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Metamorphosis of the Visual and Barbel Sensory Systems at Settlement in the Reef Fish *Upeneus tragula* (Family Mullidae)

M. I. McCormick and J. Shand

Department of Marine Biology, James Cook University, Townsville, Q. 4811, Australia, Fax. No.: 61 77 25 1570

Abstract. Settlement for a reef fish involves a major change in sensory stimuli as the fish undergoes metamorphosis and moves from a planktonic to demersal existence. This study examines the nature of structural changes in the sensory system at settlement for the goatfish *Upeneus tragula* (Family Mullidae). Pelagic-stage fish were collected using purse-seines around aggregation rafts or in light-traps off Lizard Island, on the northern Great Barrier Reef, during the summers of 1989/90 and 1990/91. Settled fish were caught from the reef using fence-nets. Changes in the visual system and outgrowths of the olfactory and gustatory system (barbels) were examined in detail, using various morphometric and histological techniques. Major changes were found to occur in both systems. An unusual double-layer of cone photoreceptors and high densities of bipolar (neural processing) cells were found in the dorsal retina during the pelagic stages; this double-cone layer was lost, and both the cone and bipolar cell densities reduced during the 6–12 h settlement period. This may represent an overall reduction in relative visual acuity. Sensory barbels, which aid in the detection and manipulation of prey items once settled, underwent rapid growth at settlement, largely due to the expansion of taste bud cells. These changes to the sensory system are likely to be linked to the changes in environmental stimuli which occur during settlement.

Introduction

For reef fish, the transition between the pelagic larva and the reef-associated juvenile may be of crucial importance in determining the abundance patterns of fish on the reef (Doherty 1992). This settlement process represents the greatest change in the suite of environmental and biological pressures that a reef fish will actively experience during its lifetime (McCormick and Molony 1992). Factors such as light regimes, turbidity, water chemistry, prey types and availability, and predator pressure are likely to differ greatly between the two environments. Structural changes which occur during the metamorphosis associated with reef-fish settlement, will interact with the existing functional capabilities of the fish to determine its response to these new sensory cues, and hence the fish's ability to capture prey and escape predators.

The few studies done to date suggest that structural and functional changes that occur at settlement are varied, and range from subtle changes in pigmentation (some Pomacentrids e.g. *Abudefduf* sp., McCormick unpublished data) to major reorganisations of the physiology, somatic and sensory systems of the fish (e.g. Pleuronectiformes, Laurence 1975, Neave 1986). The potential for high mortality at settlement and the nature of the structural, physiological and behavioural changes which occur at metamorphosis have led some researchers to refer to settlement as a "critical period" (Blaxter 1988, Browman 1989).

The present study examines the changes that occur in the sensory system of the tropical goatfish, *Upeneus tragula* (Family Mullidae), as it undergoes metamorphosis and settles into the reef environment. As for many reef fish (Sweatman 1985a), settlement in *U. tragula* is a rapid, often overnight process. In this species it constitutes a shift from a pelagic existence as an active predator in the top 6 m of the water column (Leis 1991, McCormick and Milicich in press) to becoming a benthic microcarnivore that is closely associated with the substratum. Furthermore, the pelagic stages rely largely on vision for the capture of prey items, while the juvenile stages use projections of the olfactory and gustatory systems, in the form of sensory barbels, to detect and manipulate prey. This study looks at the changes that occur in these two sensory systems during metamorphosis against the framework of ontogenetic development. This study represents a summary of the preliminary results of two collaborative studies, the details of which are documented elsewhere (McCormick 1993, Shand in press).

Materials and Methods

Study species

The freckled goatfish, *Upeneus tragula* (Richardson), is a common component of the ichthyofauna of the central and northern Great Barrier Reef lagoon. Early life stages between 8 mm standard length (SL) and settlement size (19–34 mm SL) form large schools with other pelagic goatfish species, and will actively aggregate under drifting debris (McCormick and Milicich in press). Settlement involves an obvious change in pigmentation and behaviour. The silvery ventral and lateral surfaces present in the pelagic-stage fish change to a mottled cream and brown colouration; dark bars on the caudal and dorsal fins, and a dark stripe from the front of the snout to the caudal preduncle develop.

Collection

Pelagic stage *Upeneus tragula* were collected from 0.8 to 5 km west of Lizard Island (14°41'S, 145°27'E) on the northern Great Barrier Reef, over approximately 22 m of water during the summer of 1989/90 and 1990/91. Fishes were collected in two ways: some were attracted to a series of 1 × 1 m plastic aggregation rafts moored for 2 h, and were caught with a 14 × 2 m plankton-mesh purse-seine. Others were caught using automated light-traps (Doherty 1987) moored approximately 0.8 km off the fringing reef. Fish from the whole size range available were either placed into a preservative (details below) immediately upon capture, or returned to the laboratory alive and placed into an aquarium. Overnight, some of these fish metamorphosed, changed pigmentation and settled to the bottom of the tank. These fish are regarded as having been competent to settle at the time of capture. Other fish had started to attain the characteristic reef-colouration but had not completely metamorphosed and settled. Individuals in fully- and partially-settled states were preserved for histological examination. Juvenile *U. tragula* were collected using a fence-net from the seagrass beds within Watson's Bay, on the back-reef of Lizard Island, and preserved for comparison to newly-settled fish.

Histological Preparation

Visual system: A size range of *Upeneus tragula* that were in their pelagic phase (purse-seine and light-trap samples) and newly-settled phase (fence-net samples) were preserved upon capture. The eyes were pierced and the whole animal was fixed in marine Bouins for 24–48 h. Specimens were then flushed a number of times in 70% ethanol and stored in this preservative for approximately 4 weeks before processing. Partially and newly aquarium-settled fish were similarly preserved. Histological preparation for light microscopy involved dehydrating the heads of the fishes in an alcohol series, treating them in 0.5% low viscosity nitrocellulose (LVN) in methyl salicylate (MS) for 12 h, then infiltrating them with low viscosity nitrocellulose. Lastly, the fish eyes were infiltrated and embedded in parafin wax. Radial serial sections (3–4 μm) of one eye per fish were cut and stained in haemotoxin and eosin.

Counts of the number of cells were made in each of the following layers from the radial section: cone inner segments (cones), external nuclear layer (rod and cone nuclei) and bipolar (processing) cell-layer (Fig. 1). Counts were made with an ocular graticule over a strip of the retina 52 μm wide. Ten counts were made in the dorsal and ventral retina of each fish. After correction for section thickness (Abercrombie 1946), the density of each cell type was calculated (per 0.01 mm^2).

Barbels: Cross-sections (6 μm) of the barbels were cut one-third of the total barbel length from the tip, and stained with Azan. Scanning EM had previously shown that taste bud cells were largest and in highest densities in this region of the barbel (McCormick 1993). The area of the epidermis, taste buds, connective and neural tissue, muscle blocks and cartilage were quantified directly from the histological slides using a calibrated digitizing computer package. Density of taste buds per millimetre circumference was also determined. Taste bud cells were recorded as being present in a particular section only if they possessed both a neural connection

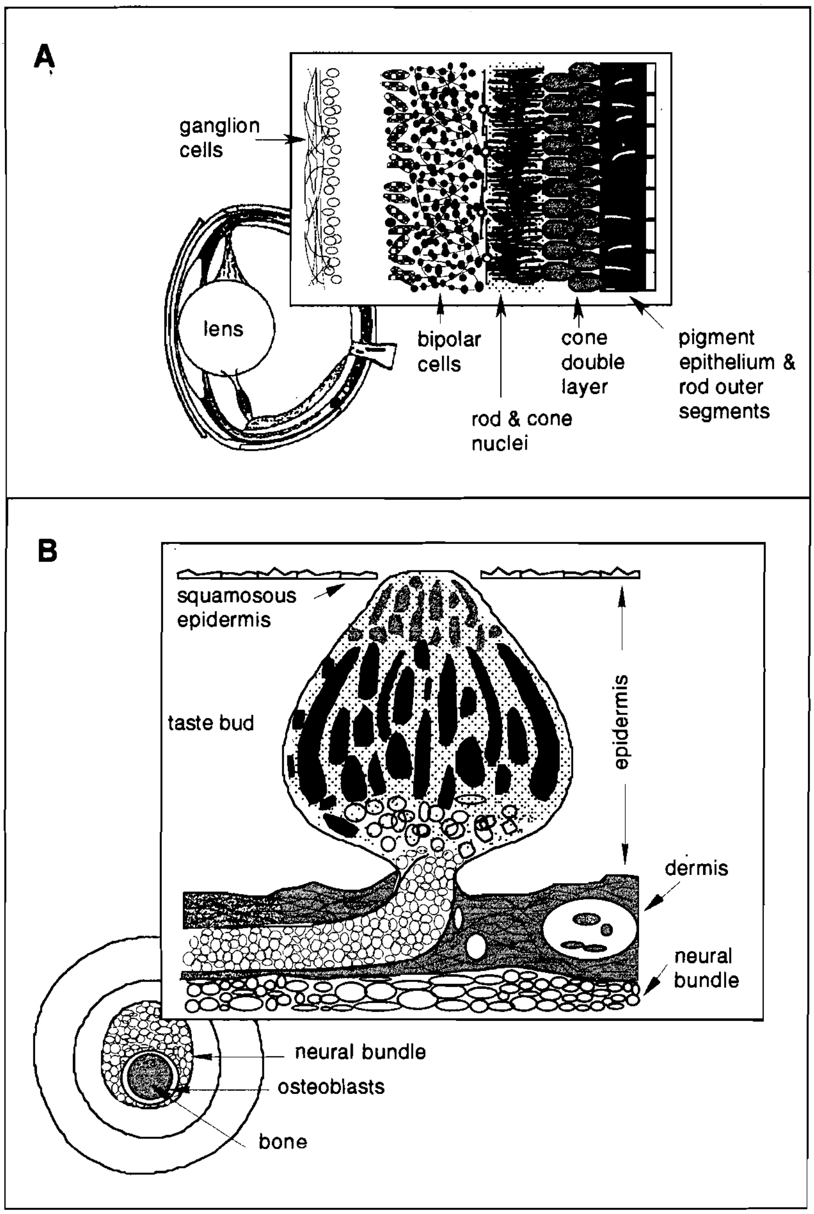

Fig. 1. General morphology of the regions of the eye and barbel discussed. A Cross-section through the eye of a pelagic *Upeneus tragula*, showing a stylised representation of the layers of the retina (note the double-cone layer). B. Cross-section through the sensory barbel, showing details of the cell layers examined.

to the main neural plexus of the barbel and the microtubules penetrated the outer squamous epidermis through a taste pore (Fig. 1b).

Results

Visual system

Examination of the histological sections showed that *Upeneus tragula* has a duplex ventral retina, typical of diurnal teleosts, and the cell-layer arrangement of this half of the retina was not found to change during development. In contrast, the development of the dorsal retina involved marked changes in the structure of the cell layers. Pelagic stage fish below 17.5 mm SL were found to have an arrangement of cells similar to that in the ventral retina, but between this size and the size at which metamorphosis occurred (in this study ~28 mm SL), the dorsal retina developed a double-layer of

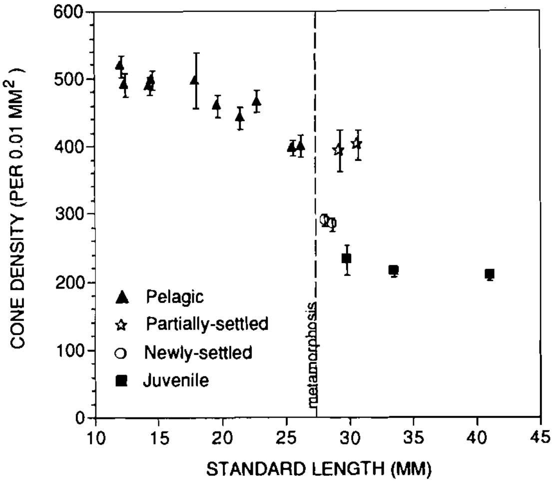

Fig. 2. Change in the density of cones (per 0.01 mm²) in the dorsal retina of *Upeneus tragula* with fish standard length (mm) (n = 10).

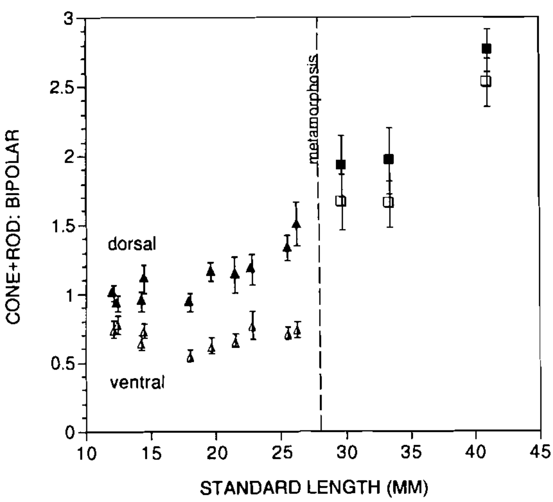

Fig. 3. Change in the number of photoreceptors (cones and rods) converging onto the bipolar (processing) cells during growth of *Upeneus tragula*. Symbols as for Fig. 2.

cones, as opposed to the single layer found previously (Fig. 1a). This double-layer is initially confined to the retinal margins, but extends to cover the entire dorsal retina prior to metamorphosis. During the 6–12 h period over which metamorphosis occurred in these fish, the structure of the dorsal retina reverted back to the cell-layer morphology of the ventral retina, with only a single layer of cone cells.

Densities of the cone cells, the receptors responsible for diurnal acuity and colour vision, decreased in density as fish length increased in both the ventral and dorsal areas of the retina. The pattern of decline was similar in both areas with an abrupt decline at settlement which was most pronounced in the dorsal retina. The results for the dorsal retina are shown in Figure 2). A comparison of the densities between partially- and newly-settled fish suggests that the rate of decrease in the cones cells maybe in the order of 25% during metamorphosis (Fig. 2).

The processing efficiency of the retina can be gauged by the number of receptor cells (cones and rods) which input information to the processing neural cells (horizontal, bipolar and amacrine cells) (Pankhurst 1989). The ratio of the photoreceptor to bipolar cells was low prior to settlement when, in both parts of the retina, there was an increase in the number of photoreceptors which are converging to each bipolar cell (Fig. 3). This trend was accentuated in the ventral retina. Calculations of theoretical visual acuity were made using cone density and lens diameter data following the formula given by Tamura and Wisbey (1963). This gives an indication of the ability of the eye to resolve fine detail by calculating a minimum separable angle in minutes of arc; thus the smaller the angle the better the acuity. For *Upeneus tragula* acuity increased during larval growth to an asymptote of 26 min, which corresponded to metamorphosis.

Barbel Development

Mid-way through the planktonic phase of *Upeneus tragula* (8 to 9 mm SL), barbels develop as thickenings along the edge of the branchiostegal membrane, originating from the hyoid complex (Leis and Rennis 1983, McCormick pers. obs.). The standard length the fish had attained when the barbels detached from the brachiostegal rays was found to be highly variable, ranging from 10 to 22 mm SL. Barbels ranged in size from 0.9 to 2 mm when first detached. During the pelagic life-history phase barbel growth was linearly related to fish length (Fig. 4, Barbel length = 0.194 SL − 1.554, r^2 = 0.852, n = 159). Barbels rapidly increased in length during the metamorphosis associated with the settlement (Fig. 4); a process which in fish that had been brought into the laboratory from the field, took 6–12 h. This was a growth spurt which may represent an increase in barbel length of up to 52% in 12 h. Upon settlement, barbel growth returned to approximately the same rate as prior to metamorphosis (Barbel length = 0.163 SL + 2.298, r^2 = 0.223, n = 12).

The cross-sectional area of the barbels showed a rapid, although less dramatic, increase during the settlement period (Fig. 5). Pre-settlement stages ex-

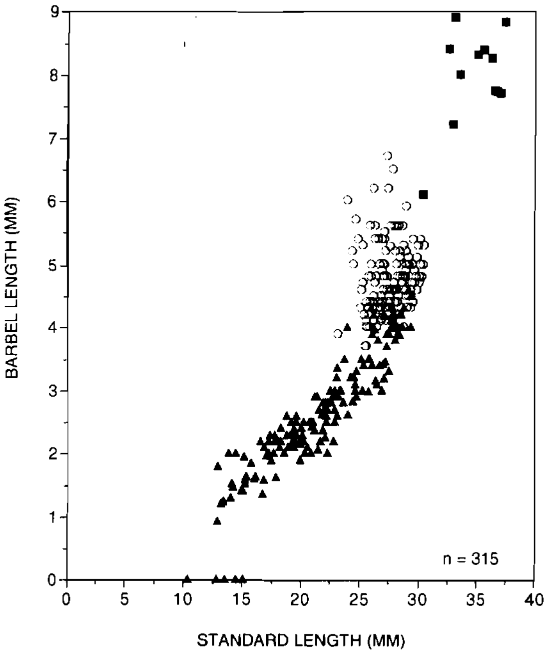

Fig. 4. Change in the length of the sensory barbels (mm) with standard length of the fish. Symbols as for Fig. 2.

hibited an exponential rate of increasing area. This was due to the rapid proliferation and differentiation of the sensory taste bud complexes, which form a major component of the epidermis. The epidermal component of the cross-sectional area increased linearly to 70% just prior to metamorphosis, increased approximately 5% during settlement, and then fluctuated around 70% during the juvenile stage (Fig. 6). This rapid increase in the epidermal area was due to a rapid increase in the size of the taste-buds within the epidermal layer. After settlement other components of the barbels, such as the neural bundle, nerve and connective tissue layer (dermis) and cartilaginous supporting rod, offset the increase in taste-bud size, thereby maintaining the epidermal contribution at about 70% of the cross-sectional area (Fig. 6).

Discussion

In many cases there exists a clear correlation between the ontogenetic timing of structural and functional changes in the sensory systems of developing fishes and changes in behaviour, which in turn coincide with ontogenetic shifts in their ecology (e.g. Blaxter 1975, Ahlbert 1976, Noakes 1978, Noakes and Godin 1988). This study is an example of the rapid changes which can occur in the visual and gustatory systems of a fish during the metamorphosis associated with settlement into the reef population. The sensory morphologies of the pelagic and demersal life-history phases are well suited to the environments in which they must efficiently attain food and survive.

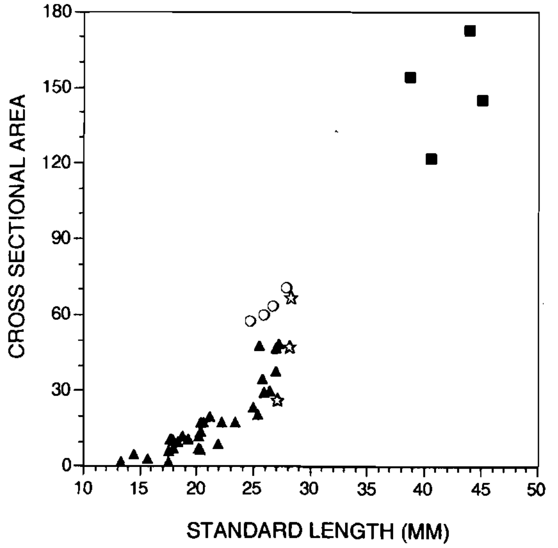

Fig. 5 Change in the cross sectional area of the barbel ($\times 10^3\ \mu m^2$) with standard length of fish. Symbols as for Fig. 2.

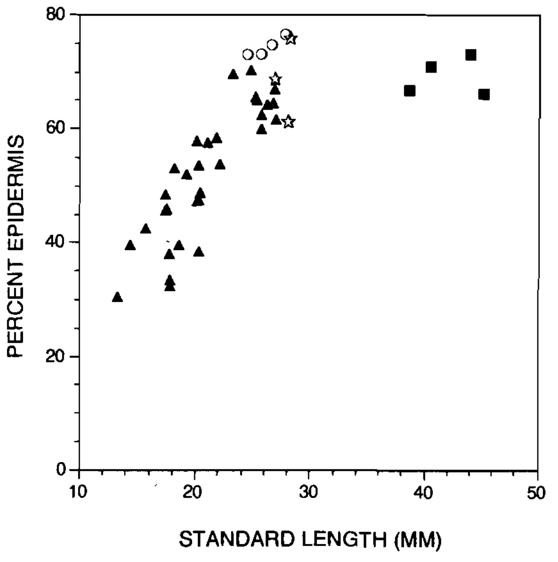

Fig. 6. Change in the percentage contribution of epidermal tissue to the total cross-sectional area of the barbel with length of fish. Symbols as for Fig. 2.

Larval and pelagic juvenile stages of *Upeneus tragula* are clupeoid in appearance and form large schools in the top 6 m of the water column during daylight hours (Leis 1991). They are planktonic predators and field observations suggest that they use a combination of visual and lateral line senses to detect prey and maintain position within the school. It may be that the high cone densities and low convergence ratios maintained in the retina during the pelagic phase are to increase capability for the visual demands of a planktivorous habit. Visual acuity in small eyes is inherently restricted by lens size. Thus as the eyes of larval fish grow there are dramatic improvements in acuity due to growth of the lens alone. A second factor affecting acuity is the density of the photoreceptors, a situation analogous to the effects of grain size on photographic film. In *Upeneus tragula* a high cone density is maintained prior to settlement which implies that the high acuity is of significance when in the pelagic phase. A number of studies of pelagic and planktivorous fish have found high cone densities associated with the visual search axis (e.g. Tamura 1957, Tamura and Wisbey 1963, Browman et al. 1990). The acuity values calculated for pre-settlement *U. tragula* are similar to those found during the growth of the planktivorous damselfish *Pomacentrus moluccensis* (Shand unpublished data). However, at settlement in *U. tragula*, acuity no longer continues to improve, whereas in *P. moluccensis* there is a further increase in acuity to a minimum separable angle of about 14 min. The acuity calculated for adult pelagic species of, for example, the skipjack tuna (Tamura and Wisbey 1963) is even greater at 2.3 min. Low ratios of the convergence of cones to neural cells as found in pre-settlement *U. tragula* will also help maintain high acuity at this stage of their lifecycle. The processing of information received by the photoreceptors begins in the neural cell-layers of the retina and there are differing degrees of summation in species occupying different reef habitats (Pankhurst 1989). A high convergence ratio would increase the sensitivity of a retina but at the expense of acuity (Lythgoe 1979).

The pelagic environment is rich in ultra-violet and upwelling blue light, while after settlement to the reef substratum the blue component will be replaced by longer wavelength reflections from the sand and algae (McFarland and Munz 1975). The displacement of cones into two layers in the dorsal retina of pre-settlement *Upeneus tragula* may be a mechanism to overcome chromatic aberrations caused by short wavelength light being refracted by the spherical lens more than long wavelengths (see Fernald 1988). The reorganisation of the cone double-layer into a single layer may be associated with a change in the spectral sensitivity of the receptors, concomitant with the change in the spectral qualities of the light environment at settlement. Indeed it has recently been found that loss of ultraviolet sensitivity during ontogentic habitat changes in the brown trout (*Salmo trutta*) and yellow perch (*Perca flavescens*) is associated with loss of small single cones from the retina (Bowmaker and Kunz 1987, Loew and Wahl 1991). Preliminary measurements of spectral sensitivity of the cones of *U. tragula* indicate that there are changes occurring at the time of settlement (Shand unpublished data).

Concomitant with this marked change in the visual system, the present study found complementary changes to the barbel microstructure. The length of the barbels increased dramatically during settlement, largely through the rapid expansion of the sensory taste-buds. These complexes comprise about 37% of the total cross-sectional area of the barbel (50% of the epidermis) at this stage. To stress the potential importance of these changes it is necessary to understand the form and function of the barbels in goatfish. Barbels are modified extensions of the first brachiostegal ray, with a central rod of bone and cartilage, and are controlled by modified muscles of the hyoid system. These are features which promote sufficient rigidity and strength to enable their use as excavating devices in the surface sediments of the reef. In an electrophysiological study of foraging in the goatfish *Parupeneus porphyreus*, Holland (1976) found that the removal of barbels adversely altered and greatly reduced arousal behaviour, and eliminated food-search behaviour. When the sense of smell was effectively removed by cauterizing the nasal rosettes of the olfactory apparatus, normal arousal and food-search behaviour was recorded. This stresses the important role of the barbels in the acquisition of prey, and that it is crucial for the barbels to be equipped for a fully functional role in the detection and manipulation of prey items as soon as the fish metamorphoses and settles.

Barbels sometimes do not separate from the branchiostegal arch until late in the pelagic life, commonly at 16–18 mm SL. In one fish, barbels were still found to be attached by a membrane connection at 22 mm SL, which represents approximately three-quarters of its pelagic existence. This suggests that wholly functional barbels are not used actively in the pelagic phase; a conclusion which is supported by tank observations of newly caught pelagic individuals.

Other researchers have found large changes in the sensory systems of marine species during metamorphosis. Most examples come from the flatfish where metamorphosis proceeds through a series of well defined stages, involving the proliferation of functioning larval structures and regression of tissues (Youson 1988). Examples are the migration of the eye in flounder (e.g. Ryland 1966), the creation of the asymmetry in the projections of the right and left olfactory bulbs in *Pseudopleuronectes americanus* (Rao and Finger 1984), or the development of the lateral line system in *Pleuronectes platessa* and *Scophthalamus maximus* (Neave 1986). None have found as rapid a change as that presented for the reef-fish in this study (e.g. Neave 1984). Obviously, further studies of the sensory systems of reef fishes are required before the generality of the magnitude and nature of these changes can be assessed.

This study has shown that major changes to the sensory system can occur at settlement in reef fishes. Youson (1988) noted that if a major remodelling of the body systems takes place after a relatively uneventful larval period, then the timing of the initiation of metamorphosis is undoubtedly of great significance to the ultimate survival of the post-metamorphic individual. To achieve some understanding of the dynamics of coral reef fish populations it is important to examine how sensory competence and the physiological status of the organism (metamorphosis often involves the mobilisation of storage reserves) interrelates with the actual timing and location of settlement onto the reef. Furthermore, since reef fish may actively choose when and where they settle (e.g. the damselfish, *Dascyllus aruanus*, Sweatman 1985b), it will be of interest to know to what extent sensory development governs settlement, and to what extent a fish can delay metamorphosis (e.g. Victor 1986) once it has reached a level of development at which the process can occur.

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