



CrossMark
click for updates

Research

Cite this article: Oke KB, Westley PAH, Moreau DTR, Fleming IA. 2013 Hybridization between genetically modified Atlantic salmon and wild brown trout reveals novel ecological interactions. *Proc R Soc B* 280: 20131047. <http://dx.doi.org/10.1098/rspb.2013.1047>

Received: 24 April 2013

Accepted: 7 May 2013

Subject Areas:

ecology, evolution

Keywords:

transgenesis, aquaculture, introgression, interspecific hybridization, *Salmo salar*, *Salmo trutta*

Author for correspondence:

Krista B. Oke

e-mail: krista.oke@mail.mcgill.ca

[†]Present address: Department of Biology and Redpath Museum, McGill University, 859 Sherbrooke West, Montreal, Quebec, Canada H3A 2K6.

[‡]Present address: School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, WA 98195, USA.

[¶]Present address: Department of Fisheries & Aquaculture, Government of Newfoundland & Labrador, St John's, Newfoundland & Labrador, Canada.

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2013.1047> or via <http://rspb.royalsocietypublishing.org>.

Hybridization between genetically modified Atlantic salmon and wild brown trout reveals novel ecological interactions

Krista B. Oke[†], Peter A. H. Westley[‡], Darek T. R. Moreau[¶] and Ian A. Fleming

Department of Ocean Sciences, Ocean Sciences Centre, Memorial University of Newfoundland, St John's, Newfoundland and Labrador, Canada A1C 5S7

Interspecific hybridization is a route for transgenes from genetically modified (GM) animals to invade wild populations, yet the ecological effects and potential risks that may emerge from such hybridization are unknown. Through experimental crosses, we demonstrate transmission of a growth hormone transgene via hybridization between a candidate for commercial aquaculture production, GM Atlantic salmon (*Salmo salar*) and closely related wild brown trout (*Salmo trutta*). Transgenic hybrids were viable and grew more rapidly than transgenic salmon and other non-transgenic crosses in hatchery-like conditions. In stream mesocosms designed to more closely emulate natural conditions, transgenic hybrids appeared to express competitive dominance and suppressed the growth of transgenic and non-transgenic (wild-type) salmon by 82 and 54 per cent, respectively. To the best of our knowledge, this is the first demonstration of environmental impacts of hybridization between a GM animal and a closely related species. These results provide empirical evidence of the first steps towards introgression of foreign transgenes into the genomes of new species and contribute to the growing evidence that transgenic animals have complex and context-specific interactions with wild populations. We suggest that interspecific hybridization be explicitly considered when assessing the environmental consequences should transgenic animals escape to nature.

1. Introduction

The production of genetically modified (GM) plants and animals has prompted considerable debate surrounding the environmental and ecological consequences should they escape [1,2]. The majority of research has assessed the performance of such organisms and their potential interactions with conspecifics and other species under experimental conditions [3–6]. In both plants and animals, the potential ecological risks are modulated by the reproductive capabilities of the transgenic organism, the fitness effects of the transgene and the potential for interbreeding with wild conspecifics. Parallel with these risks is that of introgression into gene pools of closely related species, especially between species that hybridize naturally [7]. Natural hybridization rates of wild, non-transgenic organisms are facilitated by reductions in reproductive barriers associated with mating behaviour; this has been observed in the translocations and invasions of species [8,9], including following release or escape of strains that have been exposed to domestication selection [10–12]. Successful artificial transgenic hybridization between two species of loach (genus *Misgurnus*) has been reported, yet these species are not known to hybridize naturally [13]. In contrast to plants [14], the potential ecological consequences of interspecific hybridization involving transgenic animals are unknown.

Atlantic salmon (*Salmo salar*), a candidate for growth-enhancing transgenic biotechnology for commercial production, hybridizes naturally with closely related brown trout (*Salmo trutta*). Natural levels of hybridization rarely exceed 1 per cent [15], yet translocations, as well as escapes and introductions of domesticated salmon [16–18], can increase rates to as much as 41 per cent

[17]. Hybridization between Atlantic salmon and brown trout is asymmetric, where the maternal origin of the hybrids varies among regions. The maternal species is predominately salmon within the natural geographical range of sympatry, while it is trout in areas where the species have come into secondary contact ([19] and references therein). Despite such regional differences, mortality prior to exogenous feeding is higher among hybrid offspring of trout mothers relative to hybrids with salmon mothers [19,20]. Given these biological patterns, we hypothesized that the transgene may further affect the outcome of hybridization, especially in terms of hybrid viability and hybrid growth rates.

Here we test the potential for transgene exchange through interspecific hybridization between transgenic Atlantic salmon and brown trout and whether this would generate novel ecological interactions. To do so, we created a series of experimental crosses and reared the offspring in both hatchery-like conditions and contained stream mesocosms designed to more closely emulate nature. These crosses and subsequent rearing experiments were designed to test: (i) whether the transgene can be transmitted into hybrid offspring via artificial mating; (ii) the survival of hybrid offspring; (iii) the phenotypic expression of the transgene in hybrids, particularly in relation to that observed in transgenic salmon; and (iv) the effect of direction of hybridization (i.e. whether the mother was a salmon or a trout) on phenotypic expression of the transgene. We used salmon hemizygous for the transgene, which enabled us to compare full sibling transgenic and non-transgenic hybrids (i.e. largely control for parental and other non-random genetic effects). Subsequently, to investigate potential ecological consequences of such hybridization, we undertook a replicated experiment to (v) quantify the effects of the presence of transgenic hybrids (from both maternal origins) on the growth of non-transgenic (henceforth referred to as wild-type) and transgenic salmon in stream mesocosms under food-limited conditions.

2. Material and methods

(a) Experimental fishes

Experimental crosses (see the electronic supplementary material, table S1) were produced using milt and ova from wild brown trout, wild Atlantic salmon and growth hormone (GH) transgenic Atlantic salmon. Wild salmon were captured from the Exploits River, Newfoundland, Canada, and trout from the Rennie's River, Newfoundland. Gametes from GH transgenic salmon [21] were provided by AquaBounty Farms (PEI, Canada). The gene construct used was a chimeric GH construct (opAFP-GHc2; EO-1 α line) that has been shown to greatly enhance growth [21], reduce reproductive performance among precocious mature males [22] and increase risk-taking foraging behaviours [23,24] in transgenic individuals. However, such phenotypic changes are known to be heavily influenced by gene–environment interactions [25,26]. The wild populations used in our experimental crosses were numerically large with no indications of inbreeding depression or low heterozygosity, and unaffected by the potentially confounding influences of domestication selection for growth [27]. The transgenic salmon were hemizygous, with a dominant transgene on a single chromosome that is inherited in classic Mendelian proportions [28]. Thus, families with a transgenic parent are expected to consist of ca. 50 per cent transgenic and 50 per cent wild-type offspring.

Logistical constraints on the availability and quality of transgenic sperm and ova limited the number of crosses created.

Initial plans to conduct a full-factorial half-sibling breeding design were not practical because of trauma to many gametes during shipment. Instead, seven experimental groups of fishes were available for comparisons: (i) six families of brown trout; (ii) seven families of Atlantic salmon (including wild-type and transgenic individuals); (iii) four families of hybrids from brown trout mothers (including wild-type and transgenic individuals, henceforth referred to as BT hybrids); and (iv) two families of hybrids from Atlantic salmon mothers (including wild-type and transgenic individuals, henceforth referred to as AS hybrids). To fully utilize the available gametes, four half-sibling families (two of the AS hybrid families were half-siblings with two separate salmon families) were created where gametes from two transgenic females were split to create two crosses each. Similarly, one brown trout male was also used in two separate crosses, bringing half-sibling families to six. (See the electronic supplementary material, table S1 for more information.)

(b) Transmission and phenotypic expression in hatchery-like conditions

To test for GH transgene transmission into salmon-trout hybrid offspring and to compare survival and phenotypic expression among experimental groups, 60 haphazardly selected individuals from each family were reared from the start of exogenous feeding (late March 2009) for ca. 100 days. Each family was reared in separate 25 l circular tanks, one tank per family. Fishes were fed a commercial dry feed (Corey Feed Mills, Fredericton, NB, Canada) continuously during daylight hours with automatic feeders (Lifegard Aquatics, Cerritos, CA, USA). Feeding was at satiation levels, with the weight of feed equivalent to ca. 4–6% of total biomass within a tank, which was adjusted periodically (ca. monthly) to account for changes in biomass resulting from growth and mortality. Photoperiod (controlled by automatic light timers) and water temperatures represented ambient conditions throughout the experiment. Mortalities ($n = 216$) were removed daily and every second mortality was screened for the presence of the transgene (see below).

At the start of the experiment, we quantified body mass (0.01 g) and length (0.01 mm) from 30 random individuals per family (except in one BT hybrid and Atlantic salmon family where $n = 16$ and $n = 17$, respectively) and genotyped 363 individuals that had a transgenic parent. After 100 days of rearing, we re-sampled individuals for length and weight and genotyped ca. 50 per cent of the surviving individuals ($n = 360$) following completion of the stream mesocosm experiment.

(c) Stream mesocosm environment

To test the competitive effects of hybrids on Atlantic salmon, we compared the growth rates of juvenile salmon in the presence (sympatry) or absence of hybrids (allopatry) in stream mesocosms (2.61 \times 0.25 m; see Moreau *et al.* [29] for detailed description) over a period of ca. 30 days starting in mid-July. These mesocosms, contained within the laboratory, were designed to more closely approximate natural conditions. In contrast to inanimate dry feed, fishes were fed 20.0 g of live *Artemia* nauplii per day (dry cyst weight, roughly 300 000 hatched individuals, shared among the 12 mesocosms) from drip feeders, spread over two feedings. Previous experiments using these mesocosms with the same food delivery system and where food levels were quantified suggest that food levels in the present study were probably representative of a food-limited environment of a natural stream [29]. Substrate of variable sizes and colours were added and unidirectional current forced individuals to hold position in the current as they would do in nature. Moreover, we observed characteristic territorial behaviour common in wild individuals [30], whereas density in the hatchery tanks precluded territoriality.

Thus, we consider the mesocosms to reflect 'semi-natural' conditions compared to the hatchery-like environment.

The mesocosm experiment started immediately following the 100 days in hatchery-like conditions. The sympatric treatments consisted of salmon ($n = 12$) with either BT hybrids or AS hybrids ($n = 12$). Both sympatric treatments and the allopatric treatment ($n = 24$ salmon) were replicated four times. Fishes were selected haphazardly for inclusion in the mesocosm experiment, anaesthetized and measured for initial fork length (mm; via digital photography using a Nikon D300 SLR with a 60 mm macro lens and IMAGEJ software) and initial weight (0.001 g). They were then individually marked with a passive integrated transponder tag (8 mm Biomark, Boise, ID, USA) and assigned to the experimental mesocosms after a 24 h period of recovery. We controlled for possible family effects by distributing families evenly among mesocosms. Within families, individuals were assigned to experimental streams without knowledge of their genotype, and screening for the presence of the transgene was performed following the experiment. Mortalities were recorded (2.1% of salmon, spread among streams), removed and replaced with a live individual from the same family to maintain densities. Growth rates of replacement fishes were excluded from analyses.

All fish sampling was performed under mild anaesthesia with MS-222 (Western Chemical, Inc., Ferndale, WA, USA). At the termination of the experiment, all fishes were euthanized with an overdose of MS-222.

(d) Genetic screening

We determined individual genotypes as transgenic or wild-type using the polymerase chain reaction (PCR) protocol modified from Deitch *et al.* [31]. Briefly, DNA was extracted from air-dried fin tissue (stored in 95 per cent ethanol at -20°C) with 50–200 μl of 10 mM NaOH. Samples were placed in a boiling water bath for 10 min, placed on ice for 2 min and then centrifuged at 10 000 rpm for 2 min at 4°C . PCRs were performed using recombinant Taq DNA polymerase (Fermentas International, Inc., Burlington, Ontario, Canada) and primers 2653F (5'-GCTCTTCAACATC GCGTCA-3') and 2654R (5'-ATATGGAGCAGCTTCAGGAC). These reactions (50 μl) contained 2 μl DNA, Taq DNA polymerase (2.5 U), the manufacturer's Taq Buffer with KCl ($1\times$ final concentration), 1.5 mM MgCl_2 , 0.2 mM dNTPs and 0.2 μM each of forward and of reverse primer. Thermocycling conditions consisted of 35 iterations of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min. PCRs (25 μl) were analysed using 2 per cent agarose gel electrophoresis with a visual comparison of bands using a DNA size marker. The presence of a 207 bp band was taken as diagnostic of the transgenic genotype [31].

(e) Data analyses

(i) Transmission and phenotypic expression

We used counts of mortalities as the response variable and experimental cross as a fixed categorical factor in a generalized linear model (GLM) with Poisson error structure to test for differences in mortality among crosses. Significance was assessed with an F -test to account for overdispersion. To test whether mortality occurred randomly with respect to genotype, we compared the probability of a dead individual being either transgenic or wild-type against a binomial distribution (0.5 probability of success/failure) using binomial tests.

We used a general linear mixed model (GLMM) to test for differences in growth among experimental cross and genotype. Models were formulated with experimental cross as a fixed categorical factor and genotype (transgenic or wild-type) nested in a random family factor. As individuals were too small to efficiently mark at the start of the experiment, we based analyses

on family averages. Tukey post-hoc tests on experimental cross and genotype were conducted using the 'multcomp' package in R [32].

Growth rates were standardized (Ω) to account for differences in initial size among groups (see the electronic supplementary material, table S2) following Ostrovsky [33]:

$$\Omega = (M_2^T - M_1^T) \cdot (\tau \cdot \text{time})^{-1},$$

where M_2 and M_1 are final and initial size, respectively, time is the growth interval and τ is the species-specific allometric coefficient for the relationship between growth rate and size. We set $\tau = 0.310$ and 0.308 for salmon and trout, respectively, based on experimentally determined values from the literature ([34], reviewed by [30]). Failing to account for size differences altered interpretation of growth among groups (see the electronic supplementary material, table S3).

(ii) Stream mesocosm environment

Similar to above, we used a GLMM to test for differences in juvenile salmon growth among treatments. Treatment (three levels: allopatric treatment [salmon only control], and two types of sympatric treatments [BT hybrids and AS hybrids]) and genotype (two levels: transgenic or wild-type) were included as fixed factors, with mesocosm as a random effect nested within treatment to account for potential tank effects and non-independence of growth rates. Interaction terms between treatment and genotype were tested and removed when non-significant; other factors were interpreted individually. Tukey's HSD tests were used to interpret growth differences among treatments.

Analysis of variance (ANOVA) was used to investigate if juvenile growth rate in the mesocosms differed between transgenic and non-transgenic salmon and hybrids. Specifically, we assessed standardized growth as a function of genotype, cross (salmon or hybrid) and their interaction set as fixed factors. All analyses were performed in R [35].

3. Results

(a) Transmission, viability and growth enhancement

PCR analysis confirmed successful transmission of the transgene into hybrid offspring of Atlantic salmon and brown trout, and was reflected in body size differences among groups after ca. 100 days of feeding (figure 1). Of the 363 individuals screened at the start of the experiment, 43 per cent of individuals tested positive for the transgene. Among the families of hybrids with salmon mothers (AS hybrids; $n = 60$ screened) and hybrids with trout mothers (BT hybrids; $n = 106$ screened), 37 and 42 per cent of individuals, respectively, were transgenic. Only the AS hybrid group differed significantly from expectations of 50 per cent transgene transmission ($\chi^2_1 = 4.6$, $p = 0.03$).

In laboratory conditions, rates of mortality differed significantly among experimental groups. BT hybrids died at higher rates (45%) than salmon, trout and AS hybrids (14, 11 and 6%, respectively; electronic supplementary material, figure S1; Poisson GLMM, $p < 0.001$), while mortality rates of the latter three did not differ from each other ($p > 0.1$). The direction of hybridization appeared to influence transgenic viability; 57 per cent of AS hybrid mortalities ($n = 7$; binomial test, $p = 1$) were transgenic, whereas only 27 per cent of BT hybrid mortalities were transgenic ($n = 55$; binomial test, $p < 0.001$). Lower mortality of transgenic compared to wild-type siblings was detected in every family of BT hybrids (i.e. 69, 82, 77 and 68% of mortalities were wild-type individuals

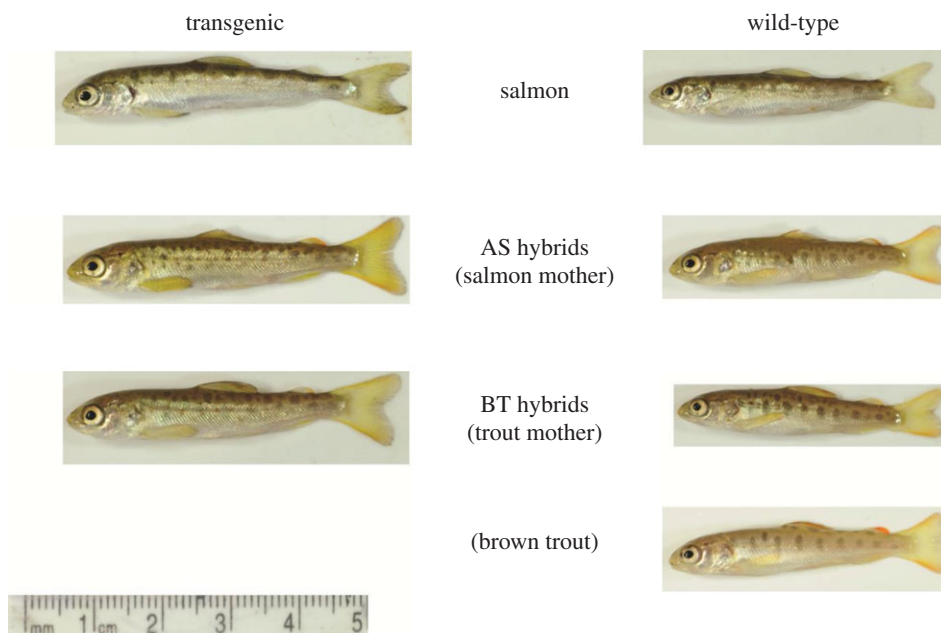


Figure 1. Representative photos of experimental groups 236 days post fertilization. From top to bottom, left to right: transgenic Atlantic salmon (fork length, 52.8 mm), AS hybrids (56.1 mm) and BT hybrids (52.5 mm), and wild-type (non-transgenic) salmon (43.9 mm), AS hybrids (46.4 mm), BT hybrids (40.1 mm) and brown trout (43.1 mm), where AS and BT denote salmon and trout maternal origin, respectively. Photos are representative of appearance and accurately depict the relative final size of groups at the end of ca. 100 days rearing in common hatchery-like conditions (see the electronic supplementary material, table S2 for more information). (Online version in colour.)

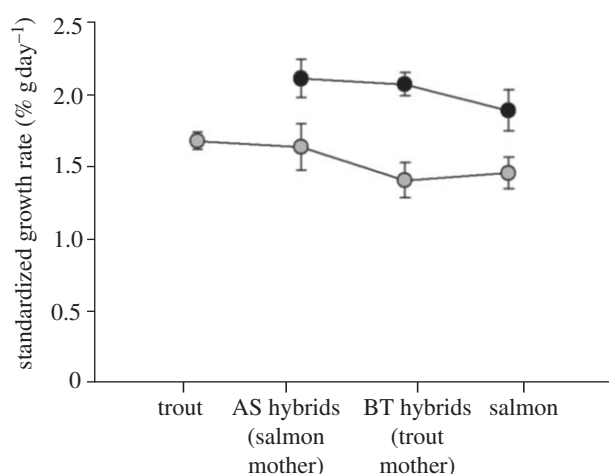


Figure 2. In common hatchery-like conditions, the standardized mass specific growth rates (% g day⁻¹) of transgenic (black circles) groups ($n = 13$) differed significantly from that of wild-type (non-transgenic, grey circles) groups ($n = 19$, $p < 0.001$). There were also significant differences in growth rates among crosses (Atlantic salmon, AS hybrids, BT hybrids and brown trout; $p < 0.001$). Error bars represent ± 1 s.e.

in four families). In summary, the patterns of mortality within BT hybrid crosses indicate that transgenic BT hybrids died at statistically lower rates than their wild-type siblings, although the mortality rate of transgenic BT hybrids (24%) was still greater than that experienced by transgenic AS hybrids (7%).

Growth rates differed among experimental crosses and genotypes after ca. 100 days of rearing (figure 2). For brevity, we report growth in body mass because analyses based on length metrics yielded similar interpretations (see the electronic supplementary material, tables S2 and S3). One family of BT hybrids had insufficiently few surviving individuals ($n = 12$) and were excluded from further analyses. GLMMs revealed

significant differences in growth among crosses ($p < 0.001$) and between transgenics and wild-types ($p < 0.001$). Among wild-type individuals, growth of trout (1.7%) and AS hybrids (1.6%) were significantly faster than that of BT hybrids (1.4%) and salmon (1.5%; $p < 0.01$). The transgene resulted in faster growth relative to wild-type counterparts in all cases (figure 2 and see the electronic supplementary material, table S3). Transgenic AS (2.1%) and BT (2.1%) hybrids grew significantly faster than transgenic salmon (1.9%, $p < 0.01$). Differences in growth were not consistent with density-dependent effects, as no relationship between mortality rates in tanks and growth rates was detected (ordinary least squares, $p = 0.87$, $r^2 = 0$).

(b) Stream mesocosm environment

Hybrids suppressed the growth (% change in mass day⁻¹) of both transgenic and wild-type juvenile salmon in stream mesocosms (figure 3; electronic supplementary material, table S4). Results for growth based on changes in length (mm day⁻¹) and condition factor ($10^3 \times \text{weight length}^{-3}$) were congruent (see the electronic supplementary material, table S5). An experimental error in identifying one family resulted in all replicates of the AS hybrid sympatric treatment containing eight hybrids and 16 salmon, rather than the intended 12 of each. Despite this, growth of both transgenic and wild-type juvenile salmon was significantly lower in sympatric treatments compared to allopatric treatments (GLMM, $p < 0.001$; figure 3). In sympatric treatments, wild-type salmon growth rate in mass was reduced by 54 per cent whereas transgenic juvenile salmon growth was reduced by 82 per cent compared to allopatric treatments. Similar growth rates between the sympatric treatments indicated that hybrid maternal origin did not affect the growth of juvenile salmon, despite a difference in the proportion of hybrids present between treatments (GLMM, $p = 0.87$; figure 3). Growth rates of juvenile transgenic and wild-type

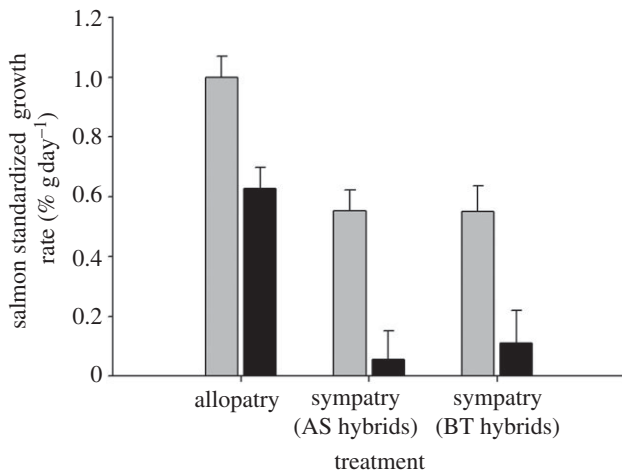


Figure 3. Hybrids suppress the growth (% g day⁻¹) of wild-type (grey bars) and transgenic (black bars) salmon in stream mesocosms ($p < 0.001$). Salmon ($n = 200$) were grown for ca. 30 days in three treatments: allopatric treatment (only salmon present), AS hybrid sympatric treatment (salmon and AS hybrids) and BT hybrids treatment (salmon and BT hybrids). Transgenic salmon grew slower than wild-type salmon ($p < 0.001$) in all treatments. Both transgenic and wild-type hybrids were present in sympatric treatments. Error bars represent ± 1 s.e.

salmon differed in both the allopatric and sympatric treatments (figure 3). Transgenic salmon had lower growth rates than wild-type salmon in all treatments (GLMM, $p < 0.001$).

Transgenic AS and BT hybrids grew 86 and 87 per cent faster than transgenic salmon in the same treatments, while wild-type salmon and wild-type hybrids grew at comparable rates (figure 4a,b). Specifically, we observed a marginally non-significant genotype \times cross interaction in the AS hybrid sympatric treatment (ANOVA, $p = 0.051$; figure 4a) and a significant interaction in the BT hybrid sympatric treatment (ANOVA, $p = 0.023$; figure 4b).

4. Discussion

To the best of our knowledge, this is the first study demonstrating transmission and ecological consequences from interspecific hybridization between a GM animal and a naturally hybridizing species. These data confirm successful transmission of a GH transgene into interspecific hybrid offspring, marking the successful first steps towards potential introgression of the transgene into the gene pool of another species. Transgenic F_1 hybrids expressed the enhanced growth phenotype; however, the direction of hybridization affected transgenic hybrid viability. The effect of the transgene on juvenile growth was environmentally dependent, with transgenic individuals growing faster than their wild-type counterparts in hatchery-like conditions, but slower in stream mesocosms. Juvenile transgenic hybrids suppressed the growth of both transgenic and wild-type juvenile salmon in stream mesocosms. Taken together, these findings suggest that complex competitive interactions associated with transgenesis and hybridization could have substantial ecological consequences for wild Atlantic salmon should they ever come into contact in nature.

Considerable differences in offspring survival were observed between genotypes (transgenic or wild-type) and among species groups (Atlantic salmon, brown trout, AS hybrids or BT hybrids) during early rearing in hatchery

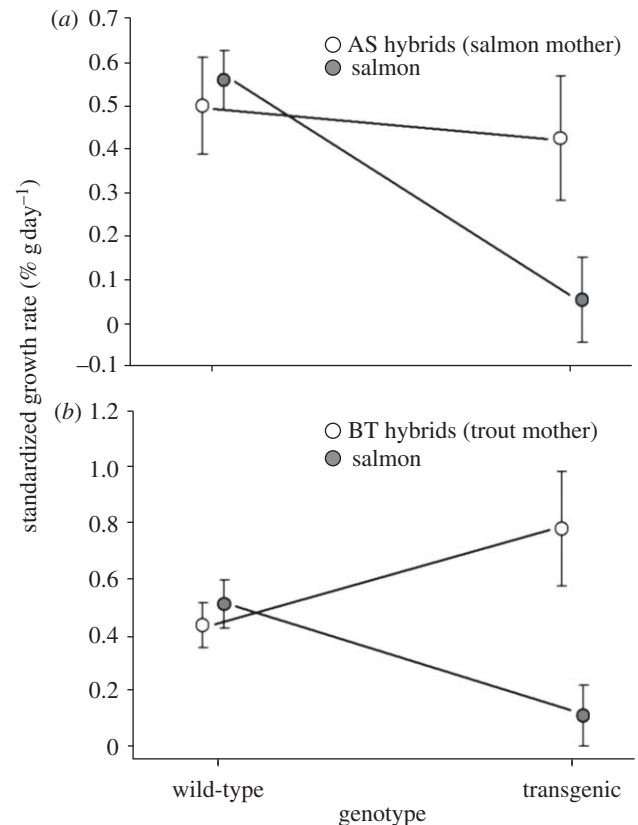


Figure 4. The growth rate (% g day⁻¹) of transgenic salmon ($n = 118$) and wild-type (non-transgenic) salmon ($n = 80$) and hybrids ($n = 78$) differed in the two stream mesocosm sympatric treatments (gene \times cross interactions). (a) AS hybrid sympatric treatment, gene \times cross interaction ($p = 0.051$), (b) BT hybrid sympatric treatment, gene \times cross interaction ($p < 0.05$). Error bars represent ± 1 s.e.

conditions. BT hybrids, whether transgenic or not, had higher mortality than all other groups, as has been observed previously [19,36]. Surprisingly, the transgenic BT hybrids had consistently lower mortality than their wild-type siblings, a pattern not seen in the AS hybrids. The underlying mechanism for this apparent interaction between transgenesis and hybrid maternal origin remains unclear. One possible explanation would be that transgenic BT hybrids died at higher rates during the egg and larval stages (prior to the start of the experiment); however, this seems unlikely as the number of transgenic BT hybrids at the start of the experiment was not significantly different than 50 per cent expectations. By contrast, AS hybrids differed significantly from 50 per cent expectations at the start of the experiment, although transgenic and non-transgenic siblings died at equal probabilities.

Results indicated that transgenesis could combine with hybridization to result in rapid growth, but heterosis, or hybrid vigour, was not apparent in juveniles. Transgenic AS hybrids and transgenic BT hybrids expressed the enhanced growth phenotype and grew faster than other groups in hatchery conditions, including transgenic salmon. However, the brown trout groups grew faster than wild-type hybrid groups, a pattern that is inconsistent with heterosis at this life stage. Wild-type BT hybrids and wild-type salmon juvenile growth rates were not significantly different, but transgenic BT hybrids grew significantly faster than transgenic salmon. Taken together, these data suggest that growth may be influenced by complex interactions between transgenesis and hybridization.

To the best of our knowledge, this is the first study reporting a competitive advantage of transgenic interspecific hybrids over a parent species. Although hybridization with non-native species is often an important conservation concern [37,38], studies that quantify the ecological effects of non-transgenic hybrids on sympatric parent species are rare (but see [39,40]). Juvenile brown trout have demonstrated competitive dominance over juvenile Atlantic salmon, and can reduce salmon growth rates in stream mesocosms ([41] and references therein). If hybrids are of intermediate dominance, this may have contributed to decreased salmon growth rates.

Although both hybrid genotypes were present in the mesocosms, we suggest that the observed decrease in salmon growth was predominately owing to transgenic rather than wild-type hybrids. Owing to chance and differential survival during the first 100 days of rearing, transgenic hybrids were on average more common in mesocosms than wild-type hybrids (on average, 64% of the hybrids in sympatric treatments were transgenic). We consider a greater influence of transgenic hybrids to be likely as transgenic salmon have higher foraging motivation than non-transgenics [23,24], which can affect access to food, growth and survival for non-transgenic salmon [5,42]. Increased foraging motivation is also probably manifested in transgenic hybrids, compounding any competitive advantage hybrids may have over salmon. Correspondingly, we observed higher growth of transgenic hybrids over transgenic salmon in the same treatments.

In contrast to the hatchery-like conditions, the stream mesocosms were food limited, which may explain some of the differences observed between salmon genotypes in response to hybrids. Transgenic salmon grew less and incurred a greater reduction in mass growth (82%) than wild-type salmon (54%) in the presence of hybrids. This is consistent with previous studies on GH transgenic coho salmon [5,24] and GH-implanted Atlantic salmon [43], where these fish perform poorly relative to their wild-type counterparts in natural or semi-natural conditions (but see [29]). Although growth in general was low, it was within the range observed elsewhere [30]. In this study, the decreased growth of transgenic relative to wild-type juveniles was probably owing to their higher metabolic demands [44] in combination with limited food availability and warm water temperatures (mean $16.9 \pm 2.5^\circ\text{C}$ during the experiment). Regardless of the mechanism, our observations of reduced growth of transgenic salmon in stream mesocosms but increased growth in hatchery-like conditions contribute to evidence suggesting a complex, dynamic interplay between transgenic growth potential and the environment [25,26].

Several caveats are worthy of discussion. First, the number of hybrid families for the two cross directions used in analyses was small, owing to logistical constraints on the availability of transgenic gametes. Family and cross effects were difficult to completely disentangle in the hatchery-like environment; we observed less variation in growth within families of brown trout and BT hybrids than among crosses, but greater variation within families of AS hybrids and Atlantic salmon. However, our results from the mesocosms are independent of potential parental influence as families were equally distributed among treatments. Thus, we interpret the differences in growth among crosses and the ecological consequences of sympatric rearing of transgenic hybrids on salmon to not be primarily the result of family effects. Second, only the juvenile stage was examined and it is possible that competitive relationships or the impact of

the transgene in the reproductive life stage in hybrids would have yielded different interpretations of their performance and viability. Even so, the juvenile life stage is known to be a critical period influencing the dynamics of salmon populations [30]. Third, we focused on the ecological interactions of transgenic hybrids on only one parent species, Atlantic salmon. A lack of hybrids precluded mesocosm experiments with the trout parental species; however, exhaustive risk assessments concerning transgenic interspecific hybridization ought to explore interactions with both parent species and other species as well [2].

(a) Implications and conclusions

We report that a foreign transgene that conveys remarkable growth advantage was successfully transmitted from GM Atlantic salmon and expressed in F_1 interspecific salmon \times brown trout hybrids. It remains unclear, however, whether the transgene could have successfully invaded the brown trout genome through viable backcrosses to the trout parental species. Despite an observed successful first step, several lines of evidence from the literature combine to suggest that introgression of the transgene into the brown trout genome via backcrossing is unlikely. First, the reproductive abilities of transgenic salmonids are reduced compared to non-transgenic fishes [22,45,46]. Second, low natural rates of hybridization [15] and poor hybrid survival [19,36] further reduce the likelihood of transgene introgression into the brown trout genome. On the other hand, poor reproductive capacity of captive salmonids improves following wild exposure [45,47–49], and the reproductive behaviour of transgenic salmonids in truly wild environments is unknown. Additionally, increased rates of salmon–trout hybridization have occurred in areas with high rates of escaped farmed or stocked heterospecific individuals ([12,15–18], but see [50]) and this has contributed to the introgression of trout genes into salmon populations and vice versa [12]. Third, only experimental backcrosses of hybrids to the salmon parental species have produced viable offspring [51,52], although evidence from wild systems suggests that hybrid backcrosses to trout can be viable [12]. Fourth, transgene introgression into the trout genome requires that a functionally intact transgene be included in the seemingly limited genetic material transferred into backcrossed offspring [12].

Despite the apparent low probability for genetic introgression into the brown trout genome, the ecological consequences of decreased salmon growth in the presence of transgenic hybrids indicate that hybridization is relevant to risk assessments. Although transgenic hybrids would probably be rarer in the wild than in our experiment, our results indicate that transgenic hybrids have a competitive advantage over salmon in at least some semi-natural conditions. Still, it is entirely unclear whether this would be observed in truly wild environments. If this advantage is maintained in the wild, transgenic hybrids could detrimentally affect wild salmon populations. Ultimately, we suggest that hybridization of transgenic fishes with closely related species represents potential ecological risks for wild populations and a possible route for introgression of a transgene, however low the likelihood, into a new species in nature.

Fish care followed the Canadian Council on Animal Care guidelines and was approved by Memorial University's Institutional Animal Care Committee (AUP 09-03-IF).

We thank C. Conway, A. Fitzpatrick, and D. Ings for assistance in data collection and fish rearing. We thank the employees of Aqua-Bounty Technologies for kindly providing the transgenic salmon gametes for our crosses. J. Hall of the CREAT Network of Memorial University of Newfoundland ran the PCR screening. This work was

funded by NSERC Discovery grant to I.A.F., K.B.O. was supported by two NSERC USRAs and funding from Memorial University's MUCEP program. Support to P.A.H.W. was provided by the Institute of Biodiversity & Ecosystem Sustainability and the Atlantic Salmon Federation.

References

- Pilson D, Prendeville HR. 2004 Ecological effects of transgenic crops and the escape of transgenes into wild populations. *Annu. Rev. Ecol. Evol. Syst.* **35**, 149–174. (doi:10.1146/annurev.ecolsys.34.011802.132406)
- Devlin RH, Sundström L, Johnsson J, Fleming I, Hayes K, Ojwang W, Bambaradeniya C, Zakaria-Ismail M. 2007 Assessing phenotypic and ecological effects of transgenic fish prior to entry into nature. In *Environmental risk assessment of genetically modified organisms: methodologies for transgenic fish*, vol. 3 (eds AR Kapuscinski, KR Hayes, S Li, G Dana), pp. 151–187. Oxfordshire, UK: CABI Publishing.
- Sundström LF, Tymchuk WE, Löhmus M, Devlin RH. 2009 Sustained predation effects of hatchery-reared transgenic coho salmon *Oncorhynchus kisutch* in semi-natural environments. *J. Appl. Ecol.* **46**, 762–769. (doi:10.1111/j.1365-2664.2009.01668.x)
- Burke JM, Rieseberg LH. 2003 Fitness effects of transgenic disease resistance in sunflowers. *Science* **300**, 1250–1250. (doi:10.1126/science.1084960)
- Devlin RH, Dandrade M, Uh M, Biagi CA. 2004 Population effects of growth hormone transgenic coho salmon depend on food availability and genotype by environment interactions. *Proc. Natl Acad. Sci. USA* **101**, 9303–9308. (doi:10.1073/pnas.0400023101)
- Howard RD, DeWoody JA, Muir WM. 2004 Transgenic male mating advantage provides opportunity for Trojan gene effect in a fish. *Proc. Natl Acad. Sci. USA* **101**, 2934–2938. (doi:10.1073/pnas.0306285101)
- Kapuscinski A *et al.* 2007 Approaches to assessing gene flow. In *Environmental risk assessment of genetically modified organisms: methodologies for transgenic fish*, vol. 3 (eds AR Kapuscinski, KR Hayes, S Li, G Dana), pp. 112–150. Oxfordshire, UK: CABI Publishing.
- Seehausen O, Takimoto G, Roy D, Joklea J. 2001 Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol. Ecol.* **17**, 30–44. (doi:10.1111/j.1365-294X.2007.03529.x)
- Grosholz E. 2002 Ecological and evolutionary consequences of coastal invasions. *Trends Ecol. Evol.* **17**, 22–27. (doi:10.1016/S0169-5347(01)02358-8)
- Davison A, Birks JDS, Griffiths HI, Kitchener AC, Biggins D, Butlin RK. 1999 Hybridization and the phylogenetic relationship between polecats and domestic ferrets in Britain. *Biol. Conserv.* **87**, 155–161. (doi:10.1016/S0006-3207(98)00067-6)
- Jensen AB, Palmer KA, Boomsma JJ, Pedersen BV. 2005 Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honeybee, *Apis mellifera mellifera*, in northwest Europe. *Mol. Ecol.* **14**, 93–106. (doi:10.1111/j.1365-294X.2004.02399.x)
- Castillo AGF, Ayllon F, Moran P, Izquierdo JJ, Martinez JL, Beall E, Garcia-Vazquez E. 2008 Interspecific hybridization and introgression are associated with stock transfers in salmonids. *Aquaculture* **278**, 31–36. (doi:10.1016/j.aquaculture.2008.03.029)
- Nam YK, Park I-S, Kim DS. 2004 Triploid hybridization of fast-growing transgenic mud loach *Misgurnus mizolepis* male to cyprinid loach *Misgurnus anguillicaudatus* female: the first performance study on growth and reproduction of transgenic polyploid hybrid fish. *Aquaculture* **231**, 559–572. (doi:10.1016/j.aquaculture.2003.09.046)
- Vacher C, Kossler TM, Hochberg ME, Weis AE. 2011 Impact of interspecific hybridization between crops and weedy relatives on the evolution of flowering time in weedy phenotypes. *PLoS ONE* **6**, e14649. (doi:10.1371/journal.pone.0014649)
- Hindar K, Balstad T. 1994 Salmonid culture and intraspecific hybridization. *Conserv. Biol.* **8**, 881–882. (doi:10.1046/j.1523-1739.1994.08030863-10.x)
- Youngson AF, Webb JH, Thompson CE, Knox D. 1993 Spawning of escaped farmed Atlantic salmon (*Salmo salar*): hybridization of females with brown trout (*Salmo trutta*). *Can. J. Fish. Aquat. Sci.* **50**, 1986–1990. (doi:10.1139/f93-221)
- Jansson H, Öst T. 1997 Hybridization between Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in a restored section of the River Dälälven, Sweden. *Can. J. Fish. Aquat. Sci.* **54**, 2033–2039. (doi:10.1139/f97-111)
- Hórreo JL, Ayllón F, Perez J, Beall E, Garcia-Vazquez E. 2011 Interspecific hybridization, a matter of pioneering? Insights from Atlantic salmon and brown trout. *J. Hered.* **102**, 237–242. (doi:10.1093/jhered/esq130)
- Álvarez D, Garcia-Vazquez E. 2011 Maintenance of asymmetric hybridization between Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) via postzygotic barriers and paternal effects. *Can. J. Fish. Aquat. Sci.* **68**, 593–602. (doi:10.1139/f2011-004)
- McGowan C, Davidson WS. 1992 Unidirectional natural hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) in Newfoundland. *Can. J. Fish. Aquat. Sci.* **49**, 1953–1958. (doi:10.1139/f92-216)
- Yaskowiak E, Shears M, Agarwal-Mawal A, Fletcher G. 2006 Characterization and multi-generational stability of the growth hormone transgene (EO-1α) responsible for enhanced growth rates in Atlantic Salmon. *Transgenic Res.* **15**, 465–480. (doi:10.1007/s11248-006-0020-5)
- Moreau DTR, Conway C, Fleming IA. 2011 Reproductive performance of alternative male phenotypes of growth hormone transgenic Atlantic salmon (*Salmo salar*). *Evol. Appl.* **4**, 736–748. (doi:10.1111/j.1752-4571.2011.00196.x)
- Abrahams MV, Sutterlin A. 1999 The foraging and antipredator behaviour of growth-enhanced transgenic Atlantic salmon. *Anim. Behav.* **58**, 933–942. (doi:10.1006/anbe.1999.1229)
- Sundström LF, Lohmus M, Johnsson J, Devlin RH. 2004 Growth hormone transgenic salmon pay for growth potential with increased predation mortality. *Proc. R. Soc. B* **271**, S350–S352. (doi:10.1098/rsbl.2004.0189)
- Devlin RH, Sundström LF, Muir WM. 2006 Interface of biotechnology and ecology for environmental risk assessments of transgenic fish. *Trends Biotechnol.* **24**, 89–97. (doi:10.1016/j.tibtech.2005.12.008)
- Moreau DTR, Fleming IA. 2012 The potential ecological and genetic impacts of aquaculture biotechnologies: eco-evolutionary considerations for managing the blue revolution. In *Aquaculture biotechnology* (eds GL Fletcher, ML Rise), pp. 319–342. Oxford, UK: Wiley Blackwell.
- Devlin RH, Biagi CA, Yesaki TY, Smailus DE, Byatt JC. 2001 Growth of domesticated transgenic fish: a growth-hormone transgene boosts the size of wild but not domesticated trout. *Nature* **409**, 781–782. (doi:10.1038/35057314)
- Fletcher GL, Shears MA, Yaskowiak ES, King MJ, Goddard SV. 2004 Gene transfer: potential to enhance the genome of Atlantic salmon for aquaculture. *Aust. J. Exp. Agric.* **44**, 1095–1100. (doi:10.1071/ea03223)
- Moreau DTR, Fleming IA, Fletcher GL, Brown JA. 2011 Growth hormone transgenesis does not influence territorial dominance or growth and survival of first-feeding Atlantic salmon *Salmo salar* in food-limited stream microcosms. *J. Fish Biol.* **78**, 726–740. (doi:10.1111/j.1095-8649.2010.02888.x)
- Jonsson B, Jonsson N. 2011 *Ecology of Atlantic salmon and brown trout: habitat as a template for life histories*. New York: Springer.
- Deitch EJ, Fletcher GL, Petersen LH, Costa IASF, Shears MA, Driedzic WR, Gamperl AK. 2006 Cardiorespiratory modifications, and limitations, in post-smolt growth hormone transgenic Atlantic salmon *Salmo salar*. *J. Exp. Biol.* **209**, 1310–1325. (doi:10.1242/jeb.02105)

32. Hothorn T, Bretz F, Westfall P. 2008 Simultaneous inference in general parametric models. *Biomed. J.* **50**, 346–363. (doi:10.1002/bimj.200810425)
33. Ostrovsky I. 1995 The parabolic pattern of animal growth: determination of equation parameters and their temperature dependencies. *Freshw. Biol.* **33**, 357–371. (doi:10.1111/j.1365-2427.1995.tb00398.x)
34. Elliott JM, Hurley MA. 1997 A functional model for maximum growth of Atlantic salmon parr, *Salmo salar*, from two populations in northwest England. *Funct. Ecol.* **11**, 592–603. (doi:10.1046/j.1365-2435.1997.00130.x)
35. R Development Team 2012 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation of Statistical Computing. See <http://www.R-project.org/>.
36. McGowan C, Davidson WS. 1992 Artificial hybridization of Newfoundland brown trout and Atlantic salmon: hatchability, survival and growth to first feeding. *Aquaculture* **106**, 117–125. (doi:10.1016/0044-8486(92)90196-R)
37. Rhymer JM, Simberloff D. 1996 Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* **27**, 83–109. (doi:10.1146/annurev.ecolsys.27.1.83)
38. Allendorf FW, Leary RF, Spruell P, Wenburg JK. 2001 The problems with hybrids: setting conservation guidelines. *Trends Ecol. Evol.* **16**, 613–622. (doi:10.1016/S0169-5347(01)02290-X)
39. Ryan ME, Johnson JR, Fitzpatrick BM. 2009 Invasive hybrid tiger salamander genotypes impact native amphibians. *Proc. Natl Acad. Sci. USA* **106**, 11 166–11 171. (doi:10.1073/pnas.0902252106)
40. Seiler SM, Keeley ER. 2009 Competition between native and introduced salmonid fishes: cutthroat trout have lower growth rate in the presence of cutthroat–rainbow trout hybrids. *Can. J. Fish. Aquat. Sci.* **66**, 133–141. (doi:10.1139/f08-194)
41. Van Zwol JA, Neff BD, Wilson CC. 2012 The effect of competition among three salmonids on dominance and growth during the juvenile life stage. *Ecol. Freshw. Fish* **21**, 533–540. (doi:10.1111/j.1600-0633.2012.00573.x)
42. Devlin RH, Johnsson JI, Smailus DE, Biagi CA, Jonsson E, Björnsson BT. 1999 Increased ability to compete for food by growth hormone-transgenic coho salmon *Oncorhynchus kisutch* (Walbaum). *Aquaculture Res.* **30**, 479–482. (doi:10.1046/j.1365-2109.1999.00359.x)
43. Sundt-Hansen L, Einum S, Neregård L, Björnsson BT, Johnsson JI, Fleming IA, Devlin RH, Hindar K. 2012 Growth hormone reduces growth in free-living Atlantic salmon fry. *Funct. Ecol.* **26**, 904–911. (doi:10.1111/j.1365-2435.2012.01999.x)
44. Cook JT, McNiven MA, Sutterlin AM. 2000 Metabolic rate of pre-smolt growth-enhanced transgenic Atlantic salmon (*Salmo salar*). *Aquaculture* **188**, 33–45. (doi:10.1016/S0044-8486(00)00332-X)
45. Bessey C, Devlin RH, Liley NR, Biagi CA. 2004 Reproductive performance of growth-enhanced transgenic coho salmon. *Trans. Am. Fish. Soc.* **133**, 1205–1220. (doi:10.1577/t04-010.1)
46. Fitzpatrick JL, Akbarshandiz H, Sakhrani D, Biagi CA, Pitcher TE, Devlin RH. 2011 Cultured growth hormone transgenic salmon are reproductively out-competed by wild-reared salmon in semi-natural mating arenas. *Aquaculture* **312**, 185–191. (doi:10.1016/j.aquaculture.2010.11.044)
47. Fleming IA, Jonsson B, Gross MR, Lamberg A. 1996 An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). *J. Appl. Ecol.* **33**, 893–905. (doi:10.2307/2404960)
48. Fleming IA, Lamberg A, Jonsson B. 1997 Effects of early experience on the reproductive performance of Atlantic salmon. *Behav. Ecol.* **8**, 470–480. (doi:10.1093/beheco/8.5.470)
49. Berejikian BA, Tezak EP, Schroder SL. 2001 Reproductive behaviour and breeding success of captively reared Chinook salmon. *N. Am. J. Fish. Manage.* **21**, 255–260. (doi:10.1577/1548-8675(2001)021<0255:RBABSO>2.0.CO;2)
50. Matthews MA, Poole WR, Thompson CE, McKillen J, Ferguson A, Hindar K, Wheelan KF. 2000 Incidence of hybridization between Atlantic salmon, *Salmo salar* L, and brown trout, *Salmo trutta* L, in Ireland. *Fish. Manage. Ecol.* **7**, 337–347. (doi:10.1046/j.1365-2400.2000.00208.x)
51. Garcia-Vazquez E, Perez J, Ayllon F, Martinez JL, Glise S, Beall E. 2004 Asymmetry of post-F1 interspecific reproductive barriers among brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). *Aquaculture* **234**, 77–84. (doi:10.1016/j.aquaculture.2004.01.017)
52. Castillo AGF, Beall E, Moran P, Martinez JL, Ayllon F, Garcia-Vazquez E. 2007 Introgression in the genus *Salmo* via allotriploids. *Mol. Ecol.* **16**, 1741–1748. (doi:10.1111/j.1365-294X.2007.03257.x)