1. Introduction

Milkfish (*Chanos chanos*), one of the Gonorhynchiformes fishes, is a euryhaline and pelagic fish which distributes in the tropic Indo-Pacific Ocean and Red Sea where the temperatures are higher than 20 ℃ (Winans, 1985). Although milkfish couldn't be fished as an industry like tuna, herring, or cod in the wild, it is believed to be one of the best suitable species for fish aquaculture (Bardach et al., 1972). Milkfish is a herbivorous species, human could obtain inexpensive animal proteins from them through aquaculture. Historically, milkfish might have been farmed in artificial pools or nature lagoons in Indonesia, Philippines, Taiwan, and Hawaii for hundreds of years (Bagarinao, 1991). After a serial of studies during 1970s to 1980s (Liao et al., 1979; Juario et al., 1984; Lee et al., 1986; Martea & Lacanilao, 1986), the seed production techniques of milkfish have been established. Today, the production of larval milkfish is mainly achieved by artificial propagation and no longer relies on collection from the wild. As an aquaculture species and a euryhaline fish, many researches have been focused on how to cultivate the milkfish (Duray & Bagarinao, 1984; Lee et al., 1986; Duray, 1995; Sumagaysay & Borlongan, 1995; Swanson, 1998; Garcia et al., 2000; Borlongan et al., 2003) and the underlying osmoregulation mechanisms (Lin et al., 2003; Chen et al., 2004; Lin et al., 2006). Yet no detailed work has been conducted on the visual biology of the fish.

Unique to the head region of milkfish is the presence of adipose eyelid which covers both sides of the ocular areas. The adipose eyelids of milkfish extend from the post-snout parts to the operculum parts, which not only cover the whole eyeballs (Stewart, 1962) but also create a chamber between eye and the eyelid on each side of the head, and inside the chamber it contains fluid. The eyelid is a clear, fleshy structure, and it is thought to be composed of fatty tissue (Rangaswamy, 1987) and in hence named as "adipose eyelid". Its function is hypothesized to offer protection to eyeball as well as to streamline the body shape, although no such work has been conducted to test the hypotheses. Adipose eyelids are found in the Orders of Elopiforme, Albuliformes, Conorhynchiformes, Clupeiformes, Cypriniformes, Characiformes, Siluriformes, Salmoniformes, Aulopiformes, Gadiformes, Mugiliformes, and Perciformes (Rainboth, 1991; Shen, 1993; Vari & Blackledge, 1996; Motomura et al., 2000; Motomura, 2003; Murray, 2004; Torii et al., 2004; Sabaj, 2005; Zanata & Vari, 2005; Nelson, 2006). The adipose eyelids are more developed and cover the whole eyes in more primitive fishes such as Conorhynchiformes and Clupeiformes. They are much reduced and divided into two parts by an elliptical aperture over the pupils in more evolved fishes, like Mugiliformes and Perciformes (Stewart, 1962). Stewart (1962) argues that adipose eyelid may have two possible functions. First, it could work as a lens, and helps eye focus. Second, it may enable the fish to detect polarized light. The second possibility had been ruled out when it was found that the adipose eyelid-removed sockeye salmon still maintained its polarized vision ability (Dill, 1971) while the first proposed function has never been verified.

In light of the limited understanding of the characteristics of the adipose eyelid, the first part of this study was intended to find the answers of the following questions: (1) The ontogenetic development processes of the adipose eyelids; (2) What are the major components of the eyelids? (3) How does the adipose eyelid protect the milkfish's eyes? (4) Do adipose eyelids participate in focusing mechanism of milkfish eyes?

Most of mammals do not have ultraviolet (UV) (280-400 nm) vision, while the UV vision could be found in fishes more commonly (Jacobs, 1992; Honkavaara et al., 2002). The UV vision provides fish with functions such as the protection, the communication, the camouflage breaking and background choice, and the color vision in fishes. For example: many fish larvae and planktivorous fishes have the ultraviolet (UV) spectrum (280-400 nm) absorption ability (Loew et al., 1993; Britt et al., 2001; Flamarique, 2005). Because the presence of the UV-protective pigments in zooplankton which could reduce UV transparency and subsequently the contrast with UV light is enhanced (Johnsen & Widder, 2001), therefore the UV-vision predators could take this UV-contrast advantage and detect the planktonic prey easily (Tovée, 1995; Britt et al., 2001; Siebeck & Marshall, 2001). In addition, UV vision could offer fishes a clue to break the camouflage of their preys and help them choose a more appropriate background to match their body colorations (Cott, 1940). This selection becomes very important to fishes whose predators have UV vision, too. Furthermore, the UV vision is considered to be a code breaker in fish communication and the UV absorption and reflectance at body coloration pattern are the codes. Fishes with UV vision could decode the signals in the body patterns and the displayed contrasting colors in UV are masked quickly with distance, only those who are close enough with UV vision could receive and understand what these codes entailed (Losey et al., 1999). In term of the color vision, the UV sensation may play a simple role by extending the spectral range and discriminating the color in more details which enhances the fish's ability to see the object of interest (Neumeyer, 1992). Despite the UV vision could offer benefits to fish, the UV light itself has some negative effects. Exposure to ultraviolet A (UVA) light (λ: 320-400 nm) of UV light could reduce animal's hematocrit, plasma protein, and plasma immunoglobulin, while the ultraviolet B (UVB) light (λ: 280-320 nm) could affect the functions of head kidney and blood phagocytes. Furthermore, the immune system of fish could be destroyed by prolonged exposure to UV light (Salo et al., 1998a; Salo et al., 1998b; Salo et al., 2000; Jokinen et al., 2001). Therefore, having UV vision could help fish to avoid over-exposure to UV light and hence reducing possible damages by UV light (Losey et al., 1999). To prevent the retina from UV damages, some fishes resolve this problem by having the UV absorption components that are mainly in ocular lenses but are rarely in the cornea or ocular humors which could block the UV light transmission (Nelson et al., 2001; Pendergrass et al., 2001; Siebeck & Marshall, 2001).

Although the UV vision could be found in different life stages of fishes, many fishes undergo a visual spectrum shift during their ontogenetic processes. The salmonids, yellow perch *Perca flavescens*, and bluegill sunfish *Lepomis macrochirus* have the UV-sensitive pigments in their juvenile stage but lose it in the adult stage. In contrast, many minnows, goldfish *Carassius auratus* L., and some damselfishes possess the UV photoreceptors only in the adult stage (Losey et al., 1999). The cellular base of the visual spectrum shift is due to the expression of different opsins in the cone cells (Cheng & Flamarique, 2004).

What causes visual spectrum shift in fish? Two possibilities offer the explanations for much of the shift. First, because different light wavelengths have unequal transmission rate in water, hence fishes live in different depths would experience different light conditions. When the fish migrates to different depths as it grows, it should shift its visual spectrum accordingly to match with the photic conditions of the new surroundings. Second, the diet shift should also coincide with spectrum shift to maximize the capture of preys. For example, the juvenile pollack (*Pollachius pollachius*) and the juvenile yellow perch (*Perca flavescens*) are planktivorous and they all have the violet and UV photoreceptors. When they grow and exhibit diet shift, the violet and UV vision disappeared (Shand et al., 1988; Loew et al., 1993).

It takes 3 to 5 years for milkfish to reach maturity with standard lengths (SL) exceeding 1 m and they are pelagic migratory fish (Bagarinao, 1994). When the breeding season comes, the adult milkfish swims from the pelagic ocean close the offshore near coral reef or small islands where the spawning sites are. Then the newly hatched young larvae start a journey which is about 10 days from the spawning grounds to the inshore water. During this trip, it is in the surf zone and they are largely transported by the current, because it lacks the swimming ability and is under insufficient nutritional conditions (Taki et al., 1987; Morioka et al., 1996). Milkfish larvae reach the shore and end the pelagic stage when its total lengths are about 10-17 mm. Here, it is called "fry" or "seed" and are caught for aquaculture use. In the larval stage, milkfish is planktivorous and its main feeding mode is by swallowing (FishBase: http://www.fishbase.org/search.php). It captures prey mostly with the aid of their vision system (Blaxter, 1988) and the eye is likely the only sensory organ to detect food at this stage, because it only could take food under lighted condition (Kawamura & Hara, 1980). When milkfish grows into juvenile stage, it undergoes metamorphosis processes (Kawamura & Hara, 1980; Kawamura, 1984), and becomes a more powerful swimmer. It then migrates to lagoons, mangrove, and estuary waters where the foods are rich and the environments are more sheltered (Bagarinao & Kumagai, 1987). Its feeding behavior also switches from swallowing to filtering

(FishBase: http://www.fishbase.org/search.php), and the diets also become more variable. Blue-green algae, diatoms, copepods, arthropods, nematodes, and detritus are all consumed by them (Bagarinao & Thayaparan, 1986). Milkfish juveniles longer than 20 mm TL already possess most of the adult characters. These juveniles then leave the nursery waters and go back to ocean. Occasionally, some milkfish live for many years in the large lagoons, atolls, or lakes and have the adult body sizes, but they could not reach sexual maturity (Bagarinao, 1994).

The ontogenetic development of milkfish from larva to juvenile is accompanied with significant changes of visual environment. In light of these changes, the second part of this study was aimed to find the answers of the following questions: (1) Since the milkfish fish larvae are planktivorous, so do they have the UV vision to find the preys? (2) What are the visual spectra range of the larval and juvenile milkfish? (3) With the habitats and diet changes during the growth of milkfish, does visual spectrum shift could be found during the ontogenetic development of milkfish? (4) Does the adipose eyelid have the UV-light filtering function to protect eyes from UV damages?

Studies on the population genetics divided the milkfish into three distinct groups: Indian Ocean group, west Pacific Ocean group (Philippines), and north central Pacific Ocean group (Hawaii) based on morphological, biochemical, or genetic characters and some distinct subpopulations also could be found within the Philippines group

(Winans, 1980; Winans, 1985; Ravago-Gotanco & Juinio-Meñez, 2004). The limited gene flow among or within three groups could be due to the nature circumstances (Williams & Benzie, 1998; Bernardi et al., 2001), or milkfish behavior (Bagarinao, 1994). Furthermore, the adult homing and spawning site fidelity are both presumed (Ravago-Gotanco & Juinio-Meñez, 2004) to contribute to the constant existence of three groups.

If the milkfish have the homing behavior, how does the visual system contribute to the overall homing behavior? The polarization vision may be the answer. What is the polarized light? According to Maxwell electromagnetic theory, light is a form of electromagnetic radiation, its proceeding direction depends on both the electric and magnetic field. The orientation of the electric field is called the electric vector (E-vector), and the ability of detecting the orientation of E-vector by the visual system is called the polarization vision. The polarized light creates the polarization pattern both in the sky and underwater, but the underwater polarized light is only available to fishes in crepuscular time periods (Flamarique & Hawryshyn, 1997). If the animal has the polarization vision, it could see the greatest polarization part, a deeper color part than others, even if the sun is obscured (Hawryshyn, 1992). Such ability is proven to contribute to the homing behavior in many animals with polarization vision ability (Von Frisch, 1947; Reppert et al., 2004; Muheim et al., 2006).

How do the fishes have polarization vision? Flamarique & Hawryshyn (1997), Flamarique & Hawryshyn (1998b), and Hawryshyn (2000) demonstrate that there are two prerequisites. First, the presence of double cones (unequal double cones) but not twin cones (equal double cones) in fishes' retinas; and second, these double cones must be arranged and to form the square cone mosaic units. In the square cone mosaic unit, the corner single cones are usually UV sensitive, but some exceptions are found (Flamarique & Hawryshyn, 1998a).

Besides the navigation function, the polarization vision also aids in the prey finding, the contrast enhancements, the camouflage breaking, the object recognition, and the signal detection and discrimination (Cronin et al., 2003; Flamarique & Browman, 2001). In theory, this should also aid in milkfish's homing ability.

The final part of this study was designed to examine the details of milkfish retina and ask the following questions: (1) How many kinds of photoreceptor cells do milkfish have? (2) Does milkfish have the square cone mosaic to detect the polarized light?