Preliminary evidence that sculpin species native to the Pacific northwest do not serve as a host in the reproductive cycle of the western pearlshell mussel (*Margaritifera falcata*)

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Introduction

In September of 2008, the U.S. Fish & Wildlife Service (USFWS) transferred 100 western pearlshell mussels (*Margaritifera falcata*) from populations in the Bear River to three creeks that lay within the boundaries of the Willapa National Wildlife Reserve (Fernandez 2007). The translocation was intended to begin establishing a healthy, self-sustaining population of western pearlshell mussels (WPM) in streams within the refuge. As a part of the translocation, the USFWS has proposed to monitor the transplanted populations on the refuge to determine survival and growth, as well as potential reproduction and recruitment (Fernandez 2007).

The larvae (glochidia) of unionid mussels such as the western pearlshell are obligate parasites on fish and other aquatic vertebrates (Parmalee and Bogan 1998). Thus, for WPM to successfully colonize an area, potential host fish are needed. Some hosts for the western pearlshell have been tentatively identified, including cutthroat trout, rainbow trout, Chinook salmon, coho salmon, sockeye salmon, speckled dace, Lohontan redside, Tahoe sucker and non-native brook and brown trout (Nedeau et al. 2003). However, some of these fish may not be suitable hosts because they slough encysted glochidia before the mussels can transform to the juvenile phase (Bigham 2002), while others may act as successful hosts in lab studies, though they don't act as hosts under natural conditions (Layzer et al. 2003). In general, sculpin can host mussel larvae (Neves et al. 1985, Zimmerman 2003). In the Pacific Northwest, native scuplin have been found to host the larvae of the western floater (*Anodonta kennerlyi*) (Martel and Lauzon-Guay 2005). Relatively little information is available concerning whether sculpin can serve as a host for WPM.

Sculpin have been sampled from and are present in the Bear River system, as well as the Headquarters Creek and North Creek (Fernandez 2008). Freshwater sculpin are not known to migrate long distances and some studies indicate that sculpins have restricted home ranges (Petty and Grossman 2007, Morgan and Ringler 1992, McCleave 1964). Because potentially infested sculpin would likely remain in proximity of the source of infestation, detection of glochidiosis among the population and simple spatial analysis may be facilitated. The purpose of this study was to determine if sculpin were acting as suitable host fish for the western pearlshell in the Bear River drainage. The primary objectives were:

1) Determine whether sculpin in the Bear River were infested with mussel larvae;

2) Determine the proportion of sculpin that were infested;

3) Determine whether infested sculpin were able to produce viable mussel juveniles;

4) Determine the proportion of sculpin that were able to produce viable juveniles.

Study Area

The Bear River is a third order stream that drains to Willapa Bay in the Coastal Range ecoregion of Washington (Figure 1). This area is characterized by highly productive, rain-drenched coniferous forests. Within the study area, four sample sites were established, with each site

being broken up into ten, 50-meter sample reaches (Figure 2, Table 1). The entire study area covers approximately 5 km of the river. The stream in this area is a low gradient channel with a series of run, riffle and pool habitats. The majority of the channel is less than 1 meter deep. The project area was surveyed previous to this investigation, and relative density and distribution of WPM were determined (Fernandez 2007). Most literature describes the spatial distribution of mussels in the context of large aggregations of relatively dense population commonly referred to as mussel beds. However, the population of WPM in the Bear River did not exhibit this pattern of distribution. Mussels tended to occur in small patches that appeared to be haphazardly distributed.

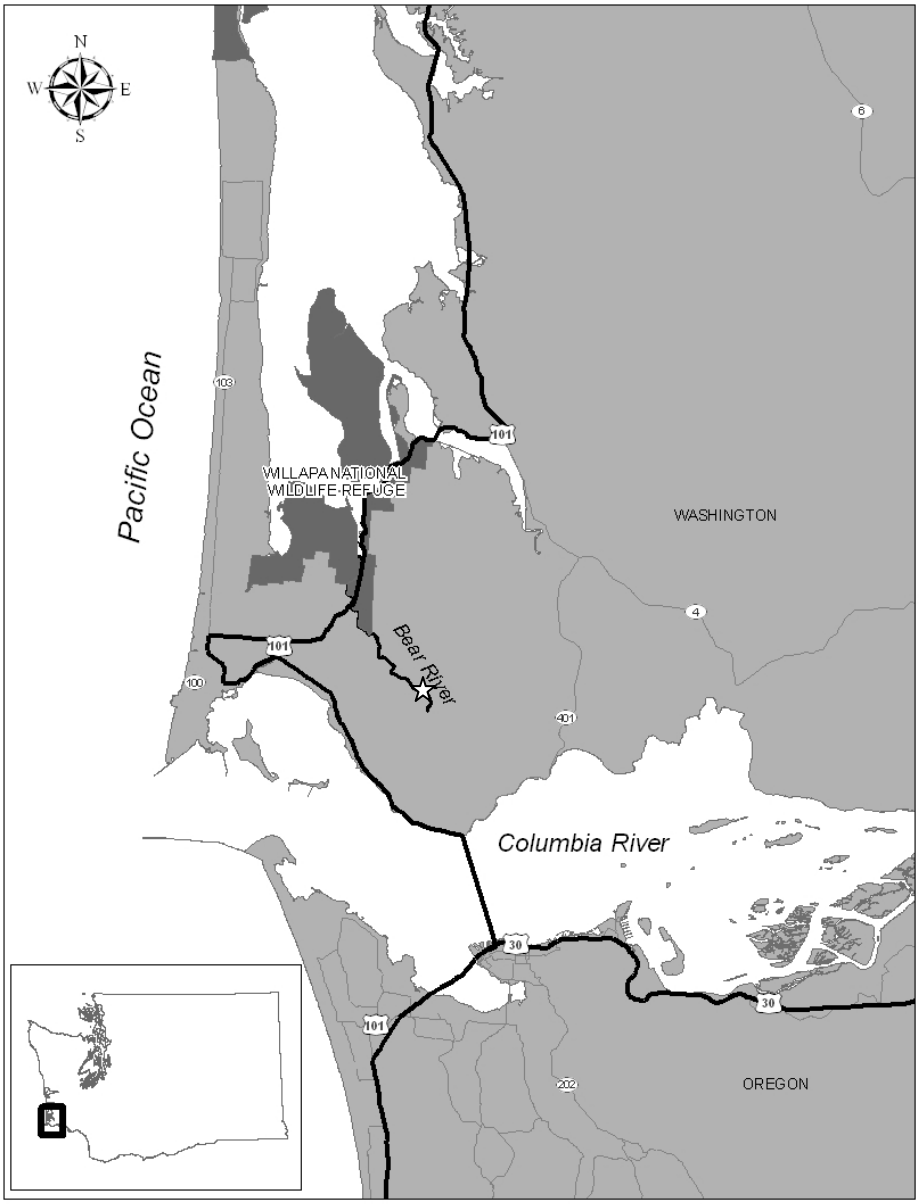

Figure 1. Location of the Bear River study area ☆ .

Mussels were most frequently found along stream margins associated with large bed roughness elements such as embedded logs. Patches ranged in size from a few square meters to approximately 20 m^2. The distribution of these patches was haphazard within the watershed. All patches were within 25 m of at least one other patch, and there were large stretches of the stream in which no mussels were observed interspersed among the aggregations of patches.

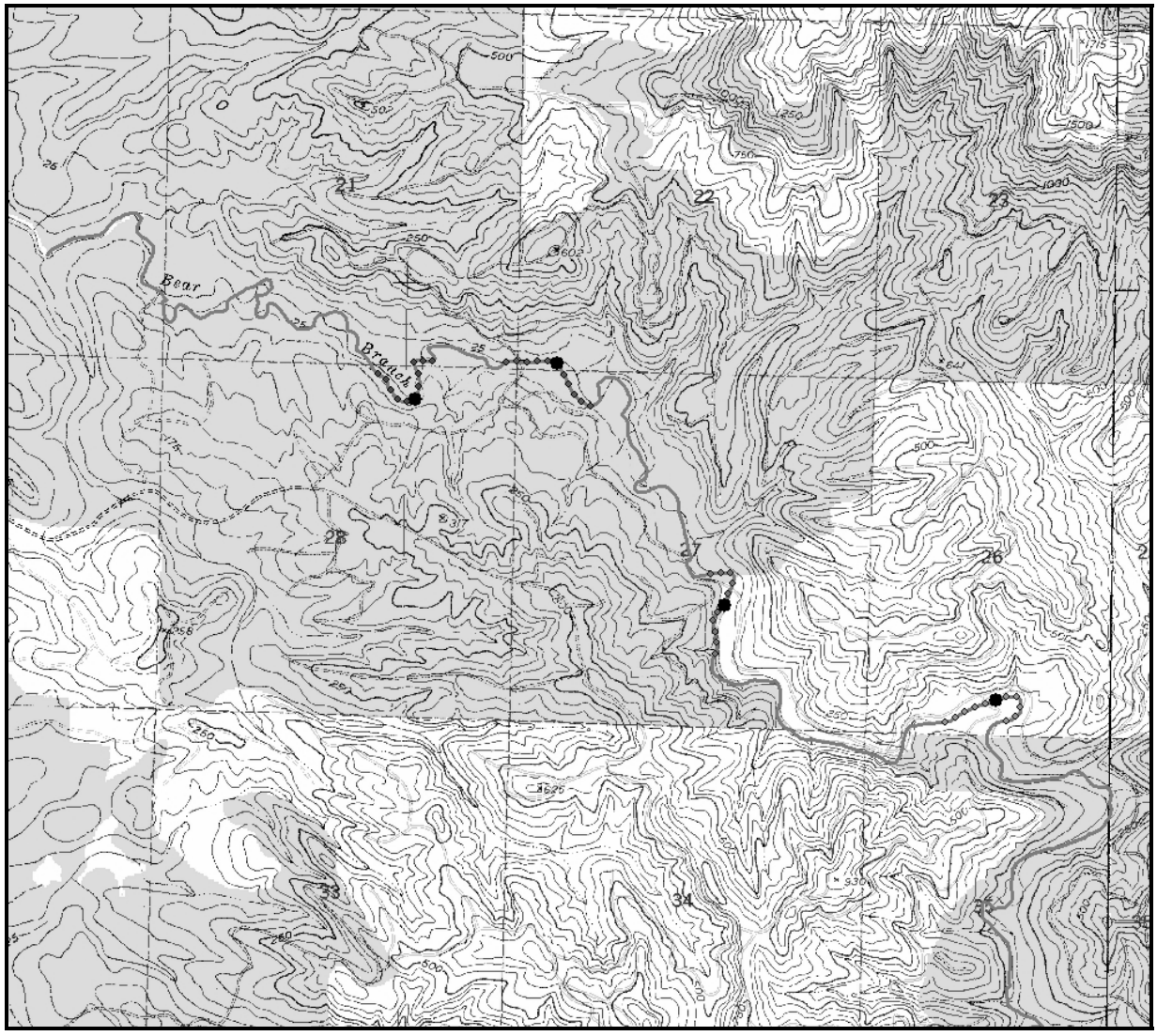

Figure 2. Location and distribution of four sample sites (large circles) and 40 sample reaches (small circles) in the Bear River.

Table 1. Location of the four sample sites and 40 sample reaches in the Bear River (LLID 1239381463343 (also see Figure 2).

Study Site	Study Reach	UTM X	UTM Y
1	1	429220.419	5131062.130
	2	429257.743	5131033.933
	3	429279.266	5130986.024
	4	429309.683	5130945.173
	5	429350.927	5130935.639
	6	429391.292	5130945.803
	7	429406.015	5131001.678
	8	429405.833	5131059.323
	9	429386.382	5131112.125
	10	429424.488	5131120.404
	11	429467.084	5131119.183
2	1	429803.027	5131111.016
	2	429849.403	5131116.285
	3	429896.328	5131112.810
	4	429943.389	5131115.436
	5	429990.391	5131119.377
	6	430035.108	5131105.657
	7	430062.945	5131057.557
	8	430083.972	5131011.019
	9	430110.065	5130965.013
	10	430145.396	5130934.569
	11	430182.856	5130911.973
3	1	430729.719	5130158.137
	2	430773.668	5130159.245
	3	430817.514	5130161.646
	4	430826.151	5130114.523
	5	430806.355	5130068.182
	6	430791.070	5130018.012
	7	430761.064	5129985.687
	8	430739.320	5129944.381
	9	430747.312	5129894.605
	10	430754.004	5129844.906
	11	430748.674	5129795.760
4	1	431789.179	5129486.066
	2	431835.353	5129507.940
	3	431879.871	5129534.498
	4	431925.613	5129555.180
	5	431972.419	5129570.331
	6	432019.051	5129586.585
	7	432066.624	5129601.446
	8	432115.574	5129601.700
	9	432131.805	5129552.966
	10	432107.675	5129510.771
	11	432069.048	5129494.138

Materials and Methods

The project was performed in three phases.

Phase I – Determining gravidity

Phase I began in mid-June, 2008. Based on available information, we assumed mussels would be reproductive for approximately 4-6 weeks (Nedeau et al 2005). Previously identified beds in the study area were surveyed weekly to determine when mussels became gravid. To determine gravidity, a minimum of 10 mussels were collected during each sampling event. Mussels were measured along the longest axis, pried open approximately 2-3 mm using a dull pocket knife, and marsupial gills were inspected. Inflated, opaque marsupial gills were assumed to be positive signs of gravidity (Haley et al 2007). If possible, glochidia were extracted from the gills of one individual to determine viability of the glochidia. Glochidia were extracted by flushing a gill tube with water using a small syringe and needle (Zale and Neves 1982). Material flushed from the marsupial gill was placed in a Petri dish and observed under a dissecting microscope. To establish viability, a few grains of salt were introduced to the dish. Viable glochidia snap shut when exposed to sodium ions (Coker et al. 1921).

Another indicator that mussels are gravid is the presence of conglutinates. Many species of freshwater mussels, including the western pearlshell, produce packets of glochidia contained within a mucosal envelope called a conglutinate. The conglutinate of the western pearlshell is a branched filamentous white mass approximately 1 cm long (Frest and Johannes 1995). As a secondary method of confirmation that gravid mussels were present, we looked for conglutinates on the substrate in proximity to WPM.

Phase II – Sculpin collections

Live sculpin (Cottus sp.) were collected from all four sample sites (Table 2). To minimize stress from handling, sculpin were not identified to species. On a relative scale, locations 1 and 4 were low mussel density. Patches of mussels were scattered and sparse and the majority of surveyed mussel patches held fewer than 20 individuals. Conversely, locations 2 and 3 were relatively high mussel density. Mussel patches occurred more frequently and the majority of surveyed patches held more than 20 individuals. In all cases, patch sizes were small ranging in size from approximately 2 m^2 to approximately 20 m^2. Sculpin were collected by electrofishing from each study location 2, 4, and 6 weeks after mussels were expected to begin expressing gravidity (approximately 7/1/08). During electrofishing, both live specimens and specimens to be sacrificed were collected. We collected a total of 40 sculpin (20 sacrificed and 20 live), from each sample site, during each of the three sampling events. To minimize any bias in our collections, we sampled five reaches at each of the four locations for a total of 20 sample reaches each event. We collected 2 sculpin, one to be sacrificed and 1 to be reared, from each sample reach. At each sample location, sampling began at the lower most sample reach. The location of each sculpin, sacrificed or retained, was georeferenced, and the total length and weight of each sculpin was measured. All electrofishing variables (i.e. voltage) were also be recorded.

Table 2. Sculpin species known or suspected to occur in the Bear River, WA. (NatureServe 2008).

Scientific Name	Common Name
Cottus aleuticus	Coastrange sculpin
Cottus asper	Prickly sculpin
Cottus bairdii	Mottled sculpin
Cottus gulosus	Riffle sculpin
Cottus rhotheus	Torrent sculpin

Phase II – Sculpin examinations

Detecting infestation

The sacrificed sculpin were preserved *in situ* in 100% ethanol and stored in individual plastic bags. Each bag was tagged with the site and reach number as well as the collection date. Sacrificed specimens were returned to the CRFPO lab where gills were to be examined for the presence of encysted glochidia. After a minimum of 24 hours in ethanol, the gills were excised from each fish rinsed and soaked in a 0.05 molar solution of KOH for at least 2 minutes (as per Layzer et al. 2003). The KOH solution causes gill filaments to become translucent, making it easier to observe encysted glochidia. Excised gills were then placed under a dissecting microscope at 10x magnification. If a glochidium was observed, it was recorded and measured under a magnification of 40x, using a digital micrometer. Glochidia were measured along the hinge line, along the longest axis parallel to the hinge line, and then along the longest axis perpendicular to the hinge line. The glochidia of Margaritiferid species are significantly smaller than glochidia of other unionid mussels (Hoggarth 1999) and mussels do not exhibit significant growth during transformation from the larval stage to the juvenile stage (Howard and Anson 1922). Since the WPM is the only Margaratiferid known to occur in the Willapa Bay basin, comparing the dimensions of glochidia collected from gravid mussels to those on encysted gills was used to confirm the identity of encysted mussels. Glochidia from WPM are < 0.15 mm in length (Spring Rivers 2007)

Assessing transformation

Live specimens were returned to the USFWS, Columbia River Fisheries Program Office (CRFPO) in Vancouver, Washington. To confirm whether WPM glochidia were present in and transform on sculpin hosts, sculpin were reared for a period of 4-6 weeks. Live sculpin were reared individually in marked containers so that each fish could be identified according to the site from which it was taken and the date on which it was collected. Initially, fish were reared in spring water at ambient temperature (mean 21ºC). The containers were not aerated but half of the water in each container was replaced every two days. Due to high initial mortality rates

during the first week in captivity, constant aeration was subsequently provided for each container and the fish were reared in 10ºC water. Fish were fed (pieces of meal worm or frozen brine shrimp) ad libitum once every two days. To isolate the sculpins from potential excysting juveniles, wire mesh was placed in the container approximately 2.6 cm from the bottom. The mesh was large enough to allow excysting mussels to fall through, while preventing the sculpin from accessing the bottom and potentially consuming any excysted mussels. Once every two days containers were emptied and the water from each container was drained through a 60-µm sieve. The contents of the sieve were rinsed into a Petri dish and observed under a dissecting microscope. If any juvenile mussels were detected, they would be observed for signs of life. Juvenile mussels are pedal feeders and frequently sweep their feet back and forth to collect food particles. Observation of such behavior would be accepted as proof that a juvenile was alive and had successfully transformed.

Analysis

To determine whether sculpin were potential host fish for the western pearlshell, occurrence of glochidiosis was evaluated. To determine the rate at which sculpin were infested, the proportion of sacrificed sculpin that were infested was calculated. To determine if infestation is related to the density of mussel beds, Fisher's Exact test was performed to compare the infestation rates of sacrificed sculpin from locations 1 and 4 to that of sacrificed sculpin from locations 2 and 3. To determine whether infested sculpin were able to produce viable mussel juveniles, the presence of live juveniles in the rearing containers was assessed during the rearing experiment. To determine the rate at which sculpin are able to produce viable juveniles, the proportion of captive-reared sculpin that produce juvenile mussels was calculated. To determine if the production of viable juveniles is related to the density of mussel beds, Fisher's Exact test was performed to compare the proportion of captive-reared sculpin from locations 1 and 4 to that produce juvenile mussels to the proportion from locations 2 and 3.

Results

Phase I – Determining gravidity

Given that a gravid mussel was observed during the first field visit to the Bear River and that mussels with partially inflated gills were observed on subsequent visits, it is likely that reproduction of western pearlshell mussels was occurring in the Bear River. During the study period, only one gravid mussel was observed on June 20, 2008 in the upstream section of Site 2. However, during all sampling events >10 % of mussels were observed with gill sections that were partially inflated (Table 2). Collection of glochidia from the one gravid mussel observed was not successful. The 2.6-cm long needle on the syringe was unable pierce deep enough into a gill tube to flush out conglutinates. Any attempt to pry the mussel open wider to facilitate gill flushing would likely have damaged the adductor muscles leading to the death of the individual. For future studies, a syringe of at least 5.2 cm is recommended.

Table 2: Occurrence of gravid mussels in Bear River, WA (06/20/08, 07/15/2008).

Date	Number Observed	Number Gravid	% Gravid	Partially Inflated	% Partial
6/20/2008	30	1	3.3%	11	36.7%
7/15/2008	19	0	0.0%	2	10.5%

Phase II – Sculpin collections

Sculpin were successfully collected during each sampling event. The fish were readily available in the sample areas which resulted in 80 fish that were sacrificed and 84 that were transported to CRFPO for rearing. All sculpin survived transportation however, in captivity, sculpin suffered a high mortality rate (85%) and those that died did so rapidly (2-7 days) (Table 3).

Table 3: Mortality of sculpin collected from the Bear River, Washington

Date	No. Collected	No. Sacrificed	Mortality	Mortality (%)	Mean Time to Death (Days)
7/15/2008	40	20	19	95%	2.3
7/21/2008	44	20	18	75%	4.8
7/25/2008	40	20	20	100%	4.6
8/5/2008	40	20	14	70%	6.4
Total	164	80	71	85%	

Phase III – Sculpin examinations

Detecting infestation

Eighty sacrificed sculpin and seventy-one sculpin that died during rearing experiment were inspected for glochidiosis. No glochidia were observed on sacrificed sculpin or sculpin that died during the rearing experiment. Therefore, the observed infestation rate was zero, and no relationships were detected between infestation rates and mussel density or proximity of sculpin to mussels.

Assessing transformation

No juvenile WPM, live or dead, were observed in filtrate collected during the rearing experiment.

Analysis

None (0%) of the sacrificed sculpin or those that died in captivity were infested with glochidia. The infestation rate sacrificed sculpin from locations 1 and 4 (0%) was the same as that of sacrificed sculpin from locations 2 and 3 (0%). None (0%) of the captively-reared sculpin produced juvenile WPM. The production of viable WPM juveniles by sculpin from locations 1 and 4 (0%) was the same as that of sacrificed sculpin from locations 2 and 3 (0%).

Conclusions

Sculpin did not appear to act as a reproductive host for *Margaritifera falcata* in the Bear River during the late spring and summer of 2008. We found no evidence of glochidia infestation on either sacrificed sculpin or sculpin that died during the rearing experiment. Given that sculpin did not appear to be infested, we were not able to determine if sculpin can produce viable juveniles. In any event, this preliminary information suggests that it may be difficult or impossible to establish WPM populations in streams whose only fish are sculpin.

Alternatively, it is possible that infestation rates were too low to be successfully detected in 2008 because it was a poor year for WPM reproduction or because reproduction occurred at a different time of the year. Mussel reproduction can be highly variable between years (Lefevre and Curtis 1912, Coker et al 1921). In the present investigation, partial inflation of gills suggests that either the mussels had recently discharged glochidia from their gills, and the gills had not yet returned to their normal flaccid state, or that the gill was beginning to charge. However, expelled conglutinates were not observed during any of the sampling events. Given these uncertainties, future sampling would benefit from 1) documenting when WPM reproduce, 2) determining whether there are environmental variables that can be used to predict the timing of WPM reproduction and 3) including fish species, such as cutthroat trout (Fuller 1974, Karnat and Millemann 1978), that are known to host WPM as a positive experimental control.

Rearing sculpin in captivity was extremely challenging. The high mortality rate among sculpin reared in captivity was likely due to a combination of factors. First, sculpins were subjected to lengthy transport (> 2 h) after collection. The sculpin were transported in 19 L buckets and there were five or more sculpin per container. The combination of crowding, lack of cover and constant jostling was likely a source of stress. Second, water temperatures in the lab were significantly warmer than water temperatures in the Bear River during the first collection effort. The transport buckets were allowed to sit for at least 8 hours in the lab so that the water could gradually warm, minimizing the impact of thermal shock. However, Otto and Rice (1977) found that maximum thermal tolerance among different populations of slimy sculpin (*C. cognatus*) differed, and was correlated with temperatures to which each population was acclimated. At the time of collection, water temperature in the Bear River on 7/15/2008 averaged approximately 10ºC (varying over the course of a day), while ambient temperature in the lab averaged approximately 21°C (varying relatively little over the course of a day). It is possible that the

water temperature in the lab was at or above the maximum thermal tolerance of sculpin populations in the Bear River. Mean survival time increased once the rearing experiment was moved to a temperature controlled room (range 9.5º-12.0°C), aeration was provided for each rearing container, and cover was provided. However, even under these conditions, 100% survival was not achieved. Thus, the ability to successfully rear sculpin in captivity needs to be improved.

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