Proximate determinants of stickleback behaviour: an evolutionary perspective

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Many factors initiate and control behaviour in threespine stickleback. Photoperiod, temperature, and other environmental variables typically have pervasive effects on responsiveness and may be exerted indirectly over a long time scale. Their effects on processes such as migration, osmoregulation, and reproductive cycling are mediated largely through the endocrine system. This aspect of stickleback biology has provided fertile grounds for study by physiologists (Guderley Chapter 4 this volume). Here I concentrate on the more immediate external factors that influence reproductive behaviour of threespine stickleback on the breeding grounds. These proximate factors tend to operate on a shorter time scale and to affect neural control of behaviour.

I focus on the threespine stickleback, *Gasterosteus aculeatus*, although findings from other species are sometimes considered as they relate to stickleback evolution and behaviour. I have also included some of the very early references on a topic whenever appropriate. This work is often overlooked because it is not published in English, is otherwise inaccessible, or is written in a style that does not conform to modern standards and is therefore assumed to be unworthy of serious attention by biologists. Nevertheless, a fair number of these works include accurate and insightful observations, and the enthusiasm and clear writing style of their authors can serve as an inspiration to workers today.

I have organized this chapter chronologically, to reflect the order in which functionally related activities typically appear during the reproductive cycle of the stickleback and to emphasize how behaviour performed at one stage often brings the fish into a situation that elicits the next stage of its reproductive cycle.

Beginning with the fish's arrival at their breeding grounds, I first discuss the factors that influence aggression and the acquisition of a breeding territory by the male. I then discuss the factors involved in his selection of a nest site and in the construction of the nest. The completion of the nest leads to the courtship phase, in which mating occurs. I consider this
from the perspective of both the male and the female stickleback, and conclude the chapter with a discussion of proximate factors involved in the male's care of his offspring to the point that they become independent.

Visual cues exert a dominant influence on behaviour of all gasterosteid fishes, and in this family vision has been the most studied sense. Although the discussion reflects this emphasis, I consider evidence for the involvement of other senses in mediating reproductive behaviour in stickleback.

This chapter is intended to serve several purposes: to describe the behaviour to which contributors refer in other chapters, to provide an up-to-date review of work on the stimuli that affect stickleback behaviour on the breeding grounds, and to present some preliminary or unpublished data that may suggest avenues for future research.

In keeping with the theme of this book, I try to maintain an evolutionary perspective in so far as this is practicable. Many of the experiments on stickleback behaviour that are discussed below, however, were conducted during a time when ethology was developing rapidly. It was therefore logical to interpret the proximate features that so dramatically influenced an animal's behaviour within the ethological context of sign stimuli and innate releasing mechanisms. These interpretations are not invalid, but another perspective, here an evolutionary one, can often provide insights that may otherwise be overlooked.

AGGRESSION AND TERRITORIALITY

The behaviour employed by male threespine stickleback to establish and maintain territories or to defend themselves and their spawn from predators has long interested biologists (e.g. Anonymous 1830; Warington 1855). The kind of intruder can profoundly influence the form of aggression. Territorial males typically direct ritualized head-down threats, paralleling, and overt attacks toward solitary individuals of the same (van Iersel 1953; Morris 1958; Li and Owings 1978a) or related (Rowland 1983a,b) species, but may respond very differently to groups of conspecifics or other predators (Huntingford et al. Chapter 10; Foster page 394 this volume). This variation suggests that aggression toward rivals and aggression toward predators may represent different phenomena, although at some level they may be causally linked (e.g. Huntingford 1976a).

In view of the central importance of rival-induced aggression for the establishment and maintenance of breeding territories, I now discuss the role of various proximate factors that are believed to play an important role in the initiation and control of conspecific aggression in threespine stickleback. These include male colour and size, the posture, movement, and location of the rival male, and previous experience with rivals.
Colour

Visual cues probably exert the strongest sensory influence on aggressive behaviour in sticklebacks. When males of closely related sympatric species were presented in watertight glass cylinders at various distances from a nest, each species evoked a different level of response from the nest-owner (Rowland 1983a,b; Fig. 11.1).

The conspicuous nuptial colour patterns that are unique to reproductive males of each species are presumed to serve as cues on which much of this species discrimination and conspecific-directed aggression is based. The most widely cited evidence of this is the experiment by Tinbergen and his co-workers (Tinbergen 1948, 1951). When a crude dummy with a red underside and a more detailed but non-red dummy were presented to territorial threespine stickleback males, the red dummy elicited the most attacks. Although the red dummy's effectiveness at eliciting attack was slightly enhanced by adding a light blue back and blue eyes, males neglected most other features (Tinbergen 1953). This led Tinbergen to conclude that it was the male colour pattern, particularly the red underside, that served as the primary feature or 'sign stimulus' eliciting territorial aggression in this species.

![Graph](image)

**Fig. 11.1** Attack rate of five territorial male threespine stickleback to three species of intruder males presented at four distances from the territorial male's nest (mean ± SE). The intruder species were the threespine stickleback, *Gasterosteus aculeatus* (GA), the blackspotted stickleback, *G. wheatlandi* (GW), and the fourspine stickleback, *Apeltes quadracus* (AQ). There is a decrease in bite rate to each intruder species with increasing distance from the nest (Page's $L = 141$ for GA, $L = 138$ for GW, and $L = 147$ for AQ, where $k = 4$ and $N = 5$ for each intruder species; all $P < 0.05$; Page 1963).
Subsequent attempts to verify the effects of nuptial colour on stickleback aggression have yielded mixed results. When Muckensturm (1969) presented males from a European population of the threespine stickleback with dummies of different colours, the most effective colour for provoking attack varied among individuals. Males did not attack red more than other colours, and when males were tested under illumination from which red wavelengths were removed by a blue filter, their aggressive reactions did not decrease (Chauvin-Muckensturm 1979). Similarly, Peeke et al. (1969), studying a Californian (USA) population of threespine stickleback, and Wootton (1971b), studying a British Columbian (Canada) population, failed to detect differences in the mean rates of attack directed by territorial males at silver dummies versus dummies that were partially or entirely red.

In other studies, the rates at which males attacked non-red dummies versus dummies with a red underside differed, but in the opposite direction. My own research on stickleback from a Long Island (New York) marine population and a Dutch anadromous population provided results that contradicted those described by Tinbergen (1953). Overall, the non-red dummy received more attacks than did the dummy with a red underside, but a small minority of males showed the opposite response (Rowland 1982a; Rowland and Sevenster 1985). In contrast, Collias (1990) reanalysed results he had obtained from Tinbergen’s laboratory in Leiden in 1947 and confirmed statistically that male threespine stickleback attacked dummies with a red underside more than those without red. But Collias’ results also revealed that the attack-provoking effect of red was less robust than had often been implied in the literature: several of his males showed little tendency to attack the red dummy more than the non-red one, even though he presented the dummies close to (7–13 cm) the males’ nests in an attempt to maximize aggression.

One plausible explanation for the different results from these studies is that red elicits fear as well as aggression in males, but that the relative increase in these two factors is context dependent (Rowland 1982a). Specifically, when the dummy is presented near the nest, as in Collias’ study, it may elicit aggression more effectively than it elicits fear, and thus operate as a ‘sign stimulus’ for aggression (sensu Tinbergen 1951). But when the dummies were placed farther from the nest, as in the studies by Rowland (20 cm from the nest) and Rowland and Sevenster (30 cm from the nest), the consequent decrease in aggression (van Iersel 1958; Fig. 11.1 this chapter) might have led to relatively more fear of the red dummy, hence relatively more biting at the non-red one. Thus, a dual effect of red colour (i.e. provocation and intimidation) could explain the discrepancies among studies.

Discrepancies among studies of nuptial colour effects in threespine stickleback could also be accounted for by differences in illumination used in aquarium experiments (Reimchen 1989). Because differences in the
relative amounts of horizontal, upwelling, and downwelling light affect hue or brightness contrast of objects viewed underwater (for reviews see Lythgoe 1979; Nicol 1989), the dimensions of aquaria and the source and placement of their illumination may affect the way nuptial colour is perceived by stickleback. Although this possibility cannot account for differences among individuals within a given study, it is an important consideration for future research design.

Another possibility is that differences in hue, saturation, or extent of red used to colour the dummies, in the procedure used to present the dummies, or in an individual's experience or genotype, affected the motivational balance between attack and avoidance behaviour in males. These differences could also have led to the conflicting results obtained in past studies. Subsequent findings that males more often abort attacks on dummies with a red underside, unusually large body size, or head-down threat posture, than on dummies lacking these features, are consistent with this hypothesis (Rowland 1983a; Rowland and Sevenster 1985).

Recent studies reveal wide interpopulational variation for many aspects of behaviour in threespine stickleback, including aggression (for reviews see chapters by Hart and Giles, Huntingford et al., Bakker, Foster and McPhail this volume). This variation could also account for some of the inconsistencies that have arisen in studies of male response to nuptial colour.

In the threespine stickleback, there is a positive association between the male's colour score and tendency to attack intruders (Rowland 1984; McLennan and McPhail 1989b). This association could explain why the red underside should be intimidating to opponents and why brighter males tend to dominate duller ones (Bakker and Sevenster 1983). The observation that male threespine stickleback in some populations maintain intense nuptial colour, even when they are guarding offspring but no longer courting females (Moodie 1972a,b; Kynard 1978a), suggests that the intimidation effect may also play a role in the nest-owner's defence of nest and offspring.

Although some accounts probably overstate the effectiveness of red for provoking attack in G. aculeatus males, this does not necessarily invalidate a sign stimulus interpretation of nuptial colour, as some imply (Reiss 1984). On balance, the response of male stickleback to the sight of a red underside in an opponent is seemingly affected in a way more complicated than implied in the original reports and even in recent textbooks on animal behaviour (e.g. Grier 1984; McFarland 1985; Drickamer and Vessey 1992).

If the red underside is interpreted broadly as an index of male condition rather than as a releaser of aggression (Rowland 1982a, 1984; Milinski and Bakker 1990), then the inter- and intrapopulational variation reported for male response to this feature is not unexpected. Just as individuals differ in their tendency to attack or avoid dummies (Pecke et al. 1969; Wootten 1971b; Rowland 1981b; Bakker and Sevenster 1983; Rowland and Sevenster 1985) and live males (Sevenster 1961; Wootten 1971b;
Rowland 1984), so too will the provoking and intimidating effects of the red underside vary among them. If intrasexual selection has played a role in the evolution of male nuptial color in stickleback, then it is probably the intimidating effect that maintains or elaborates red color in *G. aculeatus* populations. Indeed, this effect may even lead brighter males to dominate duller ones in this species (Bakker and Sevenster 1983), although the possibility that brighter males dominate merely because they behave more aggressively or are physically superior needs to be tested.

**Size**

Initially, Tinbergen (1953) concluded that intruder size had little effect on aggression in stickleback, because territorial males reacted aggressively even to a red mail truck passing in the distance outside the laboratory window. However, he also discovered that a dummy three times the size of a stickleback would provoke an attack when presented at a distance, but not when presented in the aquarium. In consequence, he acknowledged that these abnormally large objects were probably effective in provoking attack because at a distance they subtended a small angle on the retina. Thus, what may be critical in this situation is the apparent size of the intruder, in much the same way that the apparent size of prey has been found to be important in stickleback foraging behaviour (Gibson 1980; but see Hart and Gill page 232 this volume).

Although in many fishes, larger body size may confer an advantage in aggressive interactions, it has been assumed that male stickleback are similar enough in size that this factor is unimportant (Wootton 1976). In fact, most studies that have looked for a size advantage in aggressive interactions between male *G. aculeatus* failed to find a statistically significant effect (van den Assem 1967; Sargent and Gebler 1980; Bakker 1986; Fitzgerald and Kedney 1987). In some populations, however, males differ by 15 per cent or more in body weight, and in such cases heavier males tend to defeat their smaller opponents when the fish compete directly for the same territory (Rowland 1989a). Larson (1976), too, found that in a small British Columbia lake, a larger benthic form of *G. aculeatus* excluded a smaller limnetic form of the species from a vegetated area that both forms chose to occupy.

I examined the effect of body size on aggressive encounters more directly by presenting dummies of different sizes to territorial male threespine stickleback (Rowland 1983a). When the proportions of aborted attacks ('backoffs'; see Rowland and Sevenster 1985) to total attacks (backoffs + bites) were compared, it was found that males aborted a greater proportion of attacks to larger dummies than to smaller ones (Fig. 11.2). This result suggests that larger males are more intimidating than smaller males. If this is true, then size effects could confer behavioural as well as physical
Fig. 11.2  Per cent of attacks (mean ± SE) by nine territorial male threespine stickleback that were aborted when males were presented with dummies of three different standard lengths. Each male was presented in random order with each dummy once at a constant distance from the nest. There is an increase in the proportion of attacks aborted with increasing dummy size ($P < 0.01$; Page's $L = 119, k = 3, N = 9$).

advantages to the larger opponent in aggressive encounters between stickleback, especially in interspecific interactions, where size differences are often considerable (Rowland 1983a,b; Gaudreault and FitzGerald 1985).

It is not known whether males fail to attack oversized dummies (Tinbergen 1953; Rowland 1983a) because they fail to recognize them as stickleback, possibly perceiving them as potential predators, or because they are merely intimidated by them. It is possible that when the dummy is only slightly larger than the male, intimidation is the primary factor, but that when the dummy is much larger, the male instead responds as it would to a potential predator. When the dummy is several times larger than the male, the male approaches the dummy in a cautious, exploratory manner (Huntingford et al. page 278 this volume), occasionally attacking it from behind on the tail or flank (pers. obs.). This behaviour is very similar to that observed when threespine stickleback attack large prickly sculpin, Cottus asper (Pressley 1981; Foster and Ploch 1990), suggesting that the males are responding to the dummy as if it posed a predation risk.

Posture

Posture is another feature that is commonly cited as a sign stimulus for aggression in stickleback (Tinbergen 1948, 1951). Ter Pelkijk and Tinbergen (1937) reported that a dummy or a live male in a glass tube elicited more attacks from a male threespine stickleback in his territory when presented head-down than when presented horizontally. Because males threaten rivals by tilting their bodies head-down, this posture should
be more intimidating than a horizontal one. If the above argument concerning the dual (i.e., provoking and intimidating) effects of nuptial colour is valid, then males should show a corresponding response to posture.

Baerends (1985), in reviewing the evidence for sign stimuli in stickleback, questioned why the responses of males to red nuptial colour were so much more variable than were their responses to dummies in the vertical posture. I suspect that the response to posture only appears to be less variable than the response to colour because posture effects have not been subjected to the scrutiny that colour effects have received. Indeed, a re-examination of posture effects on aggression in threespine stickleback revealed lower attack rates to a head-down dummy than to a horizontal one, presumably because the males perceived the head-down dummy as more threatening (Rowland and Sevenster 1985).

Males also attacked a dummy male less when it was presented head-up (Rowland and Sevenster 1985). Head-up postures, commonly assumed by defeated males and courting females, are associated with submissiveness and have been interpreted as appeasement displays serving to inhibit aggression (Morris 1958; Bastock 1967). This effect might therefore have been responsible for the reduced attack on the head-up dummy.

The original dummy experiments on stickleback behaviour may not be strictly comparable to recent studies, and the different experimental techniques used in them may have contributed to the seemingly contradictory results. For example, in a more recent series of experiments, we presented males with stationary dummies specifically to control for the effects of movement (Rowland and Sevenster 1985). The classic experiments, however, tested for posture effects in stickleback by comparing male response to dummies or dead fish presented in various postures and moved to imitate threatening or normally swimming males (ter Pelkwick and Tinbergen 1937). Therefore, stronger aggressive responses toward the head-down dummy may better reflect a response to the combination of movement and posture than to posture alone. Indeed, ter Pelkwick and Tinbergen cautioned that their experiments did not allow them to separate the relative importance of posture and movement for evoking aggression in males, but this point seems to have been overlooked in later interpretations of their results.

**Movement**

Movement is a powerful stimulus for aggression in stickleback. In the posture experiment discussed above, a dummy, or even a dead female, attached to a wire and made to imitate the threat display of a rival (or possibly the movements of a nest-raiding fish; Foster page 394 this volume) by thrusting it head-down into the bottom provoked intense attack from males. The same dummy or dead fish manipulated to imitate the normal swimming movements of a stickleback provoked much less aggression (ter Pelkwick and Tinbergen 1937).
Fleeing stickleback also provoked chasing and biting in an opponent (van Iersel 1953). Sevenster (1961) used vertical movement and Peeke et al. (1969, pers. comm.) used horizontal movement to induce male stickleback to attack dummies. Even undirected movement increases aggression, as males intensified attacks to a stickleback held in a glass cylinder as soon as the latter moved (Rowland 1984). Thus, dummies were probably moved in the original stickleback experiments to increase the chance that males would respond to them (ter Pelk wijk and Tinbergen 1937; Baerends 1985).

Laboratory observations on freely interacting stickleback reveal that males monitor the behaviour of their opponents and respond accordingly; i.e. the response of one fish elicits a specific reaction in the other. This is especially evident for highly coordinated activities such as spine-fighting, where males carefully match their opponent's movements, rapidly chasing and circling each other head-to-tail, with a pelvic spine pointed toward their opponent (van Iersel 1953).

**Location**

Spatial context is a critical factor in animal aggression. Indeed, many of the aggressive activities of stickleback are related to territorial defence and are therefore strongly site specific. This dependence was convincingly demonstrated by an experiment in which two neighbouring males (A and B) were placed in separate glass cylinders and allowed to interact visually (Tinbergen 1953). When both cylinders were placed in A's territory, male A threatened and tried to attack male B, whereas B attempted to escape. The response of the males was reversed when the cylinders were moved into B's territory.

Van Iersel (1958) extended this experiment by presenting a stimulus male in a glass cylinder at various distances from the nest of a test male. He found that the closer the stimulus male was presented to the nest, the more intensely it was attacked by the test male. More recent work confirms this effect of distance, even on aggression provoked by intruders of another species (Huntingford 1976a, 1977; Rowland 1983a, b). This effect holds as well for other stickleback species (Fig. 11.1) and may serve to adjust male defence to the increasing danger of an intruder as it approaches the male's nest.

The differences in aggression that various species evoke from territorial males may be a question of species recognition, reflecting the degree of similarity that the territorial males share with intruders. Alternatively, the different responses to intruders of each species may reflect the kind and magnitude of threat posed to the male and his nest (Rowland 1983a; Foster and Ploch 1990).

**Tactile cues**

Three-spine stickleback have receptor nerve fibres that end in the epidermis (Whitear 1971), and these provide the skin with tactile sensitivity (Wootton
Thus, even though vision is the primary sense involved in elicitation and control of aggression in this species, tactile stimulation probably plays some role.

Tinbergen (1951) noted that touch receptors may have a specific releasing function in fighting in this species. He found that when a male strikes an opponent with his snout, this evokes a similar response in the opponent. Tinbergen, too, was able to evoke a retaliatory response from a male by imitating this tactile response with a glass rod. He therefore concluded that, although fighting as a whole is controlled by visual stimuli, the release of this specialized response in fighting stickleback is almost or entirely independent of vision.

Chemical cues
There is no evidence that external chemical cues directly elicit or control aggression in threespine stickleback. Indeed, olfaction appears to be poorly developed in *G. aculeatus* (Teichmann 1954; Wunder 1957), and the lack of response of this species even to food odours (Kleerekoper 1969) would suggest that chemical cues are unlikely to be involved in its aggressive behaviour, though they may function in group recognition (van Havre and FitzGerald 1988). Furthermore, the structure of the brain and olfactory receptors of threespine stickleback suggests that chemical stimuli play a minor role in this species’ behaviour (for references see Wootton 1976). It is, nevertheless, inadvisable to rule out the possibility of direct or indirect (Segaar et al. 1983) involvement of chemical cues in the absence of relevant behavioural data.

Experience
The influence of territorial boundaries and nest site on the focus, form, and intensity of aggression in male stickleback is well established (see above). Experience will therefore influence the aggressive behaviour of males, because it is through experience that they come to recognize the spatial relationships of their territories and nest sites.

Social experience appears to exert considerable influence on aggressive behaviour and the outcome of fights in threespine stickleback. For example, males made to lose a fight by introducing them into the tank of another male lost subsequent contests when paired against a naïve male in a neutral tank. Males made to win, however, by placing an intruding male into their territory tank, were victorious in subsequent contests with naïve males (Bakker and Sevenster 1983). Bakker et al. (1989) also found that losing had a stronger and longer-lasting effect on subsequent fight outcomes than did winning, a finding that is consistent with studies on other fishes (e.g. McDonald et al. 1968; Frey and Miller 1972; Francis 1983). Bakker et al. (1989) suggested that this is so, either because winning and losing affect the physiological mechanisms that mediate agonistic behaviour differently, or because the loser receives immediate positive reinforcement
(termination of the fight) as a consequence of its response (retreat). That is, it is the loser and not the winner whose response immediately terminates the fight.

Continuous or repeated presentation of a stimulus may reduce an animal’s responsiveness to that stimulus through habituation. Thus, territorial male stickleback decreased their attacks to males viewed behind a glass partition (van den Assem and van der Molen 1969). They also habituated if they had only intermittent view of their neighbour or could freely interact with him. Peeke et al. (1969) also found that male stickleback habituated to repeated presentation of a rival male in a glass cylinder, but if the same rival was presented at a different location in the male’s tank, or if a different rival was presented at the same location, there was a partial recovery of attack by the territory owner (Peeke and Veno 1973). Recovery of attack was greatest if a different rival was presented at a different location.

Agonistic interactions among stickleback often interfere with courtship (Wunder 1934; Li and Owings 1978a; Sargent and Gebler 1980; Borg 1985; Ward and FitzGerald 1987; Rowland 1988) or nesting activities (van den Assem 1967; Sargent 1985; Rowland 1988). The stimulus-specific nature of habituation does, however, provide a mechanism for reducing interaction among neighbours and the risk, time, and effort that such interaction incurs. Laboratory experiments reveal that reduction in aggression through habituation can increase the time that territorial males spend in courtship and nesting activities (Rowland 1988), but the extent to which habituation operates in nature is unknown.

Sevenster (1968, 1973) found that the opportunity to ‘fight’ another male separated by a glass partition served as a positive reinforcement for territorial male threespine stickleback. If this reinforcement was contingent on biting a rod suspended in their tank, males learned to perform this task. The response of males that had been conditioned to the rod suggests that some of the aggression-evoking effects of the rival become transferred to the rod during this process. Hollis (1990) has discussed how Pavlovian conditioning of aggression in male blue gouramies, Trichogaster trichopterus, could enhance their territorial defence by signalling impending aggressive encounters, and such effects may also occur in male threespine stickleback. Learning processes are therefore likely to play a role in modifying the proximate mechanisms that affect stickleback aggression in nature. A recent attempt to train threespine stickleback to decrease their use of head-down threat display, by punishing males with electric shock when they performed this response, proved unsuccessful (Losey and Sevenster 1991). In fact, seven of nine males so punished increased their use of head-down threats. This result emphasizes that, although learning provides a mechanism by which some stickleback activities can be adjusted to the environment, the biological constraints imposed on other activities will render them resistant to such modification.
NEST-BUILDING BEHAVIOUR

Once the male threespine stickleback has established a territory, he is ready to build a nest. Tinbergen (1951) stated that warm, shallow water and the presence of suitable vegetation play an important role in activating nest building. In the initial stages of this behaviour, the male picks up mouthfuls of sand from almost anywhere on the bottom and quickly ejects them. Scattered digging has been observed regularly in the field (Foster pers. comm.). In the laboratory, it results in the formation of a crater-like pattern over the bottom of the tank.

The male eventually focuses on a specific nest site and engages in more intense digging as he carries the sand farther and farther away before ejecting it. This results in the formation of a pit in which the nest will be built (Tinbergen 1951).

The choice of a nest site has been the subject of several studies. In aquaria, males often nest in the corner or near vegetation, rocks, or other objects (Hancock 1852; Warington 1852, 1855; van Iersel 1958; van den Assem 1967; Tschanz and Scharf 1971; Jenni 1972). In nature, *G. aculeatus* males may show similar attraction for nesting in cover (Black and Wootton 1970; Moodie 1972a; Kynard 1978a), but this tendency is not as marked as in some other sticklebacks (e.g. Morris 1958; Winn 1960; Courtenay and Keenleyside 1983; FitzGerald 1983). By nesting in cover, males may reduce nest predation and courtship interference by other males (reviewed by Whoriskey and FitzGerald page 192 this volume). The proximity of rocks, plants, and other objects may also serve as landmarks to facilitate the male’s orientation to his nest (van Iersel 1958, Tschanz and Scharf 1971).

It is becoming clear that stickleback populations differ in the way that males respond to proximate factors affecting nest-site selection. For example, in one Japanese population some male threespine stickleback build upright nests on a vertical shore wall (Mori 1988), a habit not seen in other populations. Mori suggested that these unusual sites may be used when high population densities prevent males from obtaining territories on the bottom. Atypical nest-site selection has also been described in other sticklebacks (Reisman and Cade 1967; McKenzie and Keenleyside 1970; Griswold and Smith 1972; Rowland 1974a,b).

Male threespine stickleback often tend to favour certain nesting substrates over others, although there exists considerable variation among populations. For example, males from a stream-resident freshwater population in the Little Campbell River, British Columbia, Canada, typically nest on mud in still water, whereas those in a parapatric anadromous population nest predominantly on sand in mild currents. When offered a choice of mud or sand in aquaria, males from both populations built nests on the substrate typical of their population, suggesting that the interpopulation difference reflected active choice (Hagen 1967; see also McPhail page 408 this volume).
Threespine stickleback that nest in brackish marsh pools with mud bottoms on Long Island, New York, USA, will nest on sand in the laboratory. Given a choice between black and off-white sand of identical texture, these males always nested in the former (10:0, \( P < 0.001 \), binomial test). Offered a choice between fine and coarse black sand, the former was favoured (9:1, \( P < 0.011 \), binomial test), suggesting that the males sought a substrate of colour and texture similar to the mud of their natural breeding grounds. The extent to which this preference is influenced by genetic factors or prior experience with such substrates is an open question.

Even during the initial stages of nest building in aquaria, Long Island males spent little time digging in fine gravel but increased their digging in sand, suggesting that the preference for the finer substrate reflects the ease with which males manipulate the finer-textured sand. Moreover, if males and their nests are less conspicuous when viewed against a dark background, the preference that these males express for the darker substrates may render them less vulnerable to predators. In the absence of further information, the possible causes and functions of substrate preferences in threespine stickleback must remain speculative.

Blouw and Hagen (1990) described a population of ‘white stickleback’ that nest exclusively in plumes of filamentous algae in shallow marine and brackish waters of Nova Scotia, Canada. Even when eggs and sperm from these fish were combined in the laboratory and the resulting progeny were reared to maturity, the male offspring would nest only on algae, indicating that the preference for this unusual nesting site by the white stickleback is inherited.

Males start to search for nest material in the latter part of the sand-digging stage (van Iersel 1953). Males that were provided with a layer of sand in which they could dig, collected and incorporated other material into the nest sooner and more frequently than did males that could see sand but were denied access to it by a plate of glass (Schütz 1980). This finding suggests that the collection of nest material is brought about by digging as well as by the pit that this activity produces. Schütz also found that sand digging was reduced if males could bring nest material into the pit. Thus sand digging, pit formation, and the collection and placement of nest material all interact to synchronize nest building in stickleback.

In the appetitive stages of gathering nest material, the male spends much time seeking and testing material (van Iersel 1953). When the male finds a strand of algae, a loose stem, or other plant matter, he sucks it up and spits it out several times in rapid succession. If it is acceptable, he incorporates it into the nest. As nest building progresses, the searching and testing phase drops out (van Iersel 1953). The male now swims directly to a source of nest material, tears off a piece, and brings it quickly to the nest. Here it is stuffed into the developing nest structure and glued with a substance that is produced by the male’s kidneys (Rinkel and Hirsch 1940).
The male applies this gluelike substance with a peculiar gliding movement as he presses his anal opening against the nest (Leiner 1929; Wunder 1930; van Iersel 1953). Each gluing bout lasts a few seconds and is performed repeatedly during the nest-building phase.

As the accumulating nest material becomes consolidated, it forms a matlike structure that is anchored to the substrate. The male continually shapes this structure by pushing into it, focusing on a point that becomes the entrance. The male bores into the nest with increasing intensity until he eventually tunnels through the entire structure. This initial act of ‘creeping through’ completes the nest and marks the transition to the courtship phase, when the male is now competent to court females (van Iersel 1953; Sevenster 1961). Here again, one can observe how internal and external factors interact to coordinate reproductive activities and produce a functional behaviour sequence in stickleback (Guiton 1960; Wootton 1976).

**Visual cues**

Visual stimuli play an important role in the initiation of nest-building behaviour in stickleback. For example, the sight of nest-building material under glass stimulated sand digging in males, whereas sandy ground under glass stimulated them to collect suitable nesting material (Schütz 1980). The selection of nest sites, too, was influenced by visual features. Tschanz and Scharf (1971) tested males in aquaria under various combinations of conditions and found that they chose subdued illumination, plants in the corner of the tank, and vertically striped background patterns.

These tests also revealed that males use objects as landmarks for more localized orientation close to the nest, but that they use differences in illumination levels to find their way back when they are farther from their nest site. When stones, plants, or other objects close to the nest are moved, males often search for their nest at a location corresponding to its position relative to the objects before they had been moved (pers. obs.).

That such males are fooled even though the nest is undisturbed and in full view emphasizes the role of visual cues for the recognition of nest site in threespine stickleback (see also Wootton 1976). Visual cues have also been implicated in selection of vegetation type in fourspine stickleback, *Apeltes quadracus* (Baker 1971), a species that in nature also selects certain plants on which to nest (Courtenay and Keenleyside 1983).

Males are selective in their choice of nest-building materials. For example, Wunder (1930) presented male threespine stickleback with nest-building material of various colours. He found that males tended to place the more brightly coloured fibres around the nest entrance, and suggested that this served to advertise the entrance. Leiner (1931) also found that a small number of males tended to place red and other brightly coloured material around the nest entrance, but was reluctant to conclude that this represented a marking mechanism.
Morris (1958) investigated this phenomenon more fully and concluded that the bright contrasting ring of colour with which males surrounded the nest entrance was a result of changes in colour selectivity during nest building. He documented interindividual differences in colour patterns, and showed that males tended to ignore red material early on but chose this colour more often as the nest neared completion and the entrance was being formed. This shift in selectivity may therefore have provided males with a mechanism to increase the conspicuousness of the nest. This could assist the male in orientating to the nest in an environment generally devoid of landmarks, and might even help induce females to enter the nest and spawn.

The limited colour range of nesting material normally available to males raises questions concerning the relevance of these preferences in nature (Wootton 1976). Material of contrasting shade, however, is more likely to be available in nature, and males might use this contrast to highlight the entrance to their nests, especially during the courtship phase. Of the ten males who built nests on black sand in the substrate colour tests discussed above, six had carried white sand from the opposite end of the aquarium and deposited it around the nest entrance, making the entrance conspicuous. This may, however, have been an inadvertent result of attempts by the males to hide the nest entrance; because only two dishes of sand were present during the substrate tests, males seeking sand from elsewhere in the tank to cover their nest could only select sand of contrasting shade (white).

If male threespine stickleback engage in nest marking, it is most likely that they would do so during the earlier stages of courtship, before they receive eggs. Field data reveal that the eggs and nest material of threespine stickleback are subject to attack from conspecifics (Whoriskey and FitzGerald page 202; Foster page 394 this volume), so it is not surprising that males in nature may try to conceal their nests by covering them with vegetation and debris (Wootton 1972a; Kynard 1978a). Indeed, nest marking has not been documented in nature, and in populations subjected to high levels of nest raiding by conspecifics, nests are cryptic even during the courtship phase (Foster 1988). Further experiments that address the question of male nest marking are clearly needed.

Males also accept or reject nest-building material on characters other than colour. Warington (1852) and van Iersel (1953) thought that males might evaluate the specific gravity of items by watching how fast they sink and rejecting those that were too heavy or too light. Perhaps by choosing material of a particular density, males are able to avoid incorporating into their nest decaying or otherwise unsuitable material. Schütz (1980) concluded, however, that the weight of material had little influence on its selection by males for nest building. Although Schütz did not investigate colour choice, he found that males did choose nesting material of certain
lengths, diameters, and surface textures, and that males learned to distinguish these features visually during the course of the nest building period. Such features are evidently important for proper nest construction in stickleback.

**Tactile cues**

Little systematic study has been conducted on the role of tactile cues in nest-building behaviour, but the effect of sand digging in the early stages of this phase (Schütz 1980) and the preferences of Long Island males for nesting substrates of finer texture were probably mediated in part through such stimuli. Males also choose nest-building material of coarse surface texture and may use tactile cues to perceive such differences (Schütz 1980). Coarse texture may facilitate binding and adhesion when material is glued into the nest. Moreover, the male presses his anogenital opening against the surface of the nest when he glues. The tactile stimuli produced by this contact probably trigger and properly orientate glue secretion.

Another opportunity for tactile stimulation occurs when males creep through the nest, a behaviour that brings the male into intimate contact with the nest tunnel. Experimental manipulation of nest structure suggests that tactile feedback does provide information to the male. For example, when a male’s nest was shortened by cutting off the hind part and removing it, the duration of creeping through decreased accordingly ('t Hart 1978). Furthermore, males whose nests were shortened began to rebuild quickly and usually restored their nests to the original length within 2 h. Although 't Hart was unable to identify the nature of the stimulation that enabled males to register the duration of creeping through, tactile and/or visual cues were probably involved in this process.

The damage sometimes sustained by nests during female entry (pers. obs.) and the importance of the nest in protecting eggs from predators (Potts 1984) emphasize the advantages of a properly constructed nest in this species. Tactile stimuli may provide essential information concerning the form, size, and strength of the nest, and can therefore affect the male’s transition to the courtship phase.

**Chemical cues**

There is little evidence that chemical cues play a role in nest-building behaviour. As the broad range of items that males accept for nest building includes such varied substances as plant, animal, and synthetic material (Schütz 1980), it would appear unlikely that males rely on chemical cues for the selection process. Even the state of decomposition of plant material, which one expects might be distinguishable through chemical cues, had no effect on the acceptance or rejection of nest-building material by male threespine stickleback (Schütz 1980).

Chemical cues are likely to be involved in selection of breeding areas.
by anadromous stickleback populations, in so far as fish distinguish and choose particular salinities (reviewed in Guderley page 92 this volume). Furthermore, the annual return of breeding stickleback to specific marsh pools on Long Island (unpubl. data), and the avoidance of temporary pools by stickleback in Quebec, Canada (Whoriskey and FitzGerald 1989), raise the intriguing possibility that breeding individuals may return to their natal breeding grounds to spawn (Hagen 1967; Kynard 1978a). This phenomenon has been well documented in salmon and results from a process of chemical imprinting (e.g. Hasler et al. 1978). A similar process might occur in stickleback, but the technical difficulties involved with marking enough stickleback fry and recapturing them as adults have so far presented an insurmountable obstacle to biologists.

COURTSHIP AND MATING BEHAVIOUR

Courtship of the threespine stickleback is one of the best-studied examples of mating behaviour in the animal kingdom. Warington's (1852) accurate but incomplete description of the attempts of a male threespine stickleback to induce a female to spawn in its nest is among the earliest published accounts of courtship behaviour in this species. Since then, many detailed descriptions and analyses of courtship in the threespine stickleback have appeared in the literature (see Wootton 1976 for references).

Stickleback courtship comprises a sequence of modal action patterns (Barlow 1968) that alternate between the male and female. The performance of a given behaviour by one member of the courting pair elicits from its partner a behaviour constituting the next step of the courtship sequence. Signals from the male thus in large part depend on the female's behaviour, and vice versa. The response chain that results from this process insures that sperm and eggs are shed in the same place at the same time (Tinbergen 1951). This synchronization is essential in sticklebacks, because fertilization is external and gametes have limited viability outside the body.

Courtship is a dynamic process that depends on the internal state of the participants and thus varies among individuals from the same population. Courtship motivation also fluctuates within the same individual over time. This fluctuation can be easily observed in males responding either to conspecifics held in a clear flask or to stationary dummies (Rowland 1984). Certain populations of threespine stickleback differ consistently with respect to the details of male courtship behaviour (Wilz 1973; McPhail and Hay 1983; Ridgway and McPhail 1984; Foster page 391, McPhail Chapter 14 this volume). Nevertheless, the behavioural components that constitute courtship are qualitatively similar, and the basic courtship patterns are conserved enough among populations that homologous components are readily identifiable. The courtship of a marine population of threespine stickleback from Long Island is representative of the species and thus
serves as the basis for the description of courtship that follows.

When the male detects a female in his territory, he approaches her in a series of horizontal darting movements that alternate toward and away from the female (Fig. 11.3(a); see also Fig. 1.4). This motor pattern is called the 'zigzag dance' (Leiner 1929; Tinbergen 1951). Zigzagging may be interspersed with bites, butts, or other aggressive behaviour directed toward the female, and the frequency and intensity of these activities vary among males (Sevenster 1961; Wilz 1973) and within the same male over time (Sevenster 1961; Wilz 1975). If the female is receptive, the male swims to
his nest, fans, glues, and performs other nest activities. The male eventually creeps through his nest, a behaviour associated with blanching of his body. This colour change makes his crimson throat and belly appear even brighter, as though the male ‘... were somewhat translucent and glowed with an internal incandescence ...’ (Warington 1852). The male returns to the female and courts her even more vigorously before returning to the nest (Sevenster 1961). Thus, the male oscillates between courting the female and performing nest activities. The process ceases when the pair spawns or the female departs.

When a sexually receptive female encounters a courting male she assumes a characteristic head-up posture. In the most intense form, the female lifts her tail and assumes a posture of lordosis, with her back forming a concave arch (see Fig. 3 of ter Pelkijn and Tinbergen 1937). With rapid, nearly imperceptible fin movements, she turns to face the courting male as he circles and weaves around the tank (Sevenster 1973). Often, the female will follow the male before he is prepared to lead her to the nest. If this occurs, the male swims under the female, rolls his body to the side, and backs up against her so that his erect dorsal spines touch her abdomen (Leiner 1930). This ‘dorsal pricking’ (Fig. 1.5) causes receptive females to hold their position on the male's territory while the male returns to his nest and engages in nest activities (Wilz 1970a,b).

Ethologists have proposed several different functional explanations for the nesting activities that males perform during courtship. These explanations need not be mutually exclusive. Moreover, the wide behavioural and morphological variation so far documented among populations of threespine stickleback raises the possibility that these nest activities might serve different functions in different populations.

The nesting activities observed during courtship were originally interpreted as displacement behaviour (Tinbergen 1951; Sevenster 1961). This causal explanation maintained that aggressive and sexual tendencies in the male normally inhibit nesting and parental behaviour, and that when the two former tendencies are balanced during courtship, they cancel each other, thereby ‘disinhibiting’ nesting and parental behaviour (Sevenster 1961).

Wilz found, however, that territorial males that engaged in these nest activities underwent a motivational shift, from an aggressive to a more sexual state. Such males decreased their attacks on the female and increased their zigzagging behaviour. Wilz therefore concluded that dorsal pricking and its associated nest activities function to synchronize male and female courtship activities.

Several workers have proposed other functions for the nest activities performed during courtship in stickleback. For example, Sevenster-Bol (1962) and McFarland (1974) suggested that these activities maintain the nest in condition to receive receptive females. Rohwer (1978) predicted that female stickleback would spawn with males that already have eggs. He
proposed that males could persuade females to spawn in their nests by behaving as though the nests contained eggs (i.e. by fanning the empty nest).

Data concerning the role of eggs in determining mate choice in threespine stickleback are equivocal (Ridley and Rechten 1981; Whoriskey and Fitzgerald 1985; Jamieson and Colgan 1989; Goldschmidt and Bakker 1990), although in other species females clearly choose to spawn in nests that contain eggs (e.g. Constanz 1985; Marconato and Bisazza 1986; Unger and Sargent 1988). The hypothesis that nest-directed behaviour is attractive to females remains to be tested.

When the male is ready to lead the female to his nest, he approaches her, turns abruptly, and rushes back to the nest. When the pair reach the nest, the male rolls his body to one side and with his dorsal surface facing the female, thrusts his snout into the entrance. In this way the male shows the nest entrance to the female (Leiner 1930; Wunder 1930; Tinbergen 1951). If the female enters the nest, she squirms into the tunnel until her head protrudes from the other end (but see 'Cues from the nest', page 336). When the female is in the nest, the male quivers his snout along her caudal peduncle several times, alternating from one side of her body to the other. Within a minute or so, the female bends her tail upwards, shivers, and deposits her eggs. After she swims out of the nest, the male squirms through, shivering momentarily over the eggs as he fertilizes them.

The act of fertilization and the presence of a fresh clutch of eggs in the nest causes a sudden decline in the male's willingness to court; this refractory period lasts for about an hour (Sevenster-Bol 1962). The refractory period probably provides an opportunity for the male to restore his nest, and perhaps permits sperm accumulation for subsequent spawnings. Fertilizations have an increasingly negative effect on the sexual tendency of males over the long term (van Iersel 1953). This decline may be associated with a gradual hormonal breakdown resulting from gonadal change or some other physiological mechanism (Sevenster-Bol 1962).

Following fertilization is a period of intense nest activity, when the male pushes the new clutch down into the nest, repairs any damage the nest may have suffered, and extends it forward to cover the newly spawned eggs. The male resumes his guard duties and drives off intruders, including females. The male's sexual tendency gradually recovers (but see below), and in about an hour he is again competent to court females and fertilize eggs (van Iersel 1953; Sevenster-Bol 1962).

The complex interactions that exist among the male, female, nest, and eggs provide numerous potential cues for eliciting and coordinating mating behaviour in stickleback. Because these cues and the mechanisms by which they are perceived play an important role in mate choice and sexual selection, their investigation is crucial to understanding the evolution of stickleback behaviour and morphology.
Colour
Preference for red

Conspicuous nuptial colour in threespine stickleback is restricted to males, and in most populations reaches maximum intensity during courtship. Nuptial colour may therefore serve an important signal function for courtship in this species. Leiner (1930) was one of the first to consider the role of intersexual selection in the evolution of nuptial colour in male threespine stickleback. By presenting receptive females to males in aquaria illuminated by monochromatic light, Leiner attempted to test the effects of male nuptial colour on females. Because males could induce females to spawn in their nests under green light (540 nm), Leiner mistakenly concluded that the red underside was unimportant to mating success and that only the zigzag dance played a significant role.

The ability of stickleback to mate in green light demonstrates only that the red nuptial colour of males is unnecessary, not that it is unimportant (ter Pelk wijk and Tinbergen 1937). Indeed, Wunder (1934) concluded earlier that female threespine stickleback selected males primarily on the basis of some aspect of nuptial colour, but this conclusion was confounded by the greater tendency for brighter males to court females and dominate duller rivals.

Experimental evidence for a direct effect of nuptial colour on female choice was first obtained by ter Pelk wijk and Tinbergen (1937), who found that receptive females followed a dummy to an artificial nest only if the dummy had a red underside. Taking advantage of a population that was polymorphic for male nuptial colour, Semler (1971) also documented an effect of red on male courtship success. He demonstrated that females choose males with red nuptial colour over a non-red (silver, mottled, or black underside) male, and that they choose non-red males with artificial red colour (lipstick or nail polish) over non-red males treated with clear lipstick or nail polish.

Although a significant proportion of females tested by Semler selected red males, 28 per cent selected non-red males. Because Semler only tested each female once, it is not known whether the difference in choice by females reflected female error or a behavioural polymorphism. The distinction is an important one for understanding the evolution of male nuptial colour.

Recent research on anadromous and resident freshwater threespine stickleback in several coastal rivers in Washington, USA, indicates that even when nuptial colour changes from red to black in a lineage, the females may still choose red over black males. In these drainages, resident freshwater stickleback in the upper reaches of the streams have diverged from the ancestral marine form (red colour) and evolved black nuptial colour, seemingly as a consequence of threat display convergence with the Olympic mudminnow, *Novumbra hubbsi* (McPhail 1969; Hagen and Moodie 1979;
Hagen et al. 1980). Female preference for red males has been partially lost, however, in the black species adjacent to a suspected zone of hybridization, suggesting that selection may favour prezygotic isolation in sympathy (McPhail 1969; Bell 1976b).

By functioning as a sign stimulus for female courtship, the red underside provides a mechanism by which female threespine stickleback recognize and choose prospective mates. In nature, however, virtually all males from which females are likely to choose (i.e. males with nests) will display some red colour. Thus, females should be able to distinguish differences in the relative expression of this trait.

A recent laboratory study of G. aculeatus from Europe revealed that females were capable of such discrimination and spent more time orientating to the brighter of two males presented simultaneously (Milinski and Bakker 1990). This preference was no longer statistically significant when males were presented under green light, suggesting that females chose males primarily by differences in colour rather than in behaviour.

McLennan and McPhail (1990) also found that females from an anadromous population in British Columbia orientated to and tracked the brighter of two reproductive males. When the females were released from the jars in which they were held during this assay, they typically mated with the brighter male, although duller males succeeded in mating in a minority of pairings. The duller males that mated did so by intruding into the territory of the brighter male and interfering with that male’s courtship attempts, suggesting that male behaviour may play an indirect role in courtship success when males compete for females.

My own observations reveal that at least the early stages of courtship can be evoked in female threespine stickleback in the absence of nuptial colour. For example, when females from a Long Island population were held in large stock tanks without males, the gravid ones often assumed the head-up posture and concave back and followed female tankmates, as in heterosexual courtship. Thus, females that are sufficiently motivated may become less selective of mates.

Preliminary experiments using dummy presentations led to similar conclusions. When I presented moving dummies on a carousel apparatus to females, few followed the one with the red underside more. Contrary to expectations, most females divided their time between red and non-red dummies (Table 11.1). Although females willing to follow dummies might have been so receptive that they no longer discriminated among them, female response to nuptial colour may vary more than past studies have recognized.

Recent study reveals that there is considerable interpopulational variation in female preference for male colour in the guppy, Poecilia reticulata (Houde 1988; Houde and Endler 1990). It is possible that threespine stickleback populations vary similarly (Rowland 1982a). Differences in experimental methodology (e.g. Baerends 1985; Rowland and Sevenster
Table 11.1 Female courtship responses (measured as mean number of following bouts per 4 min trial) to moving all-silver and red dummy males presented on a carousel apparatus. The total number of trials in which females responded to either dummy is shown in the last column.

<table>
<thead>
<tr>
<th>Female&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean follows per trial of</th>
<th>Total trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silver belly</td>
<td>Red belly</td>
</tr>
<tr>
<td></td>
<td>silver irises</td>
<td>silver irises</td>
</tr>
<tr>
<td>1a</td>
<td>2.8</td>
<td>5.8</td>
</tr>
<tr>
<td>1b</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1d</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>2a</td>
<td>4.4</td>
<td>2.8</td>
</tr>
<tr>
<td>2b</td>
<td>1.2</td>
<td>2.6</td>
</tr>
<tr>
<td>2c</td>
<td>3.5</td>
<td>2.3</td>
</tr>
<tr>
<td>3a</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>4a</td>
<td>4.4</td>
<td>6.8</td>
</tr>
<tr>
<td>5e</td>
<td>10.6</td>
<td>4.0</td>
</tr>
<tr>
<td>6a</td>
<td>1.8</td>
<td>3.8</td>
</tr>
<tr>
<td>6b</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>6c</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>6d</td>
<td>17.7</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Grand mean ± SE 4.05 ± 1.36 3.84 ± 1.08

<sup>a</sup>Numbers refer to females, letters refer to experimental trial.

1985), experience or motivational state of the subjects, and other such factors may also give rise to differences in the response of female threesspine stickleback to red colour, as discussed above for male response to this feature.

Significance of red colour

By choosing brighter red males over duller ones, females may be selecting males of high quality. Red nuptial colour in stickleback is based on carotenoids (Lonneberg 1938; Brush and Reisman 1965). Because carotenoids may be a limited dietary resource for many species, the extent or intensity of red colour may reflect an individual's ability to accumulate protein and other limited nutrients that are associated with them in nature (Rothschild 1975; Endler 1980). If bright males sire fitter offspring and provide superior parental care than do duller males, the reproductive success of females that mate with them will be greater than if they had chosen dull males.

Milinski and Bakker (1990) found that the brightness of the male's red nuptial colour and physical condition were positively correlated in a European population of threesspine stickleback. The colour and condition of preferred males deteriorated after males were infected with parasites,
and females no longer selected them (Milinski and Bakker 1990). These results support the hypothesis that animals select mates by traits that reliably indicate their bearer’s condition (Andersson 1982, 1986; Hamilton and Zuk 1982; Kodric-Brown and Brown 1984). Milinski and Bakker (1990) did not state whether male courtship intensity was correlated with male condition. They noted, however, that males in poor condition might be able to muster enough energy to court when the need arises, but are unlikely to meet the long-term demands for maintaining bright colour.

Condition factors based on weight-to-length relationships (Bolger and Connolly 1989) and colour scores (Rowland 1984) of male threespine stickleback from Long Island were subjected to a regression analysis. In contrast to the freshwater population of threespine stickleback studied by Milinski and Bakker (1990), redness of Long Island males was not correlated with condition \((r^2 = -0.097, N = 32, P > 0.05;\) unpubl. data). Perhaps the intensity of red in Long Island males is limited less by foraging ability, parasites, or general nutrition levels than by short-term (e.g. hormonal or motivational) differences among males. Under such circumstances, the conspicuousness or overall appearance of males, rather than their red colour alone, may lead females to choose them (see below).

An alternative interpretation for the association between male nuptial colour and female response is provided by the sensory exploitation hypothesis. This hypothesis maintains that the properties of signal receivers exert a major influence on the evolution of the signal (reviewed in Ryan 1990). Thus, selection is thought to act on the signaller to exploit any pre-existing bias in the perceptual system of the signal receiver. With respect to courtship in the threespine stickleback, red is regarded as having been selected from among a range of potential colours in the male because this colour is especially effective in stimulating the female’s visual system. Over time, the courtship signal comes to match the perceptual properties of its intended receiver.

Investigators studying the psychophysics of vision in threespine stickleback implied that the visual system of the female was adapted to receive the signal emitted by the male (Cronly-Dillon and Sharma 1968). By projecting stripes of monochromatic light on the walls of a chamber and measuring the threshold for the optomotor response at various wavelengths, these investigators obtained photopic (light-adapted) spectral sensitivity curves for reproductive and non-reproductive stickleback. Fish exhibited peaks of sensitivity at 500–512 nm (blue) and at 594 nm (orange). Female (but not male) sensitivity to 594 nm increased with the onset of the reproductive season. This suggested that the eyes of female threespine stickleback may adapt seasonally to enhance detection of male nuptial colour.

A major problem here, however, is that the match between male colour and female sensitivity obtained for the threespine stickleback is only approximate and may therefore be spurious. If such associations could be
extended to other species of sticklebacks, this would provide more convincing evidence for the coevolution of visual signal-receptor systems in this family of fishes. Preliminary data suggest that light-adapted female blackspotted stickleback, *Gasterosteus wheatlandi*, and female *G. aculeatus* from Long Island, New York, USA, possess the same two spectral sensitivity peaks reported by Cronly-Dillon and Sharma (1968), but that the blackspotted stickleback is relatively more sensitive to blue-green wavelengths (Baube unpubl. data). Male blackspotted stickleback develop a yellow-green nuptial colour during the breeding season (Perlmutter 1963; Reisman 1968a; McInerney 1969; Rowland 1983a), which may be taken as further evidence of the coevolution of the receptor and signal systems in sticklebacks.

Because neither signal nor receptor exists without the other, it is reasonable to assume that selection acts on both components simultaneously. Visual receptors of reproductive females must do more than detect and assess potential mates. The properties of these receptors will be shaped by ecological factors that often require compromise solutions and constrain the structure and function of signalling systems. The extent to which ecological factors affect courtship and other forms of signalling in gasterosteid fishes is the subject of current research.

Role of other colours

The majority of studies of male nuptial colour in threespine stickleback have emphasized the importance of the red underside, but the effects of this feature are likely to be confounded by the blue iris and bluish white dorsal colour constituting the other components of male colour (Titschack 1922; Wunder 1930; Craig-Bennett 1931; Ikeda 1933; McLennan and McPhail 1989a). The expression of all three colour components varies with male behaviour, and both colour and behaviour reflect the male’s reproductive state (Craig-Bennett 1931; Ikeda 1933; Reisman 1968b; Rowland 1984; McLennan and McPhail 1989b).

The blanched dorsal colour of courting male threespine stickleback provides a contrasting background that probably enhances the effectiveness of their red underside to attract females. By reversing the effects of counter-shading, a light dorsum alone will increase male conspicuousness and may in its own right serve as a sign stimulus for female courtship (van Iersel 1953).

This possibility finds support from a study of the ‘white stickleback’ that occurs sympatrically with the typical form of *G. aculeatus* in Nova Scotia, Canada (Blouw and Hagen 1990). Although the two forms mate assortatively and are reproductively isolated, females of both forms are initially attracted more to white males, even though the latter may exhibit little or no red on their ventral surface. Because breeding males develop a white dorsal and flank colour that renders them so conspicuous, the
greater attractiveness of white males to females can probably be attributed to this trait.

Although the correspondence between the intensities of the red underside and blue eyes of threespine stickleback is only approximate (McLennan and McPhail 1989a), the widespread occurrence of the latter trait in reproductive males, even in populations where males develop little or no red (Semler 1971; Reimchen 1989), suggests that blue eyes may have an important signal function in courtship. Data from a Long Island population of _G. aculeatus_ provide preliminary evidence of this. Gravid females presented with moving non-red dummies on a carousel apparatus followed the dummy with blue irises more than the one with silver irises (Table 11.2). Therefore, females may choose males with blue eyes, at least in the absence of a red belly.

McLennan and McPhail (1989a) found that blue eye colour varied less in intensity than red underside colour and suggested that red is potentially the more important signal by which females discriminate among males of their species. This is a reasonable assumption, but only choice tests can establish how much female choice depends on hue, intensity, etc. of the components of nuptial colour in male threespine stickleback. In view of the wide interpopulational variation in morphology (reviewed in Bell 1984a; Reimchen Chapter 9 this volume) and behaviour (reviewed in chapters by Hart and Gill, Huntingford _et al._, Bakker, Foster, and McPhail this volume) of this species, one might expect to find corresponding variation in female response to different components of male nuptial colour. Such differences are likely to be related to local conditions (Reimchen 1989).

**Table 11.2** Female courtship responses (measured as mean number of following bouts per 4 min trial) to moving all-silver and blue-eyed dummy male presented on a carousel apparatus. The total number of trials in which females responded to either dummy is shown in the last column.

<table>
<thead>
<tr>
<th>Female</th>
<th>Mean follows per 4 min trial of</th>
<th>Total trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silver belly silver irises</td>
<td>Silver belly + blue irises</td>
</tr>
<tr>
<td>1a</td>
<td>1.7</td>
<td>4.9</td>
</tr>
<tr>
<td>2a</td>
<td>2.8</td>
<td>6.3</td>
</tr>
<tr>
<td>2b</td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>3a</td>
<td>1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>4a</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>5a</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>6a</td>
<td>6.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Grand mean ± SE</td>
<td>3.26 ± 1.04</td>
<td>4.96 ± 0.97</td>
</tr>
</tbody>
</table>

*P = 0.016, for one-tailed Wilcoxon matched-pairs, signed-ranks test.*

*aNumbers refer to females, letters refer to experimental trials.*
Female colour

The pigmentation pattern of the breeding female stickleback, though less flamboyant than that of its male counterpart, has received some attention from biologists. Non-reproductive males and females usually appear olive tan to grey dorsally, with a silvery cast along their flanks and belly. In breeding females, especially those from marine populations, lateral colour often intensifies to a brassy silver. Van Iersel (1953; and reported in Sevenster 1961) concluded that the silver colour was a releasing stimulus for male courtship in *G. aculeatus* from the Netherlands. Results obtained from a Long Island, New York, population of *G. aculeatus* support this. When males were offered a choice between gravid dummy females painted silver or black, they courted the silver dummy more (Table 11.3). The possibility that males were reluctant to court the abnormally contrasting black dummy cannot, however, be excluded.

In the open water column, crypsis is best served by the uniform silvery flank colour (Edmunds 1974) characteristic of non-reproductive fish. When receptive females move to the shallow breeding areas to spawn, however, they become especially vulnerable to avian predators that frequent the marsh pools (Bull 1964; Whoriskey and FitzGerald 1985b; Williams and Delbeek 1989; pers. obs.). Females in some populations develop a dark vertical barring on the upper half of their flanks when they become reproductively active (Wunder 1934; Williams and Delbeek 1989; Rowland *et al.* 1991). In the shallow breeding habitat, the barring pattern disrupts the fish's outline (Nikolsky 1963), so that against the dark, non-uniform background of the tidal pools the fish becomes less visible to predators. The barring pattern may therefore serve as protective colour for the females.

Wunder (1934) first suggested that this barring pattern could be used by males when selecting among gravid females. As Wunder had, we found that female threespine stickleback developed similar pigmentation that correlated with their sexual receptivity and intensified in the presence of

<table>
<thead>
<tr>
<th>Response</th>
<th>Mean ± SE per 4 min to Silver dummy</th>
<th>Black dummy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visits to dummy</td>
<td>** 5.53 ± 5.56</td>
<td>3.74 ± 4.09</td>
</tr>
<tr>
<td>Seconds at dummy</td>
<td>* 46.32 ± 40.31</td>
<td>23.63 ± 22.70</td>
</tr>
<tr>
<td>Zigzags to dummy</td>
<td>** 24.42 ± 33.09</td>
<td>5.42 ± 9.54</td>
</tr>
</tbody>
</table>

*P < 0.02; **P < 0.01, for two-tailed probabilities on Wilcoxon's rank-sum, matched-pairs test.
courting males. Based on my own experience that barring served as a reliable cue for recognizing receptive females in a stock tank, we tested whether males might attend to this cue when courting. Indeed, Long Island males presented with a barred and an unbarred dummy courted the barred dummy more (Rowland et al. 1991).

The barring pattern of the female could serve as a courtship signal that suppresses attack and arouses sexual tendencies in the male, thereby helping to synchronize mating activities of the courting pair (Morris 1956). If this is true of barring, male responsiveness to the pattern should increase and its conspicuousness should also increase until balanced by counter-selective pressure from predation. An alternative interpretation maintains that signals advertising female receptivity may facilitate female mate choice by inciting competition among potential mates (Cox and LeBoeuf 1977; Farr and Travis 1986). In so far as the second interpretation is likely to depend on the veracity of the first, the two possible interpretations should not be considered mutually exclusive.

In species where males compete, females may mate with superior males more often than expected by chance, simply by accepting those that dominate in intrasexual interactions. If brighter, more vigorous male stickleback dominate rivals (Bakker and Sevenster 1989), this dominance will increase access to females and further enhance their reproductive success. Cox and LeBoeuf (1977) suggested that female elephant seals may even incite competition among males, and by mating with the victor produce offspring of higher fitness. Female stickleback might similarly exercise such passive choice of mates (see below).

**Behaviour**

The species specificity of certain elements of courtship, particularly those that occur during the initial stages, suggests that movement is a critical component of stickleback courtship. The differences in courtship displays commonly observed among sympatric species may have evolved in large part through selection for species identification (Tinbergen 1952; Morris 1956). Figure 11.3 illustrates the courtship dances of three sticklebacks that occur sympatrically on the north-east coast of North America. These displays are performed by males soon after they encounter prospective mates, and in conjunction with the species-distinctive nuptial colours, probably help to maintain reproductive isolation among the species (Reisman 1968b; McInerney 1969; Rowland 1970).

Leiner (1930) recognized the importance of male courtship behaviour when he observed that *G. aculeatus* females spawned with males that were denied the advantage of nuptial colour through the use of monochromatic illumination. Manipulating dummies to imitate the courtship movements of the male, ter Pelkwick and Tinbergen (1937) induced females to follow the dummies and even to enter an artificial nest. Similarly, females paid
little attention to a stationary dummy male suspended in their tank, but often adopted a head-up posture and followed the dummy when it was moved through the tank in a manner that approximated male leading movements (Rowland 1989c). The effectiveness of movement is also evident from the sudden increase in attention females direct to males that start to zigzag, even if the pair is separated by a glass partition.

The zigzag dance of the three-spine stickleback is often cited as a classical example of a fixed action pattern, even though it may vary in amplitude (Tinbergen 1951) and frequency (Sevenster 1961). If differences in courtship behaviour reflect differences in male condition, then they, like colour, might also serve as effective criteria for female mate choice. The total visual stimulation resulting from the interaction of the male's behaviour and colour may therefore provide the most important cue by which females detect and evaluate potential mates. Indeed, it is the combination of rapid movement and bright colour that provides the element of conspicuousness so characteristic of courtship display in animals (Bastock 1967).

It has been suggested that female three-spine stickleback might choose to mate with males that perform the zigzag dance at relatively high frequency (Gross and Franck 1979; Ridley 1986). There exists, however, no empirical support for this hypothesis, and in studies on three populations no such association was found (Ward and FitzGerald 1987; Jamieson and Colgan 1989; Milinski and Bakker 1990).

If it exists, an effect of male courtship vigour on female choice of mates may be obscured by a positive association between courtship vigour and brightness of the male's red nuptial colour (Rowland 1984; Ward and FitzGerald 1987; McLennan and McPhail 1989b). Females may therefore choose mates on the basis of either attribute, although Milinski and Bakker (1990) have argued that courtship intensity is an unreliable indicator of male condition because even males in poor condition may be able to court vigorously. Perhaps when carotenoids differ little among males or in a way that does not reliably indicate differences in overall quality, females attend more to other cues.

In Milinski and Bakker's (1990) study, the zigzag dance may have been inhibited by the use of small tanks (Bakker and Sevenster 1989; see also below). Consequently, the role of male courtship behaviour in female choice under natural conditions may have been under-estimated. Moreover, McLennan and McPhail (1990) illustrate how behaviour can influence male mating success indirectly through male–male interaction (see above).

In view of the wide variation discovered among populations of stickleback in nature, it is not unlikely that the relative roles of nuptial colour, behaviour, and other traits affecting mate choice will also depend on water conditions (e.g. Reimchen 1989), presence of predators (e.g. Moodie 1972b; Foster pers. comm.; this chapter), and other ecological factors.

Elements of the aggression that male stickleback use to establish and
defend territories from rivals also appear during courtship (Tinbergen 1951; van Iersel 1953; Morris 1956; Sevenster 1961). Moderate levels of aggression may have a stimulatory effect on receptive females (e.g. McPhail and Hay 1983; Ridgway and McPhail 1984), but excessive aggression may interfere with courtship (Wilz 1973; Ward and FitzGerald 1987; Rowland 1988). Moreover, the extent to which males express aggression during courtship may lead to consistent differences in courtship among populations (Wilz 1973), and these may play a role in mate selection by females.

For example, McPhail and Hay (1983; see also McPhail page 408 this volume) found assortative mating between populations of stream-resident freshwater and marine *G. aculeatus* from the Little Campbell River, British Columbia, Canada, and attributed it to quantitative differences in male courtship. Freshwater males more often zigzagged as a first response to a female, whereas marine males more often bit the female. Because this was true regardless of the type of female courted, the authors suggested that the two courtship responses reflected differences between males rather than between females from the two populations. Thus, females may assess males by comparing the ratio of zigzags to bites. McPhail and Hay also found that freshwater males zigzagged more to freshwater females than to marine females, but the mechanism by which males distinguished the two kinds of females was not ascertained.

Ridgway and McPhail (1984; see also McPhail page 421 this volume) investigated positive assortative mating between limnetic and benthic populations of threespine stickleback from Enos Lake, British Columbia, Canada. The two types of males differed in the way they approached and led females, with benthic males biting more and limnetic males zigzagging more during the initial phase of courtship. Benthic and limnetic females clearly distinguished between the two kinds of males, indicating that differences in the initial phase of courtship may play a role in the assortative mating in these two populations.

Ziuganov (1988) studied a completely plated and a low-plated species of threespine stickleback occurring sympatrically in the White Sea and Kamchatka River basins in Russia. He, too, found that males of the two species differed in the degree of aggression shown during courtship, and also in their initial approach to females, their leading behaviour, and the way they showed the nest entrance. These differences suggest that male courtship behaviour plays a role in maintaining their reproductive isolation.

Sexual selection theory predicts that in species such as the threespine stickleback, where both males and females make a substantial parental investment, both sexes will discriminate among potential mates when given a choice (e.g. Trivers 1972; Williams 1975). Tinbergen (1951) emphasized how successful reproduction in the threespine stickleback depended on sequential exchange of signals between the courting partners. The interplay of behaviour, colour, size, and other properties that occurs during the
courtship sequence thus provides both male and female stickleback with ample opportunity to evaluate prospective mating partners and to exercise mate choice, either by terminating the courtship prematurely or by spawning (Hay and McPhail 1975; see also McPhail Chapter 14 this volume).

**Posture**

Courting males of other species of sticklebacks tend to adopt a head-down posture in courtship, particularly during the leading phase (Wootton 1976), but male threespine stickleback do not appear to do so. When the male leads the female to the nest, he typically retains a horizontal posture as he dashes back to his nest, only tilting downward as he swims toward the bottom to approach the nest entrance. At this point the male rolls his body to the side and briefly assumes the unique body posture (showing) that displays his red underside to the female and points out the nest entrance.

When zigzagging, males usually maintain a horizontal position also. A male may briefly adopt a head-down or head-up posture as well, but this seems to result primarily from the male attempting to orientate himself with respect to the female as he courts her.

For female threespine stickleback, posture appears to be an important sign stimulus for eliciting male courtship. Ter Pelkewijk and Tinbergen (1937) provided an accurate illustration of the head-up posture and concave back that females typically assume when courted. Tinbergen (1951) reported that a male stickleback even courted a dead tench, *Tinca tinca*, when it was presented in a head-up posture, but the tench was made to simulate female courtship movements when it was presented to the male (ter Pelkewijk and Tinbergen 1937). It is therefore difficult to determine from these observations the extent to which posture may contribute to the elicitation of courtship in male stickleback.

A re-examination of sign stimuli in male stickleback by using stationary dummies failed to confirm the effectiveness of head-up posture (Rowland and Sevenster 1985). When Long Island males were presented with stationary dummies of gravid females, they courted those in horizontal posture more than those presented head-up or head-down. Because biting, too, was less frequent to dummy males and dummy females presented head-up, we suggested that this posture may function in courtship more as an appeasement signal to suppress attack by males than as a sexual signal (Rowland and Sevenster 1985). A subsequent study by McLennan and McPhail (1990) offered a similar interpretation.

**Body shape**

Wunder (1934) reported that male threespine stickleback mated preferentially with females of greater girth, but this observation might have reflected the greater receptivity of such females rather than male preference for them.
By comparing a distended dummy with an undistended one, Tinbergen (1948) demonstrated that the swollen belly of a gravid female stickleback served as a sign stimulus for male courtship. However, males in nature may be courted by several gravid females simultaneously (Kynard 1978a). Because such females will vary in size or egg content, males that can distinguish among them and court accordingly could improve their reproductive success.

Indeed, threespine stickleback males presented with pairs of dummies of equal length but different degrees of abdominal distension chose the more distended one (Rowland 1982b), even when distension far exceeded normal limits (Rowland and Sevenster 1985). Although stationary dummies were used in these experiments to rule out any confounding effects that female behaviour might have on male choice, similar results were obtained with males presented with pairs of live females that differed in body weight (Sargent et al. 1986).

Interestingly, males did not court the more distended or larger dummies exclusively, but distributed courtship in proportion to the dummies’ projection areas (Rowland 1989b). This indicates that males use the area of the image projected on the retina, or some closely correlated trait, as a proximate cue for comparing mates.

Even when males were presented with four dummies of different degrees

![Graph showing zigzag directions](image)

**Fig. 11.4** Number of zigzags (mean ± SE) directed to each of four simultaneously presented dummies that differed in degree of abdominal distension. The size of each dummy is expressed as per cent of the total projection area of all four dummies. All dummies were presented to 27 males at an equal distance from their nest. There is an increase in the proportion of zigzags with increasing proportion of projection area of a dummy ($P < 0.001$; Page's $L = 722.5$, $k = 4$, $N = 27$).
of abdominal distension simultaneously, they divided courtship approximately in proportion to the projection area of each dummy (Fig. 11.4). Because abdominal distension in females is associated with sexual receptivity as well as fecundity (Rowland 1982b), a perceptual mechanism that causes males to select such females would confer a reproductive advantage to males.

Males evidently attend to the shape, and not just the increased lateral projection area that results when the distension of the female's abdomen is increased. When males were presented with a superdistended (super-normal) dummy (lateral projection area = 539 mm²) and a larger but normally distended dummy (lateral projection = 661 mm²) in a single trial, they courted the supernormal distended one 45 per cent more (23.00 ± 3.10 v. 15.82 ± 2.55 zigzags per 4 min trial; mean ± SE; \( T^+ = 298, N = 27, P < 0.01, \) Wilcoxon signed-ranks, matched-pairs test). This suggests that configurational cues (Tinbergen 1951; Ewert 1980) influence courtship in male threespine stickleback. Therefore it is probably not coincidental that courting females assume a posture that displays the distended abdomen to advantage.

The superdistended dummy also presents a greater cross-sectional area, which would be visible to males as they swim around the dummies. This could confound interpretation regarding the cues to which males attended in the above experiment. More convincing evidence for the importance of configurational cues in male courtship was obtained by disrupting the normal spatial relationships of the dummy. When two normally distended dummy females were presented horizontally to males, one in the normal belly-down position and the other in an abnormal belly-up position, males courted the belly-down dummy 70 per cent more (16.57 ± 2.87 v. 9.74 ± 2.50 zigzags per 4 min; \( T^+ = 197, N = 21, P < 0.01 \)). Thus, despite the similarity in the orientation of the dummies’ body axes, projection areas, etc., males distinguished the different spatial relationships of the dummies' body contours.

Female stickleback may also use body shape as a cue in courtship, but the meagre evidence that supports this is circumstantial. For example, Mori (1984) found a marked sexual dimorphism with respect to head size in resident freshwater \( G. \) aculeatus from Japan. The ratio of head length to body length was greater in males than in females, suggesting the possibility that this attribute might provide an additional cue for sexual identification in this population. McPhail (1984; see also McPhail page 418 this volume) found considerable differences in the body shape of males from two biological species of threespine stickleback from Enos Lake, British Columbia. Ridgway and McPhail (1984) felt it likely that females use shape as a cue for selecting mates.
Body size

The increase in fecundity with body size in teleosts (Williams 1966) is well documented in threespine stickleback (Baker page 164 this volume). Male stickleback should therefore choose larger females over smaller ones. Mate choice experiments revealed that Long Island males selectively court the larger of two dummy females when the dummies are presented simultaneously (Rowland 1989b). As in the shape experiment discussed above, courtship was distributed in proportion to the projection area of the two dummies, even if the dummies exceeded the size range of real females. The supernormality effect thus apparently overrode any reluctance males might have had to approach an object large enough to be a predator.

Courtship reflected the relative sizes of the two dummies, but males were more likely to perform ambivalent behaviour (e.g. backing off) when presented with dummies whose projection area was approximately ten times the projection area of a female stickleback (Rowland 1989b). This response could have occurred because the dummy was too large to be recognized as a potential mate, or because it was perceived as a potential predator. That very large dummies (several times larger than a stickleback) were perceived as potential predators was suggested by the cautious, exploratory approach (i.e. predator inspection; Pitcher 1986, Huntingford et al. page 278 this volume) of males to the dummies (pers. obs.). In the few instances in which the approach escalated to an attack, it was directed toward the dummy's tail or flank, initiated from behind the dummy, and followed by a quick retreat. This behaviour was very like that elicited by large prickly sculpin (Pressley 1981; Foster and Ploch 1990).

Although males courted dummies that were larger than any females in the population, the distance from which they courted increased with increasing dummy size (Fig. 11.5; Baube unpubl. data). Whether the increase in courtship distance resulted from increased fear of larger dummies, or whether males tend to match the image the female casts on the retina to some expected size when courting, is uncertain. In any case, we know that size and distance interact in stickleback, because the relative attractiveness of a dummy can be increased if it is presented closer to the male. Male stickleback may therefore estimate distance or size by the angle the female's image subtends on their retina.

Students of optimal foraging theory have already proposed this 'apparent-size hypothesis' to explain how prey selection is mediated in stickleback (Gibson 1980; Wetterer 1989; but see Hart and Gill page 232 this volume). But if males attend solely to the female's projection area, one would predict dummy attractiveness to be inversely related to the square of its distance. Further data are needed to test this prediction.

Females also court larger potential mates (dummies) more actively than they do smaller ones (Rowland 1989c). When normal- and supernormal-
Fig. 11.5  Courtship distance (mean ± SE) of males presented with dummy females of identical shape but different body length, based on 19 males presented once with each dummy. The 50 mm dummy approximated the size of females from this population of stickleback. There is an increase in courtship distance with dummy size ($P < 0.01; \text{Page's } L = 244.0, k = 3, N = 19$).

Sized dummy males were moved together through the tanks of receptive females to simulate courting males, the females spent more time following the larger one. Like those of males, the females' preferences were incomplete, and they divided their following time in proportion to the projection area of the two dummies. Similarly, females from a population of relatively small body size chose males from a population of unusually large threespine stickleback when offered a choice of males from the two populations (Moodie 1982). Moodie suggested accordingly that the large males served as a supernormal stimulus for females of smaller body size.

**Distance**

Courtship response in stickleback depends on how far from the nest the male encounters the female. For example, males zigzagged more to females presented at 100 cm than at 10 or 280 cm from the nest (Symons 1965). Symons concluded that the lower rate of zigzagging at 10 cm probably resulted from the correspondingly higher amount of showing that males performed when they encountered females so close to the nest. Unpublished studies by van Iersel and by Sierksma and Sevenster (cited in Sevenster-Bol 1962) also found a reduced rate of zigzagging to females close to the nest. They attributed this reduction to an increased inhibition of sexual tendency by the increase in aggression that results when males are close to the nest (Fig. 11.1).
An interesting implication of this finding is that the use of small aquaria in laboratory studies may lead to a somewhat distorted view of stickleback courtship in nature. Indeed, Bakker and Sevenster (1989) suggested that the small size of aquaria (34 × 17 × 20 cm) used in their behavioural genetic study of stickleback may have inhibited the full expression of courtship in males because females were forced to remain close to the nest. This possibility emphasizes the need for further study of stickleback in the field, where behaviour is less likely to be affected by such spatial constraints.

The limited field observations of courtship so far reported for *G. aculeatus* indicate that males often encounter several females at a time (e.g. Kynard 1978a; Borg 1985). Under such conditions, distance could become an important factor in choosing mates. If a female is less likely to follow the male to the nest the further away the nest is when he starts courting, or if being farther from the nest increases courtship costs or vulnerability to nest raiding for the male, then he should preferentially court females nearest to his nest.

To test this possibility, we presented dummy females at two distances from the male's nest. Preliminary trials revealed that males courted a dummy twice normal size (in all linear dimensions) more than a normal-size dummy when the dummies were presented equidistant from the males' nests (Table 11.4). Males presented with two normal-size dummy females, one at 22 cm and the other at 44 cm, still courted both dummies, but spent more time courting the closer one (Table 11.4).

We then tested whether an increase in mate size would compensate for increased mate distance. Indeed, males spent about the same amount of time courting the large dummy presented at 44 cm as they did the normal-size dummy presented at half that distance (Table 11.4). This result

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**Table 11.4** Effects of distance on female size preference of male stickleback, based on single 4 min pairwise presentations of dummies to 27 males.

<table>
<thead>
<tr>
<th>Dummy pairing</th>
<th>Response (mean ± SE) to each dummy per trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visits</td>
</tr>
<tr>
<td>Normal at 22 cm</td>
<td>*** 8.11 ± 0.78</td>
</tr>
<tr>
<td>Normal at 44 cm</td>
<td>6.19 ± 0.66</td>
</tr>
<tr>
<td>Normal at 44 cm</td>
<td>7.74 ± 1.11</td>
</tr>
<tr>
<td>Large at 44 cm</td>
<td>***10.96 ± 1.46</td>
</tr>
<tr>
<td>Normal at 22 cm</td>
<td>7.00 ± 0.93</td>
</tr>
<tr>
<td>Large at 44 cm</td>
<td>5.93 ± 0.82</td>
</tr>
</tbody>
</table>

*P < 0.01; **P < 0.001 for two-tailed probabilities on Wilcoxon's rank-sum, matched-pairs test.*
is consistent with the apparent-size hypothesis discussed above, because
the size of the retinal image cast by the supernormal dummy would approxi-
mate that of the smaller, closer dummy.

This result also might imply that stickleback are unable to perceive
absolute size differences between dummies presented at different distances.
However, the binocular vision of fishes facilitates perception of absolute
size differences between objects viewed at different distances (e.g. Herter
1930), and such size constancy is retained even in monocular fish (Douglas
et al. 1988). The well-developed visual sense of stickleback (Wootton 1976)
suggests that they, too, should have the capacity to perceive absolute size
differences among objects irrespective of distance. Therefore, the equal
allocation of courtship between the large dummy at 44 cm and the normal
dummy at 22 cm may instead reflect a trade-off between the advantages
of courting a larger mate and the disadvantages of having to go farther from
the nest to do so.

The female's proximity to the nest may also reflect her readiness to
spawn. If this is true, then the male should value the closer female more
than a female farther from the nest, even if he recognizes that the closer
one is of equal size or smaller. However, stickleback have not been studied
fully enough to warrant conclusions concerning the limitations of size
constancy of vision in this species.

The effects of distance also depend on the size, shape, and orientation of
the territory, distance from neighbouring males, density of females, and
other physical and biological factors. The relevance of such information can
only be reliably established in conjunction with data collected in the field.

Experience

Sevenster (1968, 1973) demonstrated that male stickleback can be condi-
tioned to perform a simple operant response (i.e. swimming through a ring)
for the opportunity to court a female through a glass partition. There is,
however, little information on how experience may influence stickleback
courtship in more natural contexts. Males are often observed searching
and zigzagging in the area of the tank where a live or dummy female was
presented, long after the female has been removed.

Such observations suggest that stickleback learn where they are likely
to encounter mates, much as they learn where they have encountered food
(Thomas 1974; Hart and Gill page 220 this volume). Guiton (1960) reported
observations on threespine stickleback suggesting that the nest site may
itself come to acquire sexually stimulating qualities through conditioning.
When a male was induced to move his nest to a new site by repeated destruc-
tion of his nest, he responded sexually (quivering) to a dummy presented
at the old nest site, now devoid of nest or pit, but attacked the same dummy
when it was presented at the site where the new nest was being constructed.

Males must associate social stimuli with their location in the field. Thus,
by learning where females, rivals, and other intruders are likely to appear, males could adjust their behaviour, territories, and nest sites accordingly. Similarly, females could learn the location of territories and nest sites of courting males, and return to or avoid particular males or areas, depending on their experience there. If stickleback distinguish neighbours from strangers and relate this discrimination to specific locations within the territory (Peeke and Veno 1973), then perhaps they can extend this ability to the sexual context.

To what extent does learning influence courtship and mate choice in animals? Stickleback are often regarded as a species whose mating behaviour is largely innately determined (e.g. Cullen 1960), so it would be interesting to examine whether males can learn to modify their pattern of mate choice. The ability to vary mate choice might have a selective advantage. We are currently testing this hypothesis by presenting males with a pair of dummies that differ in abdominal distension (Rowland 1989b). We then attempt to shift the initial choice of males by immediately removing both dummies from the male's tank for 30 min (a presumably aversive stimulus) as soon as the male begins to court the dummy we have designated as ‘negative’. This paradigm was chosen to simulate the case where a male, by focusing courtship on the fatter but non-receptive of two females (e.g. a parasite-distended female), may lose the opportunity to mate with either one because they leave the male's territory. Preliminary results so far indicate that male choice for the more distended dummy female is resistant to modification by experience.

Peeke and Peeke (1973) noted the lack of information regarding the role of habituation in sexual behaviour of fishes. Given the relatively short period during which male threespine stickleback actively court (i.e. the courtship phase), compared with the period when they actively attack, males are likely to maximize their chances of obtaining additional matings if they persist in courting prospective mates during the courtship phase. One might therefore expect courtship behaviour to be more resistant to habituation than is the case for aggressive behaviour in this species. Courtship behaviour of stickleback may rely more directly on hormonal changes and other physiological processes (reviewed in Guderley Chapter 4 this volume) than on experiential ones for its timing and control.

Tactile cues

Male threespine stickleback often initiate courtship by biting or butting the female. This behaviour quickly drives off unreceptive females, but receptive ones face and approach males that behave this way (e.g. Sevenster 1968). This activity may test the receptivity of prospective mates and sexually stimulate those that are ready to spawn. Violent contact behaviour at the beginning of courtship is typical of males from several populations of threespine stickleback (e.g. Wilz 1973; McPhail and Hay 1983;
Male threespine stickleback also provide gentler forms of tactile stimulation to females during courtship, including dorsal pricking (discussed above) and the quivering that males direct to females that have entered the nest. If the male is removed just prior to quivering, the female fails to spawn. The female can be induced to spawn, however, by repeatedly tapping her caudal peduncle with a rod in a way that simulates the tactile stimulus normally provided by the male (Tinbergen 1951). This activity might have originated from the male's attempts to enter the nest and fertilize the eggs deposited by the female.

Tactile stimulation may also stimulate leading in threespine stickleback males, because receptive females sometimes contact the male with their snout during courtship. Indeed, contact by the female is characteristic of courtship in fourspine stickleback, *A. quadracus* (Hall 1956; Reisman 1963; Rowland 1974a), brook stickleback, *Culnea inconstans*, and blackspotted stickleback, *G. wheatlandi* (Reisman 1968a; McInerney 1969; Rowland 1970; Wootton 1976). In *G. wheatlandi*, the female repeatedly touches her snout between the male's erect pelvic fins as he vibrates his body and slowly leads her on a circuitous route to the nest. This vibration probably provides both visual stimulation to the female as the male's orange pelvic fins oscillate before her eyes, and tactile stimulation to the male and female when they contact. As dorsal pricking and the frequent nesting activities seen in *G. aculeatus* males when they court are absent in *G. wheatlandi*, the protracted leading behaviour of the latter species may represent an alternative mechanism for synchronizing male and female mating activities.

Reproductive male *G. wheatlandi* are yellow-green and inconspicuous in vegetation, so tactile signalling could provide an effective way to gain and hold the attention of a female in such habitats. Moreover, the female provides feedback to the male through such contact. If this contact is interrupted while the male is leading, he backs up and waits for her to re-establish contact or repeats the courtship sequence. Populations of *G. aculeatus* that differ in conspicuousness, or that inhabit waters that differ in visibility, should be compared to determine whether the relative contribution of tactile signalling varies accordingly.

Tactile cues are probably critical for the culmination of courtship: the fertilization of the eggs. Fertilization occurs when the male creeps through the nest just after the female has deposited her eggs there. The tactile stimuli that the male experiences during this final activity of the courtship sequence are probably necessary for the emission of sperm (Sevenster-Bol 1962).

**Chemical cues**

As noted above in the discussion of the role chemical cues in aggression, neither behavioural evidence nor the structure of the brain and olfactory receptors of threespine stickleback suggest that chemical stimuli play a
major role in this species' behaviour. Nevertheless, the possible influence of chemical cues in stickleback courtship has not been ruled out. Leiner (1930) assumed that the glue which males secreted over the nest during courtship had a sexually stimulating effect on the male himself, rather than on the female. Leiner noted that males usually glued immediately before they led the female to the nest. Although the gluing act itself may effect a motivational shift in males that results in leading (Wilz 1973) and consequent nest entry by the female, the secretion may provide some chemical stimulation to the male or female.

In some fishes, chemical cues from receptive females may stimulate courtship in conspecific males (e.g. Crow and Liley 1979; Farr and Travis 1986). The discovery that mucus from freshly laid eggs can elicit fertilization in male threespine stickleback (van Iersel 1953) suggests that chemical stimuli could be involved in other aspects of courtship behaviour in this species. Perhaps leakage of mucus or other fluids from the cloaca of gravid females provides a chemical cue by which males recognize females whose spawning is imminent. This kind of cue could virtually ensure a successful mating by the male.

In a pilot study, I presented territorial male threespine stickleback with two visually identical dummies, each fitted with a fine plastic tube that permitted water to flow slowly from the ventral opening. When water from a jar containing a receptive, gravid female was allowed to flow from one dummy, males showed no obvious difference in response to this dummy compared with response to the dummy through which plain tank water flowed. Further study is needed, however, before any conclusions can be drawn concerning a possible role of chemical cues in courtship.

Cues from the nest

The vast majority of stickleback courtships do not result in spawning, and these are often terminated by the female before she reaches the nest (e.g. Tinbergen 1954; Borg 1985). These incomplete courtships could reflect nest site preferences of females. For example, females may prefer to spawn in concealed nests (Sargent and Gebler 1980; Sargent 1982).

It is also possible that females identify and select nests on the basis of specific visual cues such as long pieces of plant material at the entrance parallel to the nest tunnel. These 'leidstengels' (Dutch for 'lead stems') are a commonly observed feature in nests of stickleback from Europe and North America (Wunder 1930; pers. obs.), and may serve to mark the nest entrance. Bright or contrasting colours around the nest entrance could serve the same purpose.

On arriving at the nest, females in captivity often fail to spawn after examining and even after entering the nest (e.g. Warington 1852; Li and Owings 1978a; Ridley and Rechten 1981; Jamieson and Colgan 1989; pers. obs.). Some authors have interpreted this failure as an expression of
selection by the female. Ridley and Rechten (1981) concluded that females preferred to spawn with males that already possessed eggs, and that females determined this by poking their snout into the nest. Others (e.g. Wootton 1976; Jamieson and Colgan 1989), however, invoked a motivational explanation for aborted nest entry and suggested that many of these incidents observed in the laboratory may have resulted from workers using females not yet ready to spawn. However, aborted nest entry has been observed in the field in stickleback from British Columbia, Canada (Foster pers. comm.), Long Island New York, USA (pers. obs.), and the Netherlands (Goldschmidt and Bakker 1990); it is not merely a laboratory artefact. Female stickleback may therefore place their snout into the nest entrance to obtain information about the condition of the nest to decide whether to abort or to complete nest entry.

PARENTAL BEHAVIOUR

Once the male has positioned the fertilized eggs in the nest and repaired whatever damage the nest may have sustained during spawning, he redirects his attention to courting. Beside courting females and guarding the nest, the male must now care for the spawn. Although Leiner (1960) described what may be rudimentary egg and nest tending by female fifteenspine stickelback, Spinachia spinachia, it is generally believed that it is exclusively the male that administers parental care in the Gasterosteidae. This phase of the reproductive cycle of G. aculeatus has been carefully analysed by van Iersel (1953).

In addition to guarding spawn, the primary paternal activity is fanning. The male hovers head-down above the nest with his head close to the entrance and oxygenates the eggs by fanning a current of water across the top of the nest with his pectoral fins (Hancock 1852; Tinbergen 1951; van Iersel 1953; Sevenster 1961; Wootton 1984a). This activity is repeated at regular intervals, depending on the number and developmental state of the eggs, the environmental conditions, and the internal state of the male (van Iersel 1953; Sevenster 1961; Wootton 1976; Whoriskey and FitzGerald page 197 this volume). The male also consumes dead or fungus-infected eggs, behaviour which may help to inhibit the spread of disease (van Iersel 1953).

Although the male's sexual tendency recovers about an hour after fertilization, recovery is less complete with each succeeding fertilization (van Iersel 1953). These internal changes, as well as those brought about by the increasing stimulation from the developing eggs, increase parental behaviour and decrease sexual behaviour and nuptial colour. This process continues until parental behaviour inhibits sexual behaviour and courtship is no longer expressed (van Iersel 1953). During the parental phase, males usually assume a duller, more cryptic colour (Craig-Bennett 1931), but in some populations red colour may be maintained or may reach a peak
during this period (Moodie 1972a). This retention of red colour lends some support to the suggestion that nuptial colour may also serve as a warning signal to potential nest predators (Moodie 1972b).

As the eggs develop, their need for oxygen increases. To accommodate this change, the male increases both the frequency and duration of fanning bouts (van Iersel 1953; Sevenster 1961). A minimum amount of time is required to expel the water from a nest at the start of each fanning bout, and the increase in bout duration is well adapted to keeping the eggs supplied with oxygen throughout the parental phase (van Iersel 1953).

The demand for oxygen reaches a peak late in the parental phase. To facilitate oxygenation of the eggs, the male increases water exchange through the nest by boring holes in the top and rim of the nest (Wunder 1930; Leiner 1931). As the eggs near hatching, the male picks and tears at the nest and sucks out sand until the nest consists of little more than a heap of algae and debris with a depression in the centre (Wunder 1928; Leiner 1930; van Iersel 1953). Observations of stickleback in the wild also reveal that some males disassemble their nests and pile sticks and rushes around them just before the fry hatch (Foster 1988). This activity improves ventilation and produces a structure that may serve as a nursery in which the newly hatched young can seek refuge, as described for *G. wheatlandi* (McInerney 1969), *Pungitius pungitius* (Morris 1958), and the brook stickleback, *Culaea inconstans* (McKenzie 1974).

The parental behaviour of the white stickleback of Nova Scotia, Canada (Blouw and Hagen 1990) has become reduced to the dissemination of eggs. Shortly after the male has crept through the nest to fertilize the newly spawned eggs, he removes them from the nest and spits them into the filamentous algae that encompasses much of his territory (Jamieson *et al.* 1992b). The abundance of algae on the breeding grounds of these fish evidently provides an environment in which the separate eggs are cryptic and can obtain enough oxygen to develop without any further attention from the male. Whether emancipation from parental care in the white stickleback has increased the maximum reproductive output of males is unknown.

Reports of parental male stickleback retrieving young date back more than a century (e.g. Hancock 1852; Warington 1852, 1855). As young wander from the nest they are pursued, sucked up into the male's mouth, and spat back into the nest. This behaviour is performed intensely on the day of hatching and for some days thereafter (Feuth-de Bruijn and Sevenster 1983; see also Huntingford *et al.* page 292 this volume).

Leiner (1930) noted that parental male stickleback often retrieve and place another's fry into their own nest if the fry are placed nearby. Indeed, satiated parental male threespine stickleback may even retrieve fry of the ninespine stickleback, *P. pungitius* (Feuth-de Bruijn and Sevenster 1983). These observations suggest that males may have difficulty distinguishing their offspring from those of others. On the other hand, males may recog-
nize alien fry as such, but adopt them as a way to reduce the probability that their own young will be taken if a predator attacks the nest (e.g. McKaye 1984).

The response of a parental male toward newly hatched fry is thought to depend on various factors, including his experience, point in the parental phase, hunger state, and the number of egg clutches he has acquired (van Iersel 1953; Feuth-de Bruijn and Sevenster 1983). The influences of the cues involved in retrieval of young and in other aspects of parental behaviour in *G. aculeatus* are therefore likely to vary accordingly.

Adult stickleback feed on a variety of prey, including conspecific eggs and fry (e.g. Hynes 1950; Semler 1971; Kynard 1978a; Worgan and FitzGerald 1981a; Hyatt and Ringler 1989b; Whoriskey and FitzGerald page 202, Hart and Gill page 232; Foster page 394 this volume). Leiner (1929) questioned why males do not devour their spawn and concluded that males must gradually develop an inhibition during the parental phase that prevents them from doing so. Leiner argued that the limited area and time available to parental males for foraging forces them to fast most of the time. But Feuth-de Bruijn and Sevenster (1983) found that parental males eagerly devoured prey (live *Artemia*), especially toward the latter part of the parental cycle, just when the eggs began to hatch. This indicated that the process that suppresses males from eating their own spawn was more specific than a general inhibition of feeding.

Feuth-de Bruijn and Sevenster (1983) also found that even though males started to retrieve young several days before the eggs hatched, hungry males would eat their own young if they had previous experience eating young. When satiated, such males switched from eating to retrieving young. Feuth-de Bruijn and Sevenster interpreted this behaviour as evidence for competition between eating and retrieving young as the parental phase progressed.

These findings are of particular relevance to the filial cannibalism hypothesis, which predicts that male stickleback may eat a small percentage of their spawn to maintain themselves in condition to care for the remaining spawn (Rohwer 1978 but see Sargent 1992; FitzGerald and Whoriskey 1992). Perhaps the interaction between hunger level and parental behaviour could limit filial cannibalism to periods of severe food deprivation, but field study is needed before any conclusions can be drawn.

The male is unable to keep up with the growing young as they stray farther and farther from the nest. Within a week after they hatch he ceases retrieving them, and instead chases and devours them as he would any other prey (van Iersel 1953). The breakdown of the nest, the fleeing response of the young to the approaching male, and other changes in external stimuli contribute to this change in behaviour, but internal factors also play a role (van Iersel 1953; Sevenster 1961; Wootton 1976).

Thus, the organization of parental behaviour in stickleback depends on an interplay between internal and external factors, and consequences of
egg development may effect changes in the external stimuli that help coordinate fanning, nursery preparation, and other paternal activities. Although the experimental work on stickleback parental behaviour has dealt primarily with the role of chemical cues in controlling fanning and recognizing spawn, visual and tactile cues have also been implicated. I now consider external factors that are thought to play a role in the elicitation and control of parental behaviour in threespine stickleback.

**Visual cues**

The contribution of vision to other aspects of stickleback reproduction makes it likely that this sense is important for mediating parental behaviour, too. For example, fanning behaviour clearly depends on visual input. Tinbergen (1951) noted that fanning could be separated into a stereotyped motor component and a more variable orientation component. The latter component depended on the position and orientation of the male’s nest; if it was tilted, the male tilted his body accordingly so that his body was orientated at the same angle relative to his nest when he fanned.

Vision also mediates egg retrieval. When males found a cluster of eggs outside their nest, they usually retrieved the eggs and replaced them in the nest (Leiner 1929; van Iersel 1953). Ter Pelkijk and Tinbergen (1937) discovered, however, that the cluster had to comprise a minimum of five eggs to release retrieval. When one or two eggs were found lying outside the nest they were eaten immediately.

Clumps of gelatin placed outside the nests of males were also retrieved, provided that the clumps were at least as large as a cluster of five or six eggs (ter Pelkijk and Tinbergen 1937). The effectiveness of these gelatin clumps in eliciting retrieval can probably be attributed partly to their resemblance to the transparent pale yellow of freshly laid eggs, because gelatin clumps coloured blue or red were not retrieved, but were carried far away from the nest. This experiment does not rule out a role for chemical cues, but if stickleback eggs produce some unique chemical stimuli, these appear to be unnecessary for egg retrieval in parental males.

Once the eggs are in the nest they are generally hidden from view, so it is unlikely that vision plays a major role in their care. The pursuit and retrieval of young by parental males do, however, involve visual cues. Males presented with day-old fry visually fixated on one of the fry as soon as it moved (Feuth-de Bruijn and Sevenster 1983). Visual cues may also help the male to distinguish young from prey or other moving objects when they are close by. In any case, the young must be taken into the mouth before they are retrieved or eaten, so the final decision regarding their fate may depend on chemical or tactile cues.
Chemical cues

Male stickleback accept and care for eggs that have been fertilized by other males (Evers 1878; Leiner 1930; van Iersel 1953). Thus male stickleback appear unable to distinguish their own eggs from those of others. Males in the laboratory sometimes steal eggs from the nests of rivals, carry the stolen eggs to their own nest, and care for them (van den Assem 1967; Wootton 1971a; Whoriskey and FitzGerald page 196; Foster page 390 this volume).

FitzGerald and van Havre (1987) directly tested for egg recognition ability in stickleback by presenting parental males with their own clutch and an alien clutch in separate mesh bags. Because males attacked their own eggs as readily as alien eggs, the authors concluded that male stickleback cannot recognize their own eggs. The possibility that the sack itself instigated attack on the eggs even though males had recognized them cannot be ruled out.

Females, however, did attempt to devour alien eggs more than eggs they had laid themselves, leading FitzGerald and van Havre (1987) to propose that egg recognition prevents females from preying on their own eggs when they participate in nest raiding. Smith and Whoriskey (1988) found subsequently that females readily attacked their own eggs if they were presented in the same bag with alien eggs. They therefore rejected FitzGerald and van Havre's hypothesis on the grounds that nests in nature usually contain clutches from several females. Whatever its function may be, the egg recognition shown by females is probably mediated by chemical cues that are easily masked or altered.

Chemical cues may also play a role in the recognition of young by parental male stickleback, but only to the species level. Male threespine stickleback readily retrieved unrelated conspecific young (Leiner 1929; Feuth-de Bruijn and Sevenster 1983) but treated young ninespine stickleback, *P. pungitius*, differently (Feuth-de Bruijn and Sevenster 1983). Ninespine young were quickly devoured if the males were hungry, but if the males were satiated they held the ninespine young in the mouth for some time and performed mumbling movements with their jaws. Several threespine males finally retrieved ninespine young, but others dropped them after testing them in this manner. This suggests that males may obtain tactile or chemical cues when they take young into their mouth, and that they accept or reject them depending on external stimuli and the males' internal state.

Perhaps the best-documented example of chemical cues in stickleback behaviour is in the control of parental fanning. When water from a bucket containing a large number of threespine stickleback was siphoned into a nest that contained eggs, the male increased the duration and frequency of fanning (van Iersel 1953). Because the rates of O$_2$ consumption and CO$_2$ production increase during development of embryos, van Iersel presumed that males gauged this when they poked their head into the nest.
entrance and then adjusted their fanning levels accordingly.

Sevenster (1961) extended van Iersel's 'bad water' experiments by charging tapwater with CO₂ and siphoning this into the nest of parental males. This, too, increased fanning, but Sevenster was unable to establish a clear correlation between the concentration of CO₂ and the extent to which fanning increased. He suggested that both increased CO₂ and reduced O₂ levels may elicit fanning and even show heterogeneous summation.

The use of direct chemical cues, rather than solely an internally generated cycle, to control fanning is advantageous because it allows for fine-tuning to local environmental conditions. Despite variations in substrate, temperature, water currents, and other environmental factors, males can maintain efficient fanning levels for a given set of conditions. Internal factors would lead the male to visit the nest periodically, increase responsiveness to eliciting stimuli, and help to suppress behaviour that might otherwise interfere with paternal care (van Iersel 1953; Sevenster 1961).

Finally, chemical factors may determine the time at which the nest is disassembled to provide increased ventilation for the developing embryos. Indeed, van Iersel (1953) found that males increased their boring and pulling at the nest more than fourfold during the 'bad water' experiments.

**Tactile cues**

Whenever the object of a response comes into close contact with the male's mouth, the possibility exists that tactile as well as chemical perception is involved in the control of that response. Therefore the prolonged holding in the mouth and eventual acceptance of ninespine young by some threespine males could be the result of similar tactile cues produced by young of the two species. The possible role of tactile cues in the male's perception of the eggs has already been noted.

**Experience**

The repeated performance of nesting activities and the general experience with the nest surroundings may enhance the efficiency with which the male performs his parental duties. If males complete multiple breeding cycles in a season, as laboratory studies show is possible (e.g. van Iersel 1953; Sargent 1985), improvement between cycles would be possible. The limited data indicate, however, that a large proportion of males in most (Whoriskey and FitzGerald page 193 this volume) but not all populations (Foster pers. comm.) fail to renest.

Particularly in populations in which males typically nest only once in the breeding season, the short duration of the breeding cycle and the rapidly changing factors associated with the developing progeny probably provide little opportunity for males to improve their parental behaviour through practice. Therefore, experience is less likely to be important for shaping parental behaviour than it is for shaping foraging and other activities that
are repeated regularly throughout a fish’s lifetime.

The experiments of Feuth-de Bruijn and Sevenster (1983) revealed, however, how experience could influence parental behaviour in stickleback indirectly by interfering with the parental male’s response to fry. When non-parental males were kept hungry and then fed conspecific fry, they came to accept them as prey. When these males mated and entered the parental phase, they devoured rather than retrieved conspecific fry, including their own, unless they were kept satiated with *Artemia* or *Tubifex*.

In many populations, few if any fish that have spawned survive to the next breeding season (Wootton 1984a). Of those that survive, we do not know whether the males are able to breed more than one season. Moreover, although it might appear unlikely that if males were capable of breeding more than one season, they could modify their parental behaviour in accordance with experience from the previous season, this possibility cannot be ruled out. Such questions emphasize the need for further study of life history in stickleback.

**CONCLUSIONS**

The vast literature on threespine stickleback, dating back more than two centuries (e.g. Arderon 1746), bears testimony to the fascination the species has long held for ethologists. The wide availability, ease of maintenance, and well-developed social behaviour of this common little fish make it an ideal system for studying the causation, control, and evolution of behaviour. These qualities have led to a proliferation of studies on the species that continues to this day.

Many of the examples discussed in this review illustrate how careful laboratory observation and experimentation can reveal much about the factors that elicit and control reproductive behaviour in threespine stickleback. This research provides insight, not only into the behaviour of stickleback, but into behaviour in general. Indeed, stickleback research has had an enormous impact on the very foundation of ethology. Such fundamental concepts as sign stimulus, releasing mechanism, fixed action pattern, response chain, and displacement activity developed in large part from observations on this fish.

Despite our detailed knowledge of stickleback behaviour, many questions remain. For example, why do males fan the nest during courtship, even before they have obtained eggs? And why do males steal eggs from the nests of rivals, transfer them to their own nests, and care for them? Do these activities merely reflect a limitation or misfiring of the mechanism(s) controlling behaviour, or do they serve a purpose for the animal? The answer to such questions may be best answered in evolutionary—behavioural ecological terms, an approach that students of stickleback behaviour strongly advocate, and which has gained momentum in the past two decades.
An evolutionary approach is crucial to a complete understanding of stickleback behaviour and is bound to generate new and important questions.

Several authors in this volume presented evidence that the behaviour of threespine stickleback is at least as evolutionarily malleable as morphological traits. The wide interpopulation variation, even among stickleback populations in close proximity, is impressive and has forced us to alter our view of 'the' threespine stickleback and of speciation processes in general.

The relationship between behaviour and evolution is, however, bidirectional. Because selection acts on the whole organism, the constraints that behavioural mechanisms impose on the species play a critical role in its evolution. It is therefore imperative that we continue to investigate the proximate determinants of stickleback behaviour at all levels of organization, from neurophysiology to ethology and animal learning. Only by adopting this broad approach can we gain a complete understanding of the stickleback's evolution.

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