

Sexual and seasonal dimorphism in adult adfluvial bull trout (*Salvelinus confluentus*)

J.M. Nitychoruk, L.F.G. Gutowsky, P.M. Harrison, T.J. Hossie, M. Power, and S.J. Cooke

Abstract: Sexual dimorphism in fishes may be obvious during the reproductive period and less clear during the nonreproductive periods. Despite being difficult to discern during the nonreproductive period, sex-related differences in body condition and shape can yield important insights into a species' behaviour and ecology. The purpose of this study was to test hypotheses about body condition and shape variation related to sex and season (nonreproductive and reproductive periods) in a population of adult adfluvial bull trout (*Salvelinus confluentus* (Suckley, 1859)), which is a poorly understood and imperiled species across much of its range. Geometric morphometric samples were collected by angling in the spring and late summer in a reservoir in British Columbia. Principal components analysis identified two principal components (PC) that were related to body condition and that varied according to season and sex. Spring-caught females were in better body condition than spring-caught males. There was a significant sex \times season interaction on body condition such that late-summer males were not different from late-summer females. Spawning bull trout exhibited a decline in body condition during the summer season. An additional PC that described head size was found to vary significantly between sexes; however, an assignment test showed that it failed to reliably distinguish between the sexes. We hypothesized that the ecology of these animals, including sex-specific behaviour, is responsible for sexual and seasonal differences in bull trout body condition and morphology. This study offers new insight into the ecology of bull trout and shows that shape data for fishes can be obtained nonlethally, which is particularly important for species that are imperiled.

Key words: bull trout, *Salvelinus confluentus*, geometric morphometrics, morphology, body shape, body condition, sexual dimorphism, Kinbasket Reservoir, threatened species, adfluvial.

Résumé : Le dimorphisme sexuel chez les poissons peut être évident durant la période de reproduction, mais moins évident à d'autres périodes de l'année. Bien qu'elles soient difficiles à déceler en dehors de la période de reproduction, les variations morphologiques et de l'état d'embonpoint reliées au sexe peuvent fournir d'importants renseignements sur le comportement et l'écologie d'une espèce. Le but de la présente étude était de tester des hypothèses concernant les variations morphologiques et de l'état d'embonpoint reliées au sexe et à la saison (période de reproduction et reste de l'année) dans une population d'adultes adfluviaux d'ombles à tête plate (*Salvelinus confluentus* (Suckley, 1859)), une espèce mal comprise et en péril dans une bonne partie de son aire de répartition. Des poissons ont été capturés à la ligne au printemps et vers la fin de l'été dans un réservoir en Colombie-Britannique afin d'obtenir des données de morphométrie géométrique. L'analyse en composantes principales a permis de cerner deux composantes principales associées à l'embonpoint et variant selon la saison et le sexe. Les femelles prises au printemps présentaient un meilleur embonpoint que les mâles pris à la même période. Il y avait une interaction significative du sexe selon la saison sur l'état d'embonpoint, qui faisait en sorte que les mâles capturés à la fin de l'été n'étaient pas différents des femelles prises à la même période. L'embonpoint des ombles à tête plate reproducteurs diminuait durant la saison estivale. Si une autre composante principale décrivant la taille de la tête variait significativement selon le sexe, un test d'affectation a toutefois démontré qu'elle ne permettait pas de distinguer les sexes de manière fiable. Nous postulons que l'écologie de ces animaux, dont les comportements dépendant du sexe, est responsable des différences sexuelles et saisonnières de l'état d'embonpoint et de la morphologie des ombles à tête plate. L'étude jette un nouvel éclairage sur l'écologie de l'omble à tête plate et démontre que des données morphologiques sur les poissons peuvent être recueillies de manière non létale, une caractéristique particulièrement importante dans le cas d'espèces menacées. [Traduit par la Rédaction]

Mots-clés : omble à tête plate, *Salvelinus confluentus*, morphométrie géométrique, morphologie, forme du corps, état d'embonpoint, dimorphisme sexuel, réservoir Kinbasket, espèce menacée, adfluvial.

Introduction

Many animals exhibit sex-related phenotypic differences during development, maturation, and reproduction (Fairbairn et al. 2008; Leonard and Cordoba-Aguilar 2010). In addition, a growing body of evidence indicates that males and females exhibit neurological and behavioural differences that extend beyond the development of primary sexual characteristics and reproduction (Shine

1989; Cooke et al. 1998; Cahill 2006). Taken together, such findings have led to novel theories about sexual selection (Andersson 1994; Maan and Seehausen 2011) and in some cases been used to inform wildlife management (Boake et al. 1996; Rode et al. 2006).

Sexual dimorphism in behaviour, shape, size, colour, and secondary-sexual characteristics are common among fishes (Dugatkin and FitzGerald 1997). Differences in male and female

Received 28 November 2012. Accepted 13 April 2013.

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phenotypes result not only from sexual selection (Douglas et al. 2001; Kitano et al. 2012), but also sex-specific differences in foraging strategy (Rijnsdorp and Ibelings 1989), energy intake (Holtby and Healey 1990), and energy allocation (Blanckenhorn 2005); all of which may manifest as shape differences during the reproductive and nonreproductive periods (Monet et al. 2006; Hanson et al. 2008). The effect of sex-specific differences in foraging strategies, energy intake, and energy allocation on between-sex variation in shape should be exaggerated in mature individuals during the reproductive season, as females prepare for egg laying and males compete for mates. In contrast, sexually selected morphological traits expressed by mature individuals may be less plastic and carried throughout the year. Identifying the nature of sex-associated differences in size, shape, and behaviour is an important consideration for fisheries management because such between-sex variation can lead to overharvesting one sex, thereby skewing the population's sex ratio and reducing its future reproductive success (DeMartini et al. 2000; Hanson et al. 2008; Chiba et al. 2012).

Bull trout (*Salvelinus confluentus* (Suckley, 1859)) is a North American charr that has experienced declines across much of its historic range (DeHaan et al. 2010). These declines have been explained in part by the generally slow growth rate of the species (Carl et al. 1989), extreme sensitivity to angling pressure, competition with invading species (McPhail and Baxter 1996), and sensitivity to changing environmental conditions (Baxter et al. 1999; Kiser et al. 2010; Selong et al. 2001). Bull trout exhibit a variety of life histories (e.g., Ladell 1991; Brenkman et al. 2001) and a significant degree of morphological variation exists within and among populations (McPhail and Baxter 1996; Haas and McPhail 2001; Rieman and McIntyre 1993). For instance, the development of a male kype (i.e., a lengthening of the lower jaw with the development of a pronounced terminally located hook that fits into a groove in the upper jaw; Morton 1965) may occur in some populations but remain absent in others (McPhail and Baxter 1996). The kype serves in male-to-male combat and as a visual signal of sexual maturity (Morton 1965). Similar to its congeners, mature bull trout often exhibit pronounced sexual dimorphism during the reproductive period from September to October (McPhail and Baxter 1996). In addition to a possible kype, reproductive male bull trout are identified by bright colouration compared with that of females, ventral reddening, brightening of the leading edges of all ventral fins, and large body size compared with females (McPhail and Baxter 1996). Reproductive female bull trout may be characterized by bright colours but lack a prominent kype (McPhail and Baxter 1996; Warnock et al. 2010). Bull trout are iteroparous (i.e., spawn multiple times after maturation) and have been shown to skip spawning when population density is high (Johnston et al. 2007; Johnston and Post 2009). Despite the conservation status of bull trout, the species remains relatively understudied compared with its congeners (Dunham et al. 2008) and has rarely been examined with respect to sexual dimorphism, particularly outside the reproductive period.

The objectives of this study were to identify body condition (herein known simply as condition) and shape characteristics of male and female adfluvial bull trout (i.e., a life history where mature individuals migrate between lake and stream ecosystems for the purpose of reproduction) during the nonreproductive period (spring) and the reproductive period (late summer). We first hypothesized (H1) that between-sex differences in morphology would be greatest in the reproductive season and most strongly related to condition than to fixed sexually selected traits such as a kype or relative body size. We also hypothesized (H2) that outside of the reproductive period, between-sex differences in body shape result primarily from fixed sexually selected traits and are less related to condition.

Materials and methods

Sampling

Bull trout were captured using rod and reel from 11 April to 25 May 2010 and from 16 August to 15 September 2010 in Kinbasket Reservoir (52°18'N, 118°27'W) in the Kootenay–Rocky Mountain region of British Columbia. Kinbasket is a large, glacially fed impoundment that is part of the Columbia, Canoe, and Wood River systems (Fig. 1). The mean surface area of the Kinbasket reservoir is 410 km² and extends approximately 190 km. Kinbasket has a mean depth of 57 m and reaches 150 m in some places.

Bull trout spawn in the autumn (Dunham et al. 2008) and begin their spawning migration in late summer. Therefore, in the spring, bull trout were sampled north from the Sullivan River, throughout the confluence of the Columbia and Canoe rivers. In late summer as bull trout migrated to their spawning habitat, sampling was done at the mouths of Horse Creek and Ptarmigan Creek in the Canoe River reach and of the Beaver River and Quartz Creek in the Columbia River reach (Fig. 1). Spawning tributaries exhibited similar flow regimes and environmental conditions during the late summer (L.F.G. Gutowsky, personal observation). In the spring, bull trout were captured by trolling using weighted hard-body minnow-like lures (#4/0 hooks; for additional details see Gutowsky et al. 2011). During late summer, sampling was conducted by casting minnow-like lures. Both sampling methods were expected to capture mature individuals.

Once captured and anesthetized in a 50 L bath of reservoir water containing 30 mL of anaesthetic (1 part clove oil emulsified in 9 parts ethanol; Blackman 2002), bull trout were photographed (by the same researcher, P.M. Harrison) laterally against a white cooler lid from a distance of approximately 55–65 cm. Images were captured using a Nikon D5200 24.1 megapixel digital camera. Photos were always taken immediately following removal of each specimen from the anesthetic bath. Each specimen was oriented with a head-to-the-left positioning, and photos were taken with the camera angled down and aimed at the central body position of each fish (Fig. 2). The camera was hand held rather than braced in a fixed position; however, the lens was kept parallel to the specimens to minimize inconsistencies in camera pitch among specimens and to ensure that the volume of each specimen was projected at a consistent angle in the photo frame. Specimens were held in place by hand such that all landmarks were visible, profile distortion in the photograph was minimized, and specimens did not slide off the platform. Specifically, fish were held in place by using one finger from each hand to provide only enough resistance to keep the specimen from sliding while also minimizing distortion of the body profile. To evaluate distortion from camera angles and to provide scale, a small envelope of known dimensions (56 mm × 87 mm) was included in every photo.

Each photo was evaluated for quality prior to the assignment of landmarks. Although every effort was made to minimize non-shape variation during sampling, 60 of 183 specimens (33%) were deemed unsuitable for use in shape analyses. Unsuitable photos were typically subject to unacceptable camera pitch (e.g., >5°), partially obscured by shadow, or otherwise had obscured profiles resulting from the environmental conditions in which sampling was conducted (e.g., rough water conditions and low light levels). Since specimen photographs were taken at various distances (but within 55–65 cm), direct measures of fish size from the photographs were calibrated using the software Image J version 1.46 (Rasband 2012). Specifically, the length (in pixels) of the scale packet was used to generate a scaling factor and this image-specific scaling factor was used to convert the centroid size of each individual from pixels to millimetres.

Measurements of mass (g) and total length (mm) were recorded for each specimen directly during sampling. Standard condition (Le Cren 1951) was calculated using these data because a regression of mass against total length suggested nonisometric growth in the

Fig. 1. Kinbasket Reservoir (52°18'N, 118°27'W) in the Kootenay Rock Mountain region of British Columbia.

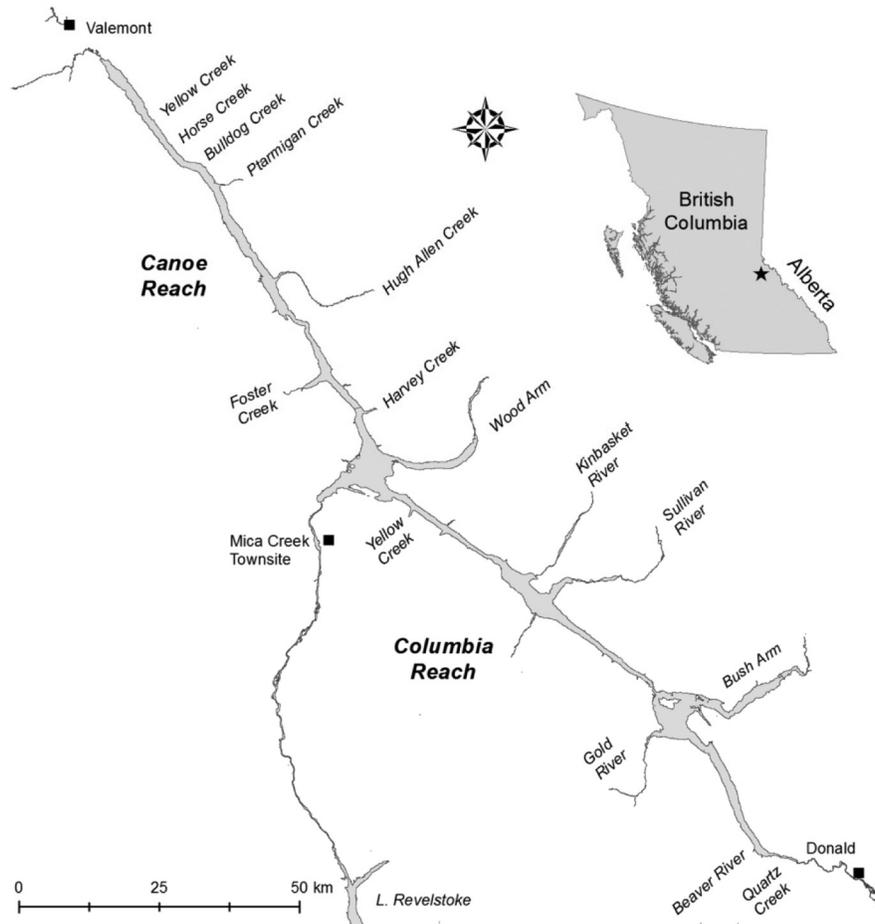
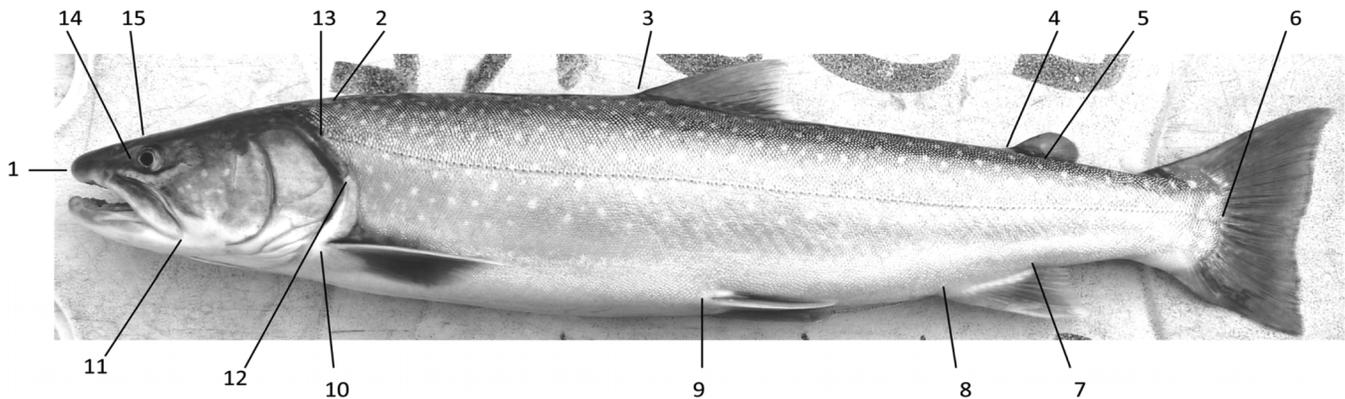


Fig. 2. Landmarking scheme chosen for morphometric analysis of body shape of bull trout (*Salvelinus confluentus*) from the Kinbasket Reservoir.



population. Standard condition is defined as $W = aL^b$, where W is mass, L is length, a is a constant, and b is an exponent value ranging from 2.5 to 4.0, with a value of 3.0 indicating fish that maintain the same shape over time (Le Cren 1951). In this study, the constant b was calculated to be 3.43. The sex of each specimen was determined by visual inspection of the gonads through a 4 cm incision in the ventral mid-body. Spring males were identified by the presence of small clear to white reproductive organs, whereas late-summer males contained relatively large white gonads. Spring females contained white, undeveloped eggs, whereas late-summer females contained relatively large and yellow eggs. All fish captured for this study were assumed to be mature, be-

cause immature bull trout (i.e., younger than 3 years) remain in upstream tributaries outside of our sampling area (McPhail and Murray 1979; Nakano et al. 1992). For the purpose of our study, we assumed that stomach contents were roughly equivalent between the sexes and seasons. We knew that ignoring stomach contents may limit our inferences; however, the assumption allowed us to minimize stress associated with gastric evacuation sampling and therefore improve the postrelease survival. After sampling, each fish was placed in a bath of ambient temperature lake water, allowed to recover from the anesthetic, and released once it showed an adequate escape response to handling.

Table 1. Sample size and total length (actual lengths or range in millimetres) for bull trout (*Salvelinus confluentus*) captured in the Kinbasket Reservoir.

	Confluence	Ptarmigan Creek	Beaver River	Horse Creek	Quartz Creek
Spring					
Females	36 (536–786)	—	—	—	—
Males	53 (434–881)	—	—	—	—
Late summer					
Females	—	3 (451, 467, 677)	2 (358, 431)	3 (474, 533, 697)	7 (541–625)
Males	—	5 (465–585)	2 (443, 820)	8 (487–767)	4 (484–621)
Total	89	8	4	11	11

Landmarking

The shape of each individual was recorded by assigning landmarks to relevant points of the body profile. Landmark data were recorded using the software TPSDig2 version 2.16 (Rohlf 2010). Landmarks were based on those used in the Monet et al. (2006) study of brown trout (*Salmo trutta* L., 1758) and included the following: 1, anterior aspect of the snout; 2, lateral extent of the head; 3, anterior insertion point of the dorsal fin; 4 and 5, insertion points of the adipose fin; 6, posterior point of the lateral line; 7, anterior insertion point of the anal fin; 8, posterior insertion point of the anal fin; 10, insertion point of the pectoral fin; 11, ventral extent of the maxilla; 12, posterior point of the operculum; 13, anterior insertion point of the lateral line on the operculum; 14, anterior point of the eye; 15, point adjacent to the head profile on a line bisecting the eye vertically (Fig. 2). Landmarks 13 and 15 were not included in the analysis by Monet et al. (2006) but were added here to help further identify deformations in head shape.

Transformation

Digitized images created using TPSDig2 (Rohlf 2010) were imported into the IMP7 line of morphometrics software (Sheets 2011a, 2011b, 2011c). A general Procrustes transformation was performed on the landmark coordinates using CoordGen7a (Sheets 2011b). This removes the influence that differences in orientation, position, or size among specimens might have on the interpretation of morphological variation (Zelditch et al. 2004).

Data analysis

Sexual and seasonal effects on condition (Le Cren 1951) were investigated using a factorial ANOVA followed by a Tukey's post hoc test. Using the program PCAGen7 (Sheets 2011c), a principal components analysis (PCA) was conducted on the Procrustes-transformed coordinates to classify variation in body shape as principal components (PCs) (Zelditch et al. 2004). PCs that explained >10% of the total variance (Quinn and Keough 2002) were then visualized using plotted vectors on landmarks (program tpsRelw; Rohlf 2010). A factorial ANOVA was conducted on each PC to examine the effects of sex, season, and their interaction on shape.

An animal's morphology may not remain constant as it grows (i.e., allometric growth), therefore fish may vary in shape simply because of differences in size. To investigate the possible allometric relationship between body shape and size, we conducted a regression on each PC separately against centroid size. For PCs that varied significantly with centroid size, we used the residuals from the regression in our subsequent analyses. This approach allowed us to remove the effect of size on shape in those PCs that showed evidence of allometry. We used a factorial ANOVA to examine the main effects of sex and season and the sex × season interaction on morphology. Significant effects were then investigated using Tukey's post hoc test. To determine whether condition had an effect on body shape, we regressed body size against each of the shape variables (PCs). Finally, we assessed the ability of the shape variation that our landmarks quantified to classify individual specimens as being either male versus female and from

the spring versus late summer using a jack-knifed assignment test in the program CVAGen7 (Sheets 2011a).

Results

Sampling

Of the 183 total number of bull trout captured and photographed in spring and summer, 123 (67%) provided photographs of suitable quality for geometric morphometric analysis. There were 36 spring females, 53 spring males, 15 late-summer females, and 19 late-summer males (Table 1). Total length ranged from 44 to 88 cm with a normal distribution across the entire sample (Table 1). The PCA yielded three PCs that explained >10% of the variation in body shape.

Evaluation of sexual and seasonal effects on body condition

Season had a significant effect on condition ($F_{[1,119]} = 30.089$, $p < 0.001$), whereas the effect of sex was not significant ($F_{[1,119]} = 1.384$, $p = 0.242$). There was a significant interaction between sex and season on standard condition ($F_{[1,119]} = 4.662$, $p = 0.033$; Table 2). A Tukey's post hoc analysis of the ANOVA results showed that spring males were in better condition than late-summer males ($p = 0.013$), spring females were in better condition than late-summer females ($p < 0.001$), spring females were in better condition than spring males ($p = 0.002$), but there was no significant difference in condition between late-summer males and late-summer females ($p = 0.563$).

Principal components analysis of body shape

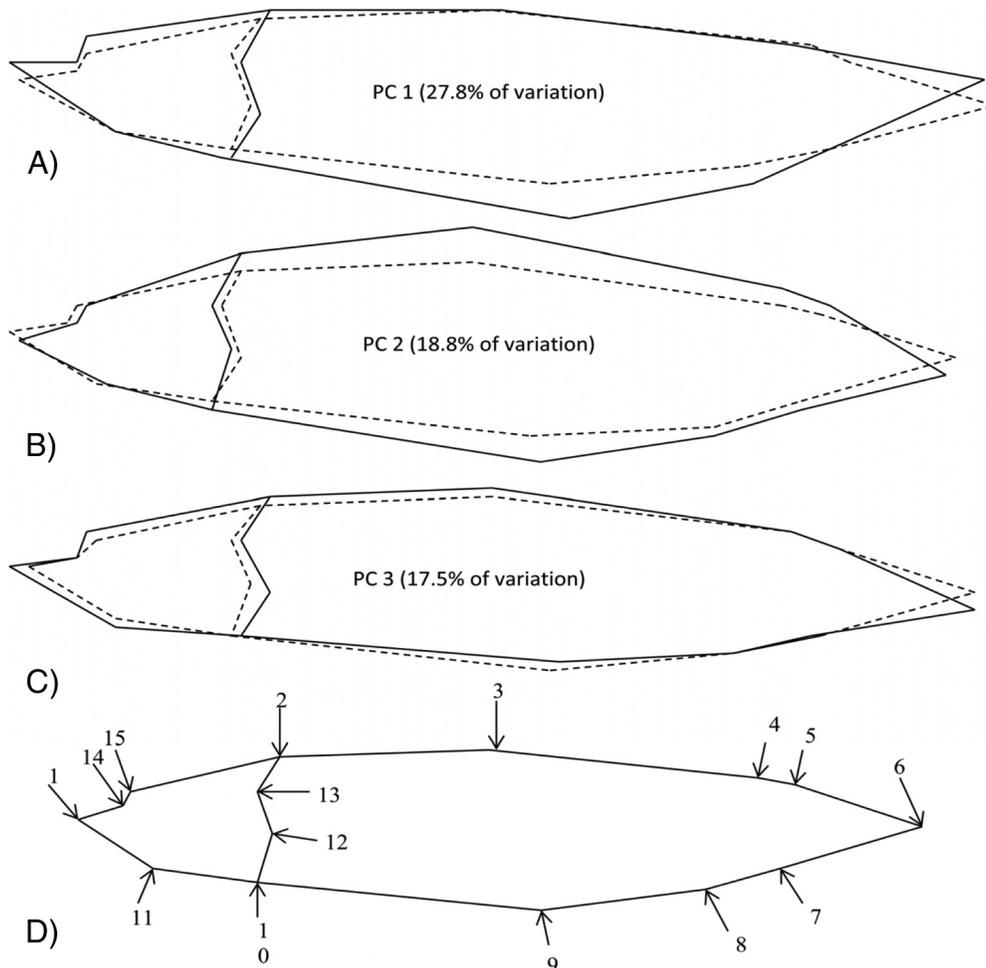
PC1 accounted for 27.8% of the shape variation within the sample. The shape variation associated with PC1 was a convex exaggeration of the body profile where landmarks 8, 9, and 10 were displaced downward, while landmark 6 and head landmarks were simultaneously displaced upward (Fig. 3A). There was a significant effect of season on the PC1 score ($F_{[1,119]} = 4.633$, $p = 0.033$). Spring bull trout typically displayed positive PC scores (solid outline; Fig. 3A), whereas late-summer bull trout displayed negative PC scores (broken outline; Fig. 3A). Neither sex ($F_{[1,119]} = 0.336$, $p = 0.563$) nor the sex × season interaction ($F_{[1,119]} = 0.236$, $p = 0.628$) had a significant effect on PC1.

PC2 accounted for 18.8% of the variation in shape and was characterized by a deepening of the body profile (Fig. 3B). Season had an effect on the PC2 score ($F_{[1,119]} = 74.451$, $p < 0.001$), whereas sex did not have an effect on the PC2 score ($F_{[1,119]} = 0.991$, $p = 0.322$). Test results for PC2 revealed a significant sex × season interaction ($F_{[1,119]} = 7.022$, $p = 0.009$). Here, spring females showed a deeper body profile than late-summer females, late-summer males, and spring males (spring females: 0.009 ± 0.002 (mean ± SE); late-summer females: -0.013 ± 0.002 ; spring males: 0.002 ± 0.001 ; late-summer males: -0.010 ± 0.002 ; $p < 0.001$). PC2 scores were greater for spring males than late-summer males ($p < 0.001$). Body depth profile did not differ between late-summer males and late-summer females ($p = 0.331$). Spring bull trout typically displayed positive PC2 scores (solid outline, Fig. 3B; 0.005 ± 0.001 , mean ± SE), whereas late-summer fish typically displayed negative PC2 scores (broken outline, Fig. 3B; -0.011 ± 0.002 , mean ± SE).

Table 2. Summary of ANOVA main effects and interactions between sex of bull trout (*Salvelinus confluentus*) and season on standard condition values and principal components (PC).

Response	Sex		Season		Interaction		Allometry		Sex		Season		Interaction	
	F	p	F	p	F	p	R ²	p	F	p	F	p	F	p
Condition	1.384	0.242	30.09	<0.001	4.662	0.033								
PC1	0.336	0.563	4.662	0.033	0.236	0.628	0.065	0.002	0.276	0.600	1.395	0.240	0.081	0.776
PC2	0.991	0.322	74.45	<0.001	7.022	0.009	0.198	<0.001	0.773	0.381	44.95	<0.001	5.191	0.024
PC3	8.167	0.005	3.475	0.065	0.054	0.815	0.001	0.356						

Note: Allometry is calculated by regressing PCs on centroid size. If significant, model residuals were further analyzed for an effect of sex, season, and their interaction using ANOVA.

Fig. 3. Superimposition of extreme body-shape deformations of bull trout (*Salvelinus confluentus*) for principal component 1 (PC1) (A), PC2 (B), and PC3 (C). Solid outlines correspond to positive PC scores and broken outlines correspond to negative PC scores. (D) For reference, a consensus shape identifies landmark locations.

Deformation along PC3 accounted for 17.5% of the shape variation in the sample. The shape variation was expressed as a slight upward displacement of mid-body landmarks located posterior to the operculum. Head size varied along PC3, while body shape and size remained relatively unchanged (Fig. 3C). There was a significant effect of sex on the PC3 score ($F_{[1,119]} = 8.167$, $p = 0.005$). Females typically displayed negative PC3 scores (broken outline; Fig. 3C), whereas males displayed positive scores (solid outline; Fig. 3C). Season did not have a significant effect on the PC3 score ($F_{[1,119]} = 3.475$, $p = 0.065$) and a sex \times season interaction was not detected ($F_{[1,119]} = 0.054$, $p = 0.815$).

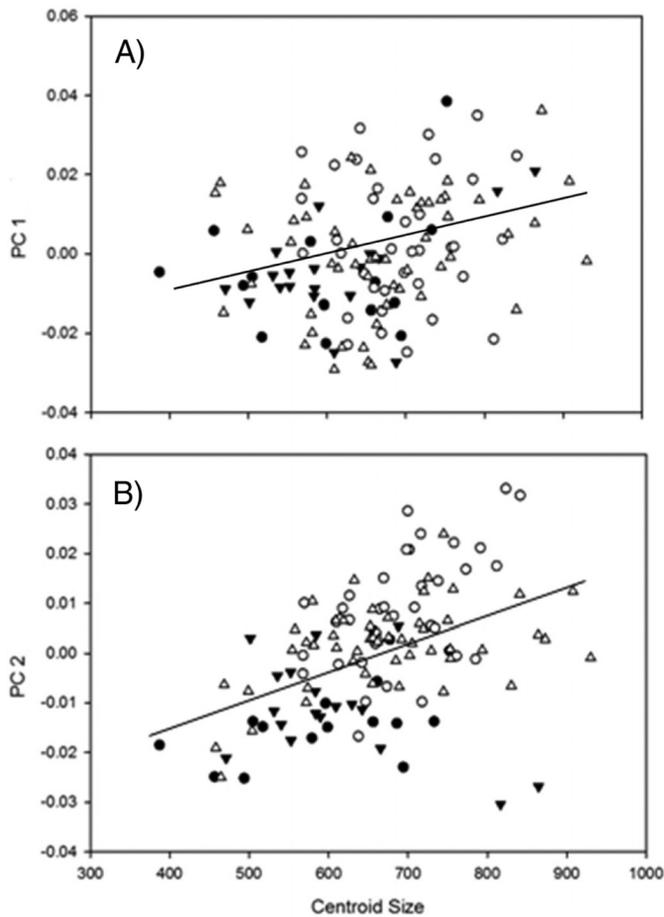
Assessing allometric effects

The regression of PC1 values against centroid size showed that body size explained a significant but small amount of the varia-

tion in PC1 scores ($R^2 = 0.065$, $F_{[1,121]} = 9.455$, $p = 0.002$; Fig. 4A). The subsequent ANOVA of grouped residuals revealed no interaction between sex and season ($F_{[1,119]} = 0.082$, $p = 0.776$) and no difference in shape variation related to sex ($F_{[1,119]} = 0.276$, $p = 0.600$) or season ($F_{[1,119]} = 1.395$, $p = 0.240$).

An analysis of PC2 values against centroid size showed that body size explained a significant proportion of the variation in PC2 scores ($R^2 = 0.198$, $F_{[1,121]} = 31.036$, $p < 0.001$; Fig. 4B). Season was determined to effect residual scores ($F_{[1,119]} = 44.952$, $p < 0.001$), whereas sex did not ($F_{[1,119]} = 0.773$, $p = 0.381$). An interaction between sex and season was detected ($F_{[1,119]} = 5.191$, $p = 0.024$). Differences in residual scores for PC2 were detected between spring females and late-summer females ($p < 0.001$; spring females: 0.007 ± 0.002 (mean \pm SE); late-summer females: -0.010 ± 0.002 (mean \pm SE)), between spring

Fig. 4. Regression plot of principal component 1 (PC1) (A) and PC2 (B) on centroid size (mm) (PC1: $R^2 = 0.20$; PC2: $R^2 = 0.06$). Open circles are spring female bull trout (*Salvelinus confluentus*), solid circles are late-summer females, open triangles are spring males, and solid triangles are late-summer males.



males and late-summer males ($p = 0.001$; spring males: 0.001 ± 0.001 (mean \pm SE); late-summer males: -0.008 ± 0.003 (mean \pm SE)), and between spring males and spring females ($p = 0.004$), but not between late-summer males and late-summer females ($p = 0.411$).

The regression of PC3 scores on centroid size revealed that there was no relationship between body size and PC3 score ($R^2 = 0.001$, $F_{[1,121]} = 0.860$, $p = 0.356$).

The regression of PC1 and PC2 residuals from the size–shape regressions against standard condition values showed no significant effect of condition on shape for PC1 ($R^2 = 0.003$, $F_{[1,121]} = 0.461$, $p = 0.499$). For PC2, a significant effect of condition on shape was detected ($R^2 = 0.225$, $F_{[1,121]} = 36.560$, $p < 0.001$). The effect of condition on shape was assessed using PC3 scores rather than residuals because we observed no evidence of allometry in PC3 (see above). There was no effect of condition on PC3 ($R^2 = 0.005$, $F_{[1,121]} = 0.643$, $p = 0.424$).

Jack-knifed assignment test

The ability to properly assign spring and late-summer bull trout to their respective groups based on landmark deformations was high, as 95.1% of the specimens were correctly assigned. The ability to distinguish between male and female bull trout based on deformations in landmarks was poor, with only 50.8% assigned correctly. The ability to properly assign each specimen to its group as spring male, spring female, late-summer male, or late-summer

female based on landmark deformations was equally poor, with only 45.5% of specimens assigned to their correct groups.

Discussion

Bull trout were in better condition during the nonreproductive season (spring) than during the reproductive season (late summer). However, female condition varied more dramatically between seasons than did male condition such that between-sex differences in condition were only evident in the nonreproductive season (spring). Analysis of body shape resulted in three distinct shape variables (PCs) that described fish morphology. The first two PCs quantified variation in the body depth and were significantly related to both condition and body size. In contrast, PC3 described a difference in relative head-to-body size and was unrelated to season, condition, or body size. Importantly, a significant difference in PC3 between male and female fish indicated that male bull trout have larger heads relative to their body size when compared with females, irrespective of season, condition, or body size. Overall, these results were somewhat contrary to the predicted relationships in that between-sex differences in condition and condition-related shape were evident only during the nonreproductive season (spring), and fixed-morphology variation between the sexes (i.e., in PC3) was not a direct result of a known sexually selected morphology in mature bull trout (i.e., the kype).

Late summer is generally expected to correspond to a time of high-energy intake and active feeding for adfluvial bull trout (Beauchamp and Van Tassell 1999). In contrast, fish were in relatively better condition during the nonreproductive season (spring) than the reproductive season (late summer). The poorer mean condition in the late summer may result from energetic costs associated with the reproductive migration of bull trout from the reservoir to their selected spawning tributary. Body condition in fish, particularly salmonids, is known to vary with food availability and water temperature (Cada et al. 1987; Currens et al. 1989; Filbert and Hawkins 1995). Despite favorable water conditions, growth rates in other salmonid species (e.g., brown trout, rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) may decline during the summer and increased during the winter (Cada et al. 1987). Water temperature has been shown to affect metabolic energy demands in stream-dwelling rainbow trout in habitats with low productivity, thereby influencing the size of energy reserves available for somatic growth (Railsback and Rose 1999). Although information on seasonal prey availability in Kinbasket is currently limited, a similar scenario may be occurring here with adfluvial bull trout. Investigating the influence of food availability on growth potential in both males and females would provide valuable information for threatened bull trout populations.

Males and females differed significantly in body condition only during the nonreproductive season (spring), and not the reproductive period (late summer) as predicted. In fish, gamete production in females and courtship behaviour in males are generally considered to be the greatest energetic costs of reproduction (Gross and Sargent 1985). Sampling in this study may have taken place prior to the onset of active male courtship (Kitano et al. 1994; McPhail and Baxter 1996); however, gamete production had begun by late summer, as mature yellow eggs were carried in sampled female specimens (L.F.G. Gutowsky, personal observation). Therefore, the between-sex differences observed here do not appear to reflect differential costs of reproduction as described in other species, e.g., largemouth bass (*Micropterus salmoides* (Lacépède, 1802)) (Hanson et al. 2008). Adfluvial bull trout may spawn sequentially and are also known to increase the number of skipped spawning events under high population density (Johnston et al. 2007; Johnston and Post 2009). Bull trout are abundant in Kinbasket reservoir, and it remains possible that some of the individuals captured during the spring had skipped spawning the previous year, resulting in the differences in condition observed here. By

reallocating resources from the production of eggs to somatic growth, female bull trout that skip a spawning event may disproportionately increase their condition in the spring. An interesting alternative explanation yet to be investigated is that important between-sex differences in the ecology of adfluvial bull trout exist outside of the breeding season.

The shape variation explained by PC1 involved a relative deepening of the body and showed a significant positive relationship with both condition and body size, but was unaffected by sex, season, or the sex \times season interaction once the effect of body size was removed. Thus, the shape variation described by PC1 was the result of allometric shape changes (i.e., differences in shape related to body size) and condition. PC2 described variation in body depth and showed a significant positive relationship with both condition and body size; however, a significant effect of season and the sex \times season interaction remained after removing the effect of size on shape. Notably, even after controlling for body size, the among-treatment pattern of variation in PC2 paralleled the treatment-related variation in condition, therefore PC2-related shape variation was likely the result of sex- and season-specific changes in condition. Despite efforts to minimize distortion of the photographed specimen, the shape variation associated with both PC1 and PC2 (depicted in Figs. 3A–3C) likely result at least partially from the “barrel distortion” effect described by Muir et al (2012). Specifically, the methodology of placing fish on a flat surface can result in apparent shape distortion such that the subject looks like it is wrapped around a barrel (Muir et al 2012). This effect is exaggerated both in longer fish and fish that are in better condition, and it therefore appears that PC1 represents barrel effects driven primarily by differences in body size, whereas PC2 represents barrel effects driven by differences in condition. Although this somewhat precludes direct interpretation of shape variation depicted by PC1 and PC2, PC2 remains a measure of the relative condition of the fish and indicates that between-sex differences in shape related to condition are likely to be more apparent during the nonreproductive season (spring).

Male and female bull trout differed significantly in PC3, which reflected sex-specific morphologies when compared with females, male fish had larger heads relative to their bodies. This difference in relative head size was unaffected by body size (i.e., allometric effects), condition, or season and thus represents a fixed sexually dimorphic morphology. Mature male brown trout sometimes possess a larger head size compared with females (Monet et al. 2006; Reyes-Gavilán et al. 1997); however, to our knowledge, this sex-specific difference in relative head size has not been reported previously in bull trout. Although other salmonids frequently exhibit secondary sexual characteristics (e.g., Beacham and Murray 1985; Quinn and Foote 1994; Klemetsen et al. 2003), the appearance of such characteristics are not always easily identified in bull trout populations (McPhail and Baxter 1996). The assignment test for sex based on shape deformations performed poorly, which indicates that while significant between-sex differences exist, there remains overlap between the sexes in phenotypic expression. Interestingly, PC3 indicated a relatively larger head overall and not specifically the presence of a known sexually selected trait (i.e., the kype).

Although sample-wide trends were absent, a kype was encountered in the three largest males captured. These three males together represented half of the specimens measuring greater than 80 cm in length, all of which were male and caught in the spring. It may be that only very large males in this system develop secondary-sexual characteristics in their head. Unfortunately, large (>700 mm) males were underrepresented in the sample ($n = 6$). The underrepresentation of the large and overtly ornamented males indicates that these fish are either uncommon in Kinbasket reservoir or that we were unable to adequately capture this size class given our sampling strategy. Although we assume that all bull trout sampled from the reservoir or tributary mouths

were mature adult individuals, the sample likely contained a mixture of year classes. If a pronounced kype only develops among large and experienced spawners, the inclusion of first- and second-year spawners could impact the ability to detect differences in secondary-sexual characteristics. Finally, it is important to note that although it may be difficult to differentiate consistently between male and female adfluvial bull trout by shape analysis, incorporating information from alternate secondary-sexual characteristics (e.g., colour, hue) will also facilitate sex discrimination.

Management implications

Accurately assessing sex ratios is important when estimating the reproductive potential of a population (Hanson et al. 2008). Although some fluvial bull trout spawning assessments have not encountered difficulty with sex determination (see Pillipow and Williamson 2004; Langston and Cubberley 2008), distinguishing between the sexes based on morphology was somewhat difficult in the adfluvial population studied here. Little is currently known about intrapopulation variation in depth distribution, horizontal movement, and prey preference among adfluvial bull trout. Given that the species is susceptible to overfishing (Carl et al. 1989) and habitat loss (Al-Chokhachy et al. 2010), the techniques employed here may provide fisheries managers with a better means to identify sex-biased angling harvest and track its effects on natural populations. In addition, geometric morphometrics are used for stock identification (i.e., the recognition of self-sustaining components of wild populations; Dwivedi and Dubey 2013). Geometric morphometrics can identify whether particular stocks are harvested disproportionately and should therefore be provided special consideration for management, which may be particularly important for imperiled species. Indeed, as demonstrated in this study, nonlethal sampling and shape discrimination can be used effectively to study imperiled species such as bull trout and can produce novel insights into a population's ecology. Critically, the methods of this study can be modified easily to study or monitor morphological variation in other species, including threatened species where lethal sampling is not feasible.

Acknowledgements

Financial support was provided by the Natural Sciences and Engineering Research Council of Canada (via HydroNet and the Collaborative Development and Research Program) and BC Hydro. S.J.C. is supported by the Canada Research Chairs Program. We thank J. Tippe of Grizzly Country Marine Contractors for his help and expertise in catching bull trout. Our gratitude is also extended to J. Molina, S. Landsman, and J. Thiem for their angling assistance. We also thank the staff of the Mica Creek camp for providing logistical support during the field component of this study. This work was approved by Carleton University's Animal Care Committee and conducted under a scientific fish collection permit obtained from the British Columbia Ministry of Environment.

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