# Water chemistry variation promotes adaptive radiation in three-spined stickleback (Gasterosteus aculeatus)

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**Abstract**

The context and cause of adaptive radiations has been widely described and explored but why rapid evolutionary diversification does not occur in related evolutionary lineages has yet to be understood. One possible answer to this is simply that evolutionary diversification is provoked by environmental diversity, and that some lineages do not encounter the necessary environmental diversity. Three-spined stickleback on the Scottish island of North Uist show enormous diversification, which seems to be associated with the diversity of aquatic habitats. Stickleback on the neighbouring island of South Uist have not been reported to show the same level of evolutionary diversity, despite levels of environmental variation that we might expect to be similar to North Uist. In this study, we compared patterns of morphological and environmental diversity on North and South Uist. Ancestral anadromous stickleback from both islands exhibited similar morphology including size and bony 'armour'. Resident stickleback showed significant variation in armour traits in relation to pH of water. However, North Uist stickleback exhibited greater diversity of morphological traits than South Uist and this was associated with greater diversity in pH of the waters of lochs on North Uist. Highly acidic and highly alkaline freshwater habitats are missing, or uncommon, on South Uist. Thus, pH appears to act as a causal factor driving the evolutionary diversification of stickleback in local adaptation in North and South Uist. This is consistent with diversification being more associated with ecological constraint than ecological opportunity.

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**Abstract**

The context and cause of adaptive radiations has been widely described and explored but why rapid evolutionary diversification does not occur in related evolutionary lineages has yet to be understood. One possible answer to this is simply that evolutionary diversification is provoked by environmental diversity, and that

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**Keywords** : Stickleback, morphometric study, North Uist, South Uist.

## Introduction

Studies of adaptive radiation have tended to concentrate, for obvious reasons, on lineages and locations in which it is known to have occurred. The circumstances associated with a failure to radiate are less well explored, yet there is much that might be learned from them about the conditions that favour divergence. Differences in ecological conditions among local populations are generally assumed to be the reason for adaptive divergence leading to adaptive radiation, reproductive isolation and speciation (Schluter 1996; Schluter 2000; MacColl 2011). In particular, according to the ecological theory of adaptive radiation (Schluter, 2000), 'ecological opportunity', the diversity of available resources not used by other taxa, is central to explaining why adaptive radiations occur. Lineages often diversify when they colonise habitats where there is little competition for constraining resources (Simpson 1953, Schluter 2000). The concentration on biotic interactions, especially competition for food, that has followed from the idea of ecological opportunity, has meant that the role of abiotic environmental variation has been less well explored. Here, we scrutinise diversification of three-spined stickleback (*Gasterosteus aculeatus* ) in lochs on two neighbouring Scottish islands, for clues about the causes of adaptive radiation.

The three-spined stickleback (hereafter 'stickleback') has served as a model organism for the study of adaptive radiation due in part to parallel diversifications of freshwater populations from marine ancestors, that have occurred in the last 10,000 years ago (Bell and Foster 1994; Schluter 2000; Jones et al 2012; Magalhaes et al. 2021). Freshwater populations have evolved conspicuous differences in morphology, physiology and behaviour (Bell and Foster 1994; Ostlund-Nilsson et al. 2006). The divergence of morphological traits through major changes in the bony armour that have repeatedly evolved in various locations, are common components of adaptive radiation of stickleback (Colosimo et al. 2004; Chan et al. 2010). Ancestral marine anadromous sticklebacks are heavily armoured with a continuous row of 30-36 bony lateral plates running from head to tail on each side (known as a complete morph), and have dorsal spines, a well-developed pelvic girdle and further spines attached to the girdle (Colosimo et al. 2005; Barrett et al. 2008). On the other hand, derived freshwater (live in freshwater year round) and saltwater-resident (inhabit coastal saltwater all year round without migration to the open sea) stickleback exhibit substantial reduction in the total plate number with either a discontinuous row of 9-28 plates (partial morph) or with 0-9 lateral plates at the anterior end (low morph), and reductions in the size of spines and girdle (Colosimo et al. 2004; Colosimo et al. 2005). In addition, other phenotypic traits such as body size and shape of freshwater and saltwater-resident stickleback show morphological transformation from the ancestral anadromous form (Moodie and Reimchen 1976; Schluter 1993; Bell et al. 1993; Schluter et al. 2004; Shapiro et al. 2004; Ravinet et al. 2015).

To date, several abiotic and biotic environmental factors have been considered as causes for these major phenotypic changes including variations in salinity, temperature, nutrient and calcium availability, stream gradient, predators and parasites (Giles 1983; Bergstrom 2002; Barrett et al. 2009; Myhre and Klepaker 2009; Marchinko 2009; Wark and Peichel 2010). For example, predation-driven selection has been reported

to influence the evolution of bony armour structures such as the lateral plates and spines within marine and freshwater stickleback populations (Marchinko 2009). Moreover, other factors such as reduced nutrients or salinity, and calcium ion deficiency have also been reported to have an association with the loss of lateral plates in freshwater populations (Giles 1983; Barrett et al. 2009; Myhre and Klepaker 2009).

The neighbouring islands of North and South Uist, in the Scottish Western Isles, appear to have much in common from the perspective of aquatic habitats and species. Both have large numbers of small to medium-sized shallow lochs, that occur over a variety of surface geology, from peat and bedrock on their eastern sides, to shell-rich machair sand on their western sides. Although fish, including three-spined stickleback, populations have been surveyed on both islands (Campbell and Williamson, 1979; Campbell, 1985), detailed studies of phenotypic variation in the stickleback have only taken place on North Uist (Giles 1983; MacColl et al. 2013; Magalhaes et al. 2016; Magalhaes et al. 2021) apparently because of a lack of such variation on South Uist (Campbell 1985).

North Uist comprises a mosaic of interconnected freshwater and brackish water lochs and lagoons which are known to have exceptional variation in their water chemistry, with high pH (~8) in the western machair lochs in the west and low pH (~6) in the east. These are associated with variation in the concentrations of alkaline metals (sodium, potassium, magnesium, calcium etc.) (Waterston et al.1979). This variation has been shown to correlate with the evolution of high diversity of stickleback populations across the island (Giles 1983; MacColl et al. 2013; Magalhaes et al. 2016; Haenel et al. 2019) and offers a unique opportunity to study the variation in phenotypes in relation to adaptation under different environmental conditions.

In contrast, environmental conditions and phenotypic variation have been poorly explored in South Uist, and there have been no reports of unusual morphological variation in stickleback. While different in topography (it has higher hills and less low-lying ground), it appears to have similar variation in aquatic environments, with both saline and freshwater lochs, and both western, machair lochs and eastern lochs on peat or bedrock. This begs the question why, on two such closely neighbouring islands, where ancestral variation is likely to be shared, and environmental variation is similar, there appears to have been little or no diversification of stickleback on one, while the other exhibits some of the most dramatic variation known in the species. Here we make a direct comparison of the morphological and environmental variation in the two islands, and relate the former to the latter, to understand what factors may promote or inhibit adaptive radiation at local scales. We hypothesised that morphological traits of three-spined stickleback would show variation between South Uist and North Uist due to differences in the environmental diversity of the lochs.

## Materials and methods

### 2.1 Study area

Two neighbouring islands with apparently similar aquatic environments were selected to make a comparative study of morphological variation in three-spined stickleback. North Uist (57°31'12"N; 7°27'42"W) is in the centre of the Western Isles of Scotland and is approximately 303 $km^2$in total area (Fig. 1B). It comprises a mosaic of peat bogs, heathland and low hills which makes it different from South Uist. South Uist (57°13'54"N; 7°02'38"W) is the second largest island of the Outer Hebrides (Fig. 1C). It is around 320 $km^2$ in total area and differs greatly between its west and east sides.

### 2.2 Sample collection

Stickleback samples were collected from 10 lochs on North Uist and 8 lochs on South Uist between the dates of $06^{th}$ – $16^{th}$ May 2019 (Fig. 1). Locations on both islands were selected to maximise likely variation in surface geology (sand versus peat or bedrock), and hence water chemistry, using Google Earth. Stickleback were caught in unbaited Gee's Minnow Traps (Gee traps, Dynamic Aqua, Vancouver, Canada), set overnight (approximately 16 hrs.) at all sites. The geolocation of each sample site (GPSmap 60CSx, Garmin, UK) and water quality parameters including temperature, absolute conductivity, salinity and pH (multi-parameter probe - Multi340/set, WTW, Germany) were recorded from all sites (Table A1 - A2). Lochs were grouped based on the pH of water: high pH >7.5, neutral pH = >7.0 - 7.4, low pH< 7.0 (Table A1 - A2).

Across all ten sampling sites of North Uist, 135 stickleback were haphazardly selected (Fig. 1B). From South Uist, 128 fish were collected in the same way from 11 sites (3 sites from loch Aroa) in eight lochs (Fig. 1C). Of the total 263 stickleback, 45 were anadromous fish sampled from both islands to estimate the ancestral state of stickleback in the Uist lochs. Fish were euthanized and preserved in 70% ethanol for morphometric study.

### 2.3.Body armour and spine data collection for morphometric analysis

To collect data on external bony skeletal structures (armour), ethanol-preserved samples were stained with Alizarin Red solution following a standard staining method (Peichel et al. 2001). Samples were stored in 40% isopropyl alcohol (propan-2-ol). After confirming the appearance of bony parts, a digital photograph of the right side of every fish was captured using a digital SLR camera (Nikon D5200) with 60 mm macro lens, digital ring flash and a tripod (to set a fixed distance). All photographs included graph paper as a scale (cm) and the measurements of standard length (from the tip of the snout to the end of caudal peduncle), first and second dorsal spine, pelvic spine (from insertion point to the tip), pelvis height and pelvis length were recorded using tpsDig2 version 2.31 (Rohlf 2010) (Fig. Appendix, A1). The total number of lateral plates was also counted from the right side of the stained photograph.

### 2.4 Data analysis

Data were collated in Excel (Microsoft) and statistical analyses were conducted using R, version 3.6.3 (R Core Team 2020).

### Variation in morphological traits between North Uist and South Uist

All measured armour traits (except number of lateral plates) were size-standardized by calculating the residuals of a regression of each trait against standard length to obtain their allometric relationship with body size. The resident (including freshwater) (hereafter 'resident') and anadromous fish were analysed separately to quantify variation in morphological data [lateral plate count (hereafter 'plate count'), standard length, residuals of 1$^{st}$ dorsal spine length (first dorsal spine), 2$^{nd}$ dorsal spine length (second dorsal spine), pelvic spine length (pelvic spine), length of pelvis (pelvis length), height of pelvis (pelvis height)]. Principal component analysis (PCA) was performed using the singular value decomposition method to explore the axis of greatest variation in the measured armour data. All variables were scaled and centred for PCA and grouped for visualisation purposes according to three variables: location (North Uist and South Uist), pH (high pH, neutral pH and low pH) and salinity (freshwater and saltwater).

A one-way analysis of variance (ANOVA) was performed to test for mean differences in each morphological trait between the two different locations (North Uist and South Uist). Modified signed-likelihood ratio tests (MSLRT) (Krishnamoorthy and Lee 2014) were used to test for differences in coefficients of variation (CV) between North Uist and South Uist for each morphological trait. Pearson's correlations were used to test for relationships between measured armour traits. To compare the mean fish length of the anadromous and resident stickleback, a Wilcoxon rank sum t-test was performed for all samples of North Uist and South Uist.

### Environmental factors affecting the variation of morphological traits in North Uist and South Uist

To quantify associations between morphological traits of resident stickleback and different environmental factors of the loch water in North Uist and South Uist, GLMs were fitted with a Gaussian distribution and identity link function. PC1 and PC2 from the PCA of armour traits were fitted as response variables, with location (North or South Uist), pH, salinity and conductivity as predictor variables. GLMs using the mean of each morphological trait (standard length, 1$^{st}$dorsal spine length, 2$^{nd}$ dorsal spine length, pelvic spine length, length of pelvis, height of pelvis and plate count) of each population as the response variable were fitted in relation to pH value, conductivity and salinity of loch water. A Scheffe post-hoc test (Scheffe 1951) for multiple comparisons of mean with unequal sample sizes was performed to observe the differences in the size of measured armour traits among four low pH lochs in North Uist.

## 3. Results

### 3.1 Variation in morphological traits between North Uist and South Uist

The first two PCs from the PCA of the armour traits for the anadromous stickleback from both North and South Uist accounted for 58% of the total variation. All measured armour traits had positive loadings for PC1 and PC2 except pelvic spine length and length of pelvis (Fig. 2). Overall, anadromous fish from North and South Uist were morphologically similar, with some exceptions. The mean height of the pelvis of South Uist anadromous fish was significantly greater than North Uist (one-way ANOVA: $F = 5.19$, $df = 1, 44$, $p = 0.02$; Table 1). The standard length of South Uist anadromous stickleback showed significantly less variation than North Uist fish (MSLRT = 4.15, $p = 0.04$) (Fig. 3).

Resident populations of stickleback from North and South Uist were significantly smaller (smaller mean standard length) than the anadromous populations ($W = 9742$, $p < 0.001$; Fig. 3). Mean standard length of the North Uist resident stickleback (mean ± S.E. 36.44 ± 0.64 mm) was significantly greater than South Uist fish (34.55 ± 0.53 mm) (one-way ANOVA: $F= 5.21$, $df = 1, 216$, $p= 0.02$; Fig. 3), although the difference in length is small.

The first two principal components of a PCA of armour traits for resident fish collected from North and South Uist explained approximately 85% of the variance among individuals (Fig. 4A). PC1 was strongly correlated with all measures of armour traits so that high values of PC1 are associated with more armour. Plate count was highly correlated with PC2 and had a stronger influence on the variation in North Uist fish than South Uist. All measured armour traits (except plate count) were strongly correlated with each other Pearson's correlation (r)>0.5 (Fig. 4B). For resident fish, despite no significant differences in trait means, there was significantly more variation (CV) in all traits (except standard length) in North Uist than South Uist (Table 1).

### 3.2 Factors affecting the variation of morphological traits in North Uist and South Uist

There was wide variation in many of the environmental factors including salinity and pH across the 8 lochs of South Uist and 10 lochs of North Uist: loch pH ranged from low (6.5, acidic) to high (~ 9, alkaline) pH of water (one-way ANOVA: $F = 10.28$, $df = 1, 216$, $p = 0.001$) in the freshwater lochs of North Uist compared to mostly neutral (~ 7) lochs of South Uist (Table A1 and A2).

There were significant associations between the first two PCs of armour traits and the pH of the 18 populations of resident fish collected from North and South Uist (Table 2). There was striking morphological variation in fish found in acidic freshwater lochs relative to other lochs (Fig. 4).

All morphological traits of the resident fish showed significant associations with pH levels in both North and South Uist (Table 2). Total plate count showed different associations with pH of water across the two locations with significantly lower number of plates in higher pH on South Uist, although overall across both North and South Uist the mean plate count was highest in the high pH populations (6.58 ± 1.41) and lowest in freshwater low pH lochs of North Uist (1.86 ± 0.27) ($F_{1,\ 17} = 11.3$, $p<0.001$; Fig. 5A). The mean standard length of resident fish was highest in freshwater high pH populations (37.02 ± 0.97 mm) and lowest in freshwater low pH (33.49 ± 0.92 mm) ($F_{1,\ 17} = 4.85$, $p = 0.044$; Fig. 5B). Among the 18 populations of resident fish of both islands, the mean length of all measured armour variables increased with increasing pH (Fig. 5C-F). One exception to the overall trend was in Tros (pH 6.63), were armour measurements were relatively large. Post-hoc tests (Scheffe Test) revealed that all measured armour traits Tros were significantly larger in length than in the three other freshwater low pH populations of North Uist ($p < 0.001$).

## 4. Discussion

Studies of adaptive radiation have tended to concentrate on what we can learn from adaptive radiations themselves, rather than their absence. In this study we have compared lacustrine populations of three-spined stickleback from isolated habitats on two neighbouring Scottish islands. North Uist has been known for decades to support a striking example of adaptive radiation in this species (Campbell 1985; Magalhaes et

al. 2021), apparently linked to variation in the abiotic and biotic environments that is indexed by variation in pH (Giles 1983, MacColl et al. 2013, Magalhaes et al. 2016, Haenel et al. 2019). In contrast, variation in stickleback on South Uist has hardly been considered, despite apparently similar variation in aquatic environments. We have shown that in fact South Uist lacks the striking morphological variation of North Uist, and this lack of evolutionary diversification is well explained by a lack of variation in the pH of water bodies.

Anadromous stickleback, that are likely to provide a good approximation for the ancestors of Uist freshwater stickleback showed similar variation in armour traits and size on North and South Uist, consistent with close common ancestry in the marine environment, and supporting the idea that differences between the two islands arise because of differences in freshwater. In contrast, resident fish collected from North and South Uist showed variation in all measured armour traits within and between populations which indicate there is adaptive radiation of phenotypic characters among populations as a result of colonization and adaptation to the freshwater environment (Bell and Foster 1994; Magalhaes et al. 2021). There was significantly more variation in armour traits in resident fish of North Uist than South Uist which demonstrates differences in evolutionary diversification of closely-related lineages.

The pH of the lochs showed strong associations with the armour traits of resident fish collected from North and South Uist consistent with several previous studies of stickleback adaptive evolution in freshwater environments (Giles 1983; Bell and Foster 1994; Spence et al. 2012; MacColl et al. 2013; Magalhaes et al. 2016). In the present study, the armour traits of resident stickleback showed substantial variation in dorsal spines, pelvic spines and pelvis length in the freshwater populations of both islands. However, North Uist stickleback exhibited much more morphological variation than the stickleback of South Uist and this variation was directly associated with greater diversity in pH of the loch water on North Uist. This suggests that the differing extent of morphological diversification between North and South Uist is a direct consequence of differences in some aspect or aspects of environment that are related to pH.

It is not immediately obvious why there should be less environmental variation on South, than North, Uist. At first sight, the surface geology of both islands, the root cause of the pH variation (Waterston et al.1979), is rather similar, with acidic, peaty lochs in the east and alkaline, machair lochs in the west. On North Uist, the acidic lochs where the most extreme phenotypes have evolved are relatively large. Adjacent, smaller lochs with similar pH have less extreme phenotypes (ADCM, personal observations). It may be that larger stickleback populations in larger lochs facilitate evolution. On South Uist the eastern lochs are generally smaller, but even the larger ones (e.g. Druidibeg) do not contain unusual stickleback. In any case, the eastern lochs in South Uist are clearly less acidic, but again it is unclear why. Druidibeg is rather shallow, with a largely rocky bottom, and it may be that a shorter residence time of water in the loch, coupled with less contact with peat, may prevent the development of more extreme acidity. The topography of South Uist is also rather different to that of the North. North Uist is generally low-lying, and catchments drain in a radial pattern, meaning that there is little variation in surface geology within catchments. In contrast, South Uist is hilly in the east and the main catchments drain to the west across the machair. The latter pattern results in a kind of 'environmental flow' (movement of water from east to west) that may reduce variation in water chemistry between lochs, at least the development of more alkaline conditions in machair lochs. These linear catchments may also facilitate gene flow between lochs on South Uist that is absent on North Uist, and this could inhibit diversification, but the restricted environmental variance alone on South Uist appears sufficient to explain the reduced morphological diversification, without invoking differences in gene flow.

It is not obvious exactly why phenotypic variation should be associated with variation in pH, although on North Uist resource availability, competition and predation regimes covary with pH (MacColl et al. 2013; MacColl and Aucott, 2014; Magalhaes et al. 2016). It seems most likely that many differences in ecology between alkaline and acidic lochs are fundamentally linked to a reduction in nutrient availability in the latter. This is consistent with the reduced size and armour that is apparent in acidic populations. Body size of some low pH populations was extremely small which mirrored the previous finding of dwarfism in stickleback in association with low pH indicative of poor resource conditions (Giles 1983; MacColl et al. 2013). The dorsal

spines and pelvic complexes were also either rudimentary or missing in most of the populations from low pH lochs (except Tros, see below) in North Uist. Previously, several studies have suggested that this striking variation in armour was due to calcium ion limitations in acidic conditions (Giles 1983; Bourgeois et al. 1994). There is no obvious correlation on North Uist between calcium availability and armour evolution, but it is likely that there is a more general shortage of nutrients in the acidic lochs, which are usually oligotrophic verging on dystrophic. This suggests that the evolution of extreme morphological variation on North Uist is driven more by ecological constraint than by ecological opportunity.

The striking exception provided by stickleback in Loch Trosavat (Tros) to the general pattern of relationship between armour traits and pH is illuminating, if anecdotal. Tros is linked to the sea by a short stream, and this means that large, migratory salmonids (Atlantic salmon,*Salmo salar* and sea trout, *S. trutta* ) and potentially other marine fish that prey upon stickleback, occur in this loch. This by itself could explain the more developed armour in Tros stickleback, but it is also likely that proximity to the sea results in Tros having a better nutrient status than most acid lochs, which could compound the effect of predation.

To conclude, the increased evolutionary variation of North Uist stickleback populations compared to South Uist populations can be explained by greater environmental variation on North Uist, especially linked to variation in the pH of loch water. Research is on-going to understand the role of pH in the ecology and evolution of Uist stickleback populations.

Table 1: One-way analysis of variance (ANOVA) for mean value and modified signed-likelihood ratio test (MSLRT) for coefficients of variation (CV) of each morphological trait [standard length, 1$^{st}$ dorsal spine length (DSL), 2$^{nd}$dorsal spine length (DSL), pelvic spine length (PSL), length of pelvis (LPS), height of pelvis (HPS) and plate count] of stickleback sample fish (217 residents and 45 anadromous) collected from two locations (North Uist and South Uist, Scotland). **Significant P values are in bold.**

| **Morphological traits** | **North and South Uist** | **North and South Uist** | **North and South Uist** | **North and South Uist** | **North and South Uist** | **North and South Uist** | **North and South Uist** | **North and South Uist** | **North and South Uist** |
|---|---|---|---|---|---|---|---|---|---|
| | **Anadromous Mean F** | **Anadromous Mean df** | **Anadromous Mean p** | **Anadromous CV MSLRT** | **Anadromous CV p** | **Resident Mean F** | **Resident Mean df** | **Resident Mean p** | **Resident CV MSLRT** |
| **Std. Length** | 1.03 | 1, 44 | 0.31 | 4.15 | **0.04*** | 5.21 | 1, 216 | **0.02** | 0.89 |
| **1$^{st}$ DSL** | 0.13 | 1, 44 | 0.72 | 0.45 | 0.50 | 3.13 | 1, 216 | 0.08 | 23.44 |
| **2$^{nd}$ DSL** | 0.26 | 1, 44 | 0.61 | 2.25 | 0.13 | 0.22 | 1, 216 | 0.64 | 112.11 |
| **PSL** | 3.99 | 1, 44 | 0.05 | 0.31 | 0.58 | 0.73 | 1, 216 | 0.40 | 84.46 |
| **LPS** | 0.59 | 1, 44 | 0.45 | 1.05 | 0.30 | 0.64 | 1, 216 | 0.43 | 50.90 |
| **HPS** | 5.19 | 1, 44 | **0.03** | 0.39 | 0.53 | 3.67 | 1, 216 | 0.06 | 100.76 |
| **Plate count** | 2.64 | 1, 44 | 0.11 | 0.17 | 0.68 | 0.92 | 1, 216 | 0.34 | 12.46 |

Table 2: GLMs of minimum adequate models with significant variables fitted for morphological traits including PCs of armour traits of resident fish collected from North Uist and South Uist in association with environmental factor (pH value). **Significance at p<0.05 is denoted in bold.**

| **Response variable** | **Predictor variable** | **Family** | **Population** | **F** | **df** | **p** |
|---|---|---|---|---|---|---|
| PC1 | pH value | Gaussian | 18 | 4.88 | 1,17 | **0.042** |

| Response variable | Predictor variable | Family | Population | F | df | p |
|---|---|---|---|---|---|---|
| PC2 | pH value | Gaussian | 18 | 5.25 | 1,17 | **0.034** |
| Avg. standard length | pH value | Gaussian | 18 | 4.85 | 1,17 | **0.044** |
| Avg. plate count | pH value | Gaussian | 18 | 11.3 | 1,17 | **0.003** |
| Avg. first dorsal spine | pH value | Gaussian | 18 | 4.46 | 1,17 | 0.050. |
| Avg. second dorsal spine | pH value | Gaussian | 18 | 4.83 | 1,17 | **0.043** |
| Avg. pelvic spine | pH value | Gaussian | 18 | 7.42 | 1,17 | **0.015** |
| Avg. length of pelvis | pH value | Gaussian | 18 | 11.3 | 1,17 | **0.004** |
| Avg. height of pelvis | pH value | Gaussian | 18 | 10.4 | 1,17 | **0.005** |

**Figure legends**

Fig.1 Sites for the collection of samples from ten lochs in North Uist and eight lochs in South Uist, Scotland (A. Map of United Kingdom B. Loch position in North Uist C. Loch position in South Uist with road map).

Fig. 2 The first two PCs of body armour traits for anadromous sticklebacks collected from North (Ash, circle) and South (orange, triangle) Uist. Traits were plate count and residual values (from body length) of 1$^{st}$ dorsal spine length (X1st.DSL), 2$^{nd}$ dorsal spine length (X2nd.DSL), pelvic spine length (PSL), length of pelvis (LPS), height of pelvis (HPS). PC1 (35.2%) describes overall variation in armour, with positive loadings of all variables. PC2 (22.75%) mainly describes variation in plate count (positive loading), but also reflects negative loadings for LPS and PSL. Ellipses represent 95% confidence levels within each data set.

Fig. 3 Mean standard length (± S.E.) of anadromous and resident fish shows little overall variation in size between North (Ash, circle) and South Uist (orange, triangle) sticklebacks. Resident stickleback of North and South Uist were significantly smaller than the anadromous fish.

Fig. 4 A. The first two principal components (PCs) of six body armour traits and standard length for resident stickleback of North Uist (circle) and South Uist (triangle). PC1 (71.3%) describes overall variation in spine length and pelvic size, while PC2 (13.8%) describes variation in plate number. B. Correlogram showing correlations between all measured armour traits including plate count, PC1, PC2 and standard length of North and South Uist resident fish. Blue colour denotes positive correlations where shade of the square box indicates the strength of the correlation. Measured traits were: 1$^{st}$dorsal spine length (X1st.DSL), 2$^{nd}$ dorsal spine length (X2nd.DSL), pelvic spine length (PSL), length of pelvis (LPS), height of pelvis (HPS). Ellipses with 95% confidence intervals for each data set describing variation in four pH groups of loch water, separating the larger cluster for samples in freshwater low pH fish than the other groups.

Fig. 5 A-F Mean (± S.E.) plate count (A) and standard length (B-F) of five armour traits in 18 freshwater stickleback populations on North (circle) and South Uist (triangle).

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## Appendix

**Table A1. Sampling locations, habitat types, sample number (N) and physicochemical parameters of eight lochs at South Uist, Scotland (FW = Fresh water, SW = Saltwater, Anad = Anadromous, Resi = Resident).**

| Sl. | Island | Loch | Location | Habitat | N | pH | Conductivity (μΣ/ςμ) | Salinity (ppt) | Da sa co tic |
|---|---|---|---|---|---|---|---|---|---|
| 1. | South Uist | a'Mhoil (Mhoi) | 57°17'11"N;7°25'06"W | FW high pH | 11 FW | 7.5 | 63.9 | 0.04 | 08. |
| 2. | | Grogarry (GroS) | 57°19'59"N; 7°22'55"W | FW high pH | 5 FW | 7.7 | 1167 | 0.746 | 08. |
| 3. | | Eadaray (Eada) | 57°15'39"N; 7°22'14"W | FW high pH | 5 FW | 7.7 | 60.5 | 0.038 | 08. |
| 4. | | West Loch Ollay (OllW) | 57°15'58"N; 7°24'01"W | FW neutral pH | 30 FW | 7.4 | 138.2 | 0.088 | 08. |
| 5. | | Stilligarry (Stil) | 57°19'09"N; 7°22'12"W | FW neutral pH | 3 FW | 7.4 | 116.5 | 0.074 | 08. |
| 6. | | a' Phuirt-ruaidh (Phui) | 57°17'48"N; 7°21'54"W | FW neutral pH | 1 Anad 11 Resi | 7.3 | 48.2 | 0.038 | 08. |
| 7. | | Druidibeg (Drui) | 57°19'26"N; 7°19'38"W | FW neutral pH | 10 FW | 7.3 | 40.9 | 0.026 | 08. |
| 8. | | Abhainn Roag (Aroa 1,2,3) | 57°17'17"N; 7°21'58"W 57°17'27"N; 7°22'33"W 57°17'30"N; 7°22'47"W | FW neutral pH Brackish water | 13 Anad 38 Resi | 7.4 7.0 7.4 | 52.2 106.8 2630 | 0.033 0.068 1.683 | 06. 08. |

**Table A2. Sampling locations, habitat types, sample number (N) and physicochemical parameters of ten lochs at North Uist, Scotland (FW = Fresh water, SW = Saltwater, Anad = Anadromous, Resi = Resident).**

| Sl. | Island | Loch | Location | Habitat | N | pH | Conductivity (μΣ/ςμ) | Salinity (ppt) | Da sa co tic |
|---|---|---|---|---|---|---|---|---|---|
| 1. | North Uist | Chadha Ruaidh (Chru) | 57°35'37"N; 7°11'44"W | FW low pH | 15 FW | 6.6 | 148 | 0.094 | 14. |
| 2. | | Scadavay (Scad) | 57°35'6"N; 7deg14'10"W | FW low pH | 12 FW | 6.5 | 130.9 | 0.083 | 15. |
| 3. | | Tormasad (Torm) | 57°33'45"N; 7deg19'1"W | FW low pH | 14 FW | 7.0 | 162.5 | 0.104 | 13. |
| 4. | | Trosavat (Tros) | 57°35'3"N; 7°24'45"W | FW low pH | 5 Anad 9 Resi | 6.6 | 165.9 | 0.106 | 14. |

| Sl. | Island | Loch | Location | Habitat | N | pH | Conductivity (μΣ/ς μ) | Salinity (ppt) | Da sa co tio |
|---|---|---|---|---|---|---|---|---|---|
| 5. | | Hosta (Hosta) | 57°37'40"N; 7deg29'18"W | FW high pH | 11 FW | 8.5 | 432 | 0.280 | 13. |
| 6. | | Grogary (Grog) | 57°36'54"N; 7deg30'40"W | FW high pH | 5 Anad 10 Resi | 8.3 | 340 | 0.220 | 16. |
| 7. | | na Reival (Reiv) | 57°36'39"N; 7°30'50"W | FW high pH | 12 FW | 9.0 | 439 | 0.280 | 14. |
| 8. | | Ard heiskir (Ardh) | 57°34'48"N; 7deg24'48"W | SW / Brackish water) | 7 Anad 7 Resi | 8.3 | 47,400 | 30.33 | 14. |
| 9. | | Fairy Knoll (Faik) | 57°38'7"N; 7°12'54"W | SW / Brackish water) | 7 Anad 7 Resi | 8.5 | 45,100 | 28.86 | 16. |
| 10. | | Loch Duin (Duin) | 57°38'35"N; 7°12'40"W | SW / Brackish water) | 7 Anad 7 Resi | 8.4 | 27,460 | 13.73 | 15. |

Fig. A1 Measurement of standard length (SL), dorsal spines (1st and 2nd), pelvic spine, pelvis (height and length) and lateral plates in a fully plated stained fish. Pelvis length was measured from the reflection in the mirror.

**Competing Interests Statement** : We have no conflicts of interest to disclose.

**Ethical Approval** : All research work in this article was reviewed by the University of Nottingham AWERB, and carried out under Home Office licence 3003415. No licence was required to collect stickleback in Scotland.

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