Assortative schooling in three-spined sticklebacks?

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Using three-spined sticklebacks, we examine the consequences of foraging in groups for the individual fish. Firstly, the number of prey eaten per fish increases with the number of fish in the school, which suggests that sticklebacks benefit from schooling. Secondly, the feeding rate increases with body size. Thirdly, when foraging alone, both small and large fish clearly prefer large prey items to small ones, but the presence of a competitor alters size selectivity. Minor changes are observed in the rates of feeding on two prey sizes when two small or two large fish are foraging together. The effect of a competitor is most notable, however, when a small fish is foraging with a large fish — the small fish switches to eating smaller prey. In the presence of a small fish, a large stickleback selects more large prey items than when foraging with a large fish. Intraspecific competitive ability thus appears to be size-dependent.

These observations suggest that, when schooling, it pays for small sticklebacks to group with small fish. On the other hand, a large fish also seems to do better in association with smaller fish than in schools of uniformly large fish. To examine the validity of this conclusion we ran two different experiments.

First, when five small and five large fish were released together into an arena, the fish split into two schools after 3–6 min. In the newly formed schools, small fish frequently consorted with small fish and large with large. After 9–10 min, the pattern was well pronounced. Second, fish of different size were allowed to chose between schools of large and small fish. Small fish tended to associate with the school of small fish while large fish were most often seen with large fish. Finally, we discuss whether these results are sufficient proof of assortative schooling (i.e., two phenotypes associating with fish of their own type) in three-spined sticklebacks.

1. Introduction

Predator avoidance and improved food acquisition have been suggested as key factors explaining schooling behaviour in teleost fish (Clark & Mangel 1986, Pitcher 1986, Magurran 1990). Indeed, there is evidence that joining a school enhances an individual's likelihood in detecting (Magurran et al. 1985, Milinski 1986, Magurran 1990) and avoiding predators (Neill & Cullen 1979, Milinski 1979, Tremblay & FitzGerald 1979, Magurran & Pitcher 1986). Also, fish in larger schools appear to locate food patches faster than fish foraging either alone or in small groups (Pitcher et al. 1982, Pitcher 1986).

Once it is established that living in groups enhances an individual's success, the next step is to determine the optimal group size for a particular environment (Wilson 1975, Caraco & Wolf 1975, Rodman 1981, Giraldeu 1988). However, the problem in school formation is not merely that of finding the optimal group size. For example, it is reasonable to assume that individuals differ in their acquisition of resources, such as food. They should thus pay attention to the kind of members in the school. If the phenotypic composition is adverse to the phenotype of the individual, it may be advantageous for it to look for schools of more favourable structure.

Ironically, the question of the phenotypic composition of fish schools has been studied within the framework of the theory of ideal free distribution, IFD (Milinski 1979, 1988, Sutherland et al. 1988). In brief, the IFD theory (Fretwell & Lucas 1970) says that competitors, which are all assumed to be *equal* in competitive ability and free to move around, should distribute themselves in proportion to habitat profitabilities. Since the decision of each competitor depends on the decision of others, none of them can gain from moving once the IFD pattern is achieved. In other words, the IFD is spatially an evolutionarily stable strategy.

It has been shown that fish distribute themselves around two feeding stations according to their differences in productivity (Milinski 1979, 1984, Sutherland et al. 1988), as predicted by the theory. However, Milinski (1984) convincingly demonstrated with three-spined sticklebacks that even fish selected to be identical nonetheless did differ in their competitive abilities. In fact the best competitor in Milinski's experiment obtained about 2–3 times as much food as the poorest competitor. This finding has later been corroborated with goldfish (Sutherland et al. 1988), and has been the subject of theoretical work by Sutherland & Parker (1985), Parker & Sutherland (1986) and Houston & McNamara (1987).

In the work reported in this paper we started from the fact that in most cases there are clear differences in food acquisition rate between individuals. In our experiments we used three-spined sticklebacks (*Gasterosteus aculeatus* L.) of two clearly distinct size categories — small and large fish. After establishing that there is a benefit to be gained by joining a school, we studied the consequences of intraspecific differences in competitive ability. That is, we focused on the consequences of having a school with a given number of fish, which differed in size. Our aim was to discover what happens in such situations to an individual's foraging success, once a food patch is located. On the basis of our findings we were able to make a prediction about the size composition of stickleback schools. The prediction was then tested with simple experiments. At the end of this paper we shall discuss whether the results obtained are evidence of assortative schooling.

2. Material and methods

2.1. General

Three-spined sticklebacks ($n=150$) were collected with fry traps from the Baltic Sea littoral. Before the experiments the sticklebacks were acclimated to fresh water for 10 days in 60 l holding aquaria (20–25 fish each, 15°C, 16h:8h light:dark rhythm). While not in use, the fish were fed *ad libitum* on live *Daphnia*, chironomid larvae and aquarium fish food.

The standard body length of the fish used in the trials ranged from 3.4 to 6.4 cm. "Small" fish averaged 4.4 cm ($SD\pm0.3$ cm, $n=50$) while "large" fish were 5.7±0.5 cm ($n=62$) long. In the feeding experiments no single fish was used more than once a day. Prior to these experiments the sticklebacks were deprived of food for about 12 hours. The experiments (made between 09:00 and 17:00 hours, under 200 lux light at ca. 20°C in a win-

dowless laboratory room) were run in a white polyethylene aquarium (60×40×22 cm) filled up to 19 cm with fresh water. As standardised prey in the trials, we used 1.7-mm and 2.2-mm *Daphnia magna* (Nuutinen & Ranta 1985).

2.2. Feeding behaviour

Three different experiments were made.

First, we scored the number of *Daphnia* eaten by individual sticklebacks (5.7 cm in length) in schools of different size. The group sizes were 1, 2, 5, 10 and 20 fish, and they were replicated in a randomized order. The pre-selected number of fish were introduced into the aquarium and a single fish was chosen at random as the focal fish. The observation started when 500 1.7-mm *Daphnia* were introduced into the aquarium. We recorded the number of prey eaten by the focal fish during a 3 min period. The sample sizes were 15 for single fish, 12 for 2 fish, 10 both for 5 and 10 fish and 8 for 20 fish.

Second, the feeding rates of single fish, differing in size, were scored in experiments in which the initial prey density was 100 1.7-mm *Daphnia* per trial. Altogether 17 individuals (size range 3.4–6.4 cm) were used. Each individual was tested 8 times.

Third, we examined prey size selection by small (4.4 cm) and large (5.7 cm) sticklebacks by providing them with 50 small (1.7 mm) and 50 large (2.2 mm) *Daphnia*. The trials lasted 5 min. In these tests we first scored prey selection when small and large fish were foraging alone. Next we put two small or two large fish into the test aquarium and recorded the feeding of a randomly selected focal individual. Finally, we tested a small and large fish together, in half of the trials we followed the feeding of the small individual and in half of the cases the focal fish was large. The trials were so replicated that each level had observations of 10 different fish.

2.3. Schooling behaviour

Small (4.4 cm) and large (5.7 cm) sticklebacks were used to examine their schooling behaviour. Two different experiments were made.

First, we used a 1.2×1.0 m aquarium filled up to 15 cm with fresh water. The bottom of the aquarium was covered with a thin layer of sand. One of the long ends of the aquarium had a small cabin (10×15 cm) connected with a sliding door to the main arena. Through this door 5 small and 5 large fish were released and the trial began. The door and a time-lapse camera (1 frame s⁻¹) were operated from a hide. The locations of the 10 fish were followed for 10 min beginning from their release. The experiment was repeated with four different groups of 5 small and 5 large fish. The number of schools in the arena was scored for every full minute of the experiment. On the basis of the information obtained, the second half (6–10 min) of each run was taken for detailed analysis. For each full minute the number of fish in the schools was counted and the composition of the schools was assessed. The observations average about 5 s around each full minute.

Second, two plexiglass tubes (diam. 15 cm) were placed 80 cm apart in a children's wading pool (diam. 1.2 m). One of the tubes contained 6 small (4.4 cm) fish, while the other one had 6 large (5.7 cm) fish. The position of the schools was randomised between trials. In the middle of the two tubes was a third tube. The experiment was begun by placing the test fish (either small or large) in the central tube. After 5 min the tube was gently lifted from a hide and the test fish was freely swimming in the aquarium. The position of the experimental fish was then scored after 5 min. Usually the fish swam, after some waiting, to the preferred school and stayed there more or less to the end of the observation period, but we here shall report only the 5-min readings. Altogether 30 different small and 38 large fish were tested.

3. Results

3.1. Feeding behaviour

The number of prey eaten by an individual stickleback increases with school size. For example, a single fish, foraging alone (for 3 min), averages 7.5 *Daphnia* eaten, while in a school of two individuals the average feeding rate is 13 *Daphnia*. The increase in the feeding rate with school size is not linear, but gradually levels off in larger
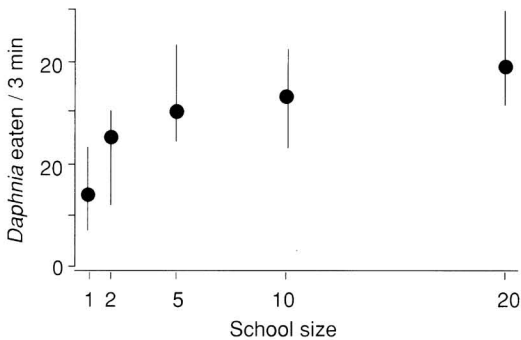
Fig. 1. Number of 1.7-mm *Daphnia magna* eaten (median with lower and upper quartiles) by stickle-backs (5.7 cm standard length) in schools of different size. The data refer to feeding rates of individual fish.
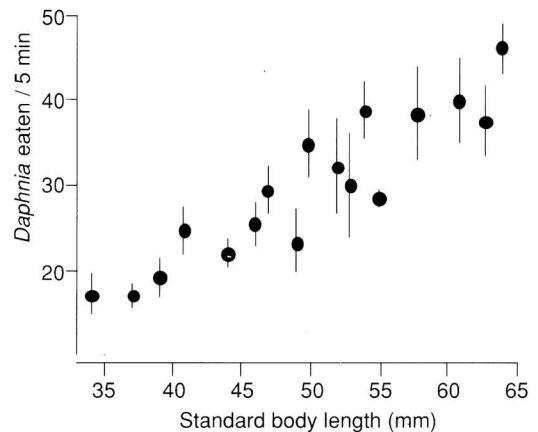

Fig. 2. Number of 1.7-mm *Daphnia magna* eaten (mean ± standard error) by 17 individual sticklebacks of different size. Each individual was tested 8 times.

schools (Fig. 1). A comparison of the numbers of prey eaten (Kruskal-Wallis ANOVA, H=43.57, df=4, P>0.001) indicates significant differences among the different school sizes. A-posteriori comparisons (Zar 1984; S-N-K -tests, α = 0.05) suggest differences between the school of one fish and the schools of 5, 10 and 20 fish, and between the school of 5 fish and 20 fish. The results thus show that for an individual fish there is clearly an advantage (in terms of foraging success) in join-ing a school of other sticklebacks.

There is a positive relationship between stick-leback body size and the rate of feeding on *Daph-nia*. The largest fish feed at about twice the rate of the smallest (Fig. 2; the correlation coefficient between body size and the mean number of prey eaten is r=0.91, df=15 P<0.001). The pattern suggests that, in a school of fish, the feeding rates realized by an individual stickleback may differ with the relation of its own size to the sizes of other members of the school.

To examine how size differences between simultaneously foraging sticklebacks affect their feeding success, we scored the prey size selection of small fish when they were foraging alone, with another small fish, and in the company of a large fish. The corresponding observations were also made for a large fish. Irrespective of body length, the three-spined sticklebacks are size-selective planktivores (Fig. 3). For a solitary small fish the percentage of large *Daphnia* eaten is about 70% of
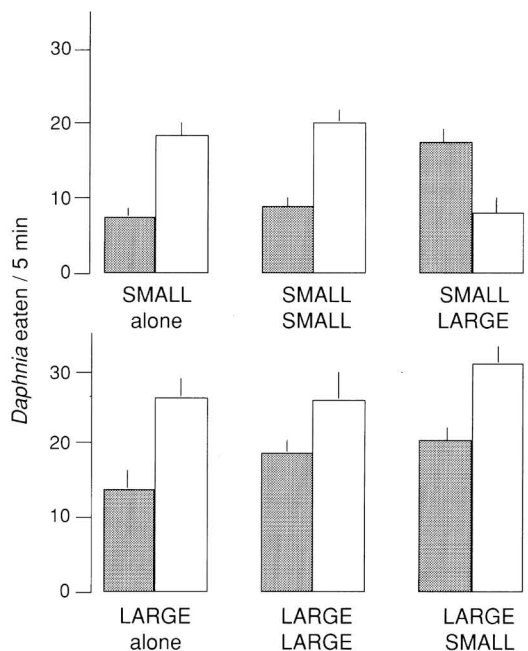

Fig. 3. Prey size selection (hatched = 1.7 mm, open bar = 2.2-mm *Daphnia magna*; mean with standard error indicated) by small and large sticklebacks when tested alone or in groups of two fish. The upper panel shows prey size selection by small (4.4 cm) and the lower panel the corresponding figures for large (5.7 cm) fish (see also Table 1).

the total prey ingested. The equivalent figure for a solitary large fish is 65%. When two small fish were foraging together no change in size selectivity was observed (69% large prey). The prey size chosen by a small fish changes sharply (30% large prey) when it is foraging with a large fish. On the other hand, for the bigger fish the proportion of larger prey is 58% in a group of two large fish, and 60% when it is foraging together with a small fish (Fig. 3).

In the following statistical tests we took the proportion of larger prey eaten (with arcsine square root transformation) as the dependent variable. Firstly, no difference was observed between single small and large fish in their prey size selectivity ($t=0.928$, $df=18$, $P>0.1$). Secondly, a comparison was made to check how the size of the competitor affects prey size selection by the focal fish. For this comparison we took only the trials with two fish (Fig. 3). Both the size of the focal fish and the size of the competitor clearly affect the prey size selection by the focal fish (Table 1).

In the two-prey experiments we again observe a slight improvement of the feeding rate when a fish is foraging with another fish: A small fish alone averages a total of 22.5 *Daphnia* eaten. In the company of another small fish the feeding rate is 27.2 *Daphnia*. When a small fish is in the company of a large fish it manages to eat 24.5 *Daphnia*. The corresponding figures for a large stickleback are 38.8 (alone), 43.5 (with another large fish) and 50.1 (with a small fish). As competition affects size selectivity and hence the total number of prey eaten, no statistical comparisons are made here.

Table 1. ANOVA table for examining how the size of the competitor (small, large) affects the prey size selection of the focal fish (small, large). The abbreviations used are *SS* = sum of squares, *df* = degrees of freedom, *MS* = mean square, *F* = *F* ratio and *P* = the level of significance.

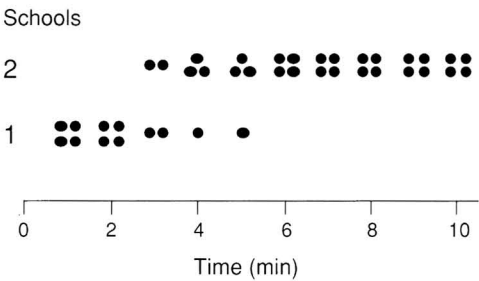| Source	SS	df	MS	F	P
Focal fish	0.122	1	0.122	17.5	0.0004
Competitor	0.702	1	0.702	100.6	<0.0001
Interaction	0.870	1	0.870	124.7	<0.0001
Error	0.251	36	0.007		

Fig. 4. Ten fish, 5 small and 5 large, were released into an arena of 1.2×1.0 m. Every full minute from the beginning of the trial the number of schools was scored until 10 min had elapsed. The experiment was repeated with four different sets of 10 fish. The black dots refer to these four replicates, e.g. at 4 min in one trial the fish were in a single group, while in three trials they formed two separate schools.

3.2. Schooling behaviour

The five small and five large fish released into the 1.0×1.2 m arena first formed into a tight school. Gradually, the cohesiveness of the group broke down and the fish invariably formed two schools. The presence of two schools in the second half of the experimental runs was consistent in the four trials (Fig. 4). The two schools differed in fish size composition. In all cases one of the schools was numerically dominated by small fish and the other by large fish. The school of small fish was slightly larger than that of the large fish, but in the two last min of the experiment both schools had five fish (Fig. 5a, b). During the first five min of the experiment the fish swam between the two schools. The movements levelled off towards the end of the 10-min runs. As a result, the fish in the two schools were rather alike in size at the termination of the trials (Fig. 5c).

The final experiment was planned to ascertain, with a different set-up, whether sticklebacks tend to form assortative schools. A fish of a given size was allowed to make a choice between two homogeneous groups of six fish of different size. In 67% of the cases (comparison of the observed frequencies against the 1:1 binomial expectation gives a p value of 0.0493) small sticklebacks associated with the school of small fish and in 73% of the trials ($P=0.0026$) large fish were found close to the school of large fish (Fig. 6).
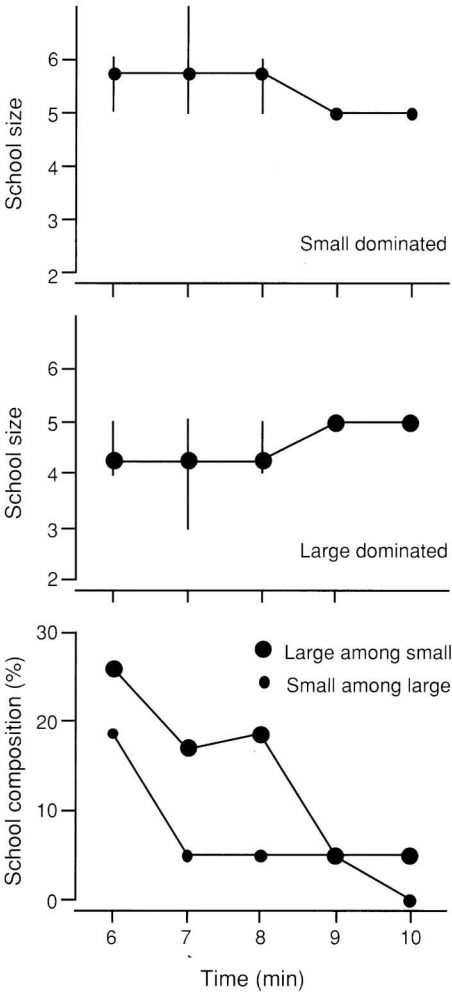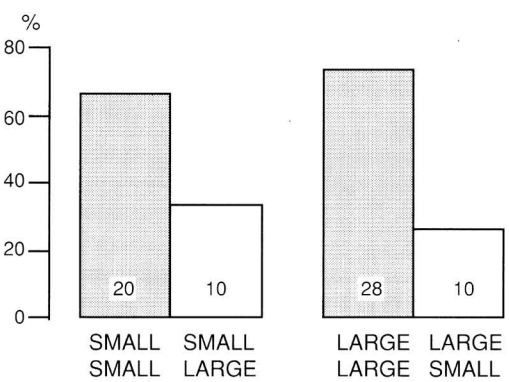
Fig. 6. Small fish were allowed to select between two schools, one composed of six small fish and the other of six large fish. In 20 out of 30 cases the small fish were found close to the school of small fish. The same experiment was repeated with large fish. In 28 cases out of 38 they were found close to the school of large fish.

Fig. 5. Ten fish, 5 small and 5 large, were released into an arena of 1.2×1.0 m. Five min after the beginning of the experiment, two distinct schools had formed (Fig. 4). The uppermost panel gives the number of fish (mean and range of four trials) for the schools dominated by small fish for every full minute to the end of the experiment. The middle panel gives the same information for the schools dominated by large fish. The bottom panel indicates the size composition of the two schools.

4. Discussion

An individual three-spined stickleback has a higher rate of food intake when it is in a group of other fish than when it is foraging alone. The gain in feeding rate is an asymptotic function, levelling off with increasing school size. Our finding parallels that by Pitcher & Magurran (1983) for goldfish. They could show that individuals in groups of five spent more time foraging than those living in groups of two. An asymptotic gain function with increasing school size was observed with three-spined sticklebacks foraging for prey on the aquarium bottom (Ranta & Kaitala, unpubl.). We conclude that, all other things being equal, it pays (in terms of prey eaten) for an individual stickleback to join a school of sticklebacks.

We have demonstrated that, when foraging alone, sticklebacks of different size differ in their food intake rates, small fish being notably less effective than large fish. Sticklebacks clearly prefer larger prey items to smaller ones. The presence of a competitor, however, alters their size selectivity. The effect of an opponent is most striking when a small fish is foraging with a large fish — the small individual switches to eating smaller prey. We also found that in the presence of a small fish a large stickleback has a more pronounced selectivity than when foraging with another large fish. Our findings are comparable with those of Milinski (1982), who observed that competition forced the less successful sticklebacks to consume a lower proportion of the more profitable prey

type. Intraspecific competitive ability in three-spined sticklebacks is clearly size-dependent.

If we assume that sticklebacks of different size have a choice while schooling, it would pay a small stickleback to join other small sticklebacks. On the other hand, a large fish would also do better in association with smaller fish than in the company of uniformly large fish. This suggestion is in conflict with our present observations. In the schooling experiments we found that small fish tend to associate with small fish and large fish are most often found with large fish. Whether this pattern is due to intraspecific competition or due to predator avoidance remains to be discovered.

We propose a simple model in which individual fish of different size form schools of uniform size structure. We hypothesise that there is selection for individuals living in schools because it amplifies their foraging returns (present study) and diminishes predation risk (Magurran 1990). The benefits of joining a school increase asymptotically, while the costs increase linearly. Hence, the net benefit function reaches a maximum at some intermediate — optimal — school size (Fig. 7).

The costs of joining a school are, as we have shown, size-dependent. The costs of joining a group of small fish is smaller than that of joining a group of large fish. Hence, the maximum difference between the benefit function and the cost function — the optimal school size — will be reached later for small fish than for large fish (Fig. 7). We observed that the feeding rate of sticklebacks is positively correlated with body size. Given a limited number of food particles available, a school composed of small fish could sustain more fish than a school of large fish in the same environment. If the gross benefit function is assumed to be equal for the two fish sizes, the optimal school size is greater for small fish than for large fish. If natural selection favours individuals that live in groups of optimal size, then assortative schooling can be expected.

What prevents large individuals from invading schools of small fish? The large individual takes its share of the resources (greater than that of any of the smaller individuals) but it also provides the school with two keen eyes ready to scan for food and predators. Obviously, for any given environment there is a great number of combina-
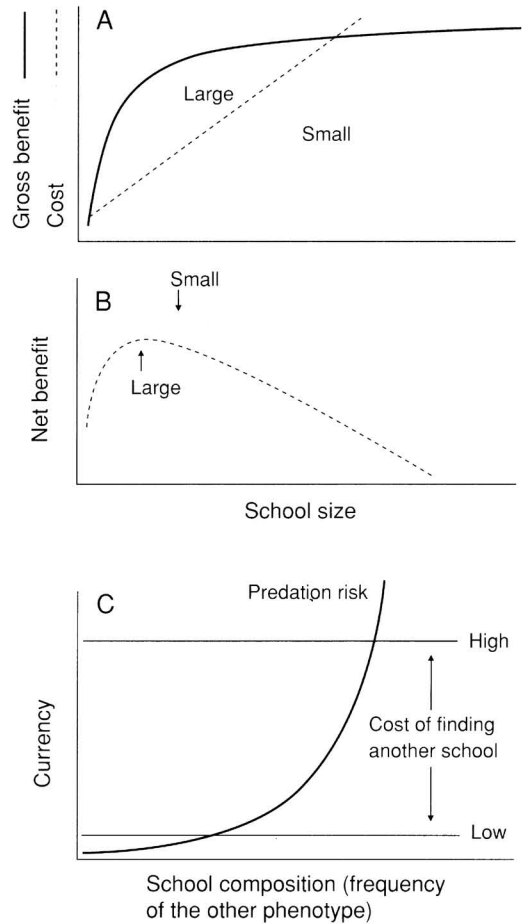

Fig. 7. — A. Gross benefit (solid line) and cost (small and large fish are indicated with separate functions) of joining schools of different size. It is assumed that the costs of joining a school are size-dependent — smaller costs for smaller fish, while the gross benefit is independent of size. — B. Calculating the difference between gross benefit and cost functions results in net benefit functions, which are different for small and large fish. Arrows indicate optimal school sizes. — C. Risk of being eaten (solid line) plotted against school composition. The "oddity score" of an individual increases from left to right (x-axis): In the school close to the origin all individuals are alike, say small. None of them are odd. While in the school at the right all the others are of same phenotype (large) but one stray individual is different (small). The stray individual is assumed to be an easy target for a predator. The two horizontal lines depict situations (from the point of view of the stray individual) in which the costs of finding another school of more favourable phenotypic composition are different. When the costs are low, it pays to leave almost any mixed school, but when the costs are high, it is more favourable to stay in a school of almost any composition.

tions of small and large individuals which could make efficient use of the available resources.

We propose that predation is the key to understanding size-assortative schooling. Consider a school composed of two phenotypes, small and large fish. When the proportion of one of the phenotypes decreases, individuals representing this phenotype become more conspicuous to predators. This is also known as the oddity effect (Landeau & Terborgh 1986). The deviating individuals are a far more easy target for the attacking predator than individuals of the dominating phenotype (Theodorakis 1989), which can benefit from the confusion effect (Ohguchi 1981, Magurran 1990). Deviating individuals have also been shown to rely less on schooling in the presence of predators (Wolf 1985). That is, whenever the costs of changing schools are smaller for an individual of the odd phenotype than the gain from staying in a school with adverse size composition, it pays to move (Fig. 7).

The model generates the following testable predictions. Firstly, with increasing population density the costs of changing school decrease. This is a consequence of the proximity of many other schools of fish. Therefore, at high densities the degree of size assortativeness should increase. Given the option, the more an individual differs from others in the school, the more willingly it should change the school. In a mixed school, as predation risk increases, the disadvantage of being odd also increases. It follows that high levels of predation risk should be associated with low size heterogeneity among individuals in schools. When no predators are present, the oddity effect disappears and the size asymmetry in intraspecific competition becomes more severe. Under these circumstances single small fish should not be observed among large fish.

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