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Macro- and microparasite infection profiles of hatchery fish before and after release from a conservation hatchery (steelhead trout, *Oncorhynchus mykiss*)[1]

Carli HALPENNY[2] & Mart R. GROSS, Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada.

Abstract: Conservation hatcheries are becoming a tool in rebuilding salmonid populations, but their contributions are strongly debated. Most studies focus on genetic, morphological, and behavioural, issues and less is known of the importance of infectious agents. The Living Gene Bank (LGB) program of the British Columbia Ministry of the Environment is a recent effort to restore collapsed steelhead trout (*Oncorhynchus mykiss*) populations on the east coast of Vancouver Island. Our study describes the change in macro- and microparasitic profiles of LGB-hatchery-produced juveniles after they leave the hatchery and enter the natural river environment and also compares them with a limited wild smolt sample. We found that the hatchery was successful in raising parr free of infectious agents, but once introduced into the river environment, the hatchery fish quickly took on a diverse profile of infectious agents. The high numbers of fish released from the hatchery and the existence of hatchery fish that stay in the river as residents increased the number of fish in the system with sub-clinical levels of infection. An ecological understanding of the role resident hatchery fish play in the spatial distribution of infectious agents as well as the temporal and seasonal variations in infectious agent prevalence and diversity will be important in determining the net effect of the LGB conservation hatchery on the wild population.
Keywords: hatchery, *Oncorhynchus mykiss*, parasite, salmonid, survey.

Résumé : Les stations piscicoles de conservation deviennent un outil pour le rétablissement des populations de salmonidés, mais leur contribution est largement remise en question. La plupart des études se concentrent sur des questions génétiques, morphologiques et comportementales et on en connaît peu sur l'importance des agents infectieux. Le programme « Living Gene Bank (LGB) » du ministère de l'Environnement de la Colombie-Britannique est un récent effort visant à remédier à l'effondrement des populations de truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) sur la côte est de l'île de Vancouver. Notre étude décrit les changements de profils de macro et microparasites des juvéniles de pisciculture LGB après leur départ de la station piscicole et leur arrivée dans l'environnement fluvial naturel. Une comparaison est aussi effectuée avec un échantillon limité d'alevins sauvages. Nous avons constaté le succès de l'élevage en pisciculture à produire des tacons sans agents infectieux, mais une fois introduits dans l'environnement fluvial, les poissons de pisciculture ont rapidement présenté divers profils d'agents infectieux. La grande quantité de poissons d'élevage relâchés et leur présence dans la rivière en tant que résidents a fait augmenter le nombre de poissons ayant des niveaux d'infection subcliniques dans le système. Une compréhension écologique du rôle des poissons de pisciculture résidents dans la distribution spatiale des agents infectieux aussi bien que des variations temporelles et saisonnières de la prévalence et de la diversité des agents infectieux sera importante afin de déterminer l'effet net de la pisciculture de conservation LGB sur les populations sauvages.
Mots-clés : *Oncorhynchus mykiss*, parasite, relevé, salmonidé, station piscicole.

Nomenclature: Behnke, 1992.

Introduction

Once abundant salmonid populations have declined dramatically (Smith & Ward, 2000; Lackey, 2003), and disease has been one of several factors cited to threaten already at-risk populations (Nehlsen, Williams & Lichatowich, 1991). Indeed, pathogens such as *Ichthyophonus hoferi*, *Ichthyophthirius multifiliis*, and *Gyrodactylus salaris* have had significant impacts on Pacific (*Oncorhynchus nerka, O. tshawytscha)* and Atlantic (*Salmo salar)* salmon populations at juvenile and adult life history stages (Johnsen & Jensen, 1991; Traxler *et al.*, 1998; Kocan, Hershberger & Winton, 2004).

Conservation hatcheries, sometimes referred to as "Living Gene Banks" (LGB), are a common tool used to rebuild salmonid populations. These hatcheries aim to preserve or increase the number of individuals in a population without changing their genetic makeup (Wightman *et al.*, 1998; Flagg & Nash, 1999; O'Reilly & Doyle, 2007). They also aim to reduce the ecological impacts of hatchery releases on wild fish (Flagg & Nash, 1999). Typically, studies have focused on the genetic, morphological, and behavioural consequences of hatchery–wild interactions (Waples, 1991; Fleming & Gross, 1993; Berejikian, Mathews &

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Associate Editor: Marco A. Rodriguez.
[2]Author for correspondence. Present address: Institute of Parasitology, Macdonald Campus, McGill University, 21 111 Lakeshore Road, Ste. Anne de Bellevue, Quebec H9X 3V9, Canada.
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Quinn, 1996; Berejikian *et al.,* 1997), and while disease issues have been discussed, they are less studied (Bakke & Harris, 1998).

Potential disease issues could arise if hatchery fish carried new or altered infectious agents into the natural environment or if the hatchery-produced fish are more susceptible to natural pathogens (McVicar, 1997; Bakke & Harris, 1998; Lafferty & Gerber, 2002). Specifically, the inappropriate use of antibiotics has led to the evolution of resistant bacteria (Aoki, 1997; McVicar, 1997; Miranda & Zemelman, 2002) and the stress associated with fish culture reduces immune function (Schreck, 1996) which has been shown to increase infection in hatchery fish (Wedemeyer, 1997). The use of preventative measures in most hatcheries reduces the possibility of disease outbreak in culture; however, it also limits the pathogenic exposure of developing juveniles, possibly preventing the development of immunity (Bakke & Harris, 1998). Thus, upon release, hatchery fish may introduce novel infectious agents or may be more susceptible and thereby amplify endemic infectious agents in the wild environment.

Whether or not disease is manifested depends upon a complex set of interactions between host, pathogen and environment that can result in outcomes ranging from chronic infection to acute disease (McVicar, 1997; Hedrick, 1998; Reno, 1998). Pathogen transmission has been documented from hatchery to wild fish and *vice versa* (Flagg *et al.,* 2000), and epidemic outbreaks after supplementation of hatchery fish into the wild have had devastating effects on both hatchery production and wild systems (Johnsen & Jensen, 1991). The success of management and conservation efforts will depend upon an understanding of the complexity of these interactions which begins with detailed field observations and an ecological focus on the study of salmonid diseases (Bakke & Harris, 1998).

Wild steelhead trout (*Oncorhynchus mykiss*) populations on the east coast of Vancouver Island and the lower mainland of British Columbia (BC) have been in decline since 1989–1990 (Smith & Ward, 2000). In the Keogh River specifically, recruitment scenarios indicate the population is currently below replacement levels (Ward, 2000), resulting in their designation as being of "extreme conservation concern" by the British Columbia Ministry of the Environment (formerly BC Ministry of Water, Land and Air Protection). To help the populations recover the Ministry developed a "Steelhead Recovery Plan" that includes an LGB conservation hatchery program, watershed restoration, and reduced fishing pressure (Wightman *et al.,* 1998).

We anticipated that the LGB program would allow the opportunity to survey macro- and microparasite levels throughout the life cycle of hatchery and wild fish, but limited sampling in the year of study (2004) resulted in only a small number of wild fish. We report here on the macro- and microparasite profiles of 5 groups of fish: LGB parr leaving the hatchery (from the 2005 release year); LGB smolts after net-pen rearing; LGB parr after 2 weeks in the wild river; LGB parr after 3 months in the wild river; and natural wild smolts.

Methods

STUDY AREA

Our study was conducted with the Keogh River steelhead trout population near Port Hardy, British Columbia on the northeastern shore of Vancouver Island. The majority of adult steelhead trouts return to this river after 2 and 3 y in the ocean. Spawning is predominantly in March; most fry emerge in June and grow as parr in the river for 1 to 4 y. Smolts migrate to the ocean from April to June, with the peak abundance in the third week of May. Earlier runs consisted of 10 000–14 000 smolts (1985–1987), but numbers declined to approximately 500 to 2000 smolts during 1994–2004, with the exception of 6000 smolts in 2003 (McCubbing & Ward, 2004).

Diagnostic surveys and virus screening have been conducted intermittently by the federal Department of Fisheries and Oceans Canada (DFO) in the Keogh River system and its headwater, O'Connor Lake, since 1975. No viruses were detected, and the most common detected pathogens were *Renibacterium salmoninarum, Aeromonas salmonicida, Myxobacteria* spp.*, Tetracapsuloides bryosalmonae, Philonema* sp.*, Ichthyobodo* spp.*,* and *Trichophyra* spp. (Fish Health Database; Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, accessed March 2004).

LIVING GENE BANK (LGB) PROGRAM AND PRACTICES

The LGB broodstock originated as a random collection of wild smolts (109–121 individuals in each of 5 y) trapped from the Keogh River in May starting in 1998. Smolts were raised to maturity in 2 to 3 y at the freshwater Vancouver Island Trout Hatchery (VITH) (Duncan, BC). Gametes were stripped from 45 to 96 adults for fertilization between single pairs, embryos were raised in incubation trays, and hatched fry were transferred to troughs for artificial feeding until September, when they were moved to net pens at O'Connor Lake. There were 5 annual smolt releases (2001–2005) into the Keogh River, which occurred at the peak of the wild migration in May and averaged 27 498 LGB smolts (range = 21 958–32 519 smolts over the period from 2000 to 2005). Fish that did not migrate to the ocean but instead remained within the river were prevented from moving upstream by a fish fence until July. In 2004, Walters (2005) found that 6% of smolts released became river residents, which resulted in an estimated 1441–1791 LGB fish in the river, now classified as LGB parr that delayed migration, matured precociously, or died (Walters, 2005).

At the VITH, preventative measures to control infectious agents were conducted. This included treatment of incoming wild smolt broodstock with injections of oxytetracyclin hydrochloride (a general antibiotic), vaccination baths for *Aeromonas salmonicida* and *Yersinia ruckeri,* as well as a salting procedure for *Salmincola* spp., *Gyrodactylus* spp., and *Trichodina* spp. At adulthood, ovarian fluid was tested for Infectious Hematopoetic Necrosis Virus (IHNV) and herpes virus; however, no viruses were detected. The VITH water supply came from a precipitation-supplied aquifer at 50 m depth and was regulated with respect to flow rate, temperature, and oxygen levels.

SAMPLING

We were restricted primarily to sampling LGB fish in their juvenile stages. We succeeded in quantifying macro- and microparasites in a small sample of wild smolts and more extensively in 4 LGB groups: LGB hatchery parr (2005 release year); LGB smolts; LGB parr after 2 weeks in the river; and LGB parr after 3 months in the river. In 2004, we collected the following 5 samples.

LGB SMOLTS

In May 2004, 80 LGB smolts were collected at random by dipnet from the tanker truck that delivered the LGB smolts from the O'Connor Lake net pens to the Keogh River for release.

LGB RIVER PARR – 2-WEEK

In June 2004, 62 LGB fish that remained in the river as resident parr were collected by trapping and angling in the lower reaches of the river. LGB river parr were distinguished from wild parr by the absence of the adipose fin, clipped from all LGB fish at the O'Connor Lake net pens.

LGB RIVER PARR – 3-MONTH

In August 2004, 68 LGB river parr were collected through trapping and angling the same locations of the lower river. These fish would have been present during our initial June 2004 sampling.

LGB HATCHERY PARR

In September 2004, 72 LGB hatchery parr from the 2005 release population were collected at VITH by dipnet immediately before their transport by tanker truck to the O'Connor Lake net pens for rearing to the smolt stage.

WILD SMOLTS

In May 2004, wild smolts were collected at the BC Ministry of the Environment's fish fence near the mouth of the Keogh River; however, sampling was halted after only 7 specimens due to low wild migration numbers. We refer to these fish as "Keogh wild smolts". We obtained an additional 38 wild smolts that had originally been collected for broodstock but we were permitted to use in this study. These fish had been taken from the river 2 or 3 weeks earlier and held at VITH; we refer to these fish as "VITH wild smolts". For infectious agents that were unlikely to have been affected by the VITH residency (*i.e., Y. ruckeri* [wild-0/7, VITH wild-1/38]*, R. salmoninarum* [wild-0/7,VITH wild-0/38]*, Listonella anguillarum* [wild-1/7, VITH wild-0/38]*, Tetracapsula bryosalmonae* [wild-0/7, VITH wild-0/38]), the sample of 45 wild smolts was statistically analyzed as one wild group; otherwise, these wild smolts were analyzed as 2 separate groups, one with 7 and the other with 38 fish.

All fish were immediately euthanized by overdose of MS-222. Mass, fork length, external copepod presence, and catch location data were recorded before transport. In addition, kidney imprint slides (USFWS, 2003) were made at the river side by extracting kidney from a small incision above the lateral line. All tools were sterilized and incisions were kept small to minimize contamination.

After each sampling day, fish were packed on ice for overnight shipment to the Fish Health Unit of the Freshwater Fisheries Society of BC (FFSBC) in Nanaimo, BC. Upon dissection, sex and gonad development were determined through visual inspection (scale of 0–5, where 0 = no development and 5 = post spawn) (Rempel *et al.*, 1983). Presence, identity, and prevalence of each infectious agent were determined by FFSBC staff as described below, in accordance with the Canadian Fish Health Protection regulations (Fisheries and Oceans Canada, 1984, revised 2004), under the supervision of Sherry Mead (Head Fish Biologist, FFSBC).

SAMPLE ANALYSIS

Fish were analyzed for microparasites (bacteria and protozoa) and macroparasites (nematodes and copepods), with a specific focus on infectious agents known for the Keogh River and O'Connor Lake system (DFO, Pacific Biological Station, historical database). A total of 8 infectious agents were discovered across the 5 fish groups (Table I). Tests for viruses were not conducted due to the historical absence of viral infectious agents in this watershed.

TABLE I. Prevalence of macroparasitic and microparasitic infection detected in Living Gene Bank (LGB) and wild steelhead trout (*Oncorhynchus mykiss*) sampled from the Keogh River, Port Hardy, British Columbia during May–August 2004. Values are percent infected (confidence interval), facultative bacteria includes *Pseudomonas* spp. and motile *Aeromonas* spp. * $n = 7$ (Keogh wild smolts only), ** LGB hatchery parr are from 2005 release year.

	Macroparasitic infections			Microparasitic infections				
Group	*Philonema* sp.	*Salmincola* sp.	Total	Facultative bacteria	*T. bryosalmonae*	*R. salmoninarum*	*Y. ruckeri*	*L. anguillarum*
Wild smolts ($n = 45$)	9 (3–21)	13 (6–27)	20 (11–34)	29* (8–65)	0	0	2 (0–13)	2 (0–13)
LGB hatchery parr** ($n = 72$)	0	0	0	0	0	0	0	0
LGB smolts ($n = 80$)	0	1 (0–7)	1 (0–7)	28 (19–38)	0	0	0	0
LGB river parr – 2 week ($n = 62$)	0	2 (0–9)	2 (0–9)	13 (6–24)	0	0	0	2 (0–9)
LGB river parr – 3 month ($n = 68$)	2 (0–9)	6 (2–15)	7 (3–16)	26 (17–38)	37 (26–49)	6 (2–15)	3 (0–7)	7 (1–13)

MACRO- AND MICROPARASITE DETECTION AND IDENTIFICATION

Macroparasitic presence was determined through external visual inspection at capture and internal visual inspection during sample dissection in the Fish Health Laboratories. Identification was based on morphological characteristics and life history literature (Margolis & Kabata, 1988; Hoffman, 1999). Microparasitic presence was determined by isolation techniques: kidney smears (bacterial); kidney imprints (protozoan) and culture of kidney tissue on Tryptic Soy Agar (TSA) (Fisheries and Oceans Canada, 1984, revised 2004), Kidney Disease Medium-2 (KDM-2), and Hestrin and Schramm (HS) media (bacterial) (USFWS, 2003). Prevalence of both macroparasites and microparasites was expressed as the percentage of all individuals in a sample group that were infected.

BACTERIAL IDENTIFICATION

Bacterial growth was isolated from culture and analyzed with presumptive tests, including Gram stain, cytochrome oxidase test, and motility testing. Final confirmation was made after isolates were tested through selective media culture, indirect fluorescent antibody testing (IFAT), and organism specific agglutination tests (Fisheries and Oceans Canada, 1984 revised 2004).

PROTOZOAN IDENTIFICATION

Kidney imprints taken before transport were stained with Leishman-Geimsa stain and inspected for presporogenic cells of *T. bryosalmonae*.

STATISTICAL ANALYSIS

Due to a low individual prevalence of *Pseudomonas* spp. and motile *Aeromonas* spp., these bacteria were grouped together as facultative bacteria.

Keogh wild smolts ($n = 7$) and VITH wild smolts ($n = 38$) were amalgamated into a single Wild smolts group ($n = 45$) for macroparasitic, *Yersinia ruckeri*, *R. salmoninarum, L. anguillarum*, and *T. bryosalmonae* infection comparisons to LGB fish. Facultative bacterial infection comparisons used only Keogh wild smolts ($n = 7$) as infection prevalence may have changed during the 2–3 weeks that VITH wild smolts spent in the VITH after collection from the wild.

Confidence intervals were calculated with the Agresti and Coull method using the binom package of R v2.6.1 statistical software (R Development Core Team, 2007) as recommended for proportions and particularly in the case of small sample sizes (Agresti & Coull, 1998). Sample groups were compared for prevalence of parasites or groups of parasites based on these confidence intervals or using contingency tables and Log-likelihood ratios in SAS v9 (SAS Institute Inc., Cary, North Carolina, USA, 2002–2004) when less than 25% of the cells had expected counts of less than 5.

Results

All fish appeared clinically healthy and showed no external signs of disease ($n = 327$); however, 30% of the individuals carried infectious agents, which differed in prevalence and type among the 5 fish groups (Table I).

MACRO- AND MICROPARASITE CHARACTERIZATION OF FISH GROUPS

LGB HATCHERY PARR (2005 RELEASE)

There were no infectious agents detected in the LGB parr.

WILD SMOLTS

Macroparasites were found in 20% of wild smolts, and facultative bacteria in 29% (Table I). By contrast, other microparasites were rare: 1 wild smolt had *Y. ruckeri,* another had *L. anguillarum*, and no wild smolts had *T. bryosalmonae* or *R. salmoninarum*.

LGB SMOLTS

Few infectious agents were found in LGB smolts. Macroparasites were rare: 1 LGB smolt had *Salmincola* spp. LGB smolts lacked other examined microparsites (*Y. ruckeri, L. anguillarum*, *T. bryosalmonae*, or *R. salmoninarum)*. Facultative bacteria were of similar prevalence in LGB and wild smolts (LGB - 28% [19–38]; wild - 29% [8–65]).

LGB 2-WEEK RIVER PARR

After 2 weeks in the river, LGB fish still had similar macroparasite prevalence (2 week - 2% [0–9]; LGB smolts –1% [0–7]), and there appeared to be fewer facultative bacteria in LGB river parr at this stage than in LGB smolts (2 week - 13% [6–24]; LGB smolt - 28% [19–38]).

LGB 3-MONTH RIVER PARR

After 3 months in the river, there was an increase in infectious agents in LGB fish. Facultative bacteria increased, exceeding those found in 2-week parr ($G = 5.39$, $P = 0.02$, df = 1), but appeared similar to the prevalence in wild smolts ($n = 7$) that had spent several years in the river (3 month - 26% [17–38]; wild - 29% [8–65]). *Tetracapsuloides bryosalmonae* and *R. salmoninarum* infection were detected for the first time in the 3-month river parr group, with a prevalence of 37% (26–49) and 6% (2–15), respectively. The prevalence of *T. bryosalmonae* was significantly higher than in the wild smolt population ($n = 45$), which had no *T. bryosalmonae* infection ($G = 29.989$, $P < 0.0001$, df = 1). Additional microparasites, *Y. ruckeri* (3% [0–7]) and *L. anguillarum* (7% [1–13]), were also detected in the 3-month river parr. Interpretation of this data was based on confidence interval comparison due to the low prevalence of these infections and the small sample size. *Yersinia ruckeri* and *L. anguillarum* had a similar prevalence in 3-month river parr and the wild smolt sample (*Y. ruckeri*: wild - 2% [0–13], 3-month - 3% [0–7]; *L. anguillarum*: wild - 2% [0–13], 3-month - 7% [1–13]).

Discussion

Our survey revealed that fish health efforts in the LGB conservation hatchery were successful in maintaining a pathogen-free environment resulting in no detected prevalence of infectious agents in LGB parr. Upon introduction into the natural environment, the macro- and microparasite prevalence in hatchery fish increased, and by 3 months, LGB river parr had a diverse parasitic fauna. *Renibacterium salmoninarum* and *T. bryosalmonae* infections were detected for the first time in the 3-month hatchery fish; however, the

possibility that this resulted from differences in environmental variables such as water temperature (Sanders *et al.,* 1978; Gay, Okamura & Kinkelin, 2001; Alcorn, Murray & Pascho, 2002) cannot be discounted as we were unable to obtain wild samples from the same time period.

There are few existing comparative surveys of salmonid pathogens in the wild. A recent survey of wild juveniles of coho (*O. kisutch*) and Chinook (*O. tshawytscha*) salmon from Pacific Northwest estuaries (Arkoosh *et al.,* 2004) shows that the prevalence of *R. salmoninarum* (6%)*, Y. ruckeri* (3%), and *L. anguillarum* (7%) in our 3-month LGB parr are within the reported ranges for Chinook (*R. salmoninarum*: 0–93%; *Y. ruckeri*: 0–8%; *L. anguillarum*: 0–31%) and coho (*R. salmoninarum*: 0–28%; *Y. ruckeri*: 0–2%; *L. anguillarum*: 0–15%) salmon. Prevalence of *T. bryosalmonae* in the wild had a documented range of 0–43% in juvenile brown trout (*Salmo trutta*) in England and Wales (Feist *et al.,* 2002) and ≤ 40% in the majority of sites sampled in Switzerland (Wahli *et al.,* 2002). Thus, the 37% prevalence detected in the 3-month LGB river parr is similar to other wild salmonid populations. Taken together, this suggests that LGB hatchery fish reached natural levels of infection within a short period of time in the wild environment.

The Living Gene Bank hatchery program released an abundance of smolts, some of which became LGB river parr (Walters, 2005). Between 22 000 and 32 500 smolts were released annually, which is 10 times the size of the wild runs during the same period (2001–2004) and twice the size of historic wild run peaks (McCubbing & Ward, 2004). In addition, the LGB river parr life history resulted in approximately 1600 additional fish in the river (Walters, 2005) that had sub-clinical levels of common salmonid infections. Thus, the release of many LGB smolts, some of which display resident life histories, increases the number of fish sub-clinically infected with endemic infections, which may be important in population regulation during periods of environmental stress (Lafferty & Holt, 2003).

In summary, no novel infectious agents were detected in the captive environment, and LGB hatchery fish had infection profiles similar to wild fish after time spent in the natural environment. However, the abundance of fish released and the resident life history exhibited by some LGB smolts increased the total number of fish sub-clinically infected with endemic infectious agents, which may be of importance during periods of increased stress. Thus, an ecological understanding of the disease interactions will be important in determining the full effect of the LGB conservation hatchery on the natural population. Specifically, a better understanding of the role the resident river parr play in spatial distribution of infectious agents and repeated surveys of wild and hatchery fish to ascertain the temporal and seasonal variations in infectious agent prevalence and diversity are needed.

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