On the spurious occurrence of Tit for Tat in pairs of predator-approaching fish

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Abstract. An experimental analysis of the movements of predator-approaching fish is presented. The experiments evaluated two competing hypotheses. (1) Predator-approaching fish play the game-theoretical strategy Tit for Tat. Alternatively, (2) the movements of predator-approaching fish superficially resemble Tit for Tat, because fish independently orient to a predator and simultaneously attempt to stay close together. Experimental subjects were mosquito fish, Gambusia affinis, approaching a green sunfish, Lepomis cyanellus. Two experiments were performed. Experiment 1 replicated results of Milinski (1987) and Dugatkin (1991), showing that Gambusia come closer to a visible predator when a mirror is oriented parallel to their direction of travel. Experiment 2 attempted to separate the effects of common orientation and social cohesion in accounting for the frequency of Tit-for-Tat-like motions in pairs of predator-approaching Gambusia. Results of experiment 2 suggest that a simple additive combination of the effects of (1) social cohesion in the absence of a visible predator and (2) orientation to a visible predator in the absence of a visible companion can account for the observed frequency of Tit-for-Tat-like motions for pairs of predator-approaching Gambusia. It is concluded that predator approach in shoaling fishes is probably a simple by-product mutualism, rather than cooperation maintained by reciprocity in a Prisoner’s Dilemma.

In 1981, Axelrod & Hamilton published an influential paper that offered a seemingly general framework for understanding the evolution of non-kin cooperation. Axelrod and Hamilton’s framework has two components: a game and a strategy. First, they argued that Flood and Drescher’s ’Prisoner’s Dilemma’ game typified the problems of achieving cooperation in a selfish world (see Mesterton-Gibbons 1992 for a precise definition of the Prisoner’s Dilemma and a review of technical issues). Second, they argued that stable mutual cooperation could be achieved in this paradigmatic game if (1) the game was played repeatedly, and (2) the players adopted a strategy they called ’Tit for Tat’. A player adopting the Tit-for-Tat strategy cooperates on the first ’play’ of a sequence and copies its opponent’s behaviour on all subsequent plays; for example, a Tit-for-Tat player cooperates on the tenth play, if its opponent cooperated on the ninth, and so on.

Predator Approach as Tit for Tat

Milinski (1987) offered what seemed to be the first plausible experimental system for the analysis of Axelrod and Hamilton’s framework. Sticklebacks, and many other small fishes, will often approach larger potentially predacious fish rather than fleeing, as the naive observer might expect. Milinski studied the phenomenon of predator approach in the stickleback Gasterosteus aculeatus. Milinski, following Pitcher et al. (1986), favoured an ‘inspection’ interpretation of this approach behaviour, in which approaching sticklebacks are thought to obtain information about the state of the predator (e.g. whether it is hungry or aggressive). With this interpretation in mind, he argued that the economics of pair-wise inspection represented a Prisoner’s Dilemma, and he therefore predicted that sticklebacks approaching a predator would play Tit for Tat. Milinski
viewed a movement towards the predator as 'cooperation' (or C in Prisoner's Dilemma jargon) and movement away from the predator as 'defection' (the non-cooperative choice, D). Thus, Milinski argued that a stickleback would tend to move forward, towards the predator, if paired with a partner that also moved towards the predator, but the same stickleback should move backwards, or stay away from the predator, when paired with a partner that also stayed away.

Milinski's apparatus consisted of a narrow aquarium with a predator at one end and a plant at the other. Milinski created 'apparent partners' by using different arrangements of mirrors. In one experimental treatment, Milinski oriented a mirror parallel to the long axis of the aquarium so that the subject's image moved towards the predator as the subject moved towards the predator. Milinski called this the 'cooperating mirror'. In the second treatment, Milinski oriented a mirror at an angle so the subject's image moved away from the predator as the subject moved towards the predator. Milinski called this the 'defecting mirror'. Milinski showed that sticklebacks experiencing the 'cooperating mirror' treatment were on average closer to the predator than the sticklebacks experiencing the 'cooperating mirror' treatment. Milinski showed that sticklebacks experiencing the 'cooperating mirror' treatment were on average closer to the predator in a 5-min interval than the sticklebacks experiencing the 'defecting mirror' treatment. Milinski interpreted this difference as evidence that predator-approaching sticklebacks were adopting Tit for Tat, or a Tit-for-Tat-like strategy. Milinski's mirror experiment has been widely replicated (Dugatkin 1988, 1991; Masters & Waite 1990). Moreover, several studies have refined Milinski's mirror technique by substituting models (Huntingford et al. 1994), trained fish (Milinski et al. 1990) or untrained fish (Huntingford et al. 1994) for the hypothetical partners provided by the mirrors in Milinski (1987).

The Critics

Milinski's claims were contested by a number of critics (Masters & Waite 1990; Lazarus & Metcalfe 1990; Reboreda & Kacelnik 1990). Although these authors raised several issues, two issues stand out. First, Milinski neither measured nor controlled the benefits associated with approach and non-approach in his experiments. Thus, although it is possible that predator-approaching fish are in a Prisoner's Dilemma, it is also possible that they are not (Lazarus & Metcalfe 1990). Indeed, recent experimental evidence supports a 'pursuit deterrence' function for predator approach (Godin & Davis 1995b), and suggests that 'approaching' individuals may suffer a lower predation risk than non-approaching fish. This evidence undermines the claim that pairs of predator-approaching fish face a Prisoner's Dilemma. Second, the prediction that a fish will move in the same direction during, say, interval 10, that its partner moved during interval 9, is not unique to Tit-for-Tat. Several authors have pointed out (Lazarus & Metcalfe 1990; Huntingford et al. 1994) that this sort of 'direction-copying' follows from the widely observed tendency of fish to stay close together (i.e. to shoal). A difficulty here, as noted by Huntingford et al. (1994), is that in the absence of an accepted quantitative model of shoaling, there seems to be no basis to experimentally differentiate move-copying due to shoaling from move-copying due to Tit for Tat.

A Diffusion Model of Shoaling

Recently, however, one of us (Stephens, in press) has advanced a relatively simple quantitative model of pair-wise 'shoaling behaviour'. This model supposes that individuals move according to a simple, possibly biased, random walk, except that they make small corrections to the probability of forward movement to move closer together. If we simplify things by considering a pair of fish moving along a number line, then the model considers two random variables, $U(t)$, representing the probabilistically determined distance separating the fish at time $t$, and $Z(t)$ representing the mean position of the pair at time $t$. Stephens showed that a random walk with social corrections implies that position $Z(t)$ will be governed by a Wiener process (i.e. a continuous time random walk). A Wiener process implies that $Z(t)$ will follow a normal probability distribution with mean $z_0 + \mu t$ and variance $\sigma^2 t$, where $z_0$ is the initial position, $\mu$ is the mean movement per unit time (with units of distance per time) and $\sigma^2$ is the variance per unit time (with units of distance squared per time). The separation variable, $U(t)$, will be governed by an Ornstein–Uhlenbeck process and will also follow a normal probability distribution with mean $u_0 e^{-\beta t}$ and variance...
where $u_0$ is the initial separation, and $\hat{\gamma}$ is a measure of the strength of the social cohesion of the pair. The parameter $\hat{\gamma}$ has net units of inverse time, because it measures ‘distance moved to correct a separation’ per ‘distance of separation’ per ‘unit time’. An important feature of the Ornstein–Uhlenbeck process is that as time gets large, the separation approaches a stationary normal distribution with mean zero, and variance $2\hat{\gamma}^2$.

Following Stephens (in press), we defined Tit for Tat as an event in which fish A moves in the same direction during the interval from $t+\tau$ to $t+2\tau$ that fish B moved during the interval from $t$ to $t+\tau$, where the experimenter determines the interval $\tau$, although we assume that this interval is relatively small. This definition operationalizes Milinski’s (1987) idea that ‘… each forward jerk … is one episode of cooperation’ (page 433), but avoids the subjectivity of deciding when a ‘jerk’ has occurred. Using this definition, we can use the ‘random walk with shoaling corrections model’ to predict the relative frequency of apparent Tit for Tat.

Figure 1 shows the results of such a calculation (reproducing Stephens’ Figure 3). The parameters of the model have been re-scaled to create dimensionless parameters to simplify the calculations and to make them independent of the scale of experimental measurements. The $\mu$ axis of Fig. 1 is a dimensionless measure of directionality, specifically

$$
\mu = \frac{\hat{\mu} \tau}{\sqrt{\hat{\sigma}^2 \tau}}
$$

which measures the direction and amount of movement in interval $\tau$ (i.e. $\hat{\mu} \tau$) relative to the amount of spatial variability that occurs in $\tau$ (i.e. $\sqrt{\hat{\sigma}^2 \tau}$). Note that $\mu=0$ implies random choice of direction, $\mu>0$ suggests movement to the right, and $\mu<0$ suggests movement to the left). The $\gamma$ axis is a dimensionless measure of the ‘social correction’,

$$
\gamma = \hat{\gamma} \tau
$$

that occurs in the experimentally defined interval $\tau$.

Figure 1 shows a striking gull-wing shape. The smallest possible frequency of Tit for Tat is $\frac{1}{2}$ in the case when there is no ‘social cohesion’ ($\gamma=0$) and no directionality ($\mu=0$). Of course, this minimal frequency is nothing more than the Bernoulli trials of two independent, unbiased random walks, i.e.

$$
P(A \text{ moves up in interval } t+1) 	imes P(B \text{ moves up in interval } t+1) +
P(A \text{ moves down in interval } t+1) 	imes P(B \text{ moves down in interval } t+1)
$$

$$
= \frac{1}{2} \times \frac{1}{2} + \frac{1}{2} \times \frac{1}{2} = \frac{1}{2}
$$

Although the figure shows that shoaling (as measured by the parameter $\gamma$) increases the likelihood of Tit for Tat as Milinski’s critics suggested, it also shows that directionality (as measured by the parameter $\mu$) can increase the likelihood of Tit for Tat. There is nothing surprising here. If two fish independently approach the same stimulus, then they will appear to copy each other’s moves, simply because they are going in the same direction.

The possibility that spurious Tit for Tat might result from the joint effects of common orientation and social cohesion seems especially relevant to the predator-approach paradigm, because subjects are provided with a stimulus (the predator) that they orient to even when alone (e.g. Godin & Davis 1995a and references therein), and a social stimulus (a partner or apparent partner) that they orient to in the absence of a predator (Masters & Waite 1990). With this logic in mind, we performed two simple experiments. First, we replicated Milinski’s mirror effect to establish the
appropriateness of our apparatus and procedures. Second, we performed a straightforward factorial experiment to estimate how the relative frequency of Tit for Tat is affected by (1) social cohesion in the absence of a predator stimulus and (2) orientation to the predator stimulus in the absence of social cohesion. In turn, we asked whether the combination of these two non-strategic effects is sufficient to explain the observed frequency of Tit for Tat when both predator and social stimuli are present.

**GENERAL METHODS**

Subjects

We studied female mosquito fish, *Gambusia affinis* (mean total body length=2.75 cm, range=2–4 cm), approaching a green sunfish, *Lepomis cyanellus* (total body length=11.5 cm). *Gambusia* are live-bearing fish native to the southeastern United States and widely introduced for the control of mosquito larvae in much of the rest of the United States. They are feral throughout the Platte river drainage in Nebraska and co-occur with green sunfish in Nebraska and throughout much of the United States. The green sunfish is a frequent predator of smaller fish (*Werner* 1977). It is primarily a sit-and-wait predator found in shallow vegetated areas (*Werner & Hall* 1977). They eat mosquito fish readily in the laboratory. Subjects were collected in south-central Nebraska in autumn 1994 and spring 1995.

Apparatus and General Procedure

We conducted our experiments in a 30 × 76 × 31-cm ‘All-Glass’ brand aquarium. We made 13 × 30-cm ‘predator compartments’ on each end by adding transparent Plexiglas partitions (Fig. 2). We sealed both compartments with silicone aquarium sealant to eliminate olfactory predator cues and filled each to a depth of 8 cm. We arbitrarily designated only one of these compartments as the predator compartment, where the green sunfish remained throughout each experiment. The sunfish was returned to its larger maintenance aquarium between experiments 1 and 2. The central ‘subject compartments’ were filled to a depth of 1.5 cm. The apparatus was designed so that the partitions between predator and subject compartments and between the two subject compartments could be made opaque or transparent. We covered the floor and walls of the aquarium with opaque white material.

During a single experimental trial, one or two female *Gambusia* were dip-netted from a nearby holding tank and transferred to the subject compartments of the apparatus. In all cases, we placed fish in individual ‘lanes’ of the apparatus; in cases where pairs of fish were studied, we placed individuals in their respective lanes haphazardly. After a 2-min acclimation period, we videotaped the *Gambusia*’s behaviour patterns for a 5-min period, using a video-camera placed 125 cm above the aquarium bottom and directly over the centre of the aquarium (Fig. 2). We used commercially available ‘tracking’ hardware and software (San Diego Instruments Poly-Track system) to obtain the *X–Y* coordinates (we defined the long-axis of the tank to be the *X* dimension) of the *Gambusia* every 0.2747 s (as close to 0.25 s as allowed by the scan frequency of the videotape). The water in the subject compartment was shallow to allow precise focusing. The aquarium floor was white to enhance contrast, because that is what the tracking software detects.

Statistical Details

Both experiments follow classical two-by-two factorial designs. In experiment 1 the experimental unit was a single fish; in experiment 2 it was a pair of fish. The experimental comparisons were between-subjects comparisons, so that a single fish or single pair experienced a single combination of treatment variables. To account for the possibility that dependent measures may change with time within a trial, we subdivided the 5-min observation period into four equal 75-s blocks. We analysed the time blocks as repeated measures within the four treatment levels (we found an effect of time in only one instance). We studied several types of dependent measures (correlation coefficients, probabilities) that are constrained to relatively narrow ranges (e.g. a probability must be between 0 and 1). Constraints of this type suggest that these dependent measures cannot themselves be normally distributed, so we assessed the validity of analysis of variance for these measures in two ways. First, we compared the quantiles of the distribution of residuals to the
Figure 2. Diagram of experimental apparatus.
quantiles of a theoretical normal distribution graphically, using a so-called ‘q–q plot’ (Cleveland 1993). Similarly, we used ‘q–q plots’ to compare the distributions of residuals with one another in an effort to identity possible heterogeneity of variances. Using these techniques, we observed no systematic departures from the assumptions of analysis of variance.

Experiment 1: The Mirror Effect

Methods

We used a transparent Plexiglas partition to separate the two subject compartments. The subject Gambusia was placed in the compartment furthest from the experimenter (Fig. 2). The adjacent compartment either had a 38-cm mirror placed parallel to the long axis of the tank abutting the ‘no predator’ end compartment, or a 23.5-cm mirror set at a 30° angle from the long axis of the tank abutting the ‘no predator’ end compartment (Fig. 2). The ‘rear’ predator partition consisted of a permanent transparent wall behind which we placed a temporary opaque Plexiglas wall. The front predator partition (behind which there really was a predator) was similar except that we placed two removable pieces of Plexiglas against it. In ‘predator visible’ treatments we began a trial with one opaque and one transparent piece of Plexiglas, so that after the acclimation period we removed the opaque sheet to reveal the transparent one (and hence the predator). In ‘predator not visible’ treatments we used two opaque pieces of Plexiglas, so removing one revealed another opaque partition. The predator was positioned in front of the subject’s lane using a V-shaped opaque Plexiglas baffle.

We randomly assigned 40 female Gambusia to one of the following four treatments done in random order: (1) mirror straight, predator visible, (2) mirror angled, predator visible, (3) mirror straight, predator not visible, (4) mirror angled, predator not visible.

Results

Table I shows an analysis of variance table for the mirror experiment. The dependent measures are the median X positions, because we believe that medians give a more robust measure of position. (We performed similar analyses with means and found an identical pattern.) The X positions were measured in cm, with 0 representing the position furthest from the predator and 50 representing the position closest to the predator (Fig. 3).

There was a significant effect of the mirror treatment: fish moved closer to the predator compartment when exposed to the straight mirror (Table I, Fig. 3). The ability to see the predator, however, had no effect on the median proximity to the predator. This result replicates the main effect reported by Milinski (1987) and Dugatkin (1988). Recall that Milinski and Dugatkin used this mirror effect as evidence that predator-approaching fishes played Tit for Tat or some Tit-for-Tat-like strategy.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>F</th>
<th>P</th>
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<tbody>
<tr>
<td>Between fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predator</td>
<td>1</td>
<td>4.237</td>
<td>0.03808</td>
<td>0.8463774</td>
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<tr>
<td>Mirror</td>
<td>1</td>
<td>2346.048</td>
<td>21.08792</td>
<td>0.0000519*</td>
</tr>
<tr>
<td>Predator × mirror</td>
<td>1</td>
<td>21.213</td>
<td>0.19068</td>
<td>0.6649611</td>
</tr>
<tr>
<td>Residuals</td>
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<td>4005.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within fish</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
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<td>0.7894785</td>
<td>0.5023258</td>
</tr>
<tr>
<td>Predator × block</td>
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<td>52.743</td>
<td>0.3188631</td>
<td>0.8117114</td>
</tr>
<tr>
<td>Mirror × block</td>
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<td>23.514</td>
<td>0.1421564</td>
<td>0.9344855</td>
</tr>
<tr>
<td>Predator × mirror × block</td>
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<td>153.748</td>
<td>0.9294896</td>
<td>0.4291694</td>
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<tr>
<td>Residuals</td>
<td>108</td>
<td>5954.797</td>
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</table>
Figure 3. A graphic representation of the results of our replication of Milinski’s mirror effect. The top two panels show the straight-mirror treatments, and the bottom two panels show the angled-mirror treatments. The left column shows the two predator-visible treatments, and the right column shows the two predator-not-visible treatments. We represent the data using Tukey-style box plots. Each box represents the distribution of the 10 median positions in the following way. The central dot shows the median; the central box measures the spread of the distribution by showing the range from the 25th to the 75th percentile of the data; the whiskers allow a visual assessment of the tails of the distribution by extending to the highest and lowest data points that are within 1.5 inter-quartile ranges of the 75th and 25th percentiles respectively. A small open circle represents a single datum that is an outlier. Within each treatment combination, we have further subdivided the data by considering the first, second, third and fourth 75-s intervals within the 5-min observation period. A vertical reference line has been drawn on each panel at the overall mean of the data.
Our failure to find a predator effect is consistent with the report of Masters & Waite (1990), although Masters & Waite did not run a predator condition. In response to Masters & Waite, Milinski (1990b) argued that fish might treat an empty aquarium as a ‘potential predator’ (see also Lazarus & Metcalfe 1990; Huntingford et al. 1994). Although our result eliminates the need to see a predator, it does not remove the possibility that something about ‘predation’, or a generalized fear of predation is involved. In our experiment, for example, it is possible that the manipulation of the predator partition induced some ‘fear’ of the predator side of the apparatus whether or not the predator could be seen after the partition was manipulated.

However, even though the median position of the fish in the ‘predator not visible’ treatments was not in the centre of the tank, predation does not need to be invoked to explain this phenomenon. In the ‘mirror straight, predator not visible’ treatment, the median location of the fish is at approximately the middle of the mirror; in the ‘mirror angled, predator not visible’ treatment, it was close to the no-predator end of the tank. These results reflect the fact that fish remain close to their apparent companion.

EXPERIMENT 2: COHESION AND ORIENTATION

Methods

We randomly assigned 40 pairs of female Gambusia (i.e. 80 individuals) to one of four treatments done in random order: (1) companion visible, predator not visible; (2) companion visible, predator visible; (3) companion not visible, predator not visible; (4) companion not visible, predator visible. We assigned 10 different pairs to each treatment. The logic of this factorial design stems from the claim that both orientation to a common external stimulus (the predator) and social cohesion can enhance the likelihood of Tit for Tat. One can think of this design as a single test condition (predator and companion visible) and three controls: ‘companion not visible, predator not visible’ controls for the effects of simple uncoordinated motion; ‘companion not visible, predator visible’ controls for the effects of common orientation to the predator in the absence of social effects; ‘companion visible, predator not visible’ controls for social effects in the absence of orientation to the predator.

At the beginning of all trials, both partitions (centre and predator) were opaque. We placed each member of the pair into a separate subject compartment (Fig. 2). After the 2-min acclimation period, we removed an opaque Plexiglas sheet from the centre partition to reveal either a transparent partition or an opaque partition as required by the treatment. We removed a second opaque Plexiglas sheet from the predator partition as quickly as possible after the centre partition was removed. Again, either a transparent or opaque partition was revealed as required by the treatment.

We videotaped the behaviour patterns of both fish for the next 5 min. We used tracking software to obtain approximately 1200 time, $X$-position, $Y$-position triplets for each fish from the videotapes. We subdivided the 5-min observation period into equal 75-s blocks of time and calculated the relative frequency of Tit for Tat (or move copying), the correlation between the $X$ positions of the fish, and the average $X$ position of the pair ($=(X_A+X_B)/2$ if we designate $X_A$ and $X_B$ as the positions of the individuals). Recall that our definition of Tit for Tat requires that we specify a time interval, $\tau$. We take $\tau$ to be $4 \times 0.2747 \ s = 1.09 \ s$, or approximately 1 s. (Although the choice of 1 s is somewhat arbitrary, we have repeated the main analyses presented here, using intervals from 0.5 s to 4 s; over this range the choice of time interval had no effect on the patterns of statistical significance we observed.) To avoid problems of interdependencies between members of a pair, we arbitrarily designated the fish in the lower lane on the video screen as the ‘copier’ and the other as the ‘copyee’. We judged a single Tit-for-Tat event to have occurred when the copier moved in the same direction during the $n+1$st second that the copyee moved during the $n$th second. We only considered data in which both fish moved. Owing to the precision of our measurements, this eliminated only a few possible data points (less than 1%).

Results

Proximity to the predator

Table II and Fig. 4 show analyses of position. We were interested in whether the Gambusia
### Table II. Analysis of variance: orientation to the predator

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Median position</th>
<th>Proportion of time within 12.5 cm of the predator</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>df</td>
<td>Sum of squares</td>
</tr>
<tr>
<td>Between pairs</td>
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<td></td>
</tr>
<tr>
<td>Companion</td>
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<td>165.166</td>
</tr>
<tr>
<td>Predator</td>
<td>1</td>
<td>1335.612</td>
</tr>
<tr>
<td>Companion × predator</td>
<td>1</td>
<td>48.882</td>
</tr>
<tr>
<td>Residuals</td>
<td>36</td>
<td>8878.464</td>
</tr>
<tr>
<td>Within pairs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
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<td>141.500</td>
</tr>
<tr>
<td>Companion × block</td>
<td>3</td>
<td>122.579</td>
</tr>
<tr>
<td>Predator × block</td>
<td>3</td>
<td>119.327</td>
</tr>
<tr>
<td>Companion × predator × block</td>
<td>3</td>
<td>457.240</td>
</tr>
</tbody>
</table>
reacted to a visible predator by approaching it, and whether the visibility of a companion modified this approach in any way. We considered two measures of position. In our first analysis, we considered a measure of the average joint position of the pair \((X_A + X_B)/2\), where \(X_A\) is fish A’s position and \(X_B\) is fish B’s position). We were concerned, however, that this pair-wise measure might be biased against the extremes of position one might expect in predator-approaching fish,

**Figure 4.** Effects of visibility treatments on position and orientation. (a) Tukey box-plots of median positions of the pair; 50 cm is closest to the predator. (b) The proportion of time spent in the quarter of the tank closest to the predator. In both panels a vertical line is drawn at the overall mean of the data.
and, more importantly, that this bias might be stronger in our ‘companion not visible’ treatments. If individual positions were correlated, as they were when the fish could see one another, then a joint measure of position would give a fair reflection of individual position. Joint measures, however, may not reflect individual position accurately when fish positions are uncorrelated, as in our ‘companion not visible’ treatments. We therefore repeated our analysis using the position of a focal individual (the lower fish on the video screen), and a direct non-parametric measure of
approach: i.e. proportion of time spent in the quarter of the tank closest to the predator. Both analyses show the same qualitative picture. The visibility of the predator had a significant effect on position, but neither time (i.e. 75-s blocks) nor companion had any effect. This evidence is consistent with predator approach. When the predator was visible, the positions were shifted towards the predator.

**Pair-wise Patterns of Movement**

We analysed two measures of pair-wise interaction, the correlation between X positions of the two individuals, and the relative frequency of Tit-for-Tat like motions (Table III, Fig. 5). Analysis of variance of the correlation coefficient showed significant effects of companion, predator and time. Positions were strongly correlated whenever the companion was visible. We saw only a relatively small (but significant) effect of common orientation in the absence of information about the companion’s position.

Our analysis of the relative frequency of Tit for Tat showed significant effects of predator and companion; the main effect of time was not significant. The key evidence, however, was the absence of a significant interaction of companion and predator. This result suggests that the elevated incidence of Tit for Tat when both predator and companion are visible does not result from a special game-theoretic strategy, but instead is a simple additive consequence of orientation to a visible predator in the absence of a companion and orientation to a companion in the absence of a visible predator. That is, there is no reason to believe that a pair of fish in the absence of a visible predator are trapped in a Prisoner’s Dilemma and are playing Tit for Tat. Similarly, it is impossible for two fish who cannot see each other but who can see the predator to play Tit for Tat. The simple additive combination of these two conditions, however, provides a perfectly adequate description of the frequency of Tit for Tat when both predator and companion are visible. One might claim that fish in our ‘companion not visible’ treatment ‘believe’ that a predator lurks behind the manipulated opaque partition, and so may play Tit for Tat in an imagined Prisoner’s Dilemma. Evidence from the previous section contradicts this view by showing that fish in ‘predator not visible’ treatments are not approaching the partition as they do when a real predator can be seen.

**Dynamic Analyses**

In this sub-section we took a more exploratory and dynamic view of our data. Specifically, we used the temporal detail of our data to look for situations in which strategic Tit for Tat and shoaling models of spurious Tit for Tat most clearly disagree.

**Transition matrices**

A number of authors (Lazarus & Metcalfe 1990; Reboreda & Kacelnik 1990; Huntingford et al. 1994) have argued that a critical examination of Tit for Tat should analyse the dynamics of behaviour. Tit for Tat is fundamentally a dynamic claim about how an individual should respond (i.e. cooperate, C, or defect, D) to its companion’s previous action. The difficulty with this commonsense argument is that general dynamic analyses of real world data can be enormously complex; indeed, ‘dynamics’ is a rapidly growing sub-discipline of mathematical statistics. We present results from one approach, the examination of transition probabilities. If we accept that a move forwards (in 1.09 s) can be considered a cooperative move (or C) and a move backwards can be considered a defect (or D), then in the i-th interval only four things can happen: DD, both fish move backwards; DC, the first fish moves backwards and the other fish moves forwards; CD, the first fish moves forwards and other fish moves backwards; and CC, both fish move forwards. If two fish really implement Tit for Tat, these states completely determine what must happen next; for example a CC in interval i must be followed by a CC in interval i+1. Extending this logic, the transition matrix

\[
\begin{array}{cccc}
\text{From} & DD & DC & CD & CC \\
DD & 1 & 0 & 0 & 0 \\
DC & 0 & 0 & 1 & 0 \\
CD & 0 & 1 & 0 & 0 \\
CC & 0 & 0 & 0 & 1 \\
\end{array}
\]
<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>F</th>
<th>P</th>
<th>df</th>
<th>Sum of squares</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correlation coefficient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between pairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Companion</td>
<td>1</td>
<td>16.96507</td>
<td>94.5717</td>
<td>0.0000000*</td>
<td>1</td>
<td>0.6904640</td>
<td>43.7738</td>
<td>0.0000001*</td>
</tr>
<tr>
<td>Predator</td>
<td>1</td>
<td>0.74635</td>
<td>4.16052</td>
<td>0.0487670*</td>
<td>1</td>
<td>0.0988517</td>
<td>6.26697</td>
<td>0.0169753*</td>
</tr>
<tr>
<td>Companion × predator</td>
<td>1</td>
<td>0.33613</td>
<td>1.87378</td>
<td>0.1795241</td>
<td>1</td>
<td>0.0110108</td>
<td>0.69806</td>
<td>0.4089468</td>
</tr>
<tr>
<td>Residuals</td>
<td>36</td>
<td>6.45798</td>
<td></td>
<td></td>
<td>36</td>
<td>0.5678441</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within pairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>3</td>
<td>1.31967</td>
<td>3.655263</td>
<td>0.0148160*</td>
<td>3</td>
<td>0.035404</td>
<td>0.843274</td>
<td>0.4730980</td>
</tr>
<tr>
<td>Companion × block</td>
<td>3</td>
<td>0.65058</td>
<td>1.802007</td>
<td>0.1511479</td>
<td>3</td>
<td>0.056389</td>
<td>1.343088</td>
<td>0.2642734</td>
</tr>
<tr>
<td>Predator × block</td>
<td>3</td>
<td>0.12192</td>
<td>0.337701</td>
<td>0.7980997</td>
<td>3</td>
<td>0.013268</td>
<td>0.316033</td>
<td>0.8137562</td>
</tr>
<tr>
<td>Companion × predator × block</td>
<td>3</td>
<td>0.06172</td>
<td>0.170962</td>
<td>0.9157981</td>
<td>3</td>
<td>0.021277</td>
<td>0.506794</td>
<td>0.6784138</td>
</tr>
<tr>
<td>Residuals</td>
<td>108</td>
<td>12.99719</td>
<td></td>
<td></td>
<td>108</td>
<td>1.511432</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the 0.05 level.
represents the dynamic predictions of Tit for Tat, where the entries in each cell represent the probabilities of a transition from the states shown in the rows to the states shown in the columns. Table IV shows averaged transition matrices for each of our four treatment combinations (these are cell-by-cell averages of the 10 different transition matrices in

**Figure 5.** Effect of visibility treatments on measures of pair-wise movement. (a) Tukey box-plots of the correlation between the X positions of members of the pair. (b) The relative frequency of Tit for Tat, measured as direction-copying over a time interval of approximately 1.1 s. In both panels a vertical line is drawn at the overall mean of the data.
each treatment combination, so the rows are not expected to sum to one. Table IV shows a glaring disagreement with strict Tit for Tat: there are no transition probabilities that come close to one. Strict Tit for Tat, in which a companion’s C is always followed by C, is probably too much to expect. Instead, we consider the entire family of ‘first-order’ Tit-for-Tat models (including generous Tit for Tat), in which c is the probability that a C will follow a companion’s C, and \( d \) is the probability that a D will follow a companion’s D. We can then write the general transition matrix for this family of first-order Tit for Tats as

Figure 5. Continued.
From $DD$ $DC$ $CD$ $CC$

<table>
<thead>
<tr>
<th>From</th>
<th>$DD$</th>
<th>$DC$</th>
<th>$CD$</th>
<th>$CC$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$DD$</td>
<td>$d^2$</td>
<td>$d(1-d)$</td>
<td>$(1-d)^2$</td>
<td>$(1-d)^2$</td>
</tr>
<tr>
<td>$DC$</td>
<td>$d(1-c)$</td>
<td>$(1-d)(1-c)$</td>
<td>$dc$</td>
<td>$c(1-d)$</td>
</tr>
<tr>
<td>$CD$</td>
<td>$(1-c)d$</td>
<td>$d(1-c)$</td>
<td>$(1-c)(1-d)$</td>
<td>$c(1-d)$</td>
</tr>
<tr>
<td>$CC$</td>
<td>$(1-c)^2$</td>
<td>$c(1-c)$</td>
<td>$c(1-c)$</td>
<td>$c^2$</td>
</tr>
</tbody>
</table>

(setting $c=d=1$ returns the ‘strict’ Tit-for-Tat matrix). It is straightforward to perform a traditional goodness-of-fit test for the Tit-for-Tat family of rules, because the basic data (from which Table IV was derived) are frequencies. The $d$ and $c$ parameters can be estimated from the top and bottom rows of an empirical transition matrix. The transition matrix itself, however, only specifies the frequencies of transitions given a specified starting state (i.e. $DD$, $DC$, $CD$ or $CC$). Therefore, to find the expected frequency of each type of transition (e.g. $DC$ to $CC$) we also need to estimate the relative frequencies of each state. The estimated $c$ and $d$ values completely determine the relative frequencies of the $DD$, $DC$, $CD$ and $CC$ states, because they specify the stationary distribution of these states (Feller 1950).

We calculated expected frequencies of each transition type by the following steps: (1) calculate the empirical transition matrix $e$; (2) find least squares estimates of $d$ and $c$ from the top and bottom rows of $e$; (3) use these estimates to calculate the theoretical transition matrix, as shown above; (4) use the theoretical transition matrix to calculate the long-run frequencies of the states (i.e. $DD$, $DC$, $CD$ and $CC$); (5) derive expected proportions of each transition type from these long-run frequencies and the theoretical transition matrix; (6) estimate observed transition counts by considering only the last 225 s of the data (allowing time for the stationary distribution to eliminate any effects of initial state). The 16 observed transition counts from step (6) are compared with predicted transition frequencies from step (5) via an ordinary chi-square goodness-of-fit test with 13 degrees of freedom removed for calculating the number of events from the data, and one removed for estimating each of the two parameters, $c$ and $d$; Table V). The observed frequencies differ from those predicted by the first-order Tit-for-Tat family in nine of 10 cases. The probability of nine or more significant departures from expectation in 10 0.05-level significance tests is approximately $1.86 \times 10^{-11} \approx (0.05)^{10} + 10 \times 0.95 \times 0.05^9$ if the underlying hypothesis is true.

The general pattern of the top and bottom rows of the transition matrix is generally consistent with Tit for Tat (Table IV). The disagreement comes with the middle rows. The data show a clear inertial pattern with $CD$ being followed by more $CD$s, and not by $DC$s as Tit for Tat predicts.
One might be tempted to give Tit for Tat credit for getting the top and bottom rows right, except that this pattern emerges even when the fish cannot see each other and so cannot be playing Tit for Tat (Table IV). This pattern suggests that an inertial component of the fishes’ normal motion creates the observed pattern.

The transition analysis is equally damning for the Stephens’ (in press) model of shoaling. The critical evidence here comes from the ‘companion not visible, predator not visible’ treatment. Following the Stephens’ model, we should see a simple unbiased random walk when the fish cannot coordinate with each other. In an unbiased random walk, behaviour at time \( i \) ought to have no effect on behaviour at time \( i+1 \). However, when we applied conventional contingency table tests of independence to the empirical transition tables, we found that all 10 pairs in the ‘companion not visible, predator not visible’ treatment showed significant departures from independence. Again, the problem is the strongly inertial nature of Gambusia movement. In a random walk, position now only depends on the position in the previous step, so it cannot account for inertia. Our data suggest an alternative model in which velocity now is determined by velocity in the previous step. The Wiener process (continuous-time simple random walk) frequently fails in this way, and is frequently patched up by modelling temporal changes in velocity (Cox & Miller 1965).

### DISCUSSION

Our results favour the view that the patterns of motion shown by predator-approaching fish reflect the simple statistical combination of individual orientation to a predator and attraction to a companion, and not the implementation of some special strategy. Moreover, the underlying patterns of motion are not well modelled by a random walk, because our fish show a strong inertial pattern (they tended to move in the direction they moved last). We believe that a model of shoaling incorporating this inertial property and the tendency to ‘stay together’ would explain the phenomena documented here.

Our results, taken together with other recent results (Godin & Davis 1995b), cast further doubt on Milinski’s (1987) claims that predator-approaching fishes play Tit for Tat in an Iterated Prisoner’s Dilemma. Godin & Davis’ experiments suggest that there may be a direct selfish benefit to predator approach (i.e. pursuit deterrence; see also Milinski & Boltshauser 1995; Godin & Davis 1995a). Our work complements Godin & Davis’ by suggesting that the observed patterns of pairwise motion can be viewed as simple additive combinations of individual actions. Moreover, mounting evidence suggests that if predator-approaching fish are playing Tit for Tat in an Iterated Prisoner’s Dilemma, they are the exception and not the rule (Scodelet al. 1959; Rapoport & Chammah 1965; Flood et al. 1983; Gardner 1983).

### Table V. Tests of the goodness of fit of Tit for Tat to transition frequencies in the ‘companion visible, predator visible’ treatment

<table>
<thead>
<tr>
<th>Pair</th>
<th>( c )</th>
<th>( d )</th>
<th>( N )</th>
<th>( \chi^2_{13} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.749</td>
<td>0.724</td>
<td>180</td>
<td>101.0</td>
<td>8.88 \times 10^{-16}</td>
</tr>
<tr>
<td>2</td>
<td>0.422</td>
<td>0.500</td>
<td>131</td>
<td>17.3</td>
<td>0.186</td>
</tr>
<tr>
<td>3</td>
<td>0.758</td>
<td>0.814</td>
<td>165</td>
<td>106.0</td>
<td>1.11 \times 10^{-16}</td>
</tr>
<tr>
<td>4</td>
<td>0.754</td>
<td>0.806</td>
<td>181</td>
<td>160.0</td>
<td>~0.00</td>
</tr>
<tr>
<td>5</td>
<td>0.817</td>
<td>0.812</td>
<td>166</td>
<td>113.0</td>
<td>~0.00</td>
</tr>
<tr>
<td>6</td>
<td>0.819</td>
<td>0.822</td>
<td>183</td>
<td>359.0</td>
<td>~0.00</td>
</tr>
<tr>
<td>7</td>
<td>0.746</td>
<td>0.770</td>
<td>195</td>
<td>227.0</td>
<td>~0.00</td>
</tr>
<tr>
<td>8</td>
<td>0.794</td>
<td>0.817</td>
<td>198</td>
<td>176.0</td>
<td>~0.00</td>
</tr>
<tr>
<td>9</td>
<td>0.888</td>
<td>0.885</td>
<td>181</td>
<td>1570.0</td>
<td>~0.00</td>
</tr>
<tr>
<td>10</td>
<td>0.724</td>
<td>0.779</td>
<td>197</td>
<td>84.6</td>
<td>1.47 \times 10^{-12}</td>
</tr>
</tbody>
</table>

\( c \) is the probability that \( C \) will follow companion’s \( C \); \( d \) is the probability that \( D \) will follow companion’s \( D \); \( N \) is the sample size (i.e. the number of pair-wise movements used in the analysis).

\( \chi^2_{13} \) is the probability that \( C \) will follow companion’s \( C \); \( d \) is the probability that \( D \) will follow companion’s \( D \); \( N \) is the sample size (i.e. the number of pair-wise movements used in the analysis).
et al. 1984; Heinrich 1988, 1989; Packer & Rutan 1988; Pruett-Jones & Pruett-Jones 1994; Clements & Stephens 1995; Grinnell et al. 1995). For example, Packer & Rutan (1988) analysed the economics of cooperative hunting in 26 species ranging from spiders to sea lions. They concluded that animals did not hunt cooperatively when there was a temptation to defect (a key component of a Prisoner’s Dilemma), but only when cooperation was in their short-term best interests. Similarly, Heinrich’s field studies of raven, Corvus corax, recruitment calls showed that ravens do not call when they are in sole possession of a defensible carcass, but instead call only when recruits can help them neutralize the defences of current carcass owners or territory residents. Moreover, these field studies closely parallel results from the laboratory with precisely controlled pay off matrices, using humans (Scodel et al. 1959; Colman 1982), rats (Flood et al. 1983; Gardner et al. 1984) and blue jays (Clements & Stephens 1995). Of course, altruistic cooperation does sometimes occur (Wilkinson 1984), but even in these rare cases the relevance of the Iterated Prisoner’s Dilemma is obscure.

In light of this evidence, we concur with Connor’s (1996) view that the most parsimonious interpretation of joint predator approach is a so-called ‘by-product mutualism’ (West-Eberhard 1989). Mutualistic cooperation differs from the Prisoner’s Dilemma in that cooperation is always the best tactic regardless of your companion’s behaviour: cooperation is simply the best selfish choice. Mutualism is called a ‘by-product’ when mutualistic cooperation results from two individuals jointly adopting the same actions they would perform if alone: for example each fish would approach if alone, but each risks slightly less by approaching jointly. In the absence of direct measurement of the game matrix the interpretation of mutualism is most parsimonious because, as we remark above, mutualistic cooperation is widely observed in nature, but clear evidence of cooperation in Prisoner’s Dilemmas is lacking. Similarly, mutualistic cooperation is readily produced in laboratory situations with controlled game matrices, but cooperation in Prisoner’s Dilemmas is markedly fragile (Clements & Stephens 1995).

Milinski (1990a, 1992, 1996; see also Dugatkin 1996) has repeatedly rejected this interpretation by arguing that if there were a selfish advantage to predator approach, then all members of a shoal should simultaneously approach. One can see the fallacy of this argument by considering a less abstract situation. Consider a pair of lionesses cooperatively hunting a gazelle. The claim that this cooperation is mutualistic does not mean that the lionesses can share their kill with a third lion without cost. Nothing about mutualism per se implies that benefits are frequency-independent. The ‘pursuit-deterrence’ function of predator approach answers this complaint, because separation from the shoal is, presumably, part of the pursuit deterrence signal.

In contrast to our view that mutualism is the most parsimonious explanation of joint predator approach, Milinski (1996) and Dugatkin (1996) claimed that tit-for-tat in an Iterated Prisoner’s Dilemma is the most plausible explanation because it makes distinct behavioural predictions, such as the existence of reciprocity, while mutualism predicts mutual cooperation but not reciprocity. Mutualism, they complain, makes no unique behavioural predictions. We have argued that the descriptive principles of social cohesion and orientation to a predator do make specific behavioural predictions (including the existence of reciprocal patterns of motion) and that our data support these predictions. Although we acknowledge that cohesion and orientation are not necessary consequences of mutualism, neither are they inconsistent with mutualism.

Mutualism and the Prisoner’s Dilemma represent two distinct economic situations (summarized by different game matrices). Any claim to support one economic situation over the other solely on the basis of observed behaviour is prone to error, simply because many economic situations predict similar behaviour. For this reason, we believe that the only unambiguous way to distinguish between competing economic models of predator approach is by objective measurement of the economics (i.e. the payoff matrices).

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