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by

Nicholas Giles

A thesis submitted for the degree of
Doctor of Philosophy

Department of Zoology
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CONTENTS

Chapter 2 STUDY SITES AND SAMPLING PROGRAM

Chapter 3 ECOLOGICAL STUDIES

Chapter 4 MORPHOLOGY

Chapter 5 BEHAVIOURAL STUDIES

LIST OF TABLES

LIST OF ILLUSTRATIONS

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SUMMARY

This thesis describes research upon the effects of predation on morphological and behavioural adaptations in seven Scottish three-spined stickleback , *Gasterosteus aculeatus* L. populations. Four of the populations (Loch Lomond, Mar burn, River Luggie, Lennox Castle reservoir) are situated on the south west Scottish mainland, and three (Loch Fada, Loch a Bharpa, Loch an Daimh) on the Isle of North Uist in the Outer Hebrides. Wherever possible the level of predation upon sticklebacks by piscivorous birds and fish has been assessed, or estimated to be high, low or absent (Table 7).

Sticklebacks in all populations have a life span of c. 18 months. Loch a Bharpa fish have the smallest mean adult body size reported for a *Gasterosteus* population; in all populations except Lennox Castle adult females are significantly larger than adult males. Lateral plate number and symmetry were highly variable in all populations except Loch Fada and Loch a Bharpa where fish are 100% lateral plate deficient. All three Uist populations show a degree of reduction of skeletal elements (dorsal spines, lateral plates, pelvic girdle); it is proposed that low environmental calcium levels have been important in the evolution of this phenomenon. Ventral spine growth patterns bore some relationships to predation risk at the study sites but Mar burn and River Luggie sticklebacks have similarly sized spines and experience very different predation risks. An index of increase in critical bodily dimension $\left(\frac{\text{dorsal spine length + ventral spine length}}{\text{Body depth}}\right)$ showed little within-population variability but highly significant inter-population differences. These differences correlated well with predation risk.

In the laboratory experiments significant inter-population differences in nesting male *Gasterosteus* aggression and sexual behaviour emerged, Mar burn males being least aggressive and showing the highest levels of sexual behaviour. Anti-predator behaviour was measured in the laboratory using Pike tests and Heron tests. Analyses of the data produced by these experiments indicates that significant differences in fright responses are present between populations and within populations between adult males and females. Such behavioural differences correlate well with the estimated risk of predation at the study sites. In general adult female Gasterosteus recover much more slowly from a frightening overhead stimulus than adult male Gasterosteus. The overhead fright response develops normally in stickleback fry reared from an early age in the absence of predators indicating that this behaviour is genetically based.

CHAPTER 1

INTRODUCTION

"Predation is an ecological factor of almost universal importance for the biologist who aims at an understanding of the habits and structures of animals" (Curio 1976). It is an established fact that predation can be a very powerful selective agency exerting significant effects upon the genetic constitution of animal populations. Cain and Sheppard (1954) have reviewed their work upon song thrush (*Turdus philomelos*) predation of the various colour morphs of the banded snail, *Cepaea nemoralis* concluding that the observed pattern of colour morph distribution in natural populations of the snail is largely determined by the effectiveness of their cryptic colouration against various vegetation types. Song thrushes were found to predate selectively conspicuously coloured snails under natural conditions, the extent of the depredation being large enough to affect significantly the relative frequencies of occurrence of the different colour morphs within the populations. Kettlewell (1955, 1956) performed the famous field experiments upon visual selection by bird predators upon the normal pale coloured form of the Peppered moth, *Biston betularia* versus the melanic form which increased in frequency in areas subject to industrial air pollution since the middle of the last century. Where smoke has blackened the tree trunks in the habitat of *B. betularia* the dark melanic form is at a considerable selective advantage over the commoner pale form, in the comparatively clean Dorset countryside the converse situation was found to be true. Predation has had such a strong effect upon the industrial populations that the relative frequency of the melanic form rose from less than one per cent to 98% in the Manchester area between the years 1848 and 1895. In Britain over 100 species of moths have been affected by "industrial melanism" (Ford 1975). Dice (1947), performed laboratory predation experiments with owls and deer mice (*Peromyscus maniculatus*) finding that mice which closely matched the background of their containers consistently evaded capture better than conspicuously coloured individuals. Ford (1975) reviews the large volume of literature relating to the importance of visually-selecting predators in the evolution of Batesian and Mullerian mimicry.

Seghers (1970) describes behavioural adaptations in Trinidadian guppy populations to variations in fish predator abundance. Where large characid and cichlid predators are present the guppies confine their feeding and courtship activities to the shallow stream edges and shoal tightly. Where only small cyprinodont predators are present the tendency to shoal is diminished and the guppies live in the open water and close to the stream beds. Seghers (1973) describes laboratory experiments where the small predatory cyprinodont *Rivulus hartii* caught more male guppies than (larger) female guppies and argues that in guppy populations sympatric with this predator an increased body size has been selected for. However no field predation measures were made. Large guppies enjoy an advantage with respect to *Rivulus* predation but are more vulnerable to the larger predators (e.g.) *Hoplias malabaricus* and *Crenicichla alta* and behavioural rather than morphological characters appear to operate where these predators occur. Field observations showed that where the larger predators occurred guppies a) showed a lower alarm threshold, b) avoided a potential predator at greater distance, and c) shoaled in shallow stream edges more than populations allopatric to these predators. Further laboratory experiments with wild and predator-naive guppies revealed significant inter-population differences in schooling and fright responses which were generally consistent with the field observations (Seghers 1973). Seghers (1974) describes experiments upon laboratory reared predator-naive guppies where he subjected fish descended from parents from a range of predation risk habitats to overhead stimuli (a black cardboard model moved over the tank). With large samples of fish reared for a minimum of 4 generations in the laboratory, significant differences in overhead fright response were recorded between high and low predation risk sites. Ballin (1973) studied the courtship behaviour of Trinidadian guppies and found that males from low predation risk sites (with high water clarity) use displays and fighting as a primary courtship strategy whereas males from high risk sites (with poor visibility) rely primarily upon tactile courtship stimuli. The broad aim of the present study is to investigate the impact of predation as a selective agency acting upon three-spined stickleback (Gasterosteus aculeatus L) populations. It seeks to establish a relationship between variation in the risk of predation experienced by Gasterosteus populations and adaptive population responses to this important selection pressure.

Gasterosteus aculeatus is found in freshwater, brackish, and marine habitats in Northern Europe, North America and parts of Asia (Wootton 1976) and throughout its range shows very considerable morphological variation. Fig. 4 (page 76) shows the key morphological features used to identify the three common morphs of the species which are separated essentially on the basis of the number and distribution of lateral bony plates (scutes) that they possess. Trachurus sticklebacks are normally anadromous, have a large adult body size in comparison with the other morphs, and possess a complete row of lateral plates extending from behind the operculum to the junction of the caudal fin. Semi-armatus sticklebacks have a small group of lateral plates above the pelvic girdle which is separate from a second group which form a caudal keel on either side of the body. Leiurus sticklebacks, normally occurring in freshwater, have a small group of lateral plates, variable in shape and number situated above the pelvic girdle and behind the operculum. All of the above three morphs together with the rare spine-deficient morph which has reduced or absent dorsal spines, ventral spines, lateral plates and pelvic girdle are regarded as belonging to a single very variable species Gasterosteus aculeatus L. In chapter 4 (Introduction, page 132) a full discussion of the morphological variability and taxonomic status of Gasterosteus is included. Freshwater populations of Gasterosteus may be monomorphic for Leiurus or (less commonly) Semi-armatus fish or may include varying proportions of Leiurus, Semi-armatus, and Trachurus sticklebacks in a polymorphic assemblage.

A large body of information exists within the literature concerning adaptation in Gasterosteus, the majority of the studies coming from North America. Hagen and McPhail (1970) considered the taxonomic status of Trachurus and Leiurus morph sticklebacks on the Pacific coast freshwaters of North America concluding that the observed phenotypic interpopulation variability is most probably the product of local selection pressures. McPhail (1969) had worked the Chehalis river system where an unusual male phenotype of Gasterosteus with a black nuptial colouration occurs both in allopatry and sympatry with the normal red-throated phenotype. McPhail hypothesised that persistent differential predation by the endemic mud minnow, Novumbra hubbsi on Gasterosteus fry concentrated close to the nest was the selective force responsible for the evolution of the black phenotype. Laboratory experiments showed that Novumbra is differentially attracted to nesting red male sticklebacks when given a choice between red and black, and that juvenile sticklebacks fathered by black males possess an innate behavioural response to Novumbra. Upon the approach of a mud minnow the "black" Gasterosteus fry suddenly dart away and then remain still, McPhail considered that this response made them significantly less vulnerable to predation by Novumbra under natural conditions. The "red" fry appeared not to react as quickly as the "black" fry and swam away more slowly from the approaching predator. Novumbra took significantly more "red" fry than "black" fry in experiments but showed no difference in the number of attacks towards shoals of each potential phenotype. Hagen, Moodie and Moodie (1972) have suggested that the black breeding colouration developed by some Gasterosteus males sympatric with Novumbra may have evolved as a mutual threat posture as the mud minnow is also a territorial fish which develops a dark brown or black breeding colouration in the breeding season.

Hagen and Gilbertson (1972) undertook a major survey of freshwater Gasterosteus populations of North West America and attempted to correlate a variety of morphological characters in the sticklebacks (lateral plate phenotype and number, body shape, dorsal and pelvic spine lengths) with physical, chemical and biological parameters associated with their habitat. Two important findings emerged from the study, namely, that populations with large modes of 7/7 plated fish (fish with 7 lateral plates on each side of the body) show a good correlation with sympatric predatory fish (trout, char, pike) and that the mean pelvic spine length is significantly greater where predators are present. Intra-population correlations between these two traits were significant in only 5 of the populations studied indicating that selection is operating to produce these effects. Geographical effects were discounted owing to the existence of sharp discontinuities of lateral plate modes over very short distances. Hagen and Gilbertson (1973) continued their work to include a longer term study of Lakes Wapato and Chelan where Rainbow Trout, Salmo gairdneri introduced for sport fishing had begun to predate the endemic Lake Wapato Gasterosteus population. The trout preyed most heavily upon Gasterosteus during the winter taking males and females of all three plate morphs present randomly from the populations.

Trout showed however a strong selective predation for lateral plate number, with 7 plated fish having a consistent significant advantage over other lateral plate classes for the three year study period. In 1968 the frequency of 7 plated fish was 56% rising to 65% in 1969 and remaining at 62% at the time of publication (1973), this increase coinciding with the peak of the recorded Rainbow Trout predation. These data are important evidence for a large shift in mean relative fitness of a lateral plate class of *Gasterosteus* through the action of selective predation. 80% of the Trout guts examined during the winter of 1968 contained *Gasterosteus* of c 20 mm total length, Trout as small as 22 cm had been preying upon the sticklebacks. The Lake Wapato *Gasterosteus* appear to have recolonised the lake (after Rotenone poisoning) from the nearby Lake Chelan where the frequency of 7/7 plated fish is 46% and the Trout predation selecting for non-7/7 individuals seems to have been responsible for the observed change in phenotypic (≡ genotypic) frequencies. Moodie, McPhail and Hagen (1973) studied the susceptibility of various *Gasterosteus* lateral plate phenotypes to predation by Northern squawfish *Ptychocheilus oregones~~e~~nsis* and Cut-throat Trout, *Salmo clarki* in the laboratory. Both predators ate 7/7 plated fish at significantly lower frequencies than would be expected from their ratio in the initial samples. However, behavioural experiments showed that 7/7 plated fish were caught more easily than 6/6 plated fish; conflicting results were obtained from tests run in August and February where during August 7/7's were caught more easily than 4/4 and 5/5 fish but in February were best at avoiding capture. This effect was consistent however and in 4 series of tests 7/7's were at a disadvantage in the summer and at an advantage in the winter months. Early experiments from within a single population showed that selective predation occurred and that therefore a lateral plate effect was present. However, several populations of sticklebacks were used for later tests and Moodie, McPhail and Hagen concluded that they could not be sure whether behavioural differences occurred between populations or plate classes (or indeed both). The adaptive significance of the variation in lateral plate number is unknown. Selection clearly operates upon a variety of characters linked to lateral plate number (MacLean 1974).

Kynard (1972) studied the Wapato Lake Gasterosteus population with a view to establishing links between male breeding behaviour and lateral plate phenotypes concluding that the genes which control the lateral plate phenotype of a fish also affect (or are linked with) genes that control ethological traits. The order of phenotypes when ranked in terms of conspecific aggression were (lowest to highest) 6/7, 7/6, 8/8, 7/7, 7/8, 8/7. Other behaviour variables linked to lateral plate phenotype were nest location and cover, depth of water over nest, number of eggs collected in nest, ability to rear and protect eggs from predators (Kynard 1972).

Moodie (1972 a) describes the Mayer Lake (Queen Charlotte Isles) Gasterosteus population which contains two distinct sub-populations, Leiurus fish which inhabit the heavily weeded inlet streams and an unusual large melanic form ("Black stickleback") which has a streamlined shape, large ventral spines and high gill raker, vertebral, and lateral plate counts. Moodie suggests that the black form is so distinctive and divergent from the normal Leiurus morph that it meets the requirements of a separate species, the black morph is known to have been present at the site for at least 39 years (in 1972).
Moodie (1972 b) puts forward the theory that the black morph has evolved through the agency of heavy predation pressure by the sympatric Cut-throat Trout. Black males develop drab breeding colours and laboratory experiments suggested that males developing red throats were more liable to be predated than drably coloured males. Female

black sticklebacks recovered from Trout stomachs had significantly shorter pelvic spines than the average for fish of their size netted from the Lake, predated males also had shorter spines but the difference was non-significant. Black sticklebacks formed the major food source of the trout examined in all months, except for occasional peaks of insect abundance, and were swallowed head-first. Moodie noted that preliminary observations suggested behaviours associated with lateral plate number influenced survival and concluded that predation is likely to have been a major selective force in the evolution of freshwater Gasterosteus populations in N.W. America.

Gross (1978 a, b) has surveyed the morphology of 77 European marine and freshwater Gasterosteus populations and related the characters studied (size and structure of dorsal and ventral spines and pelvic girdle, vertebral number, dorsal and anal fin ray and pterygiophore number, and lateral plate number) to geographical distribution and the presence or absence of predatory species. Gross regards the European distribution of Gasterosteus lateral plate phenotypes to be explained as a series of geographic trends which may be caused by any one of a range of factors including physical and biological parameters of habitat, non-selective historical events (e.g. Pleistocene ice incursions) and gradients of environmental parameters which may select for continuous changes in phenotypic occurrence throughout the range of the species. Gross (1978 b) has considered the effects of predation upon the morphometry of the spines and pelvic girdle in Gasterosteus, estimates of predation pressure relied entirely upon publications available and local information, no field work was undertaken. Marine populations (Trachurus morph) face the widest range of potential predatory species and had the largest defensive structures, freshwater populations exhibited an arched cline in spine size. Gross attributes this distribution in morphometry to his proposed distribution of major piscine Gasterosteus predators, (especially Pike and Perch) with local differences within central Europe due to site-specific predation pressure differences. Gross argues that in freshwater predation pressure upon Gasterosteus populations reaches a maximum in Central Europe and declines both to the north and south, Salmonids were not thought to be important predators. (The latter statement is challenged in chapter 4 (page 102).)

The recognition of the importance of the stickleback's spines as a means of morphological defence came with the publication of the study of Hoogland, Tinbergen and Morris (1957) which established that the stout dorsal and ventral spines of Gasterosteus are raised during predatory encounters and effectively increase the body size of the fish and inconvenience the predator. Hoogland (1951) had already documented the mechanism of locking the spines in an erect position such that no muscular effort is needed to keep the spines raised; dorso-ventral pressure merely serves to lock the spines in an erect position rather than collapsing them. Almost all extant Gasterosteus populations known contain fish with three dorsal spines and a pair of larger ventral spines which articulate with the pelvic girdle. Rarely however, populations occur where the majority of the fish have reduced numbers of spines and a reduced or absent pelvic girdle. Lateral plates are also usually absent in such fish. Sticklebacks lacking spines are likely to be at a severe disadvantage in the face of heavy predation pressure. Moodie and Reimchen (1973) describe the very variable external morphology of the Gasterosteus populations of the Queen Charlotte Islands (off Vancouver, Canada), including four populations where 1, 2 or all 3 dorsal spines are absent in 10-90% of the fish and one population (Boulton Lake) where the pelvic spines and girdle are absent in 70% of sampled fish. Most populations showing spine loss and pelvic girdle and lateral plate reduction have no outlets and apparently no other fish species present, and Moodie and Reimchen attribute the skeletal reduction to an absence of fish predators and competition allowing novel adaptations to evolve in unusual ecological environments. Moodie and Reimchen (1976) note that the Queen Charlotte Island group was glaciated c. 10,000 years ago and that whilst the mainland *Gasterosteus* have seemingly remained largely unchanged over this period, populations on the islands have diverged greatly, possibly in response to the limited diversity of the endemic fish fauna. Bell (1974, 1976) has reviewed the occurrence of pelvic girdle reduction and loss in *G. aculeatus* and by comparing fossil Gasterosteids (Pliocene Truckee formation) with modern forms has concluded that the stocks evolved along two independent phyletic lines, morphological similarity between the samples being due to parallelism rather than convergence. Parallelism in the reduction of the pelvic girdle complex has occurred within and between the genera *Gasterosteus*, *Pygosteus** and *Culaea*, Bell concluded that predator reduction is important in relation to the reduction of the pelvic girdle in these genera. *Gasterosteus* populations on the Isle of North Uist (Outer Hebrides) have recently been discovered where the sticklebacks are polymorphic for presence or absence of the pelvic girdle and dorsal spines (Campbell 1979). The external morphology of these fish is similar to that described for the Boulton Lake fish by Moodie and Reimchen (1973) and for the recent and fossil populations examined by Bell (1974). Uniquely Loch Fada (North Uist) has a *Gasterosteus* population, exhibiting reduced pelvic girdles (and ventral spines), dorsal spines, and a total absence of lateral plates, which is known to be predated by the endemic Brown Trout, *Salmo trutta* population (Campbell 1976). Campbell was studying the diet of Arctic Char and Brown Trout in Loch Fada and found spine-deficient *Gasterosteus* to be dominant in 18% of the total Trout guts examined.

(* ≡ *Pungitius pungitius*)

This finding is of great importance as it is the only record to date of fish predation acting upon a spine-deficient *Gasterosteus* population. A survey of freshwater *Gasterosteus* populations on the island undertaken as part of the present study has revealed a total of 8 spine-deficient stickleback populations, all sympatric with Brown Trout.

Variability in morphological characteristics can be attributable to genetic or environmental effects or to a combination of these factors; genetically based characters can be acted upon by natural selection. The studies of Hagen (1967, 1973 a, b) on lateral plate number, gill raker number, and body shape in *Gasterosteus* have established that these characters are under genetic control, and that the size of the dorsal and ventral spines and the bodily colouration also appear to be genetically determined. Lindsey (1962), Hagen and Gilbertson (1973), and Hay (1974) have also shown that the number and position of the lateral plates in *Gasterosteus* are under genetic control. Avise (1976) draws attention to the fact that differing *Gasterosteus* populations may vary in the genetic basis for lateral plate determination (page 81). Campbell (1979) has produced evidence that the presence or absence of the pelvic girdle in *Gasterosteus* may be genetically controlled, as has been shown for the brook stickleback *Culaea inconstans* (Nelson 1977). If the genetic determination of the morphological characters described above is a general property of *Gasterosteus* populations then inter-population variability in morphology provides an ideal opportunity for the study of micro-evolutionary adaptations within this species.

Evidence for the genetic basis of anti-predator behaviour in fish is also documented in the literature, (e.g.) Kimmel et al (1974) in the Zebra danio, *Brachydanio rerio*; Jakobsson and Jarvi (1976) in Atlantic salmon, *Salmo salar*; Pattern (1977) in Coho salmon, *Oncorhyncus* kisutch; Seghers (1973, 1974) in Guppies, Poecilia reticulata; and in the present study for Gasterosteus (page 172). Inter-population variability in behaviour towards predators is therefore of considerable interest because of its probable adaptive significance. The linking of the study of population biology and of experimental animal behaviour is an active research area in zoology and is yielding important insights into within-species adaptations to selective agencies (e.g. Krebs and Davies 1978).

The anti-predator behaviour of Gasterosteus in the laboratory toward a hunting Pike has been described in detail by Benzie (1965). The stickleback normally stops feeding, fixates the Pike, and then may sink slowly to the bottom or jump away in alarm to the bottom and then remain still for long periods often with its dorsal and ventral spines raised. Huntingford (1976) performed a series of experiments which compared the boldness of male Gasterosteus in differing reproductive conditions to a hunting Pike and the degree of conspecific aggression exhibited by the same fish. The level of both of these aspects of behaviour was found to covary (both increased) as the male fish progressed from a non-reproductive condition to nest-building and defence of a newly hatched brood of young. Huntingford discusses the possibility that this covariance might indicate shared internal causal factors in anti-predator behaviour and conspecific aggression and that this might be an adaptation to varying predation levels. She extended this line of investigation in order to test the hypothesis that high predation levels favour low levels of boldness toward a predator together with low aggression and that low predation risk fish might be expected to be bold toward a predator and more aggressive. Huntingford (in prep.) chose 12 Scottish populations of Gasterosteus including a range of potential fish predation risk categories, measuring the responses of male fish from each population toward a hunting Pike, and a conspecific male (aggression tests). Clear differences in anti-predator behaviour toward the Pike were observed, with less clear differences in levels of aggression but with the same general pattern. The above study was limited in its measurement of predation risk in the study populations and by its inclusion of fish predators alone. The present study was designed to improve upon this approach by attempting to collect information on the actual risk of predation from both fish and bird predators at 7 study sites and to test the behavioural responses of adult male and female fish and fry from each population toward predators.

The specific aims of the present study are as follows:

1. To provide information upon the life-span, diet, and predator-prey interactions in a range of freshwater Gasterosteus populations in Scotland and to identify where possible the main fish and bird predators present at each site.

2. To establish whether lateral plate number, dorsal and ventral spine size, presence and reduction or absence of the pelvic girdle complex, body size and shape vary with predation risk in these populations.

3. Given that the Loch Fada spine-deficient Gasterosteus population is known to be predated by Brown Trout to try and identify a selective agency that would account for the repeated occurrence of pelvic girdle reduction in the North Uist stickleback populations.

4. To determine whether mature male Gasterosteus in the study populations differ in their levels of conspecific aggression and reproductive behaviour and in nest site selection and territory size.

5. To establish whether inter-population differences in anti-predator

behaviour toward aerial and fish predators are apparent, and if so, whether such differences correlate with the risk of predation at the study sites.

6. To study the development of the overhead fright response in *Gasterosteus* and to determine whether inherited or experiencial factors are primarily responsible for the level of response exhibited.

7. To determine whether there are any clear behavioural differences which can be attributed to lateral plate phenotype effects rather than population effects.

By relating inter-population morphological and behavioural variation to ecological differences between the study sites it should be possible to assess the importance of predation as a selective force acting upon stickleback populations.

CHAPTER 2

STUDY SITES AND SAMPLING PROGRAM

2.1 Introduction

This thesis describes studies carried out by the author on seven Scottish *Gasterosteus aculeatus* L populations during the period September 1976 to November 1979, this time being split into two major phases: 1) September 1976 to November 1979, a detailed study of the Loch Lomond *Gasterosteus* population, and 2) December 1977 to November 1979, a comparative study of seven freshwater *Gasterosteus* populations: four on the Scottish mainland, Loch Lomond, Mar Burn, Lennox Castle reservoir, River Luggie; and three on the Isle of North Uist (Outer Hebrides), Loch Fada, Loch á Bharpa, and Loch an Daimh. During the first period data on the diet of potential stickleback predators in Loch Lomond was collected; an analysis of these data is presented in Chapter 3. Sticklebacks proved to be extremely difficult to catch from the predominantly stoney, boulder-strewn shallow littoral zone of the loch and because of the low numbers of sticklebacks obtained a broader-based comparative study was undertaken. The second phase of the project was designed to include a range of *Gasterosteus* populations which are subjected to variable predation pressure; an assessment of the risk of predation was made for each population together with concurrent studies of the *Gasterosteus* population ecology, morphology, and behaviour. During 1978 H.M. Home Office imposed a temporary ban on live anti-predator behaviour experiments such as those described in Chapter 5 in view of possible contravention of the 1912 Cruelty to Animals (Scotland) Act; permission was granted to proceed with these experiments in 1979. During 1978 therefore only anti-predator behaviour experiments involving the use of a model overhead predator (Heron tests) were performed, the tests using a live pike being carried out during the summer of 1979.

The Gasterosteus populations chosen for study were selected in order to encompass a wide range of predation risk categories, from Loch Lomond where a large number of avian and fish predators exist, to the suburban River Luggie where there are few potential predators. Of the seven study populations two exist in lotic habitats whilst the other five occupy stillwaters of varying size. Loch Fàda, Loch an Daimh, and Loch á Bharpa are situated on the Isle of North Uist in the Outer Hebrides (Fig. 13, Chapter 4). Loch Lomond, the Mar Burn, the River Luggie, and Lennox Castle Reservoir are situated on the south-west Scottish mainland. The study was limited to seven populations in order that time would be available to collect sufficient data from each whilst ensuring that a reasonable range of predation risk categories was included. This relatively small sample size does, however, raise the possibility that any differences in behaviour between populations may be the result of other characteristics on which they differ. (See Table 1 which outlines some basic chemical characteristics of the study sites.)

2.2 The Study Sites

North Uist Sites

The Isle of North Uist harbours more than 180 lochs and lochans most of which are completely unspoilt, ranging from alkaline, biologically productive west coast machair lochs to the acidic peat-bog oligotrophic waters of the central and east coast regions. The west coast band of rich vegetation based upon Atlantic shell-sand soils (machair) contrasts very sharply with the central and eastern blanket peat-bogs which are underlain by a bedrock of impervious Lewisean gneisse (Thompson, 1970). Loch Fada, Loch á Bharpa, and Loch an Daimh are all situated within the blanket bog loch complex,

Table 1. Chemical characteristics of water quality at study sites

	Ca^{2+}mg/L	Mg^{2+}mg/L	Na^{+}mg/L	K^{+}mg/L	pH	conductivity $ohms^{-1}cm^{-1}$	number of readings ($\bar{x}$)
Loch Lomond	3.82	1.09	4.42	0.47	6.9	78 x 10^6	3
Mar burn	9.2	3.4	5.0	0.9	7.1	140 x 10^6	3
Lennox Castle	7.8	2.15	5.2	0.4	6.8	146 x 10^6	3
River Luggie	30.1	22.5	76.7	12.7	7.4	2430 x 10^6	3
Loch Fada	2.25	2.35	17.5	1.5	5.8	83 x 10^6	3
Loch an Daimh	3.55	2.5	20.7	1.6	6.3	65 x 10^6	3
Loch a Bharpa	1.9	2.5	18.4	1.0	5.6	84 x 10^6	3

(Dr. R. Tippett kindly provided the Ca, Mg, N, K data for Loch Lomond) and are mostly shallow peat-stained waters with few dense stands of aquatic macrophytes *Eleocharis*, *Myriophyllum*, *Potomogeton*, *Nymphaea alba*, *Nuphar lutea*, *Carex* and *Equisetum*; all occur as sparse beds in the shallow littoral zones. The bed of all three lochs is composed of a mixture of gravel areas interspersed with wide expanses of finely divided peat silt. Inflowing streams and underground springs are very numerous and may interconnect adjacent lochs which appear on a map to be isolated. Brown Trout, *Salmo trutta*; Eels, *Anguilla anguilla*; and three-spined Sticklebacks occur in all three lochs, ten-spined Sticklebacks, *Pygosteus pungitius* occur in Loch Fada and Loch an Daimh; Arctic Char, *Salvelinus alpinus* occur in Loch Fada and Loch á Bharpa and occasional Salmon, *Salmo salar* and Sea Trout, *Salmo trutta* may occur in Loch Fada and Loch an Daimh (D. Shaughnessy, personal communication). Loch á Bharpa also contains small numbers of Rainbow Trout, *Salmo gairdneri* which have escaped from submerged aquaculture rearing cages (D. Shaughnessy, pers. comm.). North Uist has a very rich avi-fauna including several species of piscivorous birds, the following of which have been seen (by the author) fishing the three study lochs: Red-throated Diver, *Gavia stellata*; Black-throated Diver, *Gavia arctica*; Red-breasted Merganser, *Mergus serrator*; Little Grebe, *Tachybaptus ruficollis*; Heron, *Ardea cinerea*; Cormorant, *Phalacrocovax carbo*; Black-headed Gull, *Larus ridibundus*; Common Gull, *Larus canus*; Common Tern, *Sterna hirundo* and Arctic Tern, *Sterna paradisaea*.

Loch Lomond N.S. 373 957

Of the mainland sites Loch Lomond is by far the largest, 25km. long and 7km. at its widest point, classified overall as a mesotrophic lake with a long deep northern basin and a much shallower, broader and comparatively nutrient-rich southern basin. Slack (1957) describes the biology of the loch in detail. The sampling area chosen for the study was the shoreline of the Ross Peninsula which lies in the short mid-basin section of the loch and is conveniently situated near the University Field Station at Rowardennan. Two short sections of shoreline were extensively sampled to provide the sticklebacks used in this study. The first is a well defined, shallow bay, Camas an Losgainn which measures c. 200m. by 50m. with a sparse littoral flora of *Littorella uniflora* to a depth of 3m. and with dense beds of *Myriophyllum* and *Elodea* over a sand/silt bottom at depths of 3-5m. The second section of shoreline sampled lies around the headland Rubha Fhuar a Chas; here the bottom is largely sandy and *Littorella* and *Myriophyllum* grow densely at depths of 1.5m.-3m. providing plentiful cover for small fish species. Loch Lomond probably has the most diverse fish fauna of any of the freshwater Scottish lochs, to date fifteen species have been recorded (Maitland, 1972) these are: the Sea Lamprey, *Petromyzon marinus*; River Lamprey, *Lampetra fluviatilis*; Brook Lamprey, *Lampetra planeri*; Salmon; Brown Trout; Powan, *Coregonus clupeoides*; Pike, *Esox lucius*; Minnow, *Phoxinus phoxinus*; Roach, *Rutilus rutilus*; Stone loach, *Noemacheilus barbatulus*; Eel; Three-spined Stickleback; Ten-spined Stickleback; Perch, *Perca fluviatilis*; and the Flounder, *Platichthys flesus*. The avi-fauna associated with Loch Lomond is also very diverse with some 200 species recorded to date (Richmond, 1974), of these species the following occur in reasonable numbers on the loch and are potential stickleback predators: Great Crested Grebe, *Podiceps cristatus*; Little Grebe; Heron; Tufted Duck, *Aythya fuligula*; Goldeneye, *Bucephala clangula*; Red-breasted Merganser; Common Gull; Black-headed Gull; Common Tern and Arctic Tern.

The Mar Burn N.S. 442 899

The Mar Burn is a small, shallow, and for the most part fast-flowing upland stream which meets the River Endrick close to its mouth in the South Basin of Loch Lomond. In the fast-flowing sections the moss Fontinalis is the only aquatic plant offering cover for fish. Some of the deeper pools have very sparse Potomogeton beds and submerged root masses of Alder trees, Alnus glutinosa which line the banks of the stream. The 400m. long section of the Mar burn sampled during this study lies immediately below the village of Milton of Buchanan, and adjacent to the large heronry at Gartfairn wood. The fish species present in the burn are Salmon, Brown Trout, Sea Trout, Stone Loach, Minnow, Eel, Brook Lamprey and Three-spined Stickleback. The principal piscivorous birds which feed along the stream are Herons, which very commonly fish the study area, Red-breasted Mergansers (occasional sightings) and Black-headed and Common Gulls which fish the burn spasmodically but sometimes in large numbers.

The River Luggie N.S 653 745

Reference to Table 1 illustrates the essential differences between the two lotic populations under study; the River Luggie is situated in a Glasgow suburb and is comparatively eutrophic with high pH and high conductivity values. Faster flowing sections of the Luggie are densely weeded with mats of Cladophora and Ranunculus beds, the slower sections and pools support large Potomogeton beds with additional clumps of Myriophyllum, Ranunculus, Elodea and Nymphaea alba. The 500m. section of the River Luggie sampled during this project lies immediately above the confluence with the River Kelvin at Kirkintilloch. The fish species present are Three-spined Sticklebacks, Minnow, Stone Loach, Eels and occasional small Brown Trout. The only evidence of the presence of piscivorous bird species was the occasional sighting of single Black-headed Gulls flying over the river.

Lennox Castle NS 604 777

Lennox Castle reservoir, situated in the grounds of Lennox Castle

Hospital, in the Campsie Hills north of Glasgow, was chosen for study because of a complete absence of predatory fish species, Three-spined Sticklebacks are the only fish species present and have been recorded from the site for at least the past 30 years (C.A. Hopkins, pers. comm.). Avian predators are common however and Black-headed and Common Gulls fish the reservoir daily throughout the year. A small Heronry is also present in a nearby coniferous woodland and appears to be growing in size since the first recorded nesting activity in 1974 (Giles, 1981). The reservoir is small (c. $500m^2$ surface area) and heavily silted around the regions of the two inflowing streams, dense beds of Phragmites communis occur around the periphery, and the littoral zone is densely weeded with Myriophyllum and Equisetum beds.

It should be noted that it is possible that Loch Lomond sticklebacks mix with the Mar burn population and that River Luggie sticklebacks mix with the confluent River Kelvin population; the extent of this exchange is unknown. Mixing between Mar and Lomond sticklebacks is unlikely as there is a long section (c. 1 mile) of the lower burn where Gasterosteus is absent (personal observation). Trachurus morph Three-spined sticklebacks are normally anadromous and the species is therefore capable of migratory movements, often over considerable distances. Seghers (1973) has documented micro-evolutionary adaptations in Trinidadian Guppy populations where there are no obvious major barriers to gene flow.

2.3 Sampling Methods and Programs

A 40cm. diameter hand net was used to catch all of the sticklebacks used for behavioural experiments and morphological studies. The majority of fish were caught by rapidly swishing the net through any available weedbeds within reach from the shore or within wading depth (less than 1 metre). All morphological samples were preserved in

95% ethanol as formalin solutions dissolve the inner ear bones (otoliths) used for ageing the fish. Live fish were transported back to the laboratory in opaque 10L. plastic churns at a density of no greater than 12 fish per churn, a portable air pump was used to periodically aerate the containers. At all times the greatest care was taken to shield the fish from sudden violent vibrations, and rapid temperature changes.

In this thesis frequent reference is made to the terms Gasterosteus fry and Gasterosteus adults. These fish are defined as follows:

Fry: fish in their first summer and autumn of life (up to c. 6 months of age).

Adults: fish in their second summer of life (12-18 months old) which may be approaching sexual maturity, be sexually mature, or reproductively spent.

Visits to the Isle of North Uist were made at the following times: May 1977 (2 days), May 1978 (8 days), September 1978 (7 days), May 1979 (9 days), all four of the mainland populations were visited very frequently during the study period. * See appendix

2.4 Fish Care and Laboratory Conditions

All captive sticklebacks were kept in unpiped (copper-free) Loch Lomond water. Adult fish in breeding condition used for anti-predator experiments were kept in a light regime of 16 hours light/8 hours darkness, fry used in anti-predator experiments (September 1978, 1979) received 12 hours light/darkness, laboratory reared predator-naive fry were kept under natural light conditions in an outbuilding. Fish were housed communally in 30L. aquaria at a density of not more than 12 fish per tank, where possible adult males were given more space in order to reduce aggressive encounters. All fish were fed daily with either Tubificid worms or Daphnia, a constant filtration and aeration system was employed, cover was provided in the tanks by clumps of Sphagnum moss. The air temperature in the laboratory used for fish keeping and for anti-predator experiments varied in summer from $18^{o}C-25^{o}C$ and during the autumn from $12^{o}C-19^{o}C$, all of the fish used for behavioural experiments survived extremely well during their period of captivity, only fish which appeared to be perfectly healthy were used in experimental tests. All possible precautions were taken to minimise disturbance to captive fish.

A single Pike was kept for the duration of the study period in a 50L. tank with constant filtration and aeration, and was fed twice weekly on small live fish. Great care was taken to ensure that communal holding tanks and experimental tanks were at a similar temperature and under equivalent light intensities in order that fish transferred from holding tanks to experimental tanks experienced minimal stress.

CHAPTER 3

ECOLOGICAL STUDIES

3.1 Introduction

In freshwater habitats G. aculeatus become sexually mature during the early Spring. Males develop a characteristic blue eye and red throat colouration and begin to defend reproductive territories within which they construct a nest. The nest consists of a shallow depression in the substrate which is covered by a loose mat of aquatic vegetation, glued together by the male fish with kidney secretions. Maclean (1980) has observed that competition for suitable territories between mature male three-spined sticklebacks can be very intense and that this behaviour can be a limiting factor controlling the population density. When the nest is complete the male fish proceeds to attract and court gravid females, collecting several clutches of eggs within the nest and then defending the developing ova and fry until they are self-sufficient. Female G. aculeatus are capable of maturing several batches of eggs during the summer breeding season (Wootton, 1974) and may choose several different males to fertilise their eggs and care for their developing fry. The rigours of reproduction lead to a decline in bodily condition in both sexes and large numbers of dead or dying adult Gasterosteus were seen at the Mar burn, River Luggie and Loch Lomond during the late summer and early autumn period.

All seven of the populations studied conformed to the normal pattern of a protracted summer breeding season followed by a very large post-reproductive mortality of adult males and females. In the four mainland populations the first observations of nesting male fish appeared in early May in 1978 and in mid-May in 1979, this coinciding with the normal breeding season of inland Gasterosteus populations on the Isle of North Uist (R.N. Campbell, pers. comm.). Under the milder weather conditions of southern England Gasterosteus may even begin reproduction in mid to late April.

3.2 Population Age Structure

3.2.1 Methods

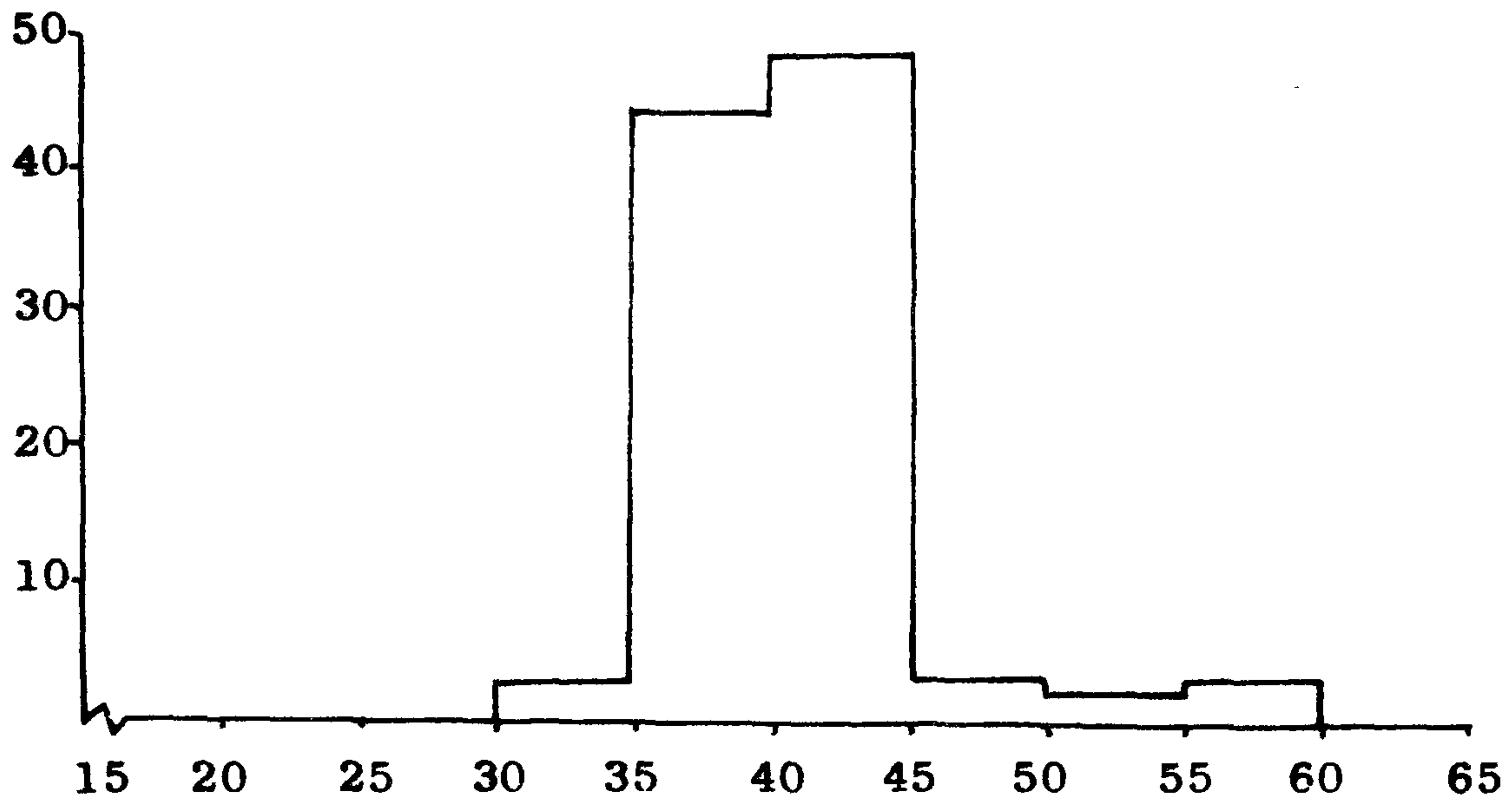
Samples of fish were collected during the early breeding season (May) and during the late summer (August and September) and preserved in 70% ethanol. Measurements of the standard length (tip of snout to base of caudal peduncle) and total length (tip of snout to posterior margin of caudal fin) were taken with finely pointed dividers and then transferred to vernier calipers. Fish were aged by examination of otolith preparations; random sub-samples (n = 20) of each sex were taken from each sample of breeding adult fish, the inner-ear bones dissected out, dehydrated, and mounted on microscope slides in Canada Balsam. Following the scheme formulated by Jones and Hynes (1950) the largest of the otoliths (the sagittae) were separated and examined as pairs from each fish with refracted light under a compound microscope at x100 magnification.

3.2.2 Results

Morphological samples collected in May 1978 and May 1979 revealed unimodal size-frequency distributions in all seven of the populations studied, mature females being consistently larger than mature males (except at Lennox Castle). No fry, or immature fish were captured at these times from any of the study populations. Fig. 1 shows a length:frequency histogram of a sample of 100 adult Mar burn fish collected in May 1978 and is typical for all populations studied.

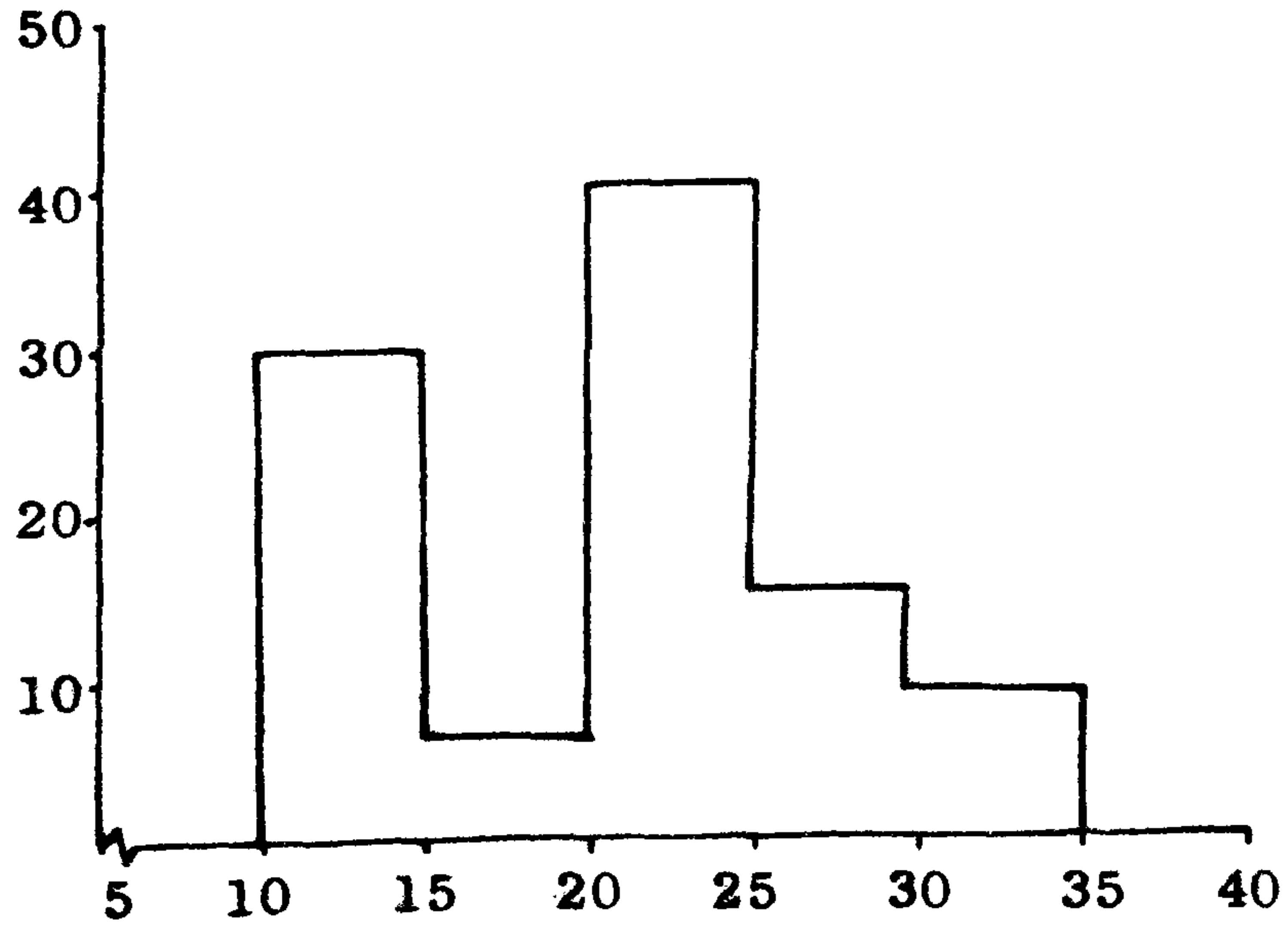
Sagittae from adult fish showed concentric clear (summer growth) and opaque (winter growth) bands which revealed that breeding adult

Fig. 1 **Length:Frequency histogram of 100 adult Mar Burn _Gasterosteus_ (May 1978)**



Standard length (mm)

Fig. 2 Length:Frequency histogram of 100 Mar Burn _Gasterosteus_ fry (August 1978)



Total length (mm)

fish from all seven populations were 1 years of age. In a fish of this age a sagitta is composed of a centrum, a clear growth band laid down in the rapid growth phase of the first summer of life, an opaque winter growth ring (which is much narrower than the summer ring), and then a peripheral clear growth band which includes the second summer's growth and maturation phase (Jones & Hynes, 1950). The use of refracted (≡ reflected) light when viewing such otolith preparations is critical, as transmitted light reveals large numbers of concentric growth bands which bear no simple relation to seasonal growth patterns. The sagittae proved to be very reliable age determining structures, and complete agreement between otoliths from the right and left hand sides of the body was found in all of the Gasterosteus dissected during the present study. Very occasionally in the May samples adult fish of both sexes were caught which were, on average 10mm. longer than the normal mean adult standard length for the population under study. Sagittae from such individuals revealed that these fish were 2 · years of age, and had survived a second winter and entered their third summer of life. Such 2 year fish occurred at a frequency of less than 1% amongst samples of breeding adults from all seven populations. It is not known whether fish which live 2 years have spawned during their second summer of life and were entering a second reproductive phase or whether they were maturing gonads for the first time upon the date of their capture.

Frequent observations of the mainland populations revealed a massive post-reproductive mortality in males and females during the period July to September. Similar events must occur in the three North Uist populations studied because morphological samples collected in September 1978 from all seven study sites were composed entirely of young of the year fish. Sagittae from September fry were composed of a centrum and a single clear summer growth band. Samples of sticklebacks from the mainland populations collected during July and August 1978 showed multimodal length-frequency distributions composed of surviving breeding adults (both sexes of which undergo several breeding cycles during the breeding season, (Wootton,(1976)) and various peaks of fry presumably corresponding to breeding waves occurring during the summer months. September samples from all populations therefore contained fry of a considerable within-population size range; Table 2 summarises such data for samples of 100 fry from each population. Fig. 2 shows a length-frequency histogram for a sample of 100 Mar burn fry collected in August 1978 and illustrates a typical sample with two fry length peaks. Assuming an average birth date of June 1st it is of interest to note that by the age of four months some male fry had developed a dull red throat colouration and that the largest fry in any sample were almost always found to be female upon dissection. Not all of the fry were dissected and sexed.

3.2.3 Discussion

All seven of the study populations therefore exhibit an annual life cycle with considerable growth and development being achieved in the first summer of life. Very few false growth checks (very narrow opaque bands caused by a short term cessation of growth) were observed in sagittae from lentic populations but both River Luggie and Mar burn fish showed occasional false checks which rendered otolith interpretation more difficult for these populations. It is possible that the greater preponderance of false checks in fish from lotic environments can be explained by the occurrence of sudden spates and temperature fluctuations within their environment.

Mann (1971) working on Gasterosteus from lotic habitats in southern England also recorded an annual life cycle with adult body

Table 2. Mean Total Length of Fry, September 1978

	N	$\bar{x}$ Total length	Range
Fada	100	25 mm.	10 - 31 mm.
Bharpa	100	23 mm.	9 - 30 mm.
Daimh	100	28 mm.	12 - 35 mm.
Lomond	100	32 mm.	10 - 37 mm.
Luggie	100	31 mm.	12 - 39 mm.
Mar	100	30 mm.	10 - 35 mm.
Lennox	100	31 mm.	12 - 35 mm.

length comparable to those shown by the mainland populations in this study. Van Mullem and Van der Vlugt (1964) studying a polymorphic anadromous Gasterosteus population on the Dutch island of Tholen also recorded an annual life cycle but with growth rates far faster than those recorded in the present study (see Chapter 3), with mature fish ascending rivers to breed at a total length of c. 60mm.. Hagen and Gilbertson (1972) working at Wapato Lake, Washington recorded an annual life cycle for Gasterosteus with 8% of the population surviving for 2, 3 or even 4 years under natural conditions; Greenbank and Nelson (1959) working at Karluk and Bare Lakes, Alaska, recorded an average life span of 2 years 4 months for Gasterosteus with occasional fish surviving a third winter. Moodie (1972) studying the Mayer Lake Gasterosteus population on the Queen Charlotte Islands off Vancouver, Canada, estimated the life span of males and females to be 2 years. Coad and Power (1973 a, b) studying Gasterosteus in the Matamek river system and in Armory Cove, Quebec recorded an average life span of 2+ years with spawning taking place in the third summer of life. Krogius, Krokhin and Menshutkin (1970) studying the pelagic fish community of Lake Dalnee, U.S.S.R. concluded that Gasterosteus lived for up to 4 years but that the growth rings seen on the sagittae did not correspond to the classification proposed by Jones and Hynes (1950) and that females matured a year later (3+ years) than males (2+ years). In their study of the Burket River (north west England) Gasterosteus population Jones and Hynes (op. cit.) described a population where males and females typically matured at an age of 2+ years with occasional individuals surviving into their third winter. Penczak (1965) concluded that most Polish Gasterosteus survive 36 months, dying after their second breeding season.

3.3 Distribution in habitat

During all sampling trips notes were made upon the distribution of sticklebacks within their habitats. During the breeding season adult female fish (gravid fish with swollen abdomens) were very rarely seen in open water (most observations carried out on mainland populations). In the Mar burn and in Loch Lomond any available aquatic vegetation was used for cover. Mar burn females were most commonly caught in Alder tree root clumps; Lomond, Luggie and Lennox Castle females in Myriophyllum, Ranunculus, Potomogeton or Elodea beds. During the visits to the North Uist study lochs in May 1977, May 1978 and May 1979 mature female sticklebacks showed similar behaviour, being caught most often in Eleocharis, Myriophyllum, Nymphaea and Nuphar beds but rarely from open water. Breeding male fish behaved very differently and often defended nest sites on open sandy bottoms in water less than 1m. deep (Loch Lomond, Loch Fada, Lennox Castle, Loch a Bharpa, Loch an Daimh, River Luggie). Mar burn males were never seen nesting in open situations, always choosing deep pools away from the stream margins on sites with maximal cover. A full discussion of male nest-site selection is included in Chapter 4. After the period of parental care stickleback fry were seen shoaling at all seven study sites; typically, shoals are composed of 50-100 fry of similar body length which maintain a tight shoal cohesion swimming close to the surface in open water feeding on small planktonic organisms. In Loch Lomond and the Mar burn during August and September the shoals disperse and fry (mean total body length c. 25mm.) live solitarily under stones and amongst vegetation in shallow littoral habitats. This behavioural change in Lomond fry coincides with the onset of predation by Perch (Chapter 3, page 57); fry continue to overwinter alone in sheltered positions.

During the sampling of fry in September 1978 no shoaling of fry above 20mm. total length was seen in any of the study populations but smaller fry, produced late in the breeding season, were commonly seen shoaling at the surface.

3.4.1 Diet

A knowledge of the diet of Gasterosteus from each of the study sites is important as it provides information on the likelihood of larval and egg predation during the breeding season and also upon the feeding areas utilised by the fish (open water, weed beds, etc.). Some evidence is available (Wootton, pers. comm.) that the level of food availability influences nest building and territorial defence in sexually mature male Gasterosteus. Fish from populations which experience high levels of egg robbing and larval predation will need to be better able to defend a developing brood of young than fish from populations where this predation pressure is reduced.

3.4.2 Methods

50 adult male Gasterosteus, 50 adult females and 50 fry from each of the study populations, were dissected and the stomach contents identified and quantified. Invertebrate prey items were separated out into watch glasses and then keyed out individually.

3.4.3 Results

The results are summarised in tables 3 and 4.

Cannabalism of larval sticklebacks occurred in two populations, by adult male and female Luggie fish and by a single adult Mar female. Egg predation was common and widespread, being observed in adult males and females from Bharpa, Daimh, Lomond and Luggie and by adult Mar males. Adult males from Daimh, Lomond, Luggie and Mar ate more Gasterosteus eggs than adult females. Egg predation was commonest amongst samples of adult male and female Lomond fish but not recorded

TABLE 3. DIET OF ADULT STICKLEBACKS IN THE BREEDING SEASON

Percentage of feeding fish containing dietary item

Dietary Item	FADA		BHARPA		DAIMH		LOMOND		LUGGIE		MAR		LENNOX	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Gasterosteus fry									4	2		2		
Gasterosteus eggs			4	4	18	6	32	26	26	12	28			
Chironomid larvae	42	48	72	68	58	42	52	14	56	98	70	88	38	18
Chironomid pupae	16	10	16	24		8			28	44	32	22	32	28
Corixids									2		4			
Plecopteran nymphs					2	6	24	22	8	8	26	18	14	18
Ephemeropteran nymphs							8	12	8	2	22			
Trichopteran larvae	4				6	22	6	10	8	6	18	26	10	12
Asellus aquaticus									20	36				
Chydorids	86	78	82	78	54	68	42	84		2				
Daphnia spp.	32	10	14	8	18	14	10	6			8		12	8
Copepods	28	26	22	16	18	34	14	18	12	16			21	12
Gammarus pulex					6	4				4	24	36		
Oligochaetes									2	4				
Gastropods									2					
Bivalves	2	4	8	12	2								12	6

TABLE 4. DIET OF STICKLEBACK FRY IN SEPTEMBER 1978

Percentage of feeding fish containing dietary item

Dietary item	FADA	BHARPA	DAIMH	LOMOND	LUGGIE	MAR	LENNOX
Chironomid larvae	72	56	38	38	76	68	48
Chironomid pupae	32	16	18		8	30	42
Plecopteran nymphs			8	6	2	38	22
Ephemeropteran nymphs				4		16	
Trichopteran larvae			18		6	8	16
Asellus aquaticus					76		
Chydorids	68	92	82	94			22
Daphnia spp.	18	12	16	8			16
Copepods	14	18	16	32	8		38
Gammarus pulex						18	
Oligochaetes					4		
Gastropods					2		

from either Fada or Lennox Castle fish. Invertebrate prey items taken by the stillwater populations were largely composed of Chydoridae, Daphnids and Copepods with Mar and Luggie fish relying more heavily on larval chironomids, Trichoptera, Ephemeroptera and Plecoptera; in the eutrophic Luggie, Asellus aquaticus replaced Gammarus pulex as the principal benthic crustacean prey. Interpopulation dietary differences are also apparent from the data for fry (collected September 1978) with the lentic populations feeding heavily on chydorids (Chydorus ovalis and Eurycercus lamellatus) and chironomid larvae, and Mar and Luggie fry taking a wide variety of chironomid larvae, Gammarus, Asellus, Plecoptera, Ephemeroptera, Trichoptera, oligochaetes and gastropods.

3.4.4 Discussion

The data discussed above closely parallel the results of other workers in this field. The diet of Gasterosteus has been studied in marine environments: Abdel Malek (1968); Lemmetyinen and Mankki (1975): and in freshwater habitats by Hynes (1950); Walkey (1967); Hagen (1967); Maitland (1965); Greenbank and Nelson (1959); Valdez and Helm (1971); Manzer (1976); Larson (1972); Coad and Power (1973); Krogius, Krokhin and Menshutkin (1970); Rogers (1968); Mann and Orr (1969).

Abdel-Malek studying Gasterosteus fry in Kandalaksha Bay, White Sea noted a marked dietary progression from copepod nauplii - copepodite stages - copepods - chironomid larvae - Gammarus locusta - Cladocerans - Nereis in maturing fry and also recorded predation of Gasterosteus eggs and larvae by sticklebacks of less than 1 year of age. Lemmetyinen and Mankki studied Gasterosteus diet as part of their broad-based investigation of the role of the three-spined stickleback in the food chains of the northern Baltic; here the dietary progression was diatoms - copepods - Cladocera - chironomid pupae - small benthic crustacea - *Nereis* - fish eggs and larvae. In freshwater populations the range of dietary items recorded has been very wide reflecting seasonal and habitat variations in invertebrate density and occurrence, and changing prey selection by maturing sticklebacks. Zooplanktonic crustacea are universally important in lentic habitats (Manzer (1976); Coad and Power (1973); Walkey (1967); Valdez and Helm (1971); Larson (1972); Krogius, Krokhin and Menshutkin (1970); Rogers (1968)), with benthic macroinvertebrates forming a varying proportion of the diet between populations studied. The most important benthic invertebrates both in terms of number and bulk are chironomid larvae, often with lesser numbers of ephemeropteran and trichopteran larvae. Larson's study (1972) revealed a particularly interesting situation where two *Gasterosteus* phenotypes ("Limnetics" and "Benthics") were spatially and trophically segregated within a single water body (Paxton Lake, British Columbia); Limnetic fish ate zooplankters and Benthic fish ate macroinvertebrates, principally *Gammarus*. Within the zooplanktonic crustacea *Daphnia* and *Bosmina* species commonly occur in *Gasterosteus* stomach contents whilst the benthic/weed dwelling *Chydoridae* (especially *Eurycercus lamellatus*) are often taken in large numbers by sticklebacks of all ages.

Lotic *Gasterosteus* populations in general eat fewer planktonic animals than lentic populations. The work of Hynes (1950), Maitland (1965), Mann and Orr (1969) and Hagen (1967) describe a wide variety of benthic macroinvertebrate prey species including the larvae of the insect families Chironomidae, Plecoptera, Trichoptera, Ephemeroptera together with *Gammarus*, *Asellus* and miscellaneous molluscs and annelids.

Of particular interest both with regard to individual food requirement and reproductive success are the consistent records of egg predation and nest-raiding by adult male and female *Gasterosteus* during the breeding season. Hynes noted that in the Birket River population feeding became sporadic in the breeding season coinciding with considerable numbers of Gasterosteus eggs and larvae in the stomach contents of breeding male fish with fewer numbers in adult females. In the Mayer Lake Gasterosteus population Moodie (1972) found stickleback eggs in the stomach contents of 33% of breeding male fish examined. Kynard (1972) studied nest-raiding behaviour in detail at Wapato Lake (Washington) and discovered that in the wild 34% of eggs examined from Gasterosteus nests were at a different developmental stage than the bulk of the eggs in the nest, indicating that they had probably been stolen from maturing egg batches in neighbouring nests.

Semler (1971) working concurrently on Wapato Lake recovered stickleback eggs from 51% overall of Gasterosteus stomachs examined; in this case also males ate significantly more eggs than females. Nesting male fish which spend almost all of their time in their territories may rely to a varying extent for food upon eggs stolen from nearby nests (Wootton, 1971); "raider packs" of non-breeding males and adult females sometimes cause total loss of nest and contents (Kynard, 1972). Male sticklebacks perform a characteristic "sneaking behaviour" when nest-raiding (Morris, 1952), losing their breeding colouration and edging forward across the bottom toward the neighbouring nest, the final dash to the nest culminating in either stealing nest material or eggs (Wootton, loc. cit.) or fertilising the newly spawned eggs deposited by the female courted by the resident male (Li and Owings, 1978). Rohwer (1978) has suggested a more complex explanation of egg-eating and nest raiding behaviour in Gasterosteus, with filial cannabalism providing a nesting male with an energetic investment toward future reproductive effort, and with stolen eggs not being eaten but collected in the nest so as to serve as possible evidence to a receptive female that loss of her own eggs will be minimal if she spawns them in the nest of an already successful male. Clearly nest-raiding and egg-eating are widespread behaviours in Gasterosteus populations possibly serving as a male courtship strategy, and certainly providing a large food input for mature male and female sticklebacks. Rohwer (loc. cit.) has suggested that females may steal and eat eggs from a nest whilst "creeping through" and spawning their own eggs.

The fact that egg predation was found to be a wide-spread phenomenon during the present study, occurring in all populations except Fada and Lennox indicates that nesting male Gasterosteus are subject to losses of reproductive output through cannabalism as well as through predation of offspring during later stages of the life cycle. This reinforces the importance of territorial defence as a part of the overall behavioural strategy evolved to allow as many offspring to survive as possible from each batch of fertilised eggs.

3.5.1 The predators of Gasterosteus

Despite the marked behavioural (Chapter 4) and morphological (Chapter 3) anti-predator adaptations exhibited by Gasterosteus the species continues to be eaten in large numbers by a wide range of natural predators. Predation upon Gasterosteus has been reported both from littoral and benthic marine habitats and from eutrophic and oligotrophic lotic and lentic freshwater habitats. A review of the literature upon known predators of Gasterosteus is necessary in order to assess the importance of predation as a selective agency acting upon stickleback populations and in order to identify likely potential predators at each of the 7 study sites.

Invertebrate predators

The importance of predatory macroinvertebrate species remains largely unknown; the large littoral leech Haemopis marmorata is known to feed upon Gasterosteus eggs (Moodie, 1972) and the water scorpion Ranatra fusca predates the smallest available stickleback larvae and fry (Hay, 1974). Reimchen (1980) found that Aeshna palmata and A. eremita nymphs also take the smallest available Gasterosteus in laboratory tests. Other potential invertebrate predators include Dytiscid beetle larvae; Odonatan nymphs and carnivorous Trichopteran larvae; adult Notonecta; Dytiscus and Dytiscus larvae have all been seen to strike at Gasterosteus fry under laboratory conditions (Benzie, 1965).

The serological method of identifying Gasterosteus antigens in the gut contents of predators (described in the appendix) could be used in future studies in order to gain an estimate of the predation pressure exerted by invertebrate species.

3.5.2 Fish predators

Of the Salmonids Salmo trutta is the most important stickleback predator in British waters. Salmo trutta exhibits two life history patterns (resident freshwater Brown Trout, and anadromous Sea Trout) both of which have been reported to predate Gasterosteus (Falkus, 1971). Campbell (1971) studied the growth rates of Brown Trout in northern Scottish lochs finding that Gasterosteus formed an important component of the Trout diet. He concluded that sticklebacks should be introduced to those lochs where an enhanced Trout growth rate would lead to an improved sport fishery. Slack (1934) found that Brown Trout in the River Test (Hampshire, England) commonly took fish prey during the months November to March with Gasterosteus occurring in 9% of the fish examined. Hunt and Jones (1972) studying the diet of Brown Trout in Llyn Alaw (Anglesey, North Wales) found that Gasterosteus formed the most important dietary component of fish larger than 430mm. total length. Frost and Brown (1967) have reviewed dietary studies on the Brown Trout from a wide variety of freshwater habitats in the British Isles and have found that fish-eating may occur in Trout of a wide variety of size categories, and that the piscivorous habit may be related to various factors including habitat type, food availability, and behaviour of prospective prey species. In the study lochs on North Uist no data are available except the single study of R.N.B. Campbell (1976) on the diet of Loch Fada Brown Trout and Arctic Char which revealed that the Brown Trout heavily predate the sympatric spine-deficient *Gasterosteus* population. It seems likely that Trout predation upon sticklebacks is a widespread and important phenomenon in the North Uist lochs because the waters are for the most part oligotrophic and support a low standing crop of alternative (benthic invertebrate) species. The Arctic Char, *Salvelinus alpinus* is primarily a planktivore (Nilsson, 1955; R.N.B. Campbell loc. cit.) but does take fish prey in certain situations. In Loch Meallt on the Isle of Skye (Inner Hebrides) large (> 19cm. total length) Arctic Char take adult *Gasterosteus* (the only other fish species present) in spring and early summer. Char of > 15cm. take stickleback fry from August onwards and also raided the stickleback nests taking pre-eyed and eyed ova (R.N.B. Campbell, pers. comm.). Adalsteinsson (1976), studying the food of Brown Trout, Arctic Char and three-spined sticklebacks in Lake Myvatn (Norway) found that Char in the oligotrophic north basin of the lake relied principally upon *Gasterosteus* as a food species whilst Char in the south basin also ate chironomids and planktonic and benthic crustacea; stickleback predation was heaviest in the winter months. De Lacy and Morton (1962) also record littoral feeding Arctic Char taking large numbers of *Gasterosteus* in Karluk Lake, Alaska, an observation repeated by Greenbank and Nelson (1959) who also found a similar situation in Bare Lake, Alaska.

The Alpine (or Dolly Varden) Char *Salvelinus malma* occurs in both anadromous and sedentary forms in western North America and Eastern Asia. Greenbank and Nelson (1959) have found *Gasterosteus* in *S. malma* stomachs from Bare Lake. Krogus, Krokhin and Menshutkin (1970) studying the diet of *S. malma* in Lake Dalnee (Russia) found that Char of 100g. body weight or less ate benthic invertebrates and immature *Gasterosteus*, but that larger Char switched to an exclusively fish diet of which sticklebacks formed the most important component.

The Cut-throat Trout, *Salmo clarki* is another North American species that occurs in saline, brackish and freshwaters and is a commonly recorded stickleback predator. Armstrong (1971) recorded *Gasterosteus* from migratory *S. clarki* at Eva Lake (south eastern Alaska), Shepard and Bilton (1953) working during the months May-September 1945-1951 in Lakelse Lake (British Columbia) found that *Gasterosteus* occurred in 8% of *S. clarki* stomachs examined. Moodie (1972) working at Mayer Lake (Queen Charlotte Islands, British Columbia) found that *S. clarki* is a diurnal predator feeding upon *Gasterosteus* of all size categories during the whole year with heaviest predation in the winter months. In the same study the Prickly Sculpin, *Cottus asper* was found to be an important predator of *Gasterosteus* eggs, fry, larvae and small adults (Moodie op. cit.).

Hagen and Gilbertson (1973) studied the effects of Rainbow Trout, *Salmo gairdneri* introduction on endemic *Gasterosteus* populations in lakes Wapato and Chelan (Washington) finding that sticklebacks occurred in c. 10% of Trout stomachs during the springtimes of 1968, 1969 and 1970 but that during the winter of 1968 the percentage occurrence of *Gasterosteus* rose to 80% of the stomachs examined. The *Gasterosteus* populations in these study lakes are annual, overwintering fry have a mean body length of c. 20mm. and are predated by Trout of 22cm. total length and larger; by springtime only the largest trout are able to take the sticklebacks (Hagen & Gilbertson, loc. cit.). In his study of the Wapato Lake Gasterosteus population Kynard (1972) made underwater observations of northern Squawfish, Ptychocheilus oregonensis predating nesting male sticklebacks.

Legget and Power (1969) found that land-locked Atlantic Salmon, predated both Gasterosteus and Pygosteus during the months June-August in Gambo Pond, Newfoundland with Gasterosteus occurring in up to 56% of the stomach samples examined. Larson (1972) studying Gasterosteus in Paxton Lake (British Columbia) has recorded that introduced Coho Salmon, Oncorhynchus kisutch predated sticklebacks heavily during the summer months.

McPhail (1969) has revealed an interesting predator-prey effect between the Olympic Mud-Minnow, Novumbra hubbsi, and three-spined sticklebacks in the Chehalis River System (north west America); Novumbra is similar in size to an adult Gasterosteus but is an important predator of Gasterosteus larvae and fry at the nest. Male Gasterosteus sympatric with Novumbra have, in some instances evolved an inconspicuous black-throated breeding colouration that appears to elicit fewer attacks upon a nest of developing offspring than when the nest is guarded by a male with the normal red throat colouration. Hagen, Moodie and Moodie (1972) have suggested that the black breeding colouration in the sympatric Gasterosteus males may serve as a mutual threat signal to the similarly coloured breeding male Novumbra.

Of the European non-Salmonid predatory freshwater fish species Pike and Perch are the most commonly reported Gasterosteus predators. In Lake Windermere (England) Pike of > 40cm. total length predate most sticklebacks (Frost (1954), Allen (1939)), this is also the case in Loch Lomond (Copland, 1956) but Healey (1956) studying Pike diet in 3 Irish lakes found that Gasterosteus occurred in fish as small as 20cm. total length. Kaukoranta and Lind (1975) recovered Gasterosteus from the stomachs of adult Pike from the estuarine waters of the Oulujoki River (Gulf of Bothnia) whilst Lemmetyinen and Mankki (1975) found Gasterosteus to be the commonest prey taken by Pike in the Finnish Archepelago. As they grow larger Perch gradually switch from a wide-ranging invertebrate diet to a diet containing a large component of small fish species; this situation has been reported by Allen (1935) from Windermere; Antosiac (1963) from lakes in the Wegorzewo district of Russia; McCormack (1970) from Windermere, and during this study, by the author, from Loch Lomond (page 57). Shafi (1974) also found that Gasterosteus form an important prey species for Perch in Loch Lomond. Mann (1978) has reported Gasterosteus predation by Perch from the River Stour, Dorset, England.

Blegvad (1917) studying the food of littoral fish species in Danish coastal waters recovered Gasterosteus from the stomach contents of Sygnathus typhle (Broad-nosed Pipefish); Ctenolabrus rupestris (Goldsinny); Crenilabrus melops (Corkwing Wrasse); Cottus scorpius (Sea Scorpion); Raja clavata (Thornback Ray); Anguilla anguilla (Common Eel); Limanda limanda (Dab); Platichthys flesus (Flounder) and Gadus calliaris (Cod). Brown and Cheng (1946) also found considerable numbers of Gasterosteus in the stomach contents of Cod from the north west Atlantic at a distance of 100 miles from land and at a depth of 220m. indicting that deep lying marine shoals of Gasterosteus occur. The potential risk of predation to Gasterosteus in marine environments is likely to be much higher than in its better documented freshwater habitats.

3.5.3 Bird predators

Piscivorous birds are important predators of Gasterosteus in freshwater and littoral marine habitats; some bird species rely almost entirely upon Gasterosteus for food during the breeding season. Madsen

(1957) studying the food of diving ducks in Danish coastal waters recorded *Gasterosteus* in small numbers from the stomach contents of Red-throated Divers, and in 80% of the Black-throated Divers examined between the months of October and February. Lemmetyinen (1973a) studying the feeding ecology of Common and Arctic Terns in the archepelago of south west Finland found that male terns of both species almost always used *Gasterosteus* during ritualised courtship feeding bouts. Pellet analysis established that *Gasterosteus* was the commonest prey species taken prior to the hatching of chicks, occurring in 93% of all samples. Young chicks were fed smaller *Gasterosteus* than those eaten by the parent birds until an age of 8 days when they were fed adult *Gasterosteus*. Lemmetyinen (1973b) noted that for Arctic Terns *Gasterosteus* forms 70-80% of all fish prey in the Finnish archepelago and the timing of Tern breeding is synchronised with the major *Gasterosteus* breeding peak in May and June when breeding sticklebacks of both sexes are heavily predated. Lemmetyinen and Mankki (1975) have assessed the importance of *Gasterosteus* in the food chains of the northern Baltic finding that sticklebacks form the main food species of Common Terns, Arctic Terns, Red-breasted Mergansers and are eaten in smaller quantities by the Goosander, *Mergus merganser*. *Gasterosteus* remains occurred in 77% of the pellets of young Arctic Terns, 85% of Red-breasted Merganser pellets, and overall formed 90% of all food items taken by Mergansers (Lemmetyinen and Mankki loc. cit.; Bagge, Lemmetyinen and Raitis, 1970). Fjeldsa (1975) studying the Black-headed Gull and Arctic Tern breeding colonies on Lake Myvatn (Iceland) noted that both species fed mainly on *Gasterosteus* during the breeding season. Bengston (1971) studied the food and feeding behaviour of diving ducks at Lake Myvatn and recorded the following predators of *Gasterosteus* eggs, larvae, fry and adults during the summer months: *Aythya marila* (Scaup); *Aythya fuligula* (Tufted Duck);

Bucephala islandica (Barrow's Goldeneye); Clangula hyemalis (Long-tailed Duck) and Mergus serrator (Red-breasted Merganser). Of these species the Red-breasted Merganser relies most heavily on sticklebacks with Gasterosteus forming 81-99% of the diet by weight, and with some Merganser chicks eating 100% Gasterosteus diet (Bengtson 1971). In Scotland Red-breasted Mergansers breed and feed on freshwater bodies in the summer and overwinter in coastal marine areas (Mills, 1962a). During a survey of the Goosander and Merganser as Salmon predators in Scotland, Mills (1962b) recovered Gasterosteus from 2.6% of stomachs of Mergansers and found none in Goosanders. Berrie (1936) in a broader survey of the diet of diving ducks in Scotland recorded that Gasterosteus is a major food item for Mergansers from some localities. Campbell (1947) found that six Mergansers examined from the islands of North Uist and Benbecula had been feeding exclusively on marine organisms. Penczak (1968) has recovered occasional sticklebacks from Goosanders in Poland, and the American Merganser (Mergus merganser americanus) is noted by Munro and Clemens (1937) and Carl and Clemens (1953) to feed exclusively on Gasterosteus both in freshwater and marine localities.

Eastman (1969) studying the Kingfisher, Alcedo althis (in Britain) noted that Gasterosteus were commonly taken by adult birds and manipulated in the bill for several seconds until the spines collapsed, the fish then being swallowed head-first. Young Kingfishers attempting to swallow sticklebacks immediately regurgitated them with their spines still raised. Owen (1955, 1960) studied the diet and reproductive success of the Grey Heron in the Thames Valley (England) finding that both Gasterosteus and Pygosteus were important dietary items. Herons took adult Gasterosteus during April, May and June and also took some of the larger developing fry during the last week of June (Owen, 1955). Schlegel (1964) reports Gasterosteus from the diet of Grey Herons in Germany where they formed an important food species. During the present study Herons were found to take adult male and female Gasterosteus from the Mar burn population; the results of this study are discussed on page 64 and detailed in the Appendix (Giles, 1981).

In any literature review of Gasterosteus predation it is important to remember that adult sticklebacks cannabalise large numbers of viable eggs during the breeding season and are therefore potentially very important in determining the defensive behaviour of an adult male stickleback caring for a brood of young. It may be that conspicuous territorial defence which is successful in excluding all rival males may be a good risk (in reproductive terms) despite the fact that the defending male will be conspicuous to avian and fish predators.

3.5.4 Reptilian predators

In certain southern Californian areas the garter snake, Thamnopis couchi hammondi is thought to exert a considerable predation pressure upon Gasterosteus populations (Bell and Haglund, 1978).

3.5.5 Mammalian predators

Aquatic mammals do not appear to take appreciable quantities of sticklebacks but both the Mink, Mustela vison in Swedish rivers and lakes (Gerell, 1968) and the Otter, Lutra lutra in Europe (Harris (1968)) are recorded as taking occasional sticklebacks. During the present study Mink were often seen around the mid-basin of Loch Lomond and along the Mar burn, Otters are common on the freshwater lochs of North Uist (D. Shaughnessy, pers. comm.).

3.6 Assessment of risk of predation at study sites

3.6.1 Loch Lomond

Fish predators

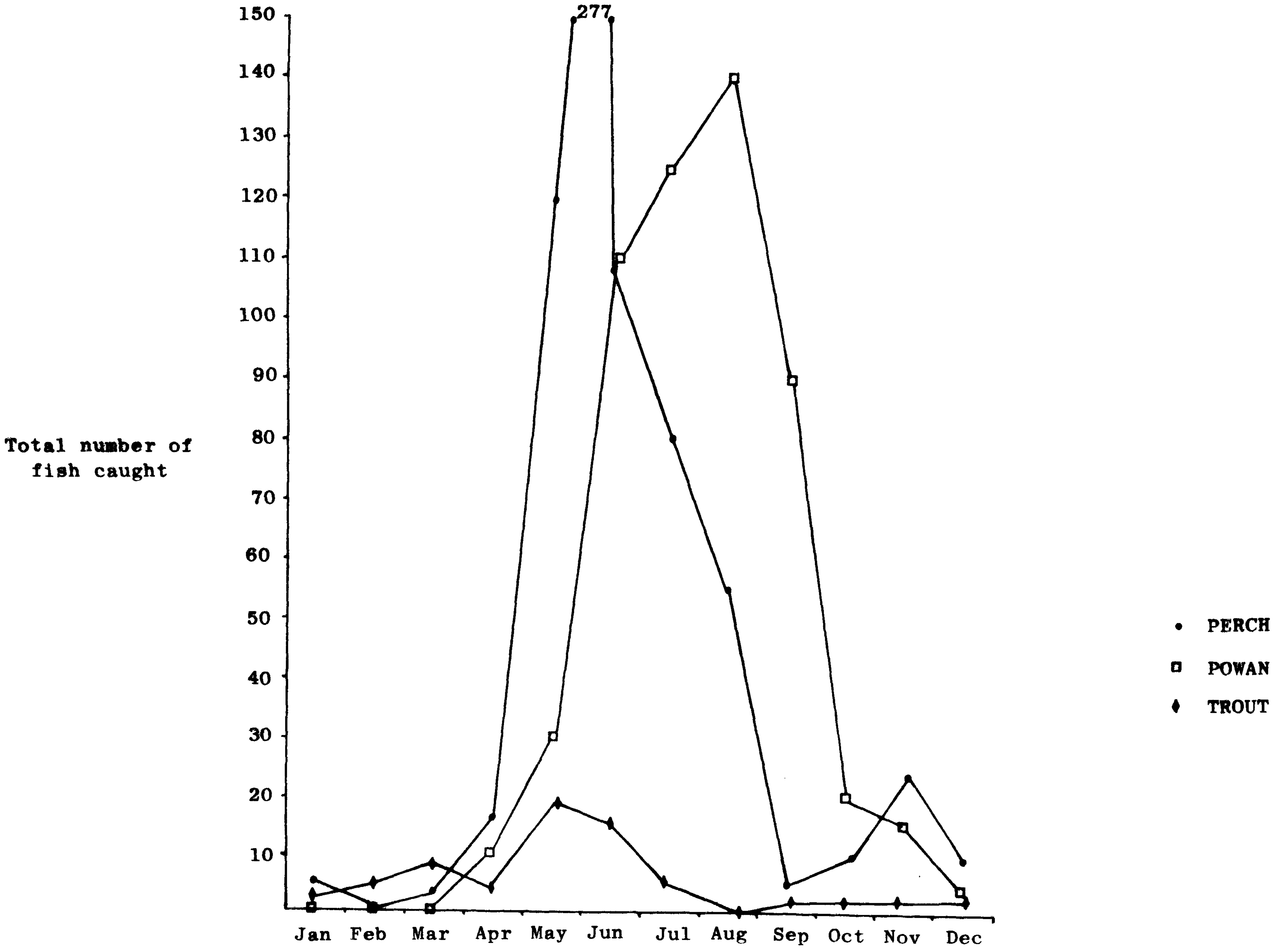
Methods

During the period January-December 1977 and September-December 1978 benthic gill nets and wire mesh perch traps (Worthington, 1950) were set weekly in Camas an Losgainn in order to sample the predatory fish species using the bay. The gill nets used were of graded mesh size (bar lengths 19mm., 26mm., 31mm., 39mm.) and were 30m. long; both nets and traps were set for a period of c. 12 hours from late evening to the following morning. As requested by the Loch Lomond Angling Improvement Association all live Salmon and Trout netted were immediately returned to the water, fish of other species were used for dietary analysis. At the beginning of the study it was apparent that regular, long-term netting and trapping coupled with the killing of predatory fish for stomach analyses would soon substantially diminish the local population density of such species. In order to avoid this situation a stomach-sampler for live fish was developed by the author, the sampler allows the stomach of a captive fish to be speedily and efficiently sampled in the field with little or no deleterious effect. The sample of the stomach contents obtained may then be stored in a small capped tube for subsequent examination, and the fish returned to the water. The sampler was tested for efficiency on Perch from Loch Lomond; an account of the construction, sampling efficiency, and method of usage of the sampler has been published (Giles, 1980) and a copy of this paper is appended to this thesis (see Appendix).

Results

Fig. 3 shows the combined netting and trapping catch data for

Fig. 3. Loch Lomond combined netting and trapping catch data 1977



the period January-December 1977, weekly data having been pooled and expressed as a monthly total, only the three commonest species are shown. Powan and Perch were caught in the largest numbers during the period May-September, adult Perch used the bay for spawning during this time, fewer numbers of Trout, Salmon, Roach, Eels and Pike were caught during the summer months. During the period October to April low numbers of Trout, Powan and Perch were consistently caught with a few Salmon and Roach. Powan were discounted as potential *Gasterosteus* predators as they are known to be planktivorous during the summer months and to switch to a diet of benthic invertebrates during the winter (Slack, 1957). From these data it was concluded that Perch were the commonest potential *Gasterosteus* predators frequenting the study area and accordingly a detailed analysis of stomach samples from a total of 590 adult Perch was undertaken. Perch of total length < 130mm. were considered to be too small to sample and were returned immediately to the water; a total of 6825 food items were identified from the sampled perch and these data are summarised in tables 5 and 6.

Table 5 gives the percentage composition of monthly dietary items consumed by number, at the onset of breeding in May. *Daphnia hyalina* and chironomid pupae occur most commonly in stomach samples with Ephemeropteran and Plecopteran nymphs and Trichopteran larvae in smaller numbers. *Asellus aquaticus* is the only benthic species taken in large numbers at this time and occurs throughout the summer in stomach samples. In June *D. hyalina*, and *Diaptomus gracilis* are eaten with *Bosmina coregoni*, with a switch to larger predatory Cladoceran species (*Bythotrephes longimanus*, *Polyphemus pediculus*, *Leptodora kindti*) in July together with a mixed component of smaller zooplankters and benthic invertebrate species. In September the majority of the Perch migrate from the littoral zone (Fig. 3) and the remaining fish show

Table 5. Percentage composition of monthly dietary items by number

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Total organisms	15	-	4	38	1717	973	1814	2193	38	21	62	10
Erpobdella octoculata L.	-	-	-	-	0.1	-	-	-	-	-	-	-
Diaptomus gracilis Sars	-	-	-	-	4.9	7.9	7.4	0.1	-	-	-	-
Daphnia hyalina Leydig	-	-	-	-	40.9	41.4	10.5	21.0	-	-	-	-
Eurycercus lamellatus Muller	-	-	-	-	-	-	-	1.7	-	-	-	-
Bosmina coregoni Baird	-	-	-	-	3.1	12.6	4.7	0.4	-	-	-	-
Bosmina longirostris Muller	-	-	-	-	0.2	3.1	11.7	1.4	-	-	-	-
Chydoridae sp.	-	-	-	-	-	-	-	0.1	-	-	-	-
Polyphemus pediculus L.	-	-	-	-	-	-	14.0	0.3	-	-	-	-
Bythotrephes longimanus Leydig	-	-	-	-	-	1.2	38.6	67.4	-	-	-	-
Leptodora kindti Focke	-	-	-	-	0.2	0.2	5.8	0.2	-	-	-	-
Asellus aquaticus L.	-	-	50.0	18.4	12.3	8.4	-	0.4	-	-	4.8	-
Gammarus pulex L.	-	-	-	-	1.0	1.9	-	-	-	-	-	-
Dipteran adults unid.	-	-	-	-	0.2	0.1	.05	0.1	-	-	-	-
Ephemeropteran adults unid.	-	-	-	-	.05	-	0.1	-	-	-	-	-
Ephemerella ignita Poda nymphs	-	-	-	-	1.3	0.6	0.2	-	-	-	-	-
Ephemera danica Mull adults	-	-	-	-	-	-	-	0.5	-	-	-	-
Ephemera danica Mull nymphs	-	-	-	-	1.1	2.8	0.2	-	-	61.9	4.8	-
Caenis horaria L. nymphs	-	-	-	-	.05	-	-	-	-	-	-	-
Leptophlebia sp. nymphs	-	-	-	-	0.1	0.2	1.3	0.7	86.8	-	1.6	-
Paraleptophlebia sp. nymphs	-	-	-	-	1.0	2.9	0.2	0.2	-	-	-	-
Centroptilum sp. nymphs	-	-	-	-	3.8	-	-	-	-	-	-	-
Plecopteran adults unid.	-	-	-	-	-	-	-	0.2	-	-	-	-
Trichopteran adults unid.	-	-	-	-	-	0.3	0.1	-	-	-	-	-
Limnephillidae sp. larvae	-	-	-	-	-	2.8	-	-	-	-	-	-
Phryganidae sp. larvae	40.0	-	-	-	.05	0.1	-	-	-	-	-	-
Chironomid pupae	-	-	-	78.8	25.8	6.1	4.6	4.6	-	-	-	-
Chironomid larvae	-	-	-	-	0.4	0.9	0.1	-	-	9.5	-	-
Chaoborus flaviscans	-	-	-	-	-	-	0.5	-	-	-	-	-
Odonata nymphs unid.	-	-	-	-	0.3	-	-	-	-	-	3.2	-
Sialis lutaria L. larvae	-	-	50.0	2.6	1.0	0.1	-	-	-	-	1.2	-
Corixidae sp. unid.	-	-	-	-	0.2	3.9	0.2	1.0	2.6	-	3.2	-
Hydracarina sp. unid.	-	-	-	-	-	-	.05	-	-	-	-	-
Perca fluviatilis L.	-	-	-	-	-	0.2	.05	-	-	-	1.6	-
Phoxinus phoxinus L.	-	-	-	-	.05	0.2	-	-	-	-	-	10.0
Gasterosteus aculeatus L.	53.33	-	-	-	0.4	0.3	0.2	.05	10.5	19.0	29.6	80.0
Pygosteus pungitius L.	-	-	-	-	0.2	0.4	0.1	.05	-	9.5	24.0	10.0
Unid. fish sp.	6.0	-	-	-	0.3	0.6	.05	.05	-	-	24.8	-
Agnatha ammocoete larvae	-	-	-	-	-	-	-	-	-	-	1.6	-
Empty stomachs	1	-	-	11	65	10	17	13	1	3	4	4

a distinct switch to a predominantly benthic diet with *Gasterosteus* fry beginning to become a more important dietary component. In October the fry of both *Gasterosteus* and *Pygosteus* are taken and the fish component of the diet becomes increasingly important throughout the winter with smaller numbers of Perch fry and Minnows being taken. From October to January both *Gasterosteus* and *Pygosteus* fry are heavily predated by Perch with a maximum of 8 *Gasterosteus* fry being removed from a single Perch. Predated *Gasterosteus* fry varied in size from 15-25mm. and had almost invariably been swallowed head-first. No adult *Gasterosteus* were recovered from sampled Perch during the study period but in May and June several adult male *Pygosteus* in breeding colouration were recovered in stomach samples together with similar numbers of *Gasterosteus* fry.

Table 6 gives the percentage by number of feeding fish in a given size category containing a given dietary item. An examination of these data shows that in general small Perch eat zooplanktonic organisms and then gradually broaden their dietary preferences as they grow to encompass a wide variety of benthic invertebrate species, sticklebacks feature largely in the diet of larger Perch. Shafi (1974) also studied the diet of adult Perch in Loch Lomond concluding that *Cyclops*, *Gammarus*, *Chironomidae*, *Corixids* and *Gasterosteus* were the food organisms taken.

Copland (1956) found that *Gasterosteus* formed 4.3% of the diet of Pike larger than 20cm. total length in Loch Lomond. During the present study only 5 pike were caught in Camas an Losgainn; their stomachs contained Powan and Trout. Whilst it remains likely that Trout predate *Gasterosteus* in Loch Lomond no assessment of the extent of this predation pressure is possible as all Salmon and Trout were returned immediately to the water upon removal from the nets.

A serological technique for identifying three-spined stickleback antigens in the stomach contents of fish predators was developed

Table 6. Percentage by number of feeding fish in size category containing dietary item

Size category	130-180mm	181-230mm	231-280mm	281-360mm
Erpobdella octoculata L.	-	0.29	0.31	-
Diaptomus gracilis Sars	4.5	2.2	3.1	-
Daphnia hyalina Leydig	9.5	8.0	3.1	-
Eurycercus lamellatus Muller	1.0	-	-	-
Bosmina coregoni Baird	4.5	0.87	3.1	-
Bosmina longirostris Muller	3.0	0.87	-	-
Chydoridae sp.	0.5	-	-	-
Polyphemus pediculus L.	3.0	-	-	-
Bythotrephes longimanus Leydig	11.5	1.74	-	-
Leptodora kindti Focke	3.5	1.74	3.1	-
Asellus aquaticus L.	10.0	26.0	12.3	-
Gammarus pulex L.	1.5	3.8	-	-
Dipteran adults unid.	1.5	.29	-	-
Ephemeropteran adults unid.	-	-	6.2	-
Ephemerella ignita Poda nymphs	2.5	3.6	-	-
Ephemera danica Mull adults	-	.29	-	-
Ephemera danica Mull nymphs	5.0	5.51	8.2	16.66
Caenis horaria L. nymphs	0.5	-	-	-
Leptophlebia sp. nymphs	2.5	1.74	-	-
Paraleptophlebia sp. nymphs	2.0	2.32	6.2	-
Centroptilum sp. nymphs	-	0.58	-	-
Plecopteran adults unid.	-	0.29	-	-
Trichopteran adults unid.	0.5	0.26	3.1	-
Limnephillidae sp. larvae	-	0.59	-	-
Phryganidae sp. larvae	-	2.9	-	-
Chironomid pupae	19.0	13.7	13.5	50.0
Chironomid larvae	5.0	2.6	3.1	-
Chaoborus flaviscans	0.5	-	-	-
Odonata nymphs unid.	-	1.16	3.1	-
Sialis lutaria L. larvae	3.5	1.45	9.3	-
Corixidae sp. unid.	1.0	0.87	6.2	-
Hydracarina sp. unid.	0.5	-	-	-
Perca fluviatilis L.	0.5	-	6.2	-
Phoxinus phoxinus L.	-	0.59	2.1	-
Gasterosteus aculeatus L.	2.0	6.6	7.1	33.33
Pygosteus pungitius L.	1.5	5.5	-	-
Unid. fish sp.	1.0	2.9	-	-
Agnatha ammocoete larva	-	0.29	-	-
Empty stomachs	42	70	8	4
Total fish	159	371	50	10

during the present study. An account of the methods used and results obtained is appended to this thesis (page 206, Appendix).

Bird predators

During the period September 1976-October 1979 all piscivorous bird species seen feeding around the Ross peninsula in the mid-basin of the loch were identified, where possible, and notes of their feeding behaviour and area were taken. Of the species list, noted on page 29, the Red-breasted Merganser was the most commonly seen piscivorous species, almost always fishing the shallow littoral zone and particularly in areas where nesting male *Gasterosteus* were known to be present in fair numbers. Herons were also often seen fishing the loch margins and along the banks of inflowing streams, also fishing in known and potential stickleback nesting areas. During the stickleback breeding season of May-late July Merganser adults and their developing broods were seen fishing the shallow littoral areas of Camas an Losgainn daily with a peak of feeding activity during the late evening and at dusk. Common and Black-headed Gulls and Common n/ Terms were seen fishing the mid-basin of the loch less commonly and tended to feed over open water as well as over shallow littoral areas. On two occasions in July 1978 adult Red-breasted Mergansers were observed (through binoculars) to take adult *Gasterosteus*, manipulating the fish for several seconds before swallowing them head-first.

3.6.2 Mar Burn

Fish predators

Maitland (1965) studied the diet of Salmon, Trout, Minnows, Sticklebacks and Stone Loach in the River Endrick and its tributaries, including a brief summary of the diet of Salmon and Trout (in August) in the Mar burn. At the time of sampling both adult *Gasterosteus* and maturing stickleback fry were present in the burn but none were recovered from the stomach contents of any of the Salmon or Trout examined (Maitland, 1965). Throughout the year large numbers of Salmon or Trout run up the Clyde estuary, through Loch Lomond and up the River Endrick to spawn; some of these fish ascend the Mar burn and must necessarily occupy the same habitat as the Mar burn sticklebacks. Mature salmon rarely feed in freshwater (Mills, 1971) but Sea Trout are known to predate Gasterosteus after entering freshwater (Falcus, 1977) and therefore pose a potential predatory threat.

Bird predators

During the period June to August 1977 frequent visits to the heronry at Gairtfairn Wood were made and food items dropped from nests were recovered and identified. Herons were seen to fish the Mar burn very frequently and in considerable numbers; early dietary information revealed that mature Roach (from the nearby River Endrick) formed the bulk of the food during those months. During the period February-August 1978 a detailed study of the diet of breeding Herons was carried out both at Gartfairn and at the much smaller Lennox Castle Heronry, a report of this study has been published (Giles, 1981) and a reprint of the paper is appended to this thesis (see Appendix). The Gartfairn Heronry is one of the largest in Scotland with a maximal number of 39 breeding pairs in recent years. Mitchell (1978) has reviewed the information available at this site to date. Gartfairn Herons were found to take a wide variety of mammalian and fish prey with adult Roach being by far the most important food species. Adult Gasterosteus were recovered from beneath nests during the months of May, June and July, forming 4% of identifiable food items in July. Both male Gasterosteus in breeding colours and gravid female Gasterosteus were found beneath nests, but despite the presence of large numbers of

Gasterosteus fry in the adjacent Mar burn none were found beneath nests during the study. During visits to the Mar burn and to the Heronry Red-breasted Mergansers, Common Gulls and Black-headed Gulls were also seen fishing.

3.6.3 The River Luggie

Fish predators

On six occasions during June and July 1978 and on four occasions during November 1978 benthic gill nets (bar length 26mm.) were set in the River Luggie across the study section. No fish at all were caught on any of these sampling occasions despite the fact that the nets were allowed to fish for 24 hour periods in each instance. Whilst sampling sticklebacks from the Luggie, small Eels, Stone Loach and Minnows were regularly caught and on two occasions small (< 10cm.) Brown Trout were caught by hand net. The River Kelvin with which the River Luggie is confluent has a large head of fish including Pike and Perch and in its upper reaches the Luggie supports a population of Brown Trout, but the study section was chosen because it has very few, if any, potential fish predators of sticklebacks.

Bird predators

Observations of piscivorous birds were similarly infrequent with only 7 sightings of Black-headed Gulls near the river in two years of regular visits.

3.6.4 Lennox Castle Reservoir

Fish predators

Lennox Castle reservoir contains no predatory fish species; an unsuccessful attempt was made to introduce Brown Trout soon after the construction of the reservoir but all of the fish died without reproducing successfully (L.C. McGillivray, pers. comm.). Gill nets were set on four occasions in April 1978 and allowed to fish for 3 days on each occasion with no fish being caught, it was therefore concluded that local information was correct and that no predatory fish were present.

Bird predators

Since 1974 a small heronry has become established in a coniferous woodland adjacent to the reservoir and it was therefore decided to study the diet of breeding Herons during the summer of 1978, over the same period as the study of the Gartfairn heronry. Weekly visits were made and all food items dropped from nests and regurgitated pellets were collected and analysed, the results of this study are appended to this thesis (Giles, 1981). Lennox Castle herons ate negligible numbers of fish (Pike and Perch) during the study period despite a local abundance of Brown Trout (in streams and rivers) and sticklebacks in the reservoir. The most important food species was the water vole, Arvicola terrestris which predominated in all food samples collected. The only important Gasterosteus predators at Lennox Castle appear to be the Common and Black-headed Gulls, both of which fish the reservoir very frequently and have been observed to take sticklebacks from just below the water surface during the summer months.

3.6.5 North Uist study lochs

Permission was applied for to net lochs Fada, Bharpa and Daimh for Trout and Char in May and September 1978 in order to investigate the diet of these species as potential Gasterosteus predators; published work and personal communications represent the information available to date however, as permission was refused. All sightings of piscivorous birds fishing the Uist lochs were recorded during field visits but insufficient time was available to study dietary preferences of individual species; predation risk is therefore inferred from a literature review of each predatory species present (pages 53 to 56). Potential invertebrate predators of Gasterosteus include Odonatan nymphs and Dytiscid larvae both of which were commonly encountered whilst sampling the Uist lochs.

Loch an Daimh

Brown Trout occur in large numbers in Loch an Daimh and in common with other Brown Trout populations in oligotrophic Scottish Highland lochs sympatric with sticklebacks they are likely to predate the resident Gasterosteus population (Campbell, 1971). Of the piscivorous birds seen fishing the loch Herons, Red-throated Divers, Black-throated Divers, Common Terns, Arctic Terns, Common Gulls, Black-headed Gulls, Red-breasted Mergansers and Little Grebes are all likely Gasterosteus predators. There is no published work on this loch.

Campbell (1949) stated that Herons were on the increase in the Outer Hebrides in the late 1940's, having only started to breed on the islands in 1902; during the present study Herons were regularly seen fishing the Uist freshwater lochs. Campbell examined small numbers of dietary remains at North Uist heronries and found evidence of marine feeding with no freshwater fish apparent.

Loch á Bharpa

Both Brown Trout and Arctic Char occur in Loch á Bharpa and both species are likely Gasterosteus predators, particularly during the winter months when insect food is at its lowest availability. The piscivorous bird assemblage is similar to that of Loch an Daimh and a considerable risk of predation is likely to be experienced both from swimming species (Grebes, Mergansers, Divers) and marginal or aerial species (Heron, Gulls, Terns). R.N.B. Campbell (1976) examined the stomach contents of 5 Char from Loch á Bharpa but found only planktonic crustacea.

Loch Fada

Campbell's study (1976) represents the only published account of the diet of Brown Trout and Arctic Char in Loch Fada. Spine-deficient Gasterosteus were found to form 2% of the spring diet of Trout and 10% of the autumn diet; overall sticklebacks were the dominant food species in 18% of the Trout guts examined. No normally spined Gasterosteus were found in any of the Trout examined (R.N.B. Campbell, pers. comm.). Loch Fada Char were found to eat mostly zooplanktonic cladocerans with lesser numbers of benthic invertebrate species, no sticklebacks were found in any of the Char examined (Campbell, 1976). As with the other two lochs large numbers of piscivorous birds were observed fishing Loch Fada, Herons, Terns and Gulls are known to nest around the shores of the loch or on its islands. Both Red-throated and Black-throated Divers nest on small lochans in the immediate vicinity of Loch Fada and Loch a Bharpa and the adult birds and developing young use the shallow littoral areas of these lochs to catch the small fish species which form their normal prey (Merrie, 1978). Table 7 summarises the information presented in this section listing the known and potential predators of adult and juvenile Gasterosteus at each of the seven study sites.

3.7.1 The incidence and significance of Schistocephalus solidus parasitism in the study populations

Schistocephalus solidus is a Pseudophyllidean cestode which is mature in the alimentary canal of piscivorous birds, and has two intermediate hosts, a procercoid infesting a cyclopoid copepod which

TABLE 7. SUMMARY OF KNOWN AND POTENTIAL STICKLEBACK PREDATORS AT THE STUDY SITES

		BIRDS		FISH	
		KNOWN	POTENTIAL	KNOWN	POTENTIAL
LOMOND	ADULTS	Merganser	Gulls, Terns, Heron, Grebes, Merganser	Pike	Trout (Eels)
	FRY	None	Gulls, Terns, Heron, Grebes, Merganser	Pike, Perch	Trout (Eels)
MAR	ADULTS	Heron	Gulls, Merganser	None	Trout (Eels)
	FRY	None	Gulls, Heron, Merganser	Stickleback	Trout (Eels)
LENNOX	ADULTS	Black-headed Gull	Common Gull, Heron	None	None
	FRY	None	Gulls, Heron	None	None
LUGGIE	ADULTS	None	Gulls	None	(Trout) (Eels)
	FRY	None	Gulls	Stickleback	Trout (Eels)
FADA	ADULTS	None	Divers, Gulls, Terns, Heron, Little Grebe, Merganser	Trout	Charr (Eels)
	FRY	None	Divers, Gulls, Terns, Heron Little Grebe, Merganser	Trout	Charr (Eels)
DAIMH	ADULTS	None	Divers, Gulls, Terns, Heron Little Grebe, Merganser	None	Trout (Eels)
	FRY	None	Divers, Gulls, Terns, Heron Little Grebe, Merganser	None	Trout (Eels)
BHARPA	ADULTS	None	Divers, Gulls, Terns, Heron Little Grebe, Merganser	None	Trout, Charr, (Eels)
	FRY	None	Divers, Gulls, Terns, Heron, Little Grebe, Merganser	None	Trout, Charr, (Eels)

is then eaten by a stickleback where the plerocercoid develops in the coelomic cavity (Hopkins and Smyth (1951), Clarke (1954)). *S. solidus* infestations occur commonly in lentic *Gasterosteus* populations (e.g. Chappell (1969), Maclean (1974), Pennycuik (1971), Greenbank and Nelson (1959), Arme and Owen (1967), Coad and Power (1973), Larson (1972)) indicating that avian predators must be taking sticklebacks in order that the parasites life cycle is completed. An example of this situation has been documented by Lemmetyinen and Raitis (1972) where Common and Arctic Terns known to predate *Gasterosteus* were also found to harbour mature *Schistocephalus* in their alimentary canals. Adult *Gasterosteus* can survive with large numbers of *Schistocephalus* plerocercoides in the coelomic cavity but various somatic stresses are created within parasitised fish; females show retarded ovarian maturation and may fail to spawn (Meakins, 1974a) and both males and females show an increased oxygen consumption and tend to swim close to the water surface (Lester, (1971). *Schistocephalus* parasitised Lennox Castle sticklebacks were often found to have very small livers (both sexes) with females having poorly developed ovaries with few or no developing ova visible. *Gasterosteus* in Priddy Pool (Somerset, England) infected with *Schistocephalus* showed a retarded growth rate and poorer bodily condition when compared with non-infected individuals (Pennycuick, 1971). In extreme cases of infestation the *Schistocephalus* energy-drain may be so great that the host stickleback dies (Threlfall, 1968). Heavy *Schistocephalus* infestation leads to gross distension of the belly of the host and hampers locomotion, this, combined with the tendency for parasitised fish to swim close to the water surface, is likely to increase the chance of the stickleback being predated by a piscivorous bird.

3.7.2 Results

Table 8 gives the incidence and mean density of Schistocephalus plerocercoides removed from morphological samples of Gasterosteus from each of the seven study sites.

Discussion

At Lennox Castle the final hosts of Schistocephalus are almost certainly the Common and Black-headed Gulls which are seen to fish the reservoir daily. The North Uist lochs pose a much more intricate problem however both because of the numbers and variety of piscivorous bird species fishing the freshwater lochs, and because of the large number of small separate Gasterosteus populations, any or all of which may be predated by the birds. The higher incidence of S. solidus in Loch Fada sticklebacks may be a good indicator of heavy avian predation or may simply be a reflection of larger numbers of gulls and terns using the loch for roosting and flocking. The occurrence of S. solidus in all three North Uist study sites (and in several of the other North Uist lochs surveyed) gives a reasonable indication that avian predation of Gasterosteus occurs on the island but provides insufficient evidence to determine whether this predation pressure may be greater at some lochs than at others.

3.8 Summary

1. Age determinations made upon otoliths, together with regular sampling of the mainland populations revealed that the usual life span of Gasterosteus at all seven study sites is approximately 18 months.

2. The five stillwater populations studied exist largely on a diet of planktonic crustacea and chironomid larvae. Gasterosteus from the two running water populations ate a wider variety of benthic

Table 8. Incidence and mean density of *Schistocephalus solidus* parasitism on *Gasterosteus*

	% fish infected	mean parasite number	Range
Loch Fada (n = 339)	10.9	1.8	0 - 4
Loch a Bharpa (n = 350)	0.85	1	0 - 1
Loch an Daimh (n = 383)	3.13	1.5	0 - 3
Loch Lomond (n = 315)	0	0	
River Luggie (n = 486)	0	0	
Mar Burn (n = 439)	0	0	
Lennox Castle (n = 411)	48	3.6	0 - 9

invertebrate species. Predation of stickleback eggs occurred in five of the study populations and cannabalism of Gasterosteus larvae at two sites.

3. A literature review of the predators of Gasterosteus revealed that Brown Trout, Arctic Char, Perch and Pike were likely to be the most important piscivorous fish encountered during the present study. Of the fish-eating birds the Grey Heron, Red-breasted Merganser, Black-headed Gull and Red-throated Diver were the greatest potential predatory threat, with the Arctic Tern, Common Tern, Little Grebe and Gull species posing a further possible threat.

4. A stomach sampler for live fish was designed and tested during the first year of the study (see Appendix). The possibility of immunological recognition of three-spined stickleback antigens in predator gut contents was also explored (see Appendix).

5. An analysis of dietary data from 590 Loch Lomond Perch is included; Perch were found to predate large numbers of Gasterosteus fry of total length 15mm.-25mm. during the months October-January. No adult Gasterosteus were recovered from Perch stomachs. Red-breasted Merganser adults and chicks fish the Loch Lomond study area very extensively, and were seen to capture Gasterosteus on two occasions in July 1978.

6. At the Mar burn Grey Herons were found to take adult Gasterosteus during the months of May, June and July. A full account of this work is appended to this thesis. Sea Trout are potential predators of Gasterosteus in the Mar burn.

7. At the River Luggie study site the threat from predatory fish and

birds is thought to be very low.

8. At Lennox Castle there are no predatory fish, Black-headed and Common Gulls were observed to take Gasterosteus from just below the water surface during the summer months. This observed avian predation correlates well with the high incidence of Schistocephalus solidus parasitism within this population. Grey Herons from a small adjacent colony fed predominantly upon Water Voles, no Gasterosteus remains were found beneath their nests (see Appendix).

9. At the North Uist study sites predation pressure data was very difficult to obtain, however Campbell (1976) has reported Brown Trout predation on Loch Fada Gasterosteus. At all three sites a large assemblage of fish-eating bird species is present and the risk of predation from this source is thought to be high.

10. The incidence of Schistocephalus plerocercoides at all seven study sites is discussed and the usefulness of this parasite as a marker of avian predation is discussed.

CHAPTER 4

MORPHOLOGY

4.1 Introduction

The aims of the morphological studies described in this chapter are firstly to determine whether adult body size and shape, lateral plate number, and spine size are related to risk of predation in the natural *Gasterosteus* populations studied. Secondly an attempt is made to identify a selective agency that can account for the repeated occurrence of pelvic girdle reduction in North Uist stickleback populations. The very variable external morphology of *G. aculeatus* has prompted a large volume of research literature over the last century. Eigenmann (1886), reviewing the American Gasterosteidae proposed six species from within the *Gasterosteus* complex; all except one (*Gasterosteus wheatlandi*, the Black-spotted stickleback) are now recognised as belonging to one very variable species, *G. aculeatus* L. Penczak (1960) reviewing his morphological studies of *Gasterosteus* in Poland defines eight distinct freshwater forms characterised by particular numbers of lateral plates and dorsal spines but regards all extant European forms as members of the single species *G. aculeatus* L. The lateral plates in *Gasterosteus* are small external bony structures analogous to teleostean scales and occur in rows along the sides of the body originating just behind the operculum. Fig. 4 illustrates the phenotypic variability exhibited by extant *Gasterosteus* morphs. Hubbs (1929) also recommended a conservative taxonomic approach to the genus *Gasterosteus* and proposed that all of the phenotypic variability exhibited by U.S.A. Atlantic Coast *Gasterosteus* populations could be encompassed within *G. aculeatus* L. except for *Gasterosteus wheatlandi* (Putnam), a marine form with distinctive low fin ray counts, absence of posterior lateral plates and restricted geographical distribution

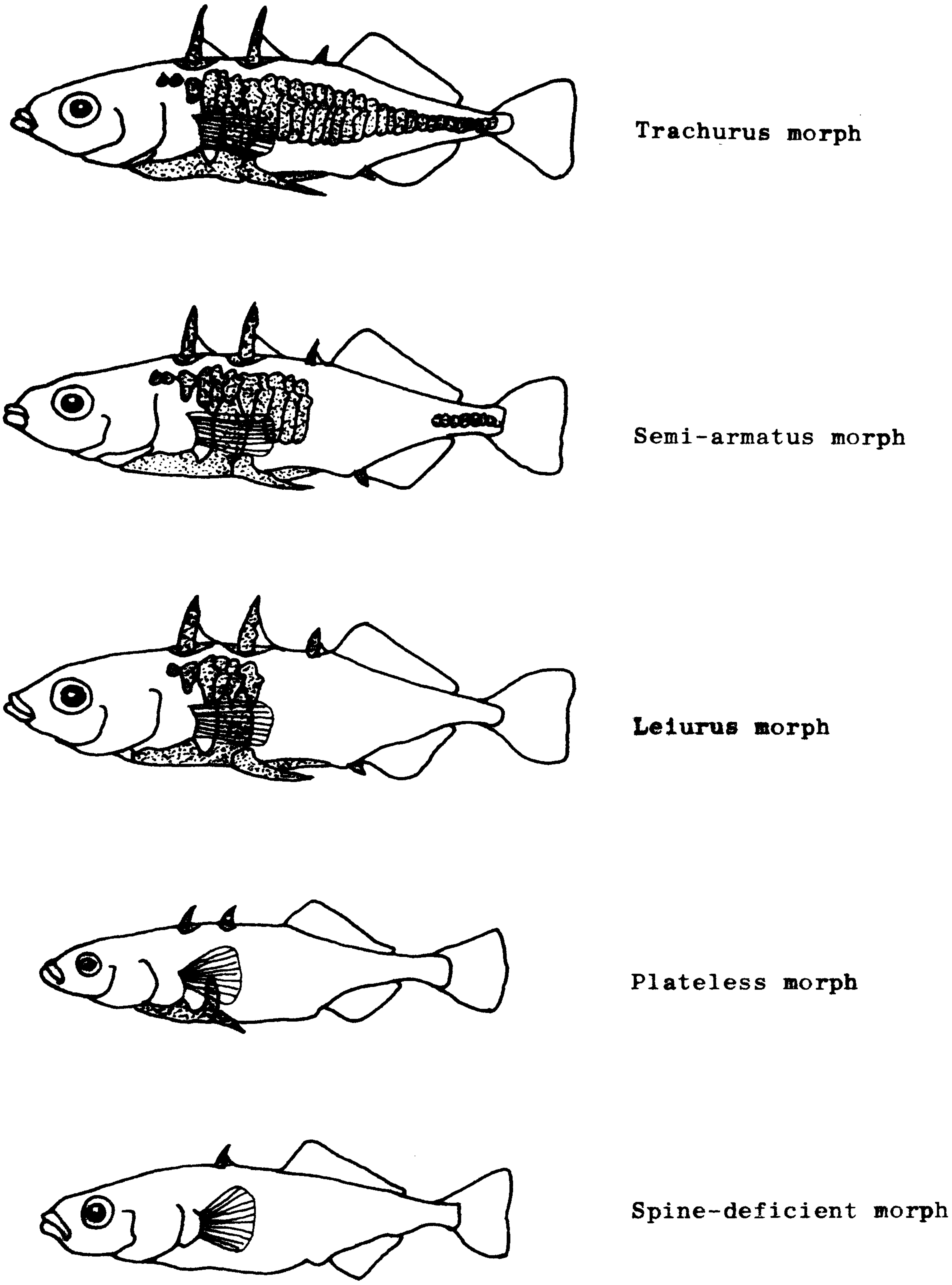


Fig. 4. Phenotypic variability in <u>Gasterosteus</u>

(Newfoundland to Massachusetts). Bertin (1925) studied morphological variability in Gasterosteus populations of French lowland freshwaters and suggested that the distribution and number of lateral bony plates could serve as a basis for sub-dividing G. aculeatus into four forms: Hologymna (no lateral plates), Gymnura (low number of anterior plates), Semiarmata (anterior plates with a caudal keel of plates), and Trachura (complete row of lateral plates from anterior to posterior of body). Muntzing (1963) used the terms Trachurus and Semiarmatus to describe forms equivalent to those of Bertin, and the term Leiurus to describe the form with low numbers of anterior lateral plates. In general, Leiurus sticklebacks occur most commonly in freshwater habitats and Trachurus sticklebacks in marine habitats but situations have been described where Leiurus, Semiarmatus, and Trachurus forms occur in estuarine, freshwater, and marine habitats (Penczak (1965), Hagen and McPhail (1970), Gross (1978), Maclean (1974), Muntzing (1963)). Trachurus sticklebacks are commonly anadromous, migrating to sea during their first winter of life, and returning to freshwater streams to spawn the following spring (e.g. Hagen (1967), Van Mullem and Van der Vlugt (1964)).

Gasterosteus populations, polymorphic for the three major morphs, occur very frequently throughout the range of the species and Muntzing (1963) has proposed two hypotheses to explain the observed variability of plate number within and between European populations:

1. that Leiurus and Trachurus are morphs of the polymorphic species G. aculeatus L and that natural selection is maintaining the observed population variability.
2. that Leiurus and Trachurus were geographically isolated during the last Pleistocene glaciation and have subsequently mixed and interbred giving rise to a complex of hybrid forms of the two morphs.

Miller and Hubbs (1969) working on *Gasterosteus* populations along the Pacific Coast of North America favour the second of Muntzing's hypotheses and recommended sub-specific status for the Trachurus, Semiarmatus, and Leiurus forms. Hagen (1967) studying *Gasterosteus* in the Little Campbell river (British Columbia) demonstrated clear ecological barriers to hybridisation between sympatric freshwater Leiurus and anadromous Trachurus sticklebacks and described a narrow hybrid zone where selection appeared to mediate strongly against hybrid survival. Using this and morphological evidence gathered from allopatric Leiurus and Trachurus populations Hagen proposed specific status for the Leiurus and Trachurus morphs. Hay and McPhail (1975) studying mate selection in *Gasterosteus* under laboratory conditions have demonstrated that in choice situations mature males and females of the Leiurus and Trachurus morphs are most likely to breed with individuals of their own morph rather than with dissimilar phenotypes. Gross (1978b) refutes environmental induction as a major factor in the evolution of meristic variability (lateral plate, vertebral, fin ray, pterygiophore numbers), favouring selective gradients in biological factors such as "stress" and predator distribution as causative factors for the observed patterns of distribution.

The first of Muntzing's hypotheses (that natural selection maintains the observed phenotypic variability) has gained increasing support in recent years with many studies elucidating the role of differing selection pressures on stickleback morphology. Heuts (1946, 1947) demonstrated that egg batches of Trachurus, Semiarmatus and Leiurus sticklebacks had markedly differing survival rates in media of differing salinity; and that a tendency for a North-South clinal trend in lateral plate number occurred indicating that temperature may also play a role in the frequency of occurrence of the different phenotypes. Hagen and McPhail (1970) strongly argue the case for environmental selection versus intergradation following introgression (Miller and Hubbs (1969)) as an explanation for the observed morphological variability of North American Pacific coast _Gasterosteus_ populations.

The nomenclature Trachurus, Semiarmatus, Leiurus is used in favour of the corresponding terms low-plated, partially-plated, and fully-plated morph advocated for North-West American freshwater _Gasterosteus_ populations by Hagen and Gilbertson (1972). The plateless morph is homologous to Bertin's Hologymna form, and to the South Californian sub-species _Gasterosteus aculeatus williamsoni_ recognised by American workers (e.g. Miller and Hubbs 1969).

Selective predation as a factor favouring certain _Gasterosteus_ phenotypes has been documented by Lea (1968); Moodie (1972); Larson (1972); Hagen and Gilbertson (1972) (1973); Moodie, McPhail and Hagen (1973); Hay (1974); Bell and Haglund (1978); and Reist (1980 a, b). Each of these studies is discussed under the relevant morphological character in this chapter and/or in chapter 5 (Behavioural Studies).

The widespread phenotypic variability of _Gasterosteus_ has prompted a proliferation of descriptive papers including the works of Penczak (1960) (1962 a, b, c) (1964); Bacescu and Mayer (1956); Muntzing (1962) (1972); Narver (1969); Garside and Hamor (1973); Coad and Power (1974 a, b); and Kynard and Curry (1976). Penczak (1960) in his review of Polish populations noted that every water body studied contained its own characteristic stickleback population. The available evidence indicates that freshwater _Gasterosteus_ populations are extremely responsive to variations in environmental selection pressures leading to a wide continuous range of adaptive extant morphs. At its most extreme this process has led to the repeated but rare occurrence in the fossil record and in present day

Gasterosteus populations of forms which lack one, two or all three dorsal spines, both ventral spines, all lateral plates, and even the complete pelvic girdle assembly (Larson (1972), Moodie and Reimchen (1973), Bell (1976)). The reduction and loss of the pelvic girdle and spines has also been reported in the 10-spined stickleback, Pygosteus pungitius, Nelson (1971), Coad (1973), and the brook ~~four-spined~~ stickleback Culaea inconstans, Nelson and Atton (1971), Nelson (1977).

The "spine-deficient morph" which has been recorded from 8 separate water bodies on the Isle of North Uist during the present study was probably first discovered by Harvie-Brown and Buckley (1888) who described a short-spined stickleback, Gasterosteus brachycentrus from the island, and re-discovered by R.N.B. Campbell in 1976 during the field work phase of his study on trout and char diet in Loch Fada (Campbell 1976). A full description of the morphology of sticklebacks from each of the study populations is given under the relevant sections of the present chapter.

Variation in morphological and meristic characters may be attributable to genetic or environmental effects or to a combination of these factors. Boag and Grant (1978) have criticised the more or less interchangeable usage of the terms genotype and phenotype in studies of avian morphology (where heritabilities (in the "broad" sense) are assumed to be equal to unity). Evidence exists for the genetic basis of several external morphological and meristic characters in Gasterosteus, forming a strong platform from which to seek evolutionary explanations for observed phenotypic variation within and between natural populations.

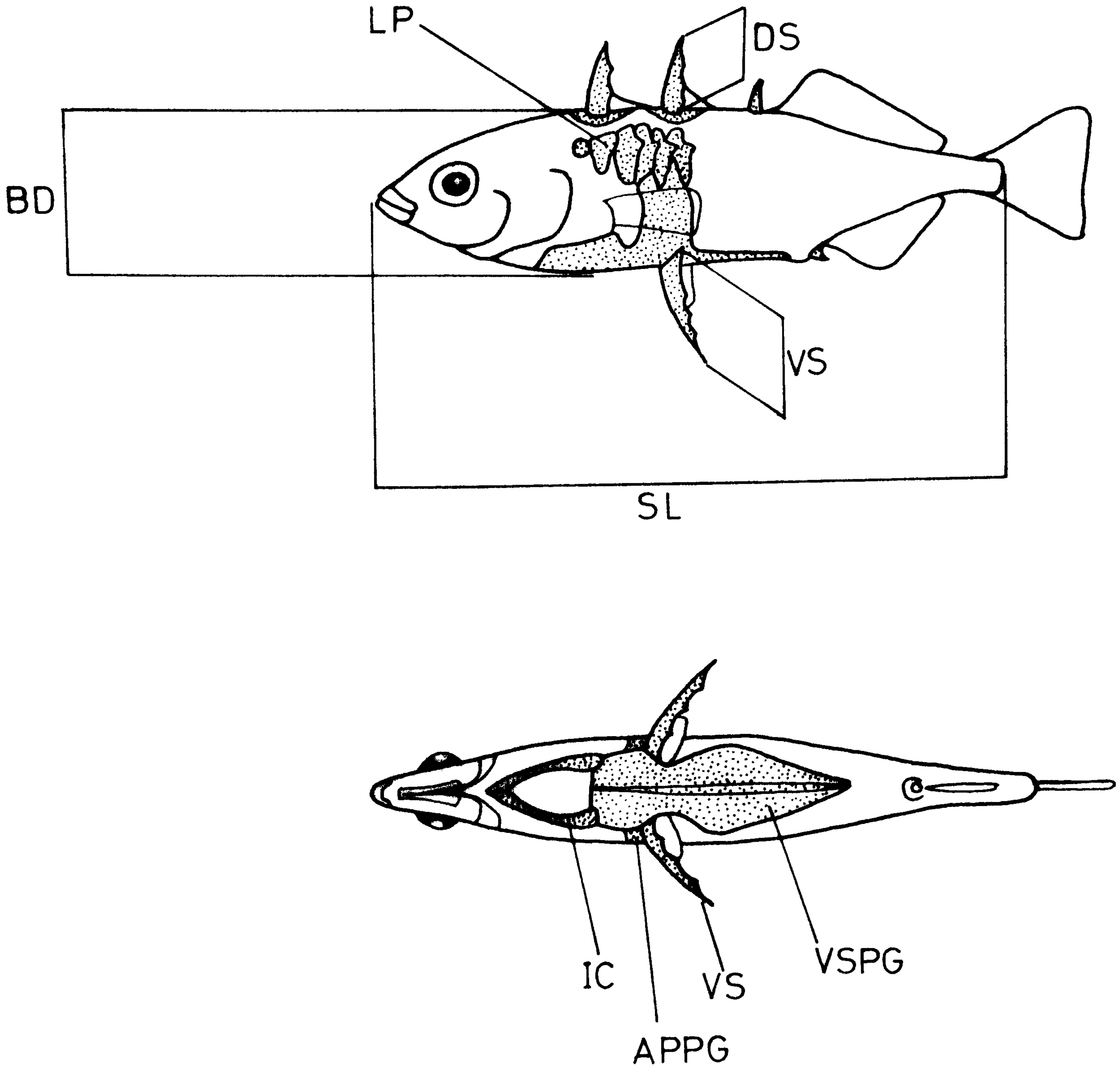
Hagen (1967) bred and reared Leiurus, Trachurus, and their hybrids in the laboratory finding that the following characters were genetically controlled: Lateral plate number; gill raker number; body shape (body depth:standard length). Other characters which were not quantified but which appeared to be genetically controlled were the size of the dorsal and ventral spines and the pattern of the cryptic bodily colouration in Leiurus fish. Hagen (1973) investigated the heritability (in the "narrow" sense; the ratio of additive genetic variance to total phenotypic variance) of lateral plate number and gill raker number in the Lake Wapato _Gasterosteus_ population. Hagen found that both characters had high heritabilities (0.5-0.84 for lateral plates, and 0.58 for gill rakers). There were no sexual differences in the inheritance of these characters. Avise (1976) has drawn attention to the fact that there may be contrasting genetic bases for plate number determination in different _Gasterosteus_ populations after demonstrating significantly different patterns of plate development in intralocality versus interlocality crosses. Lindsey (1962) notes that the lateral plate number in _Gasterosteus_ is inherited largely maternally. Hagen and Gilbertson (1973) studied the genetics of plate morphs in Lake Wapato sticklebacks concluding from the results obtained that segregation and dominance between morphs could not be explained by two or three alleles at one locus and that therefore a complex situation involving at least two unlinked loci, each with two alleles was involved. Hay (1974) found that a particular lateral plate (designated "I") positioned above the shield of the pelvic girdle in _Gasterosteus_ was under genetic control with all semiarmatus fish possessing the plate and all Leiurus fish lacking it. Some evidence for the genetic control of the presence or absence of the pelvic girdle in _Gasterosteus_ has been obtained by Campbell (1979) who crossed male Leiurus and Trachurus sticklebacks with female spine-deficient morph sticklebacks (from North Uist), all of the offspring from both crosses lacked lateral plates and c. 33% lacked a pelvic girdle. Leiurus x spine-deficient offspring back-crossed with a Trachurus male produced all spine-deficient offspring (lacking pelvic girdles) indicating that spine-deficiency is a dominant characteristic (Campbell 1979). Nelson (1977) has shown that the similar reduction in defensive structures shown by certain populations of *Culaea inconstans* is under partial genetic control. Nelson (1977) infers that variation in predation pressure is likely to be the most important selective agency involved in reduction and loss of the pelvic girdle. Nelson (1971) believes that individuals of *G. aculeatus*, *P. pungitius* and *C. inconstans* which lack pelvic skeletons were almost certainly derived from populations with full pelvic girdles. Larson's (1972) study of *Gasterosteus* in the small coastal Paxton Lake (British Columbia) has revealed the apparently unique situation of a spatial and trophic segregation of two *Gasterosteus* phenotypes ("Limnetics" and "Benthics") within an isolated still water, where the Limnetic form is normally spined with a full pelvic skeleton, but the Benthic form lacks pelvic spines, pelvic girdle, and lateral bony plates. Laboratory crosses of Limnetics x Benthics from Paxton lake would yield interesting information on the genetic basis of reduction in bodily armament in *Gasterosteus*.

4.2 Morphological and Meristic characters studied

The characters defined below were chosen for study because of their relevance to the existing literature on *Gasterosteus* morphology.

The system of measurement used during this study is similar to that of Hubbs and Lagler (1958) and is represented in Fig. 5. All samples used for analysis were stored in 70% ethanol under refrigeration. Measurements were taken from the fish using finely pointed dividers and then transferred to vernier calipers and determined to the nearest 0.1 mm.; repeated measurements made upon a small sample of fish showed the method to be entirely accurate and repeatable to within 0.1 mm. Measurements and counts were defined as follows:

Dorsal spine length - distance from the tip of the second dorsal spine



LP = Lateral Plate
DS = Dorsal Spine
VS = Ventral Spine
BD = Body Depth
SL = Standard Length

IC = Interclavicle
VSPG = Ventral Shield of
Pelvic Girdle
APPG = Ascending process of
Pelvic Girdle

Fig. 5. Morphological characters used during study

to the surface of the underlying pterygiophore.

Ventral spine length - distance from the tip of the ~~second dorsal~~ left ventral spine to the surface of the underlying pelvic girdle.

Standard body length - distance from the tip of the snout to the base of the caudal peduncle.

Body depth - distance from the base of the first dorsal spine (if present) to the anterior surface of the ventral shield of the pelvic girdle (if present).

Lateral plate number - the number of bony dermal plates on each side of the fish, counted and recorded separately.

Gill raker number - the number of gill rakers (including rudiments) on the first gill arch of the left side of the body.

Spine shape and serrations - notes were made on the thickness, and presence or absence of serrations on the dorsal and ventral spines.

Pelvic girdle - notes were made on the presence, degree of reduction, or absence of the pelvic girdle in samples from the Loch Fada and Loch a Bharpa populations. A small sample of fish were X-rayed (page 118) and others were probed with a sharp seeker in order to establish the presence of such bones.

Note - in the following sections of this chapter figures using the symbol below are included; the vertical line denotes the mean value for the character described, the horizontal line is equal to two standard errors either side of the mean value and the solid horizontal bar is equal to one standard deviation either side of the mean value (on the x axis of the figure)

4.3.1 Adult Body Size

Results

The smallest freshwater Gasterosteus reported to date were measured during the present study and are the spine-deficient, plateless fish from Loch a Bharpa (North Uist) which reach a mean mature adult standard length of 29.3 mm (♀) and 25.3 mm (♂).

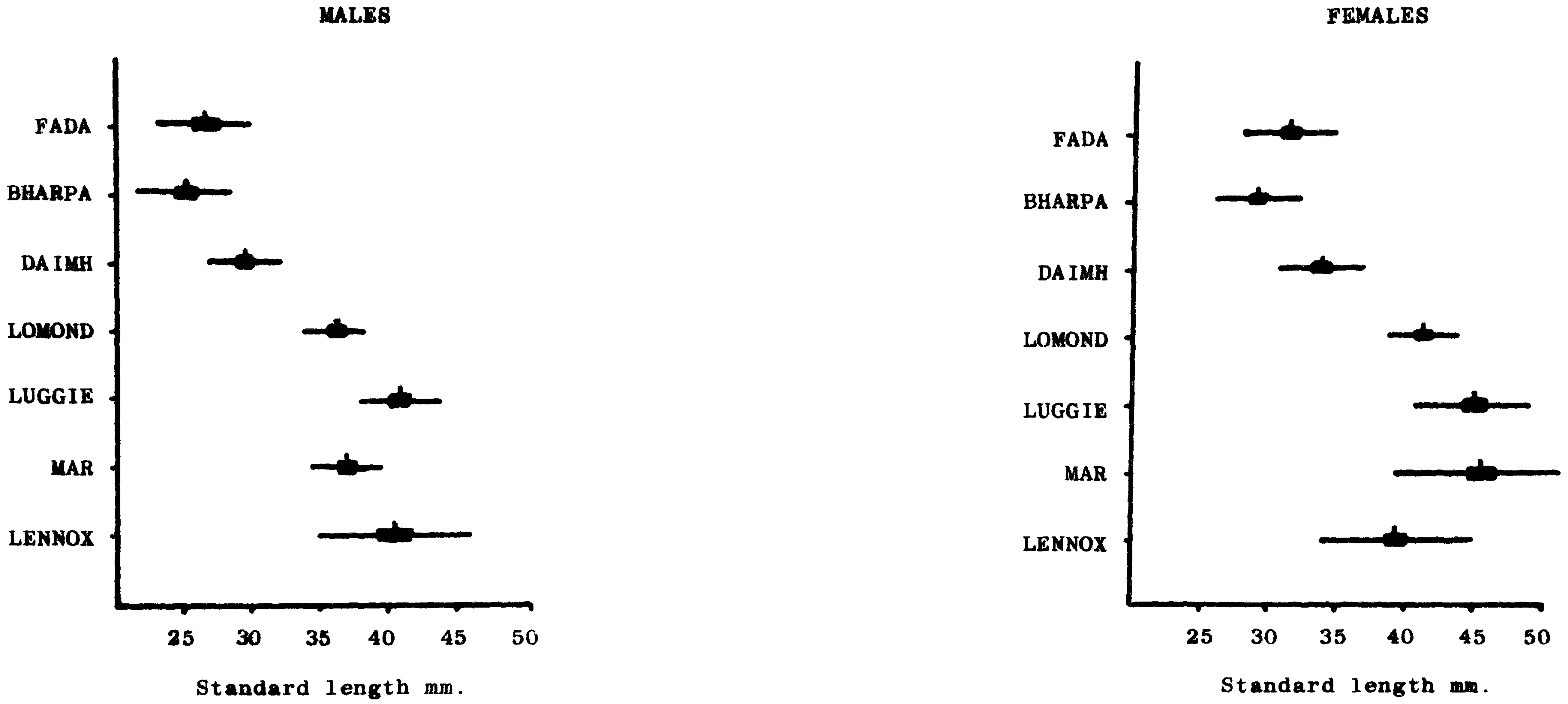
Table 9 summarises an analysis of mean adult standard length for each of the seven study populations; these data are visually represented in Fig. 6. All comparisons drawn below were made using 2-tailed t tests, sample sizes are given in the table. Body length varied considerably within and between sexes and populations; in all populations except Lennox Castle adult females are significantly larger than adult males (Table 9). The reason for the lack of this sexual difference in the Lennox Castle population may be the high incidence of Schistocephalus solidus parasitism which is known to present a considerable energy drain on the body of the stickleback host and to adversely affect the stickleback's growth rate (Arme and Owen 1967).

Mature male and female sticklebacks from North Uist populations (Fada, Bharpa, Daimh) were significantly smaller than those sampled from mainland populations ($p < .0\ 1$ in all cases). There was no significant difference in size between Fada and Bharpa males (at the 5% level) but Fada females were significantly larger than Bharpa females ($p < .01$), and both Daimh males and females were significantly larger than Fada males and females ($p = 7.5$ E - 4 and $p = 2.7$ E - 3 respectively). Between the mainland populations there was no significant difference in size for Mar and Lomond males ($p = .16$) but Mar females were significantly larger than Lomond males and females ($p = 3.8$ E - 7 and $p = 7.6$ E - 5 respectively). Luggie females were also significantly larger than Lennox females ($p = 2.0$ E - 5), but there was no

Table 9. Adult body length

	N	$\bar{x}$ Standard length mm.	2-tailed t test
Fada ♂	75	26.7	p = 1.71 E - 5
Fada ♀	100	31.5	
Bharpa ♂	75	25.3	p = 1.2 E - 4
Bharpa ♀	75	29.3	
Daimh ♂	100	29.5	p = 4.7 E - 7
Daimh ♀	75	34.0	
Lomond ♂	100	36.2	p = 5.1 E - 8
Lomond ♀	75	41.36	
Luggie ♂	75	40.86	p = 3.2 E - 5
Luggie ♀	100	45.1	
Mar ♂	75	37.4	p = 2.5 E - 6
Mar ♀	75	45.6	
Lennox ♂	75	40.4	no significant difference
Lennox ♀	75	39.6	

Fig. 6.



ADULT BODY LENGTH

significant size difference between Luggie and Lennox males (p > .05).

4.3.2 Discussion

Miller (1979) has discussed the adaptiveness of small body size in teleost fish species stressing the importance of the trophic niche of the species in relation to habitat food resources. *Gasterosteus* is a small fish by comparison with the majority of freshwater teleost species but shows very considerable inter-population differences in maximal body size throughout its geographical range. The largest freshwater *Gasterosteus* reported are the so-called "Black sticklebacks" from Mayer Lake (Queen Charlotte Islands) which reach a maximum recorded size of 116 mm standard length and a mean mature adult standard length of 89.44 mm (♀) and 80.24 mm (♂), (Moodie 1972). Large body size in female *Gasterosteus* is likely to be an adaptation to increase reproductive success as large females produce more eggs at each spawning and complete more reproductive cycles in a breeding season than small females (Wootton 1973). Rohwer (1978) has suggested that small body size in male *Gasterosteus* may be a reproductive adaptation whereby smaller males will canabalise fewer developing eggs from their nests in order to survive, and may therefore be favoured by prospective spawning females because of the lower risk of egg loss in nests attended by small males. McPhail (1977) surveying 25 lacustrine *Gasterosteus* populations has hypothesised that predation by Brook trout may lead to inherited adaptive local population responses either towards large or small body size in female sticklebacks at the time of first reproduction. Moodie (1977) has proposed that small body size may also be an adaptation shown by predated *Culaea inconstans* populations in Southern Manitoba, Canada. Size-selective predation upon *Gasterosteus* where large individuals are at high risk has been documented for Grey Herons (Owen 1960) and this study (page 221) and for Arctic Terns (Lemmetyinen 1973). Size-selective predation upon small Gasterosteus individuals has been documented for Cut-throat trout and Water scorpions (Hay 1974), Alpine Char (Krogius, Krokhin and Menshutkin 1970), Prickly Sculpin (Moodie 1972 b) and Perch (this study page 57). Sticklebacks are also known to become more important in the diet of certain predators only after the predators themselves have reached a sufficient body size to cope with the stickleback's erect dorsal and ventral spines (critical dimension), e.g. Pike (Copland (1956), Frost (1954)) and Perch (Antosiak (1963), Allen (1935), McCormack (1970) and this study (page 57)). The importance of the relative size of the dorsal and ventral spines in Gasterosteus is discussed later in this chapter (page 104).

4.4.1 Adult Body Shape

Results

In their major review of the factors affecting Gasterosteus morphology in freshwater habitats of the Pacific coast of north west America Hagen and Gilbertson (1972) used the ratio of Body length: Body depth as a single measure of body shape. A high value for this ratio indicates a slender body shape, a low value indicates a dumpy body shape. Table 10 gives the mean Body length:Body depth ratio for 100 male fish (large fry and adults) from each of the study populations, comparisons were drawn between populations using 2-tailed Mann-Whitney U tests; these data are visually represented in Fig. 7.. The Body length:Body depth ratio showed a very low variability within populations but differed considerably between populations (Fig. 7).

The data obtained show that the populations with the higher ratio value are those originating in still waters (Fada, Bharpa, Daimh and Lomond) and the low ratio values in running waters (Luggie

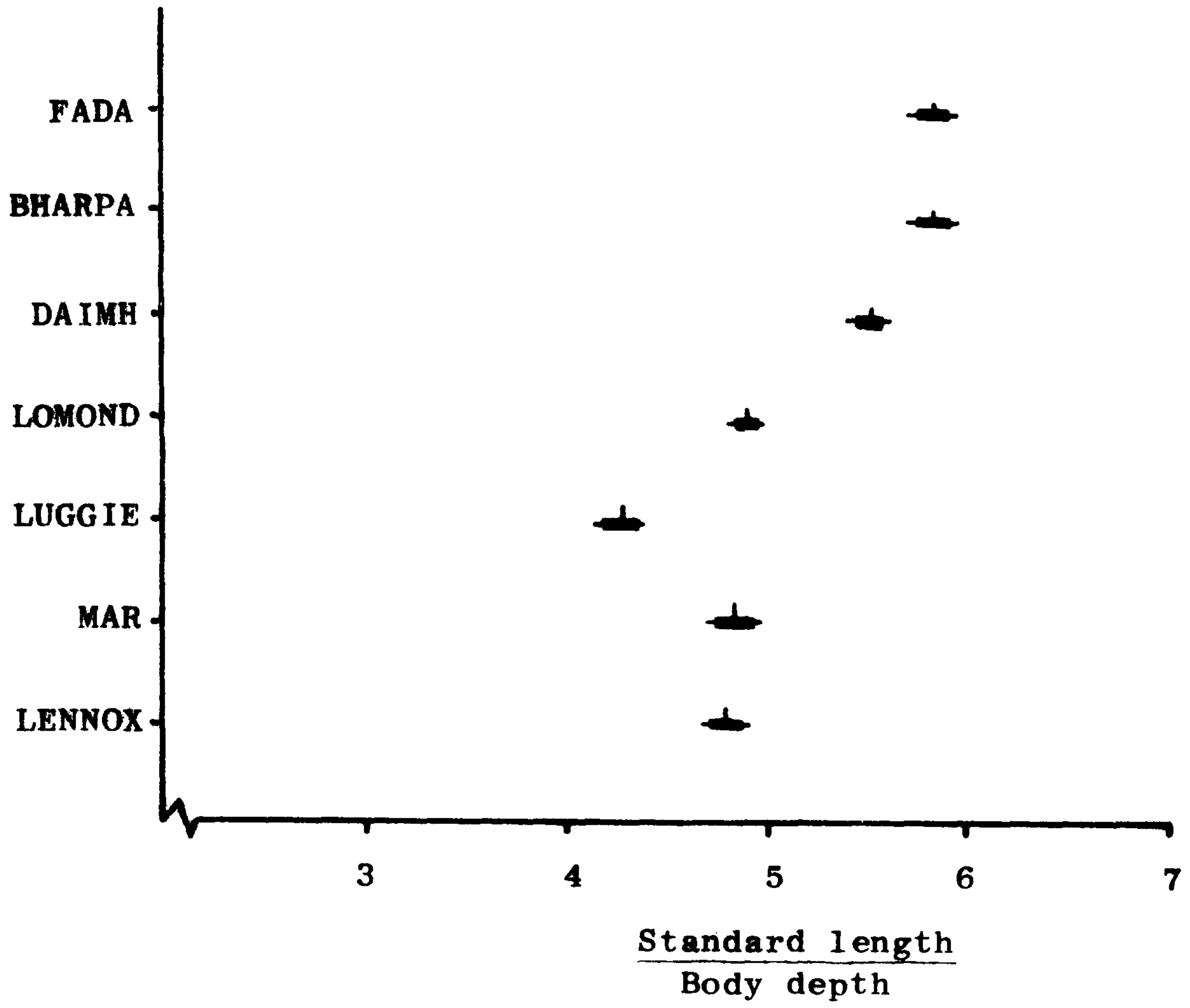


Fig. 7. Body shape in adult male *Gasterosteus*

Table 10. Adult male body shape

	N	$\bar{x}\ \frac{\text{Standard length}}{\text{Body depth}}$ ratio	2-tailed Mann-Whitney U test
Fada	100	5.8	
Bharpa	100	5.8	
Daimh	100	5.65	$p < .002$
Lomond	100	4.78	
Luggie	100	4.22	$p < .002$
Mar	100	4.71	
Lennox	100	4.64	

and Mar). The apparently anomalous low value of the Lennox Castle population is explained by bodily distortion caused by coelomic infestations of the parasite Schistocephalus solidus. The North Uist populations form a distinct group of slender shaped fish which are significantly differently shaped from Loch Lomond fish, the most similarly shaped of the mainland populations ($p < .002$). There was no significant difference at the 5% level between Lomond and Mar fish, but Luggie fish were significantly dumpier than Mar fish ($p < .002$). Body shape in fish is known to be a size-dependent characteristic, early growth phases having a larger length than depth component. Because of the size difference at maturity between the study populations it is possible that the shape differences described above are due to the relatively small size of the North Uist fish and large size of the mainland lotic populations. Table 11 presents a regression analysis of body shape:standard length for male fish from the study populations facilitating a cross-population comparison of body shape at an arbitrary standard body size ($Y = 30$ mm). An inspection of the regression analysis table reveals a similar pattern to that given in table 10 with the lowest body shape ratio values occurring in Mar and Luggie males and the highest in the lentic Uist populations.

4.4.2 Discussion

The data described above agree with that of Hagen and Gilbertson (1972) with lentic populations being slender and lotic populations dumpy.

The biological significance of the basic shape difference between lotic and lentic Gasterosteus populations is not understood. Intuitively it would be thought that a streamlined shape would be beneficial to a fish in a running water environment, but in fact the converse situation is seen to occur. The regression analysis of the

Table 11. Regression analysis of $\frac{\text{Standard length}}{\text{Body depth}}$: standard length in male Gasterosteus

	Slope	S.E. Slope	Intercept	S.E. Intercept	Y30 value
Fada	-.016	.011	6.3	.301	5.82
Bharpa	-.0118	.008	6.15	.216	5.79
Daimh	-.008	.01	5.84	.312	5.6
Lomond	-.027	.007	5.8	.241	4.99
Luggie	-.01	.004	4.82	.133	4.52
Mar	-.02	.005	5.35	.177	4.75
Lennox	-.03	.004	5.73	.161	4.83

body shape ratio:Standard body length shows that the slender shape of the North Uist fish relative to the mainland fish is not simply a function of their smaller body size. Further research is being conducted upon this subject with at least one paper in preparation (Gross 1978). Bell (1974) has speculated that streamlining may be an adaptation in predator avoidance, allowing a faster swimming speed, and has mentioned that this may be linked with the reduction in pelvic girdle anatomy seen in some *Gasterosteus* populations. The data from the present study are insufficient to test this theory since only the River Luggie fish experience a very low risk of predation. Hagen (1967) has reported that body shape in *Gasterosteus* is under genetic control.

4.5.1 Gill Raker Number

Results

Gill rakers were counted in 200 sticklebacks (adults and large fry) from each of the study populations; these data are summarised in table 12 and Fig. 8. Gill raker number was very variable in all seven of the study populations and no biological significance can be attributed to the differences seen in table 12. The size and shape of the gill rakers did however differ markedly between populations with those of Mar burn and Luggie fish being shorter and stubbier than those from the five lentic populations.

4.5.2 Discussion

A relationship between diet and gill raker number and shape in *Gasterosteus* has been described by Hagen (1967) and Hagen and Gilbertson (1972, 1973), where lentic populations existing on a primarily zooplanktonic diet tend to have more numerous and longer, thinner gill rakers than those from lotic populations eating macroinvertebrate prey. Long fine gill rakers protrude across the buccal

Table 12. Gill raker number

	N	$\bar{x}$ Gill raker number	s.d.	Range
Fada	200	14.9	1.4	9 - 16
Bharpa	200	13.5	1.5	9 - 16
Daimh	200	14.1	1.6	12 - 18
Lomond	200	13.1	1.2	10 - 16
Luggie	200	12.6	1.5	10 - 16
Mar	200	13.6	1.4	9 - 16
Lennox	200	14.2	1.3	11 - 18

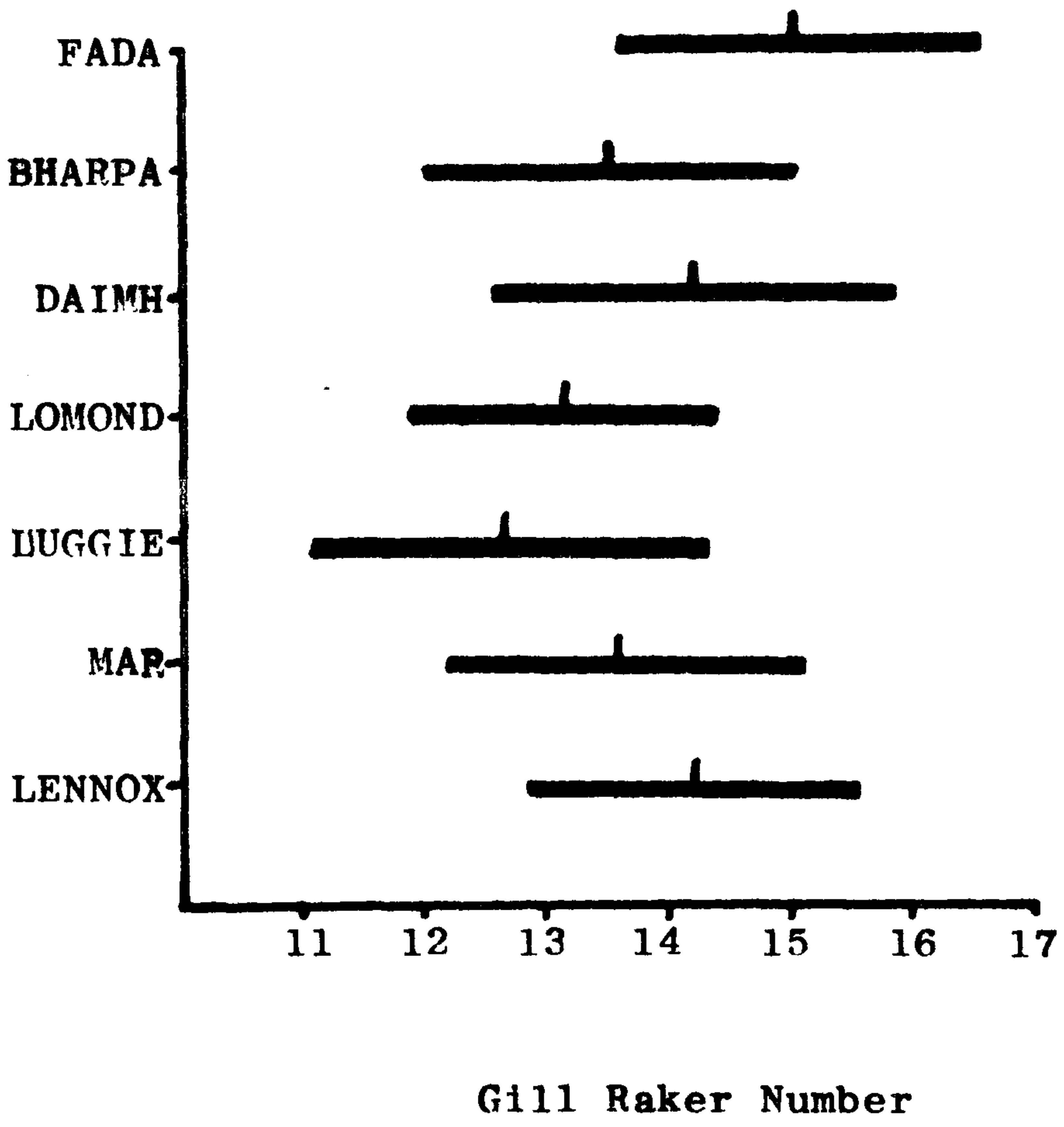

FADA
BHARPA
DAIMH
LOMOND
DUGGIE
MAR
LENNOX
11
12
13
14
15
16
17
Gill Raker Number

Fig. 8.

cavity to a greater extent than short stubby structures and may be more efficient at filtering out small planktonic organisms which form the bulk of the prey of lake dwelling and marine Gasterosteus. The Leiurus fish from the Little Campbell river (Hagen 1967) which ate a predominantly benthic invertebrate diet had a mean gill raker number of 16.4 compared with a value of 21.8 in Trachurus fish; the seven populations in the present study fell within the range exhibited by Canadian Leiurus fish.

4.6.1 Lateral Plate Number

Gasterosteus is unique amongst teleosts in having a double centre of scale formation. In Trachurus fish the anterior plates develop first, and are followed by the caudal plates which originate at a second locus, both groups then growing toward each other until they link (Igarishi 1964). Lateral plates (often termed scutes) in Gasterosteus have no proven function and are very variable in number, have a high heritability (Hagen 1973) and differ in structure between Trachurus and Leiurus fish. Plates from land-locked fish have numerous surface papillae which are absent from the plates of marine fish (Igarishi 1970). Campbell (1979) has noted that the lateral line innervating nerves are split in Trachurus sticklebacks, running either side of the caudal keel of lateral plates. In Leiurus fish where the caudal keel is absent the nerve is a single structure. Penczak (1961) has noted that damaged lateral plates in Gasterosteus are regenerated until a complete repair has been achieved.

4.6.2 Results

Table 13 and Fig. 9 summarise an analysis of left side lateral plate number from 200 adult Gasterosteus from each of the study sites. Loch Fada and Loch a Bharpa fish are 100% lateral plate deficient

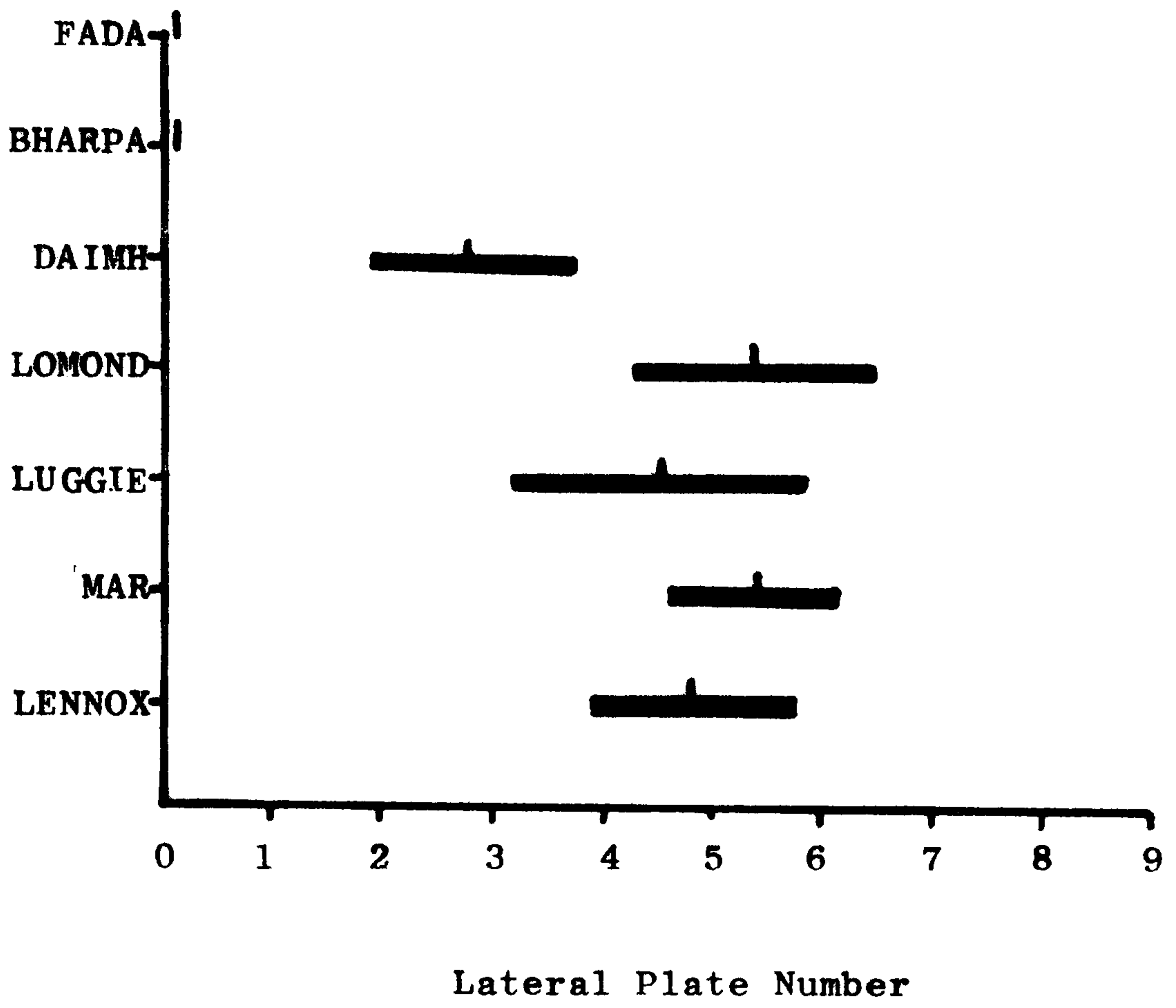

FADA
BHARPA
DAIMH
LOMOND
LUGGIE
MAR
LENNOX
0
1
2
3
4
5
6
7
8
9
Lateral Plate Number

Fig. 9.

Table 13. Lateral Plate number on left side of fish, and lateral plate symmetry

	N	$\bar{x}$	s.d.	Range	Symmetry
Fada	200	0	0	0	100%
Bharpa	200	0	0	0	100%
Daimh	200	2.7	0.9	0-5	56.7%
Lomond	200	5.4	1.1	3-9	63.6%
Luggie	200	4.5	1.3	2-9	46%
Mar	200	5.4	0.7	3-8	56%
Lennox	200	4.8	0.8	2-9	55.1%

whilst fish from the other five populations showed a high degree of variability in lateral plate number. From 18 freshwater Gasterosteus populations surveyed on the Isle of North Uist during the present study a pattern of typically low (0-5) lateral plate number emerges. Mainland populations typically have counts of 2-9 for Leiurus fish and 12-19 for Semiarmatus fish (including the plates of the caudal keel). Table 14 describes the frequency of occurrence of the lateral plate morphs recognised during the study, Fada and Bharpa being monomorphic for plateless fish; Lennox monomorphic for Leiurus; Daimh polymorphic for Leiurus and plateless fish; and Lomond, Mar and Luggie polymorphic for Leiurus and Semiarmatus fish. During the morphological analysis lateral plate numbers were counted on both sides of the fish and compared for symmetry, the result of this analysis is given in Table 13. The significance of lateral plate asymmetry in Gasterosteus is unknown but it can be seen that in all but the plateless Fada and Bharpa populations the chance of a Leiurus fish being asymmetrical for lateral plate count is very high (up to 54% amongst Luggie fish).

4.6.3 Discussion

Gross (1978) has discovered a north-south distributional arc for lateral plate number in European Leiurus populations, with fish from both extreme northern and extreme southern sites tending to have low plate counts including populations with high numbers of individuals with no lateral plates. Gross related the occurrence of the distributional arc to the presence or absence of certain predatory fish species (Pike and Perch) known to predate sticklebacks citing central European Gasterosteus populations where plate counts were, on average, highest along the arc and where Pike and Perch commonly occur. Within the British Isles the standard deviation of the mean lateral plate number was larger in extreme northern Scottish populations

Table 14. Percentage Frequency of Occurrence of Lateral plate morphs

	N	Leiurus	Semi armatus	Plateless
Fada	250	-	-	100%
Bharpa	250	-	-	100%
Daimh	368	98.65%	-	1.35%
Lomond	315	98.74%	1.26%	-
Luggie	464	95.5%	4.5%	-
Mar	474	99.58%	0.42%	-
Lennox	410	100%	-	-

(Pike and Perch absent) and smaller in southern populations where these predatory fish occur commonly; Salmonid species were thought to be unimportant predators in British waters (Gross 1978). A literature review of Trout and Char diet in Britain (page 49) reveals however that Gasterosteus is heavily predated by Salmonids in northern waters (Campbell, R.N. (1971), Campbell, R.N.B. (1976), Hunt and Jones (1972), Adalsteinsson (1976)) indicating that salmonid predation pressure in many northern Scottish sites is likely to be equivalent to Pike and Perch predation pressure in more southerly British populations. Contrary to the results of Gross (1978) from British populations, during the present study variability in lateral plate number was found to be unrelated to risk of predation by fish predators. Loch Lomond fish had highly variable plate counts (s.d. = 1.1) and are known to be predated by Perch and Pike; Luggie fish with a high variability (s.d. = 1.3) are at a low risk from fish predators; Loch Fada fish (heavily predated by Brown Trout) are 100% lateral plate deficient. Mean lateral plate number also bore no relation to predation risk either by piscivorous birds or fish, e.g. Loch Lomond ($\bar{x}$ plate number 5.4, high risk); Loch Fada ($\bar{x}$ plate number zero, high risk); Luggie ($\bar{x}$ plate number 4.5, low risk); Lennox ($\bar{x}$ plate number 4.8, high risk (Gulls)). If the mainland populations are considered alone both Lomond and Mar fish have on average more lateral plates than Lennox or Luggie fish but the significance of this difference is by no means obvious in terms of relative risk of predation.

Salmonid predators are strongly implicated as a causative factor in selection for certain lateral plate phenotypes within land-locked populations of Gasterosteus on the Pacific northwest coast of America, a theory initially propounded by Hagen and McPhail (1970) and firmly substantiated by Hagen and Gilbertson (1972). Hagen and Gilbertson studied 53 permanent freshwater Gasterosteus populations and found a mode of 7/7 plated Leiurus and Semiarmatus fish in those populations sympatric with predatory species (Squawfish, Cut-throat Trout, Rainbow Trout, Arctic Char, Dolly Varden Char, Pike), with large fluctuations in lateral plate number between populations, and low numbers of 7/7 in non-predated sites. 7/7 plated sticklebacks appeared therefore to be at a distinct advantage over other phenotypes in the face of predation by piscivorous fish species. Verification of this selective advantage was published by Hagen and Gilbertson (1973) working at Lake Wapato and Lake Chelan and studying predation upon endemic Gasterosteus by introduced Rainbow Trout (S. gairdneri); over 3 years 7/7 sticklebacks appeared consistently less often in trout stomachs than would be expected from their frequency of occurrence within the population. Sticklebacks with lower numbers of lateral plates and fish with 9 lateral plates on each side of the body were at a severe disadvantage, Simultaneously, over the three year period the proportion of 7/7 plated fish within the population rose from 56% (1968) to 65% (1969), coinciding with the peak of predation by Rainbow Trout (Hagen and Gilbertson 1973). Hagen and Gilbertson's study represents the best evidence so far obtained that natural selection by fish predators is acting to determine lateral plate phenotype frequencies in a wild Gasterosteus population. Moodie, McPhail and Hagen (1973) then studied the survival of sticklebacks of various lateral plate phenotypes and from various populations in a laboratory situation with two captive predators: Northern Squawfish and Cut-throat Trout. 7/7 fish were taken significantly less often by both Trout and Squawfish than would be predicted from their frequency in the experimental groups, but data on the anti-predator behaviour of the different phenotypes was conflicting and is discussed fully in chapter 5.

Lea (1968) has demonstrated that the full lateral plating of Trachurus sticklebacks puts them at a selective advantage over Leiurus fish by virtue of their greater mechanical strength when being predated under laboratory conditions by Northern Squawfish.

Moodie (1972) studying the Mayer lake *Gasterosteus* population found that amongst the large melanic phenotype present in the lake 8 plated (left hand side) fish occurred significantly more frequently in Cut-throat Trout stomachs than would be predicted from their relative occurrence within the population, again 7/7 plated fish showed a mode within this population. Bell and Haglund (1978) used garter snakes *Thamnophis couchi hammondi* in the laboratory to study the relative survival of differing *Gasterosteus* lateral plate phenotypes in non-reproductive condition (phenotypes were described by summing the lateral plate number from each side of the fish); fish with 5 and 7-10 plates had a higher survival rate than fish with 1, 3, 4, 11 and 12 lateral plates. Asymmetry of lateral plate number made no difference to the likelihood of survival of an individual fish.

4.7.1 Spines

Results

During the present study the length of the second dorsal spine, and of the left ventral spine, and the number of spines present was measured and counted on a total of 2723 *Gasterosteus* from the seven study populations. Table 15 presents the percentage frequency of occurrence of dorsal and ventral spines in the seven populations. All four of the mainland populations have the normal complement of 3 dorsal and 2 ventral spines, a single individual fish from the River Luggie possessed 4 dorsal spines. Broken dorsal and ventral spines were recorded from 8 adult Lomond fish and 6 adult Mar fish

Table 15. Percentage frequency of occurrence of dorsal and ventral spines

	N	Dorsal spines (3)	Dorsal spines (2)	Dorsal spines (1)	Ventral spines (2)
Fada	339	3.5%	23.5%	73%	0.29%
Bharpa	350	15%	84%	3%	3.14%
Daimh	383	98.2%	1.8%	-	97.4%
Lomond	315	100%	-	-	100%
Luggie	486	100%	-	-	100%
Mar	439	100%	-	-	100%
Lennox	411	100%	-	-	100%

indicating that these fish had successfully escaped a predator encounter. The North Uist populations are of particular interest with respect to spine number as a gradation in spine reduction can be seen from Daimh - Bharpa - Fada. Most Daimh Gasterosteus are normally spined with 0-5 lateral plates; most Bharpa fish have no ventral spines, two dorsal spines and no lateral plates; and most Fada fish also lack ventral spines, have a single dorsal spine and lack lateral plates.

Table 16 gives the results of a linear regression (least squares) analysis of ventral spine length:standard body length for fish of both sexes from the five study populations with normal pelvic skeletons. The regression lines are plotted on Fig. 10 for male fish and Fig. 11 for female fish, each line represents data from 100 fish. The equation describing these relationships is:

$$y = a + bx$$

where y = ventral spine length, a = intercept, b = slope, x = standard body length; a y value therefore gives the average ventral spine length for a given body length within each sample. In both male and female fish from all five populations the correlation of ventral spine length and standard body length is highly significant ($p < .001$ in all cases, Table 16). At a body length of 20 mm both male and female Daimh fry have the shortest spines of the five populations. Within the mainland populations ventral spine length at 20 mm body length is similar for Lennox, Mar and Luggie males, and for Lennox and Luggie females, but 20 mm male and female Lomond fry have markedly longer spines than fish from other populations, a difference particularly evident amongst males (Fig. 10).

At 40 mm body size the relative size of the ventral spines in Daimh, Mar and Luggie males and females is maintained with Daimh fish having the smallest spines in relation to body size. Lomond male and

Table 16. Linear Regression of Ventral Spine Length: Standard Body Length

	Slope	S.E. Slope	Intercept	S.E. Intercept	Correlation coefficient	Significance of correlation coefficient
Daimh ♂	.074	.010	.69	.30	.58	$p < .001$
Daimh ♀	.073	.006	.735	.21	.74	$p < .001$
Lomond ♂	.045	.012	3.2	.42	.33	$p < .001$
Lomond ♀	.071	.007	2.3	.31	.66	$p < .001$
Luggie ♂	.08	.009	1.38	.34	.65	$p < .001$
Luggie ♀	.087	.005	1.17	.21	.84	$p < .001$
Mar ♂	.089	.008	1.2	.29	.72	$p < .001$
Mar ♀	.062	.003	2.02	.16	.85	$p < .001$
Lennox ♂	.118	.006	.64	.24	.87	$p < .001$
Lennox ♀	.118	.005	.5	.18	.91	$p < .001$

Fig. 10. Ventral spine growth rate in *Gasterosteus* males

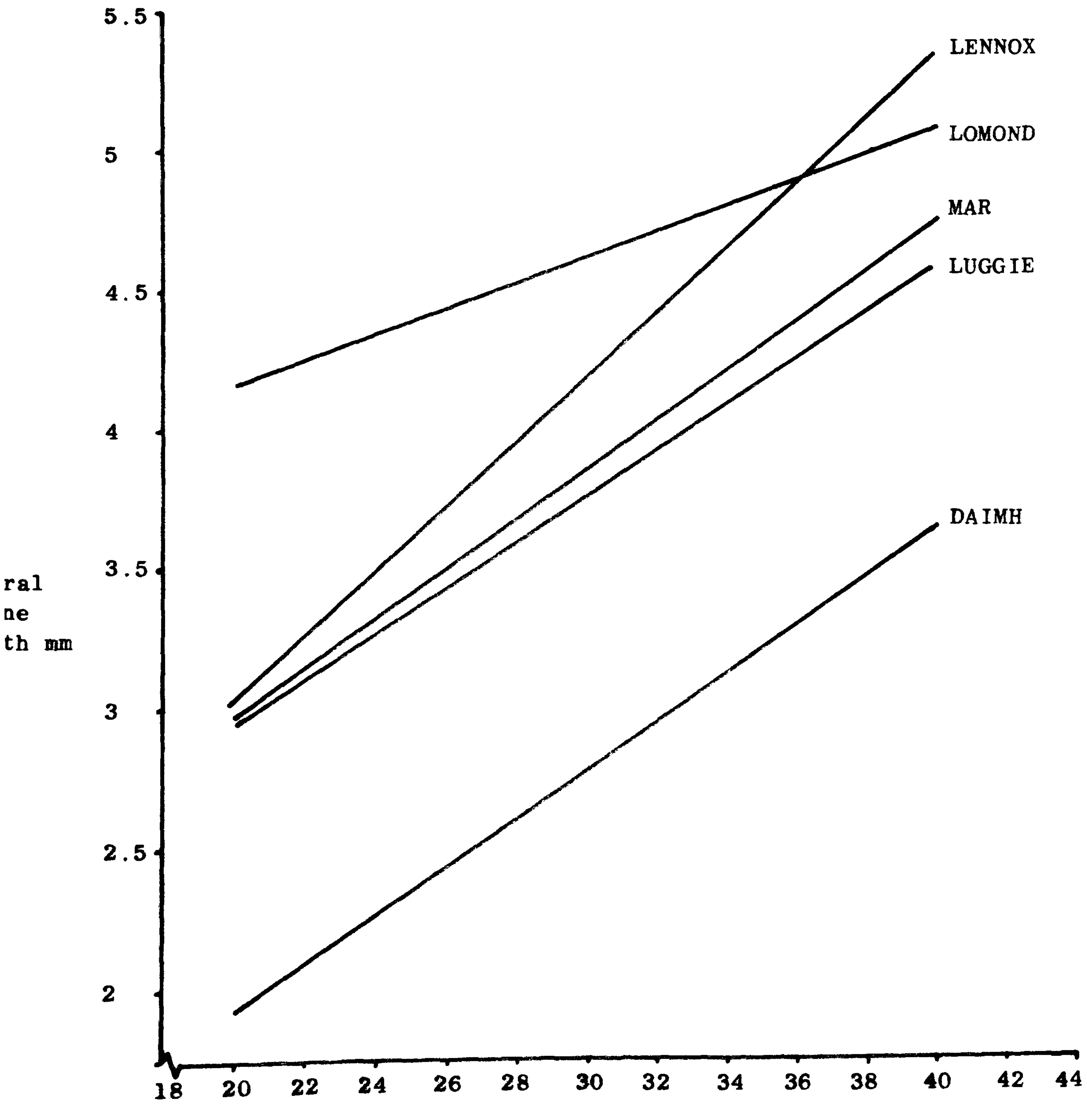
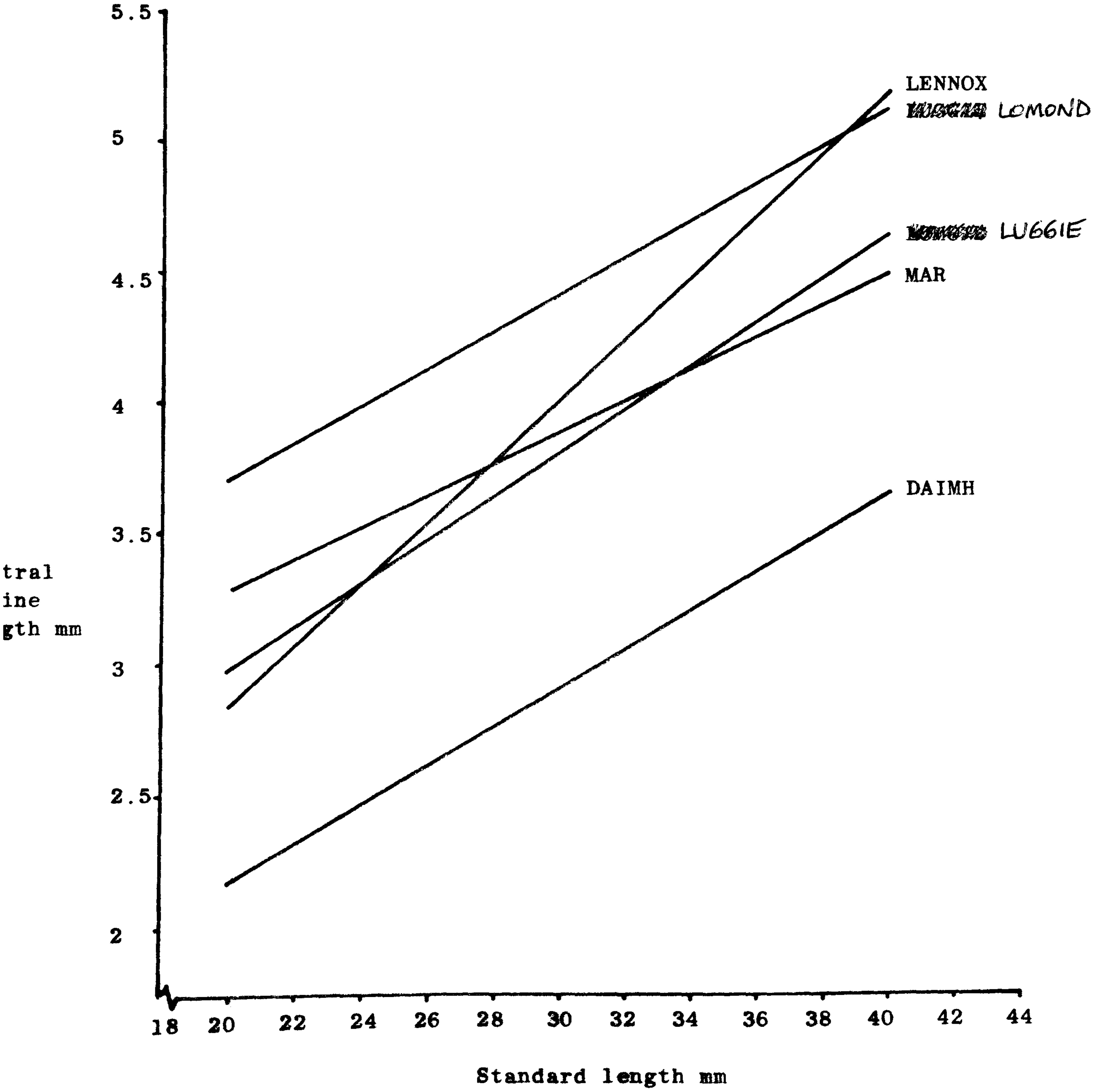


Fig. 11. Ventral spine growth rate in Gasterosteus females



female fish have markedly larger spines than these populations. Lennox Castle fish of both sexes exhibit a fast ventral spine growth rate and have the largest ventral spines at 40 mm body size.

Differences between the regression coefficients (Table 16) were tested for statistical significance using 2-tailed d tests (Bailey 1959). There were no significant differences (at the 5% level) in the ventral spine growth rate between male and female sticklebacks from the Lomond, Luggie, Lennox and Daimh populations. Mar male fish have smaller spines than females at 20 mm body length but subsequently show a significantly higher ventral spine growth rate than females ($d = 2.9$, $p < .01$). Amongst the five populations compared, Lennox male and female fish showed the highest ventral spine growth rates (despite the complete absence of fish predators from this population), Lennox males and females showed significantly higher ventral spine growth rates than Luggie males ;- ($d = 3.36$, $p < .001$), Mar males :- ($d = 2.6$, $p < .01$) and Lomond males :- ($d = 5.38$, $p < .001$). Luggie males had a significantly higher spine growth rate than Lomond males ($d = 2.3$, $p < .02$), there was no significant difference between Lomond males and Daimh males. Lomond male spine growth was significantly slower than Mar male spine growth ($d = 3.14$, $p < .002$) but there was no significant difference in spine growth in female fish from these populations.

4.7.2 Discussion

Using captive Pike and Perch as predators Hoogland, Tinbergen and Morris (1957) demonstrated that the spined *Gasterosteus* and *Pygosteus* are at a considerable advantage over small Roach, Rudd, Crucian Carp and Minnows by virtue of the protection afforded by their sharp dorsal and ventral spines. Both Pike and Perch soon learnt to avoid striking at sticklebacks presumably because of the unpleasant effects of the spines penetrating the tissues of the mouth and the subsequent extreme difficulty experienced in swallowing the stickleback. The larger, stouter spines of Gasterosteus afforded a greater measure of protection than the more numerous small spines of Pygosteus (Hoogland et al 1957). Hoogland (1951) demonstrated that no muscular effort is needed for sustained spine-raising in Gasterosteus; the base of each spine locks into a channel in the underlying pterygiophore supporting the spine such that dorso-ventral pressure serves to make the raised spine more rigid, rather than folding it down.

Moodie (1972) found that Cut-throat Trout (S. clarki) predating the large melanic Gasterosteus of Mayer lake (Queen Charlottes) selected adult female sticklebacks with significantly shorter pelvic spines than the average for a given body size within the population; predated adult male sticklebacks also had short spines but the difference was non-significant. Large sticklebacks were only predated by the largest trout indicating that the body size of the prey was critical in determining whether the predator could grasp and swallow it. Hagen and Gilbertson (1972) found that mean ventral spine length in North American freshwater Gasterosteus populations is significantly correlated with the presence or absence of sympatric predatory fish species, sticklebacks co-existing with predators having larger spines than fish from non-predated populations. In his laboratory experiments Reist (1980 a) found the spine-deficient Gasterosteus morph to be at a disadvantage to normally spined individuals when predated by small Pike (this work is discussed further on page 120). Gross (1978) in his survey of 77 European Gasterosteus populations examined the size and structure of dorsal spines, ventral spines and pelvic girdle complex concluding (as with his lateral plate number data) that a north-south arched cline in size of defensive structures exists in European freshwater populations correlated with the presence or absence of predatory fish species (especially Pike and Perch). Marine (Trachurus) Gasterosteus populations have the largest spines and are subjected to the greatest range of potential predatory species. Within the freshwater populations sticklebacks from sites with a low estimated predation pressure had significantly shorter dorsal and ventral spines and a smaller pelvic girdle than sticklebacks from high estimated predation pressure sites (Gross 1978).

The regression analysis of ventral spine growth rates during the present study revealed that at a body size of 20 mm both male and female Loch Lomond fry had markedly longer spines than fish from the other 4 populations compared. This result can be explained as an adaptive response in spine growth pattern to the known high predation risk to Lomond Gasterosteus fry from Perch especially during the months September to January.

The large ventral spines of Lennox fish can be explained in terms of predation risk, as the fry in this population have no known potential predators but the adults are at risk from Common and Black-headed Gulls. It is important to note that relative risk of predation is not a complete predictor of ventral spine size in the populations studied; Luggie Gasterosteus (low predation risk) were similar both in terms of relative spine size and spine growth rate to Mar sticklebacks which are known to be at high risk from Herons when adult.

From the range of stickleback predators known to be sympatric with the Uist populations and the known Trout predation on Loch Fada sticklebacks it is clear that no simple relationship exists between predation pressure and the reduction in defensive structures of Outer Hebridean Gasterosteus populations. This finding is contrary to that of Moodie and Reimchen (1973) who described an isolated

Gasterosteus population at Boulton Lake (Queen Charlotte Islands) where 70% of sampled sticklebacks are pelvic girdle and spine-deficient (phenotypically identical to Loch Fada fish) and where predatory fish species are absent. Moodie and Reimchen attribute the reduction in spines and pelvic girdle to a lack of predation pressure, citing examples of other Queen Charlotte Island populations which are sympatric with predatory fish and which have longer spines and normal pelvic girdles. Spine-deficient North Uist Gasterosteus populations exist in sympatry with Brown Trout and Arctic Char both of which have been reported to predate sticklebacks in large numbers. The dorsal and ventral spines of fish from the mainland populations bore marked lateral serrations which were absent from spines of the North Uist fish studied, Gross (1978) has speculated that spine serrations may help to avoid the situation whereby stickleback spines become firmly embedded in a predator's mouth.

4.8 Critical Dimension of Body

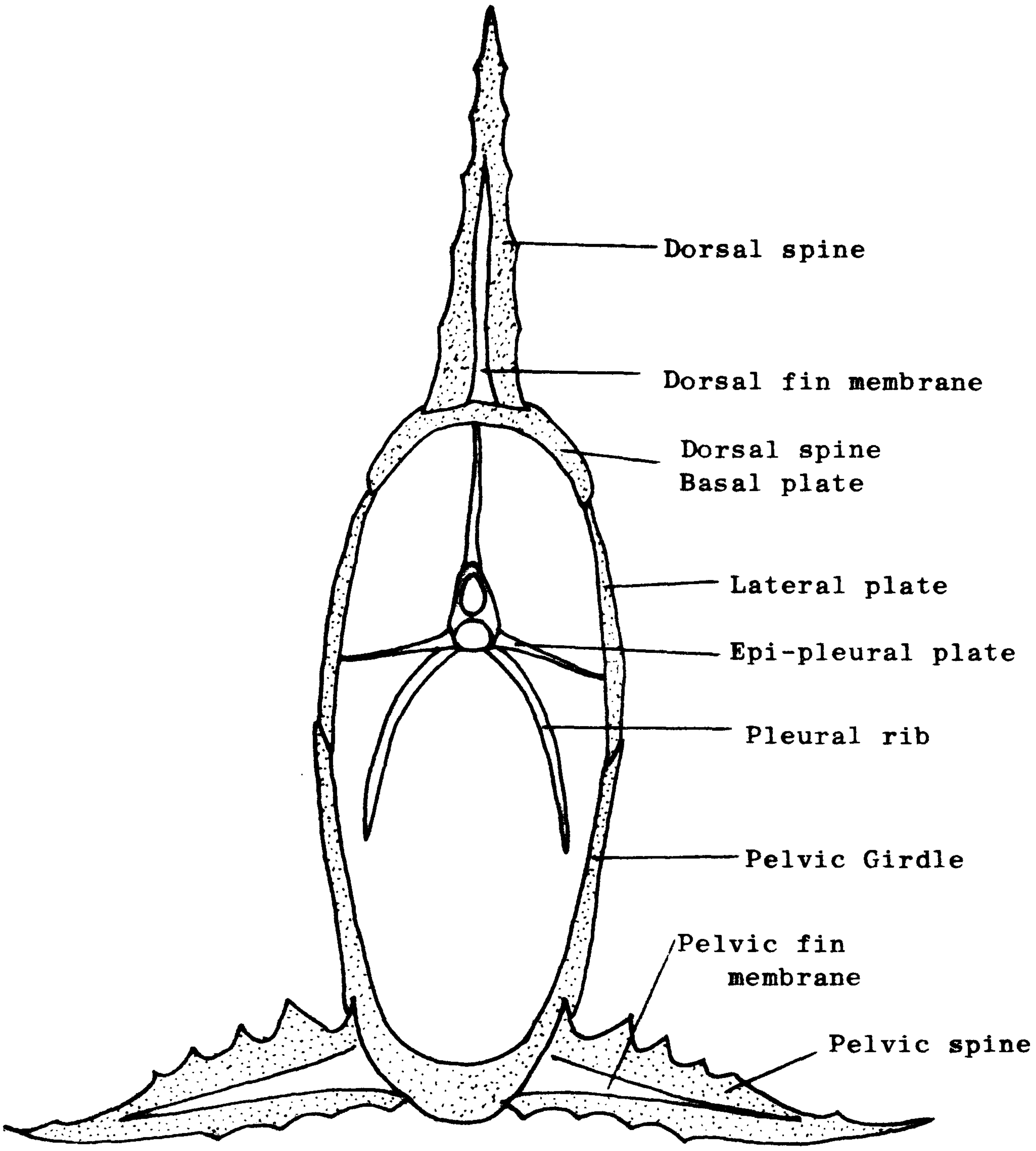
The body size of a prey species is of obvious relevance to its risk of being taken by a predator with a fixed maximal jaw gape; several teleostean genera (Gasterosteidae, Cottidae, Percidae, Labridae) have independently evolved sharp, stiff, erectable fin rays and opercular spines as a defence against predators. The dorsal spines of Gasterosteus have evolved from soft fin rays originally forming the anterior rays of the dorsal fin, the spines are articulated upon basal pterygiophores and still retain a small fin membrane along their posterior margin. The ventral spines have similarly evolved from pelvic fin rays retaining a small fin membrane along their posterior edge and articulating with the ventral shield of the pelvic girdle. The dorsal spines with their pterygiophores, the lateral plates, and the pelvic girdle complex effectively enclose the soft parts of the body of Gasterosteus in a rigid protective bone case, triangular in cross-section, and additionally braced internally by the vertebral column and epi-pleural ribs (Nelson 1971) (Fig. 12). Bannister (1967) has noted that the size and method of origin of the epi-pleural ribs is very variable in Gasterosteus. When a stickleback's spines are raised the maximal body depth and width are considerably increased, effectively protecting the fish against small predatory species. In order to quantify the magnitude of this increased effective body size 50 adult male sticklebacks were taken from each study population and a critical dimension ratio calculated. Fada fish were almost completely spine-deficient and were therefore omitted from the analysis. Bharpa fish lack ventral spines and the critical dimension ratio calculated for these fish was:

$$\frac{\text{dorsal spine length}}{\text{body depth}}$$

Daimh, Lomond, Mar, Lennox and Luggie fish are normally spined and the ratio used for these populations was:

$$\frac{\text{dorsal spine length + ventral spine length}}{\text{body depth}}$$

The results of this analysis are given in table 17; the ratios obtained were compared between populations using 2-tailed t tests. The ratio shows very low intra-population variability except amongst Lennox fish which were often infested with Schistocephalus solidus plerocercoides and thus had very variable body depths. The Bharpa fish, lacking ventral spines showed a much reduced advantage in terms of critical dimension increase when compared with the other five populations, this difference was highly statistically significant ($p = 3.4$ E $- 11$ between Daimh and Bharpa fish). Of the normally spined populations Luggie males had the smallest increase in critical dimension and have the lowest risk of predation amongst the study populations. The mean critical dimension ratio value for Luggie fish was highly significantly lower than that of Lennox males ($p = 1.2$ E $- 5$)



Diagrammatic cross-section posterior to second Dorsal spine showing relationship of axial skeleton to dorsal spines, lateral plates, and pelvic girdle in Gasterosteus aculeatus.

Fig. 12 Scale = x 10

Table 17. Critical dimension in male Gasterosteus

	$\bar{x}$	s.d.
Bharpa	.26	.05
Daimh	.83	.07
Lomond	1.05	.08
Luggie	.73	.08
Mar	.85	.08
Lennox	.93	.13

and Mar males (p = 1.9 E - 4). There was no significant difference in the value obtained for Mar and Daimh males both of which have a high risk of predation by piscivorous birds (and Trout in Loch an Daimh). Both Mar and Daimh males differed highly significantly from Lomond males (p = 4.7 E - 7, and p = 1.7 E - 7 respectively) which had the largest increase in critical dimension of any of the study populations. This result agrees with the known high predation risk of Lomond sticklebacks from Perch, Pike and Mergansers. Lennox males showed a significantly greater increase in effective size than Mar males (p = .05) but a significantly lower increase than Lomond males (p = 4.1 E - 3).

The index of critical dimension increase in Gasterosteus can thus be seen to correlate very well with the known and potential predation risk experienced by the study populations under natural conditions.

4.9 Reduction of Defensive Structures in North Uist Gasterosteus Populations

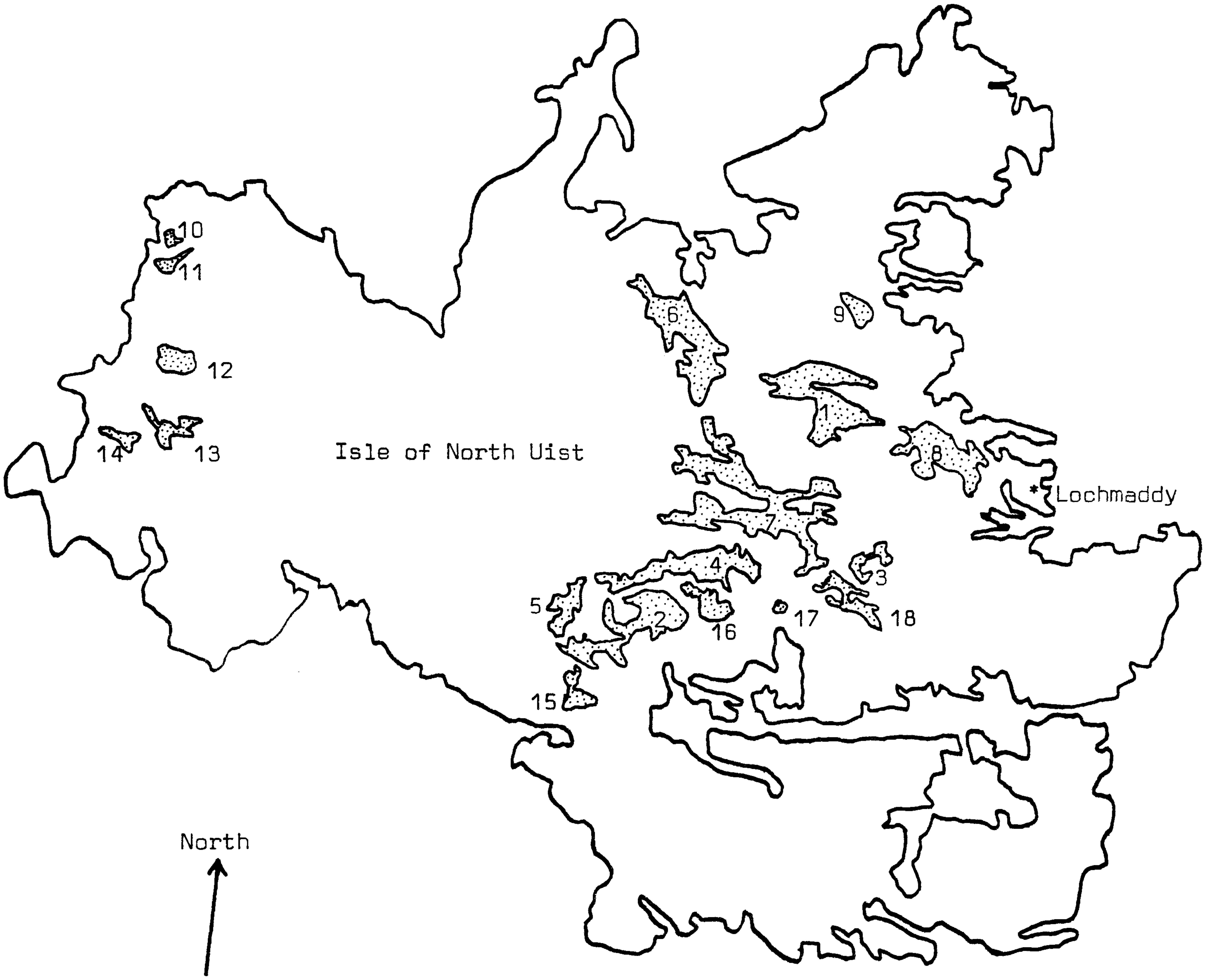
Extant Gasterosteus populations which lack lateral plates are known to occur at northern and southern extremes of the European distribution of this species (Gross 1978), in Paxton Lake (British Columbia) (Larson 1972) on certain offshore islands of the Pacific northwest coast of America (Hagen and McPhail (1970), Moodie and Reimchen (1973)) in central and southern California (often termed G. aculeatus williamsoni (Ross 1973)) and on the Isle of North Uist, Outer Hebrides. Plateless gasterosteid sticklebacks also occur in the Pliocene fossil record of the Truckee formation, Nevada where Mural (1973) recognises a form with 1, 2 or 3 dorsal spines, a reduced or absent pelvic girdle and with lateral plates only occurring rarely. Osteological evidence (particularly the evenly divided hypural plate) prompted Mural (loc. cit.) to assign this form to the genus Gasterosteus, and to name a new species Gasterosteus apodus. Mural regards present day populations with similarly reduced skeletal components (e.g. the plateless Gasterosteus population on Texada Island) as convergent populations (i.e. arising from separate genetic stock) rather than relict G. apodus populations. The fossil forms described by Mural are phenotypically identical to the spine-deficient, plateless morph from Loch Fada and Loch a Bharpa described in the present study. In the North Uist populations and in the Nevada fossil forms there is a large variability in the form of the reduced pelvic girdle which is often present as a small symetrical leaf-shaped dermal bone with no associated ventral spines. The reduced pelvic girdle is accompanied by a parallel reduction in the number of dorsal spines (table 15). The spine-deficient morph with a reduced pelvic girdle is regarded by the author as representing an extreme of morphological variability within the species Gasterosteus aculeatus L. Campbell (1979) has successfully crossed the North Uist spine-deficient morph with a Trachurus female and obtained a viable and fertile Fl generation, supporting the case for a conservative taxonomic approach to the genus. Bell (1973) has described a fossil Gasterosteus morph from the middle Pliocene ridge formation, California, where (from fragmentary evidence) it appears that again there is a reduction in dorsal spine number, an absence of lateral plates, and an incompletely developed pelvic girdle. X-ray photographs of a sample of 24 adult Fada and Bharpa sticklebacks were made at the Western Infirmary, Glasgow and showed that when sticklebacks from both populations are considered a complete range in stages of pelvic girdle reduction can be observed, from a fully developed, normally spined structure to the complete absence of even vestigeal dermal ossification. This exactly parallels the situation described by Bell (1974) where fossil Gasterosteus from the Truckee formation show a similar range of reduced pelvic anatomy, such spine-deficient forms tending to be stratigraphically separate from specimens with normal pelvic girdles. In comparing these fossil *Gasterosteus* from Nevada with the extant Paxton Lake spine-deficient *G. aculeatus* population Bell (1974) concluded that the spine-deficient *Gasterosteus* morph with reduced numbers of lateral plates had evolved in parallel along two separate phyletic lines (i.e. from common genetic stock) and that parallelism in the loss of the pelvic girdle has occurred within and between the genera *Gasterosteus*, *Pygosteus* and *Culaea*.

The reduction in defensive apparatus in sticklebacks has been attributed to several possible factors, the most common of which is a lack of piscine predation pressure (Moodie and Reimchen (1973), Hagen and Gilbertson (1972), Bell (1974), (1976)). Alternatively Bell (1974) has suggested that the loss of the dorsal and ventral spines, pelvic girdle, and lateral plates in *Gasterosteus* may make the fish more streamlined, and better able to escape from predators (no evidence was obtained in support of this theory during the present study. See chapter 5).

Nelson (1977) has studied the reduction in the pelvic girdle complex in *Culaea inconstans*, the brook stickleback, and shown from breeding experiments that the presence of the pelvic girdle is under partial genetic control, and that its reduction and loss has occurred in many localities in Ontario, Saskatchewan and Alberta. 23 sites are known from within Alberta where *Culaea* populations contain 20% or more individuals lacking a complete pelvic skeleton, in some localities 95% of all individuals sampled completely lack a pelvic girdle. Nelson has postulated that the reduction in pelvic girdle has arisen separately in various localities, and that variation in predation pressure is the causal environmental agent. Nelson assumed that the pelvic girdle loss was not an adaptation to an unknown physico-chemical variable.

Wootton (1976) cites a known instance however of a *Culaea* population with 20% individuals exhibiting a reduced pelvic girdle which is sympatric with Pike. Campbell (1976) has documented heavy predation by Brown Trout on the spine-deficient *Gasterosteus* population of Loch Fada on the Isle of North Uist. Reist (1980) has studied a polymorphic *Culaea* population at Lake Wakomao, Alberta where both spined and spine-deficient morphs occur; in laboratory experiments spined individuals (with a pelvic girdle) had a selective advantage over spine-deficient (without pelvic girdle) individuals when predated by small Pike (*Esox lucius*). Reist also found that pelvic girdle-less individuals were significantly under-represented in Pike stomach samples from the wild and has hypothesed that such fish may have behavioural adaptations aiding their survival (perhaps using weed cover more often than pelvic girdled individuals). Whilst conducting laboratory experiments with selected invertebrate predators Reist (1980 b) found that fish without pelvic girdles were as likely to be taken by *Aeshna* nymphs and *Lethocerus americanus* (water bug) as fish with a normal pelvic girdle and five dorsal spines. Reist noted that the individuals with five dorsal spines and a full pelvic girdle approached the predator more closely than other phenotypes tested. Reimchen (1980) simultaneously published his study of the polymorphic Boulton lake (Queen Charlotte Islands) *Gasterosteus* population where 80% of the samples examined lack a second dorsal spine and 64% lack pelvic spines (4.3% of the population have the normal spine compl~~i~~ement). Reimchen found that Great Northern Divers (*Gavia immer*) resident on the lake take large numbers of *Gasterosteus* and must represent a considerable predation pressure, Belted Kingfishers, *Megaceryle alcyon* were also seen to predate adult *Gasterosteus*. The nymphs of the dragonfly species *Aeshna palmata* and *A. eremita* are abundant in the littoral region of the lake and were also found to predate *Gasterosteus*, juvenile sticklebacks being recovered from 8 out of 70 Aeshna nymphs examined. Reimchen (loc. cit.) performed experiments to test the hypothesis that spines may offer a disadvantage to sticklebacks by affording purchase to the labial palp of an attacking nymph (giving spine-deficient individuals a selective advantage), however no significant differences in mortality due to predation were recorded between the spined and spine-deficient phenotypes. Reimchen noted that spineless sticklebacks on the Queen Charlotte Islands occur in shallow, bog pools of closed drainage, a description which also fits many of the peat-bog lochs and lochans of the Isle of North Uist.

Because of the anomaly in existing field observations between the reduction in defensive apparatus of Gasterosteus and the risk of predation, a preliminary survey of the freshwater sites of North Uist was undertaken in the hope of resolving the problem. Fig. 13 is a scale drawing of the island illustrating the position and approximate relative sizes of the lochs surveyed, to date 18 lochs have been sampled for sticklebacks by the author and are listed in the key to the map (page 123). Spine-deficient Gasterosteus have been caught at 8 of these sites, at the other 10 sites normally-spined Leiurus were caught; Trachurus sticklebacks are common in sea lochs around the periphery of the island. A wide range of piscivorous birds (page 66) is present over the whole island and Brown Trout are present in all of the lochs surveyed, Arctic Char are also present in at least two (Loch Fada and Loch a Bharpa) and Sea Trout and Eels have access to many of these waters, the risk of predation from both fish and avian predators was therefore estimated to be high for both the normally spined and spine-deficient populations. As more lochs were included in the survey it became apparent that the spine-deficient populations were occurring on the blanket peat-bogs of the central and eastern areas of the Island; all of the sticklebacks caught in west coast



Scale : 75 millimetres = 1 kilometre

Fig. 13. Diagram of the Isle of North Uist showing position of study lochs

Table 18

Key to study lochs on Isle of North Uist

1 = Loch Fada

2 = Loch á Bharpa

3 = Loch an Daimh

4 = Loch nan Eun

5 = Loch Huna

6 = Loch nan Gierreann

7 = Loch Scadavay

8 = Loch an Strumore

9 = Loch Vieragvat

10 = Loch an Eilean

11 = Loch Olavat

12 = Loch Hosta

13 = Loch Eaval

14 = Loch Grogary

15 = Loch Tormasad

16 = Loch na Moracha

17 = Loch nan Ceithir Eilean

18 = Loch á Bhuird

waters were normally spined Leiurus fish. As described in the introduction and study site descriptions the west coast of North Uist has a band of calcium-rich shell sand (machair) soils which supports biologically productive eutrophic lochs, whereas the blanket peat-bog areas give rise to oligotrophic waters of low productivity.

Table 19 summarises the information gained from the survey. It should be noted that the most prolific Gasterosteus morph sampled from each loch is given in the table (in some instances sample sizes were only 20 fish). The concentration of Ca^{2+} ions in Mg L^{-1} is also given for those study sites where water samples were taken. Calcium determinations were made in triplicate using the compleximetric E.D.T.A. volumetric method described by Mackereth et al (1978), the mean value is given in the table. Lochs denoted with an asterisk are waters which either lie on shell-sand soils or are subjected to maritime influences both of which greatly raise the level of available calcium ions in the water. Typical levels of Ca^{2+} ions in Machair lochs are (e.g.) 85-90 mgL^{-1} (Ratcliffe 1977) and 8.6-41.9 mgL^{-1} for other South Uist machair lochs (Waterston et al 1979); the machair of South Uist is better developed than that of North Uist. Jenkins et al (1976) have also noted that the freshwater lochs of North Uist can be classified into eutrophic waters on the machair soils of the north and west coasts of the Island, mesotrophic lochs of the lowland between the machair and the peaty hills, and oligotrophic lochs of the peatland. Reference to Table 19 shows that Leiurus sticklebacks tend to occur in the machair or maritime lochs whilst the peat moorland oligotrophic waters support spine deficient populations. However, the association is not a complete, one, with Loch nan Geirran Leiurus fish (very small body size) occurring in 1.8mgL^{-1} Ca^{2+} water and Loch an Daimh Leiurus fish in 3.55 mgL^{-1}Ca^{2+} water. No spine-deficient populations have been found in relatively calcium-rich

Table 19. Calcium concentration and phenotypic variability in North Uist Gasterosteus populations

Study Site	Predominant morph	$Ca^{2+} mgL^{-1}$
Loch Fada	spine-deficient	2.25
Loch a Bharpa	spine-deficient	1.9
Loch nan Eun	spine-deficient	2.5
Loch Huna	spine-deficient	-
Loch Scadavay	spine-deficient	-
Loch na Moracha	spine-deficient	2.5
Loch a Bhuird	spine-deficient	2.2
un-named lochan on Bogach Maari	spine-deficient	-
Loch Tormasad	Plateless	2.5
Loch an Daimh	Leiurus	3.55
Loch nan Geirrann	Leiurus	1.8
Loch an Strumore	Leiurus	- *
Loch Vieragrat	Leiurus	- *
Loch an Eilean	Leiurus	- *
Loch Olavat	Leiurus	- *
Loch Hosta	Leiurus	- *
Loch Eaval	Leiurus	- *
Loch Grogary	Leiurus	- *
Loch nan Ceithir Eilean	Leiurus	-

* denotes maritime influence on the loch or close proximity to machair area.

- denotes no sample.

waters on the island and it is therefore proposed that low calcium concentrations may have been important in the evolution of several separate populations of spine-deficient morph Gasterosteus on the Isle of North Uist.

Further evidence is available from the studies of Heuts (1944) who used the data of Bertin (1925) on the distribution of freshwater Leiurus Gasterosteus populations in France and formulated an hypothesis on the limiting role of calcium ion availability in stickleback distribution. Heuts performed experiments on Leiurus morph Gasterosteus fry and adults showing that the presence of calcium ions in the aquarium water was important in the regulation of chloride concentration in the body fluids. The presence of calcium ions appeared to lower the permeability of the surface epithelial tissues of the fish, lowering the level of chloride ion loss, an effect particularly marked in sexually mature fish.

Possible evidence supporting the calcium theory has come from a separate (unpublished) study of salmon reproduction on the Isle of South Uist by Holliday and Laird (University of Aberdeen, pers. comm.), where vertebral deformities and large scale mortalities were found amongst male salmon parr living in peat moor fed water conditions. Holliday and Laird suspect that these fish are mineral deficient, preliminary results having shown that South Uist peat has a high affinity for cations in solution (especially Zn^{2+}) and may possibly even leach ions from fish tissues. Wendelaar Bonga et al (1978) have used Trachurus sticklebacks to study calcium regulation in freshwater and found that prolactin cell activity is closely linked with hydromineral regulation. Prolactin appears to aid survival in a low calcium environment (freshwaters) by compensating for the effects of low calcium levels on water and ionic permeability of the integument. Wootton (1976, pages 123 et seq) discusses the role of prolactin

(≡ paralactin in teleosts) in enabling Trachurus sticklebacks to enter freshwater in spring to breed.

The Outer Hebridean fish fauna is represented by euryhaline species which recolonised the islands from the sea following the retreat of the last Pleistocene glacial incursion 8,000-10,000 years B.P. (Campbell and Williamson 1979). Normally spined and plated Gasterosteus (probably Trachurus morph) arriving from a marine habitat (with high calcium availability) and becoming land-locked in small oligotrophic calcium-poor freshwaters must have been faced with extreme selection pressures favouring individuals which were physiologically flexible enough to withstand the rapid environmental change. The present day North Uist Gasterosteus populations of the peat moorlands are typified by small body size, few or no lateral plates, few dorsal spines, and reduced or absent pelvic spines and girdle, all features which are non-essential skeletal components. The occurrence of fish in certain populations within the calcium-poor zone which have retained the pelvic girdle, lateral plates and spines can be explained by the counter-balancing selective pressure of predation favouring spined individuals. In Loch Fada 73% of sampled fish had only a single dorsal spine and 0.29% had a normal pelvic girdle, Campbell (personal communication) recovered no sticklebacks with obvious pelvic spines in his analysis of Loch Fada trout guts. Fig. 14 summarises the theory whereby a balanced polymorphism for spines may occur in the Fada population; low calcium-ion availability driving the population toward a reduction in skeletal components, and Trout predation maintaining a small percentage of normally spined individuals within the population. For such a theory to stand the benefits involved in economy of calcium usage must outweigh the loss of protection afforded by spines. Dacke (1979) has reviewed calcium regulation in sub-mammalian vertebrates and found that amongst teleost

Fig. 14.

low percentage of
normally spined
individuals

Trout predation

low Ca^{2+} concentration

small body size.
no lateral plates
reduced/absent pelvic girdle and spines

Hypothesised ~~Balanced Polymorphism~~ Selection pressures in Loch Fada *Gasterosteus* population species the calcium content of the scales can fall by 20% during ovarian development and be restored after breeding (Garrod and Newell, cited in Dacke 1979). Dacke concluded that the scales of modern bony fish play a major role in calcium regulation, plasma Ca^{2+} levels being finely regulated by exchanges between the environment and the hard tissues of the body. The review of calcium metabolism in fish in relation to ageing by Simkiss (1974) indicates that freshwater teleosts obtain relatively little of their calcium from their diet and are capable of absorbing and excreting calcium ions from and into the surrounding water principally via the gills and fins. It is possible that the reduction in external skeletal components in Gasterosteus (and possibly Culaea and Pygosteus) may be an adaptation reducing the loss of Ca^{2+} ions from the body, to the environment.

Amongst freshwater invertebrate animals it has been suggested that a reduction in calcification of the exoskeleton of crayfish (Genera Engaeus and Geocharax) is an adaptation to low environmental Ca^{2+} concentrations (Mills et al 1976).

4.10 Summary

1. Loch a Bharpa Gasterosteus have the smallest mean mature adult standard length reported to date.

2. In all study populations except Lennox Castle adult females are significantly larger than adult males. All three North Uist populations were smaller at maturity than the smallest of the mainland populations.

3. Male fish from the 5 stillwater populations studied had a more terete body shape than males from the 2 flowing water populations. North Uist fish were significantly slimmer than fish from any of the mainland populations. A regression analysis showed that these

differences remained true when all populations were compared at a standard body length (30 mm).

4. No clear correlation between gill raker number and diet or habitat type was found during the study, all 7 populations having very variable gill raker numbers. Lentic populations did however have longer, thinner gill rakers than the two lotic populations and gill raker shape may therefore be related to diet.

5. Loch Fada and Loch a Bharpa fish are 100% lateral plate deficient; lateral plate number was very variable in the other 5 populations studied. Lateral plate asymmetry was very common in Leiurus fish, occurring in (e.g.) 54% of Luggie Gasterosteus. No clear relationship between average lateral plate number and predation risk was apparent from the available data.

6. All three North Uist populations show a greater or lesser tendency for reduction in the number of lateral plates, dorsal spines, and degree of development of the pelvic girdle. In both the Loch Fada and Loch a Bharpa populations a complete range of morphological intergrades from full pelvic girdle development to a complete lack of pelvic girdle is present. In Loch Fada an apparently unique situation exists where spine-deficient Gasterosteus occur in sympatry with Brown Trout which are known to predate them.

7. At a body size of 20 mm Loch Lomond Gasterosteus fry have markedly larger ventral spines than any of the other populations studied. This early rapid spine growth coincides exactly with the known heavy predation pressure exerted by Perch upon juvenile sticklebacks in Loch Lomond (page 61). At 40 mm body size Lennox Castle Gasterosteus

have the largest ventral spines; these fish are known to be at risk from Common and Black-headed Gulls.

8. The relationship between ventral spine size and risk of predation is not a simple one since Luggie and Mar fish have similar ventral spine growth characteristics but significantly different risks of predation.

9. A critical dimension ratio $\left(\frac{\text{dorsal spine length + ventral spine length}}{\text{Body depth}}\right)$ showed very low intra-population variability but highly significant inter-population variation. A good correlation between predation risk and increased effective body size due to spine-raising emerged with Luggie fish (low risk) having the smallest increase and Lomond fish (high risk) the highest.

10. The rare spine-deficient morph of Gasterosteus has been found at a total of 8 separate sites on the Isle of North Uist. All 8 populations exist in sympatry with Brown Trout.

11. A preliminary review of the distribution of Gasterosteus morphs on North Uist has shown that spine-deficient populations are confined to the oligotrophic lochs of the central and eastern blanket peat-bog complex.

12. An hypothesis is put forward whereby low environmental calcium levels are thought to have exerted a strong selective effect over the last c. 10,000 years and brought about a reduction in non-essential skeletal components in North Uist Gasterosteus populations.

CHAPTER 5

BEHAVIOURAL STUDIES

5.1 Introduction

This chapter describes observations and experiments upon the behaviour of sticklebacks from the 7 study sites to determine whether mature males from the different sites differ in their reproductive behaviour and whether developing and mature sticklebacks of both sexes differ in their anti-predator responses. The development of the overhead fright response and the relationship between lateral plate phenotype and anti-predator behaviour is also investigated. Hoogland et al (1957) first described the anti-predator behaviour of G. aculeatus and P. pungitius finding that both species raised their dorsal and ventral spines upon the close approach of a small hunting Pike or Perch. The relatively larger spines of G. aculeatus were found to afford better mechanical protection from fish predators than the smaller more numerous spines of P. pungitius. Benzie (1965) analysed the anti-predator behavioural responses of both species in greater detail describing typical components of the escape response. Gasterosteus was found to behave similarly upon encountering Pike, Perch or Trout. When undisturbed in a familiar environment Gasterosteus swims slowly in short bursts using its pectoral fins and pausing frequently in mid-water; when a food item is taken the dorsal spines are usually raised simultaneously, both areas of open water and vegetation are explored freely. Upon encountering a predator normal pectoral swimming stops abruptly and is followed by one of several other possible locomotory categories (rapid retreat, freezing, sinking to bottom, etc.) (Benzie 1965).

Predators are often triggered to strike at a prey species after detecting a movement of the prey and one of the most noticeable aspects of Gasterosteus behaviour when remaining still at the bottom is that all fin and bodily movements are suppressed. The body of the stickleback is also bent into an "S" shape so as to be ready to leap away if the predator detects the hiding place of the fish.

Huntingford (1976, a) investigated the possibility that aggression shown towards conspecifics by nesting male Gasterosteus, and the anti-predator responses shown towards a hunting Pike might vary in parallel, concluding that there was a tendency for them to do so. Anti-predator behaviour and conspecific aggression were found to be positively correlated in individual fish such that highly aggressive males tended to be bolder in the face of a hunting Pike, the converse being true for timid males (Huntingford 1976, a). Huntingford (1976 a, b) suggested that this might represent an adaptation to varying predation pressure. In order to test this hypothesis she measured the levels of anti-predator response and social aggression in a number of Gasterosteus populations exposed to different degrees of predation risk. Significant differences between populations were found in both of these aspects of behaviour (pers. comm.) but her estimates of predation level were crude and some ambiguities were present in the results. The present study was therefore designed to test this hypothesis more stringently, making detailed comparisons of behaviour of fish from populations whose exposure to predators was known more accurately.

5.2.1 Nest Site Selection under Natural Conditions

At all available opportunities nest sites occupied by male Gasterosteus in the wild were noted together with measurements of the depth of water covering the nest and the distance from the nest of the nearest neighbouring occupied Gasterosteus nest. The information gathered from these observations is summarised in

Table 20. Male <u>Gasterosteus</u> nest site selection under natural conditions

	n	Mean Water Depth (Nearest 5 cm.)	Inflowing Streams	Weed Beds	Open Littoral Sites	Sheltered Littoral Sites	Deep pools/ Alder roots	Typical inter-nest distance
FADA	36	25 cm.	10	-	23	3	-	150 cm.
BHARPA	78	40 cm.	36	-	42	-	-	100 cm.
DAIMH	31	30 cm.	21	8	-	2	-	100 cm.
LOMOND	39	20 cm.	18	2	-	19	-	50 cm.
LUGGIE	48	40 cm.	-	23	7	18	-	200 cm.
MAR	30	85 cm.	-	-	-	-	30	30 cm.
LENNOX	59	>100 cm.	17	8	22	12	-	200 cm.

Table 20. It should be noted that any attended nest in open water is much more likely to be seen than a well concealed nest in a weed bed, and that deep water (> 2 m) observations were not feasible during the present study; however, all possible efforts were made to survey each of the study sites comprehensively and without bias.

Results

In Loch Fada the majority of all attended Gasterosteus nests were seen in very shallow water, open littoral sites or in inflowing streams. Inter-nest distances were rarely less than 1.5 m.

This is also the case in Loch Lomond where almost all of the observed Gasterosteus nests occurred in shallow littoral areas and in inflowing streams. Lomond fish chose sheltered littoral sites whereas Fada fish often nested in the open. Mar burn male Gasterosteus show a very different distribution of nest sites; all observed Gasterosteus nest sites in the burn were well concealed in the deepest available pools. Nests were aggregated with typical inter-nest distances being only 30 cm. Root masses from bankside Alder trees afforded sturdy overhead protection for such nest sites. At Loch an Daimh and Loch a Bharpa the differences in observed nest distribution apparently occur because of the greater availability of submerged aquatic vegetation at Loch an Daimh where more nests were found in weed beds. Inflowing streams are used by males from both populations as nest sites, water depths over nests are similar at both sites. Although many nests were found in open littoral situations at Loch a Bharpa these were in fact very inconspicuous owing to the uniform dark peat substrate. Nests were usually only located after watching territorial males for several minutes until nest-associated behaviours established the nest position beyond doubt.

At the River Luggie the majority of nests were sited in sheltered littoral sites and in weed beds; inter-nest distances were large (typically 200 cm.). At Lennox Castle nests were usually sited in water deeper than 1 metre with similar numbers of nests being located at stream inlets and in open and sheltered littoral positions.

5.2.2 Discussion

Consideration of the nest-site selection of male *Gasterosteus* under natural conditions is complicated by Kynard's finding from three natural populations in Washington State that males of differing lateral plate phenotypes preferred to nest in different locations: 14 plated (total right hand plus left hand side plates) fish out-competing 16 plated individuals for the preferred sites in vegetation at depths greater than 45 cm., (Kynard (1979)). Moodie (1972) working at Mayer Lake, Queen Charlotte Islands also found intra-population differences in nest site selection by *Gasterosteus* males of differing lateral plate phenotype, with a statistically significant trend for 6/6 - 7/8 - 7/7 - 6/7 males to nest in progressively deeper water. The distance of the nest from the nearest shelter also showed a significant difference with 7/8 - 6/6 - 7/7 - 6/7 fish nesting progressively nearer shelter (Moodie 1972). Unfortunately, during the present study not enough time was available to catch each of the nesting male sticklebacks observed in order to determine their lateral plate number.

Both Fada and Lomond male *Gasterosteus* tended to nest in shallow water (20-25 cm) along the littoral zone and in inflowing streams. The potential predation risk for both populations is similar. Fada *Gasterosteus* are known to be predated by Brown Trout, and Lomond *Gasterosteus* by Pike and Perch; at both sites diving ducks fish the 2-3 m deep littoral region extensively. Shallow water nesting by mature *Gasterosteus* males may be an adaptation to avoid such predators (Trout, Perch and Pike rarely hunt in such open shallow water owing to their own vulnerability to attack from birds (personal observations)). The Mar burn male Gasterosteus invariably nested in the deepest pools available where overhead cover gave added protection to the nest. The known high risk of predation by Herons along the burn is the most likely explanation for the aggregated nest distribution. During the breeding season it is rare to see any adult Gasterosteus in open water at the Mar burn, all sampled fish being caught amongst Alder tree root masses and other vegetation. At Lennox Castle reservoir and to a lesser extent at the River Luggie, Black-headed Gulls are a known and potential predator and the deep water nest site selection of Lennox males and sheltered nest site selection of Luggie males may be a response, at least in part to this selection pressure. At both Loch a Bharpa and Loch an Daimh inflowing springs and streams afforded ideal nesting sites where water flow probably aided the irrigation of developing eggs within nests, and where shallow water and the close proximity of the soft peat bottom gave refuge from Trout and diving duck species. The blanket peat bog which encompasses all three of the North Uist study lochs is dissected and traversed by numerous underground springs and small ditches and streams which have a bed of large peat particles interspersed with small sand and gravel patches. Gasterosteus nests at Loch a Bharpa, Loch an Daimh and Loch Fada were commonly situated in sheltered positions in the mouth of such springs and streams; when disturbed the adult male sticklebacks guarding nests darted for cover under stones and into the soft peat substrate and were then extremely difficult to locate. In general at all 7 sites male sticklebacks chose sheltered nest sites and spread themselves out within the suitable nesting areas available. At Loch Fada however nests were commonly seen in very shallow open water positions along the shoreline sometimes at a depth of < 10 cm. Whilst such nests were very conspicuous to the human observer it is possible that the majority of potential predators (Brown Trout, Arctic Char, diving ducks) are unable to exploit them and their attendant male fish as a food source.

5.3 Laboratory Experiments

5.3.1 Reactions of breeding Gasterosteus males to male and female conspecifics

Methods

During the 1978 breeding season sexually mature male fish were caught from each mainland population , transported to the laboratory and released into individual tanks (60 cm x 30 cm x 30 cm). Mature male fish from the Uist populations had already been used in Heron tests (page 148) two or three weeks before and were then housed in individual tanks and allowed to nest; it is possible that this single exposure to an overhead predator stimulus may have affected their subsequent aggressive and reproductive behaviour.

The nesting tanks were identically provisioned with an area of sandy substrate and a clump of *Sphagnum* moss, all tanks were lit naturally. The fish were fed daily on either Tubificid worms or planktonic crustacea. 12 fish from the Mar, Luggie, Lennox and Fada populations, 11 from Lomond and 8 from Bharpa and Daimh successfully built nests.

On the day following the completion of the nest the fish were tested with the following stimuli:

1. A conspecific male in breeding colours (a spine-deficient male was used to test spine-deficient fish, and a Leiurus male to test Leiurus fish) contained within a glass flask and positioned 20 cm from the nest.
2. A Gravid conspecific female (of the appropriate morph) contained within a glass flask and positioned 20 cm from the nest.

A period of 6 hours was left between the presentation of stimulus 1 and stimulus 2 for all fish, all fish were fed with tubifex worms 1 hour before being tested. The sequence of presentation of the stimuli was not randomised because the response to the male was the most important variable tested and the above procedure avoided confounding population and sequence effects. In both sets of tests the response of the nesting male to the stimulus was measured over a five minute period subsequent to the first approach to the stimulus. The behaviours recorded were defined as follows:

Bite: butting the glass flask whilst simultaneously opening and closing the mouth once.

Lunge: a sudden movement toward the flask over a distance of > 1 cm (usually followed by one or more bites).

Zig Zag: a characteristic pattern of swimming where the nesting male swims first in one direction and then back across the line of vision of the female (often followed by a lead).

Lead: swimming in a straight line from the flask toward the mouth of the nest.

The total number of bites and lunges directed toward the captive conspecific male was measured in the first tests and the total zig-zags, leads, bites and lunges directed at the captive gravid female was recorded in the second tests.

5.3.1.1 Results

Tables 21 and 22 summarise the results of these experiments; statistical comparisons between groups of fish were made using Mann-Whitney U tests. For the response to a breeding male larger inter-population differences in male aggression were found, intra-population variability being much less marked. Ranking the populations from high to low the pattern gives:

Table 21. Breeding male <u>Gasterosteus</u>: Aggression toward conspecific male

	n	Mean total bites and lunges over 5 minute test period	2-tailed Mann-Whitney U-Test
FADA	12	137 (s.d. 57)	p < .02
BHARPA	8	62 (s.d. 27)	
DAIMH	8	77 (s.d. 21)	
LOMOND	11	155 (s.d. 61)	p < .001
LUGGIE	12	80 (s.d. 25)	p < .02 p < .001
MAR	12	32 (s.d. 27)	
LENNOX	12	38 (s.d. 23)	

Table 22. Breeding male *Gasterosteus*: Sexual and Aggressive Behaviour toward gravid conspecific female

	n	Mean total bites and lunges over 5 minute test period	Mean total zig-zags and leads over 5 minute test period	2-tailed Mann-Whitney U-Test
FADA	12	17 (s.d. 20)	54 (s.d. 28)	p = .05
BHARPA	8	15 (s.d. 6)	30 (s.d. 13)	
DAIMH	8	39 (s.d. 15)	48 (s.d. 33)	
LOMOND	11	31 (s.d. 17)	60 (s.d. 26)	
LUGGIE	12	22 (s.d. 12)	31 (s.d. 16)	p < .002
MAR	12	27 (s.d. 11)	73 (s.d, 24)	p < .002
LENNOX	12	16 (s.d. 9)	29 (s.d. 14)	

Lomond > Fada > Luggie > Daimh > Bharpa > Lennox > Mar.

Lomond males were significantly more aggressive toward a conspecific male than Mar males ($p < .001$) or Luggie males ($p < .02$). Fada males were significantly more aggressive than Bharpa males ($p < .02$) or Mar males ($p < .001$). During the tests it was observed that Lomond males frequently directed zig-zags and leads toward a conspecific male before rapidly switching to short sharp bouts of aggressive lunges and bites.

Large interpopulation behavioural differences are again apparent from the results of the tests with the gravid female stimulus. Males from all populations were aggressive toward a gravid female; ranking the populations from high to low the pattern is:

Daimh > Lomond > Mar > Luggie > Fada > Lennox > Bharpa.

A comparison was then made (2-tailed Wilcoxon matched pairs signed ranks tests) between the levels of aggression (mean total lunges and bites) shown by individual males within each population toward the conspecific male and gravid female stimuli. Males from Lomond, Luggie, Fada, Bharpa and Daimh showed significantly higher aggression ($p < .01$ in all cases) toward the conspecific male than the female; males from the Mar and Lennox populations showed no significant difference in the level of aggression shown toward the two stimuli. The possibility that the reduced level of aggression directed at females is due to habituation in the tested males to repeated stimulation is made less likely due to the variability in the effect between populations.

When comparing sexual behaviour (mean total zig-zags and leads) between the populations, ranking from high to low the pattern is:

Mar > Lomond > Fada > Daimh > Luggie > Bharpa > Lennox.

Using 2-tailed Mann-Whitney U tests Mar males showed significantly higher levels of sexual behaviour than Luggie ($p < .002$) or Lennox (p < .002) males, but no significant difference when compared with Lomond males. Fada males showed significantly more sexual behaviour than Bharpa males (p = .05) but no significant difference from Daimh males. There was no significant difference between Bharpa and Daimh males.

5.3.1.2 Discussion

Clearly from the above results behavioural differences occur between populations in response to conspecific male and female stimuli.

Amongst the seven study populations tested the Mar males were the least aggressive toward a conspecific male and showed the greatest frequency of courtship behaviour toward a gravid female. Low aggression in male Gasterosteus is known to facilitate courtship (Wilz 1967) and this, coupled with a high frequency of courtship behaviour is likely to lead to a short successful courtship with minimal time spent in conspicuous zig-zag courtship swimming. By choosing concealed nest sites, reducing conspecific aggression and increasing courtship displays Mar males are likely to minimise the probability of being taken by bank fishing Herons. Aggressive responses toward females occurred in most Mar males but were of short duration before a switch to energetic courtship behaviour occurred. Mar males initially directed frequent zig-zags and leads toward a conspecific male stimulus; significantly more courtship behaviours were performed upon presentation of a gravid female (p < .01, Wilcoxon matched pairs signed ranks test).

Fada males tested in the laboratory were highly aggressive toward conspecific males and also showed high levels of courtship behaviours toward a gravid female. This is paralleled by the situation in Loch Lomond where again the laboratory tested males were found to be highly aggressive and to show high intensity courtship behaviour, choosing nest sites in shallow littoral and inflowing stream sites. If shallow water and stream nesting is an efficient way of avoiding underwater predators then the advantages gained from high levels of aggression (reduction in loss of eggs from nest) and sexual behaviour (greater reproductive output) may outweigh the dangers of being conspicuous to predators during the performance of these activities. Loch a Bharpa and Loch an Daimh Gasterosteus are exposed to a similar potential predation risk and typical inter-nest distance, conspecific aggression, and courtship behaviour are similar between the two populations. Fada male Gasterosteus experience a similar risk of predation to the above two populations but are significantly more aggressive to a conspecific male than Bharpa males. The reason for this difference is not clear but may be attributable to greater competition for nest sites. During field observations Fada males were often seen disputing territories in very shallow water and such sites may be at a premium as they are unlikely to be patrolled by Brown Trout at least during the hours of daylight.

In the laboratory nesting Luggie males were highly aggressive toward conspecific males and frequently aggressive toward a gravid female, lunges and bites frequently interspersed with zig-zag and lead sequences. Egg stealing is common amongst adult Luggie Gasterosteus and the risk of predation is low. These factors are likely to be important influences acting upon the reproductive and territorial behaviour of males such that conspicuity may be less important than territorial defence. Interestingly Luggie males still favoured sheltered nest sites and well hidden nests may be important not only in reducing the likelihood of predation of the guarding male, but also in reducing egg stealing by rival male sticklebacks.

Lennox Castle male Gasterosteus tested in the laboratory scored on average low levels of conspecific aggression and sexual behaviour in the tests. The nests built by these fish were small and tended to drift apart very readily, little nest-tending behaviour was observed from the fish during their captivity. Upon dissection 10 out of the 12 males tested were found to be parasitised by Schistocephalus solidus plerocercoides and this may in part explain their lethargic behaviour.

Many selective agencies as well as predation risk are likely to be involved in the moulding of territorial aggression and reproductive behaviour in male Gasterosteus and the interpretations offered above must be regarded as being tentative at the present state of knowledge of the study populations. The occurrence of a range of differing lateral plate phenotypes within the populations studied further complicates the picture as evidence is available that males with different numbers of lateral plates show significant differences in aggressive behaviour. During his study of the Wapato Lake Gasterosteus population Kynard found that aggression exhibited by males varied between lateral plate phenotypes with the following ranking (low to high aggression):

$$6/7 < 7/6 < 8/8 < 7/7$$

(Kynard 1972). These studies raise the possibility that proposed interpopulation behavioural differences found may be due to pleiotropic linkage of behaviour patterns to particular lateral plate phenotypes rather than local selection for behaviour at the population level. During the present study the lateral plate number of all fish used in behaviour experiments was noted but small sample sizes preclude the possibility of testing the above hypothesis. Interpopulation variability in behaviour was usually found to be greater than variability between individuals within a population but this effect may be due in part to interpopulation differences in lateral plate phenotype distribution. Further studies utilising large numbers of fish from a small number of widely differing populations need to be performed in order to test this hypothesis.

The studies of Peeke (1969) and Van dem Assem and Van der Molen (1969) upon habituation of conspecific aggression in *Gasterosteus* have highlighted the care that is needed when drawing conclusions about territorial behaviour in the wild from isolated laboratory experiments; over a 10 day test period nesting males presented regularly with a conspecific male stimulus habituated, reducing their aggressive response whilst simultaneously increasing sexual advances toward a conspecific gravid female stimulus over the test period. It is probable that Lomond and Fada males which are highly aggressive in the laboratory toward a single conspecific male presentation, would rapidly habituate to adjacent territorial males under natural conditions. This prediction agrees with Wootton's (1972) data from nesting *Gasterosteus* in the River Wear, Durham, which spent only 20-30 seconds per 20 minute observation period exhibiting overt aggression to territorial intruders. Fernald (1977) studying territorial *Haplochromis burtoni* males found that in this seemingly aggressive species only c. 1% of their time was spent in maintaining territorial boundaries. Wilz (1972) has noted that highly aggressive *Gasterosteus* males have a low success rate when courting gravid females and that a rapid motivational change from aggressive to sexual tendencies appears to occur in this species. Highly aggressive males from both Loch Lomond and Loch Fada regularly performed "displacement" nest fanning and creeping-through behaviours when modifying early aggressive reactions to a newly presented gravid female stimulus; these observations agree with those of Tinbergen and Van Iersel (1947) and Wilz (1967).

5.3.2.1 Anti-predator Behaviour Experiments

The aim of the experiments described in the following section was to quantify the anti-predator responses of fish from the 7 study populations and to relate any differences found to differences in risk of predation.

Rationale for choice of predator stimuli in laboratory experiments

Table 7 (page 69) summarises the range of known and potential Gasterosteus predators at the study sites. Of the predatory fish species Trout and Perch are chasing predators (Benzie 1965) and the Pike is a stalking/ambush predator capable of very fast short-range lunges at the prey subsequent to slow orientation and approach movements (Benzie loc. cit.).

The actual and potential avian predators encountered by Gasterosteus in the study populations can be conveniently classified into the following groups:

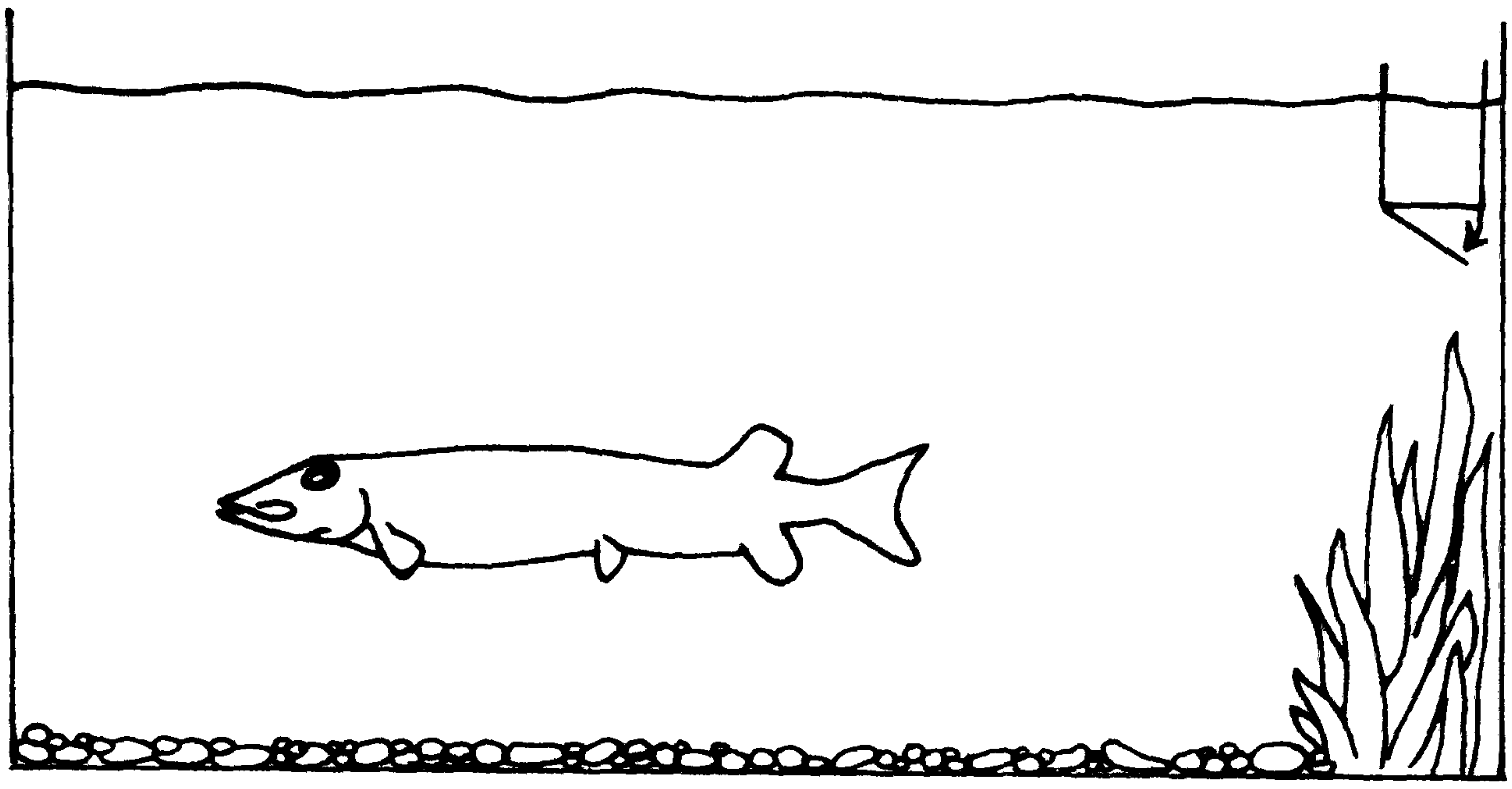
1. Stalking/Ambush/Aerial diving species, striking from above: Heron, Terns, Gulls.
2. Underwater swimming/chasing species, approaching at speed underwater: Merganser, Grebes, Divers.

Group 1 species are likely to represent a sudden darting overhead stimulus to a stickleback, which if successfully avoided is unlikely to lead to a prolonged chase. Group 2 species are fundamentally different in that they actively chase sticklebacks underwater and as such are likely to represent a similar stimulus to that of a large predatory fish giving chase. In order to investigate the fright responses of Gasterosteus toward a potential bird predator it was decided to use a model simulating the sudden overhead darting movement typified by the Heron. The major advantage of this approach is the presentation of a standard "predator" stimulus at an exact point in time. Because of the active chasing predatory behaviour of Trout and Perch the reaction of a potential prey stickleback under experimental conditions would, of necessity, be fast, agitated, and difficult to record and interpret. It was therefore decided that a small (30 cm total length) live Pike would represent the best fish-predator stimulus (as used by Benzie 1965 and Huntingford 1976 a). Before each experiment the Pike was fed with small minnows until it would still fixate upon and stalk a prey fish without actually striking at and taking it. The stalking Pike elicited a precautionary response from the sticklebacks tested and as such the experimental set-up was successful. However between-test variability in intensity of stalking behaviour by the Pike may have contributed to the variance in anti-predator behaviour exhibited by tested sticklebacks. This effect was reduced to a minimum by using the same individual Pike over the three year study period and by only performing experiments when the Pike was actively hunting. J. Tulley (pers. comm.) is currently improving this approach by using a life-like pike model representing a standard predator stimulus during each experimental test.

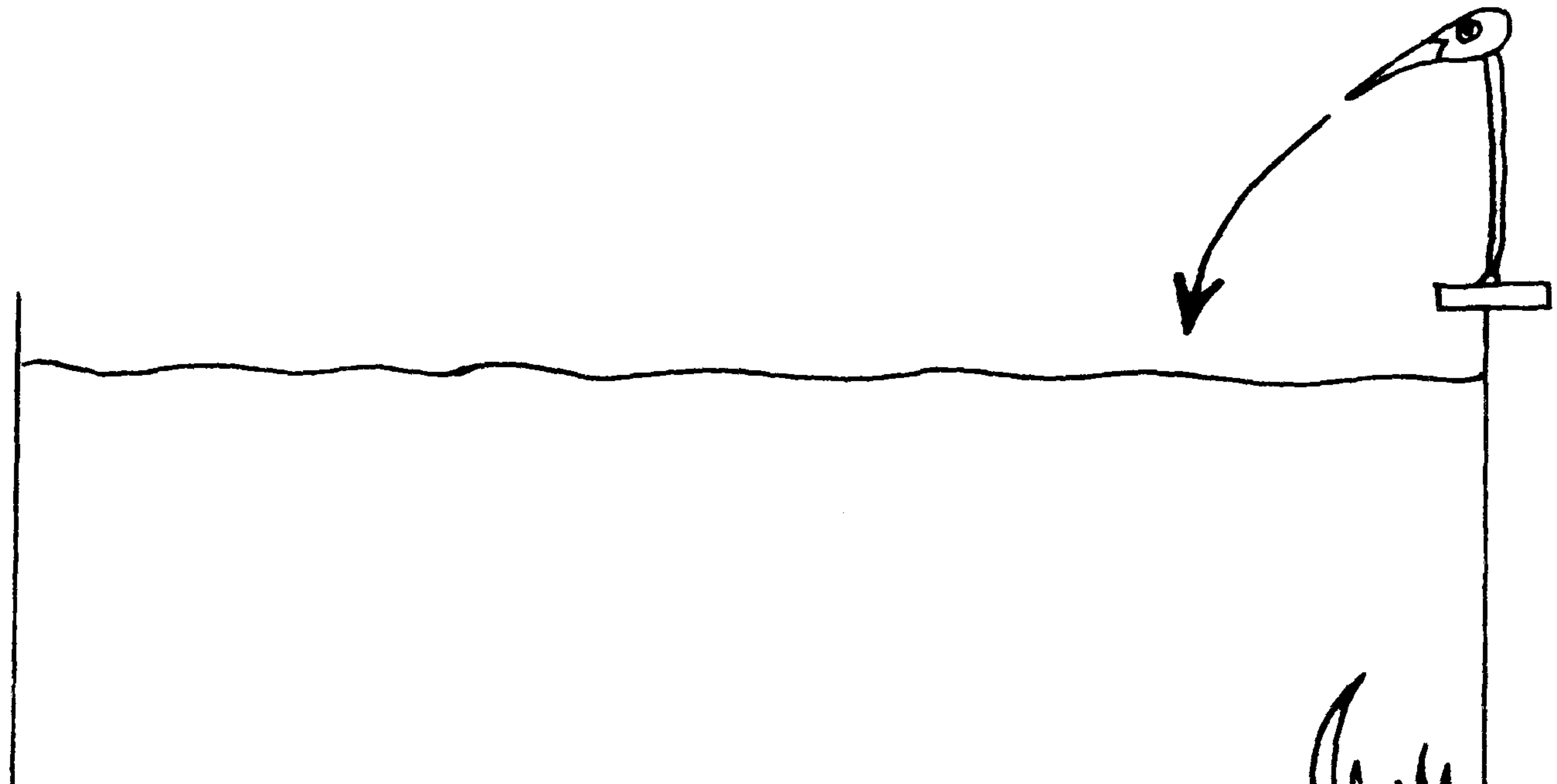
5.3.2.2 Methods

The test tanks used during the Pike and Heron experiments are represented in Fig. 15. During Pike tests the stickleback was introduced into the releasing box and allowed to settle down for 5 minutes. The trap door of the box was then lowered allowing the stickleback to swim out into the open water of the experimental tank, a 10 minute vocal recording of the subsequent behaviour of the fish began at this point. The timing of the moment of truth (definition page 153) was recorded as the first obvious response of the stickleback to the Pike, and this depended upon the activity of the Pike and the alertness of the stickleback.

During the Heron tests the stickleback was introduced into the test tank and allowed 5 minutes to settle down, the vocal commentary



Pike Test Tank



Heron Test Tank

Fig. 15. Scale = x $\frac{1}{100}$

starting at this point. For five minutes a recording of the behaviour of the stickleback was then made (the pre-moment of truth period), this period being terminated by the sudden lowering of the Heron head model performed so that the tip of the bill splashed upon the water surface; the model was then raised back to the vertical position. The sudden presentation of the overhead Heron model was termed the moment of truth. A 5 minute post-moment of truth period of stickleback behaviour was then recorded.

Timetable of anti-predator behaviour experiments

1. Inter-population behavioural variation

May-August 1978; Heron tests 12 adult males and 12 adult females from each population tested.

September-October 1978; Heron tests 12 male fry and 12 female fry from each population tested.

N.B. Fry were sexed subsequent to testing; females were usually larger and had external signs of ovarian development; a few males had precociously developed red throats.

May-August 1979; Pike tests 12 adult males and 12 adult females from each population tested.

2. Ontogeny of the fright response in Heron tests

July 1978; Heron tests 50 fry from the Mar Burn and Loch Lomond tested.

August 1978; Heron tests 50 larger fry from the Mar Burn and Loch Lomond tested.

June-July 1979; Heron tests 50 fry from Mar Burn tested (rest of sample reared predator naive).

August 1979; Heron tests 50 laboratory reared fry from Mar Burn tested.

Data recording and statistical analysis

The elements of stickleback behaviour recorded in the Pike and Heron tests are listed and defined below:

		Whether recorded in:	
Behaviour	**Definition**	**Pike tests**	**Heron tests**
Position in tank			
At surface:	Remaining at the water surface for more than .5 seconds	✓	✓
At bottom:	Laying upon the substrate, usually still, for more than .5 seconds	✓	✓
In weed:	Remaining within artificial weed clump provided in experimental tank	✓	✓
Open water:	Remaining at least 1 cm away from the water surface, substrate, or weed clump	✓	✓
Spine movements			
Dorsal spines raised:	Total or partial erection of one or more dorsal spines	✓	✓
Ventral spines raised:	One or both ventral spines raised		

		Whether recorded in	
Behaviour	**Definition**	**Pike tests**	**Heron tests**
Locomotion type			
Sneaky swimming:	Smooth type of swimming usually along the bottom using caudal and pectoral fins with dorsal and ventral spines lowered	✓	✓
Jerky swimming:	Fast agitated swimming using pectoral fins typified by abrupt stopping and starting	✓	✓
Normal swimming:	Slow bouts of pectoral swimming with frequent pauses and stoppages	✓	✓
Still:	Remaining stationary in any part of the experimental tank	✓	✓
Barage balloon:	Slow vertical ascent to water surface with little or no fin movements, facilitated by swim bladder expansion	✓	✓

		Whether recorded in	
Behaviour	Definition	Pike tests	Heron tests
Locomotion type			
Jump away:	A rapid body flexure and stroke of the caudal fin causing a fast "leap" through the water usually to a place of cover	✓	✓
Predator stimulus			
Moment of truth:	Heron tests: presentation of overhead stimulus		✓
	Pike tests: the first detectable moment in the test when the stickleback becomes aware of the presence of the Pike	✓	
Direct reaction to Pike			
Facing:	Watching the pike from a head-on position	✓	-
Monocular fixation:	Watching Pike through one eye	✓	-
Facing away:	Looking directly away with tail toward Pike	✓	-
Bite:	Biting the Pike during a Pike test	✓	-

Behaviour	Definition	Whether recorded in Pike tests	Whether recorded in Heron tests
Direct reaction to Pike			
Feed:	Ingesting any food item during the test	✓	✓
Approach:	Any direct advance toward the Pike over a distance of more than 1-2 cm.	✓	-
Retreat:	Any direct movement away from the Pike over a distance of more than 1-2 cm.	✓	-

In the Pike tests all behaviours relating to the presence and position of the Pike were recorded subsequent to the moment of truth. A cassette tape recorder was used to obtain the recordings of the stickleback's behaviour. At a later date the tapes were played back and transcribed onto computer paper tape, each behaviour recorded was punched as a number code together with a simultaneous time marker (accurate to 0.5 seconds). Data from the tape recordings was transcribed onto computer tape and the following information extracted: each experiment was split into a pre-moment of truth (m.o.t.) period and a post-m.o.t. period and the frequency of occurrence and total duration of each of the recorded behaviours was calculated for each of these periods. The frequency of performance of each behaviour pattern was then converted to a rate per minute value for the pre-m.o.t. and post-m.o.t. periods for each fish tested, and the duration of each behaviour was converted to an average proportion of each minute spent performing that behaviour during the pre-m.o.t. and post-m.o.t. periods. This procedure was necessary because the timing of the m.o.t. was very variable in the Pike tests and therefore the frequency and duration of each behaviour needed to be related to the length of the pre and post-m.o.t. periods in a given test. Summary statistics were then calculated for the frequency per minute and duration of behaviours performed by each experimental group of fish (adult males, adult females and fry from each population). This analysis produced a mean value, associated standard deviation, frequency distribution and median score for each behaviour within each experimental group of fish (e.g. 12 adult Loch Lomond male fish: Heron tests). An inspection of the frequency distributions for the frequency and duration of behaviours within groups of fish revealed that most were skewed and that very few even approximately conformed to a normal distribution, furthermore no mathematical data transformation would have normalised these distributions. A non-parametric statistical approach was therefore adopted in the subsequent analysis of this behavioural data.

5.3.3.1 Heron tests: interpopulation comparisons

Results

The results of the Heron tests on adults and fry from the seven study populations were arranged in a matrix: behaviours in columns and individual fish in rows, and reviewed by eye. By omitting the columns containing almost all zero values a simplified matrix was constructed using the following behaviours: frequency of normal swimming, remaining still, remaining in open water, feeding, remaining in weed, remaining at bottom, and duration of normal swimming, remaining still, remaining in open water, remaining in weed, remaining at bottom, all of these values being for the pre-m.o.t. periods from the experiments. The experimental groups of fish forming the columns of the matrix were 12 adult males, 12 adult females, 12 male fry, 12 female fry for each of the seven study populations. Each cell within the matrix therefore contained a mean value for the frequency or duration of a behaviour for the 12 fish within an experimental group. A similar matrix was constructed for the post-m.o.t. data using the same behaviours as above, together with the recovery time (after the m.o.t.) for each experimental run (mean value). Each data matrix (the pre-m.o.t. and post-m.o.t.) was subjected to a Friedman 2-way analysis of variance with the behaviours forming the rows and the fish the columns. The Friedman 2-way anova involves ranking each row (behaviour) across the columns (fish) and calculating the sum of the rank scores for each column; one can then test the Null hypothesis that the columns of ranks came from the same statistical population, rejection of H_o indicating a significant behavioural difference between columns. The pre-m.o.t. data matrix gave a value of $\chi^2 = 12.286$, with 27 degrees of freedom, $p > .99$, H_o was therefore accepted indicating that there are no significant differences in rank scores between columns. The post-m.o.t. data matrix gave a value of $\chi^2 = 8.27$, with 27 degrees of freedom, $p > .99$, again therefore H_o was accepted. During the performance of the experiments however there were clear observable qualitative and quantitative differences in behaviour between male and female fish and between fish from different populations; the data matrices were therefore re-analysed. The post-m.o.t. data matrix was split into two categories:

1. <u>Highly developed fright responses</u> - duration of still, remaining in weed, remaining at bottom, recovery time.
2. <u>Poorly developed fright responses</u> - frequency of normal swimming, duration of normal swimming, frequency of feeding, duration in open water.

A Friedman 2-way anova was then calculated for each matrix (behaviours in rows, fish in columns). The highly developed fright response matrix gave a χ^2 value of 72.98, with 27 degrees of freedom, $p < .001$, the poorly developed fright response matrix gave a χ^2 value of 93.85, with 27 degrees of freedom, $p < .001$, thus in both cases H_o was rejected. This finding indicates that within the complete data matrix tested originally there are opposing groups of behaviours (here termed highly developed and poorly developed fright responses) which balance each other out within columns leading to acceptance of the null hypothesis. By testing these groups of behaviours separately highly significant differences in behaviour between experimental groups of fish are found within each of the simplified matrices. Strictly the variables used in the anova should be independent; it is possible that the above result may, in part be due to correlations between the behaviour patterns included in the simplified matrix. The subsequent use of Principal Components analysis strengthens any conclusions drawn from this test.

Table 23 summarises the responses of the adult Heron-tested fish at the m.o.t; all adult males and females from Loch Lomond and the Mar Burn jumped away either to remain motionless in the weed clump or at the bottom of the test tank. Fish remaining still at the bottom relied upon their cryptic camouflage, and raised their dorsal spines; commonly fish would jump away two or three times in succession before remaining still (Humphries and Driver (1967) term this response a protean display). Adult male and female Gasterosteus from the Uist populations usually jumped away at the m.o.t. but often remained still in open water (except Daimh males and Bharpa females). The tendency to remain still in open water after jumping away at the m.o.t. may be explained by the brown peat-stained waters which form the natural habitat of these fish, where the mottled colouration of the fish may

Table 23. Responses of Adult Heron tested Gasterosteus from all populations at m.o.t.

		n	Number of fish continuing to swim normally	Number of fish remaining still	Number of fish jumping away	Number of fish jumping away to		
						open water	weed clump	bottom
LOMOND	MALES	12			12		1	11
	FEMALES	12			12	1	5	6
MAR	MALES	12			12		3	9
	FEMALES	12			12		3	9
LUGGIE	MALES	12	3	2	7		4	3
	FEMALES	12		3	9	2	3	4
LENNOX	MALES	12		1	11	1	2	8
	FEMALES	12		6	6	5		1
DAIMH	MALES	11			11			11
	FEMALES	12		5	7	3		4
FADA	MALES	12		3	9	4	2	3
	FEMALES	12			12	5	2	5
BHARPA	MALES	12		3	9	5	2	2
	FEMALES	12			12	1		11

may blend into the background water colour and render a frightened stickleback invisible to an overhead predator. Lennox fish were intermediate in their response with 11 males jumping away mostly to hide at the bottom but with 6 females and 1 male remaining still in open water at the m.o.t. Of the Luggie fish (lowest risk of predation in wild) 3 males continued to swim normally at the m.o.t. and 2 collected nest-building material during the post-m.o.t. period of the test, 5 of the 24 adult fish remained still in open water and of those that jumped away equal numbers sought refuge either in the weed clump or at the bottom. These results support the thesis that fish from heavily predated populations (e.g. Mar and Lomond) have, on average better developed fright responses than fish from low predation risk sites (e.g. Luggie). These data were insufficent to test statistically (chi square analysis with grouped data (24 fish per population) expected frequencies would be 3.7 per box, 5 being the usual minimal sample size).

The best simple measure of response to the presentation of the Heron head model (= the moment of truth) proved to be the recovery time taken for a fish to resume normal swimming in open water after stopping swimming and remaining still, or jumping away and remaining still at the m.o.t.. Table 24 summarises the mean recovery times for adult males, adult females and fry (male and female fry grouped data) ranked from high to low, left to right.

The differences described above were tested for statistical significance with 2-tailed Mann-Whitney U tests, the 5% probability level being taken as the rejection point. There was no significant difference in recovery times between Mar males, Lomond males and Lennox males. Mar males had a significantly longer recovery time than Bharpa males ($p < .002$), Fada males ($p < .002$) and Luggie males ($p < .002$). Lomond males had a significantly longer recovery period than Daimh,

Table 24. Mean recovery times (seconds) after the m.o.t.: Heron tests

Breeding males

MAR$\begin{pmatrix}\bar{x} = 436\\ s.d. = 146\end{pmatrix}$ LOMOND$\begin{pmatrix}\bar{x} = 427\\ s.d. = 166\end{pmatrix}$ LENNOX$\begin{pmatrix}\bar{x} = 398\\ s.d. = 201\end{pmatrix}$ DAIMH$\begin{pmatrix}\bar{x} = 212\\ s.d. = 88\end{pmatrix}$

BHARPA$\begin{pmatrix}\bar{x} = 125\\ s.d. = 92\end{pmatrix}$ FADA$\begin{pmatrix}\bar{x} = 102\\ s.d. = 105\end{pmatrix}$ LUGGIE$\begin{pmatrix}\bar{x} = 68\\ s.d. = 81\end{pmatrix}$

Breeding females

MAR$\begin{pmatrix}\bar{x} = 567\\ s.d. = 69\end{pmatrix}$ LOMOND$\begin{pmatrix}\bar{x} = 483\\ s.d. = 160\end{pmatrix}$ LUGGIE$\begin{pmatrix}\bar{x} = 386\\ s.d. = 227\end{pmatrix}$ BHARPA$\begin{pmatrix}\bar{x} = 244\\ s.d. = 148\end{pmatrix}$

FADA$\begin{pmatrix}\bar{x} = 236\\ s.d. = 144\end{pmatrix}$ LENNOX$\begin{pmatrix}\bar{x} = 170\\ s.d. = 167\end{pmatrix}$ DAIMH$\begin{pmatrix}\bar{x} = 86\\ s.d. = 59\end{pmatrix}$

Fry

LOMOND$\begin{pmatrix}\bar{x} = 473.5\\ s.d. = 135\end{pmatrix}$ MAR$\begin{pmatrix}\bar{x} = 338\\ s.d. = 152\end{pmatrix}$ LENNOX$\begin{pmatrix}\bar{x} = 304\\ s.d. = 182\end{pmatrix}$ DAIMH$\begin{pmatrix}\bar{x} = 256.5\\ s.d. = 166\end{pmatrix}$

FADA$\begin{pmatrix}\bar{x} = 211\\ s.d. = 138\end{pmatrix}$ LUGGIE$\begin{pmatrix}\bar{x} = 162\\ s.d. = 121\end{pmatrix}$ BHARPA$\begin{pmatrix}\bar{x} = 59\\ s.d. = 49\end{pmatrix}$

Fada, Bharpa and Lennox males ($p < .002$ in all cases). Lennox males had a significantly longer recovery period than Luggie males ($p < .002$). Daimh males had a significantly longer recovery period than Bharpa males ($p < .02$), Fada males ($p < .002$), and Luggie males ($p < .002$). There was no significant difference in recovery time between Fada and Bharpa males or Fada males and Luggie males.

There was no significant difference between Mar adult females and Lomond females or between Lomond females and Luggie females, Mar females had a significantly longer recovery period than Luggie females ($p < .02$). Mar females also had a significantly longer recovery time than Bharpa, Fada, Lennox and Daimh females ($p < .002$ in all cases). Bharpa and Fada females took significantly longer to recover than Daimh females ($p < .02$ in both cases), there was no difference between Bharpa and Fada females. Among the fry tested Lomond fry had significantly longer recovery periods than Mar fry ($p < .002$), Luggie fry than Lennox fry ($p < .05$), Daimh fry than Bharpa fry ($p < .002$) and Fada fry than Bharpa fry ($p < .002$). There was no significant difference between Daimh and Fada fry.

5.3.3.2 Discussion

Amongst the breeding adult fish Mar males and females had the longest recovery times for any of the seven study populations, these results correlating well with the known high predation risk from herons at this site (page 221, appendix). Lomond males and females were second highest in mean recovery time and are also known to be at risk from a wide variety of avian (and fish) predators. Amongst breeding males Luggie fish had the lowest recovery time and are at the lowest risk of avian predation of any of the study populations. Luggie adult females show a surprisingly high mean recovery period (386 seconds) and there is a tendency in several of the populations (Luggie, Mar, Lomond, Bharpa, Fada) for females to show a longer recovery time than males. Adult male Gasterosteus in the breeding season occupy territories which often contain an egg-filled nest liable to egg-stealing by conspecifics and other predators, and also have to attract gravid females in order to maximise their reproductive success. Adult female Gasterosteus do not exhibit parental care or territoriality and tend to be found in cover during the breeding season (page 41). Both sexes have to escape from predators and it is therefore possible that the shorter recovery period exhibited by males is linked with the need to re-occupy their territory as quickly as possible; females are not subjected to this constraint and may therefore have evolved longer recovery times. Interestingly, however, Lennox and Daimh males show considerably longer mean recovery periods than females from these populations and the above theory cannot therefore be a generalisation. The long recovery time of the Lennox males agrees well with the high potential risk of Gull predation at this site; this does not explain the low average recovery time of Lennox females however. A possible explanation for the anomaly lies in the fact that upon dissection only 3 of the 12 males tested were found to be parasitised by Schistocephalus solidus plerocercoides but all 12 of the females were heavily parasitised (1-8 larvae per fish), S. solidus is known to affect Gasterosteus behaviour (Lester 1971).

Both adult males and females from the Uist populations show relatively short mean recovery times, a behavioural strategy possibly linked to the peat-stained waters of the Uist lochs; the recovery times of the fry from these populations showed a similar pattern to those of the adults. Loch Lomond fry show a higher mean recovery time than Lomond adult male fish, this may, in part, be related to the high risk of predation that Lomond Gasterosteus experience from Perch in late Autumn and Winter.

5.3.3.3 Principal components analysis

In order to analyse the data obtained from the Heron tests economically a P.C.A. (B.M.D. program , Dixon 1973) was run on the data matrix (average frequencies and durations of behaviours per minute). Variables that were composed largely of zero scores were omitted. Initially the program calculates a Pearson product-moment correlation matrix for the behaviour scores and on the basis of these correlations it constructs a new set of mutually uncorrelated axes or components. P.C.A. is a non-inferential technique since no assumptions concerning the statistical population from which the sample was taken are made (Davies 1971). Hope (1968), Child (1970), Davies (1971) and Maxwell (1977) all give concise explanations of P.C.A., the works of Huntingford (1976) and Aspey and Blankenship (1977) provide examples of the application of P.C.A. to ethological data. Briefly P.C.A. is a method of multi-variate analysis which essentially simplifies complex heterogeneous data matrices by grouping numbers of the original variables into compound components. A P.C.A. including 16 variables will produce 16 components accounting for successively smaller proportions of the total variation (difference between individuals) within the data matrix. The first component accounts for the largest proportion of the total variance (Eigenvalue), the second component (orthogonal to the first) accounts for the next largest proportion of the total variance and so on until each successive component is only accounting for a minimal proportion of the variation present. Because the first few components may account for the majority of the total variance within the correlation matrix those components which collectively account for the remaining small proportion of variation can be ignored for some purposes. The principal components are thus compound descriptors of differences in the occurrence of the original variables within the matrix. In the present study behaviours which were highly correlated were grouped together and opposed to a behaviour grouping which differed maximally from them along a given axis (component). This process divides the variates into subgroupings which may reveal relationships between the original variables which may otherwise go undetected (Maxwell 1977). The degree to which a given behaviour contributes toward a component is termed the loading of the behaviour upon that component and can vary within the values +1 to -1; the initial sign is arbitrarily assigned and the relative signs and loadings of the subsequent behaviours are of importance. It is customary to ignore loadings between values of +.5 and -.5. Because the components are mutually uncorrelated they serve as a series of independent indices which can be used to describe the original data matrix economically (Maxwell 1977). It is important that the Pearson correlation matrix should not depend upon a small number of extreme values; this possibility was minimised during the present study by omitting behaviours with few positive scores from the matrix and by producing large sample sizes (336 fish for Heron tests, 168 fish for Pike tests). In each of the P.C.A.'s described in the following section a "varimax rotation" has been performed upon the components; this procedure seeks to maximise both the variance of the loadings of different acts on the same component and also the variance of loadings of the same act on different components. This process gives rise to either relatively large or relatively small values when compared with the original component loading (Maxwell 1977).

Huntingford (1976) describes the application of the varimax rotation technique to P.C.A.'s of behavioural data. The new maximised compound indices which emerge from the rotated components are termed factors; in the following discussion of the analysis of the Heron test and Pike test data the Factor 1 scores for each fish are used to economically describe the anti-predator behaviour of the experimental groups of fish. When interpreting the results of a P.C.A. it is usual to include those components which both account for a large proportion of the total variance and illustrate a useful descriptive relationship within the data matrix. In the P.C.A.'s performed upon data from the present study (Heron tests, pre-m.o.t. Pike tests, post-m.o.t. Pike tests) the first factor (rotated component) to emerge accounted for 25-27% of the total variance and proved to be readily interpretable in terms of the structure of the anti-predator behaviour. Subsequent factors accounting for considerably smaller proportions of the individual behavioural variation within the data matrices, were less readily interpretable in behavioural terms, and have therefore been omitted from the present discussion. The structure (behaviour loadings) of the first 5 factors which emerged from each of the 3 P.C.A.'s performed are appended to this thesis (pages 214 to 216) for the reader's reference.

Results

The factors resulting from the Heron test data P.C.A. were examined for biological significance, and the first factor which accounted for 27% of the total variance in the correlation matrix contained the behaviours described in Fig. 16. Fish which scored positively on Factor 1 performed a lot of post-m.o.t. swimming bouts in open water, pausing frequently to feed, and recovered quickly from the overhead fright stimulus at the m.o.t., i.e. they exhibited a poorly developed fright response. Fish which scored negatively on Factor 1 had a long recovery time, remaining still for long periods either at the bottom or in the weed during the post-m.o.t. phase of the experiments, i.e. they exhibited a highly developed fright response to the overhead stimulus. In the Appendix (page 210) the behaviour scores (means and standard deviations) for the principal behaviours included in Factor 1 (fig. 16) are detailed for all groups of fish tested. Factor 1 scores clearly provide a good compound measure of fright response level with which to compare the experimental groups of fish tested. The result of the P.C.A. also lends objective support to the subjective selection of behaviour groupings used in the Friedman 2-way analyses of variance. It is important to note that the pre-m.o.t. behaviour scores loaded similarly to the post-m.o.t. behaviour scores indicating that fish which reacted strongly to the predator stimulus also tended to be cautious in the unfamiliar environment of the experimental tank.

Table 25 gives the mean Factor 1 scores for each of the Heron tested groups of sticklebacks (data from male and female fry were pooled), statistical comparisons were drawn between groups using 2-tailed Mann-Whitney U tests. There were no significant within-population differences in Factor 1 scores between male and female fry from any of the seven populations. Lomond fry had significantly lower factor 1 scores (≡ higher fright response) than Bharpa, Luggie or Fada fry ($p < .002$ in all cases). Mar fry had significantly lower factor 1 scores than Luggie fry ($p < .002$). The following comparisons were non-significant at the 5% level: Lomond:Mar; Lomond:Lennox; Lomond:Daimh; Mar:Lennox; Mar:Daimh; Diamh:Lennox; Daimh:Bharpa; Daimh:Fada; Daimh:Luggie; Luggie:Lennox; Luggie:Fada; Bharpa:Fada; Lennox:Fada; Lennox:Bharpa. The post-m.o.t. behaviour of the fry was thus similar between most populations except for Lomond and Mar fry which, on average, scored negative Factor 1 scores (i.e. had better developed fright responses).

Amongst breeding males Mar fish had significantly lower Factor 1 scores than Luggie, Bharpa and Fada males ($p < .002$ in all cases) and Daimh males ($p < .05$). There was no significant difference

Fig. 16. Factor 1 (rotated first component) behaviour loadings: Heron Tests

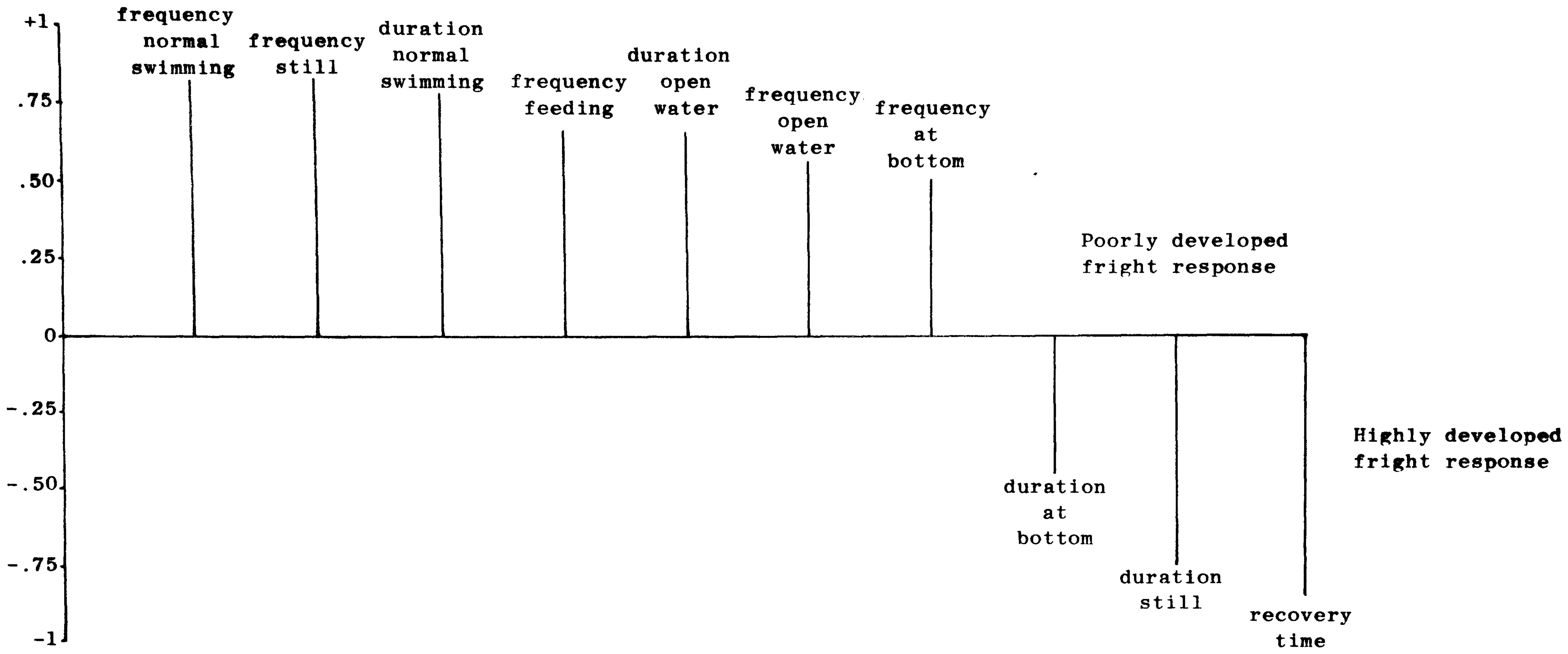


Table 25. Factor 1 scores from Principal Components Analysis of Heron test data

	FADA			BHARPA			DAIMH			LOMOND			LUGGIE			MAR			LENNOX		
	♂	♀	Fry	♂	♀	Fry	♂	♀	Fry	♂	♀	Fry	♂	♀	Fry	♂	♀	Fry	♂	♀	Fry
$\bar{x}$	.3	-.17	.54	.3	.06	.5	-.07	.49	.01	-.5	-.75	-.54	1.7	-.68	.53	-.8	-.9	-.3	-.42	-.05	.07
s.d.	.67	.79	.7	.7	.59	.42	1.14	.9	.9	1.15	.64	.55	1.4	.82	1.07	.42	.51	.94	1.01	.39	.96

between Bharpa males and Lomond or Lennox males. Lomond males had significantly lower Factor 1 scores than Luggie males ($p < .002$) and Fada males ($p < .02$), there was no significant difference between Lomond males and Daimh, Lennox or Bharpa males. Fada males had significantly higher scores than Daimh males ($p < .05$), comparisons between Fada:Bharpa and Bharpa:Daimh were non-significant.

Amongst breeding females Mar fish had significantly lower scores than Lennox, Daimh and Bharpa females ($p < .002$ in all cases) and Fada females ($p < .02$); there was no significant difference between Mar females and Lomond or Luggie females. Fada females had significantly lower Factor 1 scores than Bharpa females ($p = .05$), there were no significant differences between Fada and Daimh females or Bharpa and Daimh females.

Discussion

The differences between populations on the compound behavioural index of the first P.C.A. factor agree well with the thesis that fish from heavily bird-predated populations have better developed fright responses than fish at low risk from predators, with Mar and Lomond adults having the lowest mean scores and Luggie males the highest scores.

5.3.4.1 Development of the overhead fright response in Gasterosteus

The data resulting from the Heron tests indicate clear differences in the fright responses of adult and juvenile Gasterosteus. The following experiments were designed to allow a description of the development of the response and to provide information on whether it is primarily controlled by inherited or experiencial factors.

5.3.4.2 Methods

During the experiments performed upon the development of the overhead fright response in Mar burn and Loch Lomond Gasterosteus fry a simplified approach was adopted where the following behavioural categories were used:

Normal swimming: continuing to swim normally immediately after the moment of truth.

Still: stopping swimming at the moment of truth and continuing to remain still.

Jump away: leaping away from the overhead stimulus usually followed by a still period.

Recovery time: the period of time in seconds between stopping swimming or jumping away at the moment of truth and resuming normal swimming.

During these experiments all fish were released into the test tank and allowed to settle down for 5 minutes. The Heron model was then lowered and the response recorded. In this way a good indication of the fright response of individual fish was gained whilst the tests were short, and the data obtained in a readily analysable form.

5.3.4.3 Experiments on wild Gasterosteus fry

During the month of July 1978 samples of young Gasterosteus fry were taken from the Mar burn and Loch Lomond, brought into the laboratory, housed in large communal tanks and allowed to settle down overnight (plant cover and planktonic food were provided in all tanks). On the day following their capture a sub-sample of 50 were tested in the Heron test tank using the short test procedure explained above.

Results

The results of these tests are presented in table 26. Both

Table 26. Responses of small, Heron tested, *Gasterosteus* fry (July 1978)

	n	Mean total body length (mm)	Total still after m.o.t.	Mean recovery time (seconds)	Total swimming normally after m.o.t.	Total jumping away at m.o.t.
Lomond	50	12.5 (s.d. = 2.2)	26	5.26 (s.d. = 2.5)	24	0
Mar	50	13.8 (s.d. = 2.5)	32	5.96 (s.d. = 2.9)	18	0

groups of fish (Mar burn and Loch Lomond sub-samples) behaved similarly with approximately half of the fish continuing to swim normally after the m.o.t. and half remaining still with a short recovery period, no fish from either sample jumped away in alarm at the m.o.t.

In August 1978 further samples of Gasterosteus fry were taken from the Mar burn and Loch Lomond. A sub-sample of 50 of these larger fry from each population was Heron tested following the same procedure as above. The results of these experiments are presented in Table 27. An obvious marked change in the fright response is evident in the behaviour of the samples of the larger fry. No fish continued to swim normally immediately after the m.o.t. (cf. Table 26), small numbers of fish stopped swimming, remaining still for a mean recovery period of 16.4 seconds (Lomond) and 18.8 seconds (Mar). The majority of the larger fry jumped away at the m.o.t. and remained still for a considerable recovery period (mean 50 seconds, Lomond and mean 44.2 seconds Mar). Of the Lomond fry that jumped away 5 remained "still" in open water, 8 at the bottom, and 25 in the weed clump provided. Of the Mar fry that jumped away 5 remained still in open water, 13 at the bottom, and 27 in the weed clump.

The recovery time data for the smaller fry remaining still (Table 26) and the grouped recovery time data for larger fry remaining still, and jumping away and then remaining still (Table 27) were compared using 2-tailed Mann-Whitney U tests. A highly significant difference was found for both Mar ($p < .002$) and Lomond ($p < .002$) fry with larger fry having a longer post-m.o.t. recovery time than smaller fry.

5.3.4.4 Experiments on predator naive Gasterosteus fry

A further series of experiments was then planned in order to

Table 27. Responses of larger, Heron tests, Gasterosteus fry (August 1978)

	n	Mean total body length (mm)	Total still after m.o.t.	Mean recovery time from still (seconds)	Total swimming normally after m.o.t.	Total jumping away at m.o.t.	Mean recovery time from jump away (seconds)
Lomond	50	29.3 (s.d. = 2.5)	12	16.4 (s.d. = 13)	0	38	50 (s.d. = 42.6)
Mar	50	30.3 (s.d. = 2.9)	5	18.8 (s.d. = 3.1)	0	45	44.2 (s.d. = 19.8

discover whether the fright response to an overhead stimulus developed normally in predator naive Gasterosteus fry reared in the laboratory. In July 1979 a large sample of small stickleback fry was taken from the Mar burn, brought back to the laboratory, and housed in a large (2 M x 1 M x 50 cm) tank; the growing fry were fed daily on small planktonic crustacea. A sub-sample of 25 fish (mean total body length 10.4 mm) was Heron tested on the day following their capture; 8 fish continued to swim normally at the m.o.t., 17 stopped swimming at the m.o.t. with a mean recovery time of 4.2 seconds. A low level overhead fright response was therefore present in these young fry, which behaved similarly to the young fish sampled in July 1978. The rest of the initial sample was then raised in the laboratory, being fed daily by pouring a plankton sample down a tube to release the food underwater in the tank. In this way there was no possibility of the fry becoming conditioned to associating an overhead movement with being fed. After 2 weeks of growth in the laboratory a further sample of 25 fish (mean total length 15.3 mm) was tested; 4 fish continued to swim normally at the m.o.t., 20 stopped swimming at the m.o.t., no fish jumped away. The mean recovery time for the fish which stopped swimming was 8.47 seconds which, when compared with the July 1978 recovery times for the 13.8 mm (mean body length) fry (5.96 seconds) shows no significant difference at the 5% level (2-tailed Mann-Whitney U test). The remaining fry were reared for a further two weeks under laboratory conditions and a sample of 50 fish (mean body size 30.6 mm) were Heron tested, one fish continued to swim normally at the m.o.t., 8 stopped swimming and remained still for a mean time of 38.6 seconds, 41 fish jumped away with a mean recovery time of 56.68 seconds. Of the fish that jumped away 23 remained in open water, 9 in weed and 9 at the bottom. When the grouped recovery time data for fish which remained still, and fish which jumped away and remained still, is compared between wild (August 1978) and laboratory-reared (August 1979) fry (mean body length of both samples c. 30 mm) there is no significant difference at the 5% level (2-tailed Mann-Whitney U test).

Experiments described in the previous section include data on Heron test recovery times for 24 Mar burn sticklebacks of body lengths 35-40 mm sampled in October 1978; the mean recovery time for these fish was 338 seconds. When the recovery times from these fish are compared (2-tailed Mann-Whitney U test) with the recovery times from wild 30 mm Mar burn fry collected in August 1978 (Table 27) a highly significant difference emerges ($p < .002$) indicating that the response heightens with age/body size. This effect can be seen to continue with adult Mar males and females which have mean recovery times of 436 seconds (Heron tested males) and 567 seconds (Heron tested females).

Discussion

It therefore appears that the development of the overhead fright response in young Mar burn Gasterosteus fry occurs normally in fry which were laboratory reared from an early age, and thus independently of experience of predatory birds. The results of this series of experiments is summarised in Table 28 and indicates an inherited basis for the response. It is possible that some of the fry sampled at a mean size of 10.4 mm from the Mar burn already had experience of Herons fishing the burn and that the subsequent development of their fright response was influenced by this experience. However, the simultaneous appearance of the heightened response within all but one individual of the 30.6 mm fry indicates strongly that the behaviour has a genetic basis. It is of interest to note that the majority of wild 30 mm fry jumped away into the weed whereas the majority of

Table 28. Summary of Heron test results for laboratory reared Mar Burn fry (1979)

	n	Mean body size (mm)	Total still after m.o.t.	Mean recovery time from still	Total jumping away at m.o.t.	Mean recovery time from jumping away	Total swimming normally after m.o.t.
Newly sampled fish	25	10.4 (s.d. = 1.8)	17	4.2 (s.d. = 2.7)	0	-	8
Laboratory reared fish	25	15.3 (s.d. = 1.9)	21	8.4 (s.d. = 4.1)	0	-	4
Laboratory reared fish	50	30.6 (s.d. = 2.6)	8	38.6 (s.d. = 16)	41	56.68 (s.d. = 27.5	1

laboratory reared fry jumped away into open water; this effect is unlikely to be due to the rearing tank conditions as abundant weed cover was available within the tank.

The results of this research agree with the existing literature on the development of fright response in fishes and it appears that innate anti-predator responses may be a general property of several diverse fish genera. Kimmel et al (1974) have shown that the adult startle response (tail-flip) of the Zebra danio, Brachydanio rerio is present in a very similar form in hatchling larvae. Dill (1974) used filmed and model predator stimuli to show that this innate response is modified with experience such that escape speed and reaction distance increase in Zebra danios after simulated predator encounters. Jakobsson and Jarvi (1976) used predator inexperienced Atlantic salmon smolts to show that this species has innate avoidance reactions to specific stimuli from a hunting Pike. Pattern (1977) studied the anti-predator behaviour of small (< 45 mm) Coho salmon, Oncorhyncus kisutch fry when they encounter Torrent Sculpin, Cottus rhotheus. The Coho salmon fry had a well developed innate predator avoidance strategy which improved after a single predator experience, Pattern (1977).

5.3.5 Inter-population comparisons of Pike test results

Behavioural data from the pre-m.o.t. and post-m.o.t. phases of the Pike tests were analysed separately in order to facilitate data handling and input into the Glasgow University computer. Whilst conducting the tests the author noticed considerable variation between fish in their tendency to explore the novel environment of the test tank. In order to test whether population differences in exploratory behaviour were present the pre-m.o.t. data matrix (12 adult males, 12 adult females from each population) was split into two groupings:

1. <u>Exploratory behaviour</u>: frequency and duration of normal swimming, frequency of feeding, duration in open water.
2. <u>Timid behaviour</u>: duration of remaining still, duration at bottom, duration in weed, frequency of spine-raising.

A Friedman 2-way analysis of variance was performed upon each of the above groupings for all Pike-tested fish, for the Exploratory behaviour grouping $\chi^2 = 211.3$, with 13 degrees of freedom, $p < .001$ that all groups of fish behaved similarly. For the Timid behaviour group $\chi^2 = 148.5$, with 13 degrees of freedom, $p < .001$ that all groups of fish behaved similarly. Thus significant differences are present between experimental groups of fish in both of the pre-m.o.t. matrices tested.

A P.C.A. was run on the complete pre-m.o.t. pike data matrix (omitting all behaviours which were largely zero scores) and varimax rotation performed upon the components. The first factor emerging from this analysis accounted for 25% of the total variance within the matrix and contained the behaviours included in fig. 17 (factor loadings for each behaviour are given in the figure). It is important to note that the negatively loaded Duration in weed, Duration still and Duration at bottom behaviours have low loadings and are included as an illustration of the behaviours most closely associated with a late m.o.t. in a Pike test. In the Appendix (page 212) the behaviour scores (means and standard deviations) for the principal behaviours included in Factor 1 (Fig. 17) are detailed for all groups of fish tested. Factor 1 from this P.C.A. appears to be a measure of Exploratory (positively loaded) behaviours versus Timid (negatively loaded) behaviours agreeing with the selection of behaviours used in the Friedman anovas above. Factor 1 scores for all experimental groups of fish are presented in Table 29, differences between groups

Fig. 17. Factor 1 (rotated first component) behaviour loadings, Pre-m.o.t. data: Pike tests

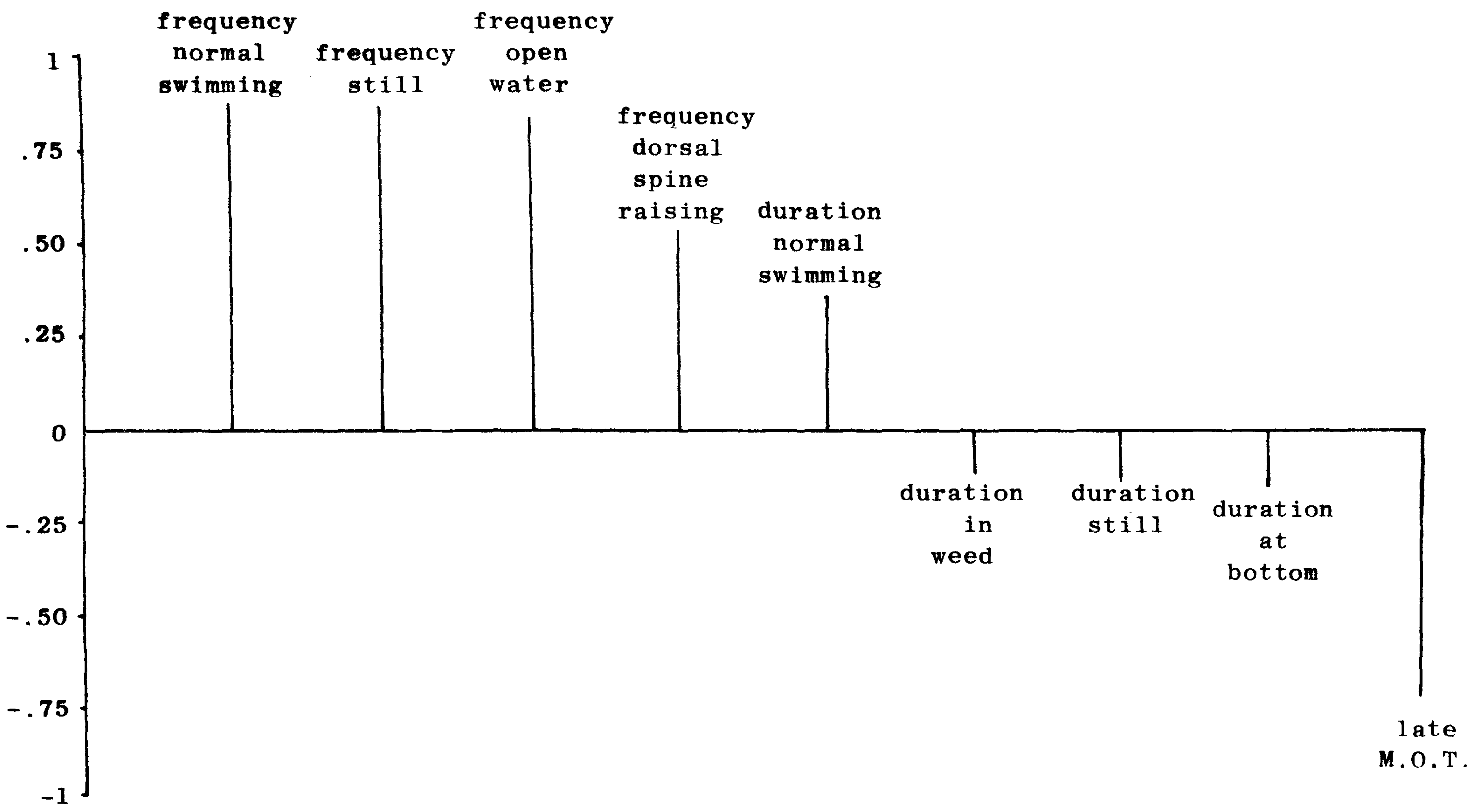


Table 29. Factor 1 scores from Principal Component Analysis of pre m.o.t. Pike test data

	DAIMH		LOMOND		LUGGIE		MAR		BHARPA		LENNOX		FADA	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
$\bar{x}$	-.04	.43	.24	-.37	.19	-.34	.21	-.53	.35	.047	-.05	-.24	.07	.028
s.d.	1.11	1.97	.9	.72	.55	1.02	.52	.45	.98	.67	1.2	.96	.79	.99

were compared using 2-tailed Mann-Whitney U tests. Because of considerable intra-population variability in behaviour before sensing the presence of the Pike none of the inter-population comparisons drawn were significant at the 5% level of probability. It is interesting to note however that, as in the Heron tests, males tended to be more adventurous (score higher values on Factor 1) than females; this is true for all populations except Loch an Daimh in this analysis.

The post-m.o.t. data matrix is of most interest however since here the information describing the anti-fish predator behaviour of Gasterosteus from each study population is contained. As before the matrix was split into two parts:

1. low level fright responses: duration of normal swimming, duration in open water, frequency of biting pike, frequency of approaching pike, frequency of feeding.
2. highly developed fright responses: duration of remaining still, frequency of retreating from Pike, duration of binocular fixation on Pike, duration in weed, duration at bottom, frequency of jumping away, frequency of sneaky swimming.

A Friedman 2-way anova was performed upon each matrix; for the low level fright responses $\chi^2 = 211$, degrees of freedom = 13, $p < .001$, for the highly developed fright responses $\chi^2 = 372$, degrees of freedom = 13, $p < .001$, thus for each matrix significant differences in behaviour are present between sexes of adult Gasterosteus and/or between populations. A P.C.A. was run on the complete post-m.o.t. Pike test data matrix (omitting all behaviours which largely contained zero values), again varimax rotation was applied to the original components to produce a set of factors. Factor 1 accounted for 27%

of the total variance within the matrix, the loadings of the behaviours comprising this factor are presented in Fig. 18. In the Appendix (page 213) the behaviour scores (means and standard deviations) for the principle behaviours included in Factor 1 (Fig. 18) are detailed for all groups of fish tested. Fish which scored positively on Factor 1 swam actively in open water, pausing frequently, and often approached the hunting Pike; such fish also frequently faced away from the Pike and fed fairly often after the m.o.t. Fish which scored negatively on Factor 1 remained still, hiding at the bottom for long periods after sensing the presence of the hunting Pike. In the Pike test P.C.A.'s behaviours loaded similarly during both the pre and post-m.o.t. test periods. A survey of the scores of the fish from the study populations revealed a trend for similar Factor 1 scores in both the pre and post-m.o.t. periods. As with the Heron test data this indicates that the fish which are cautious within the novel environment of the test tank are the individuals which exhibit the most highly developed anti-predator responses. Table 30 gives the mean post-m.o.t. Pike test Factor 1 scores for all fish tested. As in the pre-m.o.t. matrix male fish score more highly than female fish (i.e. males are less cautious); this effect is true for all seven populations in the post-m.o.t. data. The Factor 1 scores were compared between populations with 2-tailed Mann-Whitney U tests. Amongst breeding males there was no significant difference in Factor 1 scores between Lomond, Daimh, Bharpa and Fada fish, all of which scored negatively (cautious behaviour) and all of which have high known or potential fish predation risks. Mar males averaged a low positive mean Factor 1 score and have no known (but two or three potential) sympatric fish predators. Mar males scored significantly higher on Factor 1 than Lomond males ($p < .05$), Daimh males ($p < .05$), Bharpa males ($p < .05$) and Fada males ($p < .05$) and significantly lower than Luggie males ($p < .002$) or Lennox males ($p < .002$).

Fig. 18. Factor 1 (rotated first component) behaviour loadings, post M.o.t. data: Pike tests

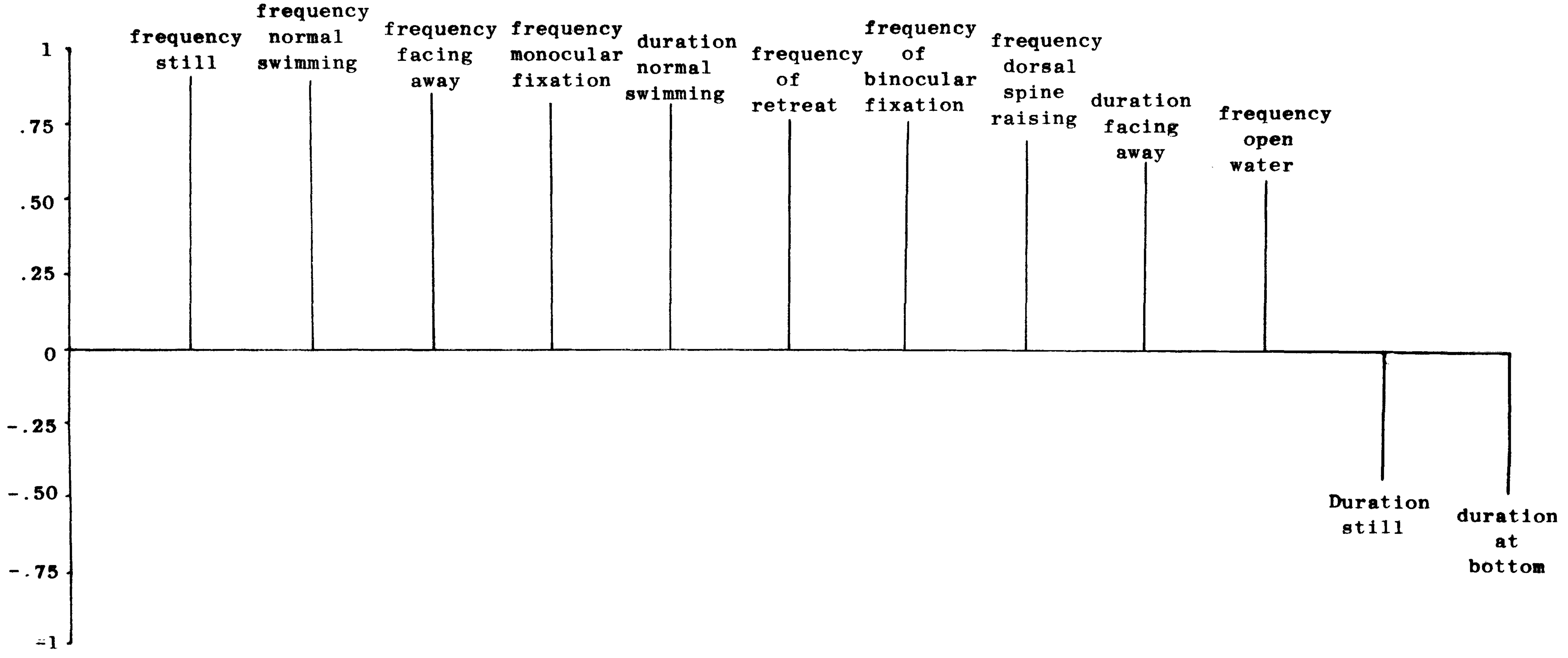


Table 30. Factor 1 scores from Principal Component Analysis of post m.o.t. Pike test data

	DAIMH		LOMOND		LUGGIE		MAR		BHARPA		LENNOX		FADA	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
$\bar{x}$	-.5	-.64	-.33	-.62	.93	.49	.017	-.29	-.45	-.6	1.89	1.38	-.43	-.7
s.d.	.32	.41	.25	.28	.7	.64	.46	.19	.33	.34	1.45	.86	.3	.19

Luggie males have few, if any, potential fish predators and scored significantly more highly on Factor 1 (≡ low level fright response) than Lomond, Mar, Daimh, Bharpa and Fada males ($p < .002$ in all cases). Lennox males have no potential fish predators and scored significantly more highly on Factor 1 than Lomond, Mar, Daimh, Fada and Bharpa males ($p < .002$ in all cases); there was no significant difference between Lennox and Luggie Factor 1 scores.

Amongst breeding females there were no significant differences between Mar, Lomond, Daimh, Fada and Bharpa fish all of which scored negatively (high fright response) on Factor 1. Luggie females scored significantly more highly than Mar females ($p < .02$) and Lomond, Daimh, Fada and Bharpa females ($p < .002$ in all cases). Lennox females (no predation risk) scored significantly more highly than females from all six of the other populations: Luggie ($p < .05$) and $p < .002$ for Mar, Lomond, Daimh, Fada and Bharpa.

Discussion

These data agree with the thesis that anti-predator behaviour is highly developed in those Gasterosteus populations studied where the known or potential risk of fish predation is high, and less well developed or even absent in those populations where the predation risk is low. During the Pike tests 2 adult male Lennox sticklebacks actually approached and bit the moving pectoral fins of the Pike indicating a complete naivety in the face of a hunting predatory fish. Loch Fada and Loch a Bharpa Gasterosteus which almost completely lack dorsal and ventral spines showed no specialised anti-predator behaviour to compensate for their lack of morphological protection. Bell (1974) suggested that spine-deficient Gasterosteus individuals may be more streamlined and better able to escape from underwater predators than normally spined individuals; during the present study no evidence was gained in support of this theory. The findings of this chapter together with those from chapters 2 and 3 are discussed in the next chapter and conclusions are drawn regarding the relationship between predation risk and adaptation at the population level in Gasterosteus.

Summary

1. Under natural conditions Gasterosteus males tended to choose sheltered, shallow water nest sites. Considerable inter-population variability in nest site location is described.

2. Under laboratory conditions nesting males from the study populations varied very significantly in their levels of conspecific aggression and sexual behaviour. Mar males showed the lowest levels of aggression toward a conspecific male, and highest frequency of sexual behaviour toward a gravid female.

3. Friedman 2-way analyses of variance were used to demonstrate significant differences in behaviour between groups of fish tested in the Heron experiments. An analysis of the response of adult fish at the m.o.t. revealed that fish from high predation risk populations (Mar and Lomond) have better developed fright responses than fish from low risk populations (e.g. Luggie).

4. The time taken to resume normal swimming after the m.o.t. (the recovery time) was found to be a good simple descriptor of the level of fright response to the Heron model.

5. A Principal Components Analysis of the Heron test data produced a first factor accounting for c. 27% of the total variance (individual differences in behaviour) within the correlation matrix. This factor

was readily interpretable in terms of the type of fright response exhibited by Heron tested fish. Factor 1 scores differed significantly between the groups of fish tested with fish from high avian predation risk sites (e.g. Mar and Lomond) having lower scores (≡ higher fright response) than (e.g.) Luggie fish where the risk of avian predation is slight.

6. Experiments with wild and laboratory reared Gasterosteus fry show that the overhead fright response becomes well developed at a body size of c. 30 mm, a size corresponding with an increased risk of Heron predation at the Mar burn. Predator naive fry developed the response normally indicating that this behaviour has a genetic basis. Experiments on adult Gasterosteus from the Mar burn indicate that the fright response heightens with age/body size.

7. Friedman 2-way analyses of variance were used to demonstrate significant differences in behaviour between groups of Pike tested sticklebacks. These differences were significant during both the pre and post-m.o.t. periods of the tests.

8. A Principal Components Analysis of the post-m.o.t. Pike test data produced a first factor accounting for c. 27% of the total variance within the correlation matrix. This factor was interpretable as a descriptor for groupings of highly developed and poorly developed anti-predator behaviours. Factor 1 scores differed significantly between populations and correlated well with the predation risks experienced by the groups of tested fish.

9. No obvious behavioural adaptations are present in spine-deficient North Uist Gasterosteus from Loch Fada and Loch a Bharpa which might compensate for their lack of morphological protection.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

This thesis describes ecological, morphological and behavioural studies performed upon seven Gasterosteus aculeatus L. populations, four on the Scottish mainland and three on the Isle of North Uist in the Outer Hebrides; wherever possible an attempt was made to quantify the risk to sticklebacks of predation by piscivorous birds and fish. The aims of the study were to answer the following points:

1. do the Gasterosteus populations studied vary in lateral plate number, dorsal and ventral spine size, presence and reduction or absence of the pelvic girdle complex, body size and shape, in relation to predation risk?

2. do mature male Gasterosteus in the study populations differ in their levels of conspecific aggression and reproductive behaviour, nest site selection and territory size?

3. are inter-population differences in anti-predator behaviour toward aerial and fish predators apparent and, if so, do such differences correlate with the risk of predation at the study sites?

4. is anti-predator behaviour in Gasterosteus principally determined by genetic or experiencial factors?

5. are there any clear behavioural differences which can be attributed to lateral plate phenotype effects rather than population effects?

The detailed results of the study are discussed in the appropriate

	PREDATION RISK		MORPHOLOGY				BEHAVIOUR				
								RESPONSE TO			
	FISH	BIRDS	MEAN LATERAL PLATE NUMBER	CRITICAL DIMENSION INCREASE	DEVELOPMENT OF PELVIC GIRDLE	Ca^{2+} Mg/L	PREDOMINANT NEST SITE CHOICE	LIVE PIKE	HERON MODEL	CONSPECIFIC MALE	CONSPECIFIC FEMALE
FADA	KNOWN HIGH	ESTIMATED HIGH	ZERO	-	.29% COMPLETE	2.25	OPEN SHALLOW LITTORAL STREAMS	HIGH ♂ HIGH ♀	LOW ♂ MEDIUM ♀	HIGH AGGRESSION	MEDIUM SEXUAL RESPONSE
BHARPA	ESTIMATED HIGH	ESTIMATED HIGH	ZERO	.26	3.1% COMPLETE	1.9	OPEN SHALLOW LITTORAL STREAMS	HIGH ♂ HIGH ♀	LOW ♂ MEDIUM ♀	MEDIUM AGGRESSION	LOW SEXUAL RESPONSE
DAIMH	ESTIMATED HIGH	ESTIMATED HIGH	2.7	.83	97% COMPLETE	3.55	STREAMS, WEED BEDS	HIGH ♂ HIGH ♀	MEDIUM ♂ LOW ♀	MEDIUM AGGRESSION	MEDIUM SEXUAL RESPONSE
LOMOND	KNOWN HIGH	KNOWN HIGH	5.4	1.05	100% COMPLETE	3.8	SHELTERED SHALLOW LITTORAL STREAMS	HIGH ♂ HIGH ♀	HIGH ♂ HIGH ♀	HIGH AGGRESSION	HIGH SEXUAL RESPONSE
LUGGIE	KNOWN LOW	KNOWN LOW	4.5	.73	100% COMPLETE	30.1	SHALLOW LITTORAL WEED BEDS	LOW ♂ LOW ♀	LOW ♂ HIGH ♀	MEDIUM AGGRESSION	LOW SEXUAL RESPONSE
MAR	ESTIMATED LOW	KNOWN HIGH	5.4	.85	100% COMPLETE	9.2	DEEP SHELTERED POOLS	MEDIUM ♂ HIGH ♀	HIGH ♂ HIGH ♀	LOW AGGRESSION	HIGH SEXUAL RESPONSE
LENNOX	KNOWN ZERO	KNOWN HIGH	4.8	.93	100% COMPLETE	7.8	WIDELY SPREAD	LOW ♂ LOW ♀	HIGH ♂ LOW ♀	LOW AGGRESSION	LOW SEXUAL RESPONSE

sections of the thesis; table 31 provides a general summary of the results obtained.

No clear relationship between the lateral plate phenotype distribution within the study populations and risk of predation emerges from the results obtained, e.g. Lomond fish (mean left hand side lateral plate number = 5.4) with high predation risk, and Luggie fish (mean left hand side lateral plate number = 4.5) with very low predation risk. This is interesting as it fails to back up the well established link between high predation pressure and 7/7 lateral plate modes in Pacific north-west coast American freshwater Gasterosteus populations (Hagen and McPhail (1970), Hagen and Gilbertson (1972, 1973)). Unfortunately, during the present study, the large numbers of Gasterosteus fry recovered from Perch stomachs (page 61) could not be counted for lateral plate number as the plates are unlikely to be fully developed in fry of 20 mm - 30 mm total body length. Bell and Haglund's (1978) laboratory study of Garter snake predation on Gasterosteus has shown that other lateral plate phenotypes (e.g. fish with a total of 7-10 plates) have a survival advantage under certain conditions.

The dorsal and ventral spines of Gasterosteus are of proven importance in the defence of the fish against a variety of fish and bird predators. Moodie's (1972) study of the large melanic Mayer Lake Gasterosteus has highlighted the subtlety of predator selection; Cut-throat Trout were found to select adult female sticklebacks with significantly shorter pelvic spines than would be expected, on average for the given size of predated fish. Such differences in spine size were not very large and indicate a delicate balance between predator size and spine size. Gross (1978) in his survey of European Gasterosteus populations found that fish from sites with low estimated predation pressure had significantly shorter dorsal and ventral spines and smaller pelvic girdles than fish from high estimated predation level sites. In this study Loch Lomond fry of 20 mm mean body length were found to have markedly longer pelvic spines than sticklebacks from the other four normally spined populations studied (page 112). This finding agrees very well with the fact that Lomond fry are heavily predated by Perch in the late Autumn and Winter months and are likely to gain considerable protection from relatively large spines at an early age. The non-predated Luggie fish were found to have a similar overall ventral spine growth rate to the Heron-predated Mar burn sticklebacks. A separate measure of the effectiveness of morphological protection in male Gasterosteus was calculated by approximating the increase in critical body dimension when the spines are raised: dorsal spine length + ventral spine length/body depth (page 113). This index proved to have a very low intra-population variability with highly significant inter-population differences. Furthermore Luggie fish (low predation risk) had the smallest increase in critical dimension whilst Lomond fish (high predation risk) had the largest increase in effective body size when raising their spines. Lishev (1950) studying the food of predatory fish in the Amur basin found that spiny Bitterling (Acanthorhodeus asmussi) were under represented in predator stomachs when compared with the spine-deficient Common Bitterling (Rhodeus sericeus). Paraketzov (1958) has found that in general, protective spines in North Pacific marine fish tend to be better developed than in North Atlantic habitats where there are fewer potential predators. This was particularly true for the Cottidae (Bullheads).

Parallelism in the reduction and loss of the pelvic girdle complex has occurred within and between the genera Gasterosteus, Pygosteus and Culaea (Bell 1974). Moodie and Reimchen (1973), Hagen and Gilbertson (1972), and Bell (1974, 1976) have all argued that a lack of piscine predation pressure is the most likely selective agency to reduce spine size, number, and pelvic girdle occurrence in stickleback populations. R.N.B. Campbell (1976) made the interesting discovery however that the Loch Fada (North Uist) spine-deficient Gasterosteus population is predated by Brown Trout and thus appears to be unique amongst the documented populations in that morphologically unprotected Gasterosteus are living in sympatry with a proven fish predator. On the island a group of populations have undergone a similar reduction in non-essential skeletal components of a known genetic origin; there are two possible reasons for such a situation:

1. Genetic drift: chance changes in the genetic constitution of small populations
2. Adaptation: to selective agencies operating upon the populations.

Berry (1979) in his excellent review of the genetics of Outer Hebridean animal populations states that chance changes are unlikely to be important in small founder populations on the Hebridean islands since if the population is small enough for random processes to be effective it will be very susceptible to extinction. It is also relevant to point out that within the peat bog Gasterosteus populations the occurrence of 8 similar cases of reduction in skeletal components (when this reduction is very rare in Gasterosteus populations) is very unlikely indeed to occur purely by random genetic drift. Berry notes in his paper that:

> "All the Holarctic forms of the three-spined stickleback (Gasterosteus aculeatus) seem to occur in the Western Isles, and are certainly unevenly distributed, which may indicate local adaptation (Campbell and Williamson 1979). Unfortunately, little is known about the factors determining the occurrence of particular forms, so it is not possible to conclude anything about their significance. However they will certainly repay

an ecogenetical study".

In section 9, chapter 4, evidence is gathered to support the hypothesis that low Ca^{2+} availability in the east coast peat blanket-bog lochs has represented a strong selection pressure, causing reduction and loss of non-essential skeletal components in Gasterosteus, (dorsal spines, lateral plates, ventral spines, pelvic girdle, small body size). The relatively calcium-rich west coast lochs based upon or associated with the Atlantic shell-sand machair soils contain Leiurus sticklebacks only, no spine-deficient individuals have been found in these lochs. All eight of the predominantly spine-deficient populations discovered in the present study occur in calcium-poor, oligotrophic peaty lochs; however in Loch nan Geirrean spined Gasterosteus occur in water with only 1.8 mg L^{-1} Ca^{2+}. Further studies to investigate the relationship between phenotypic distribution in Gasterosteus and environmental Ca^{2+} on North Uist are planned for the future. Bell (1974) has noted in his paleoecological studies that the phenotypic frequencies observed amongst Gasterosteus populations with regressed defensive structures are stable over considerable time periods. In this study (fig. 14) it has been hypothesised that the observed phenotypic frequencies in Loch Fada Gasterosteus may be a balanced genetic polymorphism where the low percentage of spined fish is maintained by Trout predation and the high percentage of spine-deficient fish by the selective pressure of low Ca^{2+} availability. Maynard-Smith (1976) states that the two major causes of genetic polymorphisms are:

1. maintenance by balanced selection pressures.
2. simple variation in neutrally selective characters.

The evidence supporting the value of spines and the pelvic girdle as anti-predator adaptations (e.g. Gross 1978) and the present study (chapter 4, page 104) amply refutes the second of these possibilities.

It has been mentioned that the standard body length of mature adult spine-deficient Gasterosteus from Lochs Fada and â Bharpa is very small. McPhail (1977) hypothesised small body size at first reproduction in female Gasterosteus as an adaptation to avoid predation by large Brook Trout (S. fontinalis). In the North Uist lochs the resident Brown Trout do not usually attain a large size however, (c. 500 g, D. Shaughnessy, pers. comm.) and water quality and food availability are more likely to be controlling factors in the body size of the sticklebacks. All three of the Uist populations studied had significantly smaller adult Gasterosteus than any of the mainland populations (page 85). In body shape the Uist fish were also significantly slimmer (standard body length:body depth ratio, page 89).

The anti-predator behaviour of Gasterosteus has been much less studied than the morphology of the species and few studies exist concerning interpopulation comparisons of behaviour in any teleostean species. The major aim of the present study was to investigate the relationship between predation risk and level of anti-predator behaviour exhibited by samples from separate Gasterosteus populations. Huntingford (1976) has suggested the possibility that high predation levels within Gasterosteus populations may lead to a reduction in social aggression and increasing timidity within the population. The results of the male aggression tests from the present study (page 139) fail to fit the above prediction for the Lomond and Fada populations (both with high predation risk and high aggression) but the Mar burn population fits the prediction very well. Mar burn breeding males are known to be at risk from Herons (page 221) and showed the lowest social aggression scores in the above tests, and also the highest numbers of courtship behaviours directed toward a captive gravid conspecific female. This overall breeding male strategy should minimise territorial fighting and help to maximise reproductive output.

The high aggression exhibited by Lomond and Fada males which had recently built nests may be related to nest-site selection (Table 20) - both prefer shallow water sites; keeping the breeding males away from most predatory fish and diving duck species. Suitable sites are often in short supply. In the courtship behaviour experiments males from all populations were found to be aggressive toward a gravid conspecific female - Mar fish surprisingly so, but the bouts of aggression tended to occur early in the test and were short and sharp, rapidly turning to sexual behaviour. Mar, Lomond and Fada males all showed short, sharp bouts of courtship behaviour; again the shallow water nest sites of Lomond and Fada males and the deep sheltered pool sites of Mar males may allow high levels of conspicuous behaviour without unduly increasing the risk of predation. Ballin (1973) studying the geographical variation in Guppy courtship behaviour from Trinidad found that male fish from headwater sites where water clarity was high, and predation risk low performed longer and more conspicuous courtship behaviour than males from turbid lowland streams where more predators were present.

The behaviour of the predatory species encountered during the present study is briefly reviewed on pages 147 and 148. Two types of experiment, Heron tests and Pike tests, were used in order to gain standard measures of the anti-predator behaviour of adult and juvenile Gasterosteus from each of the study sites. A series of Heron tests on young Gasterosteus collected from the Mar burn and Loch Lomond revealed that the fright response to a moving object overhead is much more pronounced in fry of 30 mm total length than in fry of 15 mm total length. The larger fry jumped away in alarm and remained still for a considerable period before resuming normal swimming. Predator naive Mar burn fry sampled when very small (c. 10 mm) and then reared in the laboratory showed a parallel development of the overhead fright response to that of wild fry. This result indicates that the overhead fright response in juvenile Mar burn Gasterosteus is largely inherited and may not need any predator experience to develop normally. The other possible explanation for this result is that the fry sampled when very young had already experienced Heron attacks and that their anti-predator behaviour subsequently developed as a result of these experiences. This explanation is unlikely to be the true one as the uniform development of the fright response shown by the laboratory reared 30 mm fry sample would need a similarly uniform pattern of Heron attacks at an early age. Seghers (1974) has tested the overhead fright response in predator naive guppies from parental stocks sampled from high and low predation risk populations. The experimental fish were reared for a minimum of 4 generations in the laboratory before being tested with an overhead frightening stimulus.

Seghers measured the "duration of swimming inhibition" (≡ recovery time), finding that descendants from the high predation risk population darted down farther and remained motionless longer than descendants from the low predation risk population. The differences detected were small but Seghers argues that they have high survival value since aerial bird predator-prey interactions are of short duration and involve short reaction distances (Seghers loc. cit.). Phillips (1962) used Gasterosteus when testing the overhead fright reaction of fish to different Gull models, recording the "skulking time" (= time remaining stationary) as a measure of intensity of reaction. Useful information is included on the visual field of Gasterosteus. Millinski and Heller (1978) have used Gasterosteus in order to test the hypothesis that a fish will adapt its optimal foraging behaviour after being scared by an overhead stimulus, and found that frightened Gasterosteus tended to take stragglers from a Daphnia swarm whereas undisturbed fish fed upon the densest region of the swarm.

Millinski and Heller's interpretation of this finding was that the stickleback, by modifying its feeding behaviour as above could still feed (upon stragglers) after being scared, whilst simultaneously maintaining a lookout for subsequent aerial attacks. The mean Heron test recovery time for juvenile Mar burn Gasterosteus (35-40 mm body length) sampled in October 1978 was 338 seconds, significantly longer than the wild 30 mm Mar fry tested in August 1978, indicating that the response heightens with age/body size. The effect continues to hold, as adult male and female Mar sticklebacks had mean recovery times of 436 and 567 seconds respectively. The importance of a long recovery time may increase as the stickleback grows, as avian predators often select for large prey (page 54). During the present study only adult Gasterosteus were recovered from beneath Heron nests at the Gartfairn heronry adjacent to the Mar burn, composing 4% of the July diet of the Herons surveyed (Appendix, page 221). Cook (1978) has timed the feeding behaviour of wild Herons on the Ythan estuary (Scotland) and found that adult birds made an attempt to capture a prey item once every 9.66 minutes and were 50% successful; juvenile birds made an attempt every 3.97 minutes and were 29% successful. If these data are characteristic for Grey Herons in differing habitats then the long recovery periods shown by Mar adults in the present study correlate well with a known high predation risk from a highly successful predator that pauses for considerable periods between strikes at the prey. During the present study adult Gasterosteus were seen to jump away two or three times in an erratic series of escape manoeuvres; Humphries and Driver (1967) describe such behaviour as a Protean defence whereby the behaviour is "sufficiently unsystematic to prevent a reactor from predicting in detail the position or actions (or both) of the actor".

The Friedman 2 way analyses of variance performed upon the "highly developed fright responses" and "poorly developed fright response" from the post-m.o.t. Heron test data matrix (page 156) indicate that highly significant ($p < .001$ in both cases) behavioural differences are present between the groups of experimental fish. At the m.o.t. all Mar and Lomond adults tested jumped away to remain still in the weed or at the bottom of the test tank (Table 23). There was then a gradation of degree of fright response through the populations culminating in the Luggie fish which enjoy a low potential predation risk from aerial predators and showed the least fright response at the m.o.t., 2 males even collecting nest material during the post-m.o.t. phase of the experiments. The best simple index of degree of fright response at the m.o.t. in the Heron tests was found to be the recovery time (= time taken to resume normal swimming in open water after jumping away and remaining still after the m.o.t.). An inter-population comparison of recovery times for groups of 12 experimental fish, tested statistically with 2-tailed Mann-Whitney U tests revealed that Mar, Lomond and Lennox males (high risk from Herons, Mergansers, Gulls) had the longest recovery times, with Fada males and Luggie males having the shortest recovery times. Luggie males at low risk and thus fit the short recovery period prediction well, the North Uist populations consistently showed moderate levels of fright response during the study. It is possible that even though these fish suffer a high potential predation risk the turbid, brown, peat-stained waters may camouflage them very well allowing reduced recovery times and a lowering of long term response to fish predators.

Throughout the study (Heron tests, Pike tests) there is a consistent trend for breeding male Gasterosteus to be less cautious in the predator:prey situation than adult female Gasterosteus. A possible explanation for this sexual difference in behaviour may lie in the parental care of offspring and territorial nest protection of the male. Males defend egg-filled nests against predators of all types and against conspecific egg raiders, and endeavour to attract as many gravid females as possible to the nest to spawn their eggs and complete the multiple clutch. Females have only to shed their eggs in the nest of the fittest available male and then resume feeding to mature another egg batch; both sexes must avoid being predated. This differential demand upon the male's time may have lead to the reduction in caution exhibited by breeding males which risk the loss of many eggs by remaining in hiding for too long after predator encounters. Amongst breeding females the Heron test recovery times were longest for Mar and Lomond fish and also surprisingly high for Luggie fish (low risk from occasional Gulls); however, if the above hypothesis is correct the Luggie females lose little by retaining a long recovery time, whilst the Luggie males had the shortest recovery times recorded. Amongst fry also Lomond and Mar fish had the longest recovery time; there is therefore a very good general agreement within the Heron test data for fish from high risk populations to show high level fright responses and vice versa.

No significant intra-population differences in Factor 1 scores from the Principal Components multivariate analysis between male and female fry were found in any of the populations; these data were therefore grouped within populations. Lomond fry showed the highest Heron test fright response and are known to be at risk from a wide variety of piscivorous birds (especially Red-breasted Mergansers). The known high risk for Lomond Gasterosteus fry of predation by Perch (page 61) may be linked with a highly developed general awareness of predators. Unfortunately during the present study no time was available (due to delay in permission to continue the work, page 25) to Pike test fry from the seven study populations but such tests on Lomond fry should yield highly developed anti-fish predator responses.

Mar fry also scored negatively on Factor 1 (and probably experience Heron attacks during the summer and autumn period), the post-m.o.t. Heron test behaviour was similar between fry from the other populations.

Amongst breeding adults, Mar males scored on average the lowest Factor 1 scores (≡ highest fright response) followed by Lomond and then Lennox males; these results correlate well with their high known and potential avian predation risks. Fada, Bharpa and Daimh males scored similarly (Daimh averaging a slightly negative score) and, significantly, Luggie males averaged a high positive score (≡ low fright response) correlating well with the low risk at this site. Luggie females however showed a high fright response ($\bar{x}$ Factor 1 score = -.68). This may be due to the fact, as discussed previously, that adult Gasterosteus females do not stand to reduce their reproductive output by remaining still for long periods after a predator encounter. Mar and Lomond females recorded the highest fright response ($\bar{x}$ Factor 1 scores = -.9 and -.75 respectively) again agreeing with their known high predation risk. Daimh females scored the highest positive Factor 1 scores on average and are of particular interest during this study because both in the Heron and Pike tests they are consistently less cautious than the males in the face of a predator stimulus. The converse is true for all other populations studied and no explanation can be offered for this anomaly. In the Pike tests the Principal Component analysis Factor 1 scores for groups of experimental fish again agreed well with known and potential predation risks from fish species. Lomond, Daimh, Fada and Bharpa males all scored negatively (= cautious behaviour) on average, all are at risk in the wild. Mar males were intermediate and have no known fish predators (but high Heron predation) and two or three possible potential fish predators. Luggie and Lennox males have a very low, and absent risk of fish predation, and both groups scored high positive Factor 1 values on average. This pattern was also true for adult female Gasterosteus. These data thus support the thesis that high risk of predation in the wild leads to highly developed anti-predator behaviour with significant inter-population effects. Seghers (1970, 1973, 1974) working upon natural populations of the Guppy, Poecilia reticulata in Trinidad has stressed the difficulties involved in resolving intra-specific differences in behaviour and in experimenting with non-morphological traits. Where predatory Characid and Cichlid species occurred in sympatry, Guppies were a) more restricted to the stream shorelines b) showed a greater tendency to shoal and c) had a lower alarm threshold than in those Guppy populations sympatric with the small predatory cyprinodont Rivulus hartti where the fish shoaled less and used the open water and stream bed more (Seghers 1973). Seghers concluded from his studies that Trinidadian Guppies exhibit great geographical variation in anti-predator behaviour which could be caused by genetic differences attributable to differential predation pressures; in some cases the differences were apparently maintained without a major barrier to gene flow.

The study of Moodie, McPhail and Hagen (1973) suggested a behavioural basis for their observed differential predation upon like-sized Gasterosteus of differing lateral plate number; however the sticklebacks came from a variety of populations and they state that interpopulation differences could account for their results. The three workers conducted independent laboratory tests allowing predators (Northern squawfish or Cut-throat trout) to eat 50% of a test batch of Gasterosteus and then scored the survival of the various lateral plate phenotypes under test. 7/7 plated fish were eaten significantly less often than their initial ratio in the sample would suggest, however other results were contradictory with 6/6 fish being caught less quickly than 7/7's in individual flight behaviour tests. There were also seasonal variations between August and February tests, all summer tests showing 7/7's at a disadvantage and all winter tests showing them at an advantage. In their initial experiments differing phenotypes from a single population were used and differential predation results obtained, therefore a lateral plate effect is present. In their conclusion however Moodie, McPhail and Hagen were unable to state whether the differences that they observed in susceptibility to predation were due to population or plate class differences. Fig. 19 (overleaf) gives the lateral plate phenotypes for all pike tested adult Gasterosteus from the Lomond, Daimh, Lennox and Luggie populations (Note: Lomond and Daimh fish scored negatively on the post-m.o.t. Pike test Factor 1, and Lennox and Luggie fish scored positively on this factor). A brief examination of the table reveals that Lomond and Lennox tested fish have similar lateral plate phenotypes and yet differed very significantly on post-m.o.t. Factor 1 Pike test scores. Thus, the population effect from this data appears to be stronger than lateral plate phenotype differences. However, this is not to say that intra-population lateral plate phenotype differences in behaviour are not present in the populations sampled.

Evidence from the present study suggests a genetic basis for the development of the overhead fright response in Gasterosteus (page 169), Benzie (1965) who Pike-tested predator naive (laboratory reared) Gasterosteus fry found precautionary and fright behaviours to be present in her fish. It is important to note however that experience of a Pike heightened the anti-predator behaviour of Gasterosteus fry (Benzie loc. cit.). Given that anti-predator behaviours in Gasterosteus have an inherited basis it is reasonable to propose that the interpopulation behavioural differences demonstrated during the present study represent micro-evolutionary responses to differential predation pressure in the seven study populations. Because quantitative

Fig. 19. Lateral Plate Phenotypes of Pike tested adult *Gasterosteus*

DAIMH		LOMOND		LENNOX		LUGGIE	
MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
3/0	3/3	6/6	5/4	4/5	5/5	14/15	4/4
4/4	2/2	7/6	6/7	5/6	6/5	3/4	16/17
2/1	4/3	5/5	5/4	7/7	4/4	5/4	4/5
1/2	4/3	5/6	6/6	4/4	4/3	4/4	6/5
3/3	1/1	6/6	4/4	4/6	6/6	5/5	4/3
3/4	4/3	5/5	6/6	4/4	5/5	5/5	5/5
3/4	3/2	6/7	5/5	4/4	4/4	4/3	3/4
4/4	4/4	5/6	4/5	4/4	3/4	13/13	6/6
2/2	2/2	4/4	4/5	4/4	5/4	16/17	4/4
3/4	4/3	5/5	4/4	5/6	4/4	6/6	4/4
1/1	2/3	4/4	5/5	5/6	6/5	6/5	5/5
2/2	2/2	5/6	6/7	4/4	5/5	2/3	5/4

measures of predation pressure are very difficult to obtain under natural conditions the seven study sites are classified into known and potential high and low risk categories. This falls short of the desired accuracy but does allow a ranking of the sites from high to low risk for both fish and bird predators. The qualitative basis for this ranking means that small interpopulation differences in morphology and behaviour are difficult to assign to differences in predation risk. It can be concluded however that significant differences in morphology (e.g. critical dimension of the body) and behaviour (e.g. anti-predator behaviour) between populations have been shown to be strongly influenced by the levels of predation to which these fish are exposed. Other aspects of morphology (e.g. lateral plate number) and behaviour (e.g. male aggression) bear no simple relationship to predation risk.

APPENDIX

Immunological Analysis of Perch Stomach Contents

In some instances Perch stomach samples contained large pieces of part-digested fish species with no obvious distinguishing morphological features. This, coupled with the fact that both Gasterosteus and Pygosteus occur in Loch Fada, Loch Lomond, and Loch an Daimh led to the investigation of the possibility of developing a specific Gasterosteus anti-serum for identifying stomach content remains. Dr. R.S. Phillips (Department of Zoology, University of Glasgow) kindly agreed to immunise a laboratory rabbit with a homogenised Gasterosteus tissue extract in Freinds incomplete adjuvant (a mixture of oils), and then, after a period of several weeks to administer booster injections to promote the production of anti-Gasterosteus antibodies in the blood serum. Blood samples were then periodically taken from the rabbit and centrifuged to produce a clear supernatant anti-serum containing a range of antibodies, aliquotes of antisera were stored at -25°C. The anti-Gasterosteus serum was then precipitin tested with homogenised samples of Gasterosteus, Pygosteus, Phoxinus and Salmo trutta tissue extracts, using agarose diffusion plates (Ouchterlony tests). The Ouchterlony test allows antigens and antibodies to diffuse toward each other from small wells through a 2mm. thick layer of agarose medium. During the course of these experiments the central well was filled with anti-Gasterosteus serum and five surrounding wells were filled with test antigens (test plates were prepared in duplicate). A positive precipitation reaction leads to the formation of a white opaque band in the agarose, the significant bands indicating positive reactions withstand washing in physiological saline solution. Where two adjacent wells contain identical antigens the bands of precipitated protein run into each other forming a single curved line, where two adjacent wells contain differing antigens both of which react with the anti-serum then the precipitation bands cross each other. After a three day incubation period plates are washed for a further three days in phosphate buffered saline solution to remove non-specific precipitation bands and then dried at 37°C and stained with Amido Black solution. In the experiment described above positive precipitin reactions to the anti-Gasterosteus serum were given by both Gasterosteus and Pygosteus antigens but negative reactions were recorded for Trout and Minnow antigens. Penczak (1961) has used precipitin tests to analyse immunologically the taxonomic relationship between G. aculeatus and P. pungitius obtaining similar results to those described above (weak cross-reactions between the two species). Because of the problem of cross-reacting antibodies between Gasterosteus and Pygosteus antigens a sub-sample of the Gasterosteus anti-serum was absorbed with Pygosteus antigens in an effort to precipitate out cross-reacting components and leave a specific anti-Gasterosteus serum. This procedure proved relatively successful with the resulting absorbed anti-serum producing strong specific precipitin bands with Gasterosteus antigen but only a weak positive banding with Pygosteus antigen.

Samples from Perch stomachs were then tested using the absorbed Gasterosteus anti-serum, samples containing large amounts of part-digested fish protein were frozen (Acetone and Dry Ice) and then thawed in order to facilitate further cellular breakdown and release the potentially antigenic protein component. Strong positive specific reactions were obtained using Ouchterlony tests upon samples of perch stomach contents known to contain Gasterosteus fry. In cases where Gasterosteus remains were suspected however only weak lines were produced leaving the possibility of confusion of positive Gasterosteus reactions and weakly positive Pygosteus cross-reactions. Because enough data was forthcoming from visually identifiable Gasterosteus remains in stomach samples further purification of the anti-serum was abandoned. The results from the investigation were however very encouraging and may form a basis for future vertebrate predation studies. Davies (1969) has used a similar approach to evaluate predation pressure acting upon freshwater tridad species.

The Gasterosteus anti-serum was produced from a tissue extract from an adult Leiurus morph River Luggie stickleback and it was therefore of interest to compare spine-deficient Gasterosteus antigen (Loch Fada, North Uist) reactions toward the antisera to reactions obtained from normally spined Leiurus antigen. Ouchterlony plates prepared in triplicate yielded identical results, with strong positive confluent bands between spined and spine-deficient antigen preparations. These observations indicate that the North Uist spine-deficient phenotype is still closely allied to its normally spined Leiurus counterpart with respect to immunologically active somatic constituents.

Summary statistics of data from anti-predator behaviour experiments

P.T.O.

Table 32 Post-m.o.t. behaviour scores (principle behaviours from P.C.A. Factor 1 (Fig. 16)): Heron test data

		FADA				BHARPA				DAIMH			
		Adults		Fry		Adults		Fry		Adults		Fry	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
frequency normal swimming	x̄	1.4	.89	1.5	1.4	1.1	.89	1.6	1.6	.87	1.4	.99	.94
	s.d.	.42	.38	.82	.66	.49	.35	.51	.35	.48	.81	.64	.41
frequency still	x̄	1.5	1.1	1.6	1.5	1.2	1.0	1.7	1.6	1.2	1.5	1.1	1.0
	s.d.	.43	.34	.81	.69	.46	.4	.57	.34	.55	.61	.67	.49
duration normal swimming	x̄	75	48	48	40	72	54	68	59	36	50	23	35
	s.d.	27	26	18	14	22	15	17	14	23	24	16	17
frequency feeding	x̄	1.3	.24	.41	.2	.9	.08	.91	.53	.26	.74	.20	.22
	s.d.	.85	.36	.38	.21	.42	.03	.34	.44	.34	.69	.32	.28
duration in open water	x̄	126	104	83	62	93	75	124	135	57	63	50	70
	s.d.	42	60	32	31	38	19	41	49	27	26	33	25
frequency in open water	x̄	.6	.36	.60	.43	.54	.54	42	.35	.58	.54	.47	.40
	s.d.	.34	.18	.30	.17	.21	.23	23	.15	.33	.36	.31	.19
frequency at bottom	x̄	.52	.25	.62	.4	.49	.45	.34	.23	.60	.60	.41	.37
	s.d.	.29	.17	.23	.17	.24	.23	.27	.21	.37	.28	.3	.22
duration at bottom	x̄	27	34	34	40	26	59	13	14	64	52	60	57
	s.d.	25	38	31	31	19	25	17	17	24	31	36	35
duration still	x̄	6	15	16	29	16	14	2.2	13	5.6	7.7	21	11
	s.d.	8	22	18	37	21	12	5.3	28	8.0	9.6	29	19
recovery time	x̄	102	236	171	231	125	244	50	69	212	86	307	206
	s.d.	105	144	143	118	92	148	34	62	88	59	178	142

Table 32 (cont.)

LOMOND				LUGGIE				MAR				LENNOX			
Adults		Fry		Adults		Fry		Adults		Fry		Adults		Fry	
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
.61	.39	.55	.46	2.1	.78	1.3	1.3	.48	.3	.85	1.1	.78	1.3	.59	1.6
.72	.37	.49	.33	.97	.75	.7	.66	.36	.3	.56	.65	.76	.45	.53	.66
.73	.54	.65	.53	2.2	.86	1.5	1.5	.59	.42	.89	1.3	.98	1.3	.73	1.8
.75	.38	.48	.35	.96	.78	.7	.67	.36	.4	.65	.65	.76	.46	.52	.7
24	12	.33	9.7	68	27	55	42	15	9.1	20	22	19	50	16	45
30	11	.22	8.2	31	26	21	22	13	8.3	15	17	18	20	.7	17
.1	.03	0	0	.56	.09	.29	.40	0	0	.12	.1	.22	1.4	.13	1.8
.16	.07	0	0	.57	.15	.28	.42	0	0	.2	.16	.41	1.2	.26	1.2
53	46	30	44	91	62	106	75	31	18	60	58	62	150	56	82
37	31	26	32	28	63	44	58	15	20	36	22	59	57	55	50
.33	.28	.33	.23	.89	.2	.49	.46	.25	.2	.3	.49	.26	.22	.27	.8
.25	.18	.18	.11	.48	.12	.32	.36	.08	.15	.14	.29	.18	.06	.15	.5
.38	.18	.34	.23	.5	.13	.4	.37	.15	.17	.3	.34	.25	.1	.25	.8
.24	.13	.22	.14	.35	.18	.3	.31	.13	.12	.22	.17	.19	.13	.17	.5
84	48	83	79	15	39	35	31	73	67	56	63	57	16	52	50
37	46	33	43	14	54	25	40	49	52	44	26	45	31	35	33
8.4	33	21	31	26	31	7.7	40	28	52	31	18	23	1.96	36	5
27	42	36	44	27	52	10	47	39	68	39	18	45	4.9	36	12
427	483	454	493	68	386	147	178	436	567	375	301	398	170	383	205
166	160	141	133	81	227	112	133	146	69	174	125	201	167	172	152

Table 33. Pre-m.o.t. behaviour scores (principle behaviours from P.C.A. Factor 1 (Fig. 17)): Pike Test data

		FADA		BHARPA		DAIMH		LOMOND		LUGGIE		MAR		LENNOX	
		Adult		Adult		Adult		Adult		Adult		Adult		Adult	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
frequency of normal swimming	$\bar{x}$	2.42	1.81	2.62	1.86	1.62	1.95	2.14	1.11	1.71	1.36	1.95	1.18	1.4	1.45
	s.d.	.93	1.04	.96	.65	1.04	2.15	1.1	.18	.64	.72	.46	.65	1.06	.76
frequency still	$\bar{x}$	2.06	1.82	2.51	1.92	1.59	1.52	2.08	1.13	1.75	1.36	2.0	1.39	1.43	1.45
	s.d.	.68	.98	.82	.74	1.09	1.21	1.06	.55	.56	.68	.416	.53	1.01	.78
frequency in open water	$\bar{x}$	.94	.82	1.29	.73	.81	1.16	.77	.62	1.19	.65	.91	.6	.87	.67
	s.d.	.45	.48	.74	.32	.37	1.21	.21	.276	.50	.48	.25	.32	.73	.45
duration normal swimming	$\bar{x}$	57.1	36.8	61.66	33.9	49.2	37.9	39.2	23.4	43.52	27.04	38.4	20.9	29.9	37.5
	s.d.	21	24.2	17.6	20.8	20.5	21	19.5	20.4	20.7	12.9	12.4	13.9	23.5	23
duration in weed	$\bar{x}$	1.7	0	7.87	15.4	0	9.64	4.7	27.3	1.2	4.08	17.5	21.1	1.78	9.14
	s.d.	5.8	0	19.5	34.8	0	33.4	9.9	34.6	2.45	9.3	22.4	18.5	6.17	28.4
duration still	$\bar{x}$	62.0	81.8	57.6	84.6	69.1	93.1	84.1	85.8	75.6	92.2	80.9	88.68	88.8	81.5
	s.d.	21.7	24.1	17.8	20.4	20.1	61	15.9	33.3	20.6	11.5	12.3	30.5	24.5	23.5
duration at bottom	$\bar{x}$	21.4	8.9	32	9.4	18.3	7.0	35	35.3	49.1	25.5	47.9	45.12	11.1	5.99
	s.d.	23.3	22.1	18.7	21.6	24.2	16.7	29.4	31.2	26.9	32.4	31.7	38.7	14.5	13.2
late m.o.t.	$\bar{x}$	496	370	491	378	510	409	496	528	510	590	514	637	452	488
	s.d.	269	212	216	213	243	78.8	200	282	250	272	218	232	217	268

Table 34 Post-m.o.t. behaviour scores (principle behaviours from P.C.A. Factor 1 (Fig. 18)): Pike Test data

		FADA		BHARPA		DAIMH		LOMOND		LUGGIE		MAR		LENNOX	
		Adult		Adult		Adult		Adult		Adult		Adult		Adult	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
frequency still	x̄	.379	.236	.366	.244	.33	.212	.391	.233	.95	.77	.63	.29	1.51	1.16
	s.d.	.228	.153	.162	.14	.29	.135	.169	.12	.46	.47	.31	.11	.89	.54
frequency normal swimming	x̄	.241	.095	.111	.135	.15	.103	.213	.089	.86	.72	.37	.15	1.47	1.09
	s.d.	.297	.096	.139	.15	.16	.127	.18	.156	.35	.45	.24	.15	.89	1.06
frequency facing away	x̄	.058	.08	.206	.18	.11	.041	.16	.07	.43	.29	.35	.16	.63	.57
	s.d.	.117	.12	.106	.23	.14	.079	.12	.09	.36	.19	.34	.14	.31	.24
frequency monocular fixation	x̄	.387	.28	.561	.425	.29	.299	.44	.43	1.04	.78	.64	.56	.96	.98
	s.d.	.297	.12	.281	.25	.21	.257	.20	.23	.47	.19	.38	.28	.45	.46
duration normal swimming	x̄	5.85	2.65	2.36	1.64	3.43	3.2	4.24	1.95	18.7	12.9	8.25	2.37	26.2	27.8
	s.d.	6.99	4.88	4.0	2.5	5.8	5.4	4.5	4.4	11.4	10.2	6.2	2.7	18.3	12.6
frequency of retreat	x̄	.058	.109	.25	.187	.124	.041	.183	.08	.24	.23	.35	.111	.47	.464
	s.d.	.117	.145	.14	.23	.17	.079	.086	.11	.21	.22	.32	.13	.36	.213
frequency binocular fixation	x̄	.236	.206	.24	.269	.266	.24	.293	.24	.73	.72	.41	.28	.82	.832
	s.d.	.186	.15	.22	.20	.105	.13	.226	.14	.37	.23	.32	.16	.33	.41
duration facing away	x̄	1.04	2.63	4.98	5.84	3.45	2.05	9.14	3.68	19.7	12	7.8	7.6	33.3	24.1
	s.d.	1.8	5.38	4.04	9.3	6.7	6.03	10.4	7.19	13.8	8.7	9.2	12.8	25	10.9
frequency in open water	x̄	.203	.155	.145	.127	.17	.175	.17	.128	.23	.26	.24	.23	.439	.33
	s.d.	.117	.05	.083	.07	.108	.078	.08	.149	.12	.07	.17	.11	.378	.23
duration still	x̄	101.8	123.8	115.3	119.2	120.7	123	118	129	106	120	111	118.8	106.4	95.4
	s.d.	45.2	20	14.9	5.6	10.1	24	10.8	30.7	9.98	28.9	35.7	5.08	27.9	14.8
duration at bottom	x̄	77.4	79	44.9	52.5	53.7	61.4	73.3	67.5	18.1	7.9	55.6	61.4	12.2	7.97
	s.d.	42.1	42.4	47.7	37.1	45.3	45	47.7	65.3	21.8	17	45.2	62.4	19.8	11.4

Table 35. Heron test data, Principal components analysis: Factor structure : factor loadings

	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4	FACTOR 5
Proportion of total variance explained	27%	13%	10%	9%	6%
Eigenvalue (variance explained by each factor)	8.4	3.9	2.9	2.7	2.1
PRE M.O.T.					
frequency dorsal spines	.15	.73	.07	.15	.25
frequency ventral spines	.04	.47	-.44	-.34	.08
duration dorsal spines	-.24	.7	-.11	-.13	-.02
duration ventral spines	.03	.36	-.49	-.35	.05
frequency normal swim	.49	.38	.22	.28	.53
frequency still	.42	.40	.23	.29	.52
frequency open water	.50	.27	.45	.11	.12
frequency of feeding	.64	-.04	.11	-.2	.02
frequency in weed	-.19	.26	-.21	.64	-.02
frequency at bottom	.41	.28	.63	-.07	-.09
duration normal swim	.72	-.14	.04	.12	.26
duration still	-.72	.14	-.04	-.12	-.26
duration open water	.55	-.19	-.11	-.17	.59
duration in weed	-.55	.08	-.31	.39	-.25
duration at bottom	.01	.18	.58	-.26	-.44
POST M.O.T.					
frequency normal swim	.83	.08	-.14	.15	-.23
frequency still	.83	.12	-.12	.14	-.22
frequency open water	.66	.33	.11	.15	-.41
frequency of feeding	.66	-.15	-.05	-.22	-.16
frequency in weed	.16	.20	-.25	.68	-.19
frequency at bottom	.53	.32	.33	-.12	-.45
duration normal swim	.78	-.28	-.22	-.02	-.16
duration still	-.74	.22	.22	.01	.14
duration open water	.57	-.41	-.36	-.20	.11
duration in weed	-.29	.07	-.22	.61	-.09
duration at bottom	-.45	.26	.47	-.36	.04
recovery time	-.85	.08	.19	.02	.17

Table 36. Pre-m.o.t. Pike Test data, Principal Components analysis: Factor structure : factor loadings

	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4	FACTOR 5
Proportion of total variance explained	25%	16%	13%	11%	7%
Eigenvalue (variance explained by each factor)	4.03	2.55	2.04	1.8	1.13
frequency dorsal spines	.53	.23	.46	-.001	.20
frequency ventral spines	-.01	.03	.28	.73	-.02
duration dorsal spines	.02	.06	.78	.40	.07
duration ventral spines	-.05	-.04	.24	.67	.03
frequency normal swim	.88	.12	-.19	.07	.05
frequency still	.86	.15	-.17	.07	.06
frequency open water	.84	.28	.01	-.07	.04
frequency of feeding	.21	.81	-.26	-.09	.12
frequency in weed	.13	.01	-.14	.73	-.01
frequency at bottom	.25	.88	-.12	-.03	-.04
duration normal swim	.36	.23	-.64	.05	.36
duration still	-.14	-.14	.78	-.02	.10
duration open water	.01	-.10	.10	-.17	.93
duration in weed	-.12	-.10	-.12	.70	-.21
duration at bottom	-.15	.72	.30	.03	-.40
timing of m.o.t.	-.72	.36	.04	.16	.16

Table 37. Post-m.o.t. Pike test data, Principal components analysis:

Factor structure : factor loadings

	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4	FACTOR 5
proportion of total variance explained	30%	11%	9%	7%	6%
Eigenvalue (variance explained by each factor)	8.5	3.1	2.4	2.0	1.6
frequency dorsal spines	.71	.21	.22	.14	-.10
frequency ventral spines	.31	.71	-.17	.15	.06
duration dorsal spines	-.05	.80	-.05	.002	.03
duration ventral spines	-.09	.74	-.002	-.06	.11
frequency normal swim	.91	-.04	.17	.10	-.04
frequency still	.91	-.003	.18	.09	-.06
frequency open water	.58	-.01	.49	.05	.12
frequency sneaky swim	-.21	-.03	.21	-.10	.04
frequency approach	.80	-.04	-.07	.25	-.15
frequency retreat	.78	-.06	.08	-.03	.15
frequency binocular fixation	.78	.13	-.15	.41	-.06
frequency monocular fixation	.83	.08	-.03	-.05	.03
frequency facing away	.87	-.03	-.01	-.001	.09
frequency of feeding	.59	-.16	.58	.09	-.02
frequency in weed	.08	.28	.24	-.07	.78
frequency at bottom	.15	-.06	.86	-.09	.07
frequency of jumping away	-.18	.64	.21	-.27	.29
duration normal swim	.83	-.14	.11	.04	-.04
duration still	-.43	.17	-.13	.12	-.10
duration open water	.41	-.28	-.39	.09	-.07
duration sneaky swim	-.09	-.08	-.06	.003	.23
duration binocular fixation	.01	.01	-.01	.93	-.06
duration monocular fixation	-.19	.11	.04	-.85	-.04
duration facing away	.64	-.08	-.16	-.08	.06
duration in weed	-.12	.17	-.04	-.01	.88
duration at bottom	-.47	.09	.45	-.16	-.53
duration at surface	.16	-.12	-.27	-.02	-.02
timing of m.o.t.	.006	.17	.08	-.11	.13

The seven study sites were sampled at the following frequencies

Mainland sites

Loch Lomond was sampled at least weekly over the three year period.
Lennox Castle, the River Luggie and the Mar Burn were sampled on average once every two weeks.

North Uist sites

To date a total of six trips have been made by the author ranging from one week to a fortnight in duration. Each of Loch Fada, Loch a Bharpa and Loch an Daimh were studied daily on each of the visits.

REFERENCES

ABDEL-MALEK, S.A. (1968) Feeding of young three-spined sticklebacks (*Gasterosteus aculeatus* L.) of the Kandalaksha Bay in the White Sea
Probl. Ichthyol., *8*, 74-80

ADALSTEINSSON, H. (1976) Data on fish in Myvatn Norway
Natturufraedingurinn (Iceland), *45*, 2, 154-177

ALLEN, K.R. (1935) The food and migration of Perch, *Perca fluviatilis* L. in Windermere
J. Anim. Ecol., *4*, 264-273

ALLEN, K.R. (1939) A note on the food of the Pike (*Esox lucius*) in Windermere
J. Anim. Ecol., *8*, 72-75

ANTOSIAC, B. (1963) Volzial ryb w pokarmie starszych rocznikow okonia (*Perca fluviatilis* L.) w niektorych jeziorach okolic Wegorzewo [Fishes in the food of older Perch in the lakes of the Wegorzewo district]
Rocz. Nauk. roln., *82* B(2), 273-294

ARME, C. & OWEN, R.W. (1967) Infections of the three-spined stickleback (*Gasterosteus aculeatus* L.) with the plerocercoid larva of *Schistocephalus solidus* (Muller 1776) with special reference to the pathological effects
Parasitology *57*, 301-314

ARMSTRONG, R.H. (1971) Age, food and migration of sea-run Cut-throat trout, *Salmo clarki*, at Eva Lake, South Eastern Alaska
Trans. Am. Fish. Soc., *100*, 302-306

ASPEY, W.P. & BLANKENSHIP, J.E. (1977) Spiders, snails and statistical tales: application of multivariate analysis to diverse ethological data
In Hazlett, B.A. Quantitative methods in the study of Animal Behaviour, Academic Press, New York

ASSEM, J. van dem & MOLEN, J.N. van der (1969)
Waning of the aggressive response in the three-spined stickleback upon constant exposure to a conspecific. 1. A preliminary analysis of the phenomenon
Behaviour, 36, 286-334

AVISE, J.C. (1976)
Genetics of plate morphology in an unusual population of threespine sticklebacks (Gasterosteus aceleatus L.)
Genet. Res., 27(1), 33-46

BACESCU, M. & MAYER, R. (1956)
Recherches sur les epinoches (Gasterosteus aculeatus L.) des eaux roumaines
Bul. Inst. cercet. piscic. Bucaresti, 15(2), 19-36

BAGGE, P., LEMMETYINEN, R. & RAITIS, T. (1970)
Saaristomeven resilintujen kevatravinnosta
Suom. Riista, 22, 35-45

BALLIN, P.J. (1973)
Geographic variation in courtship behaviour of the Guppy Poecilia reticulata
M.Sc. thesis, University of British Columbia, Dept. of Zoology

BANISTER, K.E. (1967)
The anatomy and classification of the order Gasterosteiformes
Ph.D. thesis, University of Newcastle upon Tyne, Dept. of Zoology

BAILEY, N.T.J. (1968)
Statistical methods in Biology
Unibooks, (English Universities Press)

BELL, M.A. (1973)
The pliocene stickleback Pungitius haynesi, a junion synonym of Gasterosteus aculeatus
Copeia, 1973, 588-590

BELL, M.A. (1973)
Pleistocene three-spined sticklebacks, Gasterosteus aculeatus (Pisces) from Southern California
J. Palaeontology, 47, 479-483

BELL, M.A. (1974)
Reduction and loss of the pelvic girdle in Gasterosteus (Pisces): a case of parallel evolution
Nat. Hist. Mus. Los. Ang. Cty. Contrib. Science, 257, 1-36

BELL, M.A. (1976) The evolution of phenotypic diversity in three-spined sticklebacks (*Gasterosteus aculeatus*)
Ph.D. thesis, University of California, Los Angeles

BELL, M.A. & HAGLUND, T.R. (1978) Selective predation of three-spined sticklebacks by garter snakes
Evolution, *32*, 304-319

BENGSTON, S. (1971) Food and feeding of diving ducks breeding at Lake Myvatn, Iceland
Ornis. Fenn., *48*, 77-92

BENZIE, V.L. (1965) Some aspects of the anti-predator responses of two species of stickleback
D.Phil. thesis, University of Oxford, Dept. of Zoology

BERRY, J. (1936) Food of diving ducks in Scotland
Rep. Avon. Biol. Res., 1934-5, 31-64

BERRY, R.J. (1979) The Outer Hebrides: where genes and geography meet
Proc. Roy. Soc. Edin., *77B*, 21-43

BERTIN, L. (1925) Researches bionomiques, biometriques et systematiques, sur les epinoches (Gasterosteids)
Ann. Inst. Oceanograph. Monaco, *2*, 1-204

BLEGVAD, H. (1917) On the food of fish in the Danish waters within the Skaw
Rep. Danish Biol. Sta., *24*, 19-72

BOAG, P.T. & GRANT, P.R. (1978) Heritability of external morphology in Darwin's finches
Nature, *274*, 793-794

BROWN, W.W. & CHENG, C. (1946) Investigations into the food of the Cod, *Gadus calliaris* off Bear Island and of the Cod and Haddock (*G. aeglefinus* L.) off Iceland and the Murman Coast
Hull Bull. Mar. Ecol., *3*, 35-71

CAIN, A.J. & SHEPPARD, P.M. (1954)
Natural selection in *Cepaea*
Genetics, *39*, 89-116

CAMPBELL, J.W. (1947)
The food of some British wildfowl
Ibis, *89*, 429-32

CAMPBELL, J.W. (1949)
The breeding of the Heron in the Outer Hebrides
Scot. Nat., *61*, 73-100

CAMPBELL, R.N. (1971)
The growth of the Brown Trout, *Salmo trutta* L. in northern Scottish lochs with special reference to the improvement of fisheries
J. Fish. Biol., *3*, 1-28

CAMPBELL, R.N. (1979)
Sticklebacks (*Gasterosteus aculeatus* L. and *Pungitius pungitius* L.) in the Outer Hebrides, Scotland
Hebridean Naturalist, *3*, 8-15

CAMPBELL, R.N.B. (1976)
Comparisons between the diet of two Hebridean loch populations of Charr (*Salvelinus alpinus* L.) one allopatric, the other sympatric with Brown Trout (*Salmo trutta* L.)
B.Sc. thesis, University of Aberdeen, Dept. of Zoology

CAMPBELL, R.N. & WILLIAMSON, R.B. (1979)
The fishes of inland waters in the Outer Hebrides
Proc. Roy. Soc. Edin., *77B*, 377-393

CARL, C.G. & CLEMENS, W.A. (1953)
The freshwater fishes of British Columbia
British Columbia Provincial Museum Handbook, *5*, 93-95

CHAPELL, L.H. (1969)
The parasites of the three-spined stickleback (*Gasterosteus aculeatus* L.) from a Yorkshire pond. I. Seasonal variation in parasitic fauna
J. Fish Biol., *1*, 137-152

CHILD, D. (1970)
The essentials of Factor Analysis
Holt, Rinehart, Winston. London

CLARKE, A.S. (1954) Studies on the life history of the pseudophyllidean Cestode *Schistocephalus solidus*
Proc. Zool. Soc. Lond., *124*, 257-302

COAD, B.W. & POWER, G. (1973) Observations on the ecology and phenotypic variation of the three-spined stickleback (*Gasterosteus aculeatus* L. 1758) and the black-spotted stickleback (*Gasterosteus wheatlandi*, Putnam 1867) (Osteichthys; Gasterosteidae) in Armory Cove, Quebec
Can. Field Nat., *87*, 113-122

COAD, B.W. (1973) Modifications of the pelvic complex in the nine-spine stickleback *Pungitius pungitius* L. of Eastern Canada, and North West territories
Le Naturaliste Canadien, *100*, 315-316

COAD, B.W. & POWER, G. (1973) Observations on the ecology of lacustrine populations of the three-spine stickleback (*Gasterosteus aculeatus* L. 1758) in the Matamek River system, Quebec
Le Naturaliste Canadien, *100*, 437-445

COAD, B.W. & POWER, G. (1974) Meristic variation in the three-spined stickleback, *Gasterosteus aculeatus* in the Matamek River system, Quebec
J. Fish. Res. Bd. Can., *31*, 1155-1157

COOK, D.C. (1978) Foraging behaviour and food of Grey Herons, *Ardea cinerea* on the Ythan estuary
Bird Study, *25*, 17-22

COPLAND, W.O. (1956) Notes on the food and parasites of Pike (*Exos lucius*) in Loch Lomond
Glasgow Naturalist, *23*, 230-235

CURIO, E. (1976) The Ethology of Predation
Springer Verlag, Berlin

DACKE, C.G. (1979) Calcium regulation in sub-mammalian vertebrates
Academic Press, London

DAVIES, R.G. (1971)
Computer programming in Quantitative Biology
Academic Press, London

DE LACY, A.C. & MORTON, M.W. (1962)
Taxonomy and habits of the Charrs *Salvelinus malma* and *S. alpinus* of the Karluk drainage system
Trans. Am. Fish. Soc., *72*, 79-91

DICE, L.R. (1947)
Effectiveness of selection by owls on Deer mice (*Peromyscus maniculatus*) which contrast in colour with their background
Contr. Lab. Vertebr. Biol. Univ. Mich., *34*, 1-20

DILL, L.M. (1974)
The escape response of the Zebra Danio (*Brachydanio rerio*). II. The effect of experience
Anim. Behav., *22*(3), 723-730

DIXON, W.J. (1973)
B.M.D. Biomedical Computer programs
University of California Press, Berkley

EASTMAN, R. (1969)
The Kingfisher
Collins, London

EIGENMANN, C.H. (1886)
A review of the American Gasterosteidae
Proc. Acad. Nat. Sci. Phila., 1886, 233-252

FALKUS, H. (1977)
Sea Trout Fishing, 2nd ed.
Witherby, London

FERNALD, R.D. (1977)
Quantitative behavioural observations of *Haplochromis burtoni* under semi-natural conditions
Anim. Behav., *25*, 643-653

FJELDSA, J. (1975)
A census of Black-headed gull *Larus ridibundus*, and Arctic Tern *Sterna paradisea* in the Lake Myvatn area of Northwest Iceland
Dansk. Ornithologisk Fovenings Tiolsskrift, *69*, (1-2), 65-72

FORD, E.B. (1975) Ecological Genetics
Chapman and Hall, London

FROST, W.E. (1954) The food of the Pike, *Esox lucius* L., in Lake Windermere
J. Anim. Ecol., *23*, 339-360

FROST, W.E. & BROWN, M.E. (1967) The Trout
Collins, London

GARSIDE, E.T. & HAMOR, T. (1973) Meristic variation in the three-spine stickleback *Gasterosteus aculeatus* L. in Eastern Canadian waters
Can. J. Zool., *51*, 547-551

GERELL, R. (1968) Food habits of the Mink, *Mustela vison* Schreb. in Sweden
Viltrevy, *5*, 120-194

GILES, N. (1980) A stomach sampler for live fish
J. Fish. Biol., *16*, 441-444

GILES, N. (1981) Summer diet of the Grey Heron
Scottish Birds, *11*, 153-159

GREENBANK, J. & NELSON, P.R. (1959) Life history of the three-spine stickleback *Gasterosteus aculeatus* L. in Karluk Lake and Bare Lake, Kodiac Island, Alaska
U.S. Fish and Wildlife Serv. Fish. Bull., No. 153

GROSS, H.P. (1978) Natural selection by predators on the defensive apparatus of the three-spined stickleback, *Gasterosteus aculeatus* L.
Can. J. Zool., *56*, (3), 398-413

GROSS, H.P. (1978) Adaptive trends of environmentally sensitive traits in the three-spined stickleback, *Gasterosteus aculeatus* L.
Z. Zool. Syst. und Evolutionsforsch, *15*, (4), 252-278

HAGEN, D.W. (1967)
Isolating mechanisms in three-spine sticklebacks (*Gasterosteus*)
J. Fish. Res. Bd. Can., *24*, 1637-1692

HAGEN, D.W. (1973)
Inheritance of numbers of lateral plates and gill rakers in *Gasterosteus aculeatus*
Heredity, *30*, (3), 301-312

HAGEN, D.W. &
GILBERTSON, L.G. (1972)
Geographic variation and environmental selection in *Gasterosteus aculeatus* in the Pacific North West America
Evolution, *26*, (1), 32-51

HAGEN, D.W. &
GILBERTSON, L.G. (1972)
Selective predation and the intensity of selection acting upon the lateral plates of three-spine sticklebacks
Heredity, *30*, (3), 273-287

HAGEN, D.W. &
GILBERTSON, L.G. (1973)
The genetics of plate morphs in freshwater three-spine sticklebacks
Heredity, *31*, (1), 75-84

HAGEN, D.W. &
McPHAIL, J.D. (1970)
The species problem within *Gasterosteus aculeatus* on the Pacific coast of North America
J. Fish. Res. Bd. Can., *27*, 147-155

HAGEN, D.W.,
MOODIE, G.E.E. &
MOODIE, P.F. (1972)
Territoriality and courtship in the Olympic mud-minnow (*Novumbra hubbsi*)
Can. J. Zool., *50*, 1111-1115

HARRIS, C.J. (1968)
Otters
Weidenfield and Nicolson, London

HARVIE-BROWN, J.A. &
BUCKLEY, T.E. (1888)
The Vertebrate fauna of the Outer Hebrides
Douglas, Edinburgh

HAY, D.E. (1974)
Ecological Genetics of three-spine sticklebacks (*Gasterosteus*)
Ph.D. thesis, University of British Columbia, Department of Zoology

HAY, D.E. & McPHAIL, J.D. (1975) Mate selection in three-spine sticklebacks (*Gasterosteus*) *Can. J. Zool.*, *53*, 441-450

HEALY, A. (1956) Pike (*Esox lucius* L.) in three Irish lakes *Sci. Proc. R. Dublin Soc.*, *25*, 51-67

HEUTS, M.J. (1944) Calciumionen en geografische Verspreiding van *Gasterosteus aculeatus* *Natuurwetensch. Tijdschr. Jg.*, *26*, 10-14

HEUTS, M.J. (1946) Physiological isolating mechanisms and selection within the species *Gasterosteus aculeatus* L. *Nature*, *158*, 839

HEUTS, M.J. (1947) Experimental studies on adaptive evolution in *Gasterosteus aculeatus* L. *Evolution*, *1*, 89-102

HOOGLAND, R.D. (1951) On the fixing mechanism in the spines of *Gasterosteus aculeatus* L. *Konink. Neder. Akad. Weten. Proc. Ser. C.*, *54*, 171-180

HOOGLAND, R.D., MORRIS, D. & TINBERGEN, N. (1957) The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as a means of defence against predators (*Perca* and *Esox*) *Behaviour*, *10*, 200-230

HOPE, K. (1968) Methods of multivariate analysis University of London Press

HOPKINS, C.A. & SMYTH, J.D. (1951) Notes on the morphology and life history of *Schistocephalus solidus* (Cestoda: Diphyllobothriidae) *Parasitology*, *41*, 283-291

HUBBS, C.L. (1929) The Atlantic American species of the fish genus *Gasterosteus* *Occ. pap. Mus. Zool., University of Michigan*, No. 200, 1-9

HUBBS, C.L. & LAGLER, K.F. (1958)
Fishes of the Great Lakes Region
Cranbrook Institute Scientific Bulletin, 26

HUMPHRIES, D.A. & DRIVER, P.M. (1967)
Erratic display as a defence against predators
Science, *156*, 1767-1768

HUNT, P.C. & JONES, J.W. (1972)
The food of the Brown Trout in Llyn Alaw Anglesey, North Wales
J. Fish Biol., *4*, 333-352

HUNTINGFORD, F.A. (1973)
A comparison of anti-predator behaviour and aggression towards conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*
D.Phil. thesis, University of Oxford, Dept. of Experimental Psychology

HUNTINGFORD, F.A. (1976)
The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*
Anim. Behav., *24*, 245-260

HUNTINGFORD, F.A. (1976)
The relationship between inter- and intra-specific aggression
Anim. Behav., *24*, 485-497

HUNTINGFORD, F.A. (1976)
An investigation of the territorial behaviour of the three-spined stickleback (*Gasterosteus aculeatus*) using Principal Components Analysis
Anim. Behav., *24*, 822-834

HUNTINGFORD, F.A. (1976)
A comparison of the reaction of sticklebacks in different reproductive conditions towards conspecifics and predators
Anim. Behav., *24*, 694-697

HYNES, H.B.N. (1950)
The food of freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review of methods used in studies of the food of fishes
J. Anim. Ecol., *19*, 36-58

IGARISHI, K. (1964) Observations on the development of the scutes in a land-locked form of three-spined stickleback, *Gasterosteus aculeatus aculeatus* Linnaeus.
Bull. Jap. Soc. Sci. Fish., *30*, 95-103

IGARISHI, K. (1970) Formation of the scutes in the marine form of the three-spined stickleback *Gasterosteus aculeatus aculeatus* (L).
Annot. Zool. Jap., *43*, 34-42

JAKOBSSON, S. & JARVI, T. (1976) Anti-predator behaviour of 2-year old hatchery-reared Atlantic salmon, *Salmo salar* and a description of the predatory behaviour of Burbot, *Lota lota*
Zool. Revy., *38*, (3), 57-70

JENKINS, D., NEWTON, I. & BROWN, C. (1976) Structure and dynamics of a Mute Swan population
Wildfowl, *27*, 77-82

JONES, J.W. & HYNES, H.B.N. (1950) The age and growth of *Gasterosteus aculeatus*, *Pygosteus pungitius* and *Spinachia vulgaris* as shown by their otoliths
J. Anim. Ecol., *19*, 59-73

KAUKORANTA, E. & LIND, E.A. (1975) The Pike, *Esox lucius* L. in the estuary of the Oulujoki river, I: Ecology
Ichthyol. Fenn. Borealis, *(1-2)*: 1-40

KETTLEWELL, H.B.D. (1955) Selection experiments on industrial melanism in the Lepidoptera
Heredity, *9*, 323-42

KETTLEWELL, H.B.D. (1956) Further selection experiments on industrial melanism in the Lepidoptera
Heredity, *10*, 287-301

KIMMEL, C.B., PATTERSON, J. & KIMMEL, R.O. (1974) The development and behavioural characteristics of the startle response in the Zebra fish
Dev. Psychobiol., *7*, (1), 47-60

KREBS, J.R. &
DAVIES, N.B. (1978)
Behavioural Ecology
Blackwell, Oxford

KROGIUS, F.V.,
KROKHIN, E.M. &
MENSHUTKIN, V.V. (1970)
The pelagic fish community in Lake Dalnee. An experiment in Cybernetic modelling
Fish. Res. Bd. Can. Translation, Series No. 1486

KYNARD, B.E. (1972)
Male breeding behaviour and lateral plate phenotype in the three-spine stickleback (*Gasterosteus aculeatus* L.)
Ph.D. thesis, University of Washington

KYNARD, B. &
CURRY, K. (1976)
Meristic variation in the three-spined stickleback *Gasterosteus aculeatus* from Auke Lake, Alaska, U.S.A.
Copeia (U.S.), 4, 811-813

KYNARD, B.E. (1979)
Nest habitat preference of low plate number morphs in three-spine sticklebacks (*Gasterosteus aculeatus*)
Copeia, 1979, 525-528

LARSON, G. (1972)
Social behaviour and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake
Ph.D. thesis, University of British Columbia (Dept. of Zoology)

LEA, G.R. (1968)
Lateral plates in the three-spine stickleback (*Gasterosteus aculeatus* L.) as a defence against predators
B.Sc. thesis, University of British Columbia (Dept. of Zoology)

LEGGET, W.C. &
POWER, G. (1969)
Differences between two populations of land locked Atlantic salmon (*Salmo salar*) in Newfoundland
J. Fish. Res. Bd. Canada, 26, 1585-1596

LEMMETYINEN, R. &
RAITIS, T. (1972)
Occurrence of *Schistocephalus solidus*, Cestoidea, Dibothriocephalidae in Common and Arctic Terns in Southern Finland
Annales Zoologici Fennici, 9, (4), 208-211

LEMMETYINEN, R. (1973) Feeding ecology of *Sterna paradisea* Pontopp. and *S. hirundo* L. in the archepelago of Southwest Finland
Annales Zoologici Fennici, *10*, 507-525

LEMMETYINEN, R. (1973) Clutch size and timing of breeding in the Arctic Tern in the Finnish archepelago
Ornis Fennica, *50*, (1), 18-28

LEMMETYINEN, R. & MANKKI, J. (1975) The three-spined stickleback (*Gasterosteus aculeatus*) in the food chains of the Northern Baltic
Merentutkimuslait Julk/Hausforskningsinst, *239*, 155-161

LESTER, R.J.G. (1971) The influence of *Schistocephalus* plerocercoides on the respiration of *Gasterosteus* and possible resulting effect upon the behaviour of the fish
Can. J. Zool., *49*, 361-366

LI, S.K. & OWINGS, D.H. (1978) Sexual selection in the three-spined stickleback. Part I - Normative observations
Zeitschrift fur Tierpsychologie, *46*, 359-371

LINDSEY, C.C. (1962) Experimental study of meristic variation in a population of three-spine sticklebacks, *Gasterosteus aculeatus*
Can. J. Zool., *40*, 271-312

LISHEV, M.N. (1950) Feeding and food relations among predaceous fishes of the Amur basin
Trudy. Amur ikhtiol. expedit., 1945-1949, *1*, 19-146

MACLEAN, J.A. (1974) Variation and natural selection in a population of sticklebacks
Ph.D. thesis, University of British Columbia

MACLEAN, J. (1980) Ecological genetics of three-spine sticklebacks in Heisholt lake
Can. J. Zool., *58*, 2026-2039

Food of diving ducks in Danish fjords
Dan. Rev. Game Biol., 3, 19-83

MAITLAND, P.S. (1965)
The feeding relationships of Salmon, Trout, Minnows, Stone Loach and three-spined sticklebacks in the River Endrick, Scotland
J. Anim. Ecol., 34, 109-133

MAITLAND, P.S. (1972)
Loch Lomond: Man's effects upon the Salmonid community
J. Fish. Res. Bd. Canada, 29, 849-860

MANN, R.H.K. &
ORR, D.R.O. (1969)
A preliminary study of the feeding relationships of fish in a hard water and a soft-water stream in Southern England
J. Fish. Biol., 1, 31-44

MANN, R.H.K. (1971)
The populations, growth and production of fish in four small streams in Southern England
J. Anim. Ecol., 40, 155-190

MANN, R.H.K. (1978)
Observations on the biology of the Perch, Perca fluviatilis in the River Stour, Dorset
Freshwater Biology, 8, 229-239

MANZER, J.I. (1976)
Distribution, food and feeding of the three-spined stickleback, Gasterosteus aculeatus in Great Central Lake, Vancouver Island, with comments on competition for food with juvenile sockeye salmon, Oncorhynchus nerka
U.S. Natl. Mar. Fish. Serv. Fish. Bull., 74, (3), 647-668

MAXWELL, A.E. (1977)
Multivariate Analysis in Behavioural Research
Chapman & Hall, London

MAYNARD-SMITH, J. (1970)
The causes of Polymorphism
J. Symp. Zool. Soc. Lond., 26, 371-383

MACKERETH, F.J.H.,
HERON, J. &
TALLING, J.F. (1978)
Water Analysis
Sci. Publ. No. 36, Freshwater Biological Association

McCORMACK, J.C. (1970)
Observations on the food of Perch (Perca fluviatilis L.) in Windermere
J. Anim. Ecol., 39, 255-267

McPHAIL, J.D. (1969) Predation and Evolution in a stickleback (*Gasterosteus*)
J. Fish. Res. Bd. Canada, *26*, 3183-3208

McPHAIL, J.D. (1977) Inherited interpopulation differences in size at first reproduction in three-spine stickleback, *Gasterosteus aculeatus* L.
Heredity, *38*, (1), 53-60

MEAKINS, R.H. (1974) A quantitative approach to the effects of the plerocercoid of *Schistocephalus solidus* Muller, 1776 on the ovarian maturation of the three-spined stickleback, *Gasterosteus aculeatus* L.
Z. Parasitenkd, *44*, 73-79

MERRIE T.D. (1978) Relationship between spatial distribution of breeding divers, and the availability of fishing waters
Bird Study, *25*, (2), 119-122

MILINSKI, M. & HELLER, R. (1978) Influence of a predator on the optimal foraging behaviour of sticklebacks (*Gasterosteus aculeatus* L.)
Nature, *275*, 642-644

MILLER, P.J. (1979) Adaptiveness and implications of small size in teleosts
Symp. Zool. Soc. Lond., *44*, 263-306

MILLER, R.R. & HUBBS, C.L. (1969) Systematics of *Gasterosteus aculeatus* with particular reference to intergradation and introgression along the Pacific coast North America: a commentary on a recent contribution
Copeia, *1*, 52-69

MILLS, B.J., SUTER, P. & LAKE, P.S. (1976) The amount and distribution of calcium in the exoskeleton of intermoult crayfish of the genera *Engaeus* and *Geocharax*
Aust. J. Marine and Freshwater Research, *27*, 517-523

MILLS, D. (1962) The Goosander and Red-breasted Merganser in Scotland
Wildfowl Trust Annual Report, *13*, 79-92

MILLS, D.H. (1962)
The Goosander and Red-breasted Merganser as predators of Salmon in Scottish waters
Freshwater and Salmon Research Series, Dept. of Ag. and Fish., Scotland, *29*

MILLS, D.H. (1971)
Salmon and Trout, A resource, its Ecology, Conservation and Management
Oliver and Boyd, Edinburgh

MITCHELL, J. (1978)
The Heronry at Gartfairn Wood, Loch Lomondside
Forth Naturalist and Historian, *3*, 56-67

MOODIE, G.E.E. (1972)
Predation, natural selection and adaptation in an unusual three-spine stickleback
Heredity, *28*, 155-168

MOODIE, G.E.E. (1972)
Morphology, life history and ecology of an unusual stickleback (*Gasterosteus aculeatus*) in the Queen Charlotte Islands, Canada
Can. J. Zool., *50*, 6, 721-732

MOODIE, G.E.E. (1977)
Meristic variation, asymmetry and aspects of the habitat of *Culaea inconstans* (Kirtland), the brook stickleback in Manitoba
Can. J. Zool., *55*, (2), 398-404

MOODIE, G.E.E.,
McPHAIL, J.D. &
HAGEN, D.W. (1973)
Experimental demonstration of selective predation on *Gasterosteus aculeatus*
Behaviour, *47*, 95-105

MOODIE, G.E.E. &
REIMCHEN, T.E. (1973)
Endemism and conservation of sticklebacks in the Queen Charlotte Islands
Canadian Field Naturalist, *87*, (2), 173-175

MOODIE, G.E.E. &
REIMCHEN, T.E. (1976)
Glacial refugia, endemism and stickleback populations of the Queen Charlotte Islands, British Columbia
Canadian Field Naturalist, 90, 471-474

MORRIS, D. (1952)
Homosexuality in the ten-spined stickleback
Behaviour, *4*, 233-261

MULLEM, P. van & VLUGT, J. van der (1964)	On the age, growth and migration of the anadromous stickleback *Gasterosteus aculeatus* L. investigated in mixed populations *Arch. Neerl. Zool.*, *16*, 111-139
MUNRO, J.A. & CLEMENS, W.A. (1937)	The American Merganser in British Columbia and its relation to the fish population *Bull. Biol. Bd. Canada.*, *55*, 1-50
MUNRO, J.A. & CLEMENS, W.A. (1939)	The food and feeding habits of the Red-breasted Merganser in British Columbia *J. Wildlife Mgmt.*, *3*, 46-53
MURAL, R.J. (1973)	The Pliocene sticklebacks of Nevada with a partial osteology of the Gasterosteidae *Copeia* (1973), 721-734
MUNTZING, J. (1962)	Ein neuer semiarmatus-Typ von *Gasterosteus aculeatus* L. (Pisces) aus dem Iznikseе *Milt. Hamburg. Zool. Mus.*, *60*, 181-194
MUNTZING, J. (1963)	The evolution of variation and distributional patterns in European populations of the three-spined stickleback, *Gasterosteus aculeatus* *Evolution*, *17*, 320-332
MUNTZING, J. (1972)	Polymorpe populationen von *Gasterosteus aculeatus* L. (Pisces, Gasterosteidae) in sekundaren intergradationszonen der Deutschen Bucht und benachbarter Gebiete *Faun. Okol. Milt.*, *4*, 69-84
NARVER, D,W. (1969)	Phenotypic variation in three-spine sticklebacks (*Gasterosteus aculeatus*) of the Chignik River system, Alaska *J. Fish. Res. Bd. Canada*, *26*, 405-412
NELSON, J.S. (1971)	A comparison of the pectoral and pelvic skeletons and of some other bones, and their phylogenetic implications in the Aulorhynchidae and Gasterosteidae (Pisces) *J. Fish. Res. Bd. Canada*, *28*, 427-442

NELSON, J.S. & ATTON, F.M. (1971) Geographical and morphological variation in the presence and absence of the pelvic skeleton in the brook stickleback *Culaea inconstans* (Kirkland) in Alberta and Saskatchewan

Can. J. Zool., *49*, 343-352

NELSON, J.S. (1971) Absence of the Pelvic complex in nine-spine sticklebacks *Pungitius pungitius*, collected in Ireland and Wood Buffalow National Park region, Canada, with notes on meristic variation

Copeia, 1971, 707-711

NELSON, J.S. (1977) Evidence of a genetic basis for absence of the pelvic skeleton in brook stickleback, *Culaea inconstans*, and notes on the geographical distribution and origin of the loss

J. Fish. Res. Bd. Canada, *34*, 9, 1314-1320

NILSSON, N.A. (1955) Studies on the feeding habits of trout and char in North Swedish lakes

Rep. Inst. Freshwat. Res. Drottningholm, *36*, 163-225

OWEN, D.F. (1955) The food of the Heron, *Ardea cinerea* in the breeding season

Ibis, *97*, 276-295

OWEN, D.F. (1960) The nesting success of the heron, *Ardea cinerea* in relation to the availability of food

Proc. Zool. Soc. Lond., *133*, 597-617

PARAKETSOV, I.A. (1958) On the protective role of body spines and sharp fin rays in fishes

Lh. Obstich. Biol., *19*, 449-456

PATTERN, B.G. (1977) Body size and learned avoidance factors affecting predation on Coho salmon *Oncorhyncus kisutch* fry by torrent sculpin *Cottus rhotheus*

Fish. Bull. U.S. Dept. Commer. Natl. Mar. Fish. Serv. Seattle *75*, (2), 457-59

PEEKE, H.V.S. (1969)
Habituation of conspecific aggression in the three-spined stickleback (Gasterosteus aculeatus L.)
Behaviour, 35, 137-156

PENCZAK, T. (1960)
New cases of variability of the stickleback Gasterosteus aculeatus
Fragm. Faun. Warsaw, 8, 403-410

PENCZAK, T. (1961)
Significance of the regeneration of lateral plates of the stickleback Gasterosteus aculeatus L.
Nature, 191, 621

PENCZAK, T. (1961)
Serological relationships between the nine-spined and three-spined stickleback
Nature, 192, 673-674

PENCZAK, T, (1962)
The stickleback (Gasterosteus aculeatus L.) from Nova Scotia, Newfoundland, and the French Island Saint-Pierre
Ann. Zool. Warsaw., 20, (17), 335-341

PENCZAK, T. (1962)
The biometry of the three-spine stickleback Gasterosteus aculeatus L. from the Nev River
Fragm. Faun. Warsaw, 10, 137-161

PENCZAK, T. (1962)
Morphometry of the stickleback Gasterosteus aculeatus L. from the Baltic Sea
Zool. Pol. Wroclaw, 12, (3), 239-246

PENCZAK, T. (1964)
Three-spined stickleback from Iceland Gasterosteus aculeatus islandicus Sauvage
Ann. Zool., 22, 441-448

PENCZAK, T. (1965)
Morphological variation of the stickleback Gasterosteus aculeatus L. in Poland
Zoologica Poloniae, 15, 3-49

PENCZAK, T. (1966)
Comments on the taxonomy of the three-spine stickleback Gasterosteus aculeatus L.
Ohio. J. Sci., 66, 81-87

PENCZAK, T. (1968)
Gasterosteus aculeatus L. as food of *Mergus merganser* L.
Przegl. Zool., *12*, 357-358

PENNYCUIK, L. (1971)
Seasonal variations in the parasite infections in a population of three-spine sticklebacks *Gasterosteus aculeatus* L.
Parasitology, *63*, 373-388

PENNYCUIK, L. (1971)
Quantitative effects of three species of parasites on a population of three-spined sticklebacks *Gasterosteus aculeatus* L.
J. Zool. Lond., *165*, 143-162

PHILLIPS, G.C. (1962)
Survival value of the white colouration of gulls and other seabirds
D.Phil. thesis, University of Oxford, Department of Zoology

RATCLIFFE, D. (1977) (editor)
A Nature Conservation Review, Vol. 2, Site Accounts
Cambridge University Press

REIMCHEN, T.E. (1980)
Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators?
Can. J. Zool., *58*, 1232-1244

REIST, J.D. (1980)
Predation upon pelvic phenotypes of the brook stickleback, *Culaea inconstans* by selected invertebrates
Can. J. Zool., *58*, 1253-1258

REIST, J.D. (1980)
Selective predation upon pelvic phenotypes of brook sticklebacks *Culaea inconstans* by Northern Pike *Esox lucius*
Can. J. Zool., *58*, 1245-1252

RICHMOND, W.K. (1974)
Birds (chapter 3 in) A Natural History of Loch Lomond
University of Glasgow Press

ROGERS, D.E. (1968) A comparison of the food of Sockeye Salmon fry and three-spine sticklebacks in Wood River Lakes
University of Washington Publications, *3*, 1-43

ROHWER, S. (1978) Parent cannibalism of offspring and egg raiding as a courtship strategy
American Naturalist, *112*, 429-440

ROSS, S.T. (1973) The systematics of *Gasterosteus aculeatus* (Pisces: Gasterosteidae) in Central and Southern California
Nat. Hist. Mus. Los. Ang. Cty. Contrib. Sci., *243*, 1-20

SCHLEGEL, R. (1964) Zur Nahrund ges Gravreihers (*Ardea cinerea* L.) an oberlausitzer karpfenteichen
Zool. Abh. Mus. Tierk Dresden, *27*, (3), 65-67

SEGHERS, B.H. (1970) Behavioural adaptations of natural populations of the Guppy *Poecilia reticulata* to predation
Amer. Zool., *10*, 489-490

SEGHERS, B.H. (1973) An analysis of geographic variation in the anti-predator adaptations of the Guppy *Poecilia reticulata*
Ph.D. thesis, University of British Columbia

SEGHERS, B.H. (1974) Geographic variation in the responses of Guppies (*Poecilia reticulata*) to aerial predators
Oecologia (Berl.), *14*, 93-98

SEMLER, D.E. (1971) Some aspects of adaptation in a polymorphism for breeding colours in the three-spine stickleback (*Gasterosteus aculeatus*)
J. Zool. Lond., *165*, 291-302

SHAFI, M. (1969) Comparative studies of populations of Perch (*Perca fluviatilis*) and Pike (*Esox lucius*) in two Scottish lochs
Ph.D. thesis, University of Glasgow, Dept. of Zoology

SHAFI, M. (1974)
Studies of populations of Perch (*Perca fluviatilis* L.) and Pike (*Esox lucius* L.) in two British lakes
The Dacca University Studies *B*, *22* (1),

SHEPHARD, M.P. &
BILTON, T.H. (1953)
Distribution of Lake Fishes (in Lakelse Lake)
Fish. Res. Bd. Can. Pacific Biol. Station Annual Report 1953

SIMKISS, K. (1974)
Calcium metabolism of fish in relation to ageing, in Bagenal, T.B. (ed.)
Ageing of Fish Unwin, London

SLACK, H.D. (1934)
The winter food of the Brown Trout (*Salmo trutta* L.)
J. Anim. Ecol., *3*, 105-108

SLACK, H.D. (1957)
Studies on Loch Lomond, Vol. 1
Blackie, Glasgow

TINBERGEN, N. &
IERSEL, J.J.A. van (1947)
"Displacement reactions" in the three-spined stickleback
Behaviour, *1*, 56-63

THOMPSON, F. (1970)
The Uists and Barra
David and Charles, Plymouth

THRELFALL, W. (1968)
A mass die-off of three-spined sticklebacks (*Gasterosteus aculeatus* L.) caused by parasites
Can. J. Zool., *46*, 105-106

VALDEZ, R.A. &
HELM, W.T. (1971)
Ecology of the three-spined stickleback *Gasterosteus aculeatus* Linnaeus on Amchitka Island, Alaska
Bioscience, 21, 641-645

WATERSTON, A.R.,
HOLDEN, A.V.,
CAMPBELL, R.N. &
MAITLAND, P.S. (1979)
The inland waters of the Outer Hebrides
Proc. Roy. Soc. Edin. *77B*, 329-351

WALKEY, M. (1967)
The ecology of *Neoechinorynchus rutili* (Muller)
J. Parasit., *53*, 795-801

WENDELAAR BONGA, S.E., GREVEN, J.A. & MEIN, C. (1978)
The relationship between external and internal calcium concentration and prolactin cell activity in a Euryhaline teleost
General and Comparative Endocrinology, *34*, (1), 91

WILZ, K.J. (1967)
The organisation of courtship behaviour in sticklebacks
D.Phil. thesis, University of Oxford, Dept. of Zoology

WILZ, K.J. (1972)
Causal relationships between aggression and the sexual and nest behaviour in the three-spined stickleback (*Gasterosteus aculeatus*)
Anim. Behav., *20*, 335-340

WOOTTON, R.J. (1971)
A note on the nest-raiding behaviour of male sticklebacks
Can. J. Zool., *49*, 960-962

WOOTTON, R.J. (1972)
The behaviour of the male three-spined stickleback in a natural situation: a quantitative description
Behaviour, *41*, 232-241

WOOTTON, R.J. (1973)
Fecundity of the three-spined stickleback, *Gasterosteus aculeatus*
J. Fish. Biol., *5*, 683-688

WOOTTON, R.J. (1974)
The inter-spawning interval of the female three-spined stickleback, *Gasterosteus aculeatus*
J. Zool. Lond., *172*, 331-342

WOOTTON, R.J. (1976)
The Biology of the Sticklebacks
Academic Press, London

WORTHINGTON, E.B. (1950)
An experiment with populations of fish in Windermere
Proc. Zool. Soc. Lond., *120*, 113-149