

Quantifying seasonality along a latitudinal gradient: from stream temperature to growth of invasive mosquitofish

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Abstract. Most ecosystems undergo substantial variation over the seasons, ranging from changes in abiotic features, such as temperature, light and precipitation, to changes in species abundance and composition. How seasonality varies along latitudinal gradients is not well known in freshwater ecosystems, despite being very important in predicting the effects of climate change and in helping to advance ecological understanding. Stream temperature is often well correlated with air temperature and influences many ecosystem features such as growth and metabolism of most aquatic organisms. We evaluated the degree of seasonality in ten river mouths along a latitudinal gradient for a set of variables, ranging from air and water temperatures, to physical and chemical properties of water and growth of an invasive fish species (eastern mosquitofish, *Gambusia holbrooki*). Our results show that although most of the variation in air temperature was explained by latitude and season, this was not the case for water features, including temperature, in lowland Mediterranean streams, which depended less on season and much more on local factors. Similarly, although there was evidence of latitude-dependent seasonality in fish growth, the relationship was nonlinear and weak and the significant latitudinal differences in growth rates observed during winter were compensated later in the year and did not result in overall differences in size and growth. Our results suggest that although latitudinal differences in air temperature cascade through properties of freshwater ecosystems, local factors and complex interactions often override the water temperature variation with latitude and might therefore hinder projections of species distribution models and effects of climate change.

Key words: climate change; Europe; Iberian Peninsula; invasive fish; latitude; river; season.

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INTRODUCTION

Most regions of the world display seasonal climates, with monthly variation in temperature, photoperiod, precipitation, and many meteorological variables, which in turn imply a myriad of changes in ecosystem processes and the abun-

dance, life history and distribution of organisms. In the tropics, average temperature and photoperiod vary little and seasonality is mostly driven by rainfall, with wet and dry seasons affecting the phenology of tropical plants and the life histories of insects, fish, birds and mammals (e.g., Wolda 1978, Lindstedt and Boyce 1985, Kaspari

and Vargo 1995, Hau 2001, Tedesco et al. 2008a). At higher latitudes, variation in temperature and photoperiod is more important (Bonan 2002, Archibald et al. 2010). The role of seasonality in ecosystem functioning and structure has long been appreciated. However, the strength of seasonality across large geographical and latitudinal gradients is much less understood for freshwater ecosystems. In particular, latitude-dependent variations in water temperature and ecosystem functioning in lowland freshwater ecosystems are not well documented, despite the importance of these factors in predicting global change effects.

For instance, most species distribution models that forecast either biological invasions (e.g., Domínguez-Domínguez et al. 2006, Herborg et al. 2007, Kulhanek et al. 2011) or the potential effects of climate change (e.g., Buisson et al. 2008, Buisson and Grenouillet 2009, Wenger et al. 2011) in freshwater ecosystems use air temperature as one of the key predictors, because water temperatures at large spatial scales are not usually available (see, however, Rahel et al. [1996] and Almodóvar et al. [2012] for some exceptions). Therefore, these models assume that water temperature and its seasonal variation are well represented by air temperature. Although water temperature generally correlates well with air temperature in headwater streams (Webb et al. 2003), this is often not the case across large geographical scales (Hawkins et al. 1997, Lewis et al. 2000) and especially in lowland river stretches, which are often strongly impacted by regulation and water abstraction (Webb and Walling 1997, Poole and Berman 2001). Webb (1996) reviewed trends in stream temperature worldwide during the 20th century and concluded that this variable is not closely related to meteorological variables, but is affected by human influences such as effluent discharges, river impoundment and riparian vegetation removal. Vanicek et al. (1970) showed that the spring-summer temperature in the Green River (Utah, USA) decreased by about 6°C after completion of the Flaming Gorge dam due to the release of cool hypolimnetic waters. Through modelling, Gu and Li (2003) showed that stream temperatures are as sensitive to flow as they are to weather. Rahel et al. (1996) showed that air and stream temperatures have different relation-

ships at different altitudes because at downstream reaches water temperature does not attain the same equilibrium with air temperature, as occurs in upper reaches, due to the greater thermal inertia of higher volumes of water as well as the influence of groundwater. These authors also illustrate how using air temperatures rather than stream temperatures can lead to bias when projecting the effects of climate change.

The possible bottom-up implications of these large-scale variations in stream temperature are not well known either, although temperature is a key abiotic factor in streams. At the ecosystem level, seasonal variation in stream temperature influences the solubility of oxygen in water, photosynthetic and respiration rates, and pH and nutrient concentrations (see e.g., Allan and Castillo 2007). However, latitude has been shown to be weakly related with benthic chlorophyll concentration in streams throughout the USA (Dodds et al. 2002) and not significantly related to primary production (Lamberti and Steinman 1997), benthic respiration, or dissolved and particulate organic matter concentrations (Webster et al. 1995).

Similarly, because most aquatic animals are poikilotherms, stream temperature affects a whole range of life processes, including metabolism, growth rates, life cycles, and distribution (Allan and Castillo 2007). Among 25 European freshwater fishes, however, most life-history traits do not exhibit significant variation with latitude within species (Blanck and Lamouroux 2007). Size-at-age of freshwater fish often does not vary with latitude (Conover and Present 1990, Belk and Houston 2002) and countergradient variation, i.e., the distribution of genotypes in nature in a manner that counteracts environmental influences on the phenotype, has been suggested as a key mechanism for explaining this lack of latitudinal trends in size-at-age data (Conover and Present 1990, Conover et al. 2009). Countergradient variation has been observed in a number of taxa, such as fish, amphibians and insects, and in a number of physiological processes, such as development, growth, feeding, metabolism and activity (Conover et al. 2009).

The objectives of this paper are: (1) to quantify the degree of seasonality along a latitudinal

Table 1. Location and features of the sampling sites.

River basin	Site code	Country	Fish sampling period	Latitude	Longitude	Mosquitofish captured
Vistre	1	France	May–November	43°36'29" N	4°12'46" E	1,359
Orb	2	France	April–November	43°15'51" N	3°18'33" E	1,044
Bordigou	3	France	February–November	42°44'60" N	2°59'40" E	1,559
Fluvià	4	Spain	February–November	42°10'47" N	3°09'07" E	1,114
Ter	5	Spain	February–November	42°01'32" N	3°09'12" E	1,377
Ebro	6	Spain	February–November	40°42'15" N	0°49'31" E	1,281
Millars	7	Spain	February–November	39°55'47" N	0°03'26" W	1,619
Xúquer	8	Spain	February–November	39°10'20" N	0°17'12" W	1,279
Algar	9	Spain	February–September	38°36'26" N	0°02'37" W	1,308
Segura	10	Spain	February–November	38°06'30" N	0°39'17" W	1,521

Notes: See Fig. 1 for a map of the geographical location of the sites. All sampling was conducted in year 2007.

gradient of river mouths for a set of variables ranging from air and water temperatures to limnological features and growth of an invasive fish species; and (2) to analyze the relationships among these variables along the latitudinal gradient. For this purpose, we studied the water features and the growth of mosquitofish (*Gambusia holbrooki*) at 10 river mouths during a 10-month period along a latitudinal gradient of more than 6° along the Mediterranean coast of Spain and France. The eastern mosquitofish *G. holbrooki* is a suitable model freshwater fish species for these objectives because: (1) it is widespread and abundant in southern Europe, in contrast to many other Iberian freshwater fishes, which are often endemic of a few river basins; (2) their life history traits have been considerably investigated; (3) in many of the same localities sampled in this study, it has been previously shown the above-mentioned weak effects of latitude on life-history traits (Benejam et al. 2009, Carmona-Catot et al. 2011), although the cause for these patterns are not known, and (4) it is a warmwater fish, which has not invaded northern Europe so far, and temperature affects its growth, reproduction, competitive ability, and survival (Meffe 1992, Pyke 2005, Benejam et al. 2009, Carmona-Catot et al. 2013). The eastern mosquitofish is a live-bearing poeciliid, native to the eastern USA that has been introduced into freshwater ecosystems worldwide. Mosquitofish produce multiple litters of live newborn during spring and summer, producing large numbers of young per litter and litters approximately every 3–5 weeks (Fernández-Delgado 1989, Reznick et al. 2006); their young can mature and reproduce in the season in which they are born. We therefore hypothesized that, although air tem-

perature should be strongly related to latitude and season, water features and mosquitofish growth might display less latitudinal variation in seasonality and depend more on local factors, in part due to the pervasive anthropogenic alteration of freshwater ecosystems and to the nonlinearity of ecological processes.

METHODS

Study area

The study was conducted at ten Mediterranean stream mouths ranging from southern France to southern Spain (Table 1, Fig. 1). All the sampling sites were less than 5 km from the sea (except Vistre at 10 km) and at an altitude of less than 20 m a.s.l. and had similar habitat features, generally consisting of reed beds (*Phragmites australis*) along their shores, shallow areas (<1.5 m deep), low water velocity, and a fine depositional substrate. Conductivity (mS cm⁻¹), pH and dissolved oxygen (to the nearest 0.01 mg L⁻¹ and %) were measured with a handheld multi-probe meter (YSI 556; Yellow Springs, Ohio, USA). Nitrate (nearest 0.01 mg L⁻¹), nitrite (nearest 0.01 mg L⁻¹), ammonium (nearest 0.01 mg L⁻¹) and phosphate (nearest 0.01 mg L⁻¹) were measured in the field with colorimetric techniques (Visocolor test kits; Macherey-Nagel, Düren, Germany). Temperature loggers (ACR SmartButton; Vancouver, Canada) were deployed to monitor hourly water temperature in all sampling sites but some were lost due to floods or malfunction and only five sites yielded more complete time series and were finally used in the analyses. Hourly air temperature data were obtained from the closest weather stations to the sampling sites (generally

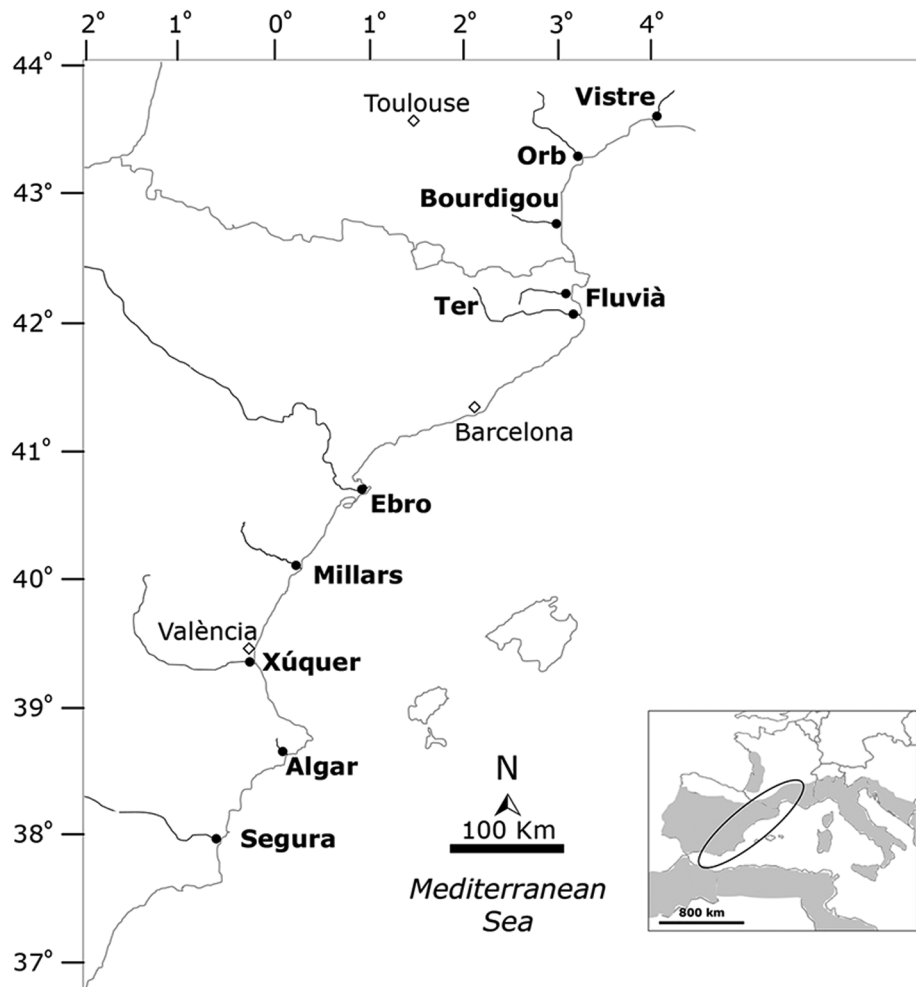


Fig. 1. Study area with the location of the sampling sites (full circles) at the ten Mediterranean river basins studied. The empty diamonds indicate large cities. The inset map shows the current distribution of mosquitofish in the western Mediterranean region (modified from Krumholz 1948).

<10 km) and were provided by Météo France and the Instituto Nacional de Meteorología (Spain). Drainage area and agricultural land use for the sampling sites were respectively obtained from Vogt et al. (2007) and the Corine Land Cover 2006 raster data (100 m) from the European Environment Agency (<http://www.eea.europa.eu/data-and-maps>).

Field and laboratory methods

Sampling was conducted monthly from February to November 2007 and samples from all sites were taken within 10 days of each month to assess the effects of seasonal variation along the

latitudinal gradient. A total of 13,036 invasive mosquitofish were collected along the shoreline using dip nets (60 cm diameter; 1-mm mesh) and preserved in situ with 96% ethanol. In the laboratory, the sex of the mosquitofish was determined by the morphology of the anal fin and by examination of the gonads (Turner 1941, Strange 1996). All the mosquitofish were measured (standard length to the nearest 0.01 mm) with a digital calliper.

The age of the mosquitofish was determined either by analyses of their scales or from length-frequency distributions. Scales were collected for analysis from the same zone below the end of the

dorsal fin of the fish; regenerated and broken scales were not included in these analyses. Six to eight scales per fish were mounted on slides and analyzed using a dissecting microscope. In a previous paper (Appendix S1 in Carmona-Catot et al. [2011]), we provide photographs of scales, with ageing criteria, for some of the same mosquitofish populations. Mosquitofish populations are invariably dominated in number (generally 85–100% of the catch) by young-of-the-year (YOY) that are a few weeks or months old; fish with an annulus (age 1) are generally observed in spring and presumably correspond to fish born in the previous summer; female mosquitofish with two annuli (age 2) are very rare (<1% of the catches) (Tedesco et al. 2008b, Carmona-Catot et al. 2011). Previous studies into length-frequency distributions of mosquitofish populations (Krumholz 1948, Fernández-Delgado and Rosso-manno 1997) described two main cohorts: one consists of individuals that are born in spring, reproduce within a few weeks and mostly die when they are few months old; the other is made up of individuals that are born in summer, are able to overwinter, reproduce early in spring and mostly die before the following summer. Very few individuals are able to survive two winters and thus have two annuli on their scales. An example of the two main cohorts (largely based on an analysis of scales) in length-frequency distributions is shown in Appendix: Fig. A3. Taking this into account and to save time, the age of the mosquitofish was derived from the length-frequency distributions for most individuals (12,140 fish, mostly age 0, i.e., less than one year old) and directly from the scales for a subsample (896 fish, particularly large-sized individuals that could be age 1 or 2). For all statistical analyses of the length-at-age data, the age estimated from scales (0, 1 or 2 years) was converted to fractional age by taking into account the capture date and the estimated birth date of the cohort; we assumed that fish captured in autumn or overwintering were born on 1 August and YOY fish captured in spring or early summer 2007 were born on 1 May, 2007 (e.g., a YOY fish captured on 1 June was 0.085 years old).

Data analyses

Variation partitioning was employed, using the “varpart” function in the “vegan” package

(Oksanen et al. 2010) of the R software environment (R Development Core Team 2012) in order to decompose the variation of the environmental variables into the two controlled factors (latitude and season) and their shared variation. Variation partitioning uses a series of partial regression analyses to estimate how much variation of the response variable is explained by a set of predictors, while controlling for another set (Legendre and Legendre 1998). Variation partitioning led to the following four fractions: a unique effect of latitude (independent of season); a unique effect of season; a joint or shared effect of latitude and season; and unexplained variation. The different partitions explained by the predictors were evaluated by ANOVA-like permutation tests, which have fewer assumptions than parametric methods, using the function “anova.rda” from the vegan package (Peres-Neto et al. 2006, Oksanen et al. 2010).

Spearman’s correlation analysis (r_s) was used to examine the relationships between the environmental variables and latitude. A principal components analysis (PCA) was conducted using the “rda” function of the vegan package to summarize the relationships among the water feature variables. The “envfit” function (Oksanen et al. 2010) was used to project month (categorical factor), latitude, percentage of agricultural use, and basin area as supplementary variables in the PCA biplot to help interpretation. Partial redundancy analysis (partial RDA), which is a multivariate analogue of partial linear regression (Borcard et al. 2011), helped to explain the effects of latitude on the set of water feature variables, after accounting for season (months). For all of these analyses, conductivity and basin area were $\log_{10}(x)$ transformed, while all the concentration variables (except O_2) were $\log_{10}(x + 1)$ transformed because in this way, the linearity and homoscedasticity assumptions of statistical analyses (linear regression analyses and variation partitioning) were better satisfied.

We used generalized additive models (GAMs) to test for site effects in the relationship between the standard length (dependent variable) and age (continuous independent variable) of the mosquitofish, i.e., to find out whether site-specific growth models were necessary. GAMs were applied using the R package “gam” (Hastie 2011), separately for female and male data

because of the obvious sexual size dimorphism (see Appendix: Figs. A5 and A6). GAMs are a semi-parametric extension of generalized linear models, which allow for highly non-linear relationships (such as, for example, in seasonal growth) between the response and the predictors by using smoothing functions whose complexity is automatically controlled (Hastie and Tibshirani 1990). We used the Akaike information criteria (AIC) and likelihood ratio tests to compare the fit of the models (see, e.g., Crawley 2007). The model that indicated a site effect on growth had the best fit (Table 4) and showed significant effects of site in the relationship between size and age. Therefore, we additionally modelled fish growth separately for each site along the latitudinal gradient. The FSA package (Ogle 2011) of the R environment was used to estimate Somers' (1988) seasonal version of the von Bertalanffy growth curve:

$$L_t = L_\infty \left(1 - \exp\left(-K(t - t_0)\right) - (CK/2\pi)\sin\left(2\pi(t - t_s)\right) + (CK/2\pi)\sin\left(2\pi(t_0 - t_s)\right) \right)$$

where L_t is the expected or average length at age or time t ; L_∞ is the asymptote for average length; K is a measure of the exponential rate of approach to the asymptotic length L_∞ (units are yr^{-1}); t_0 is the theoretical age (generally negative) at which the average length would be zero; C modulates the amplitude of the growth oscillations; and t_s is the time between time 0 and the start of the convex portion of the first sinusoidal growth oscillation (i.e., the inflection point). C corresponds to the proportion of decrease in growth at the depth of the oscillation (i.e., 'winter'): if $C = 0$, there is no seasonal oscillation and Somers' model reduces to the typical von Bertalanffy growth; if $C = 1$, growth completely stops at the "winter-point" ($\text{WP} = t_s + 0.5$); if $0 < C < 1$, growth during the winter is reduced but not stopped; if $C > 1$, average size decreases in winter, which might be due to size-dependent mortality. Somers (1988) is probably the most widely used model to describe seasonal growth in fish (see García-Berthou et al. [2012] for further explanation and examples for estimating this model with the R environment). The results for Somers' model are only reported for females

because, in general, it did not fit well to the male data, due to a more limited range of ages for males. To avoid convergence and computation problems, and to improve the estimation accuracy of the Somers' model, we used the "nls2" function of the "nls2" package (Grothendieck 2010), with a wide range of plausible values as starting values. We estimated the R^2 of the nonlinear models with function "Rsqr" of package "qqPCR" (Spiess and Ritz 2012). The growth performance index (Φ') was also calculated from the growth parameter estimates as it facilitates the comparison of growth rates because K and L_∞ estimates are often negatively correlated (Pauly and Munro 1984):

$$\Phi' = \log_{10}K + 2\log_{10}L_\infty.$$

Finally, specific growth rates (GR) in winter (from February to March) were calculated with the usual formula:

$$\text{GR} = 100 \left(\log_e(L_2/L_1) \right) / t,$$

using the lengths at the beginning (L_1) and at the end (L_2) of a period of t days (days between sampling dates).

Pearson's correlation and multiple linear regression analyses were used to test for relationships between the different growth variables and predictors (air and water monthly temperatures and latitude).

RESULTS

Seasonality effects on abiotic features along the latitudinal gradient

Seasonality explained most (92.0%) of the variation in air temperature (Fig. 2), with only 6.2% explained by latitude and 2.7% unexplained (Table 2). In contrast, seasonality was relatively less important (45.2%) and latitude more (25.3%) in explaining variation in water temperature, with a larger proportion (29.5%) remaining unexplained (Fig. 2). Seasonality explained variation in oxygen variables and pH better than latitude, but the opposite was true for conductivity and nutrient concentrations, the latter being significantly negatively correlated with latitude (see Table 3). The percentage of unexplained variation was greater than for explained variation for all variables, except water and air temperatures and nitrate concentration (Table 2). Contrary to what is

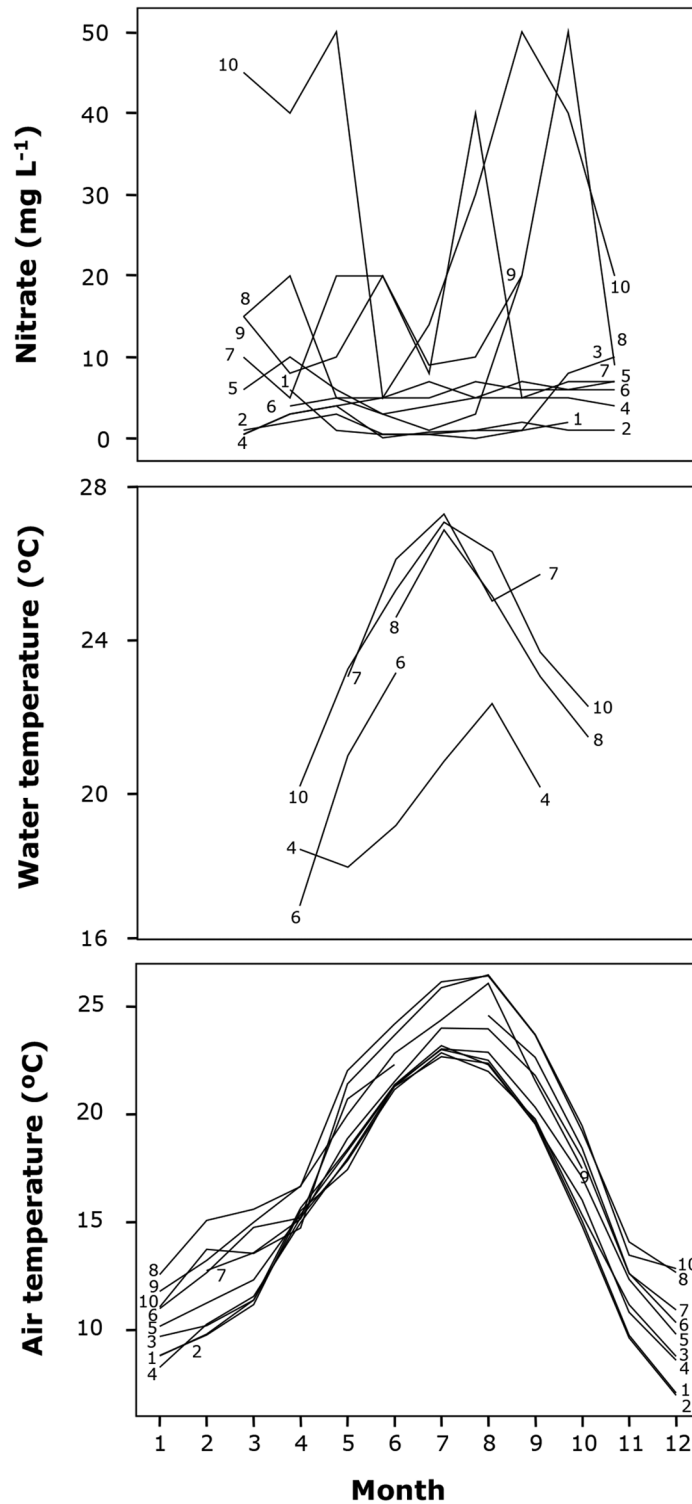


Fig. 2. Seasonal variation in nitrate concentration and water and air temperatures along the latitudinal gradient from January (Month 1) to December 2007. The numbers for the different lines indicate the sampling sites, from the northernmost (Site 1) to the southernmost (Site 10) (see Table 1 for further details on the site codes).

Table 2. Variation partitioning of air temperatures and limnological features of the study sites into: unique effects of season, unique effects of latitude, joint effects of latitude and season, and unexplained variation.

Response variable	Unique season (%)	<i>P</i>	Unique latitude (%)	<i>P</i>	Joint effects (%)	Unexplained (%)	<i>n</i>
Air temperature (°C)	92.0	0.001	6.20	0.001	0.00	2.7	113
Water temperature (°C)	45.2	0.001	25.33	0.001	0.00	29.5	29
Conductivity (mS cm ⁻¹)	0.0	0.986	3.30	0.060	0.00	100.0	93
pH	14.8	0.006	0.00	0.619	0.06	85.9	93
O ₂ (%)	10.1	0.033	0.00	0.688	0.00	90.9	93
O ₂ (mg L ⁻¹)	24.3	0.001	0.00	0.322	0.00	76.4	93
Ammonium (mg L ⁻¹)	0.0	0.924	6.32	0.021	0.00	100.0	85
Nitrate (mg L ⁻¹)	2.8	0.097	65.58	0.001	0.00	38.7	85
Nitrite (mg L ⁻¹)	1.0	0.339	34.81	0.001	0.00	67.4	85
Phosphate (mg L ⁻¹)	5.3	0.069	31.68	0.001	0.00	66.0	85

Notes: Negative percentages of explained variance (which sometimes occur with this statistical technique [Peres-Neto et al. 2006]) are indicated as zero. Permutation tests allow to analyze the significance (*P* value) of the pure fractions (*P* values < 0.05 in boldface); joint effects cannot be tested. Conductivity was log₁₀(*x*) transformed, and all the concentration variables (except O₂) were log₁₀(*x* + 1) transformed. *n* = sample size.

frequently observed when using variation partitioning (e.g., Boix et al. 2010), the joint effects (of season and latitude) were in all cases approximately zero, indicating that season and latitude acted independently of each other across the latitudinal range studied and that seasonal variation was similar in all cases.

The variance accounted for by the first two axes of the PCA of the water properties was 67.4% (see Appendix: Fig. A1). The first axis showed that O₂ correlated negatively with nutrient concentrations and conductivity. The second axis presented a positive correlation between air and water temperatures and pH. The projection of supplementary variables indicated that latitude explained most of the variation (47.8%) and was mainly related (negatively) to the first axis (i.e., nutrient concentrations), whereas the other three supplementary variables were related to the second axis; moreover,

agricultural land use or basin area were not related to latitude (Table 3). Seasonal variation was displayed in the factorial space as a circular trajectory, with the lowest temperatures within the study period with complete data being in May and October and the highest in July and August; the highest nutrient concentrations and lowest O₂ were in August and September. When the effects of season were removed with a partial RDA, latitude correlated highly with both air and water temperatures (Appendix: Fig. A2). Nutrient concentrations also correlated with latitude but there was a large proportion of unexplained variation. Oxygen variables and pH were not related to latitude (Table 3).

Seasonality effects on fish growth along the latitudinal gradient

The GAMs fitted to the relationships between standard length and age (Appendix: Figs. A5 and

Table 3. Correlation matrix of the average of environmental variables along the latitudinal gradient: Spearman's coefficient below the diagonal and *P* value above; significant correlations appear in boldface.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Latitude (°N)		0.21	0.80	0.03	0.26	0.40	0.91	0.85	0.99	0.74	0.00	0.01	0.05
2. Basin area (km ²)	-0.43		0.91	0.80	0.07	0.53	0.31	0.91	0.70	0.24	0.13	0.37	0.88
3. Agricultural land use (%)	0.09	-0.04		0.70	0.64	0.40	0.40	0.35	0.29	0.51	0.85	0.63	0.53
4. Air temperature	-0.70	0.09	0.14		0.46	0.99	0.96	0.47	0.40	0.60	0.02	0.01	0.00
5. Water temperature	-0.42	0.63	0.18	0.28		0.43	0.90	0.73	0.64	0.09	0.04	0.02	0.67
6. Conductivity (µS cm ⁻¹)	-0.30	0.22	-0.30	-0.01	-0.30		0.47	0.21	0.06	0.27	0.83	0.99	0.23
7. pH	0.04	0.36	0.30	0.02	0.05	-0.26		0.01	0.03	0.48	0.93	0.80	0.58
8. O ₂ (%)	-0.07	-0.04	0.33	0.26	-0.13	-0.43	0.75		0.00	0.13	0.88	0.83	0.91
9. O ₂ conc.	-0.01	-0.14	0.37	0.30	-0.18	-0.61	0.70	0.95		0.07	0.93	0.91	0.75
10. Ammonium conc.	-0.12	0.41	0.24	0.19	0.60	0.39	-0.26	-0.52	-0.60		0.56	0.17	0.29
11. Nitrate conc.	-0.94	0.52	-0.07	0.73	0.70	0.08	-0.03	0.05	0.03	0.21		0.00	0.07
12. Nitrite conc.	-0.76	0.32	0.18	0.79	0.73	-0.01	-0.09	-0.08	-0.04	0.47	0.83		0.04
13. Phosphate conc.	-0.62	0.05	-0.22	0.82	0.17	0.42	-0.20	-0.04	-0.12	0.37	0.60	0.65	

Notes: temp. = temperature in °C; conc. = concentration in mg L⁻¹; *n* = 10 (except when water temperature is involved: *n* = 9).

Table 4. Comparison of additive models of mosquitofish growth.

Model	R^2_{adj}	AIC	Residual df	F	P
Females					
1. SL ~ Age	0.515	53462	9117.1		
2. SL ~ Age by site	0.557	52695	9052.4	14.445	<0.0001
Males					
1. SL ~ Age	0.431	28955	5310.9		
2. SL ~ Age by site	0.482	28507	5266.1	12.507	<0.0001

Notes: See Appendix: Figs. A5 and A6 for the data. Additive models were fitted separately for females ($n = 9126$) and males ($n = 5319$) due to sexual dimorphism. Model 1 is a single smoother function for all sites and Model 2 is a separate smoother for each site. The adjusted R^2 , Akaike information criteria (AIC) and F statistics to compare the two alternative models are given.

A6) were significantly better when the sampling site was added as a categorical factor to the models (Table 4), i.e., when a different model was used for each site. The R^2 of fitted Somers' (1988) models (Fig. 3) ranged from 0.32 to 0.75, being always ≥ 0.43 except for Site 3 (Table 5). The C parameter estimates (i.e., relative amplitude of the growth oscillations) of the Somers' model were nonlinearly related to latitude ($R^2_{adj} = 0.474$; with lower AIC for a quadratic model than for a linear model; Fig. 4). These C estimates were much higher for the two northernmost populations (Fig. 4, Table 5), implying a clear decrease in the mean length-at-age of females during winter, in contrast to the other sites, which had values below or closer to 1 (i.e., growth ceases or is reduced in winter). This is also observed in the fitted Somers' models, for which a winter decrease in mean length-at-age is observed for Sites 1 and 2 whereas growth is less seasonal for the southernmost populations, i.e., Sites 7–10 (Fig. 3). A similar pattern was suggested by the GAM models for both females and males (see Appendix: Figs. A5 and A6).

Although the growth rates of mosquitofish in winter seemed related to latitude (r^2 was 0.495 for females and 0.348 for males; see Appendix: Fig. A7) and the northernmost mosquitofish (Sites 2–5) barely grew during this period, the differences in size vanished at the end of the spring (Appendix: Fig. A8). These results suggest a delayed but faster growth rate among northernmost individuals in the spring, compensating for a shorter growing season. This interpretation is also supported by the marginally significant

relationship of the growth performance index (Table 5) with latitude (females, $r = -0.58$, $P = 0.082$, $N = 6$).

DISCUSSION

Seasonality of abiotic features along the latitudinal gradient

Our data illustrate that air temperature varies strongly with season (92.0% of explained variation) and that latitude, and probably other spatial gradients, explain most of the rest of this variation. As much as 97.3% of the variation in air temperature was explained by month and latitude in a gradient of sites at very low altitudes and small distances from the sea. In our study, ranging over 6° of latitude, we did not observe a joint effect of season and latitude, although this might be observed over a larger latitudinal gradient, since the difference between maximum and minimum mean temperatures is lowest in the tropics and highest at mid-latitudes (Chown et al. 2004).

By contrast, water temperature was less seasonal (45.2% of explained variation). Compared to air temperature, a greater proportion of variation in water temperature was explained by latitude (25.3%) after accounting for seasonality, probably because of the higher thermal inertia of water compared to the air. The percentage of unexplained variation in water temperature (29.5%) was also much higher, suggesting a substantial influence of the unique characteristics of each watershed and its local factors. In our study, mean water temperature was poorly correlated with air temperature and basin descriptors such as basin area or agricultural land use. Stream temperature depends on a multitude of natural and anthropogenic factors at multiple scales (Webb 1996, Caissie 2006). In addition to climatic factors, such natural factors include the source of the water, groundwater influences, discharges, channel form and orientation and riparian canopy (Hawkins et al. 1997, Lewis et al. 2000, Poole and Berman 2001). Anthropogenic perturbations such as effluent discharges, river impoundment and riparian vegetation removal can profoundly alter stream temperature (Webb 1996). Although water temperature is in general well correlated with air temperature in headwater streams (Webb et al. 2003), previous studies have already shown that

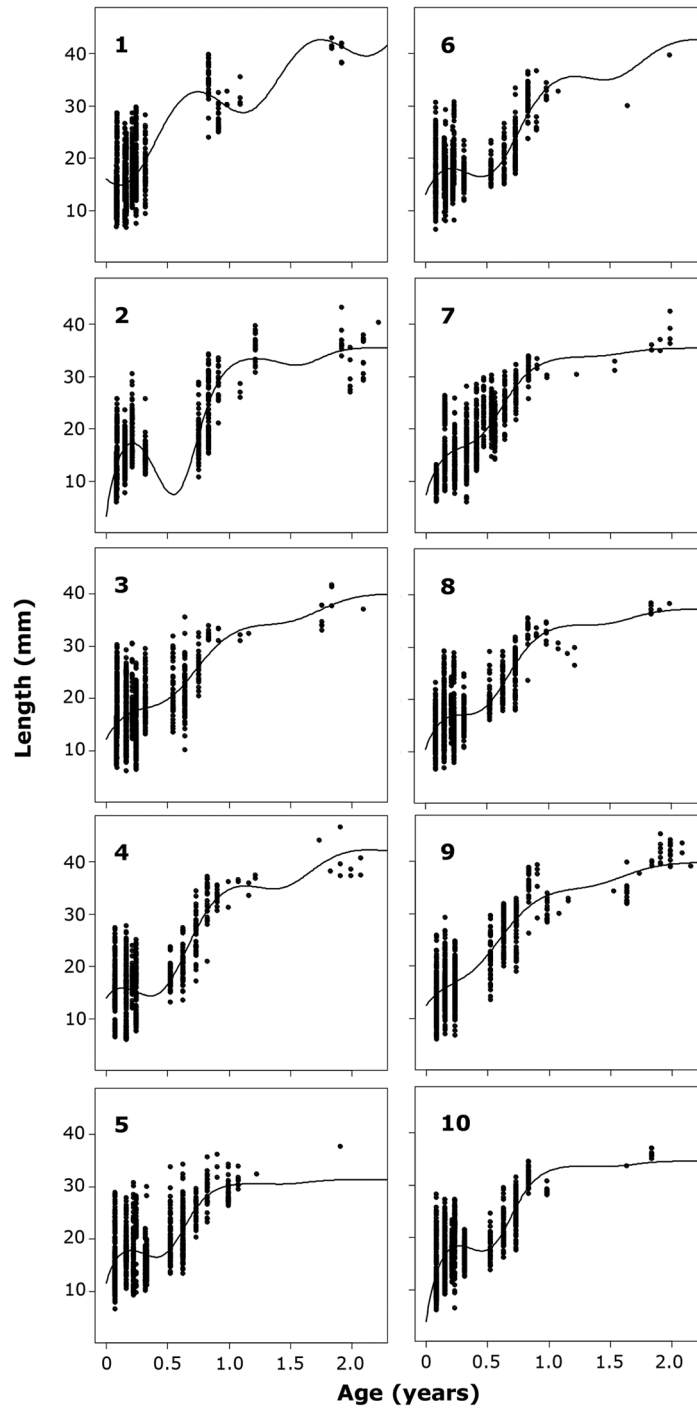


Fig. 3. Length-at-age data of female mosquitofish along the latitudinal gradient. The points are the observed data and the lines are the fitted Somers' (1988) seasonal growth models. Age estimated from scales was converted to fractional age by considering the sampling date and the estimated birth date of the cohort. The numbers in the top-left corner are the site codes (see Table 1), from the northernmost (Site 1) to the southernmost (Site 10). The parameter estimates of Somers' (1988) model are given in Table 4.

Table 5. Growth parameters of female mosquitofish along the latitudinal gradient, as estimated using the Somers (1988) seasonal version of the von Bertalanffy model.

Site	L_{∞}	K	T_S	C	R^2	n	Φ'
1	78.33	0.25	0.43	2.42	0.53	968	3.19
2	35.81	2.05	0.88	1.96	0.69	751	3.42
3	43.35	1.00	0.82	0.77	0.32	1125	3.27
4	46.06	1.04	0.25	1.32	0.59	686	3.34
5	31.43	2.79	0.80	1.24	0.43	958	3.44
6	47.43	0.91	0.83	1.36	0.43	837	3.31
7	35.63	2.35	0.29	0.79	0.58	921	3.47
8	37.93	1.73	0.80	0.97	0.55	913	3.40
9	41.71	1.28	0.71	0.61	0.75	887	3.35
10	34.67	2.74	-0.14	1.18	0.56	1080	3.52

Notes: n = number of individuals. The growth performance index (Φ') was calculated from L_{∞} and K (see text for formula). See Table 1 for site codes.

this is often not the case, particularly at large spatial scales and in lowlands (Hawkins et al. 1997, Lewis et al. 2000). Hawkins et al. (1997) in a study of temperate streams across a latitudinal gradient of 6°, which was very similar to our gradient, showed that water temperatures were barely related to latitude and in fact, were more related to differences in geomorphology and hydrology. Similarly, a recent review shows that

local trends in water temperature of western North American streams do not follow trends in air temperature (Arismendi et al. 2012). The effects of reservoirs on stream temperature are complex and include direct and indirect effects that depend on dam operation and environmental and spatial contexts (Webb and Walling 1997, Olden and Naiman 2009). Anthropogenic alteration of stream temperature may well be pervasive in Mediterra-

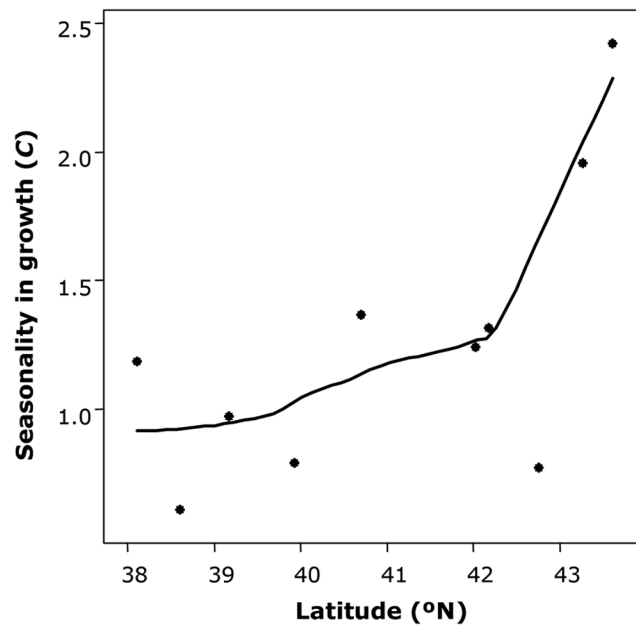


Fig. 4. Seasonality (C parameter of Somers' model) of female mosquitofish growth along the latitudinal gradient, with a fitted locally weighted regression smoother. The fitted Somers' (1988) growth models are shown in Fig. 3 and the parameter estimates in Table 4. C indicates the degree of seasonality: if $0 < C < 1$, there is reduction but not a complete cessation of growth in winter; $C = 1$ indicates complete cessation of growth in winter; and $C > 1$ indicates decreases in average size in winter (e.g., due to size-dependent mortality).

nean countries such as Spain, where over 1200 large reservoirs are in use (Carol et al. 2006) and where water abstraction is widespread (Benejam et al. 2010, Sabater and Barceló 2010). A detailed study in the Tordera stream (Benejam et al. 2010) shows that water abstraction and other local anthropogenic perturbations have profound effects on water thermal regimes (E. García-Berthou, *unpublished data*). Our results support the idea that water temperature data, monitoring and modelling should be more readily available (Webb 1996, Hawkins et al. 1997, Caissie 2006), particularly at large spatial scales, in lowlands and dry regions, to compensate for an over-reliance on air temperature data which is extensively used in modelling biological invasions and climate change effects in freshwater ecosystems.

Variations in nutrient concentrations were less well explained by latitude and seasonality than was the case with temperature variations. Nevertheless, nutrient concentrations were negatively related to latitude (as in Dodds et al. 2002), probably because the sites further south are more affected by water scarcity, altered stream flow and pollution (Sabater and Barceló 2010). Oxygen concentrations and pH were more related to season, with higher diurnal values in spring and early summer, as would be expected from higher photosynthetic activity due to the growth of macrophytes and algae (Cox 2003, Desmet et al. 2011), which was evident in the field.

To sum up, although seasonality in air temperature was very strong and similar across the latitudinal gradient we studied, it was found to be weak for other variables, including water temperatures, which were better explained by latitude and other unmeasured local factors. Similarly, seasonality was observed in oxygen concentrations and pH, but was very weak for nutrient concentrations and, although the latter displayed some latitudinal trends, most of the variation in water chemistry was related to other local factors.

Seasonality of fish growth along the latitudinal gradient

Since fish and most stream animals are poikilotherms, ambient temperatures significantly affect their physiological rates and, consequently, their growth rates. Seasonal variation in fish growth is generally related to changes in

water temperature and food resources (Magnuson et al. 1979, Wood and McDonald 1997). We observed seasonal growth in all mosquitofish populations, but stronger seasonality in the two northernmost populations. However, the relationship of growth seasonality with latitude was weak and nonlinear, as has been recently observed in other life-history traits of invasive mosquitofish (Benejam et al. 2009, Carmona-Catot et al. 2011). Previous studies have shown that latitude does affect seasonality of certain other life-history traits of mosquitofish, such as length of the reproductive season and lipid storage (Reznick and Braun 1987, Meffe and Snelson 1993).

We also observed a weak relationship between latitude and winter growth rates and overall growth performance and that any differences in size at the onset of the growing season were small and disappeared during spring. These patterns suggest that, although the growing season is shorter in the north, this does not translate into overall smaller growth rates. Since more food resources do not seem to be available in the north (perhaps even the opposite; given the significant negative relationship between nutrients and latitude and from personal observations), our results suggest that there is countergradient variation in mosquitofish growth (i.e., that northern populations have been selected to compensate for the shorter growing season with higher growth rates). However, common garden experiments or transplanting studies are needed to fully demonstrate countergradient variation, although it is known to be widespread in freshwater fish (Conover and Present 1990, Conover et al. 2009).

Although we were not able to continue the time-consuming monthly sampling and laboratory work for more than one year, many of the same localities sampled in this study were also sampled in 2004 and 2005 (Benejam et al. 2009, Carmona-Catot et al. 2011), in which we also showed weak effects of latitude on life-history traits of mosquitofish, reinforcing the reliability of our results. Moreover, up to three cohorts of mosquitofish were present in our data, so the seasonality in growth is modelled for several cohorts, which are similar to replicates.

In conclusion, although air temperature is strongly seasonal, dependent on latitude, and

less dependent on local factors, our study suggests that lowland freshwater ecosystems behave quite differently: many stream properties such as water temperature, nutrient concentrations, and fish growth display less seasonal variation, depend more on local factors (such as water abstraction, point source pollution, and other anthropogenic perturbations) and also on latitude, but in a non-linear, complex way (e.g., latitude might increase the effects of pollution through water scarcity). In contrast to terrestrial ecosystems, we know very little about the functioning of freshwater ecosystems across large spatial gradients, including easily measured variables such as water temperature (Hawkins et al. 1997, Lewis et al. 2000, Arismendi et al. 2012). The reliance on air temperature data in climate change research and species distribution modelling of freshwater ecosystems and organisms should be carefully reassessed and further efforts should be made to increase the availability of physical and chemical data (other than water-flow) from freshwater ecosystems at large spatial scales, particularly in lowland areas.

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SUPPLEMENTAL MATERIAL

APPENDIX

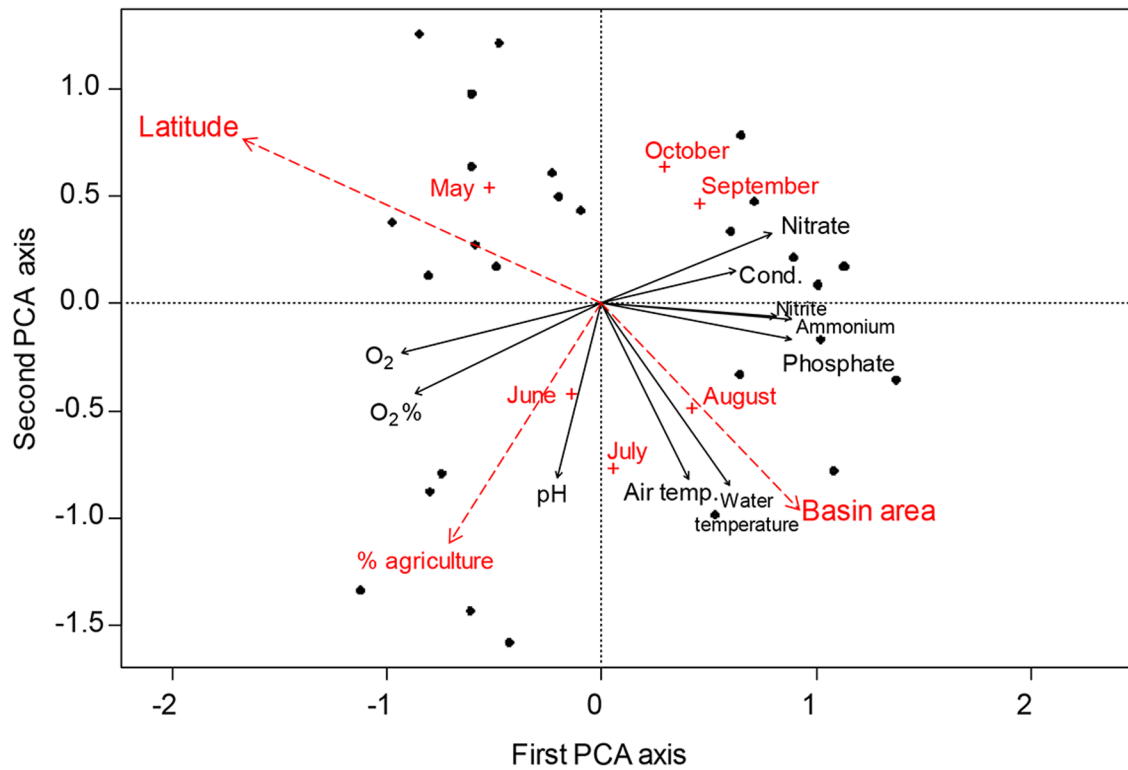


Fig. A1. Principal components analysis of the environmental variables along the latitudinal gradient. The black dots correspond to the site scores and continuous black arrows to the loadings of variables. Latitude, basin area, % agricultural land use, and sampling month (in red) were added as supplementary variables after the PCA to help interpretation; the four projected variables were significant (permutation tests, $P < 0.05$). The first PCA axis explains 46.5% of the variation and the second 21.0%.

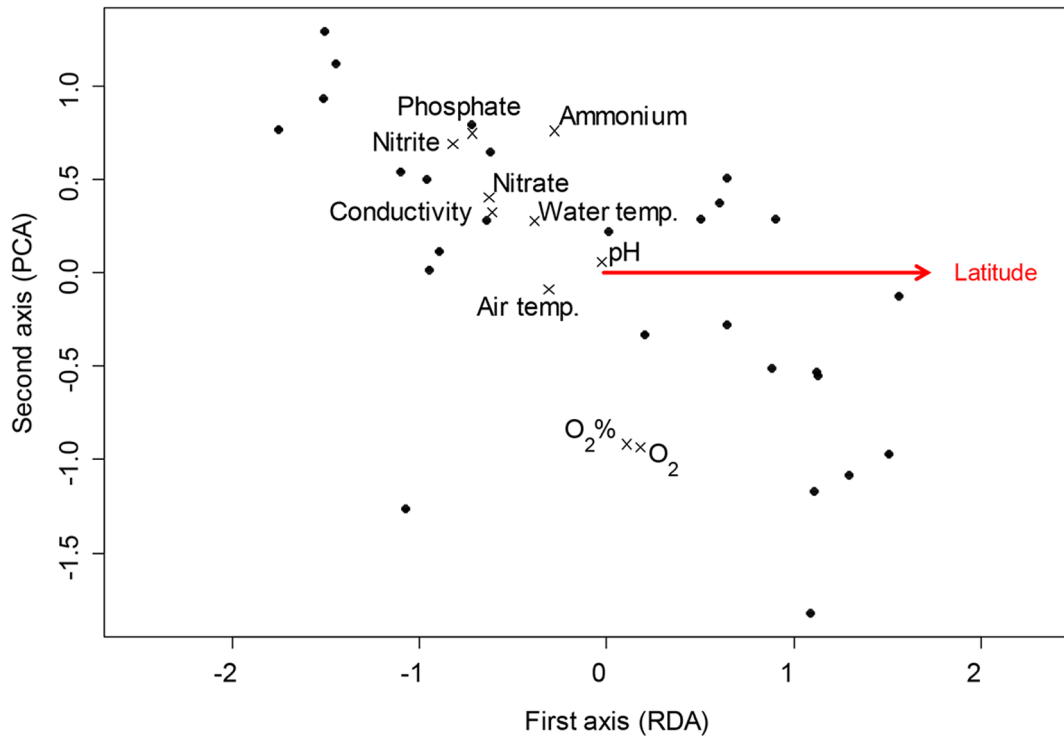


Fig. A2. Partial RDA of the environmental variables with latitude (arrow, in red) as explanatory variable, after accounting for the effects of basin area, % agricultural land use, and sampling month (covariates or conditioning variables). The black dots correspond to the site scores and crosses to response variables. 41.3% of the variation was explained by the conditioning variables, 14.4% by the first RDA axis (latitude), and 44.3% was unexplained.

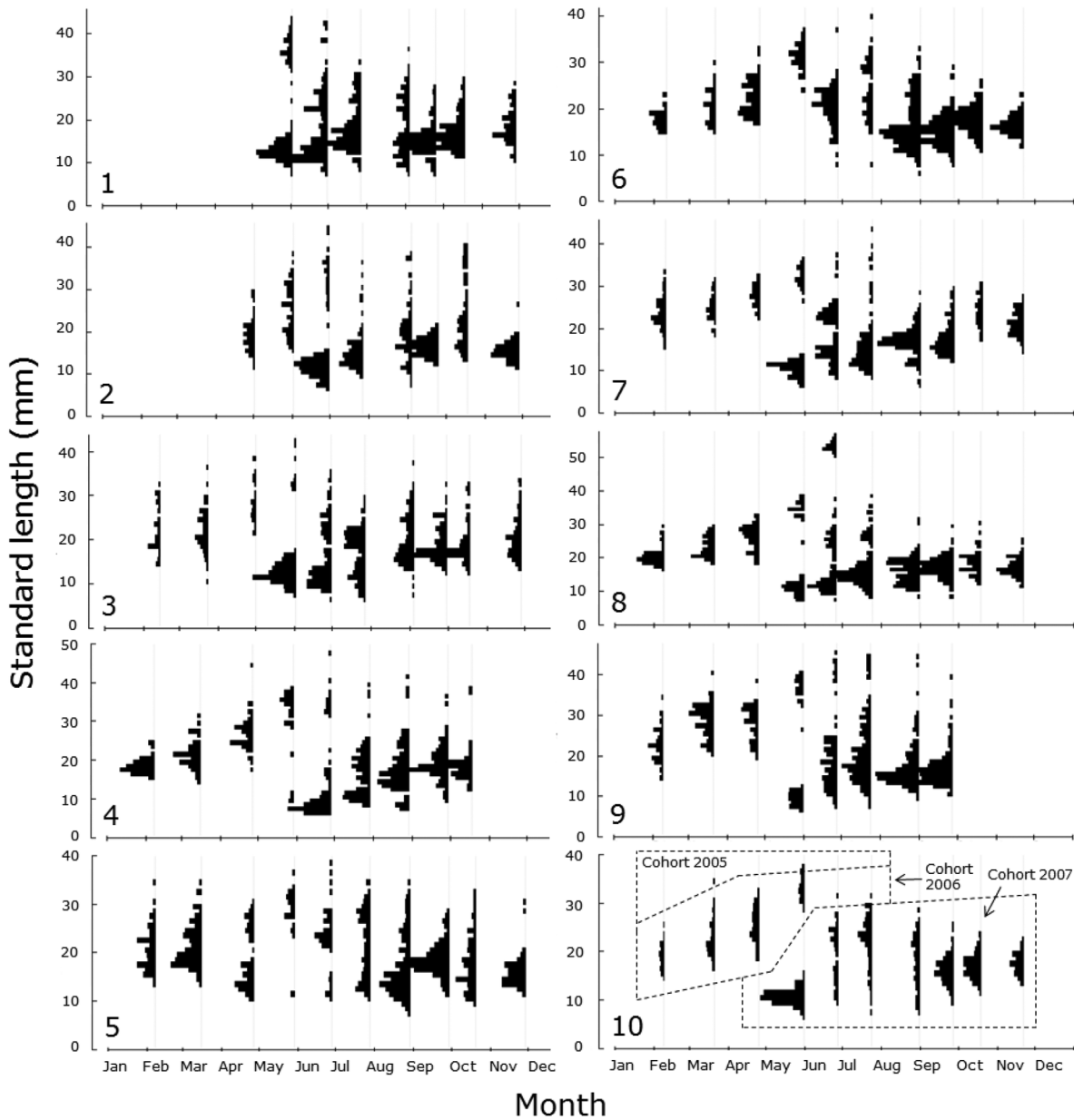


Fig. A3. Monthly length–frequency distributions of female mosquitofish by site. The numbers at the left-bottom corners are the site codes (see Table 1). The distribution for Segura (bottom-right, site code = 10) is used as an example to show the mosquitofish cohorts according to aging from scales.

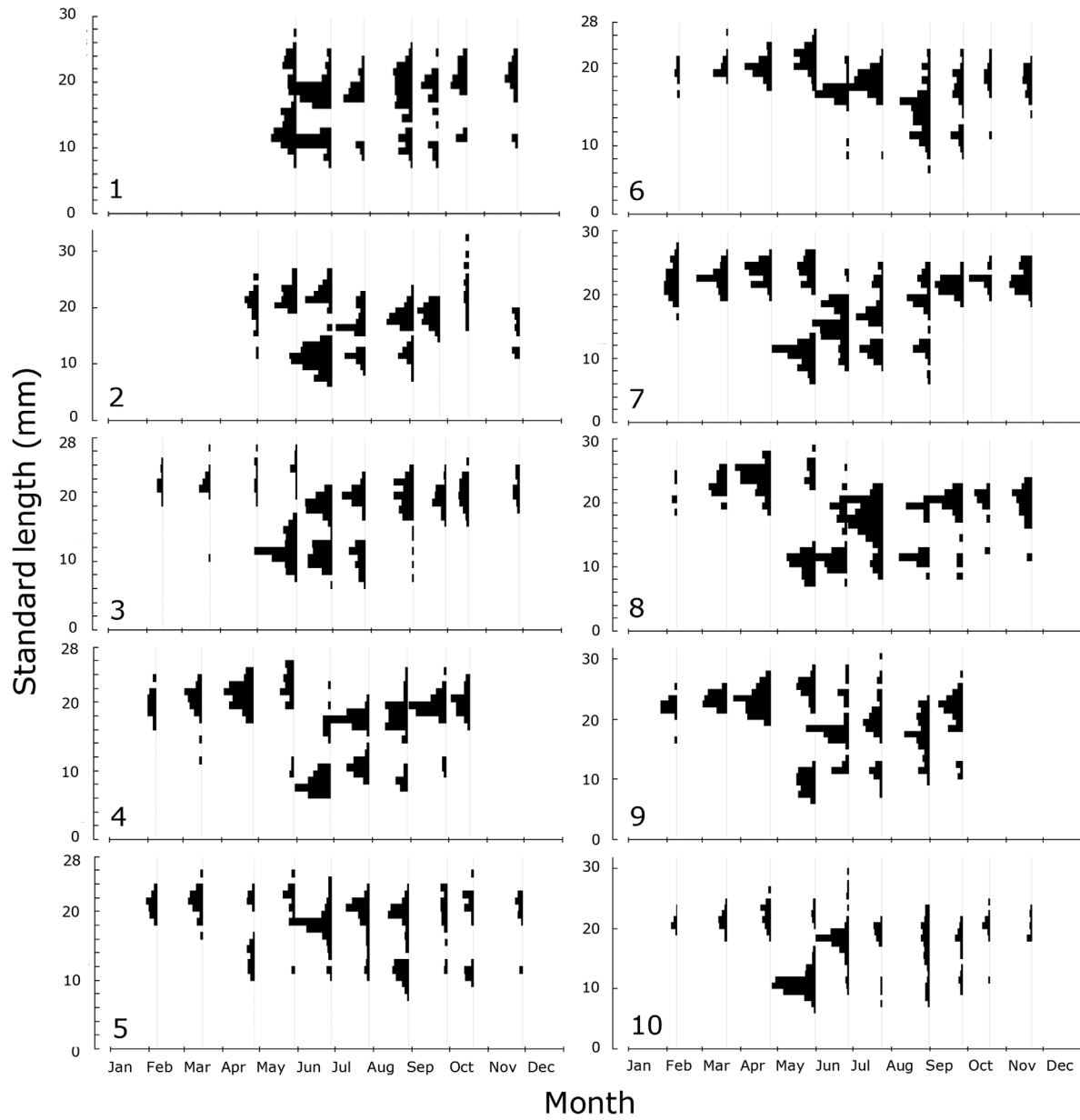


Fig. A4. Monthly length–frequency distributions of male mosquitofish by site. The numbers at the left-bottom corner are the site codes (see Table 1).

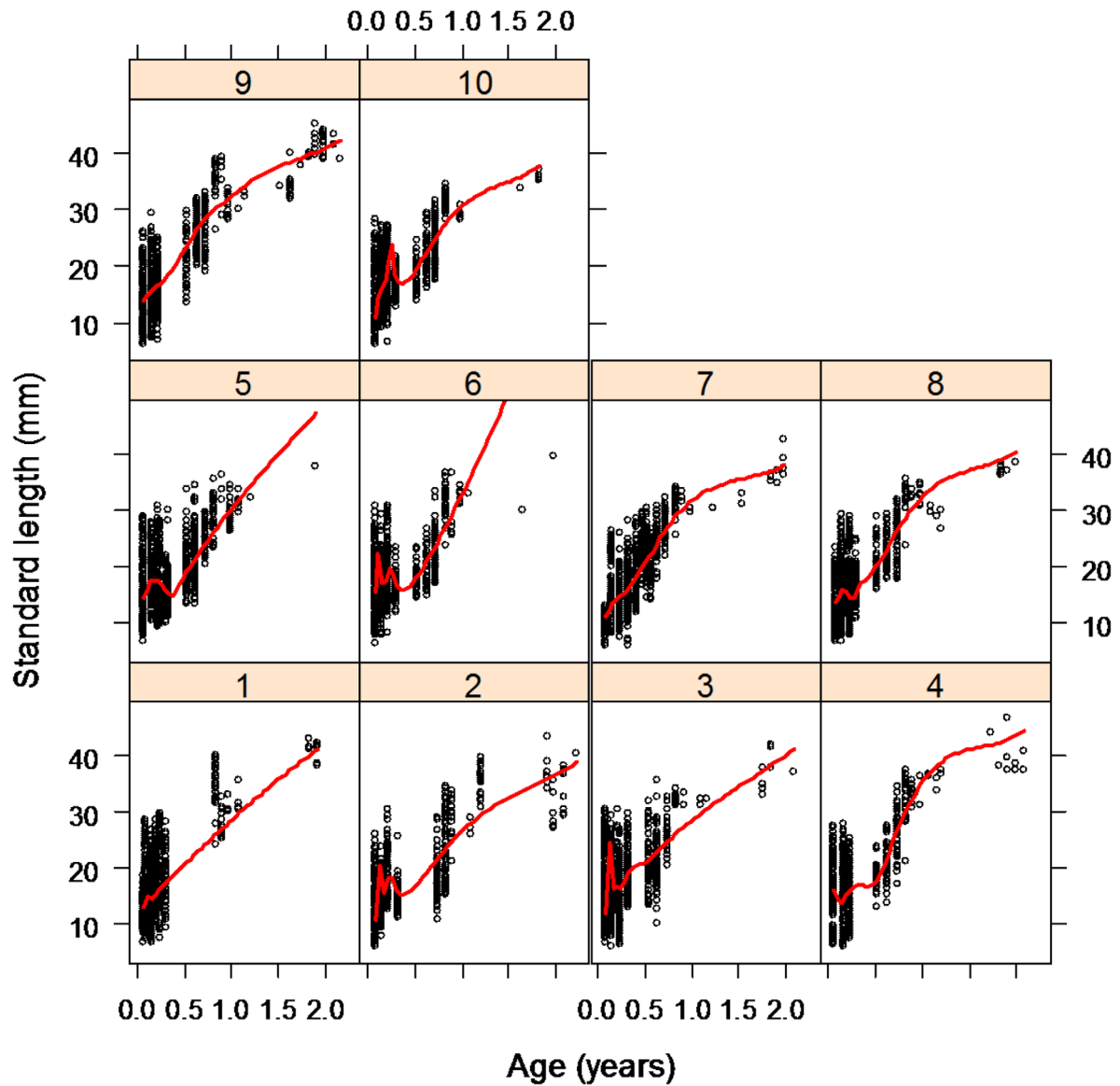


Fig. A5. Growth (length-age relationship) of female mosquitofish by site (figures on top of each scatterplot, see site codes in Table 1). The red line is the additive model (“loess” smoother with the “xyplot” function in R) and the circles the observed data.

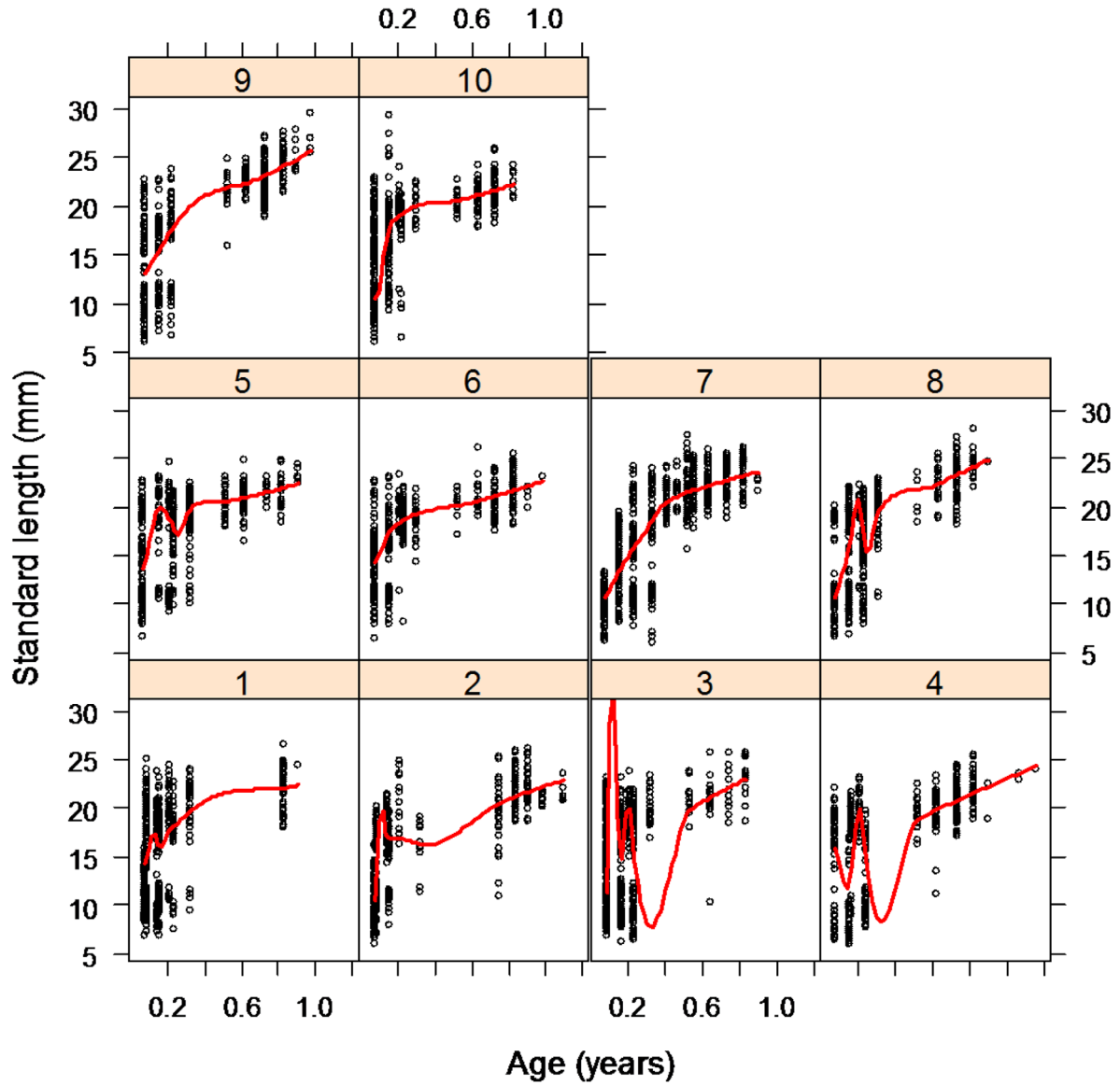


Fig. A6. Growth (length-age relationship) of male mosquitofish by site (figures on top of each scatterplot, see site codes in Table 1).The red line is the additive model (“loess” smoother with the “xyplot” function in R) and the circles the observed data.

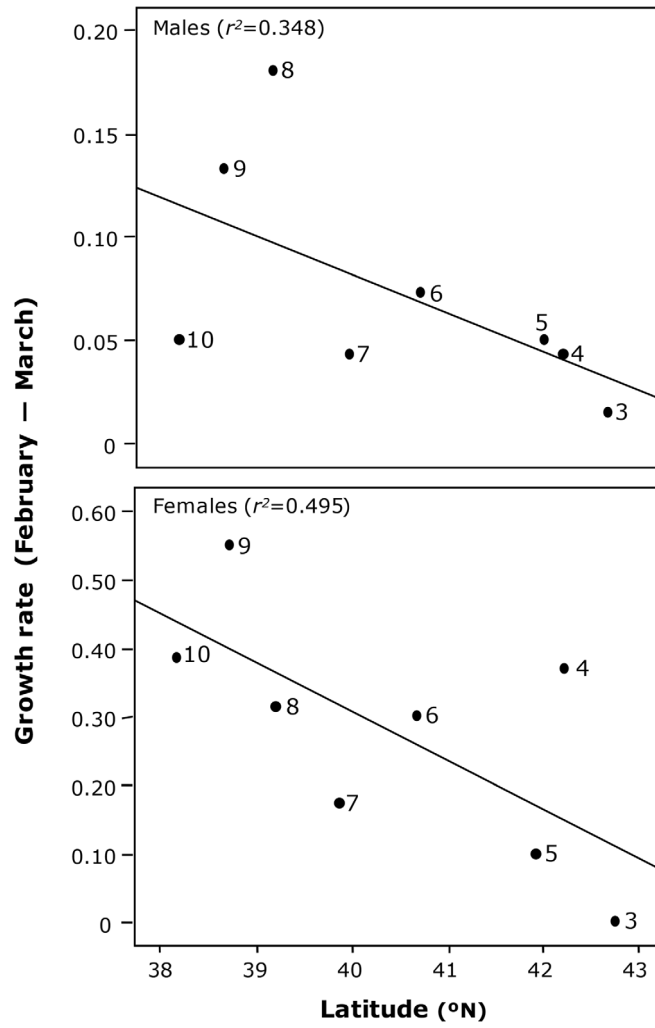


Fig. A7. Growth rates of mosquitofish between February and March along the latitudinal gradient. The numbers indicate the sampling sites (see Table 1). The top plot is for males and the bottom plot for females. *P* values of the regression analyses respectively are 0.12 and 0.05; note that the r^2 are considerable but sample size, and so statistical power, for this analyses is very low ($n = 8$).

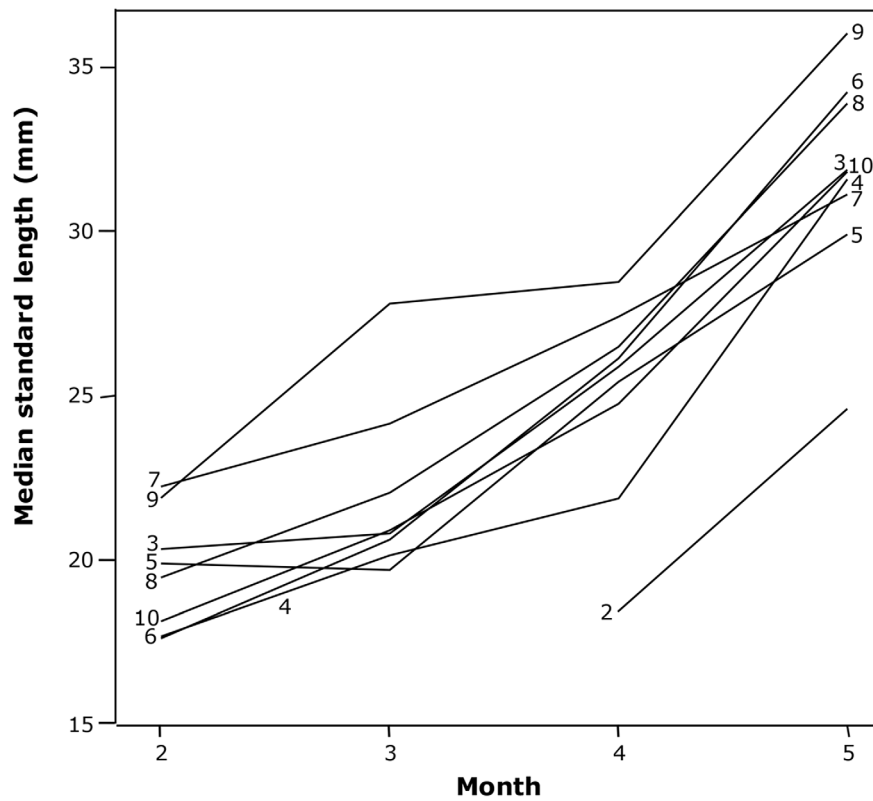


Fig. A8. Median standard length of female mosquitofish captured along the latitudinal gradient by month during late winter and early spring. The numbers are the site codes (see Table 1). Note that median size is greatest in the southern sites 7 and 9 and smallest in the northern sites 2 and 4 in winter and early spring but these differences are less clear in May (month 5).