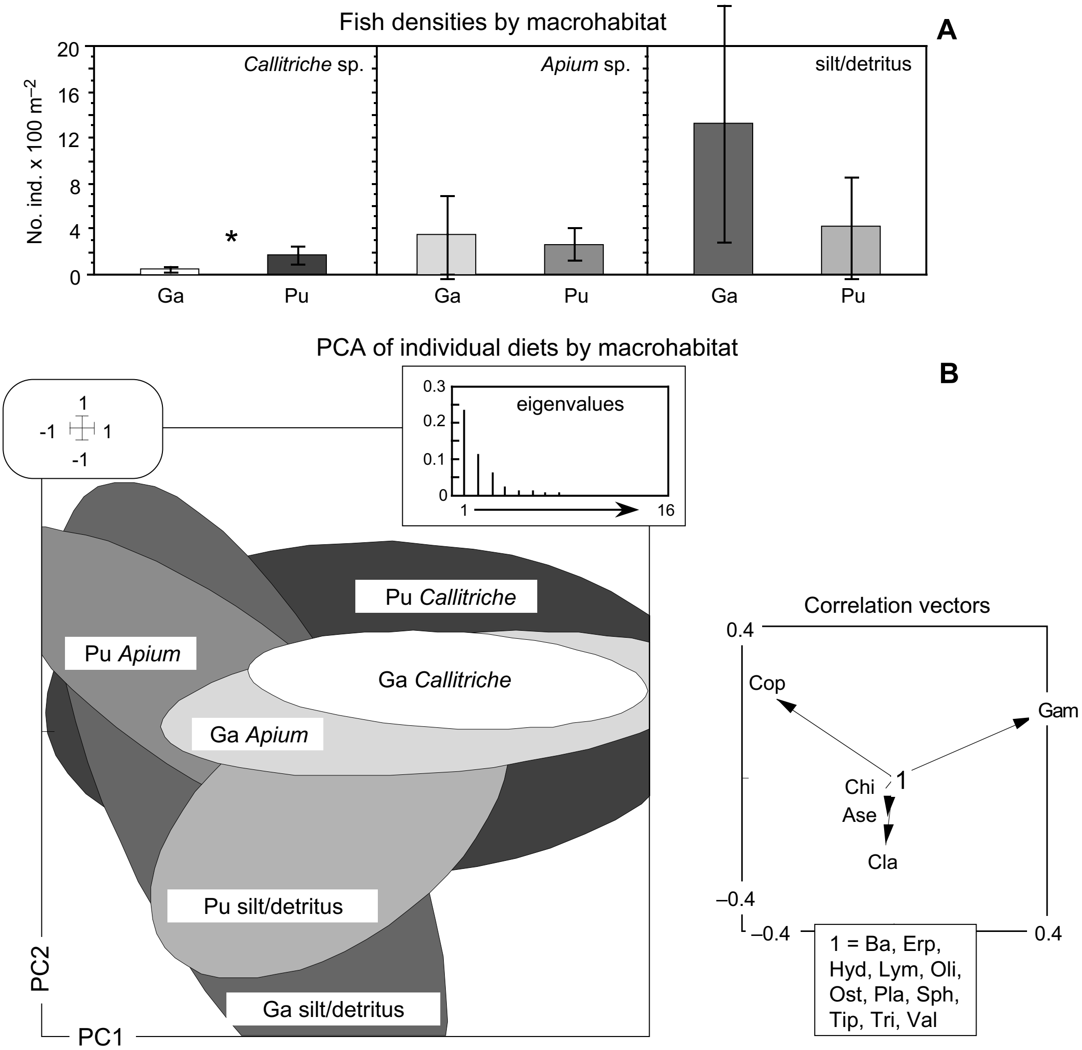

Fig. 3. — **A**: Mean density (number of individuals per m², with SE bars) of threespine (Ga) and ninespine (Pu) sticklebacks in habitats of the River Hiz sub-catchment (* = significant difference, Wilcoxon's, $P = 0.05$). — **B**: Covariance principal components analysis plot (de Crespin de Billy *et al.* 2000), which accounts for 69.8% of the variation in diet of individual specimens of Ga and Pu sticklebacks captured in three habitat types (*Callitriche* sp., *Apium* sp., silt/detritus) of streams in the River Hiz catchment in October and November 1991 (Fig. 1). Eigen values are given as an inset, and 90% contour ellipses (Green 1971) are given in the plot for specimens of each species by habitat type. The correlation vector plot (prey species codes given in Table 3) reveals most influential prey types (longest arrows) in the corresponding PCA plot (B).

smaller ninespine exploit different habitat types than their larger conspecifics, which are able to co-exploit habitats with threespine outside of the spawning period.

Only one specimen was found to have an empty stomach, a ninespine. The main prey types taken in the three habitats of the River Hiz basin were Gammaridae, Copepoda, and Cladocera, in decreasing order of importance (Fig. 3b), i.e. vector length (ter Braak 1986). Significant dietary overlap between threespine and ninespine within *Callitriche* sp. beds (Table 3) is reflected by overlapping ellipses in the PCA of individual diets for the two species (Fig. 3b) and by the pattern of significant differences in prey numbers taken (Table 3): one significant difference associated with the most overlapping diet within a habitat (*Callitriche* sp.), two significant differences with the next most overlapping diet (*Apium* sp.) and four significant differences with the least overlapping diet (silt/detritus). Some intraspecific differences in diet were also observed between habitats. This was particularly true for ninespine between *Callitriche* sp. beds and the two other macrohabitats (Table 3). Ninespine took a greater number of Gammaridae in *Callitriche* sp. beds than elsewhere, greater numbers of Copepoda in *Apium* sp. beds than elsewhere, and greater amounts of Chironomidae, Lymnaeidae, Hydrobiidae, Valvatidae, Erpobdellidae, Cladocera and Tipulidae in silt/detritus macrohabitats than in one or both of the other two macrohabitat types (Table 3). The most marked differences between macrohabitats for threespine were the numbers of Cladocera, which were taken in silt/detritus macrohabitats and not elsewhere. And, higher numbers of Chironomidae were taken in *Apium* sp. beds than in the other two macrohabitat types. Within species, Fulton's body condition factor did not differ (ANOVA, $P > 0.05$) between habitat types in either species. However, K was significantly lower ($F = 32.98$, $P = 0.0001$, df = 106) in ninespine (mean = 0.97, SE = 0.028) than in threespine (mean = 1.23, SE = 0.033).

Microhabitat use of the threespine, ninespine and bullhead in the pre-spawning period of March 1994 in St. Ippollitts Brook and the River Purwell can be accounted for (i.e. variable vector length) mainly by the proportion of sand, water velocity, the proportion of submerged macrophyte coverage, and the amount of riparian vegetation and of in-stream ligneous debris (Fig. 4a). Microhabitat preferences of threespine and ninespine stickleback differed mainly with respect to distance from bank, substratum composition, and amount of ligneous debris (Fig. 4a and b). Both threespine and ninespine

Table 3. Mean numbers (× 10) of prey taxa per fish SL, with standard error (SE), in threespine (Ga) and ninespine (Pu) sticklebacks (n = number of specimens) collected from three types of habitat (*Apium* sp., *Callitriche* sp., silt/detritus) in the River Hiz and its tributaries (11 sites in total) during October and November 1991. Significant differences ($P \leq 0.05$, Mann-Whitney U-test) between species are given in boldface, and differences between habitats for each fish species are indicated by corresponding superscript numbers for that prey type. Dietary overlap indices, within habitat types and for all habitats combined, are considered significant when ≥ 0.6 (Zaret & Rand (1971).

		Callitriche sp.				*Apium* sp.				silt/detritus			
	Code	Ga	SE	Pu	SE	Ga	SE	Pu	SE	Ga	SE	Pu	SE
Gammaridae	Gam	0.57	0.11	$0.47^{1,2}$	0.07	**0.96**	0.26	**0.18**^{1,2}	0.06	**0.15**	0.06	**0.02**	0.02
Asellidae	Ase	0	0	0.12^{1}	0.04	0.02	0.02	0.01^{1}	0.01	0.01	0.01	0.04	0.04
Copepoda	Cop	0.15	0.05	$0.29^{1,2}$	0.09	**0.35**	0.20	**2.33**^{1}	0.73	**2.98**	1.12	**0.02**^{2}	0.02
Ostracoda	Ost	0	0	0	0	0	0	0	0	0.20	0.11	0	0
Chironomidae	Chi	0^{3}	0	$0.02^{1,2}$	0.01	0.42^{3}	0.25	0.14^{1}	0.06	0.09	0.04	0.10^{2}	0.06
Lymnaeidae	Lym	0	0	$0^{1,2}$	0	0	0	$0.03^{1,2}$	0.02	**0**	0	**0.04**^{2}	0.03
Planorbiidae	Pla	0	0	0	0	0	0	0.02	0.02	0	0	0	0
Hydrobiidae	Hyd	0	0	0^{1}	0	0.01	0.01	0	0	0	0	0.02^{1}	0.02
Sphaeriidae	Sph	0	0	0.01	0.01	0	0	0.03	0.02	0.03	0.03	0	0
Valvatidae	Val	0	0	0^{1}	0	0	0	0	0	0	0	0.02^{1}	0.02
Erpobdellidae	Erp	0	0	0^{1}	0	0	0	0	0	0	0	0.02^{1}	0.02
Oligochaeta	Oli	0.02	0.02	0.01	0.01	0	0	0	0	0.03	0.02	0.02	0.02
Tricladida	Tri	**0.03**	0.03	**0**	0	0	0	0.01	0.01	0	0	0	0
Cladocera	Cla	0^{2}	0	0^{1}	0	0^{3}	0	0.01	0.01	**12.28**^{2,3}	5.14	**0.03**^{1}	0.03
Tipulidae	Tip	0	0	0^{1}	0	0	0	0	0	0	0	0.03^{1}	0.03
Baetidae	Bae	0	0	0.01	0.01	0.06	0.06	0	0	0	0	0	0
n =		10		40		10		20		20		10	
Dietary overlap				**0.91**				0.37				0.27	
All habitats combined = 0.82													

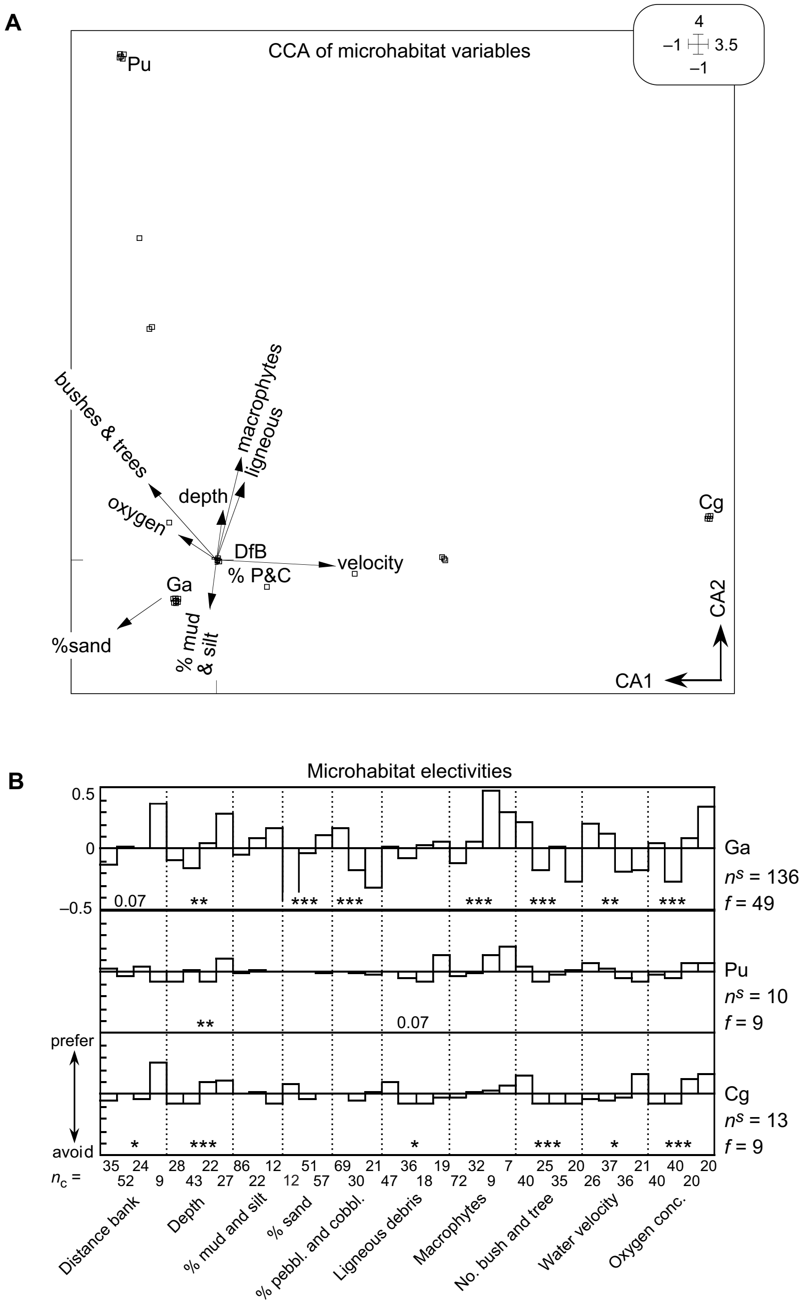

Fig. 4. — **A**: Canonical correspondence analysis triplot of samples (120) and microhabitat variables for threespine (Ga), ninespine (Pu) and bullhead (Cg) in March 1994 in St. Ippollitts Brook and the River Purwell. — **B**: Microhabitat electivities based on the same variables, with each bar being the difference between the frequency of a species in the group of samples having that category of environmental variable and the frequency of that species in all the samples (n_s = numbers of specimens captured, f = their occurrence in samples; n_c = number of times category occurred), where values approaching +0.5 indicate preferences, those approaching –0.5 avoidance. *See* Methods for category descriptions. Significant deviations from expected (Fisher Exact text) are indicated as: * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$, with P values given for near significant deviations.

preferred low velocities, though significant (Fisher Exact) for threespine only. Both species occurred significantly more often than expected in deeper parts (> 0.2 m) of the streams with elevated proportions of macrophytes (Fig. 4b). Ninespine demonstrated weak preferences for dense ligneous debris ($P = 0.07$) and higher oxygen concentrations ($P = 0.14$), whereas threespine was indifferent to ligneous debris and occurred significantly more often than expected in high oxygen concentrations. Ninespine was indifferent to substratum and distance from bank (Fig. 4b), whereas threespine preferred greater distances from the bank, which corresponds to a significant avoidance of riparian cover, and demonstrated both preferences and avoidance of substratum types (Fig. 4b): preference for elevated proportions of mud and silt, though neither significantly, occurring significantly more often than expected over sand and significantly less often than expected over pebbles and cobbles. Microhabitat use by sticklebacks was probably not influenced by bullhead, the only other fish species present at the sites, as none of the three fish species co-occurred more or less often than expected statistically (Fisher Exact), and bullhead microhabitat preference for elevated water velocities and avoidance of ligneous debris probably resulted in an absence of niche overlap (Fig. 4b).

Discussion

Threespine and ninespine sticklebacks both can occur as anadromous and resident populations (Wootton 1984), but the presence of numerous water retention structures throughout the Great Ouse basin inhibits upstream movement throughout most of this catchment. Restricted distribution of ninespine to upstream reaches close to the source suggests that any downstream migrants are unable to maintain populations downstream (Fig. 2a). Anadromous populations of threespine may exist in the lower catchment (site 50, Fig. 1), where a pair of long, man-made channels constitute part of a tidal relief system. The repartition of habitat where the two species coexist may be facilitated by plasticity in distribution behaviour, which ranges from regular to contagion but is mainly random (Fig. 2), though true randomness is thought to be extremely rare in nature (Taylor *et al.* 1978). Loose shoals of threespine stickleback are known to remember profitable food patches (Milinski 1994) and to disperse when food resources are low (Keenleyside 1955, Krause 1993), which may contribute to the variability in dispersion patterns observed (Fig. 2). Experimental studies in an artificial stream (Copp *et al.* 1998) were inconclusive as to whether competition for lentic habitat exists between the threespine and ninespine sticklebacks in very small streams. However, the proportion of time spent in the available lentic refuges was high (30%–60% of the experimental period), particularly the one at the edge of the artificial stream (> 40% of the experimental period). Based on the pattern of aggressive and avoidance behaviours observed in that experimental study, Copp *et al.* (1998) suggested that the two species were attempting to avoid each other when possible, probably due to the experimental arena being too small (despite being twice the minimum area required by a threespine to establish a territory). Threespine seemed more inclined to littoral refuges than the ninespine, which spent much time underneath a mid-stream refuge (Copp *et al.* 1998).

Threespine and ninespine sticklebacks are not expected to influence each other's diet (Walsh & Fitzgerald 1984), and we found significant dietary overlap in one habitat only (Table 3). A high incidence of empty stomachs reported previously (Hynes 1950, Wootton 1984), and attributed to sporadic foraging (Wootton 1976), was not observed in the Hiz catchment, where invertebrate densities were high (Copp *et al.* 1994); so foraging may not be sporadic when food supply is abundant. The diet composition of sticklebacks in the River Hiz catchment differed little from that reported for October in a small lowland stream, the River Birket, in Cheshire, England (Hynes 1950). St. Ippollitts threespine took a higher proportion of Cladocera and higher crustacians but a lower proportion of Copepoda than threespine in the Birket. And St. Ippollitts ninespine took a higher proportion of Copepoda and a slightly lower proportion of Chironomidae larvae than those in the Birket.

Probably the most contrasting result to previous studies was the strong preference for dense submerged vegetation demonstrated by threespine (Fig. 4), which in other English lowland streams have been reported to prefer open waters (Lewis *et al.* 1972). Both species of stickleback are found in streams with rather low oxygen levels (Hynes 1950), and they appear to be amongst the few species of fish able to sustain populations in the nutrient-rich (and presumably oxygen depleted) stretches of lowland streams in the Great Ouse catchment (Copp 1992). However, Lewis *et al.* (1972) reported a preference in sticklebacks for well-oxygenated waters, and our results for threespine corroborate this (Fig. 4).

The habitat of threespine and ninespine sticklebacks in Europe, which is based almost entirely on descriptions from England (Wootton 1984), does suggest very similar habitat preferences in the two species. Despite their primarily benthic existence, the two stickleback species appear to have little interaction with bullheads, which prefer areas with elevated water velocities (Fig. 4). The threespine's association with elevated amounts of submerged macrophytes in the River Hiz basin (Figs. 3a and 4) contrasts with weak preferences for submerged macrophytes throughout the River Great Ouse catchment (Copp 1992). Whereas, ninespine demonstrated a greater preference than threespine for elevated amounts of filamentous algae. Substratum preferences of threespine and ninespine sticklebacks do not appear to have been addresses previously (e.g.Walsh & Fitzgerald 1984, Wootton 1976, 1984). Nonetheless, the significant preference we observed in threespine for sandy bottoms in shallow waters appears to be characteristic of threespine, and of a lesser extent ninespine, microhabitat use across most of the Great Ouse catchment (Copp 1992).

The sympatry of the two species is probably most evident in their different behaviour with regard to use of macrophytes as an anti-predator refuge (e.g. McLean & Godin 1989). Ninespine initiate flight towards cover significantly earlier than threespine when confronted by a predator (McLean & Godin 1989), and tend to use available cover more than threespine when predators are absent (Copp *et al.* 1998). Differential use of cover such as vegetation beds is facilitated by the main morphological differences between the two species (Kováč *et al.* 2002): greater dorsal and ventral spine lengths in the threespine, and greater anal-fin length and post-anus distance in the ninespine (Fig. 5). The latter character is reflected in the body shape indices (Kováč *et al.* 2002), which suggest the ninespine is less manoeuvrable but potentially capable of greater bursts of speed than the threespine, with increased lift and reduced drag in larger ninespine (Webb & Weihs 1986). This is corroborated by the ninespine body condition factor *K* near 1, which according to Stahlberg and Peckmann (1987) affords greater swimming performance than in species with *K* values further from 1, such as the threespine. In experimental flume tests, threespine had lower critical water velocities and a higher mean *K* (1.33) than three other benthic stream fishes (Stahlberg & Peckmann 1987).

A similar morphologically-based distinction has been reported for the limnetic and benthic morphs of the threespine in western North America, where four ecomorphotypes have been identified: limnetic lacustrine, benthic lacustrine, stream, anadromous (Baker *et al.* 1998). Schluter (1993) indicated that the more streamlined limnetic lacustrine morph is more flexible of body than the benthic morph, being able to bend in a tighter S-shape to capture prey. As the limnetic morph of threespine has a shape that resembles the ninespine, perhaps the ninespine also benefits from a similar flexibility in capturing prey within macrophyte and algal beds. The more cruiser-form shape of the ninespine (Fig. 5) would also facilitate its movement towards and within its preferred habitat of macrophytes (Fig. 4b) and algae (Copp 1992); and the alternate inclination of its spines (Wootton 1984) may compensate for their relative shortness by clinging to the vegetation and making ninespine less easily extractable from vegetation than the threespine, which has longer but backward-inclined spines.

Thus, ninespine appears to have adapted to an existence primarily within submerged vegetation and the threespine to that adjacent to submerged vegetation or ligneous debris (Copp 1992), which are used as a refuge when needed to avoid predators. Ninespine compensate for higher predation risk, relative to the threespine (Wootton 1984), through faster escape speeds, more cryptic pigmentation, and a greater capacity to attach itself at will within vegetation beds (Kováč *et al.* 2002). Research into the swimming capacities

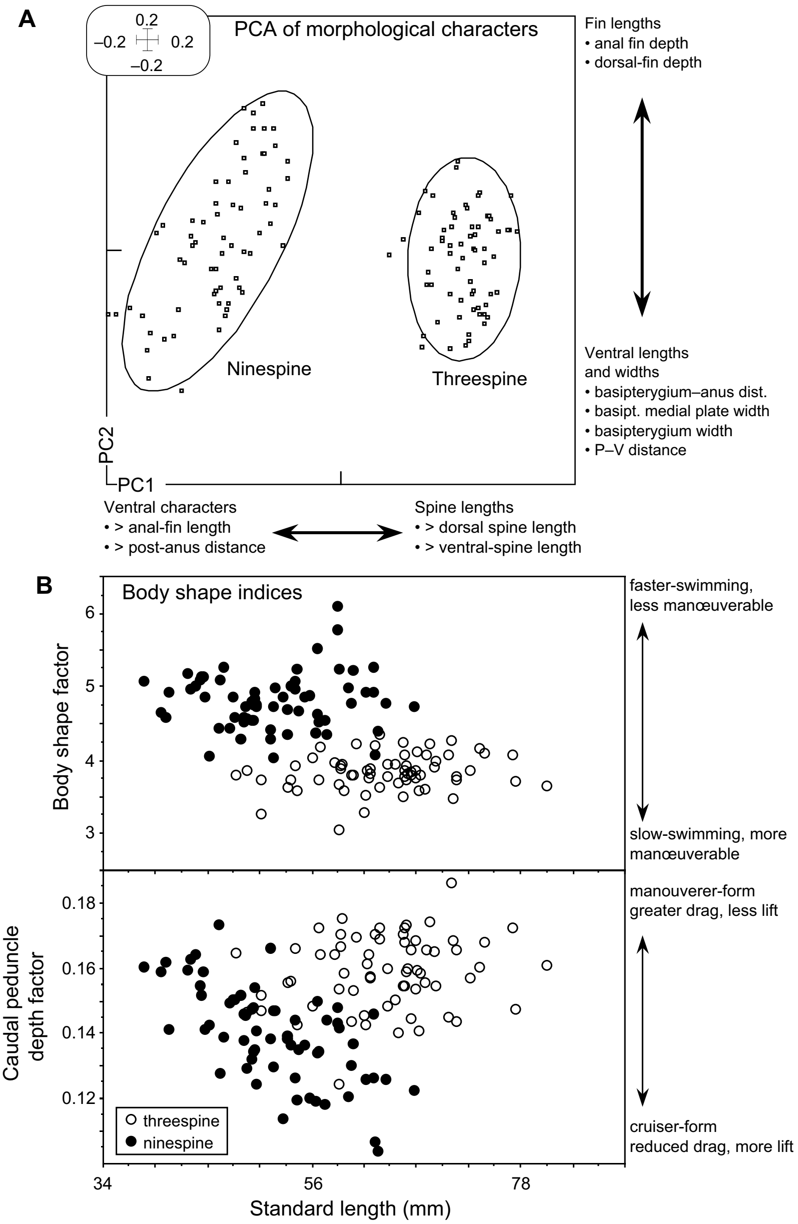

Fig. 5. — **A**: Double-centred principal components analysis of 35 mensural characters (transformed into natural log). — **B**: Body shape (standard length ÷ maximum body depth) and caudal peduncle depth (minimum body depth ÷ maximum body depth) factors for three- and ninespine sticklebacks (redrawn from Kováč *et al.* 2002).

of these two species is needed (e.g. Stahlberg & Peckmann 1987, Whoriskey & Wootton 1987), as it is relevant for understanding their evolution and distribution (Whoriskey & Wootton 1987).

The sympatry between threespine and ninespine sticklebacks has also been linked to subtle differences in reproductive strategy (Wootton 1984), and recently published studies provide much needed estimates for natural populations of clutch size and relative mass (Baker & Foster 2002) as well as egg numbers per batch, batch number per spawning season, and the proportion of reserve eggs eventually spawned (Copp *et al.* 2002). In western North America, reproductive traits in freshwater populations of threespine stickleback demonstrate great variation (Baker *et al.* 1998), though those of streams were less variable than those in pond populations (Baker & Foster 2002). Unfortunately, no similar study exists for ninespine sticklebacks. In St.

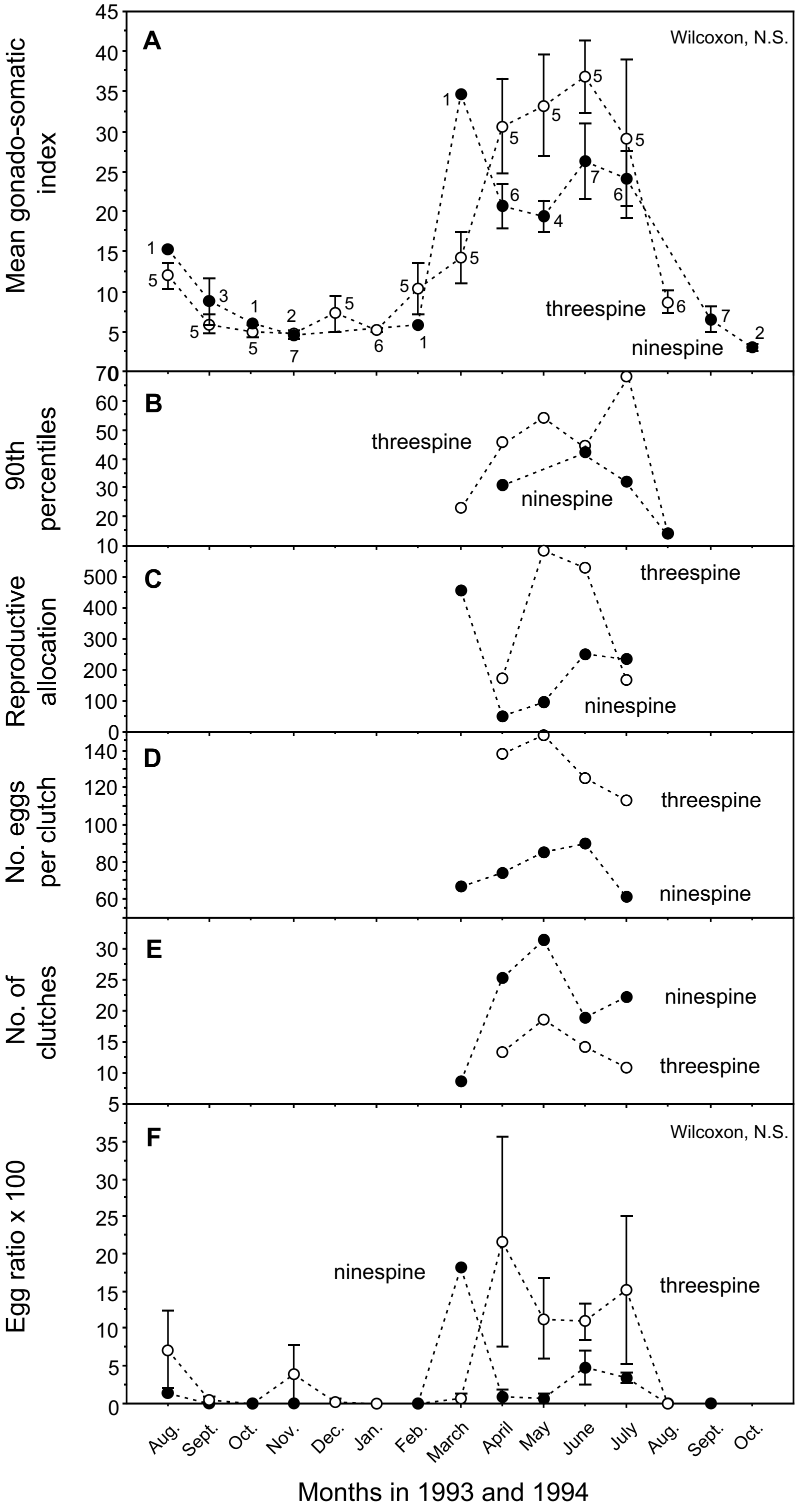

Fig. 6. Reproductive character of threespine and ninespine sticklebacks in St. Ippollitts Brook, England (redrawn from Copp *et al.* 2002), including (**A**) mean and SE of gonado-somatic index (% gonad weight per total body weight) and (**B**) their 90 percentiles, (**C**) estimated mean seasonal reproductive allocation in % body mass, (**D**) mean number of eggs per clutch, (**E**) mean number of clutches, and (**F**) mature-to-total egg ratio (mean number of mature eggs/body weight minus gut weight ÷ total number of eggs/body weight minus gut weight).

Ippollitts Brook, gonado-somatic index (GSI) of threespine and sticklebacks did not differ statistically except in males (Copp *et al.* 2002). However, higher GSI 90th percentiles in female threespine (Fig. 6) suggest a higher maximum annual reproductive allocation (Danylchuk & Fox 1994, Bertschy & Fox 1999) than in ninespine, and similarly, higher monthly estimates of mean seasonal reproductive effort were observed in threespine than for ninespine (Fig. 6). St. Ippollitts threespine also brought to maturity a higher proportion of eggs per body weight than ninespine, with a correspondingly higher mean number of eggs per batch but lower estimated number of batches than ninespine (Fig. 6).

As suggested elsewhere for other aquatic systems (Wootton 1984), threespine and ninespine sticklebacks clearly share all the major lentic habitats in small streams outside the spawning season (Tables 1 and 2, Fig. 3). Dietary and spatial overlap between the two species were greatest in *Callitriche* sp. beds (Fig. 3b, Table 3), but they otherwise appear to repartition both the spatial and dietary resources at the microhabitat scale. During the spawning season, threespine are known to prefer open areas adjacent or associated with vegetation and/or ligneous debris for spawning, potentially displacing other stickleback species such as the ninespine (Rowland 1983), which spawns in algal beds or submerged macrophytes (Wootton 1984). Our field observations in St. Ippollitts Brook confirm these general patterns in which ninespine had a slightly longer breeding season (March through July) than threespine (April through July), but the estimated mean breeding season fecundity (2060 eggs per female in threespine, 1573 eggs per female in ninespine) was not significantly different (Copp *et al*. 2002). This suggests that ninespine in St. Ippollitts Brook are able to spawn an approximately equal number of eggs per female in a given year as threespine by spawning a higher number of smaller batches over a longer period, with an overall lower growth allocation to reproduction.

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