Moderate turbidity enhances schooling behaviour in fish larvae in coastal waters

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We evaluated the effects of turbidity on school formation in ayu (Plecoglossus altivelis) [24.5 ± 2.2 mm standard length (Ls)], Japanese anchovy (Engraulis japonicus) (29.1 ± 3.1 mm Ls) larvae, which often live in turbid coastal waters, and yellowtail (Seriola quinqueradiata) juveniles (37.1 ± 2.5 mm Ls), which live in clear offshore waters. Fish were introduced into experimental tanks at one of five turbidity levels obtained by dissolving 0, 5, 20, 50, or 300 mg l$^{-1}$ of kaolin in seawater. Their behaviour was video recorded, and the nearest neighbour distance (D$_{NN}$) and separation angle ($A_s$) were compared among turbidity levels. Mean D$_{NN}$ of ayu was significantly smaller at 20 and 50 mg l$^{-1}$ than any other level of turbidity, as was $A_s$ at 20 mg l$^{-1}$ compared with 0 mg l$^{-1}$. Mean $A_s$ of anchovy was smaller at 50 mg l$^{-1}$ of turbidity than any others. In contrast, mean D$_{NN}$ of yellowtail was larger at 300 mg l$^{-1}$ than any others. These results suggest that moderate turbidities enhance schooling behaviour in ayu and Japanese anchovy larvae, whereas turbidity has an inhibitive effect on schooling of yellowtail juveniles, corresponding well to the habitat characteristics of each species.

Keywords: anti-predatory strategy, ayu, Japanese anchovy, schooling behaviour, turbidity, yellowtail.

Introduction

Larvae of some fish species are often highly concentrated in turbid coastal waters, as are typically observed in ayu (Plecoglossus altivelis) (Tago, 2002) and Japanese anchovy (Uotani et al., 2000). Ayu is an amphidromous species spending only the larval stage in the ocean (Senta and Kinoshita, 1985; Otake and Uchida, 1998), whereas Japanese anchovy is an oceanic species. The major morphological similarity of these is that both species have the larval stage of shirasu, i.e. transparent and elongate body form. Therefore shirasu larvae are likely to be adapted to turbid waters.

Most piscivorous fish rely on vision for feeding (Guthrie and Muntz, 1993) and so their feeding efficiencies decline with increased turbidity (Utne-Palm, 2002). Turbidity dramatically improved the survival rate in larvae of red sea bream (Pagrus major) [6 mm standard length ($L_s$)], ayu (6 and 23 mm $L_s$) and Japanese anchovy (6–25 mm $L_s$) when they were exposed to jack mackerel (Trachurus japonicus) juveniles as predators (Ohata et al., 2011a, b). These results suggest that turbid waters function as refuges for fish larvae from visual predators. In contrast, turbidity had no major positive effect on survival when these larvae were exposed to moon jellyfish, except the case of ayu (see below).

Schooling behaviour can provide fish an advantage of detecting predators faster, confusing predators and thus increasing the chance of escape from predators (reviewed by Pitcher and Parrish, 1993). In our previous study, the survival rate of ayu post-larvae was slightly higher in turbid conditions than in the non-turbid treatment when moon jellyfish were used as predators (Ohata et al., 2011a). In addition, we observed that ayu larvae formed a school more frequently at 50 mg l$^{-1}$ than 0 and 300 mg l$^{-1}$ of kaolin during the predation experiments (Ohata, unpublished). Therefore, turbidity might have enhanced school formation in ayu post-larvae with which they detected moon jellyfish and escaped from them more efficiently.

Schools of fish are mainly maintained by visual stimuli with, in some cases, additional information transmitted by mechanosensory...
or chemosensory systems (reviewed by Partridge and Pitcher, 1980). Fish fail to school at low light intensities, presumably due to visual limitation, as is reported in gulf menhaden (Brevoortia patronus), walleye pollock (Theragra chalcogramma), and striped jack (Pseudocaranx dentex) (Higgs and Fuiman, 1996; Ryer and Olla, 1998; Miyazaki et al., 2000). Although turbidity may well have effects on the formation of fish schools, this has not yet been verified. Therefore in the present study, the effect of turbidity on school formation was tested in the larvae of Japanese anchovy and ayu, which use turbid water for refuge from visual predators, and compared with juveniles of yellowtail (Seriola quinqueradiata). Unlike the former two species, yellowtail remains in offshore waters with low turbidity for most of their early life stage (Sakakura and Tsukamoto, 1997).

**Material and methods**

**Rearing and husbandry of fish**

Ayu post-larvae were obtained from the Fisheries Cooperative Association of Hidakagawa in Wakayama Prefecture on 24 January 2009. They were third-generation individuals from local origin broodstock. Commercial pellets (N250 Kyowa Hakko Bio Co., Ltd; www.kyowahakko-bio.bio.co.jp) were provided three times a day. After the experiment, all the fish were measured for $L_s$ after MS222 anaesthesia. The mean $\pm SD$ $L_s$ of ayu was 24.5 $\pm$ 2.2 mm ($n = 125$).

Japanese anchovy larvae were raised from fertilized eggs spawned from broodstock that were obtained with a commercial set-net in Tai, Maizuru, Kyoto, Japan (35°56′N, 135°45′E). The broodstock were collected and transferred to the Maizuru Fisheries Research Station (MFRS) of Kyoto University on 19 March and 9 April 2009, and were stocked in two black round tanks (4 m in diameter, 30 m$^3$ in volume) filled with filtered seawater. They were fed defrosted krill Euphausia sp. twice a day. Larvae were provided with rotifers (Brachionus plicatilis), Artemia nauplii and formulated food (Kyowa N250 and N400, Kyowa Hakko Bio Co., Ltd; www.kyowahakko-bio.bio.co.jp), depending on their developmental stage. Rotifers and Artemia were enriched with commercial highly unsaturated fatty acid oil (Marine Gloss, Nisshin Marine Tech, Ltd; www.nisshin-marineotech.com). After the experiment, all the fish were measured for $L_s$ after MS222 anaesthesia. The mean $\pm SD$ $L_s$ of Japanese anchovy was 29.1 $\pm$ 2.5 mm ($n = 125$).

Yellowtail juveniles were obtained from the A-marine Kindai Co., Ltd. (www.a-marine.co.jp) in Wakayama Prefecture on 23 June 2011. Juveniles were transferred to the MFRS and were reared in 500-l transparent circular polycarbonate tanks. Commercial pellets (Otohime C1 and S2, Marubeni Nisshin Feed Co., Ltd; www.mn-feed.com) were provided twice a day. After the experiment, all the fish were measured for $L_s$ after MS222 anaesthesia. The mean $\pm SD$ $L_s$ of yellowtail was 37.1 $\pm$ 2.5 mm ($n = 150$).

**Experimental procedure**

A 30 x 40 cm acrylic square tank was used for the trials of ayu larvae. The same tank was first used in Japanese anchovy and yellowtail, yet abnormal behaviours caused by stress were often observed such as biting the bottom of the tank or being attracted to the tank wall. Therefore a 30-l transparent circular polycarbonate tank was used for the latter two species. Five levels of turbidity were obtained by dissolving 0, 5, 20, 50, or 300 mg l$^{-1}$ of kaolin (Wako Pure Chemical Industries, Ltd; www.wako-chem.co.jp). Five individuals were transferred to an experimental tank filled with a depth of 5 cm water. This shallow water depth was set to minimize the difference of light intensity among turbidity levels. After adaption for 10 min, video recording of fish behaviour was conducted from above on five different turbidity levels for 5 min. Fish were not reused and new individuals at each turbidity level were used. The seawater was changed in every trial, and the kaolin was newly prepared before that. We confirmed that levels of turbidity were maintained for more than 15 min. Two fluorescent lights (18 W) were provided from under the experimental tank so that even shirasu larvae would be recognized in high-turbidity conditions. The light intensity was 250 and 1100 lux above and under the tank, respectively. Water temperature was 14.9 ± 0.4°C ($n = 25$), 21.5 ± 0.2°C ($n = 25$), and 23.9 ± 0.3°C ($n = 30$) during the experiment of ayu, Japanese anchovy, and yellowtail, respectively. These water temperatures reflected ambient temperature in the habitat of each species. Five replicates in Japanese anchovy and ayu trials, and six replicates in yellowtail were conducted in each turbidity.

Although Uotani et al. (2000) reported that turbidity of 5 mg l$^{-1}$ was chosen by Japanese anchovy larvae in their experiment, turbidity of 50 mg l$^{-1}$ is the preferred level in European anchovy (Engraulis encrasicolus) larvae in the wild (Drake et al., 2007), and has been observed in Suruga Bay where a major fishery of Japanese anchovy larvae occurs (Uotani et al., 2000). Turbidity of 300 mg l$^{-1}$ is typical in Ariake Bay, Japan (Suzuki et al., 2009).

Schooling behaviour was analysed using the nearest neighbour distance ($D_{NN}$) and separation angle ($A_s$) following Masuda et al. (2003). $D_{NN}$ was defined as the snout-to-snout distance between nearest neighbouring two individuals divided by fish $L_s$ to be standardized. $A_s$ was defined as the angle between these two individuals; $A_s$ is expected to be close to 90° when fish are randomly located, and to decrease when a parallel orientation develops. Sixteen frames were sampled in a 20 s interval for 5 min, and $D_{NN}$ and $A_s$ were measured on each of the five individuals; the average of 80 measurements were taken to represent $D_{NN}$ or $A_s$ in each trial.

**Statistical analyses**

The $D_{NN}$ and $A_s$ were compared among different turbidities using ANOVA followed by Tukey’s honestly significant difference (HSD) test. The $D_{NN}$ data were Log$_{10}$ transformed to improve the homogeneity of variance, whereas $A_s$ data had homoscedasticity (Shapiro–Wilk test). $A_s$ was also compared with 90° by one sample t-test.

**Ethical notes**

All fish larvae used as prey animals were hatchery reared and so negative impacts on the natural population should be minimum. All the experiments were performed according to the guidelines of Regulation on Animal Experimentation at Kyoto University.

**Results**

Mean $D_{NN}$ of ayu larvae was significantly smaller at 20 and 50 mg l$^{-1}$ compared with other turbidity levels ($p < 0.05$, Tukey’s HSD test; Figure 1a), and so was their mean $A_s$ at 20 mg l$^{-1}$ than 0 or 50 mg l$^{-1}$ ($p < 0.05$, Tukey’s HSD test; Figure 2a). Their mean $A_s$ was significantly smaller than 90° at 5, 20, and 300 mg l$^{-1}$ of turbidity ($p < 0.05$, one sample t-test).

Although mean $D_{NN}$ of Japanese anchovy larvae was not significantly different among turbidity levels ($p > 0.05$, ANOVA;
Figure 1b), mean $A_S$ was significantly smaller at 50 mg l$^{-1}$ compared with others ($p < 0.05$, Tukey's HSD test; Figure 2b). Their mean $A_S$ was significantly smaller than 90° at all the turbidity levels ($p < 0.05$, one sample t-test).

Mean $D_{NN}$ of yellowtail juvenile was significantly larger at 300 mg l$^{-1}$ compared with other turbidity levels ($p < 0.05$, Tukey’s HSD test; Figure 1c), whereas there was no difference in $A_S$ among turbidity levels ($p > 0.05$, ANOVA; Figure 2c). Their mean $A_S$ was significantly smaller than 90° at 0, 5, 20, and 50 mg l$^{-1}$ turbidity ($p < 0.05$, one sample t-test).

**Discussion**

Moderate turbidities (20 and 50 mg l$^{-1}$) induced schooling behaviour of ayu and Japanese anchovy larvae, whereas in high turbidity (300 mg l$^{-1}$) yellowtail juveniles reduced their schooling behaviour. These results corresponded well with the habitat characteristics of each species; ayu and Japanese anchovy larvae occur in coastal and estuarine regions with highly turbid waters whereas yellowtail live in clear offshore waters during the larval and juvenile stages. Vision, olfactory organs, and the lateral line system are important factors to understand physiological mechanism in adapting to turbid waters. Uyan et al. (2006a) reported that anchovy larvae [12–30.8 mm total length ($L_t$)] had well-developed eyes with duplex retina, grouped rods and dense retinal tapetum, making them sensitive to low light levels and enabling them to inhabit and feed in deeper waters within the photic zone. Therefore, these larvae are likely to have an efficient eye structure for utilizing low light intensities, and thus should have an advantage in school formation in turbid waters. Furthermore, Uyan et al. (2006a) suggested that olfaction may help larvae maintain the school when the light intensity is low in Japanese anchovy larvae. The lateral line system may also help the Japanese anchovy maintain the school in turbid waters. In general, adult clupeoid fish that form a large
school such as herring, gulf menhaden, and gizzard shad (Dorosoma cepedianum) have cephalic lateral line canals with many small branches (Gunter and Demoran, 1961; Blaxter et al., 1983; Stephens, 1985). Uyan et al. (2006b) reported that this was also the case in adult Japanese anchovy (9–13 cm Ls), and suggested that the dense branching of the cephalic lateral line canals ensures the sensitivity to the movement of water and might be a characteristic of schooling pelagic fish. In larval ayu, free neuromasts are located surrounding eyes and noses, the structure of which can be adapted to receive stimuli from all direction (Mukai et al., 1992). Therefore, sensory organs of Japanese anchovy and ayu provide substantial advantage for school formation in turbid waters. Furthermore, the lateral line systems may also work for the transference of predator’s information in a school. These may explain the relatively high survival rate of ayu larvae exposed to moon jellyfish at turbid conditions compared with the transparent condition in our previous study (Ohata et al., 2011a).

High-turbidity condition had a negative effect on school formation in yellowtail. This may be because visual contact among individuals was difficult in a highly turbid environment for them. High turbidity may also be stressful; indeed some individuals stopped swimming and stayed on the bottom. Turbidity is reported to cause some changes in the behaviour of fish. Enström-Öst and Mattila (2008) reported that in the presence of competitors and the stimuli of a visual predator, turbidity reduced swimming activity in northern pike (Esox lucius) larvae. Contrarily, activity of walleye (Sander vitreus) and herring increased in turbid environment (Rieger and Summerfelt, 1997; Utne-Palm, 2004). Furthermore, Meager and Batty (2007) reported that swimming activity in Atlantic cod (Gadus morhua) juveniles was non-linearly affected by turbidity. Because turbidity can either increase or decrease the activity of fish, the effect of turbidity on fish activity should be confirmed on a species-to-species basis.

The cohesiveness of fish school is different depending on developmental stages; for example both DNN and A5 reach consistently low values when Japanese anchovy attain 35 mm Ls (Masuda, 2011). Therefore the effects of turbidity on school formation may change ontogenetically from the larval to the juvenile stage. Future study should include such an ontogenetic aspect.

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