

Gonadal Changes during the Annual Reproductive Cycle of the Ascidian *Halocynthia aurantium* (Pallas)

Wang Jong Lee and [†]Gil Jung Kim

Dept. of Marine Molecular Bioscience, Gangneung-Wonju National University, Gangneung 25457, Korea



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[†]Corresponding author

Gil Jung Kim
Dept. of Marine Molecular Bioscience,
Gangneung-Wonju National University,
Gangneung 25457, Korea.
Tel: +82-33-640-2415
Fax: +82-33-640-2849
E-mail: gjkim@gwnu.ac.kr

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ORCID

Wang Jong Lee
<https://orcid.org/0000-0003-2152-8915>
Gil Jung Kim
<https://orcid.org/0000-0001-6441-2694>

Conflict of interests

The authors declare no potential conflict of interest.

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Authors' contributions

Conceptualization: Lee WJ, Kim GJ.
Data curation: Lee WJ, Kim GJ.
Investigation: Lee WJ.
Writing-original draft: Kim GJ.
Writing-review & editing: Lee WJ, Kim GJ.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

Abstract

The ascidian *Halocynthia aurantium* (sea peach), a marine invertebrate, belongs to the same genus of the phylum Chordata along with the ascidian *Halocynthia roretzi* (sea pineapple), which is one of the model animals in the field of developmental biology. The characteristics of development and reproduction of *H. aurantium* are not yet known in detail. In order to find out the spawning period of *H. aurantium*, we investigated development of the gonads during the annual reproductive cycle. Testis and ovary were both in the bisexual gonads (ovotestes) of *H. aurantium*, which is a hermaphrodite like *H. roretzi*. In *H. aurantium*, the right gonad was longer and slightly larger than the left gonad throughout the year. In each gonad, the number of the testis gonoducts was slightly higher than that of the ovary gonoducts. These features were similarly observed in *H. roretzi*. However, the number of the testis gonoducts and the ovary gonoducts in each gonad of *H. aurantium* was about half that of *H. roretzi*. The gonads of *H. aurantium* contracted during the winter and summer seasons. The gonads decreased to the smallest size around February, and then started to increase again in March. The gonads were most developed in September of the year. Therefore, it is estimated that the spawning of *H. aurantium* begins around this period.

Keywords: Ascidian, *Halocynthia aurantium*, Bisexual gonad, Spawning period

INTRODUCTION

Ascidians, also known as tunicates or sea squirts, are classified as chordates along with vertebrates because their tadpole-shaped larvae possess a notochord in the tail and a dorsal nerve cord (Kowalevsky, 1866; Katz, 1983; Nishida 2005; Lemaire, 2009). Ascidians are mainly consumed in Asia, Chile and the Mediterranean, where product is sourced from both the wild and, in the case of the *Halocynthia* and *Styela* species in high demand, from cultured populations (Nguyen et al., 2007; Lambert et al., 2016). The ascidian *H. roretzi* (sea pineapple) is not only one of the model animals in the field of developmental biology but also one of the major aquaculture varieties in Korea and Japan (Shenkar & Swalla, 2011). However, the farmed fishermen are experiencing a lot of difficulties because of the mass death of *H. roretzi* due to increase in water temperature, changes in the environment, and outbreak of the disease such as soft tunic syndrome (Kumagai et al., 2011; Kim et al., 2014; Shin et al., 2014).

The ascidian *H. aurantium* (sea peach) is a marine invertebrate belonging to the same genus of chordates with *H. roretzi*. It has been reported that *H. aurantium* inhabits the northern part of the East

Sea, the Sea of Okhotsk, the Bering Sea, and the Gulf of Alaska in the Pacific Ocean (Van Name, 1945). In Korea, it is generally distributed up to the coast of Sokcho, and there is a report that it was also collected from the coast of Samcheok, Gangwon-do (Rho & Lee, 1991). Its usual range in depth is from about 10 to 100 m, but it is estimated that it mainly inhabits at a depth of 10 to 40 m on the coast of Gangwon-do.

H. aurantium is a strong candidate, as a new cultivated variety of ascidian species. *H. aurantium* and *H. roretzi* resemble each other not only in taste but also in many characteristics of food product. They are presumed to be similar in biological characteristics. However, the reproduction of *H. aurantium* is not well known despite some studies on the physiology, the early development, and the mitochondrial genome (Inazawa et al., 1998; Kim, 2020; Kim et al., 2021). In this study, we investigated gonadal changes during the annual reproductive cycle of the ascidian *H. aurantium*.

MATERIALS AND METHODS

Adults of ascidians, *H. aurantium* (Pallas) and *H. roretzi* (Drasche), were collected in the sea near Sokcho, Gangwon-do, Korea from July 2015 to October 2016. To calculate the gonadosomatic index (GSI: gonad weight/body weight×100) of *H. aurantium*, the weight of the body and the gonad was measured using 10 individuals every month. The average size of *H. aurantium* and *H. roretzi* adults used in the experiment was 230 g and 250 g, respectively. The gonads were obtained by incision on the dorsal side (Atrial siphon side) of the Adults (Fig. 1).

RESULTS AND DISCUSSION

1. Characteristics of the bisexual gonad of *H. aurantium*

In adult of *H. aurantium*, the attached side is the posterior of the body. On the upper, the anterior side has an incurrent oral (branchial) siphon and an outcurrent atrial siphon (Fig. 1A). Oral siphon is

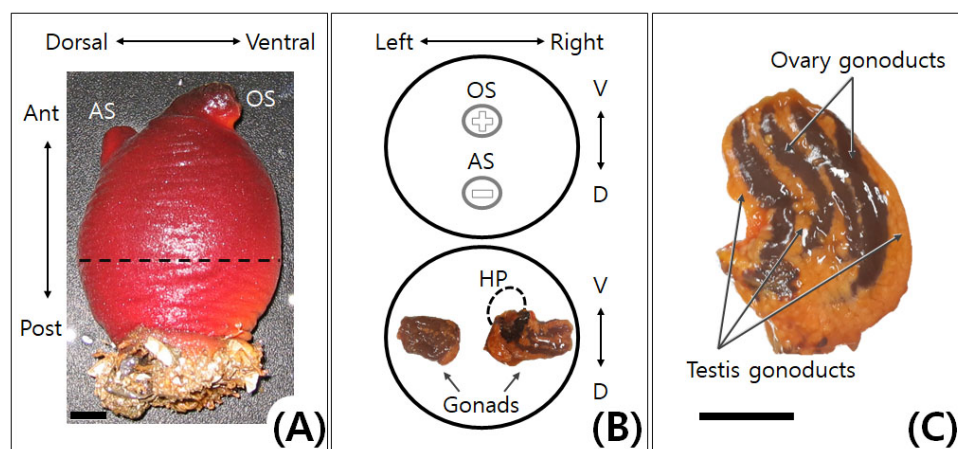


Fig. 1. The ascidian *Halocynthia aurantium*. (A) An adult with an incurrent oral siphon (OS) and an outcurrent atrial siphon (AS). Lateral view. Anterior is up and posterior is down. OS is on the ventral side. (B) Upper and lower diagrams illustrate a view of the adult from above and a transverse section which made along a broken line indicated in A, respectively. The hepatopancreas (HP, a circle of broken line) is located on the right with a gonad. (C) A bisexual gonad with the gonoducts of testis and ovary. D, dorsal; V, ventral (Scale bars = 2 cm).

located on the ventral side. When siphons are closed, oral siphon is cross-shaped, whereas atrial siphon is rod-shaped (Fig. 1B). Adult of the ascidian *H. aurantium* is a hermaphrodite like that of *H. roretzi*. The bisexual gonad of *H. aurantium* was a type of the ovotestes in which testis and ovary coexist (Fig. 1C). In *H. aurantium*, the right gonad was longer and slightly larger than the left gonad throughout the year (Fig. 1B, lower diagram). The hepatopancreas was attached only to the right gonad. In each gonad of *H. aurantium*, the number of the testis gonoducts was slightly higher than that of the ovary gonoducts (Table 1). Moreover, the number of the testis and ovary gonoducts in each gonad of *H. aurantium* was about half that of the *H. roretzi*. The gonoducts of testis and ovary were alternately located in the bisexual gonad, and the testis gonoducts were mainly located on both edges of the gonad (Fig. 1C). When the gonads were reduced, the testis gonoducts atrophied earlier than the ovary gonoducts (data not shown). These features were similarly observed in *H. roretzi* (Kim et al., 2001).

2. Seasonal changes in the bisexual gonads

To find out the spawning period of *H. aurantium*, we investigated development of the gonads during the annual reproductive cycle. Development of the gonads was determined using GSI. GSI of *H. aurantium* showed the highest level in September 2015, and then gradually decreased to the lowest level in February of the following year (Fig. 2). The GSI has gradually increased since March, and reached the highest level again in September. The average GSI in September, the highest level, was about 3.8 times higher than that in February, the lowest level. It seems to be that the gonads of *H. aurantium* contracted from winter to next summer seasons. The gonads decreased to the smallest size around February, and then started to increase in March. The gonads were most developed in September in both 2015 and 2016. Therefore, it is likely that the spawning period of *H. aurantium* begins around September.

As in the case of many marine animals, the spawning of ascidians is known to be affected by the photoperiod and water temperature, so there is a difference depending on the habitat (Bates, 2011; Shenkar & Swalla, 2011). In the East Sea of Gangwon-do, the spawning period of *H. roretzi* has been reported between December and February (Kim et al., 2001). It was reported that the spawning of *H. hilgendorfi ritteri* living in the sea near Jeju Island occurs from November to January (Choi et al., 2004). These two species belonging to the *Halocynthia* genus are a type of winter breeder. In contrast, *H. aurantium* appears to spawn in autumn. There is a possibility that the spawning periods of *H. aurantium* is likely to vary slightly depending on the depth of the habitat. The water temperature at a depth of 10 m and 30 m differs by more than 10 degrees depending on the season. Thus, it is important to know the habitat depth of *H. aurantium* used in the experiment, but it is very difficult to collect only the individuals of a certain depth in the current situation where aquaculture is not possible. There can be a difference in the spawning periods of *H. aurantium* living a depth of 10 m and a depth of 30 m or less, but the difference will not be very large. Therefore, these results will be useful for future studies on the development and reproduction of *H. aurantium*.

Table 1. The average number of the testis gonoducts and the ovary gonoducts in each bisexual gonad of ascidians *Halocynthia aurantium* and *Halocynthia roretzi*

	Gonad on the right		Gonad on the left	
	No. of testis gonoducts	No. of ovary gonoducts	No. of testis gonoducts	No. of ovary gonoducts
<i>H. aurantium</i> (n=40) ¹⁾	4.4±0.7	3.5±0.6	3.7±0.7	3.2±0.4
<i>H. roretzi</i> (n=20) ²⁾	8.4±0.7	7.4±1.1	7.4±1.2	6.8±1.4

¹⁾ In case of *H. aurantium*, 10 samples were collected in September and October, 2015 and 2016, respectively.

²⁾ In case of *H. roretzi*, 10 samples were collected in November and December 2015, respectively.

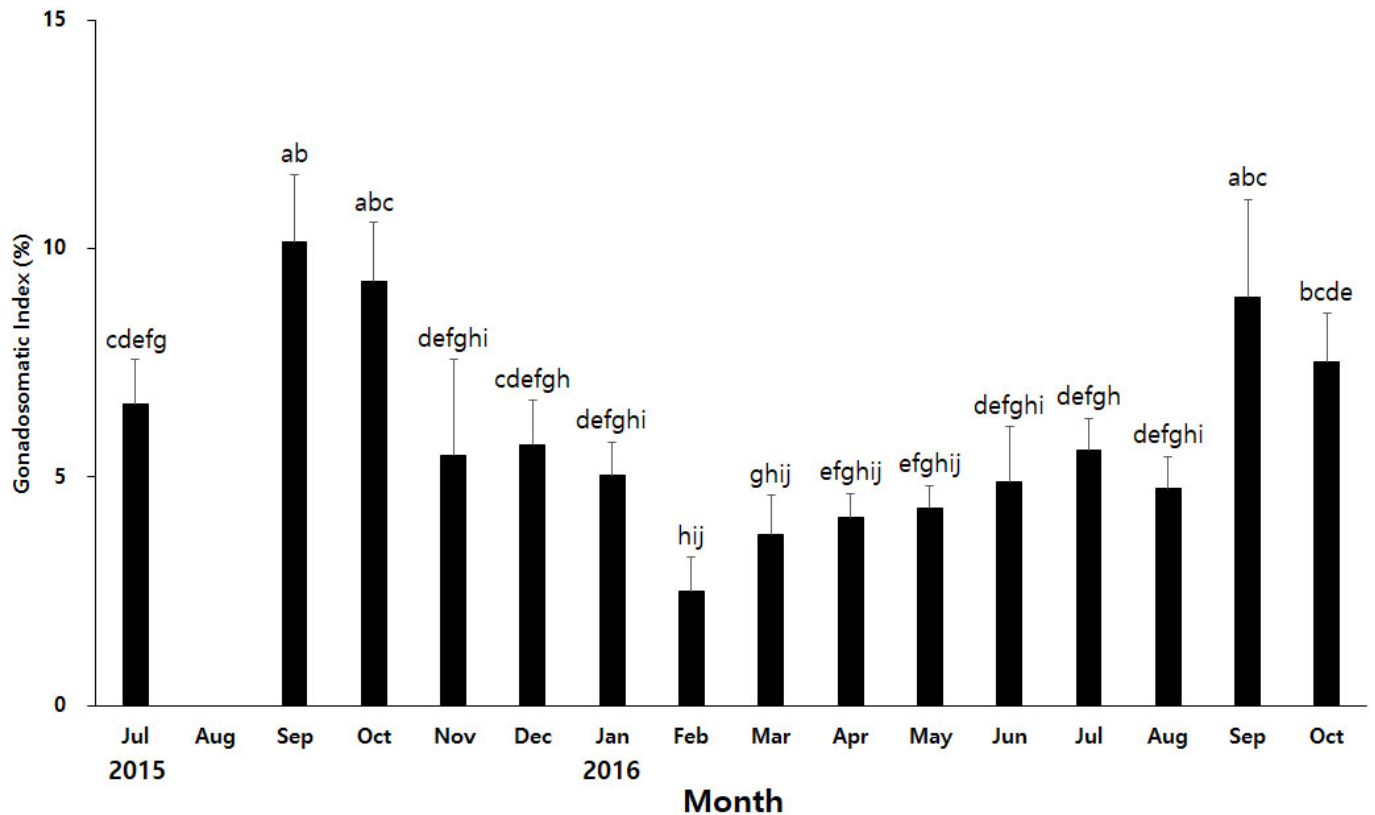


Fig. 2. Seasonal changes in gonadosomatic index (mean \pm SD, n=10) of the ascidian *Halocynthia aurantium*. Means not sharing the same superscripts are significantly ($p < 0.05$) different from each other. Samples were not available in August 2015.

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