ECOLOGY OF
FRESHWATER FISH

Spawning behaviour of amemasu charr, *Salvelinus leucomaenis leucomaenis,* with a discussion of the macroevolutionary patterns of postspawning behaviour in the Salmoninae

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Abstract – Video cameras inside underwater housings were used to record the spawning behaviour of a subspecies of the white-spotted charr, the amemasu charr (*Salvelinus leucomaenis leucomaenis*) in the Tokimae and Onbetsu Rivers, Hokkaido, Japan. Unlike other *Salvelinus* species, in which females use lateral swings of their bodies to ventilate and distribute eggs over gravel crevices (*undulating*) immediately after spawning, female amemasu charr covered their eggs with beats of their tails (*cover digging*). Cover digging right after spawning has previously been documented in *Salmo, Oncorhynchus* and *Parahucho*. Phylogenetic analysis of postspawning behaviours in the Salmoninae indicated that (i) resting then covering the eggs is plesiomorphic for the group, (ii) undulating replaced resting in the ancestor of *Salvelinus*, (iii) cover digging was advanced in the behavioural sequence to occur immediately after egg deposition in the ancestor of *Parahucho + Salmo + Oncorhynchus* and in the amemasu.

Introduction

Salvelinus species display a series of behavioural traits during spawning that separate them from other Salmoninae genera (Esteve 2005 and references therein). One of the most significant differences happens right after the spawning act when a female performs a series of slow and rhythmic swings of her body over the nest in which she has just deposited her eggs. This behaviour, called *undulating* (cf. *weaving*, Frost 1965), resembles 'swimming in place' and is thought to function in oxygenating the eggs and distributing them over the gravel crevices (Needham & Vaughan 1952; Needham 1961; James & Sexauer 1997). After a few minutes of undulating, females switch to normal tail diggings that are invariably performed a few centimetres upstream from the nest (Greeley 1932; Frost

1965). Undulating separates *Salvelinus* from salmonines that either immediately cover their eggs by beats of their tail (*cover digging*: *Salmo, Oncorhynchus* and *Parahucho*) or rest for a variable number of minutes before covering the eggs (*resting*: *Hucho* and *Brachymystax*; Esteve et al. 2009b).

Documenting the extent of intergeneric variation in spawning behaviour provides information that can be used to reconstruct phylogenetic relationships, as a starting point for investigating the developmental mechanisms underlying such behaviours and to highlight unique components in the ecology of each group, which has important ramifications for conservation and management practices. In this paper, we examine the assumption that undulating is present in all *Salvelinus* species with the exception of lake trout, which has a distinctive manner of spawning that does

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not involve nest building and egg covering (Esteve et al. 2008). This assumption is based almost exclusively upon our knowledge of North American and European *Salvelinus* species; there are few behavioural studies of Asian charr that can further test the proposition. The white-spotted charr, *Salvelinus leucomaenis*, is a freshwater salmonid characterised by both anadromous and fluvial life histories (Kawanabe 1989), restricted to rivers throughout Far East Asia including Japan, Sakhalin, Kuril and other neighbouring Islands (Arai & Morita 2005). Four subspecies have been recently described based on geographical distribution and differences in spot patterns: *S. l. pluvius* (Nikko-iwana), *S. l. japonicus* (Yamato-iwana), *S. l. imbrius* (Gogi) and *S. l. leucomaenis* (common name in Japan: amemasu: Hosoya 2002). The first three subspecies comprise endangered populations at the southern tip of the species range (Honshu Island, Japan). Amemasu, on the other hand, is widespread across the north-eastern Pacific. Literature about the spawning behaviour of the four subspecies is scarce and fragmentary. Undulating after spawning was reported from both a landlocked population of an unidentified subspecies in central Honshu Island (Maekawa et al.1994) and a population of *S. l. japonicus* (Sato & Harada 2008). The only report that exists for *S. l. imbrius* is based on observations with a single female in which the spawning pair fought with each other for 15 min and then the female started typical undulating (Kimura 1977). In this paper, we describe the spawning behaviour of amemasu from two rivers in Hokkaido.

We were initially interested in testing the hypothesis that amemasu females, like other *Salvelinus* species, undulate immediately after egg deposition. Our results, however, allowed us to go further and to investigate the macroevolutionary origin and diversification of postspawning behaviours in the Salmoninae.

Material and methods

Two Sony HDR-HC7 colour digital video cameras mounted inside underwater housings were used to monitor the mating activities of six amemasu females, associated with a variable number of males. Video signals from the cameras were transmitted via cable to a digital recorder located outside the river. Recordings took place in Hokkaido at the Tokimae (females 1–4; Fig. 1) and the Onbetsu rivers (females 5 and 6: Fig. 1). The Tokimae is a first-order stream, flowing 9 km from its source until reaching the Sea of Okhotsk in north Hokkaido. Fish were nesting in a stretch of the stream, average width 1–2 m, flowing through a dense mixed forest of elm, alder, willow, Japanese oak and bamboo grass. Masu salmon, *Oncorhynchus masou*, and pink salmon, *O. gorbuscha*, also inhabit the
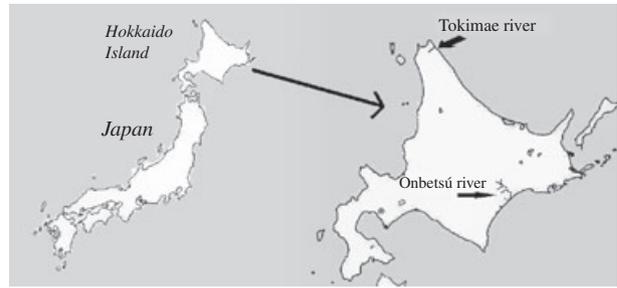
Fig. 1. Map of Hokkaido showing the two rivers where recordings took place.

Tokimae. The Onbetsu is a first-order stream, flowing 40 km from its source until reaching the Pacific Ocean in southeast Hokkaido. Recordings took place in a stretch of the stream, average width 5–8 m, flowing through a dense mixed forest of Sakhalin fir, Japanese oak, willow, Japanese larch and bamboo grass. Masu, pink and chum salmon, *Oncorhynchus keta*, also inhabit the Onbetsu.

Underwater video

Cameras were positioned approximately half a metre from the nest in which the female had previously been noted. Redds (disturbed areas of gravel containing the nests) were located in tail pool areas with decreasing water depth ranging from 0.5 to 0.2 m and increasing flow velocity. Males and females presented no noticeable sexual dimorphism, so behaviour was used to infer sex. The fish slightly behind the other, intermittently performing *quivering* (courtship behaviour consisting of low amplitude and high frequency body vibrations from head to tail) was identified as the male, while the female was the fish that regularly performed *nest digging* (female turns on her side and excavates a depression in the gravel by beats of her tail). Tape analysis was restricted to the initiation of the *spawning act* (the pair gape their mouths and tremble their bodies; Fig. 2; Video Clip S1, supporting information) and the 3 min following gamete release. Notations

Fig. 2. Pair of amemasu charr gaping during the spawning act.

Fig. 3. Female covering her eggs by beats of her tail right after spawning.

were made each time the female performed *cover digging* or *undulating* behaviours.

Evolution of the postspawning behaviour

Information about postspawning behaviour was collected from the literature and previous studies with species across the six Salmoninae genera by the first two authors (see Esteve & McLennan 2007; Esteve et al. 2009a,b and references therein). The three postspawning behaviours were initially identified as 'resting' (the female remains over the nest occasionally gently moving her tail), 'cover digging' (female bends her body and beats the gravel substrate with side-to-side flexures of her tail: Fig. 3) and 'undulating' (female performs a series of slow and rhythmic swings of her body above the nest). Categorising postspawning as the period immediately following egg deposition gives us, however, only an incomplete picture of what actually occurs after spawning because all of the salmonines perform cover digging at some

point after egg deposition. So, we expanded the temporal perspective slightly to follow individual females from the point of egg deposition to the point at which the females perform their first cover diggings. This gave us three slightly more complicated traits: 'resting then cover digging', 'undulating then cover digging' and 'cover digging only'. These traits were optimised (Farris 1970; Maddison et al. 1984) onto a Salmoninae supertree based on 34 different molecular, morphological and behavioural studies published during the last 27 years (Wilson & Williams 2010).

Results

The 3-min postspawning history of the six females is summarised in Fig. 4. Details for each female are as follows:

Female 1 (Tokimae, September 29, 2008)

Recordings started at 13:14 (local time). At that time, the female was involved in finishing nest construction. At 13:25, she performed a false spawning, 26 s later she spawned. The spawning act lasted 4 s from initiation to the end of female gaping. During spawning, three small males, possibly precocious parr, joined the pair. The female performed two cover diggings 6 and 11 s after spawning, then interspersed one more digging episode with two long undulating periods.

Female 2 (Tokimae, October 5, 2008)

Recordings with female 2 started at 8:55. At that time, the female was involved in finishing nest construction. Spawning occurred at 9:24 and lasted 4 s. During
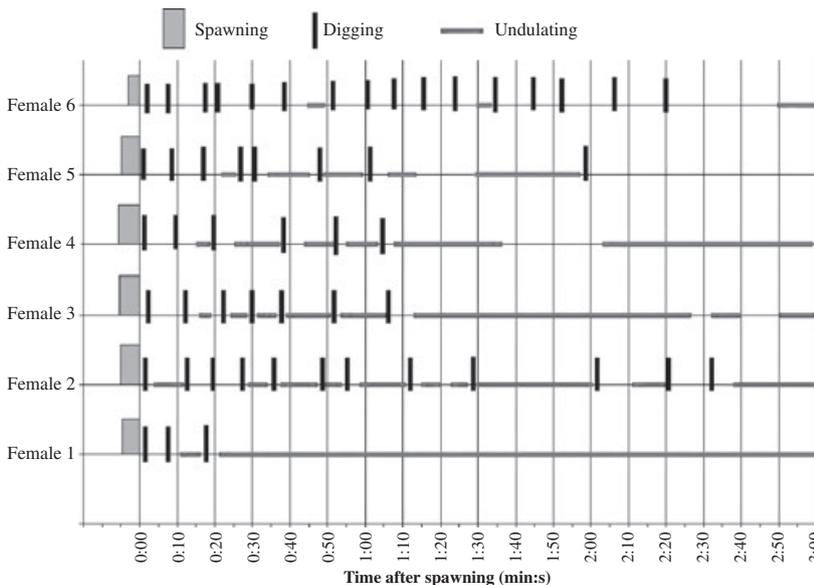

Fig. 4. Pictorial representation of the 3-min postspawning activity of six amemasu females.

spawning, two similar sized males joined the pair. The female performed one cover digging 8 s after spawning, then interspersed 10 undulatings with 12 cover diggings.

Female 3 (Tokimae, October 5, 2008)

Recordings started at 10:18, spawning occurred at 11:07 and lasted 4 s. During spawning, four additional males joined the pair. The female performed one cover digging 4 s after spawning, then interspersed seven undulatings with seven cover diggings.

Female 4 (Tokimae, October 5, 2008)

Recordings started at 12:35. At that time, the female was involved in finishing nest construction. At 12:45, she performed one false spawning, then 24 s later she performed two consecutive spawning acts (*sequential spawning* – two or more spawning acts performed on a single nest and separated by a few seconds or minutes – is another behaviour unique to *Salvelinus*; Esteve & McLennan 2007). The first spawning act lasted 3 s. The female remained motionless in the nest for 11 s and then performed a second spawning. The second spawning act lasted 5 s. During this last spawning, four males joined the pair. The female performed one cover digging 5 s after spawning, then interspersed six undulatings with five cover diggings.

Female 5 (Onbetsu, October 26, 2008)

Recordings started at 8:07. At that time, the female was involved in finishing nest construction. Spawning occurred at 8:12 and lasted 6 s. During spawning, four similar sized males and one small male joined the pair. The female performed one cover digging 7 s after spawning, then interspersed five undulatings with nine cover diggings.

Female 6 (Onbetsu, October 26, 2008)

Recordings started at 9:08. Spawning occurred at 9:24 and lasted 3 s. During spawning, five similar sized males joined the pair. The female performed one cover digging 3 s after spawning, then interspersed three undulatings with 15 cover diggings.

Evolution of the postspawning behaviour

Optimising the three different postspawning character states onto the salmonine supertree (Fig. 5) indicates that 'resting immediately after egg deposition, then cover digging' is plesiomorphic for the clade. From that plesiomorphic background, 'undulating [followed by the plesiomorphic condition of cover digging]' arose in the ancestor of *Salvelinus*, and 'cover digging immediately after egg deposition' appeared twice (is a convergent trait), once in the ancestor of *Parahucho* +
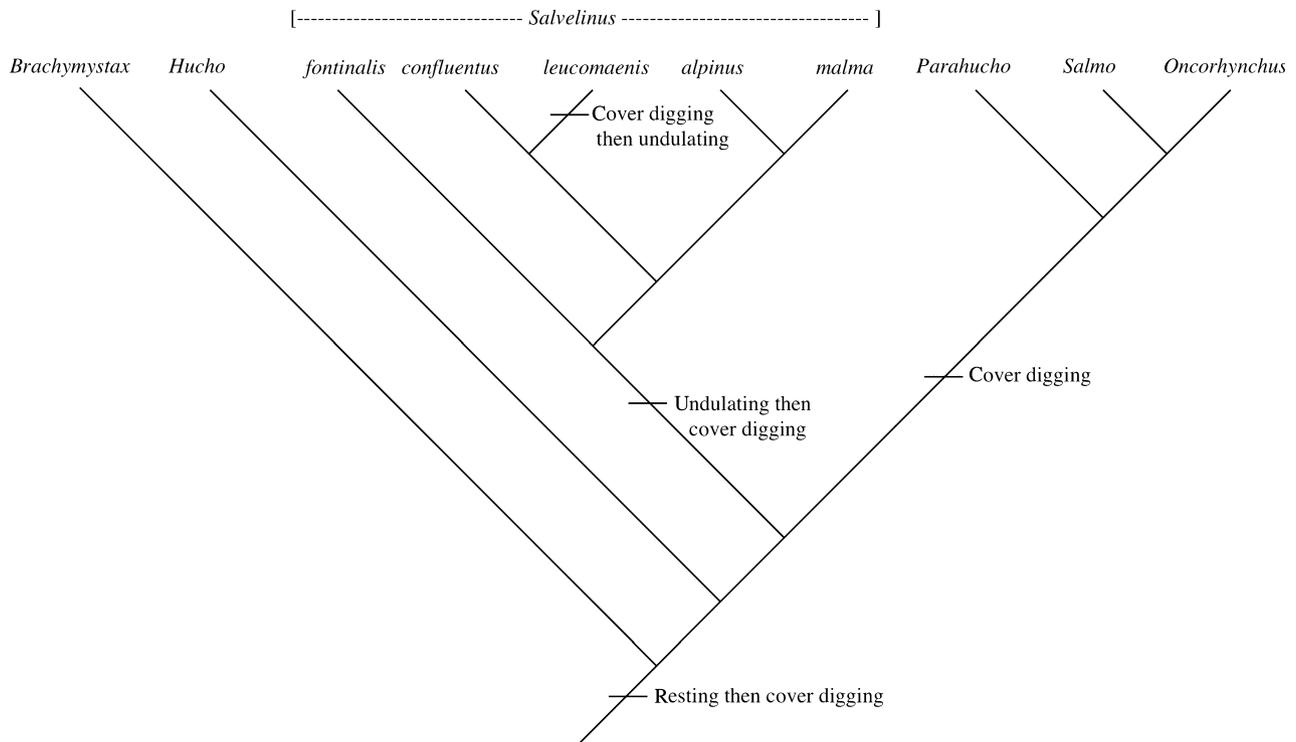

Fig. 5. Postspawning character states optimised by maximum parsimony onto the supertree for the Salmoninae. The phylogenetic tree (Wilson & Williams 2010) represents a compilation of studies using morphological, molecular and behavioural data.

Salmo + *Oncorhynchus* and again in the ancestor of *Salvelinus leucomaenis leucomaenis*.

Discussion

Amemasu spawning behaviour

In general, the spawning behaviour of amemasu resembles that of other *Salvelinus* species (Fabricius & Gustafson 1954; Groot 1996): females spend their time building and guarding nests, while males fight to monopolise the nesting female. The winner courts the female and guards her from rivals. As nest building progresses, a depression in the gravel is gradually formed. The female eventually tests her nest's depth and conditions by laying over it with her caudal fin raised, forcing her anal fin into the gravel (*probing*). Probing causes an increase in courting by the attendant male and also increases the attempts of subordinate males to approach the female. Once the nest is finished, the female signals imminent oviposition by gaping her mouth and by trembling her body. The attendant male joins her and both release their gametes over the centre of the nest depression while trembling and gaping (Fig. 2). At this point, subordinate males join the pair and release sperm (100% of the spawnings were accompanied by subordinate males). Small males at the parr stage also frequently rush in during spawning, possibly releasing sperm, feeding on eggs or both.

The conservative nature of the spawning repertoire aside, we documented a remarkable and unexpected behavioural difference between amemasu and other *Salvelinus* species: females from two geographically distant Hokkaido populations covered their eggs by beats of their tail (cover digging) immediately after spawning *then* performed the classic undulating behaviour, rather than *vice versa*. Our results were confirmed for three additional amemasu females in the Onbetsu River (K. Akiwa, unpublished observation). In all of the nine documented cases, females first performed 1–2 cover diggings, then alternated periods of active undulating with more cover diggings (Fig. 4).

Macroevolutionary patterns of postspawning behaviour origin and diversification

One very powerful way to reconstruct the evolutionary history of a particular behaviour is to trace its origin and diversification within a phylogenetic framework (Brooks & McLennan 1991, 2002). Optimising the three states of the character 'postspawning behaviour' onto the supertree for the salmonines (Wilson & Williams 2010) (Fig. 5) indicates four things. First, 'resting then cover digging' is plesiomorphic for the salmonines. Second, the behaviour cover digging was advanced in time to replace resting (it now occurs right after oviposition) in the ancestor of *Parahucho* + *Salmo* + *Oncorhynchus*. If we consider the sequence of behavioural expression to reflect a developmental pathway, then this change may be interpreted as an outcome of heterochrony (sensu Alberch et al. 1979); a change in the rate or timing of development (see McNamara & McKinney 2005 for an extensive review). What could be the function of such an ontogenetic shift? At the moment, we do not have enough information to answer this question. For example, covering the eggs immediately might have been a response to an increase in egg predation, either from conspecifics, which has been documented in at least *Salmo*, *Salvelinus* and *Oncorhynchus* (Greeley 1932; Maekawa & Hino 1990; Blanchfield & Ridgway 1999) or from other species (e. g., Scheuerell et al. 2007; Denton et al. 2009; Garner et al. 2009). To investigate this hypothesis, we need data about the levels of egg predation in the immediate seconds following oviposition across the salmonines. The hypothesis would be supported if those levels are low in *Brachymystax* and *Hucho* and show a significant increase in the remaining salmonines.

Third, a novel behaviour, undulating, replaced resting in the ancestor of *Salvelinus*, while the next part of the postspawning sequence, cover digging, remained in its plesiomorphic position; that is, digging is initiated several minutes after oviposition. During the undulating process, very often the tail, anal and ventral fins are pressed against the gravel, effectively moving loose pebbles inward towards the centre of the pit (Greeley 1932; observations in this study). Field observations indicate that undulating females completely covers their eggs in a few minutes (Fabricius & Gustafson 1954; Frost 1965); however, these anecdotal reports of function have not been corroborated by detailed experiments for any species. Even if undulating does function to cover the eggs quickly after oviposition, why undulating in *Salvelinus*, and not an advance of cover digging as in *Parahucho* + *Salmo* + *Oncorhynchus*? To answer this, we need more information about the ecological conditions in the nest area. For example, *Salvelinus* species very often breed in lakes, but for *Parahucho*, *Salmo* or *Oncorhynchus* species, still-water spawning is exceptional (Watson 1999; Esteve et al. 2009b). Perhaps, cover digging is not as effective in still waters because there is no current to move the gravel that the female displaces with her tail beats. Or perhaps, undulating and cover digging are two ways to solve the same problem (covering the eggs) given a subtly different substrate. Once again, more data, this time concerning habitat parameters such as current and substrate type, are
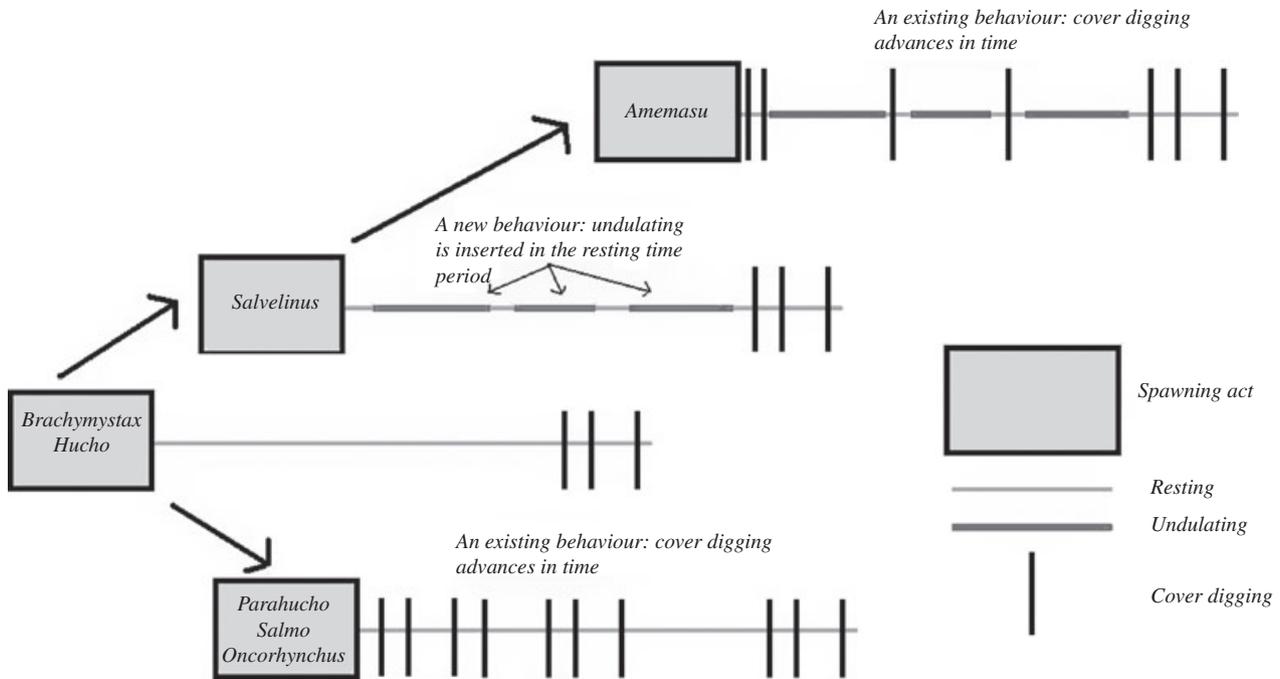
Fig. 6. Potential mechanism of the transitions in the Salmoninae postspawning behaviour.

required before we have an explanation for the observed macroevolutionary pattern.

Finally, as in the ancestor of *Parahucho + Salmo + Oncorhynchus*, the expression of cover digging has been advanced in the amemasu. Recurring peramorphosis (developing a behaviour earlier than expected) is thought to be one mechanism producing convergent (homoplasious) change throughout evolution (Fink 1982; Brooks & McLennan 2002). Interestingly, this ontogenetic shift is associated with the movement by amemasu into fast flowing waters for spawning, supporting the preceding suggestion that the effectiveness of undulating and cover digging may depend upon the velocity of water moving over the nest area. So, why should amemasu continue to undulate? We recorded a large variation in the time females spent undulating. Females 5 and 6, who showed extremely low levels of undulating (Fig. 4), spawned in the Onbetsu River where the water velocity (judged by eye) was considerably faster than in the Tokimae River. This was particularly pronounced for the area in which female 6, who barely performed any undulating movement during the 3-min postspawning period, was filmed (Fig. 4). It is thus possible that whether a female continues to perform undulating or cover digging after the initial cover digging bout is conditional upon environmental feedback (i.e., if water velocity passes a certain threshold you cover, if not you undulate). Clearly, a large number of observations and controlled experiments are necessary to corroborate this hypothesis.

In summary then, it appears that the evolution of postspawning behaviours in the Salmoninae has been complicated, involving the origin of a new character (undulating) and two convergent changes in the timing of cover digging (Fig. 6). Despite the fact that cover digging and undulating have been reported in the literature for many decades, few studies have addressed the function of those behaviours in a rigorous experimental framework. Without such data, it is impossible to move beyond macroevolutionary patterns of character origin and diversification to a more comprehensive picture of spawning behaviour evolution in these fishes. Hopefully, this study will encourage biologists to begin such studies, which will include collecting information about factors such as levels of egg predation, water velocity and substrate composition in the field, then exploring those data experimentally.

Acknowledgements

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Video Clip S1. Video showing an amemasu female (Female 1 of this study) during the spawning act and the immediate seconds after it. The female performs two *cover digs* after spawning, then starts the *undulating* behaviour for a few seconds, then digs once more and then continues *undulating* for the rest of the sequence.

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