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Elvers of tropical long-finned eel *Anguilla* sp. prefer white background in aquaria as determined behaviourally

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Abstract: In this study, given that the tropical long-finned eel *Anguilla* sp. has colour vision, we determined background colour preference of elvers of this eel in laboratory. The test aquaria covered with a pair of two-colour papers contained with a group of 10 elvers and the position of heads of elvers on each colour background was recorded 15 times for each colour pair. The background colours tested were white, black, yellow, red, green and blue. The frequencies of the head position on each colour of different pairs were analysed by the Thurstone's law of comparative judgment for paired-preference test. The elvers showed a strong bias for white background, with no significant difference in the mean z-score between the other 5 colour backgrounds. Since no negative impact of white rearing tanks on growth and stress response of other fish species were detected in previous literatures, confinement of the tropical long-finned eel *Anguilla* sp. in white background was recommended.

Keywords: tropical long-finned eel, elver, background colour preference, colour vision

Introduction

In hatcheries, common colours of commercially produced plastic tanks are white, black, red, green and blue. In the Aquaculture Department, South East Fisheries Development Center (SEAFDEC), in the Philippines, several concrete tanks are painted yellow, meanwhile white tanks are mainly used in the fish hatchery of Borneo Marine Research Institute of Universiti Malaysia Sabah. A large number of studies have been done to determine the effects of tank colour and light on feeding, growth, survival and stress response of many species of fish larvae and juveniles (reviewed in Boeuf and Le Bail, 1999; Villamizar *et al*., 2011; Kawamura *et al*., 2015). While no effect of colour was detected in half of the previous studies, yet significant effects of colour of tanks were reported in various fish species, and the effect seems to be species specific.

Experiments on the effects of colour are designed on the assumption that the test animals have colour vision. Most fish larvae are dichromatic or trichromatic at the onset of first feeding and become trichromatic or quadrichromatic after acquisition of the duplex retina (Britt *et al*., 2001). At least two types of photoreceptors are required for colour discrimination (Kelber and Osorio, 2010). In the American eel *Anguilla rostrata* (Lesuer), electroretinograms and S-potentials revealed cone peak sensitivities at 450 nm and 550 nm indicating that this eel has colour vision (Gordon *et al*., 1978). In the European eel *Anguilla anguilla* (L.), microspectrophotometry measurements revealed two different spectral classes of cones, blue-sensitive and green-sensitive cone units, implying that the European eel have the sensory capacity for colour vision (Damjanović *et al*., 2005). However, the tropical eel *A. marmorata* was reported to have a single cone type, green cone, and is colour blind (Wang *et al*., 2014). On the other hand, while the tropical long-finned eel *Anguilla* sp. has never been examined for visual pigment, we recently successfully conditioned *Anguilla* sp. elvers to discriminate blue or green colour placed among 7 different grey shades, indicating a possibility of possession of colour vision in this species (Joshua *et al*., in preparation).

In the present study, we determined background colour preference of tropical long-finned eel *Anguilla* sp. elvers in laboratory. Indonesian tropical species of anguillid glass eels have recently been exploited to be a substitute for the commercially important Japanese eel *Anguilla japonica* (Temminck and Schlegel) or European eel and are now an important aquaculture commodity (Aoyama *et al*., 2015). The technical development of these species is necessary for the

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sustainable aquaculture. Efforts are continuing to improve growth, survival, and production of these eels farmed under various conditions. In the hatchery, it is necessary to understand the behaviour of farmed animals to ensure their welfare (Mench, 1998). Kawamura *et al.* (2015) indicated the importance of choosing the correct colour for fish aggregation devices, fish tanks, structures such as breakwaters and jetties, and especially, fish tanks and cages.

Materials and Methods

Experiments were carried out under the roofed hatchery of the Borneo Marine Research Institute, Universiti Malaysia Sabah from April to May 2016.
Animal care and handling followed the guidelines for the care and use of laboratory animals set by the World Health Organization, the Malaysian Animal Handling Code of Conduct, and the National Research Council (2011).

Source of experimental elvers and husbandry

The elvers of the tropical long-finned *Anguilla* sp. were imported from Indonesia with permission by the Department of Fisheries, Malaysia. Species was identified based on morphological features; long dorsal fin and skin colouration after growing to yellow stage. The elvers were stocked in a cubic glass aquarium (100 cm × 45 cm, 40 cm water depth) equipped with water filtration system, and fed with marine sinking feed (1 mm) (Cargill Feed Sdn. Bhd., Kuala Lumpur, Malaysia) three times daily.

Three long-finned tropical eel species are reported in Indonesia; *A. celebesensis, A. borneensis* and *A. marmorata* (Arai, 2014). The eel species composition in the Poigar River estuary, Indonesia was reported that *A. celebesensis* was most abundant comprising 74.5-81.9% of all glass eels, followed by *A. marmorata* 23.8-17.7% (Arai 2014). While *A. marmorata* was excluded from our potential species based on the lack of mottled body colour pigmentation and vomerine bands morphology at 26 cm in total length, species of our eel was not identified.

Background colour preference test

For the behavioural colour preference tests, three round transparent glass aquaria with 23 cm diameter and 10 cm height were used. Their outside of half bottom and half side wall were covered with a colour paper and the other parts were covered with a different colour paper (Fig. 1). Thus, each aquarium was provided with pairs of different colour backgrounds combination; black-red, black-white, black-green, black-yellow, black-blue, white-red, white-yellow, white-green, white-blue, red-blue, red-green, red-yellow, blue-green, blue-yellow, and green-yellow.

Fig. 1: The round glass aquaria were wrapped with different combinations of colour papers for background colour preference tests.

The illuminance ranged from 70 lx to 150 lx (Light meter 401036, Extech Instruments, USA) at the water surface. The light reflectance spectra for each colour paper were measured using a spectroradiometer (HSR-8100; MAKI Manufacturing, Co. Ltd., Hamamatsu, Japan) (Fig. 2). The aquaria were arranged on a table placed in the roofed hatchery and contained dechlorinated tap water at 3 cm depth. There were 10 elvers with body weight of 2.70 to 3.36 g and total length of 13.5 to 14.7 cm, placed in each aquarium. After 25 min acclimation, the elvers were photographed at 5 min intervals with a digital camera (IXUS 160, Canon, Tokyo, Japan), positioned at 40 cm above the water surface, for 5 times (total 25 min). The number of the heads of elvers on each colour was recorded and the colour pair was changed for a

different colour pair (one session). The positions of the aquaria were rearranged using the random number table after every photographing to expose the elvers in the three aquaria to all possible ambient light conditions and avoid nuisance factors. After the first and second sessions, elvers were changed with naïve 10 specimens. The test for each colour pair was repeated 3 times. Thus, the head position on each colour background was recorded for a total of 15 times for each pair of colour papers. At the same time, the swimming behaviour of the elvers in the aquaria was visually observed and recorded by two observers.

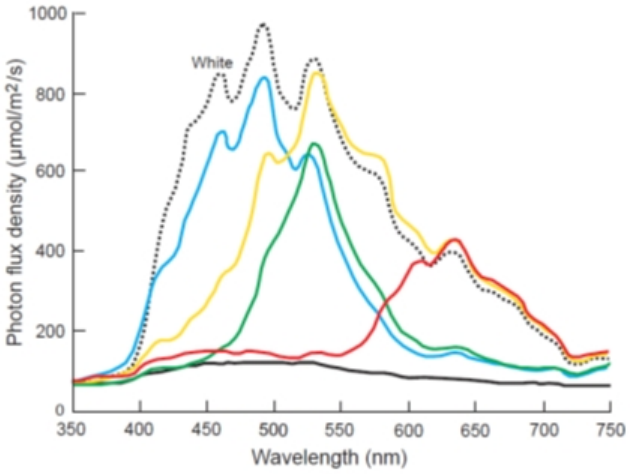

Fig. 2: Light reflectance spectra for 6 colour papers. Each colour of line represented the colour papers.

The water quality parameters were measured with a pH/ORD/EC/DO tester (Hanna Instruments, HI 9828, Washington, USA); temperature ranged from 29 to 32ºC, DO from 5.2 to 5.8 mg L^{-1}. The test was conducted during daytime from 9:00 AM to 3:00 PM for all sessions.

Data analysis

Quantitative analysis of the total number of elvers in each background colour was done by means of Thurstone's law of comparative judgment for paired-preference test (Thurstone, 1927; Kawamura *et al*., 2010). The numbers of elvers were counted for each colour of a colour pair, later transformed into proportions, and the *z*-scores were obtained from the table of cumulative probabilities of the standard normal distribution. The relative preference of fish for different colours was deduced from the mean z-score of each background colour. A negative *z*-score is below the mean, a positive *z*-score is above the mean, and a higher z-score shows a higher preference for a colour. Thus, *z*-scores imply the magnitude of relative preference. Significant difference in mean *z*-scores was confirmed with 95% confidence interval of each mean *z*-score.

Results

The elvers swam individually in random directions, mostly along the wall of the aquarium, and frequently stopped for 1 to 4 s during the observations. The result of number of head of elvers on each colour background is summarized in Table 1. Of all the 15 colour pairs, significant biases were seen between 4 colour pairs (Tab. 1). The mean *z*-score was significantly highest for white and lowest for yellow. There was no significant difference in the mean z-score among black, red, yellow, green and blue (Fig. 3), indicating the highest preference of the elvers for white background than the other 5 background colours.

Tab. 1: Summary of number of elvers on colour background presented as colour pairs to group of elvers.

	Number of elvers by colour pair (left: top)				
	Black	Red	Yellow	Green	Blue
White	89:61*	93:57**	80:70	83:67	84:66
Black		60:90*	66:84	76:74	81:69
Red			87:63	76:74	61:89*
Yellow				75:75	56:94**
Green					78:72

Note: * and ** denote significances at α = 0.05 and α = 0.01, respectively (X^2)

Discussion

The present study showed that the tropical long-finned eel *Anguilla* sp. elvers exhibited significantly biased preference for white background in laboratory. Biotelemetry studies revealed that eels essentially move at night and exhibit cryptic habits during daytime (Helfman *et al*., 1983; Parker, 1995; Baras *et al*., 1998). Elvers are also basically nocturnal (Matsui, 1993) but the difference between day and night activities is not so substantial (Tech, 2003). Therefore, preference of the elvers for white background seems not to be unexpected. In nocturnal African catfish *Clarias gariepinus* (Burchell), Bardocz *et al*. (1999) reported no effect of tank colour, white and black, on growth of larvae. Similarly reported, no effect of white tank for larval spotted sand bass *Paralabrax maculatofasciatus* (Steindachner) (Peña *et al*., 2005) and larval camouflage grouper *Epinephelus polyphekadion* (Bleeker) (James *et al*., 1997).

White tank has positive impacts in many other fish

species; greater survival in larval haddock *Melanogrammus aeglefinus* (L.) (Dowing and Litvak, 2000); greater growth and number of melanin-concentrating hormone immunoreactive cells in the brain in barfin flounder *Verasper moseri* (Jordan and Gilbert) (Yamanome *et al*., 2005); better growth in juvenile white seabream *Diplodus sargus* (L.) (Karakarsouli *et al*., 2007) and Caspian kutum *Rutilus frisii* (Kamensky) (Shahkar *et al*., 2009); better growth and survival in thinlip mullet *Liza ramada* (Risso) (El-Sayed and El-Ghobashy, 2011).

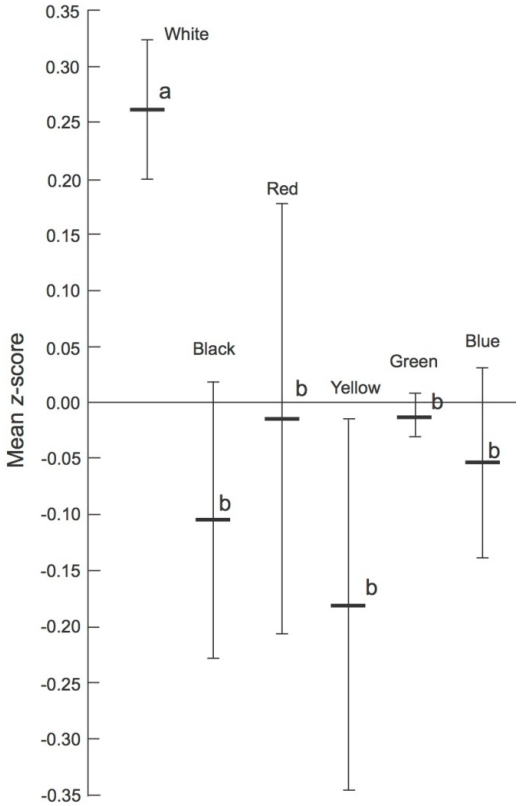

Fig. 3: Mean *z*-scores for the response of elvers of tropical long-finned *Anguilla* sp. to different colour background. Vertical bar is analysed at 95% confidence interval. Different letters denote significant differences at α = 0.05.

While some other tank colours such as red and black are reported to have negative and positive impacts on feeding and growth performance depending on fish species, negative impact of white tank has never been reported except for high incidence of jaw malformation in larval striped trumpeter *Latris lineata* (Forster) (Cobcroft and Battaglene, 2009). We recommend confining *Anguilla* sp. elvers in white tanks and associated structures for welfare of this fish, based on the background colour preference tests in the laboratory. However, growth performance and stress response of farmed fish are affected many factors other than tank colour. Höglund *et al.* (2002) reported more aggressive behaviour of juvenile Arctic char *Salvelinus alpinus* (Georgi) in white tank although there was no effect on growth. This study should be continued in raising elvers in white tank to confirm the positive impact on the elvers.

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