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Sensory development in settlement-stage larvae of Caribbean labrids and scarids: A comparative study with implications for ecomorphology and life history strategies

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Approval Sheet

This dissertation is submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Monica R. Lara

Approved April 1999

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Dedication

I would like to dedicate this dissertation to my family. They are the ones I thought of each time I was sure I wouldn't finish and they're the reason I did. I love you all.

I dedicate this in the memory of my two uncles who passed this last year Avelino Carrillo Maya and Dr. Daniel Sanchez Cornish. They did not get to see me finish but the last time I would ever speak them they each told me they were proud of me and they made me proud to be a part of them. I also dedicate this in memory of my grandfather Capt. Miguel Carrillo Ayala who, though gone many years now, will always be my inspiration.
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Abstract

The sensory capabilities of settlement-stage fishes are unknown but this information is necessary to studies of larval settlement and recruitment. The morphology of the cephalic lateral line, eye and external olfactory organ of thirteen species of settlement-stage Caribbean labroids was described. Scanning electron images of the cephalic lateral line neuromasts, lateral line canals and olfactory epithelia and histological studies of the retinas and morphological measurements of visual acuity were used to assess the level of sensory development attained at settlement. The sensory capabilities of settlement-stage fishes are discussed in relation to the possible cues settlement-stage fishes may be using to locate a reef and microhabitat within the reef system. In addition sensory development is discussed in relation to interspecific variation in settlement behavior, settlement-site selection, possible evolutionary life history strategies and morphological constraints on sensory development and settlement.
SENSORY DEVELOPMENT IN SETTLEMENT-STAGE LARVAE OF
CARIBBEAN LABRIDS AND SCARIDS

- A COMPARATIVE STUDY WITH IMPLICATIONS FOR ECOMORPHOLOGY
AND LIFE HISTORY STRATEGIES
Chapter 1.

An introduction to the problem and prospectus for study.

The larvae of most marine fishes have a planktonic stage during which they travel in the water column for a period of weeks or months (Leis, 1991). At the end of this period, many species undergo a transition from the pelagic to benthic environment during a phase known as settlement. In most cases, this transition is accompanied by metamorphosis that involves dramatic changes in morphology and behavior. These changes, which mark the transition from larval to juvenile stages, have been a topic of interest and research for scientists in a wide variety of disciplines including ecology, fishery biology, morphology, physical oceanography, physical biology and taxonomy.

For many decades, too little was known about the larval and early juvenile stages of marine fishes for the information to be of much use in fisheries models, taxonomic classification, and studies of evolution and distribution.
(Blaxter, 1984; Victor, 1986); consequently these stages were not usually represented in most of these studies. In part, the problem lies in the fact that the larvae of many species have yet to be identified and much research effort is dedicated to simply identifying and describing larvae. In addition it is difficult, expensive, and labor intensive to obtain samples of larvae that truly represent actual larval abundances. Most studies of abundance and distribution grapple with the intrinsic biases caused by collection gear design or sampling methods (see Colby, 1988; Somerton and Kobayashi, 1989; Anderson and Webster, 1992).

Direct \textit{in situ} observation of processes in the early life stages is logistically nearly impossible due to many factors, including (1) the relative rarity of encountering live larvae in the ocean; (2) the events that transpire can occur over a geographical area of hundreds of kilometers (Victor, 1987) and (3) little is known about the pelagic habitat of all stages of live larvae (reviewed in Boehlert, 1996).

In spite of these problems, the importance of information about the early life history stages of fishes has attracted an ever-increasing research effort from a diversity of disciplines. For example, from its inception and until very recently, taxonomic classification of fishes was based solely on adult characters. With the publication of the volume "Ontogeny and Systematics of Fishes" in 1984 (Moser et al., eds.), the use of larval characters to help elucidate phylogenetic relationships was established. Since then, the volume of work which involves the use of larval characters to support, refine or revise existing taxonomies based on
adult characters is relatively small (Johnson, 1993). Larval characters have been employed in taxonomic work for only a few groups of fishes including acanthurids, carapids, gobiids, myctophids, serranids, scombrids and gempylids (Johnson, 1993; Leis et al., 1997).

Fishery biologists now realize that the mechanisms of larval transport and larval supply are a major component in stock models and that these life stages can no longer be ignored (Sale, 1990). For example, stock models that include estimates of pre- and post-larval mortality depend on some reliable measurement of larval abundances and causes of mortality. In comparison to estimates of adult mortality (due to factors such as fishing pressure, predation and natural causes) estimates of larval and early juvenile mortality rates and the causes of these mortalities have been extremely difficult to ascertain. Only within the last two decades have estimates of larval fish mortality due to starvation and predation been available to fisheries modelers (Blaxter, 1984).

The roles that different factors and life stages play in recruitment have been debated in the literature. One theory suggests that recruitment is limited by space on the reef and that post-settlement processes are most important to recruitment success (Sale, 1978; 1985; Sale and Steel, 1986; Sale, 1988). Another theory suggests that space is not limiting but rather that recruitment success is limited by the availability of settling larvae. Thus the factors influencing the survival of larvae
during their planktonic stage are more important to recruitment success (Doherty, 1981, 1982, 1983; Houde, 1987; Milicich, 1994). A third theory states that post-settlement mortality due to predation is the most important determinant of adult communities (C. L. Smith, 1978; Glynn, 1988). The debate continues but the factors determining recruitment success continue to be elusive and the most likely scenario is that these factors are not mutually exclusive but contribute to differing degrees at different times (reviewed in Hixon, 1991).

The discovery that fish larvae can be transported hundreds of miles by ocean currents in a relatively short time has been used to explain species distributions and stock maintenance (Victor, 1986). The question of how larval transport and distribution occur has been divided into two parts. Large-scale transport of larvae is mediated by geophysical mechanisms such as currents, eddies, fronts and internal waves (see review by Boehlert, 1996). However, these physical mechanisms are not sufficient to explain fine scale distribution of larvae. Models based on physical oceanographic mechanisms, where larvae are treated as passive particles, have not been able to accurately predict the distributions of larvae observed in the field (cf. Walters et al., 1992; Jenkins and Black, 1994). Recent studies suggest fish larvae have more control over their movements than was formerly thought (Leis and Goldman, 1984; Leis, 1982; Breitburg et al., 1995; Leis et al. 1996; Stobutzki, 1998; Stobutzki and Bellwood, 1998).
Some researchers believe that the observed distributions of larvae can only be explained by interaction between larval behavior and physical transport (Boehlert and Mundy, 1988; Kingsford and Choat, 1986; Steffe and Westoby, 1992). The nature of these behaviors is often based on conjecture and infrequently substantiated by direct observation. Laboratory studies of behavior may not directly translate into what is actually occurring in the field (Leis, 1991); however, they provide useful information about larval capability. Studies describing larval locomotion have reported that larvae may reach sustained swimming speeds of 70 cm s\(^{-1}\) (Stobutzki & Bellwood, 1994), suggesting that larvae have the ability to swim against moderately strong currents. Information such as this suggests that larval competency cannot be ignored in studies of larval transport and distribution. Some of the behaviors which would allow larvae some degree of control over their horizontal transport include vertical migration, orientation to currents or tidal flux or use of boundary layer flow (Leis, 1986; Boehlert and Mundy 1988; Yamashita et al., 1996; Breitburg et al., 1995).

The important question that no study has addressed thus far is that of the sensory capabilities of settlement-stage fishes. If fish larvae are capable of exercising control over their movements (e.g. vertical migration and directional swimming), then they must also possess the ability to assess their environment and alter their behavior according to cues detected in their environment. When
one considers the evidence that larval fish approaching metamorphosis have the ability to select a relatively specific site in which to settle (Shulman et al., 1983; Sweatman, 1988; Booth, 1992; Wellington, 1992; Wennhage, 1994; Elliot et al., 1995), the development of some sensory ability is clearly essential. Natural selection would favor settlement into a site that would provide the proper environment to maximize the chances of survival and the ability of a larva to assess the environment prior to or shortly after settling would also be favored. Selection would therefore act to ensure the attainment of the necessary level of sensory competency by the time a larva is competent to settle to ensure environmental conditions are favorable for settlement. It is clear that in order to understand the mechanisms of larval settlement and recruitment into a population and the factors that determine successful settlement and survival, information about the capabilities of settling fishes is of paramount importance.

The processes that enable a larva to reach a specific settlement site, assess the site, and settle into it are largely unknown. Settlement occurs over a few hours to a few days, and has been observed in situ (Kaufman et al., 1992) in only one study. Most studies determine settlement patterns by censusing new recruits (Wellington, 1992; Booth, 1992). This can be problematic as it is difficult to assess post-settlement factors such as emigration, immigration and mortality occurring immediately after settlement (Wellington, 1992). Due to the difficulty in observing settlement, identifying those cues that larvae may be using to find suitable
settlement sites has proven difficult. Several interesting studies have been conducted of the influences of certain specific factors on the settlement of coral reef fishes. Selection of a settlement site has been found to be influenced by the presence of conspecifics (Shulman et al., 1983; Sweatman, 1988; Booth, 1992), physical microhabitat such as coral species (Sale, Douglas and Doherty, 1984), hole size (Shulman, 1984), and patch reef size and reef isolation (Schroeder, 1987). Elliott et al. (1995) demonstrated that juvenile anemonefishes use chemical cues to locate their host anemones and it is possible that they are using these cues to locate habitats upon settling as well. This type of study is extremely rare and the cues that are important in the settlement of any species have not been satisfactorily determined. Before any questions can be answered about what specific types of cues are being employed by settlement-stage fishes in locating a potential habitat and in selecting a settlement-site, the sensory faculties of settlement-stages must be defined. Examining the development of the sensory systems in settlement-stage fishes provides a key to the types of cues they are capable of detecting at settlement and provides a more clearly defined picture of a settling larva’s sensory environment.

Given that direct observation of settlement of larval fish is exceedingly difficult, there are two alternative avenues of research that may lend some insight into settlement processes. The observation of larval behavior when subjected to various conditions in the laboratory can provide data on larval response to stimuli.
and parameters of ability (e.g., swimming, orientation to light) which can then be extrapolated to conditions in the field. The problem lies in creating conditions in the laboratory that adequately represent those in the field. In most cases the larval environment is far too complex to duplicate in a laboratory setting and this can influence the relevance of these findings. Results of laboratory studies of behavior vary widely (Theilacker and Dorsey, 1980) and in cases where field data has become available it often differs significantly from experimental results (Cobb et al., 1989).

A second avenue of research on processes affecting settlement is the direct study of morphological structure, which provides data on the potential capabilities of larvae. Sensory ability cannot be measured directly from morphology; rather, using a comparative method, relative abilities can be inferred by comparing observed morphological development with that of conspecific adults or larvae of other species. Only one study has related sensory development to the detection of settlement cues. Major changes in all of the sensory systems in the temperate flatfish *Paralichthys californicus* were found to occur near the time of settlement (Lara, 1992).
Focus of the present study

The present study tested the hypothesis that functional changes in one or more of the sensory systems coincide with the time of settlement, and that these changes are related to aspects of the settlement event and transition into the juvenile habitat. Additionally, these changes were considered in the context of possible physical and phylogenetic constraints on sensory development. Finally, by determining the developmental state of the visual, olfactory and lateral line organs at the time of settlement, morphological sensory capacities at settlement were evaluated and the types of sensory cues settling larvae are capable of detecting were inferred.

In order to lend strength to the correlation between ecological and morphological data, the conceptual framework of the present study was modeled on the ecomorphological approach described by Reilly and Wainwright (1994). The methodology of this approach includes “community-level analyses of the correlation between measured morphological and ecological attributes” and the application of phylogenetic information to elucidate the “role of evolutionary history in shaping the form of an organism and its relationship to its environment” (Reilly and Wainwright, 1994). The most complete approach consists of three parts that allow the linkage of (1) an ecological phenomenon, such as settlement site
selection, (2) morphology, in this case the sensory systems in settling fishes, and (3) the evolution of that relationship through the application of phylogenetic information. The combined demonstration of morphological functionality, covariance of this functionality with some aspect of the ecology of an organism and an evolutionary context for the observed linkage is considered to be the most integrative and comprehensive expression of this approach.

A preliminary review of the family Labridae provided evidence that variability between species exists in settlement-site selection and sensory morphology. At least two species of labrids found in the western central Atlantic, *Thalassoma bifasciatum* and *Halichoeres bivittatus*, had been shown to differ in their settlement site specificity (Victor, 1983) and preliminary work (Lara, unpublished) showed some variation between species in the morphology of the sensory structures. Measures of theoretical functional ability (e.g. visual acuity) can be assessed from measurements of components of the sensory systems.

Certain other criteria for the selection of a target group are satisfied by the labrids. The family Labridae is very speciose consisting of at least 60 genera and approximately 500 species (Nelson, 1994). Labrid larvae are among the most abundant coral reef fish larvae both in numbers and distribution. They ranked from 5th to 13th in number and 8th to 13th in occurrence among the 89 families of fish larvae obtained in plankton samples in the Caribbean (Richards, 1984).
Although descriptions of complete developmental series of tropical fish are rare, there is some information on early life history for about half of the labrid genera (Richards and Leis, 1984) and settlement-stage larvae of these are distinguishable to species. To date, the status of the phylogenetic relationships of labrids has not been fully resolved. Currently the family is divided into several tribes, two of which have been examined separately: the Cheilinini (Westneat, 1993) and the Hypsigenyini (Gomon, 1997) are reported to be monophyletic. For the purposes of the present study comparisons between species were made at the generic level.

Many studies show that labrid larval abundance increases offshore and labrid larvae may be found many kilometers from the nearest reef (e.g. Leis, 1993; Leis and Miller, 1976; Leis and Goldman, 1984). This suggests that labrids, as well as all other species with oceanic larvae, must be proficient in returning to reefs from far offshore. This makes labrids a good model for evaluation of competency in settlement-stage fishes. The abilities that are necessary for larval fishes to locate and return to a reef have not been fully identified (reviewed in Leis, 1991).

The morphological variability in the sensory structures of labrid species was determined using electron microscopy and histological techniques. Cone and rod counts and lens measurements were used for assessment of visual acuity. Densities of sensory and non-sensory cells of the olfactory epithelium were used as a
measure of relative olfactory ability. Number and distribution of cephalic lateral line neuromasts and development of the cephalic lateral line canals were described.

The utility of the data from this study of sensory development of settlement-stage fishes is manifold. First, although various descriptions of the development of sensory structures in temperate fishes have been published (Blaxter et al. 1983; Appelbaum et al., 1983; Kawamura and Ishida, 1985; Doroshenko and Matavkin, 1986; Kawamura et al., 1990; Su and Wang, 1990; Boglione et al., 1991; Kotrschal et al., 1990; Mukai and Kobayashi, 1991; Devitsina and Kazhlaev, 1993; Senoo et al., 1994; Pankhurst and Butler, 1996; Appelbaum and Riehl, 1997) none existed for any tropical fish species at the inception of this study. In the interim, descriptions of the development of the eye in five species of tropical marine fishes have been published (Job and Bellwood, 1996; Shand, 1997). A second benefit of the present study is that it provides baseline data for the comparison of sensory development in larvae of tropical and temperate species. Thirdly, in the course of this study some basic taxonomic work was necessary. As a result two previously undesignated larval labrid morphotypes were assigned to species. Most significantly, the information about sensory ability in coral reef fish larvae will help answer questions about the mechanisms of settlement site selection. In determining the sensory tools available to the settlement-stages of fishes provides insight into what types of cues fishes may be using to locate and to
choose settlement-sites. This information is of importance to the management of coral reefs as the availability of proper settlement sites may limit successful recruitment (Sale, 1990). Finally, an understanding of sensory capability as it relates to feeding and predator avoidance in settlement-stage fishes is crucial to assessing the effects of pre- and post-settlement events on survival (Doherty 1991; Sweatman, 1993; Tupper, 1995).
Chapter 2

Distribution of cephalic superficial neuromasts and development of the cephalic lateral line canals in 12 species of larval and juvenile Caribbean labrids.

Abstract

The cephalic lateral line neuromasts and canals of settlement-stage larvae and early juveniles of 12 species of Caribbean labrids were examined using scanning electron microscopy. Ages in days after hatching and days post-settlement were determined from the otoliths. The numbers and locations of the free neuromasts as well as the state of completion of each of the branches of the cephalic lateral line system are described for each species and stage. The general state of development of the system in the settlement-stages is discussed. All but one of the cephalic lateral lines are enclosed in all of the species at settlement. Development of the cephalic lateral line system is discussed in relation to the ecology, biomechanics and evolution of larval fishes. The settlement event is
discussed in the context of this new information about the state of development of the cephalic lateral line system.
Introduction

The lateral line system in teleosts consists of sensory structures (neuromasts) capable of sensing changes in water movements in the local environment of a fish's body. The neuromasts are of two classes: free (or superficial) neuromasts and canal neuromasts. Superficial neuromasts differ from canal neuromasts in that they exist on the surface of the skin and are in direct contact with the environment, whereas canal neuromasts are situated inside a series of canals in the cranium or in modified scales on the body of the fish. These canals are punctuated by a series of pores that are open to the environment. Morphological differences exist between the two types of neuromasts (See Coombs, et al., 1988). Canal and superficial neuromasts originate from a common type of dermal placode and are innervated by a common lateral line nerve (Lekander, 1949). In addition there is evidence that the neural crest is a contributor of some neuromast cells (Collazo et al., 1994). In most cases canal neuromasts first appear as superficial and are subsequently enclosed by the developing canal.

Though the distribution of lateral line canals follows a basic pattern in all fish taxa, patterns and positions of the free neuromasts and morphology of the
lateral line canals varies widely among species. This variation exists at all
taxonomic levels and the morphology of the lateral lines is a character used to
identify taxa at the familial and subfamilial levels (Webb, 1990). Species level
variation in the distribution of the superficial neuromasts has been documented
in cottoid fishes (Vischer, 1989). The distribution of the neuromasts in different
taxa shows extensive variation and has led to inconsistency in their
nomenclature and description among authors working on different taxa or
developmental stages of fishes. I will follow the nomenclature for superficial
neuromasts and superficial neuromast lines in Coombs et al. (1988). Variation
exists as well in the order and timing of the appearance of neuromasts and
formation of the canals (Lekander, 1949). In this study I will discuss some of
these variations and commonalities in the morphology and development of the
lateral line system at the familial, generic and species levels among 12 species of
Caribbean wrasses (Fam. Labridae), and representatives of two genera in the
sister group Scaridae, and one in the outgroup Pomacentridae.

Morphological variation taken within the context of ecological variation
can elucidate possible functional aspects of these characteristics. In addition, the
study of the variation in characteristics between different species can provide
information about the phylogenetic relationships of taxa. This latter information
is important to consider when attempting to correlate morphology and ecology
as phylogenetic history can constrain these relationships. The timing of
development has several important ramifications. The time at which structures develop can be dependent upon the proper conditions for the functioning of those structures. In turn the attainment of competency at a certain stage in life will be dependent on this timing. The present study combines information about the morphology and timing of appearance of elements of the lateral line system with information about certain aspects of the function of these structures and of the ecology of the species in order to begin to address these relationships. Furthermore this information about the settlement-stages of fishes has important implications for the study of settlement as it applies to larval competency and detection of settlement cues. No previous study of the sensory competency of the lateral line in settlement-stage fishes exists.
Materials and Methods

Sample Collection

Samples were collected off Carrie Bow Cay, Belize in 1994 and 1995, Lee Stocking Island (LSI), Bahamas in 1994, Glover’s Island, Belize in 1996 and Virginia Key, Florida, USA in 1996. All pre-settlement larvae were collected using channel nets, moored plankton nets and light traps. The light traps were modified quatrefoil Plexiglas light traps (Faber, 1984). The light source was a Pelican Pro xenon dive flashlight, with 40,000 candlepower, attached to the top of each trap. Light traps were set at sunset, around the time of the new moon and were allowed to collect from one to three hours nightly.

The channel net used at LSI, Bahamas was fitted to a 1.0 m X 2.0 m rectangular frame and constructed of 2.0 mm mesh. The moored plankton nets used at Carrie Bow Cay, Belize and Glover’s Reef, Belize were constructed of 1.0 mm mesh and fitted to a 1.0 m x 0.25 m frame. All nets were set to fish just below the surface and were situated either in the middle of a large channel (at LSI, see Shenker et al., 1993) or off the end of a dock in shallow water (0.5 to 2 m depth). Channel nets were deployed before sunset and retrieved the next morning. Moored plankton nets were deployed at sunset and allowed to fish for one-hour
periods up to four hours after sunset. All collections consisted of late larvae that were presumably coming onto the reef to settle and are referred to here as "settlement-stage larvae".

Divers collected adult and juvenile fishes on SCUBA using aquarium handnets or alternatively using a pushnet in shallow water. The pushnet consisted of a square PVC frame 1.0 m x 1.0 m with a long handle fitted with a net made of 2 mm mesh. The gear was pushed along the substratum while walking over a seagrass bed or sandy bottom in water up to 1 m in depth.

The smallest juveniles collected were considered "early post-settlement" and are referred to here as "recently-settled juveniles". That they had recently settled was later confirmed from otolith data. Larger juveniles and adults are referred to here as "late juveniles" or "adults" to distinguish them from recently-settled juveniles. Any individuals larger than 30 mm total length (TL) were considered adults, as sexual maturity in at least some species of labrids is attained at a length of less than 30 mm total length (TL) (Thresher, 1984). In this study, both settlement-stage larvae and very recently settled juveniles will collectively be referred to as settlement-stage fish. Some species of labrids settle by burrowing into sediment or hiding in crevices on the reef and emerge as juveniles several days later. In the present study, the initial burial is considered to be settlement and the latter is referred to as emergence.
Fertilized eggs of *Thalassoma bifasciatum* were collected during spawning of adults in the field in Belize. These were allowed to hatch and develop in the laboratory at 20°C and were preserved at 41, 45 and 70 hours after hatching. These were used for description of the earliest developmental stages.

**Preparation of Specimens and Scanning Electron Microscopy**

Specimens were fixed overnight in a 3% solution of glutaraldehyde in 0.1 M phosphate buffer. They were then transferred to 0.1 M phosphate buffer and stored at 4°C for up to three years. Standard lengths of specimens were measured after fixation in glutaraldehyde and before post-fixation in osmium tetroxide.

Specimens were prepared for SEM using standard procedures. They were post-fixed in 1% osmium tetroxide, washed in buffer and then dehydrated through a graded ethanol series of one change each of 30%, 50% and 70% ethanol each of 30 minute duration, followed by two changes each of 90% and anhydrous ethanol each of 30 minute duration. The specimens were then critical point dried using CO₂ substitution in a Polaron E3000 critical point dryer, coated with gold-palladium, and mounted on stubs using colloidal carbon.
Scanning electron micrographs were produced on an Amray 1000 scanning electron microscope. Images were recorded on Polaroid positive/negative film or using an IXRF 5480 imaging interface frame grabber and IXRF Systems Inc. Iridium software for a Windows 95 based PC. These images were used for mapping of the neuromasts and documentation of the formation of the cephalic lateral line canals.

Analysis

Scanning electron micrographs were scanned using a flatbed scanner set at 150 dots/inch to produce an 8-bit image with 256 gray levels. The images were sharpened using Jasc Software Paint Shop Pro and annotated using Sigma Scan by Jandel.

After all required data were collected, specimens were rehydrated and the otoliths were removed (see Lara, 1992 for complete procedure). Saggita and/or lapilli were examined under transmitted light in low viscosity immersion oil at 200x using an Olympus BH2 compound microscope fitted with a Sony XC-75 CCD grayscale video camera. Images of otoliths were captured and read with the aid of a Data Translations PCI frame grabber and Adobe PhotoShop and Scion.
software for a Windows 95 based PC. The otolith increments found in juveniles of two species of labrids, *Thalassoma bifasciatum* and *Halichoeres bivittatus* has been demonstrated to correspond to daily deposition (Victor, 1982) and have been assumed to be daily in the larvae and juveniles of other species of Caribbean and Indo-Pacific labroids (Schultz and Cowen, 1994; Sponaugle, 1994; Victor, 1986; Brothers et al., 1983). In accordance with the vast body of literature in support of the daily nature of otolith deposition in larval and early juvenile fishes, increments found in the otoliths of the larvae and juveniles in the present study are assumed to be deposited daily. To obtain age in days after hatching (dah), 2 days were added to ring counts to adjust for 2 days of planktonic life in which rings are not believed to be deposited (Victor, 1986a). To obtain days post-settlement or days post-emergence, settlement marks were located and rings occurring after these marks were counted.
Results

Nomenclature

The cephalic lateral line canals and free neuromast lines that occur in settlement-stage labrids are listed in Table 2.1 and depicted in Figure 2.1. Nomenclature of the free neuromasts lines follows Coombs et al., 1988 with the following exceptions: the line of neuromasts visible on the tip of the snout is interpreted to be Lekander’s (1949) rostral pit commissure and Coombs et al.’s rostral fork replacement of the infraorbital canal. These are referred to here as the rostral line. Coombs et al.’s supraorbital rostral replacement is separated into two portions here for ease of description. The portion of the line that surrounds the nares is referred to here as the nasal line after Lekander (1949). The remaining two neuromasts located on the upper snout between the right and left anterior nares are referred to separately as the snout neuromasts (Fig. 2.2A). The position and number of neuromasts composing these lines varies between species of labrids with the exception of the two snout neuromasts. These are present in all the species and all stages of labrids examined. They were also present in the specimens of Chromis sp. These neuromasts were observed in the youngest specimens examined (T. bifasciatum and H. bivittatus 2 days after hatching) and are prominent throughout development.
Nomenclature of the lateral line canals follows Coomb’s et al. (1988). At the time of settlement, the supraorbital, infraorbital, preopercular-mandibular, main body, and supratemporal canals are all present. The number of pores may increase with growth as in the case of the preopercular canal in *Halichoeres bivittatus, Halichoeres garnoti, Halichoeres pictus* and *Thalassoma bifasciatum*, and probably in other species of labrids as well. Most species possess completely formed supraorbital and supratemporal canals at settlement. At this stage, all canal neuromasts are enclosed in the canals although in some species additional pores have yet to form. The infraorbital, preopercular and mandibular canals are incomplete at settlement in some species of labrids. The main body canal was not included in this study.

**Otoliths**

Ages are reported in total days after hatching (dah) for all specimens. Additionally, days post-settlement or post-emergence are reported for juveniles. Settlement marks in all species are consistent with the descriptions given in Victor (1986a). In some species which are known to bury themselves in sediment or hide in crevices on the reef at settlement before emerging as early juveniles, including *Thalassoma bifasciatum, Halichoeres bivittatus, Halichoeres garnoti* and
Halichoeres pictus, the settlement mark appeared as a transparent area or an area with 5-7 faint rings. Days post-emergence are reported for the juveniles of these species, which includes only the days after the fish has emerged from the sediment. In the other two species that bury themselves at settlement, Xyrichtys splendens and Xyrichtys martinicensis, there is a transition in the width of the rings from narrow to wide, which is interpreted as the settlement mark. In Doratonotus megalepis and Halichoeres radiatus the transition mark is less distinct, but includes a change in width or darkness of the rings sometimes accompanied by a darker ring at the transition. In Bodianus rufus, Clepticus parrae, and Chromis sp. there is no distinct settlement mark. These last five species have not been observed to bury themselves at settlement. Days post-settlement are reported for these species.

In Halichoeres maculipinna two of four specimens had a clear area on the otolith, indicating burial, but the other two did not. It is not known whether H. maculipinna buries at settlement and this behavior may be opportunistic in this species and dependent on the type of substratum onto which it settles. In the case of H. maculipinna, days post-settlement will be reported but for some individuals this includes the 5-6 clear rings indicative of the days in which the fish was buried.
Tables 2.2 – 2.17 list the ages and lengths of specimens of each of the species examined and for each species, a brief description is provided of the lateral line and free neuromast development with an emphasis on characters that distinguish species or genera. For some species, both settlement-stage larvae and recently settled juveniles were collected. For other species, only late juveniles and adults were available.

Discussion

Development

Canals develop around a line of free neuromasts, which lie in the position of the future canal. Canal pores start to appear at one or both ends of the neuromast line. After the canal encloses these free neuromasts, pores continue to be added along the canal at least through the juvenile stage. Recently formed pores often appear wider than pores found in older juveniles and adults. Earlier stages have fewer, wider pores in their canals and more canals or portions of canals still composed of exposed neuromasts. Later stages have a higher proportion of their canals completely enclosed, with more and smaller pores than seen in the earlier stages.
Though the number and location of lateral line canals are rather similar across all teleost species (Coombs et al., 1988) there appears to be a variation in the rate of development of the canals in the species examined in the present study. *Halichoeres maculipinna* appeared to be the most developed of the species studied at the time of settlement. Settlement-stage *Halichoeres maculipinna* possessed the most enclosed canals and the largest number of pores in those canals at this stage. The remaining *Halichoeres, Clepticus* and *Doratonotus* reached a similar state of development at settlement and all were slightly less developed than *H. maculipinna*. *Xyrichtys* and *Thalassoma* appeared slightly paedomorphic at settlement, resembling earlier stages of the other labrid species. *Thalassoma* settles before the preoperculum is fully formed, the orbit of the eye is indistinct in this species at settlement, and the epithelium of settlement-stage larvae of *Xyrichtys* lack free neuromasts, though post-settlement juveniles possess them. The scarid larvae collected appeared to be much less developed than any of the labrid species. Scarids were smaller at settlement than any species of settlement-stage larid collected. Settlement-stage scarids have indistinct orbits, few lateral line pores and an incompletely formed olfactory organ in the case of *Scarus* sp. In settlement-stage *Sparisoma* sp. the preoperculum may not yet be free of the operculum.
Evolutionary Implications

The arrangement of free neuromasts differs widely among taxa (Coombs, et al., 1988). For this reason it is at times difficult to draw parallels between neuromast lines or groups named in different studies. For this same reason however, it is interesting to look at the diversity of neuromast patterns and compare these patterns to environmental or functional information and to evolutionary relationships.

Neuromasts may be of two kinds: primary or secondary neuromasts. Primary neuromasts are derived from the neural crest or from placodes, thickenings in the epidermis of the embryo (Collazo et al., 1994). These neuromasts appear early in the development of the larva and may become enclosed in canals or remain as free neuromasts. Secondary neuromasts which are not derived from the original embryonic placodes appear later in the development of the larva and do not become enclosed in canals (Lekander, 1949).

Both the nasal and rostral lines present in labrids are composed of primary neuromasts. They are present in the earlier larval stages and remain prominent at settlement. Other neuromasts are more labile and appear later in development. The rostral line in labrids is in a position similar to Coombs et al.'s
(1988) rostral fork replacement of the infraorbital canal and the nasal line to Coombs et al.'s supraorbital rostral replacement, therefore I believe them to be equivalent to these. Replacement refers to the evolutionary replacement of lost canals with lines of free neuromasts (Coombs et al., 1988). The neuromasts "replacing" the lost canals are actually just the primary neuromasts no longer (evolutionarily) enclosed in the canals, these "replacement lines" are therefore composed of primary neuromasts. The infraorbital neuromasts are also primary and in labrids are enclosed by the infraorbital canal near the time of settlement. All of the other neuromast lines described in this study are accessory lines composed of secondary neuromasts and the variation between species and individuals in number and position of these secondary neuromasts is higher than the variation observed in primary neuromasts. The secondary neuromasts, therefore, would not appear to be as useful in elucidating phylogenetic relationships as the primary neuromasts. They are, however, useful in evaluating the effects of ecology on the evolution of labrid morphology.

The supraorbital rostral replacement may be a useful phylogenetic character in labrids and perhaps in other groups as well. The portion of this line, referred to in the present study as the snout neuromasts, appears to be a conservative element. These paired neuromasts are mentioned in the literature, or appear in micrographs or drawings, in the larvae of many species (e.g., Appelbaum and Riehl, 1997; Lara, 1992; Blaxter, 1991; Su and Wang, 1990;
Kawamura et al., 1989; Kawamura and Washiayama, 1989; Kawamura and Ishida, 1985) where they are among the earliest sensory structures to appear, and are present before the eye is fully formed. Early larvae are dependent entirely on these neuromasts and a rudimentary olfactory organ for assessment of their environment. This may explain the conservative nature of these particular neuromasts.

The portion of the supraorbital rostral replacement referred to here as the nasal line (the neuromasts surrounding the nares) is a more variable trait and the configuration of the nasal line varies at the generic level in the labrids examined. More specifically, patterns are consistent within labrid tribes and genera within tribes share a specific pattern. The pattern of nasal line neuromasts in settlement-stage labrids provides a cladistic character consistent with current classification schemes. The nasal line of all settlement-stage specimens of *Thalassoma bifasciatum*, *Bodianus rufus* and all of the *Halichoeres* species consists of five neuromasts positioned in a common pattern around the nare. The two *Xyrichtys* species have between four and six neuromasts arranged in a pattern different from that in the *Halichoeres* species, and *Clepticus* has eight neuromasts arranged in a distinctly different pattern from any of the other species. *Chromis*, a percoid (see chapter 5), has a nasal line that does not resemble that of any labrid with 10-11 neuromasts and an arrangement completely different from that in any labrid examined.
Distinction along generic lines has been described in the trunk canal morphology in labrids separating them into three groups: those with a complete smooth trunk canal including *Bodianus* and *Clepticus*, those with a complete abrupt canal including *Halichoeres* and *Thalassoma*, and those with a disjunct canal including *Xyrichtys* and *Doratonotus* (Webb, 1990). These groupings are similar to those obtained using the nasal line pattern as a trait.

**Ecological implications**

Secondary free neuromasts of the cephalic accessory lines are more labile structures than the primary neuromasts of the canals (Coombs et al., 1988). Teleosts express much higher variation in the position and number of cephalic free neuromasts than they do in the number and position of lateral line canals or the neuromasts within them. Free neuromasts apparently bud and multiply during development whereas the neuromasts within the canals do not after canal formation is complete, probably due to the morphological constraint of the canal itself (Coombs, 1988). Furthermore, Evans and Fernald (1990) state that biological innovations that appear later in ontogeny are believed to be phylogenetically more recent characters (Freeman, 1982 and Gould, 1982). If this
is applied to the lateral line system, then the common pattern of canal neuromasts seen in most teleosts is accounted for by being a common ancestral state. The interspecific variation in the free neuromasts is due to their being a more recent innovation whose variability is a result of short-term evolutionary pressure such as ecological change. In the labrids examined, the number and position of free neuromasts composing these lines varied between species, between stages, and even between individuals of the same species and stage. The free neuromast lines composed of secondary neuromasts which labrids possess include the dorsal supraorbital line, dorsal opercular line, and ventral mandibular line (the accessory preopercular line in Lekander, 1949). In general, members of the family Labridae do not have many free cephalic neuromasts but the two ends of the scale are markedly different from each other and from the bulk of the species represented by the Halichoeres, Thalassoma, Bodianus and Clepticus genera. These two ends of the spectrum are represented by Doratonotus megalepis, which appears to have many more free neuromasts on the head, especially anterior to the eyes, than other species and Xyrichtys larvae, which do not appear to have any free neuromasts. Ecologically this may be analogous to the variability seen in species of cottoids (Vischer, 1990) and other fishes (Dijkgraaf, 1962; Jakubowski, 1966 in Vischer, 1990) in which species which inhabit turbulent water possess a much lower complement of superficial cephalic neuromasts than species living in relatively quiet water. Doratonotus megalepis juveniles and adults were collected from seagrass beds in protected areas which
appears to be their preferred habitat (Farm, 1993; Bohlke and Chaplin, 1968) whereas in the present study *Xyrichtys* juveniles and adults were collected from wave swept channels. Furthermore, the lack of superficial neuromasts in *Xyrichtys* may be due to their behavior of sand burrowing which is retained in the juvenile and adult stages (Nemtzov, 1994; Baird, 1988; Victor, 1987a). Such habitual burrowing could cause mechanical damage to the delicate structures, which would evolutionarily have necessitated the enclosure of all neuromasts in canals in *Xyrichtys*. *Doratonotus megalepis*, on the other hand, have not been observed to bury in the sand at any stage (Longley and Hildebrand, 1941; present study). In the present study, specimens that transformed into juveniles while held in the laboratory were not observed to bury themselves but transformed while in the water column. Many other labrid larvae bury themselves during metamorphosis. This behavior was observed in *Halichoeres bivittatus, Halichoeres garnoti, Halichoeres pictus, Xyrichtys martinicensis* and *Xyrichtys splendens* in the laboratory (discussed in chapter 5). Perhaps this is the evolutionary trade-off that allows *Doratonotus* to possess many free neuromasts, which in turn is useful in low energy environments.
Biomechanical Implications

Two features of the development of the lateral line systems in larval fish have not been discussed in the literature prior to this study. These include the function of the neuromasts of the nasal line and the timing of formation of the lateral line canals, i.e., why the canals begin to form when they do and not earlier or later.

The neuromasts surrounding the anterior nares in all of the species studied are stout and well developed. They are some of the most easily distinguishable structures even in relatively smaller or less well preserved larvae. For this reason it is surprising that not much more than a passing reference to these structures has been found in the literature. The existence of this grouping of free neuromasts in close proximity to the incurrent nare may provide information on the direction of water flow to the nare. The fish may use this information to determine location or direction from which an odor is emanating. The nasal neuromasts appear to be polarized in slightly different directions. The sensory cilia are arranged so that water movement in different directions transverse to the nare will stimulate different neuromasts. In laboratory experiments conducted by Kleerekoper (1969) it was found that orientation of a fish toward a source of an odor is largely the result of a rheotaxic response. He
found that in the absence of directional movement of water a fish's ability to locate the source of an odor is very poor, suggesting a lack of gradient perception ability in fish. Only in experiments where differential rates of water flow existed were the experimental fish able to locate the source of an odor. Although these studies clearly showed that a rheotaxic response is necessary for a fish to locate an odor source, no mention of the mechanism for this was discussed. It is likely that the neuromasts surrounding the incurrent nare are the components of the lateral line which, when stimulated in conjunction with the stimulation of the olfactory organ, allow a directional behavioral response in the fish toward the source of an odor. Though all cephalic neuromasts may be able to aid in the detection of an odor source, due to their range of polarity and proximity to the nare, the nasal line neuromasts would allow for the greatest resolution of the direction of a current impinging on the olfactory organ.

Canal formation usually begins in fish larvae at a length of approximately 10-20 mm or more. The completion of the cephalic and trunk canals usually occurs around the time of settlement or shortly thereafter (Hoss and Blaxter, 1982; Kawamura and Ishida, 1985; Su and Wang, 1990; Blaxter, 1991; Lara, 1992). This consistency across diverse taxa suggests some ecological or morphological constraint on the timing of the development of the lateral line canals.
It has been suggested that canal formation may occur for the protection of the delicate neuromasts from mechanical damage (Dijkgraaf, 1962). As some species of labrids burrow into sand upon settlement, enclosure of neuromasts for their protection is plausible. Juvenile and adult *Xyrichtys* have been observed to habitually bury themselves to escape pursuit (Longley and Hildebrand, 1941; Victor, 1987a; present study). *Halichoeres bivittatus, H. radiatus* (Longley and Hildebrand, 1941), *H. cyanocephalus* (pers. obs.) and *T. bifasciatum* (Feddern, 1965) have been observed to bury themselves at night to sleep. However, all of these species, with the possible exception of *Xyrichtys*, have free neuromasts as well, and no evidence of mechanical damage to these was observed in any juvenile or adult specimen in this study. Canals may help protect neuromasts enclosed within them but clearly this is not a sufficient explanation for the timing of canal formation. Instead, the timing of the formation of the canals is likely the result of the transition from the larval to juvenile lifestyle and the differences in the mechanical, environmental and behavioral characteristics of larval and adult fish.

Mechanical function of the canals may be a constraint on the size at which canals can function. Models have shown that due to the behavior of water through lateral line canals there is an upper limit to the diameter of a canal above which the canal will not function without structural modifications which mimic a canal of smaller diameter (Denton and Gray, 1988). At the other end of the scale, at larval sizes, it is possible that the canals cannot function at all. Jones and
Janssen (1992) found that the sensitivity of larval *Cottus bairdi* to the movements of *Artemia* decreased as the canals formed at the end of the larval stage. The morphology of the canals differed from that of adult *Cottus* and they believed that this transitional morphology might hinder flow through the canals at this stage.

I believe the nature of water flow at the small sizes of fish larvae is sufficient to prevent the functioning of the canals. Weihs (1980) showed that a change in swimming style in larval fishes coincides with the transition of the physical environment of the fish from one in which primarily viscous forces are important (Reynolds number = Re $<$ 10) to an inertial one (Re $>$ 200). He found the transitional phase between these to occur when anchovy larvae are between 5 and 15 mm in length. This is the approximate size at which canal formation begins in many species (See Hoss and Blaxter, 1982; Kawamura and Ishida, 1985; Kawamura, et al., 1990; Su and Wang, 1990; Blaxter, 1991; Lara, 1992). Perhaps due to the physical characteristics of water flow at this larval size earlier formation of canals would be disadvantageous. Neuromasts enclosed in such canals at this size could not function properly, as flow through the canals would be restricted by their small diameter, and the high viscous forces acting on parcels of water. This would account for the consistent timing of canal formation across taxa and also for the reduced performance of the incipient canals. It is possible that this transition from low to high Reynolds number may have to
occur before lateral line canals can function properly. Therefore, the sensory efficiency of a larva would be increased if canal formation were delayed until after this transition had begun. Since the length of the larva, swimming speed, shape of the larva and temperature of the fluid are all factors which affect Re, variability in these could account for the range of lengths at which canal formation occurs across taxa.

A second potential explanation for the absence of canals in larvae is related to their behavior. The lateral line canals in adult fish serve to amplify high frequency vibrations as well as dampen “noise” in the adult environment including that created by the fish’s own swimming (Dijkgraaff, 1962). Because larvae are less active swimmers than juveniles and adults the reduction of noise caused by the larva’s swimming motions may not be as important for larvae. The possession of both free neuromasts and canals in juveniles and adults allows for a partitioning of perception of low and high frequencies by the two systems, free neuromasts perceiving the lower frequencies and canals the higher frequencies (Montgomery et al., 1995). Pelagic larvae exist in a low frequency, oceanic environment to which a system composed of only free neuromasts is suited, so again the canals may not be as important at this stage. Since larvae settle with incomplete canals, the capabilities of the free neuromast system must be sufficient for the requirements of the settling larva and in fact the higher sensitivity of the exposed neuromasts may be advantageous to the settling fish.
In conclusion, the timing of the formation of the canals shortly after settlement appears to be an optimal functional strategy. The larva is settling with most of its system composed of the more sensitive free neuromasts without the compromise of reduced function due to incipient canal formation. Rapid formation of these canals then occurs at a larger size (higher Re), once the fish is established on the reef and can benefit from the noise dampening and increased perception of high frequencies afforded by the canals.
Table 2.1  Lateral line canals and free neuromast lines present in settlement-stage labrids and their designations in Figure 2.1.

<table>
<thead>
<tr>
<th>Lateral line canals</th>
<th>Free neuromast lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supraorbital</td>
<td>Rostral</td>
</tr>
<tr>
<td>Infraorbital</td>
<td>Nasal</td>
</tr>
<tr>
<td>Supratemporal</td>
<td>Dorsal Supraorbital</td>
</tr>
<tr>
<td>Preopercular</td>
<td>Supratemporal Accessory</td>
</tr>
<tr>
<td>Mandibular</td>
<td>Dorsal Opercular</td>
</tr>
<tr>
<td>Main Body</td>
<td>Ventral Mandibular</td>
</tr>
</tbody>
</table>

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Table 2.2 *Thalassoma bifasciatum*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Fig. 2.3A)</td>
<td>27-19-T1</td>
<td>2.2C</td>
<td>9.1 mm</td>
</tr>
<tr>
<td></td>
<td>27-19-T2</td>
<td></td>
<td>9.1 mm</td>
</tr>
<tr>
<td>Tbl1</td>
<td>2.6B</td>
<td>12.5-13.5 mm</td>
<td>44 days</td>
</tr>
<tr>
<td>Tbl3</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Tbl5</td>
<td></td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Tbl9</td>
<td>2.5B, 2.5C</td>
<td>&quot;</td>
<td>47 days</td>
</tr>
<tr>
<td>Tbl10</td>
<td></td>
<td>&quot;</td>
<td>36 days</td>
</tr>
<tr>
<td>Tbl41 hr</td>
<td></td>
<td></td>
<td>41 hr after hatching</td>
</tr>
<tr>
<td>Tbl45 hr</td>
<td></td>
<td></td>
<td>45 hr after hatching</td>
</tr>
<tr>
<td>Tbl70 hr</td>
<td>2.2A</td>
<td></td>
<td>70 hr after hatching</td>
</tr>
<tr>
<td>Juveniles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Fig. 2.3B)</td>
<td>Tbj1</td>
<td>2.2D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tbj2</td>
<td>2.2B, 2.5D, 2.6A</td>
<td>55-56 days; day of emergence</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Fig. 2.4A)</td>
<td>Tba1</td>
<td>2.6C</td>
<td>90 mm</td>
</tr>
<tr>
<td></td>
<td>Tba2</td>
<td>2.5A</td>
<td>90 mm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.3B).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Has not started to form in juveniles (Fig. 2.3B).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>In larvae canal enclosed even before preoperculum is free (Fig. 2.2C). 5 pores. Free neuromasts above and between pores in adult. Adult has 24+ pores in 2-3 rows.</td>
</tr>
<tr>
<td>Nasal line</td>
<td>5 neuromasts in larvae and juveniles, &quot;Halichoeres&quot; arrangement (Fig. 2.2D). 8 neuromasts in adult (Fig. 2.5A).</td>
</tr>
<tr>
<td>Rostral line</td>
<td>In larvae very prominent (Fig. 2.5B) 4 neuromasts on hillocks evenly spaced, close together (Fig. 2.5C). In juveniles less prominent (Fig. 2.5D) and neuromasts spaced further apart (Fig. 2.6A).</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Rostral line degenerating around time of settlement.</td>
</tr>
<tr>
<td>General comments</td>
<td>Settlement-stage larvae paedomorphic (Fig. 2.6B). Adults have largest number of cephalic lateral line pores of all species examined (Fig. 2.5A). Pores vary slightly in number and position.</td>
</tr>
</tbody>
</table>

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Table 2.3  *Halichoeres maculipinna*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hml1</td>
<td>2.6D, 2.8C</td>
<td>10.7mm</td>
<td></td>
</tr>
<tr>
<td>Juveniles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hmj1</td>
<td>2.8A</td>
<td>13.0-15.0 mm</td>
<td>37-40 days; 4-5 days post-emergence</td>
</tr>
<tr>
<td>Hmj2</td>
<td>2.8B</td>
<td>“”</td>
<td>37 days; 3 days post-emergence</td>
</tr>
<tr>
<td>Hmj3</td>
<td>“”</td>
<td>“”</td>
<td>38 days</td>
</tr>
<tr>
<td>Hmj5</td>
<td>“”</td>
<td>“”</td>
<td>44 days; 7 days post-emergence</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.7A).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Forming in larva - numerous, small pores (Fig. 2.6D). Complete in juveniles (Fig. 2.8A).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Enclosed. 9 pores in juvenile.</td>
</tr>
<tr>
<td>Nasal line</td>
<td>5 neuromasts in larva (Fig. 2.4B) and juveniles (Fig. 2.8B), &quot;Halichoeres&quot; arrangement.</td>
</tr>
<tr>
<td>Rostral line</td>
<td>Larvae and juveniles 1 and 2 have 2 pairs of neuromasts. Pairs further apart from each other than neuromasts in pair (Fig. 2.8C). Juveniles 3 and 5 have only 2 neuromasts (Fig. 2.8D).</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Rostral line possibly degenerating around the time of settlement</td>
</tr>
</tbody>
</table>
Table 2.4 *Halichoeres bivittatus*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb2dah</td>
<td>2.11B</td>
<td>10.3mm</td>
<td>2 days</td>
</tr>
<tr>
<td>Hbc2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juveniles (Fig. 2.7A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hbj1</td>
<td>2.10A, 2.10C, 2.11A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hbj2</td>
<td>2.10B, 2.10D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hbj3</td>
<td>10.6mm</td>
<td>39 days; day of emergence</td>
<td></td>
</tr>
<tr>
<td>Adult (Fig. 2.9A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hba1</td>
<td>2.11C</td>
<td>80mm</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Supratemporal, Supraorbital, Preopercular, Mandibular (Figs. 2.10A – 2.10B).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Anterior portion beginning to form in juveniles (Fig. 2.10C).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Enclosed, 5 pores in juvenile (Fig. 2.10B). 13 pores in adult (Fig. 2.9A).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>5 neuromasts, &quot;Halichoeres&quot; arrangement (Fig. 2.10D).</td>
</tr>
<tr>
<td>Rostral line</td>
<td>Juveniles 1, 2 have 4 prominent neuromasts recessed into pits (Fig. 2.11A). Juvenile 3 has none.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Infraorbital canal begins to form after settlement, pores continue to be added to the Preopercular and Mandibular canals after settlement.</td>
</tr>
<tr>
<td>General comments</td>
<td>All larval specimens in poor condition of preservation. No free neuromasts preserved but canal pores can be seen (Fig. 2.11B). Not as many pores or free neuromasts are added after settlement in Halichoeres species in general (Fig. 2.11C) as compared to Thalassoma bifasciatum which gains many pores after settlement.</td>
</tr>
</tbody>
</table>
### Table 2.5 *Halichoeres garnoti*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td>Hgc1</td>
<td>2.14B</td>
<td>13.0mm</td>
</tr>
<tr>
<td>(Fig. 2.9B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juveniles</td>
<td>Hgj1</td>
<td></td>
<td>12.7mm 35-36 days; 4 days post-emergence</td>
</tr>
<tr>
<td>(Fig. 2.12A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Fig. 2.12B)</td>
<td>Hgj2, Hgj3</td>
<td>2.14A, 2.14C</td>
<td>13.6mm 12.6mm</td>
</tr>
<tr>
<td>Adult</td>
<td>Hga1</td>
<td>2.14D</td>
<td>100mm</td>
</tr>
<tr>
<td>(Fig. 2.13A)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.12A-B).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Anterior and posterior portions beginning to form in juveniles (Fig. 2.14A).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Enclosed, 5 pores in larvae (Fig. 2.14B), 9 pores in juveniles (Fig. 2.14C), ~13 pores in adult (Fig. 2.14D). Numerous free neuromasts between pores in larvae and juveniles (Fig. 2.14C).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>5 neuromasts in larvae, juveniles and adult, &quot;<em>Halichoeres</em>&quot; arrangement.</td>
</tr>
<tr>
<td>Rostral line</td>
<td>4 evenly spaced neuromasts in juveniles.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Infraorbital canal begins to form after settlement.</td>
</tr>
<tr>
<td>General comments</td>
<td>Adults have approximately the same complement of free neuromasts as late larvae and juveniles (Fig. 2.13A).</td>
</tr>
</tbody>
</table>
Table 2.6  *Halichoeres pictus*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td>Hpc3</td>
<td>2.16B</td>
<td>12.6mm</td>
</tr>
<tr>
<td>(Fig. 2.13B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juveniles</td>
<td>Hpj3</td>
<td>2.16A</td>
<td>5.0-6.0mm</td>
</tr>
<tr>
<td>(Fig. 2.15A)</td>
<td></td>
<td></td>
<td>34 days; 7 days post-emergence</td>
</tr>
<tr>
<td></td>
<td>Hpj4</td>
<td></td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>Hpj5</td>
<td></td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31 days; 4 days post-emergence</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.15A).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Anterior portion beginning to form in juveniles (Fig. 2.16A).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Enclosed. Larva has 5 pores (Fig. 2.16B), juvenile has 7 pores (Fig. 2.16A).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>5 neuromasts in larvae and juveniles, &quot;Halichoeres&quot; arrangement (Fig. 2.13B).</td>
</tr>
<tr>
<td>Rostral line</td>
<td>Larvae have 4 evenly spaced neuromasts.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Infraorbital begins to form after settlement.</td>
</tr>
</tbody>
</table>
Table 2.7  *Halichoeres radiatus*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size(SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juveniles (Fig. 2.15B)</td>
<td>Hrj1</td>
<td>2.16C, 2.16D</td>
<td>17.3 mm</td>
</tr>
<tr>
<td></td>
<td>Hrj2</td>
<td>2.16C, 2.16D</td>
<td>18.1 mm</td>
</tr>
</tbody>
</table>

**Feature**                   **Description**                                                                                                                                                                                                 |
---                            | ---                                                                                              |
Canals enclosed at settlement | No settlement-stage specimen obtained.                                                         |
Infraorbital canal            | Complete in this juvenile.                                                                       |
Preopercular canal            | This juvenile has 11 pores, more than other *Halichoeres* species examined (Fig. 2.16C).        |
Nasal line                    | Juvenile has 5 neuromasts, "Halichoeres" arrangement (Fig. 2.16D).                               |
Rostral line                  | In juveniles 4 evenly spaced neuromasts in pits (Fig. 2.16D).                                  |
Ontogenetic changes           | No larvae available for comparison.                                                             |
General comments              | Late juvenile specimen only.                                                                    |

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Table 2.8  *Halichoeres cyanocephalus*
Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>Hca1</td>
<td>2.18A</td>
</tr>
</tbody>
</table>

(Fig. 2.17A)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>No settlement-stage specimen obtained.</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Composed of two rows of pores.</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>16 pores in 2 rows, single row of free neuromasts on the preoperculum (Fig. 2.18A).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>Not observable in this specimen.</td>
</tr>
<tr>
<td>Rostral line</td>
<td>Not observable in this specimen.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>No larvae or juveniles available for comparison.</td>
</tr>
<tr>
<td>General comments</td>
<td>Adult specimen only.</td>
</tr>
</tbody>
</table>
### Table 2.9  *Bodianus rufus*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile Brj1</td>
<td>2.18B</td>
<td>20.0mm</td>
<td>51-52 days</td>
</tr>
</tbody>
</table>

*(Fig. 2.17B)*

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>No settlement-stage specimen obtained.</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Complete in this juvenile <em>(Fig. 2.17B)</em>.</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Enclosed. 9 pores. There is a row of free neuromasts above and parallel to pores. No neuromasts between pores as seen in other <em>Halichoeres</em> species examined <em>(Fig. 2.17B)</em>.</td>
</tr>
<tr>
<td>Nasal line</td>
<td>5 neuromasts, &quot;<em>Halichoeres</em>&quot; arrangement <em>(Fig. 2.18B)</em>.</td>
</tr>
<tr>
<td>Rostral line</td>
<td>4 evenly spaced neuromasts in pits <em>(Fig. 2.18B)</em>.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>No larvae available for comparison.</td>
</tr>
<tr>
<td>General comments</td>
<td>Late juvenile specimen only. Preopercular canal has more pores than in <em>Halichoeres</em>. This is probably due to the juvenile being relatively older.</td>
</tr>
</tbody>
</table>

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Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dml1</td>
<td></td>
<td>7.8-8.7 mm SL</td>
<td></td>
</tr>
<tr>
<td>Dml2</td>
<td>2.18D</td>
<td>&quot; &quot;</td>
<td>28 days</td>
</tr>
<tr>
<td>Dml3</td>
<td></td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>Dml5</td>
<td></td>
<td>7.4 mm SL</td>
<td></td>
</tr>
<tr>
<td>Dml21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juveniles</td>
<td></td>
<td>8.0mm</td>
<td>30 days; 10 days</td>
</tr>
<tr>
<td>Dmj1</td>
<td></td>
<td></td>
<td>post-settlement</td>
</tr>
<tr>
<td>Dmj2</td>
<td>2.18C</td>
<td>10.3mm</td>
<td>35-36 days; 10 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>post-settlement</td>
</tr>
</tbody>
</table>

**Table 2.10** *Doratontus megalepis*

**Feature** | Description
---|---
Canals enclosed at settlement | Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.19B).
Infraorbital canal | Anterior portion beginning to form in juvenile (Fig. 2.18C).
Preopercular canal | Enclosed in larvae (Fig. 2.19A), 6 pores in larvae and juveniles (Fig. 2.19B).
Nasal line | 5 neuromasts, "Halichoeres" arrangement (Fig. 2.18D).
Rostral line | 4 evenly spaced neuromasts in larvae (Fig. 2.18D) and juveniles.
Ontogenetic changes | Mandibular canal enclosed around the time of settlement.
General comments | Larvae and juveniles appear to have many free neuromasts all over the head and especially anterior to the eye. Larvae and juveniles have numerous "bumps" on the skin, which may be incipient neuromasts (especially in area circled in red - Fig. 2.19A).
Table 2.11 *Clepticus parrae*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juveniles (Fig. 2.20A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpj2</td>
<td>2.21A, 2.21B, 2.21C</td>
<td>5.5-7.0 mm SL</td>
<td></td>
</tr>
<tr>
<td>Cpj4</td>
<td>“”</td>
<td>“”</td>
<td>31 days</td>
</tr>
<tr>
<td>Cpj5</td>
<td>“”</td>
<td>“”</td>
<td></td>
</tr>
<tr>
<td>Cpj20</td>
<td>11.0 mm</td>
<td>28 days</td>
<td></td>
</tr>
</tbody>
</table>

**Feature** | **Description**
--- | ---
Canals enclosed at settlement | Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.20A).
Infraorbital canal | Anterior portion beginning to form in juveniles (Fig. 2.21A).
Preopercular canal | Enclosed. 4-5 pores in juveniles (Fig. 2.21A). Row of free neuromasts above and parallel to pores as in *B. rufus* (Fig. 2.20A).
Nasal line | 8 neuromasts (Fig. 2.21B), different arrangement from "*Halichoeres*" and adult *T. bifasciatum* in which there are also 8.
Rostral line | 4 evenly spaced neuromasts (Fig. 2.21C).
Ontogenetic changes | No larvae available for comparison.
General comments | Only juveniles obtained.
Table 2.12 *Xyrichtys* sp. larvae

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td></td>
<td>10.5 – 11.5mm</td>
<td></td>
</tr>
<tr>
<td>24-19-Xo1</td>
<td></td>
<td>10.5 – 11.5mm</td>
<td>58 days</td>
</tr>
<tr>
<td>24-19-Xo2</td>
<td>2.22A, 2.22B</td>
<td>10.5 – 11.5mm</td>
<td>61 days</td>
</tr>
<tr>
<td>21-19-Xy2</td>
<td></td>
<td></td>
<td>80 days</td>
</tr>
</tbody>
</table>

**Feature** | **Description**
--- | ---
Canals enclosed at settlement | Supratemporal, Supraorbital, Preopercular, Mandibular all complete in larvae (Fig. 2.22A).
Infraorbital canal | Has not begun to form in larvae (Fig. 2.22A).
Preopercular canal | Enclosed. 6 large pores in larvae (Fig. 2.22A).
Nasal line | None seen (Fig. 2.22B). See comments.
Rostral line | See comments.
Ontogenetic changes | Infraorbital begins to form after settlement.
General comments | Larvae appear to have very immature epithelium. These specimens possess no free neuromasts or keratinocytes.
Table 2.13  *Xyrichtys martiniensis*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen (Fig. 2.20B)</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juveniles Xmj1</td>
<td>2.22C</td>
<td>14.7mm</td>
<td>62-63 days; day of emergence</td>
</tr>
<tr>
<td>Xmj2</td>
<td></td>
<td>15.0mm</td>
<td>56 days; 7 days post-emergence</td>
</tr>
<tr>
<td>Xmj3</td>
<td></td>
<td>13.1mm</td>
<td>79-81 days; 3 days post-emergence</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.20B).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Anterior portion beginning to form in juvenile (Fig. 2.22C).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Enclosed. 6 pores, free neuromasts between pores (Fig. 2.22C).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>6 neuromasts, (5 in Juvenile 3).</td>
</tr>
<tr>
<td>Rostral line</td>
<td>Data not available.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Infraorbital begins to form after settlement.</td>
</tr>
<tr>
<td>General comments</td>
<td>Numerous free neuromasts on the cranium (Fig. 2.20B).</td>
</tr>
</tbody>
</table>
Table 2.14 *Xyrichtys splendens*

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juveniles Xsj1 (Fig. 2.23A)</td>
<td>2.22D</td>
<td>13.6mm</td>
<td>59 days; 6 days post-emergence</td>
</tr>
<tr>
<td>Xsj2</td>
<td></td>
<td>15.7mm</td>
<td>12 days post-emergence</td>
</tr>
<tr>
<td>Xsj3</td>
<td></td>
<td>15.6mm</td>
<td>12 days post-emergence</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.23A).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Anterior portion beginning to form in juvenile (Fig. 2.22D). Juvenile infraorbital development and free neuromast distribution is similar to <em>X. martinicensis</em>.</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Enclosed. 6 pores, free neuromasts between pores (Fig. 2.23A).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>Variable: Juvenile 1 has 6 neuromasts (Fig. 2.22D), juvenile 3 has 5 neuromasts with 2 farther from other 3 than in <em>Halichoeres</em>, juvenile 2 has only 4 neuromasts around the nare on both sides of the head.</td>
</tr>
<tr>
<td>Rostral line</td>
<td>None.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Infraorbital begins to form after settlement.</td>
</tr>
<tr>
<td>General comments</td>
<td>Numerous free neuromasts on the cranium (Fig. 2.23A).</td>
</tr>
</tbody>
</table>
Table 2.15 *Scarus* sp. larvae

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td>Scarl1</td>
<td>2.24A</td>
</tr>
<tr>
<td></td>
<td>Scarl2</td>
<td>- 5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>No pores anywhere on head in larva (Fig. 2.24A).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Has not begun to form (Fig. 2.24A).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Not fully separated from operculum, no pores (Fig. 2.24A).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>See comments.</td>
</tr>
<tr>
<td>Rostral line</td>
<td>See comments.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Juveniles not available for comparison.</td>
</tr>
<tr>
<td>General comments</td>
<td>Only examined larvae. No free neuromasts seen, specimens in poor condition. Development paedomorphic in settling larvae esp. compared to labrids e.g. in most specimens the nasal pit is not yet closed to form the two nares (Fig. 2.24A).</td>
</tr>
</tbody>
</table>
Table 2.16 *Sparisoma* sp. larvae

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sparl1</td>
<td>2.24B</td>
<td>9.0-9.4mm SL</td>
</tr>
<tr>
<td>Sparl2</td>
<td></td>
<td>9.3 mm SL</td>
</tr>
<tr>
<td>Sparl3-9</td>
<td></td>
<td>9.0 – 9.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Preopercular and Mandibular enclosed. Supraorbital complete in one larva.</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Has not begun to form (Fig. 2.24B).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Preoperculum separate from operculum in Sparl1, not in Sparl2. 4-5 pores (Fig. 2.24B).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>See comments</td>
</tr>
<tr>
<td>Rostral line</td>
<td>See comments</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Juveniles not available for comparison.</td>
</tr>
<tr>
<td>General comments</td>
<td>Only examined larvae. No free neuromasts seen, specimens in poor condition. More developed nares than <em>Scarus</em> sp. Large pores on the preoperculum (Fig. 2.24B).</td>
</tr>
</tbody>
</table>
Table 2.17  *Chromis* sp. juveniles (*scotti* or *cyanea*)

Specimens used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Plates</th>
<th>Size (SL)</th>
<th>Age (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juveniles</td>
<td>Chromj1</td>
<td>2.24C, 2.24D</td>
<td>9.6mm</td>
</tr>
<tr>
<td>(Fig. 2.23B)</td>
<td>Chromj2</td>
<td></td>
<td>7.8mm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canals enclosed at settlement</td>
<td>Supratemporal, Supraorbital, Preopercular, Mandibular (Fig. 2.23B).</td>
</tr>
<tr>
<td>Infraorbital canal</td>
<td>Anterior and posterior portions beginning to form in juveniles (Fig. 2.24C).</td>
</tr>
<tr>
<td>Preopercular canal</td>
<td>Enclosed. 8 pores (Fig. 2.24C).</td>
</tr>
<tr>
<td>Nasal line</td>
<td>10-11 neuromasts in a very different configuration from that in the labrids (Fig. 2.24D).</td>
</tr>
<tr>
<td>Rostral line</td>
<td>None.</td>
</tr>
<tr>
<td>Ontogenetic changes</td>
<td>Larvae not available for comparison.</td>
</tr>
<tr>
<td>General comments</td>
<td>Few distinguishable free neuromasts.</td>
</tr>
</tbody>
</table>
Fig. 2.1  Generalized map of the cephalic lateral line canals (in light green) and neuromast lines (in red) which occur in settlement-stage labrids. See Table 1 for nomenclature.
In figures 2.1-2.23 free neuromasts are indicated by red dots. Scale bars appear as white line, the dimensions of which are given immediately underneath, where $U = \mu m$.

Fig. 2.2  
A. Two prominent neuromasts on the snout of *Thalassoma bifasciatum* larva. B. Juvenile *Thalassoma bifasciatum* showing incomplete infraorbital canal. C. Head of *Thalassoma bifasciatum* larva showing pores of the preopercular canal present before the preoperculum is fully separated. D. Nasal line neuromasts (indicated with arrows) of juvenile *Thalassoma bifasciatum*.
Fig. 2.3  A. Larval *Thalassoma bifasciatum*. B. Juvenile *Thalassoma bifasciatum*. 
Fig. 2.4  

A. Adult *Thalassoma bifasciatum*. B. Larval *Halichoeres maculipinna*. Small canal pores are circled in red.
Fig. 2.5  

A. Head of adult *Thalassoma bifasciatum*. B. Rostral line on snout of *Thalassoma bifasciatum* larva. C. Rostral line of larval *Thalassoma bifasciatum* composed of 4 neuromasts set close together on hillocks. D. Less prominent rostral line in juvenile *Thalassoma bifasciatum*.
Fig. 2.6  
A. Two neuromasts of the rostral line in a juvenile *Thalassoma bifasciatum* showing them further apart than on larvae and located in depressions. B. Head of *Thalassoma bifasciatum* larva. C. Posterior portion of head of adult *Thalassoma bifasciatum*. D. Head of larval *Halichoeres maculipinna* showing pores of the lateral line canals including those of the infraorbital branch.
Fig. 2.7  
A. Juvenile *Halichoeres maculipinna*. B. Juvenile *Halichoeres bivittatus*. 

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Fig. 2.8

A. Head of juvenile *Halichoeres maculipinna* showing complete infraorbital canal. B. Anterior nare surrounded by neuromasts of the nasal line in a juvenile *Halichoeres maculipinna*. Two snout neuromasts can also be seen in the upper left-hand corner. C. Rostral line, nasal line and snout neuromasts of *Halichoeres maculipinna* larva. D. Rostral line of juvenile *Halichoeres maculipinna* composed of only two neuromasts.
Fig. 2.9

A. Adult *Halichoeres bivittatus*. B. Larval *Halichoeres garnoti*.
Fig. 2.10  

A. Head of juvenile *Halichoeres bivittatus*. B. Head of juvenile *Halichoeres bivittatus*. C. Anterior portion of infraorbital beginning to form in juvenile *Halichoeres bifasciatum*. D. Rostral line, nasal line and snout neuromasts in juvenile *Halichoeres bivittatus*. 

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Fig. 2.11  
A. Rostral line of juvenile *Halichoeres bivittatus*. B. Head of larval *Halichoeres bivittatus*. C. Head of adult *Halichoeres bifasciatum*. 

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Fig. 2.12  
A. Juvenile *Halichoeres garnoti* (Juvenile 1). B. Juvenile *Halichoeres garnoti* (Juvenile 2).
Fig. 2.13  

A. Adult *Halichoeres garnoti*. B. Larval *Halichoeres pictus*.
Fig. 2.14  

A. Infraorbital canal beginning to form, in juvenile *Halichoeres garnoti*. B. Head of larval *Halichoeres garnoti*.  
C. Head of juvenile *Halichoeres garnoti*. D. Head of adult *Halichoeres garnoti*. 

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Fig. 2.15  
A. Juvenile *Halichoeres pictus*. B. Juvenile *Halichoeres radiatus*.  

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Fig. 2.16  
A. Head of juvenile *Halichoeres pictus* showing infraorbital canal beginning to form. B. Head of larval *Halichoeres pictus*.  
C. Head of *Halichoeres radiatus* juvenile. D. Rostral, nasal and snout neuromasts of juvenile *Halichoeres radiatus*. 

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Fig. 2.17  
A. Adult *Halichoeres cyanoccephalus*. B. Juvenile *Bodianus rufus*.
Fig. 2.18  
A. Head of adult *Halichoeres cyanocephalus*. B. Head of juvenile *Bodianus rufus*. C. Head of juvenile *Doratonotus megalepis* showing infraorbital beginning to form. D. Rostral (upper right corner), nasal and snout neuromasts of larval *Doratonotus megalepis*. 

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Fig. 2.19

A. Larval *Doratonotus megalepis*. Area circled in red covered in numerous “bumps” which may be incipient neuromasts.

B. Juvenile *Doratonotus megalepis*.
Fig. 2.20  

A. Juvenile *Clepticus parrae*. B. Juvenile *Xyrichtys martinicensis*.
Fig. 2.21  

A. Head of juvenile *Clepticus parrae* showing infraorbital beginning to form.  
B. Nasal line of juvenile *Clepticus parrae*.  
C. Rostral line of juvenile *Clepticus parrae*. 

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Fig. 2.22  
A. Head of larval *Xyrichtys* sp.  
B. Area surrounding nares showing lack of nasal line neuromasts in larval *Xyrichtys* sp.  
C. Head of juvenile *Xyrichtys martinicensis*.  
D. Head of juvenile *Xyrichtys splendens*.  

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Fig. 2.23  
A. Juvenile *Xyrichtys splendens*. B. Juvenile *Chromis* sp.
Fig. 2.24  
A. Head of larval *Scarus* sp.  B. Head of *Sparisoma* sp.  
C. Head of juvenile *Chromis* sp.  D. Nasal line of juvenile *Chromis* sp.
Chapter 3

Morphology of the peripheral olfactory organ and densities of ciliated and microvillous receptors and ciliated non-sensory receptors in the settlement-stages and some adults of 13 species of Caribbean labroids.

Abstract

The peripheral olfactory organ of settlement-stage larvae and early juveniles and some adults of 13 species of Caribbean labrids and scarids were examined using scanning electron microscopy. Ages in days after hatching and days post-settlement were determined from the otoliths. Morphology of the external nares and the olfactory epithelium are described for these species and stages. The separation of the anterior and posterior nares occurred before settlement in the labrids but in some specimens of scarids this separation was not complete by the time of settlement. Densities of ciliated and microvillous...
receptors and ciliated non-sensory receptors were calculated. Densities of ciliated receptor cells ranged from 0.57/100 μm² in B. rufus to 38.9/100 μm² in a specimen of T. bifasciatum. Densities of microvillous receptors ranged from 3.8/100 μm² in a C. parrae juvenile to 26.6/100 μm² in a juvenile D. megalepis. The state of development of the olfactory organ in the larvae, juveniles and adults is described and compared among species and taxa. The possible role of olfaction in settlement is discussed.
Introduction

The olfactory organ of fishes is morphologically and functionally similar to that of all other aquatic and terrestrial vertebrates. In fishes the receptors of the olfactory epithelium are stimulated when they come into direct contact with certain chemicals carried in water, especially amino acids (Caprio, 1988) but also steroids, bile salts and prostaglandins (Hara, 1992b), and transmit signals to the higher-order nervous system (Hara, 1992a). The degree of olfactory discrimination that can be reached in teleosts is very high. Fishes can discriminate individuals at the species and population levels using olfaction (review in Liley, 1981; Olsén, 1992) and even kin-specific selection has been demonstrated in salmonids (Brown and Brown, 1992; Olsén and Winberg, 1996; Quinn and Busack, 1985; Winberg and Olsén, 1992).

In most teleosts the olfactory epithelium lies at the bottom of a pit which opens to the exterior via the anterior and posterior nares. The epithelium is either flat or folded into a series of lamellae. The epithelium may continuously cover the lamellae or can be patchy, alternating with nonsensory epithelium (Zeiske et al., 1982; Yamamoto, 1982). There are six types of cells that can be identified by examining the surface of the olfactory epithelium. Two of these are generally accepted to be receptor cells: ciliated receptor cells and microvillous receptor...
cells. The ciliated receptor cells can be distinguished in electron microscopic images as star shaped cells with a knob at the base and bearing four to eight cilia protruding from the knob (Fig. 3.1A). The microvillous cells appear as a smaller cluster of numerous, shorter, thinner microvilli (Fig. 3.1A). A third type of cell, the ciliated nonsensory cell, appears as a set of long cilia with all of the cilia oriented in the same direction (Fig. 3.1B). Although these cilia are generally believed to be nonsensory and serve to ventilate the olfactory epithelium, evidence for a dual function as sensory and ventilatory cells has been found as they have been demonstrated to have axons which project to the olfactory bulb (Muller and Marc, 1984). The fourth type of cell, supporting cells, occur between and around the aforementioned cells and bear very short microvilli. Indifferent epithelial cells appear as plates covered in a mazelike pattern of ridges. The sixth type of cell, the rod cell, sometimes appears. These are believed to possibly be a degenerative state of an aging receptor cell (Muller and Marc, 1984). All of these cells overlie a layer of basal cells. (For a complete description of the external olfactory organ see Yamamoto, 1982; Zeiske et. al 1992).

Few studies have been specifically aimed at determining which of the components of the olfactory system play the most important role in olfactory acuity. Though the correlation between density of receptor cells and olfactory acuity has not yet been established (Zeiske et al., 1992), it is reasonable to assume
that a higher density of receptors would be correlated to higher olfactory ability. Both the ciliated and microvillar receptor cells are capable of responding to amino acids and it is believed the receptor sites are located on the membranes of these cells (Hara, 1992b). Therefore an increase in the density of receptors, and/or of the total area covered in receptors would increase the total receptive area of the olfactory epithelium. Additionally, fishes known to have a high olfactory acuity generally have high densities of nonsensory cells (Yamamoto, 1982). These are believed to act in ventilating the olfactory epithelium thus increasing contact of water borne odorants with the receptor sites. Thus, descriptions of the olfactory epithelium and relative densities of the two types of receptor cells and of ciliated nonsensory cells may be useful in evaluation of sensory development.

Most studies of the olfactory system have concentrated on fishes that migrate to spawning grounds using olfaction, such as salmonids and anguillids. Cyprinids, atherinids and pleuronectids have also been the targets of a large proportion of research on olfaction. Olfactory studies of fishes from families other than these are few. The most extensive work to date on comparative morphology of olfactory epithelia in a variety of teleosts is a series by Yamamoto and Ueda (1979a,b,c; 1978a,b,c,d; 1977). To date, there are no studies of the
peripheral olfactory organ (i.e. external structures including the nares, olfactory chamber and olfactory epithelium) of any tropical marine perciform.

In general there are few studies of the olfactory morphology of larval fishes and only one of a larval tropical marine fish (Chanos chanos, Ostariophysi, Chanidae, Kawamura, 1984). The present study is a comparative work of the peripheral olfactory organ in the late larval and early juvenile stages (settlement-stage) and some adults of 12 species of tropical Atlantic wrasses (Labridae), a representative species in the sister group Scaridae, and one in the outgroup Pomacentridae. No previous description exists of the olfactory epithelium of the larval or settlement-stage of any tropical reef fish. No previous documentation of the state of development of the olfactory organ of any settlement-stage fish exists.

In the present study, larvae were presumed to be arriving at the reef to settle at the time of collection. Small, recently settled juveniles were also collected from the same reefs. The developmental stage of the olfactory organ at the time of settlement was assessed. A goal of this study was to determine if the olfactory sense is likely to play a role in the detection of the reef and suitable settlement sites on the reef by settling fishes. The peripheral olfactory organs in various species were compared to determine a correlation between the development or
morphology of the organ and the degree of specificity in settlement site observed among the species.

Materials and Methods

Sample collection

Samples were collected off Carrie Bow Cay, Belize in 1994 and 1995, Lee Stocking Island (LSI), Bahamas in 1994, Glover’s Island, Belize in 1996 and Virginia Key, Florida, USA in 1996. All pre-settlement larvae were collected using channel nets, moored plankton nets and light traps. The light traps were modified quatrefoil Plexiglas light traps (Faber, 1984). The light source was a Pelican Pro xenon dive flashlight, with 40,000 candlepower, attached to the top of each trap. Light traps were set at sunset, around the time of the new moon, and were allowed to collect from one to three hours nightly.

The channel net used at LSI, Bahamas was fitted to a 1.0 m X 2.0 m rectangular frame and constructed of 2.0 mm mesh. The moored plankton nets used at Carrie Bow Cay, Belize and Glover’s Reef, Belize were constructed of 1.0 mm mesh and fitted to a 1.0 m x 0.25 m frame. All nets were set to fish just below
the surface and were situated either in the middle of a large channel (at LSI, see Shenker et al., 1993) or off the end of a dock in shallow water (0.5 to 2 m depth). Channel nets were deployed before sunset and retrieved the next morning. Moored plankton nets were deployed at sunset and allowed to fish for one-hour periods up to four hours after sunset. All of these collections consisted of late larvae that were presumably coming onto the reef to settle and are referred to here as “settlement-stage larvae”.

Divers collected adult and juvenile fishes on SCUBA using aquarium handnets or alternatively using pushnets in shallow water. The pushnets consisted of a square PVC frame 1.0 m x 1.0 m with a long handle fitted with a net made of 2 mm mesh. The gear was pushed along the substratum while walking over a seagrass bed or sandy bottom in water up to 1 m in depth.

The smallest juveniles collected were considered “early post settlement” and are referred to here as “recently-settled juveniles”. That they had recently settled was later confirmed from otolith data. Larger juveniles and adults are referred to here as “late juveniles” or “adults” to distinguish them from recently settled juveniles. Any individuals larger than 30 mm total length (TL) were considered adults, as sexual maturity in at least some species of labrids is attained at a length of less than 30 mm TL (Thresher, 1984). In this study, both settlement-stage larvae
and very recently settled juveniles may collectively be referred to as settlement-stage fish.

**Preparation of Specimens and Scanning Electron Microscopy**

Specimens were fixed overnight in a 3% solution of glutaraldehyde in 0.1 M phosphate buffer. They were then transferred to 0.1 M phosphate buffer and stored at 4°C for up to three years. Standard lengths of specimens were measured after fixation in glutaraldehyde and before post-fixation in osmium tetroxide.

Specimens were prepared for SEM using standard procedures. They were post-fixed in 1% osmium tetroxide, washed in buffer and then dehydrated through a graded ethanol series of one change each of 30%, 50% and 70% ethanol each of 30 minute duration, followed by two changes each of 90% and anhydrous ethanol each of 30 minute duration. The specimens were then critical point dried using CO₂ substitution in a Polaron E3000 critical point dryer, coated with gold-palladium, and mounted on stubs using colloidal carbon.
Scanning electron micrographs were produced on an Amray 1000 scanning electron microscope at magnifications from 3300 x to 9500 x. Images were recorded on Polaroid positive/negative film. Images were produced by directing the electron beam down the shaft of the nare. In some specimens with long tubular incumbent nares, this tissue had to be removed using very fine-tipped forceps before fixation in osmium, leaving behind the exposed olfactory epithelium for further processing.

Analysis

Micrographs were input into a PC using a flatbed scanner set at 150 dots/inch to produce an 8-bit image with 256 gray levels. The images were sharpened using Jasc Software Paint Shop Pro. Area measurements and sensory cell counts were obtained using Sigma Scan by Jandel. The largest area possible was used in each image to obtain counts. Due to the difference in magnifications needed to resolve the cells, these areas ranged from 87.5 – 2229 μm². These data were used to calculate ciliated receptor cell and microvillous receptor cell densities. Densities of ciliated receptor cells are reported as number of ciliated receptor cells/100 μm². If both nares in one specimen were usable the density in each is reported, however, only one measurement from each specimen was possible in most cases. In specimens with patchy epithelium (nonsensory
epithelium interspersed with sensory epithelium) counts and areas were taken to include only the areas covered in sensory epithelium. Those specimens with patchy epithelium are noted in Table 3.2. Microvillous receptor cells were more difficult to resolve and fewer micrographs were usable for this purpose. Therefore, ranges of cell densities were ranked as high (14-27 cells/100 \( \mu^2 \)) medium (8-13 cells/100 \( \mu^2 \)) and low (0-7 cells/100 \( \mu^2 \)). For each specimen, an estimate was made of the proportion of the visible area of the epithelium covered in ciliated nonsensory cells and density was ranked as very high (90% -100%), high (70-89%), medium (40-69%), low (10-39%) and very low (0 – 9%).

After data were collected, the specimens were rehydrated and the otoliths removed (see Lara, 1992 for complete procedure). All otoliths were examined under low viscosity immersion oil using an Olympus BH2 compound microscope fitted with a Sony XC-75 CCD grayscale video camera. Images of otoliths were captured and read with the aid of a Data Translations PCI frame grabber and Adobe PhotoShop and Scion software for a Windows 95 based PC.

The lengths and ages of specimens used for study of the olfactory epithelium are listed in Table 3.1. (Refer to previous chapter for specimen designations). If both nares were used this is indicated. When otoliths were available age in days after hatching (dah) and days post-settlement or days post-
emergence are reported (refer to the previous chapter for explanation of age data). Lengths were measured using a dissecting microscope fitted with an ocular micrometer and are reported as standard length (SL) to the nearest 0.1 mm.

Results

Nares

All labrid species examined had completely formed anterior and posterior nares on each side of the head at settlement. The anterior nare of most species formed a tube, the length of which varied among the species. The posterior nares of some species and stages were partially covered with a flap of skin (Fig. 3.1C). The anterior nares of larvae and juveniles of *Xyrichtys* species were very long, and had to be removed with forceps to expose the olfactory epithelium. Conversely, larvae of *Doratonotus megalepis* possess very shallow anterior nares, which left the olfactory epithelia exposed (Fig. 3.1D). Among the juveniles, *D. megalepis* had the shallowest anterior nares.
The recently-settled juveniles of *Halichoeres* species and *Thalassoma bifasciatum* possess a posterior nare that is partially covered by a flap of skin. This flap was either absent in all other larvae or was just beginning to form at settlement as in *H. maculipinna* and *H. garnoti*. It is well developed in late larvae of *T. bifasciatum* and is entirely absent in juveniles of *D. megalepis*, *Bodianus rufus*, *Clepticus parrae*, *X. martinicensis* and *X. splendens*.

Settlement-stage scarids (*Scarus* sp.) had an incompletely formed dumbbell-shaped olfactory pit (Fig. 3.2A). Separate anterior and posterior nares had not yet formed before settlement in *Scarus* sp. This is one indication of the overall less developed state, which was observed in settlement-stage scarids as opposed to settlement-stage labrids.

*Chromis* sp. juveniles possess a shallow anterior nare and lack a skin flap over the posterior nare.

**Olfactory Epithelium**

Settlement-stage labrids possess three types of olfactory cells whose densities may be indicators of olfactory acuity: ciliated receptor, ciliated
nonsensory and microvillous receptor cells (Fig. 3.1A). Within the Labridae a wide range is observed in densities and proportions of ciliated receptor, microvillous receptor and nonsensory cells. Table 3.2 lists this information for the 14 species examined in this study. Some specimens had rod cells, some greatly enlarged at the base, and one species had ciliated receptors with cilia distinctly enlarged into a bulb at the tips. These features were not quantified but are discussed below.

Adult labroids are reported to have an olfactory organ with 8-10 lamellae (Yamamoto and Ueda, 1979). At settlement, olfactory lamellae are not formed in any of the labrid species examined in the present study. Instead, a flat layer of olfactory epithelium lies at the bottom of the nare opening. All larvae and most juveniles possess a continuous distribution of sensory epithelium. Juvenile *H. pictus*, *H. radiatus*, *H. maculipinna* and adult *H. cyanocephalus* and *H. garnoti* possess a patchy distribution of olfactory epithelium containing sensory and nonsensory ciliated cells interspersed with patches of nonsensory epithelium (Fig. 3.2B).

Densities of all three types of cells varied among species. Densities of ciliated receptor cells are shown in Figure 3.3 and ranged from 0.57/100 μm² in *B. rufus* to 38.9/100 μm² in a specimen of *T. bifasciatum*. The lowest densities of
Ciliated receptor cells were found in *B. rufus* juveniles and *C. parrae* juveniles (1.4 - 2.9/100 μm²). The olfactory epithelia of *B. rufus* and *C. parrae* are densely covered with many long cilia of nonsensory cells. This made it difficult to assess the density of ciliated receptor cells, which may have been occluded by the nonsensory cilia. In *X. martinicensis* and *X. splendens*, nonsensory cilia are so dense that receptor cells were difficult to see. Counts could be obtained only from areas where densities of nonsensory cilia were lower. In one specimen of *X. martinicensis*, density of ciliated receptor cells was 21.0/100 μm² and in one specimen of *X. splendens* the density was 9.3/100 μm². In addition, numerous microvillous receptors were observed in *X. splendens*. Thus, actual density of sensory receptors over the whole epithelium in these species may be much higher than the lowest values obtained. The low numbers of receptor cells reported here for these four species should not be taken as indicative of lower olfactory capability as areas with higher densities of receptors may simply be occluded by the dense cover of nonsensory cilia. In fact these species may actually have an acute olfactory sense as high densities of ciliated nonsensory cells are found in fishes known to have a high degree of olfactory ability such as salmon, eels and catfishes (Yamamoto, 1982).

The highest densities of ciliated receptor cells among the species examined were in juveniles of *T. bifasciatum*, 23.5 - 38.9/100 μm². Their olfactory epithelium
consists entirely of ciliated and microvillous receptors as no nonsensory cilia were found. In larval *T. bifasciatum*, many long cilia were present but the specimens were in poor condition and it was impossible to be certain whether these cilia were of sensory or nonsensory cells. However, the cilia appear to be long and grouped suggesting they were of nonsensory origin.

In three of the species, *H. bivittatus, H. maculipinna* and *H. pictus* juveniles had a much higher density of ciliated sensory cells than did the larvae. In *D. megalepis* much higher densities were found in the larvae than in the juvenile. I was unable to determine densities of sensory cells in larvae of *T. bifasciatum* and *H. garnoti* so no comparison could be made.

A few specimens could be used for counts of microvillous receptors. Among the labrids, densities of microvillous receptors ranged from 3.8/100 μm² in a *C. parrae* juvenile to 26.6/100 μm² in a juvenile *D. megalepis*. The lowest densities were found in *Chromis* sp. juveniles with 0.99-1.8/100 μm². The only apparent differences in densities of these receptors in larvae and juveniles of a species were found in *D. megalepis* and *H. maculipinna*. The juveniles of both had higher densities than the larvae. However, due to the small sample sizes, caution should be used when interpreting these results.
In five species differences exist in the relative density of nonsensory cilia in larvae and juveniles of the same species. In *H. garnoti*, *H. maculipinna*, *H. pictus* and probably *T. bifasciatum* densities of ciliated nonsensory cells were much higher in the larvae than in the juvenile specimens. In *H. bivittatus* nonsensory cilia appear to be less densely distributed in the larvae than in the juveniles. This appears to be the case with *Xyrichtys* sp. as well but the images obtained were not clear enough to be certain.

**Discussion**

**Development**

Densities of microvillous and ciliated receptor cells as well as ciliated nonsensory cells were relatively high in settlement-stage labrids. In fact densities were comparable to those found in adult fishes known to have an acute olfactory sense. Densities of receptor cells in adult fishes reported in other studies are listed in Table 3.3. It is important to note that these studies reported receptor density and do not differentiate between ciliated and microvillous receptors, therefore these densities are interpreted to be the total of both types of receptors. Only this study reports density of receptors in larval or juvenile stages of fishes.
Total densities of receptor cells (ciliated and microvillous receptors) found in larvae and juveniles of labrids fall in the high end of the range of those reported for adults of other species. The peripheral olfactory organ is anatomically complete and well developed at settlement.

Total receptive area can be increased by an increase in total area of the olfactory epithelium. The folding of the olfactory epithelium into lamellae accomplishes this in fishes later in their development. Larval and early juvenile fishes have not yet developed lamellae and the total size of their olfactory organ is constrained by their small size. It appears therefore, that larval and juvenile fish are instead maximizing the use of their olfactory area by having high densities of cells in a continuous arrangement. Continuous olfactory epithelium is found in fishes with a high olfactory ability (Yamamoto, 1982). The epithelium, which is continuous in the larvae and early juveniles of all of the labrid species studied, develops into a patchy arrangement of sensory and nonsensory epithelium in older juveniles and adults of at least some species. This would lead to a decrease in the total number of receptors were it not for the formation of the lamellae, which allows for a greater total olfactory surface area. This change from continuous to patchy may be roughly coordinated with the formation of the lamellae.
The density of ciliated nonsensory cells may change over the course of a fish's life. These cells are present only in larval and early juvenile stages of some fishes such as the atheriniforms *Nematocentris maccullochi* (Breucker, 1979) and *Belone belone* (Theisen et al., 1980). In some species of labrids, densities are higher in the larvae than the juveniles, indicating a reduction in density with age/size. In developing fish these cells may become less important in ventilating the olfactory epithelium as the accessory sacs, which are not yet formed in larvae, take over the ventilatory action (or as a fish is better able to ventilate the epithelium through induced flow by swimming faster through the water). Ciliated nonsensory cells may be more important in the larval stage as larvae cannot swim as fast as adults and due to their small size find themselves in a relatively more viscous flow field than adult fish. These nonsensory cilia are especially important in small fishes that lack a mechanical way of moving liquid, as this liquid will tend to remain in the vicinity of the sensory receptors instead of flowing over them.

Adult labrids have eight lamellae radiating from one end of the olfactory pit (Yamamoto and Ueda, 1979). Although the olfactory pit and two nares are completely formed at settlement, none of the larvae and juveniles had any distinct lamellae. In some juveniles a slight fold in the epithelium was apparent
indicating that lamellar formation does not begin until some time after settlement. In the only other study to include labrids, Yamamoto and Ueda (1979) state that "especially in Halichoeres poecilopterus, the lamellae are rudimentary" compared with those of other perciforms. The individual they examined (14 cm SL) was larger than any of my specimens and was probably an adult. From this and my results I believe the lamellae develop relatively late in all labrids and even when fully developed are not as pronounced as in other fishes. The delay in the formation of the lamellae may be related to the mechanics of moving water through the nasal chamber and over the lamellae. In species in which olfactory development has been described and which possess accessory sacs as adults, (cyclosmates, including the Labridae, Doving, 1977), these appear late in the development of the olfactory organ. It is likely that all cyclosmates must developmentally switch from primarily ciliary movement of water through the chamber to ventilation using accessory sacs. The lamellae probably begin to develop in synchrony with this developmental change, which in turn must not occur until after settlement in labrids.

Many labrids bury themselves in sediment upon settlement and emerge several days later. Bodianus rufus (Sponaugle and Cowen, 1997), C. parrae and D. megalepis (this study) are believed not to burrow at any time. Settlement in these species involves a direct and immediate transition from the pelagic larval
environment to a demersal existence in their juvenile habitat on the reef. This is supported both by observations (Sponaugle and Cowen, 1997; this study, Chapter 5) and by the lack in these species of distinct settlement marks believed to be produced in the otoliths of labrids during the time they are buried (Victor, 1986a). These species appear to have specific microhabitat preferences (this study, Ch.5; Sponaugle and Cowen, 1997; Farm, 1993; Victor, 1991), and are probably locating these microhabitats during settlement and settling directly into them (see Ch.5). Interestingly, these were also among the species with the highest densities of nonsensory ciliated cells, which may be associated with high olfactory ability (Yamamoto, 1982).

This period of burial observed in many labrids has been proposed as a strategy which allows the larva to complete metamorphosis into the juvenile stage and growth has been assumed to be rapid during this period (Victor, 1983). Whether further development of the sensory systems occurs during this period was not known prior to the present study. In *H. bivittatus, H. maculipinna* and *H. pictus* the density of ciliated receptors increased from the larval to the juvenile stage. In *H. maculipinna* and *D. megalepis* microvillous receptors increased as well. For the other species either no change occurred or both stages were not available for comparison. Further study is needed to determine if significant changes occur during this period of burial in any of the species. Densities of ciliated receptors in
settlement-stage labrids are similar to those reported for adult fishes of other species suggesting that at least as far as receptor cells are concerned near maximum densities may have already been attained by the time of settlement. The density of nonsensory ciliated cells does decrease in many species and this may be related to the attainment of an alternative method for ventilation of the olfactory epithelium.

The Role of Olfaction in Settlement

It is not clear for any fish studied to date, which of the sensory systems plays the greatest role in detecting settlement cues. It is possible that more than a single type of cue is used. In eels in which the olfactory sense has been ablated, orientation and homing still occur though not as quickly and consistently (Barbin, 1998). However, of all sensory cues, olfactory cues can be detected farthest from the source (Baird et. al, 1996).

It has been shown that fish can localize an odor source at a short distance from the source by the detection of an odor gradient. Turbulence causes the dispersal of the odorant so that detection of the gradient is no longer possible at some distance from the source of the odor. However, the localization of and
orientation of a fish to a distant odor source is possible if the olfactory cue is accompanied by a current detectable by the fish (Kleerekoper, 1982). It is possible for a fish to locate a reef using some olfactory cue whose source is on the reef, if there is some form of off-reef transport of water in the form of a current detectable by the fish. The behavior of orientation toward an odor has been studied in a limited number of species. It has been demonstrated that the rheotaxic response in some fishes such as eels (Anguilla rostrata and Anguilla vulgaris), cod (Gadus morhua) and salmon (Onchorhynchus keta and O. kisutch) occurs only after olfactory stimulation (Kleerekoper, 1982). In Anguilla rostrata a behavioral mechanism, selective tidal stream transport, is used to migrate toward a home site. The eels migrate vertically in order to maximize transport toward the home site located within an estuary. One of the cues enabling them to determine the proper tide for transport and which triggers their vertical movements is an olfactory cue (Barbin, 1998). In American eel elvers, chemosensory input will modify their normal rheotactic response to induce behavior known as filament tracking or casting in which the fish will swim in a zigzag pattern in and out of an odor plume, following it toward its source (Oliver, et al., 1996). Clearly orientational movement in these fishes is the result of a response to a chemical cue in conjunction with rheotaxis. Since the reef and its resident organisms are probably the source of settlement cues for reef fish larvae (reviewed in Leis, 1991) and larvae have been observed to orient
themselves with respect to a reef from a distance too far for visual perception of the reef (Leis et al., 1996), it is probable that the detection of a reef, and the orientational movement of settlement-stage fishes toward that reef, are accomplished by the detection of olfactory cues and orientation of the fish to an off-reef current, probably tidal (Sponaugle and Cowen, 1997), using the lateral line system.

At some distance from the reef a general "reef smell" may act as the olfactory cue, giving way to more specific odors as the fish approaches the reef. In some reef species settling fish will preferentially recruit to areas where conspecifics are detected using chemical cues or avoid areas where other species are detected (Sweatman, 1988). I and others (Sponaugle and Cowen, 1997) observed that most newly settled labrids are found on the reef in groups, with conspecifics of various sizes, or with other labrid species. Olfactory cues may allow for both the location of a reef and of conspecifics or congerenics once the fish has reached the reef. Conspecific aggregation behavior using olfactory cues has been demonstrated in various species and the cues detected are believed to be pheromones contained in fish mucus (Liley, 1981).

Some reef fish larvae may remain within a few km of the reef on which they were spawned (Kingsford and Choat, 1989; Kingsford et al., 1991). This case
occurs more often than previously thought (Kingsford and Choat, 1989). Physical and behavioral mechanisms exist which may facilitate this “retention” on a specific reef or reef group (reviewed in Leis, 1991). Some of these mechanisms, such as the slicks of internal waves (Kingsford and Choat, 1986) or tidal fronts act to extend the influence of the reef making it a larger “target” than could be detected using visual cues alone (Kingsford et al., 1991). Due to the unique faunal composition and hydrologic patterns around individual reefs, they are very likely to bear individual odor “signatures”. Olfactory cues may elicit behavior that results in the retention of larvae within those hydrographic features in the vicinity of the natal reef. These same hydrologic features can transport the fish reefward. In cases where larvae are transported to greater distances from the reef and the cues are lost, the detection of odors similar to those from the natal reef may induce the larvae to begin exhibiting settlement-facilitating behaviors such as vertical migration or horizontal swimming in search of reefward currents. In goldfish that were trained to respond to a variety of chemical stimulants, it was observed that the memory of a specific amino acid can be retained for at least three months and that of an acidic stimulant such as acetic acid for 10 months (Zippel et al., 1993). Olfactory imprinting of very specific odors may occur early in the development of a fish (Olsén and Winberg, 1996; Winberg and Olsén, 1992), even before hatching (Olsén, 1985). It would
therefore be possible for a larval fish to retain the memory of a specific odor from its natal reef during the entire larval stage.

The detection of conspecifics using olfactory cues may play an additional role in pre-settlement fishes. Olfaction is important in maintaining adult fish aggregations in darkness (Blaxter, 1988, Liley, 1981). Evidence has been found for the existence of “larval schools”. Larvae of clupeids (Leis 1986), gobiids (Breitburg, 1987, 1989), blennioids and gobiesocids (Marliave, 1977) have been found forming schools prior to settlement. In coral reef fish larvae, aggregated settlement has been reported for a serranid (Avise and Shapiro, 1986), a lutjanid (Sweatman, 1988) and various other groups including labrids (Kaufman et al., 1992). Furthermore, the samples of settlement-stage larvae collected for the present study usually consisted of numerous individuals of only a few species per sample, though the species varied between samples (Lara, unpub. data). Other researchers have noted this phenomenon (Victor, 1991; J. E. Olney, pers. com.). In the present study, groups of conspecific or congeneric labrids observed on the reef usually included small recently settled individuals of similar size. Groups of presettlement larvae of gobiesocids and tripterygiids have been observed maintaining their position under a variety of current conditions (Kingsford and Choat, 1989). This suggests that monospecific larval schools may form while the fish are planktonic, may spend the later part of the presettlement
period on or very near the reef, and settle as a group, either directly into existing
groups of adult conspecifics, or to patches of sediment or crevices on the reef,
from which they later emerge to join a group of conspecifics.

Although some species of labrids may be settling directly into groups of
conspecifics (e.g., C. parrae, pers. obs.), this is not generally the case. Most species
of labrids have been observed to bury themselves in sediment at settlement and
emerge as juveniles several days later. These include H. bivittatus (Victor, 1983),
H. garnoti, H. pictus, H. maculipinna, H. poeyi, X. martinicensis, and X. splendens
(this study). Thalassoma bifasciatum is assumed to bury itself based on the nature
of the settlement mark on its otolith (Victor, 1986a; 1986b). It is not known
whether emergence from burial occurs at night but this appears to be the case. In
the present study newly emerged juveniles of all of the above species were
always discovered in the early morning. If emergence does occur at night then
the location of conspecifics would have to be accomplished in the dark and
would still rely heavily on non-visual cues.

If indeed olfaction is used in the location of the reef by settling fishes then
alteration of the olfactory cues or of the ability of the fish to detect them could
cause a failure of pre-settlement fish to locate and settle onto a reef. This could
take several forms. Olfactory organs are exposed directly to the environment and
it has been shown that they are particularly sensitive to damage from pollutants such as herbicides and detergents (Kleerekoper, 1982). An olfactory cue (most likely biological) could be lost if the source of the cue is lost from the reef, alternatively chemicals other than those which act as natural cues could mask cue odors. It is possible that recruitment failures observed where reef environments have been altered (Richmond, 1993) are due to failure of larvae to locate the altered reef using olfaction.
Table 3.1 Larval and juvenile specimens used in the study of olfactory epithelium.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Specimen</th>
<th>Nares used</th>
<th>Standard length (mm)</th>
<th>Age in days after hatching</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bodianus rufus</em></td>
<td>Juvenile</td>
<td>Brj1</td>
<td>one</td>
<td>20</td>
<td>51-52</td>
</tr>
<tr>
<td><em>Chromis</em></td>
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<td>Chromj2</td>
<td>both</td>
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<td></td>
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<tr>
<td><em>Clepticus parrae</em></td>
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<td>Cpj1</td>
<td>one</td>
<td>5.5 - 7.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Cpj2</td>
<td>one</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Cpj3</td>
<td>one</td>
<td>&quot;</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Cpj5</td>
<td>one</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><em>Doratonotus megalepis</em></td>
<td>Larva</td>
<td>Dml3</td>
<td>one</td>
<td>7.8 - 8.7</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Larva</td>
<td>Dml21</td>
<td>both</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Dmj2</td>
<td>one</td>
<td>10.3</td>
<td>35-36; 10-11 days post-settlement</td>
</tr>
<tr>
<td><em>Halichoeres bivittatus</em></td>
<td>Larva</td>
<td>Hbl2</td>
<td>one</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Larva</td>
<td>Hbl3</td>
<td>both</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hbj1</td>
<td>one</td>
<td>10.6</td>
<td>35; 9 days post-emergence</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hbj3</td>
<td>one</td>
<td>13</td>
<td>37; day of emergence</td>
</tr>
<tr>
<td><em>H. garnoti</em></td>
<td>Juvenile</td>
<td>Hgj1</td>
<td>one</td>
<td>12.7</td>
<td>35-36; 4 days post-emergence</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hgj2</td>
<td>one</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hgj3</td>
<td>one</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td><em>H. maculipinna</em></td>
<td>Larva</td>
<td>Hml1</td>
<td>one</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hmj1</td>
<td>one</td>
<td>13.0-15.0</td>
<td>37-40; 10 days post-settlement ~44; 12 days post-settlement</td>
</tr>
<tr>
<td><em>H. pictus</em></td>
<td>Larva</td>
<td>Hpl1</td>
<td>one</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hpj2</td>
<td>one</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hpj3</td>
<td>one</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hpj4</td>
<td>one</td>
<td>5.5-6.0</td>
<td>34; 7 post-emergence</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Hpj5</td>
<td>one</td>
<td></td>
<td>31; 4 post-emergence</td>
</tr>
<tr>
<td><em>H. radiatus</em></td>
<td>Juvenile</td>
<td>Hrj2</td>
<td>one</td>
<td>18.1</td>
<td>45 - 47; 7-8 days post-settlement</td>
</tr>
<tr>
<td><em>Scarus sp.</em></td>
<td>Larva</td>
<td>Scarl1</td>
<td>both</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Larva</td>
<td>Scarl2</td>
<td>one</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td><em>Thalassoma bifasciatum</em></td>
<td>Adult</td>
<td>Tba1</td>
<td></td>
<td></td>
<td>43 - 44; 4 days post-emergence</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Tbj1</td>
<td>one</td>
<td></td>
<td>55 - 56; day of emergence</td>
</tr>
<tr>
<td>Fish Species</td>
<td>Juvenile</td>
<td>Code</td>
<td>Age (days)</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------</td>
<td>------</td>
<td>------------</td>
<td>------------------------------</td>
<td></td>
</tr>
<tr>
<td><em>X. martinicensis</em></td>
<td>Tbj3</td>
<td>one</td>
<td>11</td>
<td>51; day of emergence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tbj4</td>
<td>one</td>
<td>10.8</td>
<td>56; day of emergence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tbj5</td>
<td>one</td>
<td>11.4</td>
<td>48; day of emergence</td>
<td></td>
</tr>
<tr>
<td><em>X. splendens</em></td>
<td>Xmj1</td>
<td>one</td>
<td>14.7</td>
<td>62 - 63; day of emergence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Xmj4</td>
<td>one</td>
<td>14.4</td>
<td>66; day of emergence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Xmj3</td>
<td>one</td>
<td>13.1</td>
<td>79 - 81; 3 days post-emergence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Xsj2</td>
<td>one</td>
<td>15.7</td>
<td>***; day of emergence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Xsj3</td>
<td>one</td>
<td>15.6</td>
<td>***; 3-5 days post-emergence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Xsj4</td>
<td>one</td>
<td>16.4</td>
<td>***; 6 days post-emergence</td>
<td></td>
</tr>
</tbody>
</table>

*** Not resolved
Table 3.2 Relative density of cells of the olfactory epithelium among 13 species of labroids and Chromis sp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ciliated receptors - cells/100 μ²</th>
<th>Microvillous receptors</th>
<th>Ciliated non-sensory</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Larva</strong></td>
<td><strong>Juvenile</strong></td>
<td><strong>Larva</strong></td>
<td><strong>Juvenile</strong></td>
<td></td>
</tr>
<tr>
<td>Bodianus rufus&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.57</td>
<td>Medium</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Clepticus parrae&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1.4 - 2.9</td>
<td>Low</td>
<td>V. Low - V. High</td>
<td></td>
</tr>
<tr>
<td>Doratonotus megalepis</td>
<td>18.2 - 26.0</td>
<td>Medium</td>
<td>High</td>
<td>Medium - V. High</td>
</tr>
<tr>
<td>Halichoeres bivittatus</td>
<td>1.3 - 7.3</td>
<td>Medium</td>
<td>Low</td>
<td>Medium - V. High</td>
</tr>
<tr>
<td>Halichoeres cyanopephalus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>***</td>
<td>9.0 - 23.0</td>
<td>Low</td>
<td>V. High</td>
</tr>
<tr>
<td>Halichoeres gurnoti</td>
<td>***</td>
<td>9.0 - 23.0</td>
<td>V. High</td>
<td>Low - Medium</td>
</tr>
<tr>
<td>Halichoeres maculpinna</td>
<td>2.3</td>
<td>11.5 - 26.3</td>
<td>Medium - High</td>
<td>V. Low</td>
</tr>
<tr>
<td>Halichoeres pictus</td>
<td>5.4</td>
<td>8.0 - 10.8</td>
<td>Medium</td>
<td>V. Low</td>
</tr>
<tr>
<td>Halichoeres radiatus&lt;sup&gt;j&lt;/sup&gt;</td>
<td>***</td>
<td>11.8</td>
<td>V. High</td>
<td>High</td>
</tr>
<tr>
<td>Thalassoma bifasciatum</td>
<td>***</td>
<td>23.5 - 38.9</td>
<td>High</td>
<td>None</td>
</tr>
<tr>
<td>Xyrichtys martinecensis juveniles</td>
<td>9.3 - 21.0</td>
<td>11.5 - 26.3</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Xyrichtys splendens juveniles</td>
<td>0.83 - 9.0</td>
<td>8.0 - 10.8</td>
<td>***</td>
<td>High</td>
</tr>
<tr>
<td>Scarus sp.&lt;sup&gt;L&lt;/sup&gt;</td>
<td>7.1 - 11.1</td>
<td>Med-High</td>
<td>Low</td>
<td>Very Low</td>
</tr>
<tr>
<td>Chromis sp.&lt;sup&gt;j&lt;/sup&gt;</td>
<td>15.0 - 20.3</td>
<td>Low</td>
<td>Medium</td>
<td></td>
</tr>
</tbody>
</table>

SE = Sensory epithelium
A = Information for adult only
J = Information for juveniles only
L = Information for larvae only
*** = Not resolved
* = High -V. High within patches

Microvillous receptor density : cells/100 μ²
High = 14-27
Medium = 8-13
Low = 0-7

Ciliated non-sensory
V. High = 90% -100% coverage
High = 70-89% coverage
Medium = 40-69% coverage
Low = 10-39% coverage
V. Low = 0% - 9% coverage
Table 3.3  Receptor density in the olfactory epithelium of some teleosts.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of receptor cells*/100 μm² of olfactory epithelium</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engraulis japonica</td>
<td>22</td>
<td>Yamamoto, 1982</td>
</tr>
<tr>
<td>Oncorhyncus masou</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Histrio histrio</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Oryzias latipes</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Trachurus japonicus</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Pagrus major</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Scomber japonicus</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Fugu niphobles</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Nematocentris macullochi</td>
<td>20-25</td>
<td>Breuer, Zeiske and Melinkat, 1979</td>
</tr>
<tr>
<td>6 species of Atheriniformes</td>
<td>20-25</td>
<td>Zeiske et al., 1979</td>
</tr>
<tr>
<td>Xiphophorus helleri</td>
<td>40-50</td>
<td>Zeiske et al., 1976</td>
</tr>
<tr>
<td>Aplocheilus lineatus</td>
<td>40-50</td>
<td></td>
</tr>
<tr>
<td>Lota lota</td>
<td>7</td>
<td>Gemme and Doving, 1969</td>
</tr>
<tr>
<td>Phoxinus phoxinus</td>
<td>10</td>
<td>Teichmann, 1954 (in Kieerekoper, 1969)</td>
</tr>
<tr>
<td>Perca fluviatilus</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Noemacheilus barbatulus</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Gobio gobio</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Anguilla anguilla</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Esox lucius</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Salmo gairdneri</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Tinca tinca</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Gasterosteus aculeatus</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Leuciscus cephalus</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

* ciliated + microvillous receptors
Fig. 3.1  

A. Olfactory receptors: Ciliated receptor cell (ci) and microvillous receptor cell (mi). B. Nonsensory ciliated cells (ns). 
C. Anterior (a) and posterior (p) nares showing skin flap over posterior nare in juvenile Halichoeres bivittatus. D. Anterior (a) and posterior (p) nares of settlement-stage larva of Doratonotus megalepis.
Fig. 3.2  A. Settlement-stage scarid larvae with dumbbell shaped olfactory pit (incompletely formed nares) (arrow). B. Patchy olfactory epithelium of juvenile *Halichoeres radiatus*. 
Fig. 3.3  Densities of ciliated receptor cells in settlement-stage labrids.
Fig. 3.3  Density of ciliated receptor cells in settlement-stage fishes

Density (# cells/100 µm²)

Species

- B. rufus (J)
- D. megalepis (J)
- H. maculipinna (J)
- H. radiatus (J)
- X. splendens (J)
- Chromis sp. (J)
- C. parrae (J)
- D. megalepis (L)
- H. bivittatus (J)
- H. bivittatus (L)
- H. pictus (J)
- H. pictus (L)
- T. bifasciatum (J)
- H. gamoti (J)
- X. martinicensis (J)
Chapter 4

Morphology of the retina, photoreceptors and visual acuities of the settlement-stages and some adults of 12 species of Caribbean labroids.

Abstract

The morphology of the retina and photoreceptors of settlement-stage larvae and early juveniles and some adults of 12 species of Caribbean labrids and scarids were examined using histological techniques. The retinal structure is described in these species and stages. Larvae have a pure cone retina and unorganized mosaic and organization into a square mosaic pattern occurs during metamorphosis. Early post-settlement juveniles have an organized mosaic with structures that may enable them to detect polarized and UV light. Visual acuities were calculated for all species and stages and acuities ranged between 86.6 -29.4 minutes of arc in the settlement-stages and 16-1.8 minutes of arc in the adults.
The visual abilities of the settlement stages and the possibility of the use of vision during settlement are discussed.
**Introduction**

The resolving power or visual acuity of the eye is defined as the minimum angle formed at the eye by two objects that are perceived as separate (Douglas and Hawryshyn, 1990). Visual acuity is measured in degrees of arc and can be assessed both behaviorally and morphologically. The assessment of visual acuity from morphological measurements is based on the following: if the eye is exposed to a grating of dark and light bars, light bars will stimulate the cones and dark bars will not. In order to resolve two separate light bars, the bars must be separated from each other by one row of unstimulated cones. Thus, the minimum distance between the bars must correspond to the diameter of one row of cones. Therefore, acuity is limited by the distance between cones in the retina and this is reported as the minimum separable angle (MSA) in degrees or minutes of arc. The diameter of the lens and its distance from the retina also contribute to visual acuity in that the larger the lens, the lower the spherical aberration, and the further it is from the retina, the larger the retinal image size (Douglas and Hawryshyn, 1990).

Morphological measurements of acuity are measures of the limit of resolution of the eye. Behavioral trials show that demonstrated ability is lower than that predicted by morphological measurements of the retina and focal
length (Browman et al., 1990; Miller et al., 1993; Pankhurst, 1994; Job and Bellwood, 1996). The disparity between morphological and behavioral assessments of acuity is either due to delayed development of other structures in the eye relative to the development of the retina (Shand, 1997) or the tendency of behavioral trials to underestimate the actual acuity of the eye (Miller et al. 1993). In any case, species or stages possessing higher morphological acuities display higher behavioral acuities as well (Hairston and Li, 1982, Wahl et al., 1993; Job and Bellwood, 1996). Though not a direct measurement of actualized visual ability, morphological measurements of visual acuity are useful in comparative studies of relative ability between species and stages.

In studies of visual ability that have included larval and juvenile stages, interest has been largely focused on first feeding fishes and their ability to find food and avoid predators (Blaxter and Jones, 1967; Job and Bellwood, 1996; Kvenseth et al., 1996; Margulies, 1989; Mills et al., 1984; Miyazaki, T., 1993; Neave, 1984; Pankhurst, 1994; Sandy and Blaxter, 1980; Wahl et al., 1993). Only Shand (1993; 1994; 1997) focused specifically on settlement-stage fishes and pre- and post-settlement influences on retinal development in 4 species of tropical Pacific fishes, an apogonid, a mullid, a labrid and a pomacentrid. Other studies of visual acuity that have included the settlement-stages of fishes are listed in the results section of this chapter. With the exception of Shand's work, none of these
studies has focused specifically on the visual competency of settlement-stage fishes.

The present study consists of a description of the retinas and photoreceptors and reports morphological visual acuities in 12 species of settlement-stage and adult tropical labroids from the Caribbean. The possession of both rods and cones and low values of MSA theoretically increases the level of visual ability and the organization of the cones into a regular pattern has been found only in fishes with superior visual ability (Wagner, 1990). Specimens were examined for the presence or absence of rods and various types of cones, values of MSA were calculated and retinal mosaic patterns were described.

The purposes of this investigation were (1) to provide a basis for comparison of visual abilities predicted by the morphology of settlement-stage fishes to earlier and later stage fishes and (2) to document changes occurring in the visual system of these fishes around the time of settlement. Furthermore this information is useful in assessing the relative abilities predicted by morphology of settlement-stage labroids as compared to settlement-stages and adults of other species of fishes. Lastly, this information provides insight into the theoretical visual ability of the settlement-stages of some coral reef fish species and the implications of this for their ability to locate and settle onto reefs and survive after settlement.
Materials and Methods

Sample Collection

Samples were collected off Carrie Bow Cay, Belize in 1994 and 1995, Lee Stocking Island (LSI), Bahamas in 1994, Glover's Island, Belize in 1996 and Virginia Key, Florida, USA in 1996. All settlement-stage larvae were collected using channel nets, moored plankton nets and light traps. The light traps were modified quatrefoil Plexiglas light traps (Faber, 1984). The light source was a Pelican Pro xenon dive flashlight, with 40,000 candelepower, attached to the top of each trap. Light traps were set at sunset, around the time of the new moon and were allowed to collect from one to three hours nightly.

The channel net used at LSI, Bahamas was fitted to a 1.0 m X 2.0 m rectangular frame and constructed of 2.0 mm mesh. The moored plankton nets used at Carrie Bow Cay, Belize and Glover's Reef, Belize were constructed of 1.0 mm mesh and fitted to a 1.0 m x 0.25 m frame. All nets were set to fish just below the surface and were situated either in the middle of a large channel (at LSI, see Shenker et al., 1993) or off the end of a dock in shallow water (0.5 to 2 m depth). Channel nets were deployed before sunset and retrieved the next morning. Moored plankton nets were deployed at sunset and allowed to fish for one-hour periods up.
to four hours after sunset. All of these collections consisted of late larvae that were presumably coming onto the reef to settle and are referred to here as "settlement-stage larvae". Some species of labrids settle by burrowing into sediment or hiding in crevices on the reef and emerge as juveniles several days later. In the present study, the initial burial is considered to be settlement and the latter is referred to as emergence.

Divers collected adult and juvenile fishes on SCUBA using aquarium handnets or alternatively using pushnets in shallow water. The pushnets consisted of a square PVC frame 1.0 m x 1.0 m with a long handle fitted with a net made of 2 mm mesh. The gear was pushed along the substratum while walking over a seagrass bed or sandy bottom in water up to 1 m in depth.

The juveniles used in this study were considered "early post settlement" and are referred to here as "recently-settled juveniles". Any individuals larger than 30 mm total length (TL) were considered adults, as sexual maturity in at least some species of labrids is attained at a length of less than 30 mm TL (Thresher, 1984). In this study, both settlement-stage larvae and recently-settled juveniles may collectively be referred to as settlement-stage fish.
**Preparation of Specimens**

Specimens were fixed overnight in a 3% solution of glutaraldehyde in 0.1 M phosphate buffer. They were then transferred to 0.1 M phosphate buffer and stored at 4°C for up to three years. Standard lengths of specimens were measured after fixation in glutaraldehyde and before embedding.

Whole heads of smaller specimens and whole eyes of larger specimens were dehydrated through an ethanol series and embedded in LKB 2218-500 Historesin. Transverse serial sections 3µm thick were cut using a Reichert-Jung Supercut 2050 retraction microtome and then mounted on glass slides. Tangential sections were cut from the eyes of some larvae and recently-settled juveniles to allow observation of the mosaic pattern of the cones. Sections were stained with Harris hematoxylin and eosin-phloxine.

**Table 4.1.** lists the species examined in the present study, the number of individuals used, their standard lengths and estimated ages at settlement in days after hatching. As the otoliths could not be extracted from specimens used for study of the retina, ages at settlement for a species were obtained using otoliths extracted from other settlement-stage individuals from the same samples.
Analysis

Slides were examined under transmitted light and computer bitmap images were captured using an Olympus BH2 compound microscope fitted with a Sony XC-75 CCD grayscale video camera, connected to a PC via a Data Translations PCI frame grabber. Images of lenses were captured at 100x or 200x magnification and retinas under oil immersion at 1000x. Measurements of lens diameters and cone distances were made using Sigma Scan software by Jandel. Rods were considered to be present either when their outer segments could be seen in transverse sections or when the outer nuclear layer was composed of two distinct tiers of nuclei (Sandy and Blaxter, 1980).

Calculation of Visual Acuity

Visual acuity was defined as the “minimum angle which a stimulus can subtend an eye and still be resolved” (Neave, 1984) or “an estimate of the minimum visual angle needed to distinguish two points as separate” (Job and Bellwood, 1996). This definition is based on a hypothesis proposed by Helmholtz (1924-1925, in Neave, 1984) in which cone density is assumed to be the limiting factor in resolving two separate white bars from a grating of black and white bars. The white bars (which simulate the cones) would have to be separated by a minimum distance of one row of unstimulated cones in order to
be resolved as two separate bars. Thus an estimate of visual acuity can be
determined histologically by using the anatomical formula from Neave (1984)
derived from Helmholtz (1924-1925) based on cone density:

\[
\sin \alpha = \frac{c}{f}
\]

where \( \alpha \) = the minimum separable angle in minutes of arc (MSA), \( c \) = the
distance between the centers of adjacent retinal cone cells, and \( f \) = the focal
length of the lens. The focal length is calculated by using Matthiessen's ratio,
which is the ratio of the focal length to lens radius. This ratio ranges between 2.2
and 2.8 and averages 2.5. The latter is the figure most often used in studies of
visual acuity in larval and settlement-stage fishes (Job and Bellwood, 1996) and
which maintains a constant value throughout different stages within one species
(Fernald, 1990). Some recent studies of morphological visual acuity in fishes
(Shand, 1997; Job and Bellwood, 1996; Pankhurst 1994) used an alternative
equation, which appeared in Shand (1994). This equation is believed to be
preferable (Shand, 1994b cited in Job and Bellwood, 1996) and was used in the
present study:

\[
a(\text{degrees}) = \left(\frac{c}{2.5r}\right) \times \left(\frac{180}{\pi}\right)
\]
where $a = \text{the minimum separable angle}$ (the angle formed at the eye by
two points which can just be distinguished as being separate) and $r = \text{the radius}$
of the lens. An estimated shrinkage of approximately 5\% due to fixation in
glutaraldehyde (Hayat, 1981) was factored into the right side of the equation by
multiplying the equation by 1.05. Embedding in Historesin reportedly avoids
further shrinkage (Kotrschal et al., 1990) and in order to verify this the eyes of
several species were measured before and after embedding. No substantial
shrinkage or distension was found and correction for shrinkage due to
embedding was not necessary. A correction for the thickness of the sections was
applied to the number of cones (Abercrombie correction, Abercrombie, 1946)
with the resulting equation:

$$a(\text{degrees}) = \left[\frac{(1.05 \times 3 \ c)}{(2.5 \ r)}\right] \times \left(\frac{180}{\pi}\right)$$

The three sections that appeared to have the largest lens diameters were
selected from each specimen. The mean of three measurements of diameter was
calculated for each section and the largest of these means was chosen as the
maximum lens diameter for use in the calculation of visual acuity. Three sections
of the central retina were captured from each specimen and used for
measurements of cone distance. The central portion of the retina was chosen, as
the retina begins to curve a short distance from the central portion in the smaller
specimens (Fig. 4.1A). In addition, cone densities are believed to be highest in this area in labrids (Munz and McFarland, 1973; Collin and Pettigrew, 1988) and measures from this area would provide the maximum value for acuity of the eye. Three transects of 10 cone cells each were taken per section (nine per specimen). The length of each transect was divided by ten to give the mean cone distance (c) for that transect. The mean of the nine cone distance means obtained for each specimen was used in the calculation of visual acuity.

**Results**

**Cones and Rods**

All of the adults examined possessed retinala that contained double cones, single cones and rods. The double cones of the adults (Fig. 4.1B) were larger in diameter than those found in either juveniles (Fig. 4.1C) or larvae (Fig. 4.1D).

All of the juveniles examined had rods except for *X. martinicensis* and *X. splendens* which possessed pure cone retinas after settlement. *Halichoeres bivittatus* and *H. garnoti* juveniles appeared to have few rods and were probably only starting to acquire them after settlement. All of the juveniles examined
possessed both double and single cones. Tangential sections of all juveniles revealed a square mosaic pattern consisting of four double cones surrounding a single cone. In addition, all juveniles examined had the “additional single corner” cones (Zwischenzapfen) found in some cyprinids and salmonids (reviewed in Douglas and Hawryshyn, 1990) (Fig. 4.2A-C).

Larvae of all of the labrids examined had retinas composed of both double and single cones. In comparison with the juveniles, the mosaics of larvae appeared relatively unorganized, without the regularly spaced rows of cones observed in the juveniles of all of the species (Fig. 4.2D). The exception was *D. megalepis* larvae, in which some organization was obvious. Larvae of the two scarid species examined, *Sparisoma* sp. and *Scarus* sp., differed from the labrids in having a retina composed of very narrow double cone pairs arranged in regular rows. None of the larvae of either family possess rods, with the exception of one specimen of *D. megalepis*.

Embryonic fissures (Fig. 4.3A-C) were observed in juveniles of *T. bifasciatum*, *H. bifasciatum* and *H. pictus* and in larvae of *D. megalepis* and *Sparisoma* sp.
Histological Visual Acuities

The histological visual acuities of the 12 species of labroids are plotted in Fig. 4.4. They are divided into three stages: settlement-stage larvae, recently-settled juvenile, and adult (80-100 mm SL). Adults had acuities in the range of 11.6 - 21.1 minutes of arc. Visual acuities of juveniles fell between 29.4 and 63.4 minutes of arc. Larvae had the greatest range with visual acuities falling between 34.6 and 86.6 with the scarids, *Scarus* sp. and *Sparisoma* sp. falling at the lower end of the spectrum of visual acuity (higher MSA). The increase in cone distance with stage was small and the growth of the lens accounted for most of the increase in morphological visual acuity.

Visual acuities of larval and juvenile labroids from this study cover the lower end (higher MSA) of the range of acuities reported for all other settlement-stage fishes which have been examined (Table 4.2). Adult labrids examined in this study have acuities at the lower end (higher MSA) of the spectrum reported for other adult fishes and are close to the values reported previously for adult labrids (Table 4.3).
**Discussion**

**Visual Acuity**

In all fishes growth of the lens is usually continuous throughout the life of the fish and this increase in the size of the lens improves visual acuity (Fernald, 1990). In the labroids examined in the present study, there is a slight decrease in cone density with growth but the growth of the lens more than compensates for any decrease in acuity which results from this decrease in cone density. Consequently, visual acuity should continue to increase throughout the life of these fish. Larger fishes, with their larger eyes, can resolve smaller objects and objects further away than can larvae and small juveniles (Hairston Jr. and Li, 1982; Douglas and Hawryshyn, 1990). Morphological visual acuities measured in the labroids from the present study were on the lower end of those reported for other settlement-stage fishes and adults. Using simple geometry it was calculated that adult labroids could resolve the equivalent of two points 30 cm apart from a distance of 49 m to 89 m, recently-settled juveniles from 16 m to 35 m, and settlement-stage larvae from 12 m to 30 m. This means that in daylight a settling larva could see a small (30 cm) coral head from a distance of 12 to 30 meters. This is roughly equivalent to what a human diver can see under
exceptional conditions of water clarity and is more than sufficient for navigation around a reef environment during the day.

**Development of the Retina: Photoreceptors and Retinal Organization**

As is true for other adult shallow water species, the retina of adult labrids is composed of two types of photoreceptors: rods and cones. Most fishes have only cones in their earliest developmental stages and do not acquire rods until later in their development (Powers and Raymond, 1990). In these earlier stages the cones are the only photoreceptors available to detect light and movement. The functions of perception in lower light levels and color vision are partitioned with the appearance of the rods and differentiation of the cones into different types (Bowmaker, 1990). Engström (1963) described the cones in six species of labrids from the west coast of Sweden as being of different types, varying in length and diameter. Different cone types are maximally receptive to different wavelengths (Bowmaker, 1990). Given the variety of cone types in the labrid retina and the fact that tropical labrids are among the most colorful of fishes, it is likely that labrids are capable of broad spectrum color vision from ultraviolet to red. However, the only way to confirm this would be with wavelength-discrimination experiments (Douglas and Hawryshyn, 1990).
In various studies of the development of the retina in marine fishes, it has been observed that the first appearance of rods coincides with metamorphosis (Blaxter and Staines, 1970; Sandy and Blaxter, 1980; Guma’a, 1982; Evans and Fernald, 1990; Lara, 1992; Kvenseth et al., 1996), or that a rapid proliferation of these photoreceptors occurs near the time of settlement and/or a migration into a deeper habitat (Mas-Riera, 1991; Shand, 1997). Retinomotor movements, or the contraction and extension of the rods and cones under different light intensities, do not begin until after the appearance of the rods (Blaxter and Jones, 1967; Blaxter and Staines, 1970; Guma’a, 1982).

Rods are difficult to detect in the retinae of larval fish and although some species of freshwater fishes do acquire rods early in their development, the retinae of most larval teleosts are believed to be cone-dominated (Powers and Raymond, 1990). Indeed in the tropical labrids examined in the present study rods appear to be absent (with one possible exception) in all late larval specimens caught shortly before settlement. It is possible that there are a few rods in the retina that were not detected, but their frequency of occurrence would be very low. Rods were observed in all of the post-emergence juveniles and all post-settlement juveniles of species that do not burrow. All of these were believed to have settled within the last few days before capture indicating a rapid proliferation of the rods within a week after settlement.
Many species of labrids have been observed to bury themselves in sediment upon settlement for a period of approximately five days after which they emerge (Victor, 1983; present study). This period of burial may induce differentiation of rods and rods may begin to differentiate during burial. The only individual with rods before settlement was a single specimen of *D. megalepis*, which is a species that does not bury upon settlement but settles directly into seagrass beds. Some fishes do not complete settlement in one night but may "settle" and rise back into the water column over several days before finally becoming permanently demersal (reviewed in Leis, 1991; Kaufman et al. 1992). There is some evidence that this may occur in at least some larvae of *D. megalepis* (personal observation). Some fishes caught in light traps in this study and in others (reviewed in Victor, 1991) appear to have some juvenile pigmentation and may well have been in a similar transitional stage. Differentiation of the rods may begin during this transitional period in *D. megalepis*.

Other species of fishes also exhibit some form of transitional behavior between settlement and the active juvenile stage (reviewed in Leis, 1991) and this may provide these fishes with additional time to develop the sensory features such as rods and retinomotor responses necessary for survival in their new juvenile habitat. Many settlement-stage fishes are attracted to strong light.
introduced over the reef at night and this in fact is the basis for light traps used in the collection of these stages (Doherty, 1987). These traps are highly selective for settlement-stage fishes (reviewed in Victor, 1991). No one knows why this is so but it may be explained if the beginning of rod proliferation and retinomotor responses is restricted to the time of settlement in all of the taxa collected as it appears to be in labrids and scarids.

All settlement-stage larvae, juveniles and adults of the species examined have double cones and single cones, with the exception of the scarids Sparisoma sp. and Scarus sp., which appear to have only single cones. Retinae composed of both single and double cones in a well-organized mosaic pattern are generally found in fish which feed on fast-moving prey and inhabit shallower habitats. The fact that double and single cones are found in the retinae of labrids is not surprising. This type of retina may provide superior resolution (Wagner, 1990). The retina composed of only single cones found in settlement-stage scarids may be a result of slower retinal development in scarids. In these fishes the twinning of the cones (the process by which a single cone becomes a double cone) does not begin until after settlement suggesting that scarids may have lower resolving power than labrids at settlement and may not have the ability to detect polarized light at that time.
A lack of single cones is characteristic of deeper water species (Boehlert, 1978). Interestingly the planktonic larval stages of those species do possess single as well as double cones. The loss of the single cones occurs as maturing fish begin to move into deeper water and appears to result from their change in habitat as the loss of single cones was delayed experimentally by holding those fish in a well lit environment (Boehlert, 1978).

Pre-settlement stage labrid larvae had an unorganized pattern of cones in their retinas (Fig. 4.2D). Organization of the retina into a regular mosaic pattern of cones must occur between settlement and a few days after emergence since recently emerged juveniles have very organized and regular retinal mosaic patterns. This organization is probably occurring (at least in the species of Halichoeres and Xyrichtys) along with rod differentiation during the period of burial after settlement. In C. parrae, D. megalepis and T. bifasciatum, which do not bury at settlement, this must occur shortly after migration from the plankton into the deeper reef waters of their juvenile habitats. Kvenseth et al. (1996) observed this organization to occur at metamorphosis in Atlantic halibut (Hippoglossus hippoglossus). Organization of the retinal elements into a regular pattern is believed to increase visual acuity (Wagner, 1978; 1990), increase perception in low light (Engström, 1963), enhance motion detection (McFarland, 1991) or enable perception of polarized light (Cameron and Pugh, 1991; Land, 1991).
In regular mosaics of double and single cones, the cones can be arranged either in what is known as the row pattern or the square pattern (Fernald, 1988). In all of the recently-settled juvenile labrids examined, the cones of the retina are arranged in the square pattern. Four double cones surround a single cone (Fig. 4.2A-C), with the axes of the double cones at right angles to one another (Fig. 4.2C). This arrangement is generally found in fishes which are highly dependent upon vision (Fernald, 1988). This pattern may be characteristic of the Labridae as Engström (1963) described this pattern in adult members of 4 genera (5 species) of labrids from the west coast of Sweden.

In larvae of Sparisoma sp. and Scarus sp. the retina appears to be composed entirely of slender single cones. In tangential sections, the mosaic appears unorganized but in oblique and transverse sections the cones appear to be arranged in rows. Row mosaics seem to be common among schooling species whereas square patterns tend to be more commonly found in predatory fishes (Wagner, 1990). The ecological distinction between labrids and scarids does seem to be consistent with this pattern as scarids are herbivorous and tend to roam about the reef grazing in monospecific groups whereas labrids are primarily carnivorous fishes that feed upon mobile prey. The pattern seen in scarids may alternatively simply be a less developed state of the retina. Juvenile
and adult scarids will need to be examined in order to determine if this pattern is a developmental state.

Juveniles of the labrids in the present study possess the unique retinal elements which appear to enable fishes to detect both polarized light and UV radiation, both of which are believed to play a role in contrast enhancement (Hawryshyn and McFarland, 1987) and navigation and orientation (Douglas and Hawryshyn, 1990). Though relatively little is known about the ability of fishes to detect polarized light (reviewed in Loew and McFarland, 1991), some species have been found to respond to polarized light in behavioral tests (Waterman and Forward, 1970, 1972; Kawamura et al., 1981; Hawryshyn and McFarland, 1987; Cameron and Pugh, 1991). This ability is now believed to be a function of the arrangement of the double cones in the retina of some fishes (Cameron and Pugh, 1991). The retinae of all the recently-settled juvenile labrids examined in the present study have double cones arranged in the square pattern described above. This arrangement has been described in *Sebastes diploproa* (Boehlert, 1978), *Lepomis cyanellus* (Cameron and Pugh, 1991) and *Haplochromis burtoni* (Fernald, 1988). Cameron and Pugh (1991) described how this arrangement can enable the detection of polarized light (Cameron and Pugh, 1991; Land, 1991). Briefly, the inner segments of members of a double cone pair are contiguous and along with the elliptical shape of the pair (in cross section) the structure can act to propagate...
light of one polarization more efficiently. Two double cone pairs positioned at right angles to each other will be maximally sensitive to light with a 90-degree periodicity.

A second possible mechanism has been proposed for the detection of polarized light by some larval and juvenile fishes. Kunz (1987) and Kunz and Callaghan (1989) discuss the possibility that the embryonic fissure found in fishes, specifically *Poecelia reticulata*, *Tilapia mossambica* and *Salmo trutta*, may act in the detection of polarized light. This fissure is present in the early development of the eye and later closes and is completely fused in most adult fishes. In the brown trout *Salmo trutta*, however, the fissure remains open as an area of active growth in the adult and is proposed to allow the detection of polarized light to aid in navigation during migration (Kunz and Callaghan, 1989). Open embryonic fissures were observed in the recently-settled juvenile stages of three of the species from the current study: *H. bivittatus*, *H. pictus*, and *T. bifasciatum* and in larvae of *D. megalepis* and *Sparisoma* sp. They are likely present in the other species as well, however, only the sections from these five species were in the proper orientation to allow for its detection.

In recently-settled juvenile labrids additional single cones at the corners of a square mosaic occur. These elements are believed to lend sensitivity to UV
light (Bowmaker and Kunz, 1987; Bowmaker, 1990) and have been reported in some cyprinids and salmonids (reviewed in Douglas and Hawryshyn, 1990). They are present in the retina of some young fishes including Salmo trutta “yearling” (Bowmaker and Kunz, 1987; Kunz and Bowmaker, 1986; Kunz, 1987), and deep water Sebastes diploproa (“small pre-juveniles”, see Boehlert, 1978) and absent in the adults of these species. The advantage of having increased UV sensitivity in the juvenile stage is not known but is probably related to some aspect of the juvenile environment which is not shared by the adults. These cones may be present in the retinas of settlement-stage larvae as well. However, their existence can be verified only from their position within the mosaic (Bowmaker, 1990) as visual pigment analysis has not been successfully accomplished in labrids due to the small size of their cones and large amounts of melanin in retinal preparations (McFarland, 1991). Since the mosaic pattern is not well-organized in labrid larvae, the presence of these additional single cones could not be ascertained in the larval retina.

Changes in retinal organization, proliferation of retinal elements such as rods and UV sensitive cones, and rapid improvements in histological visual acuity have been found to be coincident in labrids with settlement from the larval planktonic environment to the juvenile reef environment. Changes in retinal structure and visual pigments occurring during settlement have been
found in a few other coral reef fishes (Shand, 1993, 1994, 1997). The first appearance of the rods in some species around the time of settlement when those species are beginning to move into deeper or murkier waters has been proposed as occurring in preparation for the new lower light environment. An alternative explanation is that the size of the eye is acting as a constraint to the further addition of retinal elements until a time when the eye has grown sufficiently to accommodate them (Kotrschal et al., 1990). If this is the case, then size of the eye is the constraint on rod formation and thus on the size of the fish at settlement. As most marine teleost species studied acquire rods at the time of settlement, an alternative hypothesis is that these morphological changes are induced by the behavioral and environmental changes a fish undergoes at settlement, and in fact, some of these changes have been artificially induced by manipulation of the environment (Boehlert, 1978). Wagner (1990) concluded that characteristics of the retina appear to be more a result of adaptation to environmental and behavioral factors than of phylogeny. One can add that physical constraints on growth and size must also be considered when attempting to explain the mechanisms underlying developmental events.
**Visual Ability of Settlement-stage Labrids**

Larval labrids approaching a reef to settle have a limit to their visual ability. Acuities are not high compared to older conspecific fishes and even as compared to settlement-stage fishes of other species. Though the retinae are composed of both single and double cones they lack the organization that appears to be required of fishes with high visual abilities. The rods have not yet begun to appear in most of these individuals and vision at low light levels is expected to be poor. Little is known about the ability of fishes at this stage to detect and use polarized and UV light. They lack the square mosaic pattern and possibly also the corner cones believed to function in the detection of polarized and UV light. Both of these appear shortly after settlement, as do the rods. All of these factors suggest that visual abilities are relatively poor in settling fishes and improve significantly shortly after settlement. This development may be occurring in some of these species while they are buried in the substratum during metamorphosis or hiding in crevices in the reef for as long as 5 to 6 days. The visual abilities of emerging early juveniles should be much higher as they not only have higher morphological visual acuities but also organized retinal mosaics and rods.
Settlement-stage fish have been assumed to be incapable of visually detecting a reef from a distance of >1 km (Leis et al., 1996) or >30 m (Stobutzki and Bellwood, 1998) during the day. Based on the visual acuities measured here, these stages have the ability to visually perceive an entire reef from these distances during the day and could be using vision to locate a reef during the day. However movement toward the reef appears to occur at night (Stobutzki and Bellwood, 1998), settlement sites are selected at night, and most larval fishes settle at night (Robertson et al., 1988). Many species of Caribbean labrids settle during the new moon although some settle during the third quarter moon (Sponaugle and Cowen, 1997). As a result of all-night sampling, it was found that the majority of settlement-stage fish appear on the reef one to two hours after sunset (Lara, unpublished data).

Most settlement-stage larvae have not yet developed dark-adapted vision. The rod cells of the retina are primarily responsible for vision at low light levels. Rod cells do not begin to appear until around the time of settlement (Blaxter and Staines, 1970; Sandy and Blaxter, 1980; Guma’a, 1982; Evans and Fernald, 1990; Lara, 1992; Kvenseth et al., 1996) and fishes do not acquire retinomotor abilities until after the rods appear (Blaxter and Jones, 1967). Though larval fishes could probably use visual cues to locate the edge of the reef from >100 meters away in daylight, their vision is expected to be poor under the nighttime, new moon
conditions under which they usually settle (Robertson et al., 1988). Therefore, visual cues are unlikely to be important in the orientation toward and navigation around the reef during settlement and I believe larvae are probably not relying on visual cues to locate conspecifics or other microhabitat features at the time of settlement. However, most settlement occurs within a few hours after sunset indicating that settlers are not far off the reef before they settle (Lara, unpublished data). It remains possible that settlers are using vision to locate the reef during the day and switching to other sensory modalities after dark.
Table 4.1  Specimens used, standard lengths and ages at settlement (in days after hatching).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number</th>
<th>SL (mm)</th>
<th>Age at settlement*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clepticus parrae juveniles</em></td>
<td>4</td>
<td>5.5-6.9</td>
<td>26-29 (35-49)</td>
</tr>
<tr>
<td><em>Doratonotus megalepis larvae</em></td>
<td>3</td>
<td>5.7-6.4</td>
<td>18-23(20-24)</td>
</tr>
<tr>
<td><em>D. megalepis juvenile</em></td>
<td>1</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td><em>Halichoeres bivittatus larva</em></td>
<td>1</td>
<td>11.3</td>
<td>32(22-26)</td>
</tr>
<tr>
<td><em>H. bivittatus juveniles</em></td>
<td>4</td>
<td>12.4-13.6</td>
<td></td>
</tr>
<tr>
<td><em>H. bivittatus adult</em></td>
<td>1</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td><em>H. cyanocephalus adult</em></td>
<td>1</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td><em>H. garnoti juveniles</em></td>
<td>4</td>
<td>13.1-14.4</td>
<td>23(23-30)</td>
</tr>
<tr>
<td><em>H. garnoti adults</em></td>
<td>2</td>
<td>80,80,100</td>
<td></td>
</tr>
<tr>
<td><em>H. pictus juveniles</em></td>
<td>4</td>
<td>5.5-5.9</td>
<td>(22-27)</td>
</tr>
<tr>
<td><em>H. poeyi adult</em></td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>Thalassoma bifasciatum larvae</em></td>
<td>4</td>
<td>8.1-9.8</td>
<td>32-54 (38-78)</td>
</tr>
<tr>
<td><em>T. bifasciatum juveniles</em></td>
<td>3</td>
<td>10.3-13.7</td>
<td></td>
</tr>
<tr>
<td><em>T. bifasciatum adults</em></td>
<td>2</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td><em>Xyrichtys sp. larvae</em></td>
<td>4</td>
<td>13.3-14.7</td>
<td></td>
</tr>
<tr>
<td><em>X. splendidens juvenile</em></td>
<td>1</td>
<td>13.6</td>
<td>50 (85-114)</td>
</tr>
<tr>
<td><em>X. martinicensis juvenile</em></td>
<td>1</td>
<td>14</td>
<td>47-58 (59-98)</td>
</tr>
<tr>
<td><em>Scarus sp. larva</em></td>
<td>1</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td><em>Sparisoma sp. larvae</em></td>
<td>4</td>
<td>8.6-9.1</td>
<td></td>
</tr>
</tbody>
</table>

*Ages at settlement were determined from individuals used elsewhere in the present study as otolith data was not obtainable for the specimens used for analysis of the visual system. In parentheses = range from Victor, 1986a.*
Table 4.2 Visual acuities of other settlement-stage fishes reported in the literature.

<table>
<thead>
<tr>
<th>Species</th>
<th>Acuity (min arc)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral reef species</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Upeneus tragula</em> (Mullidae)</td>
<td>27</td>
<td>Shand, 1994</td>
</tr>
<tr>
<td><em>Pomacentrus moluccensis</em> (Pomacentridae)</td>
<td>38</td>
<td>Shand, 1997</td>
</tr>
<tr>
<td><em>Stethojulis strigiventer</em> (Labridae)</td>
<td>90</td>
<td>&quot;</td>
</tr>
<tr>
<td><em>Apogon doederleini</em> (Apo gonidae)</td>
<td>40</td>
<td>&quot;</td>
</tr>
<tr>
<td><em>Premnas biaculeatus</em> (Pomacentridae, Amphiprioninae)</td>
<td>8</td>
<td>Job and Bellwood, 1996</td>
</tr>
<tr>
<td>Non-Coral reef species</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pleuronectes platessa</em> (Pleuronectidae)</td>
<td>22</td>
<td>Neave, 1984</td>
</tr>
<tr>
<td><em>Scophthalmus maximus</em> (Bothidae)</td>
<td>11</td>
<td>&quot;</td>
</tr>
<tr>
<td><em>Paralichthys californicus</em> (Bothidae)</td>
<td>38</td>
<td>Lara, 1992</td>
</tr>
<tr>
<td><em>Perca flavescens</em> (Percidae) (25mm)</td>
<td>~25,~36</td>
<td>Miller et al., 1993, Wahl et al., 1993</td>
</tr>
<tr>
<td><em>Atractoscion nobilis</em> (Sciaenidae) (15mm)</td>
<td>25</td>
<td>Margulies, 1989</td>
</tr>
<tr>
<td><em>Perca fluviatilis</em> (Percidae)</td>
<td>145</td>
<td>Guma'a, 1982</td>
</tr>
</tbody>
</table>

Table 4.3 Visual acuities of adult fishes reported in the literature.

<table>
<thead>
<tr>
<th>Species</th>
<th>Acuity (min arc)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labrids</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thalassoma bifasciatum</em> (30,70,130mmSL)</td>
<td>16, 9,7</td>
<td>McFarland, 1991</td>
</tr>
<tr>
<td>Other families</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Merluccius</em> -3 species (80cmTL)</td>
<td>9-11</td>
<td>Mas-Riera, 1991</td>
</tr>
<tr>
<td>10 species inc. large pelagics</td>
<td>1.8-4.0</td>
<td>Tamura and Wisby, 1963</td>
</tr>
<tr>
<td><em>Serranidae</em>, <em>Sparidae</em>, <em>Tetraodontidae</em>, <em>Scorpaenidae</em>, <em>Carangidae</em>, <em>Scombridae</em></td>
<td>4.2-15.4</td>
<td>Tamura, 1957</td>
</tr>
<tr>
<td><em>Perca flavescens</em></td>
<td>9-12</td>
<td>Wahl et al., 1993</td>
</tr>
</tbody>
</table>
Fig. 4.1  

A. Transverse section of an eye of a settlement-stage larva of *Sparisoma* sp. Arrows indicate the section of retina used for measurements of cone distance. L denotes the lens. 100x.  

B. Transverse section of the retina of an adult labrid (*Thalassoma bifasciatum*). C denotes one cone of a double cone pair. 1000x.  

C. Transverse section of the retina of a recently-settled juvenile labrid (*Thalassoma bifasciatum*). C denotes one cone of a double cone pair. 1000x.  

D. Transverse section of the retina of a larval labrid (*Thalassoma bifasciatum*). C denotes a single cone. 1000x.
Fig. 4.2  

A. Tangential section of the retina of a *X. splendens* recently-settled juvenile at the level of the cone outer segments. Square mosaic pattern can be seen. Arrows denote Zwischenzapfen cells. 400x.

B. Tangential section of the retina of *Halichoeres bivittatus* recently-settled juvenile at the level of the cone outer segments. Well-organized square mosaic pattern can be seen as well as Zwischenzapfen. 400x. C. Tangential section of the retina at the level of the cone outer segments of a *Clepticus parrae* recently-settled juvenile. Square mosaic pattern can be seen. Arrows denote intersection of two cones of double cone pair. Note that cells composing square are oriented at 90 degrees from one another. 400x. D. Tangential section of the retina of a *Halichoeres bivitattus* larva at the level of the cone outer segments. Note lack of organization into regular square mosaic (compare to juvenile in Fig. 4.2B). 400x
Fig. 4.3  

A. Whole eye of recently-settled *Thalassoma bifasciatum* juvenile. Arrow denotes embryonic fissure. 40x.  

B. Embryonic fissure in recently-settled *Thalassoma bifasciatum* juvenile. 200x.  

C. Whole eye of *Sparisoma* sp. larva. Arrow denotes embryonic fissure. 100x.
Fig. 4.4  Graph of the histological visual acuities, in minutes of arc, of settlement-stage larvae (Stage 1), recently-settled juveniles (Stage 2) and adults (Stage 3) of 12 species of labroids.
Fig. 4.4 Histological visual acuities of 12 species of labroids

- C. parrae
- D. megalepis
- H. bivittatus
- H. cyanocephalus
- H. gaminot
- H. pictus
- H. poeyi
- T. bifasciatum
- X. martinicensis
- X. splendens
- X. sp
- Scarus sp.
- Sparisoma sp.

Minimum Separable Angle (minutes of arc)

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Chapter 5

Conclusions about the development of the sensory systems in settlement-stage fishes and implications for the morphology, ecology and evolution of coral reef fishes.

Introduction

The aim of this study was to describe the sensory development of settlement-stage labrids and to determine the sensory capacity of settling fishes based on the morphology of the olfactory, visual and lateral line systems. The cues that settling larvae use to locate and choose settlement sites have been a mystery to researchers studying larval fishes and their patterns of settlement. A few studies have addressed the problem directly by field experiments involving the manipulation of possible cues (Sweatman, 1988), field observation (Elliott et al., 1995; Leis et al., 1996) or testing of physical capabilities (Stobutzki, 1998; reviewed in previous chapters). The approach taken in this study was to use
information about the morphology and developmental state of three sensory systems to assess sensory competence of these fishes at the time of settlement.

Questions about the sensory abilities of the settlement stages of fishes are relevant to the understanding of the settlement event and its role in the survival and recruitment of fishes. The answers to these questions will lead to a better understanding of the mechanisms involved in settlement, the roles that the behavior and sensory abilities of the larva play in the settlement event, and the possible alternative evolutionary strategies involved which result in different patterns of settlement. The questions that will be addressed here include:

1. Is there inter- or intraspecific variation in the sensory morphology of settling fishes, and if so, is this variation reflected in the behavior of these fishes at settlement?

2. Do developmental events involving sensory systems occur shortly before or after settlement that may be necessary for settlement or for early juvenile life?

3. Is the time of settlement constrained by the acquisition of certain sensory abilities, and is the acquisition of these abilities constrained by the size of the settling fish?
4. What is the over-arching role of phylogenetic constraints on developmental state, age, size and behavior of settlement-size fishes?

Summary of the developmental state of the sensory systems of settlement-stage labroids.

Settlement-stage labroids possess both free neuromasts and canal neuromasts of the lateral line system. This is consistent with what has been observed in other settlement-stage fishes. This study and studies of other taxa have shown that the formation of the lateral line canals usually occurs around the time of settlement.

Formation of the nares occurs shortly before settlement in other fishes (Kawamura and Ishida, 1985; Lara, 1992) and labrids have well formed nares at settlement. In settlement-stage labrids densities of microvillous and ciliated receptor cells as well as ciliated nonsensory cells are relatively high and these densities are comparable to those found in adult fishes known to have an acute olfactory sense. In the early juvenile stages lamellae are not yet formed. However, the relatively smaller area of the olfactory organ in the juveniles, as compared to the adult, appears to be compensated for by dense and continuous
coverage of the epithelium in receptors and non-sensory cilia. In these early stages ciliary movements mainly accomplish ventilation of the olfactory epithelium.

Visual acuities of settlement-stage labrids fall between 31.5 and 86.6 min of arc at settlement and these acuities are similar to those calculated for other settlement-stage fishes. Rods appear shortly before and after settlement in labrids as in various other taxa (reviewed in chapter 4). In addition to color vision, all juvenile labrids studied appear to have the morphological structures used to detect UV wavelengths and polarized light. All cone types present in the juveniles, including those cones which can detect UV light, are probably present in the retina before settlement but do not become organized into the square mosaic characteristic of juvenile labrids until after settlement has occurred. The square mosaic may play a part in polarized light detection, which may be used for navigation by the juvenile stages. The embryonic fissure, that may be involved in polarized light detection, is retained at least into the early juvenile stages and may play a role in navigation during the pelagic larval stage.
Settlement-site characteristics

Observations of behavior and distributions of labrids from the present study suggest that there are interspecific differences in settlement-site characteristics. That recently-settled individuals of different species of labrids are found in association with different areas of the reef has been observed previously (Victor, 1986c; Sander, 1994; Cowen, 1997). In the present study characteristics of the areas in which recently-settled individuals were captured were noted. These observations were collected on the reef surrounding Carrie Bow Cay (a mangrove island on the Belize barrier reef) and nearby patch reefs, and on Glovers Reef (an oceanic atoll). These characteristics included location on the reef (e.g. reef flat, reef edge at end of spurs), depth, substratum type (e.g. seagrass beds, sand flats, hard coral bottom) and associated structure (e.g. branching coral, base of large coral heads, large sponges), and whether there were conspecifics or congenerics in the area and the respective stages of these individuals (recently-settled, older juveniles or adults, determined from size and coloration). These findings are summarized in Table 5.1. Species were considered specialized if they were found in association with a particular substratum type and/or structure, depth and location on the reef. Species considered generalists were those found in more than one type of area defined by the above characteristics.
Evidence for the use of cues by fishes in locating settlement sites

Visual, olfactory, rheotactic, auditory and magnetic cues could be used by settlement-stage fishes to locate a reef and to select a site for settlement. No previous study has investigated the sensory structures themselves in settlement-stage fishes in order to determine the capacity of larval fishes to detect such cues. Using developmental data to determine which of these cues is most likely to be important is a novel direction in studies of settlement.

Settlement-stage fishes can actively select particular settlement sites both in the laboratory (Sale, 1969; Booth, 1992; Champalbert et al., 1992; Keefe and Able, 1994; Wennhage and Pihl, 1994; Lara, unpublished observations) and field (Shulman et al., 1983; Sale et al., 1984; Sweatman, 1983, 1988; Hair et al., 1994; Elliot et al., 1995). Settlement-stage fishes exhibit various behaviors that may play a part in this location and selection such as vertical movements (reviewed in Leis, 1991; Yamashita et al., 1996), orientation to small scale flow patterns (Breitburg et al., 1995), phototaxis (Doherty, 1987), rheotaxis (reviewed in chapter 3) and active swimming toward and away from reefs (Leis et al. 1996; Stobutzki and Bellwood, 1998). Their swimming ability has recently been shown to be better than what was previously believed (Stobutzki and Bellwood, 1994; Stobutzki, 1998) and the
transport of settling fishes to reefs is no longer believed to be a strictly passive phenomenon.

Possible primary cues used in the location of settlement sites

Most, if not all, settlement is believed to occur at night (Robertson et al., 1988; Victor, 1986c). Most settlement probably occurs within two hours after sunset. At the two sites in Belize during the three years of the present study, the largest total number of settlement-stage fishes were collected within two hours after sunset after which the number collected in both light traps and moored plankton nets dropped off steeply. In addition, many species, including many labrids, settle during moonless nights (McFarland et al., 1985; Victor, 1986c; Robertson et al., 1988; Milicich et al., 1992). The visual ability of settlement-stage fishes in daylight is comparable to that of a human diver under ideal conditions of visibility. That is, settling fishes can probably see the edge of the reef from a distance of <100 meters during the day (Chapter 4). In addition they may have the ability to detect UV radiation and polarized light. In most settlement-stage fishes that have been studied to date, dark-adapted vision is not acquired until after settlement. Though settling fishes could probably locate the reef during the day using vision it is highly unlikely that settlement-stage fishes are using vision
to navigate toward the reef, or to locate specific microhabitats on the reef, at the
time of settlement. It is also possible that they are using visual cues during the
day to move into juvenile habitats after initial settlement (e.g. *D. megalepis*
associating with green corner posts) but are most likely using non-visual cues to
locate areas near their preferred juvenile habitats during settlement.

The use of auditory cues in the detection of reefs and discrimination
between reef types and habitats by settling fishes has recently been proposed
(Stobutzki and Bellwood, 1998). Sound propagation is good underwater (Rogers
and Cox, 1988) and reef organisms do produce sounds that may be heard by
fishes at some distance from the reef. Directional sound detection has been
demonstrated in some adult fishes (Popper et al., 1973). Unfortunately there is no
information to date about the auditory abilities of settlement-stage fishes or
about the nature of the sound (i.e. decibels, frequencies) that would be necessary
for those larval fishes to detect the reef using hearing. Hopefully this information
will be available in the future and until then the possibility of fishes using
auditory cues cannot be accepted or refuted.

The results of the present study suggest that the most important cue used
in the detection, location and choice of settlement site by labrids is most likely a
combined rheotactic/olfactory cue. Olfaction has been demonstrated to be
important in many aspects of fish behavior (Atema et al., 1988) and settlement-stage fishes appear to have highly developed olfactory systems. Some settlement-stage reef fishes (Pomacentridae) have been demonstrated to detect chemical cues and to use these cues to aid in the location and selection of settlement sites (Sweatman, 1983; Elliott et al., 1995). This has long been known to be the case in some settlement-stage invertebrates. In the migration of salmonids back to the specific sites where they were spawned, and in the homing migrations of eels, rheotactic and olfactory cues are believed to be the most important (reviewed in chapter 3). Imprinting on specific olfactory cues occurs in these salmon during the embryonic stage (Olsén, 1985). In addition there is evidence that some adult reef fishes may be traveling back to specific spawning sites and bypassing other spawning sites on the way (Bolden, in review). It is plausible that in these fishes the selection and location of these sites is through olfactory imprinting and this could occur while still in the early larval stages.

Using their lateral line system to detect water currents, and specific olfactory cues from the reef and its biotic constituents, fishes may both detect the reef from a distance and discriminate between specific settlement sites. These olfactory cues could include substratum type and the presence or absence of other organisms including prey types, conspecifics and/or congenerics and symbionts. In addition, the detection of shear between different water masses
when combined with a coarse sun compass may allow for mesoscale long-distance orientation (Atema, 1988). Theoretically, a larval fish moving vertically through different water masses can distinguish between them using olfaction and can follow the water mass until it ends. This behavior has been observed to be used by salmon during homing and by other large fishes (reviewed in Atema, 1988). Auditory cues may be involved as well in the long distance detection of reefs, and the reef may be located during the day at shorter distances using vision, but olfactory cues are much more likely to be the basis of discrimination used to distinguish between reef types and habitats.

**Related questions**

1. *Is there inter- or intraspecific variation in the sensory morphology of settling fishes, and if so, is this variation reflected in the behavior of these fishes at settlement?*

   There are two pieces of important information that may be obtained in answering this question. If there were little variation in the developmental state of the sensory systems across taxa, this would indicate strong evolutionary selection for a specific level of development of the sensory systems at the time of settlement. Most likely this would be for a minimum level of development
required for the successful detection of specific settlement sites and necessary for the subsequent survival of the early juvenile stages. Alternatively, if there were variation in the state of development of the sensory systems among taxa, it would suggest possible variation in settlement strategies among different taxa.

Although sample sizes were small and ranges of size of larvae were narrow in the present study, there is an indication that there is low intraspecific variation of sensory developmental state in labrids at settlement. Samples of settlement-stage larvae were collected from different sites and different years yet varied little in length and developmental state of any of the sensory systems. This is true even though the larval duration varied.

*Thalassoma bifasciatum* has a highly variable planktonic duration, which may extend from 38 - 78 days. If any variation in sensory development at settlement occurs due to age, it would be most easily detected in this species. It is believed that approximate settlement size is reached at the same age in all individuals and those with an extended larval period exhibit a slower growth rate after this size is attained (Victor, 1986b). Individuals of *Thalassoma* of different larval durations should be examined to see if sensory competency is attained before settlement and if olfactory development continues or is also slowed during this extension of the planktonic stage. Specimens of *Thalassoma*
from the present study were examined and little variation in the state of development of their sensory systems occurred. Unfortunately all specimens were approximately the same age, 40-55 days after hatching, so the question could not be answered using these specimens. The examination of individuals of *T. bifasciatum* of varying larval duration is necessarily the next step in this line of investigation.

There is some interspecific variation among the Labridae in all three of the sensory systems though much less than was expected. Given that the larval stages of different coral reef fish taxa exhibit different taxon-specific habitat distributions and behavior (Leis, 1991), it is surprising that the developmental stage of the sensory systems at settlement is so similar across all taxa studied to date. This is an indication that there is some minimum requirement in developmental state, which is common to all settlement-stage fishes, and that there is strong selection for the attainment of a particular suite of sensory capacities by the time of settlement. In fact, even in unfavorable conditions in a laboratory setting, the sensory systems continue to develop even though growth is slowed (Lara, 1992).

Settlement peaks in coral reef fishes are uncoupled from peaks in spawning production (Robertson et al., 1988). Dorsey and Cowen (unpublished
data) could attribute only about 20% of the variability in settlement peaks in Barbados to peaks in egg production. Obviously there is more influencing successful settlement events than egg supply alone. The variability in settlement success of a cohort is likely to be due in large part to variability in conditions during the larval period. Unfavorable conditions such as low food availability (reviewed in Leis, 1991) will lead to a slower growth rate with many of the larvae possibly not attaining the level of sensory development necessary for settlement at the time when conditions are favorable for settlement (i.e. physical transport mechanisms, moon phase). I believe the larvae collected in moored nets and light traps in the present study have probably been intercepted on their way to settlement sites and are the individuals who have attained the necessary developmental state at the time they are in the vicinity of a suitable settlement site. This would explain the lack of major differences in the developmental state of the sensory systems across taxa of settlement-stage fishes. It is plausible that a greater degree of intraspecific variation occurs in earlier planktonic larval stages but only those individuals that are competent to settle at the appropriate time are successfully arriving at the settlement sites to be collected. The small interspecific variations which are observed may be in those features of the sensory systems which are not as crucial to successful settlement, but which represent different life history strategies related to finer scale variation in habitat selection and post-settlement survival. (This is discussed further in answer to question 5).
There are some interspecific differences that are notable either for their possible functional implications or for their use as a taxonomic character. The presence of neuromasts around the nare is probably functionally important (chapter 2). The variation in the configuration of free neuromasts around the nare in the labrids, however, is less likely to provide a functional difference between species than to be a result of phylogenetic history. For example, the presence of six rather than five neuromasts surrounding the nare probably does not provide much difference in function. Traits such as this provide among the best characters for examining phylogenetic relationships and the patterns observed are consistent with the current tribe designations of the genera (see chapter 2).

On the other hand it is highly likely that most components of complex and vital organ systems such as the visual, lateral line and olfactory systems are under high selection pressure. Most or all of the components of these systems have an important function within the system and their development is functionally tied to the other components within the system. Therefore it is highly likely that interspecific variation in these systems is a result of different environmental requirements, which have acted selectively on these systems. In these systems a correlation between form, function and environment is tenable. This is especially true in the case of variables influencing performance (Ricklefs
and Miles, 1994). Recently, retinal patterns reflecting visual capacities (Wagner, 1990) and swimming abilities (Stobutski, 1998) have been found to vary between confamilial species and this variation appears to be more a result of comparatively recent behavioral and environmental factors than of evolutionary history.

**Question 2.** Do developmental events occur shortly before or after settlement that appear to be necessary for settlement or for early juvenile life?

Obviously some features that were appropriate or sufficient for the pelagic larval environment may not be so for the settling larva or the early juvenile. At least some of these changes must be necessary for settlement or for survival in the new environment into which a settling fish is introduced. This transition in modalities could incur a vulnerable stage in which the fish is not optimally fit for either the pelagic or juvenile environment. In order to minimize this period, these changes would have to occur neither too far in advance nor too long after settlement. This would result in the short window of time in which many of these changes are observed to occur.
If there were a high amount of variability observed in the timing of the development of sensory structures with respect to the timing of a life history event such as settlement, it would imply that these structures were not necessarily related to that event. In labrids low variability in timing of the development of sensory structures and their coincidence with the settlement event implies a high degree of correlation between the developmental and settlement events.

In labrids many changes in sensory modality appear to converge on the settlement event, e.g. lateral line canals form around the free neuromasts present in the larval stage, rods and UV sensitive cones first appear in the retina, and the photoreceptors become arranged into an organized retinal mosaic. Furthermore, a behavioral strategy employed by some labrids may provide additional time for development. Some labrids such as the *Halichoeres* species and *T. bifasciatum* may be in effect “buying time” by settling into sand or hiding in the interstices of the reef for a few days before emerging as juveniles. This period of burial or hiding may provide them with additional time in which to acquire features necessary for juvenile survival. Even though feeding is not believed to occur during this period of burial, changes in the arrangement of the photoreceptors and the proliferation of rods occurred during this period. Changes in the densities of olfactory cells were observed and formation of the lateral line canals continued.
Due to the high correlation observed in labrids between the timing of the completion of certain sensory structures and the settlement event, it is likely that the completion of these structures is requisite to either the ability of the fish to settle and/or to survive in the early juvenile environment.

Question 3. Is the time of settlement constrained by the acquisition of certain sensory abilities and is the acquisition of these abilities constrained by the size of the settling fish?

It is clear that successful settlement is critical to survival yet nothing was known of the various constraints that could be acting to limit successful settlement. Though the timing of development of certain sensory structures, the settlement event, and the sizes of fishes at settlement all appear correlated, it is not known which of these is the controlling factor in the timing of the settlement event. Comparing the sizes and the development of the sensory structures in settlement-stage fishes across taxa suggests factors that may be controlling the timing and success of the settlement event.

If a high degree of variation in sizes and sensory development exists among settlement-stage fishes then it is likely that these factors are not important
to settlement. If low variability in either or both of these are found to occur in settlement-stage fishes across a variety of taxa, this would suggest that these factors are imposing some control on the timing of occurrence of the settlement event. That either a fish must attain a certain size or stage of development in order to successfully settle.

In labrids it was found that there is relatively little variation in both the sizes of the heads of the settlement-stages and the development of many of their sensory structures. In comparing this information with what is known for other species it was found that the sizes of the heads of settlement-stage fishes and their sensory development at settlement varies relatively little across a wide variety of taxa.

It is likely that the timing of the appearance or completion of sensory structures is constrained by the size of the developing fish. If, indeed, there is a minimum requirement for the state of development of the sensory structures in order for a fish to successfully settle, then the timing of settlement is in turn constrained by the size of the settling fish. Within a species there is very little variation in the size of settling fishes (Robertson et al. 1988; Wellington and Victor, 1989; Victor, 1991). Fishes of different taxa settle at different ages, and planktonic larval durations of Caribbean reef fishes vary between none and
many months (Victor, 1991). In an extensive collection of Caribbean reef fishes the smallest recorded size of a post-settlement fish was 3.8 mm SL for a chub Pareques acumineatus and the longest was for a pearlfish (Carapus sp.) at 174 mm SL. The size of the majority of fishes of settlement-stage is between 7 mm and 12 mm SL (Victor, 1991).

However, in regard to the development of the cephalic lateral line canals, eyes and olfactory organ, head size is probably more relevant than standard length. Carapids have small heads relative to their length and Pareques acumineatus have large heads relative to their body size. If head size of settlement-stage fishes is examined, variability in size between species will probably be found to be much lower than that of standard length. For those species in which total length at the beginning of canal formation (complete formation of first canal) is known, body length ranges from 7.3 mm in Scophthalmus maximus (Neave, 1986) to 51.0 mm in Trichiurus lepturus (Kawamura and Munekiyo, 1989). Interestingly, in those species in which head lengths (tip of maxilla to tip of operculum) could be estimated, this was always approximately 2.5-4.0 mm at the time of initial cephalic lateral line canal formation (Table 5.2). This consistency across diverse taxa suggests some ecological or morphological constraint on the development of the lateral line canals. Due to the size of small fish larvae they exist under a viscous regime rather than the inertial one in which
adult fishes exist. Propulsion and feeding in small organisms differ from those in larger organisms because the smaller organisms are subject not to inertial but to viscous forces (Vogel, 1981). Being small in diameter the lateral line canals in fishes with heads smaller than 3-4 mm may have Reynolds numbers too low for the canals to function properly. It may be that only after a minimum size is reached can the canals function at all. I believe this physical transition, which occurs as a result of increase in both body (head) size and velocity of swimming, is controlling the timing of formation of the lateral line canals.

Ontogeny of the visual system may be constrained by size (Kotrschal et al., 1990). The small retinal area of most larval fishes is composed of small tightly packed cones. This is perhaps a way of making optimal use of the small retinal area as cones and not rods are capable of both light and movement perception in the levels of ambient light under which most planktonic larvae exist. To obtain maximum acuity, cones are small in diameter so a higher density and lower cone distance is possible than with larger cones (Kotrschal et al., 1990). Larger cones are more sensitive however (Kotrschal et al., 1990), and apparently as lens diameter increases and influences visual acuity to a greater extent, cones are free to increase in diameter without significant reduction in acuity. With growth, rods can then be added to the retina, which increases sensitivity at lower light levels.
Adult labrids move water over their olfactory organs by pumping water using accessory sacs, which are extensions of the olfactory chamber. Labrid larvae and early juveniles do not possess accessory sacs and ventilation is accomplished mainly by means of ciliary movements of non-sensory cells distributed on the surface of the olfactory epithelium. The lack of accessory sacs in small fishes and their formation in larger individuals may be due to the necessity for economy of space in the larval head and the loss of effectiveness of ciliary action to sufficiently ventilate an increasing olfactory area. This changeover in the mechanism for olfactory ventilation appears to occur in the juvenile stages of fishes (Chapter 3) and is another example of size constraint upon timing of sensory development.

Question 4. What is the over-arching role of phylogenetic constraints on developmental state, age, size and behavior of settlement-size fishes?

Many of the characteristics of the development of the sensory systems described in this study appear to have some relationship to the transition from a pelagic to a benthic habitat. The timing of certain developmental events close to the time of settlement (e.g. completion of the nares and lateral line canals, appearance of rods, organization of the retina) suggests that they may be a set of
evolutionary adaptations which facilitate settlement and the location of favorable settlement sites or which increase the chances for survival of early juveniles. The acquisition of certain specialized sensory structures (e.g. square retinal mosaic, UV detecting cones, high densities of olfactory receptors and non-sensory cilia, retention of the embryonic fissure) may be adaptations within the Labridae that enhance their ability to successfully settle and survive through the early juvenile stages. Information provided in this study is available for too few taxa to determine whether the characteristics described in labrids are widespread among marine fishes or whether these are specializations of or within the Labridae. In addition, the monophyly of the Labridae is not established and the basal members of the family have not been defined. Thus, an extensive survey of sensory development in all labrids and their known sister groups is not presently possible. A well-corroborated phylogeny of the Labridae would facilitate the distinction of sensory characters that are ancestral in the lineage from those that are a more recent adaptation. This is important, as only the later characters can be interpreted in light of current ecological conditions and it is these more recent adaptations which would have evolved to improve the chances of successful settlement and survival of members of a species. Knowing what these specializations are would help answer some of the many questions remaining; What structures and functions have evolved to facilitate settlement and survival? What accounts for the differences in settlement success across taxa and
geographic and temporal scales? Are there a variety of settlement strategies that have evolved among fishes?

In order to move closer to the goal of interpreting settlement in marine fishes in an evolutionary context, I will discuss three topics related to evolutionary history. First, I will discuss the current status of the phylogenetic relationships within the Labroidei, and will provide twelve new characteristics based on the morphology of the sensory systems and settlement behavior of settlement-stage labroids. Secondly, it is important to determine what role evolutionary history has played in the relationships observed between sensory development, size of settlement-stage fishes, settlement behavior and the settlement event. If the constraints which evolutionary history imposes are not taken into account, spurious conclusions can be drawn about these relationships. Therefore, I will discuss these relationships based on what was learned in the present study and offer some possible evolutionary scenarios, yet these interpretations will need to be revisited as phylogenetic information becomes available. Finally, examining the information about sensory development and settlement behavior suggests that there are a variety of alternative settlement strategies which fishes may be employing and that these strategies may be related to evolutionary history. These strategies are discussed and their adaptive value awaits interpretation in the context of phylogenetic information.
Phylogeny

As presently defined, the Labroidei includes the families Labridae, Scaridae and Odacidae. The phylogenetic relationships within the labroids and among labroids and other perciform fishes are not well understood. Based on various synapomorphies of the pharyngeal jaw apparatus some include the Cichlidae, Embiotocidae and Pomacentridae in the Labroidei (Liem and Greenwood, 1981; Kaufman and Liem, 1982; Stiassny and Jensen, 1987). However, early life characters support the close relationship between the labrids, scarids and odacids but not the expanded Labroidei (Richards and Leis, 1984). Others reject the expanded Labroidei on the basis of genetic evidence (Streelman and Karl, 1997), the lack of other characters supporting monophyly (Johnson, 1993) and the probability of convergent evolution of the pharyngeal jaw apparatus (Gomon, 1997). The Labridae, Scaridae and Odacidae are considered to comprise the Labroidei. The sister-group status of the Scaridae and Labridae is supported by genetic evidence (Streelman and Karl, 1997) and the scarids have been determined to be monophyletic (Bellwood, 1994).

The Labridae are presently divided by some into several tribes of which only the Hypsigenyini and the Cheilinini have been determined to be monophyletic (Gomon, 1997; Westneat, 1993). Doratonotus is a member of the
Cheilinini and *Bodianus* and *Clepticus* are in the tribe *Hypsigenyini*. *Xyrichtys* is in the Novaculinini and *Halichoeres* and *Thalassoma* are lumped into the Julidini. The distinction along generic lines based on trunk canal morphology in labrids (Webb, 1990) supports the grouping of *Bodianus* with *Clepticus*, *Halichoeres* with *Thalassoma*, and *Xyrichtys* with *Doratonotus*. The relationships between the tribes are not known and it is not certain whether the Labridae is a monophyletic family.

**Putative characters based on the morphology of the sensory systems and settlement behavior in some members of the Labroidei.**

In the following, I list 12 putative characters related to sensory development and settlement in labrid and scarid fishes. For the reasons described above, these characters cannot be polarized and ultimately may not be informative in future phylogenetic studies of these fishes.
Character 1. Larval duration

Maximum duration in *Scarus* is less than 31 days and in *Sparisoma* between 32-69 days. For the labrids, those durations were: *D. megalepis*, and *H. bivittatus*, less than 31 days; *T. bifasciatum* and both *Xyrichtys*, over 70 days; and all other *Halichoeres* species, *B. rufus* and *C. parrae*, intermediate larval durations between 32-69 days. (Table 4.1) Most reef fishes have a larval duration of 20-30 days (Victor, 1991).

Character 2. Specificity of settlement site

As defined earlier in this chapter those species that were found in a variety of microhabitats were considered generalists. This is true even if they were usually associated with particular structures within these habitats. These species include *T. bifasciatum* and the *Halichoeres* species. A specialist is defined as a species found in a limited area with well-defined microhabitat characteristics not found over a large part of the reef. These species include *C. parrae*, *D. megalepis*, *B. rufus* and both *Xyrichtys* species. (Table 5.1). Both scarid genera settle only into seagrass beds and are considered specialists.
Character 3. Settlement behavior

Settlement was considered direct if no burial occurred at settlement and the larva moved directly from the plankton to its juvenile habitat. Labrid species with direct settlement were *B. rufus*, *C. parrae* and *D. megalepis*. Labrids that concealed themselves on the reef either by burial in sediment or hiding in crevices at settlement and did not emerge until after several days include all the *Halichoeres* species, *T. bifasciatum* and both species of *Xyrichtys* (Chapter 5). Direct settlement occurs in scarids.

Character 4. Number of nasal neuromasts

Both recently-settled juvenile and settlement-stage larval states of this character were used as preservation in some larvae was not sufficient to preserve the free neuromasts. These were not preserved in the scarids. Labrid species possessing five free neuromasts around the anterior nare were *B. rufus* (Fig. 2.18B), *D. megalepis* (Fig. 2.18D), all *Halichoeres* species (Figures 2.8B, 2.10D, 2.12A, 2.15A, 2.16D) and *T. bifasciatum* (Fig. 2.2D). These were arranged in the same pattern in all of these species. Both *Xyrichtys* species had up to six nasal line neuromasts but individuals varied in having from 4-6 (Figures 2.20B, 2.23A) and
C. parrae (Fig. 2.21B) have eight. Neuromasts may be added with growth as adult
T. bifasciatum have eight (Fig. 2.4A), but the arrangement is distinct from that in
C. parrae juveniles.

Character 5. Developmental state of the cephalic lateral line canals at settlement

The infraorbital line is the last to become enclosed in a canal during
development. At settlement all other canals were formed in all of the labrid
species. At settlement the first two free neuromasts of the infraorbital line were
enclosed in all labrid species except T. bifasciatum (Fig. 2.2B). In Sparisoma sp.
(Fig.2.24B) only the preopercular and mandibular canals were enclosed and in
Scarus sp. (Fig. 2.24A) none of the canals were enclosed at settlement.

Character 6. Number of free neuromasts

D. megalepis had an unusually high number of free neuromasts at
settlement compared to other labrid species (Fig. 2.19A). This state may be an
autapomorphy in Doratonotus. Free neuromasts were not preserved in scarid
samples.
Character 7. Length of anterior nare tube

The anterior nare of settlement-stage larvae may consist of a tube-like epidermal structure. At settlement this structure had the form of a long, thin walled tube in the *Xyrichtys* species (Fig. 2.22B). In *D. megalepis* larvae and *Chromis* sp. it appeared as a short ring-like structure (Fig. 2.8C). In all other labrids it was a tube with thick walls of intermediate length e.g. (Fig. 2.5D). The nares were not complete in scarids at settlement so their state could not be determined. *D. megalepis* juveniles have intermediate length tubes so the short length in larvae of *D. megalepis* represents an earlier developmental state and *D. megalepis* is apparently at an earlier point of development in this structure at settlement.

Character 8. Flap over posterior nare

Some settlement-stage larval and recently-settled juvenile labrids possess an epidermal flap over the posterior nare. Those species possessing this flap are all the *Halichoeres* (Figures 2.7A, 2.10D, 2.12A, 2.15A, 2.16D), species and *T. bifasciatum*. This flap is absent in *B. rufus* (Fig. 2.17B), *C. parrae*, *D. megalepis* (Fig. 2.8C) and scarids.
Character 9. State of completion of nares at settlement

The anterior and posterior nares form when the two sides of the common olfactory pit close over the top of the pit forming two openings. A completed structure consisting of the anterior and posterior nares is found in all of the settlement-stage larvae of all of the labrid species. The anterior and posterior nares are not completely formed at settlement in the scarids (Fig. 2.24A).

Character 10. Relative visual acuities of settlement-stage larvae

Visual acuity was designated as low if greater than 80 minutes of arc. Only Scarus had acuity this low. Acuity less than 60 minutes of arc was designated as high and was found in all of the Halichoeres and Xyrichtys species. Sparisoma sp. and all the other species of labrids had intermediate acuities (Fig. 4.4).
Character 11. Presence of rods before settlement

Rods were found only in the retinae of *D. megalepis* at settlement. They are either absent in all the other species or in numbers too low to be unequivocally discerned.

Character 12. Density of microvillous receptors

The only structures of the olfactory organ whose densities were consistently observed among individuals within a genus were the microvillous receptors. Densities were designated as high (14-2/μm), medium (8-13/μm) or low (0-7/μm). *D. megalepis*, *T. bifasciatum* and *Scarus* sp. had high densities of microvillous receptor cells. *B. rufus*, all *Halichoeres* species and *Xyrichtys* had intermediate densities. *C. parrae* had low densities of microvillous receptors. Densities in *Sparisoma* sp. could not be determined.
The relationship between sensory development, size of settlement-stage fishes, settlement behavior and settlement strategies

Characteristics of development and behavior within a group of fishes may represent a life history strategy that has led to the evolutionary success of that group. There appear to be two separate life history strategies among the labrids and scarids studied. *Thalassoma*, *Xyrichtys* and *Halichoeres* are characterized by most or all of the following: they have long pelagic durations (*Thalassoma* and *Xyrichtys*), slower developmental rates (*Thalassoma* and *Xyrichtys*), burial upon settlement (all three genera) and are habitat generalists (*Halichoeres* and *Thalassoma*). The opposite strategy is represented by *Doratonotus* and *Clepticus* and is characterized by the following: a shorter larval duration (*Doratonotus*), a faster rate of development (both), no burial at any life stage (both), and habitat specialization (both). (*Table 5.1* describes the settlement habitats of the early juveniles of these species).

It has been assumed that species that settle to the reef at a small size are relatively undeveloped. Fishes that are small at settlement have been observed to settle into shallow back reef areas, a behavior which is postulated to have evolved because predation on these vulnerable larvae is lower in these areas (Shulman, 1985; Victor, 1991). This is not observed in the labrids. Small size and
short larval duration are not correlated to a less developed state at settlement in labrids. Settling larvae of *D. megalepis* were the smallest and had the shortest larval duration among the labrids studied (Victor, 1986a; present study). However, they have among the highest densities of microvillous and ciliary receptors, have well developed canal systems and the highest number of free neuromasts among the labrids, and are the only species examined to unequivocally possess rods at settlement. This species is not believed to settle into sediment at settlement, as do other labrids but appears to move directly from the plankton into its juvenile habitat. Contrary to being undeveloped at settlement, the sensory structures of *D. megalepis* are more developed than in the other labrids at settlement and attain the minimum sensory development necessary for settlement over a shorter period (larval duration) and at a smaller size. *Halichoeres* species do not attain the level of sensory development which *D. megalepis* possesses at settlement until they are early juveniles.

Sensory development within a species does seem to be correlated to the size of the individual (Lara, 1992) but at least in labrids those that settle at a larger size are not necessarily more developed. These large settlers may actually need to attain a large size at settlement because their sensory systems are developing more slowly and are not sufficiently developed until they have reached a large size. Fish species with larvae that are found farther offshore tend
to have longer larval durations and also tend to be larger at settlement (Victor, 1991). Offshore transport of larval stages is believed to be a mechanism that allows them to be transported farther away from the site where they were spawned. This is believed to be a strategy to increase geographic dispersal and to carry larval fishes away from reef associated predators. *Doratonotus* and other fishes which settle at a small size may not be advected far offshore (Richards, 1984; Victor, 1991) but may be retained within the reef system during their entire larval duration. This may be a favorable strategy for species that develop rapidly and can settle at a small size after a relatively short time. Spending a long period offshore, rather than being solely an adaptation for dispersal, may actually be necessary for some slowly developing species to provide them with enough time away from reef associated predators to attain the developmental state necessary for settlement.

As for developmental state after settlement, labrids may employ a variety of strategies. *D. megalepis* settles with slightly more developed sensory systems than the other labrids and into an area that provides good concealment. These factors may increase survival after settlement without the need for burial. On the other hand the early juvenile habitats of all of the *Halichoeres* species consist of areas where there is little cover or refuge. The juveniles of *Halichoeres* are brightly colored and very conspicuous on the reef. As a result, these fishes could be seen
easily in these flat areas. Juvenile labrids are difficult to capture; they are quick and it is impossible to approach them undetected. Perhaps the period of burial allows locomotor and sensory abilities additional time to develop so that the emergent juvenile is well equipped to survive in these exposed habitats in which other fishes would be quite vulnerable. In fact, there are few other fishes in these areas aside from labrids and scarids.

Scarids appear to have an alternative settlement strategy. At settlement the scarids were very small compared to the labrids and their degree of sensory development resembled that of the earlier larval stages of labrids. They were never observed to burrow upon settlement and when introduced into a bare aquarium, settled to the bottom and began to acquire juvenile pigmentation in less than 24 hours. Recently-settled juvenile scarids were always captured in seagrass beds. Their post-settlement growth rate appears to be extremely slow (David L. Jones, unpublished data). Scarids settle into areas providing good concealment while still in a larva-like stage and slowly acquire better sensory and locomotor skills after settlement.

These two strategies, represented by the differing state of sensory development at settlement in labrids vs. scarids, might represent an evolutionary trade off. Fishes that are more developed at settlement will be better able to find
food and avoid predation in the juvenile environment, however they would have
to have acquired these abilities by the time conditions were favorable for
settlement, possibly leading to a missed opportunity to settle. Fishes with a
slower rate of sensory development would be less prepared for the perils of life
on the reef. However, if a good refuge were available to them at settlement it
would be less crucial to have attained a high sensory ability by that time and
their window of opportunity to settle would be larger. Some labrids avoid the
problems posed by either strategy. Some species have extended larval durations
during which they are competent to settle for an extended time and which may
allow them more opportunity to settle (Victor, 1986), and some conceal
themselves underground while their sensory systems continue to develop.

The sensory development discussed here is not necessarily reflected in the
length or weight of the fish. Obviously suboptimal conditions such as lack of
food will affect all growth and metabolic processes but there is evidence that the
sensory systems will continue to develop even though growth in size is slowed
(Lara, 1992). Therefore, larger size cannot be equated with better sensory
development. In fact, labrid species that are more developed at settlement tend
to be smaller. Labrids that bury themselves for up to a week at settlement are not
believed to feed during this period, and recently emerged juveniles are the same
length or smaller than pre-settlement larvae, though their sensory systems are
more developed. In the laboratory, larvae of *Paralichthys californicus* that are starved show a marked decrease in growth rate but a comparably slight decrease in their rate of sensory development (Lara, 1992).

In conclusion, in view of the life history strategy hypothesis discussed earlier in this section, *Thalassoma, Halichoeres* and *Xyrichtys* have long larval durations, burrow at settlement and emerge with highly developed sensory systems. *Xyrichtys* and *Thalassoma* also have the ability to extend their larval durations. These are all characteristics that would favor long range dispersal and increase the chances of survival during settlement and early juvenile life. *Doratonotus, Bodianus* and *Clepticus* have shorter larval durations, there is no indication that they can extend these larval durations, they do not burrow at settlement and they settle with more highly developed sensory systems. The first two characteristics favor higher retention of larvae within an area. If the absence of burrowing at settlement is a primitive condition that functions as an evolutionary constraint on certain labrid taxa, then the acquisition of a highly developed sensory system may have evolved as an alternative strategy for settlement in the first group. These alternative life history strategies may have been selected for separately in *Thalassoma-Halichoeres-Xyrichtys* and in *Bodianus-Doratonotus-Clepticus* and may be a suite of characters, which can help elucidate the evolutionary history of the labrid genera.
Table 5.1 General descriptions of occurrence and settlement habitats for 11 species of early juvenile Caribbean labrids.

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>Other references to habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodianus rufus</td>
<td>Solitary. Usually in 5-20m of water. Around large coral heads.</td>
<td>Do not burrow. Found near large coral heads (such as Montastrea cavernosa) located at the offshore ends of coral spurs. Solitary or small groups fairly high in water column (3).</td>
</tr>
<tr>
<td>Clepticus parrae</td>
<td>Edge of reef, over large coral heads at ends of spurs, in 10-20m deep water. Heterospecific groups of similar sized individuals and gobies. Near conspecific groups of larger individuals.</td>
<td></td>
</tr>
<tr>
<td>Doratonotus megalepis</td>
<td>Mixed Thalassia and Syringodium beds. 2m deep water.</td>
<td></td>
</tr>
<tr>
<td>Halichoeres bivittatus</td>
<td>Ubiquitous over reef flat sand/coral rubble, depths from 1-5m.</td>
<td>&lt;5m depth. Sargassum beds, Thalassia beds and hard reef areas, including sandy bottoms with scattered coral growth (2). Swimming low over sand/rubble substrates (3).</td>
</tr>
<tr>
<td>Halichoeres garnoti</td>
<td>Ubiquitous. Near bottom of branching corals and sponges at edge of reef patches. Around rubble, coral boulders, branching coral in boulder strewn sand/algae flats. In clumps of Eucheuma isiforme. 2-10m depth</td>
<td>Solitary and in mixed schools near sand/coral interface (3).</td>
</tr>
<tr>
<td>Halichoeres maculipinna</td>
<td>Around rubble in seagrass beds. 2-3 m depth.</td>
<td>Early juveniles solitary, very low in coral crevices. Later juveniles remain solitary (3).</td>
</tr>
<tr>
<td>Halichoeres pictus</td>
<td>Rarer than H. garnoti. In mixed schools of similar sized H. garnoti, other labrids, scarids and gobies. Also around bottoms of coral heads and sponges at edges of patch reefs. Also among clumps of algae Udotea flabellum and Dictyopteris delicatula.</td>
<td>Small schools (some mixed), relatively high in water column near areas of high vertical relief (3).</td>
</tr>
<tr>
<td>Thalassoma bifasciatum</td>
<td>Reef flat, coral rubble/sand. Associated with barrel sponges or empty conch shells. In areas of more extensive coral coverage associated with coral heads. Mixed size groups with older juveniles and adults, including other labrid species such as H. garnoti. 2-15m depth.</td>
<td>&lt;2ft depth. Flat non-living surfaces (1). Associated with coral reef areas and rocky outcrops (2). Early juveniles very low in coral crevices. Older juveniles progressively higher in water column, forming progressively larger schools (3). Mixed size schools close to substratum (4). Associated with anemone Condylactis gigantea (5).</td>
</tr>
<tr>
<td>Xyrichtys splendens</td>
<td>Bare rubble/seagrass in more protected channel than X. martinicensis. Also in clumps of algae Acanthophora spicifera.</td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2 Sizes (total length, TL; standard length, SL; estimated head length) of some fishes at the time of initial cephalic lateral line canal formation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Body Length</th>
<th>Head Length</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichiurus lepturus</td>
<td>Trichiuridae</td>
<td>51.0 mm TL</td>
<td>NA</td>
<td>Kawamura and Munekiyo, 1989</td>
</tr>
<tr>
<td>Pleuronectes platessa</td>
<td>Pleuronectidae</td>
<td>10.0 mm SL</td>
<td>2.8 mm</td>
<td>Neave, 1986</td>
</tr>
<tr>
<td>Scophthalmus maximus</td>
<td>Bothidae</td>
<td>7.3 mm SL</td>
<td>3.2 mm</td>
<td>Neave, 1986</td>
</tr>
<tr>
<td>Cottus bairdi</td>
<td>Cottidae</td>
<td>NA</td>
<td>2.4 mm*</td>
<td>Jones and Janssen, 1992</td>
</tr>
<tr>
<td>Sparus macrocephalus</td>
<td>Sparidae</td>
<td>9.5 mm SL</td>
<td>NA</td>
<td>Su and Wang, 1990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.5 mm SL*</td>
<td>~4.0 mm*</td>
<td>Su and Wang, 1990</td>
</tr>
<tr>
<td>Clupea harengus</td>
<td>Clupeidae</td>
<td>28.0-30.0 mm TL</td>
<td>2.4 mm*</td>
<td>Blaxter, 1991</td>
</tr>
<tr>
<td>Cyprinus carpio</td>
<td>Cyprinidae</td>
<td>25.0 mm SL</td>
<td>~4.0 mm</td>
<td>Appelbaum and Riehl, 1997</td>
</tr>
<tr>
<td>Doratonotus megalepis</td>
<td>Labridae</td>
<td>7.4-8.5 mm SL</td>
<td>~2.5 mm*</td>
<td>This study</td>
</tr>
<tr>
<td>Thalassoma bifasciatum</td>
<td>Labridae</td>
<td>12.5-13.5 mm SL</td>
<td>~3.5 mm*</td>
<td>This study</td>
</tr>
<tr>
<td>Xyrichtys sp.</td>
<td>Labridae</td>
<td>10.5-11.5 mm SL</td>
<td>~2.5 mm*</td>
<td>This study</td>
</tr>
<tr>
<td>Halichoeres sp.</td>
<td>Labridae</td>
<td>10.3-13.0 mm SL</td>
<td>~3.0 mm*</td>
<td>This study</td>
</tr>
</tbody>
</table>

*Most canals formed
Literature Cited


Kunz, Y. W. 1987. Tracts of putative ultraviolet receptors in the retina of the two-year-old brown trout (Salmo trutta) and the Atlantic salmon (Salmo salar). Experientia 43: 1202-1204.


VITA

MONICA R. LARA
