UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL : Development of Sensory Organs and Changes of Behaviour in the Early Larval of Mouse Grouper, *Cromileptes altivelis.*

IJAZAH : Sarjana Sains (Akuakultur)

SESI PENGAJIAN : 2004/2005

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PERKEMBANGAN ORGAN DERIA DAN PERUBAHAN PERLAKUAN LARVA PERINGKAT AWAL KERAPU TIKUS, *Cromileptes altivelis*

AUDREY DANING TUZAN

TESIS INI DIKEMUKAKAN UNTUK MEMENUHI SYARAT MEMPEROLEHI IJAZAH SARJANA

INSTITUT PENYELIDIKAN MARIN BORNEO
UNIVERSITI MALAYSIA SABAH
2007

DECLARATION

The materials in this thesis are original except for quotations, excerpts, summaries and references, which have been duly acknowledged.

AUDREY DANING TUZAN
PS04-004-012
DECEMBER 2007

ACKNOWLEDGEMENTS

I would like to express my sincere grateful acknowledgement to my supervisors, Dr. Yukinori Mukai for his invaluable suggestions, guidance, encouragement, support and patience throughout my study period. My gratitude also goes to my co-supervisor, Prof. Dr. Shigeharu Senoo.

I thank Universiti Malaysia Sabah for the assistantship and Yayasan Universiti Malaysia Sabah for providing me the scholarship to support my study. I would like to express my gratitude to Prof. Dr. Saleem Mustafa, Director of Borneo Marine Research Institute, Dr. Ridzwan Abdul Rahman, Director of Centre for Research Management and Conference (Former Director of Borneo Marine Research Institute), Prof. Datin Dr. Maryati Mohamed, Dean of Centre for Postgraduate Studies and Prof. Datuk Dr. Mohd. Noh Dalimin, Vice-chancellor, Universiti Malaysia Sabah, for their encouragement and assistance.

My sincere appreciation is also extended Institute of Tropical Biology and Conservation for allowing me to use the equipment in the institute during my experiment. I extend heartfelt thanks to Dr. Saifullah Jaaman, Dr. Siti Raehana, Dr. Shahbudin Saad, Mr. Julian Ransangan, Mr. Abentin Estim, Mr. Kennedy Aaron Agoul, Mr. Motinius Guntalib, Mrs. Rossita Shapawi, and all the staff of Borneo Marine Research Institute for their co-operation.

I wish to heartily express my indebtedness to my dear colleagues and friends; Mr. Norazmi Osman, Mr. Henry Lipan, Mr. Ha Hou Chew, Ms. Sow Sok Hui, Ms. Nguang Siew Ing, Ms. Mok Wen Jye, Ms. Chun Kia Huey, Ms. Ching Fui Fui, Ms. Bobita Golam Ahad and all the staff of Fish Hatchery, Borneo Marine Research Institute for their assistance and valuable inputs directly or indirectly for the successful completion of the thesis.

Last but not the least; I would like to express my heartfelt gratitude to my family for their love, emotional support, patience, encouragement and support throughout my difficult period.

Finally, with the support and prayer from all of you this thesis is completed.

Praise the Lord.

ABSTRACT

This study was conducted to clarify the development of the sensory organs with larval growth in mouse grouper Cromileptes altivelis for the purpose of understanding larval behaviour and improving larval rearing methods. Larvae of the mouse grouper were reared from hatching to 20 days old, sampled every day, and observed under light microscope and scanning electron microscope for morphological development of the sensory organs. Eggs were collected from the hatchery of Borneo Marine Research Institute. Larvae were fed with rotifers, Artemia nauplii, copepods and artificial powder feed. The newly hatched larvae were 1.87±0.20 mm (mean ±S.D) in total length (TL) and floated motionless in the water column. A pair of free neuromasts (15-17µm diameter and 35-40 sensory cells) was observed behind the eyes in newly hatched larvae. The inner ears of the newly hatched larvae were only oval-shaped vesicle. One-day-old larvae (TL. 2.47±0.47 mm) still showed suspended posture, and were repeatedly moving up and down in the water column. The eyes of one-day-old larvae had a lens and several layered retina, and olfactory pits were opened with epithelium cilia. Two-day-old larvae (TL. 2.60±0.39 mm) swam with a horizontal posture, the eyes were slightly pigmented, the mouth was opened, and lower jaw movements were observed. Three-day-old larvae (TL. 2.64±0.35 mm) had well pigmented eyes when they commenced ingesting rotifer Branchionus sp. Six pairs of free neuromasts were found on the head and 4 pairs on the unilateral side of the trunk of 3-day-old larvae. Free neuromasts were distributed around the eyes on the head, and the middle of the trunk. On the head the free neuromasts were oriented on lines tangential to concentric circles around the eye; hence the free neuromasts on the head could detect the stimuli from various angles. It was considered that free neuromasts of mouse grouper play an important role in avoiding predators especially until the eyes begin to function. The apical surface of free neuromasts changed in outline from a circular shape to a lozenge shape, suggesting that the shape of the cupula changes from a stick-like shape to a vane-like shape. Larvae of 5 days old start to show positive phototaxis. At 20 days old, taste buds were observed on the epithelium of the gills. The development of the sensory organs is accompanied by behavioural changes that have important implications for larval ecology at sea and mouse grouper larva rearing.

ABSTRAK

Perkembangan Organ Deria dan Perubahan Perlakuan Larva Peringkat Awal Kerapu Tikus, *Cromileptes altivelis*

Kajian ini menjelaskan perkembangan organ deria larva kerapu tikus dan bertujuan untuk memahami perlakuan larva serta memperbaiki kaedah pengkulturan larva. Larva kerapu tikus dipelihara dari peringkat mula menetas sehingga berusia 20 hari, disampel setiap hari dan larva yang disampel diperhatikan perkembangan morfologi organ derianya di bawah mikroskop cahaya dan mikroskop pengimbas elektron. Telur ikan adalah diperolehi dari hatcheri Institut Penyelidikan Marin Borneo. Larva diberi makan rotifer, naupli Artemia, kopepod dan serbuk makanan tiruan. Larva yang baru menetas mempunyai ukuran panjang keseluruhan 1.87 ± 0.20mm (purata ± S.D.) dan terapung tidak bergerak di dalam badan air. Sepasang neuromas yang bebas (15-17 µm diameter dan 35-40 sel deria) dikenalpasti di bahagian belakang mata larva yang baru menetas. Telinga hanyalah merupakan gelembung berbentuk bujur. Larva berusia sehari (TL. 2.47±0.47 mm) didapati masih berada di pertengahan kolum air dan bergerak berulang kali ke atas dan ke bawah. Mata larva yang berusia sehari mempunyai satu kanta dan beberapa lapisan retina. Pit olfaktori didapati terbuka dengan silium epitelium. Larva berusia dua hari (TL. 2.60±0.39 mm) didapati berenang secara kedudukan mendatar, mempunyai mata yang sedikit berpigmen, mulut terbuka dan rahang bawah yang bergerak. Larva berusia tiga hari (TL. 2.64±0.35 mm) mempunyai mata berpigmen sepenuhnya dan mula memakan rotifer, Brachionus sp. Larva berusia tiga hari juga mempunyai enam pasang neuromas yang bebas di kepala dan empat pasang di bahagian unilateral badan. Neuromas bebas bertaburan di sekeliling mata dan di bahagian tengah badan. Neuromas bebas didapati terdapat di bahagian kepala adalah berorientasikan garisan yang bersudut tepat kepada lingkaran di sekeliling mata yang mana neuromas bebas ini dapat mengesan ransangan dari pelbagai arah yang berbeza. Neuromas bebas yang terdapat pada larva kerapu tikus dianggap memainkan peranan yang penting bagi mengelak pemangsa khasnya sehingga mata larva mula berfungsi. Garis kasar permukaan apikal neuromas bebas berubah dari yang bentuk bulat kepada bentuk berlian segiempat. Ini mencadangkan bahawa bentuk kupula berubah dari yang berbentuk ranting kepada berbentuk bilah-kipas. Larva berusia 5 hari mula menunjukkan fototaksis positif. Pada usia 20 hari, tunas rasa dapat dilihat pada epithelium di insang. Perkembangan organ deria yang disertai dengan perubahan kelakuan membawa implikasi penting kepada ekologi larva di laut dan pengkulturan larva kerapu tikus.

ABBREVATIONS

ac	anterior canal
BH	body height
cm	centimeter
CN	ciliated non-sensory cells
CR	ciliated receptor cells
d	day
dAH	days after hatching
DO	dissolved oxygen
ED	eye diameter
GCL	ganglion cell later
hAH	hours after hatching
hc	horizontal canal
INL	inner nuclear layer
kg	kilogram
L	litre
mm	millimeter
m	metre
OGD	oil globule diameter
ONL	outer nuclear layer
ORC	olfactory receptor cell
pc	posterior canal
PE	pigmented epithelium
ppt	part pert thousand
PR	photoreceptor cell
SD	standard deviation
SEM	scanning electron microscope
TL	total length
YSH	yolk-sac height
YSL	yolk-sac length
µm	micrometer
%	percentage
°C	degree celsius

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CHAPTER 1

INTRODUCTION

1.1. Fisheries and Aquaculture Industry in Malaysia

Fisheries industry is important in Malaysia because it provides fish as a source of animal protein and also provided employment for 89,453 fishermen and 21,507 fish culturists in 2004 (Department of Fisheries, 2004). Therefore, fisheries also contribute to improving the economy of Malaysia. Total fish productions of fisheries were 1,353,187 tonnes in 1998 and 1,537,988 tonnes in 2004, and the values of fish production were RM 4.53 million in 1998 and RM 5.51 million in 2004 (Department of Fisheries, 1998; 2004).

The fishery industry includes the fishing industry and aquaculture (Department of Fisheries, 1998; 2004). The fishing industry is still the main source of the total fish production in Malaysia. In 2004, marine fishing contributed 87% of the total fish production which was (1,338,050 tonnes with a value of RM 4.80 million) (Department of Fisheries, 2004). The marine fishing sector can be divided into coastal fishery and deep-sea fishery (Department of Fisheries, 2004). The average production from the coastal fishery is about 1 million tonnes annually (Department of Fisheries, 2004). The production was 1,079,954 tonnes in 1998 and 1,060,150 tonnes in 2004 (Department of Fisheries, 1998; 2004).

Research on the natural resources showed that the coastal fishery cannot increase fish production; hence in order to increase fish production efforts must be made in the deep-sea fishery. The government of Malaysia has managed to increase the number of deep-sea fishing vessels and to controlled deep-sea fish production. However, the production of the fishing industry, including the coastal and deep-sea areas, is estimated to decrease to 900,000 tonnes by 2010 (Department of Fisheries, 2004).

On the other hand, Malaysia has a great potential to develop aquaculture. Since Malaysia has a long coastline, many islands in its waters can protect cage culture from strong wind. Recently, aquaculture in Malaysia has been developing rapidly; aquaculture productions were about 9.9% of the total production in 1998 and 13.2% in 2004. Thus aquaculture is expected to be the most important sector to increase fish supply in Malaysia. The aquaculture industry has been targeted to produce about 662,000 tonnes by 2010 (Department of Fisheries, 2004).

1.2. Groupers as Target Species for Marine Finfish Culture

Groupers are commercially important fishes because of their high market demand and value in Southeast Asian countries including Malaysia (Leong, 1998; Tucker, 1999; Sugama *et al.,* 2001). In the year 2000, the total landings of groupers in Sabah were 6,241 metric tonnes (Department of Fisheries, 2002). Within the grouper family (Serranidae), the genera *Epinephelus, Plectropomus and Cromileptes* are known as high-valued commercial fishes and are cultured all over the world (Ralston and Polovina, 1987; Leong, 1998).

At present, most commercial grouper farms still depend on wild seeds because of limited success in seed production that due to difficulties in rearing early larval stages of groupers (Kohno *et al.,* 1997; Leong, 1998; Tridjoko, 1999; Sugama *et al.,* 2001; Rimmer *et al.,* 2004; Mous *et al.,* 2006). Availability of seeds from the wild is not constant and very unreliable due to environmental and seasonal constraints. Moreover, the resources of grouper seeds have rapidly declined. Thus shortage of seed is the biggest obstacle to expanding grouper culture (Sugama *et al.,* 2001).

1.3. Mouse Grouper, *Cromileptes altivelis*

Mouse grouper, *Cromileptes altivelis,* is an expensive fish in seafood restaurants. In the live fish trade, mouse grouper is priced at up to US$70 per kg (Rimmer *et al.*, 2004). According to Senoo (2002), the market price of mouse grouper can reach RM140-180/kg, increasing up to RM160-200/kg during festive seasons. This fish has excellent flavour and high demand (Sugama *et al.,* 2001). Apart from being served as a table fish, mouse grouper is also suitable for use in the ornamental fish industry because of its attractive appearance; hence it is a target species for aquaculture in Southeast Asia (Mishina and Gonzares, 1994; Sugama *et al.,* 2001; Rimmer *et al.,* 2004; Senoo *et al.,* 2004). Mouse grouper is also known as kerapu tikus, ikan bebek, senorita, humpback grouper, barramudi cod or polka dot grouper (Randall *et al.,* 1990; Heemestra and Randall, 1993; Senoo, 2001; Senoo, 2002; Senoo *et al.,* 2004).

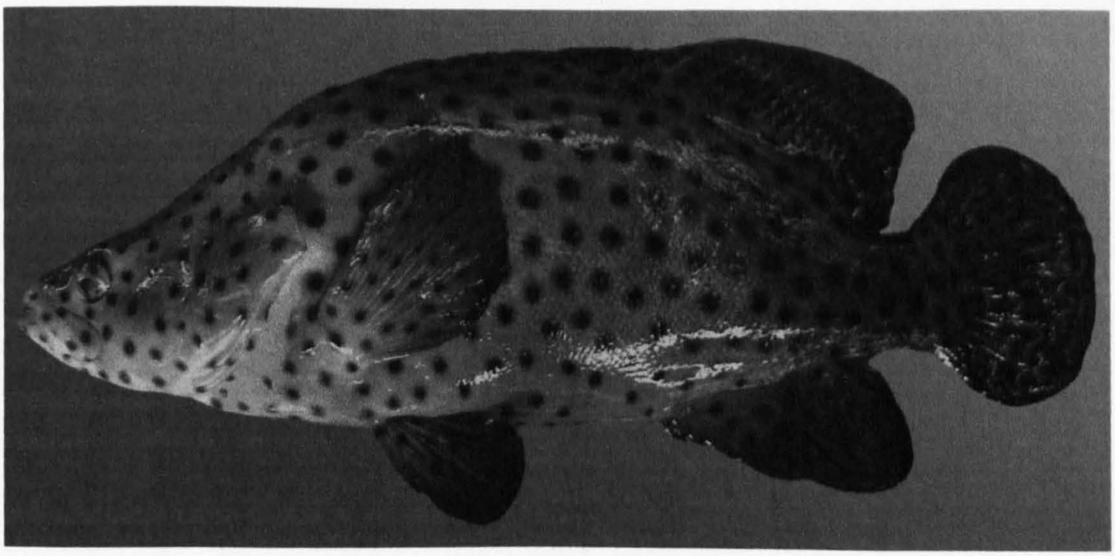

Figure 1.1: Mouse grouper, *Cromileptes altivelis,* 3.2 kg in body weight and 44.8 cm in total length.

1.4. Problems of Mouse Grouper Larval Rearing

The larval rearing of mouse grouper faces many problems. For example, until the larvae have completed metamorphosis to the juvenile stage, they are very sensitive to environmental conditions and have a high mortality (Sugama *et al.,* 2004). Therefore, careful management is required for the larval rearing of mouse grouper.

In larval rearing of mouse grouper, there are many factors related to larval mortality, for example, surface death, sinking death, entanglement of the spines, nutritional deficiency and disease (Sugama *et al.*, 2001). To improve the survival rate in larval rearing, it is necessary to eliminate or minimize the mortality from these factors.

1.5. Study on Development of Sensory Organs and Behaviour Changes

This study was conducted to obtain knowledge regarding the ecology of mouse grouper larvae as well as larval behaviour in their natural habitat in order to improve larval rearing methods. Sufficient knowledge about environmental conditions of the natural habitat and feeding habits of mouse grouper larvae is necessary for the preparation of suitable conditions in the larvae rearing tank, and the improvement of seed production methods. Ecological studies need a large number of scientists, a long period and a great deal of work. When there is insufficient knowledge on the ecology of a species, the natural habitat and feeding habits can be estimated from studies relevant to behavioural changes and the development of sensory organs with larval growth (Kawamura *et al.,* 2003).

Fish have several sensory organs such as eyes, lateral lines, olfactory organs, inner ears and taste buds. In general, the behaviour of larval fish dramatically changes with larval growth and is closely related to the development of sensory organs. The sensory organs of mouse grouper have not been studied. Therefore the study of the relationship between the development of sensory organs and changes in their behaviour is important to understand their ecology and improve larval rearing methods.

In aquaculture practices, many conditions have to be considered, such as light intensity and water flow intensity, type of feed and density of feed. By considering these conditions it is possible to prepare the best rearing conditions for mouse grouper larvae. Fundamental biological information is necessary to understand the ecology and improves the larval rearing conditions.

1.6. Objectives of Study

The objectives of this study were:

a) To describe the development of each sensory organ with larval growth
b) To describe how larval behaviour changes with larval growth.
c) To describe the relationship between sensory organ development and changes in behaviour with larval growth.

CHAPTER 2

LITERATURE REVIEW

2.1. Mouse Grouper

Mouse grouper, *Cromileptes altivelis* is a coral reef fish. This species is unique among the groupers because the head is depressed anteriorly and elevated posteriorly, rising sharply at the nape. Because of this special characteristic, this fish is known as mouse grouper (Akhbar, 2002). Mouse grouper is also known as polka dot grouper, panther grouper, barramundi cod and humpback grouper (Senoo *et al.,* 2004). This fish is classified in the phylum Chordata, class Osteichthyes, order Perciformes and family Serranidae (Akhbar, 2002; Kordi, 2001; Senoo *et al.,* 2004).

2.1.1. Morphology

Mouse grouper has a small, concave head which gives a humpback appearance (Akhbar, 2002). This fish has an oblong body and strongly compressed head (Mohsin & Ambak, 1996). The pectoral and caudal fins are round in shape and the body is covered with small and adhesive cycloid scales (Mohsin and Ambak, 1996; Akhbar, 2002). This fish can grow up to 70 cm in total length and a weight of about 4.8 kg (Kordi, 2001). Mouse grouper do not have canine teeth. The colour of the skin is creamy-grey with black spots around the body. Juvenile fish have bigger and fewer spots than adult fish (Mohsin and Ambak, 1996; Kordi, 2001; Akhbar, 2002).

2.1.2. Biology of Fish

Eggs and larvae of mouse grouper are pelagic. When they reach the juvenile stage, they become demersal fish (Kordi, 2001; Akhbar, 2002). Mouse grouper are sexually matured at 39 cm total length. This species is a protogynous hermaphrodite fish with bisexual character (Sugama *et al.,* 2001; Akhbar, 2002). Mouse grouper first sexually mature as females and later change to males (Sugama *et al.,* 2001; Akhbar, 2002). The smallest mature female is around 1 kg in body weight (Sugama *et al.,* 2001). According to Sugama *et al.* (2001), when the female reaches the weight of 2.5 kg, the sex change takes place. However, in some cases, females do not change sex even if their body weight is more than 3 kg (Sugama *et al.,* 2001). Mouse grouper is usually matured whole year (Akhbar, 2002).

2.1.3. Distribution and Habitat

Mouse grouper can be found in the western and eastern Indian Ocean, western central Pacific, eastwards to China, southern Japan to Taiwan, Philippines, Thailand, Malaysia, Indonesia, Guam and northern Australia (Randall *et al.,* 1990; Heemstra and Randall, 1993; Mohsin and Ambak, 1996; Akhbar, 2002; Rimmer *et al.*, 2004).

Mouse grouper is a coral reef species which can be found in well-developed reefs as well as dead or silty reefs (Mohsin and Ambak, 1996; Akhbar, 2002). This species stays at a depth range of 1-40 m; young fish can be found near the shore such as in coastal coral reef areas at a depth of 0.5-3.0 m (Akhbar, 2002). During larval and juvenile stages, mouse groupers can be found near estuarine areas such as silty reef areas with seagrass (Kordi, 2001; Akhbar, 2002). When the fish mature, the adult fish will move to a deeper area (7-40m) (Kordi, 2001; Akhbar, 2002).

2.2. Sensory System

Fish survival depends on the fish's ability to gain information from its environment through its senses. Table 2.1 shows the relationship of each fish sensory system with the external stimuli.

Table 2.1: Sensory system of fish

Organs	Stimuli
Eye	Light (vision)
Lateral line	Vibration (touch)
Inner ear	Vibration (hearing)
Olfactory organ	Chemical (smell)
Taste bud	Chemical (taste)

2.2.1. Eye

a. Eye Structure of Adult Fish

The primary function of fish eyes is to detect light from the surroundings (Guthrie & Muntz, 1993; Yew *et al.*, 2001; Evans, 2004). In most species of fish, eyes are considered the dominant sensory organ especially during the larval stage, as they are required for feeding, orientation, schooling and avoiding predators (Blaxter, 1968; Kawamura and Hara, 1980; Fuiman and Magurran, 1994; Rodriguez and Gisbert, 2001; Carvalho, *et al.,* 2002; Rice and Westneat, 2005).

The main optical components of eyes are the cornea, lens and retina (Figure 2.1). The fish cornea has the same refractive index as water. The fish eye has a spherical lens which provides a sufficient index of refraction for focusing images on the retina. The retina has a light-sensitive cell layer which made up of photoreceptors (rod and cone cells). Focus adjustments for near or far vision are controlled by moving the lens without changing its shape by using muscles (retractor lentis) within the eye.

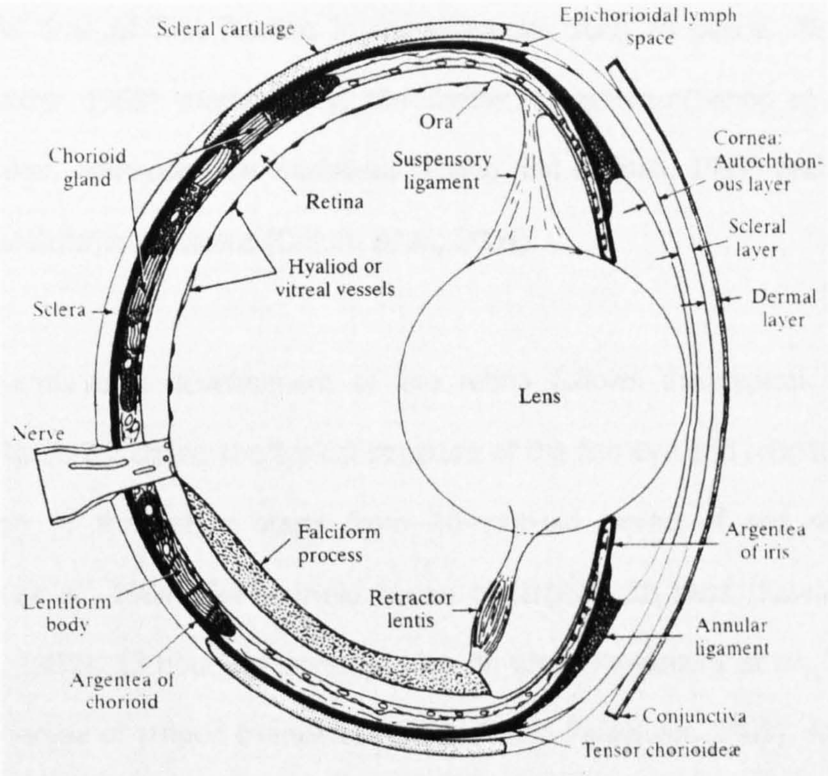

Figure 2.1: Diagrammatic vertical section of a typical teleost fish eye (Source: Bond *et al.,* 1995).

b. Eye Development

Generally, many fish species hatch with unpigmented eyes and almost certainly non-functional eyes (Blaxter, 1986), for example: halibut, *Hippoglossus hippoglossus* (Blaxter *et al.,* 1983), flounder, *Paralichthys olivaceus* (Kawamura and Ishida, 1985), largemouth bass, *Micropterus salmoides* (Kawamura and Washiyama, 1989), striped trumpeter, *Latris lineata* (Cobcroft and Pankhurst, 2003) and bluefin tuna, *Thunnus orientalis* (Kawamura *et al.,* 2003). The larvae hatch with a formed eyeball and lens, but the retina lacks of pigmentation to intercept light (Evans, 2004). The melanin (pigmentation) will gradually form in the eyes before all the yolk sac and oil globule are absorbed. In all species, the eyes are pigmented during first feeding time (Blaxter, 1986). According to Blaxter and Staines (1970), a pure-cone retina can be found at the time of first feeding in many species such as plaice, *Pleuronectes platessa* (Blaxter, 1968), marble goby, *Oxyeleotris marmoratus* (Senoo *et al.,* 1994), Atlantic croaker, *Micropogonias undulatus* (Poling and Fuiman, 1997) and Japanese flounder, *Paralichthys olivaceus* (Omura *et al.*, 2004).

The embryonic development of the retina follows the typical vertebrate sequence. Figure 2.2 shows the typical structure of the fish eye and retina. Cell layer differentiation in the retina starts from 10-hour-old larvae of red sea bream (Kawamura *et al.,* 1984), 24-hour-old larvae of largemouth bass (Kawamura and Washiyama, 1989), 13-hour-old larvae of blue fin tuna (Kawamura *et al.,* 2003) and 24-hour-old larvae of striped trumpeter (Cobcroft and Pankhurst, 2003). At the early developmental stage of the retina, the outer nuclear layer (ONL) has a single layer of cells and a thin inner plexiform layer (INL). Later, photoreceptor cells (PR) appear in the retina. According to Nag and Sur (1992), teleosts generally have two types of photoreceptor, rods and cones. In many species, cone cells (active in bright vision) start to develop before rod cells appear (active in dim vision) (Poling and Fuiman, 1997). Rod cells usually start to develop only at metamorphosis (Omura *et al.,* 2004), and continue to be added throughout life, for example in Japanese eel and Japanese flounder (Omura, *et al.,* 1997, 2003, 2004; Evans, 2004). It has been assumed that the early larval stage only has cone cells; rods being excluded from the retina until later stage (Powers and Raymond, 1990; Fernald, 1993; Shand, 1993; Higgs and Fuiman, 1996; Poling and Fuiman, 1997).

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