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

2 **winter temperature and pH**

3 C. Smith[1,2,3], G. Zięba[1], R. Spence[2,3], T. Klepaker[4] & M. Przybylski[1]

4 1 Department of Ecology & Vertebrate Zoology, University of Łódź, Łódź, Poland

5 2 School of Animal, Rural & Environmental Sciences, Nottingham Trent University, Brackenhurst, UK

6 3 Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

7 4 Department of Biology, University of Bergen, Bergen, Norway

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12 **Correspondence**

13 Carl Smith, School of Animal, Rural & Environmental Sciences, Nottingham Trent University,

14 Brackenhurst NG25 0QF, UK.

15 Email: carl.smith02@ntu.ac.uk

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

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.

315

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430

431 **Figure legends**

432 **Figure 1** Frequency distribution of three-spined stickleback lateral plate number for 2,358

433 individuals from 85 European populations.

434 **Figure 2**. (a) Posterior mean fitted probability of three-spined sticklebacks possessing lateral plates

435 as a function of pH; (b) average minimum environmental temperature (°C). Shaded areas are 95%

436 credible intervals. Black points are observed data for individual fish.

437 **Figure 3**. (a) Posterior mean probability of expressing the *complete* lateral plate morph as a function

438 of pH; (b) average minimum environmental temperature (°C); (c) standard length (mm). Shaded

439   areas are 95% credible intervals. Black points are observed data for individual fish.

440   **Figure 4**. Posterior mean fitted number of lateral plates for three-spined sticklebacks for: (a) the

441   *low* plate morph as a function of pH; (b) the *low* plate morph as a function of average minimum

442   environmental temperature (°C); (c) *low* and *complete* morphs as a function of standard length

443   (mm). Shaded areas are 95% credible intervals. Black points are observed data for individual fish.

444   **Figure 5**. Posterior mean standard length (mm) of three-spined sticklebacks as a function of nine-

445   spined stickleback standard length (mm). Shaded areas are 95% credible intervals. Black points

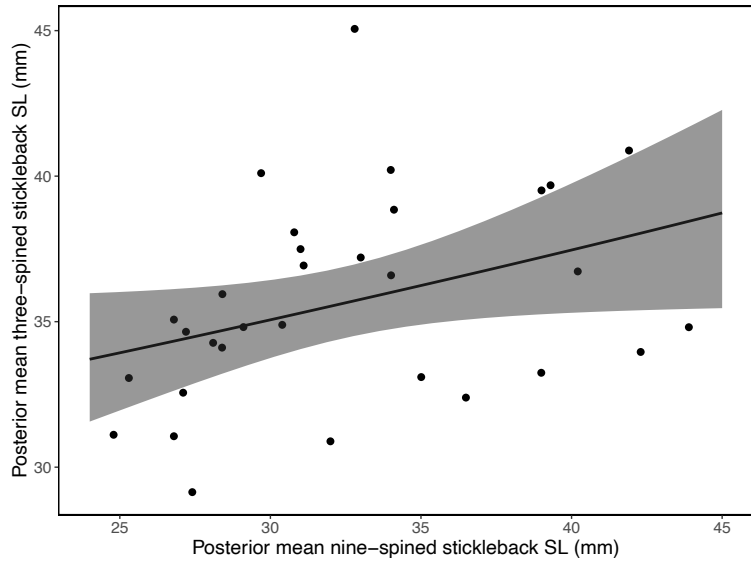446   are observed data for different populations.

Fig.1

Fig.2

(a)

(b)

Fig.3

(a)

(b)

(c)

Fig.4

(a)

(b)

(c)

Fig.5
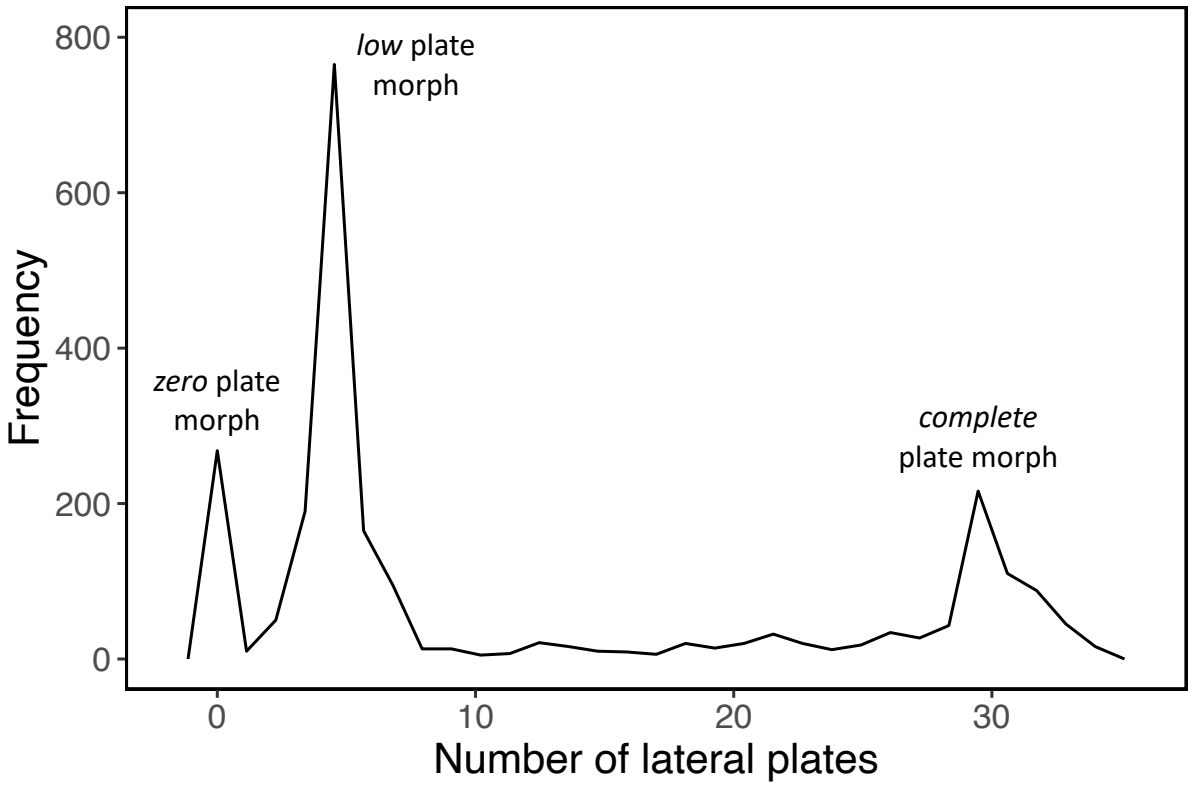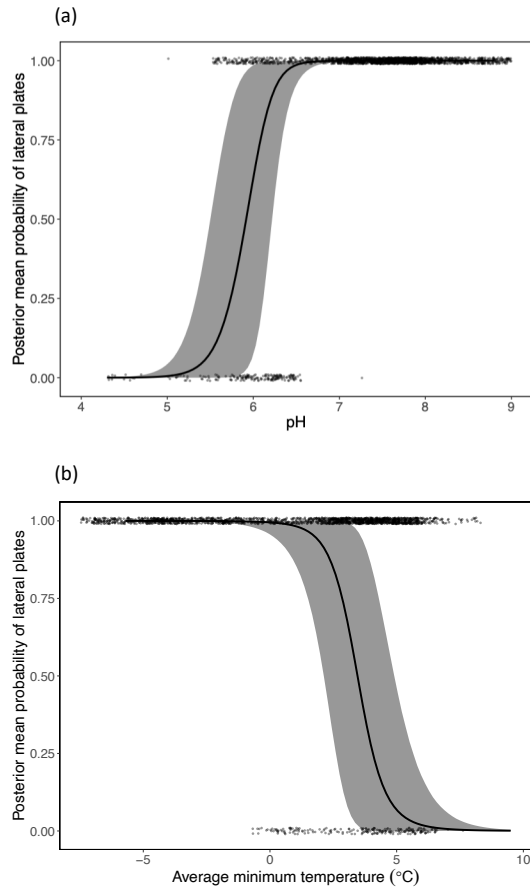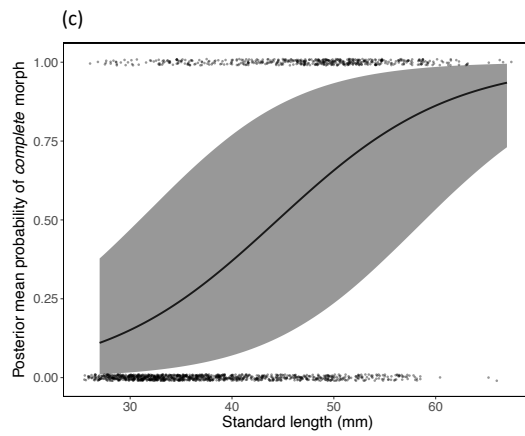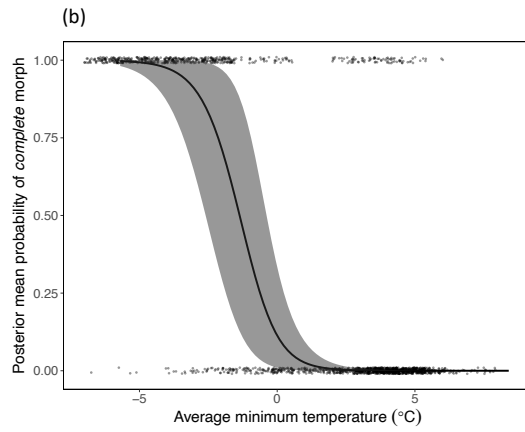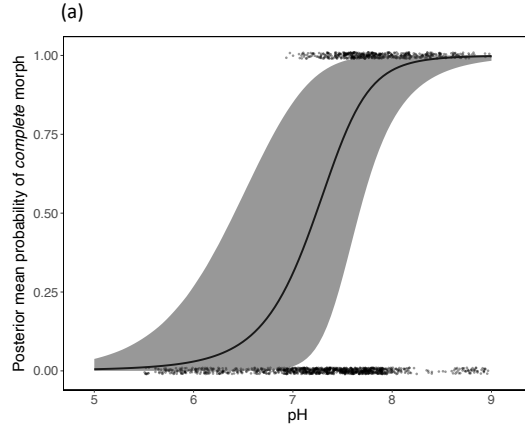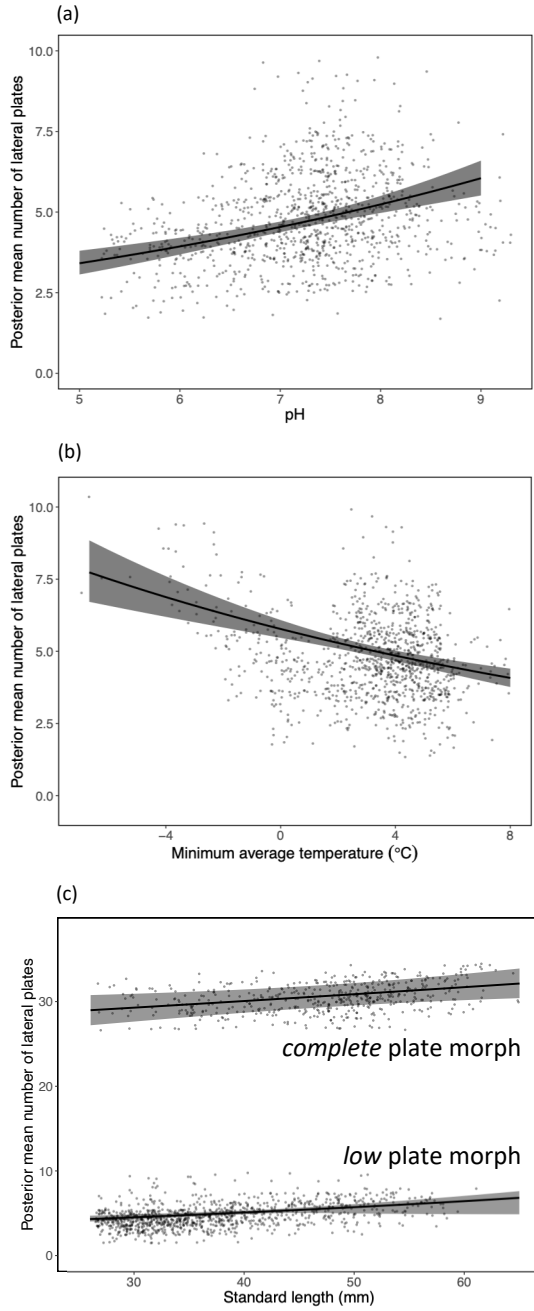
Table 1. *A priori* models for the evolution of lateral plate morph. In some cases multiple authors have proposed and tested the same model. We cite a single source for each model for brevity, giving either the first author to propose the model or the one that most fully articulated it.

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, Δ*i* 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	Δ*i*	ω	model	WAIC	Δ*i*	ω	model	WAIC	Δ*i*	ω	model	WAIC	Δ*i*	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

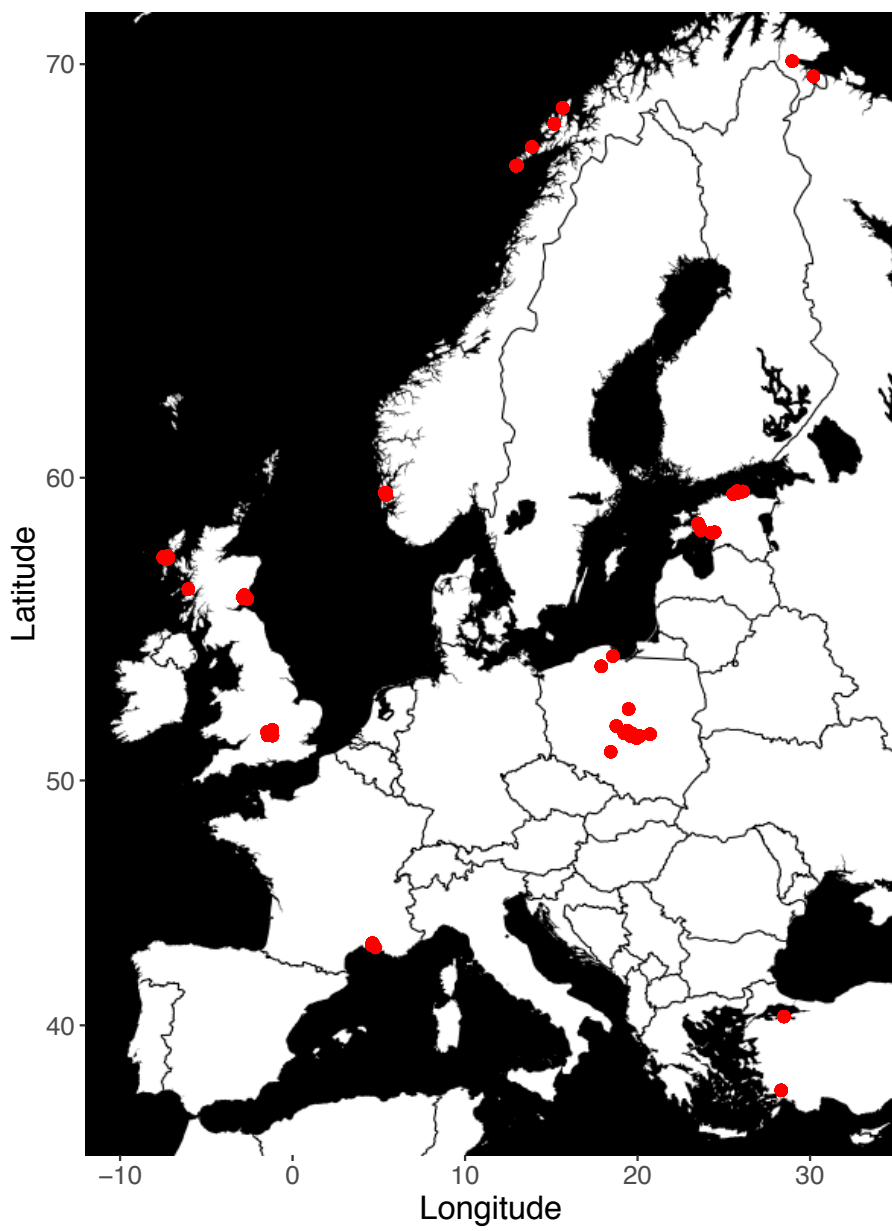Figure S1. Map showing location of sampling sites.

Figure S2. Correlation between pH and dissolved calcium concentration (mg l^{-1}) for a subset of study sites. Each point represents a discrete location.