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Improving the Food Quality of Sea Urchins Collected from Barren Grounds by Short-Term Aquaculture under Controlled Temperature

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Abstract: In west Hokkaido, there are many barren grounds where *Mesocentrotus nudus* is abundant. These sea urchins have poor commercial value due to their thin gonads, but can become marketable by intensive feeding for gonad enhancement. In general, the quality of sea urchin gonads as food products decreases as gametogenesis progresses. Mature ovaries and testes are not suitable as food products because of the unpleasant taste caused by gamete content and the melting appearance caused by gamete flow via breakage of the gonoduct. Immature to pre-mature gonads that contain predominantly nutritive phagocytes (somatic nutrient storage cells) and not copious gametes have a higher commercial value. Thus, enhancing the nutrient accumulation into nutritive phagocytes plus suppressing gametogenesis is advantageous for sea urchin aquaculture. We are developing short-term aquaculture techniques to improve the food quality of *M. nudus* collected from barren grounds under controlled temperature to suppress gametogenesis. Rearing *M. nudus* under a low temperature between summer and autumn has proved to be effective to increase the gonad size without the quality deterioration caused by maturation.

Keywords: aquaculture, food quality, gametogenesis, gonad, sea urchin, temperature

For decades, the loss of seaweed beds and the expansion of barren grounds has been a serious problem for coastal fisheries in Japan (Fujita, 2010). The bare grounds populated by sea urchins are known as urchin barrens (Pearse, 2006; Fujita *et al*., 2008). In west Hokkaido, there are many urchin barrens, where *Mesocentrotus nudus* is abundant (Fig. 1). The sea urchins in these barrens have poor commercial value because they have thin gonads (edible portion) due to deficiency of their main food, macroalgae. However, they can become marketable by intensive feeding for gonad enhancement in short-term aquaculture (Agatsuma and Nishikiori, 1991; Unuma and Kayaba, 2015). If the cultured *M. nudus* of improved quality are sold in autumn, out of the fishing season of this species, higher market price can be achieved.

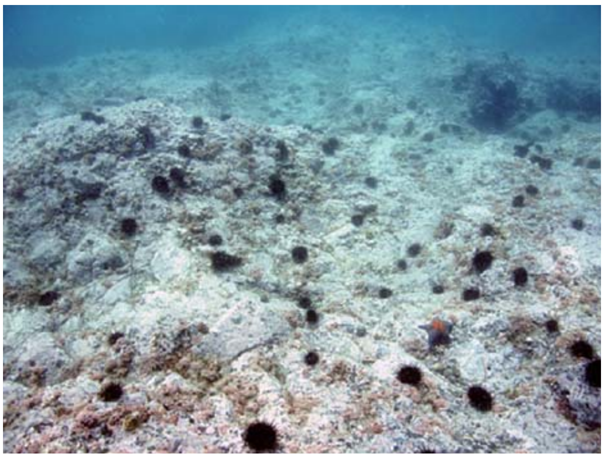

Fig. 1. An urchin barren in Suttsu, west Hokkaido. The bare ground is populated by *Mesocentrotus nudus* that has poor commercial value because of thin gonads. The photograph was taken by Kazuhiro Takahashi.

Unlike the gonads of other animals, sea urchin

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ovary and testis (both of which are equally preferred as food) play a role as a nutrient storage organ (Walker, 1982; Walker *et al*., 2013; Unuma, 2002, 2015). A thorough understanding of the unique characteristics of sea urchin reproduction should permit novel methods to improve the quality of gonads as food products in its aquaculture. We are currently conducting research and development for short-term aquaculture techniques to maximize the value of *M. nudus* collected from urchin barrens under controlled temperature and harvest them when wild *M. nudus* is scarce in the market. In this paper, we describe the theoretical background to improve the food quality of *M. nudus* by manipulating environmental conditions and give a brief overview of our ongoing research.

Features of Gametogenesis

The sea urchin has five gonads attached internally to the test (shell). A lobe of gonad consists of hundreds of gonadal acini and resembles a bunch of grapes (Fig. 2A). There are two major populations of cells inside the acinus (Fig. 2B): germ cells (GCs, from oogonia to ova in the ovary and from spermatogonia to fully differentiated spermatozoa in the testis) and somatic cells called nutritive phagocytes (NPs) that are present in both sexes (Walker, 1982; Walker *et al*., 2013). NPs store

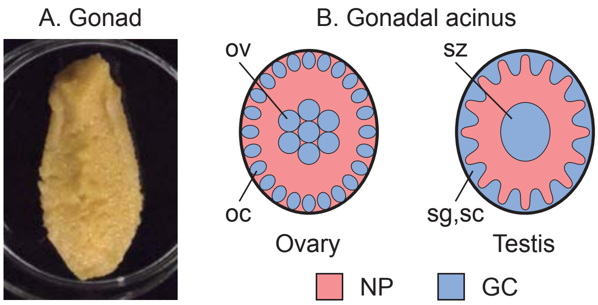

Fig. 2. Structure of sea urchin gonad. (A) A gonad of *Mesocentrotus nudus*. A lobe of gonad consists of hundreds of acini. (B) Schematic drawing of the inside of gonadal acinus. Each acinus contains two main types of cell populations: germ cells (GCs) and nutritive phagocytes (NPs). NPs are somatic cells that store nutrient necessary for gametogenesis.
oc = oocyte, ov = ripe ovum, sc = spermatocyte, sg = spermatogonium, and sz = spermatozoon.

nutrients necessary for gametogenesis and supply it to GCs (Walker, 1982; Unuma, 2002).

During the annual reproductive cycle, gonads of both sea urchin sexes pass through a predictable series of structural changes (Unuma and Walker, 2009; Walker *et al*., 2013). Fig. 3 shows histological changes in the ovary and testis of *M. nudus* during gametogenesis as classified into five stages by Fuji (1960a) with modifications (Unuma, 2002).

Stage 1. Immature gonad before gametogenesis: In both sexes each acinus is filled with NPs (eosinophilic cell populations). In ovaries, a few young oocytes are present at the periphery of the acini. Hematoxylin-stained round spots, residue from phagocytized ova (Masuda and Dan, 1977; Tominaga and Takashima, 1987), are occasionally present centrally in the ovarian lumen. In testes, detection of spermatogenic cells is sometimes difficult at this stage in paraffin sections. Instead, many hematoxylin-stained speckles, residue from phagocytized spermatozoa (Kato and Ishikawa, 1982; Reunov *et al*., 2004), are often present in NPs. These speckles, which are amorphous unlike the round spots observed in the immature ovary, are a useful feature to distinguish testes from ovaries.

Stage 2. Beginning of gametogenesis: Many developing oocytes or clusters of spermatogonia are present at the periphery of the acini, and the gonadal lumina are still filled with NPs.

Stage 3. Middle of gametogenesis: NPs are replaced with ripe ova or spermatozoa in the center of the gonadal lumina. Numerous developing oocytes or clusters of spermatogonia and spermatocytes (Ward and Nishioka, 1993; Walker *et al*., 2005) are present at the periphery of the acini. NPs are gradually decreasing in size and are present between the GCs.

Stage 4. Fully mature gonads at the end of gametogenesis: The gonadal lumina are filled with ripe ova or spermatozoa. Shrunken NPs, which have already lost nutrients, are present only at the periphery of the acini.

Stage 5. After spawning: The gonadal lumina have numerous empty spaces and a few residual ova or spermatozoa. NPs gradually phagocytize residual gametes and begin to grow as they store nutrients. After this stage, gonads return to Stage 1 and a new cycle starts.

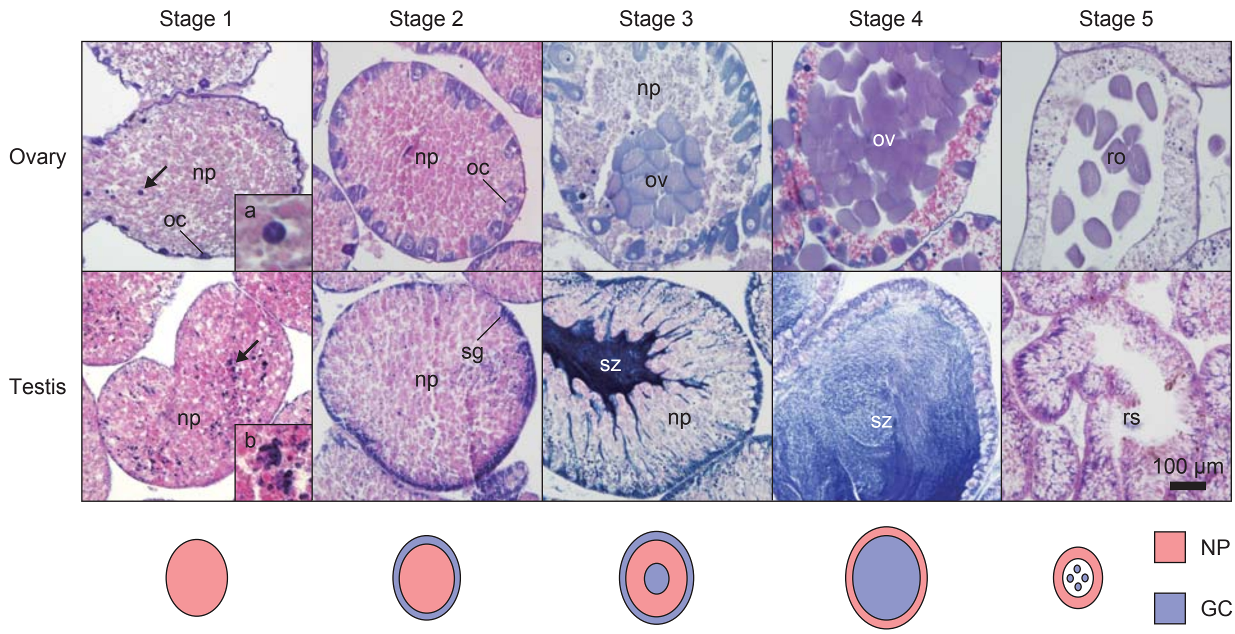

Fig. 3. Histological changes in the ovary (upper panels) and testis (lower panels) of *Mesocentrotus nudus* during gametogenesis. Paraffin-embedded sections are stained with hematoxylin and eosin. Schematic drawing below the photomicrographs shows structural features that are common between the ovarian and testicular acini. At stage 1, the gonadal lumina are filled with nutritive phagocytes. At stage 2, many developing oocytes or clusters of spermatogonia are present at the periphery. At stage 3, nutritive phagocytes are replaced with ripe ova or spermatozoa in the center of the lumina. At stage 4, the lumina are filled with ripe ova or spermatozoa. At stage 5, the lumina contain a few residual ova or spermatozoa. np = nutritive phagocyte, oc = oocyte, ov = ripe ovum, ro = residual ovum, sg = spermatogonium, sz = spermatozoon, and rs = residual spermatozoon. Inset a, round spot derived from a phagocytized residual ovum. Inset b, amorphous speckles derived from phagocytized residual spermatozoa. Scale bar represents 100 μm.

Seasonal Changes in Gonad Size and GCs/NPs Proportion

Sea urchin gonads grow in size not only because gametogenesis increases the size or numbers of GCs but also because NPs store extensive nutrient reserves before gametogenesis (Unuma and Walker, 2009; Walker *et al.*, 2013). Fig. 4 shows the seasonal changes in the gonad index (the ratio of gonad weight to total body weight) and in the proportion of GCs and NPs in *M. nudus*. This species spawns around October and gonad indices rapidly decrease (Fuji, 1960b). After spawning, the gonad index gradually increases until the next spawning. The increase before gametogenesis is attributable to the growth of NPs. NPs accumulate various nutrients, such as proteins (principally the major yolk protein; Unuma *et al.*, 2003, 2011), lipids, and carbohydrates (principally glycogen; Marsh *et al.*, 2013) and increase in size. Proliferation and development of GCs begin

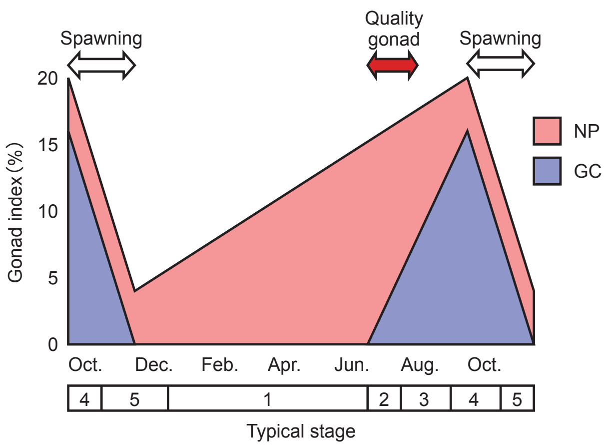

Fig. 4. Diagrammatic representation of the seasonal changes in the gonad index and in the proportion of nutritive phagocytes (NPs) and germ cells (GCs) in *Mesocentrotus nudus*. Typical gametogenic stages are indicated below the graph. Spawning occurs around October. The long-term increase in the gonad index before gametogenesis is attributable to the growth of NPs. The best season for eating gonad is around July.

three months before spawning in *M. nudus* (Unuma, 2009). After gametogenesis begins, the size of the gonad continues to increase but the proportion of NPs in the gonad rapidly decreases.

The Best Season for Eating Gonads

The best season for eating *M. nudus* gonads is around July, when NPs have grown sufficiently but the proportion of GCs is still smaller (Unuma, 2009). Their gametogenic stage is usually from stage 2 to early stage 3 in July (our unpublished observation). Before this period, the size of the gonad is too small and the color of the gonad is not attractive (brownish). After this period, the proportion of GCs becomes higher and the quality as food gradually deteriorates. The most serious problem for food quality is the melting appearance caused by the flow of gametes (Unuma, 2002; Unuma and Walker, 2009). After ripe gonads are taken out of the test, eggs or sperm flow out from the breakage of gonoduct, and the gonads cannot maintain their consistency (Fig. 5). This phenomenon considerably reduces the commercial value of the gonads. Additionally, the gametes cause unpleasant tastes such as bitterness in the mature gonads (Unuma, 2002; Unuma and Walker, 2009).

Relationship between the gametogenic stage of gonads and the food quality varies among species. Ovaries of *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus* produce an extremely bitter taste after oogenesis begin (Murata *et al*., 2002; Murata 2009, Unuma and Walker, 2009). In these species, the season for eating is limited only when their gonads are at stage 1 (Murata 2009; Unuma 2002, 2009). On the other hand, other Japanese sea urchins including *M. nudus* can be eaten until when their gonads are at stage 3, because the maturing gonads do not produce a strong bitterness (Murata 2009; Unuma 2009). In every species, the best season for eating gonads is rather short because of the problems caused by gametogenesis. Low-quality or small gonads can be obtained for a longer period of time, but superior-quality and sizable ones can be harvested for only about one or two months (Unuma, 2002).

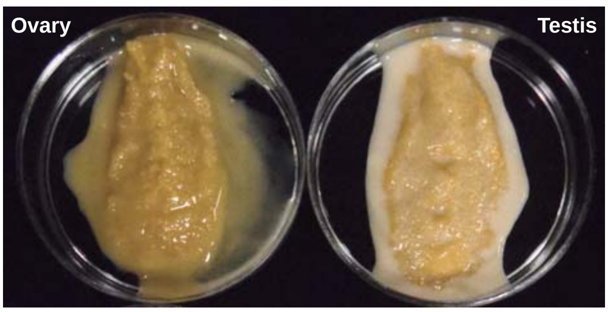

Fig. 5. Gamete flow from the mature ovary (left) and testis (right) of *Mesocentrotus nudus*. After mature gonads are taken out of the test, they appear to be melting because of flowing eggs or sperm from the breakage of gonoduct.

Improving Food Quality by Short-term Aquaculture

As we have seen, NPs are more important as food than GCs. Thus, when sea urchins are cultured for gonad enhancement, not GCs but NPs should be increased in the gonads. The sea urchins dwelling at urchin barrens cannot store much nutrient in the gonads due to deficiency of food (Agatsuma and Nishikiori, 1991). However, the sea urchins produce appropriate amount of gametes and spawn even if the food availability is low. Therefore, the schema of gonad index and GCs/NPs proportion (Fig. 4) is turned to be depressed in the sea urchins at urchin barrens (Fig. 6A). We are trying to change this schema by short-term aquaculture as shown in Fig. 6B. The aims of the culture are (1) to promote nutrient accumulation into NPs by intensive feeding and (2) to suppress proliferation and development of GCs by manipulating environmental conditions. Combination of these two aims should prolong the season for quality gonad until the spawning season when wild *M. nudus* is not fished due to quality deterioration as well as for resource management.

Environmental Factors Regulating Gametogenesis

Gametogenic cycles of sea urchins are regulated by environmental factors, such as photoperiod and water temperature. There have been many reports describing the effects of these factors on gametogenesis of sea urchins as listed in Table 1. Most of these researches were conducted as basic biology to identify factors affecting maturation, or as applied biology to develop techniques for promoting

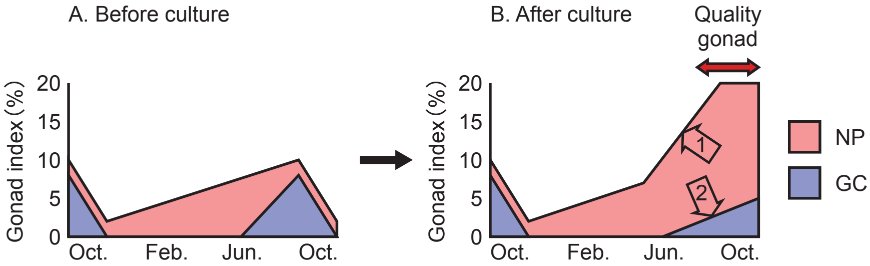

Fig. 6. Purpose of the short-term aquaculture of *Mesocentrotus nudus* collected from urchin barrens. Schema of gonad index and GCs/NPs proportion can be changed from (A) to (B) by rearing under optimized conditions. The aims of the culture are (1) to increase the nutritive phagocytes (NPs) by intensive feeding and (2) to decrease the germ cells (GCs) by suppressing gametogenesis. Combination of these two aims should prolong the season for quality gonads.

Table 1. Researches on environmental factors affecting sea urchin gametogenesis

Factors investigated	Species	literature
Photoperiod	*Eucidaris tribuloides*	McClintock and Watts (1990)
	Paracentrotus lividus	McCarron *et al.* (2010)
	Strongylocentrotus droebachiensis	Walker and Lesser (1998)
		Böttger *et al.* (2006)*
		Dumont *et al.* (2006)
		Kirchhoff *et al.* (2010)
	Strongylocentrotus purpuratus	Cochran and Engelmann (1975)
		Pearse *et al.* (1986)
		Bay-Schmith and Pearse (1987)
Temperature	*Heliocidaris crassispina*	Sakairi *et al.* (1989)
	Hemicentrotus pulcherrimus	Yamamoto *et al.* (1988)
		Ito *et al.* (1989)
		Sakairi *et al.* (1989)
	Pseudocentrotus depressus	Yamamoto *et al.* (1988)
		Masaki and Kawahara (1995)
		Noguchi *et al.* (1995)
	Strongylocentrotus droebachiensis	Garrido and Barber (2001)
	Strongylocentrotus intermedius	Kayaba *et al.* (2012)*
Photoperiod and Temperature	*Paracentrotus lividus*	Spirlet *et al.* (2000)*
		Shpigel *et al.* (2004)*
	Psammechinus miliaris	Kelly (2001)

*Conducted from a view point of suppressing gametogenesis

maturation to obtain gametes out of the spawning season for laboratory use or for seed production. For example, in some sea urchin hatcheries in Japan, out-of-season maturation of brood stock of *Pseudocentrotus depressus*, *Hemicentrotus pulcherrimus* and *Strongylocentrotus intermedius* is promoted by manipulating water temperature (Ito *et al.*, 1989; Masaki and Kawahara, 1995; Noguchi *et al.*, 1995; Sakai, 2015). However, for culturing adult sea urchins to enhance the gonads, not promotion but suppression of gametogenesis is required. To our knowledge, only four studies have been conducted from a view point of suppressing gametogenesis to obtain the gonads of better food quality. In *Strongylocentrotus droebachiensis* (Böttger *et al.*, 2006), *Strongylocentrotus intermedius* (Kayaba *et *al.*, 2012) and *Paracentrotus lividus* (Spirlet *et al.*, 2000; Shpigel *et al.*, 2004), maturation was delayed by manipulation of photoperiod, water temperature and both of them, respectively. However, the control regime of environmental conditions for culturing *M. nudus* is still to be clarified, because optimum conditions to suppress gametogenesis effectively varies among different species.

Overview of Our Research

Our final goal is to develop a land-based aquaculture system, using *M. nudus* collected from urchin barrens, controlling their gametogenic cycle, and harvesting high-quality urchins when their wild counterparts are scarce in the fish market. As an initial step, we investigated the effects of water temperature on the size and quality of the gonads in *M. nudus*.

Adult *M. nudus* collected from an urchin barren in Suttsu, west Hokkaido (Fig. 1), were reared from late June to mid October in three tanks maintained at either a constant temperature (10℃ or 15℃) or a variable temperature, similar to their natural environment (control group; 18.0℃ to 22.5℃). They were given a surplus of kelp (*Saccharina longissima*). Over the experimental period, gonad indices increased in all treatments from 7% initially to over 20% after three months. Histological observations revealed that after three months, there were no urchins with fully mature gonads in the 10℃ group, but 6% in the 15℃ group, and 39% in the control group. Sensory tests of final gonads found that the quality of the control group was inferior to that of the 10℃ and 15℃ groups, in terms of both appearance and taste. We conclude that rearing *M. nudus* under a low temperature between summer and autumn is effective to increase the gonad size without the quality deterioration caused by maturation. These results are being prepared for publication in the journal. We are further investigating the relationship between the rearing conditions and the size and quality of the gonads to determine the optimum control regime for culturing *M. nudus*.

Methods to Decrease Water Temperature

To put our findings into practice, economic methods for decreasing the water temperature in aquaculture farms are required. Using electric cooling devices is a classic method but has high energy costs. It would not be practical to use it in a conventional flow-through system. However, closed or semi-closed recirculating system may minimize the problem of energy costs in electric cooling devices.

Deep seawater drawn from a depth of over 300 m has a very low temperature (Nakasone and Akeda, 2000). Large initial investment costs are required to build a facility to draw the water. However, more than ten facilities capable of drawing deep seawater have been constructed in Japan, where it is used for aquaculture, handling of captured fishes, the food industry, medical treatment, and agriculture (Nakasone and Akeda, 2000; Kayaba *et al.*, 2012); including two in west Hokkaido. Therefore, the use of existing facilities is a practical way to adopt the deep seawater method in sea urchin aquaculture.

The use of salty groundwater for aquaculture, with a salinity comparable to seawater, is a recent development in Japan (Ebata *et al.*, 2006; Imada *et al.*, 2006). It can be obtained from wells bored near to the shore and has a stable temperature year round. In west Hokkaido, the temperature of salty groundwater is about 10℃ even in summer, although that of surface seawater rises over 20℃. The advantage of this method is that it requires small initial investment costs. However, this water sometimes contains high levels of ammonium and manganese ions (Ebata *et al.*, 2006). Such groundwater requires treatment using aeration and specified microbes to remove the ions before use (Ebata *et al.*, 2006).

Thus, there are multiple methods for decreasing the temperature of rearing water in summer. The chosen method depends on the location and scale of the aquaculture farm.

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