Evidence of Natural Reproduction of Aquaculture-Escaped Atlantic Salmon in a Coastal British Columbia River

JOHN P. VOLPE,*§ ERIC B. TAYLOR,† DAVID W. RIMMER,‡ AND BARRY W. GLICKMAN*

*Centre for Environmental Health, Department of Biology, University of Victoria, P.O. Box 3020, Stn. CSC, Victoria, British Columbia V8W N1G, Canada

†Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia V6T 1Z4, Canada

‡British Columbia Ministry of Environment, Lands and Parks, 2080-A Labieux Rd., Nanaimo, British Columbia V9T 6J9, Canada

Abstract: *We present evidence of the first successful natural spawning of Atlantic salmon (*Salmo salar*) documented on the Pacific coast of North America. Twelve juvenile Atlantic salmon composed of two year classes were captured in the Tsitika River, British Columbia. We analyzed restriction-length polymorphisms of PCR-amplified 5S rDNA and mtDNA to confirm that these individuals were Atlantic salmon. Scale analysis strongly suggested they were the products of natural spawning by feral adults. The gut contents, size, and condition of these individuals suggest that Atlantic salmon are successfully maturing in the Tsitika River, British Columbia. This event has raised concerns that the presence and possible establishment of feral Atlantic salmon may further jeopardize the continued persistence of already fragile native Pacific salmonids through competition for resources and occupation of niches that are currently underutilized.*

Evidencia de la Reproducción Natural de Salmones del Atlántico Escapados de Instalaciones de Acuicultura en un Río de la Costa de British Columbia

Resumen: *Presentamos evidencia del primer desove exitoso de salmón del Atlántico (*Salmo salar*) documentado en la costa del Pacífico de Norte América. Doce salmones del Atlántico juveniles comprendiendo dos clases anuales de edad fueron capturados en el río Tsitika, British Columbia. Analizamos polimorfismos de longitud restringida de ADNmt amplificados con PCR para confirmar si estos individuos eran salmones del Atlántico. El análisis de escamas sugiere fuertemente que estos organismos son producto de un desove natural de adultos silvestres. El análisis de contenidos estomacales, tamaño y condición de estos individuos sugiere que el salmón del Atlántico esta desarrollándose de manera exitosa en el río Tsitika, British Columbia. Este evento ha elevado la preocupación de que la presencia y posible establecimiento de salmones del Atlántico silvestres podría poner en riesgo la persistencia continuada de los de por sí ya frágiles salmones del Pacífico mediante la competencia por recursos y la ocupación de nichos que actualmente son poco utilizados.*

Introduction

International consumer demand for fresh salmon of consistent size and quality has generated a significant salmon aquaculture industry in coastal British Columbia. Approximately 76% of commercial finfish aquaculture production in 1997 (30,700 tonnes dressed, British Columbia Ministry of Agriculture and Food) was of Atlantic

§*email jvolpe@uvic.ca*

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salmon (*Salmo salar*), an exotic species. In 1997, 7,472 Atlantic salmon were reported escaped from British Columbia marine net pens (Thomson & Candy 1998). During the same period 2655 free-ranging individuals were captured at sea and 155 individuals were reported captured or sighted in fresh water (Thomson & Candy 1998). The actual number of Atlantic salmon escapes and recoveries or sightings are not known. Further, few data exist on the fate of aquaculture-escaped Atlantic salmon and on the effects their presence in the northeast Pacific may have on native fauna. This lack of knowledge is not limited to Atlantic salmon in the Pacific but is typical of most potential invasions worldwide. Vermeij (1996) points out that there is no standardized predictive model of invasion ecology. A crucial step toward establishment of any prospective invader, however, is successful reproduction in the recipient habitat (Carlton 1996; Grosholz 1996).

There have been no documented cases of successful reproduction of Atlantic salmon in the northeast Pacific, despite numerous reports of adult Atlantic salmon in both freshwater and marine coastal environments. We report the first such case.

Study Site

The Tsitika River is a moderate-sized (42-km mainstem length), remote river system on the northeast coast of Vancouver Island approximately 325 km north of Victoria, Canada (mouth: lat 50°29′N, long 126°35′E). The Tsitika River drains a watershed of 360 km^2 and supports eight species of native salmonids: anadromous summer steelhead trout–resident rainbow trout (*Oncorhynchus mykiss*), coho salmon (*O. kisutch*), sea-run cutthroat trout (*O. clarkii*), chinook salmon (*O. tshawytscha*), chum salmon (*O. keta*), sockeye salmon (*O. nerka*), pink salmon (*O. gorbuscha*), and Dolly Varden charr (*Salvelinus malma*). A partial migration barrier is located 3 km upstream from the estuary and restricts further upstream migration by chinook, chum, sockeye, and pink salmon. Of the total mainstem length, 40 km is suitable spawning and rearing habitat and is accessible to anadromous adults that can clear the barrier.

Occurrence of *Salmo* sp.

On 18–20 August 1998, what appeared to be four juveniles of the genus *Salmo* were captured in the Tsitika River. Eight more individuals were captured on 26–28 September 1998 in the proximity of the initial captures. Twenty-eight more individuals were observed but not captured during snorkel surveys, bringing the total number of the unidentified juveniles to 40. Some reaches of the river were surveyed more than once, so some individuals likely represent repeat sightings. A resident population of introduced brown trout (*Salmo trutta*) reside in the adjacent Adam River system but have not been recorded in the Tsitika River. Atlantic salmon and brown trout can be distinguished by a number physical features, but, the two appear similar as juveniles and can be confused. Thus it was important to confirm that the fish captured in the Tsitika River were not brown trout. All fish were observed or captured between 5.0 and 10.5 km upstream of the estuary (above the partial migration barrier) in head or tail riffle sections above coarse gravel and boulder substrate. In all cases, the sampled fish were observed among or in close proximity to *O. mykiss* juveniles. Rimmer (1998) provides details of the sampling sites and methods.

Methods

Tissue samples from the initial four captured individuals were used to test species identity as outlined by Taylor et al. (1996). Control samples of two known Atlantic salmon (commercial McConnell strain) and two brown trout (Adam River, British Columbia stock) obtained from a commercial farm and a provincial hatchery, respectively, were also assayed. An approximately 3.0 kilobase (kb) fragment of mitochondrial DNA (mtDNA) encompassing the cytochrome b gene, the d-loop, and a portion of the 12S ribosomal RNA (rRNA) gene was amplified by the polymerase chain reaction (PCR) with the primers "GLUDG-5" and "12SAR-3" (Palumbi 1996) in 50-μL reactions. The PCR products were incubated overnight at 37°C in the presence of the restriction enzymes *Alu*I, *Dde*I, *Hae*III, and *Rsa*I, which assay restriction site variation that differentiate Atlantic salmon from brown trout. The diagnostic sites were obtained from d-loop sequences reported by Bernatchez et al. (1992), which distinguish Atlantic salmon from brown trout. A second molecular system was employed to confirm the diagnosis. This involved PCR amplification of the 5S rDNA (biparentally inherited) region of the 5S ribosomal RNA gene by means of the Atlantic salmon–brown trout discriminatory primers "5SA" and "5SB" (Pendas et al. 1995).

Scale samples were taken from the initial four captured fish for determination of origin. Fish growth and age can be assessed by examining the circuli or concentric growth rings of the scales (International Council for Exploration of the Sea 1984). As in trees, periods of rapid growth (late spring to early fall in wild, northern-hemisphere salmonids) are characterized by wide gaps between rings. During periods of reduced or no growth (late fall to early spring) rings are laid down close to or on top of each other, forming a dense band of rings known as a "winter check" or annulus. Farm-reared fish experience little variation in seasonal growth rate, a result of a consistent diet year-round. Therefore spaces be-

Table 1. Molecular markers used to diagnose unknown fish from the Tsitika River as either Atlantic salmon or brown trout.*

Sample	*Hae*III	*Alu*I	*Rsa*I	5S rRNA
AS 1	0111	01111	111	250
AS 2	0111	01111	111	250
Tsitika 1	0111	01111	111	250
Tsitika 2	0111	01111	111	250
Tsitika 3	0111	01111	111	250
Tsitika 4	0111	01111	111	250
BT 1	1001	11111	011	300
BT 2	1001	11111	011	300

**Shown are the minimum restriction-site differences resolved by cutting the cytochrome b/d-loop/*12.S rRNA *segment of mitochondrial genome with Hae*III, *Alu*I, *and Rsa*I *(1, restriction site present; 0, restriction site absent) and the size, in base pairs, of the* 5S rDNA *(AS 1–2, known Atlantic salmon from a fish farm; Tsitika 1–4, salmon of unknown identify from Tsitika River; BT 1–2, known brown trout from a provincial hatchery).*

tween circuli are nearly equidistant and show diminished or absent winter checks. Scale patterns were interpreted by means of standard protocols (International Council for Exploration of the Sea 1984).

Length, weight, and stomach contents of the 12 captured putative Atlantic salmon were examined. The stomach contents of 12 sympatric juvenile *O. mykiss* trout of similar age were also examined for comparison. Length and weight data were recorded from an additional 66 (33 age 0 and 33 age 1) *O. mykiss* sampled in the same area.

Results

The mtDNA PCR-RFLP assays unambiguously identified the mtDNA from the four "unknowns" from the Tsitika River as Atlantic salmon. The RFLP profiles were identical to the two known Atlantic salmon and differed from the known brown trout by at least one site change at each of the four enzymes. In addition, the 5S PCR product in the Tsitika juveniles was 250 bp in size, the same size as that in the known Atlantic salmon and smaller than the 300 bp PCR product from the known brown trout (Table 1; cf. Pendas et al. 1995).

Scales sampled from the captured Atlantic salmon showed variation in the rate at which circuli were laid down, suggestive of a wild rather than domestic rearing. This was particularly apparent in fish belonging to the larger size class: their scales showed a single distinctive winter check, a feature typical of 1-year-old, wild-reared salmonids. Fish from the smaller size class lacked a winter check, indicating that they were young of the year (age 0). Independent analyses of both scales and otoliths from the same fish support these conclusions (A. J. Thomson, personal communication).

Atlantic salmon of both size classes were observed feeding freely among similar sized (but not necessarily similarly aged) *O. mykiss* juveniles. All stomachs of the eight Atlantic salmon and eight *O. mykiss* sampled for comparison were full. Almost complete overlap in prey species consumed by the two species was observed; both species fed predominantly on aquatic insect larvae (Table 2). Atlantic salmon of age 0 and 1 year were 1.5 times and 2.3 times larger by mass, respectively, than similarly aged *O. mykiss* (Table 3). Mean condition factors among the two species were comparable at both ages (Table 3).

There is no Atlantic salmon culture activity within the Tsitika River drainage. The nearest rearing facility is approximately 26 km to the northwest. The barrier at 3 km would prevent up-stream migration of juveniles from other locations. Together with these facts, our data suggest that the most parsimonious explanation for the presence of juvenile Atlantic salmon in the Tsitika River is natural reproduction of feral adults.

Discussion

Recent evidence suggests that escaped Atlantic salmon are capable of ranging significant distances from their putative escape sites in the Pacific. The northern limit of Atlantic salmon culture is located near the northern tip

Table 2. Qualitative summary of stomach content of eight *O. mykiss* (ST) and eight Atlantic salmon (AS) juveniles captured in the Tsitika River together.

				Stomach contents (% presence)			
Size class	*Species*	*Mean fork length (mm)*	*Mean weight (g)*	*mayfly larvae (H,B)**	*stonefly larvae*	*caddis larvae*	*stone cases*
Small $n = 3$	ST	63	2.8	100, 0	33	0	0
Large $n = 5$	ST	122	19.5	40, 40	0	80	100
Small $n = 3$	AS	65	3.2	66, 0	33	33	0
Large $n = 5$	AS	121	21.2	80, 0	40	60	40

**H, Heptagenaidae; B, Baetidae.*

Table 3. Length and weight (±SD) data of two year classes of juvenile Atlantic salmon and rainbow trout–steelhead captured in the Tsitika River, British Columbia, in September 1998.*

	Age 0 S. salar	*Age 0* O. mykiss	*Age 1* S. salar	*Age 1* O. mykiss
Mean fork length (mm)	63.75 ± 3.3	55.42 ± 5.81	119.25 ± 5.52	92.59 ± 10.46
Mean weight (g)	2.94 ± 0.60	1.91 ± 0.54	19.61 ± 4.01	8.67 ± 2.75
Mean codition factor	1.12	1.12	1.16	1.10
Weight (g) × 100/[fork length (cm)]^3				
n	4	33	8	33

**Ages of Atlantic salmon determined by scale analysis. Ages of* O.mykiss *determined by size.*

of Vancouver Island (approximately lat 51°N), but marine and freshwater recoveries are now well documented in Alaska (Wing et al. 1992; Thomson & Candy 1998). One adult has recently been recovered from the Bering Sea (lat 55°N, long 159°W; Brodeur & Busby 1998). Large-scale escapes from American farms in the Puget Sound region of Washington State likely contribute to the numbers of Atlantic salmon observed in coastal British Columbia (McKinnell & Thomson 1997).

It is unknown if the two juvenile cohorts of Atlantic salmon in the Tsitika River are the products of locally escaped adults (from Vancouver Island). No farm-specific tags, clips, or markers (physical or genetic) are used by the aquaculture industry, so it is not possible to determine an individual's time or place of escape when its observed or captured. Without such data it is difficult to make any inferences about the ecology of the species once escaped. At present, the only data available are gut analyses of marine-captured individuals, which suggest that escapees exhibit low feeding rates after escape (McKinnell et al. 1997). Although this may well be the case, there are no data available regarding what "normal" behaviour may be for feral Atlantic salmon in the northeast Pacific with respect to feeding rates or any other aspect of ecology. Thus, discussions regarding the long-term survivorship of the species in coastal British Columbia are speculative.

Based on our observations, we suggest that Atlantic salmon may constitute an invading species. A logical first step toward mitigation of possible negative effects would be a quantitative assessment of the competitive ability of Atlantic salmon in northeast Pacific waters. A limited number of studies have addressed competition between Atlantic salmon and *Oncorhynchus* spp. juveniles (Gibson 1981; Hearn & Kynard 1986; Jones & Stanfield 1993). No such study has been carried out under conditions specific to coastal British Columbia. The biological and physical parameters of coastal British Columbia and the northeast Pacific Ocean will likely play a significant role in determining the fate of escaped Atlantic salmon. Until the competitive potential of Atlantic salmon is assessed in this context, previous studies are of limited utility.

The repeated successful spawning (minimum of 2 years) of exotic Atlantic salmon in a Vancouver Island river indicates the potential for colonization and demands that a conservative approach be taken to the expansion of Atlantic salmon culture in the Pacific.

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