The effect of spine postures on the hydrodynamic drag in *Epinephelus ongus* larvae

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**Running headline**

Spines for drag control in grouper larvae
Abstract

Laboratory behavioural observation and computational fluid dynamics (CFD) analysis were conducted to examine whether the movement of the elongated dorsal and pelvic spines changed the hydrodynamic drag in white-streaked grouper *Epinephelus ongus* larvae. The behavioural observation in the tank revealed that the larvae extended the dorsal and pelvic spines during passive transport and retracted during swimming; the angles of the dorsal and pelvic spines in relation to the anteroposterior axis were larger during the passive transport (28.84 ± 14.27° and 20.35 ± 15.05°) than those during the swimming (2.59 ± 5.55° and 0.32 ± 6.49°). The CFD analysis indicated that the relative hydrodynamic drag acting on the larvae was approximately 1.25 times higher when the spines were extended (passive transport) than when the spines were retracted (swimming), suggesting that the grouper larvae have an ability to adjust their hydrodynamic drag depending on the behavioural context.

Key words: computational fluid dynamics analysis; drag coefficient; elongated spine;
morphology; Serranidae

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Over 90% of coral reef fishes have a dispersal life stage in which they are passively transported by ocean currents (Leis, 1991; Leis & McCormick, 2002). Since most of the juvenile and adult reef fishes are sedentary and show strong site fidelity (Zeller, 1997; Kawabata et al., 2007; Meyer et al., 2010; Claisse et al., 2011), the dispersal stage is considered to be the main factor responsible for the connectivity and population structure of reef fishes (Hamner & Largier, 2012; Simpson et al., 2013). Therefore, measuring larval dispersal is of great importance towards understanding population dynamics as well as for determining management measures such as the establishment of marine protected areas (Jones et al., 2005; Sale et al., 2005; Almany et al., 2007; Planes et al., 2009).

Even though the ocean current is the main factor for controlling dispersal, behaviour has been recently begun to be recognized as an important factor that can influence dispersal trajectories (Leis, 2006; Gerlach et al., 2007; Leis, 2007; Paris et al., 2007; Putman et al., 2012; Sponaugle et al., 2012; Simpson et al., 2013). In fact, perciform fishes have swimming, orientation and sensory abilities that can influence their dispersal trajectories (Leis & Carson-Ewart, 1999; Fisher, 2005; Lecchini et al.,
2005; Simpson et al., 2005; Leis et al., 2009). However, as far as it is known, none of
the studies have focused on the ability of organisms to control their hydrodynamic drag
so that they can adjust the distance transported by the flow and gravity, and therefore
influence their vertical and horizontal distribution.

Grouper (Serranidae) larvae have distinct morphology, which might affect
hydrodynamic drag; they have an elongated second dorsal and pelvic fin spines (Colin
et al., 1996; Kawabe & Kohno, 2009; Russo et al., 2009). Some anecdotal studies have
suggested that this distinguishing morphology is an adaptation for predator evasion
(Leis & Carson-Ewart, 1999; Kusaka et al., 2001), but other studies have suggested an
adaptation for maintaining position in the water column (Hirata et al., 2009; Kawabe &
Kohno, 2009). However, no experimental studies have been conducted on when and
how the larvae use spines and whether the use of the spines changes hydrodynamic
drag.

The objective of this study was to examine whether the movement of the
elongated spines changed the hydrodynamic drag experienced by grouper larvae.

White-streaked grouper *Epinephelus ongus* (Bloch, 1790) was chosen as a model species, because it is an abundant grouper species in the Indo-West Pacific. Since there were no data on the larval morphology of this species, the ontogenetic changes of the lengths of the dorsal and pelvic spines were investigated from the hatchery-reared specimens in advance. The postures of the spines during swimming and passive transport were then measured by a video camera in a laboratory setting. Finally, the 3D shape of the larva with elongated spines was reconstructed, and hydrodynamic drag when the spines were extended and retracted was calculated using computational fluid dynamics (CFD) analysis.

Since serranid species including this species were rarely caught by plankton nets or light traps around the Yaeyama islands (Nanami et al., 2013b), hatchery-reared fish were utilized for the experiments. Fertilized eggs of *E. ongus* were obtained from natural spawning wild broodstock kept in captivity at the Yaeyama Laboratory, Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency, Okinawa, Japan. The eggs were incubated and the 1-day-old
larvae were kept in 2.3 kL fiber-reinforced plastic tanks at a density of c. $10^4$ individuals kl$^{-1}$. They were fed with SS-type rotifers *Brachionus rotundiformis* (Thai strain) at a density of c. 20 individuals ml$^{-1}$ from 2 days post hatching (dph), and *Nannochloropsis oculata* was added at a density of c. $5 \times 10^6$ cells ml$^{-1}$. Then, SS type rotifers were substituted with S-type rotifers (Yaeyama strain) from 5 dph. *Artemia franciscana* nauplii and dry pellets (Rich, Scientific Feed Laboratory Co. Ltd., Japan) were provided in addition to rotifers from 16 and 24 dph, respectively. Rotifers and Artemia were discontinued from 26 and 40 dph, respectively. The details of the rearing protocol were similar to those of the coral trout *Plectropomus leopardus* (Lacepède, 1802) (Takebe et al., 2011). Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University with approval of the Institutional Animal Care and Use Committee.

A sample of 10 individuals were taken every day from day 0 to day 10, and at 2-4 days intervals from day 10 to day 48; as a result, 220 individuals were sampled in total. The fish were anaesthetized using tricaine methansulphate (MS-222; 100 mg l$^{-1}$) and the total length ($L_T$) and lengths of the second dorsal spine and pelvic spine were
measured to the nearest 0.0005 mm using an ocular micrometre under a stereoscopic microscope. After the measurement, specimens were fixed in 10% formalin-seawater solution.

Dorsal and pelvic spines started to grow at the same time at c. 4 mm LT (Fig. 1). Both spines were then quickly elongated until the maximum lengths were attained at c. 7-10 mm LT (Fig. 1). Maximum ratios of the spine lengths to LT were attained at c. 6-9 mm LT (Fig. 1). The dorsal and pelvic spines were thick along their full length, and their morphology was similar to other *Epinephelus* species (Kusaka *et al.*, 2001; Kawabe & Kohno, 2009); the second dorsal spines have three ridges with a number of small spinelets, and the pelvic spines have four ridges with a number of small spinelets.

In order to investigate whether the fish changes the posture of the spines depending on the behavioural context, the behaviour of 10 live larvae [8.68 ± 0.85 mm LT (mean ± standard deviation)] was recorded in a glass aquarium [590 (length) x 50 (width) x 290 (height) mm] from the side using a video camera (HDR-XR520V,
Handycam, Sony, Tokyo, Japan) at 30 frames s$^{-1}$. The aquarium was filled with seawater to a depth of 230 mm, and the water temperature was 26.5 °C. In order to establish a weak flow (c. 20 mm s$^{-1}$) in the aquarium, sea water was supplied to the tank through 2 mm holes drilled in the PVC pipe deployed at one side of the tank, and drained from the other side of the tank. When the fish was passively transported by flow or gravity, body posture was nearly parallel to the direction of the fish movement, suggesting that the relative flow direction was nearly parallel to the fish body. Individual fish were introduced into the aquarium and acclimatized for at least 5 min. The fish behaviours were then recorded for 10 minutes from the side of the tank using a video camera. One fish was recorded at a time. After the recording, the fish were anaesthetized using MS-222, and were fixed in 10 % formalin-seawater solution.

Video data were first classified into three behavioural categories: active swimming by tail beating (swimming), passive transport by flow or gravity (passive transport) and the others (e.g. turning, resting, hovering). Next, ten frames in which the fish were oriented perpendicular to the camera were extracted for both the swimming and the passive transport categories. In order to avoid multiple samplings from a short
time-series data, intervals of the consecutive frames were set over 10 seconds. Then, the angles of spines in relation to the anteroposterior axis were measured using ImageJ 1.44p (National Institutes of Health; rsb.info.nih.gov/ij).

In order to test whether there was any difference in spine postures depending on the behaviour, the angles of spines were compared between the swimming and passive transport behaviours. Since the postures of the spines were measured multiple times in each fish, general linear mixed model (LMM) (Grafen & Hails, 2002), in which each fish was regarded as a random factor, was used to compare the differences. LMM was conducted using R 3.0.1 (The R Foundation for Statistical Computing; www.r-project.org) with the R library ‘nlme’.

In order to conduct the CFD analysis to estimate the hydrodynamic drag acting on the larva, three-dimensional morphological information is needed. Therefore, the 3D shape of the larva with elongated spines was reconstructed using the 3D editor Blender 2.68 (The Blender Foundation; www.blender.org). Photographs of one larva with
elongated spines \[ L_T, 8.10 \text{ mm}; (\text{dorsal spine length}) (L_T)^{-1}, 0.45; (\text{pelvic spine length}) (L_T)^{-1}, 0.42 \] taken from the side and top were used to measure the shape of the larva.

The body was divided into 16 parts by the cross-section in the transverse plane, and the 16 points were dotted to the outline of each cross-section by assuming an elliptical transverse shape (Mchenry & Lauder, 2006). Since the structures of the dorsal and pelvic spines were complex, the spine was modelled as a cylinder with a circular cross-section. This kind of simplified morphological model allows the calculation of relative drag forces between different postures rather than the absolute drag forces acting on fish (Przybilla et al., 2010). The mean angles of the spines measured by the aquarium experiment were used to determine the postures of the spines in each behavioural mode \(28.84^\circ\) and \(20.35^\circ\) for dorsal and pelvic spines during the passive transport, and \(2.59^\circ\) and \(0.32^\circ\) for dorsal and pelvic spines during the swimming; Fig. 2).

The CFD (Gerris; Popinet, 2003) used in the analysis is a partial differential equations solver, that provided estimates of the relative rates of drag induced by the extension and retraction of the dorsal and pelvic spines during passive and active
transport. The Reynolds (Re) number used in the solver was set to the Re of the experiment, based on the length of the larva and the vertical speed during the passive transport by gravity (13.9 mm s$^{-1}$), which was c. 214. Since only the drag forces experienced by the model were of interest, the domain of the solver was set to a 1x1 box, and the drag forces were recorded every 0.1 time steps. The equation for the drag force ($F_d$) was expressed as $F_d = 0.5 C_d \rho S U^2$, where $C_d$ denotes the drag coefficient, $\rho$ denotes the water density, $S$ denotes the body surface area and $U$ denotes the flow velocity. As $\rho$, $S$ and $U$ were constant throughout the analysis, $C_d$ was used as an index for drag. Effects of the spine posture (extended or retracted) and the body orientation in relation to the flow (facing upstream or downstream) on $C_d$ were tested using a two-way analysis of variance (ANOVA) (Fig. 2). The two-way ANOVA was conducted using R 3.0.1 with the R library ‘lm’.

The behavioural observation in the tank revealed that the larvae extended the dorsal and pelvic spines during the passive transport and retracted during swimming; the angles of the dorsal and pelvic spines in relation to the anteroposterior axis were larger during the passive transport (28.84 ± 14.27° and 20.35 ± 15.05°) than those
during the swimming (2.59 ± 5.55 ° and 0.32 ± 6.49 °) (LMM, \( F_{1,189} = 404.49, 156.56, \) both \( P<0.01, \) respectively) (Fig. 2). The CFD analysis indicated that the relative hydrodynamic drag acting on the larvae was ca. 1.25 times higher when the spines were extended than when the spines were retracted (Fig. 3; two-way ANOVA, \( F_{1,157} = 1165.21, \) \( P<0.01 \)). In addition, the drag force was smaller when the fish was facing upstream, compared to that when the fish was facing downstream (Fig. 3; two-way ANOVA, \( F_{1,157} = 9.52, \) \( P<0.01 \)). These results suggest that grouper larvae have an ability to change the hydrodynamic drag depending on the behavioural context.

Since the serranid larvae started to inflate the swim bladder from the onset of the development of the elongated spines (Colin et al., 1996; Hirata et al., 2009), the larvae would likely be able to control the hydrostatic force (i.e. buoyancy) to float in the water column. However, the swim bladder is not useful when the fish quickly changes their vertical distribution (Jones, 1952). Field observation on the serranid larvae revealed that the fish actively change the position in the water column and horizontal distribution (Leis & Carson-Ewart, 1999; Leis et al., 2009). In addition, the larviculture experiment on the seven-band grouper *Epinephelus septemfasciatus* (Thunberg, 1793)
revealed that the survival rate became higher when the flow speed had been increased during the period when the fish had elongated spines (Soyano et al., 2008). This was considered to be reflection of its ecology; the ontogenetic change of spine lengths and swimming capability were associated with its inshore migration (Soyano et al., 2008). Considering these facts, it is possible that the fish uses elongated spines for controlling the hydrodynamic drag, together with the control of hydrostatic force by the swim bladder, for efficient locomotion in the water column. Further research measuring the postures of the spines during the vertical and horizontal movements in the field or in a large tank is necessary to verify this hypothesis.

There are also reports that the larvae use spines for predator avoidance; the late stage serranid larvae *P. leopardus* extended spines when the predator approached the larvae (Leis & Carson-Ewart, 1999). Considering that adaptive functions of elongated spines of other plankton species include drag control (Takahashi & Be, 1984) and predator avoidance (Morgan, 1989), it is possible that the elongated spines of grouper larvae have both adaptive functions.
*E. ongus* has a bipartite life cycle (pelagic eggs and larvae with benthic juveniles and adults), which is similar to other groupers and most reef fishes (Simpson *et al.*, 2013). Although ecological information such as age and growth (Craig, 2007), microhabitat association (Nanami *et al.*, 2013b), and reproductive ecology (Ohta & Ebisawa, 2009; Nanami *et al.*, 2013a; Nanami *et al.*, 2014) of juveniles and adults have been accumulated, no study had been conducted on the larval ecology of this species. This study revealed that *E. ongus* larvae have an ability to control hydrodynamic drag by changing the posture of their elongated spines, which would then likely affect dispersal trajectory. Further research using a hydrodynamic model that incorporates behavioural parameters, including spine postures, is clearly needed for explicitly quantifying the larval dispersal process of this species.

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Figure captions

Figure 1. The spine length and the ratio of the spine length to the total length, in relation to the total length in *Epinephelus ongus*. (a) Dorsal spine length; (b) (Dorsal spine length) (total length)$^{-1}$; (c) Pelvic spine length; (d) (Pelvic spine length) (total length)$^{-1}$.
Figure 2. The spine posture (extended or retracted) and body orientation of *Epinephelus ongus* larvae in relation to the flow (facing upstream or downstream) used in the computational fluid dynamics analysis. The mean angles of the spines in relation to the anteroposterior axis, determined by the aquarium experiment, were used to fix the postures of the spines in each behavioural mode (28.84° and 20.35° for dorsal and pelvic spines during the passive transport, and 2.59° and 0.32° for dorsal and pelvic spines during the swimming, respectively).
Figure 3. Comparisons of the drag coefficient ($C_d$) of *Epinephelus ongus* larvae determined by the computational fluid dynamics (CFD) analysis; (a) time series of $C_d$ for larvae with spines extended (upper) and retracted (lower); and (b) mean $C_d \pm 95\%$ confident intervals for larvae with spines extended (○) and retracted (□) while facing either downstream (left) or upstream (right).