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Elucidating the spawning migration and core reproductive duration of male flatfish using sperm duct volume as an index for better fishery advice and management

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ABSTRACT

The conservation and management of heavily exploited fish stocks require reliable data on various aspects of reproduction. In particular, information about spawning migration and spawning period of both sexes is essential for protecting important spawning habitats. However, studies that focus on male spawning ecology are very limited. We elucidated the male spawning migration, core duration of reproduction, and seasonal changes in the maturity of the barfin flounder *Verasper moseri*, an important target flatfish for stock enhancement programs in Japan. We conducted histological observations of the testes and utilized the sperm duct volume index to facilitate accurate determination of the male spawning period. Fish before spermiation were mainly observed off Hokkaido and partially off northern Tohoku (40.2–43.4°N) during August–December. However, fish undergoing spermiation and sperm release were not found near these areas and were observed only off southernmost Tohoku (35.8–37.5°N approximately 300 m deep) from late January to April. Very few spent fish were caught off southern Tohoku, but were frequently observed off Hokkaido during May–June. These results demonstrate that male *V. moseri* repeat widespread spawning migrations over 700 km between feeding grounds off Hokkaido and spawning grounds off southern Tohoku. Moreover, a combined analysis related to seasonal variations in the sperm duct volume and the proportion of spawning adults identified that the core duration of male reproduction was during March. Until now, reproduction of the next generation in the wild has not been confirmed, despite the continual release of seedlings and local fishery restrictions around Hokkaido. Our results underscore the necessity of a more widespread fishery management plan for *V. moseri* that encompasses the waters off Hokkaido and Tohoku to more effectively enhance reproduction.

1. Introduction

The conservation and management of heavily exploited fish stocks requires reliable data on various life cycle aspects, including their reproductive ecology (Wakefield et al., 2011). In particular, information about the spawning season and location is essential for determining time–area closures as a management measure to protect important spawning habitats (Van Overzee and Rijnsdorp, 2015).

Many flatfish species display sexual dimorphism in their growth, reproductive traits, and behavior (Bromley, 2000, 2003; Land et al., 1996; Macdonald et al., 2013; Rijnsdorp and Ibelings, 1989). Recent studies in megrim (*Lepidorhombus whiffiagonis*) and Baltic Sea turbot (*Pesetta maxima*) demonstrated sexual segregation in their distribution and habitat preferences (Florin and Franzén, 2010; Gerritsen et al., 2010). These differences result in differential sexual exploitation by spatially selective fisheries, resulting in a negative population impact by decreasing the reproductive output and

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changes to the genetic composition (Kuparinen and Merilä, 2007; Rijnsdorp and Witthames, 2005; Van Overzee and Rijnsdorp, 2015). Therefore, to establish sustainable fishery management plan, it is important to understand the reproductive ecology of both sexes. Nevertheless, studies that focus on male spawning ecology, such as migratory traits and spawning duration in the wild, are very limited.

To analyze the spawning ecology of male fish, the biological characteristics that accurately indicate the spawning state are essential. Until now, the spawning period of male fish was estimated mainly using the gonadosomatic index (GSI) and microscopic observations of the testes. However, these methods pose the problem of estimation accuracy and convenience for sample processing, respectively. In our recent study, we demonstrated that analysis of sperm duct weight is an extremely effective and convenient method for accurately identifying the spawning period of male flatfish (Kayaba et al., 2015). An analysis that incorporates sperm duct data as an index will allow specification of the core location and duration of male spawning.

The barfin flounder *Verasper moseri* is a commercially valuable flatfish that inhabits coastal waters off the northern Pacific coast of Japan. However, their population almost vanished, probably because of overfishing during the 1960s–1980s (Sasaki, 1997). To address this rapid decline in stock, a stock enhancement program, namely the release of hatchery-reared juveniles, was implemented for Hokkaido in 1987, the main habitat region of this species (Kayaba et al., 2014). As a result of these trials, commercial landings around the release areas have markedly recovered and annual landings in Hokkaido increased from <100 kg in 1980 to >170 tons in 2010 (Kayaba et al., 2014). The *V. moseri* stock enhancement program is highly prized as a successful model in Japan, and recovery of wild populations through reproduction of the next generation from stocked fish has become an important goal as the next step. However, almost all landings are now fish that have grown from released seedlings (Kayaba, 2013); they can be distinguished from wild fish as hatchery-reared *V. moseri* have an abnormal body pigmentation. In other words, the population of wild fish has remained at a negligible level, despite the continual release of seedlings over the last 25 years. Therefore, to completely restore the depleted *V. moseri* stock, an adequate fishery management plan that incorporates their reproductive ecology is required. Recently, we clarified the gonadal maturation and spawning migration of stocked female *V. moseri* (Kayaba et al., 2014; Wada et al., 2014). Accordingly, as the next step, elucidation of the spawning migration pattern and reproductive duration of male *V. moseri* is required.

The aims of this study were to investigate the spawning ecology of stocked male *V. moseri* including (1) spawning migration patterns and (2) the core location and duration of spawning by observing seasonal changes in the testes and sperm duct development. Based on these results, we propose improvement to the fishery management plan for the depleted flatfish stock that aim to accelerate spontaneous reproduction in the wild.

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 to December 2013, a total of 5325 male specimens over 150 mm total length (TL) were sampled monthly from 35 fish markets located in four research areas on the Pacific coast of Hokkaido and Tohoku (ranging from 43.4°N, 145.8°E to 35.7°N, 140.9°E), Japan (Fig. 1). Samples were not collected from fish markets south of Tohoku or the Sea of Japan because *V. moseri* have rarely been caught in these regions, which are regarded as having unsuitable habitats. Fishery information including fishing date, gear, location, and depth of the fishing grounds were recorded for each sample collection. All samples were transported to the laboratory in refrigerated or frozen condition. TL was measured to the nearest 1 mm and weight to the nearest 0.1 g. The testes and sperm ducts were separately removed and weighed to the nearest 0.1 g, as previously described (Kayaba et al., 2015). A GSI (GSI% = testis weight × 100/body weight) and a sperm duct index (SDI% = sperm duct weight × 100/body weight) were calculated, respectively. 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. Based on the histological structures of the testes and the SDI values, the gonadal development was classified into six phases, following the method of Kayaba et al. (2015). The maturity scale is outlined in Table 1. The age of each specimen was 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 1st, given that the peak female spawning period is in March (Kayaba et al., 2014).

Table 1
Maturity phases of male *V. moseri* (Kayaba et al., 2015).

Maturity phase	Testis development	Sperm duct development
I. Pre-spermatogenesis	Testes are very small. Cysts containing type A or B spermatogonia are observed only in the testes, and spermatogenesis has not yet started.	Sperm ducts are fine and undeveloped. SDI: <0.1
II. Early spermatogenesis	Spermatogenesis starts and the volume of the testes marginally increases. Cysts containing spermatocytes and type B spermatogonia are organized.	Sperm ducts are fine and undeveloped. SDI: <0.1
III. Mid-spermatogenesis	The volume of the testes markedly increases because of active meiosis. Mainly cysts containing spermatids, spermatocytes, and spermatogonia are observed.	Sperm ducts are fine and undeveloped. SDI: <0.1
IV. Late spermatogenesis	Cysts containing spermatozoa occupy the testes. Spermiation has not yet started.	Sperm ducts are fine and undeveloped. SDI: <0.1
V. Functional maturation and spawning	Spermiation and sperm release are continuously repeated. Spermatozoa are abundant in the testes.	Spermatozoa are abundant in the sperm ducts. The volume of sperm ducts is markedly large. SDI: 0.1–0.8
VI. Spent	Testes are shriveled. A small mass of relict sperm or type A spermatogonia are only observed.	The sperm ducts are largely empty and flabby but are larger than in phase I. SDI: 0.1–0.2

2.2. Estimation of male spawning duration

To estimate the duration of male spawning in *V. moseri*, time-series changes of the proportion of spawning males were assessed by applying generalized linear modeling (GLM) in its basic form:

$$\mathrm{Logit}(S[y]) = \beta_1 + \beta_2 \mathrm{D} + \beta_3 \mathrm{D}^2$$