

Colour pattern and inhibition of aggression in the cichlid fish *Astronotus ocellatus*

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Oscars *Astronotus ocellatus* defeated in combat often undergo a colour pattern change in which the normal olive-green to brown body coloration darkens to near black, interrupted with irregular white barring. Dummy oscars were used in two complementary tests which showed that the dark-and-banded colour pattern inhibits aggression in conspecifics under two different experimental designs and with two different subject groups.

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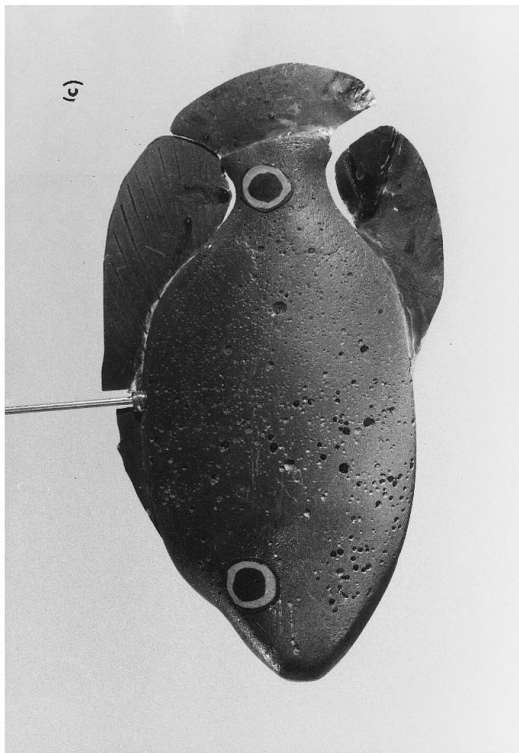
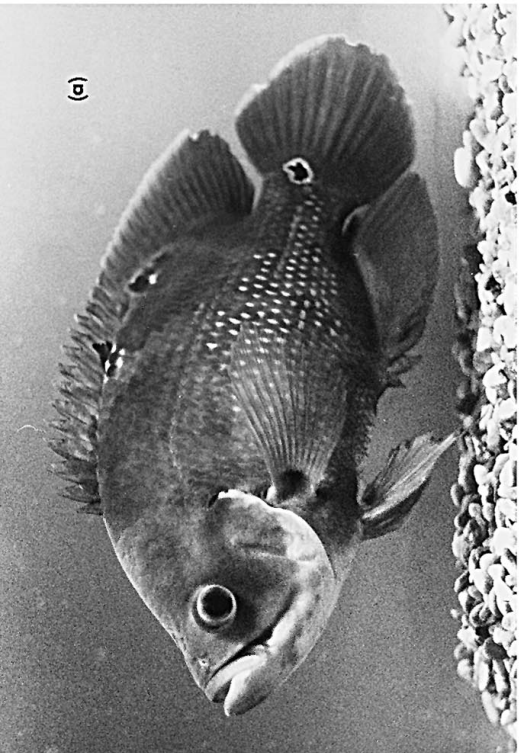
Key words: aggression; colour pattern; inhibition; cichlid.

INTRODUCTION

Teleost colour pattern changes range from instantaneous changes during social interactions to gradual changes associated with maturation, and coloration may be highly variable both within and across taxa. Studies of cichlid fishes have revealed correlations between an individual fish's colour pattern and its developmental stage, sex, status, or behaviour (Baldaccini, 1973; Fernald & Hirata, 1979; Baerends *et al.*, 1986).

Behavioural correlates with colour patterns suggest that colour pattern signalling may be an important mechanism of communication in fishes with suitable environments (i.e. favourable light and water conditions) and visual systems, although very little experimental evidence exists regarding such signalling systems. Moreover, ethological studies of some potential colour pattern signals have yielded apparently contradictory results. For example, studies of the red nuptial coloration of male three-spined sticklebacks *Gasterosteus aculeatus* L., do not consistently support the hypothesis that the red ventrum of nested males elicits aggression from conspecific males (Tinbergen, 1948; Rowland, 1982; Collias, 1990). Collectively, studies suggest that male nuptial coloration in *G. aculeatus* may have several communicative functions, and functional significance dependent upon behavioural context. Thus, studies of colour pattern signalling may require stringent control of the social and physical environment to be successful. The present study attempts to reveal the behavioural significance of a short-term colour pattern change in oscars, the cichlid fish *Astronotus ocellatus* (Cuvier), by using dummy fish to mimic two alternative colour patterns observed in this species.

The neotropical cichlid *A. ocellatus* is monogamous and sexually monomorphic, and both sexes will establish and defend breeding sites. Individuals are typically olive-green to chocolate-brown in colour, with variable yellow, orange, or red markings on the flanks, and a conspicuous, yellow-orange 'eyespot' or



ocellus on the caudal peduncle. Individuals which are startled or defeated in intraspecific combat often assume an alternative colour pattern in which the body darkens to near black while developing irregular white banding, including a band arching over the head, posterior to the eyes. In this study the behavioural significance of the change from the normal (NL) to the dark-and-banded (DB) colour pattern in *A. ocellatus* was investigated. Dummy fish were used to compare the relative strength of each colour pattern in eliciting aggression from isolated, adult oscars.

MATERIALS AND METHODS

Juvenile *A. ocellatus* were obtained from a local distributor and raised in groups of 10 in 108-l stock aquaria in the laboratory. At sexual maturity (12 cm total length), subjects were isolated in 80-l aquaria (31 × 31 × 76 cm) with 2–3 cm of gravel substrate, and allowed to acclimate to their surroundings. Each experimental tank included a corner box filter and a clay flower pot to serve as a shelter. Fish were maintained at 28° C on a 14 : 10 h light : dark cycle, and fed twice daily on a mixed diet of commercial cichlid pellets and live goldfish *Carassius auratus* (L.) or mosquitofish *Gambusia affinis* (Baird & Girard). Under these conditions, the fish entered reproductive condition rapidly, as evidenced by their excavation of spawning sites in the substratum and development of orange patches on the flanks. Subjects were allowed visual contact with fish in adjacent aquaria until 48 h before experimentation began. At that time, opaque screens were installed between aquaria to isolate the subject visually.

Two solid epoxy dummies of 125 mm total length were constructed using the method of Rowland (1979). Unpaired fins were made by photocopying fin rays on to acetate sheets. The fins were then cut from the acetate and affixed with epoxy to the dummy bodies. NL and DB colour patterns were painted on to the dummies using enamel paints, and these patterns mimicked as closely as possible the actual colour patterns observed in *A. ocellatus* (Fig. 1). The NL dummy was painted dark green (Testors Dark Green, FS 34079) with a countershading of grey (Testors Flat Gull Gray, FS 36440) on the ventrum, and an irregular patch of orange (Floquil Milwaukee Orange 110162) on the flanks. The DB dummy was painted black (Testors Black, 1149) with grey ventrum and barring, and an irregular orange patch.

Before testing began, one randomly chosen dummy was suspended in the centre of the test subject's aquarium for 10–60 min each day until bite or lateral display was elicited. This priming required from 1 to 3 days, with NL and DB dummies alternately used, and testing began the day following the first dummy-elicited bite or lateral display. Subjects were then presented dummies within only one of the following two experimental procedures.

SEQUENTIAL DUMMY PRESENTATION

Fourteen isolated individuals were presented, in random order, each of the dummies four times. One dummy was presented daily, for a period of 10 min. Observations were recorded by videocamera from a distance of 1 m. For further details of dummy presentation apparatus, see Rowland (1975). The complete series of presentations was then repeated, again in random order, for each subject, yielding a total of 16 10-min presentations to each subject. The frequencies of the attack activities charge, head bite (bite to anterior half of dummy), tail bite (bite to posterior half of dummy), and display (frontal display, lateral display) activities (as described by Baerends & Baerends-van Roon, 1950) performed during each presentation were scored from the video recordings.

FIG. 1. Alternative colour patterns expressed by *A. ocellatus*, and the dummy mimics of those colour patterns. (a) The normal (NL) pattern. (b) Same individual 30 s later expressing dark-and-banded (DB) pattern after being startled by tapping on aquarium cover. (c) NL dummy. (d) DB dummy.

TABLE I. Effect of dummy colour on dummy-elicited activities during sequential dummy presentation

Activity	Dummy	Mean (\pm S.E.)/10 min	Median/10 min	$F_{(1,12)}$	P
Time at dummy (s)	NL	314.5 (13.3)	323	0.348	0.5663
	DB	318.0 (14.5)	309		
Lateral display	NL	6.7 (0.5)	6	0.19	0.6706
	DB	6.6 (0.4)	5		
Head bite	NL	30.0 (2.6)	21	6.14	0.0263
	DB	26.4 (2.4)	19		
Charge	NL	11.5 (1.0)	9	6.73	0.0235
	DB	10.2 (1.0)	7		
Frontal display	NL	3.6 (0.7)	2	7.43	0.0184
	DB	3.2 (0.6)	1		
Tail bite	NL	5.3 (0.7)	2.5	5.08	0.0436
	DB	7.4 (1.0)	4		

NL, Normal colour pattern; DB, dark-and-banded colour pattern.

In addition, the frequency of approaches within 1 body length of the dummy (approach dummy) and the total time spent within 1 body length of the dummy (time at dummy) were recorded. Data were analysed by repeated measures ANOVA of rank-transformed data (Conover & Iman, 1981) using the SAS computer package (SAS, 1985).

PAIRED DUMMY PRESENTATION

Ten isolated individuals were presented both dummies simultaneously by suspending the dummies from a $2 \times 2 \times 40$ cm wood crossbar supported by the front and back of the aquarium. To control for potential position bias, the position of the dummies was reversed midway through the 120-min presentation. Each subject was presented dummies on two consecutive days following priming. Details of data collection were identical to those described above. Data were pooled by subject and analysed using the Wilcoxon matched-pair signed-ranks test.

RESULTS

SEQUENTIAL DUMMY PRESENTATION

The attack activities of head bite and charge, and the aggressive display activity frontal display were more effectively elicited by the NL dummy than the DB dummy (Table I). The reduced DB dummy-elicited aggression was not part of a general reduction in responsiveness towards the DB dummy. Neither time at dummy nor lateral display frequency differed between treatments. Tail bite frequency was greater during DB presentations than during NL presentations. Eleven of 14 subjects delivered more head bites to the NL dummy (Fig. 2). Eleven subjects exhibited greater charge frequency towards one of the two dummies, and nine of these 11 charged the NL dummy more frequently (Fig. 3).

PAIRED DUMMY PRESENTATION

The NL dummy elicited significantly more attack activities than the DB dummy. Eight of 10 subjects delivered more bites (Fig. 4; $P < 0.025$) and charges (Fig. 5; $P < 0.05$) to the NL dummy than the DB dummy. As in the sequential

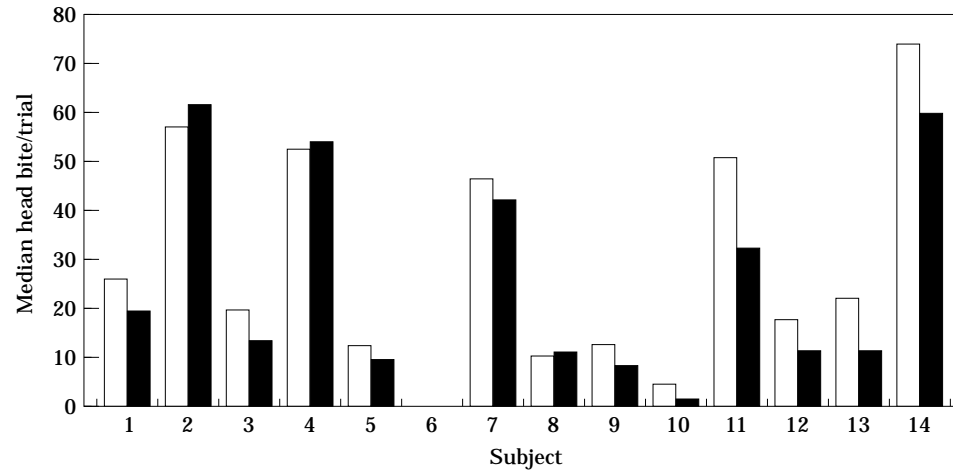


FIG. 2. Median head bite frequency 10 min trial during serial presentation of normal (□) and dark-banded (■) dummies to 14 *A. ocellatus* subjects ($P=0.0263$).

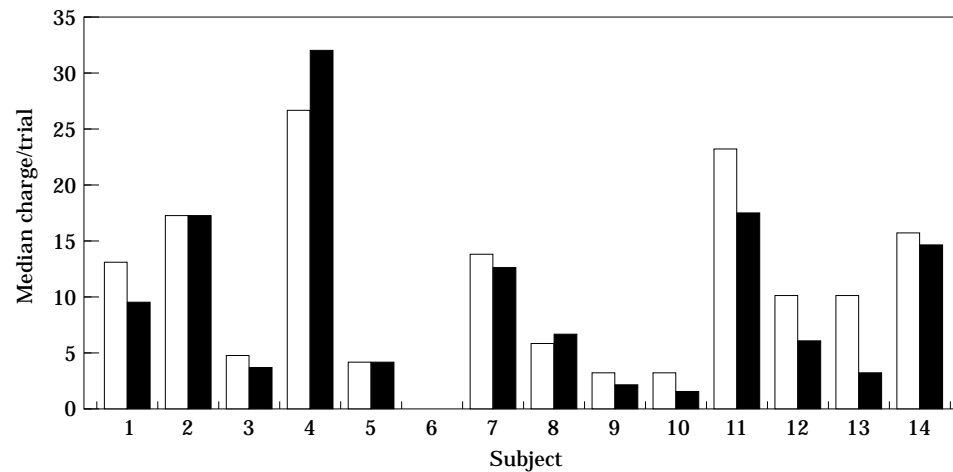


FIG. 3. Median charge frequency 10 min trial during serial presentation of normal (□) and dark-banded (■) dummies to 14 *A. ocellatus* subjects ($P=0.0235$).

dummy presentation experiment, there was no difference in the frequency of lateral display elicited by the dummies.

DISCUSSION

In the laboratory, the DB colour pattern of *A. ocellatus* was expressed when an individual was startled by a non-social stimulus, such as a hand or net introduced quickly to the aquarium, or an intense light source being directed toward the aquarium. Oscars also exhibited the DB colour pattern when defeated in intraspecific combat. Whether presented singly or pair-wise, NL dummies were attacked significantly more than DB dummies by isolated adult oscars in this

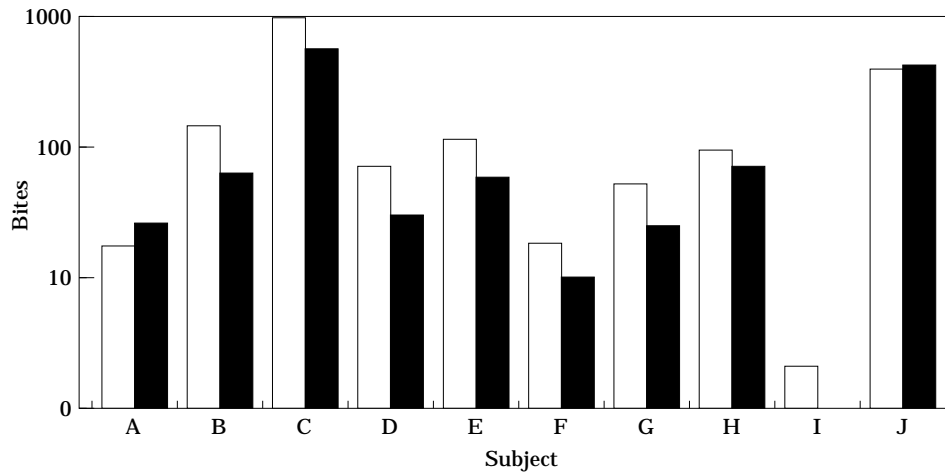


FIG. 4. Total bites during 120 min of paired presentation of normal (□) and dark-banded (■) dummies to 10 *A. ocellatus* subjects ($P < 0.025$).

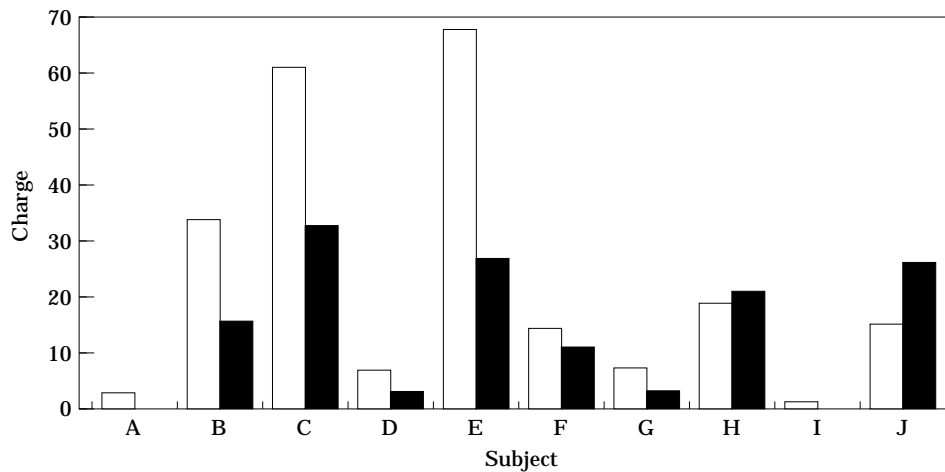


FIG. 5. Total charges during 120 min of paired presentation of normal (□) and dark-banded (■) dummies to 10 *A. ocellatus* subjects ($P < 0.05$).

study. Thus, during the aggressive interactions which characterize reproduction in this species, the DB colour pattern may function to inhibit attacking conspecifics.

One of the limitations of dummy presentation experiments is the possibility that the novelty of the dummies can elicit responses which parallel those elicited by naturally occurring stimuli, but for different reasons. In the present study, for example, decreased aggression towards the DB dummy might have resulted from low responsiveness of subjects towards a less recognizably conspecific dummy, rather than the hypothesized aggression-inhibiting quality of the dummy's colour pattern. Analysis of dummy-elicited activities in the sequential dummy presentation experiment revealed that non-attack behavioural measures,

including time at dummy and lateral display were not inhibited by the DB dummy. Tail bite, an activity which may serve a non-aggressive social function (Beeching, 1993) actually increased during DB dummy presentations. Thus, the decrease in DB dummy-elicited attack activities did not appear to be part of a more generally lowered responsiveness towards DB dummies.

Expression of the DB colour pattern results from the patterned expansion (darkening) and contraction (blanching) of dermal melanophores (Fujii, 1969). Maximal expression of the DB colour pattern can occur within 2 s in startled laboratory subjects [Figs 1(a) and 1(b)]. The details of the DB pattern were invariant within an individual, but differed among individuals, although a white band arching over the head, posterior to the eyes, was always observed, producing a pattern of light and dark bands resembling the disruptive coloration observed in many animal species (Edmunds, 1974).

Many fishes exhibit similar rapid colour pattern changes with potential for communicative function (Fujii, 1969). Fast colour pattern changes result from the action of innervated chromatophores (Bagnara & Hadley, 1973), a physiological adaptation shared with cephalopod molluscs (Barnes, 1980), but not with most other vertebrates. Teleosts exhibit sympathetic, but not parasympathetic control of melanophores, and an array of functional neurotransmitters, including norepinephrine and adenosine triphosphate (ATP). Norepinephrine and ATP stimulate melanosome aggregation and dispersion, thus skin lightening and darkening, respectively (Fujii, 1993). In addition, chromatophores bearing adrenergic receptors can respond to adrenal epinephrine, causing 'excitement pallor' or 'excitement darkening' in fishes (Fujii, 1993). The colour pattern change described in this study for *A. ocellatus* is similar to colour pattern changes described in other species (see below), and results from one or both of these physiological mechanisms.

As Fujii (1993) noted, the behavioural significance of these colour pattern changes has not been adequately explained. Correlations between behaviour and colour pattern are well documented in the Cichlidae, and several studies have attempted to demonstrate signal function in cichlid colour patterns based on these associations. For example, banded colour patterns are temporally associated with fear or flight-inducing stimuli in *Tilapia mossambica* (Peters) (Lanzing & Bower, 1974) and *Cichlasoma meeki* (Brind) (Neil, 1984). Baldaccini (1973) reported that non-territorial individuals of the cichlid fish *Tilapia mariae* (Boulenger) would flee from territorial residents when introduced into their territories, and rapidly acquire black vertical barring. In that study, *T. mariae* were found almost invariably to assume the barred pattern when being attacked or after losing a fight. Slovin & Rowland (1978) found that barred *T. mariae* dummies were attacked significantly less than dummies with the spotted pattern normally expressed by territorial individuals. Aggression-inhibiting properties have also been attributed to barring in non-cichlid species. For example, lateral bars inhibit aggression in the pumpkinseed sunfish *Lepomis gibbosus* (L.) (Stacey & Chizar, 1978). Overall, studies of colour pattern and behaviour in teleosts suggest that dark barring is a common startle or stress response, and may also function as an intraspecific signal in some species.

Two attributes of the DB colour pattern suggest how this signalling system may have arisen in *A. ocellatus*. First, the DB colour pattern may provide

crypsis by disrupting the visual borders of the fish. Thus, the NL to DB colour pattern change would be adaptive as an anti-predator tactic. A reliable autonomic colour pattern change might subsequently be utilized by conspecifics as a signal of intent or motivational state, particularly if 'honesty' is critical in signal evolution, as several models suggest (Grafen, 1990; Zahavi, 1991; Johnstone & Grafen, 1992, 1993). Second, the DB colour pattern closely resembles the typical juvenile colour pattern of *A. ocellatus*. Most cichlid juveniles exhibit some variety of vertical barring (Baerends & Baerends-van Roon, 1950). If juvenile colour patterns inhibit parental cannibalism, as Zaret (1977) suggested, then adoption of juvenile coloration by adults might effectively inhibit the aggression of attacking conspecifics.

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References

- Baerends, G. P. & Baerends-van Roon, J. (1950). An introduction to the study of the ethology of cichlid fishes. *Behaviour Supplement* **1**, 1–242.
- Baerends, G. P., Wanders, J. B. W. & Vodegel, R. (1986). The relationship between marking patterns and motivational state in the pre-spawning behaviour of the cichlid fish *Chromidotilapia guentheri* (Sauvage). *Netherlands Journal of Zoology* **36**, 88–116.
- Bagnara, J. T. & Hadley, M. E. (1973). *Chromatophores and Color Change*. Englewood Cliffs, NJ: Prentice-Hall.
- Baldaccini, N. E. (1973). An ethological study of reproductive behaviour including colour patterns of the cichlid fish *Tilapia mariae* (Boulenger). *Monitore Zoologico Italiano (N.S.)* **7**, 247–290.
- Barnes, R. D. (1980). *Invertebrate Zoology*, 4th edn. Philadelphia: Saunders College.
- Beeching, S. C. (1993). Eyespots as visual cues in the intraspecific behavior of the cichlid fish *Astronotus ocellatus*. *Copeia* **1993**, 1154–1157.
- Collias, N. E. (1990). Statistical evidence for aggressive response to red by male three-spined sticklebacks. *Animal Behaviour* **39**, 401–403.
- Conover, W. J. & Iman, R. L. (1981). Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician* **35**, 124–129.
- Edmunds, M. (1974). *Defence in Animals*. New York: Longman.
- Fernald, R. D. & Hirata, N. R. (1979). The ontogeny of social behavior and body coloration in the African cichlid fish *Haplochromis burtoni*. *Zeitschrift für Tierpsychologie* **50**, 180–187.
- Fujii, R. (1969). Chromatophores and pigments. In *Fish Physiology*, Vol. III (Hoar, W. S. & Randall, D. J., eds), pp. 307–353. New York: Academic Press.
- Fujii, R. (1993). Coloration and chromatophores. In *The Physiology of Fishes* (Evans, D. H., ed.), pp. 535–562. Ann Arbor, MI: CRC Press.
- Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology* **144**, 473–516.
- Johnstone, R. A. & Grafen, A. (1992). The continuous Sir Philip Sidney Game: a simple model of biological signalling. *Journal of Theoretical Biology* **156**, 215–234.
- Johnstone, R. A. & Grafen, A. (1993). Dishonesty and the handicap principle. *Animal Behaviour* **46**, 759–764.
- Lanzing, W. J. R. & Bower, C. C. (1974). Development of colour patterns in relation to behaviour in *Tilapia mossambica* (Peters). *Journal of Fish Biology* **6**, 29–41.

- Neil, S. J. (1984). Color pattern variability and behavioral correlates in the firemouth cichlid, *Cichlasoma meeki*. *Copeia* **1984**, 534–538.
- Rowland, W. J. (1975). The effects of dummy size and color on behavioral interaction in the jewel cichlid, *Hemichromis bimaculatus*. *Behaviour* **53**, 109–124.
- Rowland, W. J. (1979). Some methods of making realistic fish dummies for ethological research. *Behavior Research Methods and Instrumentation* **11**, 564–566.
- Rowland, W. J. & Sevenster, P. (1985). Sign stimuli in the threespine stickleback (*Gasterosteus aculeatus*): a re-examination and extension of some classic experiments. *Behaviour* **93**, 241–257.
- SAS Institute, Inc. (1985). *SAS User's Guide: Statistics*. Cary, NC: SAS Institute.
- Slovin, M. & Rowland, W. J. (1978). The effects of color patterns on the aggressive behavior of *Tilapia mariae* (Boulenger). *Behavioural Biology* **24**, 378–386.
- Stacey, P. B. & Chiszar, D. (1978). Body color pattern and the aggressive behavior of male pumpkinseed sunfish (*Lepomis gibbosus*) during the reproductive season. *Behaviour* **64**, 271–297.
- Tinbergen, N. (1948). Social releasers and the experimental method required for their study. *The Wilson Bulletin* **60**, 6–51.
- Zahavi, A. (1991). On the definition of sexual selection, Fisher's model, and the evolution of waste and of signals in general. *Animal Behaviour* **42**, 501–503.
- Zaret, T. M. (1977). Inhibition of cannibalism in *Cichla ocellaris* and hypothesis of predator mimicry among South American fishes. *Evolution* **31**, 421–437.