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Fitness component assessments of wild-type and growth hormone transgenic coho salmon reared in seawater mesocosms

Rosalind A. Leggatt [a,*], Carlo A. Biagi [a], Dionne Sakhrani [a], Robert Dominelli [a], Erika J. Eliason [b,1], Anthony P. Farrell [b], Robert H. Devlin [a,*]

[a] Fisheries and Oceans Canada, West Vancouver Laboratories, 4160 Marine Drive, West Vancouver, BC V7V 1N6, Canada
[b] Department of Zoology and The Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

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ABSTRACT

Growth hormone (GH) transgenic fish have been proposed for use in aquaculture to enhance production efficiency. As part of a risk analysis for use of such fish, the influence of GH transgenesis on the potential to persist and succeed in natural ecosystems is being examined in confined laboratory conditions. GH transgenesis can greatly accelerate growth and, in culture conditions, is associated with secondary effects such as poor swimming capacity and spawning success. However, standard culture has also been shown to negatively affect fitness components of wild-type fish, raising the question of whether culture conditions influence fitness components of transgenic fish in a similar way. To examine factors influencing the phenotype of marine-stage GH transgenic salmon (T), and to determine if genotype-by-environment interactions exist at this life stage, we grew T and wild-type (NT) coho salmon (*Oncorhynchus kisutch*) over six cohort years in 350,000 L seawater tanks (termed mesocosms) designed to minimize effects of standard culture conditions. Mesocosm rearing partially facilitated development of normal size and morphology of NT fish relative to nature-reared counterparts, but altered overall body shape, indicating mesocosm conditions do not fully mimic natural environmental effects on coho salmon phenotype. T fish reared in mesocosms had larger mass at maturity than mesocosm- or nature-reared NT fish, indicating GH transgenesis can alter maximum obtainable mass in salmon. Unlike NT, T fish obtained maximum size at maturity across environments, suggesting marine environmental conditions may affect T growth less than NT growth. Screening parents for a common disease agent (*Renibacterium salmoninarum*) improved seawater survival, and T fish had lower survival than NT fish when from unscreened parents and inconsistent relative survival when from screened parents, indicating GH transgenesis may constitute an advantage or disadvantage in terms of survival. Transgenic salmon had lower swimming capacity and aerobic scope, but similar routine metabolic rate and thermal tolerance, demonstrating transgenesis can have different influences depending on what phenotype is examined. Using an alternate strain of T fish in phenotypic comparisons did not greatly influence most fitness components, although had a strong effect on female fecundity. The inconsistent influence of GH transgenesis on different fitness components, and existence of genotype-by-environment interactions during the marine life stage, complicates extrapolation of laboratory data for transgenic fish to natural environments. However, current and previous data do not provide evidence that overall increased performance of GH transgenic salmon over wild-type fish would arise in the marine environment.

Statement of relevance: Rearing in seawater mesocosms demonstrate that growth hormone transgenesis has inconsistent effects on marine fitness components in coho salmon.

Abbreviations: bl, body length; CAER, Centre for Aquaculture and Environmental Research; CF, condition factor; COT, cost of transport; COTnet, net cost of transport; DFO, Fisheries and Oceans Canada; EPOC, excess post-exercise oxygen consumption; GH, growth hormone; LOE, loss of equilibrium; LSM, least square means; MO_2, oxygen uptake; $MO_{2\text{-max}}$, maximum obtainable oxygen uptake; $MO_{2\text{-R}}$, routine oxygen uptake; NT, non-transgenic (wild-type) coho salmon; OnH3GH1, sockeye salmon histone-3 promoter driving expression of the growth hormone 1 gene from the same species; OnMTGH1, sockeye salmon metallothionein-B promoter driving expression of the growth hormone 1 gene from the same species; PIT, passive integrated transponder; Rs, *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease; SGR, standard growth rate (mass); T, growth hormone transgenic coho salmon; T_{H3}, growth hormone transgenic coho salmon containing the OnH3GH1 transgene; T_{MT}, growth hormone transgenic coho salmon containing the OnMTGH1 transgene; U_{crit}, maximum sustainable swim speed.

* Corresponding authors.
E-mail addresses: rosalind.leggatt@gmail.com (R.A. Leggatt), robert.devlin@dfo-mpo.gc.ca (R.H. Devlin).
[1] Current address: Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara 93106, USA.

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1. Introduction

Growth hormone (GH) transgenesis is known to greatly accelerate growth rates in many fish species, and the use of this technology for aquaculture production is now approved in the USA and Canada for an Atlantic salmon (*Salmo salar*) model. While the risks of accidental escapes that could allow a transgene to introgress into natural populations is minimized in highly secure land-based facilities coupled with biological containment strategies, other scenarios where transgenic fish might more readily enter nature (e.g. open net pens, pond culture, etc.) dictate an urgent need to assess potential ecological impacts. The purpose of the present study was to contribute to such assessments for transgenic coho salmon (*Oncorhynchus kisutch*) maintained in marine land-based culture conditions where abiotic and biotic factors (e.g. water supply, lighting, rearing density, human interactions) more closely mimicked natural conditions than in previous studies.

The potential for GH transgenic fish to impact natural ecosystems is determined in part by their ability to survive, reproduce and persist within these ecosystems, i.e., fitness. GH transgenic fish are known to have altered factors than can influence fitness (hereafter termed "fitness components") compared to non-transgenic fish. For example, GH transgenic fish reared in standard culture when compared with non-transgenic counterparts reared in nature or standard culture have lower reproductive success, swimming ability, survival, and disease resistance (Bessey et al. 2004; Devlin et al. 2004a; Figueiredo et al. 2013; Jhingan et al. 2003; Kim et al. 2013; Lee et al. 2003a; Li et al. 2007; Moreau et al. 2011) while having a faster growth rate and being more aggressive (Devlin et al. 1999; Devlin et al. 1994; Du et al. 1992; Duan et al. 2011; Rahman et al. 1998, see Devlin et al. 2015). However, a strong effect of culture conditions is known to affect many fitness components in non-transgenic salmon (e.g. reproduction, body size, Berejikian et al. 2001b; Berejikian et al. 2001a; Berejikian et al. 1997; Bessey et al. 2004), and genotype-by-environment interactions have been identified when comparing GH transgenic and non-transgenic fish (e.g. juvenile growth rates and behaviour, Devlin et al. 2004b; Sundström et al. 2007, adult spawning behaviour and success, Leggatt et al. 2014). Consequently, it is very difficult to predict the fitness of GH transgenesis in nature, as well as resulting ecological effects, without better mimicking natural abiotic and biotic factors. Indeed, indications that laboratory culture conditions may be poor indicators of how fish respond to natural environments come from observations that altered laboratory rearing conditions can greatly limit size at maturity and spawning success of non-transgenic coho salmon (Bessey et al. 2004; Devlin et al. 2004a), and potentially limit growth in GH transgenic tilapia (Martínez et al. 2000).

While rearing of juvenile GH transgenic salmonids in semi-natural contained stream systems has already provided insight into how GH transgenic fry may respond to natural conditions as well as interact with ecosystem components (e.g., Sundström et al. 2007, see Devlin et al. 2015), creating contained environmental conditions that partially or fully mimic natural marine conditions of salmon (i.e., the ocean) has proven more of a challenge. To fill this void and directly assess whether or not marine rearing conditions impact fitness of GH transgenic salmon, we raised GH transgenic and non-transgenic coho salmon from the smolt stage to maturation in extremely large 350,000 L seawater tanks (termed mesocosms) where abiotic and biotic factors (e.g. water supply, lighting, rearing density, human interactions) were maintained close to natural conditions. We compared growth and survival of transgenic and non-transgenic salmon over multiple cohort years to gain an understanding of overall fitness effects of GH transgenesis in the marine environment, as well as to assess the influence of year-to-year variation on relative growth and survival of GH transgenic compared to non-transgenic coho salmon. We further examined the effect of GH transgenesis on other marine fitness components (swimming ability, swimming efficiency and thermal tolerance) that are known to influence the ability of near-mature fish to migrate to natural rivers to spawn (e.g., Eliason et al. 2011). We also studied whether fitness components were consistent between two strains of GH transgenic coho salmon to determine if fitness estimates can be inferred across GH transgenic strains within a species.

2. Materials and methods

2.1. Fish

Experiments were conducted at the Fisheries and Oceans Canada (DFO) Centre for Aquaculture and Environmental Research (CAER), West Vancouver, BC, Canada under an institutional animal care permit meeting guidelines established by the Canadian Council for Animal Care. The facility was specifically designed to prevent escape of GH transgenic fish to natural ecosystems. All coho salmon used in this study possessed a Chehalis River, BC, hatchery genetic background and transgenic lines were originally propagated within this hatchery strain. The hatchery strain is propagated at each generation using wild fish collected from nature and hatchery returns both of which are phenotypically highly similar (Chittenden et al. 2010). Unless otherwise stated, GH transgenic salmon were M77-strain fish (termed T or T_{MT}) produced by insertion of the OnMTGH1 gene construct containing GH1 driven by the metallothionein-B promoter both from sockeye salmon (*O. nerka*; Devlin et al. 2004b; Devlin et al. 1994). Some experiments also used a second line of transgenic fish (T_{H3}) that contained the same GH1 construct structure as OnMTGH1, but was coupled to a histone-3 promoter from sockeye salmon (OnH3GH1, H3-3339 line, see Leggatt et al. 2012). Transgenic fish used in this experiment were produced by crossing T parents with wild-caught Chehalis River hatchery salmon, and wild-type non-transgenic smolts (NT) were produced from wild-caught Chehalis River hatchery salmon. Fish were produced by pair or batch crosses using a minimum 7 females and 5 males. Genetic diversity and hatchery-strain background of transgenic fish lines was maintained by back-crossing to wild-caught Chehalis River hatchery salmon at each generation, in order to assess the potential impacts of the transgene in a wild population.

2.2. Rearing conditions

Post-smolt rearing of NT and T coho salmon was conducted in three replicate mesocosms designed to minimize the effects of culture (i.e., 350,000 L seawater tanks as described by Leggatt et al. 2014, see Fig. 1). In brief, mesocosm tanks were supplied with ambient temperature, flow-through, sand-filtered seawater with unidirectional inflow (approximately 460 L/min) to stimulate continuous swimming of fish at low density (<2 kg/m^3) and with natural lighting. The marine organisms that colonized these mesocosms, presumably entering as larvae through the natural water supply, included chitons (Class Polyplacophora), sea anemones (Subclass Hexacorallia), and nudibranchs (Order Nudibranchia). The mesocosm upper edges were fitted with fine mesh screens that reached 2 m above the floor, which had a primary purpose of minimizing fish disturbance resulting from human presence. Fish were implanted with Passive Integrated Transponder (PIT) tags (BioMark, Idaho USA) prior to introduction to the mesocosm. During mesocosm rearing fish were hand fed 2 times per day to satiation with size-appropriate commercial salmonid diet (Skretting Canada Ltd., Vancouver, BC, Canada). Approximately every 3 months, all fish were seine-netted out of the mesocosms and lightly anaesthetized with 50 mg/L tricaine methanesulfonate for enumeration and measurement of mass and length.

2.3. Effect of transgenic and promoter type on growth and survival

Overall size at maturity and seawater survival of T and NT fish were compared over six year classes (2007–2013). The six year classes were designated by the year that fish entered seawater and termed smolt-