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Short communication

A new method for identifying the male spawning period of fish using sperm duct volume as an index

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ABSTRACT

For conservation and management of fish stock, reliable reproductive parameters, especially information on the spawning period, are greatly needed. Until now, the spawning period of male fish was estimated mainly using the gonadosomatic index (GSI) and microscopic observation of the testes. However, these methods pose the problem of estimation accuracy and handiness for analysis, respectively. To identify biological characteristics that would allow accurate determination of spawning periods, we investigated the seasonal development of testes and sperm ducts in our model fish, the barfin flounder *Verasper moseri*. GSI (testis weight × 100/body weight) reached a maximum during November–December; however, this peak period occurred approximately two months before the onset of spawning. In contrast, the sperm duct index (SDI) (sperm duct weight × 100/body weight) sharply rose from February to March when spermiation and sperm release actively proceeded. On comparing the SDI of all gonadal development phases, it was found that only the fish that underwent spawning had significantly enlarged sperm ducts due to sperm accumulation. This finding strongly suggested that sperm duct volume is an adequate indicator for accurately identifying spawning period in male fish. Moreover, analysis using SDI takes less effort for sample processing, suggesting to be practical for continuous monitoring of stock assessment.
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1. Introduction

Conservation and management measures of heavily fished stock require reliable data on various aspects of the life cycle, including reproductive ecology (Wakefield et al., 2011). In particular, information about spawning period and location are essential for determining time–area closures as a management measure to protect important spawning habitats (Van Overzee and Rijnsdorp, 2015).

The gonadosomatic index (GSI), the calculation of the gonad mass as a proportion of total body mass, is commonly used to describe gametogenesis and estimate a crude spawning period in fish (West, 1990; Wootton and Smith, 2014). However, it is not easy to fully identify the spawning periods of male fish using GSI. Because the quantitative development of the gonads is not necessarily consistent with the progression of gametogenesis (Barnett and Pankhurst, 1999; Moulton and Burton, 1999), it is difficult to determine the onset and end of male spawning period using only GSI data. Although histological observation is the best method for accurately identifying the spawning period, it has the disadvantage of requiring much effort for sample processing. In most studies, spawning state is judged by applying pressure around the abdomen and collecting sperm (hereafter, the "hand-stripping" method) as a substitute for histological observation (Bromley, 2000; Macdonald et al., 2013). However, as the method is executed by many people, the results obtained from this method may be influenced by human errors. Furthermore, they are qualitative data and are insufficient for a detailed investigation of the spawning trait. For this reason, the identification of biological characteristics that accurately indicate the spawning state of male fish is desirable.

The sperm duct is considered to have an important physiological role in the spawning process (Nagahama, 1994;

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Weltzien et al., 2004). Differentiated immature spermatozoa in testes are released into the sperm ducts (in a process called spermiation) at the start of the spawning period. They acquire motility by the stimulation of maturation-inducing steroids and mature completely in the sperm ducts (Miura et al., 1992; Morisawa and Morisawa, 1986). In view of this physiological information, we hypothesized the following: (1) the spawning period of male fish is the range from first spermiation to final sperm release; and (2) the volume of sperm ducts expands at the start of spermiation. If so, changes in sperm duct volume will become an effective index of accurately elucidating the spawning period. However, to the best of our knowledge, no study linking fish sperm duct volume to spawning has been reported.

In this study, we used barfin flounder *Verasper moseri* (family Pleuronectidae) as a model species. This flounder is a commercially valuable bottom fish that inhabits cold seawater basins off the northern Pacific coast of Japan. Male *V. moseri* is a multiple spawner; the male repeatedly releases sperm in synchrony with female spawning for about one month (Kayaba et al., 2003). Recent studies have described the spawning ecology of the species in the wild (Kayaba et al., 2014; Wada et al., 2014). Given this basic knowledge of its reproductive traits, *V. moseri* is a suitable model for investigating fish spawning.

The objective of the present study was to identify the biological characteristics that would help in determining the spawning periods in male fish. We focused on the sperm ducts. Seasonal changes of testis and sperm duct weight were evaluated in male *V. moseri* captured during 2008–2013. The quantitative relationship between the development of testes and sperm ducts was investigated, and the efficiency of observation of sperm ducts as an indicator of spawning period was discussed.

2. Material and methods

2.1. Sample collection and processing

Some of the methods used were similar to those applied to female *V. moseri* (see Kayaba et al., 2014) and are only summarized here. From January 2008 through December 2013, a total of 4740 male specimens over 205 mm in total length (TL) were randomly sampled from 35 fish markets located on the Pacific coast of Hokkaido and Tohoku (ranging from 43.4°N, 145.8°E to 35.7°N, 140.9°E), Japan. All samples were transported to the laboratory in refrigerated or frozen condition. Their TLs were measured to the nearest 1 mm and they were weighed to the nearest 0.1 g. Their testes and sperm ducts were separately removed and weighed to the nearest 0.1 g. The sperm duct was defined as the duct from the base of the testis to the urogenital pore. When the sperm duct was extracted, both ends of the duct were held with forceps so that the contents did not leak and the duct was surgically cut outside the holding points. The sperm duct weight was the sum of the weights of the main ducts and the sperm contained inside the ducts. A GSI (GSI % = testis weight × 100/body weight) and a sperm duct index (SDI % = sperm duct weight × 100/body weight) were, respectively, calculated. For histological observations, pieces of testis were fixed in Bouin's solution. The specimens were dehydrated through a graded ethanol series and embedded in TissePrep (Fisher Scientific Inc., USA). They were sectioned to 5–6 μm thickness using a microtome, stained with Meyer's hematoxylin and eosin, and observed under a light microscope. The ages of samples were determined using the surface reading method for sagittal otoliths, as previously described (Takaya et al., 2004). The time of transition from one age group to the next was assumed to have occurred on April 1, given

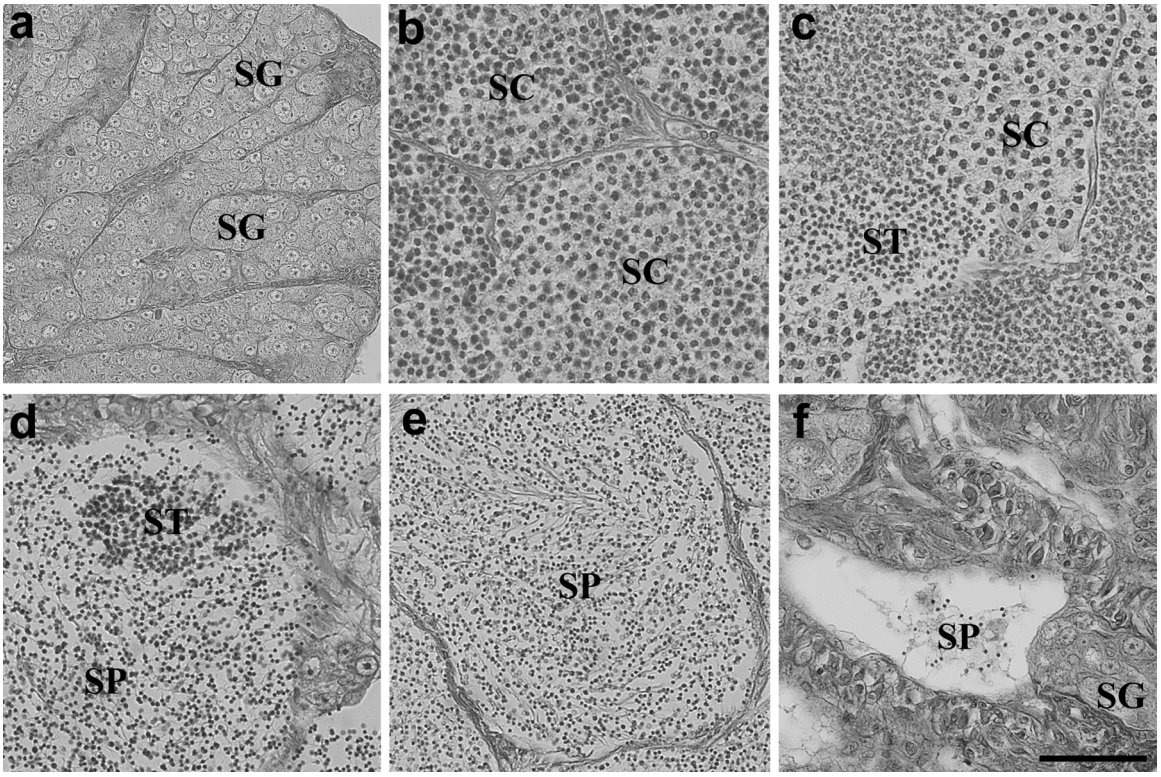

Fig. 1. *Photomicrographs of testicular developmental stages of male V. moseri.* (a) Phase I: pre-spermatogenesis phase; (b) phase II: early spermatogenesis phase; (c) phase III: mid-spermatogenesis phase; (d) phase IV: late spermatogenesis phase; (e) phase V: functional maturation and spawning phase; (f) phase VI: spent phase. SG: spermatogonia, SC: spermatocytes, ST: spermatids, SP: spermatozoa. Scale bars: 50 μm.

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