A COMPARISON OF FEEDING, SPACING, AND AGGRESSION IN COLOR MORPHS OF THE MIDAS CICHLID. II. AFTER 24 HOURS WITHOUT FOOD

by

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(With 7 Figures)

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The most common explanation for color polymorphism (polychromatism) is that it makes it difficult for predators to form a search image because of the different appearances of the morphs (*e.g.* CLARKE, 1975). This explanation might apply to the polychromatic Midas cichlid (*Cichlasoma citrinellum*), but the evidence to date points to an unusual social benefit enjoyed by the conspicuous gold colored morph. In small groups of juveniles the gold morphs regularly dominate the normal cryptic ones (BARLOW & BALLIN, 1976). We call this the *Gold Effect*; it appears to derive from the gold color inhibiting attack in the perceiver rather than the gold morphs being intrinsically more aggressive (BARLOW & WALLACH, 1976).

These data were obtained separately in small aquaria with juveniles of similar size. We wanted to create a rather more natural situation in which a number of questions could be addressed at the same time (BARLOW *et al.*, 1975). We especially wanted to see whether a much larger but still immature Midas cichlid would show inhibition of attacking small juvenile gold morphs when competing for food that was continuously present. That aspect of the experiment was uninformative, however, because the large fish guarding the feeder attacked too seldom for analysis; subtle threats sufficed to deflect approaching small fish. Consequently, we repeated the experiment, which we report here, but this time we withheld food for 24 hr and then replaced it to heighten aggression

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around the feeder; that resulted in sufficient data to test the hypothesis that the small golds would be attacked less than normals by the large guardian. We also predicted that the small golds would be able to get closer to the feeder.

Two unanticipated findings emerged from the previous experiment (BARLOW *et al.*, 1975) which we term here the *Dominance-Generalization Effect* and the *Habituation Effect*. Both are important to understanding the interactions between gold and normal morphs. And because they were post hoc hypotheses we set about to test them prospectively here.

In the Dominance-Generalization Effect, the small subordinate fish apparently learn the color of the dominant one (see also BARLOW & BALLIN, 1976). Fish who share the color of the large dominant fish are less likely to be attacked. We were especially interested in examining the interaction between the Dominance-Generalization and Gold Effects, so we alternated between gold and normal large fish.

Since the gold morphs occur at a low frequency in nature their advantage in aggressive interactions could be frequency dependent. Our previous experiment (BARLOW *et al.*, 1975) indicated this might be the case. Normal juveniles who had not been in contact with gold morphs attacked them hardly at all at first. But after a few days the normals attacked golds and normals about equally. Thus the effect of gold coloration might have been enhanced through novelty. That is, when golds are common normals seem to habituate to them. Various unpublished observations, however, led us to suspect that this Habituation Effect might disappear if competition were enhanced by withholding food for one day.

In another early experiment (BARLOW, 1973), gold and normal juveniles were shown to grow at equal rates when reared apart. But golds grew faster than normal ones when they were reared together. It was assumed that the golds' superior growth was attributable to their dominance of the normals in contests over food, but their behavior was not observed. In the present experiment we recorded aggressive behavior of all the individuals, their feeding acts, and changes in weight. The prediction was that the gold morphs would dominate the normal ones, feed more, and grow faster.

Materials and Methods

Only the major features are reported here because the Materials and Methods are mostly the same as in BARLOW *et al.* (1975).

Midas cichlid.

The Midas cichlid is abundant in the lakes of Nicaragua. Roughly 7 to 10% of the adults there lack melanophores and are consequently various shades of yellow through red due to the exposed deeper lying chromatophores. The Lake Masaya fish used here are yellowish orange and we call them 'gold'. Most Midas cichlids, however, have a cryptic pattern of gray and black with orange or red on the throat and in the eyes; such fish are called 'normals'. All fish start life as normals. If the parents are amelanic, then all the offspring eventually become amelanic; the first may metamorphose as young as 3 months and the last as old as several years (BARLOW, 1975). In this report, large golds are referred to as G, large normals as N, and small golds and normals as g and n, respectively.

Experimental arena (Fig. 1).

Three wading pools were used, each 182 by 122 cm, filled to a depth of 22 cm. A feeder and a shelter were located at one end at the apex of a V formed by two plastic-screen

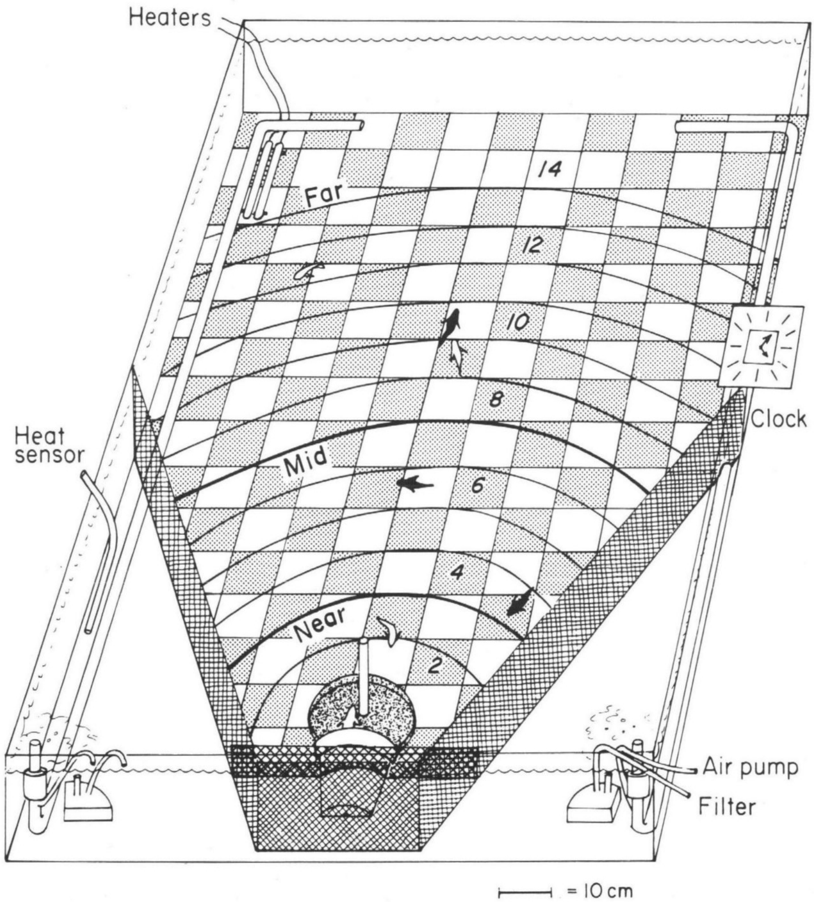

Fig. 1. The arena. The large fish is in the shelter where it controls the feeder (Zone 1). The pipes along the sides of the pool are to circulate warm water.

fences. The 3 shelters in Zone 14 in the previous experiment were removed, and an additional screen prevented fish from swimming behind the shelter in Zones 1 and 2.

When the feeder was in place it provided a continuous source of live tubificid worms. A fine-mesh screen covered the surface of the feeder, so worms could be taken only one at a time.

Experimental plan.

On Day 0 we selected a group consisting of one large immature fish plus 3 g and 3 n for each of the 3 arenas. The small fish ranged from 22.6 to 40.5 g but were closely matched within each group; in 3 of 4 conditions (2 treatment plus 2 control) the mean weights of n and g were exactly the same, and in the fourth n was on average only 2.5% heavier than g (not significantly different). The large fish was always twice the mean weight of the small ones with which it was associated. In one treatment group the large fish was N; in the other it was G. One trial was comprised of 3 groups, 2 treatment plus one control.

The treatment consisted of removing the feeder on Day 2, 24 hr prior to recording on Day 3. For the control situation the feeder was removed on Day 3 and quickly replaced. Immediately after replacing the feeder in all situations the behavior was hectic and fluctuated rapidly. Pilot studies indicated that the interactions became relatively stable after 20 min; we waited that long to commence video recording with no one in the room on midday of Day 3. Each observation period lasted 20 min. There were 10 trials, hence 10 replications of each treatment group and 5 of each control group.

Data recorded.

Position.

The video tape was stopped every 30 sec and the fish were counted according to Zone and type, *i.e.*, G or N, and g and n. For analysis, the Zones were lumped into a Near Region (Zones 1-4), a Mid Region (5-8), and a Far Region (9-14). The occurrence was calculated on a per m^2 basis to adjust for the progressively larger areas proceeding from Near to Mid to Far Regions (see BARLOW *et al.*, 1975).

Attack.

An attack was an accelerated swim aimed at another fish, approaching to within one body length. It could culminate in a butt or nip, and it could occur in a repetitive series, each rush being counted as one attack. When an attack took place we recorded where, and who attacked whom.

The attack data were not adjusted by area, as in the earlier work, because the small fish were so evenly distributed (see Distribution). However, we did adjust the attack scores of the small fish to compensate for number of fish of the same color that were available to attack. Thus g had 3 n but only 2 g to attack, so attacks by g on g were increased by a factor of 3/2, and the same was done for n attacking n.

Forage.

Forage was registered each time a fish bit at the screen surface of the feeder.

Statistical analyses.

Because the sample sizes were small, and because the variance was often large, nonparametric tests prevailed. As appropriate, these were the Mann-Whitney U Test, Wilcoxon Matched-Pairs Signed-Ranks Test, Fisher Exact Probability, and the Friedman Two-Way Analysis of Variance (SIEGEL, 1965). In some cases the variances were close so the Parametric Student's t Test was used. The critical value for significance was set at 5%.

Results

Distribution of small fish.

In our previous study we searched diligently for differences in the distribution of the small fish but found only one that was significant: When all groups were combined, small fish were slightly more common in the Far Region (which then had shelters). After an interval without food we expected g to approach closer to the food than n. However, the small fish were remarkably uniformly distributed across all three regions (Fig. 2).

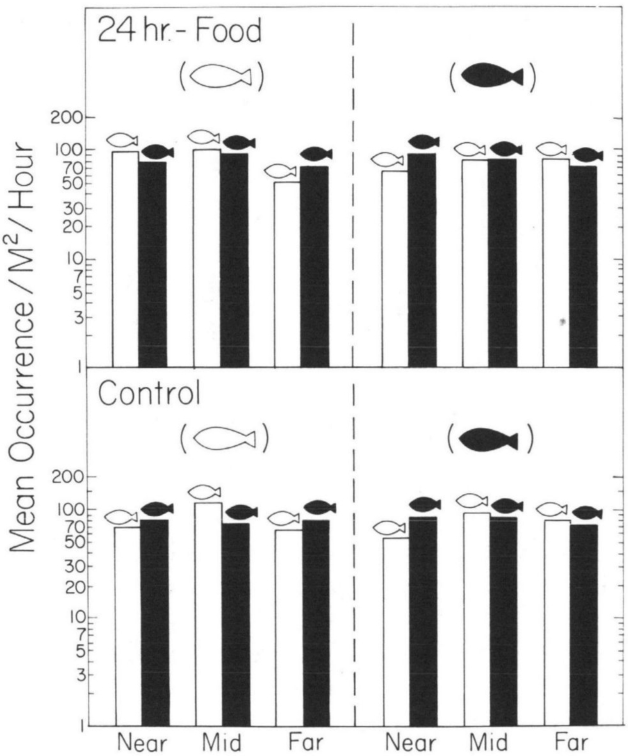

Fig. 2. The vertical bars portray the mean frequency of occurrence per small fish per m² per hr in the Near, Mid, and Far Regions of the arena. In the upper pair of panels are data for the treatment groups while in the lower panel are data for the control groups. The left two panels are for situations in which the large fish was G, and the right two panels are for when it was N. For each pair of bars the white-left one represents g, and the black-right one represents n.

Aggressive behavior.

Large fish.

The previous experiment was uninformative about the large fish because they so seldom attacked; they kept the small fish at bay with subtle threats. Similarly, the large fish in the present control groups revealed no differences and for much the same reason. One difference did emerge in the previous experiment, however: G attacked all the small fish in general more often than did N. In the present study, in contrast, the total attacks by G and N on small fish, whether in treatment or control groups, were virtually the same.

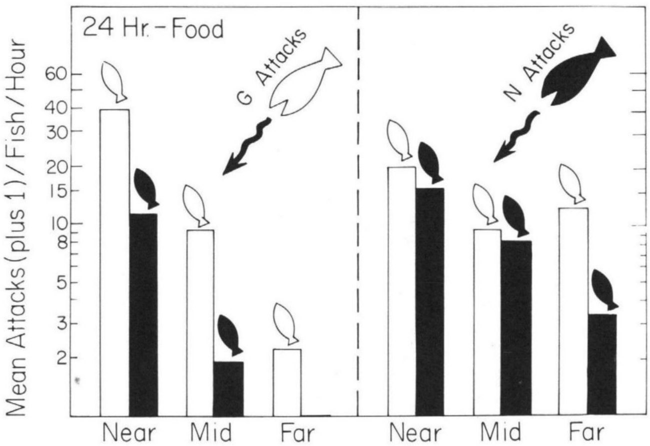

Fig. 3. The vertical bars indicate the mean attacks per large fish per hr in the treatment situation (left = G, right = N) on g (white-left) and on n (black-right). Because one bar would have been zero, the value of 1 was added to all bars for convenience of representation.

The earlier study was meant to test the following:

Hypothesis: *The large fish will attack g less than n because gold coloration inhibits attack.*

Due to the higher attack rate in the present treatment groups we were able to test the hypothesis. To our surprise we not only rejected the hypothesis but demonstrated the opposite: g were attacked *more* than n by the large fish (Fig. 3, $p = 0.025$).

Small fish.

The exchange of attacks among the small fish stands in contrast to the attacks directed to them by the large fish. We formulated essentially the same starting hypothesis:

Hypothesis: *Small fish (g + n) will attack g less than n because gold coloration inhibits attack.*

This time we accepted the hypothesis. For all 4 situations (Fig. 4 and 5, Table 1) g received significantly fewer attacks from small fish than did n. This is in agreement with the previous study.

From the earlier report we made two predictions about the interaction of the Dominance-Generalization Effect and the Gold Effect:

Hypothesis 1: *When with G, both g and n will attack n more than g because the effects of Dominance-Generalization and Gold summate.*

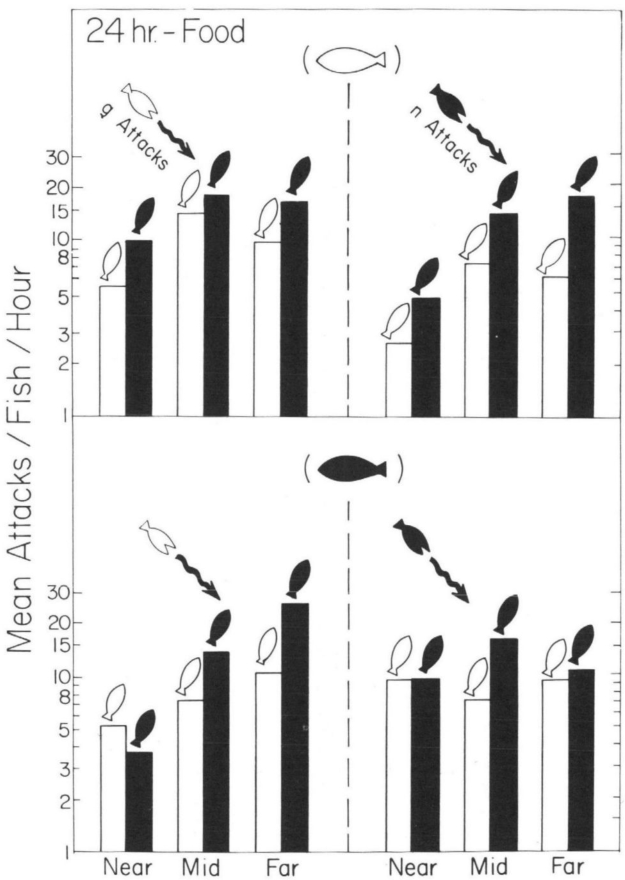

Fig. 4. The vertical bars reveal the mean frequency of attacks per hr by small fish in the treatment situation. The upper pair of panels is for small fish with G, the lower for with N. The left panels are for attacks by g, the right for attacks by n. In each pair of bars, attacks received by g are white-left; by n they are black-right.

As before, we expected the interaction to be clearer close to the large fish, in the Near Region. It was clear and significant there, but it was equally clear in all Regions (Fig. 4 and 5, Table 2). The n were attacked 2.5 times as often as g.

Hypothesis 2: *When with N, the small fish will attack g and n equally because the effects of Dominance-Generalization and Gold are in opposite directions and therefore cancel out.*

In the Near Region the prediction was borne out — g and n were attacked about equally (Fig. 4 and 5, Table 2). Overall, however, n

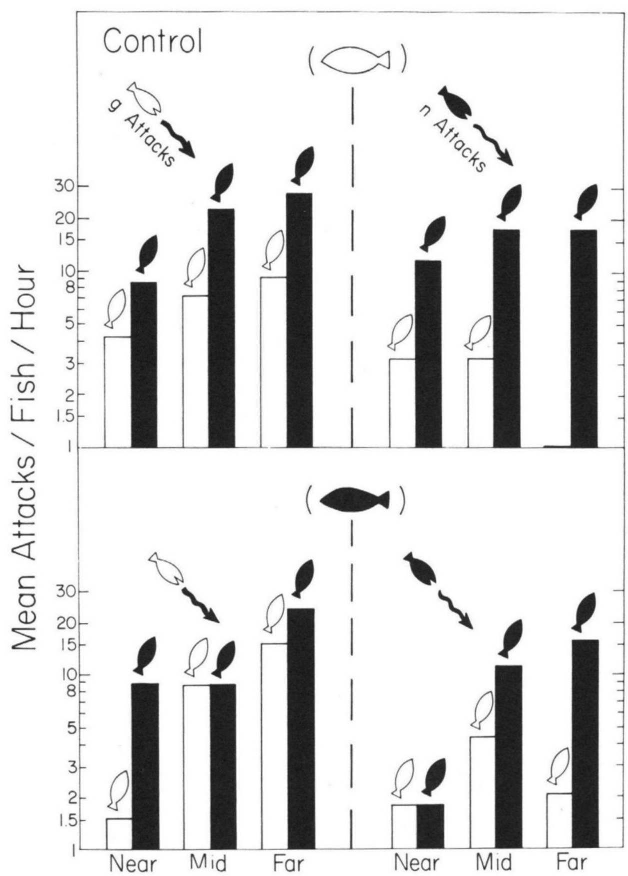

Fig. 5. The vertical bars reveal the mean frequency of attacks per hr by small fish in the control situation. The upper pair of panels is for small fish with G, the lower for with N. The left panels are for attacks by g, the right for attacks by n. In each pair of bars, attacks received by g are white-left; by n they are black-right.

TABLE 1

Attacks received by g and by n from all the small fish

Condition	Large fish	Mean total attack by n + g on g		on n	n ÷ g	p
24 hr - food	G	45.6	<	80.6	1.8	0.047
24 hr - food	N	49.9	<	78.9	1.6	0.047
Control	G	28.1	<	105.9	3.8	0.032
Control	N	33.0	<	67.9	2.0	0.048
Mean		39.2		83.3	2.1	

TABLE 2

The effects of the color of the large fish on attacks by small fish directed toward g and n

Condition	Attacker	Attacks received (mean/hr) Near region			All regions		
With G		g		n	g		n
24 hr - food	g	5.55		9.90	29.4		44.3
24 hr - food	n	2.60		4.65	16.0		36.3
Control	g	4.20		11.40	20.7		59.4
Control	n	3.20		11.70	7.4		46.5
Mean		3.89	<	9.41	18.4	<	46.6
p			0.029			0.014	
With-N							
24 hr - food	g	5.25		3.70	23.0		43.1
24 hr - food	n	9.40		9.60	25.9		35.7
Control	g	1.50		8.80	25.2		39.4
Control	n	1.80		1.80	7.8		28.5
Mean		4.49	≈	5.98	20.5	<	36.7
p			0.30			0.028	

received significantly more (1.8 ×) attacks than did g. Thus away from N the Gold Effect still prevailed. This is in contrast to the previous study in which there were no differences in attacks on g and n with N after 3 days.

We found earlier that attacks by small fish decreased in the Near Region, apparently due to the presence of the intimidating large fish. The same result was obtained in the present study (overall, $p = 0.005$). The effect was clearest for n, being significant in 3 of the 4 test situations and nearly so in the fourth.

Feeding behavior.

Large fish.

In the earlier report, the large fish seldom fed, though G fed (9.7/hr) significantly more often than did N (0.7/hr). The large fish in the present control situation fed so seldom (G = 3.6/hr, N = 0/hr; Fig. 6) that testing was not possible.

After 24 hr without food (Fig. 6) G in particular fed frequently (49.2/hr), and significantly more often ($0.02 > p > 0.01$) than did N (10.2/hr).

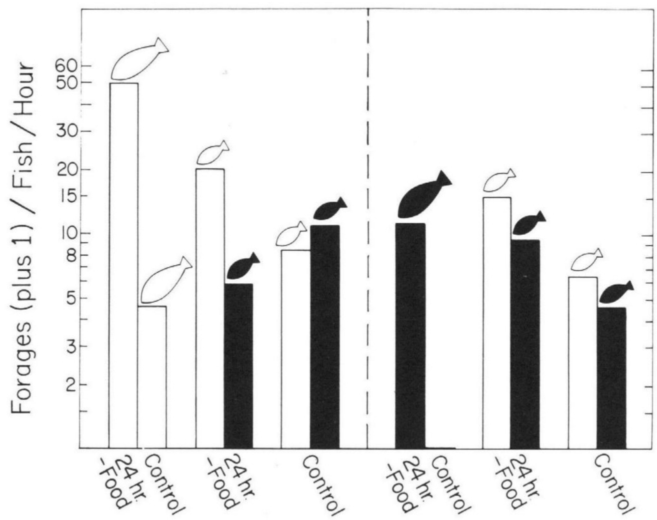

Fig. 6. The vertical bars convey the mean frequency of feeding (plus one) per fish per hr. In each panel, the far left pair of bars is for the large fish (G in the left panel, N in the right panel). The other two pairs of bars are for the small fish (treatment = left, control = right). In each pair of bars g is white-left and n is black-right. Data for the small fish with G are in the left panel, with N in the right panel.

Small fish.

Given that G and N attacked g more than n, but that g and n attacked n more than g, it was difficult to predict whether g or n should feed more after 24 hr without food. Three alternative hypotheses were tested:

Hypothesis 1: *Large fish selectively attack g, therefore g will feed less than n.*

Hypothesis 2: *Small fish attack n more than g, therefore n will feed less than g.*

Hypothesis 3: *Dominance-Generalization and Gold Effect interact such that g will feed more than n when with G, but g and n will feed equally when with N.*

The data reject the first two hypotheses but not the third (Fig. 6). When with G, g fed significantly more often ($p = 0.01$) then did n. When with N, however, there was no significant difference between n and g ($p \approx 0.30$).

Within the two control groups, as in the earlier report, there were no significant differences in feeding between g and n (with G, $p = 0.345$; with N, $p = 0.24$).

Fig. 6 seems to suggest that overall g fed more often than n. The difference, however, is not significant ($p = 0.34$).

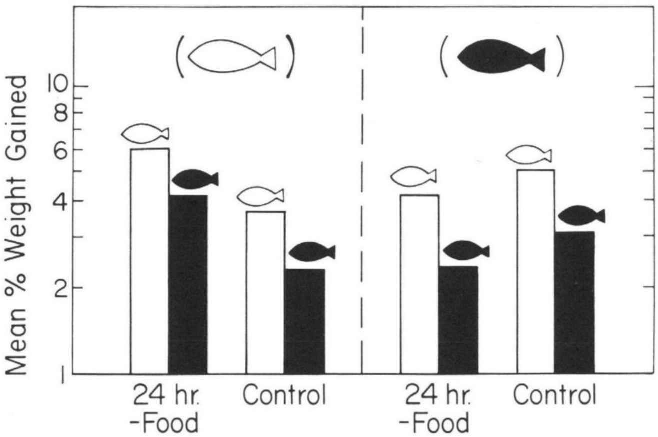

Fig. 7. The vertical bars show the mean percent increase in weight of small fish with G (left panel) and with N (right panel) in the treatment situation (left pair of bars in each panel) and in the control situation (right pair in each panel). For each pair of bars, g is white-left and n is black-right.

Changes in weight.

We calculated the percentage weight gained or lost for each fish and have presented the mean values (Fig. 7). The analysis of feeding behavior led us to predict the following:

Hypothesis: *Dominance-Generalization and Gold Effect interact such that g gains more weight than n when with g, but g and n gain equally when with N.*

The first half of the prediction was not borne out; although g appeared to have gained more weight than n when with G (Fig. 7) the difference was not quite significant ($p = 0.10$). On the other hand, there was no suggestion of a significant difference between g and n with N ($p = 0.40$).

We were impressed, nonetheless, with the consistent differences in Fig. 7. No matter which pair of comparisons one examines, the mean gain in weight is shown as greater for g than for n. Thus there appears to be a persistent difference in favor of g even if the individual paired comparisons seldom indicate significance (with N in the control groups, g gained significantly more than did n). Overall, g gained 4.8% in weight while n gained 2.9%; the difference is significant ($p = 0.029$).

Discussion

The clearest new finding in the previous study (BARLOW *et al.*, 1975) was the Dominance-Generalization Effect. It was best revealed by the pronounced drop in attacks on g by small fish when the large fish was G. and by the lack of a difference in attacks on g and n when with N. This pattern of summation and of cancellation of effects was confirmed in the present study. It is apparent in the aggressive and feeding behavior of the small fish, and it might hold for the pattern of gain in weight. The presence again of this interactive effect therefore supports both the Dominance-Generalization Effect and the Gold Effect. The responses of the large fish toward g and n, however, are the opposite of what the Gold Effect predicts.

Studies by BARLOW & BALLIN (1976) and BARLOW & WALLACH (1976), plus unpublished findings, indicate that when gold and normal morphs come into conflict the gold prevail *when all else is equal.* Further, the effect appears to derive from an inhibition of attack in the perceiver, not from the golds being intrinsically more aggressive than the normals.

The unexpected preferential attacking of g by the large fish in this report is open to three interpretations. The g could have been attacked more because they fed more, or because they attacked more, or simply because they were gold in color. All three hypotheses make essentially the same predictions in this study, which was not designed to sort them out. Had the three alternatives been anticipated, the data could have been gathered in such a way as to discriminate between the hypotheses. Nonetheless, it is possible to test the two post hoc hypotheses to determine if either is impossible.

Hypothesis: *The large fish will attack more at whichever small fish exhibits the most aggression toward other small fish.*

We did a Fisher Exact Probability Test to establish whether the differences in attacks in the Near Region by the large fish on the small ones were in the same direction as the differences in attacks by the color morphs of the small fish. (The attacks by small fish were not adjusted for availability of same color morph.) The data for the treatment groups support the hypothesis (Table 3, p = 0.026). However, we were bothered by the observation that the relationship was not consistently obvious when the means of the data were examined separately according to the color of the large fish (Table 4). When G was the large fish the pattern of the means agreed with the hypothesis: The number of attacks by G on g and n were almost the same as the attacks made by g and n, respectively. But when N was the large fish the mean data contradicted the hypothesis:

TABLE 3

The number of groups (minus ties and zero scores) in the treatment condition in which the differences in attacks by large and small fish were in the same or in opposite directions (p = 0.026)

		Actual attacks by small on	
		g > n	n > g
Attacks by	g > n	10	2
large on	n > g	1	4

TABLE 4

Mean attacks per 20 min observation period of the large fish and the actual mean attacks of 3 g and of 3 n on n plus g per observations period

	Attacks by Lrg. on			Attacks by		
Lrg. fish	g	n	g ÷ n	g	n	g ÷ n
G	13.2	3.4	3.9	13.6	5.7	2.4
N	6.3	4.8	1.3	7.2	15.8	0.46

Although not significantly different, N attacked g slightly more than n, whereas g attacked small fish only about half as often as did n. While we cannot reject this hypothesis for now, we conclude it does not square well with the data.

Hypothesis: *The large fish will attack more at whichever small fish feeds the most.*

Employing the Fisher Exact Probability Test again, we get exactly the same frequencies as in Table 3 (p = 0.026). And now the mean values are consistent with the hypothesis: with G, g fed 4.0 times as often as n, and G attacked g 3.9 times as often as n. With N, g fed 1.6 times as often as n, and N attacked g 1.3 times as often as n. The data, therefore, consistently support the hypothesis because the small fish who fed most consistently got attacked most by the large fish.

In the Results we have already shown that the hypothesis that g are attacked more because of their color is also acceptable. This hypothesis leads to an interesting consideration of proximate and ultimate factors.

That the large fish attacked g more than n could suggest that gold coloration is more evocative when the asymmetry is against the gold colored fish. This is consistent with a context model (Barlow, 1982) that predicts gold will inhibit attacking when the asymmetry is in its favor, but will elicit attacking when it is not. While seldom overtly stated, this is consistent with a large body of ethological literature on threat displays and how they work.

If the large fish do indeed differentially attack g because of its color, that offers a new insight into the temporal pattern of metamorphosis. Gold morphs are seldom observed in nature less than about 75 mm long (SL) though they occur at half that length in the laboratory. It has been suggested (Barlow, 1975) that predators set the minimum size by selecting against the smaller gold morphs. However, if large fish preferentially attack smaller gold ones, that could also select against early metamorphosis.

The g in this study, however, did not seem disadvantaged relative to n. The g were as close to the feeder, appeared to feed more, and gained slightly more weight than did n. Apparently the advantage of g over n, through aggression, was more telling than the difference in attack behavior of the large fish. But that might be specific to the particular laboratory situation. Without recourse to field studies it will only be possible to establish the conditions of size and numbers of fish of both colors that favor or handicap gold morphs.

In our previous study we found a pronounced novelty effect: The n were intimidated by g on Day 1, but by Day 3 the advantage enjoyed by g was slight or undetectable. We hypothesized, nevertheless, that habituation only masked the Gold Effect. By forcing the fish to compete for food in the present study we expected the Gold Effect to be unmasked. Our prediction was realized. However, in the control situations g and n should have received attacks about equally but they did not; the Gold Effect was obvious.

We suspect the problem with the control group was in the nature of the control situation. At night several of the tubificid worms crept out of the feeder and hid under it. When we lifted the feeder then immediately put it back, we flushed out the hidden worms. That resulted in heightened feeding activity and aggression among the small fish; their behavior was at least temporarily as hectic as that of the treatment groups. We thought they had returned to the previous control level within 20 min, but that may not have been a correct assessment.

In any event, we tentatively accept that there is a Habituation Effect, and that it interacts with a Gold Effect that will prevail when the stakes are high. It is important to test more rigorously the Habituation Effect because of its relevance to frequency-dependent selection in accounting for balanced polymorphism in nature.

For the same reason it is important to sort out, in future work, whether g elicit relatively more attacks because of their color, or because of their higher levels of feeding and aggression.

Summary

We set out to test three hypotheses: (1) Gold Effect — the coloration of the gold morph confers an advantage in hostile interactions because that color inhibits aggression. (2) Habituation Effect — fish habituate to gold fish if they are encountered frequently. (3) Dominance-Generalization Effect — the color of the dominant fish is learned, and small fish who share that color receive some protection from attacks.

The basic experiment consisted of one large juvenile dominating a feeder which was approached by small fish, 3 golds and 3 normals. In the treatment, the feeder was removed for 24 hr then replaced 20 min before recording; this was done to see if the Gold Effect would prevail over the Habituation Effect under conditions of forced competition.

Contrary to expectations, the large fish attacked the small golds more than the small normals. However, their attacks could have been evoked by the attacks or feeding behavior of the small fish. The small fish themselves showed both the Gold and Dominance-Generalization Effects. And, as predicted, the Gold Effect prevailed over the Habituation Effect after being deprived of food; but the same thing happened in the control situation, apparently due to the stimulation of revealing concealed worms when their feeder was simply lifted and immediately replaced.

The small golds appeared to feed more often than the small normals, but the difference was not significant. However, the small golds gained significantly more weight than did the small normals. Thus the small golds benefited more from their aggressive advantage over the small normals than they lost by being attacked more by the large dominant fish.

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