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Experimental Hybridization and Competition between Atlantic Salmon (*Salmo salar*) and Native Salmonid Species in Japan

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Abstract: Atlantic salmon (*Salmo salar*) is a leading aquaculture species that has been extensively bred in Norway using selective DNA marker technologies. The commercial application of a high-growth transgenic Atlantic salmon (namely AquAdvantage[®] Salmon) was approved by the United State Food and Drug Administration in November 2015. AquAdvantage salmon is a strain of Atlantic salmon that has been genetically modified by the integration of a growth-hormone regulating gene from a Chinook salmon and the antifreeze protein gene promoter from the ocean pout. The GM strain grows at least twice as fast as regular farmed Atlantic salmon. Should it become a candidate for commercial production in Japan, there is not enough available information on the biology and ecology of Atlantic salmon in Japanese waters for comparison. To remedy this in part, we evaluated the maturation of experimentally reared Atlantic salmon in Japan, and tested the potential of the species for hybridization and competition with four native salmonid species. Seasonal water temperature was a primary determinant of maturation of the Atlantic salmon. Crossed with Amago salmon, Masu salmon, and Biwa trout, most of the hybrid embryos ceased to develop between mesoderm induction and axis formation. However, a relatively low number of the Atlantic salmon and native white potted char (*Salvelinus leucomaenis*) hybrid, as confirmed through restriction fragment length polymerase analysis, survived for 2 years. In addition, we reared different life stages of Atlantic salmon and three native salmonid species in tanks or ponds to determine the extent of interspecific and intraspecific competition for food and space, as well as related changes in body mass. We found that competition was dependent on the combination of salmonid species and life stage of the different species.

Key words: Atlantic salmon (*Salmo salar*), competition, interspecific crosses, maturation, native salmonids in Japan

Introduction

The distribution of Atlantic salmon (*Salmo salar*) depends on water temperature and is native to the North Atlantic, where it is generally considered to comprise three populations: North American,

European and Baltic. As with other salmonids, the anadromous life cycle of this species is complex (Mills, 1989, 1991; Hendry and Cragg-Hine, 1997; Urke *et al.*, 2010). Young Atlantic salmon spend one to four years in their natal river. When they develop a highly evolved chemotactic system, they migrate

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toward the Atlantic Ocean and follow on sea surface currents and feed on plankton or juveniles of other fish species such as herring. After a year to up to five years of good growth, they move to the sea surface currents that transport them back to their natal river. It is a major misconception that salmon swim thousands of kilometers in the sea (Hendry and Cragg-Hine, 2000; Jonsson and Jonsson, 2007; Øystein *et al.*, 2010).

Since the mid-1960s, Atlantic salmon aquaculture has grown well beyond the native range of the species, with Norway, Chile, Scotland and Canada currently the largest producers (Marine Harvest, 2015). Atlantic salmon can now be farmed through their entire life cycle, with brood stock selected for a number of traits, including rapid growth, hardiness and good edible yield, and the adults are often cultured in marine net pens or sea cages.

Nearly one third of the total world production of Atlantic salmon occurs in regions where this species is exotic. There is an evidence of successful spawning in three streams in British Columbia, Canada, but whether escaped Atlantic salmon have established breeding populations along the North American West Coast remains uncertain (Volpe *et al.*, 2001a). Spawning of escaped farmed Atlantic salmon has not been documented in Chile or Tasmania. Outside its native range the Atlantic salmon is a poor colonizer. Because Atlantic salmon are attacked by other salmonid fishes, such as native brown trout (Heggenes *et al.*, 2002), the probability of escaped Atlantic salmon establishing populations in exotic environments seems low, although the possibility of this occurring cannot be ruled out. It is difficult to predict if or how Atlantic salmon adapt to a new environment, partly because research on the potential impacts in such a habitat is limited (McGinnity *et al.*, 2003).

Released or escaped cultured salmon have the potential to compete with the wild stocks for food, space, and breeding partners (Van Zwol *et al.*, 2012). As a result of the morphological, physiological, ecological, and behavioral changes that may occur among artificially reared salmon, their competitive ability is likely to differ from that of the wild fish. These changes are partly phenotypic and partly genotypic (Perrier *et al.*, 2013). For instance, genetic

change among hatchery-reared fish is a form of selection, with differential mortality among the genotypes, with brood stock chosen based on commercial production traits such as high adult body mass and fast growth rate (Perrier *et al.*, 2013). Escaped male salmon are often larger than the wild fish, making them more attractive to females and more successful in spawning, even though they may be less fit genetically (Hendry and Cragg-Hine, 2000).

Numerous researchers have described interspecific and intraspecific competition between Atlantic salmon and other salmonid species for all life stages (Fausch, 1998; Bremset and Heggenes, 2001; Volpe *et al.*, 2001b; Heggenes *et al.*, 2002; Van Zwol *et al.*, 2012). Most studies to date have suggested that the performance of wild steelhead, rainbow and brown trout is superior to that of Atlantic salmon, even with significant behavioral differences observed among these species. In experimental tank environments, the behavior of Atlantic salmon has been described to be influenced by that of other salmonid species in a manner similar to that occurs in natural river environments (Stradmeyer *et al.*, 2008; Berg *et al.*, 2014).

Fletcher *et al.* (2004) reported genetically modified (GM) Atlantic salmon to grow faster than non-GM farm-raised Atlantic salmon. The GM salmon contain an rDNA construct comprising a growth hormone gene from Chinook salmon, under the control of a gene promoter derived from the ocean pout (*Macrozoarces americanus*). The GM salmon have proven to grow faster before the pre-smolt stage and can reach market weight one year faster than the regular farmed Atlantic salmon (Du *et al.*, 1992; Cook *et al.*, 2000; Fletcher *et al.*, 2004; Deitch *et al.*, 2006).

AquaBounty Technologies Inc. first applied to sell a GM strain of Atlantic salmon (AquAdvantage® Salmon) in North America. Based on the proposed physical and biological containment measures, the United States Food and Drug Administration deemed that the likelihood of the GM salmon escaping from land-based facilities (in Canada and Panama), thereafter surviving in the ocean or freshwater and interbreeding with wild-type salmon, is remote. Accordingly, they issued a 'Finding of No Significant Impact' (FONSI) on November 19, 2015, thereby approving the company's application to produce the

salmon commercially.

The only report of farmed Atlantic salmon in Japan comes from Aomori Prefecture for 1983–1988 (Yoshida and Matsuzaka 1991). Thus, there is scant information describing the biology and ecology of Atlantic salmon in Japanese conditions for comparison with AquaAdvantage salmon, which would help in determining whether AquaBounty Technologies can apply for commercial production of the GM strain in Japan. Therefore, to partly remedy the lack of comparative data, we evaluated the maturation of Atlantic salmon cultured at two locations in Japan. Next, we tested the potential of the species for hybridization with four native salmonid species, and meanwhile sought to evaluate the levels of intraspecific competition and interspecific competition, with three native salmonid species, under contained conditions.

Materials and methods

Experimental salmonids

To investigate maturation of Atlantic salmon (*Salmo salar*) reared in Japan, we obtained hatched fry from the Hokkaido University Nanae Freshwater Station (Hakodate) and transferred them to the National Research Institute of Aquaculture's Tamaki (Mie Prefecture) and to Nikko inland station (Tochigi Prefecture) (the latter being in the coldest region on Honshu Island during winter). To investigate competition and the potential for crossbreeding with native salmonids, Biwa trout (*Oncorhynchus masou rhodurus*) were sourced from Lake Biwa Museum (Shiga Prefecture) and transferred to Tamaki station; Masu salmon (*O. masou masou*) and white-spotted char (*S. leucomaenis*) were sourced from Nikko station and transferred to Tamaki station; and the Amago salmon (*O. masou ishikawae*) were bred at Tamaki station.

Crossbreeding the Atlantic salmon with four native salmonid species

We monitored the water temperature for the immature Atlantic salmon cultured in outdoor tanks (15 m × 2.5 m × 1 m) at Tamaki station from January to September. The immature salmon were then transferred to an indoor 2 tons tank for rearing

between October and the following January to decrease the water temperature to approximately 10°C. A separate batch of immature Atlantic salmon were cultured indoors throughout the year at Nikko station in spring water maintained at approximately 10°C. Between late November and December, once the salmon female had matured, we squeezed eggs from females and sperm from males, and then artificially fertilized the salmon to ensure the quality of the egg and sperm. Male Atlantic salmon were matured from the end of October at Tamaki station, and we exposed the sperm of mature Atlantic salmon to the eggs of mature Amago salmon, Masu salmon, Biwa trout and white-spotted char to induce hybridization.

Competition between juvenile Atlantic salmon, and with Amago salmon and Biwa trout

To estimate intraspecific and interspecific competition between juvenile fish, ten individuals each of Atlantic salmon, Amago salmon and Biwa trout of average weight of 30 g were reared for 6 months in separate 2 m × 50 cm × 25 cm tanks with transparent fronts. In addition, we reared five Atlantic salmon with five Amago salmon, and five Atlantic salmon with five Biwa trout for 3 months. During this period, we used time-lapse video to record the position of each fish at 5 - minute intervals for 1 hour each day, and we weighed the juvenile fish approximately every 2 weeks.

Competition between the adult Atlantic salmon and three native salmonid species

To quantify interspecific and intraspecific competition between adult fish, 6 individuals each of Atlantic salmon, Amago salmon and Biwa trout of average weight of 250 g, were reared in separate 5 m × 1.5 m × 1 m ponds. Additionally, we reared 3 individuals each of Atlantic, Amago and Masu salmon and Biwa trout together for approximately 2 months. For 1 hour per day over a 2-week period, the position of each fish was recorded using two underwater video cameras. And, each day for 2 weeks, we counted the number of fish consuming the food pellets offered, using the camera footage for 30 minutes each feeding. Lastly, the fish were weighed every 2 weeks during the approximately 2 - month

period.

Results and Discussion

Maturation of Atlantic salmon reared on Honshu Island, Japan

Seasonal water temperature was an important determinant of the maturation of the experimentally reared Atlantic salmon. At the more northern Nikko inland station, the salmon could be bred year-round in relatively cold water, maintained at approximately 10 °C (Fig. 1A). However, the survival of the fry grown from artificially fertilized eggs was < 0.1% over a five-year period (2011–2015). Only at the more southern Tamaki station, in 2012, did survival of similarly derived fry reach 4%. In 2012, the water temperature between January and September ranged from 13 to

15 °C, after which it decreased to approximately 10 °C from late October to November (Fig. 1B). In order to mature Atlantic salmon at water temperature similar to that in 2015, Atlantic salmon were reared outdoor from January to September at water temperatures from 12 to 15 °C, after which they were transferred to an indoor 2 tons black breeding tank, from October to December, where the temperature was maintained at approximately 10 °C (Fig. 1C). These conditions produced survival to the fry stage.

The natural breeding grounds of Atlantic salmon are rivers in Europe and the east coast of North America (Øystein *et al.*, 2010). The bulk of individuals of the ocean-run form live in freshwater for the first 2 years of life, after which they smolt and migrate to the Atlantic Ocean to feed, grow and mature for 1 or more years. The adults utilize the ocean surface

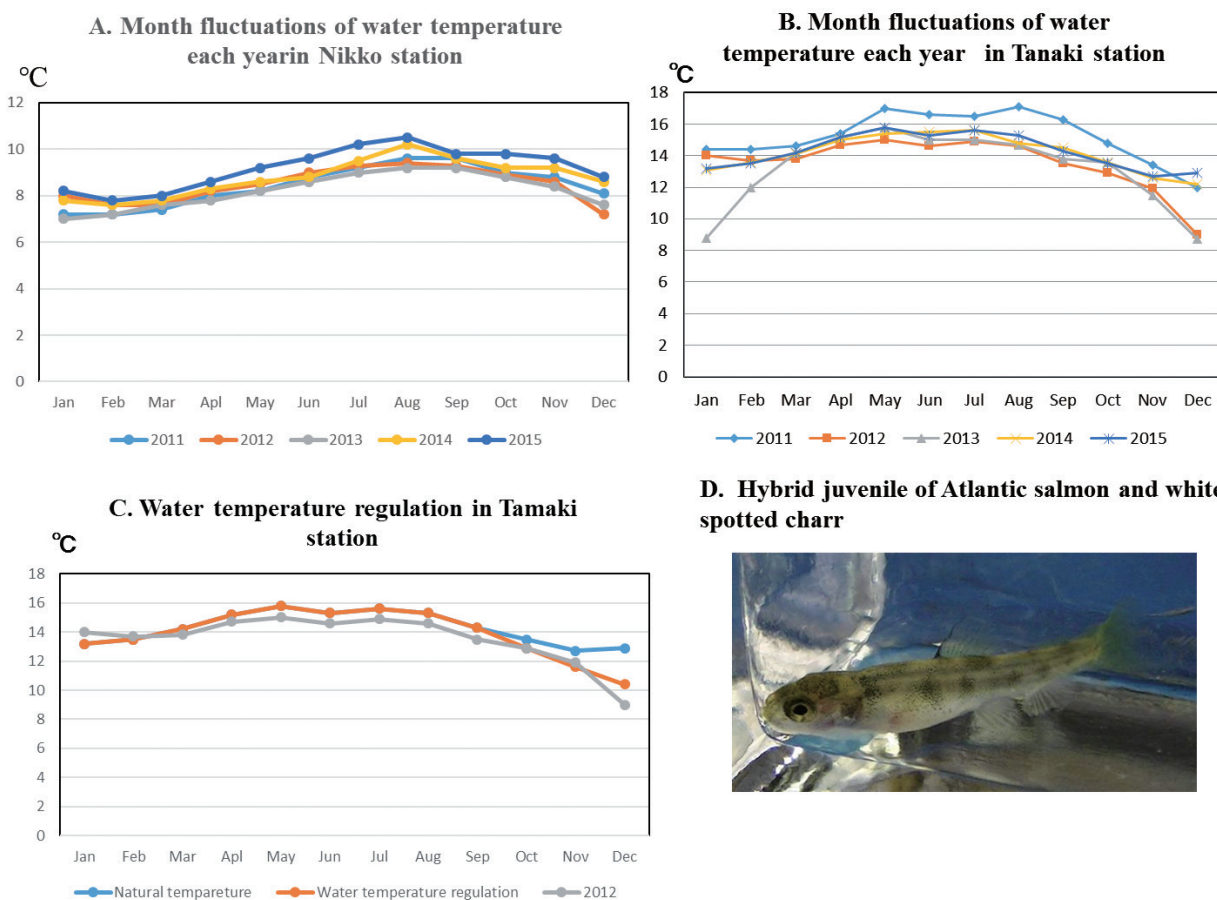


Fig. 1. Water temperature at Tamaki and Nikko stations, and an Atlantic salmon × white-spotted char hybrid fry. A: Water temperatures at Nikko station. B: Water temperatures at Tamaki station. C: Regulated water temperature in the indoor 2-t water black breeding tank at Tamaki station. D: Atlantic salmon and white-spotted char hybrid fry.

currents (Hendry and Cragg-Hine, 2000) to return to their natal river to spawn, generally from April to September (with peak spawning in October and November in Canadian waters). Atlantic salmon complete their maturation in the sea, and return to their natal river before or during summer with higher water temperatures (Bromage and Roberts, 1994; Jonsson and Jonsson, 2007). Rapid reductions in water temperature during October or November induce spawning (Taranger *et al.*, 2010; Good, 2015). In 2012, the year that we successfully managed to artificially fertilize the salmon and hatch out embryos to the fry stage, the culture water temperatures were similar what to this species would encounter in the wild in its native range. Natural populations of Atlantic salmon live at high latitudes, with most aquaculture facilities also traditionally located at high latitudes (e.g., Norway, Chile, Canada, United Kingdom, and Alaska in the US). Both experimental stations in our study, however, are situated at somewhat lower latitudes. The maturation of Atlantic salmon is understood to be a complex process variously affected by seasonal water temperature, photoperiod, growth rate while at sea, and age (Taranger *et al.*, 2010). Future research on these aspects will be required to determine the total appropriate conditions without water temperature for the artificial breeding of Atlantic salmon in Japan.

Hybridization between the Atlantic salmon and four salmon species native to Japan

We attempted to crossbreed Atlantic salmon with four native salmonid species, at both Tamaki and Nikko station. Most cross-fertilized embryos of Atlantic salmon with Amago salmon (*Oncorhynchus masou ishikawae*), Masu salmon (*Oncorhynchus masou masou*), Biwa trout (*Oncorhynchus masou rhodurus*), and white-spotted char (*Salvelinus leucomaenis*) died between mesoderm induction and axis formation because mesoderm was induced and cell cycle rate closed to that of somatic cells at the stage. However, some embryos of female white-spotted char crossed with male Atlantic salmon did hatch. These hybrid fry have been previously identified using restriction fragment length polymorphism (RFLP) analysis of a PCR-amplified fragment of the 16S rRNA gene and the second

intron of the aromatase gene (Masaoka *et al.*, 2015). In our experiments, the surviving fry achieved a relatively short body length and had a slightly irregular body form (Fig. 1D); however, we did not determine to what life stage the hybrids might have survived or whether they could have ever reached maturity.

Ban *et al.* (2013) crossed Atlantic salmon with several salmonid species native to Japan with a low level of success, and almost all the hybrid embryos were gynogenetic. Likewise, those authors reported an even lower level of success with hybrid embryos of male Atlantic salmon crossed with female Japanese char. We similarly observed poor survival of the Atlantic salmon and white-spotted char hybrid fry. Thus far, the potential for natural hybridization between these species in Japan appears to be unlikely, indicating that escaped farmed Atlantic salmon have little potential to impact the genetic fitness of the wild salmonid stocks in Japan.

On the other hand, interspecific hybridization between Atlantic salmon and brown trout was possibly the first hybrid fish ever described; natural hybridization of these species was mentioned by Willughbeii in 1686 (Makhrov, 2008), and artificial hybridization of these species has been known since the early 19th century (Leaniz and Verspoor, 1989; Jansson and Ost, 1997). Today, the hybridization of Atlantic salmon and brown trout provides a good model for studying the factors and consequences of interspecific hybridization among salmonids (Garcia-Vazquez *et al.*, 2004; Quilodr  n *et al.*, 2003; Ban *et al.*, 2013).

Interspecific competition among juveniles

Prior to our experiments, no published information was available on competition between Atlantic salmon and salmonid species native to Japan. To evaluate competition for food and space, and the resulting differences in body mass, we reared two different life stages (juvenile and before maturation) of Atlantic salmon together with three native salmonid species.

Most of the Atlantic salmon juveniles (average weight 20 g) reared in a 2 m × 50 cm × 25 cm aquarium gained weight during the same seasonal period but with different individual growth rates.

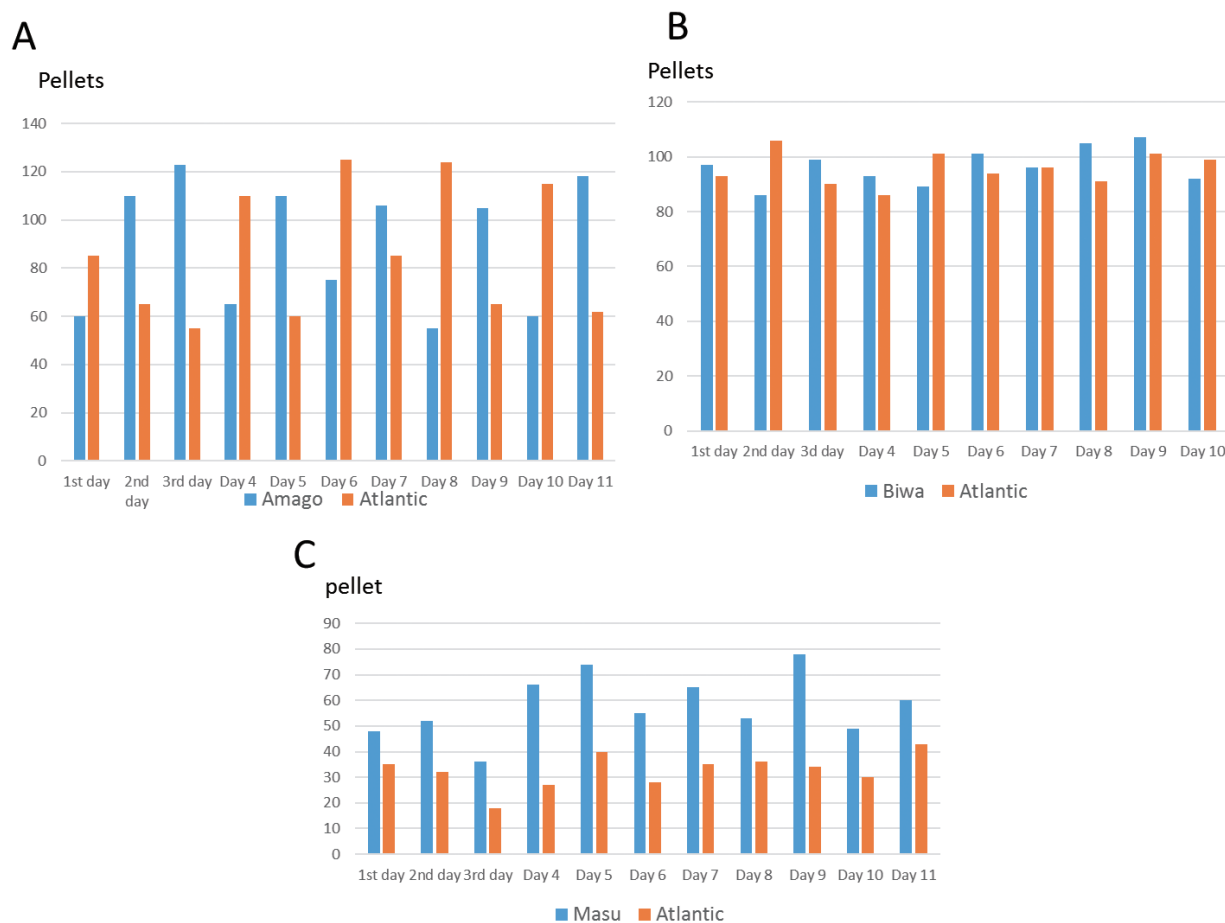


Fig. 2. Food competition between Atlantic salmon, Amago salmon, Biwa trout and Masu salmon. A: Competition for food between Atlantic salmon and Amago salmon. B: Competition for food between Atlantic salmon and Biwa trout. C: Competition for food between Atlantic salmon and Masu salmon. X-axis: time (days) from commencement of experiment; Y-axis: total number of food pellets consumed by 3 individual salmon.

This also occurred among ten juvenile Amago salmon (average weight 20 g) reared in a single-species tank, however their individual growth rates were greater than that of the juvenile Atlantic salmon. Interspecific competition for food between juvenile Atlantic salmon and Amago salmon appeared weak; intraspecific competition for food among juvenile Amago salmon appeared stronger than what was observed among Atlantic salmon. Among ten Biwa trout juveniles (average weight 30 g) reared in one aquarium, the weight gain of one was considerably large, and that of another much less so pronounced. Finally, intraspecific competition for food appeared stronger among the juvenile Biwa salmon than among either Amago salmon or Atlantic salmon.

Kept in sympatry, most of the five individuals each of Atlantic salmon and Amago salmon gained weight during the same period, but with overall differences in growth likely caused by intraspecific competition among one or the other of the species. Thus, interspecific competition between juvenile Atlantic salmon and Amago salmon appeared weak. When we reared five individual Atlantic salmon together with five Biwa trout, the weight of one Biwa trout individual increased substantially, whereas the weights of the other juvenile Biwa trout and the Atlantic salmon increased relatively slowly. The largest Biwa trout was aggressive toward the Atlantic salmon to the point of damaging the fins of the latter. Moreover, the Atlantic salmon appeared to avoid the Biwa trout by aggregating in a corner of

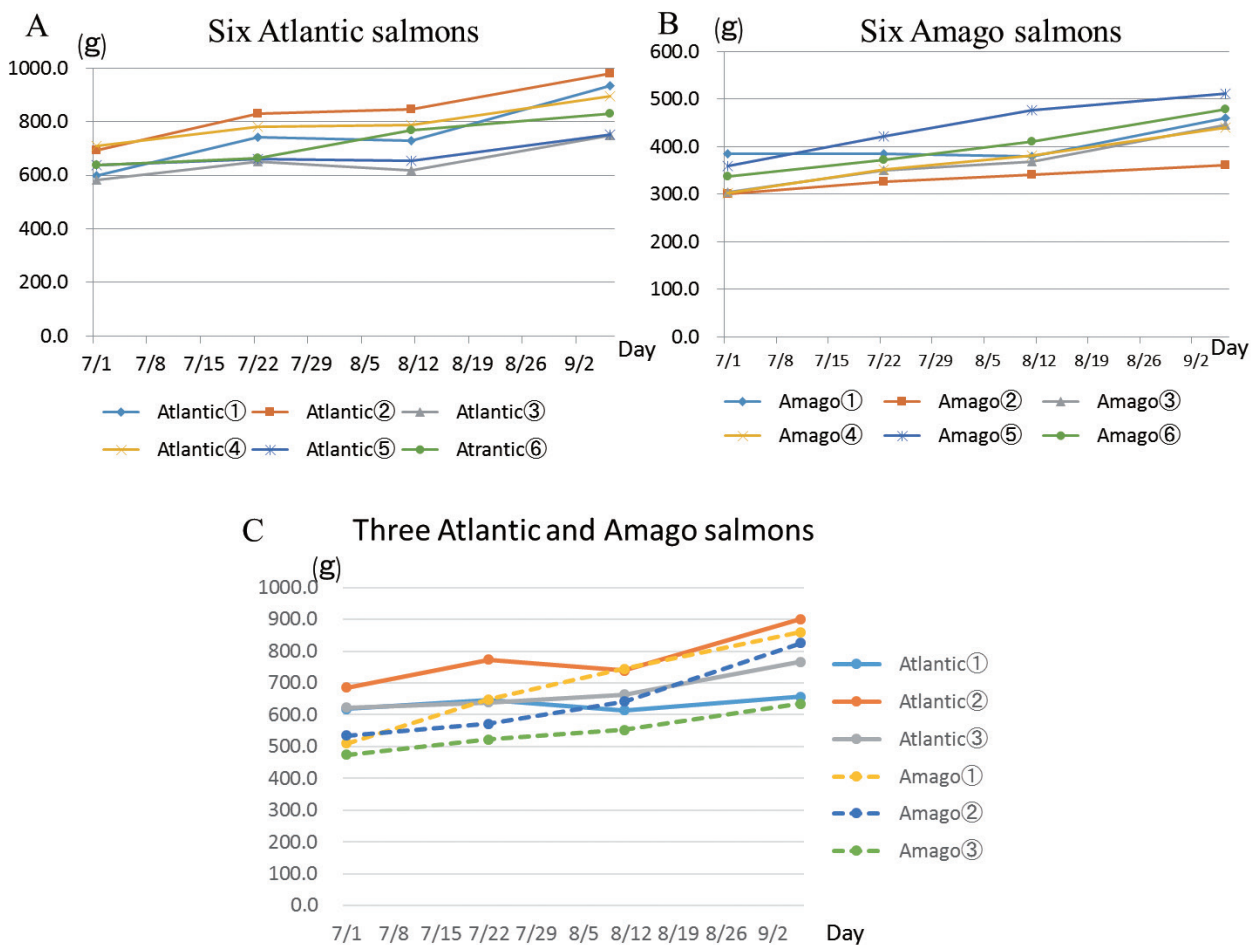


Fig. 3. Body weight change in the competition test between Atlantic salmon and Amago salmon. A: The body weight change of 6 individual Atlantic salmon reared in a single-species pool. X-axis: time (day); Y-axis: body weight (g). B: The body weight change of 6 individual Amago salmon reared in a single-species pool. C: The body weight change of 3 Atlantic salmon and 3 Amago salmon reared and fed together in one pool.

the aquarium. Thus, we surmise that interspecific competition between juvenile Biwa trout and Atlantic salmon was strong in the containment conditions.

Intra and Interspecific competition among sub-adults

When we investigated outdoor inter- and intraspecific competition between Atlantic salmon and Amago salmon, or Biwa trout or Masu salmon before maturation, Atlantic salmon raised in single-species ponds increased weight at the same period with little difference in growth rate (Fig.3A, 4A, 5A). Subadult intraspecific competition was deemed weak. We also deemed intraspecific competition between subadult Amago salmon was weak because Amago individuals increased their weight with little

difference in growth rate (Fig 3B). Biwa trout raised in a single-species pond gained weight slowly (Fig 4B). For the six Masu salmon raised in a single-species pond, the weight increased considerably in three individuals and decreased slowly in one individual (Fig 5B). Our results suggest stronger intraspecific competition in Masu salmon than Amago salmon and Biwa trout.

We investigated interspecific competition by observing three individual Atlantic salmon reared together with three Amago salmon, three Biwa trout, or three Masu salmon, before maturation, in 5 m × 1.5 m × 1 m outdoor ponds. When we cultured Atlantic salmon and these native species of salmon in one pond, the amount of food that Atlantic salmon could eat changed depending on the salmon species

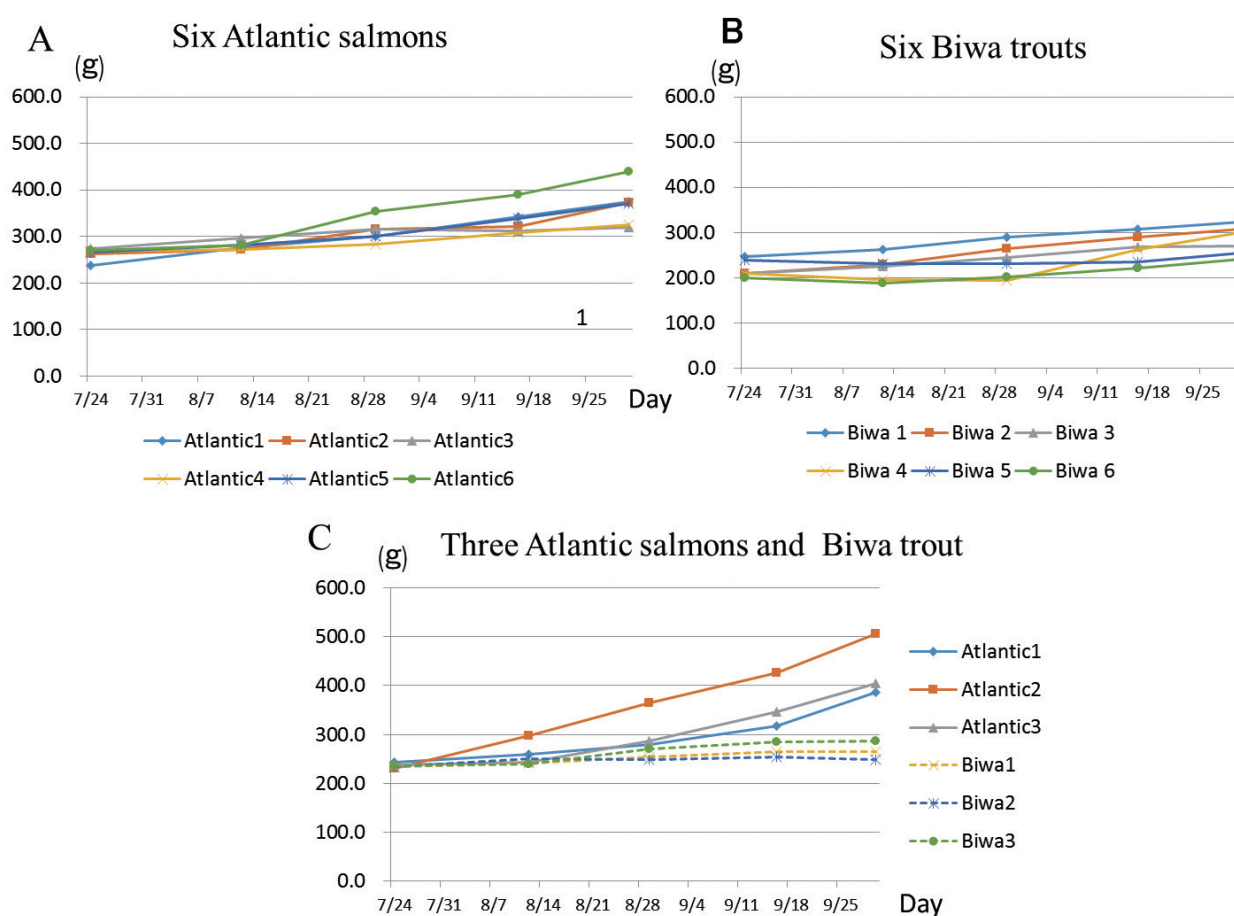


Fig. 4. Body weight change in the competition test between Atlantic salmon and Biwa trout. A: The body weight change of 6 individual Atlantic salmon reared in a single-species pool. X-axis: time (day); Y-axis: body weight (g). B: The body weight change of 6 individual Biwa trout reared in a single-species pool. C: The body weight change of 3 Atlantic salmon and 3 Biwa trout reared and fed together in one pool.

combined with Atlantic salmon (Fig.2). We found that the overall growth of the Amago salmon exceeded that of the Atlantic salmon regardless of the fish being kept in separate single-species ponds or in sympatry (Fig. 3). Kept together in one pond, the Amago salmon tended to distribute themselves throughout the pond, whereas the Atlantic salmon appeared more restricted in the space use. Based on these observations, we surmise that interspecific competition was limited between these two species at this life stage, although we recognize that Amago salmon tended to dominate the Atlantic salmon in sympatry.

Reared together in one pond, the three Atlantic salmon and three Biwa trout consumed a comparable number of food pellets each day (Fig. 2B). However,

the growth of Atlantic salmon exceeded that of Biwa trout (Fig. 4C). Kept together in one pond, the two species tended to divide themselves in the habitat. Hence, we surmised a minimal level of interspecific competition between these species at this life stage, but recognized that Atlantic salmon would possibly dominate Biwa trout with age and growth.

When three individual Atlantic salmon and three Masu salmon were reared together, we observed that Masu salmon tended to occupy the center of the pond and fed easily, whereas the Atlantic salmon tended to stay near the bottom and in shade. Also, kept in sympatry, Masu salmon consumed more food pellets than Atlantic salmon (Fig. 2C) and their body mass increased advantageously (Fig.5C). We conclude that interspecific competition between Masu salmon and

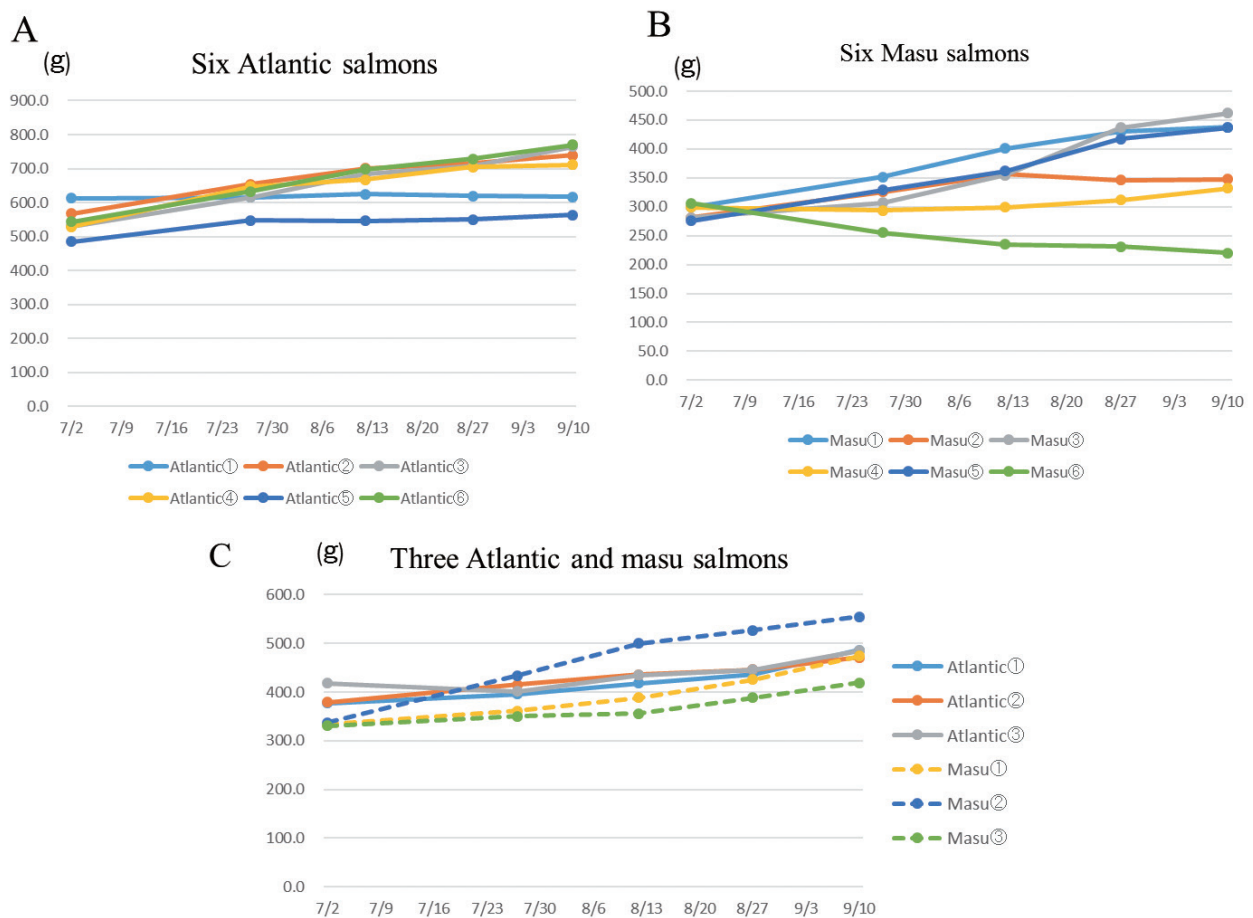


Fig. 5. Body weight change in the competition test between Atlantic salmon and Masu salmon. A: The body weight change of 6 individual Atlantic salmon reared in a single-species pool. X-axis: time (day); Y-axis: body weight (g). B: The body weight change of 6 individual Masu salmon reared in a single-species. C: The body weight change of 3 Atlantic salmon and 3 Masu salmon reared and fed together in one pool.

Atlantic salmon was strong in the experimental conditions, and that the level of competition depends on the combination of species at this life stage (sub-adult).

Many accounts of interspecific and intraspecific competition describing dominance, feeding behaviors, and growth rates of Atlantic salmon and other salmonid species exist, particularly as the habitat niche of Atlantic salmon overlaps with that of brown trout and rainbow trout, especially in juvenile stage (Fausch, 1998; Bremset and Heggenes, 2001; Volpe *et al.*, 2001b; Stradmeyer *et al.*, 2008; Van Zwol *et al.*, 2012). Most reports suggest that brown trout dominates and maintain a favorable growth rate regardless of which other salmonid species are present, and also that rainbow trout will dominate

Atlantic salmon and consume more food than Atlantic salmon. Habitat segregation is an important means for juvenile Atlantic salmon and brown trout to avoid competition in rivers and lakes (Berg *et al.*, 2014), with Atlantic salmon juveniles tending to inhabit deeper waters than brown trout (Heggenes *et al.*, 2002). Small, naturally reproducing populations of Atlantic salmon may exist in some rivers in Vancouver Island on the Pacific coast of Canada, where they are believed to segregate within their habit when in sympatry with other salmon species, such as steelhead trout (Maitland and Campbell, 1992; Volpe *et al.*, 2001b). In this study, the Atlantic salmon reared in a pond with either Amago salmon or Biwa trout occupied the deeper areas, seemingly to avoid the other species, and ate only food pellets

fallen on the pond floor. Moreover, when reared with Masu salmon, Atlantic salmon were typically chased away from the food pellets.

Our objectives were to determine whether Atlantic salmon could mature in containment conditions in Japan Honshu Island and to evaluate the potential of the species to hybridize and compete with native salmonids. We demonstrated that water temperature was important for the maturation of Atlantic salmon up to the pre-smolt stage, yet small numbers of salmon fry from artificially fertilized eggs survived for two years. Additionally, of four native salmonid species that we attempted to hybridize with Atlantic salmon, only crosses with white-spotted char produced a hybrid that had the potential to survive for at least 2 years. We also observed variable degrees of interspecific competition for food and space in the contained settings, with the level of competition depending on the combination of salmonid species and their life stage. Based on the observational data so far, we propose that escaped Atlantic salmon cultured in Japan would pose a negligible competitive threat to the native salmonid species. These observations can assist the decision making of the government departments and commercial companies in the event that Atlantic salmon, particularly AquAdvantage salmon, are ever commercially cultured in Japan.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Annotated bibliography

- (1) Du S.-J., Gong Z.-Y., Fletcher L., Shears M. A., King M.-J., Idler D. R. , and Hew C.-L., 1992: Growth enhancement in transgenic Atlantic salmon by the use of an "all fish" chimeric growth hormone gene construct. *Biotechnology (N Y)* **10**(2), 176–181

We have developed an "all fish" growth hormone (GH) chimeric gene construct by using an antifreeze protein gene (AFP) promoter from ocean pout linked to a chinook salmon GH cDNA clone. After microinjection into fertilized, nonactivated Atlantic salmon eggs via the micropyle, transgenic Atlantic salmon were generated. The presence of the transgene was detected by polymerase chain reaction (PCR) using specific oligonucleotide primers. A number of these transgenic fish showed dramatic increases in their growth rate. At 1 year old, the

average increase of the transgenic fish was 2 to 6 fold and the largest transgenic fish was 13 times that of the average non-transgenic control.

- (2) Deitch E. J., Fletcher G. L., Petersen L. H., Costa I. A., Shears M. A., Driedzic W. R., and Gamperl A. K., 2006: Cardiorespiratory modifications, and limitations, in post-smolt growth hormone transgenic Atlantic salmon *Salmo salar*. *J. Exp. Biol.* **209**, 1310–1325

In recent years, there has been a great deal of interest in how growth hormone (GH) transgenesis affects fish physiology. However, the results of these studies are often difficult to interpret because the transgenic and non-transgenic fish had very different environmental/rearing histories. This study used a stable line of size-matched GH Atlantic salmon (*Salmo salar*) that were reared in a shared tank with controls (at 10°C, for ~9 months) to perform a comprehensive examination of the cardiorespiratory physiology of GH transgenic salmon, and serves as a novel test of the theory of symmorphosis. The GH transgenic salmon had a 3.6 faster growth rate, and 21 and 25% higher values for mass-specific routine and standard oxygen consumption (MO₂), respectively. However, there was no concurrent increase in their maximum MO₂, which resulted in them having an 18% lower metabolic scope and a 9% reduction in critical swimming speed. This decreased metabolic capacity / performance was surprising given that the transgenics had a 29% larger heart with an 18% greater mass-specific maximum in situ cardiac output, a 14% greater post-stress blood haemoglobin concentration, 5 – 10% higher red muscle and heart aerobic enzyme (citrate synthase or cytochrome oxidase) activities, and twofold higher resting and 1.7 higher post-stress, catecholamine levels. However, gill surface area was the only cardiorespiratory parameter that was not enhanced, and our data suggest that gill oxygen transfer may have been limiting. Overall, this research: (1) shows that there are significant metabolic costs associated with GH transgenesis in this line of Atlantic salmon; (2) provides the first direct evidence that cardiac function is enhanced by GH transgenesis; (3) shows that a universal upregulation of post-smolt (adult) GH transgenic salmon cardiorespiratory physiology, as suggested by symmorphosis, does not occur; and (4)

supports the idea that whereas differences in arterial oxygen transport (i.e. cardiac output and blood oxygen carrying capacity) are important determinants of inter-specific differences in aerobicity, diffusion-limited processes must be enhanced to achieve substantial intra-specific improvements in metabolic and swimming performance.

(3) Volpe J. P., Anholt B. R., and Glickman B. W., 2001: Competition among juvenile Atlantic salmon (*Salmo salar*) and steelhead (*Oncorhynchus mykiss*): relevance to invasion potential in British Columbia. *Canadian. J. Fish. Aquat. Sci.* **58**, 197–207.

Atlantic salmon (*Salmo salar*) are routinely captured in both freshwater and marine environments of coastal British Columbia (Canada). Recent evidence

suggests that this species is now naturally reproducing in Vancouver Island Rivers. Our objective was to quantify the performance of each species in intra- and inter-specific competition by assessing the competitive ability of Atlantic salmon sympatric with native niche equivalent steelhead – rainbow trout (*Oncorhynchus mykiss*). Significant behavioural differences, particularly with respect to agonism, were observed between species; however, the status of an individual as resident or challenger was the best predictor of performance. Resident fish always outperformed challengers, regardless of species. Thus, we suggest that Atlantic salmon may be capable of colonizing and persisting in coastal British Columbia river systems that are underutilized by native species, such as the steelhead.