Journal of the Korea Academia-Industrial cooperation Society
Vol. 18, No. 1 pp. 49-54, 2017

https://doi.org/10.5762/KAIS.2017.18.1.49
ISSN 1975-4701 / eISSN 2288-4688

Naturally Collection and Development until Yolk Absorption of Domestic Walleye Pollock *Theragra chalcogramma* Fertilized Eggs and Larvae

Seo Joo-young[1], O-Nam Kwon[2*]
[1]Gangwon-Do Deep Ocean Water Fisheries Resources Center, [2]GABI Ltd. Co.

국내 명태 *Theragra chalcogramma* 자연채란과 난황흡수까지의 난 발생

서주영[1], 권오남[2*]
[1]강원도 해양심층수수산자원센터, [2]강원양식생물연구소(주)

Abstract We collected and reared Theragra chalcogramma walleye pollock brood-stock for use in natural spawning tests and undertook to obtain domestic pollock via fertilized egg capture, development of fertilized eggs, and absorption of yolk sac after hatching. Whole pollock were caught with trammel and set nets and immediately placed in a deep-sea water tank. Adults were the most common pollock age group (43.0%; n = 86) among the 254 pollock captured in March 2014 with 57.9% (n = 147) being captured off Southern Gosung, Korea. The main spawning period of pollock is February (spawning phase of 91% of pollock). From the deep-sea tank, we collected 1640 mL of naturally fertilized eggs (~820,000 eggs) from 12 spawning events occurring between February 4 and 22 2015. The floating/ live eggs were maintained in deep-sea water tanks at 5.5 ± 0.2℃. Egg size was 1.5 ± 0.03 mm. Six hours after fertilization the eggs were at the 2 cell stage, and the eggs hatched approximately 340 hours after collection. At hatching, larval length and yolk sac area were 5.2 ± 0.25 mm and 9.5 ± 1.00 mm2 (100%), respectively. Four days after hatching, the yolk sac area was 2.2 ± 0.53 mm2 (23.1 ± 5.55%). This is the first report of collection of naturally fertilized eggs from pollock and their subsequent hatching while held in an indoor deep-sea water tank. The results suggest that such collection could assist in the recovery of pollock resources and the possibility of domestic rearing of cultivated larvae.

요 약 우리는 자연에서 포획된 명태 어미를 이용하여 자연채란 시험을 하였으며, 이 과정에서 국내 명태 포획과 수정란의 발생 및 부화 후 난황흡수에 대한 정보를 얻는 것이 목적이다. 모든 명태는 삼중자망과 정치망에서 잡혀 당일 해양심층수 수조에 수용하였다. 이 어미 명태는 총 254마리에서 43.0% (86마리)가 2014년 3월에, 57.9%(147 마리)가 고성 남부지역에서 포획되었다. 그리고 주 산란기는 2월이었지만 (91.0% 개체들이 산란기), 6월까지 지속되었다. 포획 후 2015년 2월 4일에서 22일 사이에 12번의 자연채란으로부터 1,640 mL (~820,000개)의 수정란을 확보하였다. 자연채란된 수정란에서 14% (~115,000개)는 발달하지 않고 폐사하였다. 이 살아있던 부유란들은 5.5±0.2℃의 해양심층수에서 사육 관리하였다. 이들의 크기는 1.5±0.03 mm였다. 자연채취 후 6시간이 경과한 수정란은 2세포기, 24시간 경과 후에는 포배기 단계로 발생하였다. 그리고 자연채란 후 340 시간 경과 시 전장 5.6±0.21 mm로 부화하였다. 이들 부화한 자어 길이와 난황의 면적은 5.2±0.25 mm와 9.5±1.00 mm^2였다. 그리고 4일이 경과하였을 때 난황은 2.2±0.53 mm^2 (부화 당시 대비 23.1±5.55%)로 조사되었다. 결과적으로 우리의 연구결과는 국내에서 첫 명태 자연채란 사례로 해양심층수 수조에서 이루어졌다는 것이 매우 의의가 있다고 할 수 있다. 그리고 이를 통한 인공종묘생산과 자원회복의 가능성을 확인하였다고 할 수 있다.

Keywords : Deep-sea Water, Natural Egg Collection, Seedlings, *Theragra chalcogramma*, Walleye pollock

This research was a part of the project titled " Development of seedlings and releasing technology of Eastern coastal walleye pollock" funded by the Ministry of Oceans and Fisheries, Korea.
*Corresponding Author : O-Nam Kwon (GABI Ltd. Co.)
Tel: +82-33-610-2038 email: onamkwon@yahoo.com
Received September 5, 2016 Revised (1st October 11, 2016, 2nd November 11, 2016)
Accepted January 6, 2017 Published January 31, 2017

1. Introduction

The annual global harvest of pollock *Theragra chalcogramma* is the second highest of all fish species and have shown continuously increasing catches from 2003 to 2012[1]. But a total of 1.7 million tons were harvested in the Bering Sea in 1987, and since then the catch has declined [2]. At that time, only 0.16 million tons were harvested in the early 1980s in Korea, though Nogari (young pollock) and pollock were classified as different species in the 1980s [3]. Based on the distribution of their meristic characters, Kim and Huh [4] concluded that the two were the same species, emphasizing the necessity of resource protection. In Gangwon Province in South Korea, the annual harvest of pollock was 1,500 tons in 2000 [5]. By 2013, the annual harvest had declined dramatically to 0.5 tons. The decline was thought to be caused by overharvest, by-catch, and habitat degradation as a result of climate change and pollution [6]. Some studies have suggest overfishing as the main reason [3]. However, there are few data to point towards any of these causes as the primary spawning ground is located in Wonsan in North Korea [3]. In 2014, there was a complete ban on the capture of live Korean pollock.

The pollock are a multi-spawning species, as are other members of the Gadidae family, including cold-water haddock *Melanogrammus aeglefinus* and whiting *Merlangius merlangus* [7,8], and warm-water fishes such as dragonet *Callionymus enneactis*, *Hemiramphus brasiliensis*, *H. balao* [9], Mediterranean sardine *Sardina pilchardus* [10]. Seedlings of pollock is most commonly carried out by obtaining fertilized eggs from naturally spawning rather than batch egg collection using the abdominal pressure method. Northern anchovy spawn daily at 28 - 30°C [11] and are fertile for up to 30 d [12]. Pollock commonly spawn at 5°C and have a longer spawning interval or total spawning period than northern anchovy. For the first time in Korea, this paper has examined the pollock's egg collection process through the naturally spawning for the pollock which were caught in Feb 2013 ~ Jan 2014 and bred at the Fisheries Resource Center for Deep-sea Water of Gangwon province. Also the purposes of this study were confirmed the informations of the domestic pollock capture, development of fertilized eggs and absorption of yolk sac after hatching eggs on the seedlings.

2. Materials and Methods

2.1 Adult pollock collection

The adult fish for the natural spawning were selected from Korean pollock caught during the period February 2014 - January 2015 in Goseong and Donghae, Gangwon Province. The fish were caught using stationary nets and gill nets and transferred live to the Fisheries Resource Center for Deep-Sea Water in Gangwon Province. We used three in dividuals that were caught in 2014 (two males, one female; 45-60 cm total length [TL] ; 450 - 600 g body weight [BW]) and one individual that was captured in January 2015 (female; 55 cm TL; 650 g BW). The breeding tank was 6 m in diameter and 2 m depth, and was filled with deep-seawater (2 - 5°C). Their fish were fed squid *Todarodes pacificus* and sand lance *Ammodytes personatus* that were cut into pieces. A net (40 natural mesh size 300 μm) was installed in January 2015 at the drain outlet of the breeding tank to collect eggs following spawning (Fig. 1).

2.2 Natural fertilization in tank

Every morning, the egg-collecting net was checked the presence of fertilized eggs and floating and fertilized eggs were collected. The eggs were kept to divide in the surface water and the deep-sea water.

Before being maintain in the tank, the eggs were classified as dead eggs or normally fertilized eggs in a 1L mess-cylinder 2 -3 times.

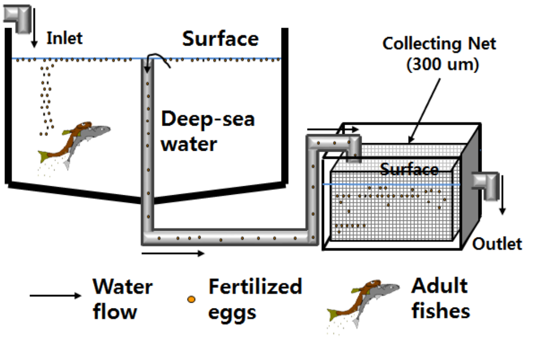

Fig. 1. Schematics of the system for collecting naturally fertilized eggs of Korean pollock, *Theragra chalcogramma* at the Fisheries Resource Center for Deep-Sea Water in Gangwon Province.

2.3 Pollock egg development

The fertilized eggs were transferred to the Marine Biology Center for Research and Education at Gangneung-Wonju National University, the East Sea Fisheries Research Institute of the National Fisheries Research Development Institute, and the Fisheries Resource Center for Deep-Sea Water in Gangwon Province.

And their natural spawned fertilized eggs was confirmed a procedure up to hatching and yolk sac area through management in 5.5±0.2℃ of marine deep-sea water in Gosung, Gangwon province.

3. Results

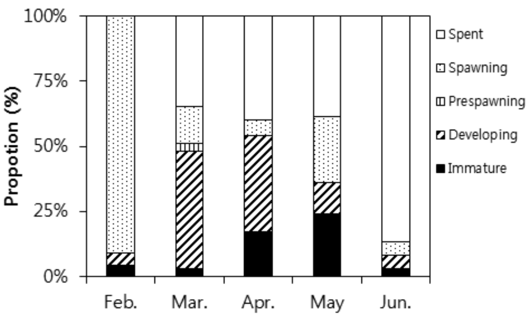

Fig. 2. Maturity condition (%) of Korean coastal walleye pollock *Theragra chalcogramma* female based on macroscopic examination.

Table 1. Number of catching (individuals) and proportion (%) of Korean coastal walleye pollock *Theragra chalcogramma* caught in Gangwon province at February to June 2014

	February	March	Apral	May	June	Sub sum
NG	2 (4.8)	4 (4.7)	1* (2.1)	2 (4.9)	21 (56.8)	30 (11.8)
SG	34* (81.0)	53 (61.6)	25 (52.1)	25* (61.0)	10 (27.0)	147 (57.9)
SC	6 (14.3)	27* (31.4)	11 (22.9)	10 (24.4)	5 (13.5)	59 (23.2)
YY	-	1 (1.2)	8 (16.7)	-	1 (2.7)	10 (3.9)
GN	-	1* (1.2)	2 (4.2)	4 (9.8)	-	7 (2.8)
DS	-	-	1 (2.1)	-	-	1 (0.4)
Sub sum	42 (21.0)	86 (43.0)	48 (24.0)	41 (20.5)	37 (18.5)	254 (100)

Caught pollock were not separated with female or male.
"*"indicated a month caught live pollock on a fixed shore net in each region.
Abbreviations (NG, SG, SC, YY, GN and DS) were indicated Northern Gosung, Southern Gosung, Sokcho, Yangyang, Gangneung and Donghae/Samchuck, respectively. Number in parenthesis indicated percentages of whole caught pollock in a month, but subsum was ratio of caught pollock in whole period.

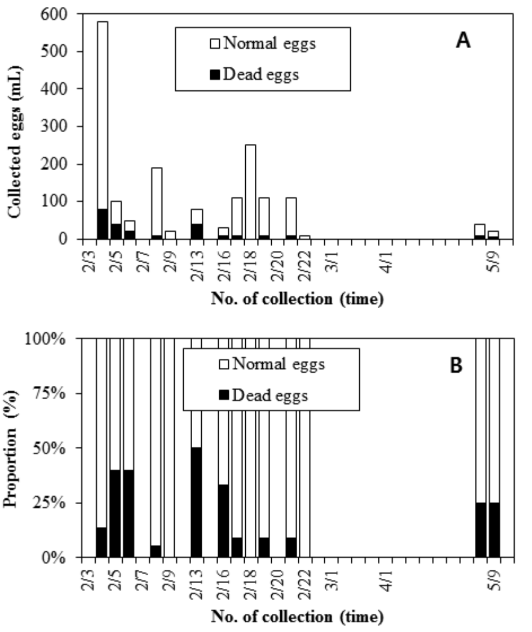

Fig. 3. Proportion (%) of fertilized (normal) eggs and dead eggs (mL) collected daily following natural spawning events in Korean coastal walleye pollock, *Theragra chalcogramma* on indoor tank. A and B were quantities (mL) and proportion (%) of collected eggs.

3.1 Adult pollock collection

We collected naturally fertilized pollock eggs on 12 occasions during February 2015. The fertilized eggs were separated from the dead or unfertilized eggs in a measuring cylinder based on differences in buoyancy (Fig. 2). Collected pollock caught mainly SG and SC. Also the pollock caught on March, especially. And the pollock in March at SG occupied to 20.9% out of total 254 pollocks (Table 1).

3.2 Natural fertilization in tank

On February 4, 2015, 580 mL of eggs were collected, of which 90 mL were dead. The fertilized eggs were 1.56 mm in diameter and we calculated there were 50,000 eggs per 100 mL. Spawning and fertilization occurred irregularly at 1 - 4 d intervals. A total of 1,640 mL of fertilized eggs were collected over a 19 d period. From those, we obtained 1,410 mL of eggs (705,000 eggs)(Fig. 3).

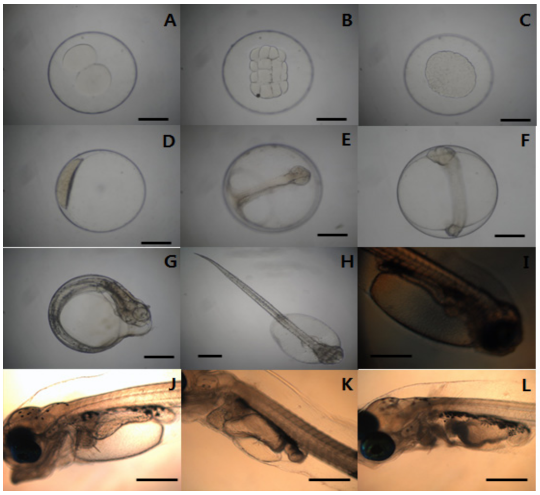

Fig. 4. Photographs of preserved Korean coastal walleye pollock *Theragra chalcogramma* eggs and larvae. (A) 2 cell (stage 2) ; (B) 16 cell (stage 5) ; (C) 32 cell (stage 6) ; (D) Blastodermal cap (stage 7); (E) germ ring (stage 13); (F) tail around yolk (stage 17) ; (G) Hatching egg; (H) Hatched larva; (I) larva of 0 day after hatching ; (J) larva of 2 day after hatching; (K) larva of 4 day after hatching; (L) larva of 6 day after hatching. The stages determined by Blood et al. (1994).

3.3 Pollock egg development

The development of the fertilized eggs showed in Fig. 4 and Table 2. The eggs became to 2 cells stage into 6 hours after natural spawning, and developed blastodernmal cap stage after 24 hours after the spawning. And then the eggs became up to germ ring stage into 110 hours after natural spawning, and developed up to around yolk stage into 175 hour after natural spawning. And it finally hatched after 340 hours after natural spawning. Hatched larval length was 5.2±0.25 mm. And the area (mm^2) of yolk sac after hatching showed in Table 2. The area was 9.5±1.00 mm^2 (100%) at just hatching , and then decrease until 2.2±0.53 mm^2 (23.1+5.55%) in forth days after hatching. Finally, that of sixth days diminished until 0.3±0.27 mm^2(3.2±0.64%) (Table 3).

4. Conclusion

Walleye Pollock, *Theragra chalcogramma* are subject to high harvest pressure through the world and are known as the "people's fish" in Korea.

However, the annual harvest has declined dramatically since 2000, triggering the need for conservation measures. So it is necessary to study for the recovery of pollock domestic resources. Pollock are multi spawning species during a spawning period. Northern anchovy *Engmulis mordar* spawn up to 20 times during their breeding period and can fertilize eggs 19 - 28 h after a prior spawning event [13]. Similarly, the dragonet *Callionymus enneactis* can spawn daily when water temperature is 28 - 30°C.

Postovulatory follicles are not present 15 h after spawning and are only clearly distinguishable for up to 3 h after spawning [11]. This state persists for ~30 d, and may last much longer under natural conditions, during which time small oocytes are resorbed. Because pollock live at depths of 200 - 1,200 m and 2 - 4°C it is hypothesized that they may spawn multiple times over a longer period than the species described above.

For species that spawn multiple times in a season, fertilized eggs should be collected using the natural spawning method, as used in this study, rather than the abdominal pressure method used by most culturists. We successfully reared four fish (female: male = 1: 1) until breeding age and obtained ~ 700,000 normally fertilized eggs from 12 spawning events. Expansion of artificial rearing may provide options for supplementing wild production of the severely depleted Korean pollock stocks.

The development procedure of walleye pollock, *T. chalcogramma* eggs were concretely expressed in Blood et al. [14]. And in Japan [15,16], Alaska [17,18] was carried out eggs development and hatching of pollock's fertilized eggs related with temperature.

Table 2. Midpoint in hours (h) of stage of development of Korean coastal walleye pollock *Theragra chalcogramma* eggs and hatched larvae incubated at 5.5±0.2 ℃*

Marks of Fig. 3	no. of Stage due to Blood et al. [3]	Developmental stage	Elapsed time (hour)
A	2	2 cell	6 hr
B	5	16 cell	11 hr
C	6	32 cell	23 hr
D	7	Blastodermal cap	46 hr
E	10	Middle germ ring	110 hr
F	17	Early tail around yolk	175 hr
G	21	Late tailaround yolk	340 hr
H		Hatch	

* The stages determined by Blood et al. (1994)

Table 3. Variation of yolk sac area (mm^2) after hatch of Korean coastal walleye pollock *Theragra chalcogramma* larvae incubated at 5.5±0.2 ℃*

Day after hatch	0	2	4	6
Yolk sac area (mm^2) (%)	9.5±1.00 (100)	5.8±0.21 (60.9±2.21)	2.2±0.53 (23.1±5.55)	0.3±0.27 (3.2±0.64)

* Parenthesis is percentage (%) of yolk sac area compared with yolk sac of just hatched pollock larvae.

The tendency was similar with our results as size, developmental shape and hatching time and so on. But they could not suggest relating with first feeding of diet, yolk absorption and so on, because the researches were just resources estimation and management of natural resources. Our result could suggest that first feed supply after forth days after hatch, because of enough diminishment/ absorption of yolk sac areas. Therefore we could confirmed the possible chances for resource recovery of domestics pollock and possibility for artificial seedlings in domestics.

5. Acknowledgments

This research was a part of the project titled "Development of seedlings and releasing technology of Eastern coastal walleye pollock" funded by the Ministry of Ocean and Fisheries, Republic of Korea.

References

[1] FAO. "The status of world fisheries and aquaculture" p 223, Rome, Italy. 2014.

[2] K. M. Bailey, "An empty donut hole: the great collapse of a North American fishery", Fisheries Society, vol. 16, no. 2, article 28, 2011.

[3] S. Kang, J. H. Park, S. Kim, "Size-class estimation of the number of walleye pollock *Theragra chalcogramma* caught in the southwestern East Sea during the 1970s-1990s", Korean Journal of Fisheries Aquatic Science, vol. 46, pp. 445-453, 2013.
DOI : http://dx.doi.org/10.5657/KFAS.2013.0445

[4] W. S. Kim, S. H. Huh, "Meristic and morphometric observation on Nogari and Alaska pollock", Journal of the Oceanological Society of Korean, vol. 13, pp. 26-30, 1978.

[5] East sea Fisheries Research Institute (EFRI). "Report of the oceanographic observation and fishing condition in the East Sea of Korea in winter", 2000. p. 58, East Sea Regional FRI Oceanography and Marine Fisheries Resources News 32., Gangneung, Republic of Korea. 2000.

[6] Y. Lee, D. Y. Kim, "Measuring surface water temperature effects on the walleye pollock fishery production using a translog cost function approach", Environmental and Resource Economics Review, vol. 19, pp. 897-914, 2010.

[7] C, Loots, S. Vaz, B. Planque, P. Koubbi. "Understanding what controls the spawning distribution of North Sea whiting (*Merlangius merlangus*) using a multi-model approach" Fisheries Oceanography, vol. 20, no. 1, 18-31, 2011.
DOI: http://dx.doi.org/10.1111/j.1365-2419.2010.00564.x

[8] I. R. G. Hislop, A. P. Robb, J. A. Auld, "Observations on effects of feeding level on growth and reproduction in had dock. *Melunogrummus ueglefinus* (L.) in captivity", Journal of Fish Biology, vol. 13, pp. 85-98, 1978.
DOI: https://doi.org/10.1111/j.1095-8649.1978.tb03416.x

[9] R. S. McBride, P. E. Thurman, "Reproductive biology of *Hemiramphus brasiliensis* and *H. balao* (Hemiramphidae): Maturation, spawning frequency, and fecundity", Biology Bulletin, vol. 204, pp. 57-67, 2003.
DOI: https://doi.org/10.2307/1543496

[10] K. Ganias, "Ephemeral spawning aggregations in the Mediterranea sardine, *Sardina pilchardus*: a comparison with other multiple-spawning clupeoids" Marine Biology, Vol, 155, pp. 293-301, 2008.
DOI: http://dx.doi.org/10.1007/s00227-008-1027-7

[11] T. Takita, T. Iwami, S. Kai, I. Sogabe, "Maturation and spawning of the dragonet, *Cullionymus enneuctis*. in an aquarium", Japanes Journal of Ichthyology, vol. 30, pp. 221-226, 1983.

[12] J. R. Hunter, B. J. Macewicz, "Measurement of spawning frequency in multiple spawning fishes. In: An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy (*Engraulis mordax*)",R. Lasker, ed. U.S. Department of Commerce, National Oceanic and Atmospheric Administration Technical Report National Marine Fisheries Service, Washington DC, U.S.A. vol. 36, pp. 79 - 94, 1985.

[13] J. R. Hunter, R. Leong, "The spawning energetics of female northern anchovy, *Engruulis mordar*", Fishery Bulletin, vol. 79, pp. 215-230, 1981.

[14] D. M. Blood, A. C. Matarese, M. M. Yoklavich, "Embryonic development of walleye pollock, *Theragra chalcogramma*, from Shelikof Strait, Gulf of Alaska", Fishery Bulletin, vol. 92, pp. 207-222, 1994.

[15] T. Nakatani, T. Maeda, "Thermal effect on the development of walleye pollock eggs and their upward speed to the surface", Bulletin of Japanese Society of Scientific Fisheries, vol. 50, pp. 937-942, 1984.
DOI: https://doi.org/10.2331/suisan.50.937

[16] I. Yabe, A. Kusaka, T. Hamatsu, T. Azumaya, A. Nishimura, Water mass structure in the spawning area of walleye pollock (*Theragra chalcogramma*) on the Pacific coast of Southern Hokkaido, Japan, Bulletin of the Japanese Society of Fisheries Oceanography, vol. 75, no. 4, pp 211-220, 2011.

[17] T. I. Smark, J. T. Duffy-Anderson, J. K. Horme, E. V. Farley, C. D. Wilson, J. M. Napp, Influence of environment on walleye pollock eggs, larvae, and juveniles in the Southeastern Bering Sea, Deep=-Sea Research II, Vol, 65-70, pp. 196-207, 2012

[18] J. P. Stahl, "Maturation of walleye pollock, *Theragra chalcogramma*, in the eastern Bering sea in relation to temporal and spatial factors", Master thesis, University of Alaska Fairbanks, U.S.A. 2005.

Joo-Young Seo [Regular member]

- Feb. 2006 : Gangneung-Wonju National Univ., MS
- Feb. 2009 : Gangneung-Wonju National Univ., PhD
- Apr. 2009 ~ Dec. 2010 : Hokkaido Univ. Post-Doctor
- Mar. 2011 ~ current : Gangwon-Do Deep Ocean Water Fisheries Resources Center, Fisheries Resercher

<Research Interests>
Aquaculture, Fish diets

O-Nam Kwon [Regular member]

- Aug. 2001 : Gangneung-Wonju National Univ., MS
- Feb. 2006 : Gangneung-Wonju National Univ., PhD
- Jul. 2006 ~ Aug. 2008 : Hokkaido Univ. Post-Doctor
- Nov. 2009 ~ current : GABI Research Chief

<Research Interests>
Aquaculture, Propagation, Seedlings