1   **Three-spined stickleback armour predicted by body size, minimum**

2   **winter temperature and pH**

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Abstract

Similar phenotypes evolve under equivalent environmental conditions through parallel evolution. Because they have repeatedly invaded and adapted to new freshwater environments, the three-spined stickleback (*Gasterosteus aculeatus*) offers a powerful system for understanding the agents of selection in nature that drive parallel evolution. Here we examine the ecological and environmental variables responsible for morphological variation in three-spined stickleback populations across its European range. We collected fish from 85 populations, encompassing much of the European latitudinal range of the species and including lowland rivers and lakes, coastal lagoons, and moorland ponds. We measured biotic and environmental variables at all sites along with morphological traits for 2,358 individuals. Using an information theory approach, we identified body size, minimum average winter temperature and pH as primary predictors of stickleback armour evolution, challenging current hypotheses for stickleback morphological diversification and demonstrating the fundamental role played by body size and scaling in mediating responses to selection. Stickleback lateral plate phenotype represents a potentially powerful tool for monitoring change in climate variables across the northern temperate region.

Introduction

Similar phenotypes are expected to evolve under the same environmental selection pressures through parallel evolution (Haldane, 1932; Bolnick *et al*., 2018). However, identifying the ecological and abiotic agents of selection that underpin parallel evolution is often difficult. Parallel evolution is a feature of the three-spined stickleback (*Gasterosteus aculeatus*) complex, which shows substantial inter-population phenotypic variation in morphology, behaviour, physiology and life-history traits (Wootton, 1976, 1984; Bell & Foster, 1994). The evolutionary history of the three-spined stickleback is one of repeated, independent invasions of freshwater habitats by marine populations. These invasions are characterized by a reduction in anti-predator 'armour', comprising lateral bony plates, pelvic girdle and spines, and dorsal spines, as well as other aspects of their biology (Bell & Foster, 1994). Their wide distribution, tolerance of a wide range of environmental conditions, and striking phenotypic variability makes the three-spined stickleback an ideal model for investigating selective forces underpinning parallel evolution.

Freshwater populations of three-spined sticklebacks distributed over large geographical distances typically converge on a *low* lateral plate morph, with <10 plates on each side of the anterior portion of the body, along with dorsal spines, pelvic girdle and pelvic spines. The separate elements of the armour function in concert, with anterior lateral plates bracing the dorsal and pelvic spines, which thereby resist compression while limiting ingestion by gape-limited vertebrate predators (Reimchen, 1994). Marine sticklebacks are almost exclusively represented by a *complete* morph, with >30 lateral plates along the entire body along with robust dorsal and pelvic spines (Reimchen, 1994). Posterior lateral plates appear to function in preventing skin puncture by toothed predators (Reimchen, 1994). A *partial* morph also occurs, expressing an intermediate but variable number of lateral plates. This morph is commonly, though not exclusively, encountered in brackish water (Wootton, 1976). The rate of evolutionary response shown by three-spined sticklebacks in

56 freshwater environments appears rapid, with a reversal in plate morph dominance possible in as few

57 as 10 generations (e.g. Bell *et al*., 2004; Kitano *et al*., 2008).

58   Parallel phenotypic evolution in three-spined sticklebacks is reflected by genomic

59 parallelism, with 242 genomic regions associated with repeated adaptation to fresh water (Jones *et*

60 *al*., 2012). The *ectodysplasin A* (EDA) locus has been specifically implicated in lateral plate morph

61 evolution, with ancient variants of the EDA locus reused across multiple freshwater populations;

62 approximately 70% of variation in plate number and size is associated with variation in EDA

63 (Cresko *et al*., 2004; Colosimo *et al*., 2005), though the association between variation in EDA and

64 lateral plate morph is not universal (Pujolar *et al*., 2017). The contemporary model for stickleback

65 morph evolution involves the repeated, independent establishment of freshwater populations from

66 ancestral, panmictic populations of *complete* lateral plate marine fish following the end of the last

67 glacial period (*ca*. 11,700 cal years BP) when numerous freshwater environments in the northern

68 hemisphere became accessible with the retreat of the Laurentide and Scandinavian ice sheets

69 (Carlson, 2013). Under this model, *complete* morph marine three-spined stickleback populations are

70 assumed to possess a pool of standing genetic variation, with otherwise rare alleles experiencing

71 strong selection once fish enter fresh water where they rapidly increase in frequency (Barrett, 2010).

72   Several hypotheses have been proposed to explain the selective advantage of armour loss

73 associated with colonization of fresh waters by the three-spined stickleback. These include the

74 effects of temperature, salinity, predation, geographic location, dissolved calcium and phosphorus

75 availability, proximity to the marine environment, water density, parasitism, competition, water

76 turbidity and body size (Table 1). However, there are striking deviations from the typical pattern of

77 lateral plate morph evolution. Notably the *complete* morph occurs widely in fresh waters in eastern

78 Europe, the east coast of North America and sites in Japan (Wootton, 1976, 2009; Hagen & Moodie,

79 1982). In contrast, some populations on the west coast of North America, Norway, Scotland, Ireland

80   and Iceland express greatly diminished armour development, typically with <3 lateral plates of

81   reduced size and thickness, and in some cases with plates and spines entirely absent (Giles, 1983).

82   We addressed the ecological basis to variation in three-spined stickleback lateral plate

83   number using phenotypic and ecological data collected from across the European range of the

84   species. Using an information theory approach (Burnham & Anderson, 2002), we compared a series

85   of *a priori* alternative models of plate loss based on previously published studies (Table 1) to identify

86   the ecological and abiotic agents of selection that underpin parallel evolution in this species. We

87   focused on plate number, rather than other skeletal elements, because lateral plates show greater

88   variation than dorsal spines and pelvic girdle, appears more sensitive to environmental variation and

89   can be unambiguously quantified.

90

91   **Materials and methods**

92   **Fish collection and analysis**

93   Field collection of three-spined sticklebacks was undertaken across Europe using dip nets and Gee

94   minnow traps. A total of 2,358 individuals from 85 locations in England, Estonia, France, Norway,

95   Poland, Scotland and Turkey were collected (Table S1, Fig. S1). Where they co-occurred with three-

96   spined sticklebacks, samples of nine-spined sticklebacks (*Pungitius pungitius*) were also collected.

97   Collected fish were killed with anaesthetic (benzocaine) and fixed in 4% buffered formalin. Samples

98   were restricted to adult fish >27 mm standard length (SL) to ensure morphological characteristics

99   were fully developed (Bańbura, 1989). Nine populations with <16 individuals were dropped from

100   the analysis (Table S1). After fixing, fish were transferred to 70% ethanol for 24 hours, stained with

101   0.08% alizarin red in 1% KOH for 24 hours, rinsed in water for 24 hours, and stored in 70% ethanol

102   (Brubaker & Angus, 1984). For each fish, a record was made of SL and the number of lateral plates

103   on its left flank. The presence of the parasite *Schistocephalus solidus* (Platyhelminthes) was also

104 noted.

105

106 **Environmental data**

107 A water sample was collected at each sample site in one-litre sample bottles from below the water

108 surface at a depth of approximately 1 m. Dissolved calcium concentration was measured by atomic

109 absorption spectrometric methods (ISO 7980:1986), phosphorus concentration by ammonium

110 molybdate spectrometric methods (EN 1189:1996), and pH was measured electrochemically (ISO

111 10523:2008). An accredited commercial laboratory (ZWiK, Łódź) performed all water chemistry

112 measurements. Estimates of dissolved calcium and phosphorus were not obtained for all sites, but

113 in these cases, pH was measured directly in the field using a multi-probe portable water quality

114 meter (Eutech, PCSTestr 35). Dissolved calcium and pH were correlated among sites (Fig. S2).

115 Consequently, pH was used as a proxy for dissolved calcium in models to maximise the number of

116 sites included in the analysis. Habitat variables were also scored at each site, these were; water

117 turbidity (either high, <0.8 m Secchi depth, or low >0.8 m) and vegetation cover (either high, >5%

118 of water body area, or low <5%). The presence/absence of predatory fishes was determined by

119 electrofishing (EFGI 650, BSE Bretschneider Specialelektronik, Germany) at sites in Poland.

120 Detailed fish faunal lists were available for sites in France and England. In Scotland and Norway,

121 angling records were available for all sites. All marine sites (North Poland, Estonia, Turkey) were

122 assumed to be support marine fish predators. The presence/absence of predatory dragonfly

123 (Odonata) and beetle (Coleoptera) larvae and nine-spined sticklebacks was ascertained by dip

124 netting in vegetation at the lake/river bank. For every sampling location the elevation above sea

125 level was estimated from Google Earth using Web-based software (www.freemaptools.com).

126 Average minimum winter temperature data for each site over a 30-year period from 1961–1990 were

127 obtained from the nearest meteorological station (Table S1).

128

Data analysis

129

130   Data were modelled using R (version 3.5.3; R Development Core Team, 2019) with models fitted

131   in a Bayesian framework using Integrated Nested Laplace Approximation (R-INLA; Rue *et al*.,

132   2017). Using an information theory approach, 15 alternative models were formulated comprising

133   variables proposed as having importance as agents of selection for lateral plates in previously

134   published studies (Table 1). Default priors were put on all model parameters and best-fitting models

135   were identified using Watanabe-Akaike Information Criterion (WAIC) (Vehtari *et al*., 2017).

136   Data for lateral plate number were multimodal and zero inflated, with three discrete

137   distributions for modal plate numbers of 0, 5 (range 1-8) and 30 (28-34) plates (Fig. 1). To

138   accommodate this data structure, we fitted two models. We compared fish without lateral plates to

139   those possessing plates using a binary (Bernoulli) process to model zero and positive lateral plate

140   counts, while data for *low* and *complete* morphs were modelled using a hurdle model. Hurdle models

141   are partitioned into two parts, with a binary process modelling probability of an event, and a second

142   process modelling the magnitude of an event (Hilbe, 2014). In the case of plate counts, we modelled

143   data using a negative binomial distribution to accommodate overdispersion in the data (Smith &

144   Warren, 2019), fitting models to *low* and *complete* morphs separately. This approach enabled us to

145   fit models that explained: 1. the probability of possessing lateral plates, 2. the probability of

146   expressing the *complete* rather than *low* plate morph, 3. the number of plates expressed by the *low*

147   plate morph, 4. the number of plates expressed by the *complete* plate morph. Data for fish expressing

148   the *partial* morph were excluded from the analysis, since they occurred at low frequency (6% of

149   specimens). Including *partial* morph fish as either *complete* or *low* plate morph did not result in a

150   qualitative change to the outcome of the analysis. Exploratory analyses demonstrated differences

151   among populations in the number of lateral plates. To accommodate this effect, a random intercept

152   for fish population of origin was included in models to introduce a correlation structure between

153   observations for different fish from the same population. Similarly, to accommodate potential

154   dependency in the data due to temperature data for different populations deriving from the same

155   meteorological station, this factor was included as an additional random term in models.

156   As a follow-up to the main results, three-spined stickleback body size was modelled with a

157   gamma GLMM, which assumed body size was strictly positive and continuous. The full model

158   included salinity, turbidity, flow regime (lentic/lotic), vegetation cover, presence of predatory

159   invertebrates, predatory fishes, nine-spined sticklebacks and *Schistocephalus solidus*, average

160   minimum temperature, pH, and elevation, with an optimal fixed structure identified with a backward

161   selection procedure based on WAIC. Population and month of sample collection were random

162   intercepts in the model. To characterize the relationship between the size of three- and nine-spined

163   sticklebacks among populations a gamma GLM was fitted to the SL of both species in 33 populations

164   where they co-occurred.

165

166   **Results**

167   The best-fitting models to predict the probability of possessing lateral plates were models M15 and

168   M06 (Table 2). Both showed a comparably good fit to the data, though M15 showed higher

169   weighting. Model M15 comprised three explanatory variables, with pH positively associated (Table

170   3, Fig. 2a) and average minimum temperature negatively associated with the probability of

171   expressing plates (Table 3, Fig. 2b). There was no statistically important association between the

172   probability of expressing plates and SL (Table 3). The model predicted that all fish from locations

173   with pH values <5 would be plateless, while from a pH of 5.0-6.5 there was a switch in the

174   probability of the *zero* morph from 1 to 0. Above a pH of 6.5 all fish were predicted to possess lateral

175   plates. Model predictions matched the data, though with a single specimen from Turkey, exhibiting

176 the *zero* morph at a pH exceeding 7.0 (Fig. 2a). Similarly, below an average minimum temperature

177 of -1 °C, sticklebacks were predicted to express lateral plates (Fig. 2b).

178   The best-fitting hurdle model for the probability of expressing a *complete* rather than *low*

179 plate morph was again model M15, though with models M13, M14, M04 and M10 showing good

180 fits to the data, though with lower weighting (Table 2). The probability of expressing the *complete*

181 morph was positively associated with pH (Fig. 3a, Table 4), negatively with average minimum

182 temperature (Fig. 3b, Table 4), and positively with SL (Fig. 3c; Table 4). The number of plates

183 expressed by both the *low* and *complete* morphs was also best predicted by model M15 (Table 2).

184 In the case of the *low* plate morph, pH (Fig. 4a), average minimum temperature (Fig. 4b) and SL

185 (Fig. 4c) all proved statistically important in the model (Table 5). In the case of the *complete* plate

186 morph, only SL was a statistically important predictor of plate number (Fig. 4c; Table 4).

187   The best-fitting model for three-spined stickleback SL, identified by backward selection,

188 showed a statistically important negative association with the presence of nine-spined sticklebacks

189 and average minimum temperature, and a positive association with pH (Table 5). There was a

190 statistically important positive relationship between mean SL of three- and nine-spined sticklebacks

191 among populations (Table 6; Fig. 5).

192

193 **DISCUSSION**

194 The repeated independent evolution of identical traits in similar environments implies natural

195 selection (Bolnick *et al*., 2018). We modelled the expression of lateral plate armour in the three-

196 spined stickleback from a range of habitats across its European range, showing a consistent pattern

197 within habitats with the trait predicted by pH, average minimum temperature and body size.

198   Two environmental variables were consistent in predicting morph and plate number; pH and

199 average minimum temperature. Stickleback populations without lateral plates (the *zero* morph),

200   were predicted by pH values <6.5 (Fig. 2a), though with a single specimen from southern Turkey,

201   at the southern geographic limit of the distribution, showing the *zero* morph at a pH exceeding 7.0.

202   The *zero* morph is globally rare, and typically associated with low pH sites on the west coast of

203   Europe and North America, where they experience mild winter temperatures (Hagen & Moodie,

204   1982; Wootton, 1984). It is also associated with small body size. In the Scottish Hebrides the *zero*

205   morph has been described as a 'dwarf' form, with sexual maturity achieved at <30 mm SL in some

206   populations (MacColl *et al.*, 2013). The *zero* morph contrasts with the *complete* morph in fresh

207   water, which is associated with high pH, low winter temperatures and large body size (Fig. 3a-c).

208   While an association between pH and lateral plate morph is clear, the mechanism by which

209   pH affects lateral plate evolution is opaque. Dissolved calcium and pH are correlated, and we used

210   pH as a proxy for dissolved calcium in the present study (Table 1, Fig.S2). In the Hebrides, plateless

211   and spine-deficient sticklebacks are confined to lochs with low dissolved calcium concentrations

212   and correspondingly low pH (Giles, 1983; Spence *et al.*, 2013; Klepaker *et al.*, 2016). An extreme

213   reduction in plates may result from calcium limitation; calcium is required for the normal

214   development and maintenance of the skeletal system of vertebrates. However, an additional

215   consideration is that both pH and dissolved calcium correlate with productivity in fresh water (Moss,

216   2010). This means that any apparent effect of dissolved calcium on plate morph may be mediated

217   by feeding conditions and, therefore, body size. Since body size predicted plate number, and body

218   size was predicted by pH (Table 5), the relationship between plate morph and pH may reflect a more

219   general pattern associated with resource availability and body size, rather than direct selection from

220   low pH or limited calcium availability.

221   An association between stickleback plate morph and average minimum temperature has been

222   long recognized, though never satisfactorily explained (Heuts, 1947; Hagen & Moodie, 1982;

223   Wootton, 2009). The *complete* morph is characteristic of sites that experience low winter

224   temperatures, both marine and fresh water, on the eastern and northern fringes of continents

225   (Wootton, 1976; Hagen & Moodie, 1982). In contrast, the *low* morph is associated with milder

226   winter temperatures in the south and west of the geographic range of the species, while the *zero*

227   morph is associated with mild maritime climates at the southern and western periphery of the

228   distribution. This pattern was captured in our analysis, which demonstrated a negative relationship

229   between average minimum temperature and the probability of expressing the *complete* morph (Fig.

230   3c). Thermal regimes reflect latitude and altitude and, as such, geographical gradients are often

231   tightly linked to metabolism and phenotypic traits. Average minimum temperature predicted

232   stickleback body size (Table 5), a prediction of Bergmann's rule (Bergmann, 1847). The causal

233   explanation for Bergmann's rule is unclear but applies across a range of endotherms and ectotherms,

234   including fishes (Partridge & Coyne, 1997; Belk & Houston, 2002). In the case of ectotherms, higher

235   resting metabolic rates may incur higher energetic costs that compromise growth. Selection for large

236   offspring body size at low temperatures may also play a role (Pettersen *et al*., 2019). Within

237   generational plasticity and trans-generational epigenetic effects may also contribute to this pattern.

238   Female sticklebacks that develop at low temperatures tend to produce fewer but larger offspring

239   (Shama, 2015), and males raised at high winter temperatures achieve a smaller body size (Kim *et*

240   *al*., 2016). Hence, like pH, the relationship between plate morph and temperature may be mediated

241   through body size.

242   Body size reliably predicted lateral plate morph, as well as lateral plate number within

243   morphs (Fig. 3c,4c). A model identified pH and temperature, as well as co-occurrence with nine-

244   spined sticklebacks, as predictors of body size (Table 5). The presence of nine-spined sticklebacks

245   was negatively associated with three-spined stickleback body size, suggesting that competition from

246   nine-spined sticklebacks might limit three-spined stickleback growth (MacColl *et al*., 2013).

247   However, the mean size of three and nine-spined sticklebacks was correlated among populations

248   (Fig. 5), implying body size in both species is a function of local conditions. Overall then, variance

249   in lateral plate number was explained directly by body size, or by variables with consequences for

250   body size. An implication is that lateral plate morph in three-spined stickleback is a function of

251   scaling (*sensu* Schmidt-Nielsen, 1984).

252   Body size is the most important attribute of an organism, imposing limitations on structure

253   and function (Bartholomew, 1981). As the scale of biological systems change, so anatomical and

254   physiological attributes must be altered to enable an organism to function (Schmidt-Nielsen 1984).

255   If three-spined stickleback morph evolution is a product of scaling rules, a consequence is that

256   selection on armour must experience scaling constraints. Hence while lateral plates may be the

257   targets of selection, for instance from predators, this selection must occur within limitations imposed

258   by scaling relationships; a case of Thompsonian evolution (Thompson, 1917) constraining

259   Darwinian adaptive evolution (Brock, 2000). Under this model of lateral plate evolution, the

260   *complete* plate morph is predicted to be associated with environments that favour large body size,

261   such as marine environments and fresh waters experiencing low winter temperatures. The *low* morph

262   is predicted in environments that limit body size; primarily freshwater environments and sites with

263   mild winter temperatures, such as on the Mediterranean coast of Europe and Asia Minor where the

264   *low* morph is ubiquitous. The *zero* morph, without lateral plates and highly reduced or missing dorsal

265   spines and pelvic girdle and spines, is predicted in resource-poor environments experiencing mild

266   temperatures, exemplified by acid lochs and peat bogs in the Hebrides (Giles, 1983). Thus, a

267   conceptual model of stickleback plate evolution would involve a potential role for multiple

268   environmental variables in shaping lateral plate number, but with their effects mediated through

269   body size.

270   If body size predicts lateral plate number, this raises the question of what scaling constraints

271   generate this relationship. One possibility is that variation in lateral plate number has no Darwinian

272   adaptive advantage and solely reflects scaling (Thompson, 1917). Alternatively, natural selection

273   may drive changes in body size, with plate number reflecting optimal mechanical efficiency at a

274   given body size (Bonner & Horn, 2000). In aquatic animals scaling effects have consequences for

275   hydrodynamic resistance to movement (Schmidt-Nielsen, 1984). Because three-spined sticklebacks

276   are neutrally buoyant, they expend a negligible amount of energy supporting their bodies in water.

277   However, energy is needed to offset the resistance of swimming through water; i.e. to overcome

278   drag. Drag comprises two components. Friction drag derives from the force required to overcome

279   the viscosity of water while pressure drag stems from the requirement to displace water during

280   movement. Friction drag is a function of fluid density, surface area and speed of the moving object,

281   and a drag coefficient (Schmidt-Nielsen, 1984). Small fish swim slower than large fish in the ratio,

282   all things equal, of the square-root of body length, in accordance with Froude's Law (Thompson,

283   1917; Videler, 1993). Small fish also incur a relatively greater cost of swimming in comparison with

284   larger fish, a relationship that applies both within and among species (Videler, 1993). In the three-

285   spined stickleback the lateral plates increase the drag coefficient; lateral plate number correlates

286   negatively with swimming velocity after correcting for body size (Bergstrom, 2002). Selection can

287   compensate for lower swimming speed and efficiency in small fish by minimizing the drag

288   coefficient. In sticklebacks this can be achieved through armour loss, since armour demonstrably

289   compromises hydrodynamic efficiency (Walker, 1997; Bergstrom, 2002). Thus, sticklebacks that

290   experience selection for reduced body size will simultaneously experience selection for plate loss to

291   avoid compromising swimming speed and energy expenditure. A prediction is for a trade-off

292   between armour development and growth rate in sticklebacks, which has been demonstrated

293   experimentally (Barrett *et al*., 2008).

294   Below a threshold body size selection appears to converge on the *low* plate morph, an armour

295   configuration that provides robust protection from predators, while facilitating rapid and efficient

296 swimming at a smaller body size. It is noteworthy that the most striking examples of rapid

297 contemporary change in morph frequency, from domination by the *complete* plate morph to *low*

298 morph following invasion of marine fish to freshwater sites (e.g. Bell *et al*., 2004), and the reverse

299 following environmental change (Kitano *et al*., 2008), can be explained on the basis of selection for

300 changes in body size. We further propose that in the case of populations reduced to unusually small

301 body sizes, like those in acid lochs, complete loss of pelvic apparatus, spines and lateral plates

302 appears to be favoured under selection as a mechanism to further reduce friction drag and thereby

303 maintain sufficiently rapid escape responses to enable fish to avoid predators. An implication of our

304 findings is that elevated temperatures will tend to shift selection pressures on stickleback plate

305 morph, potentially driving plate loss (Des Roches *et al*., 2019). As such, stickleback lateral plate

306 phenotype offers a powerful tool for monitoring change in climate variables.

307

308 **CONCLUSION**

309 We used an information theory approach to identify body size, average minimum temperature, and

310 pH as the primary predictors of the parallel evolution of stickleback lateral plate phenotype. Our

311 results indicate that lateral plate armour development is underpinned by selection for body size,

312 which has implications for swimming velocity and predator avoidance. Temperature-linked changes

313 in stickleback plate morph offers a tool for monitoring temperature trends across the northern

314 temperate region.

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434

Figure legends
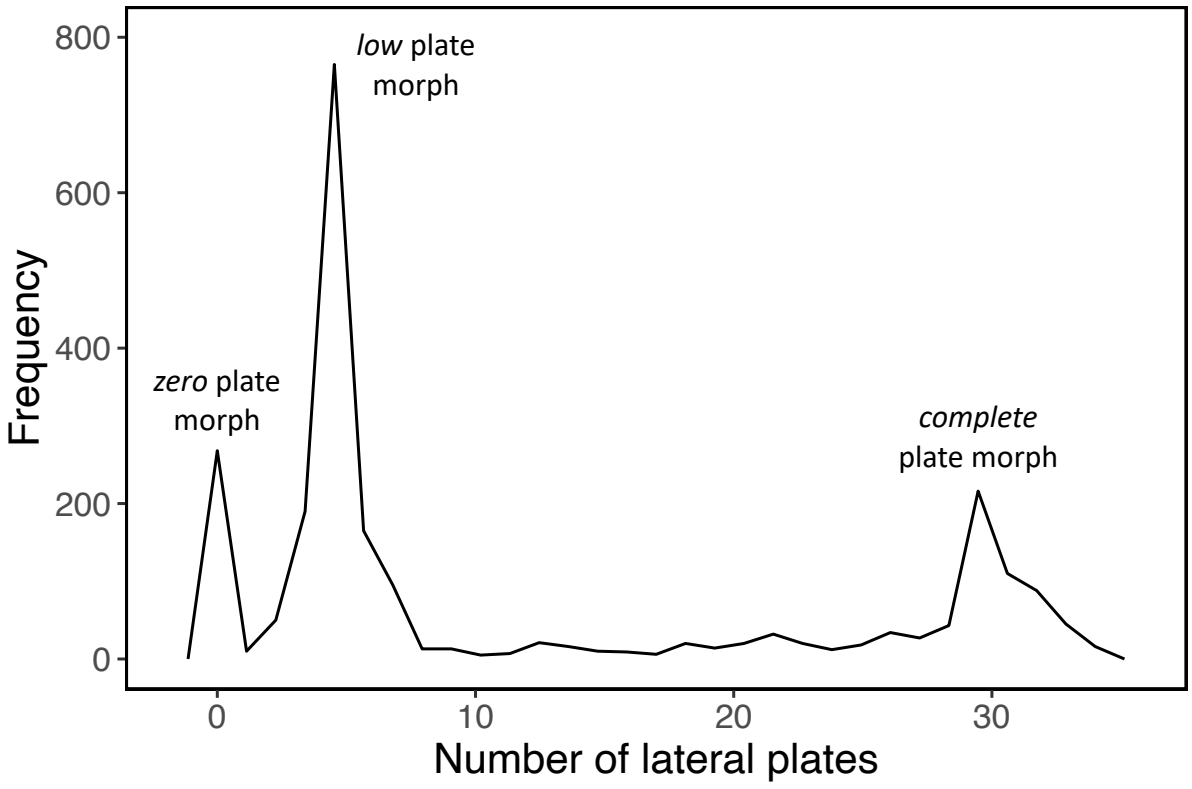
Figure 1 Frequency distribution of three-spined stickleback lateral plate number for 2,358 individuals from 85 European populations.

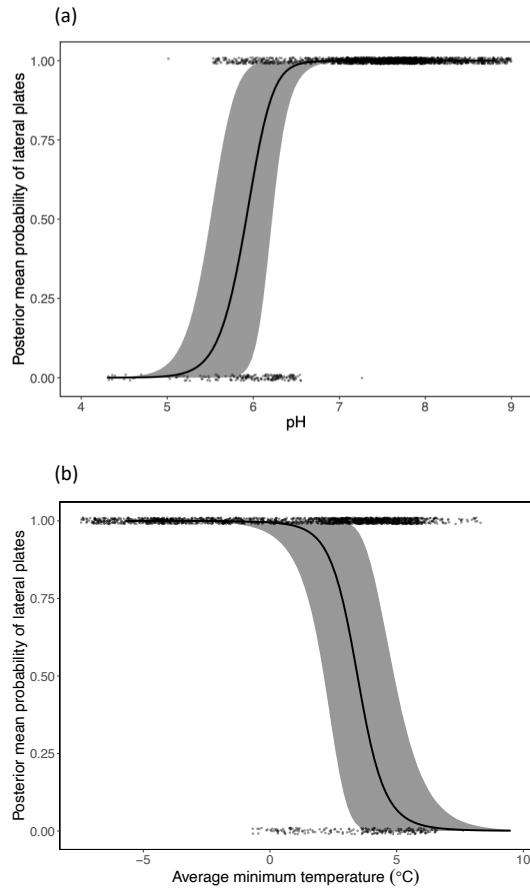**Figure 2**. (a) Posterior mean fitted probability of three-spined sticklebacks possessing lateral plates as a function of pH; (b) average minimum environmental temperature (°C). Shaded areas are 95% credible intervals. Black points are observed data for individual fish.
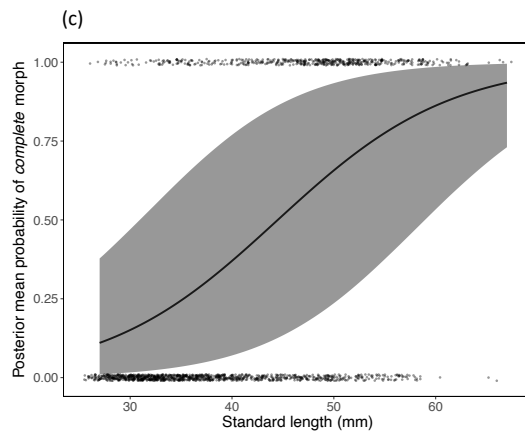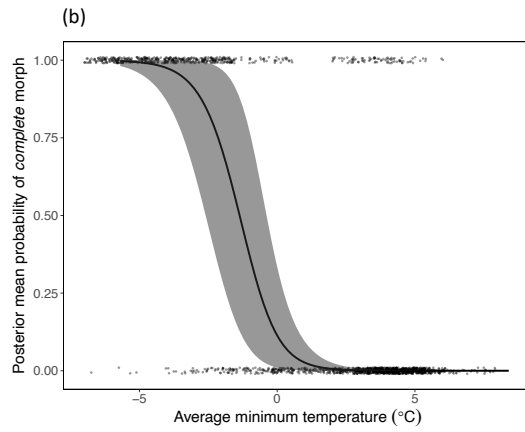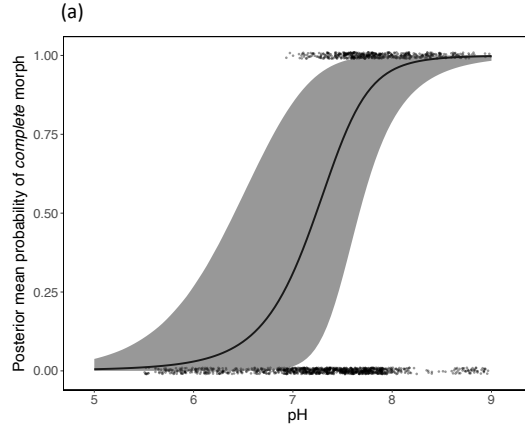
Figure 3. (a) Posterior mean probability of expressing the *complete* lateral plate morph as a function of pH; (b) average minimum environmental temperature (°C); (c) standard length (mm). Shaded areas are 95% credible intervals. Black points are observed data for individual fish.

Figure 4. Posterior mean fitted number of lateral plates for three-spined sticklebacks for: (a) the *low* plate morph as a function of pH; (b) the *low* plate morph as a function of average minimum environmental temperature (°C); (c) *low* and *complete* morphs as a function of standard length (mm). Shaded areas are 95% credible intervals. Black points are observed data for individual fish.

Figure 5. Posterior mean standard length (mm) of three-spined sticklebacks as a function of nine-spined stickleback standard length (mm). Shaded areas are 95% credible intervals. Black points are observed data for different populations.

Fig.1

Fig.2

(a)

(b)
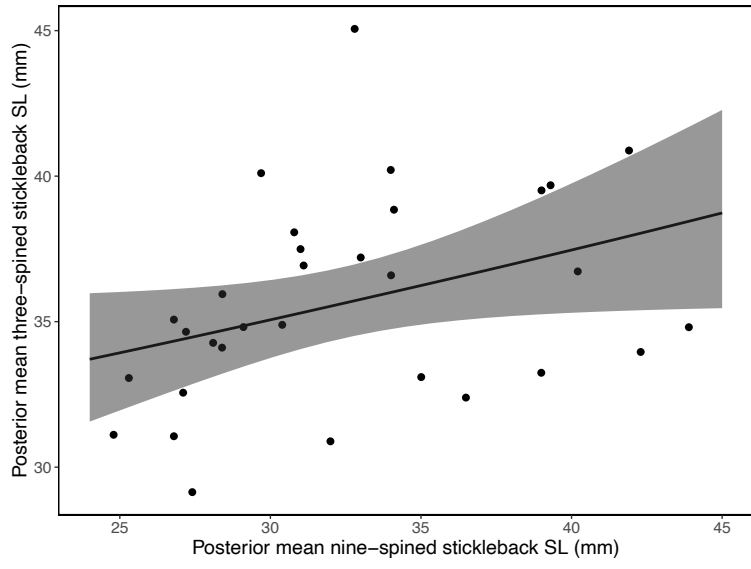
Fig.3

Fig.4

(a)

(b)

(c)

Fig.5

Table 1. *A priori* models for the evolution of lateral plate morph. In some cases multiple authors have proposed and tested the same model. We cite a single source for each model for brevity, giving either the first author to propose the model or the one that most fully articulated it.

model	fitted model	source	model description
M01	temperature + salinity	Heuts (1947)	average minimum temperature, salinity
M02	presence/absence of fish predators	Hoogland *et al.* (1956)	vertebrate predation
M03	latitude x longitude	Münzig (1963)	geographical location
M04	temperature	Wootton (1976)	average minimum temperature
M05	presence/absence dragonfly larvae	Reimchen (1994)	invertebrate predation
M06	pH	Giles (1983)	dissolved calcium
M07	elevation	Raeymaekers *et al.* (2007)	coastal-inland gradient
M08	salinity	Myhre & Klepaker (2009)	water density
M09	presence/absence *Schistocephalus solidus*	Morozińska-Gogol (2011)	parasitism by *S. solidus*
M10	presence/absence *Pungitius pungitius*	MacColl *et al.* (2013)	competition with *P. pungitius*
M11	turbidity + presence/absence of fish predators	Reimchen *et al.* (2013)	light spectra and vertebrate predation
M12	pH + presence/absence of fish predators	Spence *et al.* (2013)	dissolved calcium, vertebrate predation
M13	pH + presence/absence of fish predators + turbidity	Klepaker *et al.* (2016)	dissolved calcium, vertebrate predation, light spectra
M14	presence/absence of fish predators + *P. pungitius*	Magalhaes *et al.* (2016)	vertebrate predation, competition with *P. pungitius*
M15	temperature + standard length + pH	this study	average minimum temperature, body size, dissolved calcium

Table 2. Best-fitting models for expression of lateral plates, expression of either a *low* and *complete* morph, and for the number of plates expressed by *low* and *complete* morphs. WAIC is Watanabe-Akaike Information Criterion score, Δi is delta WAIC, ω is WAIC weighting.

Expression of lateral plates				Expression of *low* or *complete* morph				Number of *low* morph plates				Number of *complete* morph plates			
model	WAIC	Δi	ω	model	WAIC	Δi	ω	model	WAIC	Δi	ω	model	WAIC	Δi	ω
M15	194	0.0	0.56	M15	160	0.0	0.26	M15	3864	0	1.00	M15	2760	0.0	0.47
M05	196	2.0	0.20	M13	160	0.6	0.19	M01	3883	19.1	0.00	M07	2764	3.8	0.07
M12	198	4.0	0.09	M14	161	0.8	0.17	M08	3887	22.9	0.00	M10	2764	4.1	0.06
M02	199	5.0	0.04	M04	161	1.4	0.13	M05	3888	24.5	0.00	M08	2764	4.2	0.06
M06	199	5.0	0.04	M10	161	1.6	0.12	M12	3888	24.7	0.00	M09	2765	4.8	0.04
M10	199	5.0	0.04	M09	163	3.0	0.06	M13	3890	26.1	0.00	M01	2765	4.8	0.04
M09	200	6.0	0.03	M12	163	3.3	0.05	M04	3892	27.7	0.00	M05	2765	5.1	0.04
M14	202	8.0	0.01	M02	165	5.0	0.02	M11	3892	28.6	0.00	M04	2765	5.1	0.04
M07	220	26.0	0.00	M05	168	8.2	0.00	M03	3893	29.1	0.00	M14	2765	5.1	0.04
M08	220	26.0	0.00	M08	172	12.5	0.00	M02	3894	30.5	0.00	M06	2765	5.2	0.04
M13	224	30.0	0.00	M06	177	16.9	0.00	M07	3894	30.5	0.00	M02	2765	5.2	0.04
M03	245	51.0	0.00	M07	219	59.3	0.00	M14	3895	30.8	0.00	M12	2766	6.1	0.02
M11	266	72.0	0.00	M11	225	65.1	0.00	M06	3895	30.9	0.00	M03	2766	6.1	0.02
M04	468	274.0	0.00	M03	229	69.1	0.00	M10	3895	31.4	0.00	M11	2767	6.9	0.01
M01	8622	8428	0.00	M01	4092	3932	0.00	M09	3896	32	0.00	M13	2768	8.1	0.01

Table 3. Posterior mean estimates for the expression of lateral plates in three-spined sticklebacks modelled with a Bernoulli GLMM fitted using INLA. Stickleback population of origin and nearest meteorological station were fitted as random intercepts. CrI are the Bayesian credible intervals. Credible intervals that do not encompass zero indicate statistical importance.

Parameter	Posterior mean	Lower CrI	Upper CrI
Intercept	-53.43	-108.30	-26.22
SL	0.04	-0.06	0.15
Temperature	-2.89	-6.68	-1.00
pH	10.35	5.26	21.18

Table 4. Posterior mean estimates for the expression of lateral plates by *low* and *complete* morph three-spined sticklebacks for the best-fitting hurdle model using INLA. Stickleback population of origin and nearest meteorological station were fitted as a random intercepts. Frequency data were fitted to a Poisson distribution. CrI are the Bayesian credible intervals. Credible intervals that do not encompass zero indicate statistical importance.

Parameter	Occurrence model			Frequency model (*low*)			Frequency model (*complete*)		
	Posterior mean	Lower CrI	Upper CrI	Posterior mean	Lower CrI	Upper CrI	Posterior mean	Lower CrI	Upper CrI
Intercept	-4.78	-7.88	-2.77	1.55	1.49	1.63	3.42	3.40	3.44
SL	1.29	0.88	1.72	0.10	0.07	0.15	0.02	0.01	0.04
Temperature	-6.44	-10.20	-4.14	-0.10	-0.21	-0.04	0.01	-0.01	0.02
pH	3.47	1.42	6.37	0.12	0.07	0.23	-0.01	-0.02	0.02

Table 5. Posterior mean estimates for three-spined stickleback standard length as a function of the presence of nine-spined sticklebacks, average minimum temperature and pH, modelled using a gamma GLMM. Stickleback population of origin and month of collection were fitted as random intercepts. CrI is the 95% Bayesian credible interval. Credible intervals that do not encompass zero indicate statistical importance.

Model parameter	Posterior mean	Lower CrI	Upper CrI
Intercept	3.67	3.58	3.74
Nine-spined sticklebacks(present)	-0.12	-0.19	-0.05
Temperature	-0.13	-0.16	-0.09
pH	0.05	0.03	0.08

Table 6. Posterior mean estimates for the SL of three-spined sticklebacks as a function of nine-spined stickleback SL, modelled with a gamma GLM fitted using INLA. CrI are the Bayesian credible intervals. Credible intervals that do not encompass zero indicate statistical importance.

Parameter	Posterior mean	Lower CrI	Upper CrI
Intercept	3.220	2.887	3.552
Nine-spined stickleback SL	0.011	0.001	0.021