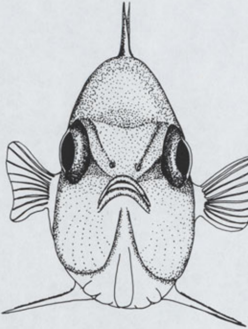
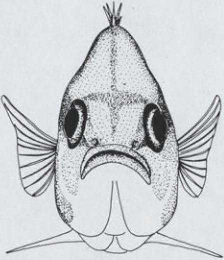


A. GASTEROSTEUS



B. PYGOSTEUS

FIG. 2. 2. STICKLEBACKS WITH THEIR SPINES RAISED

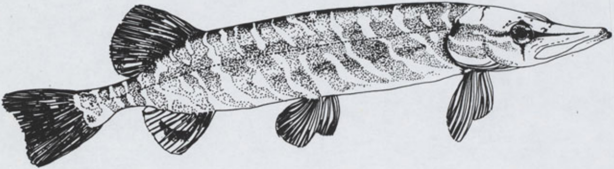


FIG. 3.1. PIKE

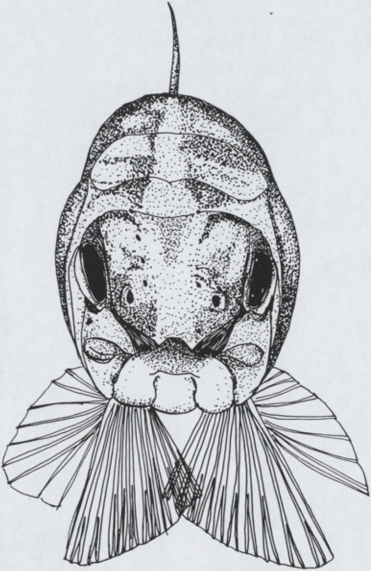


FIG. 3.2. FRONTAL VIEW OF PIKE

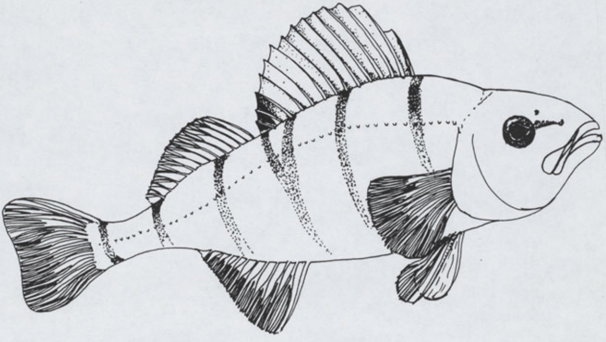


FIG. 3.4. PERCH

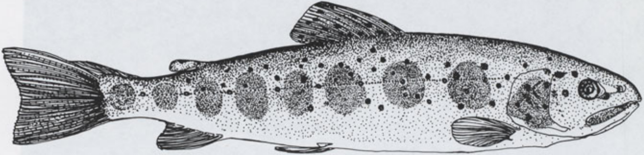


FIG. 3.6. BROWN TROUT

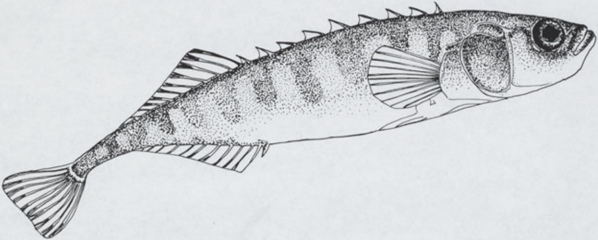


FIG. 4.3. Head-up, tail-down posture.

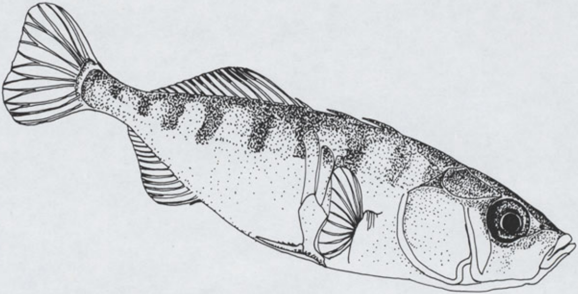


FIG.4.1. HEAD-DOWN ORIENTATION
IN BINOCULAR FIXATION.

SOME ASPECTS OF THE
ANTI-PREDATOR RESPONSES
OF TWO SPECIES OF STICKLEBACK

by

VIVIENNE LOUISE BENZIE

(Lady Margaret Hall)

A thesis submitted for the degree of
Doctor of Philosophy
in the
University of Oxford

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ABSTRACT

Hoogland, Morris and Tinbergen (1957) demonstrated that the spines of two species of sticklebacks, *Gasterosteus aculeatus* and *Pygosteus pungitius*, are an effective defence against the pike (*Esox lucius*) and perch (*Perca fluviatilis*) as predators, and the present study was undertaken to compare the fright behaviour (i.e. responses to a predator) of the same two species.

The risk to the eggs and newly-hatched young has been dealt with separately from predation upon the independent fish. Amongst aquatic predators the pike, the perch and the trout (*Salmo trutta*) appear to be the only important enemies of sticklebacks, and their habits, hunting behaviour and diet have been described. The risk of aerial predation by *Ardea cinerea* and *A. purpurea* and *Larus ridibundus* also seems to be appreciable, and other birds have been reported to eat sticklebacks.

The responses of sticklebacks to their fish predators have been described in detail and grouped into two major categories: the escape responses and precautionary behaviour. The precautionary behaviour, which reduces the chance of a fish provoking the predator to attack, is more developed in *Pygosteus*.

Introduction into a new environment elicited from both species of stickleback behaviour which shows them to be alarmed, but whereas *Gasterosteus* explored freely, *Pygosteus* hid in the weed and dashed from clump to clump without exploring the open areas of the tank. The actual responses of naïve, laboratory reared sticklebacks

to a predator were recorded. All fish tested were below breeding age. When groups which had some prior experience of the predator were compared with inexperienced fish it was found that experience affected the subsequent responses; the experienced fish showed more precautionary behaviour and responded at a greater distance. There was a difference between the species, both as experienced and as inexperienced fish.

Since both species of stickleback normally have, after hatching, a period of parental care during which the young show similar responses to the parent as they later show to a predator, the effect upon the fright responses of raising the fish as orphans was tested. Orphan experienced groups did not differ from normal experienced fish; inexperienced orphan *Gasterosteus* were more easily frightened than normal fish. *Gasterosteus* artificially given a longer period of experience of their father than is usual (six weeks instead of up to two weeks) were correspondingly less responsive than normal fish. The orphan *Pygosteus* tested differed very little from the normal *Pygosteus*.

These differences between the species are thought to be due to differences in the developmental history of the two species. *Gasterosteus* has a much longer period of parental care, whereas in *Pygosteus* parental care does not extend beyond the stage at which the young fill their swim bladders (up to four days). It is suggested that the period of parental care served to habituate the young somewhat to the presence of a large fish near them, so that orphan fish remained more responsive than those normally reared. The difference was less for *Pygosteus* since the period of parental care is less.

From these experiments, in which all fish tested were of a size the predator could eat, despite the spines, it was found that *Pygosteus* was better protected against a predator by its behaviour than was *Gasterosteus*. Where the predator-prey size ratio was smaller (as in the experiments of Hoogland et al.) the spines were an effective defence which favoured *Gasterosteus* over *Pygosteus*, but in predator encounters where the predator is proportionally larger the behavioural defence was the most important factor.

Sticklebacks (mainly *Gasterosteus*) form a significant element in the diet of some populations of pike, perch, trout, herons and black-headed gulls in the wild, but in the absence of more precise information on their relative abundance the relative risk of each species is not known.

Acknowledgments

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To Dr. J. M. Cullen, who supervised this work, go my most sincere thanks for all his help and encouragement.

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Finally, I would like to thank my colleagues who have so often helped in the collection and maintenance of fish and other aspects of this work.

CONTENTS

Chapter I

INTRODUCTION

The problem of predation is basic to the study of the life-history of any animal, whether it is itself predator or prey in any given context. Virtually all species are eaten by other animals at some stage in their life-history, and adaptations against being discovered or being eaten are universal. Among vertebrates, fish are particularly liable to these dangers. Since relatively few species have the advantage of parental protection, and almost all must survive the development from a small, vulnerable egg to a tiny larva which must quickly establish a satisfactory feeding habit, no matter what size the fish may later attain, or to what age it may survive, this is a most pressing problem. The young larvae or eggs provide a source of food for many slightly larger organisms, and Nikolsky (1963) describes a life cycle in which newly-hatched fry are eaten by the copepods on which the slightly older fry themselves predate. Fish are notable for the tremendous number of tiny eggs they produce, and only a small proportion of the eggs laid normally survive to the age of breeding. Though by no means the only source of this mortality, predation is an important agent in the wild.

Birds and mammals differ from fish in that they have substituted parental care of some sort (yolk supply, auxiliary feeding, protection) for this tremendous fecundity. Nevertheless, Errington (1946) has argued that the population regulation of many vertebrates is the result of predators taking those individuals which are outside their optimal habitats, because they have been forced out by competition with others of their kind. This emphasises not only the adaptedness of these species against their predators, but the link between this adaptedness and the appropriate habitat. Furthermore, since most animals have a variety of predator species, often with different hunting methods, a stereotyped escape response may be less effective than reactions tailored to the various predator situations.

The observations of Kruuk (1963) on the complex of anti-predator responses of breeding black-headed gulls (Larus ridibundus) reveal the subtleties of responsiveness possible, with the adult birds attacking predators which threaten the young, fleeing from predators which specialise on adult birds, and showing intermediate responses to predators capable of harming both adults and the brood. Both in the egg stage and as newly-hatched birds, the brood exhibits cryptic coloration as an additional defence, and the young show suitable behavioural responses by remaining very still when threatened. The limitations of even this complex system are emphasised by Kruuk. The weaknesses which arise are due to the following factors: the conflict between anti-predation and other requirements, the fact that protection against one predator may make the gulls more vulnerable to another, the development of counteradaptations in the predators, the discrepancy between the present-day breeding habitat and the one for which the behaviour seems to have been adapted, physiological and behavioural inadequacies, and lastly, the apparent failure of individuals.

There are many mechanisms of defence against predators in fish including chemical (distastefulness, poisonous structures), visual (camouflage or, in the case of distasteful or poisonous species, warning coloration), and electrical discharges. Spiny structures (Nikolsky, 1963; Lagler et al., 1962) are very widespread among the Acanthopterygii, and may involve the pectoral and dorsal fins most commonly (e.g. Percidae, Scorpaenidae and the catfishes) and the opercula (sculpins, perch). Associated with these structures there may also be the poison glands already mentioned. Sometimes symbiotic or semi-parasitic relationships between a fish and another species such as a siphonophore, sea anemone, or holothurian confer protection on the fish by virtue of the partner's unpleasant qualities, or because it provides a hiding place. Each of these defensive devices requires appropriate behaviour.

Even the most simple escape, that of swimming away from the predator, is often not on a straight course but by an erratic zig-zag path. Since large fish can in general swim faster than smaller ones (Bainbridge, 1958), a small fish will be ill-advised to rely on outswimming its pursuers. In open water, where there is nowhere to hide, many prey fish live in schools which can be seen to afford some degree of protection (Breder, 1959). Among reef fishes the advantages of this habit against predators have been described by Eibl-Eibesfeldt (1962). When a predator attacks, a school may break up and scatter, or else it A.A. Nikoyaeta
may close up to form a more aggregated mass (Eibl. loc. cit.; Allen, 1920). In aquarium tests, using *Pollachius virens* L. as prey, Radakov (1958, quoted in Nikolsky, 1963) showed that single fish are more rapidly consumed than fish from a school, despite the higher concentration in the

latter case. Nikolsky (1963) points out other advantages of the shoal: a shoal can detect a predator over a greater distance (although it is, of course, itself more noticeable than an individual fish) and can therefore escape more easily.

It is clearly of advantage to the prey to be able to detect predators before they come within attacking range. Visual stimuli probably play the main role here in most species and the multiplicity of eyes afforded by a school may be one reason why it is an effective defence. Chemical cues are known sometimes to be important. The best known examples concern the alarm substance. This is a species-specific chemical released when the skin of certain fish is injured, and was first described for the minnow by von Frisch (1941). It is now known that the alarm substance is a part of the defence of a large number of fish of the superfamily Cyprinoidea and in some of the Siluroidea (Schutz (1956), Pfeiffer (1963)). Since both these superfamilies make up the bulk of the Ostariophysi, which comprises ~~2/3~~ two thirds of all freshwater species, the success of these predominantly unprotected non-predaceous fish may be attributed in part to the efficiency of their early-warning system (Pfeiffer, 1963). As Pfeiffer points out, such a system is of value at the specific level rather than at the individual level. That this alarm system helps to warn prey fish against areas where a fellow has already been caught has been seen in the wild and in the laboratory by von Frisch (1941), but in tests the strength of the response is characteristically variable (von Frisch, Schutz and Pfeiffer, loc. cit.). Furthermore, a less intense response is given if fish are tested soon after a strongly positive test, so that some form of habituation would seem to be operating. If so, it is difficult to see how this warning system could be useful in stagnant or slow-moving waters. Göz (1941) showed that minnows can, at least under laboratory conditions, identify olfactory stimuli from their predators, the pike, perch (and also salmon), although this response is best shown in the presence of alarm substance, and von Frisch (1941) showed that this sensitivity to the smell of pike augmented the response of minnows to alarm substance when presented with it, not before it.

While there is a mass of circumstantial evidence for the effectiveness of these protective devices, few of them have been studied in relation to actual predation. The spiny bitterling (*Acanthorhodeus asmussi*) which makes up 70% of bitterling catches, and the common bitterling (*Rhodeus sericeus*), which comprises the other 30% in natural populations, are together preyed upon by marine fish, but in the total bitterling contents of predators only 19% was the spiny bitterling, 81% was the common one (Lishev, 1950; quoted by Nikolsky, 1963). Despite its relative abundance, the spiny bitterling is effectively protected from predation by its spines. The opercular spines of the sculpins, which roughly double the width of the head, are effective against predators to such an extent that spiny sculpins up to only 18 cm long are eaten by skates of the F. Rajidae, which eat smooth sculpins up to 42 cm in length, and fish of the F. Scorpaenidae eat spiny sculpins of up to 12 cm, smooth ones up to 25 cm, (Paraketsov 1958, quoted in Nikolsky, 1963) so that by doubling the spread of the head these fish have effectively halved the sizes up to which they can be eaten.

The present investigation arose from the study of Hoogland, Morris and Tinbergen (1957) on the protection conferred on the three and ten-spined sticklebacks, *Gasterosteus aculeatus* and *Pygosteus pungitius*, by their spines. They found that the three-spined stickleback was less easily eaten by pike and perch than the ten-spined stickleback, and that each was shunned when minnows (*Phoxinus phoxinus*) were also given. The preference largely vanished when the spines were cut off, proving that it was the spines which conferred the advantage on the species.

With this demonstration that sticklebacks were protected from predation by their spines, and that the anatomical equipment differed for the two species, it was decided to study their behavioural responses to find out whether there were corresponding differences in their response to a potential or known predator, and to explore some of the factors affecting the responses to predators.

Throughout this study the term "fright" is used as delimited by Barlow (1961).

Chapter 2

2.1 LITERATURE

Since the early work of Leiner (1929, 1930, 1931, 1934) and Wunder (1930, 1931) there has been a number of studies of sticklebacks, most of them on breeding behaviour, and most of them on *Gasterosteus*. In an unpublished thesis Hall (1956) has given general accounts of the behaviour of all five species of sticklebacks more fully than any published reference, and Morris (1958) deals with *Pygosteus* in detail, and compares it in part with *Gasterosteus*. These authors also review the literature on the behaviour of sticklebacks up to that time. More recent investigations have been concerned with special topics, Baggerman (1957, 1960, 1964), Hoar (1958, 1963) and Segaar (1961) on the physiological control of reproductive behaviour; Sevenster (1961) on the mechanism of displacement activities, Tugendhat (1959, 1960a,b,c) and Beukema (1963) on the feeding behaviour, Sevenster-Bol (1962) on motivational changes within the breeding cycle. Keenleyside (1958) described schooling, and development of the eggs has been studied by Swarup (1958). The many works on the life history and migrations of sticklebacks are referred to in the appropriate section.

2.2 SOURCE OF FISH

All the fish used in this study were collected in the vicinity of Oxford, or were bred from that stock. The major source of three-spined sticklebacks was the R. Glyme at Stratford Bridge, about ten miles north of Oxford, and these were supplemented by fish taken from the Oxford Canal. This canal was also the source of many ten-spined

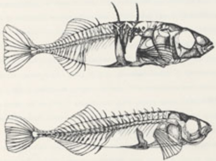


FIG. 2.1. SKELETON OF STICKLEBACKS

UPPER — *GASTEROSTEUS ACULEATUS*

LOWER — *PYGOSTEUS PUNGITIUS*

sticklebacks, but a supplementary source was an old quarry pool near Abingdon. This pool dried up during the summer of 1964.

The local three-spined sticklebacks are *Gasterosteus aculeatus* L. f. gymnura brevis (Bertin, 1925) synonymous with *Gasterosteus aculeatus* Race A, (Heuts, 1947). The characteristics of this form are given in the descriptions of both species to follow. The ten-spined stickleback is *Pygosteus pungitius* L. f. brevis (Bertin, 1925).

2.3 ANATOMY OF THE SKELETON IN STICKLEBACKS

All the sticklebacks have modified the anterior rays of the dorsal fin and the first anal fin-ray and the pelvic fins as spines. The number and size of dorsal spines is characteristic for a species. *Gasterosteus* has three dorsal spines, of which the first two and the pelvic spines are larger than those of *Pygosteus*. The anal spine in the *Pygosteus* collected in the Oxford area is larger than in the local *Gasterosteus*. Associated with the dorsal spines there is a subcutaneous system of base plates which is almost as extensive in Oxford populations of *Gasterosteus* as in *Pygosteus* (Figure 2.1). The pelvic spines articulate with the modified pelvic girdle which extends for some length ventrally and laterally, being more extensive in *Gasterosteus*, which has a system of lateral plates in addition.

Both species have a set of basal plates dorsally and ventrally associated with the dorsal and anal fin-rays even extending behind these. (Lindsey, 1962, described this as an unusual feature among teleosts.) Together with the extremely well developed vertebral spines dorsally and ventrally in the caudal region these provide an unusually firm skeletal system in this region. Furthermore there is a short series of small plates laterally on the slender caudal peduncle of *Pygosteus* (the carina). The stronger nature of the caudal skeleton of *Pygosteus* has not been remarked upon before. Together with the pectoral and pelvic girdles this forms a much more substantial skeleton than is seen in other small teleosts, e.g. the minnow, although the overall armouring of *Pygosteus* is less than that of *Gasterosteus*. The ecological variation of the sticklebacks was thoroughly described by Bertin (1925), when he divided the species into several races. Heuts (1947) has continued this work on *Gasterosteus*, distinguishing two races which differ physiologically: race A which occurs in freshwater, has few lateral plates (mode at about 5), is smaller overall and has a different vertebral number from race B, which occurs in brackish and seawater, has many lateral plates, extending down to the tail (mode 32), and is larger overall. Heuts also studied phenotypic variations within these two races and artificially produced variations in fin ray numbers and vertebral counts (Heuts, 1947, 1949). Lindsey (1962a,b) has also done similar work both with *Gasterosteus* and *Pygosteus*. He does not refer to relative sizes of spines in these comparisons, but McPhail (1963) has described variation within the genus *Pygosteus* in Canada and mentions that the brackish water forms have stouter spines than the freshwater forms. He suggests that the Canadian population derives from two surviving populations which spread out after glaciations, but the variation

Table 2.1

Measurements of sticklebacks from marine and inland areas, (taken from Hall (1956), Appendix).

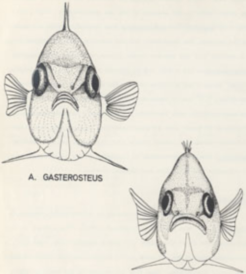
	PYGOSTEUS		GASTEROSTEUS	
	Oxford	New Brunswick	Oxford	New Brunswick
total length in mm. without caudal fin	45.0	45.5	38.5	37.5
snout - 1st dorsal spine	3.50	2.40	2.85	2.90
snout - ventral spine	2.25	2.30	2.00	2.20
snout - anal spine	1.50	1.70	1.42	1.50
caudal peduncle	8.20	5.60	7.70	7.50
length 1st dorsal spine	30.00	22.70	10.00	8.25
last " "	30.00	15.20	10.00	8.50
length ventral spine (pelvic)	12.80	9.10	6.70	6.90
dorsal fin rays	11	9	10	10
anal fin rays	10	7	7	9

These measurements are based on data from two individuals of each type.

in spine size occurs between the marine tolerant form and the inland form, rather than in the history of the two populations. Hall (1956) worked with *Pygosteus* and *Gasterosteus* from the Oxford area and also from St. Andrews, New Brunswick, and she mentions that the Oxford form had a shorter tail and shorter dorsal spines, while the New Brunswick *Gasterosteus* also had a full set of lateral plates, thus corresponding to Heuts' races A and B respectively. She appends data on these variations (Table 2.1) and it seems that the variation within a genus on these characters is much less than the difference between genera. On the evidence it seems safe to generalise that *Gasterosteus* has longer and stouter dorsal and pelvic spines than *Pygosteus*, and in addition *Gasterosteus* has more protection to its body, as the pelvic girdle is more extensive and there are some lateral plates.

2.4 SPINE RAISING

The mechanism of spine raising in sticklebacks, especially *Gasterosteus*, has been studied by Hoogland (1951). The sockets in the base plates into which the spines fit have a complex form. Pairs of antagonistic muscles are attached to the spines, so that their movement can be finely controlled. When they are raised to the maximum extent the spines are lifted into their sockets and form a solid mechanical system which requires no muscular effort to maintain it. This is the position shown in Figures 2.1 and 2.2 A & B. The dorsal spines point vertically, the pelvic spines point out at right angles to the body, forming a horizontal line, and the first anal ray points directly down. This system provides the maximum obstruction, since in *Gasterosteus* the



A. GASTEROSTEUS

B. PYGOSTEUS

FIG. 2.2. STICKLEBACKS WITH THEIR SPINES RAISED

longest dorsal spine is placed at the highest part of the body, with the pelvic spines immediately below. When the spines are lowered from this position they are lifted out of their sockets by their muscles. The opposing muscle system makes possible a smooth raising and lowering of the spines as the fish moves about, the height to which they are raised varying with the motivation of the animal. Just the dorsal spines may be raised, or one or both of the pelvic spines may be raised as well, according to the circumstances. The spines are only mechanically locked into their position of maximum raising when the animal is grasped.

This description is based on the Hoogland, et al. (1957) account for *Gasterosteus*. They state that the spine raising mechanism of *Pygosteus* is similar to that for *Gasterosteus*, and a comparison of alizarin stained skeletons of both species confirms their similarity in the shape and disposition of all the skeletal elements, as well as the strength of the fully raised spine-system.

The conditions under which the spines are raised will be discussed later.

2.5 LIFE HISTORY OF STICKLEBACKS

2.5.1. Introduction

Some aspects of the life history of sticklebacks have been the subject of several rather intensive studies, and for this reason it will only be dealt with in general terms here. The works of Hall (1956) and Morris (1958) are particularly concerned with this.

2.5.2.1. *Gasterosteus*. The three-spined sticklebacks studied bred in shallow side streams, the breeding season extending from about March to June or July. In that time the males adopt territories, where they are conspicuous because of their bright blue-green eyes, vermilion throat and belly, and the iridescent sheen of the body, which is quite pale at times in the male's sexual cycle. These territories are in open areas in the shallow water, and from his territory the male chases off all intruders, and begins to build a nest on the ground from plant fragments and algal strands. The appearance of the nest varies with the materials available and the substrate, but its form is always the same, a flat mat of fragments glued together by a secretion of the male's kidneys, with a tunnel bored through it longitudinally, and a round opening at one end. This opening is called the mouth and is the area at which the male directs most of his nest-tending activities, and to which he attempts to lead any ripe females which he encounters as they swim past. After she has laid her eggs the female is driven away from the male's territory. Several clutches of eggs from separate females may be collected over a period of days, then the male pays all his attention to the continued defence of his territory and the maintenance of the nest and eggs, ventilating them with a current of water and removing mouldy eggs.

When the eggs hatch the male cares for the young by retrieving them and returning them to the nest area, either on the nest or in the pit he has made at the nest entrance. Within a week or two the young become independent of their father and move away from his territory. The male may then begin a new nesting cycle, so that he rears several broods of young in a season. The young form small schools and feed on tiny organisms in the vegetation along the shallow streams in which they were hatched. Also in this vegetation there are ripe and spent females and some non-breeding fish such as young fish late in the season. Only the males are territorial, the other fish are in schooling groups, except perhaps really ripe females which may remain near a male's territory. In later summer the young of the year, now anything up to 3.5 cm. long, move down the side streams into slightly deeper water, so that they collect in great schools in the streams. In August and September they are commonly found under the edges of weed masses in the main river, where the current is less fierce. The winter is spent in the main river, and in the spring the migration into the sidestreams begins again.

Van Mullem and van der Vlugt have recently (1964) published a thorough study of the growth and migration of mixed populations of *Gasterosteus aculeatus* races A & B. They found that the fish attain sexual maturity after one winter, and live for about a year and four months. There was a sex difference in lengths, although there was no length difference between the forms or races. Growth is from about 30 mm. at three months to about 60 mm. at 11 months. They followed the young fish after their first three months in the ditches, through their migration into the canals during the next three months, then into the Ooster Schelde about a month later. They overwintered in the sea and began the return trip during the early spring. Their results correspond with and augment the observations of earlier authors. From this description of the migration of mixed populations it is possible to recognise similar trends in the local Oxford population of f. [illegible] *trachurus*, with its short migrations between shallow and deeper waters.

2.5.2.2. *Pygosteus*. The *Pygosteus* collected during the breeding season lived in a pool which had plenty of plant growth and shallow verges. In spring the males adopted territories around the verges, and built their nests among the dense plant growth. The nests are made of plant fragments woven into a curved tubular structure, or a rounder mass, all fastened with the male's kidney secretion. They are usually at about mid-water (i.e. about 5-15 cm. below the surface) but may extend down just above the ground, with the entrance slightly lower than the exit.

Territorial males are a uniform velvety black when aggressive, the only contrast being the white pelvic spines, while the aggressive females, which also adopt territories, are black with a very silver abdomen (Morris, 1958, p.15. Figs. VIII & VII). The male courts a female, and when she is ripe she abandons her territory, and follows him to the nest to lay. Like *Gasterosteus* the male can secure several clutches of eggs over a few days before he switches to entirely parental activities. The territory is defended throughout, the only intruders of the same size allowed being ripe females during the period of courtship, but as soon as a female is spent she is driven off most vigorously. When the young hatch the male retrieves them and returns them to the nest area, or to a "nursery" which he constructs in the form of a loose nest. When the young leave the male is free to begin a new nesting cycle. As in *Gasterosteus* the females are able to produce several clutches of eggs at intervals of about eight days.

The young disperse through the dense vegetation but can be netted out if the net is forced through vigorously. As they grow they move out into deeper water, and it is possible to see numbers of them moving in any area, but they do not school like *Gasterosteus*. No observations have been made on the life-history of *Pygosteus* living in moving water, beyond the fact that they were breeding in waters which ran into the Oxford Canal, since they were netted among the *Gasterosteus* below the banks.

2.6 MAINTENANCE OF FISH

Fish collected from the local sources were stored in two large zinc baths which were coated with biologically inert Bitumastic paint, with a gravel substrate, and were kept in a cool room with eight hours illumination daily so that they would not become sexually mature (Baggerman, 1957). When they were required for breeding purposes the fish were put in smaller tanks under less crowded conditions, with sixteen or eighteen hours light a day, and plenty of vegetation in the tanks. The temperature was not controlled, but varied from about 10°C. to 25°C. The vegetation used was *Myriophyllum spicatum*, *Vallisneria spiralis*, *Fontinalis antipyretica* (willow moss), and *Callitriche verna* (starwort). There was also usually a covering of duckweed to the tanks (*Lemna* sp.) and clumps of a filamentous green alga,which was used for nest building. The rearing tanks were almost all 45 x 25 x 25 cm., and this was the space available to the young after their father was removed. Broods were reared to testing age in these tanks unless they were very large, when so many fish would become crowded.

Illumination of all tanks was from neon strip lighting or from bulbs suspended individually over the tanks. Observations were made in tanks of many sizes, but the largest tanks were the most useful for tests with the predators and most of the experiments reported here were made in tanks 100 to 200 cm. long. The fish tested all ranged between 2.5 and 4.0 cm. in total length.

In the preliminary observations the observer sat unscreened in front of the tank, but under these conditions *Pygosteus* would remain fearful and *Gasterosteus* would often come to the front and investigate any slight movements. Consequently later observations were made from a collapsible hide (4 x 4.5 x 1.75 ft.), covered in black cloth, and observations were made through a long narrow slit in the front, covered with a double layer of black nylon net. Through this screen the sticklebacks seemed oblivious to the observer, though occasionally pike would watch the observer for a time.

Sticklebacks were fed on *Tubifex* worms, or on *Artemia* nauplii when they were newly hatched. The young transferred directly to feeding on *Tubifex.* The three predator species were fed on minnows and despined sticklebacks. The trout also ate *Daphnia* and *Tubifex*, and the perch ate mealworms.

2.7 Special techniques

For the description of the skeleton,dead fish were fixed in formalin, macerated in potassium hydroxide, stained with Alizarin Red S., and cleared and stored in glycerol.

Model pike were made by casting a mould from a dead pike and preparing forms in cream latex. Two were painted in the cryptic pattern of the pike, and one was given large glass eyes.

2.8 STATISTICS

A non-parametric statistical test, the Mann-Whitney U-test, has been applied to the data on distances (Siegel, 1956). All probabilities quoted are for one-tailed tests since the direction of the difference is always determined,and the probability levels are taken from Siegel's tables. Where one of the totals is less than 20 the statistic is U, if over 20, the statistic is z, and these are given in all the Tables.

The only other test regularly applied is the chi-square test for two independent samples. The χ^2 value and the associated probability is given beside the quantities compared on all the Tables, and an absence of a value means that the results are not significantly different.

2.9 FISH WHICH OCCUR IN THE SAME LOCALITIES AS STICKLEBACKS

Several works giving lists of the fish fauna of a locality which also supports sticklebacks have contributed to the list below, which is almost certainly not complete. The lists used were those of Swynnerton and Worthington (1940), Hartley (1947, 1948), and McCormack (1965).

None of these lists included *Pygosteus pungitius*.

Esox lucius	pike
Perca fluviatilis	perch
Salmo trutta	trout

Salmo salar	salmon
Phoxinus phoxinus	minnow
Anguilla anguilla	eel
* Coregonus wartmanni	Schelly (McCormack ref.)
Cottus gobio	bullhead
Nemacheilus barbatula	loach
Carassius carassius	Crucian carp
Gobio gobio	gudgeon
Tinca tinca	tench
Leuciscus leuciscus	dace
Rutilus rutilus	roach
Scardinius erythophthalmus	rudd
Abramis brama	bream
Blicca bjoerkna	whitebream
Alburnus lucidus	bleak
Acerina cernua	ruffe
Platichthys flesus	flounder
Osmerus eperlanus	smelt
Salvelinus alpinus lonsdalii	char
* Coregonus clupeoides stigmaticus	Skelly (Swynnerton and Worthington ref.)

Chapter 3

PREDATORS AND THEIR BEHAVIOUR

3.1 Introduction

A variety of different animals feed on sticklebacks at various stages in their life-cycle. The adults are eaten by several species of birds and fish. In addition they may suffer the attentions of a large group of organisms such as *Argulus*, blood-sucking leeches and internal parasites, including the tapeworm *Strongylocentrus*, which achieves enormous proportions inside the abdominal cavity, and many other smaller parasites. The smaller young and eggs suffer from an even larger range of fish, including their own species, and from predatory insects as well as from almost any aquatic animal which happens upon them. There can be no clear-cut definition of the boundary between predator and parasite, but for the purpose of this study we shall take *predators* to be animals larger than the size of an adult stickleback (about 5 cm.) which are capable of killing a stickleback which has reached the free-swimming stage, independent of its father's care. This definition has been framed to leave out the complex of hazards to the eggs and young which will briefly be considered first.

3.2 PREDATORS ON THE EGGS AND YOUNG FISH

3.2.1. Older Sticklebacks

In spite of the extremely aggressive territoriality of the male stickleback, in which the nest is most vigorously defended, egg-robbing still occurs. This is seen occasionally in the laboratory; in a crowded tank many individuals may participate. In an analysis of the gut contents in natural populations of sticklebacks, Hynes (1950) found that a high proportion of _Pygosteus_ and _Gasterosteus_ had eaten eggs of their own species during the breeding season, suggesting that egg-robbing is even more prevalent in the wild. The young fish are also eaten.

3.2.2. Other Fish

Any of the other fish listed in Chapter 2 as occurring together with sticklebacks is a potential predator on the eggs and young. The fact that there seem to be no records of this is of no value since it is both difficult to identify eggs and very young fish, and also such delicate prey would soon be rendered even more unrecognisable by the processes of digestion. Almost all the fish listed are recorded as eating a wide variety of small food items, at least at the early stages in their life-histories. The male stickleback defends his territory against all comers, but he is most ferocious when he has eggs or young. Apart from keeping his own species and other fish of that size at bay, the male will attack intruders many times his size. In a film sequence Tinbergen has shown a territorial male stickleback attacking a newt and a freshwater crayfish, each of which was much larger than the fish. The attack was direct contact, not threatening behaviour, and I have seen this also against fish which are unfamiliar to the stickleback, such as guppies and goldfish. The guppies were about the same size, the goldfish were about twice the size and three times the depth of the victorious stickleback.

Newts, which hunt their prey by its smell, are a persistent danger to the eggs, which are only hidden visually by the nest, but the male sticklebacks always kept away individuals they were tested with, although the newts were larger than the defender.

3.2.3. Insects

Adult *Notonecta* and *Dytiscus*, a dragonfly nymph and a large *Dytiscus* larva were all seen to stalk and attempt to catch young fish, and doubtless must succeed occasionally, but *Notonecta* was seen to catch only sick or abnormal sticklebacks, especially those with a defective swim-bladder. The dragonfly nymph never caught a young stickleback because they always jumped away just as the labium began to flick forward while they investigated this motionless insect. Young minnows and quite large tadpoles were easily caught. The *Dytiscus* larva stationed itself, abdomen held up at a right angle to its body, head facing forwards and motionless. It remained very still just below the surface, on weed strands, and caught and held with its powerful mandibles *Gasterosteus* of about 3 cm. total length when it was itself about 5 cm. long. The fish died with their spines up, pumped dry by the larva which changed the position of its mandibles occasionally.

In addition adult *Dytiscus* were often seen to approach nests which contained eggs of both *Pygosteus* and *Gasterosteus*. They were nudged and pushed away by the aggressive males of both species, which normally left food if the beetle came to eat at the same place, and avoided it as they moved about.

3.2.4. Snails as potential predators of eggs

During the parental phase, after egg-laying, the male threespined stickleback keeps an area of the substrate around his nest free of food, faeces, snails, insects such as caddis larvae, and débris. *Pygosteus* males attack *Gammarus* and snails which stray on to their nests, actually keeping them off since an untended nest is soon festooned with snails. On several occasions when I had removed a male from his tank when I though his eggs had hatched I later found snails clustered densely over the dead eggs, eating them. An experiment in which two developing eggmasses, one of each species, were placed on the clean sandy substrate of a well aerated tank which contained many snails (*Physa* spp., *Limnaea peregra*), proved that while the snails would eat the dead eggs they did not touch developing eggs, even on the same mass. Eggs left in the same way in a tank without snails soon died, but after three days some eggs in the snail tank were still developing. The scavenging activities of the snails had in this case been beneficial to the developing eggs, which die very rapidly when they are not well ventilated.

3.2.5 Discussion

Apart from the variety of animals capable of predating stickleback eggs, during the early stages in its life history the stickleback is at considerable risk from a variety of predators, and because of its avoidance of open water may be vulnerable to the attacks of rapacious insects which lie in wait in the vegetation. Their rapid avoidance of any strange movement must stand the young

FIG.3.1. PIKE

in good stead, but the eggs are dependent upon their father's protection. As he will fight to protect the eggs and very early young, it is in the first free-living stages after leaving the nest, when the spines are also undeveloped (see Chapter 7), that the greatest risk of juvenile mortality must lie.

3.3 PREDATORS UPON THE INDEPENDENT STICKLEBACK

3.3.1 Introduction

The type of associations of fish in which sticklebacks are found have been listed by workers such as Swynnerton and Worthington (1940), Hartley (1947, 1948) and Maitland (1965). Several studies have been made of ecological relationships or food habits in such natural populations, and it seems that pike, perch and trout are the main predators on sticklebacks (Allen, 1935, 1939; Hartley, 1947, 1948; Frost, 1954). Each of these has been used in the laboratory during this study, with special attention to the pike.

3.3.2 The Pike

As a subtly camouflaged slender shape (Figure 3.1), lurking in the weedy recesses of the bank or hovering in mid-water, the pike is a familiar sight in canals, river and lakes. A solitary hunter, he patrols slowly from one section of a stream to another, sometimes stalking an unwary fish moving freely in the open, sometimes darting after an escaping prey. Izaak Walton (1676) refers to this solitary habit and calls the pike the tyrant of the rivers. It is seldom that more than one pike is seen in a stretch of water, yet the pike does not exhibit a well marked aggressive territoriality (Fabricius and Gustafson,1958), although aggressive postures (their Fig.1) are exhibited between males at spawning time. In the laboratory an individual of about 20 cm. kept in a tank 30 x 33 x 185 cm. behaved with the same aggressive posture to a smaller model pike when it was put in the tank.

In this tank the pike's behaviour seems to correspond with that seen in the open river. The weed masses are used as resting stations, the pike often remains with the head held so that the horizontal black line through the eye and along the snout is parallel to the ground, the body curved downwards in an arc, with the tail just touching the ground. All the fins are erect, but their movement may be reduced to a slight rhythmic movement of the pectoral and pelvic pairs. In the absence of prey the pike occasionally takes up a horizontal position and swims languidly to another area of the tank where it may scan the vegetation, or go to rest again. Periodically it patrols restlessly about the tank, keeping to a relatively fixed course though it may be varied over a period of days.

When fish are introduced into the tank with the pike they are stalked and eaten; the stalking and snapping sequence has been described by Hoogland, Morris and Tinbergen (1957). The first signs of the pike's attention being engaged are the eye movements by which it fixates the prey, then turns its body towards it, taking up a position suitable for binocular fixation, stalks it to within a short distance, which increases with the size of the pike, then leaps and snaps its mouth open and shut at the end of the leap, so that the prey is sucked in with great force. If the fish is not taken head-first and is large enough to be difficult to swallow, or else if it is a stickleback of adult size, it is held in the jaws and turned until it is facing head-downwards into the pike's oesophagus. Swallowing follows but this is not always a rapid process. Sticklebacks are intractable prey because of the spines which are locked into position as soon as they are grasped, and pike would repeatedly regrasp them with the jaws and turn them before even trying to swallow them. One small pike (up to 17 cm.) used regularly in tests as a predator on sticklebacks, sometimes managed to swallow three-spined sticklebacks after turning them on their right side, head-first down his oesophagus, so that as they struggled in the stomach for up to ten minutes after being swallowed, the points of the two long dorsal spines showed as bulges on the left side of the pike's body wall. All the pike used in these experiments were fed on despined and juvenile sticklebacks so that they would continue to stalk and eat them, since normally pike quickly learn to avoid sticklebacks. For this reason the distinction (in the Hoogland et al. (1957) scale of attack) between leaping (to attack prey) and snapping (to grasp it) is valuable, as a fully fed pike or one experienced with normal sticklebacks with spines will leap at a stickleback but stop just short of it without harming it. This form of attack was the behaviour exploited in my own experiments on the response of sticklebacks to pike.

As the movements used in the locomotion of the pike are also extensively described by Hoogland et al. (1957) the details will not be described apart from mentioning that the pike swims with extreme control of all its movements, so that it is able to stalk its prey without the benefit of any cover, using scarcely perceptible rapid lateral undulations of the hind edge of the dorsal, anal and caudal fins with some pectoral and pelvic control. This controlled swimming is used to good effect in a common hunting sequence where the pike scarcely moves until its prey faces the opposite direction, when it moves up very fast, only to move very slowly once the prey can see it again. It is equally delicate in backing. When a pike misses its prey it keeps absolutely still exactly as it stopped, on the very spot where the prey had been, and it may remain so for up to a minute or longer, although the eyes quickly swivel to re-locate the prey. If it is successful in seeing its prey the pike will fixate and stalk it once more, so that with the deliberate nature of its second attempt the unsuccessful first attempt does not prejudice the result as a hasty second dash would do. This deliberately controlled locomotion is of vital importance to the success of the pike's hunting.

So far brief mention has been made of the use of the eyes in hunting. The relative development of the visual and other areas of the brain in the pike and the specialisation of the eyes, led

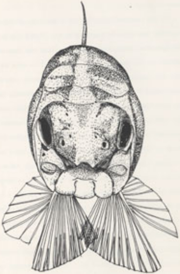
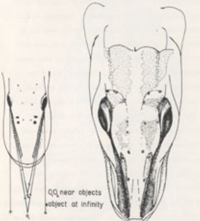


FIG. 3.2. FRONTAL VIEW OF PIKE

Polyak (1957) to conclude that the pike is a highly specialised visual hunter with binocular vision. The head is formed so that the large and mobile bulging eyes, on their hydrostatic cushions in the optic cup, can be swivelled to face forwards, while a pair of grooves, "sighting grooves", from the front edge of each eye extend convergently down the snout (Figure 3.2a~~——~~a). Combined with a thickening of the cornea in the corresponding rostral area and an anterior pupillary notch in the iris, which allows light rays to impinge directly on the retina from the forward region, this makes an excellent system for forward vision. In addition Polyak has examined the retina and describes it as mainly diurnal in function with a disposition of cones which suggests a fair degree of visual resolution over the whole wide field of view of the pike. A locality sufficiently differentiated to act as a "posterior area", comparable to the "central area" of other vertebrates, is present in the posterior corner of the retina, precisely where an anterior pencil of rays strikes, after coming through the anterior pupillary notch. This suggests that the possibility of binocular stereoscopic vision presented by the gross anatomy of the eye region is accommodated for in the retinal specialisation, and as the hunting behaviour has been described as relying upon binocular fixation for the whole sequence, Polyak's tentative conclusions in this respect are confirmed. Furthermore, he interprets the large dimension of the numerous cones as conferring a low threshold and high responsiveness to chromatic stimuli, so that the pike is not only equipped to respond to moving

FIG. 3.3 DORSAL VIEW, HEAD OF PIKE.



POLYAK (1957), FIG 451, VIEW FROM ABOVE THE HEAD OF PIKE SHOWING SIGHTING GROOVES WITH THE ANTERIOR PENCILS OF LIGHT RAYS USED IN BINOCULAR STEREOSCOPIC VISION.

stimuli in its wide field of view, which includes a forward stereoscopic field, but it is also responsive to colour cues.

The pike can easily adjust to certain changes of position of the prey in the horizontal plane. If the prey moves to right or left of the pike's aiming line it shifts its aim accordingly; if it moves away the pike can move after it. Braum (1963) found this compensation is shown in the first hunting movements of post yolk-sac pike. However, the pike has more difficulty if the prey comes too close, as it then has to back off, somewhat awkwardly, until the fish is at the correct range again, which seems to correspond to the point in Figure 3.3, redrawn from Polyak (1957, Figure 451, p.810), showing the maximum and minimum convergent lines drawn through the sighting grooves of the pike, lending some support to this interpretation of their function. An upward or downward shift of the prey is also somewhat difficult for the pike to accommodate. Pike respond to visual stimuli, and especially to movement, though the possibility that olfactory or other cues might play some role in alerting or orientation cannot be excluded.

It is known that a pike will orient towards a still minnow and that it will snap rapidly and almost continuously at a moving prey whether it is normal, headless and/or finless, or half a minnow. (Meesters, 1940. All the minnows were behind glass, so the responses were entirely visual.) This behaviour has also been shown by the pike I used. A fish which remains still on the ground, in full view, may escape notice, while a starved pike can be induced to eat anaesthetised or dead fish - normally ignored - if they are thrown into the water with some force so that they move through it instead of gently floating or sinking. In the case of anaesthetised fish, which lie very still, the pike is attracted to them by their slight opercular movements, and as they move a little more during this recovery they are snapped up.

The food for the pike has been the subject of a number of studies, those most relevant here being: Allen (1939), Frost (1954), Hartley (1948), Healey (1956). Sticklebacks form a substantial part of the food in some areas but all references are to *Gasterosteus*. The significance of the interesting absence of records for *Pygosteus* will be discussed later.

In ~~Lake~~ Windermere the young pike eat small prey fish; small perch are the preferred food, although *Gasterosteus* and *Phoxinus* are taken in roughly equal numbers (Allen, 1939). This indicates some selection of the sticklebacks as minnows are much more numerous in the lake as a whole, though they may not be equally available on the pike feeding grounds. Frost (1954) has shown that overall the char, perch, and trout are the most important food of the pike in ~~Lake~~ Windermere. She found that the stickleback is the most important minor item, with a percentage frequency of occurrence a third that of perch and trout, less than a quarter that of char. Pike between 20 and 60 cm. total length contained sticklebacks, while those below this size fed on Entomostraca and insect larvae, and those above 60 cm. ate large fish. In the 20 to 29 cm. range the pike made their heaviest predation upon minnows (34% frequency of occurrence, only 3% for sticklebacks); in the 30 to 49 cm. range minnows and sticklebacks figure equally at about 8%, and above this range the numbers of minnows eaten were negligible, although sticklebacks were most heavily predated by the 50 to 59 cm. range of pike. When the dates of capture of the pike are considered it is found that sticklebacks are taken mainly in May and June, which corresponds with part of their breeding season. An examination of Frost's seasonal breakdown of the most important food fish of the pike shows that all are taken most abundantly at their spawning season.

The pike of three Irish lakes were the subject of Healey's (1956) study. In one lake there are no sticklebacks and in another some are eaten, but the most extensive study was on Lough Glore where sticklebacks were a staple item in the diet of pike above 15 cm. in total length between April and July, and they were regularly eaten by pike up to 50 cm. long. During the winter months sticklebacks were still taken. The mean monthly percentage occurence of sticklebacks in pike of all sizes in Lough Glore is given in Table 3.1

Table 3.1

Size of pike cm.	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64
mean % occurrence of sticklebacks (empty stomachs included)	84%	56%	59%	48%	26%	25%	21%	8%	7%	10%

In older pike the percentage of perch and trout in the stomach contents increased.

As large pike take larger prey, such as large perch and trout, and fewer sticklebacks, this could be at least partly due to

FIG. 3.4. PERCH

the larger pike moving into deeper water, away from stickleback haunts, and also to a preference for larger prey.

3.3.3 The Perch

The perch is another widespread river and lake fish, which, however, differs from the pike in its habits. Quoting from Walton (1676) "... they are not like the solitary Pike but love to accompany one another, and march together in troops." It is common to see large numbers of perch in a single locality, and their relatively shorter and thicker bodies do not lend themselves to delicate and controlled stalking as is so characteristic for the pike (Fig. 3.4). Perch can be seen darting about turning this way and that, examining weed masses with rapid movements of the whole body.

In the laboratory the perch propels itself by rhythmic movements of the pelvic and pectoral fins when investigating the tank. Fast movement is by means of lateral beats of the tail, the paired fins being used in braking. The perch's method of locomotion contrasts strongly with the normal and stalking movements of the pike, in that the perch makes extremely jerky movements and tends to sink between them. The eyes are well developed and mobile, although not quite as much as those of the pike. They face somewhat upwards and forwards. As it swims about the fish constantly scans the environment, either by converging both eyes forward on an area in front of and above its snout, or else it rolls a little to one side, using one eye to search the lateral field. The whole body has to be tilted forwards to examine the ground with both eyes during feeding or stalking. The monocular field is used only when investigating in a general manner.

When quiescent, the perch rests on the ground, propped by its paired fins, so that the head faces upwards. The first dorsal fin is lowered and the bands on the body are very clearly marked.

When hunting behaviour begins (in the absence of prey, this is a routine prowling of the tank) the first dorsal fin is raised and the lateral bands and dark circum-orbital ring fade to a level which varies with the lighting in the tank. In a brightly lit tank only the spot at the hind edge of the first dorsal fin and the eye itself remain in dark contrast. An investigation of the tank consists of slow swimming with a very keen examination of the surface and any weed masses. The slightest movement will attract the perch's attention, so that it will binocularly fixate a prey almost in front of it by swivelling the eyes. While stalking, the fish lowers the conspicuous first dorsal fin, as when it swims fast, and then manoeuvres itself into a position 4 - 8 cm. from the prey, usually a little below it. After approaching the prey the perch stops to reorientate itself for the final assault, but the clumsiness of the braking almost always warns the stickleback or minnow, at least under laboratory conditions, so that the perch has to catch a fleeing prey. To catch a quiescent fish a sudden leap is made and the prey vanishes, sucked forcibly into the pharyngeal cavity, but a moving fish is a much more difficult target. A perch often misses even a quiescent prey which jumps after the predation leap has begun, because the final pause and location of the prey determines

FIG. 3.5A. LATERAL VIEW, PERCH HEAD.

FIG. 3.5B. FRONTAL VIEW OF PERCH

the extent of the final leap - there is apparently no compensation once it has begun. In this respect the perch again differs from the pike, which can compensate in the horizontal plane for changes in the position of its quarry. If it misses, the perch makes a rapid second leap, followed by more and faster attempts after each failure, so that the prey is often lost through this apparent impatience.

Deelder (1951) and Hoogland et al. (1957) have mentioned the perch's habit of edging around to grasp its prey from the head end - a useful move since the perch cannot turn its prey as can the pike, but must spit it out and recapture it. In aquaria perch experience some difficulty in catching a moving prey which is familiar with the tank, unless they manage to corner it by heading it off.

The anatomy of the visual system of the perch was not studied by Polyak (1957), but he extensively described *Lepomis*, a North American sunfish which looks rather similar to the perch. This description aided me in the examination of the visual anatomy of the perch. The eyes of the perch have already been mentioned, and they are set on the sides of the head facing more forward and upward than those of the pike. The eye movements are easily detected, and the presence of two short, very shallow grooves from the front corner of the eyes forward onto the snout, suggests a convergent development for the most efficient uptake of light rays for binocular stereoscopic vision (Fig. 3.5a). In addition there is a pronounced bulge in the cornea in the appropriate position to correspond with that seen in the pike. The frontal view of a perch shows all these features

(Fig. 3.5b). I have no information about the retina, but general observations show that the perch appears to be extremely sensitive to movement over a field which is not as extensive laterally or ventrally as that of the pike, but extends further vertically above the snout.

In their natural environment perch hunt in groups and they move about at different depths at different seasons in lakes such as Windermere (Allen, 1935; Le Cren, 1958). Allen found that the Windermere perch underwent two feeding changes in their life, being plankton feeders up to about 14 cm., then feeding on the bottom fauna until about 18 cm. when they change to a fish diet. He reports that perch between 16.5 and 18.5 cm. total length eat the same proportion of *Gasterosteus* and *Phoxinus* (10% frequency of occurrence in stomachs examined) but over 18.5 cm. they eat 14% *Gasterosteus*, 20% *Phoxinus*. As large perch prefer larger prey, and minnows grow to a greater size, this may explain these figures. It seems that in ~~Lake~~ Windermere, at least in the early stages of the perch's and the pike's fish-eating life, *Gasterosteus* is being taken in greater numbers relative to *Phoxinus* than would be expected from the overall abundance of the species in the lake, but this may well be due to habitat and/or habit differences. The latter suggestion is supported by Frost's (1954) records, as the bulk of the sticklebacks eaten were taken in May and June, the peak of their breeding season, when the males are particularly conspicuous through their coloration and behaviour. No record has been kept of the sex of the sticklebacks caught (Frost, 1965 pers.

comm.; Banks, 1965 pers. comm.; Cragg-Hine, 1965 pers. comm.).

The study made by Deelder (1951) of the stunted perch in the Dutch polders shows that thee fish remain at between 13 and 14 cm. long, although perch living in deeper streams without a lot of weed cover grew normally. The fish-food of perch in Holland is roach and smelt which will hide in weed to escape perch, and Deelder mentions that a perch on its own will seldom catch roach, some team-work and open water is required. The stage at which the polder perch remain stunted is the stage at which Allen's Windermere perch change from a mid-water invertebrate to a bottom fauna and them a fish diet.

Among the fish of Haweswater, Westmorland, there is the same change to an almost completely fish diet, but the perch are larger by this time (over 25 cm.), those between 20 and 25 cm. feeding largely on insects, with some fish, (Swynnerton and Worthington, 1940). By contrast with these studies Healey's (1954) study of the food of perch in three Irish lakes indicated that there the perch were feeding on the available food supply. The size of the fish prey was apparently not important. Her figures for Lough Glore suggest that the larger the perch were, the more likely they were to eat sticklebacks (Table 3.2), those of 30 cm. and over feeding on them more commonly.

FIG. 3.6. BROWN TROUT

Table 1.2

Percentage of fish in each size group of perch (empty stomachs included).

Lough Glore.

size range of perch in cm.	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22	22-24	24-26	26-28	28-30	30-32	32-34	34-36	36-38	38-40
mean % occurence of Gasterosteus in stomachs (empty stomachs included)				4	3	6	8	13	13	10	5	7	22	29	25	33	

On the evidence presented the perch is almost as well equipped visually, except in the size of the field of vision, as the pike, but is inferior when its locomotion is considered, and it does not seem to specialise on sticklebacks during its fish-eating stage, although quite large quantities may be eaten.

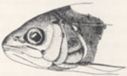
3.3.4. The Brown Trout

Young trout are aggressive and adopt and defend territories in the stream or river where they live (Kalleberg 1958). Within these territories, which are held for some considerable time, certain places, "stations", are preferred as a resting position, and are most vigorously defended. Both territory and station are defined by Kalleberg, and they are used here in his sense.

A brown trout of about 15 cm. total length (Fig. 3.6) was kept in the laboratory for some time in a well aerated tank 30 x 33 x 160 cm., planted with scattered weed clumps and with floating weed masses. This fish adapted very well to these conditions, although it was not in moving water, and used to spend a lot of time, like the pike and perch, remaining very still below weed in several preferred areas in the tank, moving from one to another periodically or patrolling a variable beat. These places correspond to Kalleberg's stations, although no clear preference was observed, possibly because there was no current. Unlike the pike and perch, the trout would often return to a station as soon as it had grasped its prey. Another difference is that the trout always hovered in the water, even when quiescent, its movements being controlled by occasional movements of the paired fins and some use of the median fins. The trout normally moves by tail beats, using its paired fins as brakes and stabilisers (Harris, 1937; Wickler, 1960). Its movements are nicely controlled, intermediate between those of the perch and the pike.

A trout is a catholic feeder and will snap at almost any moving object at the surface or in the water (Swynnerton and Worthington, 1940; Hartley, 1948; Müller, 1954; Nilsson, 1955 and 1957; Maitland, 1965). Fish are also stalked quite skilfully and are often taken unawares at the first jump. The hunting behaviour shows the same sequence as that for pike and perch; attention engaged by some movement, orientation of the body so that the eyes face forwards to the prey, an approach and a final leap directly forwards, with grasping and swallowing. If it misses, the trout resembles the perch in the haste with which it pursues and attempts to capture the prey, sometimes making four or five jumps in rapid succession before it succeeds. The trout has a smaller gape than the pike or perch and is, therefore,

FIG. 3.7A. LATERAL VIEW TROUT HEAD



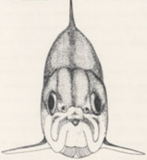


FIG. 3.7B. FRONTAL VIEW OF TROUT

more limited in the size of prey it can accommodate. Sticklebacks were caught by the aquarium trout, which would immediately spit them out and recapture them, just as the perch does, since it also could not turn prey in the mouth, but the trout was more persistent than the perch and often held a stickleback by the caudal region. In the laboratory this trout did more damage to sticklebacks than either pike or perch managed, although it ate fewer. The pharyngeal teeth were used to rasp away the unprotected caudal musculature, so that the fish died afterwards.

The visual system of the brook trout (*Salvelinus fontinalis*) and the brown trout have been described by Polyak (1957). From the anterior corner of the laterally placed eyes shallow grooves extend directly forward to the upper jaw; these are sighting grooves which correspond to those of the pike and perch (Fig. 3.7a). At the anterior corner of the eye there is a frontal corneal bulge which, together with a small anterior pupillary notch in the iris (as described in the pike, Polyak, 1957), acts as a system for the collection of the maximum number of rays from the forward direction (frontal view in fig. 3.7b). Once again the eye is very mobile, and it is inferred that the trout scans a field roughly equal to that available to the pike. The retina has several regions at which the cones are particularly dense, ("areae") (Polyak, 1957) and Polyak considers that the structure of the retina confers diurnal sensitivity of vision over a specialised area around the periphery of the retina, while the central territory has a lower acuity. Without observations on a living animal, Polyak could only speculate that there might be

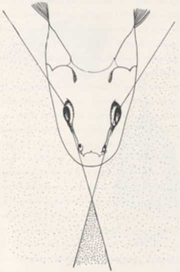


FIG. 3.8. FIELD OF VIEW OF TROUT

an anterior common binocular fixation point, scanning the field somewhere just in front of and above the head, but my observations on the living fish support this interpretation. (The field of vision of a trout is given in Fig. 3.8, based on measured angles given by Nikolsky, 1963.) In this way the trout scans for surface insects and then transfers to an anterior common fixation point, with an adjustment of the position of the body before the final stalking and/or leaping.

In the wild, trout are said to eat *Gasterosteus* but it is a minor feature of their diet. Nilsson's (1955, 1957) work in particular emphasises the varied nature of the diet of trout. His work on lake and stream trout shows that lake trout were eating fish, while the stream trout ate very little fish, but subsisted mainly on insect drift downstream. The trout stream territories are feeding territories (Kalleberg, 1958) and Kalleberg estimates from their size that the trout would not be able to live on food taken exclusively from that area, but would rely on the drift fauna. The trout's visual system is adapted to respond to cues over a wide field, so that it is well fitted to benefit from drifting prey.

The trout in Lake Windermere (Allen, 1938) rarely eat fish until they are about 40 cm. long. Trout over 40 cm. mainly ate fish between 10 - 15 cm.long, so that their likelihood of eating sticklebacks is slight. Hartley (1948) lists the prey of a community of fishes from the River Cam and the Shepreth Brook over a period of three years, and gives a wide range of food, including fish, taken from thirty trout, but only one stickleback was eaten. Trout of over 25 cm. in Haweswater ate a high proportion of fish, stickleback and minnow being important here, but the smaller trout ate insects (Swynnerton and Worthington, 1940).

From these records it is concluded that trout prey on sticklebacks incidentally, most fish-eating trout being so big that even the largest sticklebacks are below the size preferred.

3.3.5. Discussion

The pike is an appreciable predator upon sticklebacks at a relatively brief period in its life-history, the perch will eat sticklebacks when it reaches a fish-eating size, and trout may do so, although this is much more rare. There is no evidence at present that other British freshwater fish attack adult sticklebacks with any regularity. Cragg-Hine (1965, pers. comm.) gave the data presented below for predators collected in Willow Brock, a tributary of the Northampton R. Nene.

Table 1.1

Predator species	number of stomachs containing food	number of stomachs with *Gasterosteus*	number of stomachs with *Pygosteus*
Chub	141	1	-
Perch	189	1	-
Pike	94	1	-
Eels	82	-	1

Data provided by Cragg-Hine on stickleback predation, R.Nene.

Both *Pygosteus* and *Gasterosteus* were present, but the data show that sticklebacks are a negligible food item in that locality.

In her study of the food of eels in Lake Windermere, Frost (1946) found nosticklebacks were eaten by the specimens examined, but Banks (1965, pers. comm.) working in similar areas to Cragg-Hine, reports *Gasterosteus* to have been eaten by eels, chub, pike and perch, but no *Pygosteus* was found,although it occurs in the same waters. He also records that the pike which had eaten sticklebacks were collected between March and June, so that again the pike are eating sticklebacks during their breeding season, although the total numbers involved are not appreciable when considered with the resident population numbers.

The fact that *Gasterosteus* becomes more liable to predation by pike and perch during its breeding season suggests that the change of habit entailed, and perhaps the increased conspicuousness of the nesting male in both appearance and behaviour, endanger the species. Unfortunately there is no record of the sex of sticklebacks eaten by pike or perch (Frost, 1965, pers. comm.), so that it is not yet possible to assess the relative risk of predation for the sexes during the breeding season. *Pygosteus* remains in dense weed for the breeding season, and although the colour and habits of both males and females changes, it is never as vulnerable to predatory attacks as *Gasterosteus*.

In the laboratory pike, perch and trout quickly learned to ignore normal sticklebacks and had to be specially fed with minnows and despined sticklebacks to keep them responsive for the experiments. Even then tests often had to be suspended for a day or more after a fish had tried to eat a normal stickleback and had experienced difficulty, because it would ignore all subsequent specimens until fed again on despined fish. In the experiments of Hoogland, Morris and Tinbergen (1957) the pike and perch learned to avoid normal sticklebacks, and ate minnows for preference. Ten-spined sticklebacks were eaten before the three-spined ones were taken, as long as the spines were intact, but if kept very hungry the predators could learn to cope with even normal *Gasterosteus*. Despined fish were almost all eaten immediately. Nevertheless, field records have shown that pike, perch and trout eat sticklebacks at least during some stage in their life history, but they are taken somewhat less than would be expected by their apparent abundance ~~availability~~. If the spines were the only protection it would be expected that *Pygosteus* would be more heavily predated than *Gasterosteus*, but surprisingly, the opposite is the case. Presumably, this lower predation of *Pygosteus* is due to its cryptic habits, and it is to investigate this that the present study has been undertaken.

3.4 AERIAL PREDATION

3.4.1. Introduction

There is some evidence of predation upon sticklebacks by birds, notably the black-headed gull and the heron. It was with this in mind that Phillips (1962) used stickleback fright responses to overhead models in his study of the survival value of the white coloration of gulls and other birds.

3.4.2 The Black-headed Gull, *Larus ridibundus*

In an investigation of the feeding habits of the black-headed gull Creutz (1963) used analyses of crop contents and the food taken by neck-ringed birds, as well as crop pellets. Gasterosteus figured highly in the crop contents for 1961, but was not recorded from the pellets. As the skeleton of sticklebacks is relatively strong and distinctive its absence from pellets may be taken as evidence that they were not taken as food by the birds which supplied the pellets. Fifty percent of the adult gulls had taken fish (total from all samples); the total percentage by weight of fish food for adults was twenty percent. More than twice as many carp as sticklebacks were eaten. The sticklebacks were caught between July and November, that is, after the peak of the breeding season.

In experiments involving the feeding of nine species of fish to the gulls, Creutz found that the smooth fish were always taken before spiny fish of the same total length. He also drew up a table of optimal, maximal and refused (too large) sizes of fish from these experiments. All the optimal sizes were larger than the length of an adult stickleback. In a later paper, Creutz (1965) incorporates additional data. He lists the food-containing gizzards of black-headed gulls which included Gasterosteus among their contents; the Gasterosteus contribution, expressed as percentage of birds containing sticklebacks, shows a slight annual change (3% in 1960; 15% in 1961; 28% in 1962; 21% in 1963; 20% in 1964). Sticklebacks are never the main food of these gulls, but as each gizzard contained an average of up to six specimens they have some importance. Pygosteus was not available in the hunting localities (Creutz 1965, pers. comm).

Table 3.4

Small Prey fed to Nestling Grey Herons (From Appendix 1, Owen, 1960).

	WYTHAM	BUSCOT	HIGH HALSTOW
Minnows	158	195	-
Roach	21	13	-
Rudd	-	-	10
Sticklebacks	285	162	1031
Eels < 15 cm.	-	-	52
Bullheads	13	11	-
Stone loach	-	68	-
Goby	-	-	11
Palaemonetes varians	-	-	1059
Crangon vulgaris	-	-	38
Dytiscus sp. larvae	21	3	31
Agrion splendens	41	1	2
Rest: Crustacea, Insects, Amphibia	25	17	17
	565	470	2251

3.4.3. Herons, *Ardea cinerea* and *Ardea purpurea*

Both Owen (1960) and Creutz (1964) found that the food of *Ardea cinerea* could be divided into two categories, large prey defined by Owen as eels over 15 cm., all other fish over 10 cm. plus mammals and birds, and small prey which is on Owen's definition smaller fish plus Amphibia, insects and crustacea. Creutz gives the heron's optimal food size as 14 cm., smaller prey only being taken when the birds are very hungry and lack larger prey, in which case large quantities of sticklebacks are devoured (17-23 specimens per bird).

Owen's (1960) data are of particular value since his herons fished from waters where both species of stickleback occurred. His data for three localities are given in Table 3.4. At High Halstow large prey was not readily available and larger numbers of small prey were taken. The birds of the other two localities seem to be feeding from similar sources which differ from the High Halstow area in the fish available.

Both species of stickleback are listed together but Owen mentions that *Gasterosteus* always outnumbers *Pygosteus*, reaching a proportion of 2:1 in the prey at the High Halstow station. In 1953 there was a sudden increase in the number of sticklebacks taken at the end of June, which Owen attributes to the fact that the young of the year are by them big enough to be caught. Before the end of June all sticklebacks recorded were adults, but during the last week of June the majority were young of the year (Owen, 1955). In 1960, Owen lists the total items of each food given to the nestling herons:

among the 2251 small prey given to the young at the High Halstow station, 46% were sticklebacks. At Wytham it was 51% of a total of 564 items, 15% of 470 items at Buscot. By the end of June less fish food was taken and Owen attributes this to the inaccessibility of fish since the streams became choked with the summer plant growth.

In their survey of the food of the nestling purple heron (*Ardea purpurea*) in Holland, Owen and Phillips (1956) found that *Gasterosteus* and some few *Pygosteus* were among the small prey taken.

Creutz (1965) fed a variety of fish to grey herons by presenting them alive in dishes of water. The herons would eat sticklebacks but had some difficulty in swallowing them.

On the evidence presented it seems that herons are appreciable predators upon sticklebacks at least while they are collecting food to feed their young, and once again this is over the late March to Late June period, which covers the long breeding season of the sticklebacks, and includes the earlier young of the year.

3.4.4 *Other birds*

There are records of other birds eating sticklebacks but none of them specialise upon them to the extent shown for black-headed gulls and herons.

The double crested cormorant (*Phalacrocrax auritus auritus*) in North Dakota is recorded by Lewis (1929) as having eaten *Gasterosteus*, but it is an incidental food. In a survey of the shags and cormorants of Cornwall, Steven (1933) records among the food just two *Spinachia*, no *Gasterosteus*, and similarly Lumsden and Haddow (1946) record *Spinachia* in *Phalacrocorax aristotelis* of the Clyde Sea area. The cormorant of the Netherlands, (*Phalacrocorax carbo sinensis*), feeds on *Gasterosteus* which is extremely abundant on their Ijsselmeer hunting grounds (van Dobben, 1952). Sticklebacks are grasped just behind their head region and turned by "nibbling" movements of the bill. In van Dobben's opinion, sticklebacks and smelt were infrequently taken, despite their abundance. When they were taken these sticklebacks were in with catches of other fish, as well as in lafge masses exclusively composed of *Gasterosteus* and including up to over 100 individuals, "apparently collected with great patience". (van Dobben, 1952).

The American merganser (*Mergus merganser americanus*) in British Columbia is recorded by Munro and Clemens (1937) as eating a lot of *Gasterosteus* and in Palmer (1962) some species of grebe are recorded as having eaten *Gasterosteus*. The goldeneye, *Bucephala clangula*, occasionally eats small fish including *Gasterosteus aculeatus* (Olney and Mills, 1963; Madsen, 1954). There are records of occasional sticklebacks in the food of a number of fish-eating birds such as grebes, divers, kingfishers, terns, storks and sawbill ducks (Witherby et. al., 1943-1944; Palmer, 1962).

It is not unlikely that there are other birds which occasionally eat sticklebacks but it seems unlikely that any of them will prove to be a major predator, even to the extent of the black-headed gull and the heron. It is surprising that more sticklebacks are not eaten by fish-eating birds, unless their spines and/or behaviour are protecting them, since *Gasterosteus* in particulaf is such an abundant fish.

Chapter 4

RESPONSES TO ACTUAL AND POTENTIAL FISH PREDATORS

4.1 Description of responses

Outside the breeding season sticklebacks commonly move about in schools, the behaviour of the school changing according to circumstances. **Keenleyside** (1958) has described this well and shows how the schooling becomes more marked when the fish are slightly alarmed. I will not deal with it further, but will describe the behaviour of individuals.

A stickleback normally swims rather jerkily, propelling itself a few centimetres at a time with synchronised backward thrusts of the pectoral fins and it then glides with the pectorals held close to the body until it stops, braking with expanded pectorals. Between these short swims the fish may hover more or less stationary in the water, maintaining its position with the pectorals beating alternately and with a slight movement of the caudal fin which apparently compensates for any yawing and rolling produced by the pectoral movement.

This method of swimming contrasts with that of many fish in which the pectoral (and pelvic) fins are mainly involved in braking and steering, while the tail and/or other median fins provide the main thrust (Harris, 1937, 1953). The admirable control which sticklebacks exert over their movements is particularly well demonstrated when they examine objects on the ground, since they are able to tilt smoothly at any angle from the horizontal hovering position. Lateral deflections of the dorsal and anal fin are used for extra control of stability at these times. Sticklebacks seldom feed from the surface (supported by field observations by Hynes, 1950) but are quite capable of tilting the body upwards to inspect the surface or take food from it.

The normal swimming behaviour of the sticklebacks, alone or in groups, consists of unhurried short swims with occasional quick changes of direction giving a general impression of jerkiness. The fish may swim at any depth in the tank but if near the ground they frequently tilt nose-down to investigate material lying there, (feeding behaviour has been described by Tugendhat (1960a, b, c)). Interspersed with this slow means of progression, are faster spurts with the pectoral fins held close to the body, the tail providing the propulsive force.

This description holds for both *Pygosteus* and *Gasterosteus*. The species differ in that *Pygosteus* has a longer body (mainly in the caudal region) and less rigid skeletal system and is therefore capable of more sinuous movement. Where reactions are shown by only one of these species the generic name will be given, otherwise "stickleback" will refer to both species.

A1 Encounter.

When a stickleback encounters a predator it *stops feeding*, or whatever it is doing, to watch it, either monocularly or binocularly. At this stage the fish *sinks* slowly towards the ground, usually to about half the depth of the water or below. Not only individuals, but a whole school may do this together.

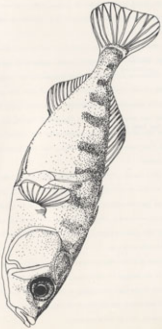


FIG.4.1. HEAD-DOWN ORIENTATION IN BINOCULAR FIXATION.

This is the movement which Phillips (1962) described as the "slow reaction" when testing the responses of sticklebacks to predator-like models moving above the water. Morris (1958) lists "sinking" in the general behaviour of *Pygosteus* but this differs in important respects from the movement described by Phillips and will be discussed later.

During the *monocular fixation* the predator may be directly abeam or slightly in front of or behind this, while the animal hovers motionless. A group of fish examining the same object may take up positions with the predator at different angles.

With *binocular fixation* the fish usually tilts slightly downwards so that it looks at the predator over its snout (Fig. 4.1). During binocular fixation the tail is sometimes flexed to one side.

Associated with the switch from undisturbed behaviour to these orientation responses there is a change in the rate of movement of the pectoral and caudal fins, and of the operculum. The caudal fin moves as usual at much the same rate as the pectoral fins, going from between 2 and 4 beats per second up to rates reaching 5 beats per second during fixation, while the operculum changes from an initial rate of <1 to 2 beats per second up to 3 beats per second. The fast movement of the alternating pectoral fins goes with a decrease in the amplitude of their beat and this led Morris (1955, 1958) to call this response "flickering", which he described as a variant of hovering while in situations of "emotional stress". Hall (1956) refers to this, and in her description includes this with backing

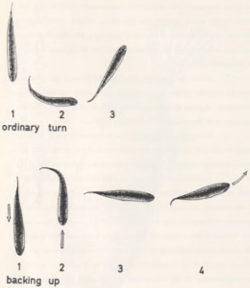


FIG. 4.2. TURNING AWAY

away as a part of the retreat of a stickleback from some alarming stimulus. As this change in fin and opercular rates is associated with a wide range of responses I use the term "flickering" simply to describe the fin component of this movement.

After a period of inspection the fish may turn away and resume more normal activities, but after prolonged binocular inspection it often <u>backs-up</u>, i.e. swims backwards using its pectoral fins, keeping the predator binocularly fixated, the angle of downwards tilting being increased as it backs. In addition the tail may be actively flexed to one side at a right angle during this movement, this being particularly common in <u>Pygosteus</u>, where it also occurs when the fish touches a wall or dense weed. After backing-up a short distance - usually less than the body length of the stickleback - the fish may resume a more horizontal position as it turns one to one-and-a-half right angles to move off, thus avoiding swimming directly away from the predator and losing sight of it, and also avoiding approaching it more closely during the turn (Fig. 4.2).

A fish which is alarmed has other ways of swimming, which I have called slow and fast swimming.

<u>Slow swimming</u> occurs in two forms, the first of these is <u>smooth swimming</u>, in which the fish glides very smoothly, usually quite close to the ground, with the pectoral and caudal fins flickering at high speed with a correspondingly restricted amplitude [illegible]. This eliminates the jerky component of "normal" swimming. The opercular beat is also accelerated but its amplitude is normal. This mode of swimming can

FIG. 4.3. Head-up, tail-down posture.

also be combined with a head-up, tail-down posture (Fig. 4.3). When the fish is very alarmed a shuddering form of slow swimming occurs, the head-up posture being maintained. The jerkiness arises through a reduction in the frequency of the pectoral and caudal fin beats with no corresponding increase in amplitude, while the opercular beat both slows down and decreases in amplitude, and the pectoral fins now move in phase instead of out of phase. This will be called <u>jerky swimming</u> distinct from normal swimming which is also jerky but in a quite different way, as described above. Slow swimming is an effective method of locomotion since a pike, perch or trout is less likely to attack a slowly moving prey. The jerky component is not understood, since to my eye at least, it increases the conspicuousness of the stickleback. Moreover, it often happens that a fish which has remained very still is not seen by the pike but when it begins to move in this slow jerky manner it is snapped up immediately. It is possible that such swimming is a compromise between a smooth slow retreat and the freeze response, when fright motivation is not quite high enough to elicit the freeze response directly.

The head-up posture may be combined with more normal swimming and monocular fixation and is the same as that described as the submissive posture in intraspecific encounters (Morris, 1958; Hall, 1956). Morris and Hall described the conditions in which this posture is elicited and have suggested that it may be appeasement behaviour, since it is the opposite orientation from that used in threat. When comparing the five species of stickleback, Hall (1956) mentions the head-up posture for Pygosteus only and describes it as a "reaction rarely seen in agonistic situations except in "beaten-up" fish in which escape motivation is very high indeed and the tendency to attack is completely absent".

During predator tests under varied conditions, with both species of sticklebacks, I have seen this reaction, though it is more common in Pygosteus, for which it is figured. Morris (1958) calls this the tail-down posture in Pygosteus, as the rather flexible tail stalk is actually bent downwards. I can corroborate Hall's (1956) interpretation of the posture as occurring when the fish is strongly motivated to escape. Its function in the predator situation is not known. Kruuk (1964, p.120) has noted for the black-headed gull the similarity between responses given in intra-specific encounters and as anti-predator behaviour, and suggests that they are due to the same underlying motivation, which may also be the case for the head-up posture of sticklebacks.

Rapid escape from another fish may be by means of fast swimming, in which the tail provides the propulsion. The path travelled by the fish has slight lateral wiggles corresponding to the tail beats. (Film sequences confirmed this.) This is the same as the rapid swimming described in normal behaviour, except that it is quicker, and in addition the deflections in the path may become so great that a rapid erratic zig-zag path is followed which increases the chance of escape. This is the response shown by Phillips' (1962) fish in their violent reaction to moving overhead models.

Sometimes the fast swimming is preceded or followed by one or more lightning *jumps*. A quick strong flick of the tail causes a jump, which usually ends with the fish slightly head-down, having changed its position in three dimensions, and continuing to fixate the predator. One or more jumps may be followed by fast swimming or re-fixation of the predator, and also a jump may lead directly into fast swimming so an impression is received of an extremely long jump. By very rapidly changing the position of the fish in three dimensions the jump is very effective as a temporary escape from the attack of a predator. Combined with fast swimming it is even more successful as an escape measure.

When a fish suddenly encounters a predator at very close range it is likely to *freeze*, becoming suddenly very still with very reduced fin and opercular movements. The opercular beat-rate and amplitude are reduced, while the fins move through a very reduced amplitude or become quite still as the animal slowly rises to the surface or sinks to the ground, with the body remaining perfectly horizontal. *Gasterosteus* rise, or sink, while freezing, *Pygosteus* sinks, and it is in this context that Morris (1958) has described sinking. He mentions a complete cessation of fin movements during the fall but in my experience of both *Gasterosteus* and *Pygosteus*, pectoral fin movements usually continue until the rise or fall is arrested, at the surface or the ground, while the caudal beat may cease at the onset of freezing, or when the pectoral fins stop. Morris also mentions that freezing may follow swimming to the bottom of the tank during escape. *Gasterosteus* occasionally shows this reaction during escape, but it is usually shown when there is no weed, or when cover is far away or not dense enough to shield the fish from a persistent predator. It is much more common for *Pygosteus* to freeze and it will even use as cover tiny pieces of loose vegetation lying on the ground. By suppressing all movements, including slowing down and decreasing the amplitude of the respiratory beat, the prey fish presents a minimal stimulus to release attack by a predator.

As long as the predator is close this posture is maintained, and it may be some time after the predator has gone before the stickleback begins to move. The first sign of recovery is a resumption of a normal frequency and exaggerated amplitude of opercular beat. When the fish does move away it begins with jerky slow swimming, often crawling along the ground. This response may also be shown by a fish which has dived into weed, in which case the weed supports it. The behaviour described by Phillips (1962) as "skulking" is probably freezing. The effectiveness of freezing is emphasized when it is noted that those fish which attracted the attention of the pike as they began to move after freezing were quickly snapped up and eaten.

A fish which has shown a fixation response to a predator may subsequently ignore it or move away but it may also move towards it. An *approach* is usually made in a series of short swims as in normal locomotion, the predator being fixated binocularly throughout. If the predator should swim away while being watched the fish may *follow*, either maintaining a fixed distance or closing-up.

By enabling the animal to make a closer survey of an unusual object in its environment, the approach increases the efficiency of the stickleback's exploitation of the environment, as long as it is still possible for the animal to escape if the object of attention proves to be dangerous. Curiosity, which this approach behaviour closely resembles, is a common form of behaviour among many species of animals and is the subject of a great deal of research (see Berlyne, 1960). After closer inspection the stickleback may lose interest in the predator and move elsewhere, or it may retreat in one of the ways already described.

This retreat may be merely to a greater distance from the stimulus, the fish resuming its previous behaviour, with perhaps a periodic fixation of the predator. After one of the responses described above, or straight after fixation a fish often goes directly to cover such as weed. It may remain out of sight for some time before eventually peering out cautiously, or it may merely hover alongside cover but still in full view. *Pygosteus* will bury only its head in weed, leaving the body outside, leading Morris (1958) to accuse it of being more ostrich-like than the ostrich. It is striking to see *Pygosteus*, in full view of an actively hunting pike, frozen and in contact with weed.

At other times a frightened fish may not go to the weed at all, but move about the tank, usually just below the surface, keeping away from the predator but keeping it fixated, often binocularly. This behaviour is called "floating" by Morris (1958),

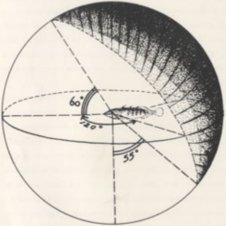


Figure 4.4

The visual field of *Gasterosteus*

Mapped by N. Tinbergen and W. Kloos by presenting single *Daphnia* to a Three-spined Stickleback. Taken from Phillips, 1962.

who lists it among the agonistic responses of *Pygosteus*, being characteristic of subordinate individuals. He does not mention the head-down orientation which accompanies it in interspecific encounters.

The visual field of *Gasterosteus* was mapped out approximately by N. Tinbergen and the late W. Kloos and is indicated in Fig. 4.4 (taken directly from Phillips (1962)). When it maintains the head-down "floating" posture the stickleback reduces the chance that a fish predator can approach unseen, but is practically unprotected against aerial predation, unless it uses weed as cover. The prevalence of this response, shown in the open during tests, was probably an artefact due to the restricted chances of escape.

Interspersed with all the other reactions of sticklebacks to stimuli present in their tanks are *yawning* and *stretching* movements. They occur (i) occasionally during normal behaviour when no disturbance is detected (being described as comfort movements in this context),

(ii) when a fish is about to approach something which has recently been introduced into the tank,

and (iii) when it pauses after each short swim in this approach, to a predator. Yawning precedes the approach and either yawns or stretching occur once the fish has begun to move.

These movements have been described in similar circumstances by Tugendhat (1960a,b) and by Beukema (pers. comm.) during the feeding behaviour of *Gasterosteus*. In my experiments these reactions only occured when the predator was introduced into the same tank as the test fish, but separated by a glass screen, so that the stickleback made the first approach, and on every such occasion escape reactions followed this approach. Tugendhat (loc. cit.) has demonstrated the link between yawning and stretching and the conflict between approaching and avoiding an object just before the approach was begun, and this seems to apply to my observations of this behaviour.

Besides the reactions involving the whole body and fins, alarm responses of the sticklebacks often involve the erection, partial or complete, of the dorsal and/or pelvic spines. The spine systems and their control were described earlier; sticklebacks use their spines in both inter and intra-specific encounters. In inter-specific encounters the dorsal spines will be raised first with increasing alarm, and when the reaction becomes more intense the pelvics are also raised (Morris 1958, Hall 1956).

In *Gasterosteus* the dorsal spine raising itself can be graded, rapid fluctuations in their raising and lowering preceding longer bouts of spine-raising. The extent to which the spines are raised also varies. Once a *Gasterosteus* has begun to raise its dorsal spines it will increase the duration and extent of this as it approaches the predator, and spine raising also increases during the test period with the predator: in these respects dorsal spine raising in *Gasterosteus* is scaled in exactly the same way as the escape responses, but it is carried out partly independently of those responses and it is convenient to treat it separately.

Pygosteus has shorter dorsal spines than *Gasterosteus* and raises them very readily, keeping them up continuously even at what seems from its behaviour to be a low level of alarm.

Both sticklebacks raise the pelvic spines when very near the predator, or at a very sudden movement by the predator. If the animal is side-on to the predator the pelvic spine towards it may be the only one raised, as described for intraspecific encounters by Morris and Hall. In these agonistic situations territorial *Gasterosteus* males may raise a single pelvic spine when threatening an adjacent male, or when engaged in a fight which has taken him over the edge of his own territory.

Spine-raising in intraspecific agonistic situations differs in some respects from the interspecific responses. In *Pygosteus* raised dorsal spines alone characterises a subordinate fish, ventral spines alone a dominant (aggressive) fish, and both systems raised indicates an intermediate status (Morris, 1958). The spine-raising of *Gasterosteus* is described by van Iersel (1953, p.7) as the exact opposite of this, but Hall (1956) correlates the raising of both systems of spines with escape motivation. There is a partial secondary linkage in the male between spine raising and an activated sexual tendency (Hall loc. cit.).

The raising of single pelvic spines is an unusual feature, and the dorsal spines of *Pygosteus* act in concert (Morris, loc. cit.) and are either raised or lowered. The dorsal spines of *Gasterosteus* may be raised to varying extents up to fully extended (by muscular action, not locked into position), and the same is true of the pelvic spines of *Pygosteus* (Hall, loc. cit.).

The thresholds for the raising of spines differ, and whereas in *Gasterosteus* the dorsal spines will be raised before the pelvic spines, Hall (loc. cit.) gives the opposite order for *Pygosteus* in intraspecific encounters. During the present study with predators *Pygosteus* raised its dorsal spines before its pelvic spines.

Apart from these agonistic situations, sticklebacks commonly raise their dorsal spines during sudden stops when there is no reason to infer an increase in the state of alarm. This is easily seen when sticklebacks are feeding actively in midwater, catching prey such as *Daphnia* or falling *Tubifex*. Their dorsal spines flick up at every stop, as their fins are used in sudden braking. This spine raising is seen in fish which have habitually fed on moving midwater food such as *Artemia* nauplii, copepods or *Tubifex* held at the surface, so that it is unlikely that such prey is at all frightening. When *Tubifex* worms lie on or in the ground the fish take them in the normal way, but if they snatch up the food in haste the dorsal spines flick up during this stop. When animals which are not feeding show a continual raising and lowering of the dorsal spines - almost a flickering - as in jerky swimming, or during a hesitant approach to a predator, this can be attributed to the state of alarm in the stickleback rather than to the type of locomotion.

During her experiments Tugendhat (1960a,b) noticed that the dorsal spines were raised when the fish stopped abruptly, this spine-raising being most frequent in the feeding area and in the part of the tank nearby during experiments on the effect of electric shock on feeding responses. It was also higher in the thwarted feeding situation (Tugendhat, 1959), where a glass plate was put over the food. These conditions favoured the sudden stopping of initiated feeding movements. There is no description of the precise method of swimming in these circumstances, but I think that the spine-raising Tugendhat saw was most likely to be due to mild or increasing alarm, rather than merely a response to the sudden stop of a rather fast swimming spurt.

When a stickleback is grasped by a predator all the spines are locked into position (Hoogland, 1951; Hoogland et al., 1957; also Chapter 2), this being also the maximum extent to which they are ever raised. Apart from a brief locking during yawning and stretching this is the only time at which the spines are held up by mechanical rigidity rather than by direct muscular control. A close examination of frames from a film of pike catching sticklebacks did not show the spines locked before the moment of grasping, but it occurred within 1/16th of a second, the time difference between two frames.

During all these possible responses the colour pattern of the stickleback does not alter unless the background changes. It is entirely for camouflage (this refers only to non-breeding sticklebacks, the colour changes of the male vary according to circumstances). In this respect sticklebacks differ from the cichlids (Baerends and Baerends-van Roon, 1950) and Badis badis (Barlow, 1963) in which the colour changes can be correlated with the level of motivation of the animal, and lag some seconds behind any motivational changes. Other fish show colour changes with fright so that some darken, others become pale. (Odiorne 1957, in Brown 1957).

It will have become clear from the description of the responses above that many of them occur as a direct response to stalking and attack by predators. These actions, which include slow swimming (both smooth and jerky varieties, in horizontal or head-up position), jumps, fast swimming and freezing, will be grouped as escape responses, since they remove the animal from the immediate danger area. Another group, called precautionary responses*, keep the animal at some distance from the predator, and less likely to release its attack. These involve hiding in cover, either moving about there or freezing. Also in this category are the circumspect swimming movements by which the stickleback keeps at some distance from the predator. Slow smooth swimming is the most usual method of swimming when this response is given. The

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* The term precautionary is not very satisfactory but the word which might seem more suitable, avoidance, has already been used in several other contexts. Barlow (1961) uses "avoid" or "avoidance" to describe the fleeing behaviour of *Badis badis*, while he separates off hiding and the freeze reaction as "concealing". Morris (1958) uses the term "avoidance" to describe the behaviour shown by adjacent territory holders in *Pygosteus*, and he groups fleeing and hiding responses together, which agrees with my grouping of escape and precautionary responses as the fright responses. In his very elaborate study of the organistion of behaviour in *Pelmatochromis subocellatus kribensis* Heiligenberg (1963, 1964) separates fleeing and hiding rather widely in his multiple drive system, fleeing being incorporated in a centre for general activity.

Table 4.1

Responses given by sticklebacks to a predator and their functional interpretation

RESPONSES	FUNCTION
feeding behaviour stops	
monocular fixation binocular fixation approach	Investigatory responses
sinking backing-up slow swimming (smooth or jerky) fast swimming jump or jumps freeze	Escape responses
remaining at a distance from the predator hiding in cover	Precautionary responses

animal is defined as having taken fright when it first starts to show precautionary responses, so that no matter whether it has been stalked before, or has approached before, once it begins to hide, or avoid the predator, it has "taken fright". These two groups of responses clearly help to protect the fish against its enemies. The same is probably true, to some extent, of the investigatory responses, by informing the fish about potential dangers in its environment (fixation, monocular or binocular, and approach). Table 4.1 gives the responses in their functional groups.

All the descriptions of behaviour given up to this point have referred to the presence of a predator without reference to its nature. The behaviour described above can be seen in a more or less complete form when a stickleback encounters a pike, perch, or other fish predator in the tank, but it also occurs in response to a variety of other objects including large conspecifics, alarming stimuli outside the tank, unusual objects introduced into the tank, or even moving shadows and drifting weed fragments. Any strange objects, particulafly when large and moving, must be potential sources of danger for small fish, to be avoided or at least investigated, and for the sake of convenience any of the class of stimuli evoking this "predator syndrome" is called a predator, however innocuous it may in fact be.

Phillips (1962) described the behaviour of sticklebacks to moving bird-models above the tank, which evoked various degrees of alarm. He confined the fish in a glass-walled cell without cover, and under these conditions the main responses shown by *Gasterosteus* to the aerial "predators" were to sink, to swim rapidly, or to freeze ("skulking"), actions which I also observed to fish predators. *Pygosteus* was not used very extensively in Phillips' tests since it almost invariably showed the freeze response.

During tests which lasted for fifteen minutes, it was not unusual for a fish which took violent evasive action in the first minute still to be motionless at the end of that period. After the pike was removed these fish quite quickly resumed more active behaviour, first swimming slowly about the tank rather near the ground. The resumption of feeding is a sign that fright is subsiding (Tugendhat, 1960a,b) and this happened within half an hour of removing the pike. With a gradual spreading of the fish higher and higher in the water a more normal distribution is reached but some effect may persist for days and the fish take fright more easily at minor stimuli to which similar fish which have not lived with a pike do not respond. Fish which had twelve hours experience of the pike did not move freely in their own tank for up to two days afterwards.

4.2 Causal organisation of the responses

During encounters with actual or potential predators sticklebacks often showed only some of the reactions listed above. Their reactions depended partly upon their distance form the predator, and also upon its behaviour. Violent movements by the predator provoke fast swimming to cover, less violent movements elicits slow smooth or even normal swimming away. With very slight or gently rhythmic movements (such as fin movements in a hovering or stalking pike) a temporary fixation often occurs, and the stickleback may approach the predator.

Similarly any particular movement by the predator, such as a flick of the tail, can provoke fast swimming if the stickleback is very close, but smooth swimming or only fixation as the distance is greater. In this way it is possible to grade many of the responses on a scale of alarm or fright. Such a scale is represented by the series:

sinking
monocular fixation
binocular fixation
backing-up
smooth swimming away
jerky swimming away
jumping away and fast swimming

Within some of these categories of behaviour a further grading is possible according to the same criteria. The hearer a fish is to a predator the longer and more numerous its jumps will be, and the longer the spurts of fast swimming will be. When a fish approaches the predator, the closer the predator, the slower the approach. The duration of binocular fixation before backing-up is longer if the predator's movements are barely perceptible, and it varies with the distance from the predator.

The end point for this scale, at least for fish in aquaria, can be taken as freezing, although this reaction does not occur at a distance in response to a violent movement by a predator. Freezing occurs when the element of surprise is very high, as when

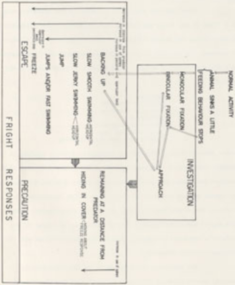


FIG.4.5 SCHEMATIC RELATIONSHIP BETWEEN RESPONSES

a fish is stalked and appears suddenly to become aware of the predator when it is at close range, or when they meet coming around the same dense block of weed without an earlier encounter during the test. As described before, fish which have been hotly pursued also show this reaction in the open (mainly *Pygosteus*) or when they have dived into weed.

These responses have been grouped on functional grounds as escape responses (Table 4.1). Those which have been called precautionary, and are maintained for some time, can also be scaled relative to each other. For instance, the more extreme the alarm the more likely it is that the fish will hide in the weed rather than remain beside it; and, once in weed, the more likely it is to become motionless in the freeze reaction. The length of time before the fish peeps out of the weed again also increases with greater alarm.

Together with the Investigatory Responses the Escape and Precautionary responses are given in Figure 4.5. The arrows are given to indicate likely sequences of events, but it is not to be assumed that all responses are shown before the animal takes Fright. Interspecific differences have been mentioned where they occurred but they should be summarised here.

(i) *Pygosteus* is less likely to approach a predator than *Gasterosteus*.

(ii) It differs from *Gasterosteus* in having a lower threshold for raising the dorsal spines, and has no gradation in the height to which they may be raised.

(iii) It has a special thigmotactic response, so that it more often touches weed, the wall, or the ground - *Gasterosteus* seldom rests directly on the ground.

(iv) The head-up posture is much more readily shown by *Pygosteus*, and it has the tail-down component which *Gasterosteus* does not show.

(v) When Frightened *Pygosteus* will creep up a corner of the aquarium or up the weed in a vertical (or near-vertical) head-up position, and it may freeze in this position in weed after pursuit. I have never seen *Gasterosteus* assume this posture.

(vi) When looking out of weed after taking fright in order to locate possible predators, both species look out below a weed mass or strand, in the head-down posture, but the angle of inclination is often much steeper in *Pygosteus* than in *Gasterosteus*, so that the visual field scanned is a maximum with respect to Figure 4.4.

From the relative frequency and form of the responses described, and using the criteria by which they were themselves scaled in terms of increasing fright, Morris' (1958) suggestion that *Pygosteus* is more easily frightened can be substantiated.

Chapter 5

EXPLORATION OF A STRANGE ENVIRONMENT

5.1 Introduction

When introduced into new surroundings a stickleback behaves in a characteristic manner. At any transfer to a new tank - or even at a change in the home tank - the fish remain low in the water, moving very little to begin with, but gradually moving more freely until finally their behaviour appears to be undisturbed. When weed is present in these tanks the fish tend to go to it, though *Pygosteus* is more likely to enter weed than is *Gasterosteus*.

Table 5.1

Mean time for fish to move from open to weed cover.

Gasterosteus	126"	$z = 4.59 \quad p < 0.00003$
Pygosteus	40"	

It also moves more quickly (Table 5.1) and shows less distinction between different types of weed cover than does *Gasterosteus*. A novel situation must be for a small animal a potentially dangerous one, and there are similarities between a stickleback's behaviour after encountering a predator and its behaviour in a strange tank, even when no predator is present. For this reason the way in which a stickleback would explore a strange tank was investigated more systematically.

5.2 Method

A tank 165 x 33 cm. filled to a depth of 20 cm., was marked into three longitudinal "rows" of 11 cm. width by two glass rods, and into transverse "sections" each 16 cm. long, by vertical

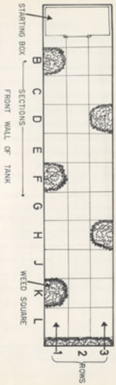


FIG. 5.1. PLAN OF TANK USED IN EXPLORATION EXPERIMENT.

THE STARTING BOX AND TERMINAL HEDGE WERE EXCHANGED FOR HALF THE TESTS AND THE LETTERING WAS REVERSED ACCORDINGLY.

markings at the front and back walls of the tank (Fig. 5.1). Each section was designated by a letter of the alphabet (excluding I), and in the alternating sections B. D. F. H. K. a patch of weed (Callitriche) extending from the ground to the surface, was planted in one "square" (11 x 14 cm), the B. F. and K. squares being at the front wall of the tank, D. and H. at the back. The end wall at M. was planted with the same weed, this being the only section which had weed from front to back.

The whole tank was illuminated by a 233 cm. fluorescent lamp set at 36 cm. directly above the middle row, so that all areas were equally lit. For half the tests the position of the starting box and the lettering were reversed.

As usual, all the fish to be tested were non-reproductive, about half being taken from local streams, the other half having been reared in the laboratory. At the start of the experiment the individual fish to be tested was placed in a metal box (30 x 16 x 30 cm.) at the A end of the tank, this box being painted to match the sandy substrate, and without any weed or corner shadows. After two minutes the 10 x 18 cm. door of this box, giving on to the middle row of the tank at ground level, was raised by means of a pulley and observations were made from a darkened hide for fifteen minutes from the time at which the fish left the starting box. If a fish had not emerged from the starting box in five minutes it was discarded.

The time to the nearest second at which the fish entered and left each square of all sections, the distance it travelled in one burst of swimming, and its general behaviour, including the depth at which it swam and the number of feeding movement initiated, were recorded. When the passage across a square was very rapid no time was allotted to it. This makes all mean values for times slightly low, but I think it would give a false impression of accuracy to tie values to such rapid movements.

The end of a test was recorded when a fish had traversed the full 135 cm. of the tank and reached section L. for the first time, or after 15 minutes, whichever was the shorter. Initially it was intended to compare the behaviour of single fish with fish exploring in groups, but this turned out to be difficult, so that the observations were made on one fish at a time. A proportion of the fish put into the starting box did not emerge, or emerged but hid in a patch of weed throughout the period of the test. This proportion was much higher in *Pygosteus* than in *Gasterosteus*, so that, of 60 *Gasterosteus* tested, 40 completed the run, but 92 *Pygosteus* had to be tested to get 36 complete runs.

put in results.

5.3 *Results*

Almost all the *Pygosteus* entered the tank from the starting box by a rapid jump along the ground, and most of their movements in the tank were at ground level or just above it. *Gasterosteus* swam slowly to the doorway, looked out for some time, then swam out slowly or normally. Only a few individuals jumped to the weed. Another difference between the species lay in the depth at which they moved about. *Pygosteus* moved at ground level and just above it, the

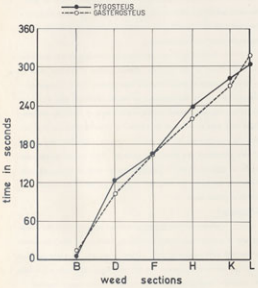


FIG. 5.2. Mean time of arrival for first time in each section.

highest any individual went to was half-depth. *Gasterosteus* moved at between half and quarter-depth of the water above the ground, but five fish moved about high in the water once they had entered the tank. When they entered new regions of the tank they went lower in the water temporarily. This slight sinking with alarm has already been mentioned (Chapter 4).

During the test a fish often temporarily swam back into one or more of the sections it had already passed through. These were called re-entries of sections and should be distinguished from first entries. For the completed runs only the times of first entries into each section is given as mean and median values for both species in Table 5.2, the mean values being graphed in Figure 5.2.

Table 5.2

Times of first entering sections with weed, i.e. alternate sections

		B.	D.	F.	H.	K.	L.
Gasterosteus	Mean	12"	101"	163"	221"	270"	314"
	Median	5"	50"	85"	153"	222"	235"
Pygosteus	Mean	4"	123"	164"	239"	282"	303"
	Median	2"	55"	113"	175"	209"	212"

Neither the mean nor the median values for these times differ between the two species, but both are given because the distributions were skewed. As may be seen from Figure 5.2, both species move down the tank at the same average rate. (There were no differences between the wild and the laboratory-reared fish.)

Table 5.3

USE OF WEED

(i) First entries to weed sections

		B.	D.	F.	H.	K.
Gasterosteus	No. of entries of weed squares	11	4	8	5	4
	No. of entries of non-weed squares	29	36	32	35	36
	% entering weed	38%	10%	20%	13%	10%
Pygosteus	No. of entries of weed squares	24	13	21	11	12
	No. of entries of non-weed squares	12	23	15	25	14
	* % entering weed	68%	36%	58%	31%	33%

(ii) Duration of first visits to weed squares

Mean values	B.	D.	F.	H.	K.
Gasterosteus	**75"	17"	16"	17"	13"
Pygosteus	**104"	34"	55"	33"	9"

** z = 2.98, p<0.001

Median values					
Gasterosteus	65"	12"	17"	10"	14"
Pygosteus	70"	30"	23"	20"	11"

(iii) Duration of first visits to non-weed squares

Mean values	B.	D.	F.	H.	K.
Gasterosteus	***14"	16"	19"	13"	6"
Pygosteus	***3"	9"	****19"	9"	2"

*** z = 7.0 p<0.00003

**** But for a single stay of 3' 30" the mean value would be 4".

Median values	B.	D.	F.	H.	K.
Gasterosteus	10"	10"	11"	10"	5"
Pygosteus	0"	7"	5"	3"	0"

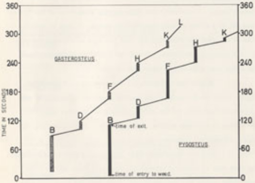
* It must be made clear that Table 5.3 deals with all those first entries to sections which were made into the weed square contrasting them with the entries to non-weed (or open) squares. A fish entering a section at one of these two open squares and later entering the weed square there is not scored as having a first entry to that weed square.

Their relative use of weed is shown in Table 5.3. The species showed a difference in the time spent in the weed. If each fish entered the weed or open squares at random the expected figure for the use of weed squares would be 33%. Instead Table 5.3 shows that the species differ greatly in the number of entries to weed; *Gasterosteus* tends to avoid it after the first section and *Pygosteus* is attracted to weed, especially at the start. It should be added that *Gasterosteus* may really have been less aversive to weed than these figures imply. It appeared that *Gasterosteus* behaved as if the "cover" provided by a clump of weed included its shadow, so that it extended 5 to 10 cm. from the clump itself, that is, into an adjacent square, but for *Pygosteus* the animal was only in "cover" when touching weed, and often even when it was surrounded by it.

In part (ii) of Table 5.3 the duration of these first visits to the weed square of the section is given. This can be compared with Part (iii) of the Table, which gives the same data for the visits to the open squares of the section. Again these are different between the species.

Thus the Table shows that *Pygosteus* is more likely to visit the weed when it enters a section for the first time, and it will stay there longer than *Gasterosteus*. If it does not enter the weed however, it swims through the section more quickly. Comparison of parts (ii) and (iii) in the Table shows that, except for section B *Gasterosteus* spends about as long in a square with weed as it does in the open squares, while *Pygosteus* dashes quickly through open squares

FIG. 5.3. Mean time of first entering weed section and mean duration of first visit.



and spends much longer in weed. In other words, it moves in spurts from weed patch to weed patch, but Gasterosteus explores the tank more smoothly. This is also shown in Figure 5.3, which plots the mean time from the start of the test that alternate sections of the tank were first entered, and the mean duration of this first entry (also given in Table 5.3). This does not take into account whether the time in the section was spent in the weed or not.

Another difference between the species was in the incidence of rapid crossings of a section at the first visit. The duration of these was defined as less than 5 seconds, the percentage of such first trips being given in Table 5.4.

Table 5.4

Percentage of fish making a rapid passage through weed sections of tank at first entry to each section. (Less than 5 seconds.)

	B.	D.	F.	H.	K.
Gasterosteus	0	8%	5%	10%	28%
Pygosteus	31%	19%	19%	33%	64%

These trips are rare for Gasterosteus and tend to occur later in the exploration, so that by the time section K. is reached a maximum of 28% is travelling quickly. Pygosteus has a high initial rate as the animals dive rapidly out of the starting box to a weed square, and in addition the incidence of rapid trips increases towards the end of the run, with 64% dashing across section K. In spite of this preponderance of rapid trips the mean duration of stays in weed is much higher for Pygosteus, though it may dive across one section

Table 5.5

DURATION OF VISITS TO SECTIONS

(i) First visits

Mean values	B.	D.	F.	H.	K.
Gasterosteus	30"	16"	19"	13"	8"
Pygosteus	105"	19"	38"	19"	4"

Median values	B.	D.	F.	H.	K.
Gasterosteus	15"	10"	15"	10"	5"
Pygosteus	15"	10"	10"	5"	0"

Medians of stays of 5" and over

Gasterosteus	15"	10"	15"	10"	5"
Pygosteus	65"	12"	20"	11"	10"

(ii) All visits

Mean values	B.	D.	F.	H.	K.
Gasterosteus	30"	16"	13"	11"	7"
Pygosteus	65"	16"	35"	17"	4"

Median values	B.	D.	F.	H.	K.
Gasterosteus	15"	10"	10"	8"	5"
Pygosteus	30"	5"	5"	5"	1"

Medians of stays of 5" and over

Gasterosteus	25"	15"	10"	10"	5"
Pygosteus	108"	10"	15"	11"	10"

Table 5.6

ALL ENTRIES TO SECTIONS CONTAINING WEED

(i) Entries to weed squares

Section	B.	D.	F.	H.	K.
Entries of weed square	36	22	18	14	7
Gasterosteus					
Entries of non-weed squares	87	89	73	57	44
% of entries to weed	29%	20%	20%	20%	14%
Entries of weed squares	40	25	30	20	8
Pygosteus					
Entries to non-weed squares	27	48	33	33	30
% of entries to weed	60%	34%	48%	38%	21%

(ii) Duration of all visits to weed squares

Mean values

Section	B.	D.	F.	H.	K.
Gasterosteus	44"	24"	18"	16"	7"
Pygosteus	105"	37"	65"	25"	12"

Median values

Section	B.	D.	F.	H.	K.
Gasterosteus	27"	20"	15"	14"	3"
Pygosteus	85"	25"	23"	18"	15"

(iii) Duration of all visits to open squares

Mean values

Section	B.	D.	F.	H.	K.
Gasterosteus	25"	10"	12"	10"	8"
Pygosteus	7"	5"	3"	5"	3"

Median values

Section	B.	D.	F.	H.	K.
Gasterosteus	15"	7"	7"	5"	5"
Pygosteus	2"	5"	0"	3"	0"

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to go to the weed square in the next. At the start of the test *Gasterosteus* individuals surveyed the tank from the doorway before entering section B. more sedately, and the whole pattern of exploration is more relaxed.

In the same way as the results have been treated for *first* visits to sections data for *all* visits to sections - return trips and visits to other squares in the same section involving a transfer between the weed and the open - are given in Table 5.5. In both cases the large number of quick trips made by *Pygosteus* shortens the median values, since, compared with *Gasterosteus*, rapid trips across squares are much more numerous. Nevertheless the medians for section B. in Table 5.5 (ii) are significantly different (U test, $z = 2.28$, $p < 0.01$). If the values below 5 seconds are excluded the medians for both species are similar to the means. Comparing the duration of first visits with that of all visits it is seen that the only difference is in the time spent in section B. by *Pygosteus*, the first visit being almost twice as long as the average value.

So far no mention has been made of the data on the return trips made during the exploration, to be called back-tracking, except when considering the duration of all visits in the last Table. In the same way as Table 5.3 was drawn up for first visits to sections Table 5.6 has been drawn up for all visits. The only real difference between Tables 5.3 and 5.6 is the relative shortening of visits to the weed square of section B, and the shortening of visits to the open squares, except in the first section, by both species.

Table 5.7

TOTAL NUMBER OF VISITS TO SECTIONS INCLUDING RETURN TRIPS

Gasterosteus

Section	B.	D.	F.	H.	K.
Weed	36	22	18	14	7
Non-weed	87	92	73	57	44
Total	123	114	95	71	51
%	29%	19%	20%	20%	14%
Mean no.	3.08	2.85	2.38	1.78	1.28

Pygosteus

section	B.	D.	F.	H.	K.
Weed	40	25	30	20	8
Non-weed	27	48	33	36	30
Total	67	73	63	56	38
%	60%	34%	48%	36%	21%
Corrected Mean no.	1.81	1.97	1.70	1.51	1.03

	B.	D.	F.	H.	K.
Difference	1.27	0.88	0.68	0.27	0.25
% of *Pygosteus* score	70%	45%	40%	18%	24%

In Table 5.7 the total number of visits to all weed sections is given. The bottom row of figures compares for each species the mean number of trips to each section, the totals for *Pygosteus* having been brought up from their actual value for 36 individuals to a total for 40 individuals, so that a direct comparison can be made with *Gasterosteus*. The Table shows that, as would be expected from the fact that the fish explored the tank from one end, they were more likely to re-enter sections near the starting box than those at the other end of the tank, as the test was stopped as soon as the far end was reached for the first time. It also shows that there was a marked difference in the number of re-entries by the two species, with *Gasterosteus* making considerably more return trips. It is likely that if a longer record had been kept the difference between the species would have been even greater as every *Pygosteus* which completed the run hid in the line of weed at N., peeping out part of the time, indeed, many seemed to dive for the "hedge" from up to 30 cm. away. *Gasterosteus* never entered the "hedge" and immediately after reaching section L. individuals often went back down the tank, usually in the middle row, which was often used in their exploration. *Pygosteus* individuals crossed the middle row between weed squares, but only a few explored along it, and judging by their more relaxed behaviour and short time to complete the test, they were bold individuals.

As twenty *Gasterosteus* and fifty-six *Pygosteus* did not complete the course their behaviour should be mentioned. The first and final weed squares entered by these fish are given in Table 5.8.

Table 5.8

First and final weed squares entered by fish which did not finish

First resting place	B.	D.	F.	H.	K.	
Gasterosteus	18	2				
Pygosteus	40	14	2			
Final resting place						
Gasterosteus	9	8	-	3		33% not finished
Pygosteus	25	15	14	2		61% not finished

These figures show that the *Gasterosteus* individuals began as normally as the ones which finished, but scarcely moved away during the test, and the *Pygosteus* showed a slightly greater spread down the tank after a similar start. For both groups about half the fish remained where they first went to cover and about a fifth moved about without finishing, so that the only significant difference between the species, with respect to the fish which did not finish the test, is in the significantly higher proportion of *Pygosteus* which failed to finish ($p < 0.01$).

5.4 Discussion

This experiment has shown that while the overall rate of exploration was the same for both species the method was different. *Pygosteus* imidividuals went into weed more often than *Gasterosteus* did, and stayed there longer. They also made more rapid trips across sections, often even through weed, and doubled back on their tracks less. Although *Gasterosteus* enters the weed squares it uses the cover of the shadow cast by the weed rather than pressing against it and wriggling into it as *Pygosteus* does.

Gasterosteus explored freely and smoothly, paying no special attention to any part of the tank apart from scanning it carefully, *Pygosteus* sneaked from one weed area to another, with a final leap into the terminal line of weed. In addition there is the difference, already mentioned, in the depth at which the two species explored the tank. Among the fish which did not complete the test many *Pygosteus* spent the time actively crawling into and through their weed mass without ever scanning the tank, whereas the *Gasterosteus* which did not finish spent most of their time beside the weed masses rather than in them. They mostly moved in and out at the base of their weed, occasionally looking out at the tank or the weed nearest to them, then going back to the base of their own weed mass. A few *Gasterosteus* explored freely but did not go to section L.

With all these differences in the exploring methods it is all the more surprising that the two species should have explored at the same rate, and that the rate should have been approximately constant. It is concluded that each new section of the tank represents an only slightly decreased risk down the tank, since the uneventfulness of the preceding investigations does not affect the rate of spread, but only the time spent in the weed. The back-tracking can be taken as a measure of the relative security of the sections already explored. Gasterosteus is less frightened in this respect, since it makes more return trips.

In the last Chapter the fright behaviour of sticklebacks in the presence of a predator was described and the behaviour of the fish used in the present experiment can be compared. A few Gasterosteus showed the relaxed opercular rate and pectoral bent, but most fish - and all the Pygosteus - showed the flicker rate, at least when moving into the open. For Gasterosteus this response waned with time. When a fish examines its new environment visually it uses the binocular fixation position, but when it moves afterwards it swims off forwards. During the experiment no fish was seen to back up. When moving the fish swam normally, and during exploration both fast and slow swimming were seen, but no jerky slow swimming or jumps were seen. Some dorsal spine-raising was seen, especially in Pygosteus, which also used the head-up posture and swam very close to the ground. Such alarm responses as spine-raising could be seen to wane with time and the further investigation of the tank, so that it occurred less in areas explored before. This supports the view that the investigation of initial sections of the tank represents more risk to the animal than the later sections. Feeding movements were occasionally seen (Gasterosteus only), but they never occurred at the beginning of a test, and once they were initiated several might follow, the animal moving about busily over the substrate, nipping at tiny pieces of loose vegetation. By their other behaviour these Gasterosteus showed themselves to be relatively little afraid. Pygosteus was never seen to eat anything in the open, though several fish which moved actively in a weed mass nipped occasionally at the weed.

The characteristic behaviour seen in this experiment is indicative of mild to substantial alarm, the responses of *Pygosteus* being the more severe, as evidenced also by the large number not moving from the weed in which they first took refuge. *Gasterosteus* explored the tank, *Pygosteus* dashed through it.

5.5 Explorations by more than one fish

It had been planned to repeat this experiment with pairs and larger groups of fish. In the pilot experiment on pairs 26 sets of *Gasterosteus* were tried in 30-minute tests. Thirteen sets failed to emerge, three pairs emerged and two of them completed the run, two single fish emerged and completed the run and six pairs emerged as two independent fish and explored separately, three of them finishing. When twenty pairs of *Pygosteus* were tested the results were even more disappointing. Out of thirteen assorted runs, two singles and one pair moving independently finished the run, ten ended at section 3. Despite these discouraging results this experiment was repeated with sixteen pairs of *Gasterosteus*, fourteen pairs of *Pygosteus*.

The nine pairs of *Gasterosteus* which completed the course took much longer than the single specimens of the main experiment, but on such small numbers the pairs which wandered busily and extensively had an enormous effect on the mean score. The mean and median times of first entering weed sections are given in Table 5.9. (Compare with Table 5.2).

Table 5.9

Exploration rates of pairs of *Gasterosteus*

SECTION	B.	D.	F.	H.	K.	L.
Mean	7"	141"	218"	384"	501"	559"
Median	5"	70"	165"	183"	320"	385"

The mean time of completion is not significantly different from that for all the *Gasterosteus* singles used in the experiment described before, but this may well be because so few pairs completed the course. Within this experiment the ground covered during the test by pairs is greater than that for the five single fish which completed (that is, they entered more squares).

Half the *Pygosteus* pairs stayed at the B. weed square, moving to and fro actively through the weed, often touching each other. Two pairs finished the course - one pair went from weed square to weed square down one side of the tank, in fifteen minutes, the other pair visited all the weed squares in 5' 30".

On the strength of these meagre results I came to the conclusion that the presence of a companion did not accelerate the rate of exploration, but rather decreased the chance of the run being completed. Those pairs of *Gasterosteus* which did complete the test moved about freely and actively, eating tiny fragments of vegetation from the ground, and they did not seem to be concerned with reaching the far end at any stage. One might interpret the result by saying that the single fish explored the tank in search of a companion. Once a fish is in company it ceases to explore.

Chapter 6

Effect of experience on reaction to predators

6.1 INTRODUCTION

Among the factors which appeared to affect the reaction of sticklebacks to predators such as a pike, it seemed that the previous experience of predators might be important, and an experiment was designed to test this. Preliminary observations had also suggested that the normal young stickleback's experience of being chased by its father, snapped up and spat into the nest, might have some of the characters of predation for the young. To examine the possible effect of the father on the fright responses to pike, some broods of sticklebacks were reared without fathers from the time of hatching, while another large brood was "overfathered" - left an unusually long time with the father. Besides the parental effect there was the possibility of a change of response to pike with age, and this too was tested. All these experimental broods - orphans, overfathered, and normals - were tested at one of two ages (8 and 13 weeks) and each group to be tested was divided into two sub-groups, one of which received experience of the pike, and one of which did not.

To simplify the presentation and analysis of the results the effects of experience of the pike will first be considered, with the effects of age included as a separate section, and later the effect of parental experience will be considered.

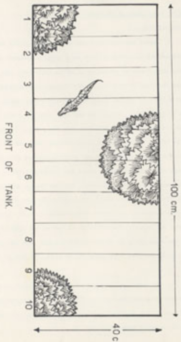

FIG. 6.1. PLAN OF TESTING TANK.

6.2 METHOD

The fish used were broods of Gasterosteus and Pygosteus raised in the laboratory and tested in their first three months of life, when they were between 2.5 and 4 cm. long. At this age they do not become sexually mature. The pike used was about 12 cm. long and grew up to 20 cm., but at all stages it was capable of eating these sticklebacks. When testing of sticklebacks was about to commence they were transferred to holding tanks in the same room as the testing tank. After they had settled down here they were netted out into a paraffin lined 50 ml. beaker, and then poured gently into the weed in a corner of the testing tank. This proved to be the best way of transferring fish since it lessened the rapid dashing from wall to wall which otherwise often occurs when a fish is suddenly transferred to a strange tank without any special care. It was especially important that such rapid and uncontrolled movements should not occur in the present experiment.

Observations were carried out in a tank illuminated from above by two 40 watt electric bulbs. The plan of the tank is given in Figure 6.1. The weed masses were dense clumps of Callitriche, the central mass being set in the gravel substrate. The two end masses extended from the surface to between 5 and 8 cm. from the ground. The depth of the water was 20 cm. A screen with gauze covered slits stood about 40 cm. from the front of the tank during each test, through which the observer watched, for the most part without the fish paying him any attention.

The normal broods of fish were all raised with the father until they became independent of him, at about ten days old, at which time he was removed. Each experiment involved fish from a single brood. Before the individual tests began, the group to be tested was halved: one half was put into the observation tank, which contained the pike, to give them predator experience. To ensure that a minimum of these fish would be eaten, the pike was given several minnows or despined sticklebacks immediately beforehand, as under these circumstances it will still stalk fish but seldom attacks them. The other half of the group was put in a second tank, of the same dimensions and with the same disposition of weed, so that under testing conditions any differences between the two sub-groups could be attributed to the difference in their experience of the pike. The fish were left in the "pike experience" tank, or the control tank, for twelve hours, of which the first two hours were in light, the next eight in darkness. All the sticklebacks were then netted out and placed in identical holding tanks, 42.5 x 31 x 31 cm., with some weed (Callitriche) for cover, and illuminated from above so that the light was equivalent to that in the testing tank. The fish were left for a settling down period of two or three days before testing was begun in the observation tank. The pike was,as far as possible, satiated with minnows for the duration of the tests.

Individual sticklebacks were tested singly by being introduced into the tank with the pike. They were poured into the weed at one of the front corners from a 50 ml. beaker. Each test lasted for fifteen minutes from the introduction of the test fish, and this introduction was always delayed until the pike faced another direction, so that the prey fish could settle itself a little before it encountered the pike. The testing was carried out with the pike-experienced fish alternating with inexperienced ones. If a fish was caught testing was suspended for some time, as the pike always experienced some difficulty in swallowing normal sticklebacks, and subsequently tended to ignore them (Hoogland et al., 1957). Tests in which the pike remained still behind vegetation throughout, or fluttered against a wall for long periods, were discarded.

A record of the behaviour of both fish was kept, their positions being referred to the 10 cm. grid marked on the front wall of the tank.

6.3 RESULTS

6.31 Definitions

Before considering the behaviour observed in both sub-groups of test animals several terms or categories to be used require some explanation.

The number of fish tested includes fish which remained hidden in the weed into which they were introduced, but excludes fish which moved in areas out of sight of the pike, as these clearly had no opportunity to react to the pike.

The term encounter is used to denote any occasion on which the stickleback was seen to respond to the pike's presence. As already described, this response may merely involve a cessation of activity as the stickleback looks at the pike. Such a sudden arrest of movement is quickly noticed by the observer, and it may continue for up to 30 seconds or more before any further action is observed. An encounter is taken to have ended when the fish stops fixating, e.g. when the predator moves out of sight.

The behaviour following the arrest of movement used to define an encounter has been termed a *reaction*, or a *response*. The description of these reactions is given in Chapter 4. Several encounters, with their following reactions, may occur, but sooner or later the fish went to cover, or consistently avoided any closer contact with the pike, by stopping all movement when the pike was near, or by moving to the opposite end of the tank, and it was then classed as having *taken fright*. Taking fright usually occurs after an encounter with the pike, and in the experiments to be described it is convenient to distinguish the response of fish accordingly as they took fright at their first encounter, or after further encounters.

A fish is said to be *taken* when it is grasped, irrespective of whether it is later swallowed. All fish taken were eaten, with the exception of two *Gasterosteus*.

During a test the pike sometimes stalked the stickleback, but once a stickleback had made an escape response it seldom allowed itself to be stalked a second time. Thus the number of sticklebacks which were stalked gives a measure of their lack of alertness. The numbers of fish taken are included in the Tables of responses given by test fish since these are obviously a measure of the effectiveness

Table 6.1

Results of experiment on the effect of prior experience of the pike

Gasterosteus (Reared in the lab. under normal conditions)

	Experienced		Inexperienced		Signi-ficance levels
	Number	%	Number	%	
Total tested (4 broods)	80		86		
Taken	5	6%	14	16%	not sig.
Took fright at first encounter with pike	67	84%	29	34%	$\chi^2 = 40.51$ $p < 0.0001$
Mean distance at which first encounter was made	39 cm		19 cm		$s = 6.074$ $p < 0.0001$
Total which went to weed at taking fright	57	71%	39	45%	$\chi^2 = 10.49$ $p < 0.01$
Number which approached pike	10	13%	27	32%	$\chi^2 = 7.61$ $p_2 < 0.01$
Stalked by pike	31	39%	56	65%	$\chi^2 = 10.52$ $p < 0.01$

Note: fish tested at different ages are combined in this Table

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of the experimental treatments.

6.3.2. RESULTS: *Gasterosteus*

6.3.2.1. *Gasterosteus* normally reared.

From an examination of Table 6.1, which gives the results for the normally reared *Gasterosteus* tested, it is seen that more experienced fish take fright at their first encounter with the pike during the test period, and that they do so at a very significantly greater distance. Twice as many pike-experienced fish used the weed as a hiding place after taking fright.

The fish which had more than one encounter before they took fright made their first encounters at the same average distance from the pike as did the rest of their group (43 cm., 21 cm. for experienced and inexperienced fish respectively), in other words, the fish which took fright at the first encounter did not do so because they were closer to the predator than the fish which took fright only at later encounters.

More than twice as many pike-inexperienced as experienced fish actually approached the pike at some stage after their first encounter, and in both cases the average distance of their first encounters matched the other members of their respective groups (37 cm., 18 cm.), so that once again the difference was not due to a difference in the nature of these first encounters. However they approached to different average distances from the pike, the inexperienced fish going up to even 3 cm. (mean value 7 cm.) from the pike (compared with a mean of 17 cm. for the experienced fish).

Table 6.2

Reactions given by fish at their first encounter with pike during individual test

Gasterosteus (normally reared)

	Experienced		Inexperienced		Significance levels
	Number	%	Number	%	
Total tested	80		86		
Precautionary					Precautionary behaviour $\chi^2 = 23.14$ $p < 0.001$
Stayed in weed	5	6% 30%	0	6%	
Moved into weed at sight of pike	25	31%	5	6%	$\chi^2 = 16.43$ $p < 0.001$
Fleeing					
Crept to cover or swam off slowly	21	26%	14	16%	not sig.
					combined not sig.
Jumped and/or swam off fast	21	26%	31	36%	not sig.
Swam off "normally"	2	3%	3	4%	
Investigation					
Stood and watched	2	3%	3	4%	combined $\chi^2 = 3.89$, $p < 0.05$
Approached pike	107	9%	19	22%	not sig.
Taken	7	9%	17	20%	

These are the 1st reactions & apparently do not tally with 6.1 but the weed category refers to reaction subsequent to that defined as (...).

Have I defined creeping?

The pike's behaviour must be mentioned briefly, as he stalked more of the inexperienced fish. It is possible that this was because the inexperienced fish moved about more freely in the testing tank before they encountered the pike. Experienced fish often scanned the tank from below the weed, or moved slowly about, close to the bottom. In addition, a higher proportion of inexperienced fish allowed themselves to be stalked by the pike after they had once become aware of him in the tank.

As many more pike-experienced fish reacted by taking fright at their first encounter with the pike the actual reactions at this first encounter were tabulated for the two sub-groups (Table 6.2). The Table shows that while 30% of the experienced fish either did not emerge or moved into weed to hide as soon as they encountered the pike, only 6% of the inexperienced fish responded similarly ($p < 0.001$).

Table 6.3

Mean distances at which some responses were given at first encounters during pike-experience test

Gasterosteus normally reared

	Experienced	Inexperienced	
Moved into weed at sight of pike	57 cm.	24 cm.	$z = 2.24$, $p < 0.01$
Crept to cover or swam off slowly	35 cm.	24 cm.	$z = 1.68$, $p < 0.05$
Jumped and/or swam off quickly	16 cm.	14 cm.	
Approached pike	37 cm.	18 cm.	$z = 2.05$, $p < 0.02$

Table 6.4

GASTEROSTEUS

A. First reactions when pike was not moving at time of first encounter (Stalking included for comparison with approach figures.)

	Experienced		Inexperienced		
	Number	%	Number	%	
Number reacting to non-moving pike	25	31%	15	17%	not sig.
Approach made by stickleback	5	20%	7	46%	$\chi^2 = 2.03$ not sig.
Stalked by pike	2	8%	10	67%	$\chi^2 = 12.7$ $p < 0.001$

B. First reactions when pike was moving at time of first encounter

	Experienced		Inexperienced		
	Number	%	Number	%	
Number reacting to ~~non~~-moving pike	55	69%	71	83%	$\chi^2 = 3.6$ not sig.
Approach made by stickleback	7	13%	26	37%	$\chi^2 = 7.96$ $p < 0.01$
Stalked by pike	25	45%	35	49%	

The first line of Table 6.3 shows that, in addition, the distances at which they reacted are very different. This is purely precautionary behaviour, but in the next categories escape responses also occur (where the animal has engaged the attention of the predator and must get away, or when it is suddenly very close to it), as many of the encounters are at close range. The two categories which follow, the creeping or slow swimming away, and the jumping or fast swimming away, are each composed of several separate forms of retreat which can conveniently be lumped, but the "jump" category occurs at a shorter range from the pike than the slow retreat and is therefore a more intense escape response (Chapter 4). Both inexperienced and experienced fish jump away at the same distance from the pike, so there is no differentiation in the nature of this response with experience, but experienced fish become more likely to give the longer range responses. In addition, inexperienced fish are more likely to show investigatory responses ($p < 0.05$).

The behaviour of the pike affected experienced and inexperienced fish differently. Table 6.4 shows how experienced and inexperienced *Gasterosteus* reacted to moving and non-moving pike in their first encounters during the test, and also the nature of the reactions. Experience appears to increase the chance of a stickleback reacting to a non-moving pike, though not to a moving one, and to make a fish less likely to approach a non-moving pike or one which had moved. However, on the limited data available these conclusions cannot yet be substantiated statistically. Significantly fewer

Table 6.5

Responses of fish of different ages tested with pike   Gasterosteus

A. Eight weeks old

	Experienced		Inexperienced		Significance levels
	Number	%	Number	%	
Total tested (3 broods)*	53		55		
Taken	4	8%	12	22%	not sig.
Took fright at 1st enc. with pike	44	83%	18	33%	$\chi^2 = 25.9$ $p < 0.001$
Mean distance at which 1st enc. was made	** 46 cm		*** 24 cm		$z = 5.75$ $p < 0.00003$
Total which went to weed at taking fright	36	68%	22	40%	
Number which approached pike	6	11%	21	38%	$\chi^2 = 9.50$ $p < 0.01$
Stalked by pike	19	36%	36	66%	$\chi^2 = 9.32$ $p < 0.01$
Mean length of fish	2.4 cm		2.4 cm		

B. Thirteen weeks old

	Experienced		Inexperienced		Significance levels
	Number	%	Number	%	
Total tested (2 broods)**	27		31		
Taken	1	4%	2	6%	
Took fright at 1st enc. with pike	23	85%	11	35%	$\chi^2 = 12.52$ $p < 0.001$
Mean distance at which 1st enc. was made	** 26 cm		*** 11 cm		$z = 2.76$ $p < 0.003$
Total which went to weed at taking fright	21	78%	17	55%	not sig.
Number which approached pike	4	15%	6	19%	
stalked by pike	13	48%	20	65%	
Mean length of fish	2.7 cm		2.7 cm		

* in each case one brood is half a single large brood , the others are separate broods

** comparing 8 and 13 week experienced fish, $z = 3.45$, $p < 0.0003$

*** comparing 8 and 13 week inexperienced fish, $z = 13.44$, $p < 0.00003$

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experienced fish approached the pike after they had first encountered the non-moving pike, and fewer experienced fish approached the pike after they had first encountered it moving. Whereas experienced fish retreat at this encounter, inexperienced fish are more likely to watch subsequently, and approach if the pike stops moving. It is here that the difference between the two sub-groups in their jump reactions is demonstrated. Experienced fish jump when they are stalked, but rarely when the pike moves while they are facing it. Instead they retreat slowly and smoothly, while the inexperienced fish give a small jump, after which they may approach the pike, so that they are likely to have more than one encounter if they resume their former behaviour without showing any more signs of fright.

6.3.2.1.1.2. Age differences

Comparing both the pike-experienced and inexperienced fish of the two age groups (Table 6.5) there is a significant difference in the distance from the predator at which experienced and inexperienced fish made their first encounter, the thirieen week old fish responding at a shorter distance.

There were no other statistically significant age differences between these two age-classes, but this difference in the distances at which the fish first reacted, with the older fish responding at a shorter range, means that they were at a greater risk from the predator, both as experienced and as inexperienced fish, than were the younger fish.

The most obvious way in which a difference in the age of

Table 6.6

(i) Distance from pike at which broods of different ages made their first encounter Gasterosteus

Brood	Age at testing	Pike experienced	inexperienced
	8 weeks old		
A		41 cm.	17 cm.
B		45 cm.	27 cm.
C		50 cm.	20 cm.
	13 weeks old		
A		26 cm.	15 cm.
D		30 cm.	10 cm.

(ii) Mean latency of emergence from weed of the same broods

Brood	Age at testing	Pike experienced	inexperienced
	8 weeks old		
A		39"	27"
B		10"	5"
C		70"	24"
	13 weeks old		
A		97"	73"
D		84"	60"

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the fish might affect them is their size, but a comparison of the broods used showed, surprisingly, that the difference between the average lengths of eight week old and thirteen week old fish was negligible.

A further possible source of variation could be genetic differences between the separate broods tested. When they were examined for such variations the data for the four separate broods showed no such differences. The mean distances at which they made their first encounter are given in Table 6.6 (i). There is clearly an age-effect but there is no significant difference which could be attributed to a brood effect. There was a brood difference in the mean latency shown by the fish (Table 6.6 (ii)), all the fish from a single brood remaining for more or less characteristic time in the weed before moving out of it, with a shorter stay for the inexperienced fish, but in the single brood (A) tested at each age level these latencies were not constant. This character, though stable at any testing period, was so variable between broods that it was not used as a criterion in any of the tests.

no sig diff
a between
broods tests?

The effect of prior experience on the responses of normally reared Gasterosteus is thus clearly seen, the differences between the inexperienced and experienced fish being shown best in the distances at which they respond, and in some of the responses given. A difference in age affected the distance at which the animals responded.

Table 6.7

Results of experi ment on the effect of prior experience of the pike

Gasterosteus orphans with normal Gasterosteus for comparison

	Normal*				Orphan				
	Experienced		Inexperienced		Experienced		Inexperienced		Sig. levels
Total tested (2 broods)	80		86		31		30		
Taken	5	6%	14	16%	1	3%	7	23%	
Took fright at first encounter with pike	67	84%	29	34%	27	93%	12	40%	$\chi^2 = 13.29$ $p < 0.001$
Mean distance at which 1st enc. was made	39 cm		19 cm**		45 cm		32 cm**		$s = 2.99$ $p < 0.001$
Total which went to weed at taking fright	57	71%	39	45%	23	79%	7	23%	$\chi^2 = 13.68$ $p < 0.001$
Number which approached pike	10	13%	27	32%	3	10%	12	40%	$\chi^2 = 5.99$ $p < 0.02$
Stalked by pike	31	39%	56	65%	14	45%	19	63%	

* Data for normal fish, the same as Table 6.1

** Orphan inexperienced fish differ from normals. $s = 2.70$, $p < 0.004$

6.3.2.1.2. Gasterosteus orphans

The last experiment showed that experience of a pike affected the reactions of young Gasterosteus to pike later. During the first two weeks of life, as its power of swimming develops, the young stickleback becomes better and better at evading its father, who tries to catch it and spit it into the nest, and it therefore seemed possible that orphan sticklebacks (i.e. reared without any father) would react differently in their first encounter with the pike.

Several broods of Gasterosteus were reared in the laboratory to hatching stage as before, but the male was removed as soon as the young had hatched, so that they were raised without any possibility of parental influence upon their behaviour. Two of these broods, one eight weeks old, the other thirteen weeks old, were tested in the same way as the normal broods to measure their fright responsiveness to pike. Table 6.7 compares orphan and normal fish, with and without experience of the pike. Considering first the fish which had not been with the pike beforehand, the orphans reacted to the predator at a greater distance, but were less likely to go to weed to hide. A comparison of the inexperienced fish in Tables 6.8 (control) and 6.2 shows that the orphans' immediate response to the pike was less likely to involve fast movement away from the pike than the response of normally reared fish. The experienced orphan fish showed overall more movement into the weed at these first encounters. Orphan inexperienced fish were a little more likely to approach the pike than were normals.

Table 6.8

Reactions given by fish at their first encounter with pike during individual test

Gasterosteus orphans

	Experienced		Inexperienced		Significance levels
	Number	%	Number	%	
Total tested	31		30		
Precautionary					
Stayed in weed	2	7% 58%	0		* combined Precautionary behaviour $\chi^2 = 7.83$ $p < 0.01$
Moved into weed at sight of pike	16	52%	6	20%	$\chi^2 = 5.48$ $p < 0.02$
Fleeing					
Crept to cover or swam off slowly	6	19%	4	13%	not sig.
Jumped and/or swam off fast	5	16%	4	13%	
Swam off "normally"	0		3	10%	
Investigation					
Stood and watched	0		4	13%	combined investigatory responses $\chi^2 = 10.10$, $p < 0.01$
Approached pike	1	3%	8	38%	
Taken	1	3%	1	3%	

There were no significant differences between the two broods of different ages.

The effect of experience of the pike on orphans was very much as for normally reared fish, except that, even after experience, orphans were less likely to use weed to hide in than experienced normals.

In summary, we may say that the orphans differ most from normals when they have had no experience of the pike. They do not hide in weed as readily as normals which have presumably learned this means of avoiding their father. On the other hand, the inexperienced orphans respond to the pike at a greater distance, as if the normal fish had habituated to the presence of a large companion, their father, near at hand.

6.3.2.1.3. Gasterosteus "overfathered"

Having examined the effect of experience of the pike on both normally reared and orphan young the possibility was recognised that an unusually long exposure to the father might also affect the development of the fright behaviour. One large brood of fish (more than 100 individuals) was left with its father in a tank 86 x 30 x 30 cm. The young became independent of their father before they were a week old but were kept in the tank with him until six weeks after they had hatched ("overfathered"). At first the male tried continually to round up his young and keep them on the nest area, but they constantly eluded him, and remained near the vegetation adjacent to the nest area. After about a week of this (at about thirteen days old)

Table 6.9

Results of experiment on the effect of prior experience of the pike

Overfathered Gasterosteus compared with normals

	Normal*				Overfathered				
	Expd.		Inexpd.		Expd.		Inexpd.		
	Number	%	Number	%	Number	%	Number	%	
Total tested (1 brood)	80		86		42		43		
Taken	5**	6%	14***	16%	10**	24%	21***	59%	$\chi^2 = 4.70$, $p < 0.05$
Took fright at 1st enc. with pike	67	84%	29	34%	30	71%	8	19%	$\chi^2 = 9.84$, $p < 0.01$
Mean distance at which 1st enc. was made	39 cm		19 cm⁺		37 cm		14 cm⁺		
Total which went to weed at taking fright	57	71%	39	45%	27	64%	17	40%	
Number which approached the pike	10	13%	27	32%	0	0	7	17%	
Stalked by pike	31	29%	56	65%	21	50%	38	88%	$\chi^2 = 4.29$, $p < 0.05$

* Table 6.1

** comparing expd. normal and overfathered, $\chi^2 = 6.57$, $p < 0.02$

*** " inexpd. " " " $\chi^2 = 10.24$, $p < 0.01$

⁺ " Nonal and overfathered, $z = 2.18$, $p < 0.02$

Note: Fish tested at different ages are combined

Table 6.10

Reactions given by fish at their first encounter with pike during individual test.

Overfathered Gasterosteus

	Experienced		Inexperienced		Significance levels
	Number	%	Number	%	
Total tested	42		43		
Precautionary					
Stayed in weed	0		0		
Moved into weed at sight of pike	5	12%	0		
Fleeing					
Crept to cover or swam off slowly	11	26%	3	7%	$\chi^{2*} = 12.78$ $p < 0.001$
Jumped and/or swam off rapidly	18	43%	9	21%	
Swam off normally			2	5%	
Investigation					
Stood and watched			3	7%	$\chi^{2**} = 7.77$ $p < 0.01$
Approached pike			6	14%	
Taken	8	19%	20	47%	$\chi^2 = 6.05$ $p < 0.02$

* experienced fish showed significantly more fleeing responses.

** inexperienced fish showed significantly more investigation responses.

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the young were moving freely in all parts of the tank, whereas before this they had avoided the male, and the male was building a new nest. From then on he continually attempted to attack any young coming near the nest. These young avoided actually being hit by the male, but would remain within a few centimetres of him at any time, and when he attacked they jumped away only a few centimetres to be out of range.

The male was removed at the end of six weeks, and the young immediately spread over the nest area which he had defended so vigorously for so long. When they were eight weeks old a sample was removed and tested with the pike in the same way as the normal and orphan broods had been. A second sample was tested at thirteen weeks old. The combined results are given in Table 6.9.

The overall picture is of a difference between experienced and inexperienced fish in their responses to the pike, these differences being similar to those already described for the normally reared fish, and the orphans.

The most interesting feature of this overfathered brood was how they compared with normals (Table 6.1 and 6.5) and orphans (Tables 6.7 and 6.8), particularly the inexperienced fish, in their first encounters with the pike. The clearest difference was in the reduced readiness of overfathered fish without pike experience to take fright on encountering the pike for the first time (Table 6.10 gives the first reactions of the overfathered fish for comparison with Tables 6.2 and 6.8).

There were almost no Precautionary responses, escape

Table 6.11

Responses of fish of different ages tested with pike

Overfathered *Gasterosteus*

A. Eight weeks old

	Experienced		Inexperienced		
	Number	%	Number	%	
Total tested (1 brood)	22		23		
Taken	1^1	5%	9	39%	$\chi^2 = 4.43$ $p < 0.05$
Took fright at 1st encounter	21^2	96%	8^5	35%	$\chi^2 = 15.46$ $p < 0.01$
Mean distance at which 1st enc. was made	41^4cm		15cm		
Total which went to weed at taking fright	18^3	82%	11	48%	$\chi^2 = 3.70$ not sig.
Number which approached pike	0	0	2	9%	
Stalked by pike	8	36%	18	78%	$\chi^2 = 6.02$ $p < 0.02$
Mean length of fish	2.6cm		2.6cm		

B. Thirteen weeks old

	Experienced		Inexperienced		
	Number	%	Number	%	
Total tested (brood as above)	20		20		
Taken	9^1	45%	12	60%	not sig.
Took fright at 1st encounter	9^2	45%	0^5	0	$\chi^2 = 7.99$ $p < 0.01$
Mean distance at which 1st enc. was made	33cm^4		13cm		
Total which went to weed at taking fright	9^3	45%	6	30%	
Number which approached pike	0	0	5	25%	$\chi^2 = 3.66$ not sig.
Stalked by pike	13	65%	20	100%	$\chi^2 = 6.23$ $p < 0.02$
Mean length of fish	3.4cm		3.6cm		

1 significantly different. $\chi_c^2 = 10.71$ $p < 0.01$
2 " " $\chi_c^2 = 10.71$ $p < 0.01$
3 " " $\chi^2 = 6.42$ $p < 0.02$
4 " " $\chi_c^2 = 4.09$ $p < 0.$[illegible]03
5 " " $\chi^2 = 4.57$ $p < 0.05$

responses were common and the amount of investigation was about normal. Another difference between overfathered and normal fish (not shown in Table 6.9) is the latency of emergence from the weed after being introduced there at the start of the test. Alone among all the sticklebacks tested, the overfathered fish always moved into the tank immediately, although their use of weed at taking fright as eight weeks old fish is normal, that is, the same as that for normally reared fish.

Table 6.11 gives the same data as Table 6.9, broken down into the two age-groups. Whereas the eight week old fish behaved like normal fish, except in the distance at which the inexperienced group responded to the pike, the thirteen week old fish responded less at their first encounter, reacted at a shorter distance, and more were eaten.

Thus the overfathered fish behaved in a relatively unalarmed way in the presence of the pike, particularly those tested at thirteen weeks, and this overconfidence is confirmed by the high proportion of these fish which were taken by the pike during the test periods.

6.4.1. DISCUSSION OF RESULTS FOR *GASTEROSTEUS*

It may be concluded that prior experience of the pike does affect the response of *Gasterosteus* at a subsequent encounter, and prior experience sensitises a stickleback to a non-moving pike. Experienced fish respond at a greater distance and are more likely to retreat to the weed at their first encounter, that is, they show more precautionary behaviour**, Significantly fewer inexperienced fish show precautionary behaviour at their first encounter, and when they** do so they are less likely to hide in weed, so that they are both initially and subsequently at a greater risk than the experienced fish. In addition they are more likely to approach the predator before they show lasting fright responses, so that the risk is increased.

The real nature of the risk is shown by the number of sticklebacks taken during tests. Although the numbers taken in any test were small, 58 fish were taken in all, significantly more of them being inexperienced ($p < 0.01$). While the degree of hunger of the predator was difficult to standardise on different days, the experimental design, with the experienced and inexperienced sticklebacks being tested alternately, ensured that the differential predation indicated a real difference in risk to the sticklebacks, at least under the conditions of the experiment.

During the pre-training period, when half the brood was placed in the control tank, half in the testing tank with the pike, the behaviour of both sub-groups was watched. While the control fish rapidly overcame their initial relatively mild alarm at being placed in a new tank, and moved in the open in all levels of the water, even searching for food on the ground, the fish in the other tank soon encountered the pike as they began to explore the tank tentatively, and at its first definite movement they moved quickly away from that area. If the pike stalked or snapped at a fish the disturbance was sufficient to alarm the rest, so that it was usual for all or most of the fish in this tank to retreat to cover within a few minutes of the start of the twelve hour period. Usually they

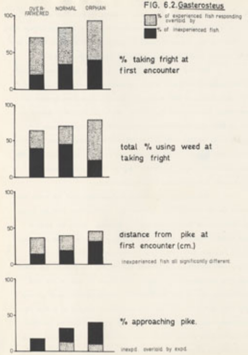

FIG. 6.2. Gasterosteus

would watch the pike from below the weed at first, and some moved into the open in parts of the tank away from the pike, but even these activities were quickly superseded by hiding. At the end of the twelve hour period the control fish were easily netted from their tank, where they moved in the open in schools, but the fish from the pike tank could only be retrieved by removing the weed and sorting them out of it. This showed that prior experience with the pike made fish more likely to use the weed as cover, and even hunting up control fish to remove them from the tank did not approach the effect the pike had on the experienced fish.

The orphan fish differ from the normals in several respects. Figure 6.2 summarises the effects of parental rearing conditions on the responses to predators for the most sensitive behaviour scores, and shows that in each respect normally reared fish are intermediate between orphans and the overfathered brood. The presence of the male, presumably because of the way he tends the young and the way they gradually come to avoid him, reduces the risk of the young from predators by making them more likely to use weed to hide in after an encounter, and less likely to approach the pike. On the other hand, they are at a greater risk through responding to the pike at a shorter range and being less likely to show precautionary behaviour as a result of this first encounter. The last effect might be some kind of habituation to the presence of a large companion. The fact that no experienced and few inexperienced overfathered fish investigated the pike indicates that it is the novelty of the large moving fish which elicits this response.

Differences in the responsiveness of fish of different ages were variable. Normally reared fish tested at thirteen weeks old responded at shorter distances than those tested at eight weeks old, but the corresponding orphan groups showed no significant change. The overfathered fish followed the trend for the normal fish and were so unresponsive to the predator at thirteen weeks old that 37% of them were eaten, and none of the inexperienced fish responded at their first encounter. The size of the fish tested was in the same range for both age groups (except in the overfathered brood), and all fish tested were of a size that the pike could eat.

Overall, it is clear that the correct response to the predator, described as precautionary behaviour, is quickly acquired by *Gasterosteus* when it has experience of a pike, no matter what the rearing conditions, but that the early history of the fish affects their responses, and consequently their risk, at their first encounter. The fact that the older fish were at a greater risk (i.e. responded when the pike was closer) may just be due to the greater length of time they had lived without any such experience, a situation most unlikely to occur in the wild. The broods raised in the laboratory to the age of testing were kept in groups from the one family, without the society of larger members of their species, while in the wild sticklebacks of different sizes are often to be found close together, so that small fish would certainly have been exposed to larger individuals of their kind, to say nothing of the other species of fish,

Table 6.12

Results of experiment on the effect of prior experience of the pike Pygosteus, with Gasterosteus for comparison

	Gasterosteus**				Pygosteus				
	Experienced		Inexperienced		Experienced		Inexperienced		
Total tested (2 broods)	80		86		33		35		
Taken	5	6%	14	16%	0	0	4	10%	
Never emerged from weed					16	49%	2	6%	χ^2 = 13.78, p < 0.001
Took fright at 1st enc. with pike	67	84%	29	34%	17/17	100%	22/33	67%	χ^2 = 5.45, p < 0.02
Mean distance at which 1st enc. was made	39 cm		19 cm*		45 cm		35 cm*		
Total which went to weed at taking fright	57	71%	39	45%	31	94%	29	83%	not sig.
Number which approached pike	10	13%	27	31%	1	3%	6	17%	
Stalked by pike	31	39%	56	65%	9/33	27%	17/35	49%	χ^2 = 2.41 not sig.

Note: Fish tested at different ages are combined in the Table

* inexperienced fish respond at significantly different distances, p < 0.001

** Data from Table 6.1

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some of them actual or potential predators, which are common in stickleback waters.

6.322 RESULTS Pygosteus

6.3.2.2.1.1 Pygosteus normally reared

The effects of pike experience on the fright responses of Pygosteus were studied just as in Gasterosteus, but it was not possible to obtain as many broods suitable for testing. Two broods were reared with their father in the same way and under the same conditions as the four broods of Gasterosteus tested, and the male was removed when they became independent of him. Even the limited results (Table 6.12) indicated some clear differences between the species and they will be reported here. As in Gasterosteus the effects of pike experience were investigated on normal and orphan fish tested at eight and thirteen weeks. The definition of terms used is given just before the section on Gasterosteus.

Broadly speaking, the effect of giving experience of a pike changed the behaviour of Pygosteus in the same way as was seen for Gasterosteus. The experienced fish were more likely to take fright at the first encounter, but there is no significant difference between the experienced and inexperienced fish in the number going to weed, or in the number approaching the pike. In the experienced group of Gasterosteus 3% approached the pike before they took fright. Inexperienced Pygosteus went to weed at taking fright more often than inexperienced Gasterosteus ($p < 0.001$). In fact in all these ways inexperienced Pygosteus behaved like experienced Gasterosteus.

Table 6.13

Reactions given by fish at their first encounter with pike during individual test

Pygosteus

47

16 + 17.

	Experienced		Inexperienced			
	Number	%	Number	%		
Total tested	33		35			
Precaution		76%		14%		Precautionary behaviour $\chi^2 = 23.6$ $p < 0.001$
Stayed in weed	16	49%	2	6%	$\chi^2 = 13.18$ $p < 0.001$	
Moved into weed at sight of pike	9	27%	3	9%	not sig.	
Escape		24%		60%		Escape responses $\chi^2 = 7.48$ $p < 0.01$
Crept to cover or swam off slowly	4	12%	17	49%	$\chi^2 = 8.93$ $p < 0.01$	
Jumped and/or swam off fast	3	9%	3	9%		
Froze*	1	3%	1	3%		
Investigation						
Approached pike	1	3%	6	17%		
Taken	0	0	4	11%		

* this response is not on Tables for Gasterosteus as it did not occur

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Of other possible differences between the species the distance at which the first encounter was made by inexperienced fish was found to be significantly greater in Pygosteus.

A small proportion of pike-inexperienced Pygosteus followed the pike after the first encounter (included in the approach category), but this is more than twice as likely to happen when inexperienced Gasterosteus are tested.

The responses shown by these Pygosteus at their first encounter with the pike are given in Table 6.13 (directly comparable with Table 6.2 for Gasterosteus). A high proportion of the experienced fish never emerged from the weed during the test, and, together with the fish which moved into the weed as soon as they saw the predator, make up 76% of the experienced fish tested. By contrast, only 14% of the inexperienced Pygosteus showed these precautionary responses at their first encounter. There is also a difference in the proportion of Pygosteus showing escape responses, since most of the experienced fish responded with precautionary behaviour. The percentage of experienced fish showing precautionary behaviour at their first encounter is much higher for Pygosteus than it is for Gasterosteus ($p < 0.001$).

Among the inexperienced fish, the main difference between Pygosteus and Gasterosteus lies not in the proportions showing precautionary responses, but in the relative frequencies of jump responses and slow swimming retreats. Pygosteus is more likely to swim slowly, Gasterosteus to jump (jumps, $p < 0.02$; slow swimming, $p < 0.01$).

Table 6.15

Responses of fish of different ages tested with pike

Pygosteus

A. Eight weeks old

	Experienced		Inexperienced		
	Number	%	Number	%	
Total tested (1 brood)	15		16		
Taken	0	0	2	13%	
Never emerged	3		0		
Took fright at first enc. with pike	12	100%	11	69%	
Mean distance at which 1st enc. was made	47cm		37cm*		not sig. different
Total which went to weed on taking fright	13	87%	11	69%	
Number which approached pike	0		3	19%	
Stalked by pike	7	47%	12	75%	

B. Thirteen weeks old

	Experienced		Inexperienced		
	Number	%	Number	%	
Total tested	18		19		
Taken	0		2	11%	
Never emerged	13	72%	2	11%	$\chi^2 = 12.34$ $p < 0.001$
Took fright at 1st enc. with pike	5/5	100%	11	58%	
Mean distance at which 1st enc. was made	47cm		43cm*		not sig. different
Total which went to weed at taking fright	18	100%	18	95%	
Number which approached pike	1	6%	3	16%	
Stalked by pike	2	11%	5	38%	

* inexpd. fish differ significantly at 0.01 level
inexperienced 13 week old compared with experienced 8 week old: no sig. difference.

Table 6.14

Distance from pike at which some first responses were given

Pygosteus

	Experienced	Inexperienced
Went to weed immediately	78 cm.	69 cm.
Crept off slowly	28 cm.	24 cm.
Jumped or swam fast	14 cm.	17 cm.

The distances at which the most common of these responses occured (Table 6.14) are not significantly different between experienced and inexperienced fish. The high number of inexperienced *Pygosteus* responding to the pike at their first encounter, and retreating to the weed, resembles the behaviour of experienced rather than inexperienced Gasterosteus. Whereas inexperienced *Gasterosteus* differed significantly from experienced fish in the nature of their responses, the only significant difference between these categories in *Pygosteus* lies in the distance at which the fish responded to the predator.

6.3.2.2.1.2. Age differences

The two broods of normally reared *Pygosteus* tested were of different ages, and the combined data given in Table 6.12 are broken down into these two age groups in Table 6.15. The only significant difference between the two ages lay in the number of experienced fish which never emerged from the weed, and the distance at which the older inexperienced fish responded. Experienced *Pygosteus* responded at the same distance from the pike as experienced *Gasterosteus*, inexperienced *Pygosteus* responded at a significantly greater distance

Table 6.16

Results of experiment on the effect of prior experience of the pike

Orphan Pygosteus, with normal Pygosteus for comparison

	Normal Pygosteus*				Orphan Pygosteus				
	Expd.		Inexpd.		Expd.		Inexpd.		
	Number	%	Number	%	Number	%	Number	%	
Total tested	33		35		16		14		one brood
Taken	0		4	11%	0		0		
Never emerged	16	49%	2	6%	4	25%	2	14%	
Took fright at 1st enc. with pike	17/17	100%	22/33	67%	11/12	92%	8/12	67%	
Mean distance at which 1st enc. was made	45 cm		35 cm**		43 cm		27 cm**		U = 8.5 p < 0.001
Total which went to. weed at taking fright	31	94%	29	83%	11	69%	7	50%	
Number which approached the pike	1	3%	6	17%	0		1	7%	
Stalked by pike	9	27%	17	49%	3	19%	9	64%	

* Data correspond to Table 6.12

** inexperienced fish significantly different at $p < 0.0001$ ($z = 3.78$)

than inexperienced Gasterosteus. Both categories of the older Pygosteus showed more precautionary behaviour, and the inexperienced older fish responded at a greater distance than the younger fish.

Since the experimental evidence rests on only two broods of fish nothing can be said about possible brood variation in Pygosteus beyond the fact that two other broods reared behaved continually in such a frightened manner that they could not be tested.

6.3.2.2.2. Pygosteus orphans

Several broods of young were reared in the laboratory, and the male was removed in each case when the young had hatched. These orphan fish were reared to the age of eight and thirteen weeks before being tested in the same way as all the other broods.

Only one brood of fish was tested, at eight weeks old. One other brood, thirteen weeks old, survived to testing, but proved to be quite unsuitable for testing as they were extremely frightened in their rearing tank.

The results for the brood tested are given in Table 6.16. The only significant difference between the pike-experienced and inexperienced groups lay in the distance from the predator at which the fish made their first encounter, and these distances were identical with those for normally reared fish of the same age. There was a possible difference in the number of inexperienced fish using weed when they took fright at their first encounter (Fisher exact probability test, $p = 0.06$). Although this difference matches the trend shown by inexperienced orphan Gasterosteus it is clearly not yet reliable.

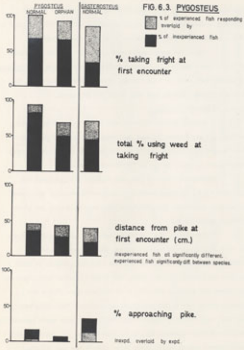

FIG. 6.3. PYGOSTEUS

Pygosteus orphans do not differ from normally reared fish as much as *Gasterosteus* orphans do (compare graphs in Figs. 6.2 and 6.3).

6.42 DISCUSSION FOR *PYGOSTEUS* AND COMPARISON WITH *GASTEROSTEUS*

In Figure 6.3 the responses of *Pygosteus* under various experimental conditions are shown together with normal *Gasterosteus* for comparison. Prior exposure to the pike affects the subsequent responses elicited from *Pygosteus*, so that experienced fish respond at a greater distance from the predator. Although there were some slight differences in the responses of experienced and inexperienced fish, these were not statistically significant, except that overall the experienced fish were more likely to show precautionary responses. In short, the experienced fish were behaving like experienced *Gasterosteus*, and the inexperienced fish were virtually the same in all respects except their proportion of precautionary responses at the first encounter, and the distance at which they responded.

Pygosteus reared as orphans show the same responses as normally reared fish in all respects except in the reduced use of weed by inexperienced fish taking fright at their first encounter. They are at all times at much less risk than normally reared *Gasterosteus*, and do not resemble the orphan *Gasterosteus*, which are at an even greater risk than normal fish in some respects.

Age differences in the fish tested appear to have less effect than in *Gasterosteus*, but as this is based on a comparison between two broods only, too much importance should not be attached to this. The only significant differences were in the smaller number of experienced orphan fish which did not emerge from the weed, and the shorter distance at which the inexperienced fish responded, although it was the same as for the normal fish of the same age.

Although both *Gasterosteus* and *Pygosteus* show the same range of fright responses when exposed to a predator, inexperienced *Pygosteus* respond at a greater distance from the predator than inexperienced *Gasterosteus*, and all *Pygosteus* show a much greater use of the weed as a hiding place. In this respect even inexperienced *Pygosteus* show more precautionary behaviour than *Gasterosteus*, although the actual response distances for experienced fish are the same. The reduced risk to *Pygosteus* from the pike is shown by the smaller proportion taken during the tests. Combining experienced and inexperienced fish 12% of 227 normal and orphan *Gasterosteus* were taken, while only 4% of the 90 normal and orphan *Pygosteus* were taken, a suggestive but not a significant difference.

Differences in the rearing of *Gasterosteus*, and also an age difference, affect the subsequent responses more than the same differences in the rearing of *Pygosteus*, which appears to remain relatively timid no matter what the early history may be.

When age differences were considered, the older *Gasterosteus* differed from the younger in responding at a closer range, so that they appeared to be less responsive to the pike or less easily frightened. By contrast, the older *Pygosteus*, appeared to be more frightened by the predator than the young fish were, since more experienced fish did not emerge from weed during the test. While the experienced fish responded at the same distance as experienced normals, the inexperienced fish responded at a significantly greater distance than inexperienced normal *Pygosteus*. Once again *Pygosteus* shows more precautionary behaviour, with a high responsiveness, so that it is possible to say that at all times *Gasterosteus*' behaviour places it at a greater risk from the predator, especially at the first encounter. This encounter is usually sufficient in both species to produce precautionary behaviour in later encounters, but *Gasterosteus* is still at a greater risk, since it retreats to weed less. Although it may be argued that in the field *Gasterosteus* would be more likely to leave the area where the predator is, while *Pygosteus* would hide from the predator, this relatively greater risk would still exist, since a moving prey attracts the attention of the pike, which can run down its quarry.

Chapter 7

DEVELOPMENT OF YOUNG STICKLEBACKS

7.1 Introduction

The early part of the breeding cycle of the male stickleback has been investigated extensively, but there is little published information on the care of the young after hatching, the best accounts being van Iersel (1953), Hall (1956), and Morris (1958). The parental behaviour of the male and the behaviour of his family differ somewhat in the two species, and may be summarised as follows.

7.2.1. *Gasterosteus*

7.2.1.1. Parental care

A male *Gasterosteus* will secure several clutches of eggs over a period of two or more days if suitable females are available, but in order to be able to date the changes in the behaviour of parent and young it was preferable to have all the eggs laid on the same day, so that for the observations to be described here each male was given several ripe females on the same day. This method of multiple clutches was followed as van Iersel (1953) found a greater hatching success in males with more than one clutch of eggs. After the female has spawned the male settles the eggs more firmly into the nest and cares for them by ventilating them by fanning at the nest entrance. Any dead eggs are promptly removed. In the laboratory, at temperatures of between 18° and 20°C the young took eight to ten days to hatch, in agreement with the data of van Iersel (1953, p.58). Swarup (1958) hatched eggs in six to eight days at 18° to 19°C.; his eggs were not kept in a nest tended by their father, but loose in Petri dishes. This suggests that in normal circumstances, in spite of the father's fanning, the nest does not provide the optimal environment for rapid development. This is probably the cost to be paid for the protection from predation which the nest affords.

Some days before the eggs hatch the male makes several openings in the roof of the nest, and during the day before hatching he prepares a pit by increasing the depth of the hollow in the sand in front of the mouth of the nest. This nest pit has been considered by Hall (1956 p.311) to be the functional equivalent of the "nursery" of *Eucalia* and *Pygosteus*. The nest usually measures about six by four centimetres, and the nursery pit is at this stage about three by three centimetres. Also during the day before hatching or immediately after hatching, the male may pull at the walls of the nest structure, teasing it up into loose strands (Hall, 1956, p.311).

The male increases the amount of time spent fanning as hatching approaches (van Iersel, 1953), and I have even seen newly hatched young swept out of the nest and lying on the substrate facing the back of the nest, as the male fanned almost continuously at the entrance. Half an hour later he had discovered them and returned them to the nest.

At this time the male's main activities are the defence of his territory, feeding, and the fanning of the nest and retrieving of the young. At about the time of hatching (taken as Day 0) the male becomes more aggressive and increases the size of his territory, (Morris, 1958, p.42). At this time he may spend very little time in feeding, and a considerable time in fanning at the original nest entrance, although the young quickly spread over the surface of the nest. To retrieve the young the father must pick them up in his mouth, in precisely the same way as he feeds, but he then spits out the young fish on to the surface of the nest, or, more often, at the nursery pit. A careful watch has been kept on many males, in some cases over several broods, and no undisturbed male has ever been seen to eat his young. By feeding the male at or on the nest it became evident that the inhibition of eating the young was not due to their proximity to the nest, and a male is capable of eating food and retrieving his young as consecutive activities on the same site.

For the first day or two (Days 0 and 1 - the hatching day is taken as Day 0*) the male keeps his young on the nest or in the pit at the nest entrance, where all the little fish lie on the ground in close-packed rows, facing the same way, directly into or away from the male's fanning current. The fanning wanes in the two days after hatching (van Iersel, 1953), although the male retrieves the young assiduously, and is even more aggressive towards intruders than before (Morris, 1958, p.43). During Days 2 and 3 the task of keeping the young together is made much more arduous as they have filled the swim bladder and now swim easily. As well as he can, the male keeps them in the vicinity of the nest, although during the next four days they

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*The hatching period for a clutch lasts about 12 hours. With multiple clutches some may hatch on Day 1, but often the clutches were laid within an hour of each other and the young hatched together.

spread more and more over the surrounding area. By about Day 9 the young fish move at all levels in the water and the male does not catch them, although he may still be able to round them up to spend the night on the old nest. This is because, although the male can no longer catch his young, he attempts to catch them as he patrols around his territory, and they avoid him by jumping towards the nest. It seems as if they learn the location of the nest during their first few days when they are kept near it, and later they use it as a retreating place. When the light is failing they tend to remain more still, so that by night-fall they are gathered up on the nest, where the male also spends the night from the time the eggs are laid until the brood is finally dispersed. By Day 8 or 9 the nest is a frayed and tossed oval of vegetation, ringing a sandy pit, as well as having the now extensive nursery pit outside. Between Days 7 and 14 (usually about Day 10) the young become independent of their father and can no longer be collected into his nest at night, though I have seen males trying to retrieve young of 14 days and older into the nest, and he begins a new nesting cycle.

7.2.1.2. Behaviour of the young

Brood phase.

Already, at the time of hatching, the cryptically coloured Gasterosteus young are seen to move the tail quite vigorously as they lie on their sides; the yolk sac interfereswith their balance (Swarup, 1958). There is a continuous rapid pectoral beat, and these movements have been thought to aid ventilation (van Iersel, 1953, p.19, but see Wickler, 1960, p.57). From hatching, the young are capable of single short jumps of two to three centimetres, by means of strong beats of the tails. After such a jump they sink to the bottom again, and in these very young fish other jumps do not usually follow immediately. But jumps occur quite often, especially when the male is absent. Another response seen from the start is facing into a current, the behaviour shown by a newly-hatched brood described earlier. While the male is fanning and the young face this current, the likelihood of jumps being made is much less, but when the male moves away or stops fanning this restraint is removed. Also it is common for the young fish to dive from the nest surface or the nursery pit into the actual nest material, which the male has teased out, especially when they have been disturbed.

As the yolk reserves are depleted jumps become more frequent, and the male has to retrieve his young more and more. He concentrates them in close proximity by returning them to the nursery pit, which becomes larger and deeper as the days go by, partly as a result of the male removing more sand and partly because, as he blows back each young fish retrieved, he displaces a little more sand. By Day 2 the yolk has mostly been used up so that the young can move more freely and it is common to see them spreading over the ground by the nestpit. At some time late in Day 2, or early in Day 3, the young fish are seen to be jumping higher in the water and they swim to the surface in a series of poorly controlled spiralling jumps. They open the mouth at the surface to take air into the swimbladder, but they often dive down very fast on to the nest area again. More than one trip may be needed to fill the swim bladder. Each such trip involves the avoidance of the father on the way up, or the young fish is summarily returned to the nest. As soon as the swim bladder is full the young are capable of controlled swimming in a horizontal position, although full control does not come for some days, after the development of the median fins. At first the young fish keep in a group at 2 - 3 cm. above the nursery pit area, which is now about 6 cm. in diameter, but during the next few hours they spread out. By circling his territory and constantly retrieving or attempting to retrieve young, the male keeps them rounded up over the nest area.

By Days 7 to 9 the young fish swim extremely well and, as already mentioned, become independent at about Day 10. van Iersel (1953 p.20) reported that young become independent at a week at the most.

From the day of hatching young fish were seen to give very small jumps when *Artemia* nauplii were near, but it was Day 2 before they actually ate any. From then on they ate large quantities of this food, and by the time of independence many fish had begun to eat small *Tubifex*. The eyes are very well developed at hatching, and the hunting of food is visual. The characteristic head-down grasping posture (Tugendhat, 1960a) is adopted as soon as horizontal swimming is established, and is used immediately for ground prey, including *Tubifex*.

7.2.1.3. Responses to their father

From hatching, the cryptically coloured young *Gasterosteus* sink low, flat to the substrate, or burrow into the nest material, as their father passes directly above them. During the very early phases, when the yolk sac is very large and they are not very manoeuverable, the young do not show any other responses, although they will jump away if anything is brought very near to the head. Within the next three days the passing of the male overhead still elicits this response, but, from about this age but not before, when he noses down at his young, which is both his feeding and his retrieving orientation, they jump a few centimetres before lying low. The same jumping response is shown by even the youngest fish when a needle or a pipette is brought to 3 - 5 cm. from the head. To this artificial stimulus the distance at which the fish reacts increases with its age in the first few days. This response was seen many times to a slightly older baby stickleback which was actively searching for *Tubifex*, whereas other fish of the same younger brood elicited no such reaction, even when they were also feeding.

During subsequent days, and especially after the young have filled the swim bladder and swim well, this jumping away response is often seen to the nearby male. The jumps become longer, and almost always take the young in the direction of the nest. As the male passes overhead the young also sink a few centimetres, so that in response to the patrolling male they are kept low in the water and concentrated over the nest area, although they are by now seldom caught. The end of the brood phase seems to be marked by the successful avoidance of the father.

Evidence for the survival value of this parental behaviour is suggested by the observation that the few males which spend very little effort in retrieving their young are rather timid, and in tanks containing more than one male they have a lower brooding success. In a tank containing six breeding males, in which the competition for space was very keen, only the most aggressive male reared his full brood, and when it dispersed it remained in small groups in the vegetation. The other males lost their brood at the egg stage, or when the young fish begun to move about, and the few young which reached independence were lost immediately.

In a tank with a single male, the end of the brood phase is marked by the young's spread to all levels and all areas of the tank, in contrast to the spread below the surface or over the ground, up to about six centimetres above ground level, which is the limit of their range earlier. The later spread into all levels in the water is even only if the male is removed, otherwise he is avoided, so that the young collect in areas which he does not normally frequent. In a tank in which there is more than one territory the young 'vanish' into cover in interstices between territories and in weakly defended areas. They are later (after about three to five days) seen to be in small schools around the tank, this schooling having been established after the swim bladder was filled.

From the time of hatching the well developed system of chromatophores functions to keep the young cryptically coloured to match any background.

7.2.1.4. The anatomy of the young fish

As far as it concerns their swimming ability and protection, the anatomy of the young *Gasterosteus* will be described briefly. More details are given by Swarup (1958).

When they hatch the young fish have a median fin-fold from just behind the operculum dorsally right around to the yolk-sac below. The pectoral fins are also present. By the fourth day after hatching the caudal fin rays start to develop, and the tail is complete in the adult form by just over two weeks after hatching. The dorsal and anal fins begin to appear in the fin-fold about nine days after hatching, and develop rays at about two weeks, when they become separate from the caudal fin. The pelvic rudiments are first seen at about ten days after hatching, and it is about three weeks before the dorsal spines are formed well, though they first appear as thickenings at two weeks, and they are not of the adult length until between four and five weeks have passed. The pre-anal portion of the median fin-fold persists as a keel until the fish is between three and four weeks old.

As the mechanical protection afforded by the dorsal and pelvic spines is not available to the young fish until they are about five weeks old, they must rely for protection in their early life upon their ability to avoid predators.

7.2.2. *Pygosteus*

7.2.2.1. *Parental care*

As in *Gasterosteus*, the male *Pygosteus* tends the eggs, but in a nest which is usually situated above ground level.

When the male has successfully courted a female and has a clutch of eggs, he settles them in the nest and fans them occasionally. Although he will care for and hatch eggs laid over two or three days, for the purpose of the observation of development ripe females were offered on the day of the first laying only, as for *Gasterosteus*.

The first indication that the eggs have hatched comes when the male stays by his nest all the time, and searches over the ground below it. This is between 4 and 8 days after laying, usually about six days (at 18° - 19°C.). Hall (1956) gives 4 to 7 days. When the nest is deteriorating or is very close to the ground, the male is likely to build a new loose nest structure above it, and he transfers the young, so that they lie on the surface of it. This is what Morris (1958 p.131) has called the nursery. The young remain rather still, occasionally jumping out, and any which go to the ground in a series of spiralling jumps, or just fall passively, are soon picked up by the male and returned to the mouth or surface of the nest, or on to the nursery.

On Day 3 the tiny young have depleted their yolk and are more active. During Day 3 or 4 they make a series of swimming jumps so that they spiral up to the surface to fill their swim bladder, several visits often being necessary, although these young remain at the surface between gulps. After this the male cannot easily catch them, and they remain hiding in the vegetation, near the surface. It is between Days 7 and 10 that they move down through the water and begin feeding at ground level, and if the male is still in the tank they may continue to hide.

7.2.2.2. Behaviour of the young

Brood phase

Newly-hatched *Pygosteus* are smaller than *Gasterosteus* (ca. 4.5 and 6 mm. total lengths, respectively) and both species have yolk sacs of about the same absolute size. They move much less, and can be seen to hang motionless in the algal threads of the nest, head up, tail vertically down, with the yolk sac resting against a strand of alga. This is such a characteristic sight, no matter where the nest is built, that I suspected the presence of larval adhesive organs on the yolk sac, but could see no trace of such specialisation by direct observation. (No histological investigation was made to check this more precisely.) Some small movements, made by the tail, occur very occasionally as the young settle more firmly on the vegetation, but it is not unusual to see only one or two movements in 15 minutes' watching of the whole nest, whereas *Gasterosteus* young are constantly moving. Some jumps are made by *Pygosteus*, and in the course of these some young fall out of the nest, but if a brood of *Gasterosteus* was put in a *Pygosteus* nest it would shower down in a very short time.

It is only in the day preceding the filling of the swim bladder that more activity is seen, and once the swim bladder is filled they swim as well as the corresponding *Gasterosteus*. At this stage *Pygosteus* is particularly cryptic in its habits, and it is usual for the young fish to "vanish"in the tank, aided by their well developed system of chromatophores, which functions from the time of hatching, as in *Gasterosteus*.

The feeding habits of the young fish are similar to those of Gasterosteus, except that they do not snap at *Artemia* nauplii until about Day 2, when the yolk is diminished and they begin to feed. After the swim bladder is filled they remain at the surface and feed on any small organisms below the surface film, and when they move down to the lower levels and the ground they begin to eat larger prey, including *Tubifex*, for which they search in the same way, using the head-down orientation. With this change in habits associated with the assumption of the ground feeding habit, the young can be seen more easily again. This is somewhat before the time at which the protective dorsal spines develop.

7.2.2.3. Responses to father

As there is no parental care after the young have filled the swim bladder and swum to the surface there can be no such detailed observation of the relationship between young *Pygosteus* and their father at this time as is possible for *Gasterosteus* under natural circumstances. However, isolated young responded to the approach of a needle or a pipette in the same way as described for *Gasterosteus*. It was noticed that,unlike the *Gasterosteus* young, *Pygosteus* moved away from the glass front of the tank and hid in the vegetation when I approached the tank. They became much bolder and no longer showed this response, at the time when they began to feed at ground level, although they seldom become as bold as young *Gasterosteus*.

7.2.2.4. Anatomy of the young fish

The development of the fins follows the course described for *Gasterosteus*, and at two weeks after hatching the dorsal and anal fins are developing, the caudal fin is heterocercal, and these three fins are still connected by the median fin fold, which, however, is broken at the anus, so that the keel is formed. It persists until about three and a half weeks. The dorsal spines are beginning to develop at two weeks, and by three weeks have their adult height, which is just the height of the still-continuous fin-fold.

7.2.2.5. Later development of *Pygosteus*

Broods of *Pygosteus* reared in the laboratory varied in their later behaviour. Some broods were apparently quite normal in their behaviour in that an unusual stimulus would cause them to hide or to watch from a corner, but they would come forward immediately for food, and did not remain frightened for too long after a shock. Other broods seemed to be permanently frightened, so that they never fed when watched, but stood side-on to the observer, or hid. Such broods never improved even after several months and could not be used for any tests. They usually included a high proportion of young with defective swim bladders, and showed an even greater disparity in the extreme sizes within the brood than is normally found. Similar difficulties were very rare for *Gasterosteus*.

With this tendency to show greater fright under ordinary circumstances, it was a surprise to find that young *Pygosteus* kept together in the same tank for a long time developed, by their third month, territorial behaviour like that of the adult fish at the onset of the breeding season, yet their light régime had been kept to 8 hrs. daily so that this could not be attributed to a precocious onset of sexual maturity.

The most aggressive fish became black dorsally, like aggressive females (see Morris, 1958, Fig. 2 VII), and they showed the full range of aggressive and threat behaviour characteristic of adults. When any frightening thing occurs young sticklebacks normally "crowd" in a corner, in a school, but these fish remained on their territories. When an adult male three-spined stickleback was put in the tank it was attacked by the most aggressive young fish, although it was many times larger than any of them. This same adult male was tested before and after this test with non-territorial young fish, and it attacked and chased them ceaselessly. The aggressive young fish were transferred to another tank for observation and promptly lost their aggression. When, after some days, they were still docile, they were tested with the aggressive adult male stickleback again, and fled before his attacks. After they were restored to their original tank they failed to re-establish territories, and showed no further strong aggression, supporting the view that it was not of a sexual origin.

In each case in which this precocious aggressive behaviour was observed (four times) the tank was somewhat crowded - eight or more fish in a 40 x 30 x 30 cm. tank - with little vegetation. Perhaps

FIG. 7.1. GASTEROSTEUS

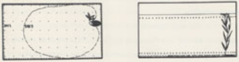
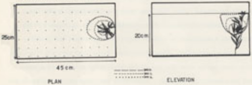


FIG. 7.2. PYGOSTEUS



RATE OF SPREAD OF ORPHAN FISH

in normal habitats, where the young are spread sparsely in dense cover, there is little chance to see the operation of an aggressive spacing-out mechanism, occurring long before the young reach breeding age, such as is suggested by this behaviour seen in the laboratory. Such behaviour was never observed in the more numerous crowded broods of Gasterosteus nor in most Pygosteus broods.

The schooling shown by Pygosteus in the laboratory is much less marked than that of Gasterosteus, which always lives more in the open, and which moves so freely that the young of any brood must be widely dispersed soon after they leave the territory of their father. During the breeding season territoriality can be induced in some female Gasterosteus (Leiner, 1931b, Morris, 1958). The behaviour described for these females is like that of Pygosteus females, but Morris only induced this territoriality in one Gasterosteus female by keeping her in a tank with two territorial males. These conditions are more crowded than the field situation.

7.3 Development without a father

With several broods of each species the father was removed as soon as the eggs had hatched, since it had been found that to remove him earlier endangered the eggs. The ground of each tank (for all broods) was marked out into 4 cm. squares by green glass beads at the corners, so that the rate of spread of the young could be measured. This is given as both surface and plan views for both species in Figures 7.1 and 7.2.For this purpose an attempt was made to induce Pygosteus to nest at ground level, but this was once again unsuccessful.

7.3.1. *Gasterosteus*

On the day of hatching, that is Day 0, some orphan *Gasterosteus* reached the opposite corner of the tank, 50 cm. off, and by the next day all were spread out over the tank, on the ground. During this day, or early on Day 2, they filled the swim bladder, and for the next few days swam either just below the surface or just above the ground. There was no concentration at the nest at any time after the young had dispersed and no aggregation at night; and by about Day 7 to 10 these young moved about freely in all levels in the water, as did the normally reared ones.

All the young faced into a current and collected in the corners and angles in which the current was least. In the absence of a current orphan young spread out evenly over the tank, normal young had their movements controlled to some extent by the presence of their father. The orphan *Gasterosteus* developed schooling just like normally reared fish.

7.3.2. *Pygosteus*

Pygosteus reared as orphans spread over the nest and the weed around it faster than normal broods, but their movements were much more restricted than those of orphan *Gasterosteus*. During Day 0 they moved out of the nest cavity onto the surface of the nest, in the vicinity of its mouth (the end at which the male had fanned it), and they moved upwards into the weed above to some slight extent. By Days 1 and 2 some young were just below the surface, hanging on algal threads or *Lemna* roots, and the rest were spread over a wider area round the nest (Figure 7.2 illustrates this spread over successive days). During Day 3 the swim bladder was filled and all these young could swim well. They apparently vanished, only to be seen occasionally in the *Lemna* roots, or, if a hand was passed above the surface of the tank, they sank a few centimetres when disturbed. Between Days 7 and 10, just as in *Gasterosteus*, they spread down through the water, and moved in all levels of the tank. This is the same stage at which the spread through the levels of the water occurs in young reared with their father.

7.3.3. Discussion of the effect of rearing fish as orphans compared with a normal rearing

When reared without their father orphan young of both of both species move about more freely and fill the swim bladder earlier than normally reared young. *Pygosteus* young still differ from *Gasterosteus* in having more sedentary habits, and spread up initially from the nest towards the surface, rather than spread out horizontally over the ground. *Gasterosteus* spreads rapidly and actively over the ground and only rises to the surface when it goes up to fill the swim bladder. After this *Gasterosteus* orphans may go down to the ground again, to swim about it, or they may spread across the tank below the surface. Unlike *Pygosteus* these young move between surface and ground considerably, although neither species spends any time between these extremes until a few more days have passed (Days 7 to 10).

In the absence of the male the young of neither species congregate at night, those of *Pygosteus* spending the night on fegetation above the ground level, while *Gasterosteus* remains at ground

Table 7.1

DIFFERENCES IN THE DEVELOPMENT AND BEHAVIOUR OF YOUNG STICKLEBACKS OF TWO SPECIES

	GASTEROSTEUS	PYGOSTEUS
Nest site	in open on ground	in weed above ground
Egg size	larger eggs	
Time to hatching	8 - 10 days	4 - 8 days
Length at hatching	0.6 cm.	0.45 cm.
Behaviour of young	active, will spread over ground	less active, hang in vegetation, will spread out and up from nest
Feeding	begin at Day 2	begin Day 3 or later
Time of filling swim bladder	Day 2 (or Day 3)	Day 3 (or Day 4)
Subsequent behaviour	dive back to nest, kept together by male although they can elude him gradual spread on male's territory, nights spent with father until	spread below surface, independent of male
Independent life	about Days 7 - 14 (ca. 10)	about Days 3 - 4
Spread of young through all levels of water	about Days 7 - 10	about Days 7 - 10
Development of spines begins	Day 10 - 12	Day 12
Complete	4 - 5 weeks	3 weeks

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level or at the surface. This contrasts with the normally reared *Gasterosteus* broods, where the young spent the night on the ground in the nest area. The effect of the male's parental care seems to be to confine the young to the nest area from the moment of hatching. There is also a difference between the species in the parental behaviour of the male, since the *Pygosteus* male does not retrieve his young after they have filled the swim bladder, while the *Gasterosteus* male keeps his young together for some time after this. At this time there is an additional difference in the behaviour of the young, since, whereas an orphan *Gasterosteus* may remain at the surface for some time after filling the swim bladder, young reared with their father dive back on to the nest area very rapidly when theyhave been up to the surface. Subsequently *Gasterosteus* is more likely to go to the ground, orphan *Pygosteus* remain at the surface, so that this is another interspecific difference independent of parental care.

7.4 Comparison of the two species

The main differences between the young of the two species are summarised in Table 7.1, and the details will be discussed in this section. The nest sites differ between the species, and in an attempt to make the study of the development of the behaviour of the young more directly comparable, some *Pygosteus* were left to nest in tanks provided with alga only, so that they would have to build their nests on the ground. Many did not build, but a few did build nests at ground level, although some of these then built another nest on top, thus achieving some elevation above the ground. In the two cases where males successfully raised eggs in these ground nests the behaviour of the young was the same as that already described for normal nests, although there was some indication that they were less successful in filling the swim bladder than normal broods. In any case it was noticed that many more laboratory reared *Pygosteus* than *Gasterosteus* had defective swim bladders.

The egg sizes for the two species are in the same range. Swarup (1958) quotes 1.2 - 1.7 mm. diameter, with variation within and between females, for *Gasterosteus*. Morris (1958 p.118) mentions that *Gasterosteus* eggs are much larger than those of *Pygosteus*, but, while there is a difference in average size, there is considerable overlap.

The *Gasterosteus* male differs from the rest of the family Gasterosteidae in his very high level of fanning. It may be suggested that a nest of decaying vegetation (alga, plus plant débris) built at ground level would decay rapidly, to the detriment of the eggs, and could also silt-up, unless the male was very assiduous in ventilating it, but against this may be set the experimental observation of van Iersel (1953) that developing eggs hatched normally if the male's fanning level was cut by 25%. Yet the nest is not an optimal environment for speediest development if the observation of Swarup (1958) is considered, since he reared isolated eggs to hatching stage in two days less than they take in a nest at the same temperature. Wunder (1930) describes the sudden decay of nests, partly correlated with the sudden decomposition of the glue secreted by the male when building the nest.

Pygosteus has a shorter period of development and hatches as a smaller and more helpless fish. The males of both species care for their young by first providing them with a nursery area, the nest or a newly constructed nursery for *Pygosteus*, a pit at the mouth of the nest for *Gasterosteus*. The retrieval behaviour differs in appearance since *Pygosteus* has constantly to move from the nest to the ground, scanning for fallen young. They are often "fielded" before they land.

The behaviour of the young differs. *Pygosteus* remains very still until about Day 3. *Gasterosteus* young move, jump, orient to a current, and jump when *Artemia* is pipetted towards them, although the mouth is not fully formed at hatching (Swarup, 1958) and they do not eat Artemia until Day 2. *Pygosteus* show no response to *Artemia* until their yolk is almost gone, about Day 3 or even later.

Pygosteus does not fill the swim bladder until between Days 3 and 4. From then on *Pygosteus* spread over the surface, wherever there is vegetation. The male has already kept them in a nursery well off the ground, and as soon as they can swim moderately well - that is, after filling the swim bladder - they are independent of him. In a crowded tank they may stay above the male's territory for some time, although under these conditions in the laboratory few, if any, young survive in such a brood. *Gasterosteus* spiral up, fill the swim bladder, and dive down on to the nest again. The development of their behaviour, as they avoid capture by the father, yet remain at the nest site, is very marked, and over a period of days they move out of the male's territory, keeping in small schools. At about Day 10 they also become independent of their father.

For both species between 8 and 11 days after hatching (Days 7 to 10) marks the emergence of the young into all levels of the water, instead of just the surface and the ground.

In their later growth the development of the fins is about the same for both species, but the dorsal and pelvic spines of *Gasterosteus* do not reach the adult proportions until they are about four or even five weeks old. In *Pygosteus* the dorsal spines are no higher than the juvenile median fin-fold, and are complete at about three weeks. *Pygosteus* grows faster in its first year than *Gasterosteus* (Jones and Hynes, 1950).

When the orphan young are compared with the normally reared fish of the same species they are different in several respects. In the absence of the father young *Gasterosteus* spread over the whole tank floor particularly quickly, and they fill the swim bladder a day earlier than normally reared fish. Orphan *Pygosteus* move up through the water, towards the surface, from the nest, but their rate of spread horizontally is much less than that for *Gasterosteus*. They also fill the swim bladder earlier than normally reared fish. After the swim bladder is filled the activities of orphans of both species are very similar. At first the young remain near the surface, feeding around the *Lemna* roots, although *Gasterosteus* may feed at the ground. By about Days 7 - 10 the young of both species, orphan or normally reared, move about more freely in the tank and are seen at all levels in the water. The effect of parental care appears to be to keep the newly-hatched young on the nest, to such an extent that the young do not fill the swim bladder until about a day later than they could have done. Subsequently *Pygosteus* would be on their own in any case, but for *Gasterosteus* parental care has already affected the young so that normally reared young dive back on to the nest area after filling the swim bladder, and the male keeps his young together for several days more. In her comparative study of the five species of stickleback Hall (1956) has discussed as far as possible the parental cycle and the early care of the young. She had no information on this stage of the life cycle in *Spinachia*, beyond the fact that the male builds the nest and fans the eggs for about three weeks until they hatch. *Apeltes* builds a nest in the vegetation, rather like a rudimentary *Pygosteus* nest, to which it adds as it secures more clutches of eggs. The male ventilates these eggs by sucking water over them, so that, when the eggs hatch on about their eighth day after laying, the newly-hatched young are sucked out of the nest and spread around in the vicinity. The male only rarely tries to catch the young as they come spinning out of the nest, and he lets them go immediately. These young rest motionless on the substrate unless they are disturbed, so that they resemble orphan *Pygosteus* in this respect. Hall (loc. cit. p.291) writes: "The reaction of a newly-hatched young (*Apeltes*) when it is alarmed is first to dash violently away from the source of the disturbance, and then to subside once more, either on to the ground, or into a plant. About two days after emerging from the nest the young inflate their swim bladders by gulping air at the surface, and after this they abandon their sedentary mode of life and swim about as freely as an adult." It seems that these young are as capable of avoiding danger as are *Pygosteus* and *Gasterosteus*, but they are not initially as active as the latter. *Apeltes* lives and breeds (the exact site is not known) in marine or brackish conditions mainly, and in the inter-tidal zone it may well be advantageous to the young to be dispersed immediately, into deeper water with less risk of buffeting by the waves and being thrown up into areas which dry up periodically. At least it would be difficult for the male to keep his young together.

Eucalia also builds a nest like *Pygosteus* (Hall, loc. cit.) and the male fans the eggs for the five to eight days before they hatch. Hall describes the early behaviour of the young. As they hatch they attach themselves to the roof or side of the nest, so that they hang here, tail down, holding on by the mouth, for several hours. The male goes on fanning until all the eggs have hatched, although he retrieves any young which are dislodged, and spits them back into the nest, where they attach themselves once more to the roof and walls. When all have hatched the male stops fanning and builds a nursery above the nest, to which he transfers the young. Those young which become dislodged gyrate upwards and lodge in the nursery. During the day after the transfer to the nursery the activity of the young increases to such an extent that the male is no longer able to catch them, and as a result they gradually disperse. This description might have been written of the behaviour of the father *Pygosteus* and his young, *Eucalia* is so similar.

Pygosteus young may hatch a little sooner than those of

Eucalia and *Apeltes*, although this difference may merely be due to the different temperatures at which they were kept. However, *Gasterosteus* seems to have a slightly longer egg period, in larger eggs, and it hatches a larger and more vigorous young fish. This, together with the extended period of parental care, and the high level of aggression of the male *Gasterosteus* in the parental phase (Morris, loc. cit.), can be attributed to the specialisation of this species to a more open mode of life, since it is obviously advantageous for vulnerable young fish to be cared for until they swim easily, have estoblished their feeding behaviour, and have developed their special protective spines, which are larger than those for any other species, and take longer to develop. With this care the young spend only about three weeks on their own before their spines develop fully.

Chapter 8

8.1 General Discussion

In considering the defensive devices of sticklebacks it is convenient to discuss separately the adaptations of the newly-hatched young (dependent phase) and of the older fish (independent phase).

8.21 Dependent phase

During the extremely vulnerable stages in the life-history of a fish, as eggs and newly-hatched young, some protection is, in certain taxonomic families, supplied by parental care from one or both parents, with the parents defending an area and bringing the young back there when they stray. This is most elaborately developed in the cichlids, where the adults protect the eggs and young, and in which there is special behaviour which keeps the young together (schooling) and, in some cases, near the parents, especially when a predator threatens (this sometimes includes a sort of imprinting, Baerends and Baerends-van Roon, 1950). In *Betta sp.* and other anabantids, young are spat back into the nest while they are very young, although this parental care is not as prolonged as in the cichlids (Forselius, 1957). As in the sticklebacks the centrarchids (Noble, 1934; Breder, 1936; Carter-Miller, 1963) have a paternal family system in which the male cares for his eggs and/or young. In *Cottus gobio* (Morris, 1954) the male cares for the eggs but does not retrieve the young. In *Pygosteus* parental care extends only over the few days before the young fill the swim bladder, but it goes on for some days longer in *Gasterosteus*, by which time the young are quite well developed, move very actively, and have established their feeding habits, although their dorsal and pelvic spines are not fully developed at this stage. The end of this phase in *Gasterosteus* is marked by the dispersion of the young, but until this time the male rounds up his brood at dusk each night, and they all rest together on the nest area during darkness. Presumably this protects the young against being scattered and perhaps preyed upon by nocturnal hunters in the darkness when the largely visual stickleback father could do little to protect them.

Young *Gasterosteus* also have their own escape responses to a large moving object, including their ever-present father. At first they burrow down into the nest material, later they give a short jump away. The latter response is difficult to release at first but becomes more conspicuous as the young swim better. With this improvement in swimming the young begin to move into weedy patches on the edges of the male's territory, and they often avoid being retrieved by sinking a little into the weed as the male patrols nearby. By learning the position of hiding places in the vicinity of the nest area the young acquire additional protection during this phase of parental care. *Gasterosteus* reared with their father dive back on to the nest area after filling the swim bladder, whereas orphans show no such site attachment, so that even in their first three days *Gasterosteus* learn the position of the nest. From the first *Pygosteus* young are more quiescent, relying on concealment in the vegetation in which they may hang, and during the days after hatching they spread vertically in the vegetation, while the more active *Gasterosteus* spread out extensively in the horizontal direction from the nest, over the ground. The behaviour of *Pygosteus* young is much more like that reported for the other sticklebacks studied (Hall, 1956), and it seems probable that the greater activity of young *Gasterosteus* has evolved along with the extended period of parental care.

8.2.2. Independent phase

8.2.2.1. Response to predators

There is a smooth transition in the responses of the young to their father or other larger fish as they achieve more skilful locomotion, until finally the only difference between them and independent fish is that the father still protects the area around them from predation.

Responses to the predators may be grouped as those which give the stickleback more information (fixation, head-down posture, approach, following) and those which reduce the chance of the predator attacking successfully. The latter act by reducing the chance of detection (precautionary behaviour by keeping in weed, keeping at a distance), or by reduction of movement (slow swimming, freezing). The predator can be caused to reorientate (sinking, zig-zag swimming, jumping), and there is also simple avoidance by fast swimming. Bending the tail and an increase in the respiratory rate may be taken as preparations to escape. Spine raising during all these responses presumably prepares for locking the spines into position, which increases the chance of being released once grasped (Hoogland et al., 1957).

Yawning and stretching have no obvious protective function, though it is conceivable that the use of the muscles prepares the animal for active flight.

Some of the above responses are to be seen in a strange environment, where the exploring animal moves slowly, low in the water, with its spines half raised or fluctuating. Obviously for an animal to exist in nature it must explore new areas, even if they may harbour predators.

A predator such as the pike has counter-adaptations to the defences of its prey, and it exploits the chinks in the stickle-backs' behavioural armour by skilfully stalking, even in the open, by sneaking up fast when the prey faces in the opposite direction, and by waiting, almost motionless, for a longer period of time than the duration of fright in the prey. In this way an animal which has been badly frightened, and is beginning to hazard more normal activities, is suddenly picked off by the pike. For this method of hunting the pike is relying on its excellent camouflage and the slightest protection of traces of shadow from floating weed fragments, as well as on the delicacy of its movements. The pike's use of weed not only gives it cover for stalking but also presumably helps to conceal it from its own predators. This lurking beneath weed was noted in the laboratory. In the field, where the light can come only from above, there would be deeper shadow below weed available to escaping or watchful fish. Neither the perch nor the trout is capable of the stalking shown by the pike and it is characteristic for these predators to snap up their prey unawares or to pursue it until it escapes or is cornered.

8.2.2.2. Factors affecting the response to predators

Responses to pike, perch, or trout in the laboratory are basically similar, and differences seem mainly to reflect the different hunting behaviour of predators. The pike was the main predator used in the quantitative tests, but in a pilot study it was found that inexperienced young fish responded in the same way to aggressive male sticklebacks, to young pike, to young perch, to a tench and to large minnows. They showed no response to a model pike. This suggested that the young were reacting to the stimulus situation "Large body, actively moving".

Experience was shown to affect the behaviour, so that fish would respond to a non-swimming pike more often (Table 6.4A) pp F.97 and experienced fish were more likely to respond to eye movements and other small actions by the predator. The young fish kept with a variety of predators soon learned the measures necessary to avoid them - they stayed away from the territory of a male stickleback, ignored the minnows and the tench, and were watchful with the pike and perch, the response to the pike being the more intense. (No investigation has been made of how far experience gained from the pike might be generalised to affect responsiveness to other predators.) As a result of experience of the predator, moreover, sticklebacks became more likely to take more effective precautionary behaviour, i.e., they used the weed more, reacted at a greater distance and to slighter cues from the predator.

The above experiments showed that experience gained two or more days earlier affected the response to a pike, but it was also found that different rearing conditions had an effect. This was shown best in Gasterosteus, for which there was most information. Orphans reared without fathers showed some changes improving survival (more likely to take fright, and at a greater distance) but others diminishing it (less likely to use weed, more likely to approach pike). Fish kept too long with their father showed exactly the opposite effects, except that they were no more likely than normally-reared fish to use weed as a retreat (Figure 6.1). As already discussed it seems that these effects might be due to some kind of learning by the young during the parental phase of

(a) how to avoid their father when he comes to gather them up, and

(b) habituation to a large fish nearby, even when it attempted to snap them up.

Under the laboratory conditions the fish tested including the "normals" - were kept only with their own kind for two or three months before their first exposure to the pike. In more natural conditions, when young sticklebacks are likely to see fish of many sizes of their own and other species, such differences as have been ascribed to differences in rearing might well disappear. Experience of the pike given even to broods with abnormal parental experience quickly smoothes over any differences, so that they behave like experienced normal fish.

8.2.2.3. Species differences

Differences in the anti-predator responses between the species have already been mentioned for the behaviour of the young before they become independent. Though there is basically the same behavioural repertoire for the two species, these differences persist in the independent fish. In their responses to an unfamiliar environment, Pygosteus individuals and pairs spent more time in cover, and deeper in it, and they swam more rapidly across open spaces between clumps of weed. In response to predators they used more intense alarm reactions (on the scale used) and were less likely to investigate the predator.

That these differences affected their risk from predators can be confirmed by the data from the experiments, which showed that 12% of the 227 Gasterosteus tested and 4% of the 92 Pygosteus tested, were taken. These differences are significant at $p \leq 0.02$, using the Fisher Exact Test. In these experiments the sticklebacks were considerably smaller than their pike predator (prey/predator size ratio 3.0 to 8.0) and once taken, all but two of the Gasterosteus and all the Pygosteus, were swallowed, showing that the spines afforded virtually no protection when the size differential was as large as this. However, with predators of a similar size (prey/predator size ratio ca. 1.5 to 4.0), Hoogland et al. (1957) showed the effectiveness of the spines of adult sticklebacks against pike and perch. Of the fish grasped by the pike there was a 60% chance for Gasterosteus that they would be let go, compared with a 10% chance for Pygosteus (calculated from Hoogland et al., loc. cit., Figure 13). With the perch as predator both species had a high chance of escape after grasping, 90% for *Gasterosteus*, 55% for *Pygosteus*, so that against the perch the spines of *Pygosteus* and *Gasterosteus* confer much more protection than they give from the pike (Figure 6, loc. cit.). As far as protective behaviour is concerned, *Pygosteus* appears to be more difficult for a pike to catch than *Gasterosteus* (three times); once caught it is less likely to be let go on account of its spines (seven times). How these two effects will balance out will obviously depend largely on the relative size of prey and predator, and probably there are other factors, such as the availability of alternative prey.

There is disappointingly little information in the literature on the relative abundance of *Pygosteus* and *Gasterosteus* in nature. Hartley (1947) collected extensively in several different areas,and reports that *Gasterosteus* occurs patchily, although in some abundance where it does exist. From my own observations around Oxford, and from the proportions of the two species taken by Jones and Hynes (1950), and Cragg-Hine (1965, pers. comm) *Gasterosteus* not only occur in more places than *Pygosteus*, but also, where both are found, the ratio is roughly 2 - 3 *Gasterosteus* to one *Pygosteus*. As shown already in Chapter 3, *Gasterosteus* is much more common in the reported diet of the various fish and bird predators. There is not sufficient detailed evidence to decide at present what the relative risk of the two species may be in nature.

The spines afford some protection to *Gasterosteus*, but, as mentioned, their effect diminishes when the predator becomes very much larger than the stickleback. Healey's data from one locality

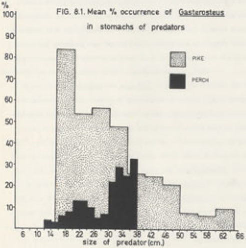
FIG. 8.1. Mean % occurrence of Gasterosteus in stomachs of predators

(1954, 1956), given together as Figure 8.1 show that as perch get larger they are more likely to take sticklebacks. However the reverse is true for pike above a certain size (and for large perch in other habitats, see Chapter 3). How far these trends apply over a much greater size range, and in other localities is uncertain, but it would seem understandable in terms of hunting efficiency that a very large predator will cease to spend time catching small prey, even if the defensive mechanisms of the prey - such as the spines - do not in the least deter him. This means that the protective devices which a species evolves against predators need to be suited only to a limited size range of predators, and this is what the two species of stickleback studied here seem to have done. Both depend in part on behaviour and on spines, but the spines are more important for Gasterosteus than for Pygosteus, while the relative importance of behaviour is greater for Pygosteus.

References

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

________ 1938. Some observations on the biology of the Trout (*Salmo trutta*) in Windermere.
J. Anim. Ecol. 7:333-349.

________ 1939. A note on the food of Pike, *Esox lucius*, in Windermere.
J. Anim. Ecol. 8:72-75.

Allen, W. E. 1920. Behaviour of loon and sardines.
Ecology I: 309-310.

Baerends, G. P. and J. M. Baerends-van Roon. 1950. An introduction to the study of the ethology of Cichlid fishes.
Behaviour, Suppl. I. 243pp.

Baggerman, B. 1957. An experimental study on the timing of breeding and migration in the three-spined stickleback (*Gasterosteus aculeatus* L.)
Archs. néerl. Zool. 12:105-317.

________ 1960. Factors in the diadromous migrations of fish.
Symp. Zool. Soc. Lond. 1:33-60.

________ 1964. On the endocrine background of stickleback behaviour.
Archs. néerl. Zool. 16:159-163.

Bainbridge, R. 1958. The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat.
J. exp. Biol. 35:109-133.

Barlow, G. W. 1961. Ethology of the Asian Teleost, *Badis badis*.
I. Locomotion, maintenance, aggregation and fright.
Trans Ill. St. Acad. Sci. 54:175-188.

__________ 1963. Ethology of the Asian Teleost *Badis badis*.
II. Motivation and signal value of the colour patterns.
Anim. Behav. 11:97-105.

Berlyne, D. E. 1960. Conflict, Arousal, and Curiosity. McGraw-Hill.

Bertin, L. 1925. Recherches bionomiques, biométriques et systématiques sur les Épinoches (Gastérostéidés)
Annls. Inst. océanogr., Monaco II : 1-204.

Beukema, J. J. 1963. Experiments on the effects of the hunger state and of learning process on the risk of prey of the three-spined stickleback, *Gasterosteus aculeatus*.
Archs. néerl. de Zool. 15:358-361.

Braum, E. 1963. Die Ersten Beutefanghandlungen junger Blaufelchen (*Coregonus wartmanni* Bloch) und Hechte (*Esox lucius* L.)
Z. Tierpsychol. 20:257-266.

Breder, C. M. 1936. The reproductive habits of the North American Sunfishes (Centrarchidae)
Zoologica 21:1-47.

__________ 1959. Studies on social groupings in fishes.
Bull. Am. Mus. nat. Hist. 117:393-482.

Carter Miller, H. The behavior of the pumpkinseed Sunfish Lepomis gibbosus (Linnaeus), with notes on the behavior of the other species of Lepomis and the pigmy sunfish, Elassoma evergladei. Behaviour 22:88-151.

Creutz, G. 1963. Ernährungsweise und Aktionsradius der Lachmöwe (Larus ridibundus L.) Beitr. Vogelk. 9:3-58.

__________ 1964. Ernährungsweise, Nahrungsauswal und Abwehr des Graureihers (Ardea cinerea L.) Zool. Abh. Mus. Tierk. Dresden. 27:29-64.

__________ 1965. Stichlinge als Vogelnahrung. Orn. Beob. 62: 24-26.

Deelder, C.L. 1951. A contribution to the knowledge of the stunted growth of Perch (Perca fluviatilis) in Holland. Hydrobiol. 3: 357-378.

Eibl-Eibesfeldt, I. 1962. Freiwasserbeobachtungen zur Deutung des Schwarmverhaltens verschiedener Fische. Z. Tierpsychol. 19:165-182.

Errington, P. L. 1946. Predation and vertebrate populations. Q. Rev. Biol. 21:144-177, 221-245.

Fabricius, E. and K.-J. Gustafson, 1958. Some new observations on the spawning behaviour of the Pike, Esox lucius L. Rep. Inst. Freshwat. Res. Drottningholm 39:23-54.

Forselius, S. 1957. Studies of Anabantid fishes I. II. III. Zoologiska Bidrag. Från Uppa., 32:93-598.

Frost, W. E. 1946. The food of eels from the Windermere catchment area. J. Anim. Ecol. 15:43-53.

Frost, W. E. 1954. The food of pike, *Esox lucius* L., in Windermere
J. Anim. Ecol. 23:339-360.

Göz, H. 1941. Über den Art - und Individualgeruch bei Fischen.
Z. Tierpsychol. 29:1-45.

Hall, M. F. 1956. A comparative study of the reproductive behaviour of the sticklebacks (Gasterosteidae).
D.Phil. thesis, Oxford.

Harris, J. E. 1937. The mechanical significance of the position and movements of the paired fins in the Teleostei.
Publ. Carnegie Inst., Pap. Tortugas Lab. 31:171-189.

__________ 1953 Fin patterns and mode of life in fishes. In "Essays in Marine Biology" Oliver and Boyd.

Hartley, P. H. T. 1947-48. The natural history of some British freshwater fishes. Proc. zool. Soc. Lond. 117:129-206.

__________ 1948. Food and feeding relationships in a community of freshwater fishes.
J. Anim. Ecol. 17:1-13.

Healey, A. 1954. Perch, *Perca fluviatilis*, in three Irish lakes.
Scient. Proc. R. Dubl. Soc. 26:397-410.

__________ 1956. Pike, *Esox lucius*, in three Irish Lakes.
Scient. Proc. R. Dubl. Soc. 27:51-68.

Heiligenberg, W. 1963. Ursachen für das Auftreten von Instinktbewegungen.
Z. vergl. Physiol. 47:339-380.

Heiligenberg, W. 1964. Ein versuch zur ganzheitbezogenen Analyse des Instinktverhaltens eines Fisches (*Pelmatochromis subocellatus kribensis* Boul., Cichlidae).
Z. Tierpsychol. 21: 1-52.

Heuts, M. J. 1947. The phenotypical variability of *Gasterosteus aculeatus* L. populations in Belgium.
Meded K. vlaam Acad Wetens. 9:5-63.

__________ 1949. Racial divergence in fin-ray patterns.
J. Genet. 49: 183-191.

Hoar, W. S. 1958. The evolution of migratory behaviour among juvenile Salmon of the genus *Oncorhynchus*. J. Fish. Res. Bd. Can. 15: 391-428.

__________ 1963. The endocrine regulation of migratory behaviour in anadromous Teleosts.
Proc. 16th Int. Congress Zool. 3:14-20.

Hoogland, R. D. 1951. On the fixing-mechanism in the spines of *Gasterosteus aculeatus* L.
Koninklijke Nederlandse Akademie van Wetenschappen 54C:171-180.

__________, Morris, D. and N. Tinbergen. 1957. The spines of Sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defence against predators (*Perca* and *Esox*).
Behaviour 10:205-236.

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.

Jones, J. W. and H. B. N. Hynes 1950. The age and growth of *Gasterosteus aculeatus*, *Pygosteus pungitius* and *Spinachia vulgaris*, as shown by their otoliths.
J. Anim. Ecol. 19:59-73.

Kalleberg, H. 1958. Observations in a stream tank of territoriality and competition in juvenile Salmon and Trout (*Salmo salar* L. and *Salmo trutta* L.).
Rep. Inst. Freshwat. Res. Drottningholm 39:55-98.

Keenleyside, M. H. A., 1955. Aspects of schooling behaviour of fish.
Behaviour 8: 183-246.

Kruuk, H. 1963. Predators and anti-predator behaviour of the Black-headed gull (*Larus ridibundus* L.).
Behaviour Suppl. 11 300pp.

Lagler, K. F., J. E. Bardach, R. R. Miller. 1962. Ichthyology.
Ann Arbor.

Le Cren, E. D. 1958. Observations on the growth of Perch (*Perca fluviatilis* L.) over twenty-two years, with special reference to the effects of temperature and changes in population density.
J. Anim. Ecol. 27:287-334.

Leiner, M. 1929. Ökologische studien an *Gasterosteus aculeatus*.
Z. Morph. Ökol. Tiere 14:360-399.

__________ 1930. Fortsetzung der Ökologischen Studien an *Gasterosteus aculeatus*.
Z. Morph. Ökol. Tiere 16:499-540.

Leiner, M. 1931a. Ökologisches vom *Gasterosteus aculeatus* L. Ergänzende Mitteilungen zu den Abhandelungen von W. Wunder und M. Leiner. Zool. Anz. 93:317-333.

________ 1931b. Der Laich und Brutpflegeinstinkt des Zwergstichlings, *Gasterosteus* (*Pygosteus*) *pungitius* L. Z. Morph. Ökol. Tiere 21: 765-788.

________ 1934 Die drei europäischen Stichlingen (*Gasterosteus aculeatus* L., *Gasterosteus pungitius* L. und *Gasterosteus spinachia* L.) und ihre Kreuzungsprodukte. Vergleichende Betrachtung ihrer Laichzeit, ihrer Körperformen und ihrer Brutpflegetätigkeit. Z. Morph. Ökol. Tiere. 28:108-154.

Lewis, H. F. 1929. The natural history of the Double-crested Cormorant, *Phalacrocorax auritus auritus*. Cornell University thesis, Ithaca. N.Y.

Lindsey, C. C. 1962(a) Experimental study of meristic variation in a population of Threespine Sticklebacks, *Gasterosteus aculeatus*. Can. J. Zool. 40:271-312.

________ 1962(b) Observations on meristic variation in Ninespine Sticklebacks, *Pungitius pungitius*, reared at different temperatures. Can. J. Zool. 40:1237-1247.

Lishev, M. N. 1950. The feeding and feeding inter-relationships of the predatory fishes of the Amur River basin. Trans. Ichthyol. Exped. to the Amur I. (Reference in Nikolsky, 1963).

Lumsden, W. H. R. and A. J. Haddow. 1946. The food of the Shag (*Phalacrocorax aristotelis*) in the Clyde Sea area. J. Anim. Ecol. 15:35-42.

McCormack, J. 1965. Observations on the Perch population of Ullswater. J. Anim. Ecol. 34:463-478.

McPhail, J. D. 1963. Geographic variation in North American Ninespine Sticklebacks, *Pungitius pungitius*. J. Fish. Res. Bd. Canada 20:27-44.

Madsen, F. J. 1954. On the food habits of diving ducks in Denmark. Danish Rev. Game. Biol. 2:157-266.

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.

Meesters, A. 1940. Über die Organisation des Gesichtfeldes der Fische. Z. Tierpsychol. 4:84-149.

Morris, D. J. 1954. The reproductive behaviour of the River Bullhead (*Cottus gobio* L.) with special reference to the fanning activity. Behaviour 7:1-32.

__________ 1955. The reproductive behaviour of the Ten-spined Stickleback (*Pygosteus pungitius* L.). D. Phil. thesis, Oxford.

__________ 1958. The reproductive behaviour of the Ten-spined Stickleback (*Pygosteus pungitius* L.) Behaviour Suppl. 6, 154pp.

Müller, K. 1954. Investigations on the organic drift in North Swedish streams.

Rep. Inst. Freshwat. Res. Drottningholm 35: 133-148.

Munro, J. A. and W. A. Clemens. 1937. The American Merganser in British Columbia and its relation to the fish population.

Bull. biol. Bd. Can. 55:1-50.

Nikolsky, G. V. 1963. The Ecology of Fishes. Translated from the Russian. Academic Press.

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.

__________ 1957. Feeding habits of Trout in a stream of Northern Sweden.

Rep. Inst. Freshwat. Res. Drottningholm 38:154-166.

Noble, G. K. 1934. Sex recognition in the Sunfish, *Eupomotis gibbosus* L.

Copeia 4: 151-155.

Odiorne, J. M. 1957. Color changes. Chapter 8 in Physiology of Fishes, Vol.2., ed. M. E. Brown. 1957. Academic Press.

Olney, P. J. S. and D. H. Mills. 1963. The food and feeding habits of Goldeneye, *Bucephala clangula* in Great Britain.

Ibis 105:293-300.

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.

Owen, D. F. and G. C. Phillips. 1956. The food of nestling Purple Herons in Holland.
British Birds 49:494-499.

Palmer, R. S. 1962. Handbook of North American Birds. vol.1 Loons through Flamingoes.
Yale University Press.

Parakestov, I. A. 1958. The protective significance of spines and thorns in fishes.
Zh. Obshch. Biol. 19, no.6 (reference from Nikolsky, 1963).

Pfeiffer, W. 1963. Alarm substances.
Experientia 19:113-168.

Phillips, G. C. 1962. Survival value of the white coloration of gulls and other sea birds.
D. Phil. thesis, Oxford.

Polyak, S. 1957. The Vertebrate Visual System.
Univ. of Chicago Press.

Radakov, D. V. 1958. On the adaptive significance of the shoaling behaviour of young coalfish (*Pollachis virens* L.).
Vop. Ikhtiol. no.11. (reference from Nikolsky 1963).

Schutz, F. 1956. Untersuchungen über die Schreckreaktion bei Fischen und deren Verbreitung.
Z. vergl. Physiol. 38:84-135.

Segaar, J. 1961. Telencephalon and behaviour in Gasterosteus aculeatus
Behaviour 18:256-287.

Sevenster, P. 1961 A causal analysis of a displacement activity.
Behaviour Suppl. 9, 170pp.

Sevenster-Bol, A. 1962. On the causation of drive reduction after a consummatory act (in Gasterosteus aculeatus) Archs. néerl. Zool. 15: 175-236.

Siegel, S. 1956. Non-parametric Statistics for the Behavioral Sciences.
McGraw Hill.

Steven, G. A. 1933. The food consumed by Shags and Cormorants around
the shores of Cornwall (England).
J. mar. biol. Ass. U.K. 19:277-292.

Swarup, H. 1958. Stages in the development of the stickleback Gasterosteus aculeatus L.
J. embryol. Exp. Morph. 6:373-383.

Swynnerton, G. H. and E. B. Worthington. 1940. Note on the food of
fish in Haweswater (Westmorland).
J. Anim. Ecol. 9:183-187.

Tugendhat, B. 1959. Studies of the effects of thwarting in sticklebacks.
D. Phil. thesis, Oxford.

__________ 1960(a). The normal feeding behaviour of the Three-spined
Stickleback (Gasterosteus aculeatus L.)
Behaviour 15:284-318.

__________ 1960(b). The disturbed feeding behaviour of the Three-spined
Stickleback. I. Electric shock is administered in the food area.
Behaviour 16: 159-187.

__________ 1960(c). Feeding in conflict situations and following
thwarting.
Science 132:896-897.

van Dobben, W. H. 1952. The food of the Cormorant in the Netherlands.
Ardea 40:1-63.

van Iersel, J. J. A. 1953. An analysis of the parental behaviour of the male Three-spined Stickleback (*Gasterosteus aculeatus* L.)
Behaviour Suppl. 3. 159pp.

________________ 1958. Some aspects of the territorial behaviour of the male Three-spined Stickleback.
Arch. néerl. Zool. 13 Suppl.:383-400.

van Mullem, P. J. and J. C. van der Vlugt. 1964. On the age, growth and migration of the anadromous stickleback, *Gasterosteus aculeatus* L., investigated in mixed populations.
Archs. néerl. Zool. 16:111-139.

von Frisch. 1941. Über einen Schreckstoff der Fischhaut und seine biologische Bedeutung.
Z. vergl. Physiol. 29:46-145.

Walton, Izaac. 1676. The Compleat Angler (Fifth Edition).

Wickler, W. 1960. Die Stammesgeschichte typischer Bewegungsformen der Fisch-Brustflosse.
Z. Tierpsychol. 17:31-66.

Witherby, H. F., Jourdain, F. C. R., Ticehurst, N. F., and B. W. Tucker. 1943-44. The Handbook of British Birds.
Witherby.

Wunder, W. 1930. Experimentelle Untersuchungen am dreistachligen Stichling (*Gasterosteus aculeatus* L.) während der Laichzeit.
Z. Morph. Ökol. Tiere 16: 453-498.

Wunder, W. 1931. Brutpflege und Nestbau bei Fischen.
Ergebn. Biol. 7:118-195.