

content of noradrenaline in the gall bladder neck than in the body or fundus. Immunocytochemical studies have shown a similar distribution pattern of NPY immunoreactive nerves to that reported for adrenergic nerves¹¹ and this is distinct from nerves containing vasoactive intestinal polypeptide, substance P, somatostatin, enkephalin and bombesin-like immunoreactivity¹¹. The distribution was also different from cholinergic nerves, mainly in that no NPY-immunoreactive ganglia were found in the tissues¹¹.

Sympathetic function appears to play an important role in neural control of biliary function as stimulation of beta adrenergic receptors results in smooth muscle relaxation¹⁵ and stimulation of thoracic splanchnic nerves antagonises cholecystokinin-induced gall bladder contraction¹⁷. In view of the close links of NPY with sympathetic function, it appears that this peptide may play an important role in the control of gall bladder motility, especially as the structurally related peptide, porcine pancreatic polypeptide¹³ has already been shown to relax biliary smooth muscle¹⁸. The presence of high concentrations of NPY within the biliary tract should stimulate further investigation of its physiological role.

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Arousal and fright responses and their habituation in the slippery dick, *Halichoeres bivittatus*

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Summary. Behavioral arousal and fright responses of *Halichoeres bivittatus*, occurred in aquaria to a moving shadow and a 'tap' stimulus. Arousal was characterized by changes in the beat of pectoral fins, dorsal fin erection and eye movements, whereas in fright, adduction of pectoral and dorsal fins and rapid forward movement occurred. Serial stimulus presentation caused the fright response to be replaced by arousal which habituated in that the proportion of behavioral components exhibited decreased during the process.

Behavioral arousal and fright and their physiological correlates have been extensively studied in domesticated cyprinids⁴. In these fish arousal is associated with slight changes in orientation or an increase in stabilizing movements. Thus, changes in pectoral and/or tail fin movement and erection of the dorsal fin often occur. Novel stimuli may elicit arousal or the more violent response of fright. This escape response consists in cyprinids of a rapid lateral flexion of the tail or tail-flip. Both arousal and fright habituate in cyprinids, and if the initial responses are of fright then these habituate to be replaced by arousal, which itself habituates⁵. *Halichoeres bivittatus* is a diandric protogynous hermaphrodite, and with the other labrids its reproductive biology has been extensively studied⁶. The labrids are extremely active and alert fish and this makes them ideal subjects for testing the generality of descriptions of behavioral arousal and fright, and the motor components of these responses.

Material and methods. Adult (phase 4) *Halichoeres bivittatus* were collected for aquarium observations using minnow traps baited with squid. Individuals were placed for 36 h in a 0.7 × 0.3 × 0.3 m outdoor aquarium, shaded from direct sunlight, filled with fresh aerated seawater and enclosed in brown paper. Stimuli (or equivalent pre-experimental non-stimulus control observations) were presented at 30-sec intervals and

changes in the movement or position of eyes, head, body, tail, pectoral fins and dorsal fin were recorded from the prestimulus 5 sec to the 5 sec after stimulus initiation. Overall responses of the fish were considered to fall into 2 categories: realignment or reorientation and rapid flight. The former was interpreted as arousal, the latter as fright. The stimuli were: serial presentation of a 10 × 20 cm black card moved over the aquarium at ≈ 8 cm/sec for 4 sec and serial presentation of a consistent sharp tap on the end of the aquarium. Waning of the

Table 1. Components of behavioral arousal and fright in *Halichoeres bivittatus*: mean \pm SEM of proportion of responses with each component

Component	Arousal	n	Fright	n
Head movement	39.8 \pm 10	7	97 \pm 2.5	4
Eye movement	62.5 \pm 8.6	7	54.2 \pm 21	4
Change in pectoral beat	57.3 \pm 10.2	7		4
Adduction of pectorals		7	90.6 \pm 8.1	4
Dorsal fin erection	50.3 \pm 12.2	7		4
Adduction of dorsal fin		7	100 \pm 0	4
Tail movement	21.1 \pm 7.9	7	97.0 \pm 2.5	4
Body movement	18.8 \pm 5.4	7	100 \pm 0	4

entire response or a component of it was considered to have occurred on the last stimulus eliciting a reaction, prior to 2 consecutive stimuli with no response.

Results and discussion. Fish in the aquarium would normally cruise by 'labiform', pectoral locomotion⁷. In the control non-stimulus situation where 7 fish were each observed for 20 periods of 10 sec at 25-sec intervals, 2 fish each only showed one spontaneous arousal response during 1 of 20 mock stimuli. Thus, in the stimulus situation, any change in the stimulus compared to the prestimulus 5 sec was considered as a response. Stabilizing or orienting movements of fish, described as behavioral arousal were most evident to the moving shadow. The proportion of arousal responses in which particular components were exhibited (table 1) illustrates that eye movement, change in pectoral fin movement and dorsal fin elevation were most frequently evident. Head movement could occur though body and tail movements were infrequent. Occasionally a fish would stop swimming for a brief period with the pectoral held, unmoving, at right angles to the body. Earlier serial presentations of either stimulus produced more components of responses than did later stimuli (fig. 1, a and b). Rapid flight responses were often made to early stimulus presentations. They often included burrowing attempts and were most frequently elicited by the 'tap' stimulus. 4 of the 7 fish showed fright responses which in over 90% of cases involved withdrawal of pectoral and dorsal fins and the passage

of a rapid wave of movement down the body from head to tail. This quickly drove the animal forward in the water. Eye movements were observed in 50% of responses (table 1). Unlike arousal responses which showed varying numbers of components, fright responses usually involved all the typical components mentioned above.

The response to a novel stimulus was either arousal or fright, though the latter was the more common response to a 'tap'. Repeated presentations of a stimulus caused responses to wane, fright being replaced by arousal which then itself declined. The decline in response is indicated by the number of fish responding to either stimulus (fig. 2, a and b) though responses to the tap were more resistant to decline and the last response in a stimulus series was always one of arousal. Not only did the proportion of fish responding decline, but if the components of both arousal and fright were grouped together, so did the proportion of components exhibited by fish (fig. 1, a and b). This was true for either stimulus.

Table 2. Habituation of the components of the responses of *Halichoeres bivittatus*, to repeated presentation of a moving shadow and a tap stimulus. Mean \pm SEM of number of trials for habituation

Component	Moving shadow	n	Tap	n
Head movement	3 \pm 0.86	7	4 \pm 1.06	7
Eye movement	8.33 \pm 1.07	7	1.17 \pm 0.9	7
Pectoral movement change	3.14 \pm 0.94	7	4.6 \pm 1.07	7
Dorsal fin change	3.0 \pm 1.12	7	4.14 \pm 1.08	7
Tail movement	2.3 \pm 0.87	7	1.86 \pm 0.9	7
Body movement	2.29 \pm 0.77	7	1.57 \pm 0.6	7

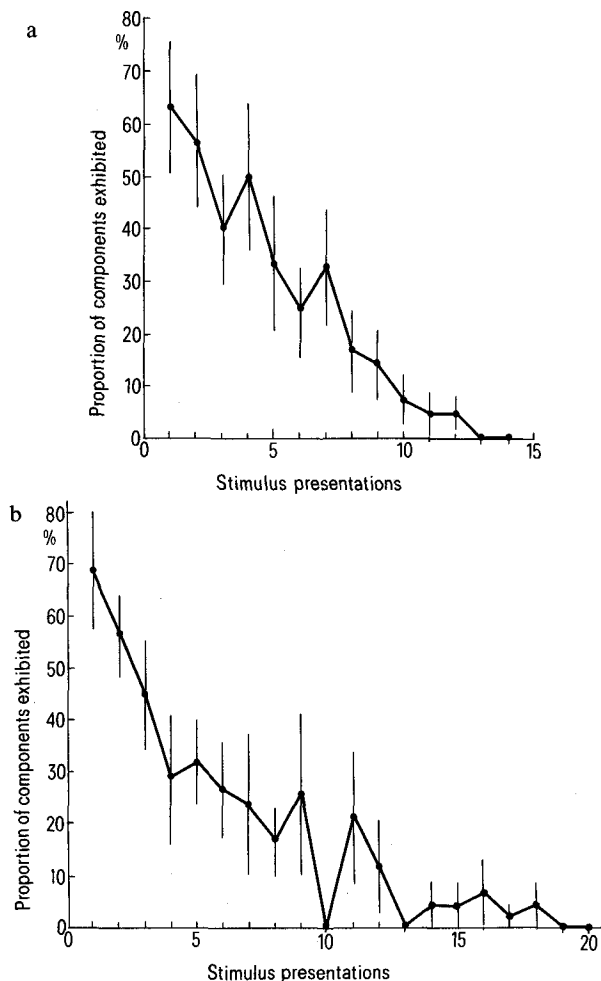


Figure 1. Behavioral components of responses to a a moving shadow stimulus; b a 'tap'. Mean \pm SEM of proportion of components studied exhibited by 7 fish on serial presentation of the stimulus.

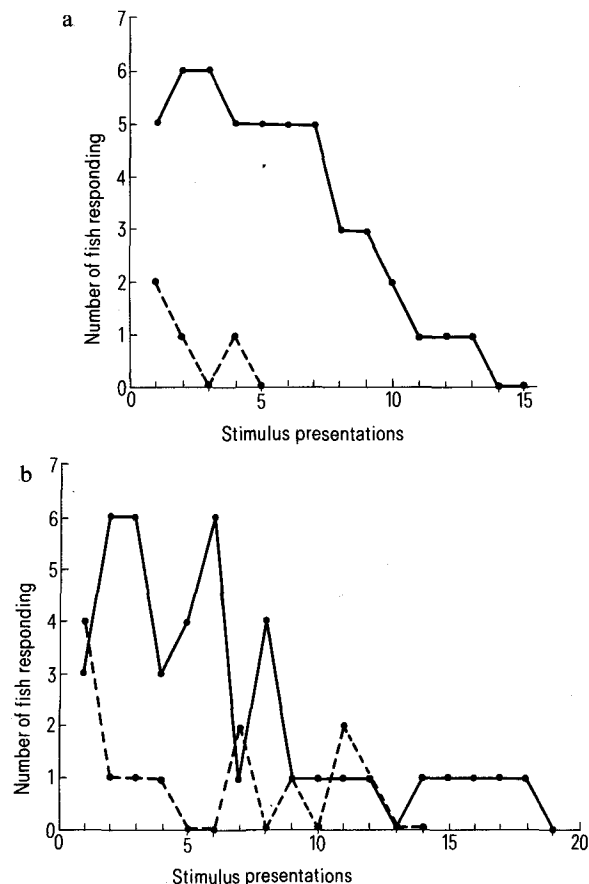


Figure 2. Numbers of fish responding with arousal and fright, to presentation of a a moving shadow stimulus; b a 'tap'. Solid line = arousal, pecked line = fright, n = 7.

This differential resistance to decline of the various components of the responses can be demonstrated by the number of trials required for their habituation (table 2). With the moving shadow stimulus, eye movements are the most resistant to decline, followed by changes in pectoral fin movements, dorsal fin position and head movements. The rates of waning of these components differ using a Friedman's 2-way analysis of variance ($\chi^2 = 13.9$, $n = 7$, $p < 0.02$). The tap stimulus produced a similar result, pectoral and dorsal fin and head movement tending to be the most resistant to decline ($\chi^2 = 8.9$, $n = 7$, $p < 0.2$).

Arousal responses shown by the slippery dick, *Halichoeres bivittatus* are typified by a change in pectoral fin movements and erection of the dorsal fin. Orientation of the head, eyes and body may also occur if the stimulus is visual. These components of arousal are similar to those described for goldfish⁸⁻¹⁰ and roach⁴.

Early stimuli in a serial presentation can evoke fright responses, especially if the stimulus is a tap. Here, pectoral locomotion ceased, both pectoral and dorsal fins were adducted whilst a wave of contraction passed down the body from head to tail, driving the animal forward. Burrowing behavior often ensued. These vigorous sinusoid movements may be a natural extension of the cyprinid-type tail flip response⁸ when performed by this slimmer, more elongate species. Early fright responses disappear to be replaced by arousal, as observed previously⁵, though the muscular components of fright are maintained during its expression. The same is not true of the arousal response in which the components of the behavior are reduced during repeated stimulus presentation. Those components which are the most consistent responses to a novel stimulus, whether this be a moving shadow or a tap, are also those most resistant to decline. These are erection of the dorsal fin and change in beat of the pectorals, which take some 3-4 trials to decline. If the stimulus is a moving shadow then detection of the stimulus, indicated by eye movements, may occur for some 8

presentations even though a generalized arousal response is not apparent.

The present work has established a similarity between the behavioral responses of the marine perciform, *Halichoeres* and the previously studied cyprinids. Whereas fright responses are of an 'all' or 'none' nature, arousal responses can differ in the number of behavioral components they entail. Both, however, decline on repeated stimulus presentation so that the response is appropriate both to the environmental circumstances and the previous experience of the individual studied.

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Host cell reactivation capacity of different strains of *E. coli* B resistant or sensitive to ozone

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Summary. Host cell reactivation capacity for ozonated or irradiated phage was determined for different strains of *E. coli* either more sensitive or resistant to ozone than the wild type strain. The results suggest that the *ozr* gene product could be involved in the same repair pathway for ozone-induced lesions on DNA as the *polA* gene. The possible involvement of a specific endonuclease for these lesions is also considered.

Ozone is a natural constituent of clean air at high altitudes and is generated in the stratosphere by reaction of oxygen atoms with molecular oxygen. On the other hand, ozone is the major oxidant found in photochemical smog in the lower atmosphere. This gas is formed in polluted atmospheres by photolysis of NO₂ in the presence of hydrocarbons produced by traffic and chemical plants.

Various toxic effects of ozone on biological systems have been described in recent literature. This strong oxidizing agent has been shown to cause aberrations in the growth of plants², membrane damage in erythrocytes³ and histological changes in animal organs⁴. It has been shown to be capable of inducing chromosomal aberrations in *Vicia faba*^{5,6}, in human cell culture⁷ and in the peripheral lymphocytes of human subjects⁸ while similar levels induced chromatid-aberrations in peripheral lymphocytes from Chinese hamsters⁹. In addition, a con-

siderable number of reports concerning the radiomimetic^{10,11} and mutagenic¹²⁻¹⁴ effects of this gas have been accumulated. Furthermore, ozone has been reported to damage viral¹⁵⁻¹⁷ and bacterial¹⁸⁻²⁰ nucleic acids. On the molecular level, ozone has been shown to cause lipid peroxidation²¹, covalent protein cross-linking²² and degradation of nucleobases by attacking primarily guanine²³⁻²⁵.

Some previous work has emphasized the fact that *E. coli* could repair ozone DNA-damage^{26,27} and that DNA polymerase I could be involved in the repair process^{28,29}. Recently, several mutants of *E. coli* either more sensitive or resistant to ozone than the wild type have been isolated³⁰⁻³². Published data on cell survival and genetic mapping with these different mutants³⁰⁻³² indicate the location in the *malB* region of *E. coli* of 3 genes (*ozrA*, *ozrB* and *ozrC*) that could be involved in the repair of ozone-induced lesions on DNA.