

Research



Cite this article: Crossin GT, Devlin RH. 2017 Predation, metabolic priming and early life-history rearing environment affect the swimming capabilities of growth hormone transgenic rainbow trout. *Biol. Lett.* **13**: 20170279.
<http://dx.doi.org/10.1098/rsbl.2017.0279>

Received: 11 May 2017

Accepted: 8 August 2017

Subject Areas:

behaviour, ecology, biotechnology, evolution

Keywords:growth hormone, transgenic, risk assessment, mesocosm, *Oncorhynchus***Author for correspondence:**

Glenn T. Crossin

e-mail: gtc@dal.ca

Physiology

Predation, metabolic priming and early life-history rearing environment affect the swimming capabilities of growth hormone transgenic rainbow trout

Glenn T. Crossin¹ and Robert H. Devlin²¹Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2²Fisheries and Oceans Canada, West Vancouver Laboratory, Centre for Aquaculture and Environmental Research, 4160 Marine Drive, West Vancouver, British Columbia, Canada V7 V 1N6

GTC, 0000-0003-1080-1189

The period of first feeding, when young salmonid fishes emerge from natal stream beds, is one fraught with predation risk. Experiments conducted in semi-natural stream mesocosms have shown that growth hormone transgenic salmonids are at greater risk of predation than their non-transgenic siblings, due partly to the higher metabolic demands associated with transgenesis, which force risky foraging behaviours. This raises questions as to whether there are differences in the swim-performance of transgenic and non-transgenic fishes surviving predation experiments. We tested this hypothesis in wild-origin rainbow trout (*Oncorhynchus mykiss*) that were reared from first feeding in semi-natural stream mesocosms characterized by complex hydrodynamics, the presence of predators and oligotrophic conditions. Using an open-flume raceway, we swam fish and measured their capacity for burst-swimming against a sustained flow. We found a significant genotype effect on burst-performance, with transgenic fish sustaining performance longer than their wild-type siblings, both in predator and predator-free stream segments. Importantly, this effect occurred before differences in growth were discernable. We also found that mesocosm-reared fish had greater burst-performance than fish reared in the controlled hatchery environment, despite the latter being unexposed to predators and having abundant food. Our results suggest a potential interaction between predation and metabolic priming, which leads to greater burst capacity in transgenic trout.

1. Introduction

With the ever-advancing pace of gene technologies, there is a persistent drive to manipulate the phenotypes of commercially viable plant and animal species to accelerate food production cycles and increase yield. In many countries, various transgenic plants have become commonplace in agriculture, justified by the need to feed the planet's burgeoning human population [1]. Until recently, no government had approved the use of transgenic animals for human consumption, due mostly to uncertainties concerning the potential effects on human health and impacts on the environment. However, in 2016 the first federal approvals for the commercial production and sale of growth hormone (GH) transgenic Atlantic salmon (*Salmo salar*) were granted in the USA and Canada. Unlike transgenic plants, which are usually produced from domesticated strains and show limited capacity to establish self-sustaining populations in the wild, transgenic fishes are usually derived from wild-origin progenitors, which can easily breed with wild conspecifics or hybridize with closely related species. This reality has been an ongoing source of scientific and social concern, for breaches to containment

Table 1. Biological characteristics and details concerning the rainbow trout used for burst-swimming trials.

	rearing environment	family	genotype	predator treatment	number surviving ^a	number swum	length (mm) ± s.d.	mass (g) ± s.d.
core analysis	mesocosm	A	transgenic	pred.	8	7	38.1 ± 8.04	0.44 ± 0.19
				no pred.	32	22	37.7 ± 4.71	0.52 ± 0.25
		non-transgenic	pred.	34	34	35.2 ± 3.27	0.46 ± 0.15	
			no pred.	81	13	36.9 ± 5.11	0.58 ± 0.22	
relative controls	mesocosm	B	non-transgenic	pred.	48	5	39.4 ± 3.29	0.70 ± 0.16
				no pred.	79	5	36.0 ± 3.54	0.48 ± 0.15
	hatchery	A	transgenic	no pred.	74	6	36.5 ± 0.84	0.43 ± 0.03
				non-transgenic	no pred.	75	7	34.9 ± 3.76
		B	non-transgenic	no pred.	78	15	34.3 ± 2.85	0.38 ± 0.12

^aThose surviving are from an initial group of 100 fish per experimental group [7]. Most, but not all, fish in each group were swum, as time permitted.

systems could permit transgenic individuals to escape from culturing facilities into the wild [1]. Although the recent approvals for transgenic salmon in North America stipulate that those produced are made sterile via triploidy, which reduces the risk of introgression if escape should occur, such methods are not always 100% effective [2], and so legitimate concerns remain over the impacts that GH-transgenic salmonids could have on the environment.

Hypothetically, the most probable ways that GH-transgenic salmonids could find their way into the environment are the escape of adults and juveniles from contained land-based production facilities or open-water net pens, and the spawning of such escapees with wild conspecifics. There have been several studies that have assessed these risks using semi-natural stream mesocosms [3–7]. The period of first feeding, when salmonid fishes emerge from natal stream beds to begin their free-swimming life, is one fraught with predation risk. These studies show that GH-transgenic fishes are highly susceptible to predation during this life-history phase, as the high metabolic demands associated with extraordinary growth force risky foraging behaviours (but see [8]). However, whether transgenesis should confer a deleterious, neutral or beneficial effect to growth and survival, experiments show that some transgenic individuals can ultimately avoid predation and survive, and so the ecological risks posed by transgenic fishes remain.

One trait important for survival and predator avoidance is burst-swimming capacity. Although it can be defined various ways [9], burst capacity can be measured as the maximum duration that a fish can sustain anaerobic swimming [9]. When one considers the fishes that survive the natural mesocosm predation experiments referenced above, an important question arises: do transgenic and non-transgenic fishes that survive the period of first feeding by avoiding predation possess intrinsically different burst-swimming capabilities?

In this article, we compared the burst-swimming performance in a family of juvenile GH-transgenic rainbow trout (*Oncorhynchus mykiss*) and their wild-type non-transgenic siblings. Swim tests were performed on fish that survived a first feeding, predation experiment conducted in replicated, semi-natural stream mesocosms [7]. Our models examine potential genotype-by-environment interactions on burst capacity [5]. To put our results into context, we also present burst data from a second family of trout that were also exposed to predator treatments in mesocosms, but in the absence of transgenic

siblings as this family was all non-transgenic. Finally, we also present burst results from fish in both families that were reared in a controlled, predator-free, hatchery environment. Our mesocosm results suggest a potential interaction between predation and metabolic priming, which enhances the swimming phenotype of transgenic trout.

2. Material and methods

Transgenic strains of rainbow trout were produced via microinjection of a growth hormone gene construct (OnMTGH1) into eggs of wild females [10,11]. After rearing, mature males hemizygous for the gene construct were crossed with multiple wild females to produce a population with equal proportions of offspring that were non-transgenic or hemizygous transgenic. This construct is known to induce extraordinary growth in this population of trout [10], but approximately seven and eight weeks after first feeding—our trout were tested only five weeks post first feeding. Genotypes were verified using a transgene-specific polymerase chain reaction assay [12]. These fish are henceforth called wild-type non-transgenic (NT) or GH-transgenic (T). Trout from a single genetic family (family A) maximized our ability to identify transgene effects, thus minimizing background genetic influences. Although we have not determined the structure of this transgene, we do know that it is located at a single genomic site based on Mendelian segregation (50 : 50).

In a study reported elsewhere [7], fry from family A were exposed to a 25 day stream mesocosm predation experiment. At the end of this experiment, the T- and NT-survivors from replicated predator and predator-free stream segments were assessed for burst-swimming capacity using an open-flume raceway. Similarly, T- and NT-siblings of these trout were reared simultaneously in controlled hatchery tanks, without predators and with food provided ad libitum. Burst capacity of these hatchery fish was also assessed. Additionally, a second family of trout (family B, all-NT) were also swum. Family B trout were subjected to a parallel experimental mesocosm treatment, simultaneously, as for those in family A, but rather than a 50T : 50NT ratio all fish were NT. Table 1 provides details about all of the fish used in our swim trials. The main analysis is of the genotype and predation effects on family A in the mesocosm. Family A's hatchery-reared siblings and mesocosm-reared NT-fish from family B are both used as points of reference against which genotype and treatment effects can be interpreted in family A.

The swimming area of the flume measured 100 × 17 × 4 cm [9]. Mesh grates were placed at the front and rear to keep fish

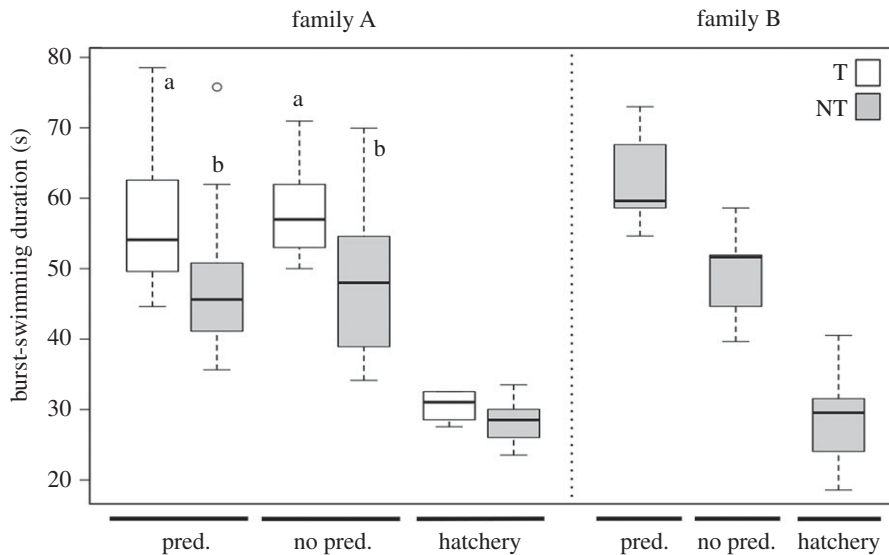


Figure 1. Boxplots showing the burst-swimming performance of GH-transgenic (T) and non-transgenic (NT) rainbow trout siblings, reared in different environments and predation regimes. Differing letters indicate statistically significant contrasts within family A, which is the focal group in which gene-by-predation interactions were examined (see Results). Burst results from a second family of all-NT-fish are also presented for comparison, as are results from hatchery-reared fish from both families.

within the flume. Two honeycomb flow-straighteners were used to ensure laminar flow. Unfiltered water was supplied from a wild stream, which was partially diverted into the facility (12–19 September 2010). Water velocity during trials was maintained at 67 cm s^{-1} [9]. Each swim trial was recorded with a wide-angle video camera.

For each swim trial, fish were haphazardly selected from either the family A mesocosm, the family B mesocosm (all-NT) or the hatchery (families A and B). Individuals were placed at a starting position 30 cm downstream from the flume's front. After a 30 min acclimation period, in which the fish held position against a moderate current of 15 cm s^{-1} , the current was increased to 67 cm s^{-1} to induce burst-swimming. We timed burst duration until the fish fatigued. Fatigue was defined as reduced swimming speed and fallback to the most downstream end of the flume. Individuals were tested once. They were then anaesthetized, weighed ($\pm 0.01 \text{ g}$), measured (nearest millimetre) and fin-clipped for molecular genotyping (PCR [7]).

A linear mixed model (*lme4* package, R Project) was used to compare the burst capacity in the core group of fish from family A, where genotype, treatment and stream segment were fixed-effects, body length a covariate and tank nested within treatment a random effect. Linear regression was used to show the relationship between fish length and burst capacity.

3. Results

A total of 114 trout were assessed for burst capacity (table 1). In family A, there was a significant genotype effect, such that burst duration was higher in the T mesocosm-reared fish relative to their NT-siblings, irrespective of predator treatment and after controlling for variation in body length (figure 1; $n = 76$, genotype $t_6 = -2.131$, $p = 0.0317$, treatment $t_6 = 0.182$, $p = 0.979$, genotype \times treatment $t_6 = -0.761$, $p = 0.449$, length $t_6 = 5.273$, $p < 0.001$). Burst capacities of the all-NT family B fish, and fish from both families reared in the hatchery, are presented as points of reference (i.e. relative controls) to aid the interpretation of the genotype effect in family A (figure 1).

Although there was no length difference between T and NT-fish (ANOVA by genotype, rearing environment and

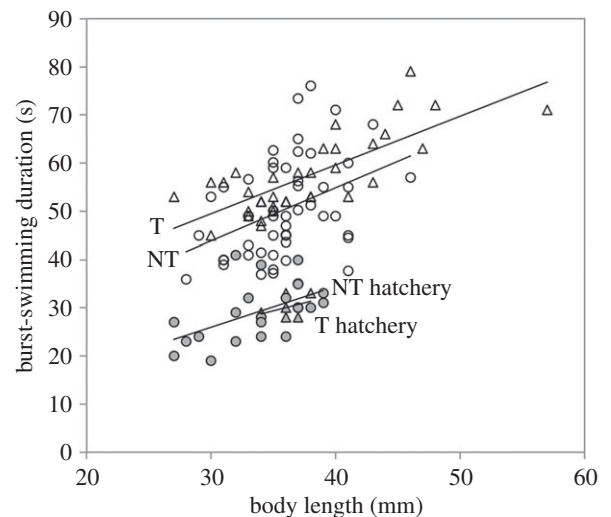


Figure 2. Relationship between body length and burst-swimming capacity in GH-transgenic (T) and non-transgenic (NT) rainbow trout, reared in different environments and predation regimes.

family: $n = 114$; $F = 2.129$, $p = 0.082$), there were significant positive relationships between individual length and burst capacity (figure 2).

4. Discussion

The ability of young fishes to invoke anaerobic metabolism and fast predator-avoidance behaviours is important to fitness. Our results show that despite the higher susceptibility of GH-transgenic trout [7] and other salmonids [3–5] to predation, predation can nevertheless allow some dominant phenotypes to emerge, via enhanced burst-swimming capacities. This is illustrated in figure 1, in which the transgenic trout from family A show greater burst capacity compared to their NT-siblings, irrespective of predator treatment. Importantly, these differences emerged at a developmental stage *before* a significant growth effect could be detected between T- and

NT-salmonids. This suggests two things. Firstly, in the absence of predation, food limitation itself seems to have deleterious effects on the survival of T-fish (table 1), presumably because T-fish cannot satisfy the basic metabolic demands of transgenesis. However, in this case, the survivors may have benefited from GH-metabolic priming, which enhanced swimming ability to increase the likelihood of prey-capture in a naturally oligotrophic environment. Coupled with their greater food-conversion efficiencies and motivation to acquire food resources [11], this would explain how some T-trout persisted when most were dying. Secondly, under predation pressure, T-fish also suffered high mortality (table 1), from exposure via risky foraging behaviours, but here too, survivors had greater burst capacities. In this context, predation was probably the selecting agent, although it is possible that metabolic priming could have contributed to this. The NT-fish from both treatments had lower burst capacities, as predation and metabolically driven mortality was differentially directed towards their T-siblings living alongside them, and so there was less selection for burst capacity in the NT-fish. By contrast, results from the all-NT family B show that burst capacity was enhanced to a level similar to the T-fish in family A (figure 1). Here, predation appears to have been the stronger selective agent, as GH-metabolic priming would not have occurred in the NT-fish. Collectively, our results suggest that both predation and metabolic priming can affect the swimming performance of rainbow trout, in a context-dependent manner.

Finally, our results also show very clearly how rearing environment influences swimming phenotype, as all fish reared in the static hatchery environment, free from predation and with abundant food, had much lower capacity for burst-swimming (figure 1).

Our results have important implications for ecological risk assessment. Although other studies have found reduced swimming capabilities in juvenile T-fishes [13], as well as reduced survival in complex stream environments, this does not mean that the ecological impacts of transgenic fishes born in nature are negligible as individuals would be

swiftly removed from the population. Our results show that despite their low initial fitness [7], those transgenic trout that *survive* the early life-history mortality bottleneck could attain higher fitness at older life-stages, when predation pressure naturally relaxes [14]. We recognize that caution is warranted, as population-level impacts of GH-transgenesis are best determined on lifetime, rather than stage-specific, fitness [15]. Ultimately, the degree of transgenic introgression into wild populations, and the relative fitness of other physiological and behavioural traits, will determine how populations respond [16]. However, our results suggest a potential mechanism which might allow some transgenic trout to persist in nature during a life-history stage when mortality is naturally high. An important question remains: how relevant is this risk, when variable selection pressures will inevitably arise at other life-history stages? Further studies are needed to estimate the lifetime fitness costs of GH-transgenesis in trout and other salmonids.

Ethics. Research protocols were approved by Fisheries and Oceans Canada, Pacific Region Animal Care Committee (AUP 10-015). Mesocosm experiments were conducted at the Centre for Aquaculture and Environmental Research, West Vancouver, British Columbia. This federal research facility has multiple containment screen systems to prevent the escape of genetically modified fish to the wild.

Data accessibility. The data used for this study have been deposited in Dryad as <http://dx.doi.org/10.5061/dryad.bm5b6> [17].

Authors' contributions. G.T.C. and R.H.D. conceptualized the study, wrote the paper and approved the final version; G.T.C. collected and analysed the data; both authors are accountable for integrity of the data.

Competing interests. We have no competing interests.

Funding. This research was supported by funding from the Canadian Regulatory System for Biotechnology (grant no. 61740). Additional support was provided by a Natural Sciences and Engineering Research Council Visiting Fellowship.

Acknowledgements. Thanks to Lina Crossin and Eduardo Martins for their assistance in sampling fish, and to Scott Hinch for the use of his swim flume.

References

- Devlin RH, Sundström LF, Leggett RA. 2015 Assessing ecological and evolutionary consequences of growth-accelerated genetically engineered fishes. *BioScience* **65**, 685–700. (doi:10.1093/biosci/biv068)
- Devlin RH, Sakhrani D, Biagi CA, Eom K-W. 2010 Occurrence of incomplete paternal-chromosome retention in GH-transgenic coho salmon being assessed for reproductive containment by pressure-shock-induced triploidy. *Aquaculture* **304**, 66–78. (doi:10.1016/j.aquaculture.2010.03.023)
- 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, Löhmus M, Johnsson JI, 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)
- Sundström LF, Löhmus M, Tymchuk WE, Devlin RH. 2007 Gene-environment interactions influence ecological consequences of transgenic animals. *Proc. Natl Acad. Sci. USA* **104**, 3889–3894. (doi:10.1073/pnas.0608767104)
- 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)
- Crossin GT, Sundström LF, Vandersteen WE, Devlin RH. 2015 Early life-history consequences of growth-hormone transgenesis in rainbow trout reared in stream ecosystem mesocosms. *PLoS ONE* **10**, e0120173. (doi:10.1371/journal.pone.0120173)
- Sundström LF, Devlin RH. 2011 Increased intrinsic growth rate is advantageous even under ecologically stressful conditions in coho salmon (*Oncorhynchus kisutch*). *Evol. Ecol.* **25**, 447–460. (doi:10.1007/s10682-010-9406-1)
- Pon LB, Hinch SG, Wagner GN, Lotto AG, Cooke SJ. 2007 Swimming performance and morphology of juvenile sockeye salmon, *Oncorhynchus nerka*: a comparison of an inlet and outlet fry population. *Environ. Biol. Fish.* **78**, 257–269. (doi:10.1007/s10641-006-9094-4)
- Devlin RH, Biagi CA, Yesaki TY, Smailus DE, Byatt JC. 2001 Growth of domesticated transgenic fish. *Nature* **409**, 781–782. (doi:10.1038/35057314)
- Devlin RH, Yesaki TY, Biagi CA, Donaldson EM, Swanson P, Chan WK. 1994 Extraordinary salmon growth. *Nature* **371**, 209–210. (doi:10.1038/371209a0)
- Fitzpatrick JL, Akbarshandiz H, Sakhrani D, Biagi CA, Pitcher TE, Devlin RH. 2011 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)

13. Deitch EJ, Fletcher GL, Petersen LH, Costa IAFS, 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)
14. Fleming IA, Hindar K, Mjølnerød IB, Jonsson B, Balstad T, Lamberg A. 2000 Lifetime success and interactions of farm salmon invading a native population. *Proc. R. Soc. B* **267**, 1517–1523. (doi:10.1098/rspb.2000.1173)
15. Muir WM, Howard RD. 1999 Possible ecological risks of transgenic organism release when transgenes affect mating success: sexual selection and the Trojan gene hypothesis. *Proc. Natl Acad. Sci. USA* **96**, 13 853–13 856. (doi:10.1073/pnas.96.24.13853)
16. Muir WM, Howard RD. 2002 Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. *Transgen. Res.* **11**, 101–114. (doi:10.1023/A:1015203812200)
17. Crossin GT, Devlin RH. 2017 Data from: Predation, metabolic priming and early life-history rearing environment affect the swimming capabilities of growth hormone transgenic rainbow trout. Dryad Digital Repository. (<http://dx.doi.org/10.5061/dryad.bm5b6>)