Diver observations on spawning of *Hexagrammos agrammus* in Tongyeong, Korea

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Abstract

The season and characteristics of spawning of the greenling *Hexagrammos agrammus* that dwell along the Tongyeong coast were investigated by dive observations. Eleven spawning grounds were identified between November 11th 2013 and January 25th 2014. The fertilized eggs of *H. agrammus* were assigned to developmental stages I, II, III, and IV and, based on these observations, the spawning season was estimated to be from the end of October to mid-January. *H. agrammus* uses various marine algae on shallow bedrock areas as spawning substrates that provide good camouflage. Two to seven egg masses are fertilized around the holdfast of the algae at a depth of 1.2–4.0 m. Species-specific characteristics of *H. agrammus* during the spawning season, including strong parental care for the fertilized eggs were observed.

Key words: *Hexagrammos agrammus*, spawning season, spawning substrate, SCUBA, egg masses
Introduction

Hexagrammos agrammus (Hexagrammidae) is a winter-spawning fish that distributes along the entire coasts of Korea, Japan and northern China (Chung and Kim, 1994). It inhabits shallow regions with high densities of marine algae and bedrock with mixed sandy and muddy substrates. Six species are recognized in the genus Hexagrammos among which H. agrammus is a temperate species whose fertilized eggs form demersal and adhesive egg masses (Chyung, 1977). Hexagrammos males establish breeding territories only during their breeding seasons and care for egg masses spawned by multiple females, until hatching (Munehara et al., 2000).

Although many ethological studies have examined freshwater and coral reef fishes, the spawning characteristics of marine fishes that spawn during winter remain largely unknown. Although hexagrammids spawn on shallow rocky bottoms there have been few reports of their spawning characteristics. Spawning behavior and interspecific breeding were reported by Munehara et al. (2000), and breeding habitat selection was studied in three species of Hexagrammos (Kimura and Munehara, 2010). A single published study documented the use of underwater observations to investigate spawning characteristics of H. agrammus and H. otakii during winter in Korea (Lee et al., 2013). Underwater visual observations techniques have been widely applied for reef fish assessments since they are non-destructive and easily applicable methods for estimating species richness and abundance (Edgar et al., 2004; Kulbicki et al., 2007).

The purpose of this study was to describe the characteristics of fertilized eggs of H. agrammus observed by SCUBA diving at the coast water off Tongyeong during the presumed spawning season and to determine the precise spawning time and regional differences in spawning characteristics and habitats of H. agrammus.
Materials and methods

We investigated the spawning grounds of *H. agrammus* in coastal water off Tongyeong in depth layers of 1-10 m from October 2013 to February 2014 (Fig. 1), with special emphasis on the spawning season during November and December (Kim, 2003). The number of diving trials during the survey period was as follows; 1 time in October, 5 times in November, 4 times in December, 4 times in January, 3 times in February. Observations were carried out for a total 1,360 minutes in sessions of approximately 40 minutes by teams of two divers. The divers swam parallel to each other at minimum horizontal distances of 5 m apart, each monitoring the range of 3 m to each side of him by proceeding to 250 m ahead (about 1,500 m²). The characteristics of the spawning grounds of *H. agrammus* and of the fertilized eggs were observed *in situ*. The marine algae around the egg masses were then moved to the laboratory to further investigate the attachment of the fertilized eggs and the community structure of algae in the area. Fertilized eggs were classified into four developmental stages (Fukuhara, 1971): stage I (0–5 days post-fertilization; appearance of many oil globules and yolk granule), stage II (6–11 days post-fertilization; formation of eye lens, increase in number of melanophores on the embryonic body, motility), stage III (12–20 days post-fertilization; increase in number of melanophores), and stage IV (21–31 days post-fertilization; active motility, embryo just before hatching) The stages of development were used to estimate the time frame of spawning. We used a pH-conductivity meter (SG23-SevenGo Duo™; Mettler-Toledo Inc., Columbus, OH) for water temperature and salinity measurements and a dive computer (ZOOP; SUUNTO, Vantaa, Finland) for depth measurement. Pictures and videos under the water were taken with a digital
camera (DSC-RX100; Sony Corp., Japan). Identifications of fish during dive observations were made according to Kim et al. (2005) and confirmed later from the video records. Identification of collected marine algae followed Lee and Kang (2002).

Results and discussion

The developmental stages of fertilized eggs observed underwater varied among egg masses, presumably reflecting differences in their times of fertilization (Fig. 2E–L; Table 1). Fukuhara (1971) carried out rearing experiments on fertilized eggs of *H. agrammus* at 10–12°C, which is similar to the temperature range of Tongyeong coastal water in December. Based on these data and the egg mass stage, we estimated the elapsed time since fertilization of the eggs. Ochiai and Tanaka (1998) reported that the time to hatching was 31–36 days at 11°C and about 20 days at 17°C. In this study, fertilized eggs found in November, when the average water temperature was 15.5°C, were considered to take 20–26 days until they hatched, and for eggs fertilized in January, when the water temperature was 7.6°C, the developmental period was estimated as more than 36 days. A marked decrease in water temperature was observed from 18°C in November to 7°C in January.

We identified 11 spawning grounds between November and January (5 in November, 3 in December, 3 in January; Table 1), however no egg mass was found in October and February. A stage IV egg mass, fertilized at least 20 days previously, was found on November 10th, suggesting that spawning had taken place on October 20th. We found both stage II and stage IV egg masses on January 25th; the fertilization day of the stage II egg mass (6–11 days earlier, at the water temperature of 8°C) would, therefore, have been in mid-January. We suggest that the spawning season of *H. agrammus* at experimental
site is between the end of October and mid-January. However, further investigation is
needed to confirm the start of spawning season by intensive diving trials in October
because egg mass spawned in October was found in November. It is generally known that
spawning season of *H. agrammus* is between November and December (Kim, 2003). In
order to estimate the spawning season of fishes more precisely, combination of both
conventional method such as condition factor, gonadosomatic index (GSI) and
hepatosomatic index (HSI), and direct observations by SCUBA diving is needed.
Furthermore, parental care of eggs by male fish may continue into the beginning of
February because stage II eggs were found on January 25th. For determination of closed
season for *H. agrammus*, the period of parental care of eggs after spawning season should
also be considered.

Chung and Kim (1994) analyzed annual changes in the average GSI and HSI for 398
*H. agrammus* captured intertidally in Busan, which is located approximately 75 km
northeast of Tongyeong. The GSI of female *H. agrammus* increased rapidly from 1.11 in
September to 2.23 in October and to a maximum of 4.31 in November. It then decreased
sharply to 1.54 in December and to the very low value of 0.46 in January. The HSI also
shows similar changing pattern to the GSI. These peaks in the GSI and HSI, and the
greater number of spawning grounds and egg masses observed in November in the
present study indicate that November is the peak spawning time. The low GSI value
observed in January was similar to that in non-spawning months but we found three
spawning grounds and 15 egg masses. Although fewer than during the peak in November
(5 spawning grounds and 18 egg masses), this observation confirms that spawning
continued into January. Moreover, the absence of stage I egg masses and the presence of
stage II and IV egg masses (5–9 days and 16 days post-fertilization, respectively) on
January 25th, indicates that spawning by *H. agrammus* continued until mid-January.

Spawning of *H. agrammus* occurs over bottom areas consisting of bedrock and variably sized stones and pebbles. Several species of marine algae are attached to the bedrock and are well irrigated by a smoothly flowing water. The depth of water in the spawning grounds was 1.2–4 m (average 2.6 m) (Fig. 2A, B; Table 1). *H. agrammus* uses various marine algae for spawning substrates. In this study, 2–7 egg masses (average 4) of diameter 2–5 cm are attached to the holdfast of the algae; they are well hidden and consequently difficult to find (Fig. 2B–H; Table 1). Similarly, Kanamoto (1976) stated that the egg masses of *H. agrammus* are well-camouflaged. Sometimes, the egg masses were regularly spaced, which is consistent with the report by Munehara et al. (2000) that an average of seven egg masses were spawned together on marine algae in shallow water.

Fourteen species of marine algae were present in the spawning grounds and the adjacent area: *Undaria pinnatifida*, *Codium fragile*, *Caulerpa okamurae*, *Ulva pertusa*, *Ahnfeltiopsis flabelliformi*, *Lomentaria catenata*, *Prionitis cornea*, *Rhodymenia intricata*, *Callophyllis adnata*, *Acrosorium polyneurum*, *Gelidium amansii*, *Grateloupia filicina*, *Grateloupia turuturu*, *Plocamium telfairiae*. Among them 10 species belonged to the red algae. The most common algae used as attachment substrates for *H. agrammus* were *Prionitis cornea* (72%) and *Caulerpa okamurae* (18%); *Ahnfeltiopsis flabelliformi*, *Grateloupia turuturu*, *Lomentaria catenata*, and *Gelidium amansii* were also used (Fig. 2C–H; Table 1). Kanamoto (1976) reported that *H. agrammus* spawn mainly on *Gelidium amansii* but, in the present study, *Prionitis cornea* was preferred. This suggests that they may not have a fixed preference except that they prefer red algae.

Lee et al. (2013) reported that males of *H. otakii* on the southern coast of Korea do not vigorously protect and guard their fertilized eggs; when divers approached the eggs,
males often hovered at a distance of 2–3 m, and, in some cases, a guardian male was not present. In contrast, males of *H. agrammus* in the present study and on the western Korea (Lee et al., 2013) showed aggressive behaviors when divers approached within 1 m of the eggs. The observed difference in protective behavior on the fertilized eggs between two species is similar to that of Kanamoto (1976). Therefore, *H. agrammus* appears to exhibit stronger protective behaviors for their fertilized eggs than *H. otakii* does. In addition, *H. agrammus* spawns onto the holdfast of the marine algae where the fertilized eggs are well hidden and color-camouflaged against the bedrock of the western and southern Korean coast. On the contrary, *H. otakii* spawns onto the exposed bedrock. Subsequently, the differences in breeding territory between two species might be related to the differences in protective behaviors on the fertilized eggs. Nuptial coloration of male *H. agrammus* during spawning season provides excellent camouflage; while hiding in nearby marine algae or bedrock areas, they are able to change their color to match their surroundings (Fig. 2A, B; Table 1). Munehara et al. (2000) reported that the color of the ventral fins of male *H. agrammus* changes to black. Slightly different changes in coloration were observed in this study, possibly reflecting differences in their environment.

Kurita et al. (1995) reported that 78% of male *H. agrammus* more than one year old protected fertilized eggs in December and 100% did so in January. However, male *H. agrammus* younger than one year old mate randomly and do not protect their eggs. In this study, we found fertilized eggs in the spawning grounds on January 4th 2014 but we did not observe male fish protecting fertilized eggs. Presumably, males less than one year old participated in mating in our study.

Confirming the spawning status of specific fish through direct observations by SCUBA diving could minimize ecocide caused by fishing tools such as trawl nets, and is
a useful and eco-friendly method for observing spawning characteristics such as behavior and habitat preference. These types of studies could provide crucial data for setting standards for the protection of spawning grounds and for the determination of closed seasons for specific fish populations.

References


Munehara H, Kanamoto Z and Miura T. 2000. Spawning behavior and interspecific

Table 1. Characteristics of spawning grounds, the guardian male, and egg masses of *H. agrummus*

<table>
<thead>
<tr>
<th>Station</th>
<th>Date</th>
<th>Environmental characteristics</th>
<th>Guardian male</th>
<th>Egg mass</th>
<th>Algae around egg clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Depth (m)</td>
<td>Temp. (°C)</td>
<td>Total length (cm)</td>
<td>Response to divers</td>
</tr>
<tr>
<td>1</td>
<td>Nov 10</td>
<td>3</td>
<td>18</td>
<td>20-25</td>
<td>- Guard the eggs at the lower part of them on the rock</td>
</tr>
<tr>
<td>2</td>
<td>Nov 17</td>
<td>1.2</td>
<td>18</td>
<td>25-27</td>
<td>- Keep an eye on the diver at a crevice in the rock</td>
</tr>
<tr>
<td>3</td>
<td>Nov 23</td>
<td>3</td>
<td>13</td>
<td>18-20</td>
<td>- Keep an eye on the diver at a ghost net on the rocky area</td>
</tr>
<tr>
<td>4</td>
<td>Nov 30</td>
<td>3</td>
<td>13</td>
<td>20-22</td>
<td>- Keep an eye on the diver at a crevice in the rock</td>
</tr>
<tr>
<td>5</td>
<td>Dec 08</td>
<td>2.5</td>
<td>12</td>
<td>15-17</td>
<td>- Keep an eye on the diver at a ghost net on the rocky area</td>
</tr>
<tr>
<td>6</td>
<td>Dec 15</td>
<td>2.3</td>
<td>10</td>
<td>20-23</td>
<td>- Guard the eggs at the lower part of them on the rocks</td>
</tr>
<tr>
<td>7</td>
<td>Jun 04</td>
<td>4</td>
<td>8</td>
<td>-</td>
<td>- No male fish found</td>
</tr>
<tr>
<td>8</td>
<td>Jun 25</td>
<td>2.5</td>
<td>7</td>
<td>17-20</td>
<td>- Guard the eggs at the lower part of them on the rock</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>2</td>
<td>8</td>
<td>20</td>
<td>- Guard the eggs at the lower part of them on the rock</td>
</tr>
</tbody>
</table>

*: Algae used as spawning substrate.
Fig. 1. Map showing the location of the study site (●: Research point by SCUBA Diving).
Fig. 2. Images of the spawning grounds and egg masses of *H. agrammus*. A and B: guardian fishes in nuptial coloration (black arrows); B–H: egg masses *in situ* (white arrows); I–L: egg masses under light microscope.