**Title**

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 $^{o}$ and 20.35 ± 15.05 $^{o}$) than those during the swimming (2.59 ± 5.55 $^{o}$ and 0.32 ± 6.49 $^{o}$). 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

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

75 elongated spines changed the hydrodynamic drag experienced by grouper larvae.
76 White-streaked grouper *Epinephelus ongus* (Bloch, 1790) was chosen as a model
77 species, because it is an abundant grouper species in the Indo-West Pacific. Since there
78 were no data on the larval morphology of this species, the ontogenetic changes of the
79 lengths of the dorsal and pelvic spines were investigated from the hatchery-reared
80 specimens in advance. The postures of the spines during swimming and passive
81 transport were then measured by a video camera in a laboratory setting. Finally, the 3D
82 shape of the larva with elongated spines was reconstructed, and hydrodynamic drag
83 when the spines were extended and retracted was calculated using computational fluid
84 dynamics (CFD) analysis.
85
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87 Since serranid species including this species were rarely caught by plankton
88 nets or light traps around the Yaeyama islands (Nanami *et al.*, 2013b), hatchery-reared
89 fish were utilized for the experiments. Fertilized eggs of *E. ongus* were obtained from
90 natural spawning wild broodstock kept in captivity at the Yaeyama Laboratory,
91 Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute,
92 Fisheries Research Agency, Okinawa, Japan. The eggs were incubated and the 1-day-old

93 larvae were kept in 2.3 kL fiber-reinforced plastic tanks at a density of *c.* $10^4$ individuals
94 $kl^{-1}$. They were fed with SS-type rotifers *Brachionus rotundiformis* (Thai strain) at a
95 density of *c.* 20 individuals $ml^{-1}$ from 2 days post hatching (dph), and *Nannochloropsis*
96 *oculata* was added at a density of *c.* $5\times10^6$ cells $ml^{-1}$. Then, SS type rotifers were
97 substituted with S-type rotifers (Yaeyama strain) from 5 dph. Artemia *franciscana*
98 *nauplii* and dry pellets (Rich, Scientific Feed Laboratory Co. Ltd., Japan) were provided
99 in addition to rotifers from 16 and 24 dph, respectively. Rotifers and Artemia were
100 discontinued from 26 and 40 dph, respectively. The details of the rearing protocol were
101 similar to those of the coral trout *Plectropomus leopardus* (Lacepède, 1802) (Takebe et
102 al., 2011). Animal care and experimental procedures were performed in accordance with
103 the Guidelines for Animal Experimentation of Nagasaki University with approval of the
104 Institutional Animal Care and Use Committee.
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107 A sample of 10 individuals were taken every day from day 0 to day 10, and at
108 2-4 days intervals from day 10 to day 48; as a result, 220 individuals were sampled in
109 total. The fish were anaesthetized using tricaine methansulphate (MS-222; 100 mg $l^{-1}$)
110 and the total length ($L_T$) and lengths of the second dorsal spine and pelvic spine were

111 measured to the nearest 0.0005 mm using an ocular micrometre under a stereoscopic
112 microscope. After the measurement, specimens were fixed in 10 % formalin-seawater
113 solution.
114
115
116 Dorsal and pelvic spines started to grow at the same time at *c*. 4 mm $L_T$ (Fig. 1).
117 Both spines were then quickly elongated until the maximum lengths were attained at *c*.
118 7-10 mm $L_T$ (Fig. 1). Maximum ratios of the spine lengths to $L_T$ were attained at *c*. 6-9
119 mm $L_T$ (Fig. 1). The dorsal and pelvic spines were thick along their full length, and their
120 morphology was similar to other *Epinephelus* species (Kusaka *et al.*, 2001; Kawabe &
121 Kohno, 2009); the second dorsal spines have three ridges with a number of small
122 spinelets, and the pelvic spines have four ridges with a number of small spinelets.
123
124
125 In order to investigate whether the fish changes the posture of the spines
126 depending on the behavioural context, the behaviour of 10 live larvae [8.68 ± 0.85 mm
127 $L_T$ (mean ± standard deviation)] was recorded in a glass aquarium [590 (length) x 50
128 (width) x 290 (height) mm] from the side using a video camera (HDR-XR520V,

129 Handycam, Sony, Tokyo, Japan) at 30 frames $s^{-1}$. The aquarium was filled with seawater
130 to a depth of 230 mm, and the water temperature was 26.5 $^{o}$C. In order to establish a
131 weak flow (*c.* 20 mm $s^{-1}$) in the aquarium, sea water was supplied to the tank through 2
132 mm holes drilled in the PVC pipe deployed at one side of the tank, and drained from the
133 other side of the tank. When the fish was passively transported by flow or gravity, body
134 posture was nearly parallel to the direction of the fish movement, suggesting that the
135 relative flow direction was nearly parallel to the fish body. Individual fish were
136 introduced into the aquarium and acclimatized for at least 5 min. The fish behaviours
137 were then recorded for 10 minutes from the side of the tank using a video camera. One
138 fish was recorded at a time. After the recording, the fish were anaesthetized using
139 MS-222, and were fixed in 10 % formalin-seawater solution.
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142 Video data were first classified into three behavioural categories: active
143 swimming by tail beating (swimming), passive transport by flow or gravity (passive
144 transport) and the others (e.g. turning, resting, hovering). Next, ten frames in which the
145 fish were oriented perpendicular to the camera were extracted for both the swimming
146 and the passive transport categories. In order to avoid multiple samplings from a short

147 time-series data, intervals of the consecutive frames were set over 10 seconds. Then, the
148 angles of spines in relation to the anteroposterior axis were measured using ImageJ
149 1.44p (National Institutes of Health; rsb.info.nih.gov/ij).
150
151
152 In order to test whether there was any difference in spine postures depending
153 on the behaviour, the angles of spines were compared between the swimming and
154 passive transport behaviours. Since the postures of the spines were measured multiple
155 times in each fish, general linear mixed model (LMM) (Grafen & Hails, 2002), in which
156 each fish was regarded as a random factor, was used to compare the differences. LMM
157 was conducted using R 3.0.1 (The R Foundation for Statistical Computing;
158 www.r-project.org) with the R library ‘nlme’.
159
160
161 In order to conduct the CFD analysis to estimate the hydrodynamic drag acting
162 on the larva, three-dimensional morphological information is needed. Therefore, the 3D
163 shape of the larva with elongated spines was reconstructed using the 3D editor Blender
164 2.68 (The Blender Foundation; www.blender.org). Photographs of one larva with

165 elongated spines [$L_T$, 8.10 mm; (dorsal spine length) $(L_T)^{-1}$, 0.45; (pelvic spine length)
166 $(L_T)^{-1}$, 0.42] taken from the side and top were used to measure the shape of the larva.
167 The body was divided into 16 parts by the cross-section in the transverse plane, and the
168 16 points were dotted to the outline of each cross-section by assuming an elliptical
169 transverse shape (Mchenry & Lauder, 2006). Since the structures of the dorsal and
170 pelvic spines were complex, the spine was modelled as a cylinder with a circular
171 cross-section. This kind of simplified morphological model allows the calculation of
172 relative drag forces between different postures rather than the absolute drag forces
173 acting on fish (Przybilla *et al.*, 2010). The mean angles of the spines measured by the
174 aquarium experiment were used to determine the postures of the spines in each
175 behavioural mode (28.84 $^{o}$ and 20.35 $^{o}$ for dorsal and pelvic spines during the passive
176 transport, and 2.59 $^{o}$ and 0.32 $^{o}$ for dorsal and pelvic spines during the swimming; Fig.
177 2).
178
179
180 The CFD (Gerris; (Popinet, 2003) used in the analysis is a partial differential
181 equations solver, that provided estimates of the relative rates of drag induced by the
182 extension and retraction of the dorsal and pelvic spines during passive and active

183 transport. The Reynolds (Re) number used in the solver was set to the Re of the
184 experiment, based on the length of the larva and the vertical speed during the passive
185 transport by gravity (13.9 mm $s^{-1}$), which was *c*. 214. Since only the drag forces
186 experienced by the model were of interest, the domain of the solver was set to a 1x1 box,
187 and the drag forces were recorded every 0.1 time steps. The equation for the drag force
188 ($F_d$) was expressed as $F_d = 0.5C_d\rho SU^2$, where $C_d$ denotes the drag coefficient, $\rho$ denotes
189 the water density, *S* denotes the body surface area and *U* denotes the flow velocity. As $\rho$,
190 *S* and *U* were constant throughout the analysis, $C_d$ was used as an index for drag. Effects
191 of the spine posture (extended or retracted) and the body orientation in relation to the
192 flow (facing upstream or downstream) on $C_d$ were tested using a two-way analysis of
193 variance (ANOVA) (Fig. 2). The two-way ANOVA was conducted using R 3.0.1 with
194 the R library 'lm'.
195
196
197 The behavioural observation in the tank revealed that the larvae extended the
198 dorsal and pelvic spines during the passive transport and retracted during swimming;
199 the angles of the dorsal and pelvic spines in relation to the anteroposterior axis were
200 larger during the passive transport (28.84 ± 14.27 $^{o}$ and 20.35 ± 15.05 $^{o}$) than those

during the swimming (2.59 ± 5.55 $^{o}$ and 0.32 ± 6.49 $^{o}$) (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 *c*. 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)

219 revealed that the survival rate became higher when the flow speed had been increased
220 during the period when the fish had elongated spines (Soyano *et al.*, 2008). This was
221 considered to be reflection of its ecology; the ontogenetic change of spine lengths and
222 swimming capability were associated with its inshore migration (Soyano *et al.*, 2008).
223 Considering these facts, it is possible that the fish uses elongated spines for controlling
224 the hydrodynamic drag, together with the control of hydrostatic force by the swim
225 bladder, for efficient locomotion in the water column. Further research measuring the
226 postures of the spines during the vertical and horizontal movements in the field or in a
227 large tank is necessary to verify this hypothesis.
228
229
230 There are also reports that the larvae use spines for predator avoidance; the late
231 stage serranid larvae *P. leopardus* extended spines when the predator approached the
232 larvae (Leis & Carson-Ewart, 1999). Considering that adaptive functions of elongated
233 spines of other plankton species include drag control (Takahashi & Be, 1984) and
234 predator avoidance (Morgan, 1989), it is possible that the elongated spines of grouper
235 larvae have both adaptive functions.
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237

238 *E. ongus* has a bipartite life cycle (pelagic eggs and larvae with benthic
239 juveniles and adults), which is similar to other groupers and most reef fishes (Simpson
240 *et al.*, 2013). Although ecological information such as age and growth (Craig, 2007),
241 microhabitat association (Nanami *et al.*, 2013b), and reproductive ecology (Ohta &
242 Ebisawa, 2009; Nanami *et al.*, 2013a; Nanami *et al.*, 2014) of juveniles and adults have
243 been accumulated, no study had been conducted on the larval ecology of this species.
244 This study revealed that *E. ongus* larvae have an ability to control hydrodynamic drag
245 by changing the posture of their elongated spines, which would then likely affect
246 dispersal trajectory. Further research using a hydrodynamic model that incorporates
247 behavioural parameters, including spine postures, is clearly needed for explicitly
248 quantifying the larval dispersal process of this species.

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393 **Figure captions**

394

395

396 **Figure 1.** The spine length and the ratio of the spine length to the total length, in

397 relation to the total length in *Epinephelus ongus*. (a) Dorsal spine length; (b) (Dorsal

398 spine length) (total length)$^{-1}$; (c) Pelvic spine length; (d) (Pelvic spine length) (total

399 length) $^{-1}$.

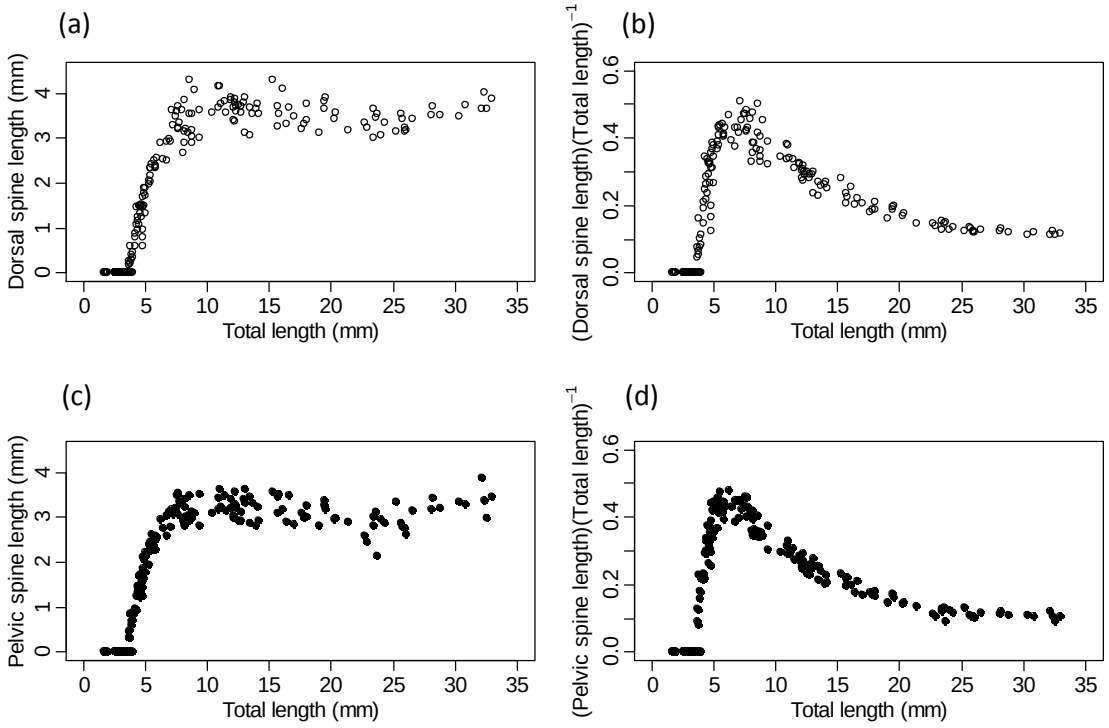


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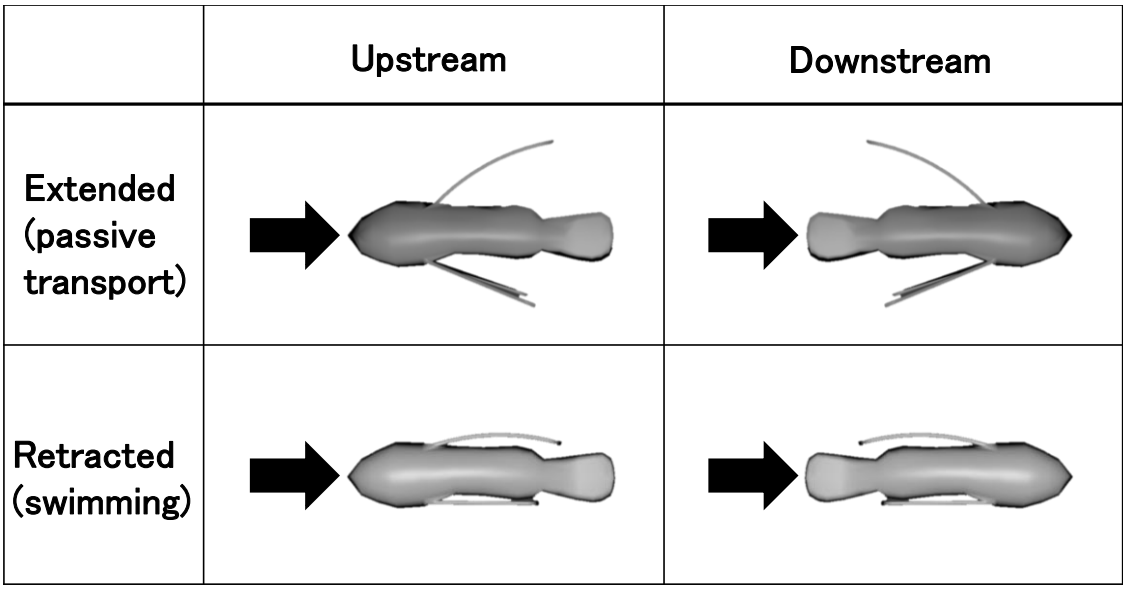
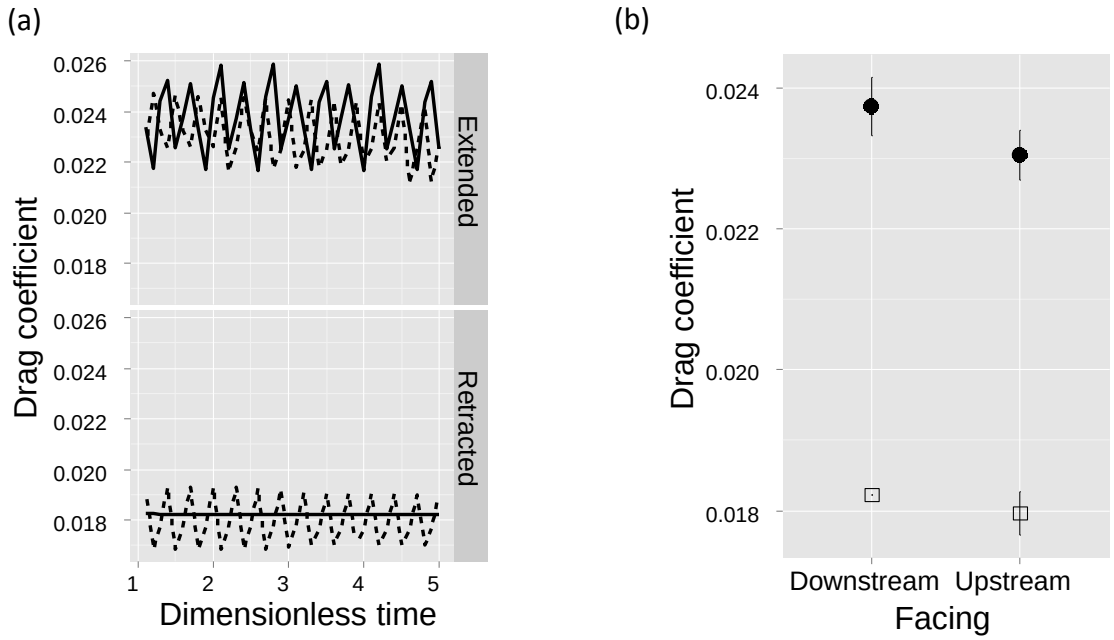
**Figure 2.** The spine posture (extended or retracted) and body orientation of *Epinephelus ongus* larvae in relation to the flow (facing upstream or downstream) used in the computational fluid dynamics analysis. The mean angles of the spines in relation to the anteroposterior axis, determined by the aquarium experiment, were used to fix the postures of the spines in each behavioural mode (28.84 $^{o}$ and 20.35 $^{o}$ for dorsal and pelvic spines during the passive transport, and 2.59 $^{o}$ and 0.32 $^{o}$ for dorsal and pelvic spines during the swimming, respectively).



411 **Figure 3.** Comparisons of the drag coefficient ($C_d$) of *Epinephelus ongus* larvae
412 determined by the computational fluid dynamics (CFD) analysis; (a) time series of $C_d$
413 for larvae with spines extended (upper) and retracted (lower); and (b) mean $C_d$ ± 95 %
414 confident intervals for larvae with spines extended (○) and retracted (□) while facing
415 either downstream (left) or upstream (right).

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