

# Underwater Observations of Spawning of *Hexagrammos agrammus* off the Tongyeong Coast, Korea

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## Abstract

We observed seasonality and other characteristics of spawning in the greenling *Hexagrammos agrammus* off the coast of Tongyeong. Eleven spawning grounds were identified between November, 2013 and January, 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, algal spawning substrate, 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 reproductive season to determine the spawning period; we examined regional differences in the spawning traits and habitats of the species.

## Materials and Methods

We investigated the spawning grounds of *H. agrammus* in coastal waters off Tongyeong (128°26' E, 34°49' N) 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  $\leq 5$  m apart. Each diver scanned a 6-m wide track that was 250 m long (total area scanned: 1,500 m<sup>2</sup>). 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 a dive computer (ZOOP; SUUNTO, Vantaa, Finland) 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 (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.

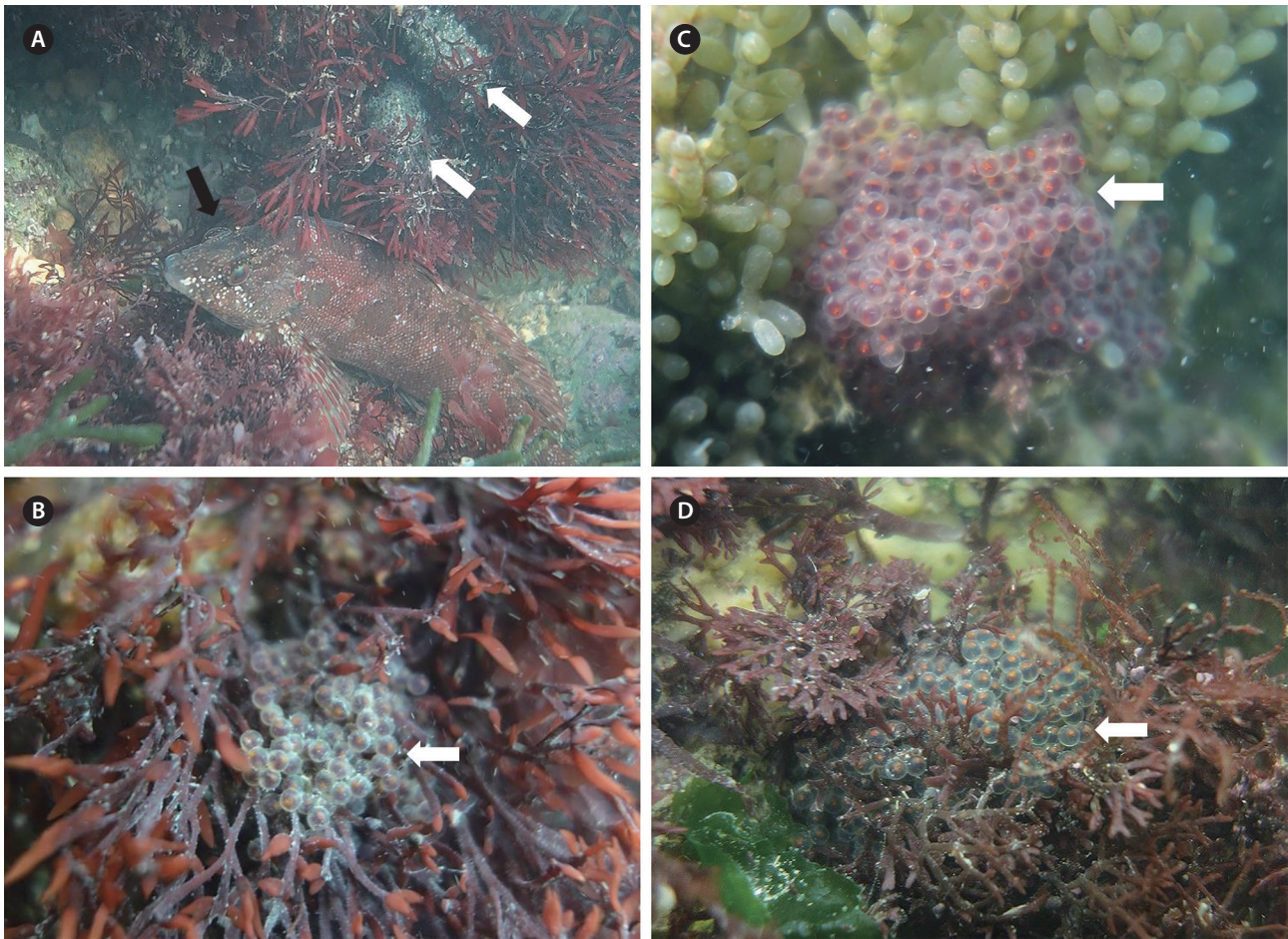
We identified 11 spawning grounds between November and January (five in November, three in December, three in January; Table 1), but found no egg masses in October or February. A stage IV egg mass fertilized  $\geq 20$  days previously was found on November 10, suggesting an October 20 spawning date. 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; Table 1). *H. agrammus* uses various seaweeds as spawning substrata. We found 2–7 egg masses (mean: 4) in





**Fig. 1.** A, *Hexagrammos agrammus* in nuptial coloration is taking care of the two egg masses (white arrows) on *Prionitis cornea*; B, the egg masses on *Prionitis cornea*; C, the egg masses on *Caulerpa okamurae*; D, the egg masses on *Ahnfeltiopsis flabelliformis*.

the diameter range of 2–5 cm attached to seaweed holdfasts, where they were well hidden and difficult to find (Fig. 1A–1D; 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. 1B–1D; Table 1). Kanamoto (1976) reported that *H. agrammus* spawns

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

**Table 1.** Characteristics of spawning grounds, the guardian male, and egg masses of *Hexagrammos agrammus*

| Station | Date   | Environmental characteristics |            |                   | Guardian male   |                    |                     | Egg mass |                      |  |  |
|---------|--------|-------------------------------|------------|-------------------|---|--------------------|---------------------|----------|----------------------|--|--|
|         |        | Depth (m)                     | Temp. (°C) | Total length (cm) | Response to divers  | Nuptial coloration | Aggressive activity | Number   | Stages               | Location   | Algae around egg clusters  |
| 1       | Nov 10 | 3                             | 18         | 20-25             | - Guard the eggs at the lower part of them on the rock<br>- Well camouflaged  | Red                | O                   | 4        | I<br>II<br>III<br>IV | - Holdfast of algae on the rocks   | <i>Undaria pinnatifida</i><br><i>Ahnfeltiopsis flabelliformis</i> *<br><i>Lomentaria catenata</i><br><i>Prionitis cornea</i> *<br><i>Codium fragile</i><br><i>Rhodymenia intricata</i> |
| 2       | Nov 10 | 2.7                           | 18         | 20-22             | - Keep an eye on the diver at a crevice in the rock<br>- Well camouflaged   | Red                | X                   | 3        | II<br>III            | - Holdfast of algae in Crevices  | <i>Prionitis cornea</i> *<br><i>Callophyllis adnata</i><br><i>Acrosorium polyneurum</i><br><i>Gelidium amansii</i>   |
| 3       | Nov 17 | 1.2                           | 18         | 25-27             | - Keep an eye on the diver at the pothole in the rocky area<br>- Distinguishable body color                                       | Brown              | X                   | 3        | II<br>I<br>IV        | - Holdfast of algae at the peak of large rocky area with good current circulation                                  | <i>Lomentaria catenata</i><br><i>Acrosorium polyneurum</i><br><i>Caulerpa okamurae</i> *   |
| 4       | Nov 23 | 3                             | 13         | 18-20             | - Keep an eye on the diver at a ghost net on the rocky area<br>- Run away when diver come closer                                  | Red                | X                   | 6        | I<br>II<br>III       | - Holdfast of algae on the rocks<br>- 50cm distance between 5 of egg masses and 1 of egg mass                      | <i>Prionitis cornea</i> *<br><i>Grateloupia filicina</i><br><i>Gelidium amansii</i><br><i>Codium fragile</i>   |
| 5       | Nov 30 | 3                             | 13         | 20-22             | - Keep an eye on the diver at a crevice in the rock<br>- Distinguishable body color<br>- The fishing line was sawing in its mouth | Brown              | O                   | 3        | I<br>II<br>IV        | - Holdfast of algae on the rock  | <i>Ahnfeltiopsis flabelliformis</i><br><i>Prionitis cornea</i> *<br><i>Acrosorium polyneurum</i><br><i>Lomentaria catenata</i>   |
| 6       | Dec 08 | 2.5                           | 12         | 15-17             | - Keep an eye on the diver at the pothole in the rocky area<br>- Distinguishable body color                                       | Brown              | X                   | 6        | I<br>II<br>III<br>IV | - Holdfast of algae on the large rocky area with good current circulation<br>- Regular distance between egg masses | <i>Grateloupia filicina</i> *<br><i>Prionitis cornea</i> *<br><i>Caulerpa okamurae</i> *<br><i>Gelidium amansii</i>  |
| 7       | Dec 15 | 2.3                           | 10         | 20-23             | - Guard the eggs at the lower part of them on the rocks<br>- Well camouflaged   | Red                | X                   | 2        | I<br>III             | - Holdfast of algae on the rocks   | <i>Ahnfeltiopsis flabelliformis</i><br><i>Lomentaria catenata</i><br><i>Prionitis cornea</i> *<br><i>Ulva pertusa</i><br><i>Grateloupia filicina</i><br><i>Plocamium telfairiae</i>    |
| 8       | Dec 15 | 2.3                           | 10         | 20-23             | - Guard the eggs at the lower part of them on the rock<br>- Well camouflaged  | Red                | X                   | 3        | I<br>III             | - Holdfast of algae on the rocks   | <i>Ahnfeltiopsis flabelliformis</i><br><i>Lomentaria catenata</i><br><i>Prionitis cornea</i> *<br><i>Rhodymenia intricata</i><br><i>Grateloupia turuturu</i>                           |
| 9       | Jan 04 | 4                             | 8          | -                 | - No male fish found  | -                  | X                   | 4        | I<br>II<br>IV        | - Holdfast of algae on the rocks   | <i>Lomentaria catenata</i><br><i>Prionitis cornea</i> *<br><i>Caulerpa okamurae</i>  |
| 10      | Jan 25 | 2.5                           | 7          | 17-20             | - Guard the eggs at the lower part of them on the rock<br>- Well camouflaged  | Red                | X                   | 4        | II<br>IV             | - Holdfast of the algae on the big or small rocky area with pebbles  | <i>Gelidium amansii</i> *<br><i>Acrosorium polyneurum</i><br><i>Callophyllis adnata</i>  |
| 11      | Jan 25 | 2                             | 8          | 20                | - Guard the eggs at the lower part of them on the rock<br>- Well camouflaged  | Red                | X                   | 7        | II<br>IV             | - Holdfast of the algae on the big or small rocky area with pebbles  | <i>Lomentaria catenata</i> *<br><i>Acrosorium polyneurum</i><br><i>Callophyllis adnata</i><br><i>Grateloupia turuturu</i>  |

\*Algae used as spawning substrate.

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 of bedrock, they were able to change their color to match their surroundings (Fig. 1A; 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

This work was supported by the Gyeongsang National University Fund for Professors on Sabbatical Leave, 2014.

## References

- Chung EY and Kim SY. 1994. On the maturity and spawning of the greenling, *Hexagrammos agrammus* (Temminck et Schlegel). *Kor J Ichthyol* 6, 222-236.
- Chyung MK. 1977. The Fishes of Korea. Iljisa, Seoul, Korea. pp 727.
- Edgar GJ, Barrett NS and Morton AJ. 2004. Biases associated with the use of underwater visual census techniques to quantify the density and size-structure of fish populations. *J Exp Mar Biol Ecol* 308, 269-290.
- Fukuhara O. 1971. On the egg development and larval stage of *Agrammus agrammus*. *Aquacul Sci* 19, 159-165.
- Kanamoto Z. 1976. On the ecology of Hexagrammidae fish. 1) Habitats and behaviors of *Agrammus agrammus* (Temminck et Schlegel) and *Hexagrammos otakii* (Jordan et Starks). *Jpn J Ecol* 26, 1-12.
- Kim IS, Choi Y, Lee CL, Lee YJ, Kim BJ and Kim JH. 2005. Illustrated book of Korean fishes. Kyo-Hak Pub Co, Seoul, Korea. pp 615.
- Kim MS. 2003. Ecology of Fishes. Academy Pub Co, Seoul, Korea. pp 386.
- Kimura M and Munehara H. 2010. The disruption of habitat isolation among three *Hexagrammos* species by artificial habitat alterations that create mosaic-habitat. *Ecol Res* 25, 41-50.
- Kulbicki M, Sarra-megna S, Letourneur Y, Wantiez L, Galzin R, Mou-Tham G, Chauvete C and Thollot P. 2007. Opening of an MPA to fishing: natural variations in the structure of a coral reef fish assemblage obscure changes due to fishing. *J Exp Mar Biol Ecol* 353, 145-163.
- Kurita Y, Sano M and Shimizu M. 1995. Reproductive cycle and body size and age at maturity of the hexagrammid fish *Hexagrammos agrammus* in central Japan. *Fish Sci* 61, 1-6.
- Lee YP and Kang SY. 2002. A Catalogue of the Seaweeds in Korea. Cheju National University Press, Jeju, Korea, 662.
- Lee YD, Kim JS, Jung JH, Shim WJ and Gwak WS. 2013. Direct observations of spawning characteristics on the Hexagrammidae fishes in Korean coastal waters using SCUBA diving. *J Kor Soc Oceanogr* 18, 1-6.
- Munehara H, Kanamoto Z and Miura T. 2000. Spawning behavior and interspecific breeding in three Japanese greenlings (Hexagrammidae). *Ichthyol Res* 47, 287-292.
- Ochiai A and Tanaka M. 1998. Ichthyology (Part II). Koseisha-koseikaku Tokyo. pp 1139.