

NATURAL MARKS FOR IDENTIFYING INDIVIDUAL FISH IN SMALL POPULATIONS OF AT-RISK WESTSLOPE CUTTHROAT TROUT

Caitlin M. Gifford¹, and David W. Mayhood²

¹BSc Environmental Science Program, University of Calgary, 2500 University Drive NW, Calgary, Alberta T2N 1N4

²FWR Freshwater Research Limited, 1213-20th Street NW, Calgary, Alberta T2N 2K5

Abstract—Noninvasive marking methods are highly desirable for identifying individual fish in small populations of at-risk trout species. We used photographs of unique natural spotting patterns and other individual morphological marks to study abundance and fall movements in a remnant population of threatened Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* in an Alberta mountain stream. We show that unique natural marks are stable and suitable for short-term studies up to at least 6 weeks duration for large juvenile- to adult-size Cutthroat Trout, that with effort large juveniles can be recognized as adult fish at least 2 years later, and that individual adults are readily recognizable over periods of at least 2 years. We used a simple spreadsheet and *ad hoc* sort routines to assist in matching the identifying marks from recapture runs. Using individual natural marks, we determined that the large juvenile to adult abundance in fall 2010 was approximately 108 (95% CL 48-270) in this 4-km stream segment, that some individuals moved downstream up to 1.6 km in late fall, and that about half the population was likely to use a single waterfall plunge-pool as an overwintering site. Using data from the literature and our abundance estimate, we calculated that this population has less than a 25% probability of persisting for 40 generations. Natural marks show considerable promise as a means of recognizing individual Westslope Cutthroat Trout while keeping handling injuries and stress to a minimum. We recommend further development of the approach to manage at-risk, critically-small remnant trout populations as well as using it in conjunction with other minimally-invasive techniques such as underwater photography.

INTRODUCTION

Westslope Cutthroat Trout (WCT; *Oncorhynchus clarkii lewisi*) were once widespread and abundant in the Bow and Oldman River drainages of Alberta, but the current distribution of genetically-pure native populations is now severely contracted from the historical range (Cleator *et al.* 2009). Alberta populations of genetically-pure native WCT are listed as “threatened” under the federal *Species at Risk Act* in Canada (Government of Canada 2013), and under the regulations of the Alberta *Wildlife Act* (Province of Alberta. 2013). The few remnant populations are small, highly fragmented and limited to headwaters of the Bow and Oldman River drainages due to losses from hybridization, habitat reduction, and historical overexploitation (Cleator *et al.* 2009). Introduced Rainbow Trout (RT; *Oncorhynchus mykiss*) have damaged the genetic integrity of WCT through introgressive hybridization throughout the subspecies range (Allendorf and Leary 1988). When WCT and RT exist sympatrically they commonly form a fully

introgressive hybrid population called a hybrid swarm in which the characteristics unique to the native fish are lost (Allendorf *et al.* 2001). A competitive advantage for WCT may exist in headwater streams where cooler water temperatures limit RT and hybrids (Rasmussen *et al.* 2010, Rasmussen *et al.* 2012). However, these small refugium stocks are also vulnerable to extirpation from stochastic events and a variety of anthropogenic threats (Cleator *et al.* 2009).

Techniques are needed that allow these sensitive populations to be managed with minimal mortality. Most methods available for trout population studies use invasive marking techniques and require lethal sampling to obtain reliable structures for age estimation. Such approaches threaten the typically small populations they are intended to assist. Some form of external, noninvasive method for marking individual fish would permit analysis of abundance, movements and growth, critical information needed to manage small, vulnerable stocks.

¹Corresponding author: giffordcaitlinm@gmail.com present address: TERA Environmental Consultants, a CH2M Hill Company, Suite 1100-815 8 Ave SW, Calgary, AB T2P 3P2.

Morphological markings are widely used to identify individual vertebrates and invertebrates from whales (Wurzig and Jefferson 1990) and felids (Kelly 2001) to octopus (Huffard *et al.* 2008). In salmonids, natural marks have been used to identify individual Brown Trout *Salmo trutta* (Bachman 1984), European Grayling *Thymallus thymallus* (Persat 1982), and Chinook Salmon *Oncorhynchus tshawytscha* juveniles (Merz *et al.* 2012). To our knowledge, external morphological marks have not been used to identify individual Cutthroat Trout.

Diverse spotting patterns are found in WCT. Here we (1) show that these patterns can be used as natural marks to identify individual fish; and (2) use the individual marks to estimate abundance and detect fall movements in a small at-risk population.

METHODS

Evan-Thomas Creek (50° 52' N, 115° 07' W), a tributary of the Kananaskis River in the Bow River basin of southwestern Alberta, Canada, holds a rare remnant stock of genetically-pure native WCT (Nine diagnostic markers, N = 34, mean WCT > 0.99; Alberta Fish and Wildlife unpublished data). We studied the creek above the Highway 40 bridge and below the waterfall approximately 4 km upstream. No WCT have been found upstream of the 3-m vertical drop waterfall as it is a major barrier to upstream movement. The creek below the Highway 40 bridge has been channelized, creating unfavorable trout habitat that tends to isolate the upstream population.

We sampled WCT by angling with flies during two widely-spaced periods; five occasions between September and October 2010 and in the fall of both 1997 and 1999. We photographed with a tripod-mounted digital camera each fish on the left and right sides, measured fork length to the nearest millimeter, recorded injuries and previous marks, then revived and released specimens in calm water, noting condition at release. We recorded the position of each capture with a geographic positioning system (GPS) receiver (Garmin GPSmap 60Cx).

Our 2010 results encouraged us to examine detailed field notes and archival color slides of WCT captured in Evan-Thomas Creek from three consecutive dates in October 1997 as well as two dates in September 1999 that were 12 d apart. In 1997 and 1999, the same methods were used as 2010 except fish were photographed on the left side only with a handheld single-lens reflex film camera, fork

length measured to the nearest millimeter, the location recorded with a Garmin GPS 38 and the adipose fin of each fish was clipped before release.

Trout photographs were analyzed using a variety of computer photo applications, especially a public domain image analysis system, Image J (Rasband 2011), to identify individually distinct spotting and marking patterns. For the 2010 fish, shape and position of markings on the entire body of the left side were manually compared between individuals of similar fork length (± 15 mm) after each sampling event. When two captures were found with identical markings on the left side, the images of both captures on their right side were also compared. If both the left and right side photographs had identical marking patterns, the fish were treated as recaptures. The 1990s photos were digitized with a scanner and analyzed in the same method as the 2010 photos, but within years, and on the left side only. If a match could not be made in the 1999 samples, the 1997 photos were searched. In addition, we divided the photographed fish into 21 fields using a truss diagram (Strauss and Bookstein 1982) defined by landmarks readily identifiable in most photos (top corner of operculum, insertions of pectoral, pelvic, anal, dorsal and adipose fins; upper and lower caudal lobe insertions). We found 10 fields consistently visible on most photos, and recorded spot numbers in each field for each fish in a spreadsheet database.

Google Earth (<http://www.google.com/earth/index.html>) and a topographic map were used to measure distance and direction traveled by recaptured individuals. Population estimates and 95% confidence limits were calculated by Schnabel, Peterson, and Schumacher-Eschmeyer methods (Ricker 1975). The number of unique individuals captured provided a measure of the absolute minimum population size. If calculated as described by Ricker, the upper 95% confidence limit of the Schumacher-Eschmeyer population estimate is a negative in our data, so the limits do not bracket N. Instead, the reciprocal of $1/N$ was used to calculate the upper limit of N in Ricker's equations 3.13 and 3.14.

RESULTS

Of the 39 WCT captured in the fall of 2010, 35 had unique spotting patterns. Four fish had identical markings, on both sides, as a previous capture (e.g.; Figure 1), identifying them as recaptures.

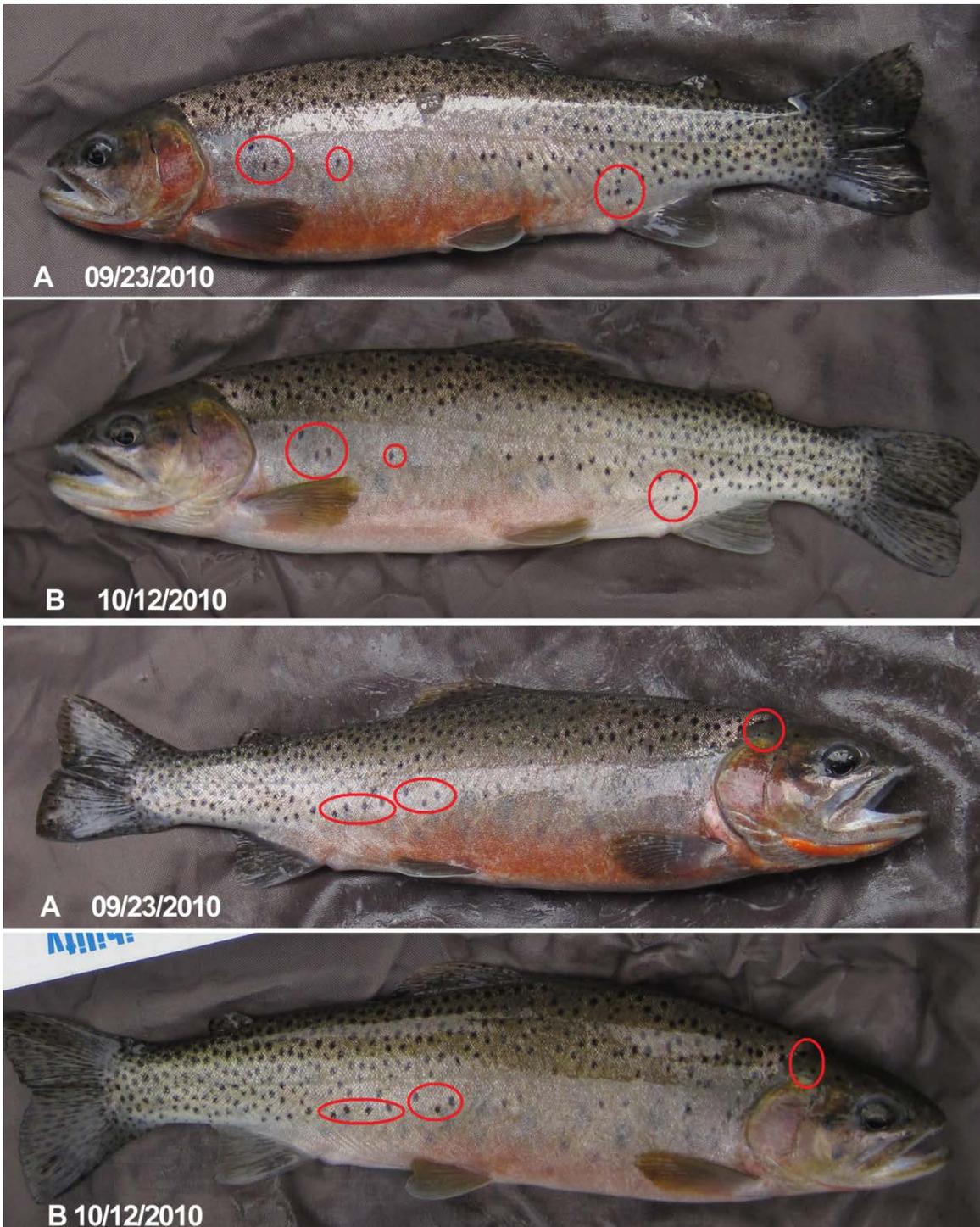


Figure 1. Identification of recaptured individual Westslope Cutthroat Trout from left and right side photos of initial capture in Evan-Thomas Creek on September 23, 2010 (A) and recapture on October 12, 2010 (B). Key identification patterns are circled in red.

In 1997, 10 fish were caught between 28 and 30 October. On the latter date one fish was identified in the field from the clipped adipose, markings and size as a recapture from October 28, but was not photographed again (the original purpose of that study did not call for a second photo). The remainder of the October 1997 specimens had individually unique spotting patterns.

In September 1999, 37 trout were captured. Of these, all but four had fully unique spotting patterns. Four fish captured on September 29, 1999 had missing adipose fins, indicating that they were recaptures. The spotting patterns of two of these recaptures matched to fish captured 12 days earlier. One more specimen, a distinctively large fish (> 400 mm long), more than 65 mm longer than the next largest caught in the 1997-1999 sampling, had a spotting pattern identical to a trout of similar length caught almost 2 years earlier on October 29, 1997 at the identical location.

One 239-mm specimen captured September 29, 1999 proved to be problematic. It had a missing adipose fin showing that it was a recapture, but initially could not be matched in photos to any previously-caught fish; it had a unique set of spots. Its adipose clip had completely healed, the overlying skin being pigmented with the green ground color of the rest of the dorsum, and spotted black. It was clearly an old wound. On closer inspection it was matched to a much smaller (144 mm) specimen captured almost 2 years earlier on October 28, 1997. The smaller fish had spots, but fewer spots than the larger specimen. Those spots matched spots on the larger fish in a corresponding location, forming identical patterns (Figures 2), thereby identifying the larger fish as that same individual recaptured almost 2 years later, having grown 95 mm in fork length and added more spots.

Spot counts by area defined in the truss diagrams varied widely among all fish and between capture

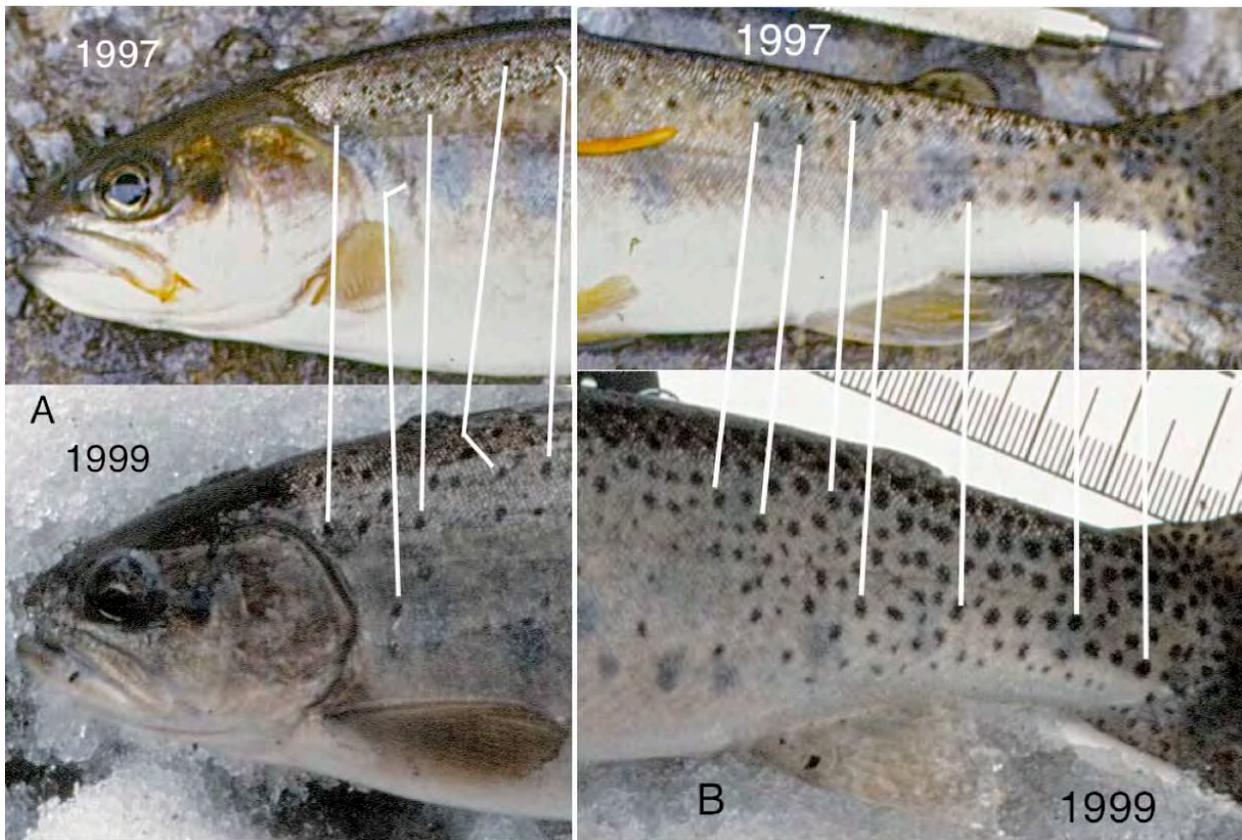


Figure 2. Left, head, and right, caudal region of Westslope Cutthroat Trout captured October 28, 1997 at 144 mm fork length (top), recaptured September 29, 1999 at 239 mm (bottom). Lines connect spots on the 1997 fish to spots in identical locations on the 1999 fish. Nearby patterns on the top photo are visible and identical among new spots in the bottom photo. The fish in the top photo had the adipose removed on collection; that in the bottom photo had a completely-healed scar where the adipose fin should be.

and recapture photos of recaptured specimens due to the varying orientations of the fish in the photos. Two areas below the lateral line between the pectoral and pelvic fins varied widely among individuals, and were useful as sorting criteria to limit the number of photos of possible matches that had to be inspected.

Four different methods place the fall 2010 population of Evan-Thomas Creek between 77 and 147 individuals, and certainly not less than 35, the number of unique individuals caught (Table 1). All four estimate methods assume a closed population. Of the total 35 fish captured in 2010, 17 (49%) were found in the plunge pool below the falls. The 1997 and 1999 sampling did not provide sufficient recaptures within a short time to permit us to estimate abundance.

Table 1. Population estimates of Westslope Cutthroat Trout in Evan-Thomas Creek in fall 2010 by four methods, with 95% confidence limits.

Estimate Method	N	95% confidence limits
Schumacher-Eschmeyer	147	69 - 272
Schnabel (Chapman Adjustment)	108	48 - 270
Petersen (Chapman Adjustment)	77	34 - 194
Absolute Minimum	35	n/a

Two recaptures in 2010 were caught 1,640 m and 1,260 m downstream of their original capture point, the plunge pool below the falls. The other two 2010 recaptures and the recaptured fish in 1997 were found in the same location as the initial capture. Three of the four recaptures in 1999 had not moved from the location of their first capture; two of these were found in the same locations they occupied almost 2 years earlier. The single remaining recapture in 1999 had moved 225 m upstream over a 12-d period.

We solved for N_e in Equation 1 of Soulé (1980), a rule-of-thumb estimating time-to-extinction of populations < 50 , setting generations to 40 and assuming a probability of persistence of 1%, since in very small populations it must be low but not 0 for any mixed-sex adult population greater than 1. This gave us an estimate of persistence probability for populations with < 50 adults, and with the above data from Reed *et al.* (2003) allowed us to relate probability of persistence to population size by another rule-of-thumb (Equation 1): $y = 40 \log_{10} x - 57$, (1)

where y is probability of persistence (%) for 40 generations, and x is adult population size.

Estimated from Equation 1 for the fall 2010 population of 108 mostly adult-size WCT in Evan-Thomas Creek, there is less than a 25% probability that this stock can persist for at least 40 generations.

DISCUSSION

In the 2010 study, we reasoned that fish of similar length with identical spotting patterns on both sides of the body—in a population in which most individuals had spotting patterns that could not be matched—must be the same individuals. This seems highly likely, but without independent evidence of recapture it is not incontrovertible. We addressed this issue by examining the 1997 and 1999 archival photographs and accompanying field notes, in which independent evidence of recapture was available from adipose fin clips administered when fish were collected. When in later sampling runs we encountered specimens with the adipose fin removed, we had independent evidence of recapture. Photos of these recaptured fish in each case could be matched to a previously-caught fish by its identical spotting pattern on the left side. Fish without adipose clips had spotting patterns on the left side that were unique, and could not be matched. This is good evidence that spotting patterns in this population of Westslope Cutthroat Trout are unique to individual fish, and that their spotting patterns can be used as individual markers.

Spotting patterns appear to be stable over at least 2 years for large adults. The largest trout collected, could be matched by its left-side spotting pattern, and by its adipose clip, to a photo of a fish captured almost 2 years earlier in the same location. We suggest further that juveniles will have a reduced set of spots with patterns that persist into adulthood and can be detected in the adults, even though the adults have additional spots. We found such evidence in one of the few juvenile-sized fish (144 mm fork length) captured in 1997, whose suite of spot patterns was visible within the more elaborate spotting pattern of an adult-sized (239 mm fork length) trout caught two years later in 1999. The adult fish had a well-healed scar in place of its missing adipose fin, showing that it had been clipped prior to the season in which it was caught, and that it was a recaptured fish. The only spotting pattern match that could be made was with the 1997 juvenile.

These observations suggest that spotting patterns may be useful as individual markers for periods longer than a single season for large juveniles and adults.

Two issues remain to be resolved in using spotting patterns routinely as natural markers to identify individual cutthroats. First, pattern-matching by visual inspection can be tedious, time-consuming, and only reasonable for small datasets of the size used in this study. Our approach using sort routines in a spreadsheet database of spot counts by area can work, but requires that the spots be counted, another tedious and time-consuming procedure. The system developed by Merz *et al.* (2012) for young Chinook Salmon, or by Kelly (2001) for cheetah may be adaptable for use on lateral views of Cutthroat Trout. Second, while our evidence of long-term pattern stability is suggestive, it is based on just two fish and requires verification.

The best Petersen population estimate of several possible, and that reported here, used 21 and 23 September as the marking run and 12, 14 and 24 October as the census runs, which gave the largest number of recaptures and best met the assumption of one brief marking event followed by a prolonged census event (Ricker 1975). Schnabel and Schumacher-Eschmeyer estimates are multiple census estimates which better fit our study design. The Schumacher-Eschmeyer estimate is close to the Schnabel estimate, and confidence limits for the 2 estimates are nearly identical. The best single estimate is the Schnabel estimate due to its assumption of random sampling and increasing number of marked fish, which are closely satisfied by the data. The confidence limits for the three mark-recapture estimates all widely overlap, lending confidence that the most likely population size is within the range 77 - 147. The population cannot be lower than 35, the number of unique fish captured, which is effectively identical to the lower 95% confidence limit of 34 for the Petersen estimate.

Population size is a good predictor of population persistence (O'Grady *et al.* 2004). Adult abundances in the thousands are ordinarily required to ensure long-term persistence of vertebrate populations (Reed *et al.* 2003, Reed 2005). Reed *et al.* (2003) estimated that approximately 5,800 adult animals are needed for a 95% chance of persistence over 40 generations, 4,700 for 90% persistence, and 550 for a 50% chance of persistence. At very low numbers, inbreeding effects become important (Soulé 1980). The fall 2010

population of adult-size WCT in Evan-Thomas Creek has less than a 25% probability of persistence over 40 generations.

ACKNOWLEDGMENTS

Zach Southgate, Zach Vant Erve, Dermot Kinsella, Brent Piché (2010) and Mike Sawyer (1999) assisted with field collections. Jennifer Earle provided genetic data on the Evan-Thomas population. CMG completed the 2010 work as her major undergraduate project in the B.Sc. Environmental Sciences Program at the University of Calgary (UC ESP). UC ESP (2010) and the Alberta Government's Parks Venture Fund (1997-99) provided partial funding.

LITERATURE CITED

- Allendorf, F. W., and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* 2:170-184.
- Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problem with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution* 16:613-622.
- Bachman, R. A. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. *Transactions of the American Fisheries Society* 113:1-32.
- Cleator, H., J. E. Earle, L. Fitch, S. Humphries, M. Koops, K. E. Martin, D. Mayhood, S. Petry, C. J. Pacas, J. D. Stelfox, and D. Wig. 2009. Information relevant to a recovery potential assessment of pure native westslope cutthroat trout, Alberta population. Fisheries and Oceans Canada, Canadian Science Advisory Secretariat Research Document 2009/036, revised February 2010, iv+26 p.
- Government of Canada. 2013. Order amending Schedule 1 of the Species at Risk Act. SOR/2013-34. *Canada Gazette Part II* 147:625-655.
- Huffard, C. L., R. L. Caldwell, N. DeLoach, D. W. Gentry, P. Humann, W. H. MacDonald, B. Moore, R. Ross, T. Uno, and S. Wong. 2008. Individually unique body color patterns in octopus (*Wunderpus photogenicus*) allow for photoidentification. *PLoS One* 3:e3732. doi:10.1371/journal.pone.0003732
- Kelly, M. J. 2001. Computer-aided photograph matching in studies using individual identification: An example from Serengeti cheetahs. *Journal of Mammology* 82:440-449.
- Merz, J. E., P. Skvorc, S. M. Sogard, C. Watry, S. M. Blankenship, and E. E. Van Nieuwenhuysse. 2012. Onset of melanophore patterns in the head region of chinook salmon: A natural marker for the reidentification of individual fish. *North American Journal of Fisheries Management* 32:806-816.

- O'Grady, J. J., D. H. Reed, B. W. Brook, and R. Frankham. 2004. What are the best correlates of predicted extinction risk? *Biological Conservation* 118:513-520.
- Persat, H. 1982. Photographic identification of individual grayling, *Thymallus thymallus*, based on the disposition of black dots and scales. *Freshwater Biology* 12:97-101.
- Province of Alberta. 2013. Wildlife Act: wildlife regulation. Alberta Regulation 143/1997. Alberta Queen's Printer, Edmonton, AB. 323 p.
- Rasband, W. 2011. ImageJ64 1.46a. Public domain image analysis software, National Institutes of Health, USA. <http://imagej.nih.gov/ij/> (September 2010)
- Rasmussen, J. B., M. D. Robinson, and D. D. Heath. 2010. Ecological consequences of hybridization between native westslope cutthroat (*Oncorhynchus clarkii lewisi*) and introduced rainbow (*Oncorhynchus mykiss*) trout: effects on life history and habitat use. *Canadian Journal of Fisheries and Aquatic Sciences* 67:357-370.
- Rasmussen, J. B., M. D. Robinson, A. Hontela, and D. D. Heath. 2012. Metabolic traits of westslope cutthroat trout, introduced rainbow trout and their hybrids in an ecotonal hybrid zone along an elevation gradient. *Biological Journal of the Linnean Society* 105:56-72.
- Reed, D. H. 2005. Relationship between population size and fitness. *Conservation Biology* 19:563-568.
- Reed, D. H., J. J. O'Grady, B. W. Brook, J. D. Ballou, and R. Frankham. 2003. Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biological Conservation* 113:23-34.
- Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* 191:1-382.
- Soulé, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. pp. 151-169. *in* M. E. Soulé, and B. A. Wilcox, editors. *Conservation biology: an evolutionary-ecological perspective*. Sinauer Associates, Inc., Sunderland, MA. xv+395 p.
- Strauss, R. E., and F. L. Bookstein. 1982. The truss: body form reconstructions in morphometrics. *Systematic Zoology* 31:113+135.
- Wurzig, B., and T. A. Jefferson. 1990. Methods of photo-identification for small cetaceans. Report of the International Whaling Commission Special Issue 12:43-52.