SCUBA Observations of Spawning in *Hexagrammos agrammus* off the Tongyeong Coast, Korea

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Abstract

We used direct diving observations to determine the seasonality and other characteristics of spawning in the greenling *Hexagrammos agrammus* off the coast of Tongyeong. Eleven spawning grounds were identified between November 11, 2013 and January 25, 2014. The fertilized eggs of *H. agrammus* were assigned to developmental stages I, II, III, and IV. Based on this classification, we showed that the spawning season extended from the end of October to mid-January. *H. agrammus* used diverse seaweed species attached to shallow bedrock as spawning substrata that provided good camouflage. Two to seven egg masses were fertilized around the holdfasts of individual seaweeds at depths of 1.2–4.0 m. We identified species-specific reproductive traits of *H. agrammus* during the spawning season, including strong parental care of the fertilized eggs.

Key words: *Hexagrammos agrammus*, Spawning season, Spawning substrate, SCUBA, Egg masses

Introduction

*Hexagrammos agrammus* (Hexagrammidae) is a winter-spawning fish that occurs along the entire coastlines of Korea, Japan and northern China (Chung and Kim, 1994). The species inhabits shallow waters in sites where seaweeds are abundant, and the substrata comprise bedrock interspersed with mixed sandy and muddy sediments. *Hexagrammos* contains six congeners. *H. agrammus* is a temperate water species; it produces fertilized eggs masses that are demersal and adhesive (Chyung, 1977). *Hexagrammos* males establish breeding territories during the reproductive season and care for the egg masses spawned by multiple females through hatching (Munehara et al., 2000).

Many ethological studies have examined freshwater and coral reef fishes, but the spawning characteristics of marine fishes that reproduce during winter remain largely unknown. Although hexagrammids spawn on readily accessible shallow rocky bottoms, few reports of their spawning traits have been published. Spawning behaviors and interspecific breeding were reported by Munehara et al. (2000), and breeding habitat selection was evaluated in three congeners of *Hexagrammos* (Kimura and Munehara, 2010). Only one study has described direct underwater observations of winter spawning in *H. agrammus* and *H. otakii* in Korea (Lee et al., 2013). Underwater visual observation techniques have been applied widely in investigations of reef fishes, because the methodology is non-destructive and facilitates estimations of species richness and abundances (Edgar et al., 2004; Kulbicki et al., 2007).

The aim of this study was to provide a description of the fertilized eggs of *H. agrammus*. We obtained our data by direct SCUBA diving observations off the coastline of Tongyeong. Dives were performed during the putative spawning period; we examined regional differences in the spawning traits and habitats of the species.

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Materials and Methods

We investigated the spawning grounds of *H. agrammus* in coastal waters off Tongyeong (128°26ʹE, 34°49ʹN) at depths of 1–10 m from October 2013 through February 2014. Observations were especially concentrated during the spawning season, which extended from November to December (Kim, 2003). The numbers of diving observations made during the survey period were as follows: one in October, five in November, four in December, four in January, three in February. Overall, our two-person teams logged 1,360 min of observation during dives that lasted ca. 40 min each. The divers swam parallel to one another ≤ 5 m apart. Each diver scanned a 6-m wide track that was 250 m long (total area scanned: 1,500 m²). Thus, we observed the features of the spawning grounds and the fertilized eggs *in situ*. The seaweeds in the vicinity of the egg masses were collected and transported to the laboratory, where we examined the attachment of fertilized eggs and determined the specific identities of the algae.

Fertilized eggs were classified into four developmental stages (Fukuhara, 1971): stage I (0–5 days post-fertilization; many oil globules and yolk granules present); stage II (6–11 days post-fertilization; eye lens formed, and the number of melanophores on the embryonic body increased; embryos were motile); stage III (12–20 days post-fertilization; melanophore number increased); and stage IV (21–31 days post-fertilization; embryos were actively motile immediately prior to hatching) Classification into the stages of development allowed us to estimate the timing of spawning. We used a pH-conductivity meter (SG23-SevenGo Duo™; Mettler-Toledo Inc., Columbus, OH, USA) to measure water temperature and conductivity meter (SG23-SevenGo Duo™; Mettler-Toledo Inc., Columbus, OH, USA) to determine water depth. Still images and videos were captured underwater using a digital camera (DSC-RX100; Sony Corp., Tokyo, Japan). We used information provided by Kim et al. (2005) to identify the fishes underwater; these identities were later checked by examining our video recordings. We used the keys provided by Lee and Kang (2002) to identify the seaweed species.

Results and Discussion

The developmental stages of the fertilized eggs observed underwater varied among egg masses, presumably reflecting differences in the times of fertilization (Fig. 1E-1L; Table 1). Fukuhara (1971) raised embryos from the fertilized eggs of *H. agrammus* at temperatures of 10-12°C, which were similar to those that we measured in the Tongyeong coastal water in December. We therefore used Fukuhara’s (1971) data to estimate the time since fertilization for each of the egg mass stages that we observed. Ochiai and Tanaka (1998) reported that the time to hatching was 31–36 days at 11°C and ca. 20 days at 17°C. We measured a water temperature of 15.5°C in November and accordingly calculated the time to hatching from fertilization as 20–26 days. In January, the temperature was 7.6°C, and we therefore calculated a time to hatching that exceeded 36 days.

We identified 11 spawning grounds between November and January (five in November, three in December, three in January; Table 1), but found no egg masses in October or February. A stage IV egg mass fertilized ≥ 20 days previously was found on November 10, suggesting an October 20 spawning date. We found both stages II and IV egg masses on January 25; the stage II egg mass (spawned 6–11 days earlier at a water temperature of 8°C) was likely fertilized in mid-January. We suggest that the spawning season of *H. agrammus* at our observation site extended from the end of October to mid-January. However, further intensive diving observations are required to confirm the start of the spawning season in October, because the egg mass spawned in October was found in November. The spawning season of *H. agrammus* is generally believed to extend to between November and December (Kim, 2003). More precise estimation will require a combination of conventional methodologies, such as calculations of the condition factor, the gonadosomatic index (GSI) and the hepatosomatic index (HSI) and direct SCUBA observations.

Our observation of stage II eggs on January 25 may indicate that the care of eggs by male fish continues through the beginning of February. The closed season for *H. agrammus* harvesting should include the entire period of parental care after spawning.

Chung and Kim (1994) analyzed annual changes in the average GSI and HSI for 398 *H. agrammus* individuals captured intertidally along the Busan coast, 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, reaching a maximum of 4.31 in November. The index decreased sharply to 1.54 in December and to a very low value of 0.46 in January. Changes in the HSI tracked a similar pattern. These peaks in GSI and HSI and the large numbers of spawning grounds and egg masses observed in November in the present study indicated that this was the month of peak spawning. The low GSI value measured by Chung and Kim (1994) in January was similar to those measured in non-spawning months; however, we found three spawning grounds and 15 egg masses in January. Although our counts in January were lower than those at the peak in November (5 spawning grounds and 18 egg masses), we were able to confirm that spawning continued into January. Furthermore, 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 for stages II and IV, respectively) on January 25 indicates that the spawning of *H. agrammus* continued until mid-January.

*H. agrammus* spawns over rocky seafloors where several seaweed species are attached to the bedrock. Water depths in the spawning grounds were in the range of 1.2–4.0 m (mean: 2.6 m) (Fig. 1A, 1B; Table 1). *H. agrammus* uses various seaweeds as spawning substrata. We found 2–7 egg masses...
(mean: 4) in the diameter range of 2–5 cm attached to seaweed holdfasts, where they were well hidden and difficult to find (Fig. 1B–1H; Table 1). Kanamoto (1976) also noted that the egg masses of *H. agrammus* are well-camouflaged. The egg masses we found were sometimes regularly spaced, in agreement with Munehara et al. (2000), who reported that an average of seven egg masses were spawned together on seaweeds in shallow water. Fourteen species of seaweeds were present in the spawning grounds and adjacent areas that we evaluated: *Undaria pinnatifida, Codium fragile, Caulerpa okamurae, Ulva pertusa, Ahnfeltiopsis flabelliformis, Lomentaria catenata, Prionitis cornea, Rhodymenia intricata, Callophyllis adnata, Acrosorium polyneurum, Gelidium amansii, Grateloupia filicina, Grateloupia turuturu, and Plocamium telfairiae*. Of these, 10 species were members of the Rhodophyta. The most common algae used as attachment substrata were *Prionitis cornea* (72%) and *Caulerpa okamurae* (18%). *Ahnfeltiopsis flabelliformis, Grateloupia turuturu, Lomentaria catenata,* and *Gelidium amansii* were also commonly used (Fig. 1C–1H; Table 1). Kanamoto (1976) reported that *H. agrammus* spawns
Table 1. Characteristics of spawning grounds, the guardian male, and egg masses of *Hexagrammos agrammus*

<table>
<thead>
<tr>
<th>Station</th>
<th>Date</th>
<th>Depth (m)</th>
<th>Temp. (°C)</th>
<th>Guardian male</th>
<th>Egg mass</th>
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<tr>
<td>1</td>
<td>Nov 10</td>
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<td>18</td>
<td>20-25</td>
<td>Red</td>
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<td>2</td>
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<td>3</td>
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<td>1.2</td>
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<td>Brown</td>
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<td>4</td>
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**Environmental characteristics**

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**Guardian male**

- Guard the eggs at the lower part of them on the rock
- Well camouflaged
- Nuptial coloration: Red
- Aggressive activity: ○
- Holdfast of algae on the rocks

**Egg mass**

- Holdfast of algae in Crevices
- Holdfast of algae at the peak of large rocky area with good current circulation
- Holdfast of algae on the rocks
- Holdfast of algae on the large rocky area with good current circulation
- Holdfast of algae on the rocks
- Holdfast of algae on the rocks
- Holdfast of algae on the rocks
- Holdfast of algae on the rocks
- Holdfast of algae on the rocks
- Holdfast of algae on the rocks

**Location**

- Undaria pinnatifida
- Ahnfeltiopsis flabelliformis
- Lomentaria catenata
- Prionitis cornea
- Codium fragile
- Rhodymenia intricata
- Prionitis cornea
- Callophyllis adnata
- Acrosorium polyneurum
- Gelidium amansii
- Prionitis cornea
- Grateloupea filicina
- Gelidium amansii
- Codium fragile
- Infantaria catenata
- Prionitis cornea
- Caulerpa okamurae
- Gelidium amansii
- Grateloupea filicina
- Gelidium amansii
- Grateloupea turuturu
- Caulerpa okamurae
- Gelidium amansii
- Infantaria catenata
- Prionitis cornea
- Caulerpa okamurae
- Grateloupea turuturu
- Grateloupea turuturu
- Gelidium amansii
- Acrosorium polyneurum
- Callophyllis adnata
- Grateloupea turuturu

**Algae around egg clusters**

- *Undaria pinnatifida*
- *Ahnfeltiopsis flabelliformis*
- *Lomentaria catenata*
- *Prionitis cornea*
- *Codium fragile*
- *Rhodymenia intricata*
- *Prionitis cornea*
- *Callophyllis adnata*
- *Acrosorium polyneurum*
- *Gelidium amansii*
- *Prionitis cornea*
- *Grateloupea filicina*
- *Gelidium amansii*
- *Codium fragile*
- *Infantaria catenata*
- *Prionitis cornea*
- *Caulerpa okamurae*
- *Gelidium amansii*
- *Grateloupea filicina*
- *Prionitis cornea*
- *Caulerpa okamurae*
- *Gelidium amansii*
- *Infantaria catenata*
- *Prionitis cornea*
- *Caulerpa okamurae*
- *Gelidium amansii*
- *Grateloupea turuturu*
- *Caulerpa okamurae*
- *Gelidium amansii*
- *Infantaria catenata*
- *Prionitis cornea*
- *Caulerpa okamurae*
- *Gelidium amansii*
- *Grateloupea turuturu*

*Algae used as spawning substrate.*
mainly on *Gelidium amansii*, but that was not the case in the present study. Thus, the fish may not have fixed preferences, other than an obvious tendency to use red algae.

Lee et al. (2013) reported that males of *H. otakii* on the southern coast of Korea do not vigorously protect or guard their fertilized eggs; when divers approached the eggs, males often hovered 2–3 m away from the egg masses. In some cases, they (*loc. Cit.*) observed no guarding males. In contrast, males of *H. agrammus* in western Korea (Lee et al., 2013) and at the site we evaluated exhibited aggressive behaviors when divers approached within 1 m of the eggs. Kanamoto (1976) also observed differences in protective behavior between the species. Thus, *H. agrammus* males generally provide stronger protection of fertilized eggs than do males of *H. otakii*. Spawning on seaweed holdfasts, where the zygotes and embryos were well hidden and color-camouflaged, provided an additional level of protection for *H. agrammus* eggs along the western and southern Korean coasts. In contrast, *H. otakii* spawns on exposed bedrock. The differences in breeding territory between the two species may be related to differences in the level of protection they provide of fertilized eggs. Nuptial coloration of male *H. agrammus* during the spawning season provided excellent camouflage; while hiding among the fronds of nearby seaweeds or areas or bedrock, they were able to change their color to match their surroundings (Fig. 1A, 1B; Table 1). Munehara et al. (2000) reported that the color of the ventral fins of male *H. agrammus* changed to black. We noted slightly different changes in coloration, which may have been a reflection of differences between environments.

Kurita et al. (1995) reported that 78% of male *H. agrammus* individuals > 1 year of age protected fertilized eggs in December, and all males provided protection in January. However, male *H. agrammus* < 1 year of age mated randomly and did not protect their eggs. In this study, we found fertilized eggs in the spawning grounds on January 4, 2014, but no male fish were guarding these eggs. We surmised that males < 1 year of age had mated on this occasion.

The data we collected by direct observation during SCUBA diving should contribute to the reduction of damage to stocks caused by fishing during the reproductive season. Our methods had minimal environmental impact but nevertheless provided crucial data for setting closed season dates for specific fish populations.

**Acknowledgments**

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**References**


