A Population Study of Threespine Sticklebacks *(Gasterosteus aculeatus)* in the Reed College Canyon During the Summer of 1995.

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Abstract:

The high degree of phenotypic plasticity in Threespine Sticklebacks *(Gasterosteus aculeatus)* has been well documented and studied for a number of years. The presence of sticklebacks in the Reed College Canyon (Portland, Oregon) has allowed this first order study to begin to uncover some of the most basic facts about the population itself, its phenotypic variation and morphological expression. When coupled with known physical facts of the Canyon, valuable insights into the species and the local ecology will be gained. A mark- recapture method was employed and a tentative estimate of a population size of 19,578 individuals was reached using a simple Lincon-Type Index model. The physical isolation of the sticklebacks in the Canyon is briefly addressed. Results on the distribution of sticklebacks and their densities are tentative and further investigation is needed. There is evidence from age class investigations (using lengths) that the life cycle for this population is of one to two years, though exceptions may exist. Morphologically, all examined individuals belonged to the low plated form but there are indications that there may be a dimorphism within the population, termed here "blue morph" and "gold morph". The significance of this observed morphological differentiation is discussed together with a variety of possible explanations. The absence of any males with red throats in the sample is also discussed in light of the fact that there was an area of the canyon which was not sampled (giving possible insights into nest site choice by males in the Canyon) as well as the possibility there may be evolutionary or genetic reasons for this observation.

Introduction:

The Threespine stickleback (*Gasterosteus aculeatus*) is perhaps one of the most important animals ever to be studied by biologists. The species complex it comprises has yielded many answers to behavioral, evolutionary and ecological questions, and has pointed the direction for many theoretical and experimental advances.

Some of the earliest interest in the group centered around systematics and taxonomic divisions, reaching as far back as the 1700's (Linnaeus, 1758 *In:* Bell and Foster, 1994) and from those initial studies until the most recent discoveries in paleobiology or evolutionary genetics perhaps the most valuable lesson has been that there is much more to be learned and understood. It is in this spirit of humility and awe that the present study proposes to begin to uncover some of the most important aspects of a single group of threespine sticklebacks in the Reed College Canyon.

1.1: Geographic and Ecological Distribution.

A complete detailing of the known aspects of the biology of *Gasterosteus aculeatus* would require at least a large book, and certainly what remains to be discovered could fill many more. Some basic facts, however, are essential for a full understanding of the significance of the results described here. The emphasis for these purposes will be placed on aspects of the ecology, evolution and phenotypic expression of the species.

The threespine stickleback is a widely distributed fish, being found in both the Atlantic and Pacific oceans of the northern hemisphere. These marine populations are distributed across the northern rim of the Atlantic (from the coasts of northern Europe to north eastern Canada) and in the Pacific from the waters of Japan, around western Siberia then down the Pacific coast of Canada and the United States.

There are freshwater populations that are widely distributed inland from these coastal ranges, notably in northern Europe and the Americas. There are also reports of isolated freshwater populations in south western Syria, Turkey and North Africa (Reagan 1909; Krupp and Coad, 1985 *In* : Bell and Foster, 1994)

The fact that the range of *G. aculeatus* spreads over both salt and freshwater environments leads to the basic distinction of the species into "marine", "freshwater" and migratory "anadromous" populations. Investigations into differences between these distinct groups have yielded insights into the life history of sticklebacks and the differences in freshwater, anadromous and marine sticklebacks (Snyder and Dingle, 1988; Snyder 1989; Snyder and Dingle, 1990). Size at first reproduction is one aspect which has received attention: while it was previously known that there are interpopulational differences (McPhail, 1977; Greenbank and Nelson, 1959) the salinity of the environment has consequently been shown to be one of many factors that affect life history.

The large amount of within species variation has confused ichthyologists for many years and serves to illustrate what perhaps is the most important evolutionary feature of the threespine stickleback: the extreme plasticity of the phenotypic characters within and across populations of *Gasterosteus aculeatus* .

1.2: The Phylogeny of the Threespine stickleback.

Perhaps the most important and well documented aspect the biology of *G. aculeatus* is the baffling complex of phenotypic expressions created by the exceptional plasticity of the species. Most of the work on sticklebacks has centered on one or another aspect of the variability observed, and from this pattern evolutionary biologists have inched closer to understanding an aspect of natural selection regimes, or have fitted another piece into the mental map of the ecological structure of a particular pond.

One of the earlier modern complete treatments of sticklebacks by Wooton (1976) goes to great lengths to try and make sense of the pattern created by the distribution of different plate morphologies (see section 1.3), drawing correlations with aspects of the abiotic environment (e.g. salinity, temperature and latitude) as well as the biotic one (e.g. predation pressure and competitive interactions). Initially, plate morphology had been used as a taxonomic determinant, the variants being considered different species. After Wooton and others' attempt to employ it as a tool in zoogeography, plate morph has finally been recognized as a powerful instrument in exploring ecological genetics. This has remained as a major subject of study in threespine sticklebacks.

The results of using *Gasterosteus aculeatus* in studies of this sort have been ground- breaking. Reproductive character displacement was shown to have occurred in a population of sticklebacks with different behavioral responses to mating (Bell, 1976). As an extension to this, there has also been experimental evidence of adaptive radiation driven by interspecific competition (Schluter, 1994), ultimately clarifying our understanding of the causes of diversification and the nature of competitive interactions. The discoveries in studies of different aspects of stickleback life have been no less impressive, but are too many to cite here.

Besides these theoretical and experimental advances, perhaps the most intriguing model for explaining the evolutionary history of sticklebacks is that proposed by Bell and Foster (1994). The argument is that the *G. aculeatus* species complex may be described as a phylogenetic raceme, where the central core of the raceme represents a common and persistent ancestral form of stickleback (Fig. 1.1).

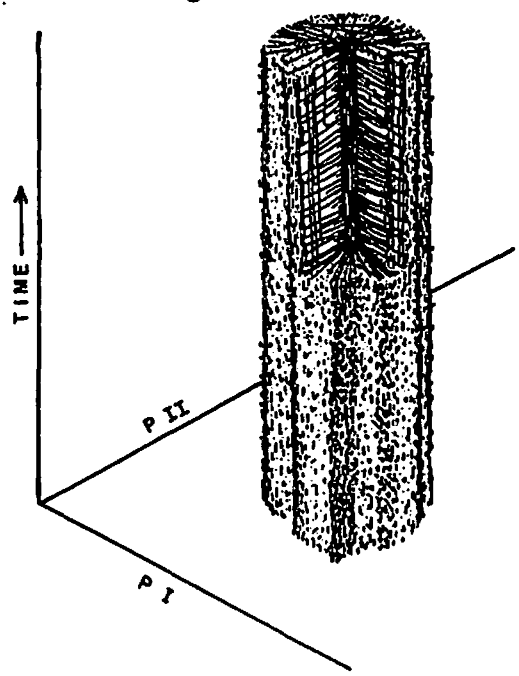

Figure 1.1: Simplified representation of the phylogenetic raceme of the Threespine stickleback species complex. PI and PII represent two- dimentional phenotypic space. (From: Bell and Foster, 1994).

Foster and Bell outline the evidence supporting the evolutionary directionality of marine to freshwater radiation in the species and further substantiate this argument with the proven phenotypic stability of marine populations. They conclude that there have been repeated invasions of freshwater by marine sticklebacks, which will then diverge rapidly from each other and from the marine form. In addition to this rapid divergence, however, these populations become extinct rapidly once they become isolated in an "inland" freshwater habitat. The factors that contribute to the ecological instability and relatively short life of these rapidly diverging freshwater populations are many, such as the elimination of the lake by a glacier, or due to the difficulty of movement between bodies of water.

This work deals with a single isolated population of threespine sticklebacks, and the results contained must be viewed especially with the fact that not only are morphological traits and phenotypes affected by this divergence of freshwater populations, but behavioral, life history and physiological expressions all have the potential to be altered. While these are not being specifically investigated, the potential for their exploration is very great. Of the traits that are being targeted it is also worth noting that many have genetic underpinnings (e.g.: Hagen, 1973; McPhail, 1977; Hagen and Gilbertson, 1972), highlighting the possible existence of other idiosyncratic characteristics of this population , associated or not.

Future work, both theoretically and experimentally, should combine the observed phenotypic patterns and begin to construct associations between the various observed variability in traits. This approach to understanding the processes of evolution is referred to as "phenotypic integration " (Bell and Foster, 1994) and some of the results of this approach will be discussed more fully below.

1.3: Variability in the *Gasterosteus aculeatus* species complex.

The great degree of variability observed in many of the phenotypic traits of the *Gasterosteus aculeatus* species complex has already been mentioned, and now a more detailed outlining of specific characters involved is required, showing the "major dimensions of phenotypic variation in *G. aculeatus* " (Bell and Foster, 1994).

Possibly as a consequence of its ready observability, body form variation was one of the first variable characteristics to be investigated (e.g.: Hoogland et al, 1957). It has already been mentioned how one aspect of body form (plate morphology) was initially employed as a way to distinguish between sticklebacks populations and as a tentative taxonomic division. Different aspects of stickleback phenotypic variation and their importance to the present work will be presented below.

Plate morphology, a central distinguishing factor in works such as Wooton's (1976) has been traditionally centered on classifying populations of *Gasterosteus aculeatus* into three different forms: Trachus, Semiarmatus and Leiurus. In this paper, following the suggestions of Bakker and Sevenster (1988) the alternative and less confusing terminology of "fully plated", "partially plated" and "low plated" for describing plate morphology will be employed (Fig. 1.2). The separating of these different forms into species seems to be a difficult endeavor, and other characters are more commonly accepted as being definitions of good biological species.

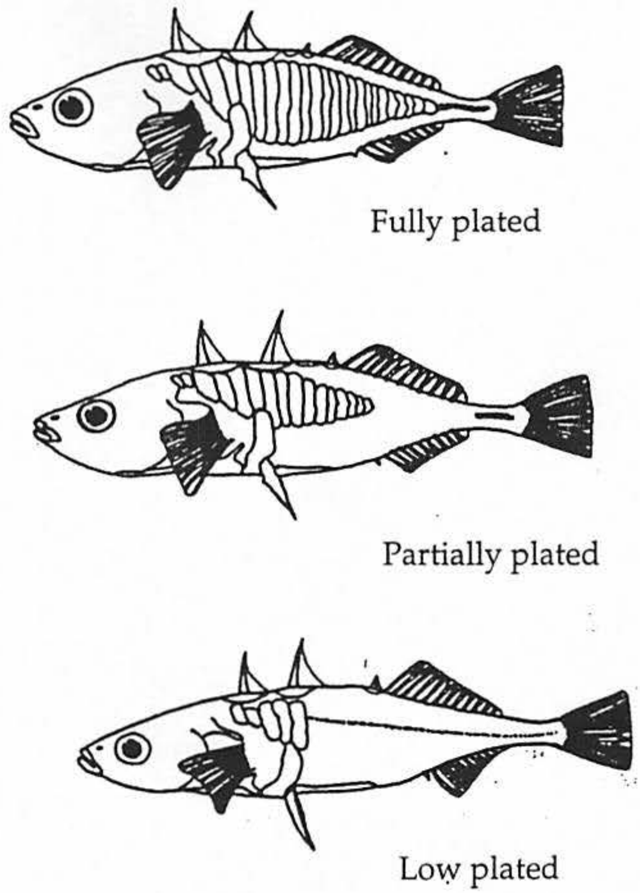

Figure 1.2: The three different plate morph- types of the Threespine stickleback species complex showing the extent of the lateral bony plates (scutes)
(From: Wooton, 1976).

In addition to plate form, there is also variability in plate number. While this particular character is not of particular interest to this study, researchers have found that sticklebacks with a plate number of seven have a selective advantage in terms of predatory pressure from other fish species. (e.g.: Hagen and Gilbertson, 1972). Explanations for higher observed frequencies of seven-plated sticklebacks in areas of higher predation range from the possibility of a behavioral link associated with the seven-plated phenotype which may increase fitness with regards to predation (Wooton, 1976) or that natural selection strongly favors this condition due to frequency dependent mating success (Gilbertson, 1980). While there is no strong evidence for the evolutionary mechanism involved, most agree that there is no geographic or clinal pattern to the observed variation.

Recently, one of the most important aspect of stickleback variation to be investigated has been the distinction between Benthic and Limnetic forms, with the realization that these represent specialization for alternative trophic niches (Bentzen and McPhail, 1984). The evolutionary implications are, of course, enormous and are being continually investigated (e.g.: Schluter, 1994). It is also being speculated that trophic specialization may be one of the most important and revealing distinctions in the species complex, being coherently associated to a large number of other known variable characters (See Fig. 1.3), such as snout length, gill raker number, body color and so on, which have already been shown to exhibit strong intra and inter population differences.

All these characters can also be seen to have strong relationships to life histories in populations of *G. aculeatus* (Foster et al, 1992). While life history is itself a variable phenotypic character, perhaps with a genetic underpinning (Caswell, 1983), all the separate factors that influence its expression or exist in concert with it have yet to be fully understood. Overwintering environments (Snyder and Dingle, 1990) and migratory patterns (Snyder, 1991), to mention just a few of the observed effects, have been shown to have significant impact on life histories. Fundamentally, it is probably true that these effects are not separate but can be understood as cohesive patterns of a large number of variables producing the observed associations, highlighting the importance of the phenotypic integration approach described by Bell and Foster (1994).

Finally, it is worth noting that in realizing that the expressions of the different populations within the *Gasterosteus aculeatus* species complex usually represent a gradient, as opposed to distinct and exclusive classes, stressing the taxonomic difficulty in separating variants. The variability has been shown also to extend to behavioral (e.g.: FrizGerald, 1983; Huntingford, 1976) , physiological (e.g.: Campeau et al, 1984) and ecological (e.g.: Bell, 1984; Larson and McIntire, 1993) expressions.

This paper is a first order study of the stickleback population in the Reed College Canyon and reveals aspects of the size and distribution of the

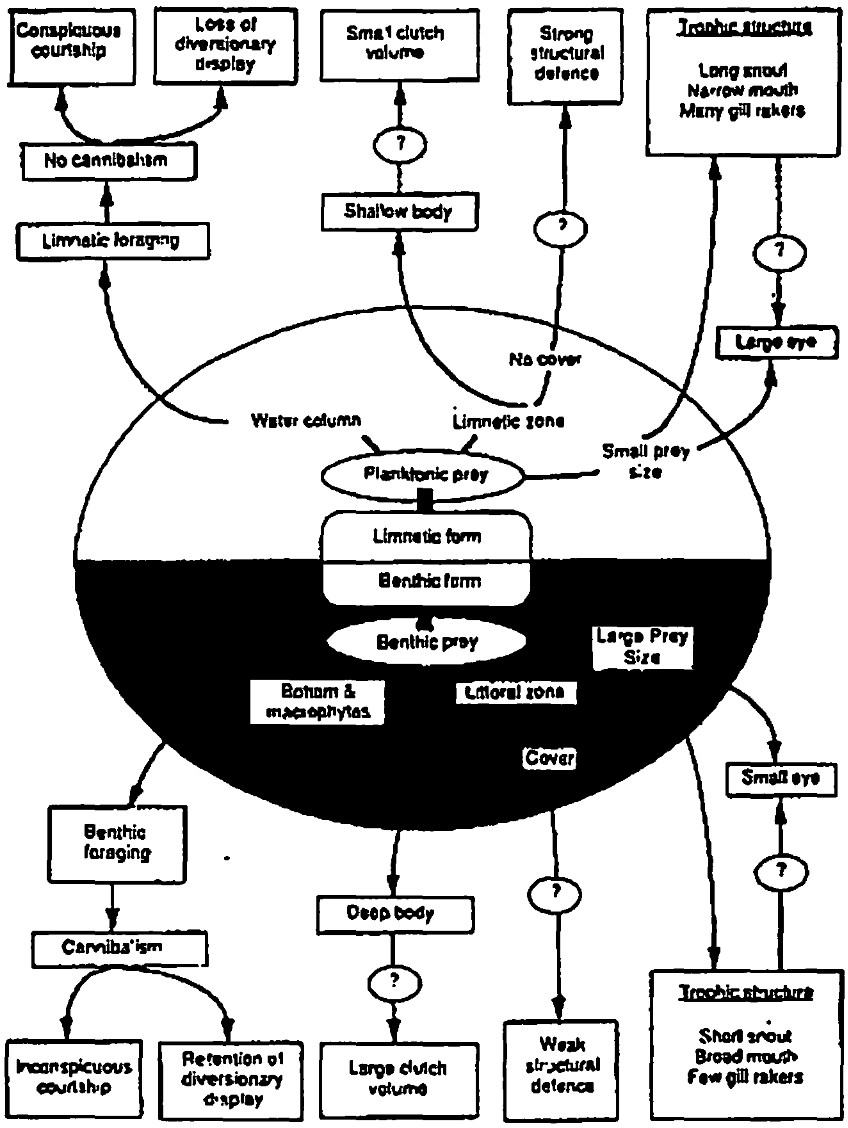

Figure 1.3: A schematic representation of the known evolutionary consequences assosiated with planktivory (limnetic form) and benthic feeding (benthic form). Arrows with question marks are hypothetical or poorly studied, unmarked arrows have strong empirical support. (From : Bell and Foster, 1994).

animals themselves. In addition to this, some of the most poignant and important aspects of the phenotypic characteristics of the population are determined and described, along with some interpretations of their significance.

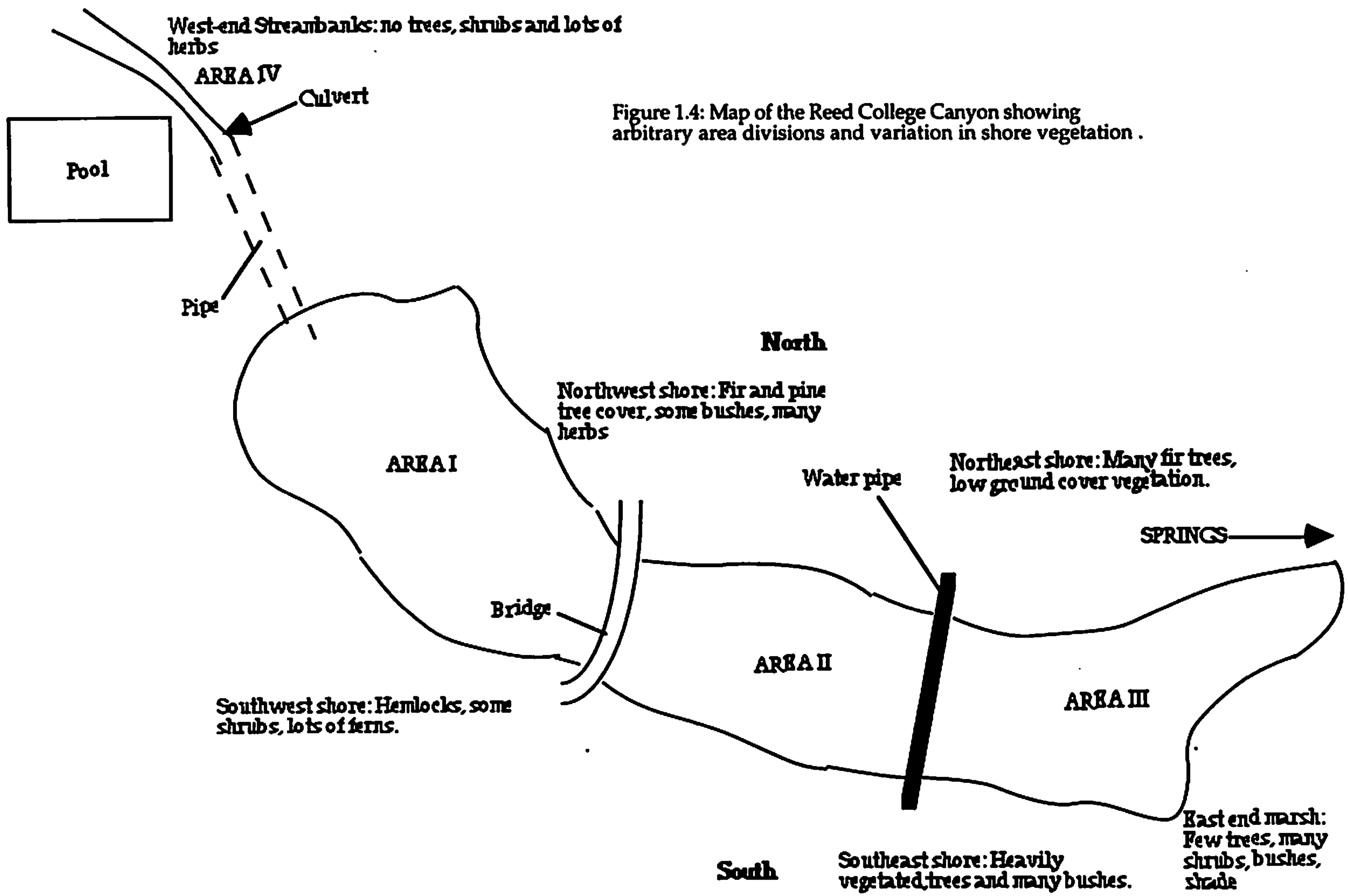

Figure 1.4: Map of the Reed College Canyon showing arbitrary area divisions and variation in shore vegetation .

Experimental Methods:

2.1: Sampling, Randomization and Trapping methods.

Sticklebacks were collected in the Reed college canyon over three major collecting sessions during the summer of 1995. For the first session (May 22-24) three traps were set per day (n=9 traps): one in each of three arbitrarily defined areas, which split the canyon into three roughly equal parts. During the second session (June 19- 22) four traps were set daily (n=16 traps): one in each of the three areas and one in a random part of the stream on the other side of the tunnel which allows water to escape from the canyon into the Johnson Creek drainage system. This stream was sampled to test for the presence of sticklebacks at different reaches of the course in order to determine the extent of the isolation of the population in the Canyon.

The third session (July 17, 19 and 21) saw 10 traps set per day (n=30 traps) in one of the three areas, so that over three days all areas were sampled once.

Areas at which fish were collected were determined by traveling a randomly determined number of meters along paths located on either side of the areas then placing the minnow trap a certain distance perpendicularly away from the bank (both numbers were determined by Permutator™). During the first two sessions (three traps set per day only), the order of the side (North or South) of each area to be sampled was also determined using the same random number generator. For the third session five traps were set on each side of the area being sampled.

The traps ("Gee's improved wire minnow traps"- see Fig. 2.1) were bated with dog food (Purina Puppy Chow™) wrapped in aluminum foil. Holes were made in the foil, and the traps were left out for around twenty-four hours. Just before collecting data were recorded from the physical environment surrounding the trap.

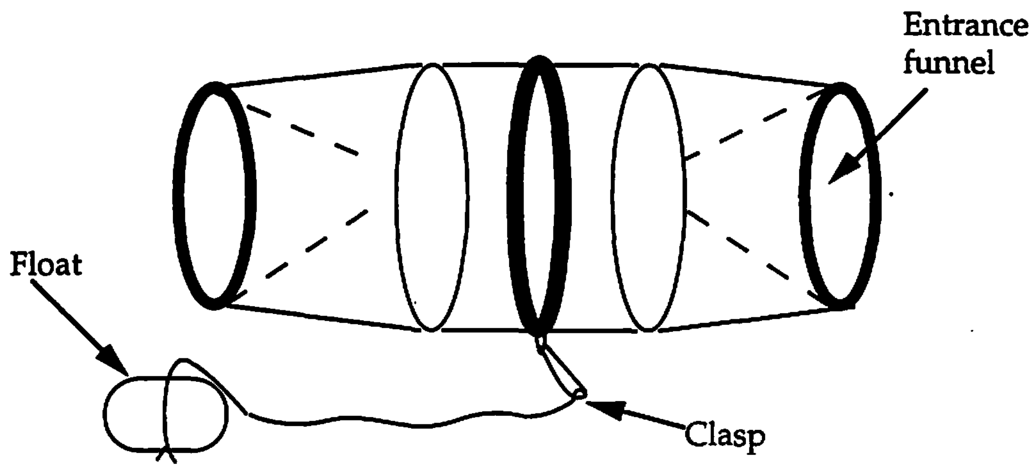

Fig. 2.1: "Gee's improved wire minnow trap"- schematic diagram.

Water temperature in trap (by placing a thermometer in the mesh of the trap without moving it), depth of the water (by standing a length of PVC tubing marked off at 10cm intervals next to the trap then estimating depth) and the physical surroundings (especially amount of cover, sunlight exposure and underwater vegetation, if any) were all recorded. After this background information had been collected, the sticklebacks were removed from the trap.

All the fish in each trap were emptied into a 5- gallon bucket filled with water. Sticklebacks were removed and placed in marked ziploc™ bags then brought to the lab for sessions 1 and 2. Data collected during the third session was recorded in the field.

2.2: Data collected.

For sessions one and two, sticklebacks collected in the field were brought back to the lab in marked closing plastic bags. They were acclimated (by placing the bags in the tank with water at a specific temperature) then released into the 10 gallon tanks in a temperature controlled room (at 15°C), filled with about 7 cm of Canyon water and allowed to rest for a few hours.

A group of fish from one of the traps was brought into the laboratory in large fingerbowls, then each fish was removed from the water, briefly dried on a soft paper towel and weighed on an electronic balance. It was then quickly placed on a measuring board with a 0-15 cm ruler affixed in it. The overall length and weight were recorded while the fish recovered in another fingerbowl. A single pelvic spine was removed with sharp scissors for the first session and both spines were removed for the second session (Fig. 2.2). The removal of pelvic spines was chosen as the method for marking due to its simplicity, easy detection and because it was the least traumatic option for marking such small fish. The implications to the individual's survival are also not many: while it is known that spine length varies with relation to predation (Wooton, 1976) and that spines in general play an important role in the defensive complex of *Gasterosteus aculeatus* (Hoogland et al, 1957), removal of a pelvic spine is likely to have a less significant effect on the stickleback's

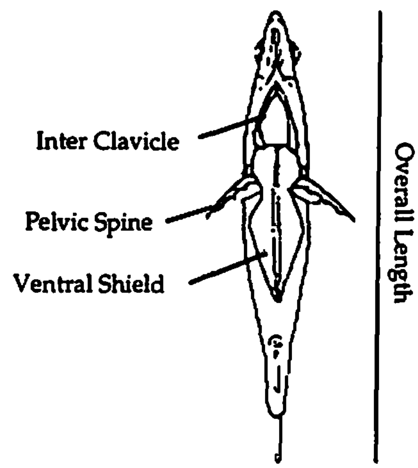

Figure 2.2: Selected ventral morphological characters and overall length of threespine sticklebacks studied in the Reed College Canyon. (From: Giles, 1986).

fitness than the removal of a dorsal spine. In lab tests, removal did not seem to affect the fitness of the stickleback significantly.

Once all the fish for a particular session had been measured and marked (over around three days), they were released at the point of capture after appropriate acclimation (by allowing the bags to rest in the water for a few minutes).

The procedure for the third session called for the sampling of a greater number of fish, and all the data collected was recorded in the field. Ten traps (five per side) were placed for about 18 hours in one of the three areas at randomly determined positions. Fish from one trap were placed in the bucket filled with canyon water after the physical conditions of the surroundings had been recorded as described above, then were individually measured with the measuring board. Each was then checked for any of the markings from the other two sessions then returned to the lake. Ten traps were set in each of the three areas for the third session.

A subsample of the total number of sticklebacks were examined more closely for morphological details.

2.3: Additional Observations.

A series of additional aspects of the Canyon and of the collected individuals were also examined closely for reference upon the application of assumptions implicit in the population size model. Morphological examination was also important for the eventual reporting of phenotypic details.

The first set of observations was in relation to the physical isolation of the stickleback population in the Canyon. In order to determine the existence of migration or emigrations the water flow was followed upstream and its source determined as accurately as possible. The pipe leading out to the culvert below the Canyon was also examined closely for the possible stickleback movement in or out of the study area through there. The setting of a minnow trap in the stream below was also an attempt to examine stickleback density in this area.

During the third session, the coordinates of the traps where recaptures were recorded were matched against coordinates of areas where marked sticklebacks were released in an attempt to roughly determine the possible extent of movement upon release.

2.4: Data Analysis.

An estimate of absolute size of the adult population of sticklebacks in the Reed College canyon was calculated using a Simple Lincon- Type index where:

Total Population/Original # marked= Total second sample/Total # Recaptured

or,

$$\hat{N} = \frac{an}{r}$$

Where:

$\hat{N}$ = The estimated pop. size
n= Total # in second sample
a= Total # marked
r= Total recaptures

(*From:* Southwood, 1966)

Besides the other assumptions implicit in this model (see Discussion), it is also being assumed that the minnow traps are sampling sticklebacks above a threshold size which represents the adult population only.

The variation in the overall length of sticklebacks sampled by each of the traps was examined by producing a point chart and labeling each of the traps with numbers from 1 to 49. After determining that there was a large amount of variation in the overall lengths of fish both within each trap as well as across all traps, the same results were graphed in the same way, with the difference that they were split by the various different variables (water depth, temperature, area, session and side), and the most significant clustering was observed in the plot split by area (Fig. 3.1) indicating the possibility of a significant effect of area on the lengths of the fish in the traps.

An analysis of variance (ANOVA) was carried out on the mean lengths of the sampled sticklebacks from each trap, using both side and area as variables in order to yield a fuller understanding of the interaction of these two geographic variables. The means were used in an attempt to produce a more conservative estimate of the actual effects of side and area on the lengths of sticklebacks sampled and as a way to minimize the within trap variation.

The age classes existing in the Canyon population of *G. aculeatus* were determined by the analysis of frequency distributions of the lengths of fish in each of the sessions.

The growth rate for the age class considered to be "adult" within each sampling session was also examined by eliminating the "juveniles" (overall length of under about 4.0 cm) then testing for significant change in the mean overall lengths of the adult sticklebacks both graphically (Fig. 3.3) and formally (with a t-test).

Other possible correlations of water depth and temperature with mean length, weight and count per trap were also tested.

2.5: The Study Site.

The Reed College canyon is a part of the Crystal Springs/ Johnson Creek drainage system in south east Portland, Oregon. Its waters originate from at least two springs from the Portland Terraces area and they eventually drain into the Willamette then Columbia rivers (Moreira, 1995).

While the canyon now holds a large (150,000 ft^2 surface area- Dunne et al, 1995) and relatively slow moving body of water, it was originally a stream, which in 1910 ran through pasture land and later through a small wooded area (Dembrow, 1995). In 1929 the canyon was dammed originally to produce an area for the building of an outdoor pool, which probably began the process of eutrophication and silting which is currently underway. The ecological conditions in the "Reed lake" are reasonably healthy, despite the occurrence of freak ecological events like unprecedented algal blooms during the last summer and this one (Dunne et al, 1995). The environmental impact of such events and imbalances have yet to be fully investigated.

Much attention has been directed at the Canyon by Reed thesis students and other researchers, but the aquatic system has received practically no attention to date. It is therefore hard to place the present population of sticklebacks in any historical perspective not only because of the absence of any systematic research before this, but also because of the many effects of human activity on the Canyon's ecology. One well known example of this sort of impact is the fact that the canyon has been subject to a yearly day of care, known as "canyon day". Though the focus of this activity is presently to remove debris and invasive plant species, its original intent was more for the enhancement of the recreational aspects of the area (Dembrow, 1995).

The environment is heterogeneous with variation in vegetation, water depth and other factors (Fig. 1.4). It is also known that there may be distinct ecological conditions on the North and South sides of the canyon, light intensity being one of the factors which has been shown to vary significantly (Manoukis and Moreira, 1995). Conditions in the canyon are generally favorable to sticklebacks, however. First, while it has been shown that the phenotypic plasticity displayed by the species complex is extended to temperature preferences (e.g. Jordan and Garside, 1972; Lachance and Magnan, 1986 and Garside et al, 1976) and optimums vary across populations, it is generally true that a temperature range of 9-16°C are common, which includes the temperatures recorded in the canyon (Dunne et al, 1994; personal observation). The amount of sedimentation, slow water flow and shallowness are consistent with the findings of Mori (1993) and others with respect to ideal nest building conditions for male sticklebacks. The abundance of vegetation cover is also widely believed to improve the reproductive success of sticklebacks, though the amount of predatory pressure in this region has yet to be investigated. It is known that some predators do exist, such as crayfish (personal observation) and various birds have been recorded (Moreira, 1995), such as great blue herons (*Ardea herodias*) and the green backed heron (*Butorides striatus*).

Recent surveys of the vertebrate aquatic life in this area have not included the Canyon itself, but species from the Salmonidae, Cyprinidae, Catastomidae, Centrarchidae, Ictaluridae, Petromyzonteae and Cottidae families as well as threespine sticklebacks have been found in the stream below, from the culvert below the lake to the confluence with Johnson Creek (Boatner, 1993).

Results:

3.1: Population Size.

Using the data collected from all three sessions, the population estimate would be of 19,578 adult sticklebacks in the Reed College Canyon over this summer season. The assumptions used for reaching this estimate are outlined in the Discussion. An examination of the release site for marked sticklebacks as compared to the recapture sites for the same group was carried out and no readily observable pattern was detected.

3.2: Distribution of Sticklebacks.

One of the first aspects of the Canyon habitat to be examined was the possibility that there may have been migrations or emigrations of sticklebacks (see section 2.3). Migration to the east (upstream) is impossible for any considerable distance mostly due to the fact that the Reed College canyon is fed by springs originating in an underground aquifer. There are no connecting bodies of water upstream.

As for the situation at the westernmost extreme of the Canyon, where water flows through a long pipe into a culvert, the picture is not entirely clear. A small number of sticklebacks were captured at the uppermost reaches of the stream below the Canyon (n= 5, over three trapping sessions) but none were observed or captured by dipnetting or trapping further down. A juvenile stickleback was once observed being pulled into the mouth of the pipe by the strong flow of water, but no quantitative work was carried out to determine the number of sticklebacks captured in this fashion.

There was a large amount of variation in the lengths of sticklebacks captured in different traps as well as differing variances within traps. The clearest effect on length distributions within traps appears to be the area effect (Fig. 3.1), and the full implications of this observation will be discussed below. The analysis of variance for the effects of area and side on mean overall length of stickleback in each trap is shown in figure 3.2.

ANOVA Table for Mean length

	DF	Sum of Squares	Mean Square	F-Value	P-Value
North or South	1	.153	.153	1.867	.1790
Area (I, II or III)	2	.872	.436	5.312	.0087
Side * Area	2	.693	.347	4.224	.0212
Residual	43	3.530	.082		

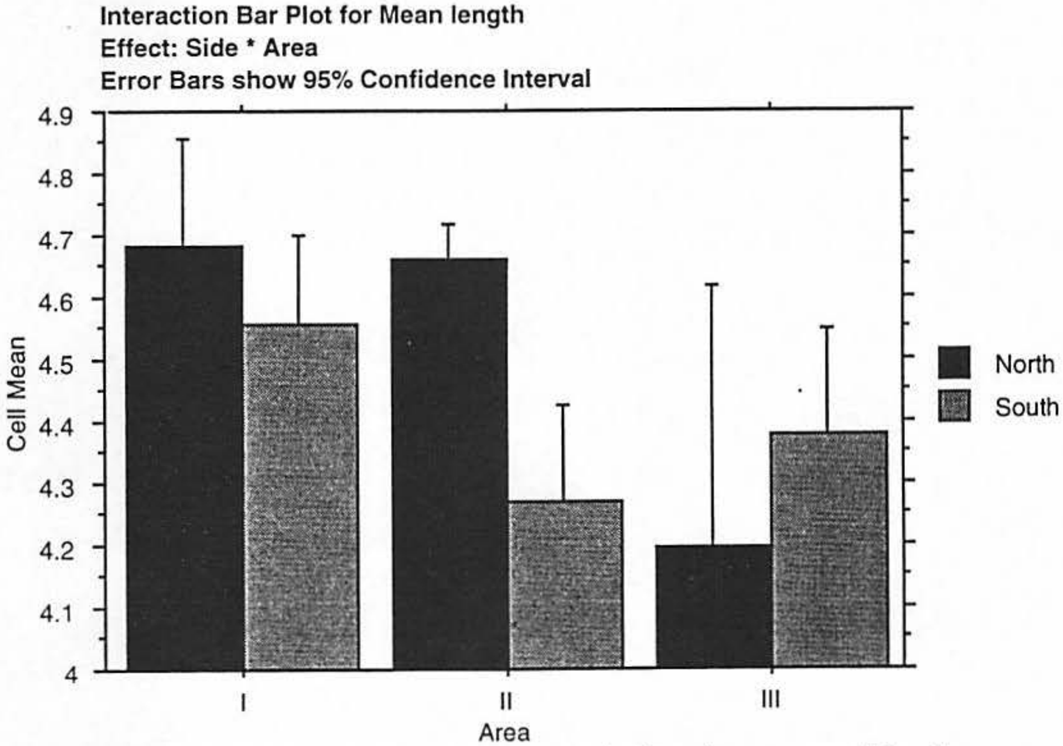

Fig. 3.2: Analysis of variance for the effects of area and side on the mean overall length of stickleback captured within each trap during the summer of 1995 in the Reed College Canyon.

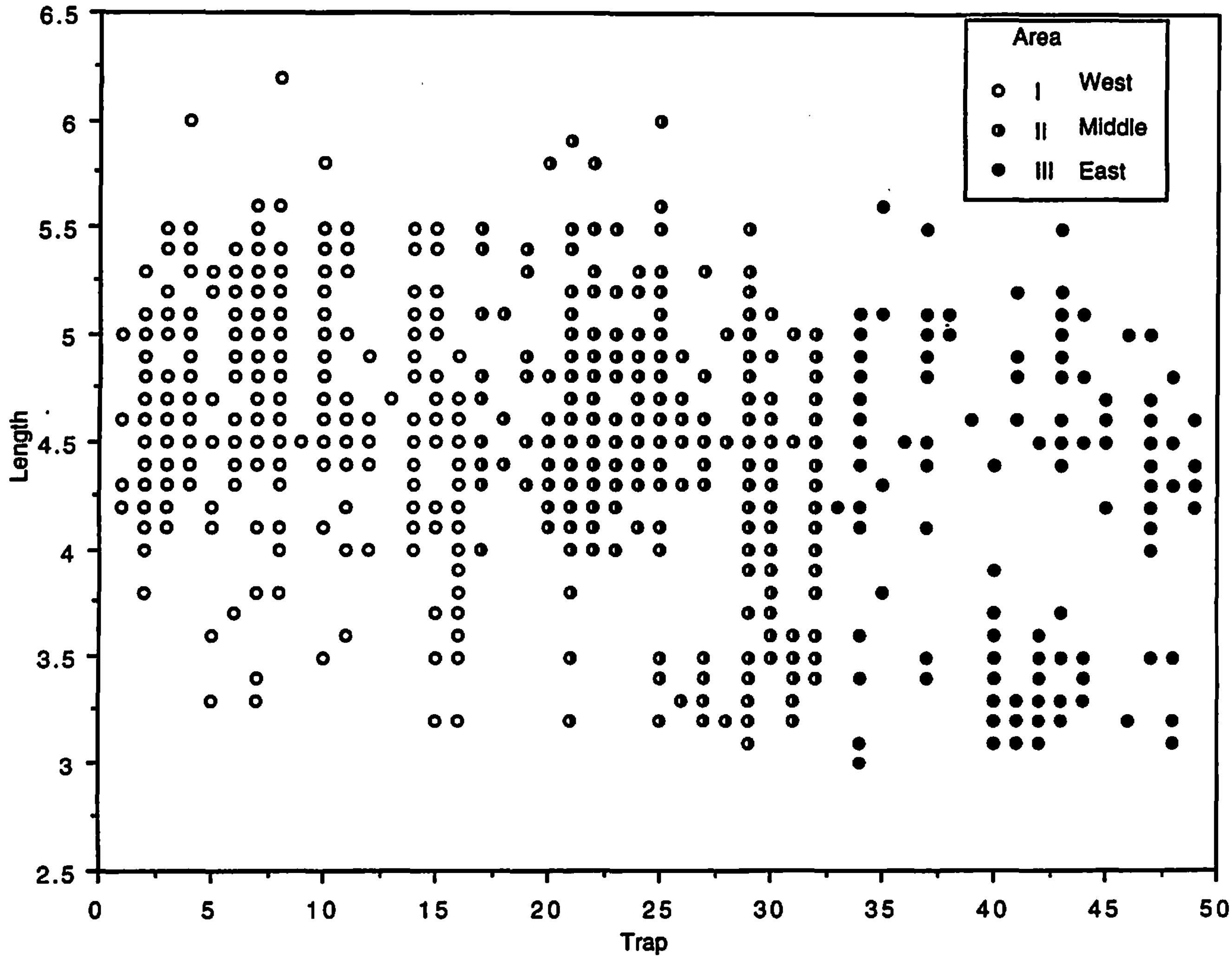

Figure 3.1: Point distribution showing realtive abundances of sticklebacks captured in each area and variation in length per trap. The number assigned to each trap is random. Note the higher proportional concentration of smaller sticklebacks in area two and particularly area three.

3.3: Age classes and Growth Rate.

The age classes existent during the three collecting sessions can be easily discerned by using the lengths as age indicators (e.g.: Gilbertson, 1980), since there is a direct relationship between the length and age of the individual. Fig 3.3 shows the frequency distributions for the three sessions and estimated number and age of the age groups within the population. Formal testing (with t-tests) for change in the mean length of adult sticklebacks across the three sessions did not show any significant changes.

3.4: Plate Morphology and Other Phenotypic Observations.

A subsample (n=12) of the 1,175 collected sticklebacks was examined for plate morphology. All the examined individuals belonged to the low- plated plate morphology phenotype. These were individuals especially set aside for this purpose (n=7) and sticklebacks that perished during the process of marking, measuring and weighing (n=5).

The individuals examined point to the existence of two distinct morphotypes (color and an associated morphological character) in the canyon, termed here "gold morph" and "blue morph" (names after Gilbertson, 1980). The gold morph individuals displayed stouter, thicker bodies than the other form and had a wider ventral shield in the pelvic girdle, while the blue morph individuals tended to have smaller, more triangular representations of this structure (Fig. 3.4). In the blue morph the reduced width of the ventral

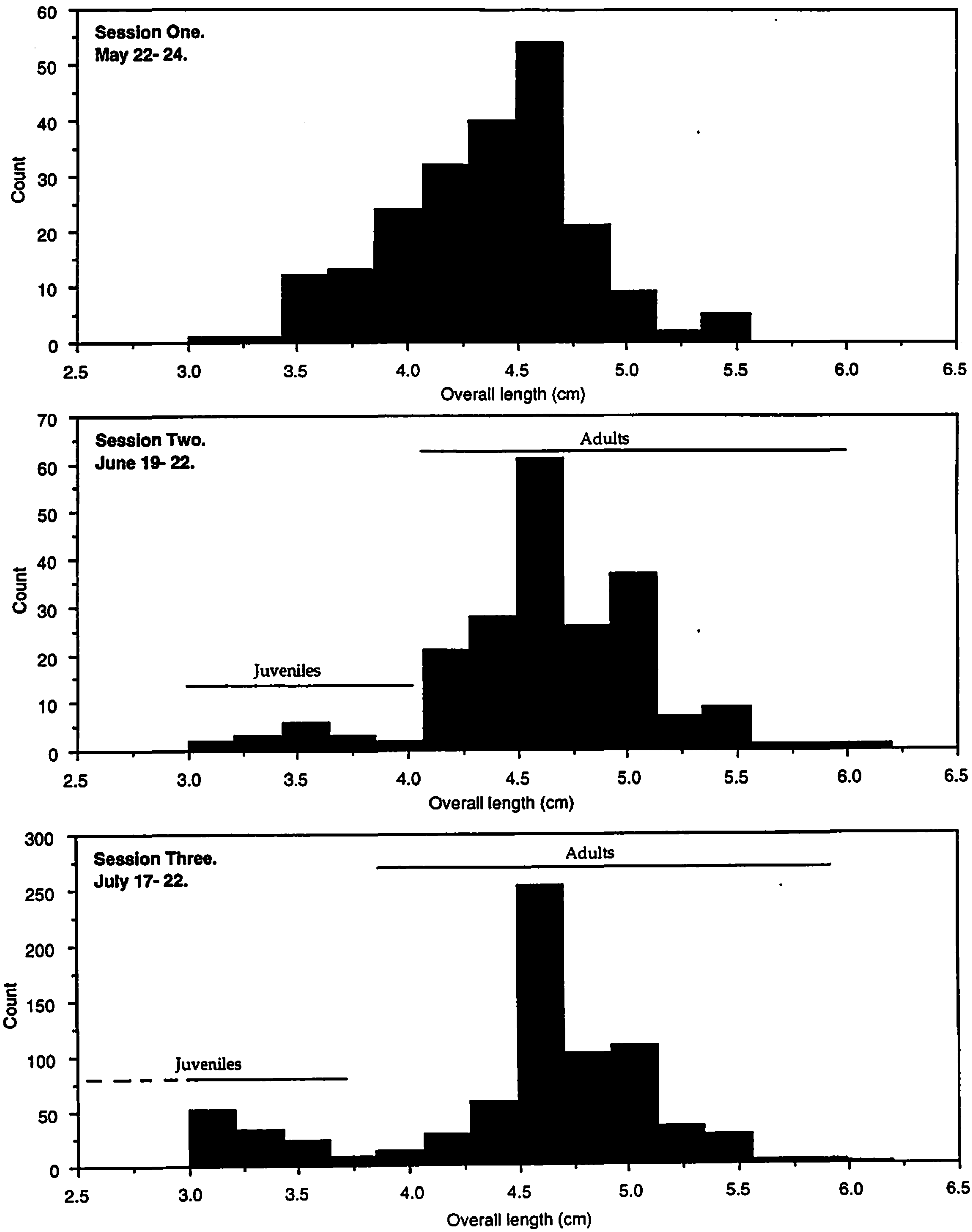

Figure 3.3: Frequency Distributions showing overall lengths of sticklebacks sampled from the Reed College Canyon during three sessions in the summer of 1995.

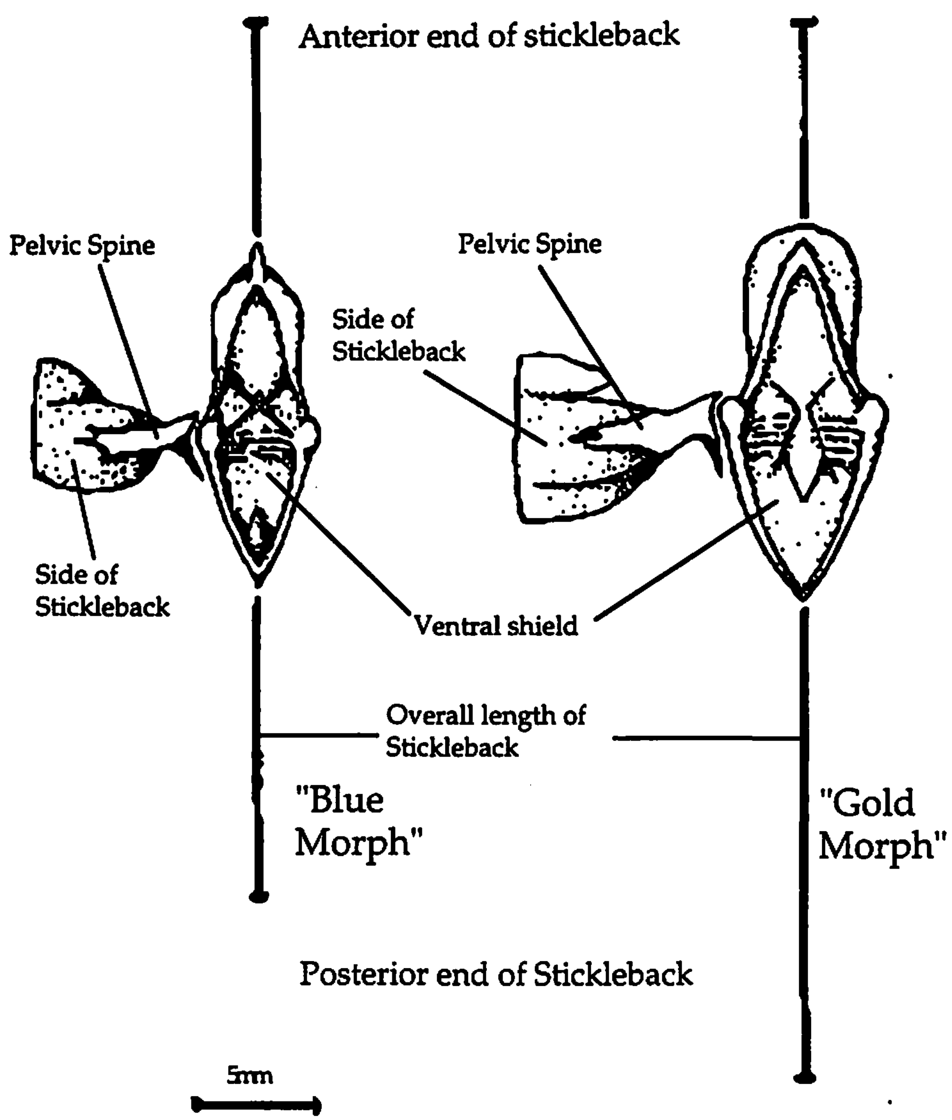

Figure 3.4: Representation of the ventral shield in an example of the blue and gold groups of Threespine Sticklebacks in the Reed College Canyon, captured during the summer of 1995. Note the different positioning of the ventral structure relative to the overall length of each individual and the differing morphologies of the shields themselves. (Diagram by Sallie Schott).

shield was often so extreme that the pelvic spines were overlapping and difficult to access.

Another unexpected but significant observation was that none of the sampled sticklebacks displayed the typical red coloration developed by reproductively active males in their underside (Assem, 1967). Some of the individuals did have a slight blue tinge around the eyes and over the sides, another indicator of reproductive activity (ibid).

3.5: Other Possible correlations.

There were no significant relationships between the number of sticklebacks or the mean length and weight of individuals in a trap with water depth or the temperature of the water in which the traps were set.

Discussion:

4.1: Population Estimate and Distribution.

The population estimate reported in the Results section is a very tentative one. The model used to calculate it is the most simple of its kind and includes six major assumptions (Southwood, 1966), some of which are probably not completely satisfied by the actual conditions in the Reed College Canyon.

The fist assumption is that marked animals are not affected by marking and that the marks are not lost over the study period. This condition was probably satisfied by the careful release of the sticklebacks into the environment and the observation of marked animals in the lab (no physical effect was observed over two weeks) Also, no predatory fishes were observed and there are no reports of any existing in the canyon itself, so it may be assumed that the predation pressure is moderate to low, meaning that the loss of one or both pelvic spines would not have a noticeable effect. There is, however, the possibility of an effect occurring on the stickleback's behavior as a result of being removed from the Canyon for up to three days- males may lose breeding territories to other individuals for example.

The second assumption is that marked animals will distribute themselves completely randomly within the population. Gilbertson (1980) also attempted a mark- recapture population assessment with threespine sticklebacks and concluded that there was no evidence supporting the hypothesis that sticklebacks were creating home- ranges around the site where they had been released and thus were not dispersing randomly. This is still a possibility, however, and merits further attention. For the present study, the comparison of release areas with points of recapture showed no immediately obvious pattern, but the non- specificity of the marking technique precluded a more powerful method for answering this question.

The third assumption of Lincon Index type models is known as "equal catchability", and has two components: that individuals of all age classes and both sexes are captured in the proportions in which they occur and that all individuals are available for capture regardless of their position in the habitiat. It is known that the minnow traps are not sampling any juvenile sticklebacks below a size of about 3.0 cm, as evidenced by the absence of any sticklebacks this size in the recorded data. It is known that there were sticklebacks below this size, as evidenced by the capture of individuals between two and three centimeters by dipnetting late in the summer (Manoukis, personal observation). Furthermore, the cut-off is also clear in the frequency distribution for the lengths of sampled fish in session three (Fig. 3.3) where there are no fish below three centimeters but around fifty individuals at just over that size.

It is important to note that this fact does not necessarily render the estimate useless: it simply restricts it primarily to the adult population, especially since the first two sessions saw mostly adult fish being captured. As for the second component of the third condition, position in the canyon should not affect catchability since the randomization process allows the possible sampling of all parts of the habitat.

Another assumption included in Lincon- index type models is that sampling is done at discrete intervals that are small in relation to the actual time used for sampling. This condition is generously satisfied by the 28 to 30 day intervals between the generally three to four day sampling periods.

The last two conditions are the most important for the validity of this method of population estimate: that the population is an isolated one or that migration and emigration can be quantified and that there are no births or deaths within the system, and if there are that these may be quantified also.

The first of these was investigated by examining the possibilities for the movement of sticklebacks out or into the canyon (see section 2.3 and 3.1) and movement seems highly unlikely, except for the movement of juveniles out of the canyon by means of the underground pipe into the culvert below the canyon. This effect is likely to be too small to be noticeable.

The question of births and death rates is the most serious flaw in the design, though the number of births is probably not of central importance since the juvenile population was not widely sampled. It is likely, however, that there has been a high rate of mortality, not only from evidence from the frequency distributions (Fig. 3.3- a decrease in the relative number of "adult" sticklebacks with respect to "juveniles" from session two to three) but also from the inferences about the life history of the population in the canyon (see section 3.2): If the sticklebacks are indeed on a yearly or two- year cycle there will necessarily be a significant drop in the number of adults after each breeding.

While these factors certainly render the population estimate presented a blunt one, the figure is useful as a reference estimate for the adult population of *Gasterosteus aculeatus* in the Reed College Canyon during the summer of 1995.

The experimental methods and the data collected effectively limit the ability to determine anything more than the most basic facts about the distribution of sticklebacks in the Canyon and provide a reduced certainty about any conclusions. As a first order study, however, some factors may be discussed.

Correlating the several variables (water depth, temperature, number of fish in a trap, lengths and weights of the fish) to the independent variables (session, side and area of the canyon) showed a clear pattern only in the distribution of large and small fish in relation to area (Fig. 3.1): the pattern with respect to side is unclear. It is apparent from the data that most of the juvenile sticklebacks (below about 4.0cm overall length from the length frequencies in figure 3.3) were captured in areas two and three, though in area two they appear more on the south side and in area three were observed most in the north side (Fig. 3.2). This inversion certainly allows for the tentative interpretation that sticklebacks in the canyon may be not only grouping by size but also moving to and from different parts of the canyon in groups.

There have been a number of studies which have highlighted the spatial distribution of *G. aculeatus* in relation to a variety of factors. It is known, for example, that during the reproductive season male sticklebacks will set up territories near the banks of the body of water and defend them, while females will school towards the deeper water then travel through a number of male territories, being courted and laying their eggs in the nest prepared by the owner of one of the territories (Wooton, 1976; Snyder, personal communication 1995). The degree of confidence in the effect of sides is low, as reported above, and demonstrates the need for more and more specific research.

More relevant to the present study are the results presented by Picard et al (1989), where habitat segregation by age groups was observed in the St. Lawrence estuary, Canada. The observed pattern is strikingly familiar after the observations in the Reed College Canyon: adults moved from the more open waters towards shallower waters (presumably to set up nests and territories) and that catches of adults in all these areas declined significantly as the abundances of young born that year grew. It was known that this population was on a one to two year life cycle, much like the results presented suggest about the population in the Canyon.

There were a series of important differences in the sticklebacks from the St. Lawrence estuary, perhaps the most important being that they comprised an anadromous (migratory) population, where as migration in the Canyon population seems unlikely (see section 3.2). The overall pattern, though more pronounced in sticklebacks living in open areas is probably valid for the subject population of this study.

The implications of the increased abundance of juvenile sticklebacks in areas two and three (Fig. 3.1) are most significant in light of the fact that there was an area of the Canyon that was not sampled due to the design of the experiment. The area directly to the east of the easternmost edge of area three was not sampled due to the unevenness of the terrain, absence of passable paths near the water and irregular pattern of water: there are large tracts of only mud or very shallow water in many parts. This area is also where the spring waters feed the Canyon, and this combination of factors will become important in the next section.

4.2: Phenotypic Expression of the Threespine sticklebacks in the Reed College Canyon.

One of the clearest results of this study is the information regarding the life history of the population of *Gasterosteus aculeatus* in the Reed College Canyon. Lengths in fish in general have long been known to be related to their age (e.g. Everhart and Youngs, 1953). In sticklebacks, Gilbertson (1980) related the age of individuals (as defined by three different methods) to their lengths and found a consistent relationship between the two and good agreement across the methods, as shown in figure 4.1. These findings coincide well with the data from this investigation remembering that standard length (from the tip of the snout to the last vertebrae) is lower than overall length (from the tip of the snout to the end of the tail). This accounts for the discrepancy in defining as age zero (born this season) as being below 25mm in Gilbertson's work and in this study individuals below about 37mm representing that group. There are probably also populational differences and variation in the time of the breeding season during which data was collected for the two studies.

Method:	Age 0	Age I	Age II	Age III
Visual	**<25**	**25-37**	**38-51**	**>51**
Graphic	<25	25-36	37-50	>50
Direct Aging	<25	25-38	37-49	>49

Fig. 4.1: Comparison of length- age distributions derived by three methods for a sample of threespine stickleback in Lake Aleknagik, Alaska in 1970 (From Gilbertson, 1980). Note: "Visual" refers to aging by visual inspection of length frequency and is equivalent to the method used here.

The studied group of sticklebacks seems to be typical in that they are existing on a one to two breeding cycle schedule. Figure 3.3 shows what are believed to be the different age classes, and if the sticklebacks are on a yearly breeding cycle it seems likely that the oldest fish live to be two years old and breed twice, though most of the adults live to breed only once. This result is consistent with the literature, though anomalous populations with sticklebacks of up to eight years of age have been found (Bell, 1984).

The movement of the generations of sticklebacks are also especially pronounced in figure 3.3, where the mean length of the adults is seen to remain somewhat constant at 4.5cm, larger fish becoming relatively less abundant (assumedly dying off) once they become larger than the mean size. It is also clear from figure 3.3 that juveniles were becoming relatively more abundant and growing large enough to be sampled by the end of the summer. By July, the cut- off at 3.0 cm (any smaller fish could not be trapped in the minnow traps) clearly shows that there is probably a whole group of juveniles below that size already in existence, and most likely represent a large number of threespine sticklebacks.

The observation of only low plated sticklebacks in the canyon is consistent with the general observation that the low plated form is commonly observed in exclusively freshwater populations (Wooton, 1976), which has been attributed to osmoregulatory mechanisms and different physiological characteristics, though exceptions have been observed (Guderley, 1994).

The unexpected fact that none of the 1,175 sampled sticklebacks displayed the typical red throat coloration indicating reproductive readiness in males may be interpreted in a number of ways. It is highly unlikely that this observation signifies the absence of the red throat characteristic in this particular population, and equally unlikely is the possibility that none of the sampled sticklebacks were males. This second option may be discarded not only due to the large number of sampled individuals, but also due to the observation of bluish tinge under the eyes of a number of sticklebacks, which is another indicator of reproductive activity in males, as discussed above (Section 3.4).

A more conservative explanation is that the absence of sampling in the easternmost extreme of the Canyon is responsible for the absence of red throats: it is possible that this area is where most males set up their territories, a hypothesis confirmed by the optimal nest- building conditions of fresh springwater, slow water flow and relatively low flow rates (Mori, 1993). This possible restriction of reproductive areas to a particular part of the habitat may be particularly interesting when matched against the differences in the physical environment in the eastern end. An investigation into such physical differences may provide some revealing insights into nest site selection and the factors that drive it in this species. There have already been some factors that are notably different in the east part of the canyon: the water tends to be more shallow (Dunne et al, 1995) and may have as much as a ten degree centigrade temperature difference to the westernmost reaches the colder temperatures being observed to the east (Dunne, personal communication, 1995).

The final phenotypic observation to be discussed here is perhaps the most intriguing: the possibility of the existence of two distinct sets of associated morphological characteristics, the "blue" morph and "gold" morph of threespine sticklebacks in the Reed College canyon.

Qualitative and repeated observations suggest that the gold morph sticklebacks tend to have wider bodies (and therefore a possible difference in length to width ratio) than the blue group and tend to have a much wider, more rectangular ventral shield, in addition to a strong yellowish golden color as opposed to the dull bluish tinge and somewhat more transparent skin of the blue morph *G. aculeatus* .

The implications of these observations, especially if they are ever supported by quantitative measurements in future research, may be far reaching with respect to our understanding of the threespine sticklebacks in the Canyon. It is possible that these morphological manifestations are not genetically determined and are produced by environmental factors, such as nest site choice. The existence of a genetic underpinning may be easily shown through controlled crosses, and it is strongly suspected that such an experiment would confirm the hereditary nature of these associated phenotypic characters.

If both blue and gold morph can be shown to be genetically determined, there is the possibility that they represent a simple or a group of dominant/ recessive loci and will this case may be proven through Mendilian crosses and the examination of the resulting phenotypes. If indeed the dominance of one phenotype can be proven, then the actual genetic mechanism that allows for such a combination of physical traits to be combined will no doubt be of great interest and expand our understanding in this area.

The other possibility is of the existence of a evolutionary mechanism being at work in the existence of two distinct phenotypes in the Canyon. Ecological character displacement (Schluder, 1994) and trophic nitche specialization (Bentzen and McPhail, 1984) have already been proven for this species and the potential presence of such an ecological scenario may provide one of the very few opportunities to detect and experiment in the field. The importance of observing such a larger scale evolutionary event in nature will undoubtedly substantiate years of theoretical work and debate in biological circles.

The blue and gold phenotypes are not likely to be the result of a sexual dimorphisim, based on the observation of gravid sticklebacks in both groups. Since no quantitative data has been collected and no sexing experiments carried out, the certainty of the blue and gold expressions not being related to sex is low. There is the possibility that the bluish tinge observed in reproductive males represents itself very strongly in this population and leads to the skin of the stickleback having a bluish pallor. This additional uncertainty is a reminder that all the above discussion may be purely intellectual and that the appropriate experimental design and data collection to answer questions about "blue" and "gold" have not been executed in this field session but warrant further investigation.

As a final note it should be remembered that especially since the possibilities for advance through field investigations of this population and of *Gasterosteus aculeatus* in general are so great that any such work must be undertaken with the greatest care and rigor possible. Great attention to the known problems and difficulties of field research will ensure that the knowledge gained may be validated by the widest group of individuals and ultimately be incorporated into future decisions, having a positive impact on our policies and actions on this campus and around the planet.

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