

Biology and habitat use of three-spined stickleback (*Gasterosteus aculeatus*) in intermittent Mediterranean streams

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Abstract – We present the first data on three-spined stickleback (*Gasterosteus aculeatus*) life history and habitat use in seasonal Mediterranean streams, analysing populations from NE Iberian Peninsula. Stickleback populations were strictly annual, with reproduction events involving exclusively 1+ fish. Somatic growth was concentrated in two periods, one in spring and early summer, and another in autumn and winter. During summer there was a clear stop in the somatic growth, coupled with low values of somatic condition. This growth pattern contrasts both with that of other Mediterranean stream fish species and that of stickleback in other European locations. Stickleback populations occupied mainly intermediate locations along fluvial gradients. The presence of abundant aquatic vegetation was identified as one of the main factors related with both stickleback presence and the abundance of its populations, while the latter was also negatively related with that of invasive fish and crayfish species. Stickleback tended to occur in native-dominated fish communities, being almost absent from lower stream reaches, which bear high abundances of invasive fish species.

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Key words: Mediterranean streams; summer drought; somatic condition; fish growth; habitat use; threatened fish

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Introduction

The three-spined stickleback (*Gasterosteus aculeatus*) is one of the most widely distributed freshwater fish species in the world, with an almost holarctic distribution range (Wootton 1984; Froese & Pauly 2008). Along its huge distribution range, the three-spined stickleback (henceforth stickleback) displays a wide variety of life-history strategies. Some populations are sedentary, inhabiting freshwater environments throughout the year, while others perform migratory movements, whether anadromous or along water courses. Age and size at maturity and longevity are also variable features, with some populations maturing only after individuals surpass their second winter and others being strictly annual (Wootton 1984; Bell & Foster 1994). In Europe, the stickleback is often an abundant species, even numerically

dominant, in littoral marine, brackish and inland fish assemblages.

Because of its widespread distribution and abundance, the stickleback has been considered as a species of low conservation concern. However, it has suffered sharp declines and multiple local extinction events in some areas within its range, particularly in freshwater populations at the southern edges of its distribution (Foster et al. 2003) as those occupying the Mediterranean bioclimatic region in Europe (Kottelat & Freyhof 2007). The biodiversity loss associated with these population extinctions might be especially important, as various studies have noted genetic differentiation of both Mediterranean and Black Sea stickleback populations relative to the more abundant central and northern European ones (Mäkinen et al. 2006; Cano et al. 2008). Foster et al. (2003) argued that genetically distinct populations should be consid-

ered evolutionarily significant units (*sensu* Crandall et al. 2000) and be effectively protected in order to conserve the evolutionary heritage of the stickleback adaptive radiation. In the Iberian Peninsula, both in Portugal and Spain, the species is listed as endangered following IUCN (International Union for Conservation of Nature) criteria, after having disappeared from most of its past distribution (Doadrio 2002; Cabral et al. 2005). These declines have been especially strong in Mediterranean climate areas, while stickleback seems to be still widespread and abundant in some areas under Atlantic climatic conditions in NW Iberian Peninsula (Fernández et al. 2006).

Despite intensive study of the stickleback as a model in ethological and evolutionary studies (Wootton 1984; Bell & Foster 1994), few studies have examined stickleback in the southern limits of its distribution range, where the species faces its main conservation problems. Most of the available information on stickleback in Mediterranean areas comes from Camargue (France), a large coastal wetland and agricultural area in the delta of the Rhône River (Crivelli & Britton 1987; Poizat et al. 2002). Similar studies are lacking in running waters with a Mediterranean flow-regime, although there is inherent interest in plasticity of life-history traits in such harsh environments, and such information is critical for informed conservation.

In this work, we analyse the biology and habitat use of stickleback populations inhabiting streams with a typical Mediterranean flow-regime in Catalonia (NE Spain). Our specific objectives were to: (i) report the first data on the population structure, growth and variation in somatic condition among stickleback populations in Mediterranean seasonal streams; (ii) identify the main habitat characteristics related to the presence and abundance of stickleback; and (iii)

analyse the relationships of stickleback with other fish species and invasive crayfish.

Materials and methods

Study area

This study was conducted in three different catchments, Daró, Onyar and Sèquia de Sils, in Girona (Catalonia, NE Spain), draining a mostly siliceous area (Fig. 1). Streams in these catchments run from medium altitude forested mountain areas (600–700 m elevation) to lowland alluvial plains with irrigated croplands and tree (mostly *Populus*) plantations. The Daró was originally an independent basin, running into the Mediterranean Sea through a small marsh area, but is now artificially connected to the larger Ter River through a water diversion that maintains a constant flow in the Daró's lower reaches. The Onyar is a natural tributary of the Ter River, joining it in the city of Girona. The Sèquia de Sils (henceforth simply Sèquia) is a tributary of the Tordera River, to the south of the Ter. All three catchments are relatively homogeneous in terms of drainage area, lithology and land use (Pou-Rovira et al. 2007).

Climate in the study area is typically Mediterranean, with an alternation of predictable dry and hot periods and much more unpredictable wet periods with frequent flash floods (Gasith & Resh 1999). All streams in the study area cease their superficial water flow during summer, with water being retained only in a few isolated pools or runs that tend to occur where bedrock appears near the surface, mostly concentrated in the foothill sectors. The effects of summer droughts are exacerbated by water extractions for agricultural (mainly corn) irrigation and urban supply. In average years, summer water flow in the lower reaches is maintained almost

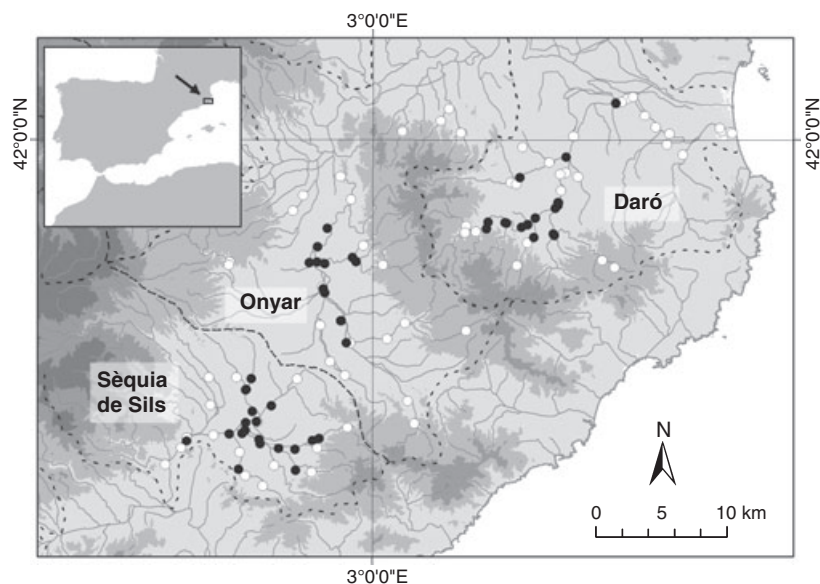


Fig. 1. Location of the study area, showing the 118 sites in which fyke net surveys were performed. Black dots indicate sites in which three-spined stickleback was present and white dots denote sites in which the species was not detected. The main three drainages in the area and their limits (broken lines) are also shown. North arrow indicates true north.

exclusively through the effluents of sewage plants, which bear an important organic load, or through punctual water diversions (as in the lower Daró). As a result of these water inputs, the lower reaches of the three basins maintain some water flow throughout summer. The studied streams are mostly unregulated, harbouring only some small weirs (Pou-Rovira et al. 2007). Potential stickleback predators in the study area include fish (especially eel, *Anguilla anguilla*, which is present throughout the area), birds (mainly little egret, *Egretta garzetta*; night heron, *Nycticorax nycticorax*; and kingfisher, *Alcedo atthis*), mammals (the invasive American mink, *Mustela vison*) and viperine snake (*Natrix maura*), which is locally abundant.

Fish surveys and habitat characterisation

We performed two complementary fish surveys to address our main objectives. To characterise stickleback growth, reproduction and somatic condition we collected individuals in 60 sites on 97 occasions, using a variety of fishing methodologies, including fyke nets, dip nets and backpack electrofishing (SAMUS 725G, Samus Special Electronics, <http://www.electro-fisher.com>). Although these surveys were not seasonally replicated in a systematic fashion they covered every month (except October) and could thus be used to analyse temporal variation in fish size and condition, or to identify reproduction events. Captured stickleback were measured (fork length to the nearest millimetre), weighed (to the nearest 0.01 g) and, when possible, sexed attending to external sexual dimorphism. Whenever we made large stickleback captures we only measured and weighed a subsample of 100–200 individuals.

To study the characteristics of stickleback populations and their relationships with other fish and crayfish species and with habitat features, we performed standardised fish sampling between April and September in 118 sites (in 2006 and 2007; Fig. 1). In these surveys, we used unbaited fyke nets with a single wing (of approx. 1 m), two funnels and a 3.5-mm mesh size. This type of fyke net is an efficient passive method for monitoring small-stream fish populations (Clavero et al. 2006). We usually set three fyke nets per site (mean 3.67; range 2–10) leaving them for 24 h. Captured fish were identified to species, counted, measured (stickleback were also weighed) and returned to the water. We also counted captured red swamp crayfish (*Procambarus clarkii*), an invasive North American species widely distributed in the Iberian Peninsula and often abundant in the study area, in which native white-clawed crayfish (*Austropotamobius pallipes*) is presently absent. Fish and crayfish captures were expressed as catch per unit of effort (CPUE, individuals \times trap⁻¹ \times day⁻¹).

Table 1. Environmental variables recorded in each of the 118 sites surveyed by means of fyke nets. Transformations are also given whenever they were used for further statistical analyses.

Environmental variables	Values	Transformation	Mean (\pm SD)
Conductivity ($\mu\text{S} \times \text{cm}^{-1}$)	Continuous	log ₁₀	650.2 \pm 342.9
pH	Continuous	–	7.62 \pm 0.34
Mean width (m)	Continuous	Square root	3.53 \pm 2.57
Mean depth (cm)	Continuous	Square root	0.46 \pm 36
Maximum depth (cm)	Continuous	Square root	1.01 \pm 0.58
Bedrock	Coded 0–5	–	0.46 \pm 1.01
Boulders	Coded 0–5	–	1.89 \pm 1.41
Cobbles and gravel	Coded 0–5	–	2.03 \pm 1.07
Sand	Coded 0–5	–	2.81 \pm 1.38
Silt	Coded 0–5	–	1.79 \pm 1.49
Tree cover	Coded 0–5	–	3.24 \pm 1.43
Leafy debris	Coded 0–5	–	1.31 \pm 1.04
Woody debris	Coded 0–5	–	1.53 \pm 0.78
Roots	Coded 0–5	–	1.01 \pm 0.85
Periphyton	Coded 0–5	–	1.06 \pm 1.11
Filamentous algae and bryophytes	Coded 0–5	–	1.46 \pm 1.57
Submerged macrophytes	Coded 0–5	–	0.61 \pm 0.87
Helophytes	Coded 0–5	–	1.76 \pm 1.34
Natural dykes	Coded 0–5	–	0.79 \pm 1.11

At each sampling site, we recorded 19 habitat variables (Table 1). Many of these variables, especially those related to cover proportions, were codified in a 0–5 semi-quantitative scale, where: 0, 0%; 1, 1–10%; 2, 11–25%; 3, 26–50%; 4, 51–75%; and 5, 76–100%. These proportions were visually estimated and usually averaged between at least two observers. To assess the relative proportion of different substrate types (using the 0–5 scale), we used a simplified substrate classification with five levels: silt (<1/8 mm in diameter; i.e., materials able to be suspended in water); sand (1/8–2 mm); gravel (2–64 mm); cobble and boulders (>64 mm) and bedrock.

Data analyses

The stickleback population structure was graphically analysed through the variation of length–frequency histograms constructed for six bimensual periods and the monthly mean fork length. Somatic condition of stickleback was calculated using Fulton's condition factor (Mills & Eloranta 1985; Copp & Kovac 2003): $K = W \times 10^5 \times FL^{-3}$, where W is the wet weight (in g) and FL is the fork length (in mm). The monthly variation in stickleback somatic condition was assessed through one-way ANOVA, using K as the dependent variable and month as factor.

Main habitat gradients present in our study area were defined by means of partial principal components analysis (PCA) on a matrix of 118 study sites \times 19 habitat variables. Basins (Daró, Onyar and Sèquia) were included as covariates in order to extract environmental gradients common to the whole study area.

We assessed habitat use of stickleback following two complementary approaches. First, we compared habitat characteristics of sites where stickleback were present with those of the sites where the species was not detected (henceforth absences). We conducted one-way ANOVA using the components (factor scores) extracted by the PCA, the abundance of invasive fish species and the abundance of red swamp crayfish as dependent variables, in order to detect possible influences of habitat gradients on the presence of stickleback. We excluded, from these analyses, 27 sites in which no fish were detected (usually low-order small brooks). In a second approach, we related the relative abundance of the species (CPUE, \log_{10} -transformed) with habitat characteristics from sites where stickleback were detected. Simple correlation analyses were used to test the associations between stickleback relative abundance and the habitat gradients defined by the PCA, the abundance of invasive fish species and the abundance of red swamp crayfish. Then we used these variables to build a multiple regression model with stickleback abundance as the dependent variable. We followed a manually conducted backward stepwise procedure to construct minimum adequate models (MAM). Retention of independent variables in the models relied on statistical significance ($P < 0.05$), which is a more restrictive criterion than information criteria (i.e., Akaike information criterion) that tend to leave more independent variables in final models (Crawley 2002).

Finally, we performed a partial canonical correspondence analysis (CCA) to summarise the variation in fish and crayfish communities, and the role of stickleback, in relation to habitat characteristics. CCA is a direct gradient multivariate analysis in which species data can be directly related to a set of environmental variables, through a combination of ordination and multiple regression techniques. The

species matrix submitted to the CCA consisted of 107 sites (excluding sites without fish or crayfish catches) \times the $\log_{10}(\text{CPUE}+1)$ of 11 species (10 fish + 1 crayfish). Rare non-native species (<5% occurrence) were pooled as a single 'species' (other invasive species or OIS; Table 2). The environmental matrix used in the CCA included the 19 variables listed in Table 1. Environmental variables were selected using manual forward selection, which tests the significance of the variables through Monte Carlo permutations ($N = 499$), and only variables with $P < 0.05$ were retained (ter Braak & Smilauer 1998). The statistical significance of the CCA's first axis and of the whole ordination were also tested through Monte Carlo permutations ($N = 1000$), testing whether these final solutions explained more variance than expected by chance. The three different basins were included as covariables in order to control for the possible spatial structure of our data. We used the biplot scaling, which is recommended for gradient lengths up to 4 SD (ter Braak & Smilauer 1998), as preliminary detrended correspondence analysis produced a first axis of 3.8 SD units in length. We also used the downweighting of rare species. Statistical analyses were performed using SPSS 15.0 and CANOCO 4.5 programs.

Results

Population structure, somatic growth and condition

We measured 6177 and weighed 2243 stickleback individuals during the year-round stickleback collections. Stickleback populations were mainly composed of young-of-the-year (YOY) individuals, which almost disappeared in early summer, after the end of their first breeding season (Fig. 2a). We only caught a few individuals that survived their second summer, most of them females, although we did not get any

Table 2. Frequency of occurrence of fish and crayfish species captured during the fyke net surveys in 118 sites and their relative abundance in relation to the total catch. Species codes and their status (N, native; I, invasive) are also given. Marked species (*) were pooled for further analyses under a single category (other invasive species, OIS).

Latin name	Common name	Status	Species'		
			acronyms	% occurrence	% abundance
<i>Gasterosteus aculeatus</i>	Stickleback	N	GAC	43.2	57.8
<i>Barbus meridionalis</i>	Mediterranean barbel	N	BME	37.3	11.5
<i>Anguilla anguilla</i>	Eel	N	AAN	31.4	1.9
<i>Squalius laietanus</i>	Catalan chub	N	SLA	15.3	1.1
<i>Gambusia holbrooki</i>	Mosquitofish	I	GHO	22.0	4.7
<i>Lepomis gibbosus</i>	Pumpkinseed	I	LGI	22.0	7.3
<i>Cyprinus carpio</i>	Carp	I	CCA	7.6	1.4
<i>Luciobarbus graellsii</i>	Ebro barbel	I	LGR	6.8	0.2
<i>Scardinius erythrophthalmus</i>	Rudd	I	SER	5.9	0.3
<i>Carassius auratus</i> *	Goldfish	I	OIS	2.5	>0.05
<i>Tinca tinca</i> *	Tench	I	OIS	1.7	>0.05
<i>Micropterus salmoides</i> *	Largemouth bass	I	OIS	1.7	>0.05
<i>Misgurnus anguillicaudatus</i> *	Weatherloach	I	OIS	0.8	>0.05
<i>Pseudorasbora parva</i> *	Topmouth gudgeon	I	OIS	0.8	>0.05
<i>Procambarus clarkii</i>	Red swamp crayfish	I	PCL	68.6	13.7
	Total			118 sites	21,741 individuals

CCA, canonical correspondence analysis.

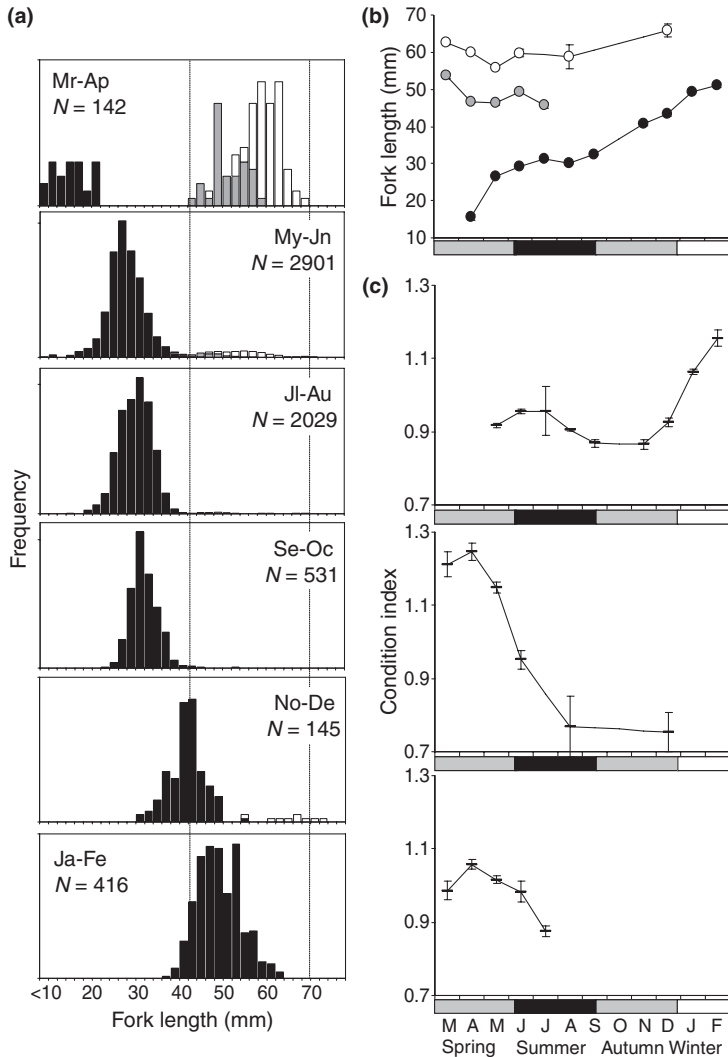


Fig. 2. Seasonal growth and somatic condition of three-spined stickleback. (a) Size frequency distribution of captured stickleback is shown in a bimonthly basis. Histogram intervals are 2 mm. Black bars represent immature (i.e., unsexed) individuals, grey bars represent males and white bars represent females. Broken vertical lines are shown to facilitate the linkage between January–February and March–April data. (b) Monthly evolution of mean (\pm SE) size of stickleback is shown separately for immature individuals (black dots), males (grey dots) and females (white dots). (c) Monthly variation in the mean (\pm SE) somatic condition (measured with the Fulton's condition factor) of immature, female and male stickleback is shown.

clear evidence of 2+ individuals taking part in reproduction events. YOY were first detected in early spring and showed an initial rapid growing phase, attaining 30 mm fork length at the beginning of the summer. However, somatic growth of juveniles almost stopped during summer months (mean fork length in September was only slightly higher than that in June), but increased again in autumn and winter (Fig. 2b). Individuals reaching their first reproductive season also seemed to experience a stop in their somatic growth, as there was no clear temporal increase in adult female or male mean sizes (Fig. 2b).

Fulton's condition factor clearly varied among the different months ($P < 0.001$ for juveniles and females; $P = 0.001$ for males). The somatic condition of juveniles reached minimum values during summer and early autumn months, coinciding with the stop in somatic growth, progressively increasing thereafter until the end of the winter. Adult somatic condition suffered a sharp decline during and after spring-time reproduction

events, both in males and females, reaching minimum levels, as for juveniles, in summer (Fig. 3c).

Habitat gradients and stickleback populations

The PCA of habitat variables produced three habitat gradients (PC1, PC2 and PC3) that altogether explained 60.8% of the variance contained in the original dataset. PC1 described an upstream–downstream gradient; PC2 was related to the presence of submerged vegetation; and PC3 was associated with changes in substrate coarseness (Table 3). There was a clear difference between the positions of occupied and unoccupied locations along PC2 (Table 4), the former having more cobbles and gravels, more filamentous algae and bryophytes, less silty substrates and less woody and leafy debris.

After selecting only locations occupied by stickleback, abundances were positively related to submerged macrophyte cover ($P < 0.001$) and filamentous algae-bryophytes ($P = 0.001$), the proportion of

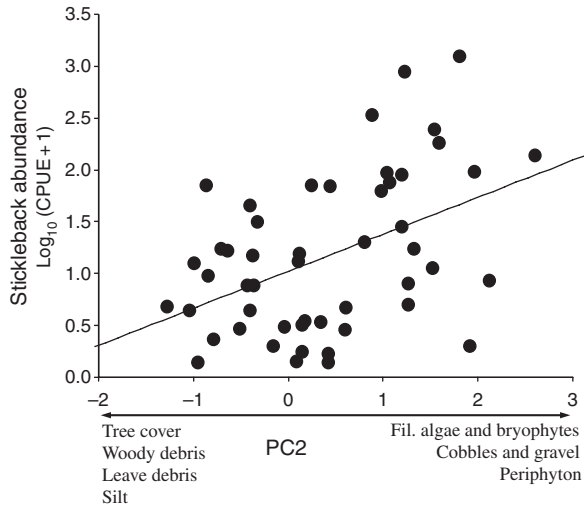


Fig. 3. Relationships between stickleback abundance and the environmental gradient defined by principal component 2. Stickleback abundance tended to increase towards areas with cobble and gravel substrates, abundant submerged vegetation and scarce riverine forest cover.

Table 3. Main environmental gradients (PC1, PC2 and PC3) defined in the study area by means of partial principal components analysis (PCA). The three studied basins were included in the analysis as covariates. Factors loadings with absolute value >0.3 are marked in bold.

Environmental variables	PC1	PC2	PC3
Conductivity	0.50	0.21	-0.12
pH	0.38	0.09	-0.10
Mean width	0.54	0.11	-0.08
Mean depth	0.22	-0.05	0.16
Maximum depth	0.49	-0.14	0.12
Bedrock	-0.49	-0.20	0.31
Boulders	-0.70	0.21	0.35
Cobbles and gravel	-0.60	0.32	-0.12
Sand	-0.14	0.26	-0.78
Silt	0.81	-0.33	0.32
Tree cover	-0.66	-0.52	-0.08
Leafy debris	-0.56	-0.39	-0.08
Woody debris	-0.15	-0.49	-0.06
Roots	-0.58	-0.35	-0.07
Periphyton	-0.15	0.32	0.27
Filamentous algae and bryophytes	-0.32	0.72	0.21
Submerged macrophytes	0.17	0.21	0.12
Helophytes	0.63	0.26	0.07
Natural dykes	-0.68	-0.27	0.30
Eigenvalues	6.27	3.20	2.08
Cumulative per cent of explained variance	33.0	49.8	60.8

boulders ($P = 0.008$) and maximum depth ($P = 0.04$), while they were negatively related to the proportion of silt ($P = 0.05$). As in the presence-absence analyses, the habitat gradient defined by PC2 was strongly and positively related to stickleback abundance (Fig. 3). Stickleback were also found to be less abundant in locations with high crayfish densities (Table 4). The final MAM resulting from the multiple regression analyses explained 27.3% of the variation in stickleback

abundance and included, as significant independent variables, PC2 ($b = 0.38$; $P < 0.001$) and the abundance of invasive species ($b = -0.29$; $P = 0.042$). Thus, after accounting for the relationship between stickleback abundance and PC2 (Fig. 3), the species tended to be scarcer in locations with a high abundance of invasive fish species.

Habitat gradients and fish communities

Only 9 of the original 19 environmental variables were retained in the final solution of the CCA, which, as well as the first axis, the Monte Carlo test proved to be significant ($P < 0.001$ in both cases). The covariates included in the analysis (i.e., the three different basins) accounted for 14.9% of the variation in the species dataset, with environmental variables accounting for a further 31.0%, 72% of which was accounted for in the first and the second axes. The first axis of the CCA (*eigenvalue* = 0.361; 56.6% of the species-environment relationship) clearly segregated locations dominated by invasive fish species from those dominated by freshwater native ones (excluding eel; Fig. 4a). Invasive species dominance was associated to downstream areas, with high water conductivity and proportion of silt, wide channels and low proportion of bedrock (Fig. 4b). Both eel and crayfish had intermediate values along CCA's axis 1, suggesting a random-fashioned use of the upstream-downstream gradient.

The second axis of the CCA (*eigenvalue* = 0.098; 15.4% of the species-environment relationship) was mainly related to the variation in native-dominated communities (Fig. 4c). This axis segregated locations dominated by native cyprinids (barbel and chub), characterised by the high proportion of bedrock and abundant leafy debris (i.e., more forested stretches), from those dominated by stickleback, which were associated with high abundance of submerged vegetation. Stickleback and crayfish scored towards opposite extremes along CCA's axis 2, a segregation that was related to the presence of aquatic vegetation.

Discussion

Population structure, somatic growth and condition

In the study area the stickleback has an almost strictly annual life cycle, with all individuals maturing after their first winter, and most of them dying after reproduction. A small proportion of individuals survived their second summer, as we caught three adult males between August and September and up to nine adult females as late as December, but they did not seem to participate in reproduction events as 2+ fish. In Mediterranean wetlands in southern France,

	Mean absences (<i>N</i> = 41)	Mean presences (<i>N</i> = 50)	<i>t</i> -value	<i>P</i>	Correlation with stickleback CPUE	<i>P</i>
PC1	0.26	-0.07	1.57	0.120	-0.24	0.096
PC2	-0.36	0.41	3.70	>0.001	0.45	0.001
PC3	-0.04	0.00	0.20	0.843	0.11	0.462
Invasive fish CPUE	0.38	0.43	0.37	0.712	-0.21	0.147
Crayfish CPUE	0.49	0.69	1.65	0.103	-0.28	0.049

PC, principal component; CPUE, catch per unit of effort.

Table 4. Comparisons of the mean values of the main environmental gradients in the study area (see Table 4), the abundance of invasive fish and the abundance of red swamp crayfish between sites with and without recorded stickleback presence. On the right side, Pearson correlation coefficients between all these variables and stickleback abundance, using only the 50 sites where stickleback presence had been recorded.

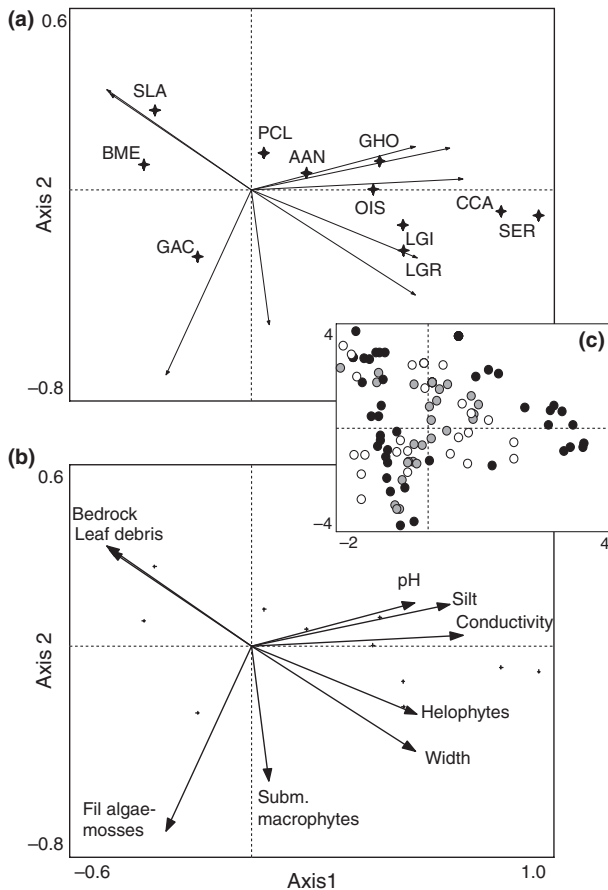


Fig. 4. Graphic summary of the results from the partial canonical correspondence analysis (CCA) relating freshwater fish and crayfish communities to environmental variables in 107 study sites. (a) Species scores in the space defined by CCA axes 1 and 2. Species codes are given in Table 3. (b) Vectors showing the relationships between environmental variables and CCA axes 1 and 2. The nine variables shown were selected through a forward selection procedure from an original pool of 19 variables. (c) Scores of the 107 study sites include in the analysis. Black dots, Daró basin; grey dots, Sèquia de Sils basin; and white dots, Onyar basin.

Crivelli & Britton (1987) described a similarly annual population, but in this case no living adults were recorded after May. A long-term study of a stickleback population inhabiting a backwater in mid-Wales also showed that life cycle was mostly annual, although the proportion of individuals that survived at least until their second autumn was often over 10% (Wootton

et al. 2005). Other works, mostly from more northern locations, have described longer-living stickleback populations. Jones & Hynes (1950) reported both stream and lake populations reaching age 3+ in northern England, while in Iceland sexual maturation was shown to occur only in the third year of life (Sandlund et al. 1992). Baker et al. (2008) described a wide range in the mean breeding age of female stickleback from Alaska, which varied between 1+ and 3–4+. Anadromous stickleback populations also display late maturation, often at age 2+ with a low proportion of 1+ individuals participating in reproduction events (e.g., Dufrense et al. 1990; Baker et al. 2008).

In our study area, stickleback growth was concentrated in two different periods, one in spring and early summer and another in autumn–winter. There was a well-defined low growth phase during summer months (Fig. 2b), coinciding with the harshest period of summer droughts in Mediterranean streams (Gasith & Resh 1999). Growth also seemed to stop after reproduction events (February–April), when mean sizes of both males and females even started decreasing. This latter pattern could be related to earlier reproduction of larger individuals that would afterwards lead to increased mortality rates (Jones & Hynes 1950). Both periods of slow or null body growth coincided with periods of low somatic condition, although patterns in body condition changes were more or less delayed by one month with respect to those in growth rates (Fig. 2b,c).

The growth pattern of stickleback in Mediterranean environments clearly contrasts with those reported elsewhere. In the Camargue wetlands, in southern France and also under Mediterranean climate conditions, stickleback growth slowed during summer, although this pattern was only evident in terms of body weight, as mean body size showed a constant increase at least from April to January (Crivelli & Britton 1987). The summer stop in somatic growth detected in our study area could be highlighting the harsher environmental conditions found in intermittent Mediterranean streams in comparison with the relatively more stable wetlands. In more northern latitudes stickleback growth is concentrated in the first spring and summer of life, almost stopping during winter

(e.g., Pollux et al. 2006). Continuous growth during winter allows stickleback in our study area to reach large sizes at the time of reproduction. In September–October mean length was 32.4 mm, which is slightly larger than individuals from the Camargue (29 mm; Crivelli & Britton 1987) but smaller than those from Wales (36 mm; Wootton 2007) or the Meuse estuary (40 mm; Pollux et al. 2006). However, in February–March stickleback from streams in our study area were clearly larger (mean length for males and females combined was 54.6 mm) than those from the Camargue (39–45 mm, data from two different cohorts) or Wales (41 mm). This high grow rate in Mediterranean locations during winter, especially intense in intermittent streams, could be interpreted as some kind of compensatory growth, which is defined as a high growth rate phase following a period of growth depression (Wootton 2004). This growth depression is typically caused by low food availability (Ali et al. 2003), but in isolated summer pools in Mediterranean streams food shortages are combined with high water temperatures (often above 25 °C) and low oxygen concentrations (Magalhães et al. 2002).

Interestingly, other native freshwater fishes in Iberian Mediterranean streams experience neither a low growth summer phase comparable with that of the stickleback nor a continuous growth phase in winter. In fact, the growth of native cyprinids and cobitids is usually continuous in spring and summer and slows down or even stops during winter (e.g., Torralva et al. 1997; Oliva-Paterna et al. 2002). The freshwater blenny (*Salaria fluviatilis*) shows some growth deceleration during summer, but growth is also reduced in winter (Vinyoles & de Sostoa 2007). The stickleback growth pattern is therefore exceptional among Mediterranean stream fish. Having evolved in colder environments, stickleback are able to grow in the relatively mild Mediterranean winters, but the harsh conditions imposed by summer droughts might put the species near its tolerance limits.

Habitat, communities and stickleback

The most consistent associations between stickleback presence or abundance and habitat features were the preference for stream stretches with an important aquatic vegetation cover and the relative avoidance of both strongly forested stretches and areas where silty substrates dominated (Table 4 and Fig. 3). The selection of areas with abundant aquatic vegetation should be in part linked to the reproductive behaviour of stickleback. At the beginning of the breeding season stickleback males defend a territory where they build a nest, using mainly plant materials glued together with a protein secretion (Bell & Foster 1994). Choice of nest site by male stickleback has been shown to be

variable, although in the presence of predators vegetated sites are usually preferred (Candolin & Voigt 1998) and some works have even noted a strict preference for such vegetated sites (Cleveland 1994). As has been shown for other fish species, YOY may also prefer submerged vegetation, which affords protection from predators (Rodewald & Foster 1998; Clavero et al. 2005) and increased availability of trophic resources (Rozas & Odum 1988; Diehl & Komijow 1998).

The negative relationship between the abundances of red swamp crayfish and stickleback (Table 3) as well as the separation of the two species along CCA axis 2 (Fig. 4) could be mediated by the stickleback's preference for vegetated sites. Red swamp crayfish have a high potential to transform aquatic ecosystems, often reducing dramatically the abundance of submerged macrophytes and leading to strong decreases in water transparency (Gómez-Yurita & Montes 1999; Rodríguez et al. 2005), processes that have been pointed out as one of the main threats for Iberian stickleback populations (Doadrio 2002). However, red swamp crayfish might be unable to achieve high densities in intermittent streams such as those of our study area, and thus, although its negative effects have been detected, there can be a certain level of coexistence between this species and stickleback.

The first axis of the CCA showed a clear segregation of native- and invasive-dominated communities, with the latter occupying mostly lower reaches (Fig. 4). These lower reaches are the only stretches in the area that maintain running waters in summer through artificial inflows (Pou-Rovira et al. 2007). This produces an attenuation of the natural drought and flood cycles in Mediterranean streams, which favours invasive species that have evolved in more stable aquatic environments (e.g., Clavero et al. 2004). The dominance of invasive fish species in Mediterranean areas with altered flow regimes has been noted both in Europe (e.g., Godinho & Ferreira 2000) and California (e.g., Marchetti & Moyle 2001). The high abundance of invasive fish species in the lower reaches of the studied streams could be related to the scarce presence of stickleback in them, and could thus limit their possible role of refugia during summer droughts. This idea is reinforced by the multiple regression model that, after accounting for the effects of environmental gradients, showed a negative relation between the abundances of stickleback and invasive species. Previous local extinctions of stickleback populations have been related to the introduction of invasive fish species, both in the Iberian Peninsula (Fernández-Delgado et al. 2000; García-Berthou & Moreno-Amich 2000) and in North America (Foster et al. 2003; von Hippel 2008). However, the mechanisms involved in the negative effects of invasive

species (e.g., predation, competition, reproductive interference) are often unclear, and deserve further work, ideally in controlled experimental environments.

This work presents the first data on stickleback biology and habitat use in intermittent Mediterranean streams. Mediterranean aquatic ecosystems are highly variable both temporally and spatially and at different scales (e.g., both in intra- and inter-annual bases) (Gasith & Resh 1999). Thus, it would be necessary to obtain comparable data on stickleback biology from different Mediterranean locations, as well as to initiate long-term monitoring programmes (e.g., Wootton 2007) in order to better understand the life-history adaptations of stickleback to such unstable environments. This information should be a useful tool for the conservation of stickleback populations in Mediterranean streams, which have suffered sharp and widespread declines.

The interest to preserve stickleback populations is enhanced by their exceptional value to infer evolutionary processes (Foster et al. 2003). The stickleback is a highly variable taxon that has colonised a wide variety of freshwater environments in multiple colonisation events occurring from millions of years ago until the present (Baker et al. 2008). This has resulted in repeated and independent adaptive radiation from an oceanic ancestor that has given rise to many forms varying in external features and life history (Bell & Foster 1994) and has provided an exceptional case study to analyse evolutionary radiation and speciation (e.g., McKinnon & Rundle 2002; Foster & Baker 2004). Foster et al. (2003) argued that the value of each stickleback population is elevated by its membership in the adaptive radiation. Southern populations of the species are exceptional in that they do not come from postglacial colonisation events and are among the oldest extant freshwater stickleback populations. They should thus be an important component of the adaptive radiation of the species. The current imperilled status of Mediterranean populations should motivate the society to conserve them and avoid further irreplaceable losses within the stickleback evolutionary heritage.

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