

Differential reproductive allocation in sympatric stream-dwelling sticklebacks *Gasterosteus aculeatus* and *Pungitius pungitius*

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Abstract. Fecundity and growth allocation to reproduction were investigated in stream-dwelling threespine *Gasterosteus aculeatus* L. (leiurus) and ninespine *Pungitius pungitius* (L.) sticklebacks of St. Ippollits Brook, Hertfordshire (England), between August 1993 and October 1994. We compared oocyte size distributions as well as the relative fecundity of females containing mature oocytes, compared growth allocation to gonadal growth and overall reproductive allocation from estimates of total egg supply, the number of reserve oocytes eventually spawned, the proportion of reserve oocytes not spawned, the total number of oocytes spawned, and the number of egg clutches per season. Notable differences in reproductive strategy were observed between the two species. Ninespine sticklebacks were smaller in size than threespine, but compensated for this through a smaller size at first maturity, an almost equal seasonal fecundity (number of spawned oocytes) accompanied by a higher number of unspawned oocytes (i.e. maintenance of a low mature-to-total oocyte ratio), an extended spawning period, a higher number of clutches, and an overall lower reproductive allocation.

Key words: threespine, ninespine, clutch size, egg batch, clutch number, fish eggs, oocyte size, seasonal fecundity

Introduction

The study of growth allocation to reproduction, under both experimental (e.g. Wootton 1973a, Ali & Wootton 1999) and natural conditions (e.g. Wootton 1973b, Craig & Fitzgerald 1982), can provide insights on intraspecific adaptations to environmental conditions (Fox & Crivelli 1998) as well as the interspecific adaptations that permit sympatry (Craig & Fitzgerald 1982). Threespine *Gasterosteus aculeatus* L. and the ninespine *Pungitius pungitius* (L.) sticklebacks are sympatric in lakes, estuaries and streams (Wootton 1976, 1984). Of particular interest is their sympatry in small streams in that available lentic habitat in such streams is relatively limited, which could incite competition for suitable habitat (Copp et al. 1998), especially during reproduction. The ecological interactions of sticklebacks in streams/rivers have received inadequate study (Lewis et al. 1972, Delbeek & Williams 1987, Lavin & McPhail 1993, Prenda et al. 1997, Copp et al. 1998) and the fecundity of ninespine has received much less attention than the threespine (Coad & Power 1973b, Wootton 1976, 1984, Baker 1994).

Of the various measures of fecundity, the most important are per spawning, per breeding season and per lifetime, though information on the latter two for sticklebacks is limited (Wootton 1984), particularly for European populations (Baker 1994). The recruitment

of reserve cells as mature oocytes resembles that of fish year-classes into a population. Thus, the principles of frequency analysis employed to fish populations (e.g. P e r s a t & C h e s s e l 1989) are equally applicable to egg maturation (e.g. M i l l s 1987). This approach should permit the estimation of clutch size, the number of clutches per season, which has not been reported for any natural population (B a k e r 1994), and seasonal fecundity. F o x & C r i v e l l i (1998) have emphasised that estimates of fecundity may provide a relative measure of annual reproductive effort in populations from the same site or geographic region, but these estimates are probably unreliable for comparing populations in different areas (see also M i l l s 1987), so they have proposed a formula for comparing reproductive allocation between populations from different regions and even between species. The aim of our study was to compare the reproductive biology of stream-dwelling threespine and ninespine stickleback. Our specific objectives were to: 1) compare the sizes and size distributions of oocytes, 2) compare the relative fecundity of females containing mature oocytes, 3) determine the relationships between fish size and clutch size, 4) compare the allocation to gonadal growth, and 5) to estimate the reproductive allocation (sensu F o x & C r i v e l l i 1998) of the two species from estimates of total egg supply, the number of reserve oocytes eventually spawned, the proportion of reserve oocytes not spawned per female in a season, the total number of oocytes spawned (seasonal fecundity), and the number of egg clutches per season. Comparative studies of reproductive growth allocation in another nest-guarding species, the pumpkinseed *Lepomis gibbosus*, have revealed that the slow-growing populations of southern France compensate for smaller size (and thus lower oocyte production) with an increased number of spawning events over an extended spawning period (F o x & C r i v e l l i 1998). A similar strategy could be proposed for the ninespine to compensate for its relatively smaller size (W o o t t o n 1984), and this issue is discussed in the light of our results.

Study Area, Material and Methods

Between August 1993 and October 1994 inclusive, specimens were collected from St. Ippollitts Brook (Nat. Grid Ref. TL 194 281) once a month (approximate 4-week intervals) due to resource limitations. St. Ippollitts is a small stream (mostly <1 m wide) that feeds into the River Purwell, a small tributary of the River Ivel (Latitude: 52 °N), which drains part of the southern extent of the River Great Ouse basin. Water depth in St. Ippollitts Brook varied according to rain events but was not observed to exceed 0.5 m during 2 years of field sampling on the brook. The shape of stream bed was natural, with 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 some silt deposits amongst beds of *Apium* sp. occasionally mixed with *Callitriche* sp. and *Ranunculus* sp. The study stretch was lotic and had relatively few lentic, silted patches and water velocities were generally >5 cm·s⁻¹, except at the margins and behind debris in mid-channel. The specimens were captured by electrofishing (DC at 120 pulses·s⁻¹, 500 V, 2–3 Amp) using a portable apparatus (Deka 3000) modified for small fishes (C o p p & G a r n e r 1995). Sampling was by catch-per-unit effort electrofishing over a designated stretch of the brook continuously for about 45 minutes on each sampling date. Water quality measurements were available for February to July 1994 only. Mean values (n = 6) in that time varied little for pH (mean = 7.74, SE = 0.03), conductivity in μs·cm⁻¹ (mean = 754.2, SE = 13.9), dissolved oxygen in mg·l⁻¹ (mean = 9.68, SE = 0.75).

Because of the brook's small size and the possibility of repeated sampling rapidly depleting the population, a maximum of 15 specimens per species was retained for laboratory analysis on each sampling occasion. Similarly, smaller specimens (<30 mm SL) were not measured or retained so as to reduce mortality due to handling as well as impacts on the subsequent year class. Retained specimens were killed with an overdose of benzocaine, then preserved in 4 % formaldehyde.

In the laboratory, all specimens of ninespine were processed, whereas a sub-sample (10–11 specimens) of the more abundant threespine was examined. The fish were measured for SL to the nearest 0.05 mm, weighed to 0.05 g and the sex determined by dissection. The gonads were removed from each specimen, the gonads weighed to the nearest 0.05 g, and the dissected body re-weighed to obtain the eviscerated body weight (e.g. B a k e r et al. 1998). The number of oocytes was determined by direct count. Oocyte diameter measurements were taken from three sub-samples of oocytes, totalling an average of 100 eggs (SE = 4.1, n = 65) per female threespine and 144 eggs (SE = 8.2, n = 44) per female ninespine. Measurements were made using a calibrated ocular micrometer. Five size classes of oocytes were identified using size-frequency (histogram) analysis, with the small (non-vitellogenic) oocytes (< 0.6 mm diameter) considered to be reserve cells.

Absolute (number of oocytes per female) and relative fecundity (number of oocytes per eviscerated weight) were calculated based on vitellogenic oocytes only (classes 1–3, ≥ 0.9 mm diameter) to permit comparison with published data (e.g. B a k e r 1994). However, because oogenesis is believed to continue throughout the breeding season (Wootton 1984), the four largest size classes of oocytes (≥ 0.6 mm diameter) were assumed to represent clutches, originally derived from the reserve cells, that would eventually be spawned. The cut-off diameter between non-vitellogenic cells and developing propagules (0.6 mm) corresponded to that (0.7 mm) reported in a more comprehensive study of egg development in Alaskan lake populations of threespine stickleback (B a k e r et al. 1998). Using only those females with oocytes of ≥ 0.6 mm diameter, the relationship between clutch size (F) and size (SL, body weight and eviscerated weight) was examined using log-log linear regressions (B a k e r 1994, B a k e r et al. 1998; see also W o o t t o n 1994), subsequently estimating the clutch size for a 53.1 mm SL female.

To have a more accurate picture of reproductive allocation, we calculated for each female fish with mature oocytes the 'mature to total egg ratio' (MTER): MTER = (mean number of mature oocytes per eviscerated weight / total number of oocytes per eviscerated weight) x 100.

The gonadosomatic index (GSI = 100 x [gonad weight/eviscerated weight]) was calculated separately for males and females. For the peak months of reproduction (June and July 1994), we calculated the 90th percentiles (monthly and for the spawning season) from the GSI distributions for females, which have been demonstrated as a useful indicator of maximum annual reproductive allocation (D a n y l c h u k & F o x 1994, B e r t s c h y & F o x 1999). Further, we used F o x & C r i v e l l i ' s (1998) formulae to estimate the mean reproductive allocation (RA) of the two species:

$$RA (\% \text{ body mass}) = 100F_b W_e N_b / W_l$$

where F_b is the mean number of oocytes in the largest oocyte size class (estimated clutch fecundity), W_e is the mean biomass of a spawned egg, N_b is the mean number of spawning periods for a female, and W_l is the mean total body mass of a female. F_b was estimated for

the months April to July, as GSI values and oocyte sizes suggested that spawning took place between these periods; as per Fox & Crivelli (1998), females with a low number of large oocytes were ignored in the analysis (skewness was not considered because all specimens had a large number of reserve oocytes, class 5).

W_e was estimated using mean values taken from subsamples for threespine (oocyte weight = 0.0049 g) and for ninespine (oocyte weight = 0.0051 g) comprised entirely or partially of class 1 (≥ 1.7 mm) oocytes. In subsamples also containing eggs of smaller size classes, the total weight of class 1 oocytes (from which weight per oocyte was derived) was calculated through a process of elimination: 1) subsamples containing class 5 oocytes were common and a reliable mean weight per oocyte was estimated thereof; 2) the mean weight per oocyte of class 4 oocytes was estimated from sub-samples containing classes 4 and 5 only, the latter distinguished and eliminated using the mean value per oocyte derived as described above; 3) mean weights per oocyte for subsequent size-classes (3, 2, and 1) were derived in the same manner. The monthly mean number of oocytes per clutch was calculated using the mean oocyte numbers for each of the larger (1–4) oocyte size classes present in that monthly sample; to avoid spurious results, no calculation was made for monthly samples for which no near-mature oocytes (classes 1 and 2) were present in the gonads. The mean numbers of oocytes for each size class (all months combined) was calculated in the same manner, yielding the overall mean number of oocytes per clutch for each species. For each species, the mean number of clutches spawned per female, N_b , was estimated by dividing the estimated total number of spawned oocytes per female for a given month by the estimated overall mean number of oocytes per clutch.

The total number of spawned oocytes per female for a given month was derived as follows: 1) total egg supply (TES) was estimated by summing the mean numbers of oocytes

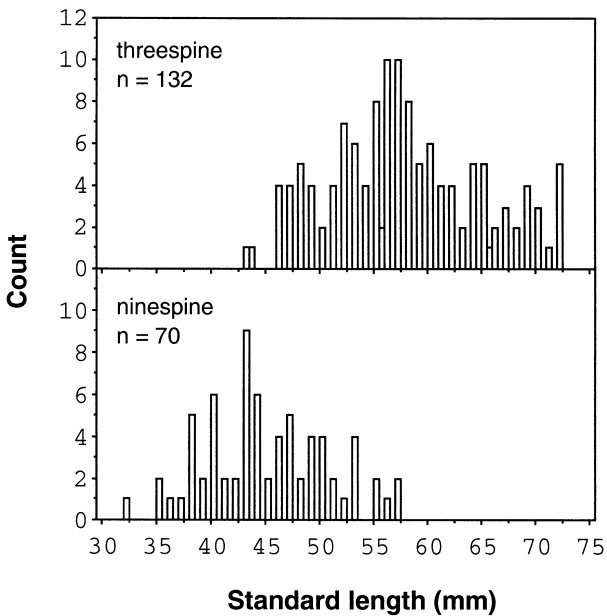


Fig. 1. Standard length frequency distributions for threespine and ninespine sticklebacks from St. Ippollits Brook (Hertfordshire, England) from August 1993 to October 1994 inclusive, using 0.5 mm size classes.

in all size classes (classes 1–5) for that monthly sample; 2) the mean number of oocytes remaining in the gonad (MERG) was estimated by determining the proportion of the gonad effectively used in reproduction; for this we compared the ratio of gonad-to-eviscerated body mass, as well as the gonad weights, in specimens from August to November inclusive (post-spawning period) with those from April to July inclusive (during spawning); these two calculations gave similar results (within 3 %), so the mean of the two was used to calculate the proportion of oocytes per female (20% for threespine, 35% for ninespine; i.e. $TES * 0.20$ and $TES * 0.35$, respectively) expected to be spawned as well as that remaining at the end of the spawning season; 3) the mean number of reserve oocytes per female (MRE) that matured and were eventually spawned was estimated by subtracting the mean number of oocytes remaining in the gonad from the estimated total egg supply (i.e. $TES - MERG$); and 4) the estimated total number of spawned oocytes per female was calculated as the sum of oocytes from classes 1 to 4 plus MRE.

Correlation and regression analyses were used to evaluate relationships between fecundity and certain body characters (e.g. SL, body weight). Comparisons of oocyte size and number per female between species and between months within species were made using analysis of variance (ANOVA), and Wilcoxon's signed-rank test was used to compare the estimates of reproductive allocation between species.

Results

Threespine were captured throughout most of the study stretch, whereas ninespine tended to occur more sporadically, in particular at locations associated with dense bank-side vegetation (Copp et al. 1998); one area of particular note included reeds *Carex* sp. and wild iris *Iris* sp. adjacent to a faster flowing ($\approx 10 \text{ cm}\cdot\text{s}^{-1}$) stretch. In total, 132 threespine (69 female and 63 male) and 70 ninespine (41 female and 29 male) were examined, extending from 43 to 75 mm SL in threespine and from 32 to 55 mm SL in ninespine (Fig. 1). Age classes could not be derived from the length-frequency distributions (Fig. 1), though there appears to be a partial, second mode in threespine (starting at about 65 mm SL) that could suggest the presence of 2+ specimens. In total, 21 threespine and 20 ninespine females contained ripe eggs. The smallest threespine with ripe eggs was 52 mm SL (three specimens), whereas for ninespine the smallest was 35.0 mm SL (one specimen). Size classes of oocytes apparent in the distribution of all eggs combined were reiterated in the monthly distributions for each species (Fig. 2). The oocyte size classes for both species were thus: class 1 (≥ 1.7 mm diameter), large mature oocytes; class 2 (1.3–1.69 mm) smaller mature oocytes; class 3 (0.9–1.29 mm), maturing oocytes; class 4 (0.6–0.89 mm), early maturing oocytes, and class 5 (< 0.6 mm diameter), reserve cells. The smallest size class (5) of oocytes was present throughout the year in threespine (Fig. 3), and presumed to be present in ninespine also though none were captured during in January and February 1994. Unspawned mature oocytes were found in the gonads of threespine much later after the reproductive season in 1993 than ninespine, but the larger size classes (1 and 2) of mature oocytes occurred earlier (March) and later (August) in ninespine than threespine during the 1994 season.

Absolute fecundity was not related significantly to SL in threespine, but it was with respect to eviscerated weight ($\log AF = 0.644 \log EW + \log 2.831$, adjusted $r^2 = 0.049$, $df = 63$, $F = 4.324$, $P < 0.05$) and to total body weight ($\log AF = 0.618 \log BW + \log 2.732$, adjusted $r^2 = 0.071$, $df = 63$, $F = 5.916$, $P = 0.02$). But in specimens containing mature

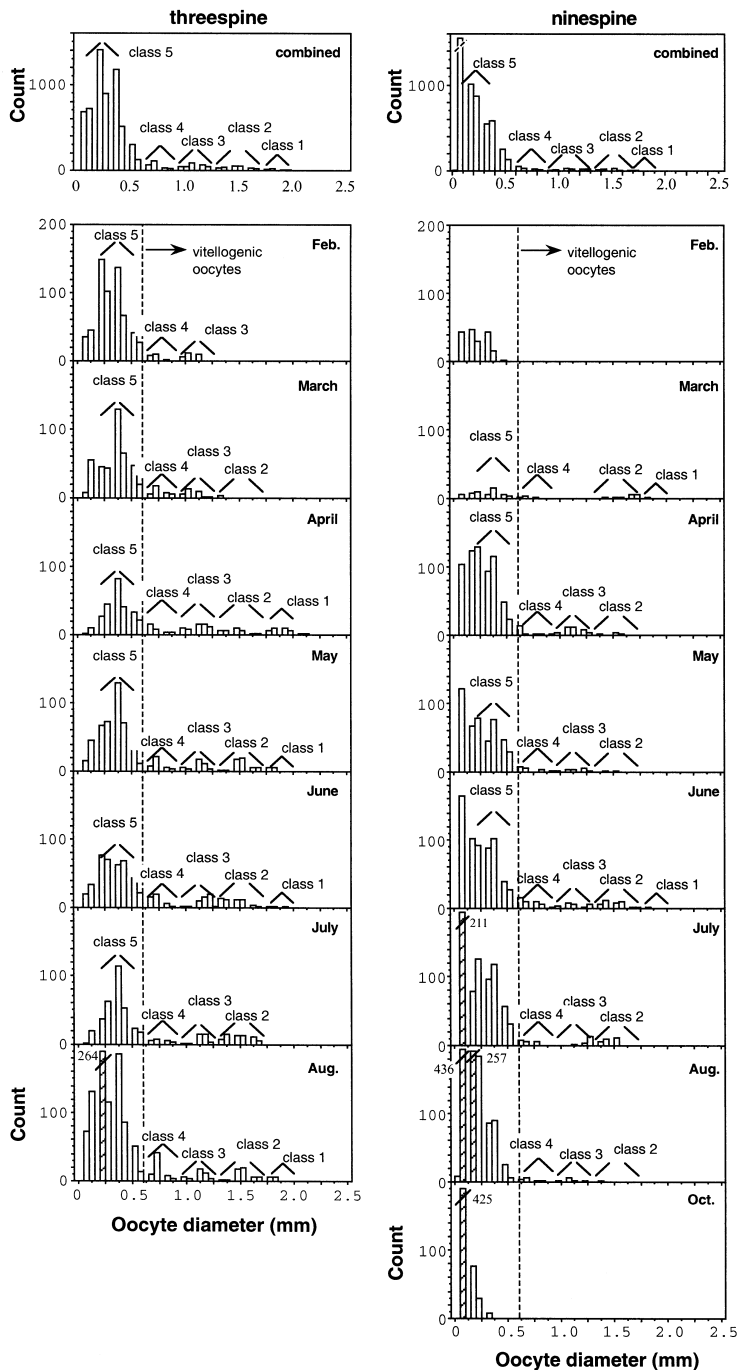


Fig 2. Combined and monthly frequency distributions of egg diameters in threespine and ninespine sticklebacks from St. Ippollits Brook (England) from August 1993 to October 1994 inclusive. To aid comparison, the proposed egg size classes from the combined distribution (Gp 1: ≥ 1.7 mm; Gp 2: 1.3–1.69; Gp 3: 0.9–1.29; Gp 4: 0.6–0.89; Gp 5: < 0.6 mm) are reiterated on the monthly distributions.

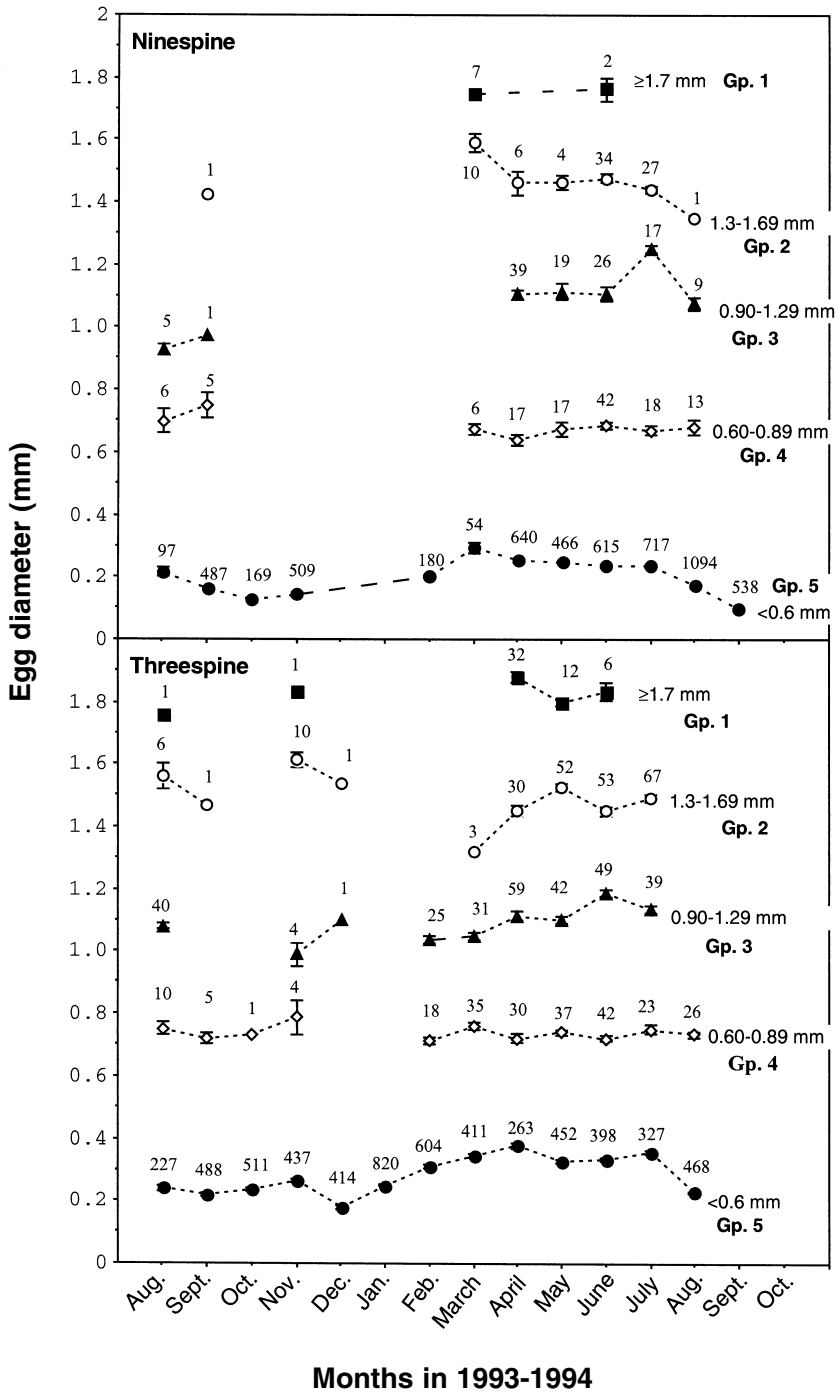


Fig. 3. Mean diameters (with SE bars) for five egg size classes in mm (Gp 1: ≥ 1.7 mm; Gp 2: 1.3–1.69; Gp 3: 0.9–1.29; Gp 4: 0.6–0.89; Gp 5: < 0.6 mm) of three and ninespine sticklebacks from monthly samples in 1993–94 from St. Ippollits Brook (see Fig. 2). The number (n) of oocytes is given above each mean value.

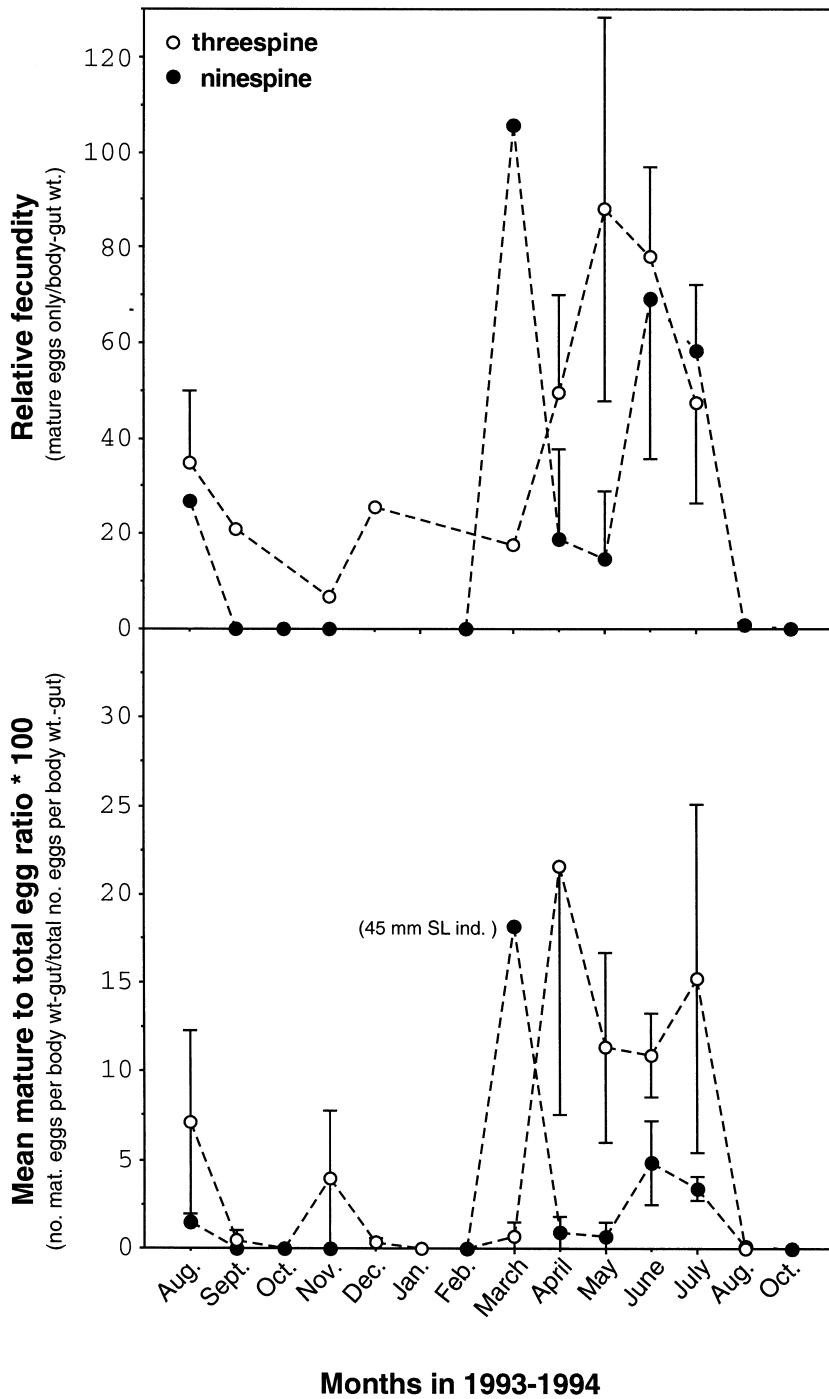


Fig. 4. Relative fecundity (females with mature eggs only) and ratio of mean number of mature-to-total oocytes (all females), both with SE bars, for three and ninespine sticklebacks from monthly samples in 1993–94 from St. Ippollitts Brook (England). The one ripe female ninespine captured in March is indicated with its standard length (SL).

oocytes, absolute mature fecundity (AMF) was not related to SL or eviscerated weight, but was with respect to total body weight ($\log\text{AMF} = 1.554\log\text{BW} + \log 0.949$, adjusted $r^2 = 0.301$, $df = 21$, $F = 10.485$, $P < 0.005$). In ninespine, absolute fecundity was related significantly to SL ($\log\text{AF} = 2.772\log\text{SL} - \log 1.31$, adjusted $r^2 = 0.283$, $df = 39$, $F = 16.78$, $P = 0.0002$), eviscerated weight ($\log\text{AF} = 0.94\log\text{EW} + \log 3.258$, adjusted $r^2 = 0.253$, $df = 39$, $F = 14.512$, $P = 0.0005$), and total body weight ($\log\text{AF} = 0.766\log\text{BW} + \log 3.124$, adjusted $r^2 = 0.192$, $df = 39$, $F = 10.487$, $P = 0.0025$), but in specimens containing mature oocytes, absolute mature fecundity was not significantly related to SL, eviscerated weight or total body weight.

Monthly mean relative fecundity was generally similar in the two species (Fig. 4), though with great variability in both species. However, the monthly mature-to-total egg ratio was lower in ninespine females; this suggests that ninespine bring a smaller proportion of oocytes, relative to body weight, to maturation from their reserve stock (Fig. 2) than do threespine females.

Within species (Fig. 5), mean SL of females and males differed significantly (2-way ANOVA: threespine, $F = 91.817$, $df = 1$, $P = 0.0001$; ninespine, $F = 6.343$, $df = 1$, $P = 0.02$), but eviscerated weight did not differ between sexes in ninespine as it did in threespine (overall, $F = 49.627$, $df = 12$, $P = 0.0001$; between months, $F = 1.87$, $df = 12$, $P = 0.05$; between sexes and months $F = 1.857$, $df = 12$, $P = 0.05$). Between species, the monthly mean SL of females (Fig. 5) differed significantly (2-way ANOVA, $F = 99.187$, $df = 1$, $P = 0.0001$), as was the case for eviscerated weight both overall ($F = 134.233$, $df = 1$, $P = 0.0001$) and between species and months ($F = 1.904$, $df = 10$, $P = 0.06$). Males of the species also differed overall in SL ($F = 57.637$, $df = 1$, $P = 0.0001$) and eviscerated weight ($F = 50.309$, $df = 1$, $P = 0.0001$).

Allocation to gonadal growth between the two, based on the GSI of all females studied, did not differ significantly between species (Fig. 5), though the probability was on the low side (2-way ANOVA: $F = 1.589$, $df = 10$, $P = 0.127$). When only the reproductive months were considered (March to July), GSI did not differ between sexes within either species. The 90th %iles for female GSI suggest that the maximum annual reproductive allocation (sensu Fox & Crivelli 1998) is greater in threespine than ninespine (Fig. 5). Mean male GSI did not differ between species, with that in threespine reaching over 6% of the eviscerated weight in April (4.8 % if calculated using total body weight) whereas that in ninespine was highest in August (1993 and 1994), reaching over 9 and 10 % of eviscerated weight (7.4 and 8.0 % if calculated using total body weight, respectively).

Based on the data for all specimens studied, the numbers of oocytes per female was significantly higher in ninespine than threespine (2-way ANOVA: overall, $F = 11.763$, $df = 1$, $P = 0.001$; between species and months, $F = 2.156$, $df = 10$, $P = 0.03$). This is reflected in the estimates derived from oocyte frequency analysis (Table 1), with the estimated total number of oocytes per female (classes 1–5) being slightly higher in ninespine (mean = 2069.3, SE = 420.9, $n = 5$) than in threespine (mean = 1926.0, SE = 180.1, $n = 4$). The estimated number of remaining oocytes per female was also higher in ninespine (mean = 724.3, SE = 147.3) than in threespine (mean = 385.2, SE = 36.0), with the estimated total number of spawned oocytes per female (seasonal fecundity) correspondingly lower in ninespine (mean = 1572.6, SE = 278.4) than in threespine (mean = 2060.9, SE = 182.0). The estimated number of clutches per female (N_b) was lower in threespine (mean = 14.3, SE = 1.7) than in ninespine (mean = 21.3, SE = 3.8), but with the

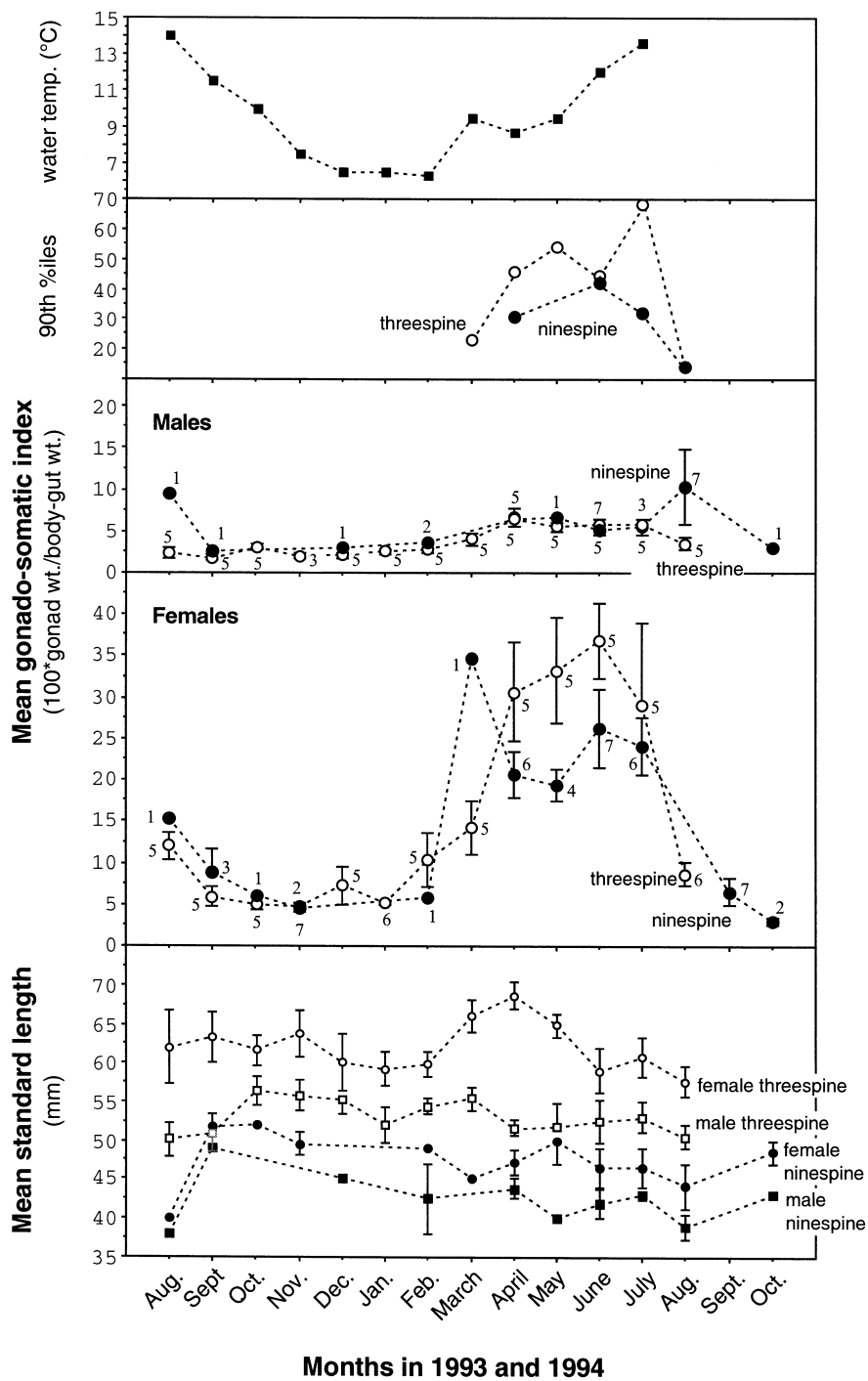


Fig. 5. Water temperature (on day of sampling), monthly female 90th %iles for GSI during the spawning period (all females), as well as monthly mean gonadosomatic index (GSI) and monthly mean standard length (both with SE bars) for three and ninespine sticklebacks (all males and females) in 1993–94 from St. Ippollitts Brook (England).

reproductive allocation (RA) being overall higher in threespine (mean = 362.8, SE = 111.0) than in ninespine (mean = 216.1, SE = 71.5), though the estimates varied greatly between months (Table 1). The estimated number of oocytes per clutch (clutch size) peaked in May for threespine and in June for ninespine. Estimated mean clutch size was greater in threespine (mean = 131.0, SE = 7.7) than ninespine (mean = 75.4, SE = 5.4), as was that adjusted for size (threespine mean = 141.2, SE = 21.3; ninespine mean = 92.3, SE = 2.6). The estimated and size-adjusted values for numbers of oocytes per clutch differed at the 90% level (Table 1), with the size-adjusted values being higher than the estimated values for both species; this suggests that the reproductive allocation parameters may be conservative estimates.

The estimated number of clutches (N_b) decreased with increasing temperature in threespine, though not significantly ($r = -0.624$, $P = 0.26$); no such pattern was observed in ninespine. None of the estimated reproductive allocation parameters were correlated with the water temperature (Fig. 5), pH, conductivity or oxygen values recorded except at 90% significance for the estimated mean clutch size in threespine with respect to water temperature ($r = -0.914$).

The monthly mean number of clutches was not correlated with mean body size (SL or any weight) in threespine or ninespine. The log-log relationship between clutch size and SL was significant in ninespine ($F = 14.16$, $df = 22$, $P = 0.0011$, $r^2 = 0.392$, slope = 2.312, intercept = -1.942), but not in threespine ($r^2 = 0.016$, $P = 0.56$, slope = 1.084, intercept = 0.169). Whereas, the log-log relationships between clutch size and eviscerated weight were significant for both threespine (log Clutch = $1.12\log EW + \log 1.532$, $r^2 = 0.174$, $F = 4.631$, $df = 22$, $P = 0.0426$) and ninespine (log Clutch = $0.821\log EW + \log 1.864$, $r^2 = 0.376$, $F = 13.273$, $df = 22$, $P = 0.0014$).

Discussion

The absolute and relative fecundity of three- and ninespine sticklebacks in St. Ippollitts Brook corresponded well with values reported elsewhere (Coad & Power 1973a, 1973b, Griswold & Smith 1973, Craig & Fitzgerald 1982, Baker 1994). Our estimated mean number of oocytes per clutch (classes 1–4, Table 1) was also in line with the reported mean (122, min-max = 86–172) for stream-dwelling threespine (Baker 1994), but no comparative data are available for the ninespine. The sizes of mature oocytes in St. Ippollitts Brook were similar to values observed elsewhere for threespine (e.g. 1.5 and 2.0 mm, Léger 1941; 1.31 mm, Wallace & Selman 1979; 1.5 and 1.8 mm, Brylinska et al. 1991; mean for stream populations = 1.59 mm, 1.30–1.81, Baker 1994) and for ninespine (Nelson 1968), though Craig & Fitzgerald (1982) reported a lower value (1.14 mm) as did Coad & Power (1973b) for stage IV eggs (0.70 mm). Our estimates of mean weight per mature oocyte for threespine (Table 1) were intermediate between that (0.0011 g per oocyte) calculated from the mean values given in Table 1 of Craig & Fitzgerald (1982) and that (0.0025 g per oocyte) given by Wootton (1976). Our estimated mean weight per mature oocyte for ninespine was about half that (0.0122 g per oocyte) derived from data in Craig & Fitzgerald (1982), and this does not appear to be due to preservation, as our storage medium was the same (4% formaldehyde).

SL at first maturity in St. Ippollitts Brook population was on the high side with respect to the reported range of values for stream-dwelling populations (25–58 mm SL, Baker 1994); comparative information for ninespine remains unavailable, but our value of 40 mm

Table 1. Mean eviscerated weight (Wt) of females with ripe oocytes (≥ 0.6 mm diameter), number of oocytes per female in threespine and ninespine sticklebacks from St. Ippollitts Brook by egg size class in 1994, monthly mean number of oocytes per clutch (egg classes 1–4, excluding zero months), and estimated total number of oocytes (Est. tot. no. oocytes). Estimates from the tabular data are: number of oocytes (eggs) remaining in the ovary at the end of the year (Aug–Nov), number of spawned reserve oocytes per female (F), seasonal fecundity (oocytes/F = total number of oocytes in gonad – unspawned reserve oocytes), number of clutches spawned per female (N_b), and reproductive allocation (RA) in % body mass, with the mean (SE in parentheses). From the raw data, the size-adjusted clutch sizes (no. oocytes/clutch estimated from the log-log regression of clutch size against eviscerated weight) are also presented. The estimated mean weights per mature egg, W_e , used in the calculation of RA were: threespine = 0.0048 g, ninespine = 0.0051 g.

adjusted eggs/clutch month	n	Egg size classes				class 5 (<0.6 mm)				mean no. eggs per clutch	Reproductive allocation			Size no. Log						
		class 1 (≥ 1.7 mm) mean SE	class 2 (1.3–1.69 mm) mean SE	class 3 (0.9–1.29 mm) mean SE	class 4 (0.61–0.9 mm) mean SE	class 5 (<0.6 mm) mean SE	Est. tot. no. eggs Gps 1–5	remain- ing eggs (Aug– Nov)	reserve eggs/F		Estimated seasonal fecundity	N_b	RA(%)							
Threespine																				
March	1	3.649	-	-	284.5	-	331.9	-	1422.4	-	2038.8	407.8	1631.0	2247.4	-	-				
April	5	4.915	80.3	51.2	36.0	283.3	116.5	136.6	55.4	1102.6	355.7	138	1654.0	330.8	1323.2	1874.6	13	173.9	203	
May	5	3.485	37.58	29.5	185.3	106.3	236.9	138.5	131.6	46.6	1949.5	441.4	148	2540.9	508.2	2032.7	2624.1	19	582.1	138
June	5	2.911	23.18	23.2	195.3	47.9	145.7	48.0	137.4	29.5	1397.1	251.4	125	1898.7	379.7	1519.0	2020.6	14	526.1	112
July	5	2.891	-	-	91.6	70.4	109.4	61.00	138.4	120.6	1158.3	501.7	113	1497.7	299.5	1198.1	1537.6	11	169.1	112
class means:	47	131	-	-	194	-	136	-	classes 1–4 mean =	131.5	-	-	14(1.6)	362.8	(111.1)	141.5	(21.3)	-	-	-
Ninespine																				
March	1	1.441	61.3	-	87.5	-	-	-	472.6	-	67	673.9	235.9	438.0	639.3	9	456.1	99		
April	6	1.281	-	-	16.4	16.4	150.9	31.7	54.4	15.6	2326.6	204.8	74	2548.2	891.9	1656.3	1878.0	25	48.8	90
May	4	1.428	-	-	28.2	28.2	119.7	33.7	106.4	40.2	2909.4	455.9	85	3163.5	1107.2	2056.3	2310.4	31	92.5	98
June	5	1.283	7.7	7.7	105.1	39.9	83.2	29.2	82.0	6.6	1425.7	209.4	90	1703.6	596.3	1107.4	1385.3	19	247.5	90
July	4	1.211	-	-	85.1	4.6	51.7	11.6	45.9	13.6520745	310.1	61	2257.2	790.0	1467.2	1649.9	22	235.5	86	
class means:	61*	64	-	-	101	-	68	-	classes 1–4 mean =	75†	-	-	21(3.8)	216.1	(71.5)	92†	(2.6)	-	-	-

* single value, as June value combined with class 2 clutch.

†, § Pair-wise comparisons: estimated mean number of oocytes per clutch vs. log-regression size adjusted (Wilcoxon's, both: $P = 0.07$).

SL is much lower than that of threespine from the same brook. The gonadosomatic index of female threespine (Fig. 5) developed in a manner similar to that reported in W o o t t o n (1984) for a population in the River Rheidol (Wales), though the peak GSI in St. Ippollitts females lasted longer and reached just over 30 % of eviscerated weight, which corresponds to mean values of just under 20% if calculated using total body weight as per W o o t t o n (1984). Female GSI in ninespine was about 30% lower than that of threespine between April and July inclusive, whereas ninespine male GSI was similar to that of threespine except for much higher values for ninespine in August (Fig. 5). The highest GSI values of male threespine from St. Ippollitts Brooks (equating from 4.0 to 4.8 % of total body weight, Fig. 5) were well above the 1.5% maximum reported by W o o t t o n (1984) for males in the River Rheidol.

Reproductive allocation

Differences in the reproductive strategies of three- and ninespine sticklebacks have already been suggested (C r a i g & F i t z g e r a l d 1982, W o o t t o n 1984), though this has been derived from limited information for ninespine. Our study revealed notable differences in reproductive strategy between the two species (Figs. 2–5, Table 1). Egg maturation patterns of both species in St. Ippollitts Brook were similar (Fig. 2), but ninespine appears to have begun spawning earlier and spawned longer, with large oocytes (classes 1–3) present in ninespine gonads from March to August 1994, whereas none or few mature oocytes were found in threespine during March and August (Fig. 2 & 3). Our estimated number of clutches for threespine corresponds with the range of 15–20 clutches given by W o o t t o n (1984). Up to 15 clutches have been reported by W o o t t o n (1976) for females kept at 20°C (16L 8D), however the water temperature in St. Ippollitts Brook was never above 15°C when we sampled. As the estimated numbers of spawned oocytes was largely similar in the two species (Table 1), the higher estimated number of clutches produced by female ninespine appears to have been possible by the maintenance of a lower mature-to-total egg ratio (Fig. 4) and the spawning of smaller clutches over a longer period, resulting in an overall lower mean reproductive allocation than estimated for the threespine (Table 1).

This reproductive compensation by the smaller-sized ninespine resembles a strategy reported for slow-growing populations of pumpkinseed in southern France (F o x & C r i v e l l i 1998); also a nest-guarding species, the Camargue pumpkinseed spawn more frequently over an extended period to compensate for smaller size compared to larger, more northerly populations in Canada. F o x & C r i v e l l i (1998) indicated that oocyte production is lower in the smaller Camargue pumpkinseed than in larger northern populations of Canada. For St. Ippollitts sticklebacks, our results suggest that oocyte number is not affected in ninespine, which instead maintains a lower mature-to-total egg ratio (Fig. 4) than the larger threespine.

Although our manner of estimating clutch number does provide much-needed field-based information on these species, it is approximate. Firstly, gonad weight during the summer may be sensitive to the maturation stage of the maturing clutch of eggs, with gonad weight increasing up to a spawning event, then decreasing abruptly. Secondly, as the spawning season progressed, some sampled females could have already spawned, i.e. specimens may have been sampled just after an abrupt drop in gonad weight. Finally, there is a reproductive cost in terms of female survival; those still alive in November could have been females that spawned less and thus still had large gonads, resulting in an underestimate of the proportion of total egg number actually spawned.

Frequency analysis has previously been applied to recruitment issues, such as by Mills (1987) to identify egg clutches in minnow *Phoxinus phoxinus* and by Persat & Chessel (1989) to identify recruitment bottlenecks in roach *Rutilus rutilus* and nase *Chondrostoma toxostoma*. Our use thereof to estimate egg recruitment for reproductive allocation (Table 1) would seem at least partially supported by the similarity in our estimated RA and those (range: 82–285) estimated by Fox & Crivelli (1998) for threespine using information derived from Fletcher & Wootton (1995). As with other small-bodied, multiple spawners such as minnow and pumpkinseed, sticklebacks are constrained by their size and must bring sequential clutches of oocytes from reserve to maturation for spawning (Mills 1987). Ninespine sticklebacks are generally smaller than threespine (Fig. 5, Table 1) and appear to compensate for this through a smaller size at first maturity, an extended spawning period, a higher number of smaller clutches, the maintenance of a low mature-to-total egg ratio, and subsequently overall lower reproductive allocation. The smaller clutch size and more extended spawning period by smaller females in ninespine than threespine suggests a bet-hedging response in ninespine, perhaps reflecting greater adult mortality (Stearns 1976) and/or competitive interactions with the threespine during the spawning season. However, any such competition remains to be demonstrated at the experimental level (Wootton 1984) and warrants further study, in particular in small streams (Copp et al. 1998).

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LITERATURE

- ALI, M. & WOOTTON, R.J., 1999: Effect of variable food levels on reproductive performance of breeding female three-spined sticklebacks. *J. Fish Biol.*, 55: 1040–1053.
- ASSEM, J. van den, 1967: Territory in the three-spined stickleback *Gasterosteus aculeatus* L., an experimental study in intraspecific competition. *Behav. Suppl.*, 16: 1–164.
- BAKER, J.A., 1994: Life history variation in female threespine stickleback. Chpt. 6. In: Bell, M.A. & Foster, S.A. (eds), *The Evolutionary Biology of the Threespine Stickleback*. Oxford Univ. Press, Oxford: 144–187.
- BAKER, J.A., FOSTER, S.A., HEINS, D.C., BELL, M.A. & KING, R.W., 1998: Variation in female life-history traits among Alaskan populations of the threespine stickleback, *Gasterosteus aculeatus* L. (Pisces: Gasterosteidae). *Biol. J. Linn. Soc.*, 63: 141–159.
- BERTSCHY, K.A. & FOX, M.G., 1999: The influences of age-specific survivorship on pumpkinseed sunfish life histories. *Ecology*, 80: 2299–2313.
- BRYLINSKA, M. (ed.), 1991: Ryby słodkowodne Polski [Freshwater fishes of Poland]. Państwowe Wydawnictwo Naukowe, Warsaw, 355 pp. (in Polish).
- COAD, B.W. & POWER, G., 1973a: Observations on the ecology of lacustrine populations of threespine stickleback *Gasterosteus aculeatus* (L., 1758) in the Matamek River System, Québec. *Natur. Can.*, 100: 377–386 (not seen, cited from Craig & Fitzgerald 1982).
- COAD, B.W. & POWER, G., 1973b: Observations on the ecology and meristic variation of the ninespine stickleback *Pungitius pungitius* (L., 1758) of the Matamek River System, Québec. *Amer. Midl. Nat.*, 2: 498–503.
- COPP, G.H. & GARNER, P., 1995: Evaluating microhabitat use of fish larvae and juveniles with Point Abundance Sampling. *Folia Zool.*, 44: 145–158.

- COPP, G.H., EDMONDS-BROWN, V.R. & COTTEY, R., 1998: Behavioural interactions and microhabitat use of a stream-dwelling sticklebacks *Gasterosteus aculeatus* and *Pungitius pungitius* in the laboratory and field. *Folia Zool.*, 47: 275–286.
- CRAIG, D. & FITZGERALD, G.J., 1982: Reproductive tactics of four sympatric sticklebacks (Gasterosteidae). *Environ. Biol. Fish.*, 7: 369–375.
- DANYLCHUK, A.J. & FOX, M.G., 1994: Seasonal reproductive patterns of pumpkinseed (*Lepomis gibbosus*) populations with varying body size characteristics. *Can. J. Fish. Aquat. Sci.*, 51: 490–500.
- DELBEEK, J.C. & WILLIAMS, D.D., 1987: Food resource partitioning between sympatric populations of brackish water sticklebacks. *J. Anim. Ecol.*, 56: 949–967.
- FLETCHER, D.A. & WOOTTON, R.J., 1995: A hierarchical response to differences in ration size in the reproductive performance of female three-spined sticklebacks. *J. Fish Biol.*, 46: 657–668.
- FOX, M.G. & CRIVELLI, A.J., 1998: Body size and reproductive allocation in a multiple spawning centrarchid. *Can. J. Fish. Aquat. Sci.*, 55: 737–748.
- GRISWOLD, B.L. & SMITH, L.L. Jr., 1973: The life history and trophic relationship of the nine-spine stickleback, *Pungitius pungitius*, in the Apostle Islands area of Lake Superior. *Fish. Bull.*, 71: 1039–1060 (not seen, cited from Wootton 1976).
- LAVIN, P.A. & McPHAIL, J.D., 1993: Parapatric lake and stream sticklebacks on northern Vancouver Island: disjunct distribution or parallel evolution? *Can. J. Zool.*, 71: 11–17.
- LÉGER, L., 1941: La ponte et les oeufs de nos poissons d'eau douce. In: Leçons de l'Institut de pisciculture de Grenoble. *Imprimerie Allier père et fils, Grenoble: 17 pp.*
- LEWIS, D.B., WALKLEY, M. & DARTNALL, H.J.G., 1972: Some effects of low oxygen tensions on the distribution of the threespine stickleback *Gasterosteus aculeatus* and the ninespine stickleback *Pungitius pungitius*. *J. Fish Biol.*, 4: 103–108.
- MILLS, C.A., 1987: The life history of the minnow *Phoxinus phoxinus* (L.) in a productive stream. *Freshwat. Biol.*, 17: 53–67.
- NELSON, J.S., 1968: Deep-water ninespine stickleback *Pungitius pungitius* in the Mississippi drainage, Crooked Lake, Indiana. *Copeia*, 1968: 326–334.
- PERSAT, H. & CHESSEL, D., 1989: Typologie de distributions en classes de taille: intérêt dans l'étude des populations de poissons et d'invertébrés. *Acta Oecol. Oecol. Gen.*, 10: 175–195.
- PRENDA, J., ARMITAGE, P.D. & GRAYSTON, A., 1997: Habitat use by the fish assemblages of two chalk streams. *J. Fish Biol.*, 51: 64–79.
- STEARNS, S.C., 1976: Life-history tactics: a review of the ideas. *Quart. Rev. Biol.*, 51: 3–47.
- WALLACE, R.A. & SELMAN, K., 1978: Physiological aspects of oogenesis in two species of sticklebacks, *Gasterosteus aculeatus* L. and *Apeltes quadracus* (Mitchill). *J. Fish Biol.*, 14: 551–564.
- WOOTTON, R.J., 1973a: Fecundity of the three-spined stickleback, *Gasterosteus aculeatus* (L.). *J. Fish Biol.*, 5: 683–688.
- WOOTTON, R.J., 1973b: The effect of size of food ration on egg production in the female three-spined stickleback *Gasterosteus aculeatus* L. *J. Fish Biol.*, 5: 89–96.
- WOOTTON, R.J., 1976: The biology of sticklebacks. *Academic Press, London*, 387 pp.
- WOOTTON, R.J., 1984: A functional biology of sticklebacks. *Croom Helm, London*, 145 pp.
- WOOTTON, R.J., 1994: Energy allocation in the threespine stickleback. Chpt. 5. In: Bell, M.A. & Foster, S.A. (eds), *The Evolutionary Biology of the Threespine Stickleback. Oxford Univ. Press, Oxford: 114–143.*