

PREDATOR HANDLING FAILURES OF LATERAL PLATE MORPHS IN *GASTEROSTEUS ACULEATUS*: FUNCTIONAL IMPLICATIONS FOR THE ANCESTRAL PLATE CONDITION

by

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Summary

The completely plated morph in the threespine stickleback (*Gasterosteus aculeatus*) is considered to be the ancestral plate condition and is the predominant morph inhabiting marine waters and numerous northern freshwaters. Evolutionary aspects of this distribution have been widely addressed yet functional mechanisms remain obscure. Experiments described here using the common piscivore (*Oncorhynchus clarki*) show that the posterior plates of *G. aculeatus* interfere with the swallowing abilities of the piscivore, possibly by disrupting pharyngeal jaw retraction, and this leads to increased escape opportunities of the stickleback. The advantage of the completely plated condition is most expressed at higher ratios of prey diameter to predator mouth diameter and appears to have a defensive effect comparable to that of dorsal and pelvic spines. This attribute, combined with the physical protection that plates offer against puncturing, would be particularly beneficial where there is high probability of capture by toothed predators. Such a selection regime appears to characterize the predominantly limnetic and pelagic habitats where marine stickleback are found and may account for the wide geographical distribution of the completely plated morph and its persistence from the Miocene.

Keywords: functional morphology, *Gasterosteus*, lateral plates, stasis, predator-prey.

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Introduction

Persistence and stasis of ancestral traits occur in the fossil record of a diversity of marine taxa (Stanley & Yang, 1987). The constancy of form, occasionally across major biophysical boundaries in the geological record, has led to an emerging view that morphological and epigenetic constraints fundamentally limit the capacity for change (Gould & Eldridge, 1977; Oster & Alberch, 1982; review in Schlichting & Pigliucci, 1998). The persistence of species with ancestral traits may be facilitated in peripheral habitats such as the oceanic depths with reduced species composition but it is not clear how ancestral traits will persist in highly competitive surface or shallow water habitats. Studies of predator-defences in marine gastropods show evidence for continuity of predator-prey interactions in the fossil record (Vermeij, 1986) which would suggest that stasis in these traits may reflect long-term stability of the adaptive landscape rather than constraints (Charlesworth *et al.*, 1982; Hecht & Hoffman, 1986; Levinton, 1988).

The threespine stickleback (*Gasterosteus aculeatus*) has been a useful model for evaluating a broad range of evolutionary processes (Bell, 1984) and may offer insight into factors influencing stasis. This species is widespread in northern marine and freshwater waters, including littoral and pelagic habitats. Bony lateral plates on these fish, which are highly variable among freshwater populations, are largely monomorphic for the completely plated morph throughout their distribution in marine waters of the Atlantic and Pacific oceans (Heuts, 1947; Münzing, 1963; reviews in Wootton, 1976, 1984; Bell, 1984). Analyses of the fossil record indicates that the completely plated condition has continuity back to the Miocene and probably represents the ancestral condition in this taxon (Bell, 1977, 1981).

Several hypotheses have been proposed to account for the occurrence of the complete morph in *G. aculeatus*. In marine populations, elevated salinity may favour this morph, perhaps due to genetic linkage between plate number and ion regulation (Bertin, 1925; Heuts, 1947). Among freshwater populations, however, there is no correlation between relative salinity and incidence of the complete morph (Hagen & Gilbertson, 1972). Low water temperatures are correlated with presence of the complete morph in marine waters (Bertin, 1925; Heuts, 1947) as well as freshwaters (Munzing, 1972; Hagen & Moodie, 1982) but it is unclear how this relates functionally to number of bony plates. Elevated frequencies of the complete morph in large

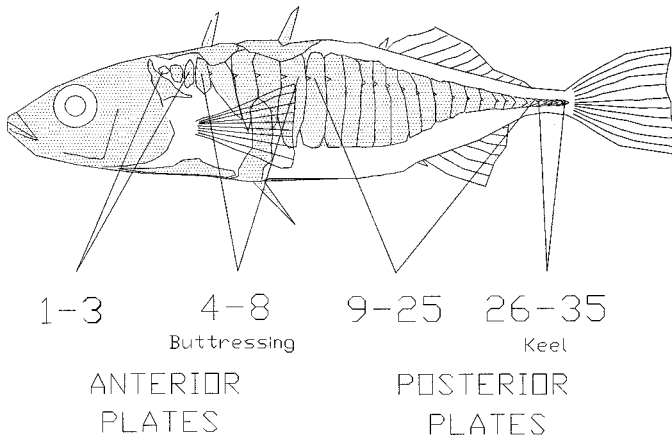


Fig. 1. Schematic of lateral plates on the complete morph of threespine stickleback. Unshaded areas show unprotected integument.

rivers of western Europe could be historical markers of late Pleistocene distribution (Münzing, 1963, 1972) and signify levels of introgression and hybridization between marine and freshwater populations (Miller & Hubbs, 1969; however, see Hagen & McPhail, 1970). These hypotheses each have a common assumption that the presence of plates along the length of the trunk is not directly functional but rather selectively neutral and/or correlated to other functional attributes. If this is correct, then the wide distribution and long term persistence of the completely plated morph would largely represent developmental constraints.

Plates on *Gasterosteus* are important during predator defence and potentially these traits extend from long-term associations with predators. The plates can be partitioned into functional-specific zones including anterior and posterior regions of the trunk (Fig. 1). Plates on both regions reduce the amount of epidermal puncturing by toothed predators and this increases probability of survival among stickleback which have escaped during handling by the predator (Reimchen, 1992). Biomechanical studies demonstrate that anterior plates from positions 4 through 8 buttress the dorsal and pelvic spines from deflection during predator handling (Reimchen, 1983). Combination of these two functional mechanisms will contribute to the wide distribution of the complete morph.

During a recent series of experiments using large-spined, low plated stickleback and a common predator (*Oncorhynchus clarki*), I observed that

increased cross-sectional diameter of the stickleback (with erect spines) was directly correlated to increased escape rate of the stickleback and increased handling time by the trout (Reimchen, 1991a, b). Yet there was considerable residual variance in handling time for any given prey size and it appeared that number of lateral plates might influence predator success as the fish with the highest number of plates required the longest handling time (Reimchen, unpubl. obs.). To examine this possibility more thoroughly, I obtained stickleback from a population which had both low plated and completely plated individuals. An additional characteristic of these fish was their small dorsal and pelvic spines relative to those present in the first locality. Because spines are very effective in reducing the handling success of predatory fish and large spines are more effective than small spines (Hoogland *et al.*, 1957), I anticipated that the stickleback with small spines would be more readily swallowed compared with long-spined stickleback, independent of the number of posterior bony plates. In this study, I test predator handling success primarily in relation to number of lateral plates and secondarily in relation to the spine length.

Methods

General experimental procedures follow those described in Reimchen (1991a). Stickleback were obtained from two localities, Drizzle Lake and Delkatla Pond, both located on the northern regions of the Queen Charlotte Islands, western Canada (morphometrics in: Reimchen *et al.*, 1985; Gach & Reimchen, 1989). Drizzle Lake stickleback are all of the low morph and usually have from two to seven lateral plates (mode 5) and are subject to predation by a broad diversity of avian and salmonid piscivores (Reimchen, 1994). Delkatla stickleback are polymorphic but generally have from seven to ten plates or more than 30 plates. This population, found on an estuary, appears to be recently derived from completely plated marine ancestors and is currently subject to predation by predatory fish and birds (Reimchen, unpubl. data). In the first series of experiments, I used stickleback from Drizzle Lake and Delkatla Pond in order to assess the combined effects of spine size and number of plates on handling success. In the second series of experiments, only Delkatla fish were presented in order to compare prey which differed predominantly in plate number. Before each trial for both series of experiments, I scored plate morph and measured body length (standard length, SL). In each trial where I used fish from two populations or of different morphs, I chose fish of similar body size. Stickleback were added individually to 120 l glass tanks each containing one cutthroat trout. The sticklebacks were usually attacked within several seconds and the outcome recorded. For all trials, I noted total handling time (s) and number of escapes following initial capture. Handling time represented the interval from first capture to completion of swallowing or to final rejection of the prey when the trout showed no additional interest in the stickleback. In some trials, I was able to observe and record the

orientation of the stickleback in the mouth; this is a factor in handling success of the trout (Reimchen, 1991b).

I obtained trout from two lakes in the region (Drizzle Lake, Mayer Lake) and in both of the localities, low plated stickleback are the dominant prey of the trout (Moodie, 1972; Reimchen, 1990). During the first series of experiments, I used four trout (SL: 16, 19, 21, 25.5 cm), all from Drizzle Lake, while in the second series, I used one trout from Drizzle Lake (SL: 25 cm) and two additional trout from Mayer Lake (SL: 32, 42 cm).

I compared the ratio of cross-sectional diameter of the stickleback with spines erected (PD) to maximum gape of the trout (MD). I measured PD (± 0.5 mm) on preserved specimens by placing the stickleback with erect dorsal and pelvic spines through graded reference holes (1 mm units). From these measurements, I derived the following regressions (Delkatla, PD = $0.41 + 0.38 \times \text{SL}$; Drizzle Lake, PD = $-1.49 + 0.46 \times \text{SL}$). For Delkatla fish, I compared regression lines of PD against SL between the low morph ($N = 42$) and the complete morph ($N = 40$) and these were not significantly different from each other ($F = 0.72$, $p = 0.4$, ANCOVA). Gape of cutthroat trout was estimated from the equation MD = $-1.347 + 1.11 \times \text{SL}$ (Reimchen, 1991a).

Data on handling time were log-transformed and analysed with ANOVA. Plate number was coded into three groups (A = Drizzle low morph, 3-6 plates, B = Delkatla low morph, 5-10 plates, C = Delkatla complete morph, > 30 plates). For comparing differences in handling time, I performed an analysis of covariance (handling time as dependent variable, PD/MD as a covariate, plate morph as the factor). I also compared means with multiple range tests (Least Significant Difference). Data on number of escapes and post-capture alignment in relation to plate grouping were tested with log-likelihood ratios. All statistical procedures were run using SPSS[®] for Windows[™] (Norusis, 1993).

Results

During the first series of experiments, which included both populations, average handling time was highest for the complete morph from Delkatla ($\bar{x} = 133$ s), intermediate for the low morph from Delkatla ($\bar{x} = 73.8$ s) and lowest for the low morph from Drizzle ($\bar{x} = 25.0$ s). Analysis of variance on log-transformed handling time was significant ($F_{2,148} = 6.0$, $p < 0.004$) and multiple range tests (LSD) show significant differences between the complete morph from Delkatla and the low morph from Drizzle Lake but not between the complete and low morph from Delkatla.

Handling time by trout is very sensitive to prey size (Reimchen, 1991a) and this might account for the higher handling time of the Delkatla stickleback if these fish were larger than Drizzle stickleback. However, SL of fish in the three groups was similar ($\bar{x} = 44.0$, 43.0 and 43.9 mm for Delkatla complete, Delkatla low and Drizzle low respectively, $F = 0.3$, $p = 0.77$). Furthermore, cross-sectional diameter of the prey (PD) (complete morph

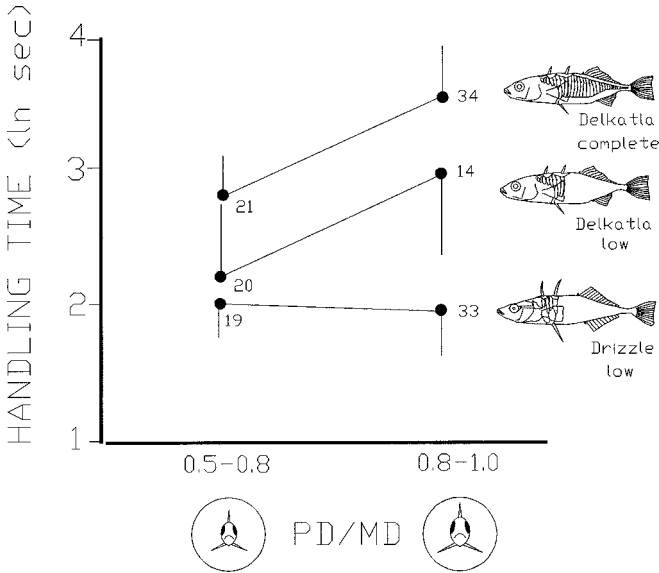


Fig. 2. Handling time ($\bar{x} \pm 1$ SE) versus PD/MD grouping for plate morph and locality (experiment 1). PD/MD is ratio of prey diameter to mouth diameter of the trout. Sample sizes shown adjacent to means.

$\bar{x} = 17.3$ mm, low morph 17.0 mm, $F = 1.0$, $p = 0.32$) was significantly smaller than in Drizzle stickleback ($\bar{x} = 18.7$ mm, $F = 9.4$, $p < 0.003$). Consequently, the elevated handling time in the Delkatla stickleback is not due to their larger size.

As the ratio of PD to mouth diameter of the trout (MD) moves towards unity (*i.e.* PD = MD), handling efficiency greatly declines (Reimchen, 1991a) and I suspected that any handling differential among the morphs would be accentuated at the higher ratios. The data appear to be consistent with this (Fig. 2) but regression analyses of handling time against PD/MD for each of the plate morphs indicates no significant differences in the regression slopes ($F < 1.9$, $p > 0.16$).

An additional factor which can contribute to differences in handling time is initial strike position of the predator. When the strike by a predatory fish occurs on the posterior region of the prey, the handling period is significantly longer and more often unsuccessful than when strikes are on the head (Reimchen, 1991b). I observed strike positions on 46 Delkatla completes, 25 Delkatla lows and 31 Drizzle stickleback of which there were 78%, 56%

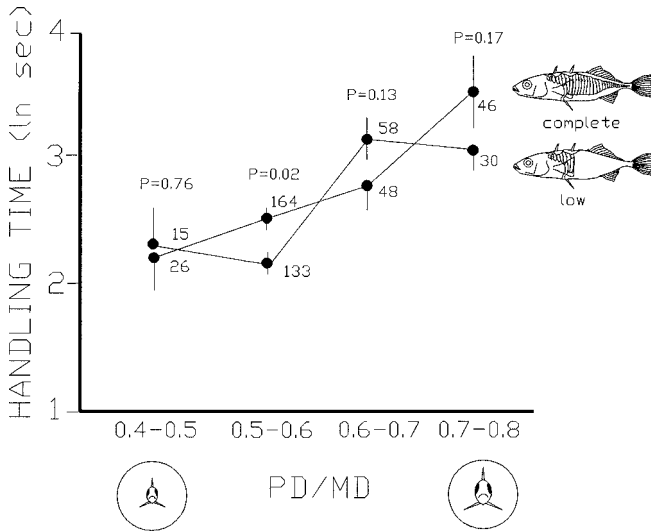


Fig. 3. Handling time ($\bar{x} \pm 1$ SE) versus PD/MD grouping for plate morph from Delkatla (experiment 2). Numbers show sample sizes and statistical probabilities for unpaired *t*-test.

and 68% tailfirst attacks on the three groups respectively; analyses of raw data shows no significant effect (log-likelihood $\chi^2_2 = 3.8, p = 0.15$).

In the second series of experiments, I excluded Drizzle Lake stickleback and presented only the low plated and the complete morphs from Delkatla. Results from these experiments, when partitioned among four PD/MD groups, show only a single group (PD/MD 0.5-0.6) with statistically significant differences in which the complete morph required higher handling times than the low morph (Fig. 3). There were no differences in the slope of the regression lines ($F = 0.0, p = 0.97$) and comparisons of intercepts (ANCOVA) showed a marginally but non-significantly higher handling time for the complete morph than for the low morph (complete = 16.9 s, low = 13.7 s, $F = 2.08, p = 0.15$).

The high incidence of repaired injuries on stickleback in some natural populations demonstrates that escape during capture by predators is a common occurrence (Reimchen, 1988). As such, escape frequency rather than handling time may provide a more realistic assessment of potential selective differences among plate morphs. In both series of experiments, I compared numbers of escapes for the different morphs. Among 151 trials during the first series of experiments, 36.9% of the Delkatla complete morph, 29.4% of the Delkatla low morph and 19.2% of the Drizzle low morph

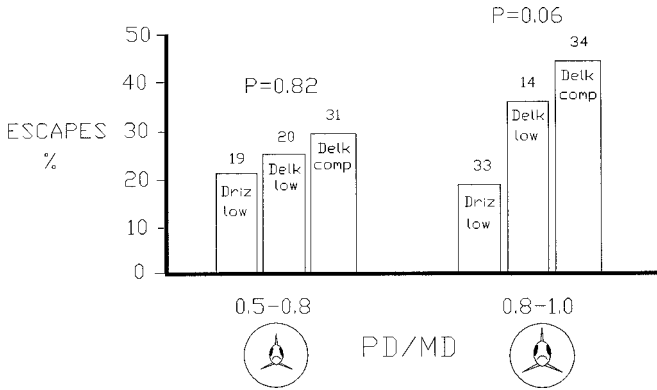


Fig. 4. Frequency of escapes for PD/MD grouping for plate morph and locality (experiment 1). Numbers show sample size and statistical probabilities for log-likelihood χ^2 .

had one or more escapes. However, these differences were not statistically significant (log-likelihood $\chi^2 = 4.51$, $p = 0.11$). Dividing the data into low and high PD/MD groups again shows a comparable ranking among the three plate morphs with the greatest differences occurring in the high PD/MD group (Fig. 4) but the differences are not statistically different. Among 518 trials in the second series of experiments, using only Delkatla stickleback, there was a similar ranking but with more discrete results as escape occurred in 7.7% of the trials with the complete morph and 1.8% of those with the low morph (log-likelihood $\chi^2 = 10.9$, $p < 0.001$). Partitioning these data among PD/MD groups (Fig. 5) demonstrated only a marginal and non-significant difference at low PD/MD ratios but a much stronger and significant increase in escape rate of the complete morphs at higher PD/MD ratios.

Stickleback often escape and are recaptured on multiple occasions during individual trials and such data might provide a supplemental comparison of handling success. For Delkatla stickleback, there were a total of 110 escapes in 299 trials with the complete morph and 25 escapes in 317 trials with the low morph ($\chi^2 = 48.5$, $p < 0.001$). However, this result could be misleading if many of the multiple escapes occurred during a few trials with very high PD/MD ratios or high handling periods. To address this limitation, I compared average number of escapes against different handling periods for the Delkatla plate morphs. These data (Fig. 6) exhibit a consistent trend in which the complete morph has marginally higher escape frequencies during each handling period but the differential is accentuated with increased handling time.

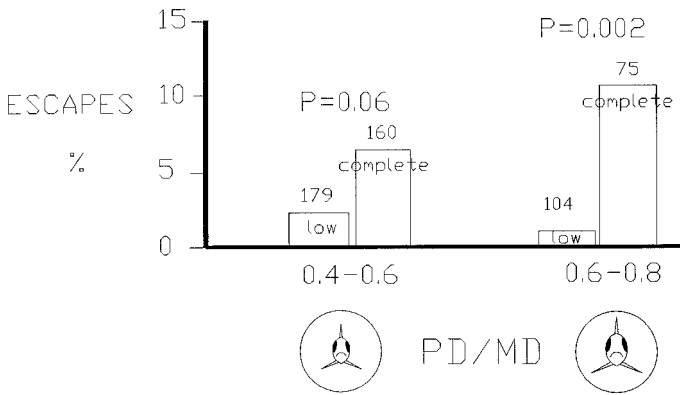


Fig. 5. Frequency of escapes for PD/MD grouping for plate morphs from Delkatla (experiment 2). Numbers show sample size and statistical probabilities for log-likelihood χ^2 .

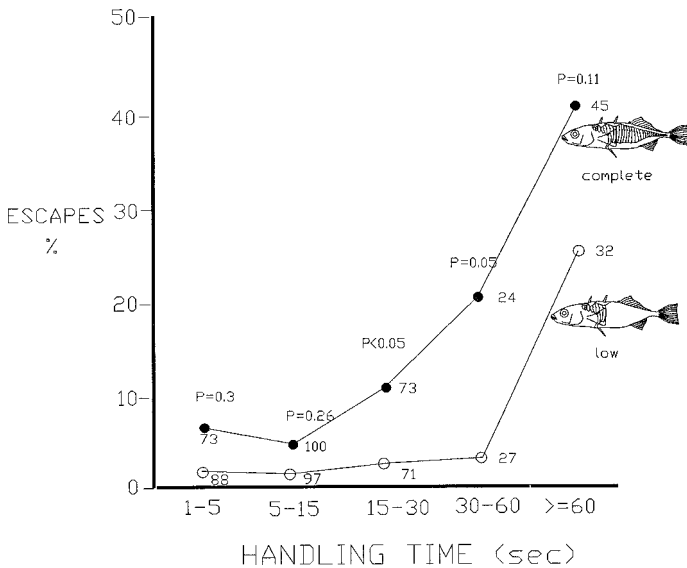


Fig. 6. Frequency of escapes against handling time for plate morphs from Delkatla (experiment 2). Numbers show sample size and statistical probabilities for log-likelihood χ^2 .

Discussion

Previous biomechanical studies on *G. aculeatus* have demonstrated two functional attributes to the lateral plates: (1) four of the anterior plates provide the major structural buttressing for the dorsal and pelvic spine (Reim-

chen, 1983) and (2) each plate reduces the extent of epidermal lacerations during capture by toothed piscivores and this results in higher survival rate of stickleback with greater numbers of plates (Reimchen, 1992). Results from the present study demonstrate a third function. Posterior plates are associated with increased handling failures of the predator and this effect is accentuated as stickleback size reaches the maximum gape of the predator.

The actual mechanism for the differential escape rate of the plate morphs has not yet been thoroughly established but it appears to be associated with pharyngeal jaw activity. Escape of the stickleback occurred both during the initial capture phase when the trout attempts to fracture or depress the erect dorsal and pelvic spines and during the second phase when the trout attempts to swallow the stickleback. In many bony fishes, including trout and other Salmonidae, the prey are raked into the esophagus from the oral cavity with repeated protraction and retraction of the pharyngeal jaws which occur at the posterior region of the oral cavity (Liem & Kaufman, 1984; Lauder, 1985; Galis, 1993). The activity of these internal jaws is not easily monitored as their motion is not coupled with mandibular jaw action and can occur when the mouth is closed. I suspected that pharyngeal jaw action might produce a weak audible stimulus and lowered a hydrophone in the tank during a feeding event. Rhythmic scratching was detectable during the swallowing phase and presumably reflected pharyngeal raking on the bony plates of the stickleback for it abruptly ceased when the stickleback was swallowed or expelled from the mouth. Based on the prolonged pharyngeal jaw activity when a completely plated morph had been captured, I suggest that posterior lateral plates directly interfere with pharyngeal jaw action and that increased number of lateral plates increase the extent of this interference. This can result in longer handling time and increased escape rate of the stickleback. That swallowing difficulty and elevated escape rate was greater when stickleback cross-sectional diameter increased relative to the maximum gape of the trout is consistent with this hypothesis.

Alignment of the prey in the mouth is an important factor in predator handling efficiency (Reimchen, 1991b) and may influence the selective differential between the completely plated and low plated morph of stickleback. From 50 to 75% of the strikes of the trout were on the mid- and posterior trunk of the stickleback and this can result in tailfirst alignment in the mouth which in turn leads to prolonged handling time. As the pharyngeal jaws rake the fish tailfirst into the esophagus, the erect but posteriorly angled dorsal

and pelvic spines can lodge against the palate or branchial arches blocking further movement of the stickleback into the esophagus. In this alignment, pharyngeal raking occurs on the trunk posterior to the spines. On a low plated fish, this is likely to lead to epidermal lacerations on the trunk and increased probability of mortality if the stickleback escapes (*e.g.* Reimchen, 1992). Increased number of posterior plates would minimize epidermal lacerations during pharyngeal raking and increase the post-capture survival.

One of the most prominent features of *G. aculeatus* are the large dorsal and pelvic spines and these probably constitute the first line of defence against predators. In one of the seminal papers on prey defences in fishes, Hoogland *et al.* (1957) demonstrated that spines increase the effective diameter of the stickleback and injure the soft parts of the predator's mouth, both of which lead to increased escape opportunities of the stickleback. In these experiments, pike (*Esox*) and perch (*Perca*) were presented with a range of prey that differed in the extent of spine expression. *G. aculeatus*, with their large dorsal and pelvic spines, were eaten at lower frequency than the nine-spined stickleback (*Pungitius*), which have comparatively small spines, and these were eaten less frequently than non-spiny prey including minnow (*Phoxinus*), roach (*Rutilus*) and rudd (*Scardinius*). Removal of dorsal and pelvic spines on *G. aculeatus* greatly increased the palatability of the stickleback although not to the equivalent level of the species lacking spines. Given this outcome, it was surprising that in my experiments, Delkatla stickleback with short dorsal and pelvic spines and with low PD/MD ratios had higher post-capture escape rate than Drizzle Lake stickleback of equivalent body size with large and robust dorsal and pelvic spines and high PD/MD ratios. One of the additional distinctions between these two populations was the presence of posterior plates on Delkatla stickleback and these plates appear to increase handling failures of the predator. If so, then some of the differences in escape rate observed by Hoogland *et al.* (1957) among the five species which were attributed to differences in spines could have been influenced by lateral plates as among the prey offered to the predators, the least preferred (*i.e.* *G. aculeatus*), was the only prey species with bony plates on the trunk.

The completely plated morph of *G. aculeatus* appears to exhibit a geographical association with water temperature. Among freshwater populations, this morph tends to be more prevalent in northern latitudes (Bertin, 1925; Munzing, 1963, 1972; Wootton, 1976 for review) and on the eastern edges of the continents where winter temperatures are lower (Hagen

& Moodie, 1982). Among southern populations as in California, the complete morph tends to be more common in colder streams but thermal factors are less predictive than stream gradient (Baumgartner & Bell, 1984). One of the potential selective mechanisms coupling plates and temperature is the ratio of pursuit to capture success by the predator, as this can lead to alternate defence adaptations (Reimchen, 1994). Prey defense following capture by a toothed predator favours increased complement of plates (Reimchen, 1992, 1997) and therefore any ecological variable or behavioural trait which leads to increased likelihood of capture should produce an adaptive regime favouring the completely plated morph. One such ecological variable could be low water temperature. Metabolic rates in an ectotherm will be reduced at lower temperatures and this will lead to reduced burst swimming performance (Webb, 1978). Any such reduction which results in an increased capture rate by a larger-bodied predator with greater thermal inertia, is likely to generate a selective advantage to the complete morph.

Variability in the complete morph may also result from differential predation on plate number. Bañbura *et al.* (1989) examined pike (*Esox lucius*) stomachs and found that stickleback with 33-35 plates were eaten less frequently than those with 30-31 plates compared with population frequencies. These results are consistent with predictions from my study as they demonstrate the potential for increased escape rate for higher plate numbers. Yet the results could also be explained by differential capture of these phenotypes rather than differential escape after capture if there were behavioural differences among morphs (Huntingford, 1993). Lateral plates comprise a meristic series corresponding to the underlying body segmentation and, as such, on a completely plated morph, fish with fewer segments will have correspondingly fewer plates. Because differences in number of body segments can alter burst acceleration (Swain & Lindsey, 1984; Swain, 1992), the differential predation observed by Bañbura *et al.* (1989) could be a consequence of differential swimming performance rather than differential plate number.

One of the major trends to emerge from large-scale collections of *G. aculeatus* was the prevalence of the completely plated morph in marine waters (Wootton, 1976, for review). Elevated salinity may be one of the selective mechanisms for the occurrence of the complete morph in these habitats (Bertin, 1925; Heuts, 1947). An additional feature of marine habitats

other than salinity, is the overwhelming predominance of the limnetic zone and great depth of the water column relative to that found in even the largest freshwater lakes. Threespine stickleback occur on the continental shelves but also in open surface waters of the Atlantic and the Pacific Oceans where they are exposed to a diverse range of toothed predators (Gross, 1978; Reimchen, 1994). Salmonids (*Oncorhynchus* spp.) are known to forage on stickleback 1000 km from the nearest land (Quinn & Light, 1989; Deagle *et al.*, 1996). There is little shelter available in open water habitats and a small fish would be at high risk of capture compared with littoral or benthic habitats where shelter is often available and where escape during pursuit is possible. Under open water conditions, the completely plated morph should have higher fitness than the low morph given the increased likelihood of escape during capture (this study) and the increased likelihood of survival following escape due to reduced risk of epidermal puncturing (Reimchen, 1992). Such a selective regime would contribute to the prevalence of the completely plated morph in marine waters. It would also account for some of the freshwater distribution of this morph. In recent geographical surveys of 180 freshwater lakes on the Queen Charlotte Islands (Reimchen *et al.*, 1985; Reimchen, 1989, unpubl. obs.), the complete morph was common in only three localities and these were characterized by their large size, steep shore profile, lack of littoral zone, high water clarity and prevalence of open water piscivores. These are the same general ecological features as those observed in open water marine habitats.

This study suggests a potential functional mechanism for the stasis of the ancestral plate condition in marine *G. aculeatus*. Most modern bony fishes (Euteleostei) use the pharyngeal jaw apparatus for prey handling (Lauder, 1983) and these fish have been common in marine waters from the Upper Cretaceous (Carroll, 1988), well before the first Miocene appearance of *Gasterosteus* (Bell, 1977). As stickleback comprise a common element in the diet of numerous marine piscivores, it is reasonable to hypothesize that the persistence of the complete morph from the Miocene, and its presence in some cold freshwater lakes extends from long-term continuity of an adaptive landscape mediated through post-capture handling failures in bony fishes.

References

- Bañbura, J., Przybylski, M. & Frankiewicz, P. (1989). Selective predation of the pike *Esox lucius*: comparison of lateral plates and some metric features of the three-spined stickleback *Gasterosteus aculeatus*. — *Zoologica Scripta* 18, p. 303-309.
- Baumgartner, J.V. & Bell, M.A. (1984). Lateral plate morph variation in California populations of the threespine stickleback, *Gasterosteus aculeatus*. — *Evolution* 38, p. 665-674.
- Bell, M.A. (1977). A late Miocene marine threespine stickleback, *Gasterosteus aculeatus*, and its zoogeographic and evolutionary significance. — *Copeia* 1977, p. 77-282.
- (1981). Lateral plate polymorphism and ontogeny of the complete plate morph of threespine sticklebacks (*Gasterosteus aculeatus*). — *Evolution* 35, p. 67-74.
- (1984). Evolutionary phenetics and genetics: the stickleback, *Gasterosteus aculeatus* and related species. — In: *Evolutionary genetics of fishes* (B.J. Turner, ed.). Plenum Publishing Corp., New York, p. 431-528.
- Bertin, L. (1925). Recherches bionomiques, biometriques et systematiques sur les Epioches (*Gasterosteidae*). — *Annales de l'Institut Oceanographique, Monaco* 2, p. 1-204.
- Carroll, R.L. (1988). *Vertebrate paleontology and evolution*. — W.H. Freeman and Co., New York.
- Charlesworth, B., Lande, R. & Slatkin, M. (1982). A neo-Darwinian commentary on macroevolution. — *Evolution* 36, p. 474-498.
- Deagle, B.E., Reimchen, T.E. & Levin, D.B. (1996). Origins of endemic stickleback from the Queen Charlotte Islands: mitochondrial and morphological evidence. — *Can. J. Zool.* 74, p. 1045-1056.
- Gach, M. & Reimchen, T.E. (1989). Mitochondrial DNA patterns among endemic stickleback from the Queen Charlotte Islands: a preliminary survey. — *Can. J. Zool.* 67, p. 1324-1328.
- Galis, F. (1993). Interactions between the pharyngeal jaw apparatus, feeding behaviour, and ontogeny in the cackled fish, *Haplochromis piceatus*: a study of morphological constraints in evolutionary ecology. — *J. Exp. Zool.* 267, p. 137-154.
- Gould, S.J. & Eldredge, N. (1977). Punctuated equilibria: the tempo and mode of evolution reconsidered. — *Paleobiology* 3, p. 115-151.
- Gross, H.P. (1978). Natural selection by predators on the defensive apparatus of the three-spined stickleback, *Gasterosteus aculeatus* L. — *Can. J. Zool.* 56, p. 398-413.
- Hagen, D.W. & Gilbertson, L.G. (1972). Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific northwest, America. — *Evolution* 26, p. 32-51.
- & McPhail, D.M. (1970). The species problem within *Gasterosteus aculeatus* on the Pacific coast of North America. — *J. Fish. Res. Bd. Can.* 27, p. 147-155.
- & Moodie, G.E.E. (1982). Polymorphism for plate morphs in *Gasterosteus aculeatus* on the east coast of Canada and an hypothesis for their global distribution. — *Can. J. Zool.* 60, p. 1032-1042.
- Hecht, M.K. & Hoffman, A. (1986). Why not neo-Darwinism? — *Oxford surveys in evolutionary biology* 3, p. 1-47.
- Heuts, M.J. (1947). Experimental studies on adaptive evolution in *Gasterosteus aculeatus* L. — *Evolution* 1, p. 89-102.
- Hoogland, R., Morris, D. & Tinbergen, N. (1957). The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as a means of defence against predators (*Perca* and *Esox*). — *Behaviour* 10, p. 205-237.

- Huntingford, F.A. (1993). Development of behaviour in fishes. — In: The behaviour of teleost fishes (T.J. Pitcher, ed.). Chapman and Hall, London, p. 57-83.
- Lauder, G.V. (1983). Functional design and evolution of the pharyngeal jaw apparatus in euteleostean fishes. — *Zool. J. Linn. Soc.* 71, p. 1-38.
- (1985). Aquatic feeding in lower vertebrates. — In: Functional vertebrate morphology (M. Hildebrand, D.M. Bramble, K.F. Liem & D.B. Wake, eds). Belknap Press, Harvard University Press, Cambridge, Massachusetts, p. 210-229.
- Levinton, J. (1988). Genetics, paleontology and macroevolution. — Cambridge University Press, Cambridge.
- Liem, K.F. & Kaufman, L.S. (1984). Intraspecific macroevolution: functional biology of the polymorphic cackled species *Cichlasoma minckleyi*. — In: Evolution of fish species flocks (A.E. Echelle & I. Kornfield, eds). University of Maine at Orono Press, Maine, p. 203-216.
- Miller, R.R. & Hubbs, C.L. (1969). Systematics of *Gasterosteus aculeatus*, with particular reference to intergradation and introgression along the Pacific coast of North America: a commentary on a recent contribution. — *Copeia* 1969, p. 52-69.
- Moodie, G.E.E. (1972). Predation, natural selection and adaptation in an usual stickleback. — *Heredity* 28, p. 155-167.
- Münzing, J. (1963). The evolution of variation and distributional patterns in European populations of the three-spined stickleback, *Gasterosteus aculeatus*. — *Evolution* 17, p. 320-332.
- (1972). Polymorphe Populationen von *Gasterosteus aculeatus* L. (Pisces, Gasterosteidae) in sekundären Intergradationszonen der Deutschen Bucht und benachbarter Gebiete. — *Faunistisch-Ökol. Mitt.* 4, p. 69-84.
- Norusis, M.J. (1993). SPSS for Windows, Release 6. — SPSS Inc., Chicago.
- Oster, G. & Alberch, P. (1982). Evolution and bifurcation of developmental programs. — *Evolution* 36, p. 444-459.
- Quinn, T.P. & Light, J.T. (1989). Occurrence of threespine sticklebacks (*Gasterosteus aculeatus*) in the open North Pacific Ocean; migration or drift? — *Can. J. Zool.* 67, p. 2850-2852.
- Reimchen, T.E. (1983). Structural relationships between spines and lateral plates in three-spine stickleback *Gasterosteus aculeatus*. — *Evolution* 37, p. 931-946.
- (1988). Inefficient predators and prey injuries in a population of giant stickleback. — *Can. J. Zool.* 66, p. 2036-2044.
- (1989). Loss of nuptial color in threespine sticklebacks (*Gasterosteus aculeatus*). — *Evolution* 43, p. 450-460.
- (1990). Size-structured mortality in a stickleback-trout community. — *Can. J. Fish. Aquat. Sci.* 47, p. 1194-1205.
- (1991a). Trout foraging failures and the evolution of body size in stickleback. — *Copeia* 1991, p. 1098-1104.
- (1991b). Evolutionary attributes of headfirst prey handling and swallowing in piscivores. — *Can. J. Zool.* 69, p. 2912-2916.
- (1992). Injuries on stickleback from attacks by a toothed predator (*Oncorhynchus*) and some implications for the evolution of lateral plates. — *Evolution* 46, p. 1224-1230.
- (1994). Predators and evolution in threespine stickleback. — In: Evolution of the threespine stickleback (M.A. Bell & S.A. Foster, eds). Oxford University Press, Oxford, p. 240-276.

- — (1997). Predator-induced cyclical changes in lateral plate frequencies of *Gasterosteus*. — *Behaviour* 132, p. 1079-1094.
- —, Stinson, E.M. & Nelson, J.S. (1985). Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. — *Can. J. Zool.* 63, p. 2944-2951.
- Schlichting, C. & Pigliucci, M. (1998). Phenotypic evolution: a reaction norm perspective. — Sinauer Associates, MA.
- Stanley, S.M. & Yang, X. (1987). Approximate evolutionary stasis for bivalve morphology over millions of years: a multivariate, multilinesage study. — *Paleobiology* 13, p. 113-139.
- Swain, D.P. (1992). The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. — *Evolution* 46, p. 987-997.
- — & Lindsey, C.C. (1984). Selective predation for vertebral number of young sticklebacks, *Gasterosteus aculeatus*. — *Can. J. Fish. Aquat. Sci.* 41, p. 1231-1233.
- Vermeij, G.J. (1986). Evolution and escalation. — Princeton University Press, Princeton, NJ.
- Webb, P.W. (1978). Temperature effects on acceleration of rainbow trout, *Salmo gairdneri*. — *J. Fish. Resh. Bd. Can.* 35, p. 1417-1422.
- Wootton, R.J. (1976). The biology of the sticklebacks. — Academic Press, London.
- — (1984). A functional biology of sticklebacks. — University of California Press, Berkeley and Los Angeles.
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