Journal of Freshwater Ecology

ISSN: 0270-5060 (Print) 2156-6941 (Online) Journal homepage: http://www.tandfonline.com/loi/tjfe20

Physiological consequences of hybridization: early generation backcrossing decreases performance in invasive bigheaded carps

Stephanie A. Liss, James T. Lamer, Greg G. Sass & Cory D. Suski

JOURNAL OF FRESHWATER ECOLOGY, 2016
VOL. 31, NO. 4, 543–554
http://dx.doi.org/10.1080/02705060.2016.1188426

ARTICLE

Physiological consequences of hybridization: early generation backcrossing decreases performance in invasive bigheaded carps

Stephanie A. Liss[a,b*], James T. Lamer[a,b,c], Greg G. Sass[d] and Cory D. Suski[b]

[a]Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, IL, USA; [b]Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA; [c]Department of Biological Sciences, Western Illinois University, Macomb, IL, USA; [d]Wisconsin Department of Natural Resources, Escanaba Lake Research Station, Boulder Junction, WI, USA

Hybridization can influence a range of characteristics and outcomes for an organism; however, relatively little is known about evolutionary consequences on nutritional performance. Information on hybrid nutritional performance would provide an understanding of how hybrids interact with their environment and insights into mechanisms affecting survival. Our goal was to test for relationships between hybridization and nutritional performance in invasive bigheaded carps (*Hypophthalmichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), and their hybrids in the Marseilles reach of the Illinois River, Illinois, USA. Silver carp showed better nutritional performance relative to bighead carp. Early generation bighead and silver carp hybrid groups were intermediate to both parental lines, thereby, reducing their nutritional performance, and advanced generation bighead and silver carp groups were nutritionally more similar to their respective parental species. Differences in gill raker morphology and feeding habits among bigheaded carps and their hybrids are plausible mechanisms explaining observed nutritional performance patterns. In addition to providing unique insights about how hybridization influences the nutritional performance of wild organisms relative to parentals, our findings may have management implications for bigheaded carps if interbreeding persists over time and reduced nutritional performance is further manifested by continued hybridization.

ARTICLE HISTORY
Received 22 December 2015
Accepted 16 April 2016

KEYWORDS
backcrossing; bighead carp; genetics; invasive species; nutrition; outbreeding; silver carp

Introduction

There are many definitions of a ‘species.’ For example, the biological ‘species’ concept is based on reproduction and refers to any distinct population division of any organism that can produce fertile offspring (Waples 1991; Turner 1999). Species can also be defined in terms of morphological similarities based around a single lineage (Turner 1999). Hybridization is the genetic crossing of two or more divergent and classifiable species and may occur naturally in closely related taxa or taxa coexisting in a habitat (Hubbs 1955; Epifanio & Nielsen 2001; Seehausen 2004). Numerous traits may be affected by hybridization, including growth, size at maturity, metabolic activities, and survival (Hutchings & Fraser 2008; Davies et al. 2012). Hybrids have exhibited inferior performance (reduced vigor and fitness), expressed intermediate traits, or showed superior performance (improved vigor or heterosis, fecundity, and accumulation of adaptive traits) relative to parental species (Stelkens & Seehausen 2009; Rosas et al. 2010; Soltis 2013). There are also documented declines

CONTACT Stephanie A. Liss s.liss4@gmail.com; sliss2@illinois.edu

in the performance and fitness of wild fish populations due to interbreeding with cultivated conspecifics (McGinnity et al. 2003; Muhlfeld et al. 2009). Other studies have documented the anthropogenic promotion of traits (growth and disease resistance) from crossbreeding (Green & Smitherman 1984; Bryden et al. 2004). Should hybridization continue, interbreeding may lead to a 'hybrid swarm' (complete merging of two taxa) or to the extinction of one taxon (Scribner et al. 2001). Although research has often focused on offspring viability, relatively little is known about the influences of hybridization on nutritional condition (i.e. health status).

Nutritional condition can serve as an indicator of individual performance, as nutritional metrics describe a balance between fitness and energy allotment (Congleton & Wagner 2006). Nutritionally deficient individuals have experienced reduced growth, fitness, and/or survival relative to their well-fed counterparts (Gingerich et al. 2010; Wagner et al. 2010). Additionally, physiology links an organism and its population to the environment (Ricklefs & Wikelski 2002; Cooke & Suski 2008). Nutrition can vary with habitat (e.g. temperature and habitat quality) or with the interaction between an organism and its environment (e.g. ability to obtain prey or avoid predation). Thus, quantifying nutritional status can provide insights into hybrid performance relative to their parents and may provide critical information for invasive species that hybridize, such as bighead carp, *Hypophthalmichthys nobilis*, and silver carp, *Hypophthalmichthys molitrix* (hereafter, bigheaded carps; Lamer et al. 2010, 2014, 2015).

Bigheaded carps coexist in their native Asian range. In the United States, bigheaded carps are federally recognized as an injurious invasive species and have become some of the dominant fishes in the Mississippi River Basin (MRB, Kolar et al. 2007; McClelland et al. 2012). Previous research has suggested that bigheaded carps may negatively affect invaded habitats (Irons et al. 2007; Sass et al. 2010; Calkins et al. 2012; Sass et al. 2014). Bigheaded carps have hybridized in native and non-native habitats, with an estimated 44% of individuals in the MRB existing as hybrids (Lamer et al. 2010, 2014, 2015). Physiological performance of hybrids relative to their parents is unknown, and quantifying differences could have significant implications for better understanding and managing these invaders. Therefore, bigheaded carps serve as model organisms to better understand nutritional performance characteristics of hybridization in wild organisms, and to test whether hybridization affects their continued success as invaders. Our objective was to test for relationships between the degree of hybridization and nutritional performance in wild-caught bigheaded carps in the Marseilles reach of the Illinois River, Illinois, USA.

Methods

Field analysis

Fish collection methods were previously described in Lamer et al. (2015). Briefly, 77 *Hypophthalmichthys* spp. individuals were collected near Morris, Illinois, USA (river km 423) in the Marseilles reach of the Illinois River. This reach of the Illinois River was selected for study because it had been recently colonized and hybridization of bigheaded carps has been documented there, thus, providing the greatest probability to collect and detect hybridized individuals (Sass et al. 2014; Lamer et al. 2015). Further, environmental characteristics and the zooplankton community of this reach were similar to other invaded Illinois River reaches (Sass et al. 2014). All bigheaded carps were captured by commercial fishermen contracted by the Illinois Department of Natural Resources using monofilament trammel and gill nets (7.62−10.16 cm inner bar mesh, 2.4−5.3 m deep) during October 2012. October was selected to sample bigheaded carps because it represents a time period of relative physiological stasis when bigheaded carps are not reproductively active. Reproductive activity may introduce variability in nutritional performance indices. The duration of each net set varied and all fishes captured were used for nutritional and genetic analyses regardless of putative identification (Lamer et al. 2010).

Initially, bigheaded carps were identified in the field based on gill raker appearance (comb-like = bighead carp, sponge-like = silver carp, twisted or club-like = hybrid bigheaded carps; see Lamer et al. (2010) for photographs). Following removal from gill and/or trammel nets, we collected blood from the caudal vessel using either a 2.5 or 3.8 cm needle (BD PrecisionGlide™ needles, gauge 22) with a 1 mL syringe (BD Slip Tip™ sterile syringes, volume 1 milliliter [mL]) pre-rinsed in heparin saline (Houston 1990). One mL of whole blood was collected and placed into a microcentrifuge tube and spun at 6600 RPM for at least three minutes to separate plasma from erythrocytes. Plasma was extracted using a transfer pipette and placed into two additional 1.5 mL microcentrifuge tubes. Plasma and erythrocytes were then flash frozen in an insulated box charged with liquid nitrogen (Suski et al. 2006). Samples were then transported to the University of Illinois at Urbana-Champaign, Illinois, USA where they were stored in a $< -75\,^{\circ}$C freezer until processing.

Following blood sampling, 1×1 cm fin clips were biopsied from the distal end of the caudal fin from all fish and placed into a labeled microcentrifuge tube containing 95% EtOH. Total length (mm) and weight (g) were recorded for each fish. A strong positive correlation was observed between total length and weight in our dataset ($r^2 = 0.88$), which is common for fishes (Froese 2006); therefore, only total length was included as a variable in our analyses.

Laboratory analysis

We analyzed plasma alkaline phosphatase (ALP, international units (U/L), calcium (mg/dL), cholesterol (mg/dL), lipase (U/L), and triglycerides (mg/dL) using commercially available kits; QuantiChrom Alkaline Phosphatase Assay Kit (DALP-250), QuantiChrom Calcium Assay Kit (DICA-500), EnzyChrom Cholesterol Assay Kit (ECCH-100), QuantiChrom Lipase Assay Kit (DLPS-100), and EnzyChrom Triglyceride Assay Kit (ETGA-200), respectively, (BioAssay Systems, California, USA). We measured protein (g/dL) with a hand-held protein refractometer (AST model 1250, Thomas Scientific, New Jersey, USA; Wells & Pankhurst 1999), which is certified for use in the range of 0−12 g/dL. ALP is a cell-membrane-associated glycoprotein found in all tissues. Elevated ALP activities in blood are related to increased processing of energy substrates by the liver (Congleton & Wagner 2006). Similarly, protein can respond to changes in body condition, with nutritionally deprived individuals showing depressed protein reserves (Farbridge & Leatherland 1992). Lipase has been shown to increase when acting as a transporter of nutrients during triglyceride hydrolysis. Triglyceride and calcium concentrations may become elevated after feeding. Cholesterol responds to changes in body energy reserves. Together, ALP, calcium, cholesterol, lipase, protein, and triglycerides in plasma have been shown to represent fish nutritional status (Wagner & Congleton 2004; Congleton & Wagner 2006; Guerreiro et al. 2012).

Deoxyribonucleic acid (DNA) was extracted from fish tissues using the Agencourt DNAdvance genomic DNA extraction kit (Beckman Coulter, Massachusetts, USA) according to the manufacturer's instructions. Genomic DNA was eluted from the magnetic particles in 150 µL of elution buffer. Reactions were performed in 96-well plates. Genomic DNA was tested for quantity and quality using the Qubit 2.0 Fluorometer (Life Technologies, New York, USA) and agarose gel electrophoresis, respectively. DNA aliquots were genotyped at 57 species-diagnostic single nucleotide polymorphisms (SNPs; Lamer et al. 2015) using the MassARRAY 4 analyzer system (Sequenom Inc., California, USA) to call SNP genotypes. The posterior distribution of individual assignment was delineated into hybrid categories implementing the algorithm as computed by NewHybrids version 1.1 beta (Anderson & Thompson 2002). The following classes were set using 'Jeffreys-type priors' (Anderson & Thompson 2002) in NewHybrids; parental species (bighead carp [BH] or silver carp [SV]), F_1 hybrids (F_1), first-generation backcrosses (B × BH or B × SV), second generation backcrosses (B × 2BH or B × 2SV), F_2 hybrids (F_2), third-generation backcrosses (B × 3BH or B × 3SV), fourth-generation backcrosses (B × 4BH+ or B × 4SV+), and all allelic probabilities indicative of a hybrid x hybrid cross were manually assigned to an (F_x) category. Initially, 100,000 replicates were used with a burn-in time of 50,000 steps. Methods for assignment and category delineation follow Lamer et al. (2015).

Statistical analyses

We separated individuals into six distinct genotypic groups. The groups were as follows: an advanced generation hybrid group of individuals more genetically similar to parental bighead carp ($n = 13$; B × 2BH and B × 4BH+; hereafter, AGBH), an advanced generation hybrid group of individuals more genetically similar to parental silver carp ($n = 33$; B × 2SV, B × 3SV, B × 4SV+, and F_x; hereafter, AGSV), an early generation hybrid group of individuals more genetically similar to parental bighead carp ($n = 6$; B × BH and F_1; hereafter, EGBH), an early generation hybrid group of individuals more genetically similar to parental silver carp ($n = 5$; B × SV and F_1; hereafter, EGSV), a parental bighead carp group ($n = 6$; hereafter, BH), and a parental silver carp group ($n = 16$; hereafter, SV). Our sample sizes reflect the natural distribution of hybridization in this river reach at this time (Lamer et al. 2010, 2015) and, because these were wild-caught individuals with no prior knowledge of genetic identity, sample sizes of hybrid classes were variable.

Non-parametric statistical significance tests on ranked sum values were used in the analysis of genetic groupings, as assumptions of analysis of variance (ANOVA) models could not be met (e.g. non-homogeneity of within-treatment variances). We used a Kruskal–Wallis ANOVA (Hollander & Wolfe 1973) to compare the six genetic groupings, by testing for the influence of genetic grouping (independent variable) on total length and all physiological indices of nutritional condition (dependent variables). If a Kruskal–Wallis test identified a significant difference ($p < 0.05$) among genetic groupings for any parameter, we used a Dunn's *post hoc* analysis of pairwise comparisons to test for differences on average ranks for unequal sample sizes (Dunn 1964). We performed all statistical analyses using Excel (2010 and 2013; Real Statistics Resource Pack; Microsoft, Redmond, Washington, USA). We used the null hypothesis of no variation in nutrition (i.e. physiological parameters) or total length among genetic groups at the $\alpha = 0.05$ level.

Results

The six nutritional parameters examined and total length were highly variable among bigheaded carps genetic identification groups (Figure 1(A)–(G)). Of the seven conditional indices examined, Kruskal–Wallis tests identified three significantly different parameters among the genetic identification groups: plasma lipase ($H = 47.5$, 5 df, $p < 0.0001$), plasma protein ($H = 47.4$, 5 df, $p < 0.0001$), and plasma triglycerides ($H = 53.1$, 5 df, $p < 0.0001$). Subsequent Dunn's tests identified the lowest quantities of plasma lipase in BH, although there were no differences observed among the BH and AGBH, EGBH, or EGSV individuals. Lipase activities in SV and AGSV were only greater than AGBH and BH (Dunn's test, $p < 0.05$; Figure 1(A)). The greatest amounts of plasma protein were found in AGSV and SV, with the EGSV group intermediate to the parentals, and the AGBH, BH, and EGBH individuals having the least amount of protein (Dunn's test, $p < 0.05$; Figure 1(B)). Concentrations of plasma triglycerides were greatest in SV, but these did not differ from AGSV. The AGSV had similar concentrations to EGSV, but had greater triglyceride concentrations than AGBH, BH, and EGBH (Dunn's test, $p < 0.05$; Figure 1(C)). There were no significant differences among genetic groups for plasma ALP ($H = 3.3$, 5 df, $p = 0.6$; Figure 1(D)), calcium ($H = 6.3$, 5 df, $p = 0.3$; Figure 1(E)), cholesterol ($H = 5.0$, 5 df, $P = 0.4$; Figure 1(F)), or total length ($H = 6.6$, 5 df, $p = 0.3$; Figure 1(G)).

Discussion

Wild-caught, invasive bigheaded carps coexisting in the Marseilles reach of the Illinois River exhibited statistically significant differences in nutritional condition. Parental silver carp showed significantly greater quantities of lipase and concentrations of protein and triglycerides in plasma relative to parental BH, indicating that SV are in better nutritional condition than BH. Lipase is an enzyme involved in the early stages of triglyceride hydrolysis and functions to break down triglycerides for

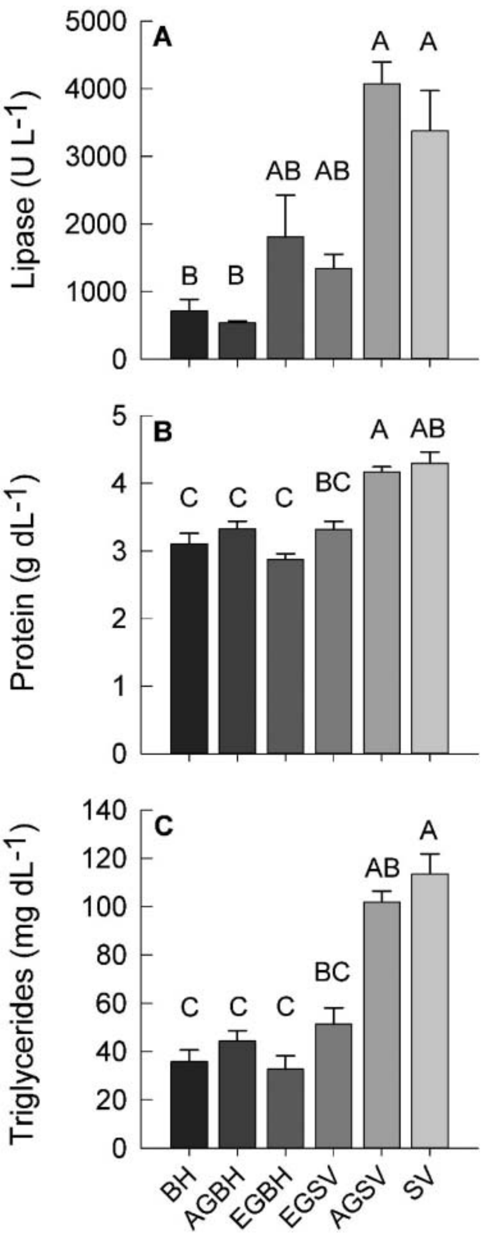

Figure 1. Results of the relationships between plasma (A) lipase (U/L), (B) protein (g/dL), (C) triglycerides (mg/dL), (D) ALP (U/L), (E) calcium (mg/dL), (F) cholesterol (mg/dL), and (G) total length (mm) among genetic identification groupings (parental bighead carp *Hypophthalmichthys nobilis* = BH [$n = 6$], advanced generation bighead carp = AGBH [$n = 13$], early generation bighead carp = EGBH [$n = 6$], early generation silver carp = EGSV [$n = 5$], advanced generation silver carp = AGSV [$n = 33$], parental silver carp *H. molitrix* = SV [$n = 16$]), sampled near Morris, Illinois on the Marseilles reach of the Illinois River, USA, (river km 423). Bars represent the mean with error bars denoting one standard error about the mean. Figures depicting groupings with dissimilar letters indicate significant differences ($p < 0.05$) according to Dunn's *post hoc* pairwise comparisons (e.g. panels A, B, C). Figures depicting groupings without letters indicate no significant ($p > 0.05$) differences (e.g. panels D–G).

mobilization (Babin & Vernier 1989; Wagner & Congleton 2004; Congleton & Wagner 2006; Mohammadizadeh et al. 2012). Triglyceride hydrolysis can increase the amount of lipase in plasma because it acts as a transporter of nutrients (Babin & Vernier 1989; Mommsen et al. 1999). Plasma protein is important for fish maintenance and growth, and is negatively correlated with food

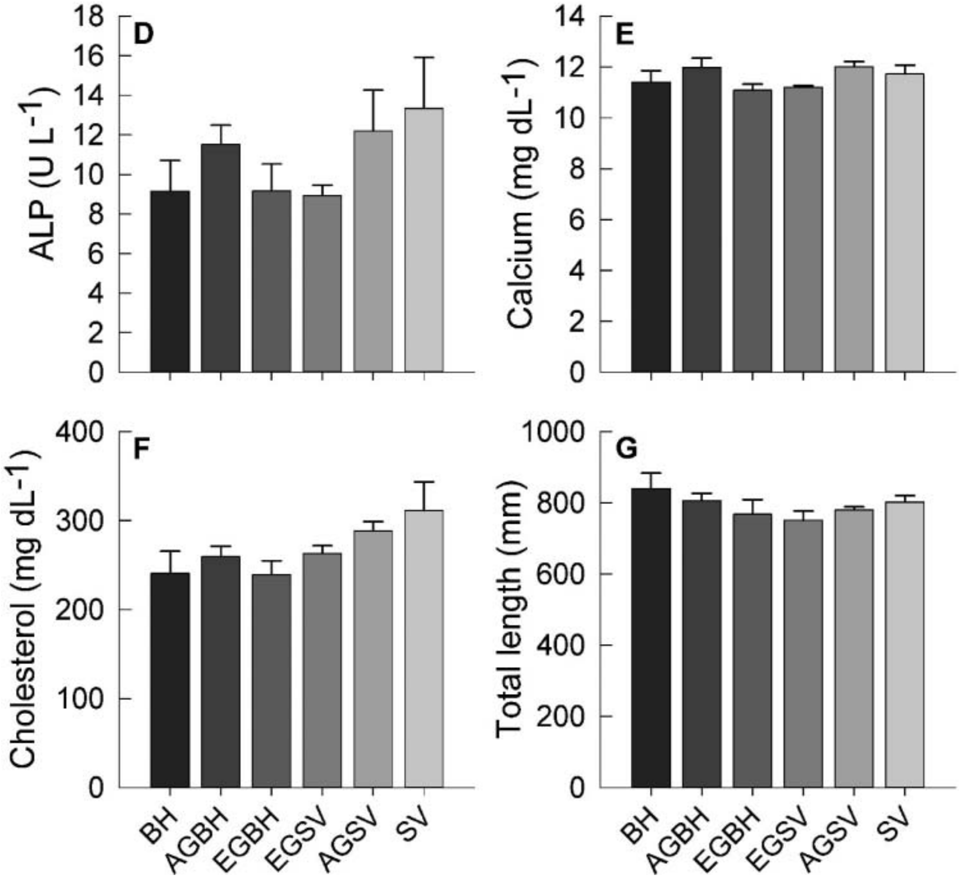

Figure 1. *(Continued).*

deprivation (Kaushik 1995; Wagner & Congleton 2004; Congleton & Wagner 2006; Svobodová et al. 2009). Triglycerides are the primary energy stores in fishes and have been shown to decline during starvation and to increase after feeding (Congleton & Wagner 2006; Morales et al. 2012). A plausible explanation for our observed nutritional differences was likely related to gill raker morphology and feeding preferences. Silver and bighead carp are pump, filter-feeding fishes that use their gill rakers to retain food particles from inhaled water (Bitterlich 1985; Kolar et al. 2007; Sampson et al. 2009; Sass et al. 2010; Calkins et al. 2012). Bighead carp gill rakers are long, straight, and comb-like allowing them to consume suspended food particles down to 17 µm (Kolar et al. 2007; Lamer et al. 2010; Sampson et al. 2009), while silver carp gill rakers are sponge-like allowing them to consume food particles as small as 3−4 µm (Omarov 1970; De-Shang & Shuang-Lin 1996).

The Illinois River is a highly disturbed ecosystem that receives excessive agricultural nutrient inputs (categorizing it as eutrophic) and also has dense concentrations of phytoplankton, zooplankton, and bacteria (Koel & Sparks 2002; Donner 2003; Wahl et al. 2008; Houser & Richardson 2010). It is plausible that elevated plasma triglyceride concentrations and lipase activities in SV relative to BH are due to the ability of SV to filter smaller particles than BH (Omarov 1970; Opuszynski & Shireman 1991; De-Shang & Shuang-Lin 1996). Silver carps have access to a wider range of prey and are able to consume food on a near-continuous basis as they respire compared to BH. In contrast, BH consume larger food particles that may be more energy-rich, but are likely less abundant (Mittelbach 2002, Wahl et al. 2008, Sass et al. 2014). Bighead carp are typically the initial invaders into new habitats between the bigheaded carps (Irons et al. 2007). After BH population growth stabilizes, SV population growth has been shown to increase exponentially (Irons et al. 2007; Sass et al. 2010). Silver carp may become the more successful species among the two, and exploit more resources, because of their increased feeding breadth (Irons et al. 2007; Sass et al. 2010; Sass et al. 2014). Our results suggest that SV are in better nutritional condition than BH in the Marseilles reach of the Illinois River, with plausible differences likely driven by gill raker morphology and feeding habits.

Hybrids of the EGBH (B × BH, F_1) and EGSV (B × SV, F_1) groups had more intermediate nutritional conditions to BH or SV, compared to AGBH (B × 2BH, B × 4BH+) and AGSV (B × 2SV, B × 3SV, B × 4SV+, F_x). Individuals from the EGBH group exhibited concentrations of triglycerides and protein in plasma analogous to BH, while fish from the EGSV group had triglycerides and protein concentrations intermediate to BH and SV. Plasma lipase activities in EGBH and EGSV were intermediate between parental groups. Individuals from the AGBH and AGSV groups were more nutritionally similar to their respective parental species. Hybrids from the AGBH group were nutritionally similar to BH in concentrations of protein, triglycerides, and quantities of lipase. Similarly, AGSV individuals were more nutritionally analogous to SV for those three nutritional metrics. Our nutritional indices (protein, triglycerides, and lipase) indicated that the nutritional condition of bigheaded carps is affected by hybridization. Hybrid individual performance can be inferior, intermediate, or superior to their parents (Seehausen 2004; Stelkens & Seehausen 2009; Rosas et al. 2010). Hybrid vigor (i.e. heterosis) occurs when offspring have increased growth, size, and/or yield per offspring compared to the parents (Birchler et al. 2010; Chen 2010; Rosas et al. 2010). Hybrid vigor may also result in a 'hybrid swarm' if hybrids can survive and interbreed, or backcross with parental species, leading to the complete merging of two taxa (Scribner et al. 2001). Early generation BH × SV hybrid offspring typically possess gill rakers that are twisted, wavy, fused, or deformed, which is phenotypically intermediate between the parental species (Kolar et al. 2007; Lamer et al. 2010). As bigheaded carps become further backcrossed, the intermediate gill raker phenotype observed in the hybrids may have developed during gene conversion, through an unequal expression of maternal and paternal alleles in the offspring (Chakraborty 1989; Chen 2010). The fraction of alleles contributed to the offspring from each parent is rarely 50% (Mallet 2007). This could explain why individuals in the EGBH group were not statistically different from BH individuals based on triglycerides, lipase, and protein concentrations. Similarly, EGSV hybrids were not statistically different from SV for protein or lipase. Our results may also indicate that individuals in AGBH and AGSV groups were becoming more genetically similar to their respective parental species based upon nutritional concentrations of plasma protein, triglycerides, and lipase activities. This is not surprising because hybrids (e.g. AG hybrids) will become more genetically similar to their parents with each successive backcross. The twisted, club-like gill rakers of EGBH and EGSV group hybrids likely function similar to BH gill rakers and prevent both groups of EG hybrids from accessing the smaller-sized food particles that SV can consume. In contrast, the intermediate gill raker phenotype of AGBH and AGSV hybrids likely begins to function more like that of BH and SV, respectively. This may be why all three of the nutritional metrics we tested were statistically similar for AGBH and BH, and two of the three nutritional metrics were statistically similar for AGSV and SV. As such, AGBH and AGSV may be nutritionally similar to BH and SV, respectively, while EGBH and EGSV hybrid offspring may be nutritionally superior to BH, but inferior to SV.

The majority of the randomly sampled individuals in our study were hybrids (with the greatest number of hybrids in the AGSV group), suggesting a high occurrence of hybridization with SV in locations of dense bigheaded carp populations, similar to the Illinois River. Lamer et al. (2015) estimated that about 44% of individuals exist as hybrids in the MRB. Bigheaded carps' hybrids may be successful in the wild, as the selection against hybrids could be ineffective or may not exist (Seehausen 2004). Hybrids from the AGBH and EGBH groups were more similar to BH and displayed decreased feeding indices of protein and triglycerides compared to SV. Individuals from the EGSV group exhibited inferior recent feeding indices relative to SV, as demonstrated by decreased triglyceride concentrations. Furthermore, our results may potentially indicate that EGBH and EGSV hybrids were being out-competed by AGSV hybrids, but not AGBH hybrids. This is potentially due to improved feeding capabilities of SV and their more closely related AGSV hybrids, which is similar to our findings. However, SV population biomass is greater than BH biomass in the Illinois River (Garvey et al. 2012). There were more AGSV backcrosses than AGBH backcrosses in our study, and this may be attributed to a higher abundance of SV existing in the Marseilles reach of the Illinois River compared to BH. Irrespective of the potential mechanisms leading to differences in hybrid nutrition, our study provides evidence that individuals in the AGSV group are more comparable to SV (and are, therefore, in better nutritional condition) compared to AGBH, EGBH, or EGSV group hybrids that are more analogous to BH (AGBH and EGBH) or more intermediate to the parental species (EGSV) in this reach of the Illinois River.

Previous research with hybrid individuals has specifically examined the condition of F_1 hybrids (first hybrid progeny of two taxa) because of their potential to show the greatest vigor or inferiority (Muhlfeld et al. 2009; Rosas et al. 2010). Because our study was performed in a natural setting, the sample sizes may appear to be limited, but our dataset was dictated by natural conditions where we had no prior knowledge of genetic identity. Larger sample sizes of some genetic classes may have been difficult to obtain or were not available because morphological identification of BH, SV, and their hybrids is difficult to ascertain in the field or are simply not well represented in this colonizing population (Lamer et al. 2010). As such, there were only two F_1 hybrid individuals and uneven numbers of hybrids across each genetic identification group. This may have influenced our results, albeit our samples are reflective of the present genetic identity of the population of this reach using the most state-of-the-art genetic identification techniques. The small sample sizes of the EGBH and EGSV in the current study may have also affected our interpretation of the differences between the two groups. However, their nutritional indices typically fell intermediate to BH, SV, AGBH, and AGSV, and our main conclusions revolve around the differences between those four groups. Although we only sampled one known location of bigheaded carps' hybrids at one single point in time, it was from a location known to contain a high proportion of bigheaded carps and their hybrids (Lamer et al. 2015). Nutritional trends for bigheaded carps may differ under varying environmental conditions and across seasons (Liss et al. 2013); however, the nutritional parameters selected for this study were chosen because they represent a long-term body energy reserve nutritional component (Wagner & Congleton 2004; Congleton & Wagner 2006). Additionally, a long-term study by Sass et al. (2014) observed a strong and consistent pattern between the presence of bigheaded carps and the zooplankton community of the Illinois River. Prior to bigheaded carps' invasion and in reaches with low abundances of bigheaded carps, cladocerans and copepods comprised of a greater proportion of the zooplankton community. Following invasion and in Illinois River reaches with high densities of bigheaded carps, rotifers dominated zooplankton community composition, and cladocerans and copepods were rare (Sass et al. 2014). Sass et al. (2014) compiled zooplankton data seasonally during 2009−2011 and showed a clear relationship between bigheaded carp presence and zooplankton community composition. Thus, it is unlikely that an annual or seasonal change in zooplankton community composition or the selection of one sampling site influenced our results in 2012. Conclusions from Sass et al. (2014) (e.g. significant and consistent dominance of one type of zooplankter (rotifers) after the introduction of bigheaded carps) described several sample sites within the Illinois River, supporting the relevance of our conclusions to more locations than our single sample site. Further, SV (and presumably, AGSV hybrids) may be more efficient consumers of rotifers and in better nutritional condition than BH (Spataru & Gophen 1985; Opuszynski & Shireman 1991; De-Shang & Shuang-Lin 1996; Sampson et al. 2009; Lamer et al. 2010; Calkins et al. 2012). Future studies should compare stomach contents from genetically different groups of bigheaded carps to test for potential differences in feeding. Despite these minor caveats, our study illustrates that nutritional performance can decline in EGBH and EGSV hybrids in the wild.

Based on our data, diverse environments may offer performance advantages to SV and AGSV. For bigheaded carps coexisting in oligotrophic habitats (i.e. habitats showing reduced phytoplankton concentrations) or eutrophic environments (replete with phytoplankton), we would expect SV and AGSV hybrids to outperform BH, AGBH, EGBH, and EGSV hybrids.

Silver carp numbers and biomass are increasing exponentially and they tend to become the dominant species of bigheaded carps in co-invaded habitats (Sass et al. 2010; Garvey et al. 2012). This may suggest that SV are outperforming BH, as indicated by their population growth. Sass et al. (2014) found that rotifers dominated zooplankton community composition in the Illinois River after the invasion of bigheaded carps, and in reaches with high densities of these species. Silver carp likely consume rotifers more efficiently, and may outperform BH based on their feeding abilities. Nutritional condition is an important indirect fitness cost that can provide valuable insights into the ecological expense of hybridization. Our results clearly illustrate a decrease in individual nutritional condition in EGBH and EGSV hybrids; however, if hybridization continues, it may benefit this invasive species as AGSV are more similar to parental SV and AGBH are more similar to parental BH.

The objective of our study was to test for relationships between hybridization and nutritional performance in wild-caught, invasive bigheaded carps. Novel patterns in blood-based nutritional constituents of bigheaded carps and their hybrids were observed. Parental SV showed improved nutritional performance relative to BH. Nutritional status of EGSV hybrids was intermediate to both parental lines, and AGBH and AGSV hybrids were nutritionally more similar to their respective parental species. Overall, our analyses suggest that early generation backcrossing decreases nutritional performance for hybrid bigheaded carps in the Marseilles reach of the Illinois River. Although our study documents previously unknown characteristics about the nutritional status of bigheaded carps and their hybrids, there are still many questions about the nutritional constituents of these fishes across broader temporal and spatial scales. Fine-scale data on wild-caught bigheaded carps and hybrid nutritional condition provided by our research suggest that feeding habits and morphological characteristics likely contribute to the nutritional differences found in these invaders and their hybrids.

Acknowledgements

The authors thank the Illinois Natural History Survey, the Illinois Department of Natural Resources, the commercial fishers, and the staff at Western Illinois University's Kibbe Biological Field Station for data collection assistance. This research was supported by The Illinois Department of Natural Resources and the United States Fish and Wildlife Service Federal Aid in Sportfish Restoration Project [F-101-R]. This research was also supported by the University of Illinois Graduate College, College of Agricultural, Consumer and Environmental Sciences, and Department of Natural Resources and Environmental Sciences.

Disclosure statement

The authors perceive no conflicts of interest.

Funding

The Illinois Department of Natural Resources and the United States Fish and Wildlife Service Federal Aid in Sportfish Restoration Project [project number F-101-R].

References

Anderson EC, Thompson EA. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics. [Internet]. [cited 2016 May 17]; 160:1217–1229. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1462008&tool=pmcentrez&rendertype=abstract

Babin PJ, Vernier J-M. 1989. Plasma lipoproteins in fish. J Lipid Res. 30:467–489.

Birchler JA, Yao H, Chudalayandi S, Vaiman D, Veitia RA. 2010. Heterosis. Plant Cell. [Internet]. [cited 2013 Mar 2];22:2105–2112. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2929104&tool=pmcentrez&rendertype=abstract

Bitterlich G. 1985. Digestive enzyme pattern of two stomachless filter feeders, silver carp, *Hypophthalmichthys molitrix* Val., and bighead carp, *Aristichythys nobilis* Rich. J Fish Biol. 27:103–112.
Bryden CA, Heath JW, Heath DD. 2004. Performance and heterosis in farmed and wild Chinook salmon (Oncorhynchus tshawytscha) hybrid and purebred crosses. Aquaculture. [Internet]. [cited 2013 May 31];235:249–261. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0044848604000730
Calkins HA, Tripp SJ, Garvey JE. 2012. Linking silver carp habitat selection to flow and phytoplankton in the Mississippi River. Biol Invasions. [Internet]. [cited 2013 Jan 10];14:949–958. Available from: http://www.springerlink.com/index/10.1007/s10530-011-0128-2
Chakraborty R. 1989. Can molecular imprinting explain heterozygote deficiency and hybrid vigor? Genetics. [Internet]. [cited 2016 May 17]; 122:713–717. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1203745&tool=pmcentrez&rendertype=abstract
Chen ZJ. 2010. Molecular mechanisms of polyploidy and hybrid vigor. Trends Plant Sci. [Internet]. [cited 2013 Mar 10];15:57–71. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2821985&tool=pmcentrez&rendertype=abstract
Congleton JL, Wagner T. 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. J Fish Biol. [Internet]. [cited 2011 Oct 21];69:473–490. Available from: http://doi.wiley.com/10.1111/j.1095-8649.2006.01114.x
Cooke SJ, Suski CD. 2008. Ecological restoration and physiology: an overdue integration. Bioscience. 58:957–968.
Davies R, Mathers KE, Hume AD, Bremer K, Wang Y, Moyes CD. 2012. Hybridization in sunfish influences the muscle metabolic phenotype. Physiol Biochem Zool. [Internet]. [cited 2013 May 23];85:321–331. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22705483
De-Shang L, Shuang-Lin D. 1996. The structure and function of the filtering apparatus of silver carp and bighead carp. Acta Zool Sin. 42:10–14.
Donner S. 2003. The impact of cropland cover on river nutrient levels in the Mississippi River Basin. Glob Ecol Biogeogr. 12:341–355.
Dunn OJ. 1964. Multiple comparisons using rank sums. Technometrics. 6:241–252.
Epifanio J, Nielsen J. 2001. The role of hybridization in the distribution, conservation and management of aquatic species. Rev Fish Biol Fish. 10:245–251.
Farbridge KJ, Leatherland JF. 1992. Temporal changes in plasma thyroid hormone, growth hormone and free fatty acid concentrations, and hepatic 5'-monodeiodinase activity, lipid and protein content during chronic fasting and re-fasting in rainbow trout (Oncorhynchus mykiss). Fish Physiol Biochem. 10:245–257.
Froese R. 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J Appl Ichthyol. [Internet]. [cited 2014 Jul 9];22:241–253. Available from: http://doi.wiley.com/10.1111/j.1439-0426.2006.00805.x
Garvey JE, Sass GG, Trushenski J, Glover DC, Charlebois PM, Levengood J, Tsehaye I, Catalano M, Roth B, Whitledge G, Small BC, Tripp SJ, Secchi S. 2012. Fishing down the bighead and silver carps: reducing the risk of invasion to the Great Lakes. Final Report to the U.S. Fish and Wildlife Service and the Illinois Department of Natural Resources. Southern Illinois University, Carbondale.
Gingerich AJ, Philipp DP, Suski CD. 2010. Effects of nutritional status on metabolic rate, exercise and recovery in a freshwater fish. J Comp Physiol B. [Internet]. [cited 2011 Oct 25];180:371–384. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19936760
Green BW, Smitherman RO. 1984. Relative growth, survival and harvestability of bighead carp, silver carp, and their reciprocal hybrids. Aquaculture. 37:87–95.
Guerreiro I, Peres H, Castro-Cunha M, Oliva-Teles A. 2012. Effect of temperature and dietary protein/lipid ratio on growth performance and nutrient utilization of juvenile Senegalese sole (Solea senegalensis). Aquac Nutr. [Internet]. [cited 2013 Jan 24];18:98–106. Available from: http://doi.wiley.com/10.1111/j.1365-2095.2011.00884.x
Hollander M, Wolfe DA. 1973. Nonparametric statistical methods. New York (NY): Wiley.
Houser JN, Richardson WB. 2010. Nitrogen and phosphorus in the Upper Mississippi River: transport, processing, and effects on the river ecosystem. Hydrobiologia. [Internet]. [cited 2012 Nov 7];640:71–88. Available from: http://www.springerlink.com/index/10.1007/s10750-009-0067-4
Houston AH. 1990. Methods for fish biology. In: Schreck CB, Moyle PB, editors. Blood and circulation. Bethesda (MD): American Fisheries Society; p. 237–334.
Hubbs CL. 1955. Hybridization between fish species in nature. Syst Zool. 4:1–20.
Hutchings JA, Fraser DJ. 2008. The nature of fisheries- and farming-induced evolution. Mol Ecol. [Internet]. [cited 2013 May 30];17:294–313. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17784924
Irons KS, Sass GG, McClelland MA, Stafford JD. 2007. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, U.S.A. Is this evidence for competition and reduced fitness? J Fish Biol. [Internet]. [cited 2011 Aug 23];71:258–273. Available from: http://doi.wiley.com/10.1111/j.1095-8649.2007.01670.x

Kaushik SJ. 1995. Nutrient requirements, supply and utilization in the context of carp culture. Aquaculture. [Internet]. [cited 2016 May 16]; 129:225–241. Available from: http://linkinghub.elsevier.com/retrieve/pii/004484869400274R
Koel TM, Sparks RE. 2002. Historical patterns of river stage and fish communities as criteria for operations of dams on the Illinois River. River Res Appl. [Internet]. [cited 2012 Mar 26];18:3–19. Available from: http://doi.wiley.com/10.1002/rra.630
Kolar CS, Chapman DC, Courtenay Jr. WR, Housel CM, Williams JD, Jennings DP. 2007. Bigheaded carps: a biological synopsis and environmental risk assessment. (Special Publication). Bethesda (MD): American Fisheries Society.
Lamer JT, Dolan CR, Petersen JL, Chick JH, Epifanio JM. 2010. Introgressive hybridization between bighead carp and silver carp in the Mississippi and Illinois Rivers. North Am J Fish Manage. 30:1452–1461.
Lamer JT, Ruebush BC, Arbieva ZH, McClelland MA, Epifanio JM, Sass GG. 2015. Diagnostic SNPs reveal widespread introgressive hybridization between introduced bighead and silver carp in the Mississippi River Basin. Mol Ecol. [Internet]. [cited 2016 May 17];24:3931–3943. Available from: http://doi.wiley.com/10.1111/mec.13285
Lamer JT, Sass GG, Boone JQ, Arbieva ZH, Green SJ, Epifanio JM. 2014. Restriction site-associated DNA sequencing generates high quality single nucleotide polymorphisms for assessing hybridization between bighead and silver carp in the United States and China. Mol Ecol Resour. 14:79–86.
Liss SA, Sass GG, Suski CD. 2013. Spatial and temporal influences on the physiological condition of invasive silver carp. Conserv Physiol. 1:1–13.
Mallet J. 2007. Hybrid speciation. Nature. [Internet]. [cited 2013 Feb 28];446:279–283. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17361174
McClelland MA, Sass GG, Cook TR, Irons KS, Nerissa N, O'Hara TM, Smith CS. 2012. The long-term Illinois River fish population monitoring program. Fisheries. 37:340–350.
McGinnity P, Prodöhl P, Ferguson A, Hynes R, Maoiléidigh NO, Baker N, Cotter D, O'Hea B, Cooke D, Rogan G, et al. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. Proc R Soc B. [Internet]. [cited 2013 May 24];270:2443–2450. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1691531&tool=pmcentrez&rendertype=abstract
Mittelbach GG. 2002. Fish foraging and habitat choice: a theoretical perspective. Volume 1. Hart PJB, Reynolds JD, editors. Oxford: Blackwell Publishing Ltd.
Mohammadizadeh M, Afkhami M, Bastami KD, Ehsanpour M, Soltani F. 2012. Determination of some biochemical values in the blood of Liza klunzingeri from the coastal water of the Persian Gulf. Afr J Biotechnol. [Internet]. [cited 2013 Jan 24];11:3022–3025. Available from: http://www.academicjournals.org/ajb/abstracts/abs2012/9Feb/Mohammadizadeh et al.htm
Mommsen TP, Vijayan MM, Moon TW. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fish. 9:211–268.
Morales AE, Perez-Jimenez A, Furne M, Guderley H. 2012. Starvation, energetics, and antioxidant defenses. 1st ed. Abele D, Vazquez-Medina JP, Zenteno-Savin T, editors. Oxford: Blackwell Publishing Ltd.
Muhlfeld CC, Kalinowski ST, McMahon TE, Taper ML, Painter S, Leary RF, Allendorf FW. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. Biol Lett. [Internet]. [cited 2013 May 23];5:328–31. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2679930&tool=pmcentrez&rendertype=abstract
Omarov MO. 1970. The daily food consumption of the silver carp *Hypophthalmichthys molitrix* (Val.). J Ichthyol. 10:425–426.
Opuszynski K, Shireman JV. 1991. Food passage time and daily ration of bighead carp, *Aristichthys nobilis*, kept in cages. Environ Biol Fishes. [Internet]. [cited 2016 May 17]; 30:387–393. Available from: http://link.springer.com/10.1007/BF02027982
Ricklefs RE, Wikelski M. 2002. The physiology/life-history nexus. Trends Ecol Evol. 17:462–468.
Rosas U, Barton NH, Copsey L, Barbier de Reuille P, Coen E. 2010. Cryptic variation between species and the basis of hybrid performance. PLoS Biol. [Internet]. [cited 2013 Apr 15];8:1–12. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2907293&tool=pmcentrez&rendertype=abstract
Sampson SJ, Chick JH, Pegg MA. 2009. Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi rivers. Biol Invasions. [Internet]. [cited 2011 Sep 1];11:483–496. Available from: http://www.springerlink.com/index/10.1007/s10530-008-9265-7
Sass GG, Cook TR, Irons KS, McClelland MA, Michaels NN, O'Hara TM, Stroub MR. 2010. A mark-recapture population estimate for invasive silver carp (*Hypophthalmichthys molitrix*) in the La Grange Reach, Illinois River. Biol Invasions. [Internet]. [cited 2012 Mar 26];12:433–436. Available from: http://www.springerlink.com/index/10.1007/s10530-009-9462-z
Sass GG, Hinz C, Erickson AC, McClelland NN, McClelland MA, Epifanio JM. 2014. Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. J Gt Lakes Res. 40:911–921.
Scribner KT, Page KS, Bartron ML. 2001. Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. Rev Fish Biol Fish. 10:293–323.
Seehausen O. 2004. Hybridization and adaptive radiation. Trends Ecol Evol. [Internet]. [cited 2014 Jul 11];19:198–207. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16701254

Soltis PS. 2013. Hybridization, speciation and novelty. J Evol Biol. [Internet]. [cited 2013 May 23];26:291–293. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23324011

Spataru P, Gophen M. 1985. Feeding behaviour of silver carp *Hypophthalmichthys molitrix* Val. and its impact on the food web in Lake Kinneret, Israel. Hydrobiologia. 120:53–61.

Stelkens R, Seehausen O. 2009. Genetic distance between species predicts novel trait expression in their hybrids. Evolution (N Y). [Internet]. [cited 2014 Sep 27];63:884–897. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19220450

Suski CD, Killen SS, Kieffer JD, Tufts BL. 2006. The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: implications for live – release angling tournaments. J Fish Biol. 68:120–136.

Svobodová Z, Smutná M, Flajshans M, Gela D, Kocour M, Máchová J, Kroupová H. 2009. Differences in biochemical profiles among spawners of eight common carp breeds. J Appl Ichthyol. [Internet]. [cited 2013 Jan 25];25:734–739. Available from: http://doi.wiley.com/10.1111/j.1439-0426.2009.01271.x

Turner GF. 1999. What is a fish species? Rev Fish Biol Fish. 9:281–297.

Wagner T, Congleton JL. 2004. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). Can J Fish Aquat Sci. 61:1066–1074.

Wagner T, Jones ML, Ebener MP, Arts MT, Brenden TO, Honeyfield DC, Wright GM, Faisal M. 2010. Spatial and temporal dynamics of lake whitefish (Coregonus clupeaformis) health indicators: linking individual-based indicators to a management-relevant endpoint. J Gt Lakes Res. [Internet]. [cited 2012 Mar 22];36:121–134. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0380133009001452

Wahl DH, Goodrich J, Nannini MA, Dettmers JM, Soluk DA. 2008. Exploring riverine zooplankton in three habitats of the Illinois River ecosystem: where do they come from? Limnol Oceanogr. 53:2583–2593.

Waples RS. 1991. Definition of “species” under the Endangered Species Act: application to Pacific Salmon. Seattle, WA: National Marine Fisheries Service, Northwest Fisheries Center, Coastal Zone and Estuarine Studies Division.

Wells RMG, Pankhurst NW. 1999. Blood glucose and lactate, and plasma protein as stress indicators in fish. J World Aquac Soc. 30:276–284.