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Growth, Development and Visual Ontogeny of Two Temperate Reef Teleosts *Pagrus auratus*, (Sparidae) and *Forsterygion varium*, (Tripterygiidae).

Patricia Melva Pankhurst.

For Sam and Michael
Arohanui
Abstract

Growth, development and behaviour were examined in artificially reared larval Pagrus auratus and Forsterygion varium, from the time of hatching. Yolk-sac larval P.auratus hatched at a small size (2.00mm SL), without functional eyes, mouth or digestive tract, and for three days spent long periods at rest. Growth was initially rapid but slowed by 3 days as yolk reserves neared depletion. By days 4-5, the mouth had opened, eyes were functional, yolk was depleted, and a rudimentary gut had formed. Larvae were now able to maintain a horizontal swimming mode and were actively searching for and attacking prey. First feeding was observed in some larvae. Growth was retarded during the transition from endogenous to exogenous nutrition and then increased as feeding proficiency improved. Yolk-sac F.varium hatched at a larger size (4.78mm SL), with functional eyes and jaws. Larvae were able to maintain a horizontal swimming mode from hatching. First feeding was observed from the first day after hatching. F.varium larvae grew steadily from the time of hatching.

Ocular morphology was examined in larval, juvenile and adult P.auratus and F.varium. There was a 96 fold increase in eye size, from 0.23mm diameter in a 4 day old larval P.auratus (3.4mm SL) to a maximum diameter of 22mm in an adult of 333mm body length. F.varium displayed a 26 fold increase in eye size, from 0.28mm diameter in the smallest larva (5.00mm SL) to a maximum eye diameter of 7.2mm in a 110mm long adult. Larval fish had pure cone retinae, however putative rod precursor cells were present from hatching in F.varium and from 18 days in P.auratus. Juvenile and adult fish had duplex retinae with cones arranged in a square mosaic in which 4 twin cones surround a central single cone. Hypertrophy of cone ellipsoids with increasing eye size, resulted in maintenance of a closely packed array in fishes of all sizes. The appearance of retinomotor movements was coincident with the development of a duplex retina in both species.

Theoretical spatial acuity (calculated as a function of cone spacing and focal length of the lens) was poor in the smallest larval fish (2° 1’ and 1° 8’ minimum separable angle
in 4 and 1 day old *P. auratus* and *F. varium* respectively) but improved to asymptotic values in adults (3'- 4', and 9' in *P. auratus* and *F. varium* respectively). Behavioural acuity (determined using the optokinetic response) of 4 day old larval *P. auratus* (37° 30') and 1 day old *F. varium* (29°) was very much lower than histological estimates. Behavioural acuity improved to 8° 8' in 16 day old *P. auratus* and 4° 18' in 14 day old *F. varium*, but did not attain theoretical estimates for fish of that size (55' and 54').

A rudimentary retractor lentis muscle was first apparent in larval fish 1 week after hatching, and was coincident with the formation of a posterior lentis space. Presumably larval fish eyes were incapable of accommodative lens movements until this time. A relative measure of Matthiessen's ratio (distance from lens centre to boundary of the pigmented retinal epithelium/lens radius) measured histologically, decreased from 4.2 and 2.7 in 3 day old *P. auratus* and newly hatched *F. varium*, to 2.2 and 2.3 in larvae 22 and 16 days of age respectively. This suggests that growth of the retina and lens were not symmetrical in the eyes of very small larval fish. If Matthiessen's ratio holds for little eyes, then they will initially be strongly myopic. This may account in part for the mismatch between behavioural and theoretical acuity.

Perceptive distances of first feeding larval *P. auratus* and *F. varium*, estimated for prey items equal in dimensions to maximum jaw widths, were very small (0.2mm and 0.4mm for prey 0.15mm and 0.2mm in size respectively), but increased with increasing body size to 2.1mm and 4.0mm for prey 0.3mm in size, at 16 and 14 days of age respectively. These data have implications for larval feeding in the wild.
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General Introduction

Teleosts which are pelagic spawners characteristically produce many small eggs, from which larvae hatch at an early stage of development after a relatively short incubation period. No parental care is given and eggs and larvae are left at the mercy of the oceans. In contrast, substrate spawners often produce fewer larger eggs, and may provide parental care at some stage during the early life history stages (Balon, 1984). Larvae of larger eggs generally hatch at a larger size and are more developed at the time of hatching. As a consequence, larger larvae are able to feed sooner than small larvae, and the generally larger yolk volume of larger sized larvae bestows considerable advantage in terms of a longer period in which to establish exogenous feeding (Miller et al., 1988). In the present study two species of marine teleost fishes, one a pelagic spawner *Pagrus auratus* (Sparidae), and the other a substrate spawner *Forstergion varium* (tripterygiidae), were chosen to compare the growth and development of the larvae as examples of pelagic and substrate spawned teleost fishes.

*P. auratus* (commonly known as snapper), is commercially fished in New Zealand and Australian waters. Previously named *Chrysophrys auratus* in Australia and New Zealand, and *Pagrus major* (red sea bream) in Japan, snapper have been recently designated as a single species *Pagrus auratus* (Bloch and Schneider) with independent and reproductively isolated populations in Japan and Australasia (Paulin, 1990). Snapper are large demersal carnivores, which are found in coastal waters and estuaries, and in New Zealand, snapper reach lengths of approximately 70cm - 80cm, and ages of 55-60 years (Horn, 1986). In northern New Zealand, sexual maturity is reached at 3-4 years of age, at a length of 20-23cm (Scott, 1991). Snapper are daily spawners, and females produce up to six million small pelagic eggs during a spawning season which lasts from October to January (Crossland, 1977; Crossland, 1981; Scott, 1991). Egg staging data suggest that broadcast spawning occurs in the late afternoon and early evening (Scott, 1991), and no parental care is given thereafter. Snapper eggs are 0.8 - 1.0mm in diameter, with a yolk diameter of approximately 0.8mm. Poorly developed yolk-sac larvae
hatch out after 36-45h at 20-18°C (Cassie, 1956). There are no validated data regarding the duration of the larval planktonic phase in New Zealand snapper, however, in Japanese red sea bream, the larval phase lasts for about 30 days after which juveniles migrate from offshore oceanic waters and take up a demersal habit in enclosed bays and estuaries (Tanaka, 1985; Fukuhara, 1991).

*F. varium*, the variable triplefin, is a small, diurnal, benthic teleost, found on shallow rocky reefs along the entire New Zealand coastline. *F. varium* is short lived (up to three years of age) and attains maximum lengths of 115mm (males) and 105mm (females) in northern New Zealand. Both sexes reach maturity within the 1st year, at a mean SL of 67.8mm (females) and 71.4mm (males). The spawning period lasts from May to October, during which time between 200 and 3,000 eggs per female are laid, in repeat spawning episodes. Eggs (1.12 - 1.30mm diameter, with yolk diameter of 1.1mm) are laid in "nests" on broken rock surfaces with 1-4 females contributing to each "nest" (Thompson, 1979; 1980). Male triplefins guard the nests during the incubation period which lasts between 7-20 days, after which well developed eyed larvae, hatch (Thompson, 1979, 1980; Ruck, 1980). Each male may brood as many as 14 successive nests during the spawning season. Larval triplefins are found in close proximity to coastal reefs (Kingsford and Choat, 1989), and juveniles settle out onto the reefs from August to December, approximately 3 months after hatching (Thompson, 1979).

Growth in length, morphological development, and the ontogeny of behaviours, are examined in laboratory reared larval snapper and triplefins from the time of hatching to 28 and 16 days of age respectively. Snapper and triplefin larvae, like other larval fishes, must make a transfer from yolk nutrition to exogenous feeding a short time after hatching. Feeding is thought to be visually mediated, at least in the earliest larval stages of most teleost fishes (Blaxter, 1986) and if this is so, the eyes of larval snapper and triplefins must be functional at the commencement of feeding. With a few exceptions, larval fishes have pure cone retinae at the time of first feeding and this will limit feeding to the photophase (Blaxter, 1986). The very small eye size of larval fishes places constraints upon visual acuity. This is a consequence of small eye and lens size (with short focal
length) resulting in a lower number of cones per visual angle than in larger eyes (Johns and Easter, 1977). Further improvement in spatial acuity, by increasing cone density and decreasing cone size, is limited by constraints set by the size at which light spills into adjacent photoreceptors (Lythgoe, 1980). Consequently visual acuity of larval fishes during the first feeding period is very low (Neave, 1984) and this is reflected in the very short food perception distances of some larval fishes at the onset of feeding (Blaxter and Staines, 1971). The eyes of fishes continue to grow throughout life, and it is largely a consequence of increasing eye size that visual acuity improves during growth (Johns and Easter, 1977; Johns, 1981). In the present study visual morphology was examined with increasing eye size in larval, juvenile and adult *P. auratus* and *F. varium* to examine changes in spatial visual acuity, estimated from cone spacing within the retina, and focal length of the lens. Histological estimates of visual acuity in larval snapper and triplefins are compared with behavioural acuity, determined using the optokinetic response. Particular attention has been paid to the ontogeny of visual function in view to the ramifications for feeding competence of *P. auratus* and *F. varium* during the critical first feeding period.
Chapter One

Growth, Development, and Behaviour of Artificially Reared Larval Pagrus auratus and Forsterygion varium.

Introduction

Teleost larvae have limited yolk at hatching and must make a successful transfer from yolk nutrition to exogenous nutrition soon after hatching (Blaxter, 1969; Vladimirov, 1975; Li and Mathias, 1987). The timing of first possible feeding is determined by the functional development of the organ systems (eyes, jaw structure, digestive tract, motor systems) and also development of behaviours involved in feeding. Larvae which hatch at a large size are generally more developed than small larvae, and this greater size bestows considerable advantage in terms of flexibility in the timing of first feeding. Larvae of a greater size are able to feed sooner. In a review Miller, et al., (1988) showed that time to first possible feeding was a negative function of larval size at hatching, such that the smallest species examined (white croaker, 1.8mm at hatching) fed after 3 days (d) and the largest species (garfish, 13.5mm at hatching) fed only 5h after hatching. In addition they reported that the time to "irreversible starvation" (also called "point of no return", Blaxter and Hempel, 1963; Yin and Blaxter, 1987), when starving larvae were unable to feed even when offered food, was a positive function of larval size at hatching. The generally greater yolk volume of large larvae provides more time to find food, and initiate feeding before the onset of "irreversible starvation". Small larvae on the other hand, are more susceptible to starvation because of their more limited yolk reserves and therefore shorter period (termed a "window of opportunity", Miller et al., 1988) in which to initiate feeding. In addition, the relatively small jaw gape of smaller larvae places constraints upon the size range of food available to them. This is compounded by the early ontogenetic stage of development at hatching in small larvae.

F. varium produces eggs 1.1-1.3mm diameter, from which eyed larvae 5.35-6.35mm long hatch out after an incubation period of 7-20 days and immediately swim to
the surface (Thompson, 1979, 1981; Ruck, 1980). Snapper on the other hand, produce small pelagic eggs of 0.8-1.0mm diameter (Cassie, 1956). Small (2.1 mm long), undeveloped larvae hatch out after an incubation period of 36-45h at 20-18°C (Cassie, 1956). Snapper larvae might, as a result of both their smaller size and earlier stage of development at hatching, be expected to be more susceptible to mortality associated with the transition from yolk nutrition to exogenous nutrition, than the larvae of the variable triplefin. This chapter examines the period of transition from yolk nutrition to exogenous feeding in cultured larval snapper and triplefins, in terms of morphological development, behaviour and growth.

Larval rearing requires the capacity to manipulate reproduction of captive breeder stock, and to provide starter food of an appropriate size which meets the nutritional requirements of larval fish.

**Brood stock husbandry for production of gametes**

A supply of viable fertilized eggs is central to larval culture and also development of mariculture. Spontaneous spawning of captive parent breeder stock is the most desirable option as it involves guaranteed supply with minimal handling (low stress effects of fish) and eliminates the need for labour intensive strip spawning and fertilization techniques. Manipulation of environmental variables (temperature and photoperiod) has been successfully used to induce natural synchronized spawning in some captive species. A 150 day photoperiod and temperature regime established for red drum breeder fish, allows for year round supply of eggs (Colura, *et al.*, 1991). In red sea bream the spawning season can be advanced and extended by 1 month by elevation of tank temperatures.

However, some species may not spawn in captivity under any conditions. Oocytes of some species (for example, goldfish, carp, gilthead sea bream, and sea bass), may develop to the final stages of vitellogenesis and then undergo rapid atresia. In others, (northern pike, grey mullet) sperm production is inhibited in captive fish (Zohar, 1989). Where lack of spawning is due to the failure of the fish to ovulate (in turn due to absence
of a preovulatory gonadotropin (GTH) surge), hormone manipulation can be used to induce final oocyte maturation and ovulation. Artificially ovulated fish may then spawn spontaneously, or handstripping and artificial fertilization may be required.

Older maturational techniques (reviewed by Zohar, 1989) generally used injection of pituitary extracts or mammalian GtH (luteinizing hormone (LH) and human chorionic gonadotropin, hCG). For example, female red drum breeder fish in the final stages of oocyte maturation, can be induced to ovulate within 24-30h at 25°C, by an intramuscular injection with hCG (500-600IU.kg⁻¹ body weight)(Colura, 1991).

More recent studies have shown that synthetic analogues of GtH releasing hormone (GnRH) are highly effective ovulatory agents. In a number of species, GtH release from the pituitary is under inhibitory control by a GtH release inhibitory factor (GRIF). Current studies suggest that GRIF is probably dopamine, with the result that efficacy of GnRH treatment is greatly increased when GnRH is administered in conjunction with a dopamine antagonist (Peter et al., 1987; Zohar, 1989).

Natural spawning of the New Zealand population of *P. auratus* has been reported in the Napier Aquarium (Smith, 1986) and Kelly Tarlton's Under Water World (pers.obsv.), indicating that spontaneous spawning does occur. There are, however, no data on the environmental manipulation of reproductive activity in captive N.Z. snapper, and no information exists regarding minimum water volume requirements for spontaneous spawning of captive fish. Spontaneous spawning does not occur in wild fish returned to the Leigh Laboratory and held in volumes of up to 5000l at densities of approximately 20 fish per m³. Mature male snapper remain spermiated for days or weeks after capture, however, freshly captured female snapper only sometimes ovulate in captivity, and then for a period of only 1-2 days post capture. At greater times after capture, wild female snapper undergo gonadal atresia and a marked fall in plasma levels of gonadal steroids (Carragher and Pankhurst, 1991). This is thought to be a result of stress imposed by capture. Stress is also known to cause falls in GtH, gonadal steroids, and the development of gonadal atresia in other species (Stacey et al., 1984; Sumpter, et al., 1987). Artificial ovulation can be induced with an intra-peritoneal (ip) injection of hCG, in
female snapper that fail to ovulate naturally on the day of capture (Pankhurst and Carragher, 1992), but ovulated fish do not spawn spontaneously. Snapper eggs for the present study were obtained both from wild fish that ovulated on the day of capture and females induced to ovulate by injection with hCG. Eggs were then handstripped and artificially fertilized.

Freshly captured triplefins on the other hand, readily set up new territories in laboratory aquaria, and undergo spontaneous spawning within a few days. In addition, triplefin nests attached to pieces of broken rock, were collected by SCUBA divers from rocky reefs in the vicinity of the Leigh Laboratory.

**Larval Fish Rearing**

The rotifer *Brachionus plicatilis*, has been used as a live feed for marine fish larvae for 25 years (Fukusho, 1991). Its dimensions make it a suitable starter food for both marine fish and crustacean larvae. *B. plicatilis* is hardy and relatively easy to mass culture. Strains of *B. plicatilis* vary in length from 120-320μm (Adult lorica length), and tolerate a wide range of environmental temperatures (3-40°C) and salinities (1-97g/kg) (Talbot et al., 1990). In Japan, two subspecies of rotifer are mass cultured for the commercial rearing of larval red sea bream. The S-type rotifer (*B. plicatilis rotundiformis*) is small (150μm lorica length) and tolerates high temperatures, and the L-type rotifer (*B. plicatilis hepatomus*) which is larger (250μm lorica length), tolerates low water temperatures (Fukusho, 1991). Microalgae, yeasts and bacteria are suitable diets for the mass culture of *B. plicatilis* (Talbot et al., 1990). Rotifers cultured on microalgae, are costly to produce but generally have a high dietary value for larval marine fish, being rich in highly unsaturated (w-3) fatty acids (HUFA)(Fukusho, 1991). *Saccharomyces cervisiae* (compressed bakers yeast), is a cheap feed for rotifers, however rotifers mass cultured on bakers yeast alone lack HUFA and must be enriched with microalgae and/or a dietary supplement high in HUFA, prior to feeding out to larval fish (Foscarini, 1988; Fukusho, 1991; Talbot et al., 1990).
Non living diets (frozen rotifers, fish meat, and artificial micro-diets) have as yet proved unsuccessful as starter feeds for larval red sea bream. Rapid decomposition of non living diets in the rearing tanks, results in the accumulation of a thick oily surface slick. This oily slick is an impenetrable barrier for red sea bream larvae, which must initially inflate their swim bladders about 5 days after hatching, by gulping air at the surface (Foscarini, 1988). Failure of cultured red sea bream larvae to inflate their swim bladders, has been implicated in increased mortality and a high incidence of the bent-spine skeletal abnormality known as lordosis (Kitajima, et al., 1981). Frozen rotifers are used after swim bladder inflation, around 15-20 days after hatching, and thereafter a hierarchy of diets is sequentially introduced including live Artemia nauplii, frozen Artemia, frozen krill, fish meat and fish eggs (Foscarini, 1988).

In the present study live rotifers, cultured on a diet of mixed microalgae, were used as a starter food for both snapper and triplefin larvae. Production of rotifers on a microalgal diet has the advantage of a high dietary value for larval fish, but is labour intensive and relatively costly to produce. Therefore a small-scale algal-rotifer culture system, which consequently limited the scale of larval fish rearing trials, was set up for the present study.

1.1 Materials and methods

1.1.1 Broodstock husbandry and egg production

Snapper

Sexually mature male and female snapper were captured by longline from the University of Auckland R.V. Proteus, in the Hauraki Gulf, New Zealand during December 1988 and November to December 1989. Fish were transported to holding tanks at the University of Auckland’s Leigh Laboratory where fertilized eggs were obtained in two ways. (1) Naturally ovulated females were stripped and eggs fertilized with milt from running ripe males. (2) Maturing but unovulated females were anaesthetized with 0.02%
2-phenoxyethanol (BDH), and injected ip with 1000U.kg\(^{-1}\) body weight hCG (Sigma), and held at ambient temperature (17-22\(^{\circ}\)C) and photoperiod in flow through 500l tanks. HCG induced ovulation within 24h in responsive fish, and eggs were stripped and fertilized as before. Fertilized eggs were incubated in brood baskets (32cm diameter) with PVC walls, and a 200\(\mu\)m mesh floor. Brood baskets were suspended in 240l aquaria with continuous water flow. Newly hatched larvae were transferred into rearing tanks.

**Triplefins**

Sexually mature male and female *F. varium* were hand netted by scuba divers from June to August 1990, from inshore rocky reefs at Leigh and Ti Point, Northland, New Zealand. Fish were transferred into 500l aquaria at the Leigh Laboratory, where they were maintained under ambient temperature and photoperiod. Fertilized eggs were obtained from natural spawning of captive broodstock, or collection by SCUBA diving, of benthic "nests" attached to broken rock surfaces. Eggs still attached to their rock substrate, were incubated in 240l aquaria with continuous water flow at ambient temperature (12.5-15.8\(^{\circ}\)C). Once hatching had commenced, all eggs were brushed off the nest, washed with filtered, UV-sterilized seawater and transferred into the larval rearing tanks. This mechanical disturbance, without which hatching success in the laboratory was low, produced mass hatching within 2-3 hours.

### 1.1.2 Larval rearing techniques

**Rotifer Culture**

The strain of *B. plicatilis* used in the present study (maximum lorica length 250\(\mu\)m) was a subculture obtained from MAFFISH, Mahanga Bay, Wellington. *B. plicatilis* were reared on concentrated cultures of microalgae, *Dunaliella* sp. and *Isochrysis galbana* which were also obtained from MAFFISH, Mahanga Bay, Wellington. Micro algae were grown in "f/2" enriched seawater medium (Guillard and Ryther, 1962)(Appendix 1), using a semi sterile culture system (Fig.1-1) under continuous fluorescent light at 20\(^{\circ}\)C (Fig.1-2). Precise determination of algal cell concentration in the production cultures proved to
Fig. 1-1. Flow diagram of the rotifer culture system.

Rotifer Culture

Stock Cultures
40 ml autoclaved f/2 medium
7 days

Intermediate Cultures
250 ml autoclaved f/2 medium
7 days

Production Cultures
6 litres U.V. sterilized, filtered sw
plus stock nutrients
3-5 days

Rotifers

Larval Fish
Fig. 1-2. Photograph of the algal-rotifer culture system.

Large carboys are production cultures of micro algae and 2 litre flasks contain rotifer stock cultures.
be unnecessary. Five to 10 ml of concentrated rotifer culture (100-200 rotifers.ml⁻¹) were added when algal cultures were too dense to see through. Rotifers were harvested when they had just begun to "clear" the algal cultures (3-5 days) and were washed in a 60µm sieve with filtered UV-sterilized sea water before feeding out to the larval fish.

Larval culture

Larvae were reared in 240l, 0.5m deep plastic tanks containing static filtered (30-50µm) UV-sterilized sea water. The tanks were gently aerated under ambient temperature and photoperiod. Dead larvae and debris were siphoned out and approximately 20% of the water volume was exchanged per day. Rotifers were introduced into the larval rearing tanks at a stocking density of 5-10.ml⁻¹, from the day of hatching (triplefins), and days 1-2 after hatching (snapper). Rotifer densities in the rearing tanks were measured daily by counting three replicate 1ml samples using a dissecting microscope, and densities were adjusted as necessary. Microalgae were added to the larval rearing tanks each day to provide food for the rotifers. Larval stocking density was not determined.

Four rearing trials of *P. auratus* and 3 rearing trials of *F. varium* larvae were conducted. Tank temperature was recorded 2-5 times per day, between the hours of 8am and 9pm. Date, duration, numbers sampled and temperature range of rearing trials for *P. auratus* are given in Table 1-1, and for *F. varium* in Table 1-2.

1.1.3 Larval growth and morphology.

Larvae were sampled randomly from the rearing tanks, with trials ending when all larvae were sampled. Snapper larvae were fixed overnight in 5% gluteraldehyde in sucrose 0.1M phosphate-phosphate buffer (pH 7.4) at 4°C, washed x3 in sucrose buffer and transferred into 70% ethanol for microscopic examination and subsequent storage. This fixation results in negligible length-shrinkage in red sea bream (*Oozeki et al.*, 1988). Triplefin larvae were anaesthetized in 0.02% 2-Phenoxyethanol(BDH) in sea water, prior to microscopic examination.
Table 1-1. Rearing conditions and sampling protocol for *P. auratus* rearing trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Date</th>
<th>Duration</th>
<th>n</th>
<th>Temp(^1)</th>
<th>Eggs(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dec-Jan 1988-1989</td>
<td>22 days</td>
<td>108</td>
<td>17.8-22.0</td>
<td>Induced</td>
</tr>
<tr>
<td>2</td>
<td>Nov 1989</td>
<td>14 days</td>
<td>442</td>
<td>17.1-19.3</td>
<td>Induced</td>
</tr>
<tr>
<td>3</td>
<td>Nov-Dec 1989</td>
<td>28 days</td>
<td>174</td>
<td>17.3-21.4</td>
<td>Natural</td>
</tr>
<tr>
<td>4</td>
<td>Dec 1989</td>
<td>16 days</td>
<td>150</td>
<td>17.0-21.7</td>
<td>Induced</td>
</tr>
</tbody>
</table>

1 Temperature range °C.

2 Egg source. a) Induced; fish were induced to ovulate with hormone treatment (see text for details), b) Natural; fish ovulated naturally.

Table 1-2. Rearing conditions and sampling protocol for *F. varium* rearing trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Date</th>
<th>Duration</th>
<th>n</th>
<th>Temp(^1)</th>
<th>Eggs(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>June 1990</td>
<td>10 days</td>
<td>88</td>
<td>14.0-15.6</td>
<td>wild</td>
</tr>
<tr>
<td>2</td>
<td>July 1990</td>
<td>12 days</td>
<td>167</td>
<td>13.5-15.1</td>
<td>wild</td>
</tr>
<tr>
<td>3</td>
<td>Aug 1990</td>
<td>15 days</td>
<td>117</td>
<td>13.0-15.3</td>
<td>captive</td>
</tr>
</tbody>
</table>

1 Temperature range °C.

2 Egg source. a) Wild; eggs spawned in the wild, b) Captive; eggs spawned in the Laboratory by captive broodstock.
Standard Length (SL, tip of snout to end of notochord) was measured with an eyepiece micrometer on a Nikon dissecting microscope. Numbers of *P. auratus* and *F. varium* sampled from rearing trials are given in Tables 1-3 and 1-4, respectively.

### 1.2 Results

#### 1.2.1 Snapper

*Morphological development and behaviour*

Snapper produced spherical pelagic eggs 0.8-1.0mm diameter (Fig. 1-3). Yolk sac larval snapper (mean SL 2.06mm) hatched 36-48h post fertilization at 18-20°C. The larvae were positively buoyant, and were held inverted at the surface with the head displaced laterally by large yolk and oil droplet reserves (Fig. 1-4a). Differentiating optic cups were just visible at this stage (Fig. 1-5a). Tail flicks associated with hatching continued for up to 12h and produced a spiral motion at the surface of the water.

By two days post hatch (mean SL 2.08mm) larvae had righted themselves, become negatively buoyant and predominantly held a "resting posture". This involved sinking slowly with head down and the body held at an angle of 45° to the water surface (Fig. 1-4b). Undulations of the body and tail produced an eccentric spiral swimming motion which periodically returned the larvae to the surface. Larvae now displayed "startle responses" to a water surface blow or when approached with a pipette. Yolk reserves were visibly reduced (Fig. 1-5b,c).

On days two to three post hatch (mean SL 3.03mm) the gut had formed a simple loop, the mouth was open and the eyes were partially pigmented (Fig. 1-6a). Yolk and oil droplet reserves were further reduced, and pectoral fins (not visible in micrographs) had started to grow. The larvae were more active but the "resting posture" was still prevalent. Body/tail undulations produced a less pronounced spiral swimming motion.

By days four to five post hatch (mean SL 3.17mm) larvae maintained a horizontal swimming position during the day, searching for and attacking prey (Fig. 1-4c). Early
Table 1-3. Numbers of fish sampled from *P. auratus* rearing trials 1-4.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
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</thead>
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<td>35</td>
<td>32</td>
<td>9</td>
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<tr>
<td>0.5</td>
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<td>40</td>
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<td>0</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
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<td>28</td>
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* Starved larvae
Table 1-4. Numbers of fish sampled from *F.varium* rearing trials 1-3.

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<td>15</td>
<td>0</td>
</tr>
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<td>0</td>
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</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>13</td>
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</tbody>
</table>
Fig. 1-3. Pelagic eggs of *P. auratus* (diameter 0.8-1.0mm) a) late stage eggs, and b) at hatching.
Fig. 1-4. Behavioural features of cultured snapper larvae.  

a) At hatching larvae were positively buoyant, and were held inverted at the surface with the head displaced laterally by large yolk and oil droplet reserves.

b) Larvae 1-2 days post hatching, predominantly held a "resting posture" which involved sinking slowly with the head down and the body held at an angle of 45° to the water surface.

c) Larvae 4-5 days post hatching maintained a horizontal swimming position during the day, and actively searched for, and attacked prey.
Fig. 1-5. Cultured *P. auratus* larvae. a) newly hatched, b) 1 day post hatching, and c) 2 days post hatching. G - gut, OD - oil droplet, y - yolk. Scale bar is 0.5mm.
Fig. 1-6. Cultured *P. auratus* larvae. a) 3 days post hatching, and b) at first feeding (4 days old). G - gut, L - liver, OD - oil droplet, R - rotifer.
Scale bar is 0.5mm.
attempts at feeding involved the behaviours of a) swim-search, b) stop and visually fix on prey, and c) lunge-snap at prey. At night they returned to the "resting posture". The pectoral fins were greatly enlarged, the eyes were fully pigmented and the yolk absorbed. A small residual oil droplet remained. First feeding (ingested rotifers in gut) was seen in some larvae (Fig. 1-6b). Initial swim bladder inflation occurred at this time.

By six days post hatch (mean SL 3.2mm) larvae were still near the surface. The oil droplet was completely absorbed.

During days seven to eight post hatch larvae were distributed throughout the tanks. Successfully feeding larvae had thickened guts and enlarged livers (Fig. 1-7a). The feeding repertoire now involved a) swim-search, b) stop-fix on prey, c) reject prey or d) contraction of the body into an "S" bend, followed by a propulsive lunge and snap at prey. There was a substantial die off in all trials at this time. This coincided with the time of death in rearing trials where larvae were not fed. Starved larvae became moribund and maintained the "resting posture" except when approached with a pipette or probe. The gut and liver of starved larvae were shrunken and the body emaciated (Fig. 1-7b).

By 14-24 days post hatch there was increased somatic growth (Fig. 1-8a,b). Tail flexion had occurred by 18-24 days and fin ray elements were apparent (not visible in micrographs). Larvae were feeding throughout the tank during the day, returning to the "resting posture" at night. Larvae grew to a maximum SL of 6.3mm by day 28.

**Growth**

Early growth of larval snapper (Fig.'s 1-9 to 1-12) could be divided into 3 phases. (1) A phase of wholly endogenous nutrition lasting for 3 days. This was characterized by an initial rapid length increase, prior to mouth opening and first feeding. (2) An intermediate phase of transition from endogenous to exogenous nutrition occurred from three to six days and was characterized by retarded increase in length that coincided with depletion of yolk and oil droplet reserves, mouth opening, eye pigmentation, and swim bladder inflation. (3) A third phase of accelerated growth occurring from about 8 days onward in
Fig. 1-7. Cultured *P. auratus* larvae. a) 7 days post hatching, and b) starved larva 7 days post hatching, with emaciated liver (L) and gut (G). Scale bar is 0.5mm.
Fig. 1-8. Cultured *P. auratus* larvae. a) 14 days post hatching (scale bar is 0.5mm), and b) 24 days post hatching (scale bar is 1mm). G - gut, L - liver, SB - swimbladder.
Fig. 1-9. a) Growth of cultured larval *P. auratus* from rearing trial 1. Values are mean ± or - SE (n values are given in Table 1-3). Arrows indicate timing of 1) mouth opening, 2) eye pigmentation and yolk absorption, 3) oil droplet absorption, and 4) death of starved larvae. 
b) Mean daily temperatures for rearing trial 1.
Fig. 1-10. a) Growth of cultured *P. auratus* from rearing trial 2. Values are mean ± SE (n values are given in Table 1-3). Arrows indicate timing of 1) mouth opening, 2) eye pigmentation and yolk absorption, 3) oil droplet absorption, and 4) death of starved larvae.

b) Mean daily temperatures for rearing trial 2.
Fig. 1�. a) Growth of cultured larval *P. auratus* from rearing trial 3. Values are mean + or - SE (n values are given in Table 1-3). Arrows indicate timing of 1) mouth opening, 2) eye pigmentation and yolk absorption, 3) oil droplet absorption, and 4) death of starved larvae. b) Mean daily temperatures for rearing trial 3.
Fig. 1-12. a) Growth of cultured larval *P. auratus* from rearing trial 4. Values are mean + or - SE (n values are given in Table 1-3). Filled circles are fed larvae and open circles are starved larvae. Arrows indicate timing of 1) mouth opening, 2) eye pigmentation and yolk absorption, 3) oil droplet absorption, and 4) death of starved larvae. b) Mean daily temperatures for rearing trial 4.
fed larvae, with nutrition being entirely exogenous. Daily growth rates and regression coefficients during this phase of entirely exogenous growth are given below in table 1-5.

**Table 1-5.** Daily growth rates and linear regression coefficients for larval *P. auratus* rearing trials 1 to 4, during the phase of exogenous feeding.

<table>
<thead>
<tr>
<th>Trial</th>
<th>r</th>
<th>Growth (mm/day)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td>0.55</td>
<td>0.08</td>
</tr>
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<td>3</td>
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</tr>
<tr>
<td>4</td>
<td>0.76</td>
<td>0.10</td>
</tr>
</tbody>
</table>

1.2.2 Triplefins

*Morphological development and behaviour*

*F. varium* produced spherical benthic eggs (mean diameter 1.1mm) which attached to rock substrate by sticky tendrils. Late stage eggs contained eyed embryos (Fig. 1-13) which hatched out after an incubation period of approximately two weeks at 12.5-15.8°C, and immediately swam to the surface.

Newly hatched larvae (mean SL 4.78mm) had large pectoral fins, a functional mouth, oil droplet and large yolk, and a long straight gut (Fig. 1-14a). In addition, larvae had a spherical "green organ" (not visible in photomicrographs) adjacent and posterior to the yolk sac, and red pigmented embryonic blood. The red blood pigmentation disappeared by day 1 post hatching. First feeding was observed at this time. Larvae were constantly active during the day, searching for and attacking prey. The larval feeding repertoire involved a) swim-search, b) stop and fix on prey with a pronounced side to side or forward and back movement, c) reject prey, or d) contraction of the entire body into an "S" bend, followed by a propulsive lunge and snap at the prey. Larvae responded with "startle responses" to a surface blow or approach with a probe or pipette.
Fig. 1-13. Benthic eggs of *F. varium* (mean diameter 1.1mm) with late stage, eyed embryos (14 days gestation).
Fig. 1-14. Cultured *F. varium* larvae. a) newly hatched, b) at six days, and c) ventral view at 6 days. G - gut, GL - gills, OD - oil droplet, R - rotifer, Y - yolk. Scale bar is 0.5mm.
By three to four days post hatch the oil droplet was absorbed in some larvae. The "green organ" was becoming clear.

By six days post hatch (mean SL 6.33mm), both the yolk and oil droplet were absorbed (Fig. 1-14b,c). In addition the "green organ" was no longer visible.

Larvae starved from hatching died on days eight and nine post hatching. Starved larvae became moribund, sinking slowly with the head down for about one day prior to death. There was no co-incident die off in the rearing trials (as observed in snapper) at this time.

_F. varium_ larvae of all ages remained near the surface of the rearing tanks day and night. Larvae grew to a mean SL of 7.9mm ± 0.12, by day 15.

**Growth**

There was little variability between trials in length/age data of triplefin larvae, and so data for rearing trials 1 to 3 were pooled. _F. varium_ larvae grew steadily from hatching (Fig. 1-15). Growth rate was 0.17 mm per day (pooled data, d0-d15), and the regression line (\( y = 5.25 + 0.17x \)) had a correlation coefficient of 0.90.

### 1.3 Discussion

#### 1.3.1 Growth

The transition from endogenous to exogenous nutrition in snapper was associated with the rapid co-ordinated development of the organs connected with the search and capture of prey. This was accompanied by slow or nil somatic growth. A similar phenomenon also occurs in anchovy larvae (Lasker _et al._, 1970), red drum larvae (Lee _et al._, 1984), American shad larvae (Wiggins _et al._, 1985), larval rabbitfish (Kohno _et al._, 1988), and is apparent (but not reported) in growth data for cultured red sea bream given by Baek (1986) and Fukuhara (1991). The period of slow growth during the feeding transition, coincided with a high rate of mortality and low feeding incidence in shad and rabbitfish larvae (Wiggins _et al._, 1985; Kohno, 1988). In red drum larvae feeding was
Fig. 1-15. Growth of cultured larval *F. varium*. Values are mean ± or -
SE for data pooled from trials 1, 2 and 3 (n values are given in Table 1-4).
Arrows indicate timing of 1) first feeding, and 2) death of starved larvae.
Temperature range was 13-15.2°C.
initiated on d3 after hatching, however larval body weight declined until after the period of yolk absorption (d4-d6) when body weight increased exponentially, presumably as feeding proficiency increased. Daily mortality data are not available for snapper in the present study, however there was a massive die off in all snapper rearing trials on d7-8 after hatching. Apparently these larvae had failed to initiate exogenous feeding as the mass mortality coincided with the time of death, in those fish which had been starved from hatching.

Widths of daily otolith ring deposition indicates that a similar period of slow growth occurs in wild snapper larvae from d7-11 (Dr J. Zeldis, MAFFish Research Division, Wellington NZ, pers. com.). The difference in the precise timing of this period of slow growth between wild and cultured fish (present study), could be explained by somewhat lower sea water temperatures in the wild (mean 17-19°C). In cultured snapper rearing trials 2 and 4 (present study), low culture temperatures at the time of feeding transition coincided with a prolonged period of slow growth.

Growth rates of larval snapper (present study), increased after the period of feeding transition. This period was also characterized in shad and rabbitfish larvae, by a rapid increase in feeding incidence and, also lower mortality (Wiggins et al., 1985; Kohno et al., 1988).

The apparent difficulty experienced by many larval teleosts in making the transition from endogenous yolk nutrition to exogenous feeding did not seem to be the case for the triplefin *F. varium*. In contrast to snapper, triplefin larvae displayed linear growth from hatching. There was also no apparent episode of mortality associated with the transition to exogenous feeding. It is difficult to ascertain whether a linear growth function from hatching occurs in other teleosts with similarly developed larvae at hatching, because growth data are often given from about the time of yolk absorption and not from hatching (Table 1-6).

Maximum growth rates of larval snapper after the period of feeding transition, were the same as the growth rate of triplefin larvae, from the day of hatching (0.17mm per day). However, growth rates of other teleosts vary considerably (Table 1-6). Growth
Table 1-6. Growth rates of cultured and wild teleost fish larvae.

<table>
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<th>Larvae</th>
<th>Temperature (°C)</th>
<th>Age (days)</th>
<th>Growth (mm.day⁻¹)</th>
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<td>7-28</td>
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<tr>
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<td>0-15</td>
<td>0.17</td>
<td>B. plicatilis.</td>
</tr>
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<td>Wild snapper²</td>
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<td>0.25-0.27</td>
<td>wild plankton.</td>
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<td><em>Tigriopus japonicus</em>,</td>
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<td></td>
<td></td>
<td></td>
<td>fish, shellfish.</td>
</tr>
<tr>
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<td>2-75</td>
<td>0.52-0.54</td>
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<td><em>Artemia salina</em>, clam.</td>
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<tr>
<td>Herring⁵</td>
<td>7-12</td>
<td>7-90</td>
<td>0.22</td>
<td><em>Balanus</em> sp. nauplii,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>A. salina</em>, wild plankton.</td>
</tr>
<tr>
<td>Plaice⁵</td>
<td>7-12</td>
<td>4-50</td>
<td>0.16</td>
<td>as above.</td>
</tr>
<tr>
<td>Red drum⁶</td>
<td>24-28</td>
<td>0-6</td>
<td>0.06</td>
<td>B. plicatilis, A. salina</td>
</tr>
<tr>
<td></td>
<td>24-28</td>
<td>6-15</td>
<td>0.2</td>
<td>as above.</td>
</tr>
<tr>
<td>Anchovy⁷</td>
<td>17.5</td>
<td>0-7</td>
<td>0.14</td>
<td>A. salina,</td>
</tr>
<tr>
<td></td>
<td>17.5</td>
<td>7-34</td>
<td>0.50</td>
<td>wild plankton.</td>
</tr>
</tbody>
</table>

rates of both snapper and triplefins were lower than those of wild-caught snapper larvae (Atkinson, 1987) and cultured red sea bream (Fukuhara & Kishida, 1980; Baek, 1986). Nutritional demands mean that cultured red sea bream require sequential introduction of additional, larger food items to the starting diet of live rotifers from about 15-20 days, to achieve maximal growth rates (Fukuhara and Kishida, 1980; Baek, 1986; Foscarini, 1988). It is likely that the purely rotifer diet used to culture the larvae contributed to the lower growth rates in the present study. It is also possible that water quality may have affected growth rates in the present study. During the culture of red sea bream water exchange rates were gradually increased from 10-30% on day 5, to more than 280% per day by day 250 (Foscarini, 1988). The limited algal/rotifer production system used in the present study precluded high rates of water exchange (approximately 20% per day), because of the resultant food dilution.

1.3.2 Morphological development and behaviour

Snapper larvae hatched at a smaller size and an earlier stage of development than triplefin larvae. Triplefin larvae maintained a continuous horizontal swimming mode from hatching. In contrast, yolk sac snapper larvae initially displayed poor motor ability and were unable to maintain equilibrium. At this stage they must be especially vulnerable to predation, which is considered to be the major cause of mortality of fish eggs and larvae (Vladimirov, 1975; Hunter, 1981; Bailey and Houde, 1989). For example, predation rates of larval jack mackerel and bay anchovy were estimated to be 50-80% and 86.6% of the larval stocks per day, respectively (Bailey and Houde, 1989). There are however, no data available on predatory losses of yolk-sac snapper or triplefin larvae.

Development of sensory and locomotor systems during ontogeny affects the ability of larvae to avoid predators. Capture success of both invertebrate and vertebrate predators decline as fish larvae increase in size (Batty, 1989; Margulies, 1989; Bailey and Houde, 1989; Blaxter and Fuiman, 1990). Startle responses (also called C-starts) were observed in snapper larvae from day 1 post hatching and in triplefin larvae from the day of hatching. C-starts are short latency escape behaviours described for a number of
species (Eaton and DiDomenico, 1986), which in very young larval teleosts are almost certainly initiated via stimulation of superficial neuromasts (Blaxter and Fuiman, 1989), probably in response to small-scale, local water currents (Blaxter, 1987). C-start reactive distances were reported to be small, less than one body length in 7 day old herring and 4 day old cod (Blaxter and Fuiman, 1989). While startle responses provided little protection for very young larvae against attack by older conspecifics or predatory fishes (Blaxter and Fuiman, 1990), it seems likely that they are appropriate escape responses for raptorial invertebrate predators that feed by suction, or ciliary currents, or those that create water disturbances as they swim (Bailey and Houde, 1989). Ambush raptorial invertebrate predators include; cyclopoid copepods, chaetognaths, euphausids, amphipods and various gelatinous zooplankton, and together with those invertebrate predators that use passive ambush techniques to trap prey, are considered to be important consumers of fish larvae (Bailey and Houde, 1989).

At hatching, snapper larvae have four pairs of free neuromasts (Ling, 1990), while triplefin larvae have 10 pairs along the trunk and up to 4 pairs around the head (pers. obsv.). Newly hatched triplefin larvae might be more responsive to predatory attack than snapper larvae, because they hatch with functional eyes (chapter 2) and a more developed superficial lateral line. Snapper larvae hatch with undeveloped eyes (chapter 2) and presumably for yolk-sac larval snapper, startle responses via stimulation of the superficial neuromasts, provide the only defence against predatory attack.

In the absence of food, red sea bream (Baek et al., 1986) and snapper (present study) failed to grow after yolk-sac absorption and died on d7 and d7-8 respectively. Triplefins starved from hatching died 7-9 days after hatching. A histological examination of herring and plaice larvae (Ehrlich et al., 1976) showed that body reserves were successively mobilized and catabolized during starvation with the gut and liver being affected first, and the brain and locomotor systems last. As body tissues were metabolized, swimming ability declined with the result of further loss in searching ability (Li and Mathias, 1987) and presumably increased susceptibility to predation.
Feeding was first observed in larval snapper from d4-5, and in triplefin larvae from d1 after hatching. Snapper and triplefins, denied food at the onset of first feeding, starved to death in 3-4 days and 6-8 days after the time of first possible feeding, respectively. However the "window of opportunity" available for the onset of first feeding may be somewhat less than this. During progressive starvation larvae reach a state of "irreversible starvation" when although alive they are too weak to feed (Miller et al., 1988). This seems to be the case for starving snapper and triplefin larvae which became moribund about one day prior to death, sinking slowly in a vertical position, with the head down.

The results of the present study are consistent with the suggestion that the susceptibility of first feeding larvae to starvation is highly size dependent. In a review of 72 species of marine and fresh water teleosts, Miller et al., (1988) established that each 1mm increase in length at hatching opens the "window of opportunity" to first feeding by 2.5 days. Snapper larvae fall into the lower range of size at hatching of the species reviewed (1.6mm smallest species - 17.6mm largest species). As a result snapper have little flexibility in terms of time to establish first feeding, and they must be more susceptible to starvation in the wild than first feeding triplefin larvae.
Chapter Two

Retinal Growth and Morphology

Introduction

The eyes of fishes continue to grow throughout life. Growth of the teleost eye is negatively allometric with respect to body length, such that larger fish have relatively smaller eyes (Lyall, 1957a; Johns and Easter, 1977). Changes in eye size with increasing body length can be very dramatic. For example, the retinal area of the cichlid *Haplochromis burtoni* increases twenty seven times in just six months (Fernald, 1985). The lens grows in proportion to the retina and consequently optical and retinal fields remain symmetrical and constant as the eye grows (Easter et al., 1977; Fernald, 1989). Post embryonic growth of the teleost retina involves both continued neurogenesis, and the spreading of existing neural elements as the retina stretches (Johns and Easter, 1977; Raymond, 1985; Fernald, 1985). Cones, ganglion cells and cells of the inner nuclear layer are added by annular addition at the circumferential retinal margin (Lyall, 1957a; Blaxter and Jones, 1967; Johns, 1977; Sandy and Blaxter, 1980), whereas rods are added from precursor cells across the entire retinal surface (Blaxter and Jones, 1967; Johns and Fernald, 1981; Raymond and Rivlin, 1987). Retinal function in the growing fish eye is apparently maintained, despite the physical constraints imposed upon eyes of increasing size and the fact that new cells and synaptic connections are being inserted along side existing neural elements (Easter, 1983; Noakes and Godin, 1988; Fernald, 1989). Before considering how this is achieved, I will briefly review the structure of the teleost retina.

The adult retina

The retina is a hemispherical sheet of neural tissue. The specialised neurons which respond to light (the photoreceptors) are on the outer (sclerad) surface of the hemisphere. There are usually two types of photoreceptors, rods and cones. Where they
occur together (called a duplex retina), rods and cones can usually be distinguished on the basis of morphology of the outer segments and synaptic terminals, and the relative position of cone and rod nuclei to each another. The outer segments of the rods and cones are embedded in the pigmented retinal epithelium, whereas the nuclei are located on the outer nuclear layer. In the duplex retina, cone nuclei are closely associated with the external limiting membrane. Rod nuclei however, are closer to the internal layers (vitread). Photoreceptor cells contact cells of the second order neurons via receptor terminals in the outer plexiform layer. The second order neurons (amacrine, horizontal and bipolar cells) constitute the inner nuclear layer. Cells of the inner nuclear layer in turn make synaptic contact with each other, and make lateral projections to other visual cells and the ganglion cells via the inner plexiform layer. Ganglion cells are located on the inner surface of the retina. Axons of the ganglion cells make up the optic fibre layer which exits the retina as the optic nerve. The entire retina is pervaded by the supporting cells, Muller and neuroglia (Johns, 1981; Ali and Klyne, 1985; Nicol, 1989).

The photoreceptors
1) The outer segment

The photoreceptor outer segments extend up into the pigmented retinal epithelium (Fig. 2-1). The pigmented retinal epithelium contains pigment granules (melanosomes), which are thought to prevent backscattering, and thus image quality loss, by absorbing much of the incoming light. The membranous discs of the photoreceptor outer segments contain the visual pigments that interact with photons to initiate the visual process. The rod discs are free floating, isolated from the plasma membrane, except at the very base. Another feature sometimes seen in rods are incisures between stacks of floating discs. Calycal processes, arising from the base of the outer segment, are generally present. There may be a small accessory outer segment. Cones on the other hand, have membranous discs which are continuous with the plasma membrane. Both calycal processes and accessory outer segments are present.
**Fig. 2-1.** Schematic diagram of photoreceptor types in the teleost retina:

Double cone (DC), long single cone (LSC), short single cone (SSC) and a rod (R). AOS - accessory outer segment, C - centriole, COS - cone outer segment, CP - calycal process, E - ellipsoid, ELM - external limiting membrane, M - Muller cell, MD - myoid, MS - melanosome, ONL - outer nuclear layer, OPL - outer plexiform layer, PN - nucleus of the pigmented retinal epithelium, REP - pigmented retinal epithelium, ROS - rod outer segment.
2) The inner segment

The inner segment is connected to the accessory outer segment by a modified cilium, which in rods may run from the base, right through the outer segment. Below the ciliary stalk, the ciliary tubules are connected to a centriole (basal body).

The ellipsoid is a swelling of the inner segment adjacent to the ciliary stalk, containing mitochondria. Cone ellipsoids generally contain more mitochondria than rod ellipsoids. The ellipsoid is connected to the photoreceptor nucleus by a contractile bridge known as the myoid. The myoid is responsible for retinomotor movements in response to changes in illumination. In species exhibiting retinomotor shifts, cone myoids contract in light and elongate in darkness. The converse occurs in rods.

Basal regions of the myoids of rods and cones are separated by Muller cells to which they are attached laterally by *zonulae adherentes* to form the external limiting membrane. The photoreceptor cells synapse with cells of the inner nuclear layer (bipolars and horizontals) via swollen synaptic terminals in the outer plexiform layer. Rod synaptic terminals are generally small and round (called spherules) and contain only a few synaptic ribbons, whereas cone synaptic terminals (called pedicles) are flattened on their vitread surface, and contain many more synaptic ribbons.

*Cone types*

Single and double cones are prevalent in the teleost retina. Triple and quadruple cones have been reported, but are less common (Lyall, 1957b; Engstrom, 1963; Ahlbert, 1976). Both short and long single cones may occur. Double cones which are structurally dissimilar, are called "paired doubles", whereas those which are structurally indistinguishable have been designated "twins".

Cones are often arranged in very specific patterns, called cone mosaics. Very common arrangements found in teleosts are 1) parallel rows of double cones, alternating with rows of single cones, or 2) square units where, for example, four double cones surround a central single cone. Cone mosaic units are considered to be physiological
units, with all the photoreceptors in the unit being electrically coupled, so that the combined input descends onto a single ganglion cell.

Rods are not generally arranged in regular patterns, however, in the specialized retinae of deep sea fishes, rods can be gathered into optically isolated, and tightly packed bundles (grouped rods), or there may be an orderly repeat (stacked) pattern of rod photoreceptor outer segments (Lyall, 1957b; Engstrom, 1963; Ahlbert, 1976; Ali and Klyne, 1985; Nicol, 1989).

**Embryonic growth**

Embryonic development of the retina proceeds in a similar fashion in all vertebrates. The presumptive neural retina forms from an outgrowth of the primitive neural tube which invaginates to form a hemispherical cup of undifferentiated neuroepithelial germinal cells (Ali and Klyne, 1985). Differentiation of the presumptive neural retina starts from the centre (the fundus) and proceeds in concentric waves of differentiation to reach the edge by about the end of the embryonic stages. In birds and mammals, the entire retina has differentiated by this time. Cellular addition ceases and the retina now has its full complement of neural elements. However, in amphibians and fishes, a circumferential zone of undifferentiated germinal cells (the circumferential germinal zone) remains at the retinal margin, and the retina continues to grow after embryonic stages.


**Post embryonic growth: maintenance of visual function.**

**Optical properties**

As the eye enlarges the lens grows in proportion to the retina (Easter, *et al.*, 1977; Fernald, 1989). Fish lenses are approximately spherical and optically symmetrical. Spherical lenses of uniform refractive index, produce poor, spherically aberrant images, because rays at different distances from the optic axis are focussed at different distances from the lens (Powers and Easter, 1983; Fernald, 1989). Fish lenses have a refractive
index gradient which produces very good images right up to the edges, (Fernald and Wright, 1983). The central core of the fish lens, a hard sphere of dense protein, has a nearly uniform refractive index and therefore produces spherically aberrant images. However, newly added tissue at the lens surface has a lower refractive index than the core, mainly due to low protein content (Fernald and Wright, 1983). As a result, a refractive index gradient occurs between the lens core and the outer cortex, and is maintained with lens growth. As cells are added at the lens surface, the cells adjacent to the core lose water and become compacted into the ever enlarging spherically aberrant central core. It appears that the increasing thickness of the lens cortex may act to correct the refractive index gradient and thus maintain the properties of a nearly aplanatic lens during continued growth (Fernald and Wright, 1983; Fernald, 1989)

**Photopic visual acuity**

Photopic visual acuity is a function of cone spacing, focal length of the lens, and convergence of cones onto higher order neurons. Spatial acuity can be maximized by high retinal grain (numbers of cone cells per unit retinal area) and low convergence of cones onto ganglion cells. However, during growth retinal expansion gradually spreads cones apart, despite continued neurogenesis of cones at the retinal margin (Lyall, 1957a; Johns and Easter, 1977; Fernald, 1985). Visual acuity is maintained by virtue of continued increase of the retinal magnification factor (a function of focal length of the lens), whereby an image of given angular subtense projects onto an increasingly larger area of retina as the eye grows (Johns and Easter, 1977; Johns, 1981; Easter, 1983). Consequently, although retinal grain becomes coarser, the angular density of cones (cells per unit visual angle) increases as the eye enlarges. Therefore, theoretical spatial visual acuity increases as the eye grows (Blaxter and Jones, 1967; Guma’a, 1982; Neave, 1984). Behavioural measurements of visual acuity confirm acuity increases with increasing body size (Rahman, *et al.*, 1979; Wright and O’Brien, 1982; Hairston *et al.*, 1982; Breck and Gitter, 1983; Neave, 1984).
The eyes of larval fish, which generally have pure cone retinas at the time of first feeding, are subject to the quite severe physical constraints imposed upon very small eyes. Despite high cone density, theoretical spatial acuity of the eyes of larval fish is low (Blaxter and Jones, 1967; Blaxter, 1975; Guma’a, 1982; Neave, 1984; Margulies, 1989). This is a consequence of small eye and lens size (with corresponding short focal length) resulting in a lower number of cones per visual angle than in larger eyes. Further improvement in spatial acuity, by increasing cone density and decreasing cone size is limited by constraints set by size at which light spills into adjacent photoreceptors (Lythgoe, 1980).

**Scotopic visual sensitivity**

Scotopic sensitivity is a function of rods. Rod development usually occurs after cones and often accompanies a habitat shift from a pelagic to a benthic, or demersal life style, at the end of the larval phase. Uniquely, rods are added right across the retinal surface from a population of special germinal precursor cells (Blaxter and Jones, 1967; Johns and Fernald, 1981; Raymond and Rivlin, 1987). Rod numbers per unit area remain fairly constant as the adult retina enlarges (Fernald, 1985) and it seems likely that rod addition is required to maintain sensitivity during growth (Raymond, 1985). Behaviourally measured scotopic visual thresholds have been shown to be constant for a wide range of fish sizes (Powers and Bassi, 1981; Allen and Fernald, 1981).

**Ontogeny of vision in snapper and triplefins**

Snapper larvae hatch at a very small size and at an earlier stage of development than larval triplefins (chapter 1). Most larval fishes are thought to be visual feeders (Blaxter, 1986) and if this is so, the eyes of both species are expected to be functional at the time of first feeding (day 4-5 in snapper, and day 1 in triplefins). A study of ocular morphology of red sea bream, confirmed that the larval retina of the Japanese sub-population of *P. auratus* is functional at the time of first feeding (3 days of age)(Kawamura et al., 1984). There are however, no data on retinal morphology of New Zealand snapper
or *F. varium*. In very small snapper and triplefins, visual function is expected to be poor, limited by the physical constraints imposed upon very small eyes.

Adult snapper can be extremely long lived (55-60 years) attaining maximum lengths of up to 800mm, although the majority of growth occurs during the first 5-8 years (Horn, 1986). Adult triplefins on the other hand, are small reaching maximum lengths of 115cm (Northland, New Zealand) during a maximum life span of only three years (Thompson, 1979). Eye size in teleosts increases with increasing body size (Lyall, 1957a; Johns and Easter, 1977) and as a consequence the absolute eye size of adult snappers is much greater than that of the very much smaller triplefins, with the associated consequences for visual acuity, due to different retinal magnification factors.

In the present study, visual morphology was examined with increasing eye size in larval, juvenile and adult stages of *P. auratus* and *F. varium*. Changes in visual cell morphology and morphometry, were used to estimate changes in both the photopic and scotopic visual systems during growth. Behavioural estimates of acuity in goldfish (Breck and Gitter, 1983) approach the limit set by intercone spacing (Stell and Harosi, 1976; Johns and Easter, 1977). If one accepts that acuity is limited by grain of the retinal mosaic then the simplest hypothesis is that minimum separable angle (MSA) will correspond to intercone spacing. Theoretical spatial acuity (MSA) in the present study, was estimated as a function of cone spacing (space between centres of adjacent cones), and the focal length of the lens (Neave, 1984). Some estimates of acuity calculate minimum resolveable angle as the distance between two cones, which are separated by at least one unstimulated cone (Tamura and Wisby, 1957), and it should be noted that this gives twice the minimum separable angle calculated here (Northmore and Dvorak, 1979). An index of scotopic sensitivity in snapper and triplefins, was determined, in terms of light path-length through the visual pigment, rod density, and convergence of rods onto bipolar cells (Pankhurst, 1989).

Retinal morphology and estimates of theoretical visual acuity and sensitivity during growth of snapper and triplefins, are compared and discussed in the light of current understanding of vision in teleosts.
2.1 Materials and Methods

2.1.1 Fish capture and husbandry

Adult and juvenile triplefins were hand netted by SCUBA divers during May to October, 1989-1990, from inshore rocky reefs at Ti Point and Leigh, Northland, New Zealand. Adult snapper were captured by long-line, at various times during 1988-1990, from Okakari Point and Kawau Bay, Northland, New Zealand. Juvenile snapper were captured by bottom-trawl, from the University of Auckland's R.V. Proteus, in Kawau Bay, in the Hauraki Gulf, New Zealand, during February 1989. Larval snapper and triplefins were sampled from rearing trials previously described in chapter 1. Triplefin larvae were sampled from two additional rearing trials conducted in June and July 1988 (temperature range 13.7-14.8°C), and June and July 1989 (temperature range 13.8-15°C), which utilized the culture techniques described in chapter 1.

2.1.2 Histology

Fifteen snapper (3.4 - 333mm body length) and 19 triplefins (5.0 - 118mm body length), were sacrificed during the day to examine changes in ocular morphology with increasing body size. In addition a size range of each species were sacrificed at night, after three hours exposure to total darkness, to investigate the presence or absence of retinomotor shifts.

Juvenile and adults were anaesthetised in 0.02% 2-phenoxyethanol (BDH) in seawater, and killed by spinal transection. Standard length (SL) of larvae, total length (TL) of juvenile and adult triplefins, and forklength (FL) of snapper juveniles and adults were measured prior to fixation. The eye diameters of adults and juveniles were measured in the dorso-ventral plane. The eyes were removed and the corneas punctured to allow penetration of the fixative. A notch was cut in the dorsal iris to enable subsequent orientation. Whole larval fish, and the eyes of juveniles and adults, were fixed overnight in cold (4°C) 5% gluteraldehyde in 0.1M phosphate-phosphate buffer (pH 7.4). Following fixation, the lenses were removed and measured. Larvae and all eyes
except those of adult snapper were then dehydrated, embedded in JB4 plastic resin (Biorad) and sectioned at 1-2.5μm on a sorval JB4 microtome. Sections were stained with JB4 polychrome stain. Adult snapper eyes were dehydrated, embedded in paraffin wax and sectioned at 5-7μm. Wax sections were stained with Mallory-Heidenhain stain. Whole larvae were serially sectioned in the sagittal or transverse planes. Transverse and tangential sections were obtained from the eyes of juvenile and adult fish. In the larvae, where the lenses were sectioned with the eyes, the eye and lens diameters were taken as the largest diameters measured from transverse serial sections.

2.1.3 Electron microscopy

Whole larval snapper and enucleated eyes of adult snapper were either fixed overnight at 4°C in 5% gluteraldehyde in 0.1M phosphate-phosphate buffer at pH 7.4, or fixed for one hour at room temperature in a combination of gluteraldehyde (2%) and formaldehyde (2%) in 0.1M phosphate-phosphate buffer at pH 7.4. Tissue was briefly washed in sucrose-phosphate buffer, post fixed for 1h in phosphate buffered 1% OsO₄, and dehydrated in an ethanol-acetone series prior to embedding in EMbed-812 resin (Electron Microscopy Sciences)(Appendix 2). Ultra thin sections were cut using a LKB Nova ultramicrotome, stained with uranyl acetate and lead citrate (Appendix 2), and examined using a Philips 301 transmission electron microscope.

2.1.4 Morphometry and cell counts

All cell counts were made from histological material under oil immersion, at 1000 X magnification from the central retina, using a Nikon Optiphoto light microscope fitted with a calibrated micrometer. Cell counts were made from transverse sections of the retina of 6 larval (5.0-6.7mm SL), 1 juvenile (36mm), and 6 adult triplefins (86-118mm TL), and 7 larval (3.4-5.9mm SL), 3 juvenile (55-103mm), and 4 adult snapper (175-333mm). Five adjacent 0.05mm strips of retina were counted in the juveniles and adults. The curvature and dimensions of the larval eyes limited the number of adjacent strips that could be counted from the larval sections. Accordingly one, two, and three adjacent 0.05mm
retinal strips were counted from transverse sections of 4-9 day old snapper (3.4-3.5mm SL), 1-16 day old triplefins (3.9-6.7mm SL) and 12-22 day old snapper (4.05-5.9mm SL), respectively.

Diameters of cone ellipsoids were measured from tangential sections of central retina.

2.1.5 Theoretical scotopic sensitivity

Scotopic sensitivity was assessed in juvenile and adult snapper and triplefins, in terms of light path-length through the visual pigment, rod density (mm⁻²), and convergence of rods onto bipolar cells (Pankhurst, 1987). Light path length through the visual pigment was taken as the distance from the external limiting membrane to the boundary of the pigmented retinal epithelium. Five adjacent measurements were taken from the central retina in a size range of fish of each species.

2.1.6 Theoretical acuity

Theoretical visual acuity (Minimum Separable Angle, MSA) was calculated as a function of cone density and focal length of the lens from:

\[ \sin \alpha = \frac{1.11}{10d \times F}, \]

where \( \alpha \) is the minimum separable angle, \( F \) is the focal length of the lens estimated from Matthiessen's ratio (\( F = 2.55 \times \text{lens radius} \)), and \( d \) is the cone density (numbers/100\( \mu \text{m}^{-1} \) of retina), with adjustment for shrinkage (Neave, 1984). For the purposes of acuity estimation, double cones were counted as single units.
2.2 Results

2.2.1 Snapper

*Retinal Morphology*

At hatching, the presumptive retina was a simple hemispherical sheet of undifferentiated neural epithelium (the optic cup), with a spiral of unspecialised cells, the developing lens, within (Fig. 2-2a). There was extensive mitotic activity in the presumptive retina (visible on the micrograph as dark spots) on day 0 and day 1 after hatching.

By 2 days post hatching the central retina had begun to separate into neural layers interposed between connecting fibre layers (Fig. 2-2b). Tall columnar photoreceptor nuclei (presumptive cones) were present in the outer nuclear layer on day 2, and rudimentary outer segments were visible in transmission electron micrographs, as small outgrowths on the sclerad surface of the cells (Fig. 2-3a). A few melanosomes were scattered across the pigmented retinal epithelial layer.

On the third day post hatching there were many bacilliform melanosomes in the pigmented retinal epithelium (Fig. 2-3b). The photoreceptor outer segment discs had started to form and were continuous with the plasma membrane, indicating that the developing photoreceptors were probably cones (Fig. 2-3c). A few mitochondria were present in the developing cone ellipsoids adjacent and vitread to the ciliary stalk (Fig. 2-3b). At this stage the optic fibre tract was connected to the optic tectum. *Zonulae adherentes* had formed between adjacent photoreceptors and Muller cells to form the external limiting membrane at the level of the inner segments, sclerad to the photoreceptors. Developing cone pedicles were present within the outer plexiform layer, however, synaptic ribbons were not yet evident (Fig. 2-3d).

At 4 days of age the lens was crystalline and the circumferential zone of undifferentiated cells was visible at the retinal margin (Fig. 2-2c). There were now many more mitochondria in the cone ellipsoids, and synaptic ribbons were present in the photoreceptor pedicles (Fig. 2-4ab).
Fig. 2-2. Light micrographs of transverse sections through the eye of larval *P. auratus* at a) hatching, b) two days after hatching, and c) at 4 days of age. CGZ - circumferential germinal zone, G - ganglion cell layer, INL - inner nuclear layer, L - lens, OC - optic cup, ONL - outer nuclear layer. Arrowheads indicate mitotically active cells. Scale bars are 50µm (a,b and c).
Fig. 2-3. Electron micrographs of differentiating cone photoreceptors in the retina of *P. auratus* larvae. (a) Two days after hatching, the developing photoreceptor outer segments are visible as outgrowths on the scleral surface of the cells (arrows). (b) Three days after hatching the lamellal discs of the developing photoreceptors have formed from infoldings of the plasma membrane adjacent to the ciliary stalk. Melanosomes (Ms) are present in the pigmented retinal epithelium and a few mitochondria (M) are present in the developing cone ellipsoids. (c) Lamellal discs are continuous with the plasma membrane (arrow), indicating that the developing photoreceptors are cones. (d) Cone pedicles (P) are visible on day 3 but synaptic ribbons and vesicles have not yet formed. CS - ciliary stalk, CP - calical process, N - cone nuclei. Scale bars are 1μm (a,b,c and d).
Fig. 2-4. Electron micrographs of cone photoreceptors in the retina of *P. auratus* larvae 4 days after hatching. *Zonulae adherentes* (arrows) between adjacent photoreceptors define the external limiting membrane (a) and synaptic ribbons (white arrowheads) and vesicles are prominent in the cone pedicles (b). M - mitochondria, N - cone nucleus, P - cone pedicle. Scale bars are 1μm (a and b)
By 18 days post hatching, there was a second population of small darkly staining nuclei in the outer nuclear layer. These were presumed to be rod precursor cells (Fig. 2-5a). In a few cases, rod outer segments appear to have differentiated (Fig. 2-5b). The presumptive rods had smaller ellipsoids containing fewer mitochondria than cone ellipsoids. The precise nature of the membranous discs within these presumptive rod outer segments (whether continuous with the plasma membrane as in cones, or free floating as in rods), could not however be discerned from the transmission electron micrographs.

Juvenile and adult snapper had duplex retinae and displayed retinomotor shifts to changing illumination. In the light adapted retina this involved contraction of cone myoids and elongation of rod myoids so that cone outer segments were closest to the incoming light. There was a concomitant migration of melanosomes of the pigmented retinal epithelium in a vitread direction to completely envelope the rod outer segments. Conversely in the dark adapted retina, melanosomes of the pigmented retinal epithelium were withdrawn in a sclerad direction, cone myoids elongated and rod myoids contracted, to bring rod outer segments into more vitread position. Rod numbers had increased dramatically as indicated by the proliferation of nuclei in the outer nuclear layer (Fig. 2-6a). Both short single and twin double cones were now present (Fig. 2-6a) and these were arranged in a regular mosaic of four twin double cones surrounding a central single cone (Fig. 2-6b).

**Morphometry and cell counts**

The eyes of snapper continued to grow with increasing body size (Fig. 2-7). There was a 96 fold increase in eye size, from 0.23mm diameter in a 4 day old larva (3.42mm SL), to a maximum of 22mm diameter in an adult of 333mm FL. Cone numbers were highest in larval fish (17 cells.0.05mm⁻¹ retina) and decreased with increasing eye size to as low as 5 cells.0.05mm⁻¹ retina in adults. Rod numbers increased dramatically from 0 in larval snapper to between 95 and 118 cells.0.05mm⁻¹ retina in settled metamorphosed juveniles, and thereafter ranged between 111 and 156 cells.0.05mm⁻¹ retina. The
**Fig. 2-5.** Photomicrographs of the retina of a larval *P. auratus* at 18 days of age. (a) Presumptive rod precursor cells (arrows) occur vitread to cone nuclei in the outer nuclear layer (ONL). (b) In a few cases, rods appear to have differentiated. These cells are characterized by smaller ellipsoids with fewer mitochondria (M) than the cones. G - ganglion cell layer, INL - inner nuclear layer. Scale bars are 10μm (a) and 2μm (b).
Fig. 2-6. Photomicrographs of (a) a transverse section through the outer nuclear layer of the light adapted retina of an adult *P. auratus*. Cone photoreceptor outer segments (arrows) are immediately sclerad to double cone (DC) and single cone (C) ellipsoids. Melanosomes of the pigmented retinal epithelium cover both cone and rod outer segments (ROS). (b) A tangential section through the cone ellipsoids in the light adapted retina of adult *P. auratus*. Cones are arranged in a square mosaic in which 4 double cones (DC) surround a central single cone (SC). Scale bars are 25µm (a and b).
Fig. 2-7. Changes in density of cone, bipolar, rod and ganglion cells (number/0.05mm retina\(^{-1}\)), and eye size (mm), with increasing body length (mm) in *P. auratus*. Body length was measured from snout to end of notochord in larvae, and snout to the fork of the caudal fin in older fish. Values for cell counts are mean ± SE (n=5) for juveniles and adults, mean ± SE (n=3) for larvae (body length 4-6mm), and are a single value for the smallest larva (body length < 3.5mm).
numbers of bipolar cells increased from between 27-29 in larval snapper, to a maximum of 36 cells.0.05mm⁻¹ retina in juveniles, and declined thereafter to a minimum of 11 cells.0.05mm⁻¹ retina in the largest adult. Ganglion cell numbers were highest in larval fish, and decreased steadily with increasing body size. The angular density of both rods and cones continued to increase with increasing body size (Fig.2-8). Cones were closely packed in fish of all sizes, however, the size of both single and double cone ellipsoids increased with increasing eye size (Table 2-1).

Acuity and sensitivity

Convergence ratios of rods : bipolar cells increased from 3.1 in a 55mm FL juvenile to a maximum of 14.8 in the largest adult (333mm FL)(Table 2-3). Density of rods (.mm⁻²) ranged between 3.6x10⁶ and 9.8x10⁶, in juveniles and adults. Outer segment length of juvenile and adult rods varied between 199 and 313μm.

Theoretical acuity was lowest in 4 day old larvae (2⁰ 1' MSA) and improved rapidly with increasing body size to an asymptotic value of 3'-4' MSA in adults (Fig. 2-9).

2.2.2 Triplefins

Retinal Morphology

Triplefin larvae had pure cone retinae at hatching. The optic nerve was connected to the optic tectum, the pigmented retinal epithelium was well pigmented and the lens was crystalline. Differentiation of the central retina was complete (Fig. 2-10), however, a circumferential zone of undifferentiated germinal cells remained at the retinal margin (Fig. 2-11a). The larval retina had large columnar cone nuclei in the outer nuclear layer, and prominent cone ellipsoids and outer segments. In addition, a second smaller population of small, darkly staining nuclei was present in a more vitread position to the cone nuclei. Their position suggested that they were rod precursor cells (putative rods)(Fig.2-11b). No rod-like outer segments were visible in light micrographs, however only examination using a transmission electron microscope would confirm this, and this was not undertaken for triplefin retinas.
Fig. 2-8. Changes in angular density (numbers.10' visual arc^{-1})
of cones and rods with increasing body length (mm) in *P.auratus*.
Visual angle was calculated using $2.55 \times r$ (lens radius), as an estimate
of focal length.
Table 2-1. Mean cone ellipsoid diameters (µm ± SE, n=15) for a size range of *P. auratus*.

<table>
<thead>
<tr>
<th>Body Length (mm)</th>
<th>Single Cones</th>
<th>Double Cones</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.05</td>
<td>2.0 ± 0.07</td>
<td>-</td>
</tr>
<tr>
<td>125</td>
<td>3.7 ± 0.09</td>
<td>6.7 ± 0.10</td>
</tr>
<tr>
<td>265</td>
<td>6.9 ± 0.12</td>
<td>13.0 ± 0.17</td>
</tr>
</tbody>
</table>

Table 2-2. Convergence ratios of rods : bipolar cells, mean rod outer segment length (external limiting membrane to boundary of the pigmented retinal epithelium, values are mean ± SE, n=5), and mean rod density (mm⁻²) of juvenile (55, 65, and 102mm FL) and adult (175, 265, 306 and 333mm FL) *P. auratus*.

<table>
<thead>
<tr>
<th>Body Length (mm)</th>
<th>Convergence rods:bipolars</th>
<th>Photoreceptor Length (µm)</th>
<th>Rod Density (mm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>3.14</td>
<td>144.4 ± 1.2</td>
<td>4.9 x 10⁶</td>
</tr>
<tr>
<td>65</td>
<td>2.60</td>
<td>-</td>
<td>3.6 x 10⁶</td>
</tr>
<tr>
<td>103</td>
<td>3.79</td>
<td>147.8 ± 2.26</td>
<td>5.6 x 10⁶</td>
</tr>
<tr>
<td>175</td>
<td>5.41</td>
<td>111.2 ± 1.74</td>
<td>8.3 x 10⁶</td>
</tr>
<tr>
<td>265</td>
<td>4.98</td>
<td>115.0 ± 1.22</td>
<td>5.0 x 10⁶</td>
</tr>
<tr>
<td>306</td>
<td>8.87</td>
<td>126.4 ± 1.02</td>
<td>7.6 x 10⁶</td>
</tr>
<tr>
<td>333</td>
<td>14.75</td>
<td>122.2 ± 1.71</td>
<td>9.8 x 10⁶</td>
</tr>
</tbody>
</table>
Fig. 2-9. Changes in theoretical acuity (minimum separable angle, MSA, in minutes) with body length (mm) in *P. auratus*. 
Fig. 2-10. Photomicrograph of a transverse section through the eye of a newly hatched *F. varium* larva. CNS - central nervous system, D - dorsal, DC - double cone, G - ganglion cell layer, INL - inner nuclear layer, L - lens, ON - Optic nerve, ONL - outer nuclear layer, V - ventral. Arrows indicate the position of the outer plexiform layer (OPL). Scale bar is 50μm.
Fig. 2-11. Photomicrographs of (a) a transverse section of the retinal margin in a larval *F. varium*. A circumferential germinal zone (CGZ) of undifferentiated retinal precursor cells remains at the retinal margin to proliferate cells of the ganglion cell layer (G), inner nuclear layer (INL) and outer nuclear (ONL). (b) The outer nuclear layer of the retina of larval *F. varium*, with collumnar cone nuclei (CN) and cone outer segments (COS) immediately sclerad to the cone ellipsoids. Presumptive rod nuclei (arrows) occur in a vitread position with respect to cone nuclei. (c) A tangential section of cone ellipsoids in a juvenile *F. varium*. The cones are arranged in a square mosaic (box) in which 4 double cones surround a central single cone. Scale bars are a) 50μm b) 10μm c) 25μm.
Juvenile triplefins displayed a duplex retina, with rods and both single and twin double cones. In addition, cone photoreceptor outer segments were now arranged in a highly ordered rectangular mosaic in which four double cones surrounded a central single cone (Fig. 2-11c). Single cones and twin double cones were present in the adult retina and had the same mosaic arrangement as the juveniles (Fig. 2-12c,d).

Retinomotor shifts occurred in juvenile and adult, but not larval triplefins. Cone myoids contracted in the light adapted retina, and rod myoids elongated so that cone outer segments were closest to the incoming light (Fig. 2-12a). In addition, melanosomes of the pigmented retinal epithelium migrated in a vitread direction to completely envelope the rod outer segments. Conversely, in the dark adapted retina, melanosomes of the pigmented retinal epithelium were withdrawn in a sclerad direction, cone myoids elongated and rod myoids contracted, to bring rod outer segments into a more vitread position (Fig. 2-12b).

Morphometry and cell counts

Eye size increased with increasing body size (Fig. 2-13), such that there was a 26 fold increase in eye size from 0.28mm in the smallest larva (5.0mm SL) to a maximum diameter of 7.2mm in an adult of 110mm TL. Cone numbers were highest in larval fish (20 cells.0.05mm\(^{-1}\) retina), and decreased with increasing eye size to as low as 5 cells.0.05mm\(^{-1}\) retina in adults. Rod numbers increased dramatically prior to, or at the time of juvenile settlement (60 cells.0.05mm\(^{-1}\) retina), and thereafter ranged between 66-80 cells.0.05mm\(^{-1}\). The numbers of both ganglion and bipolar cells were highest in larval fish, and decreased with increasing body size to be relatively constant in adults (5-8 and 27-33 cells.0.05mm\(^{-1}\) retina respectively). When expressed as numbers per unit visual arc, the density of both rods and cones increased from hatching to a maximum in adults, at which time cell numbers remained approximately constant (Fig. 2-14).

Cones were closely packed in fish of all sizes, however, the size of both single and double cone ellipsoids increased with increasing eye size (Table 2-3).
Fig. 2-12. Photomicrographs showing retinomotor shifts in a) a light adapted adult retina of *F. varium* and b) a dark adapted adult retina of *F. varium*. In the light adapted retina, cone myoids are contracted and rod myoids elongated so that cone outer segments (COS) are closest to the incoming light. Conversely in the dark, rod myoids contract and cone myoids elongate to bring rod outer segments (ROS) into a more vitread position. In the dark adapted retina, melanosomes of pigmented retinal epithelium are withdrawn into a sclerad position. In the light, melanosomes migrate in a vitread direction to envelop cone and rod outer segments. Tangential sections (c), and transverse sections (d) through the cone ellipsoids in the retina of adult *F. varium*. C - single cone ellipsoid, DC - double cone ellipsoid, INL - inner nuclear layer, ONL - outer nuclear layer, P - pigment granules of the pigmented retinal epithelium. Scale bars are 50μm (b, magnification a = b), and 10μm (c and d).
Fig. 2-13. Changes in density of cone, bipolar, rod and ganglion cells (number/0.05mm retina⁻¹), and eye size, with increasing body length (mm) in *F. varium*. Body length was measured from snout to end of notochord in larvae, and snout to end of caudal fin in older fish. Values for cell counts are mean ± SE (n=5) for juveniles and adults, mean (n=2) for larvae (body length < 8mm).
Fig. 2-14. Change in angular density (number.10' visual arc\(^{-1}\)) of cones and rods with increasing body length (mm) in *F. varium*. Visual angle was calculated using 2.55 \(x\) \(r\) (lens radius), as an estimate of focal length.
Table 2-3. Mean cone ellipsoid diameters (μm ± SE, n=15) for a size range of *F. varium*.

<table>
<thead>
<tr>
<th>Body Length (mm)</th>
<th>Single Cones</th>
<th>Double Cones</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>1.9 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>7.3</td>
<td>1.9 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>58.5</td>
<td>3.0 ± 0.03</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>80.6</td>
<td>3.9 ± 0.10</td>
<td>7.5 ± 0.1</td>
</tr>
<tr>
<td>110.0</td>
<td>4.0 ± 0.07</td>
<td>7.0 ± 0.1</td>
</tr>
</tbody>
</table>

Table 2-4. Convergence ratios of rods : bipolar cells, mean rod outer segment length (external limiting membrane to boundary of the pigmented retinal epithelium, values are mean ± SE, n=5), and mean rod density (mm⁻²) of a juvenile (36mm) and adult *F. varium*.

<table>
<thead>
<tr>
<th>Body Length (mm)</th>
<th>Convergence rods:bipolars</th>
<th>Photoreceptor Length (μm)</th>
<th>Rod Density (mm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>1.15</td>
<td>87.5 ± 1.4</td>
<td>1.5x10⁶</td>
</tr>
<tr>
<td>86</td>
<td>2.40</td>
<td>70.5 ± 5.0</td>
<td>2.6x10⁶</td>
</tr>
<tr>
<td>93</td>
<td>2.43</td>
<td>96.5 ± 1.3</td>
<td>2.6x10⁶</td>
</tr>
<tr>
<td>100</td>
<td>2.53</td>
<td>97.0 ± 1.2</td>
<td>2.4x10⁶</td>
</tr>
<tr>
<td>104</td>
<td>2.50</td>
<td>-</td>
<td>1.8x10⁶</td>
</tr>
<tr>
<td>110</td>
<td>2.30</td>
<td>95.5 ± 2.2</td>
<td>1.7x10⁶</td>
</tr>
<tr>
<td>118</td>
<td>2.58</td>
<td>106.5 ± 2.2</td>
<td>2.4 x 10⁶</td>
</tr>
</tbody>
</table>
Acuity and sensitivity

Convergence ratios of rods: bipolar cells increased from 1.2 in a 36mm juvenile to between 2.3 and 2.6 in adults (Table 2-4). There was a concomitant increase in density of rods.mm⁻² from 1.5 x 10⁶ in juveniles, to a maximum of 2.6 x 10⁶ in adults. Rod outer segment length varied between 70.5μm and 106.5μm in juveniles and adults.

Theoretical acuity was lowest in the smallest larvae (1⁰ 8') and improved with increasing body size to an asymptotic value of about 9' in adults (Fig. 2-15).

2.3 Discussion

2.3.1 Retinal morphology

The retinæ of snapper larvae were undifferentiated at hatching, and at a much earlier stage of development than triplefin larvae. Development of the photoreceptors in the snapper retina proceeded in a similar fashion to other teleosts (Johns, 1977; Fernald and Johns, 1981; Kunz et al., 1983; Branchek and Bremiller, 1984). Differentiation began in the central retina, first with the appearance of nuclear layers interposed between connecting fibre layers, and was followed by budding of the presumptive photoreceptor cells to form the outer segments (day 2). Studies on rainbow trout and the viviporous guppy (Kunz et al., 1983; Schmitt and Kunz, 1989) demonstrated that budding of photoreceptor outer segments first involved interdigitation of apical processes of the presumptive photoreceptors, with the developing pigmented retinal epithelium.
Cytoplasmic buds then started to protrude through the external limiting membrane. An apical centriole became the basal body and the connecting cilium rose up from it to form the prospective ciliary stalk. In a few teleosts, rods differentiate first (Blaxter and Staines, 1970; Pankhurst, 1983) or at the same time as cones (Kunz et al., 1983). However, more commonly, cones differentiate first (Blaxter and Jones, 1967; Blaxter and Staines, 1970; Sandy and Blaxter, 1980; Guma'a, 1982; Johns, 1982; Neave, 1984; Branchek and Bremiller, 1984; Kawamura, 1984; Raymond and Rivlin, 1987), and this was the case in
Fig. 2-15. Changes in theoretical acuity (minimum separable angle, MSA, in minutes) with body length (mm) in *F. varium*.
snapper and triplefin larvae. A single row of differentiated photoreceptors, with columnar nuclei and cone shaped outer segments was present in light micrographs of the retina of snapper on day 3, and in triplefins at the time of hatching. Electron microscope examination of snapper photoreceptor lamellae discs (day 3) showed that the photoreceptors were cones. Triplefin larvae at hatching had a second very much smaller population of undifferentiated nuclei (presumptive rods precursors), vitread to the row of larger presumptive cone nuclei. In the absence of electron microscope examination, the nature of the presumptive rods and cones could only be surmised by their position relative to one another (Johns, 1982; Raymond, 1985; Raymond and Rivlin, 1987), with the usually larger cone nuclei located more sclerad to the smaller presumptive rod precursors.

At the time of first feeding the eyes of snapper (day 4-5) and triplefin (day 1) were presumed to be functional, as most larval fish are thought to be primarily visual feeders (Blaxter, 1986). Morphology of the eyes of larval snapper and triplefins at the time of first feeding appears to support this. Newly hatched triplefin larvae, and snapper larvae 3 days after hatching, had well developed cone retinae in which the optic nerve connected to the optic tectum. However electron micrographs of snapper cone pedicles showed that synaptic ribbons and associated synaptic vesicles were not present until day 4, indicating chemical transduction of information from the photoreceptors at this time. In addition, the lens core of larval snapper, previously a homogeneous spiral of cells, was not crystalline until day 4 after hatching. During early development, the inner cellular layers of the growing teleost lens elongate and lose much of their water content. Various crystallins and protein fractions are laid down and it is thought that these concentrated refractile proteins are responsible for the transparency and high refractive index, of the lens (Nicol, 1989). As the cornea, vitreous humour and aquatic environment essentially have the same refractive indices, the dioptric power of the fish eye is invested entirely in the lens (Powers and Easter, 1983) suggesting that the eyes of snapper larvae at 4 days of age, and triplefin larvae at hatching, were capable of image forming.
Development of the eyes of larval red bream was more rapid than New Zealand snapper in the present study. Kawamura \textit{et al} (1984) reported that at 36 hours post hatching, the eyes of red sea bream were well pigmented, had differentiated cone photoreceptors and the optic nerve connected to the optic tectum. First feeding occurred on the 3rd day after hatching. This accelerated rate of growth relative to larvae in the present study, may be a developmental characteristic of the Japanese sub-population of \textit{P. auratus}, or may in fact be a temperature effect, as temperature has a potent control over the rate of larval development. However, Kawamura \textit{et al.}, (1984) gave no data regarding culture temperature.

A small population of putative rod precursor cells was present in the retina of snapper larvae at 18 days of age (6.0mm SL). Kawamura \textit{et al} (1984) reported that rods were first present in red sea bream at 11.0mm and further, that the appearance of rods (ascertained by estimation of the ratio of one ellipsoids:nuclei of the outer nuclear layer) was coincident with the first appearance of twin cones. In both snapper and triplefin larvae, twin cones formed some time after the first appearance of putative rod precursors. Certainly at the time of settlement, metamorphosed juveniles of both snapper and triplefins possessed duplex retinae in which the double and single cones were arranged in a regular square mosaic.

The mosaic arrangement of snapper and triplefins (present study), and red sea bream (Kawamura, \textit{et al} 1984) that was established in the juvenile stages remained unchanged in adults. However, cone types and mosaic arrangements are known to change during ontogeny in some fishes (Lyall, 1957b; Engstrom, 1963; Boehlert, 1978; Bowmaker and Kunz, 1987). In a number of species there is a loss of single cones, which is often coincident with the movement into deeper or more turbid water (Lyall, 1957b; Boehlert, 1978; Bowmaker and Kunz, 1987). For example, the surface dwelling prejuveniles of \textit{Sebastes diploproa} have a regular ordered mosaic of single and double cones. Loss of single cones is first seen in the peripheral retina of prejuveniles. Adults of the species, which undergo a habitat shift to a benthic environment at 250-500m depth, have only double cones. Less frequently, triple cones occur in the retinae of adult
They are thought to be transitional elements, as single cones are most probably lost by fusion with double cone elements (Boehlert, 1978). Double cones and poorly organised cone arrays have been associated with less acute vision in low light intensity environments (Boehlert, 1979). In support of this, mid and deep water teleosts captured at trawl depths of 500-1000m, had retinae with extremely low double cone density or had pure rod retinae (Munk, 1966; Locket, 1977; Pankhurst, 1987). The highly ordered single and double cone mosaic organization found in many shallow water fishes, is thought to be necessary for highly acute visual perception (Engstrom, 1963; Boehlert, 1979; Fernald, 1989), the discrimination of movement (Lyall, 1957b; Engstrom, 1963; Ahlbert, 1976) and border enhancement (Browman et al., 1990), as well as the resolution of colour (Fernald, 1985; 1989). Stell, (1976), Bowmaker and Kunz, (1987) and Fernald, (1989), have elucidated the chromatic identity of the various single and double cone elements which make up mosaic units in the retinae of goldfish, cichlids and trout respectively. Single cones and individual double cone elements have photopigments of differing absorbance maxima. Therefore mosaic arrangements ensure that a regular array of colour detectors is distributed across the retina.

Young trout have a square mosaic in which four double cones surround a central single cone. In addition they have four corner 'additional' single cones which have absorbance maxima of 355nm (near U.V.)(Bowmaker and Kunz, 1987). Bowmaker and Kunz (1987) suggest that U.V. vision may be important for the detection of planktonic prey in the very shallow water environment of larval and yearling trout. U.V. cones are subsequently lost when yearlings move to deeper water. There are no data concerning the chromatic identity of the various cone elements which constitute a mosaic unit in the retinae of snapper or triplefins. Neither snapper nor triplefins, possess the additional corner single cones, which have been identified as U.V. sensitive cones in young trout. The square mosaic arrangement of triplefin and snapper retinae appear to be consistent with their "shallow" water habitats.
2.3.3 Acuity

Like those of other teleosts, the eyes of snapper and triplefins continued to grow with increasing body size. The absolute density (cells per unit area) of all cell types in the neural retina, with the exception of rods, decreased with increasing eye size, which supports earlier reports that eye enlargement involves both stretching of the neural retina and cellular addition (Johns, 1981; Fernald, 1985). The relative contribution of neural addition to retinal growth in one year old goldfish (Johns, 1981) and the cichlid Haplochromis burtoni (Fernald, 1985), were estimated to be 20 and 40% respectively.

Decreasing cone density in snapper and triplefins was accompanied by an increase in cone outer segment diameter. Despite quite large changes in cone density, increasing cone diameter resulted in the maintenance of a closely packed cone array in fishes of all sizes. Hypertrophy of cones with increasing eye size would appear to be an integral part of retinal growth in many teleosts (Lyall, 1957; Blaxter and Jones, 1967; Johns and Easter, 1977; Boehlert, 1979; Guma’a, 1982; Pankhurst and Montgomery, 1990). Cone enlargement may enhance sensitivity of the photopic system by increasing photon capture areas (Munz and McFarland, 1973).

Although numbers of cones decreased with increasing eye size, the angular density of cones in both snapper and triplefins increased. This is a consequence of changes in the retinal magnification factor whereby an object of given angular subtense projects onto an ever larger retinal area as the eye grows, and is a function of increasing size of the lens. As a result, theoretical spatial acuity of snapper and triplefins increased with increasing body size, to an asymptote in adults, when increasing size of the lens is presumably matched by decreasing cone density.

Similar changes in spatial acuity with growth have been reported in other fishes (Blaxter and Jones, 1967; Johns and Easter, 1977; Guma’a, 1982; Hairston et al., 1982). For the purposes of comparison, MSA values derived from Tamura and Wisby (1963), which gives twice the angular subtense calculated in the present study, have been corrected. Theoretical acuity of adult triplefins (8-10") was low compared to other diurnal benthic carnivores which ranged from 1-8' (corrected, Pankhurst, 1989). Using the
criteria given by Pankhurst (1989), triplefins have both absolutely and relatively small eyes compared to other species from the same reef habitat. The relatively low acuity estimates of adult triplefins, compared with other shallow marine diurnal species, probably reflect the constraints imposed upon acuity by small eye size. Triplefins appear to be visual feeders (unpublished observations) with generally large, conspicuous, slow moving prey which includes hermit crabs, crabs, gastropods and amphipods (Thompson, 1979). This is consistent with their modest theoretical acuity. Snapper on the other hand have both absolutely large and relatively large eyes. Theoretical spatial acuity of adult snapper was between 3 and 5' (present study) and ranks snapper amongst the highest of New Zealand diurnal demersal carnivores examined (Pankhurst, 1989). Due to the large eye size of adult snapper, spatial acuity remains high despite very large increases in cone diameter.

The low levels of theoretical acuity of larval snapper and triplefins (2° 1' and 1° 8' MSA at four and one day of age, respectively) are similar to values reported for other larval species at first feeding. Herring larvae had theoretical acuity of 1° 36' (corrected, Blaxter and Jones, 1967), plaice and turbot larvae 1°, and 1° 30' respectively (Neave, 1984), perch larvae 2° 15' (corrected, Guma’a, 1982), and white sea bass larvae 1° 30' (Margulies, 1989). Low acuity of larvae appears to be partly a function of small eye size. Larval snapper and triplefins have cones of small diameter (2.0 and 1.9 μm, respectively) which are very tightly packed (34 and 40 cells.0.1mm⁻¹ retina). Similarly, in 9 other species of larval fish, mean cone ellipsoid diameter varied between 1.2 and 2.8 μm and cone density ranged between 32 and 56 cells.0.1mm⁻¹ (Blaxter and Staines, 1970). Further increases in retinal packing are presumably proscribed by the physical limit to photoreceptor size (1-2 μm) below which light spills into adjacent photoreceptors (Lythgoe, 1980; Ali and Klyne, 1985). The retinal grain of larval fish, including snapper and triplefins, appears to have been maximized within these physical constraints.

Theoretical estimates of visual acuity are a function of cone spacing and focal length of the lens, which in the present study was estimated using Matthiessen’s ratio of 2.55 x r(radius of the lens). Behaviourally determined acuities of adult fishes approach
theoretical estimates set by cone spacing (Northmore and Dvorak, 1979; Breck and Gitter, 1983; Powers and Easter, 1983). It seems generally accepted that the finest resolution for retinal images is determined by the distance between centres of adjacent cone photoreceptors, however, acuity estimates do not take into account the size of ganglion receptive fields (convergence of cones : ganglions), or higher order processing (Browman et al., 1990). This may explain the relatively small differences between theoretical and behavioural estimates of visual acuity in adult fish reported in other studies (Powers and Easter, 1983; Browman, et al., 1990). In addition, there may be errors associated with the use of Matthiessen’s ratio to estimate focal length of the lens. Matthiessen’s ratio is in fact an average value from a measured range of 2.4-2.82 (Matthiessen, 1880). This ratio measured optically in a variety of other fish has been shown to be quite variable (see chapter 3, table 3-1). Accordingly the estimates of theoretical visual acuity may be a little higher or lower than values given here. For example, theoretical estimates of visual acuity of one day old triplefin larvae varied between 1° 3' and 1° 24' over the measured range of Matthiessen’s ratio (2.1-2.8), compared with a value of 1° 8' given in the present study.

Behaviourally determined estimates of acuity in larval fishes are very much lower than theoretical estimates (Neave, 1984). This disparity will be investigated further in chapter 3.

2.3.3 Scotopic sensitivity

The juveniles of both snapper and triplefins have duplex retinae and this means that the metamorphosed juveniles can respond to visual stimuli at lower light intensity levels than the larvae. In the case of red sea bream, the advent of the duplex retina at the end of the larval phase, is coincident with an ontogenic shift to a demersal habitat in estuaries and enclosed bays (Kawamura, 1984), in which juveniles feed almost entirely upon prey species which are concentrated in patches near the bottom (Tanaka, 1985). During the juvenile phase in triplefins, and throughout juvenile and adult stages in snapper, both rod density and convergence of rods onto first order retinal neurons
increased. In adult triplefins addition of rods appears to just keep pace with retinal enlargement. Behavioural experiments have determined that rod sensitivity thresholds in other species remain constant during growth, despite the continued addition of rods (Powers and Bassi, 1981; Allen and Fernald, 1981). It has been concluded that continued rod addition is probably required to maintain sensitivity thresholds during eye enlargement, however, the mechanism by which this is achieved is unknown (Powers and Easter, 1983).

Pankhurst (1989) used morphological criteria to rank scotopic sensitivity in 31 shallow water, temperate teleosts from New Zealand. Results indicated that most nocturnal and crepuscular species had high theoretical sensitivity (scotopic rank 9-12) whereas most diurnal species had average (scotopic rank 6-8) or low sensitivity (scotopic rank 3-5) (Pankhurst, 1989). Using the same ranking system, the sensitivity of juvenile and adult snapper and triplefins were consistent with other temperate diurnal species (scotopic ranks varied between 6 and 7 in snapper, whereas triplefins had a scotopic rank of 5).

Retinomotor movements were absent in the pure cone larval eyes of triplefins and snapper, first appearing in juveniles, when the duplex retina had developed. This is consistent with earlier findings by Blaxter and Jones (1967), Blaxter and Staines, (1970), Ali (1975), and Guma’a (1982), that the onset of retinomotor shifts, coincide with the development of rods. Rods function only in very dim light and become saturated in daylight. Nicol (1989) suggests that the alternate movements of rods and cones, maximizes light absorption of rod outer segments in the dark, and cone outer segments in the light. In the light adapted state, retinomotor movements may protect rods from harmful levels of illumination as well as reduce backscatter of light emerging from cone outer segments, thus preventing image quality loss by light spilling into adjacent cones.
Chapter Three

Visual Function of Larval Fishes

Introduction

Many larval fishes, including larval snapper and triplefins, have pure cone retinae at the time of first feeding, which effectively limits visual function to the photophase. It has been shown that light is required for feeding in many larval fishes (Arthur, 1976; Blaxter, 1986) and consequently larval fishes are considered to be primarily visual feeders. There are, however, a few reports of larval fish feeding at very low light intensities (Blaxter, 1986) and in larvae of the mottled sculpin, the superficial lateral line has been implicated in feeding in total darkness (Jones and Janssen, in press).

Prey size selection by planktivorous fishes has been shown to be a direct function of the increasing visual resolution of the fishes eyes with growth (Li, et al., 1985). Factors which determine visual acuity are cone spacing within the retina, and the focal length of the lens (a function of lens diameter). In addition, there may be some acuity loss associated with convergence of cones onto higher order neurons. Cone spacing increases during growth, however this is more than offset by increasing lens size, such that theoretical acuity increases with increasing eye size, to an asymptotic value in adults (see chapter 2). The dioptric power of the teleost eye is invested entirely in the spherical lens, because the cornea, intra-ocular fluids and aquatic environment have the same effective refractive index (Powers and Easter, 1983; Nicol, 1989). Consequently the absolute limit of visual detection in teleosts, is set by the resolving power of the lens, the accommodative state of the eye and the intercone spacing within the retina. The resolving power of the teleost lens is approximately 10 times greater than can be resolved by the cone mosaic (Northmore and Dvorak, 1979; Fernald and Wright, 1985b) and therefore resolution of the lens does not limit visual acuity, even in very small fishes (Fernald and Wright, 1985b). Spherical lenses of uniform refractive index produce poor images because light rays, at different distances from the optic axis, are focussed at
different distances from the lens (Spherical aberration). Teleost lenses do not suffer these imperfections because a refractive index gradient exists between the lens core and cortex, so that light rays passing through the lens are brought into focus at a common point (Fernald and Wright, 1983). Chromatic aberration of 1.8-5.3% of the focal length, has been reported in teleost fishes (reviewed by Fernald and Wright, 1985a). Fernald and Wright (1985a) measured chromatic aberration of $1.9 \pm 0.3\%$ of the focal length, in the cichlid *Haplochromis burtoni* of a range of sizes, and determined that the chromatically aberrant images still fell on the photoreceptor outer segment layer. In addition, it was apparent that the coloured blur fringes, produced by longitudinal chromatic error in these lenses, would not be resolved by the retinal cone mosaic. Consequently, chromatic aberration of fish lenses is unlikely to be a factor in image resolution.

In addition to the optical and neural properties of the eye, visual detection at any time will depend upon refractive state of the eye. The refractive state is the degree to which the plane of focus coincides, or fails to coincide, with the photoreceptor outer segments in the retina. The lens and retina of teleost eyes are usually symmetrically disposed and bear a constant relationship to each other during growth (Easter *et al*, 1977; Fernald and Wright, 1987). This relationship is described by $R = M x r$, where $R$ is the focal length of the lens (distance from the lens centre to the level of the photoreceptor outer segments in the retina), $r$ is the lens radius and $M$ is a species-specific constant, otherwise known as Matthiessen's ratio (Table 3-1). There is little consensus about the plane of focus within the retina of the relaxed teleost eye. Reports range from myopia (plane of focus vitread to the photoreceptor layer) to hyperopia (plane of focus behind the eye). Much of the perceived variation in measured refractive state has been attributed to the techniques used to determine refractive state (reviewed by Fernald and Wright, 1985b). A major difficulty arises from the fact that the site of retinoscopic reflex in the teleost retina is not obvious. Sivak, (1974) reported that retinoscopic measurements, and anatomical measurements obtained from frozen sections, do coincide, and that the retinoscopic measurements are in fact made from the photoreceptor layer. However,
Table 3-1. Measured values of Matthiessen’s ratio (M) for various teleost fishes.

<table>
<thead>
<tr>
<th>Species</th>
<th>M</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldfish</td>
<td>2.36</td>
<td>(Charman &amp; Tucker, 1973)</td>
</tr>
<tr>
<td>Goldfish</td>
<td>2.12-2.46</td>
<td>(Easter et al., 1977)</td>
</tr>
<tr>
<td>Saithe</td>
<td>2.43</td>
<td>(Sadler, 1973)</td>
</tr>
<tr>
<td>Cod</td>
<td>2.56</td>
<td>(Sadler, 1973)</td>
</tr>
<tr>
<td>Flounder</td>
<td>2.17-2.47</td>
<td>(Sivak, 1982)</td>
</tr>
<tr>
<td>Cichlid</td>
<td>2.25</td>
<td>(Fernald &amp; Wright, 1983)</td>
</tr>
<tr>
<td>Plaice</td>
<td>2.51</td>
<td>(Hansen, 1988)</td>
</tr>
<tr>
<td>Blenny</td>
<td>2.38</td>
<td>(Hansen, 1988)</td>
</tr>
<tr>
<td>Goby</td>
<td>2.34</td>
<td>(Hansen, 1988)</td>
</tr>
</tbody>
</table>
errors will arise if the lens retractor muscle is not in a fully relaxed state when measurements are made. As a consequence small lens displacements can produce large variations in the focal distance measured, especially in small eyes.

Changes in refractive state are brought about during dynamic accommodation by displacement of the lens within the optic cup. The accommodative system of teleost fishes generally involves a single lens retractor muscle (the campanula halleri, or retractor lentis muscle), the lens, and its associated ligaments. The lens is suspended by a suspensory ligament from the dorsal iris and is attached via a ventral ligament to a retractor lentis muscle. The retractor lentis muscle is itself firmly attached by a short ligament to the ventral iris (Somiya, 1987). A pigmented covering on the retractor lentis muscle is thought to prevent axial displacement of the lens during contraction (Somiya, 1987). The lens is enclosed by the anterior chamber which is occupied by the aqueous humour, and the posterior chamber which contains the gelatinous vitreous humour. The anterior surface of the vitreous is membranous and is separated from the lens by a narrow space filled with a viscous fluid which allows the lens to move during dynamic accommodation (Nicol, 1989). Accommodative lens movements can occur along the pupillary axis in some teleosts, but more commonly occur along the pupillary plane with only a small axial component (Tamura, 1957; Fernald and Wright, 1985b; Somiya, 1987; Nicol, 1989). Accommodative lens movements are often directed at regions of retinal specialization (Tamura, 1957; Fernald and Wright, 1985b). For example, the primary axis of accommodation is naso-temporal in the eyes of the African cichlid *Haplochromis burtoni*. When the retractor lentis muscle is relaxed, the temporal retina (area of high cone density) is focused for near vision whereas contraction of the retractor lentis muscle, adjusts the focus of the temporal retina for far vision (Fernald and Wright, 1985b).

There are no data regarding the refractive state of early post-embyronic larval fish eyes. Larval white sea bass (Margulies, 1989) and herring (O'Connell, 1981) were initially incapable of accommodative lens movements because the retractor muscle did not form until some time after the onset of feeding. Presumably the refractive state in the eyes of very small larval fishes, prior to formation of the accommodative system, will be
dependent upon the degree to which the static focal plane does, or does not coincide with the cone receptor outer segments.

Theoretical estimates of visual resolution of larval eyes based upon intercone spacing within the retina are low (Blaxter and Jones, 1967; Guma'a, 1982; Neave, 1984; Margulies, 1989). Estimates of spatial visual acuity (chapter 2) of first feeding larval snapper (2° 1° MSA) and triplefins (1° 8° MSA) are consistent with the low acuity reported for the larvae of other species.

Thoretical acuity estimates of adult fishes are generally several times greater than behavioural estimates of acuity (Browman, et al, 1990; reviewed by Powers and Easter, 1983). Overestimation of theoretical acuity is probably a function of signal loss due to convergence onto ganglion cells within the retina, and higher order processing. Behavioural estimates of acuity in larval plaice at hatching, and larval turbot at 2 days of age, were very much lower (42° and 21° MSA respectively) than theoretical estimates based upon retinal morphology alone (1° and 1° 30' MSA respectively)(Neave, 1984).

However behavioural acuity improved rapidly to approach theoretical estimates at approximately 35 and 25 days after hatching in plaice and turbot larvae respectively. Rahmann and Jeserich (1979) also reported a rapid improvement in behavioural acuity in rainbow trout at the time that the larvae "swim up" from the nursery gravel beds. A number of authors have attributed the rapid improvement in behavioural acuity, observed at about the commencement of exogenous feeding in larval turbot, plaice and trout, to a period of rapid synaptogenisis in the optic tectum and cerebellum (Rahmann and Jeserich, 1979; Neave, 1989; Margulies, 1989; Schmitt and Kunz, 1989). The inference is that the development of the retina precedes that of the optic tectum and in consequence, behavioural acuity improves and approaches theoretical estimates as the integrative function of the optic tectum increases. However, a striking feature of the eyes of early post-embryonic stages of larval fishes is the large depth of the retina with respect to the lens diameter. Matthiessen's ratio has not been measured in very small larval fishes and an uninvestigated possibility, is that the mismatch of theoretical and behavioural estimates of acuity in larval fishes may in part be a result of refractive error.
If this is so, then changes in the refractive state of the eyes during early development, may in part, account for the improvement in behavioural acuity with ontogeny.

The present study attempts to expand the current understanding of visual capabilities in two marine larval fishes, with special emphasis upon the critical period of first feeding. Behavioural acuity of larval snapper and triplefins was determined using the optokinetic response (Shaw and Tucker, 1965; Shaw and Sachs, 1967; Rahman and Jeserich, 1979; Neave, 1984) and was compared with theoretical estimates described in chapter 2. The development of the accommodative mechanism was examined in the eyes of larval snapper and triplefins, from the time of hatching. Fernald and Wright (1985b) found that there were difficulties associated with the direct measurement of the focal length in very small eyes (smallest lens radius 0.5mm). This was because pupil aperture limited retinoscopic measurements, and focal length estimates made from the section of frozen tissue were quite variable, probably because small errors associated with lens movement during freezing produced potentially large errors in the measured focal length. For the present study it was considered that the very small eye size of first feeding larval snapper and triplefin larvae (lens radii 0.036mm and 0.049mm respectively) precluded the use of either of the above techniques. Instead, a relative measure of focal length, as defined by Matthiessen's ratio (R/r, where R is the distance from the lens centre to boundary of the pigmented retinal epithelium and r is the lens radius), was measured along the optical axis, from histological material in a size range of larval fish. This was an attempt to ascertain whether the retina and lens grew in proportion in very small eyes, and was only possible because the accommodative system and posterior chamber did not develop until after the period of interest. The lens was tightly apposed to the retina, and lens displacement during fixation could be discounted. It was assumed that shrinkage was constant over the size range of larval eyes examined, allowing detection of relative changes in refractive state.

Larval visual characteristics have to be related to the conditions under which they operate in the wild. Size of prey dominates prey selection in marine larval fishes with prey size increasing as the larvae grow (Arthur, 1976; Stepien, 1976; Hunter, 1981).
Larger prey have a greater nutritional content than small prey, and although in the ocean many more small than large prey are consumed, a few large prey can make a major contribution to growth. For example small prey (40% mouth width) comprise 50% by number of prey consumed by Pacific mackerel, but contribute to only 10-15% of total volume consumed (Hunter, 1981). One study showed that prey size selection was optimised in marine larval fish when primary and secondary planktonic production was high, but larvae fed less discriminately and selected all prey sizes available to them, when planktonic production in the ocean was low (Rajasilta and Vuorinen, 1983). In addition, prey selection by marine larval fish is probably affected as much by prey visibility (ie visible size), as absolute size of the plankton. Prey visibility has been shown to influence prey selection in larger planktivorous fishes (Wright and O’Brien, 1982). Most first feeding larval fish consume microzooplankton in the 30-200μm size range (Arthur, 1976; Hunter, 1981). The upper limit of prey size is limited by jaw width of the fish, and the critical dimension of prey, which was found to be the maximum width of the prey including appendages (Hunter, 1981).

There are no data available regarding the composition or size range of prey ingested by first feeding snapper or triplefin larvae. This is despite extensive sampling both of wild snapper larvae and the associated natural assemblages of microzooplankton within the Hauraki Gulf (New Zealand), by the New Zealand Ministry of Agriculture and Fisheries Research Division (Wellington, New Zealand). Data collection to determine prey size selectivity and dietary composition of snapper larvae has posed problems. Firstly, very few first feeding larval snapper were captured. The apparent "undersampling" of small larvae by plankton trawl techniques may be a result of extrusion of very delicate, early larval stages, through the plankton mesh. Secondly the few intact first feeding larvae collected displayed a high incidence of empty or evacuated guts (Pers. comm. Dr J. Zeldis, MAF Fisheries Research Centre). Accordingly, a different approach was used in the present study. Maximum jaw widths were used as a best estimate of the upper limit of ingestable prey size. Reactive distances (distance at which a predator first perceives a prey and reacts to it) were predicted for larval snapper and triplefins using
behavioural estimates of visual acuity, and jaw width as an upper size limit of prey. Incidence of feeding (% of larvae with food present in the gut) was determined in larval snapper that were maintained in a normal light:dark regime, and another group that were maintained in total darkness to ascertain whether larval fish required light for feeding.

Attempts were made to measure reactive distances of larval fishes to prey items of known size, using single frame video analysis. However this proved unsuccessful for the small larvae involved in the present study, for similar reasons reported by Blaxter and Staines (1971) in attempting to use cinematography techniques. Difficulties arose from the small field of view and shallow depth of field of the video at the magnifications required to resolve both the predator and prey. This was coupled with a requirement for a relatively large water volume to initiate a feeding response in the larval fish, and as a result no feeding events were captured on the videotape.
3.1 Materials and Methods

3.1.1 Histology

Fixation, embedding and sectioning techniques are described in chapter 2.

*Accommodative mechanism*

Morphological development of the retractor lentis muscle and lens suspensory ligaments, were examined in eyes of a range of sizes of larval snapper and triplefins.

*Estimation of Matthiessen’s ratio*

Measurements of the relative focal length of larval eyes, as defined by the ratio $R/r$ where $R = $ distance from the lens centre, along the optical axis to boundary of the pigmented retinal epithelium, and $r = $ lens radius, were made in the following way. Serial transverse sections (1-2.5µm) of whole larval snapper and triplefins in which the lenses were sectioned along with the eyes, were examined. Photomicrographs were taken of the largest diameter meridian section of one eye, in each of 8 larval triplefins (0-16 days) and 6 larval snapper (3 to 22 days). The centre of the lens was located on 25 x 16 cm photomicrographs, and the distance from the lens centre to the boundary of the pigmented retinal epithelium was measured along the optical axis.

*Maximum jaw width*

Serial transverse sections of whole larval snapper and triplefins were examined to assess changes in jaw width with increasing body size. Jaw widths were measured in 5 successive transverse sections using 6 triplefin larvae (5.0-7.0mm SL) and 5 snapper (3.4- 5.8mm SL). Maximum jaw width was taken as the maximum width of the buccal cavity as defined by the developing osteological structures, which have been described for the Japanese sub-population of *P. auratus* by Matsuoka (1985). The jaw widths of larval snapper were measured immediately anterior to the orbits (Fig. 3-1), whereas the maximum jaw dimension of triplefin larvae was more antero-nasal (see results).
Fig. 3-1. Osteological development of the cranium and jaws of red sea bream (a) at a total length of 2.90mm and (b) at 5.9mm total length. (After Matsuoka, 1985). Vertical lines indicate the approximate position at which maximum jaw width measurements were taken from serial transverse sections of *P. auratus* (present study). Stippled area is cartilage (a,b), and open area is ossified bone (b). (1) Lateral view of neurocranium, (2) ventral view of neurocranium, and (3) lateral view of upper and lower jaws and opercular bones. AN - angular, AUC - auditory capsule, EC - ethmoid cartilage, Enp - endopterygoid, Et - epiphysal tectum, Etp - ethmoid plate, Hm - hyomandibular, Ma - maxillary, MC - Meckel's cartilage, OP - occipital process, OPE - opercle, Pa - palatine, Pm - premaxillary, Scl - scleral, RC - rostral cartilage, SOB - supra orbital bar, Tr - trabecula. Scale bars are 0.2mm.
3.1.2 Behavioural Acuity

Behavioural acuity of larval snapper and triplefins was determined using the optokinetic response. Snapper larvae were reared from eggs that were obtained from natural ovulation of female broodfish captured by long-line at Okakari Point (Northland, New Zealand), on three separate occasions during November and December 1990. Fish were manually stripped and eggs artificially fertilized with milt from captive, ripe males. Eggs were incubated and larvae cultured using the larval rearing techniques described in chapter 1. Rearing conditions are given in Table 3-2.

Table 3-2 Rearing conditions of larval *P. auratus* used in optokinetic experiments.

<table>
<thead>
<tr>
<th>Brood</th>
<th>Date</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nov-Dec,1990</td>
<td>16.5-18.0</td>
</tr>
<tr>
<td>2</td>
<td>Dec,1990</td>
<td>18.3-20.5</td>
</tr>
<tr>
<td>3</td>
<td>Dec,1990</td>
<td>18.3-21.0</td>
</tr>
</tbody>
</table>

Triplefin larvae were sampled from larval rearing trials 1, 2 and 3 (described in chapter 1), during June to August 1990.

The optomotor apparatus (Fig. 3-2) consisted of a plexiglass disc which was rotated at speeds between 0-8 rpm by a variable speed, reversible electric motor. The stimulus consisted of equal width black and white stripes, produced on paper sheets using a laser printer and held on the rotating disc by PVC cylinders of 6 and 12cm diameter. Stripe widths were determined from;

\[
\alpha = 2 \times \arctan \frac{0.5H}{RD},
\]

where \(H\) = stripe width, \(RD\) = reactive distance, and \(\alpha\) = angle to subtend visual angles of between 10° and 40° at the side of the animal chamber (Breck and Gitter, 1983). A grey paper cylinder was used in control trials. The animal chamber (a glass tube, 2cm
Fig. 3.2. The optomotor apparatus used for determination of behavioural acuity. It consists of a plexiglass disc which is rotated at speeds of between 0.8 to 8 rpm by a variable speed, reversible electric motor. The animal chamber (open arrows) is suspended by a horizontal arm, in a stationary position in the centre of the rotating stimulus cylinder (black arrowhead). Stimulus stripes subtend visual angles of between 1° and 40° at the side of the animal chamber.
diameter), was suspended in a stationary position in the centre of the rotating stimulus cylinder. Stimulus stripe widths and corresponding visual angles at the side of the animal chamber are given in table 3-3.

Table 3-3 Stimulus stripe widths (H) which subtended visual angles (α) at the side of the animal chamber when placed on 6cm and 12cm* diameter rotating stimulus drums.

<table>
<thead>
<tr>
<th>Angle (α) subtended (°)</th>
<th>Stripe width H (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>14.5</td>
</tr>
<tr>
<td>30</td>
<td>10.7</td>
</tr>
<tr>
<td>20</td>
<td>7.1</td>
</tr>
<tr>
<td>15</td>
<td>5.3</td>
</tr>
<tr>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>1.7</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>1</td>
<td>0.9*</td>
</tr>
</tbody>
</table>

Larvae were sampled from the rearing tanks using a beaker. One larval fish was introduced into the animal chamber at a time using a wide-mouthed pipette. Extreme care was taken during transfer of the larval fish as rupture of the delicate larval finfold resulted in death. Fish were allowed to settle for approximately one minute before optokinetic trials began.

A positive response consisted of whole body pursuit of the moving stripes, which varied both in speed and direction. Trials started with the largest stripe width and slowest speed (less than 1 rpm). Responses were noted at increasing speeds and with direction change, at each stripe width. If a positive response was elicited, larvae were tested with increasingly smaller stripe widths until no response was elicited. Minimum separable
angle (MSA) was taken as the smallest stripe width to which a positive response was recorded. Once the smallest stripe width had been determined, larvae were again tested at a larger stripe width to establish whether the larva was failing to respond because of exhaustion. Any trials in which larval fish fatigued, or died prior to achieving the nil response criterion, were rejected. Larvae were tested with the grey control cylinder between successive change in stimulus stripe width. A positive response was never elicited by the grey control cylinder.

Fish were tested from the time of first feeding (day 1 in triplefins and day 4 or 5 in snapper), and on alternate days thereafter to 14 or 16 days post hatching in triplefin and snapper larvae respectively. Repeat transfer of larval fish proved unsuccessful and as a consequence each fish was tested only once. Three or four larvae could be tested during daylight hours, on any one day. Optokinetic trials which reached the nil response criterion were pooled with data from previous trials so that an average of 4 and 5 responses was obtained at each age tested, in snapper and triplefin larvae respectively.

Optokinetic experiments were conducted in a constant temperature room (14°C ± 1, for triplefins, and 18°C ± 1 for snapper) in which overhead fluorescent lights provided illumination of 1.4-1.6 μE.m⁻².s⁻¹, in the animal chamber. This was comparable to surface illumination in the rearing tanks (1.5 μE.m⁻².s⁻¹), in which the larvae actively fed.

3.1.3 Reactive distances

Reactive distances (RD) of snapper (4-16 days post hatching) and triplefin larvae (1-14 days post hatching) were calculated for a size range of prey items from;

\[ \alpha = 2 \times \arctan 0.5 \frac{H}{RD} \]

where \( \alpha = \) MSA (degrees) obtained using the optokinetic response, and \( H = \) prey width (mm) (Breck and Gitter, 1983). The size range of prey widths (150, 200 and 300 μm) correspond to maximum jaw widths measured in a size range of larval fish.
3.1.4 Larval feeding

Snapper larvae were sampled by beaker (150ml) from rearing trial 4 (December 1989, described in chapter 1), on the 4th, 5th, 6th, 9th and 13th days after hatching. All fish were sampled during the day between 11am and 2pm, which allowed for a minimum of 5 hours feeding in the photophase (15:9h artificial L:D cycle, at light intensity of 1.5μE.m⁻².s⁻¹), prior to sampling. Fish were anaesthetized in the beaker by addition of 1-2ml of 0.02% 2-Phenoxyethanol (BDH). When fish had stopped swimming (1-2 minutes) they were individually transferred into a petri dish using a wide-mouthed pipette, and examined under a Nikon binocular dissecting microscope for presence or absence of food in the gut. Special care was taken so as to minimise disturbance of the larval fish during sampling in an effort to reduce the likelihood of spontaneous gut evacuation.

On the 6th day after hatching, approximately 50 larval snapper were transferred into a 5 l aquarium in total darkness for 24h, to assess whether larval fish were capable of feeding in the dark. Food density (5-10.ml⁻¹) and water temperature were the same as those in the rearing tanks. After 24 hours in the dark, 20 snapper larvae were removed from the aquarium, anaesthetized (as above) in the dark, and examined as before, for presence or absence of food in the gut. Normal illumination was restored to the remaining "dark treated" larvae, which were left a further 2 hours before being anaesthetized and examined as before.

Triplefin larvae have a long straight gut and slow peristaltic contractions continued in anaesthetized fish with the result that gut contents were almost always evacuated within 1-2 minutes. Several techniques were tried to overcome these difficulties, including immersion of larvae into cold fixative (5% gluteraldehyde in sucrose 0.1M phosphate-phosphate buffer). However, this induced violent body contractions and immediate gut clearance. The prevalence of gut evacuation in larval triplefins precluded the estimation of feeding incidence in this species.
3.2 Results

3.2.1 Snapper

*Accommodative mechanism*

Rudimentary development of a single lens retractor muscle was visible in the ventro-temporal retina of first feeding snapper larvae (4 days of age) as a pigmented outgrowth of the ventral iris (Fig. 3-3a). This was presumed to be the developing pigmented covering of the retractor lentis muscle. The lens at this stage was closely apposed to the optic fibre layer of the retina except for a small retro-lental space at the retinal margin. An anterior chamber was visible between the cornea and lens (Fig. 3-3b). A discrete retractor lentis muscle was present in 7 day old snapper larvae (Fig. 3-3c). A transparent ligament attaching the lens to the retractor lentis muscle in the ventral retina and a dorsal suspensory ligament, were recognisable in 10 day old larvae in conjunction with an enlarged retro-lental space. Twenty two days after hatching the posterior chamber had enlarged in the peripheral retina (Fig. 3-3d), however, the lens remained tightly apposed to the optic fibre layer in sections made through the fundal region of the retina.

*Matthiessen’s ratio*

The relative focal length of the lens decreased from 4.19 in a 3 day old larval snapper (1 day prior to first feeding), to a minimum of 2.2 in a 22 day old larva (Fig. 3-4).

*Jaw width*

The buccal cavity of snapper larvae of 4, 7 and 12 days of age, was framed by the ethmoid cartilage on the ventro-medial surface of the cranium and Meckel’s cartilage on the lower jaw. The maximum jaw width was taken as the distance between the cartilage plates on the lower jaw (Fig. 3-5a). The skeletal elements in older snapper larvae (18 and 22 days of age) had started to ossify. Here, the maximum jaw width was taken as the distance between the developing angular bones and Meckel’s cartilage on each side of
Fig. 3-3. Photomicrographs of the developing accommodative mechanism in the eyes of larval *P. auratus*. (a) 4 days after hatching rudimentary development of the retractor lentis muscle is seen as a pigmented outgrowth (presumptive pigmented covering of the retractor muscle) of the ventral iris (arrow). (b) The anterior chamber (arrow), the space between the cornea (C) and lens (L), is present in the eye of 4 day old snapper. (c) A discrete retractor lentis muscle (arrow) is visible in 7 day old larval *P. auratus*. (d) 22 days after hatching the posterior chamber (PC), which is occupied by the vitreous humour, has expanded around the retinal margin and the retractor lentis muscle and pigmented covering (arrow), have enlarged. I - iris, ON - optic nerve. Scale bars are 50μm (a), 10μm (b), 25μm (c) and 100μm (d).
Fig. 3-4. Changes in the ratio of $R/r$, where $R$ is the distance from the lens centre to the boundary of the pigmented retinal epithelium, and $r$ is the lens radius, with increasing age in *P. auratus*. 
Fig. 3-5. Camera lucida drawing of transverse sections through the cranium and jaws of (a) a 7 day old larval *P. auratus*, in which maximum jaw width was taken as the distance between Meckel's cartilage (MC) in the lower jaw, and (b) a 22 day old larval *P. auratus*, where maximum jaw width was taken as the distance between the angular bone (An) and Meckel's cartilage of the lower jaw. An - angular bone, CNS - central nervous system, Et - epiphysal tectum bone, Etp - ethmoid plate, Olf - olfactory tissue, Ma - maxillary cartilage, MC - Meckel's cartilage. Ossified bones are black, cartilaginous skeletal elements are stippled. Scale bars are 170μm (a) and 340μm (b).
the lower jaw (Fig. 3-5b). Jaw widths ranged from 152µm at first feeding (3.4mm SL, 4 days old) to a maximum of 373µm in a 22 day old larva (5.7mm SL) (Fig. 3-6).

Behavioural acuity

Behavioural acuity of larval snapper was initially very much lower than acuity determined histologically (Fig. 3-7, theoretical acuity data from chapter 2). Behavioural acuity improved with growth from \(38^0 \pm 2^0\) MSA in 4 day old larvae, to \(8^0 8' \pm 36'\) at 16 days of age. This was still lower than the theoretical estimate of acuity (55' at 18 days).

Reactive distance

The estimated reactive distance of first feeding snapper larvae (4 days post hatching, maximum jaw widths of 150µm), was 0.2mm for a prey width of 150µm. Estimates of reactive distance increased, both with increasing body size and prey width, to a maximum of 2.1mm for a prey width of 300µm width in a 16 day old snapper (maximum jaw width 300µm)(Table 3-4).

Incidence of feeding

The incidence of feeding in larval snapper increased from 21% in 4 day old larvae, to 100% in 9 and 13 day old larvae (Table 3-5). After 24 hours in total darkness only 2 of 20 larvae had food present in the gut. Almost half (48%) of dark-treated larvae had resumed feeding 2 hours after return to the light.

3.2.2 Triplefins

Accommodative mechanism

The anterior chamber, enclosed by the cornea, lens and iris was present in the eyes of triplefin larvae from the time of hatching. A single retractor lentis muscle was present in the ventro temporal retina of larval triplefins from 7 days after hatching (Fig. 3-8b). At this time the lens was closely associated with the optic fibre layer of the retina (Fig. 3-8a). A very small retro-lental space (posterior chamber) was present at the retinal
Fig. 3-6. Changes in maximum jaw widths (micrometres) of larval *P. auratus* with age. Values are mean ±SE from 5 successive transverse sections of a single larval fish.
Fig. 3-7. Changes in behavioural and theoretical acuity (MSA) in larval *P. auratus*, with increasing age. Behavioural acuity values are mean + or - SE, and theoretical values are for a single larval fish.
Table 3-4. Reactive distance (RD) of larval snapper, *P. auratus*, for prey of varying dimensions, calculated using behavioural estimates of visual acuity (MSA), and maximum jaw width (JW) as a measure of maximum possible prey dimension.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>MSA (degrees)</th>
<th>JW (μm)</th>
<th>RD (mm) for prey widths of</th>
<th>150μm</th>
<th>200μm</th>
<th>300μm</th>
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<td>-</td>
</tr>
<tr>
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<td>0.4</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>150</td>
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<td>1.0</td>
<td>2.1</td>
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Table 3-5. Percentage of larval snapper with food present in the gut at various times after hatching.

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<th>Age (days)</th>
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<th>sample size</th>
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<tr>
<td>5</td>
<td>46</td>
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<tr>
<td>6^1</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>6^2</td>
<td>48</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>13</td>
<td>100</td>
<td>5</td>
</tr>
</tbody>
</table>

1 Percentage of larvae feeding during 24 hours in total darkness.
2 Percentage of dark treated larvae which had resumed feeding 2 hours after return to the light.
Fig. 3-8. Photomicrographs of transverse sections through the dorsal (a) and the ventro-temporal retina (b,c) in a 7 day old larval F. varium. The retractor lentis muscle (RLM, white arrowhead) and its pigmented covering (P) are visible in the ventral retina. The lens is closely associated with the retina (black arrows) except for a small retrolental space (posterior chamber, PC) immediately posterior to the iris (I) at the retinal margin. CGZ - circumferential germinal zone. Scale bars are 25μm (a,b) and 10μm (c).
margin between the lens and the circumferential germinal zone (Fig. 3-8c). The retro-lental space had not enlarged in triplefin larvae up to 16 days of age and neither were a dorsal or ventral lens suspensory ligament visible.

*Matthiessen's ratio*

The relative focal length of the lens decreased from a maximum value of 2.68 in a newly hatched larva to minimum values of 2.14 and 2.26 in 10 and 16 day old larvae respectively (Fig. 3-9).

*Jaw width*

The developing skeletal structure of the cranium and jaws was similar in triplefin larvae of all sizes (1-16 days). The roof of the buccal cavity was framed by cartilage of the developing ethmoid plate in the ventral cranium, and the lower jaw was defined by Meckel's cartilage (Fig. 3-10). The maximum jaw width was taken as the distance between the cartilaginous plates in the lower jaw. Jaw width of a first feeding larval triplefin (5.00mm SL, 1 day old) was 206μm and increased to maximum values in 7.00 mm (SL) larvae, of 294μm and 276μm at 10 and 16 days of age respectively (Fig. 3-11).

*Behavioural acuity*

Behavioural acuity of larval triplefins, was initially 28° ± 3° MSA at one day of age. This compared to a theoretical estimate of spatial acuity (chapter 2) of 1° 8' (Fig. 3-12). Behavioural acuity improved to 4° 18' but was still lower than the theoretical value (54') at 14 days of age.

*Reactive distances*

The reactive distance of first feeding triplefin larvae (1 day post hatching), with maximum jaw widths of 200μm, was 0.3mm for a prey width of 150μm and 0.4mm for a prey width of 200μm (Table 3-6). Estimates of reactive distance increased, both with
Fig. 3-9. Changes in the ratio of $R/r$, where $R$ is the distance from the lens centre to the boundary of the pigmented retinal epithelium and $r$ is the lens radius, with increasing age in *F. varium*.
Fig. 3-10. Camera lucida drawing of a transverse section through the cranium and jaws of a 10 day old larval *F. varium*. Maximum jaw width was taken as the distance between Meckel’s cartilage (MC) in the lower jaw. CNS - central nervous system, Olf - olfactory tissue. Scale bar is 288\(\mu\)m.
Fig. 3-11. Changes in maximum jaw widths (micrometres) of larval *F. varium* with age. Values are mean ± SE from 5 successive transverse sections of a single larval fish.
Fig. 3-12. Changes in behavioural and theoretical acuity (MSA) in larval *F. varium*, with increasing age. Behavioural acuity values are mean + or - SE, and theoretical values are for a single larval fish.
Table 3-6. Reactive distance (RD) of larval triplefins, *F. varium*, calculated using behavioural estimates of visual acuity (MSA), and maximum jaw width (JW) as a measure of maximum possible prey dimension.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>MSA (degrees)</th>
<th>JW (μm)</th>
<th>RD(mm) for prey widths of</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>150μm</td>
</tr>
<tr>
<td>1</td>
<td>28.6</td>
<td>200</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
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<td>200</td>
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</tr>
<tr>
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<tr>
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<td>6.6</td>
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</tr>
<tr>
<td>14</td>
<td>4.3</td>
<td>300</td>
<td>2.0</td>
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</table>
increasing body size and prey width, to a maximum of 4.0mm in 14 day old triplefins (maximum jaw width 300μm).

3.3 Discussion

Behavioural acuity of 1 day old triplefin larvae (28° 36' MSA) and 4 day old larval snapper (37° 30' MSA) was low compared to histological estimates of 1° 8' and 2° 1' respectively. Neave (1984) reported similar findings in larval plaice and turbot. Behavioural acuity of newly hatched plaice and 2 day old turbot larvae was 42° and 21°, compared with theoretical acuities of 1° and 1° 30' respectively. Similarly, trout larvae did not show an optokinetic response until 10 days post hatching when the highest behavioural acuity was 30° (Rahmann, et al, 1979). As in the present study behavioural acuity of larval flatfish and trout improved very rapidly and later slowed to approach an asymptote (Rahmann et al 1979; Neave, 1984). By 14 and 16 days after hatching behavioural acuity of triplefin (4° 18') and snapper larvae (8° 8') in the present study more closely approached, but still did not reach theoretical values (54' and 55' respectively).

As previously discussed (chapter 2), errors may arise from the use of 2.55 as an estimate of Matthiessen's ratio to calculate focal length of the lens in estimating theoretical acuity. However, the range of Matthiessen's ratio measured in teleost fishes (Table 3-1) is not large enough to account for the very large differences between behavioural and theoretical estimates of acuity of larval fish. For example, theoretical estimates of acuity in one day old larval triplefins varied between 1° 24' and 1° 3' depending upon the value of Matthiessen's ratio chosen (range 2.1-2.8).

Behavioural measures of acuity in adult fishes are often several times greater than theoretical estimates based upon cone spacing alone (Powers and Easter, 1983; Browman, et al., 1990). This is thought to be because theoretical estimates do not take into account signal convergence within the retina (ganglion receptive fields), or higher order processing (Browman et al., 1990). However, the difference is much more marked
in first feeding larval fishes, with behaviour estimates generally being an order of magnitude lower than theoretical estimates of acuity. It is suggested that visual acuity during early larval development, may be dependent upon the degree of differentiation of the optic tectum, and that the rapid improvement in behavioural acuity of larval fish, at or soon after the onset of first feeding, is attributable to a period of rapid synaptogenesis in the optic tectum (Rahmann, et al., 1979; Margulies, 1989; Schmitt and Kunz, 1989).

Another possibility is that the disparity between theoretical and functional acuity may in part be due to refractive error in the developing larval eyes. Matthiessen's ratio decreased from 4.19-2.2 in snapper larvae over 22 days, and from 2.67-2.1 in triplefin larvae over 16 days. The decreasing value of Matthiessen’s ratio indicates that the relative position of the lens and retina are changing with growth. Presumably, Matthiessen’s ratio attains a constant value at some time after hatching. This appears to be the situation in post-larval fishes of other species. Easter et al., (1977) measured the focal length in a size range of goldfish (2-6cm), both with a retinoscope in intact eyes, and from photographs of frozen tissue. They found that the lens and retina grew in proportion and that Matthiessen’s ratio, was a constant 2.12-2.46 depending upon the axis of measurement. However the relationship was not examined in the eyes of early postembryonic larval stages. Fernald and Wright (1983) investigated the optical properties of the lens in a size range of African cichlid fishes. Using laser beams to illuminate the isolated lens, they established that lenses produced finely focused images, regardless of lens sizes, in fish with lens radii equal and greater than 0.5mm.

If the optical properties of fish lenses are constant throughout growth, then a changing value of Matthiessen’s ratio, as described in the present study, will result in a change in the point of focus within the neural retina during growth. The rapid improvement in functional acuity of larval fish observed in this and other behavioural studies (Rahmann et al 1979; Neave, 1984), might be explained by a refractive error (myopia) which presumably would decrease during ontogeny as the retina and lens diameter attained constant proportions.
The effect of a changing Matthiessen's ratio and the resulting refractive error, would not be a problem if the amplitude of lens movement were sufficient to accommodate the refractive error in these very small larval eyes. However, the accommodative system was poorly developed in larval snapper and triplefins. A discrete retractor lentis muscle was not present until 7 days post hatching; 3 and 6 days after the time of first possible feeding in snapper and triplefin larvae respectively. Suspensory ligaments were not visible in light micrographs in the retinae of larval triplefins to 16 days of age. However, formation of the dorsal and ventral lens ligaments, 10 days after hatching in snapper larvae, was concomitant with the expansion of the retrolental space at the retinal margin. Presumably the retrolental space expanded as the vitreous (produced by the retina during development Nicol, 1989), was secreted. Formation of the posterior chamber appeared to progress from the retinal margin towards the central retina during ontogeny, but was incomplete in 22 day old snapper larvae. Triplefin larvae up to 16 days of age, and snapper larvae up to 22 days of age, were therefore presumed to be incapable of accommodative lens movements and this compounds the problem of a refractive error in the earliest larval stages.

Behavioural acuity of snapper and triplefin larvae (present study), trout (Rahmann, et al, 1979) and larval plaice and turbot (Neave, 1984), demonstrate that visual resolution of larval eyes at the time of first feeding is very low, but increases with increasing body size. Light is required for feeding in many larval teleosts (Arthur, 1976), at least in the early larval stages when the threshold light intensity required for feeding responses, was on average about 0.1 lux (reviewed Blaxter, 1986). If feeding is visually mediated, and snapper data (present study) suggest it is, then changing visual function has major ramifications for feeding competence of larval fishes.

Feeding by larval fishes is constrained by the availability of food, the ability to ingest it, and the ability to detect it. Density of plankton of an appropriate size for larval fishes, is on average very low in oceanic waters (13-40 nauplii.l⁻¹ and 1-7 copepods.l⁻¹). However, there is evidence to suggest that distribution of zooplankton can be highly
patchy, which may explain feeding success of fish larvae, in water masses which otherwise appear to be incapable of sustaining them (Hunter, 1981).

Prey size dominates prey selection by larval fishes, with size of prey increasing with body size (Shirota, 1970; Arthur, 1976; Hunter 1981). Jaw widths provide a good estimate of the upper limit of prey size available to larval fish (Hunter, 1981). For example, the ratio of prey width to mouth size at first feeding in anchovy and Pacific mackerel, was close to unity for Artemia nauplii and anchovy eggs, and was 0.6 for the rotifer B. plicatilis. The maximum jaw widths of first feeding snapper (3.4mm SL) and triplefins larvae (5.0mm SL) were 152µm and 206µm respectively, which are in keeping with the size-range of prey, 30-200µm, reported for other first feeding larval fishes (Shirota, 1970; Hunter, 1981). Maximum jaw widths increased to 373µm in a 22 day old snapper (5.8mm SL) and 294µm in a 10 day old triplefin larva (7.0mm SL). Shirota, (1970) found that there was a close relationship between mouth size and size range of natural prey ingested by larval fishes, such that the upper limit of prey size increased with increasing body size. Accordingly it is likely that the size range of prey ingested by larval snapper and triplefins increases with increasing jaw dimensions. There are considerable nutritional gains to ingesting larger prey items. Hunter (1981) reported that one 500µm copepod provided the same calorific content as ten 200µm copepods. Therefore a few larger prey items can make a relatively large contribution to larval growth.

Teleost larvae have limited yolk at hatching and must make a successful transfer from yolk nutrition to exogenous feeding soon after hatching (Blaxter, 1969; Vladimirov, 1975; Li and Mathias, 1987). Snapper and triplefin data (chapter 1) support an earlier review by Miller et al (1988), which showed that time to first possible feeding was a negative function of larval size at hatching, and that the generally greater yolk volume of larger larvae, provides more time to find food and initiate feeding before the onset of "irreversible starvation" (also called the "point of no return", PNR). Snapper larvae were smaller and less developed than triplefin larvae at the time of hatching. First feeding was observed in some snapper larvae on days 4-5 post hatching, and larvae denied food from hatching, died at approximately 8 days of age. On the other hand, triplefin larvae started
feeding 1 day after hatching, and larvae that were denied food, starved to death at 7-9 days of age. Both snapper and triplefin larvae became moribund, sinking slowly with the head down, for about 1 day prior to death by starvation. As a consequence, the "window" available for the onset of exogenous feeding was just 2-3 days (33-66 degree-days at rearing temperatures ranging from 17-22°C) in snapper larvae, whereas the period available to establish exogenous feeding in triplefin larvae was considerably longer, lasting for between 5 and 6 days (65-93 degree-days at rearing temperatures ranging from 13-15.6°C).

Behavioural acuity of snapper larvae was $38^\circ \pm 2^\circ$ MSA at the time of first possible feeding, and only made a modest improvement to $24^\circ \pm 3^\circ$ at 6 days of age. Reactive distance estimated for first feeding snapper (4 days of age) was just 0.2mm, improving to 0.4mm at 6 days of age (optimal prey size of 150μm), which means that prey items must be very close indeed before snapper larvae are able to detect them. Snapper larvae therefore appear to have quite severe temporal and visual constraints during the critical period when they are establishing exogenous feeding. This is compounded by a small jaw width which will limit the size range of prey available to them. It would appear then, that first feeding snapper larvae are highly susceptible to starvation mortality. Triplefin larvae on the other hand, do not appear to suffer such rigid constraints during the first feeding period. Triplefin larvae have a longer "window" period in which to initiate feeding. In addition, visual resolution of triplefin larvae, as indicated both by behavioural acuity and reactive distance estimates, was considerably better than snapper larvae at the time of first possible feeding. Behavioural acuity of triplefin larvae during the first feeding period (day 1-6 after hatching), improved considerably from $29^\circ \pm 3^\circ$ MSA to $13^\circ \pm 1^\circ$, whereas reactive distance improved from 0.4-0.9mm for prey of optimal size.

Reactive distance estimates of snapper and triplefin larvae at the onset of first feeding (0.2 and 0.4mm for prey of optimal size), are shorter than reactive distances of other first feeding marine teleosts (Blaxter and Staines, 1971; Blaxter, 1986). Blaxter and Staines (1971) estimated "Perceptive Distance" of first feeding larval herring (8-11mm body length), plaice (6-7mm body length) and pilchard (4-5mm body length) to be
approximately 3.5mm, 3.5mm and 1.0mm respectively. Herring and plaice larvae were fed Artemia nauplii which are larger (200μm -700μm) than the upper limit of prey size determined for first feeding snapper and triplefin larvae, whereas the smaller larval pilchards were offered natural zooplankton in a size range smaller than Artemia (size not specified). Blaxter and Staines (1971) presented these data with the reservation that the perceptive distance estimates were subjective observer assessments of feeding events. (Cinematography techniques were abandoned for the same reasons that video analysis of larval feeding events proved unsuccessful in the present study). Accepting these reservations, and the larger size of prey ingested by herring and plaice, the reactive distance estimates of first feeding snapper and triplefins appear to be very small. However, by 16 and 14 days of age, reactive distance of snapper and triplefin larvae had increased to 2.1mm (prey size 300μm wide) and to 4.0mm (prey size 300μm) respectively. These data correspond well with reactive distances of 3.5mm, 4.0mm and 2.0mm in herring, plaice and pilchard larvae at 15 days of age (Blaxter and Staines, 1971).

The consequences of increasing reactive distance are enormous in terms of increased probability of prey detection. Fish search a volume that is a square, or cubic function of the reactive distance (O'Brien et al 1976; Wright and O'Brien, 1982). Taking the more conservative estimate (search volume = RD^2), then a 10 fold increase in reactive distance in snapper (4-16 days of age) and a 13 fold increase in reactive distance in triplefins (1-14 days of age), equates to increases in the respective search volumes of 2.5 x 10^3 and 1.0 x 10^4.

Larval survival is based upon a playoff between numbers of offspring produced, and the energy bestowed upon individual larvae (Leiby, 1984; Pankhurst and Conroy, 1987). At one extreme, reproductive effort is directed towards production of many, nutrient poor, small-volume eggs, which are broadcast into the oceans. Larvae of these "pelagic spawners" characteristically hatch at a small size and early stage of development. At the other end of the continuum, reproductive effort is directed towards maximizing investment in a smaller number of nutrient rich, larger volume eggs which are
often protected in some manner by the parents, and are more developed and a larger
size at hatching (Balon, 1984). Pankhurst and Conroy (1987) plotted the relationship
between egg volume and relative fecundity in a number of fresh water and marine
teleosts. Snapper and triplefins fall into the middle of this range, with snapper placed
more toward the "high fecundity" end of the relationship (relative fecundity 4-6 x 10^6 eggs
. kg^-1 body weight), whereas triplefins are displaced more toward the "energy bestowal"
end of the relationship (relative fecundity between 4 x 10^4 and 2 x 10^5 eggs . kg^-1 body
weight). Snapper are pelagic spawners and produce many small pelagic eggs (absolute
seasonal fecundity ranging between 5.7 x 10^5 and 2.3 x 10^7 in mature females, Scott,
1991). Small, vulnerable, larvae hatch at an early stage of development, after a very
short incubation period and there is no parental care given. Triplefins, on the other hand
have a more modest egg production ( average absolute fecundity of 1.6 x 10^3 (estimated
by Thompson, 1979), however male triplefins guard the benthic nests during a more
protracted incubation period. Guarding of the nests must significantly decrease the
likelihood of predation upon triplefin eggs, while the valuable gain to larval triplefins, of a
larger sized egg and longer incubation period, might be the greater size and degree of
development at the onset of feeding. This difference in parental investment is finely
reflected in the differences in development and feeding competence in larval snapper and
triplefins.
References


transition from endogenous to exogenous nutrition. *Progressive Fish-Culturist* 47, 87-93.


Stock Solution A

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Distilled water to 1l

Stock Solution B

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Distilled water to 100ml

Stock Solution C

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<td>Vit H (biotin)</td>
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Distilled water to 100ml

Stock Solution D (Optional)

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Distilled water to 1l

For F Medium (F/2 Medium is half the following concentrations): Per 500ml autoclaved, filtered (0.22μm) sea water (algal stock cultures), or per 500l UV-sterilized, filtered (30μm) sea water (production cultures), add:

- 500 l Soln A
- 50 l Soln B
- 50 l Soln C
- 100 l Soln D (optional)
Appendix 2. Electron microscope fixation and embedding protocol.

**Primary fixation Method 1.**

Glutaraldehyde (25%) 5ml
0.1M Phosphate buffer, to 25ml
pH 7.4 (2g sucrose/100ml)

**Primary fixation Method 2.**

Glutaraldehyde (25%) 2ml
Formaldehyde (10%)* 5ml
0.1M Phosphate buffer, to 25ml
pH 7.4 (2g sucrose/100ml)

* Formaldehyde must be made up fresh. Heat 2g paraformaldehyde in 50ml distilled water (in fumehood) to 60°C and then add a few drops of 1N NaCl until it clears.

(This fixative does not keep for more than a few days.)

**Phosphate - Phosphate Buffer**

Solution A 0.1M NaH₂PO₄.2H₂O 15.601g.l⁻¹
Solution B 0.1M Na₂HPO₄ 14.196g.l⁻¹
Mix solutions until pH = 7.4 (approximate ratio of 1:5, solution A : solution B)

**Secondary Fixation**

Dilute 4% OsO₄ to 1% with 0.1M phosphate buffer.

Fix for 1-2h. Wash in distilled water.

**Dehydration and Embedding**

Dehydrate in an ethanol series
30, 50, 70, 90% (10 minutes each)
100%, 2 changes (10 minutes each)
acetone, 2 changes (20 minutes each)
2:1 acetone : Embed resin (3-4 hours)
1:2 acetone : Embed resin (overnight)

Cure for 48 hours at 60°C in Embed-812 resin.
EMbed - 812 resin (Electron Microscopy Sciences)

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</table>

Electron Microscopy Stains

Methanolic Uranyl Acetate

1g uranyl acetate in 10ml of 50% methanol
Shake, then leave to settle.
Keep in dark.

Lead Citrate (Venable and Coggleshall, 1965)

Dissolve lead citrate 0.03g
1N fresh NaCl 1.0ml
Dilute to 10ml with CO₂ free H₂O.
Filter before use.

Electron Microscopy Stain Procedure

methanolic uranyl acetate 10 min
methanol rinse, blot dry
lead citrate 5 min
distilled water rinse, blot dry