Visual crypsis as a possible function of polymorphic shell coloration in the infaunal clam *Ruditapes philippinarum*

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ABSTRACT

The infaunal clam *Ruditapes philippinarum* exhibits highly polymorphic shell coloration, but the function of the coloration remains uncertain. Here, a hypothesis that such shell coloration functions to enhance visual crypsis (i.e., background color matching) in juveniles (<15 mm in shell length) was tested with a combination of a field survey and laboratory experiments. Shell and background colorations were expressed as mean brightness values. Firstly, the association between shell and background brightness was investigated. For this, a field survey for two sympatric subpopulations with distinct substrates was conducted on an intertidal sandflat in western Kyushu, Japan. Secondly, the visual-crypsis hypothesis was tested experimentally using half-valve clam shells filled with paste of raw clam meat as prey and the pufferfish *Takifugu niphobles* as a visually hunting predator, in a tank with one to two dark-colored substrates and one light-colored substrate. Our field survey showed that shell brightness significantly differed between the two sympatric subpopulations of juvenile clams and was positively associated with background brightness. Our laboratory experiments indicated that prey items with comparatively light (dark) coloration on dark- (light-) colored substrate were consumed by predators more immediately and at a higher rate than in the color-matched combinations. Consequently, shell–background color matching could help juvenile clams avoid attack from visually hunting predators. The results provide a new insight into effective management planning for this clam species, creating sand habitats with a more matched coloration.

Keywords: Color-polymorphic prey, Manila clam, Visual predator, Grass pufferfish, Cryptic coloration, Background color matching

35 **1. Introduction**

36 Visual crypsis (i.e., background color matching) is an effective antipredator defense which is
37 prevalent among various prey animals (Ruxton et al., 2004; Quicke, 2017). Such a function enables
38 prey animals to conceal themselves and to avoid attacks from visually hunting predators (for
39 examples of aquatic prey animals, see Hughes and Mather, 1986; Donnelly and Whoriskey, 1993;
40 Palma and Steneck, 2001; Manríquez et al., 2008; Ryer et al., 2008). Therefore, if their survival is
41 strongly affected by visually hunting predators, prey animals with cryptic coloration will have
42 advantage in establishing a population through background color matching. Despite its importance,
43 the concept of background color matching has been largely overlooked in both species conservation
44 and resource management practices (Donnelly and Whoriskey, 1993; Baling et al., 2016).

45 The globally-distributed infaunal clam *Ruditapes philippinarum* (Adams & Reeve) (Toba et al.,
46 1992; Vincenzi et al., 2011; Humphreys et al., 2015; Talley et al., 2015; Cordero et al., 2017)
47 exhibits a highly polymorphic shell coloration which is largely determined genetically (Peignon et
48 al., 1995; Huo et al., 2017), but the function of the coloration remains uncertain. Their habitats
49 extend from intertidal to shallow-subtidal zones often covering a wide range of sediment types (i.e.,
50 from muddy sand, through sand, to gravel sand; and also patches of shell fragments) (Kondo, 1987;
51 Takeuchi et al., 2013; Takeuchi et al., 2015; Talley et al., 2015). *Ruditapes philippinarum* clams with
52 shell lengths < 15 mm were defined as juveniles, since their smallest mature shell length is
53 approximately 15 mm (Toba et al., 1992). In Japan, *R. philippinarum* clams are a commercially
54 important species. To support the establishment of clam populations, adding allochthonous substrates,
55 such as bivalve-shell fragments (Sakurai et al., 2012), offshore dredged sand (Nakahara and Nasu,
56 2002), and artificial gravel (Ikushima et al., 2012), to sandflats has been conducted.

57 Visual predation by birds and fish is an important source of mortality of *R. philippinarum* clams,
58 especially in their juvenile stage (Toba et al., 1992; Nakahara and Nasu, 2002; Kimura, 2005;

59 Shigeta and Usuki, 2012; Takahashi et al., 2016). *Ruditapes philippinarum* clams are sometimes
60 dislodged from the sediments by abrupt sediment erosion induced by hydrodynamic disturbance
61 (Kakino, 2000; Takeuchi et al., 2015), and clams exposed onto the sediment surface will undergo an
62 elevated predation risk. In this context, juveniles may be more at risk than adults due to the former's
63 more limited burrowing depths (Stanley, 1970; Kondo, 1987). Takeuchi et al. (2015) found that
64 juvenile *R. philippinarum* clams burrow into the sediments more rapidly under a light condition than
65 under a dark one. The authors concluded that this result can be explained by an adaptive behavioral
66 trait against visually hunting predators.
67 Furthermore, polymorphic shell coloration of *R. philippinarum* clams may function to enhance
68 visual crypsis, as shown by some studies with other shelled mollusks [for chitons, see Rodrigues and
69 Absalão (2005) and Mendonça et al. (2015); for gastropods, see Reimchen (1979), Byers (1989), and
70 Byers (1990); for bivalves, see Smith (1975) and Whiteley et al. (1997)]. For example, Whiteley et
71 al. (1997) showed a positive correlation between shell and background colorations in the
72 shallow-burrowing bivalve *Donacilla cornea* (their size: up to 20 mm in shell length) from an
73 intertidal sandy beach of Korinos, northern Greece.
74 The objective of the present study was to test the visual-crypsis hypothesis, using a combination
75 of a field survey and laboratory experiments. Juvenile clams were used for this study, and shell
76 coloration was quantified in terms of brightness (for the definition, see Section 2.1.2). Firstly, to
77 study the association between shell and background brightness, a field survey for two sympatric
78 subpopulations with distinct substrates was conducted on an intertidal sandflat. Secondly, to test the
79 visual-crypsis hypothesis, laboratory experiments were performed using the pufferfish *Takifugu*
80 *niphobles* (Jordan & Snyder), which is known as one of the most important predators for juvenile *R.*
81 *philippinarum* clams (Shigeta and Usuki, 2012). The results revealed that shell–background color
82 matching can help juvenile *R. philippinarum* clams avoid attack from visually hunting predators,

83 which provides a new insight into effective management planning for this clam species based on the
84 concept of color matching.
85
86 **2. Materials and methods**
87 *2.1. Field survey*
88 *2.1.1. Study area*
89 The study area is located on an intertidal sandflat in western Kyushu, Japan (32° 47.2′ N, 130°
90 35.5′ E; see fig. 1 in Takeuchi et al., 2015). Tidal level fluctuates in a semidiurnal cycle. The annual
91 means of predicted tidal ranges at spring and neap tides are 3.97 and 1.70 m, respectively, at the
92 Japan Meteorological Agency's tidal gauge station (32° 45′ N, 130° 34′ E) located ca. 5 km south of
93 the sandflat. The whole area of the sandflat is ca. 4.15 km^2, with the maximum distance from the
94 uppermost shore to low water spring tide level being 2.7 km. On the sandflat, the spatial distribution
95 range of comparatively large-sized *Ruditapes philippinarum* clams with shell lengths > 20 mm is
96 limited to the low-tide zone (1409–2129 m seaward from the uppermost shoreline), although that of
97 small-sized clams with shell length ≤ 10 mm extends over the whole intertidal zone (Takeuchi et al.,
98 2013; Takeuchi et al., 2015). Most of the sandflat is covered with blackish sand from Mount Aso, an
99 active volcano. The field survey was conducted at two sites with distinct substrates. One site
100 [hereafter, Site A (32° 47′ 12.6″ N, 130° 35′ 25.9″ E)] was covered with the autochthonous, blackish
101 sand. The other site [hereafter, Site B (32° 47′ 16.8″ N, 130° 35′ 30.1″ E)] was covered with whitish
102 sand which had been dredged from an offshore seabed and dumped over a part (80 m × 100 m) of
103 the sandflat, for enhancing the recruitment of *R. philippinarum* clams (Oshima Fisheries Cooperative
104 Association, personal communication). The two sites were ca. 1200 m seaward from the uppermost
105 shoreline and were 169 m apart from each other.
106

107 *2.1.2. Sampling and subsequent sample processing*

108 A field survey for clam-shell and sediment colorations and grain-size composition was conducted
109 at a spring low tide on 23 August 2017. At each sampling site, 25 samples for clam-shell coloration,
110 one sample for sediment coloration, and one sample for grain-size composition were collected.
111 Coloration of shell and background was expressed as brightness values of gray-scale color. This
112 value ranges from 0 (= black) to 255 (= white) and increases with brightening. Shell-color
113 configurations (e.g., plain, mottled, or banded coloration) were not discriminated, and the shell
114 coloration of each specimen was determined by the mean brightness value over its shell surface.

115 To take a sample for clam-shell coloration, sediments of the top 8-cm layer were scooped up
116 using a 23-cm × 15-cm rectangle shovel, and after sieving with a 2-mm mesh, retained materials
117 were fixed with 10% neutralized seawater formalin. In the laboratory, *R. philippinarum* clams were
118 sorted from each sample. Of them, juvenile clams with shell lengths < 15 mm were used in the
119 subsequent analysis. The shell brightness of each specimen was quantified as follows: (1) each
120 specimen was placed on a stage (5-cm length, 3-cm width, and 1-cm height) which was centered in a
121 11-cm × 11-cm square tray (3-cm height) containing freshwater (1.5-cm depth above the stage), with
122 its right valve directed upward; (2) a digital image of each specimen, with a color chart (i.e., a
123 10-mm × 10-mm standard color chart composed of 3 × 3 cells of red, green, blue, black, gray, white,
124 yellow, purple, and cyan colors; CasMatch, Bear Medic), was taken under two light sources, using a
125 digital camera (PENTAX K-70, RICOH) mounted on a copy stand (distance from the camera lens to
126 the stage = 22 cm); (3) color correction based on the black, gray, and white colors of the chart and
127 trimming were made for each image by using Adobe Photoshop Elements 15; and (4) RGB (red,
128 green, and blue) pixel values were obtained from each image using imageJ 1.48v
129 (https://imagej.nih.gov/ij/) and were converted into a brightness value (*Br*) using the following
130 equation: $Br = (R + G + B)/3$, where *R*, *G*, and *B* are means of red, green, and blue pixel values (=

131 discrete values ranging from 0 to 255), respectively. Clams with shell brightness < 127.5 were
132 defined as dark-colored prey; the other side group as light-colored prey. Shell length of each
133 specimen was measured from the image to the nearest 0.1 mm, using imageJ 1.48v. Whether mean
134 shell brightness and number of individual clams differed significantly between the two sampling
135 sites was tested by a generalized linear model analysis with a null model likelihood ratio test
136 (assuming a gamma and Poisson error distributions, respectively, and a log-link function). These
137 analyses were performed on "R" (R Core Team, 2015).
138 To take a sample for sediment coloration, surface sediments to a depth of 1.5 cm were collected
139 3 times using a 10-cm × 10-cm quadrat frame and were combined into one sample. The sediment
140 sample was fixed with 10% neutralized seawater formalin in order to prevent their color from being
141 degraded by growth of algae and/or microorganisms. The coloration of each sediment sample was
142 quantified as follows: (1) each sample was well mixed and put into a 11-cm × 11-cm square tray
143 (3-cm height); (2) freshwater was filled to 1.5-cm depth above the sediment surface, using a siphon
144 without sediment disturbance; (3) taking an image and the subsequent color correction were
145 conducted using the same method as mentioned above; and (4) to calculate the mean brightness
146 value of each sediment sample, brightness values in 99 frames (1-cm × 1-cm) randomly selected
147 from the image were averaged.
148 To take a sample for grain-size composition, sediments of the top 3-cm layer were collected
149 using a 10-cm × 10-cm quadrat frame. Grain-size composition was determined using a vibratory
150 sieve shaker (AS200, Retsch) with a sieve mesh-size series of 4.0, 2.8, 2.0, 1.0, 0.5, 0.25, 0.125, and
151 0.063 mm. Following the same procedures used in Takeuchi et al. (2016), median grain size (mm),
152 mud content [the proportion of particles with diameters < 0.063 mm to total weight (%)], and sorting
153 coefficient (σ_I) for each sediment sample were obtained. The value of σ_I indicates the uniformity of
154 grain-size distribution, where $\sigma_I > 1.0$ and $\sigma_I < 0.5$ mean that sediments are poorly and well sorted,

155 respectively.

156

157 *2.2. Laboratory experiment*

158 *2.2.1. Experimental design*

159 Three laboratory experiments (hereafter, abbreviated as Exps I, II, III) using half-valve clam
160 shells filled with paste of raw clam meat as prey and the pufferfish *Takifugu niphobles* as a visually
161 hunting predator were performed during the period from early August to late October 2017 (for
162 details of the experimental setup, see Appendix A). The experiments were conducted to examine
163 whether matching/mismatching between shell and substrate brightness affects their survival rate (or
164 time). Therefore, dead clam shells, instead of live clams, were used to exclude their reburrowing
165 activity. The shell lengths and brightness of prey items (half-valve shells) are summarized in Table 1.
166 Across the experiments, the proportions of the light- and dark-colored prey items were set varied
167 (Fig. 1). Mean standard length (±SD, *N*: number of specimens) of specimens of the pufferfish was
168 109.1 (±8.2, $N = 7$) mm for Exp I, 111.8 (±9.7, $N = 21$) mm for Exp II, and 102.2 (±10.6, $N = 18$)
169 mm for Exp III. In each experiment, two to three distinct substrates were used [i.e., (1) sand plot,
170 hereafter SA; (2) shell hash (fragments) plot, the imitation of a shelly patch (i.e., shell fragments
171 accumulated in a depression), hereafter SH (i.e., oyster shell fragments covering a 10-cm × 20-cm
172 area of SA); and (3) gravel sand plot, hereafter GS]. Mean brightness, median grain size, mud
173 content, and sorting coefficient (σ_I) of each substrate were 54.2, 0.58 mm, 0.01%, and 0.75 for SA,
174 186.5, 4.51 mm, 0.06%, and 0.62 for SH (only for shell fragments), and 36.9, 2.90 mm, 0.07%, and
175 0.97 for GS, respectively.

176 The laboratory experiments were designed as follows: (1) Exp I (15 trials in total) with two
177 substrate types (SA, SH), in which the light- and dark-colored prey items were used unequally (light
178 > dark); (2) Exp II (32 trials) with two substrate types (SA, SH), in which the two-colored prey items

179 were used nearly equally; and (3) Exp III (23 trials) with three substrate types (SA, SH, GS), in
180 which the two-colored prey items were used nearly equally. Experimental setup is shown in Fig. 2.
181 The experiments were performed using a large rectangular tank (length × width × height: 1.7 × 0.8 ×
182 0.4 m) with seawater of 20-cm depth. On the tank bottom, 12 trays (length × width × height: 34 × 24
183 × 6 cm) were placed in a 2 × 6 arrangement. Each tray had one of the three distinct substrates (SA,
184 SH, GS), and four prey items (half-valve shells) were set haphazardly within a centered 10-cm ×
185 20-cm area on the substrate. For each experimental trial, two specimens of the pufferfish were
186 introduced into the tank and allowed to swim freely throughout the tank for 1.5 h. Up to four trials
187 were performed during each daytime period (07:00–19:00) because of the diurnal activity of the
188 pufferfish *T. niphobles* (Watanabe and Ota, 2009). Before starting each trial, *T. niphobles* specimens
189 were unfed for more than one night. Seawater was renewed completely after the last trial of each day
190 and was aerated by using an air pump for a night until the start of the first trial the next day. During
191 the experiment, water temperature was maintained using the room air conditioner. Mean water
192 temperature and salinity were 23.0°C and 33.3 practical salinity unit (hereafter, psu omitted) for Exp
193 I, 24.4°C and 32.5 for Exp II, and 23.7°C and 31.0 for Exp III, respectively [these were measured
194 using a handheld conductivity meter (Pro 30, YSI)]. During the experiment, the experimental tank
195 was under the light of four LED (light-emitting-diode) lamps (LEN-F10D-BK, NICHIDO). The
196 intensity of illumination at the center of each tray ranged from 420 to 660 lux (mean ± SD = 545.8 ±
197 92.6 lux; number of trays = 12). The behavior of *T. niphobles* specimens was recorded from above
198 using three fixed digital camcorders (HDR-XR500V and HDR-CX500V, Sony) to cover the entire
199 area of the experimental tank bottom. Each recording was started before the introduction of *T.*
200 *niphobles* specimens into the tank. From the video images, “survival” time (i.e., period from the start
201 of each trial to predation) of each prey item was measured to the nearest 1 sec. Data from trials with
202 consumption rates of less than 25% (= more than 36/48 prey items “survived”) were not used for the

subsequent analysis. Data from prey items that were flipped over by pufferfish-generated water flows were regarded as invalid.

2.2.2. Data analysis

To test whether the pufferfish disproportionately consumed a common morph (known as frequency-dependent selection), the index of preference for dark-colored prey [*PD* = proportion of consumed dark-colored prey items to the total of consumed prey items ($P_{D\text{consumed}}$)/proportion of provided dark-colored prey items to the total of provided prey items ($P_{D\text{provided}}$)] was evaluated. In this analysis, data from trials in which no dark-colored prey items (i.e., <127.5 in shell brightness) were used or in which all prey items were consumed were not used. *PD*s at the times of 0.5, 1.0, and 1.5 h were calculated. Whether each *PD* differed significantly from 1 (= no frequency-dependent selection) was tested using a one-sample *t*-test.

Two statistical modellings using (1) binary values indicating whether each prey item was consumed by predators within 1.5 h (= 1) or not (= 0) and using (2) continuous values for "survival" time of each prey were performed. For each case, the following five generalized linear mixed models (GLMMs) with the random effect of experimental trials were considered: GLMM 1, with the fixed effects of shell brightness and substrate type and their interaction; GLMM 2, with the fixed effects of shell brightness and substrate type; GLMM 3, with the fixed effect of shell brightness; GLMM 4, with the fixed effect of substrate type; and Null model, with no fixed effects. The former case assumed a binomial error distribution and a logit-link function, and the latter case assumed a gamma error distribution and a log-link function. From each set of five GLMMs, the best-fit model was selected based on Akaike's information criterion (AIC) (Akaike, 1973). If there are no effects of shell brightness and substrate type on a response variable, a null model will be selected as the best-fit

226 model. Model construction was performed using “glmer” function in “lme4” package (Bates et al.,
227 2015) of “R” (R Core Team, 2015).
228
229 **3. Results**
230 *3.1. Association between shell and background brightness*
231 Both the grain-size composition and brightness of surficial sediments differed between the two
232 sampling sites. Surficial sediments of Site A were mainly composed of dark-colored muddy sand
233 [i.e., median grain size, mud content, and sorting coefficient (σ_I) were 0.16 mm, 14.9%, and 1.36,
234 respectively, and mean brightness was 35.2]. On the other hand, surficial sediments of Site B were
235 mainly composed of light-colored sand [i.e., median grain size, mud content, and σ_I were 0.48 mm,
236 0.2%, and 1.15, respectively, and mean brightness was 138.7].
237 Shell brightness also differed between the two sympatric subpopulations of juvenile *Ruditapes*
238 *philippinarum* clams from the two sampling sites (Fig. 3). Mean shell brightness (±SD, *N*: number of
239 specimens) of clams from Site A and Site B were 112.9 (±33.1, $N = 565$) and 123.6 (±35.3, $N = 158$),
240 respectively, and this difference was significant (a likelihood ratio test, $P < 0.001$). Proportional
241 abundance of light-colored clams at Site A and Site B were 35.9 and 46.8%, respectively.
242 The number of individual clams per sample (inds per 0.0345 m^2) was higher at Site A than Site B.
243 The number of individuals (mean ± SD, *N*: number of samples) ranged from 2 to 93 (22.6 ± 21.6, N
244 = 25) at Site A, and from 1 to 19 (6.3 ± 4.8, $N = 25$) at Site B. A significant difference was detected
245 by a generalized linear model analysis with a null model likelihood ratio test ($P < 0.001$).
246
247 *3.2. Visual crypsis*
248 The results from 11, 20, and 19 trials of Exps I (15 trials in total), II (32 trials), and III (23 trials),
249 respectively, were used in the subsequent data analysis. Data from trials with low consumption rates

250 (for the definition of those rates, see Section 2.2.1) were not explored. The raw data on experimental
251 results are given in Appendix B. Proportional frequency of prey items consumed by predators (two
252 specimens of the pufferfish *Takifugu niphobles* in each experimental trial) within 1.5 h was the
253 highest on SA (sand), followed by GS (gravel sand) and SH (shell hash) (Fig. 4a). Mean
254 consumption rates (±SE, *N*: number of experimental trials) were 88.6 (±5.4, *N* = 11) and 34.8 (±9.1,
255 *N* = 11) % on SA and SH in Exp I, 96.2 (±1.9, *N* = 20) and 76.8 (±4.9, *N* = 20) % on SA and SH in
256 Exp II, and 89.3 (±5.3, *N* = 19), 70.5 (±5.9, *N* = 19), and 76.4 (±6.9, *N* = 19) % on SA, SH, and GS
257 in Exp III, respectively. "Survival" time of prey items consumed within 1.5 h was the shortest on SA,
258 followed by SH and GS (Fig. 4b). Mean "survival" times (±SE, *N*: number of prey items) were
259 1890.1 (±95.9, *N* = 234) and 2378.3 (±149.2, *N* = 92) sec on SA and SH in Exp I, 1246.7 (±61.0, *N* =
260 458) and 1892.7 (±81.1, *N* = 365) sec on SA and SH in Exp II, and 1240.7 (±78.6, *N* = 270), 1713.4
261 (±98.8, *N* = 211), and 1715.6 (±91.3, *N* = 230) sec on SA, SH, and GS in Exp III, respectively.
262 No frequency-dependent selection was confirmed in the experiments (Fig. 5). The mean value
263 (±SD, *N*: number of experimental trials) of the index of preference for dark-colored prey (*PD*) was
264 1.07 (±0.48, *N* = 39) for the time of 0.5 h, 1.01 (±0.33, *N* = 43) for the time of 1.0 h, and 1.02 (±0.22,
265 *N* = 42) for the time of 1.5 h. These values did not differ from 1 significantly (one-sample *t*-test, *P* >
266 0.3).
267 Prey items with a coloration conspicuous on the background were consumed by predators at a
268 higher rate than prey items with cryptic coloration in general (Fig. 6a,b,c). For example, in Exp III,
269 mean consumption rates for the dark-colored prey on SA, SH, and GS were 82.8, 86.2, and 65.0%,
270 respectively, whereas those for the light-colored prey on SA, SH, and GS were 94.5, 58.6, and 86.9%,
271 respectively. In the GLMM analysis for the probability of predation, GLMM 1 was selected as the
272 best-fit model through the three experiments (Table 2). This model indicates that with the exception
273 of SA in Exp I, the probability of predation decreases with increasing shell brightness on SH,

whereas the probability decreases with decreasing shell brightness on SA and GS. The models for GS and SH had steeper slopes than that for SA.

Consumed prey items with a coloration conspicuous on the background were detected by predators more easily than those items with cryptic coloration in general (Fig. 6d,e,f). In the GLMM analysis for "survival" time, GLMM 4 was selected as the best-fit model for Exp I where the light-colored prey items were used more frequently than the dark-colored prey items (Table 3). On the other hand, GLMM 1 was selected as the best-fit model for Exps II and III where the light- and dark-colored prey items were used nearly equally. The former model indicates that prey items on SA are detected by predators more easily than prey items on SH regardless of the shell brightness of the prey. The latter model indicates that "survival" time increases with increasing shell brightness on SH, whereas the time increases with decreasing shell brightness on GS. The time was generally short (= ca. 1000 sec) on SA regardless of the shell brightness of the prey.

4. Discussion

Our field survey shows association between shell and background brightness. Shell brightness significantly differed between the two sympatric subpopulations of juvenile *Ruditapes philippinarum* clams from Site A and Site B (Fig. 3) and was positively associated with background brightness. Site A had a comparatively dark-colored (volcanic) muddy sand substrate which is autochthonous to the sandflat. On the other hand, Site B had a light-colored sand substrate which had been introduced from offshore sediments to support the establishment of a clam population there. The mean shell brightness of juvenile clams from Site B was significantly higher than that of Site A (a likelihood ratio test, $P < 0.001$). It would be implausible that clams could have chosen the most suitable substrate in terms of color matching degree by their active migration, due to their simple vision using photoreceptor cells (Morton, 2008). Therefore, the difference in mean shell brightness between

298 the two sympatric subpopulations was probably due to short-term (≤1 year) selective predation
299 within a range of morphs which were largely determined by a population genetic trait. In addition,
300 the individual density of juvenile clams at Site B (= 183.2 inds m^{-2}) was about one-fourth of that at
301 Site A (= 655.1 inds m^{-2}), even though the former site appears to have more suitable substrate (i.e.,
302 higher sand content) for *R. philippinarum* clams than the latter site (Toba et al., 1992; Saito et al.,
303 2007; Vincenzi et al., 2011; Boscolo Brusà et al., 2013; Bidegain et al., 2015). This result is possibly
304 due to an intense predation induced by shell–background color mismatching (cf., Donnelly and
305 Whoriskey, 1993). This speculation remains to be substantiated. The results from the present field
306 survey point to possible importance of the background color matching concept that have been
307 overlooked in a conventional method of adding allochthonous substrates to sandflats to support the
308 establishment of a clam population (Nakahara and Nasu, 2002; Ikushima et al., 2012; Sakurai et al.,
309 2012).
310 The importance of the background color matching concept is supported by our laboratory
311 experiment. The inconsistency in the results of model selection between Exp I and Exps II and III
312 was probably due to small sample size for the dark-colored prey in Exp I. Shell-color configurations
313 (e.g., plain, mottled, or banded coloration) were not discriminated, and the shell coloration of each
314 specimen was expressed as the mean brightness value over its shell surface. Despite that limitation,
315 the results indicated that prey items in color-mismatched combinations [i.e., comparatively light-
316 (dark-) colored prey items on dark- (light-) colored substrate] were consumed by visually hunting
317 predators, the pufferfish *Takifugu niphobles*, more immediately and at a higher rate than prey items
318 in color-matched combinations (Fig. 6). A similar tendency is known for some predatory fishes
319 (Okamoto et al., 2001; Arakawa et al., 2007; Ryer et al., 2008). For example, Japanese sea bass
320 (*Lateolabrax japonicus*) preferentially bites lures with a conspicuous body color against a
321 background color provided in a laboratory experiment (Okamoto et al., 2001). Cryptic color morphs

could be more adaptive than conspicuous color morphs, and hence, the former morphs would become dominant in a population through crypsis-mediated predation. Such an inference is consistent with the interpretation for the results of the present field survey. These results showed that shell–background color matching can help juvenile *R. philippinarum* clams avoid attack from visually hunting predators.

Even the lowest level of mean "survival" time, however, might be enough for juvenile *R. philippinarum* clams to start reburrowing. Indeed, mean "survival" time predicted from our statistical models was ca. 1000 sec (Fig. 6d,e,f), whereas live juvenile clams can usually start reburrowing within ca. 100 sec under a light condition (Takeuchi et al., 2015). On the other hand, the time of the first attack in each experimental trial was largely determined by the "motivation" of specimens of the pufferfish. That time varied from 14 to 4213 sec. Therefore, it should be noted that "survival" time addressed in the present study cannot be applied directly to live clams' burrowing behavior.

The shell–background color matching effect seems to be reinforced by coarse-grained background. Our statistical models for probability of predation suggested that the shell–background color matching effect was more evident on a coarse-grained background, i.e., SH (shell hash) and GS (gravel sand), than on a fine-grained background, i.e., SA (sand) (Table 2, Fig. 6a,b,c). This result might be due to visual confusion in the predator through prey' masquerading as inedible objects (i.e., gravel, shell fragments) or disruptive coloration of prey items, as with other marine invertebrate prey animals (Whiteley et al., 1997; Merilaita, 1998; Palma and Steneck, 2001; Todd et al., 2006; Manríquez et al., 2008). Consequently, our result suggests that to support the establishment of a clam population, adding coarse-grained shell fragments or gravel to a sandflat is potentially more effective than fine-grained offshore dredged sand, in a case with well shell–background color matching.

346 There is no frequency-dependent selection in the focal prey–predator system.
347 Frequency-dependent selection is often recognized as an important aspect in considering prey
348 polymorphism (Ruxton et al., 2004; Quicke, 2017). In such selection, prey items with a common
349 morph is consumed disproportionately more frequently than prey items with a rare morph regardless
350 of degree of prey' visual crypsis. For example, Smith (1975) suggested that for the wedge clam
351 (*Donax faba*), clams with the commonest color morph are consumed by predators at a higher rate
352 than clams with other rare morphs when the population density is comparatively low. By contrast,
353 the author also suggested that when the population density is comparatively high, clams are selected
354 by crypsis-mediated predation. In addition, Shigemiya (2004) experimentally demonstrated that the
355 pufferfish *T. niphobles* can exhibit frequency-dependent selection on artificial prey items (composed
356 mainly of fish paste) with two color morphs (i.e., dark brown and pale brown) when prey items are
357 uniformly arranged in space. The present study, however, confirmed that there was no preference for
358 the dark-colored prey in predation behavior of *T. niphobles* specimens regardless of
359 shell-color-morph frequencies (i.e., Exp I vs. Exps II and III: the light- and dark-colored prey items
360 were used unequally or nearly equally; see Fig. 1) (Fig. 5).
361 In conclusion, visual crypsis (i.e., shell–background color matching) is a possible function of
362 polymorphic shell coloration in juvenile *R. philippinarum* clams. Our findings provide a new insight
363 into effective management planning for this clam species, creating sand habitats with a more
364 matched coloration. To understand the ecological significance of the findings, further studies on (1)
365 the contribution of shell–background color matching toward the *in situ* survival rate of a juvenile
366 clam population and (2) spatio-temporal variability of the contribution depending on the relative
367 importance of visually hunting predators compared with predation by non-visually hunting predators
368 are required.
369

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506

507 **Figure captions**

508 **Fig. 1.** Shell-brightness-frequency distribution of prey items (half-valve shells) used in each of the
509 three laboratory experiments (a, Exp I; b, Exp II; c, Exp III). Dark- and light-gray bars stand for
510 valid and invalid prey items for data analysis, respectively. The boundary shell brightness between
511 the light- and dark-colored prey items is indicated by a vertical dashed line in each panel. N_D and N_L
512 indicate the numbers of the dark- and light-colored prey items valid for data analysis, respectively. *N*
513 = number of specimens; number of the valid specimens for data analysis is indicated in brackets.

514

515 **Fig. 2.** Schematic diagram of the experimental setup (a, side view; b, top view for Exps I and II; c,
516 top view for Exp III; d, side view). A rectangular tank (length × width × height: 1.7 × 0.8 × 0.4 m)
517 with seawater of 20-cm depth was used for the experiment. Four LED (light-emitting-diode) lamps
518 and three digital camcorders were fixed around the tank. On the tank bottom, 12 trays (length ×
519 width × height: 34 × 24 × 6 cm) were placed in a 2 × 6 arrangement. (e) Each tray had one of the
520 three distinct substrates [i.e., SA (sand), SH (shell hash), GS (gravel sand)].

521

522 **Fig. 3.** Comparison of shell brightness of juvenile clams between Site A and Site B in the study area.
523 Shell-brightness-frequency distributions of juvenile clams from Site A (a) and Site B (b). *N* =
524 number of specimens. (c) Probability-density plots of shell brightness of juvenile clams from Site A
525 (dark-gray-filled area) and Site B (white-filled area). The overlapped part between both plots is
526 indicated as semi-transparent.

527

528 **Fig. 4.** Consumption rate (a) and "survival" time (b) of prey items on SA (sand), SH (shell hash), and
529 GS (gravel sand) in each of the three laboratory experiments (Exps I, II, III). Each bar and error bar

represent mean and SE (standard error), respectively. *N*s in panels (a) and (b) = numbers of experimental trials and specimens, respectively.

Fig. 5. Plots of the proportion of consumed dark-colored prey items to the total of consumed prey items ($P_{D\text{consumed}}$) versus the proportion of provided dark-colored prey items to the total of provided prey items ($P_{D\text{provided}}$), at the times of 0.5 h (a), 1.0 h (b), and 1.5 h (c) for each of the three experiments. In the comparison, prey items with shell brightness < 127.5 were defined as dark-colored prey, and data from the trials in which no dark-colored prey items were used or in which all prey items were consumed were not used. In the case of no frequency-dependent selection, the index of preference for dark-colored prey ($PD = P_{D\text{consumed}}/P_{D\text{provided}}$) is 1 (oblique line). N = number of experimental trials.

Fig. 6. Effects of shell brightness (range: 0–255) and substrate type [SA (sand), SH (shell hash), GS (gravel sand)] on the probability of predation (range: 0–1; a,b,c) and "survival" time (d,e,f) of prey items in each of the three laboratory experiments (a and d, Exp I; b and e, Exp II; c and f, Exp III). The dashed, gray solid, and black solid curves in each panel represent the best-fit models for SA, SH, and GS, respectively.

548 **Table 1.** Summary of shell length, brightness (range: 0–255), and number of specimens used in the experiments. Those values for the specimens used in the
549 statistical analyses are noted in brackets.

Experiment name	Number of experimental trials	Substrate type	Number of specimens	Shell length (mm)		Brightness	
				Minimum–Maximum	Mean ± SD	Minimum–Maximum	Mean ± SD
Exp I	15 (11)	Sand	360 (264)	5.8–10.1	7.8 ± 1.0	35.9–235.6	175.6 ± 42.0
				(5.8–10.1)	(7.7 ± 0.9)	(35.9–235.6)	(169.6 ± 45.0)
		Shell hash	360 (264)	5.8–11.1	7.8 ± 0.9	36.6–235.9	177.4 ± 42.0
				(5.8–11.1)	(7.7 ± 0.9)	(36.6–235.9)	(172.4 ± 44.1)
		Whole samples	720 (528)	5.8–11.1	7.8 ± 0.9	35.9–235.9	176.5 ± 42.0
				(5.8–11.1)	(7.7 ± 0.9)	(35.9–235.9)	(171.0 ± 44.6)
Exp II	32 (20)	Sand	768 (476)	5.9–12.8	9.0 ± 1.3	21.8–236.4	146.2 ± 63.3
				(6.1–12.8)	(9.0 ± 1.3)	(30.2–236.4)	(146.1 ± 61.4)
		Shell hash	768 (473)	5.9–12.5	8.9 ± 1.3	25.2–240.8	144.3 ± 63.1
				(5.9–12.5)	(9.1 ± 1.4)	(25.2–240.8)	(145.2 ± 61.2)
		Whole samples	1536 (949)	5.9–12.8	9.0 ± 1.3	21.8–240.8	145.3 ± 63.2
				(5.9–12.8)	(9.0 ± 1.3)	(25.2–240.8)	(145.7 ± 61.3)
Exp III	23 (19)	Sand	368 (302)	6.4–12.9	9.7 ± 1.4	46.5–241.5	155.5 ± 54.7
				(6.4–12.9)	(9.7 ± 1.4)	(46.5–241.5)	(155.7 ± 55.4)
		Shell hash	368 (299)	6.5–13.3	9.3 ± 1.4	42.0–239.7	155.2 ± 52.0
				(6.5–13.3)	(9.4 ± 1.4)	(42.0–239.7)	(154.6 ± 53.3)
		Gravel sand	368 (300)	6.5–12.8	9.4 ± 1.1	47.4–239.8	152.4 ± 58.2
				(6.5–12.8)	(9.4 ± 1.1)	(47.4–239.8)	(152.8 ± 58.3)
		Whole samples	1104 (901)	6.4–13.3	9.5 ± 1.3	42.0–241.5	154.4 ± 55.0
				(6.4–13.3)	(9.5 ± 1.3)	(42.0–241.5)	(154.4 ± 55.7)

550

551 **Table 2.** Five generalized linear mixed models (GLMMs) including a null model used to detect effects of shell brightness (SB; range: 0–255) and substrate
552 type (sand; shell hash; gravel sand) on probability of predation (range: 0–1). The case with no fixed effect is listed as "null". Akaike's information criterion
553 (AIC) for each model is indicated; ΔAIC means residual from AIC of the best-fit model.

Experiment name	Model name	Response variable	Fixed effects	Random effect	Linear predictor (y)	AIC	ΔAIC	Best-fit model
Exp I	GLMM 1	Probability of predation	SB; Substrate; Interaction	Trials	If Substrate = "sand", $y = 3.9702 – 0.0065SB$ If Substrate = "shell hash", $y = 2.5127 – 0.0200SB$	420.9676	0	Accepted
	GLMM 2	Probability of predation	SB; Substrate	Trials	If Substrate = "sand", $y = 5.7506 – 0.0167SB$ If Substrate = "shell hash", $y = 1.9964 – 0.0167SB$	422.4544	1.4868	
	GLMM 3	Probability of predation	SB	Trials	$y = 2.6154 – 0.0117SB$	638.1052	217.1376	
	GLMM 4	Probability of predation	Substrate	Trials	If Substrate = "sand", $y = 2.7769$ If Substrate = "shell hash", $y = –0.8589$	436.6191	15.6515	
	Null model	Probability of predation		Trials	$y = 0.5943$	650.5344	229.5668	
Exp II	GLMM 1	Probability of predation	SB; Substrate; Interaction	Trials	If Substrate = "sand", $y = 1.2698 + 0.0227SB$ If Substrate = "shell hash", $y = 4.9069 – 0.0211SB$	515.3808	0	Accepted
	GLMM 2	Probability of predation	SB; Substrate	Trials	If Substrate = "sand", $y = 5.5395 – 0.0109SB$ If Substrate = "shell hash", $y = 3.1226 – 0.0109SB$	582.4506	67.0698	
	GLMM 3	Probability of predation	SB	Trials	$y = 3.5787 – 0.0093SB$	677.5618	162.1810	
	GLMM 4	Probability of predation	Substrate	Trials	If Substrate = "sand", $y = 3.7041$ If Substrate = "shell hash", $y = 1.4208$	614.5092	99.1284	
	Null model	Probability of predation		Trials	$y = 2.1127$	704.2193	188.8385	
Exp III	GLMM 1	Probability of predation	SB; Substrate; Interaction	Trials	If Substrate = "sand", $y = 1.5731 + 0.0117SB$ If Substrate = "shell hash", $y = 5.0001 – 0.0229SB$ If Substrate = "gravel sand", $y = –0.6837 + 0.0175SB$	621.7116	0	Accepted
	GLMM 2	Probability of predation	SB; Substrate	Trials	If Substrate = "sand", $y = 2.9298 + 0.0004SB$ If Substrate = "shell hash", $y = 1.1360 + 0.0004SB$ If Substrate = "gravel sand", $y = 1.6199 + 0.0004SB$	704.7826	83.0710	
	GLMM 3	Probability of predation	SB	Trials	$y = 1.7436 + 0.0004SB$	751.6426	129.9310	
	GLMM 4	Probability of predation	Substrate	Trials	If Substrate = "sand", $y = 2.9935$ If Substrate = "shell hash", $y = 1.2002$ If Substrate = "gravel sand", $y = 1.6826$	702.8362	81.1246	
	Null model	Probability of predation		Trials	$y = 1.8018$	749.6912	127.9796	

554

555 **Table 3.** Five generalized linear mixed models (GLMMs) including a null model used to detect effects of shell brightness (SB; range: 0–255) and substrate
556 type (sand; shell hash; gravel sand) on "survival" time (sec). The case with no fixed effect is listed as "null". Akaike's information criterion (AIC) for each
557 model is indicated; ΔAIC means residual from AIC of the best-fit model.

Experiment name	Model name	Response variable	Fixed effects	Random effect	Linear predictor (y)	AIC	ΔAIC	Best-fit model
Exp I	GLMM 1	"Survival" time	SB; Substrate; Interaction	Trials	If Substrate = "sand", $y = 7.3240 - 0.0002SB$ If Substrate = "shell hash", $y = 7.8876 - 0.0001SB$	5347.026	3.978	
	GLMM 2	"Survival" time	SB; Substrate	Trials	If Substrate = "sand", $y = 7.3194 - 0.0001SB$ If Substrate = "shell hash", $y = 7.8987 - 0.0001SB$	5345.030	1.982	
	GLMM 3	"Survival" time	SB	Trials	$y = 7.6149 - 0.0010SB$	5395.531	52.483	
	GLMM 4	"Survival" time	Substrate	Trials	If Substrate = "sand", $y = 7.2955$ If Substrate = "shell hash", $y = 7.8760$	5343.048	0	Accepted
	Null model	"Survival" time		Trials	$y = 7.4546$	5394.234	51.186	
Exp II	GLMM 1	"Survival" time	SB; Substrate; Interaction	Trials	If Substrate = "sand", $y = 6.9727 - 0.0008SB$ If Substrate = "shell hash", $y = 6.6189 + 0.0058SB$	13235.600	0	Accepted
	GLMM 2	"Survival" time	SB; Substrate	Trials	If Substrate = "sand", $y = 6.5545 + 0.0022SB$ If Substrate = "shell hash", $y = 7.1179 + 0.0022SB$	13282.880	47.280	
	GLMM 3	"Survival" time	SB	Trials	$y = 6.7931 + 0.0024SB$	13366.000	130.400	
	GLMM 4	"Survival" time	Substrate	Trials	If Substrate = "sand", $y = 6.8614$ If Substrate = "shell hash", $y = 7.4433$	13301.620	66.020	
	Null model	"Survival" time		Trials	$y = 7.1465$	13387.780	152.180	
Exp III	GLMM 1	"Survival" time	SB; Substrate; Interaction	Trials	If Substrate = "sand", $y = 6.6907 + 0.0007SB$ If Substrate = "shell hash", $y = 6.4736 + 0.0059SB$ If Substrate = "gravel sand", $y = 8.0544 - 0.0036SB$	11285.290	0	Accepted
	GLMM 2	"Survival" time	SB; Substrate	Trials	If Substrate = "sand", $y = 6.6860 + 0.0007SB$ If Substrate = "shell hash", $y = 7.2526 + 0.0007SB$ If Substrate = "gravel sand", $y = 7.3811 + 0.0007SB$	11347.860	62.570	
	GLMM 3	"Survival" time	SB	Trials	$y = 7.1532 + 0.0004SB$	11469.790	184.500	
	GLMM 4	"Survival" time	Substrate	Trials	If Substrate = "sand", $y = 6.7989$ If Substrate = "shell hash", $y = 7.3644$ If Substrate = "gravel sand", $y = 7.4873$	11348.080	62.790	
	Null model	"Survival" time		Trials	$y = 7.2163$	11468.420	183.130	

Appendix A. Details of the experimental setup

Half-valve shells used in the laboratory experiment as prey were prepared as follows: (1) collecting juvenile *Ruditapes philippinarum* clams from two intertidal sites (32° 49.6′ N, 129° 46.9′ E; and 32° 39.2′ N, 130° 16.2′ E); (2) boiling them to open shells; (3) removing their soft tissue; and (4) dividing each bi-valve shell into right- and left-valve shells. Shell brightness and length of each half-valve shell were measured using the same way mentioned in the text (see Section 2.1.2). In the experiment, each half-valve shell was filled with raw-clam-meat paste that was made from edible, live *R. philippinarum* clams.

Specimens of the pufferfish *Takifugu niphobles* used in the laboratory experiment as predator were collected by angling at the two site (32° 39.2′ N, 130° 16.2′ E; and 32° 36.7′ N, 130° 11.2′ E) of the southern coast of Shimabara Peninsula in Ariake Sound. They were transported to the laboratory within ca. 2.5 h. While transporting, the specimens were kept in containers (length × width × height: 49 × 34 × 30 cm and 71 × 33 × 27 cm) with field-collected sand and seawater, aerated by an air pump. In the laboratory, the specimens were kept in the same containers. About half the seawater was exchanged once daily. Water temperature at mean of 24°C was maintained using the room air conditioner.

Three distinct substrates [i.e., SA (sand), SH (shell hash), GS (gravel sand)] used in the laboratory experiment were prepared as follows: (1) for SA, sediments were collected at the site (32° 39.2′ N, 130° 16.2′ E), and after washing off silt and clay particles and sieving with a 1-mm mesh,

577 passed materials were used; (2) for SH, oyster shells (*Crassostrea gigas*) were collected at the site
578 (32° 49.6′ N, 129° 46.9′ E) and crushed to pieces, and after sieving with a 1-mm mesh, retained
579 materials were used; and (3) for GS, sediments were collected at the site (32° 49.6′ N, 129° 46.9′ E),
580 and after sieving with a 1-mm mesh, retained materials were used. Brightness and grain-size
581 composition of each substrate were measured using the same way mentioned in the text (see Section
582 2.1.2).
583
584 **Appendix B**
585 Supplementary data to this article can be found online at URL.
586

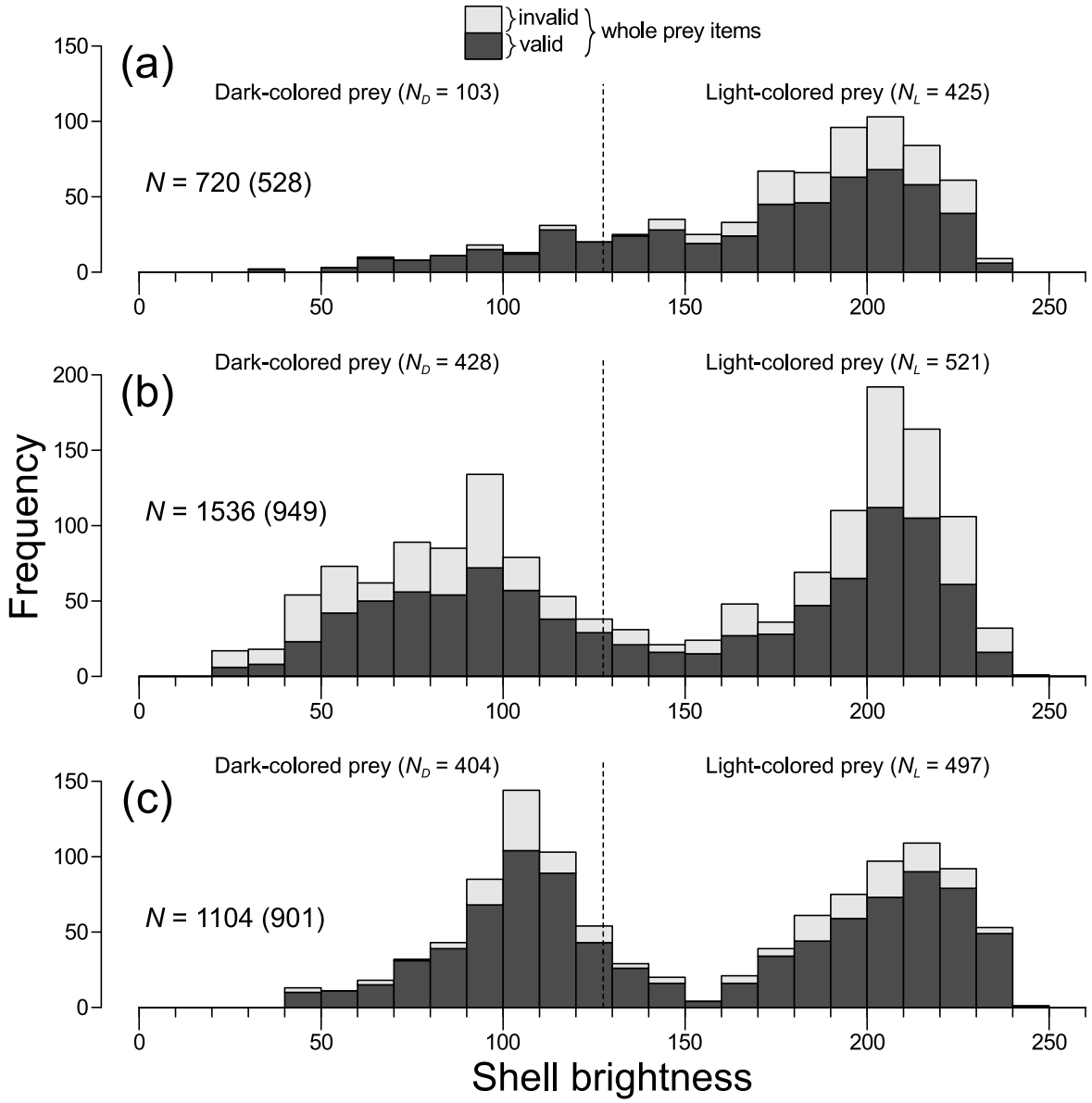

Fig. 1 (Takeuchi et al., revised)

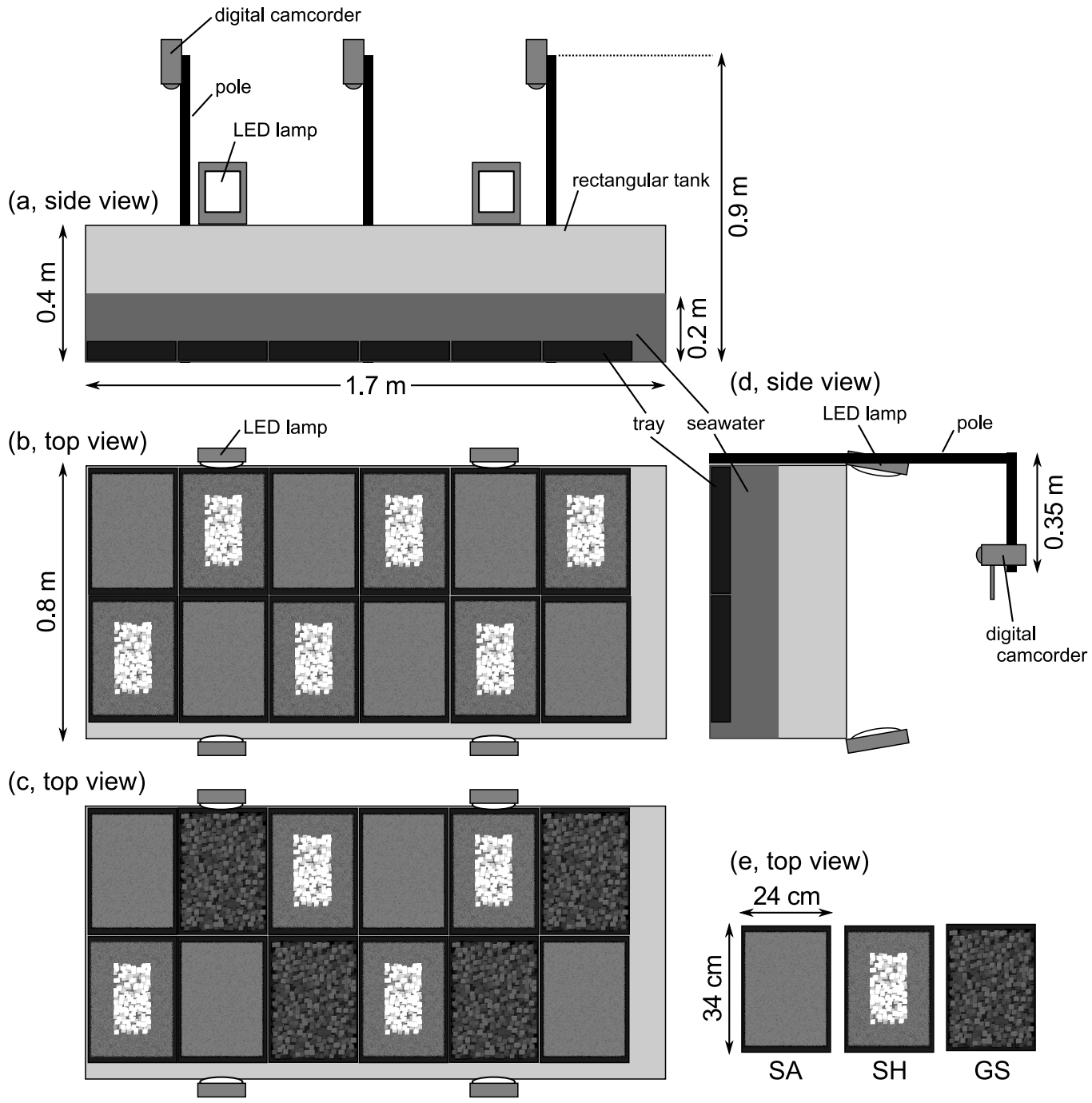

Fig. 2 (Takeuchi et al., revised)

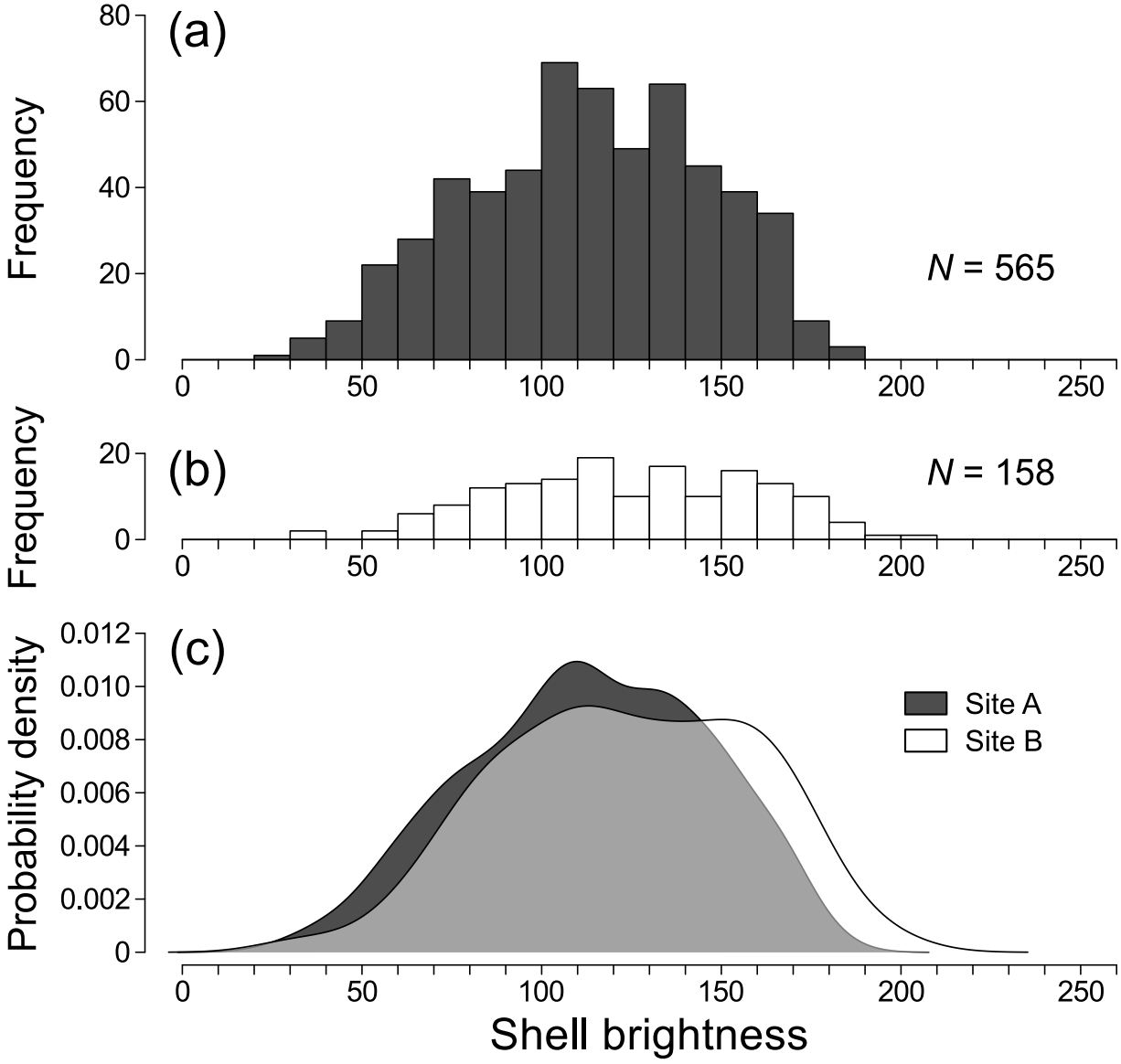

Fig. 3 (Takeuchi et al., revised)

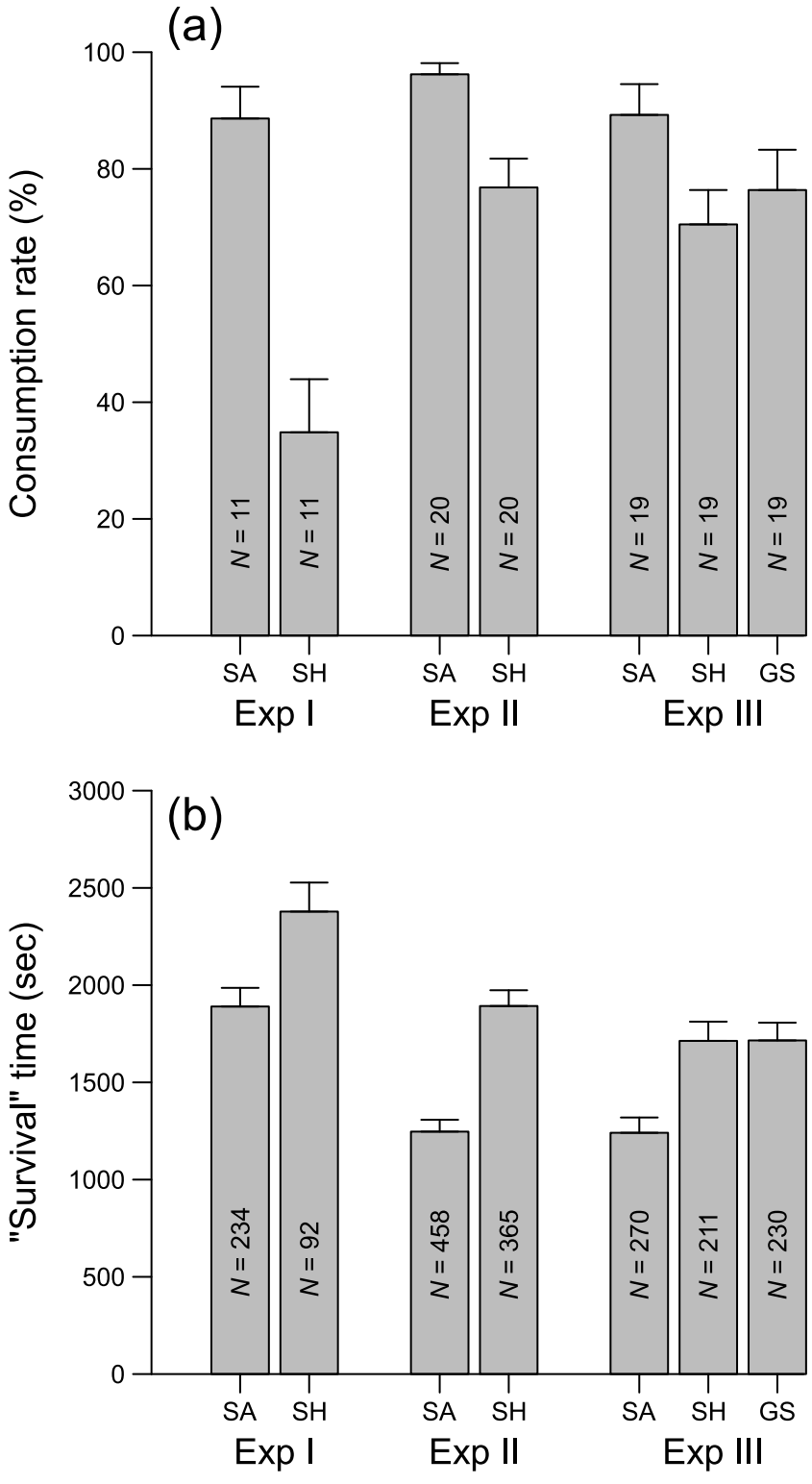

Fig. 4 (Takeuchi et al., revised)

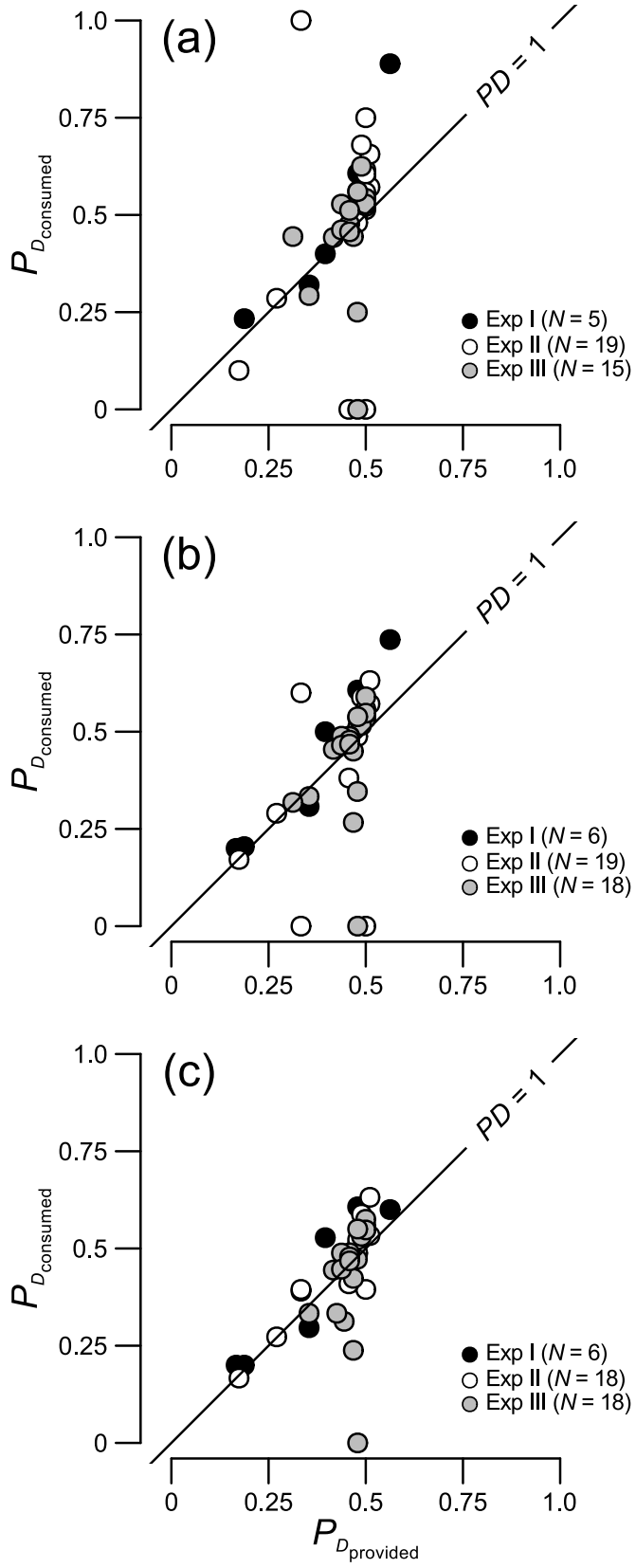

Fig. 5 (Takeuchi et al., revised)

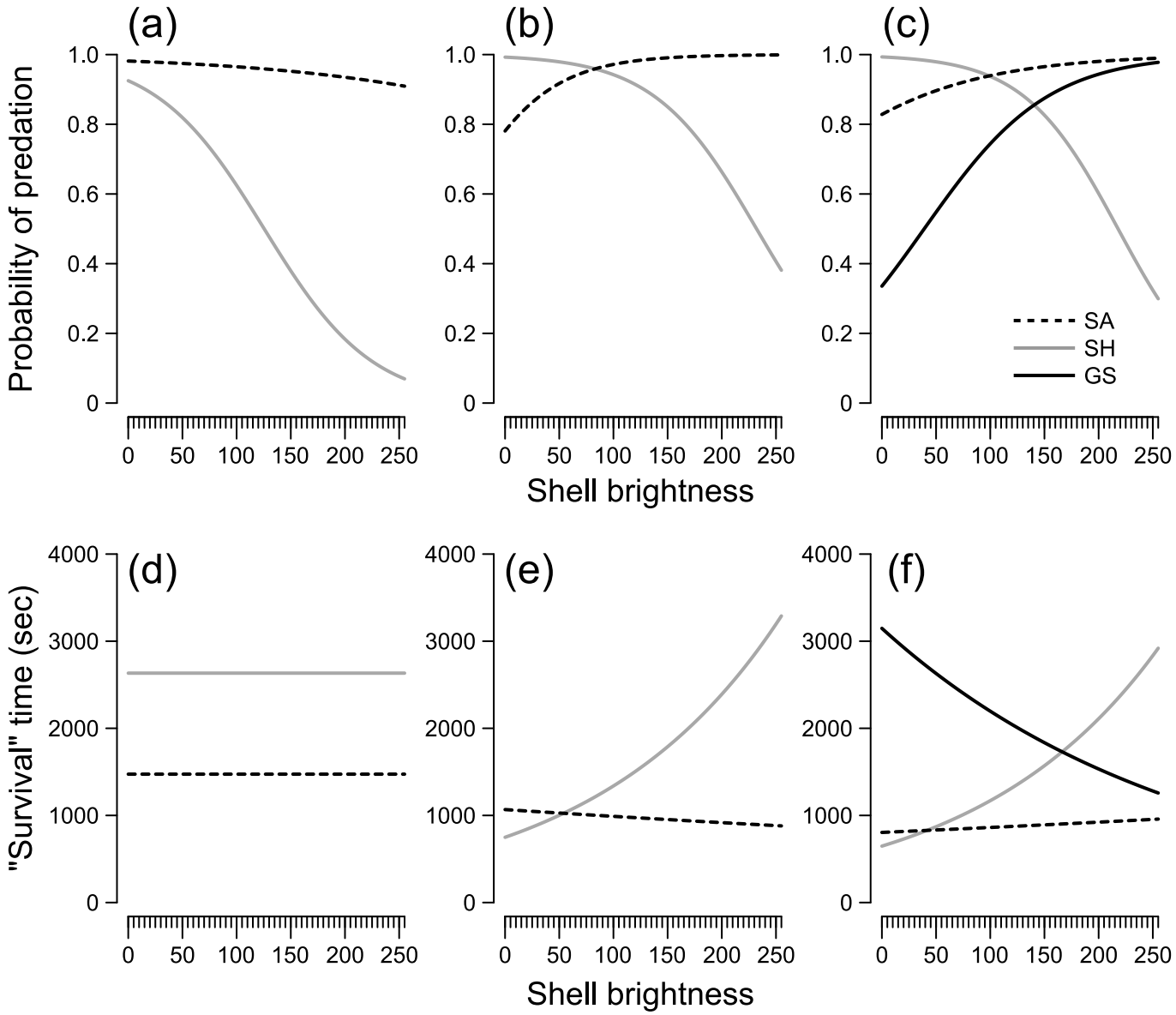

Fig. 6 (Takeuchi et al., revised)