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Invasive Species as Sentinels: Measuring Health Outcomes in Silver Carp (*Hypophthalmichthys molitrix*) during Removal

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Invasive species threaten ecosystems with destruction of native habitat, introduction of novel diseases, and enhanced competition with native wildlife subsequent to reduced predator control, leading, in many cases, towards efforts to actively remove individuals. While these effects are frequently studied, minimal research has investigated the individual or population health of the invasive species themselves. In this study, we describe multiple health outcomes of Silver Carp (*Hypophthalmichthys molitrix*), an invasive species in the Illinois River, as a component of a long-term monitoring and removal program using electrofishing, gillnets, and trammel nets. Between April–October 2018, Silver Carp were collected from two reaches of the Illinois River, examined, blood was collected for hematologic measurements, and cranial kidneys collected for histologic examination of melanomacrophage centers. Collection location impacted body condition, as Silver Carp closer to the leading edge of invasion were heavier than those from more established populations. Silver Carp caught by nets had lower packed cell volumes than those caught by electrofishing. The Health Assessment Index (HAI) showed that 52% of livers and 53% of kidneys were grossly abnormal. The HAI comes with a caveat that validation protocols are required to implement this technique effectively. Hematology and histology are more likely to be useful in species for which reference ranges exist. Overall, invasive species contain a wealth of information on health outcomes that could be used to monitor ecosystem health, but techniques used for monitoring must be adapted to the species, management needs, and removal methods.

WILD animals have been used as sentinels for ecosystem health, including detection of pathogens on the landscape and degradation of habitat through fragmentation, chemical contamination, and introduction of non-native plants and animals (Rabinowitz and Conti, 2013; Moore et al., 2014; Adams et al., 2018). Long-term monitoring programs can improve detection of these impacts by fisheries and wildlife managers (McClelland et al., 2012; Counihan et al., 2018; Nieman et al., 2021); however, long-term monitoring programs are expensive and logistically challenging due to changing personnel, funding sources, political climate, and access to populations of animals over time.

Invasive species are defined by a presence outside of their native range, with the ability to expand, often causing environmental and economic damages by disrupting food webs and displacing native species (Blackburn et al., 2011). Invasive species have been examined for their negative impacts on the health of native species, especially regarding parasite relationships (Dunn, 2009; Hershberger et al., 2010; Poulin et al., 2011; Hatcher et al., 2012; Young et al., 2017). For example, *Myxobolus cerebralis*, the myxosporidean parasite of salmonids that is responsible for Whirling Disease, has spread worldwide in large part due to the introduction of infected trout species for sportfishing (Whipps et al., 2004; Peeler and Feist, 2011). Heavy metal bioaccumulation has been explored in predatory invaders like the Indo-Pacific lionfish (*Pterois* spp. [Squadrone et al., 2020]). An ulcerative skin condition has been described in Indo-Pacific lionfish, which may impact lionfish population stability, although the potential effects of this disease on native animals is still unclear (Harris et al., 2020). Disease events in invasive populations may be underreported due to limited diagnostics or unknown cause of death. In many cases, invasive species are actively removed and culled from their invaded ranges (Evangelista et al., 2015; David et al., 2018; Love et al., 2018; Rogosch and Olden, 2021), and biological samples from these animals could be collected and utilized for long-term monitoring programs.

The Silver Carp (*Hypophthalmichthys molitrix*) is a cyprinid fish from Asia that has been intentionally and unintentionally introduced into ecosystems worldwide (Kolar, 2007). This species has a history of being a highly successful invader due to rapid growth rates, high fecundity, generalist diet, and low trophic position (Kolar, 2007). However, little is known about other aspects of health of Silver Carp in introduced ranges. There is one report of natural infection by *Lactococcus lactis lactis* in a single Silver Carp associated with a fish kill event in Mississippi, USA, in 2011 (Khoo et al., 2014), as well as a report of *Streptococcus dysgalactiae dysgalactiae* in a single Silver Carp collected from a Silver Carp-only fish kill in Louisiana, USA, in 2018 (Hawke et al., 2021). Within the Illinois River watershed, USA, plasma total solids and plasma cortisol in Silver Carp do not vary spatially in response to land use or water quality, even when individuals are sampled across a number of watersheds, at different densities of individuals, and approaching the edge of the range (Suski et al., 2013; Liss et al., 2014). However, Jeffrey et al. (2019)

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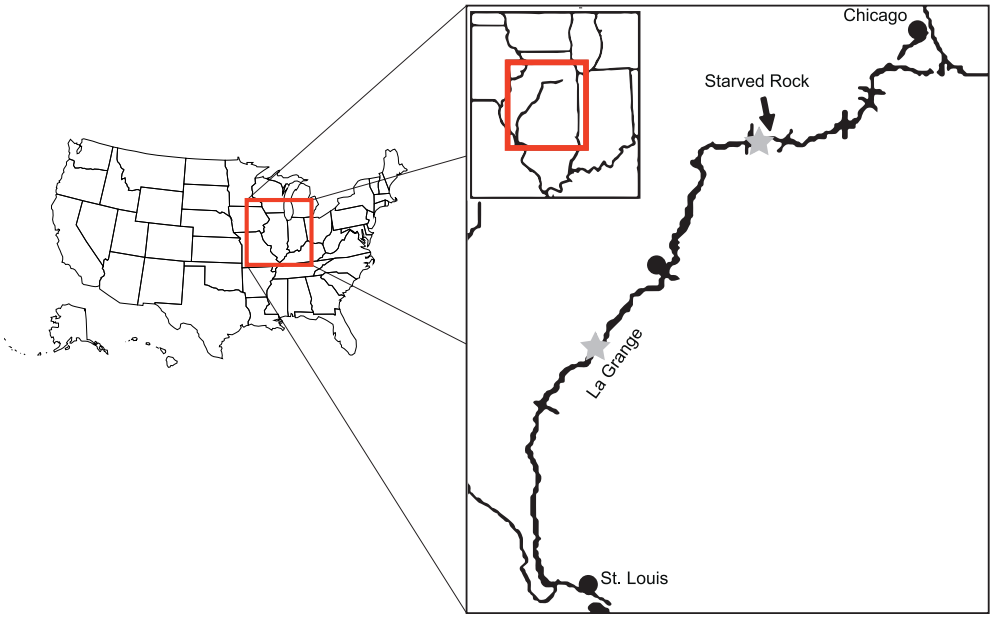

Fig. 1. Map of the Illinois River, Illinois, USA. The two reaches sampled in this study were the La Grange reach and the Starved Rock reach between April–October 2018.

showed that Silver Carp at their invasion front in the Illinois River have gene expression profiles that suggest they are experiencing higher levels of pollution than Silver Carp in downstream reaches of the river, corresponding with a recent report that upstream reaches have higher concentrations of pharmaceuticals and other pollutants (Battaglin et al., 2020; Curtis-Quick et al., 2021). Together, this evidence suggests that, while Silver Carp could be a useful sentinel species for long-term monitoring in their invaded range, health parameters are likely to vary based on distance from the invasion front.

Silver Carp are actively being removed from their invaded ranges in North America using a variety of methods, including electrofishing and gill and trammel netting (Bouska et al., 2017; Butler et al., 2019). As these removal programs are already in place, long-term monitoring of health could be a beneficial addition to current protocols. However, it is important that the samples collected are easy to collect and interpret, and that they are not influenced heavily by capture method. For example, gill and skin biopsies are a common diagnostic tool in fish health assessments (Soto et al., 2019; Seeley, 2021), but these diagnostics can be impacted by trauma induced by gill netting and time fish have spent in the boat prior to collecting tissue. In this study, we collected samples for a health assessment index, hematology, and histology to determine if these sampling methods could be useful for monitoring Silver Carp health as a complement to current removal efforts.

MATERIALS AND METHODS

Study sites and fish collection.—The Illinois River flows southwest from its origin at the confluence of the Des Plaines and Kankakee Rivers until its confluence with the Mississippi River, USA, and is divided into reaches by a series of locks and dams (Fig. 1). The La Grange Reach, which stretches from the La Grange Lock and Dam near Beardstown, Illinois, to the Peoria Lock and Dam near Peoria, Illinois, has had an established population of Silver Carp since at least late 2009 (Sass et al., 2010, 2014). On one day per month between April–October 2018, the Illinois Natural History Survey collected Silver Carp from this reach between river mile (RM) 120.0 and 128.0 near Havana, Illinois, by electrofishing with pulsed direct current from a boat. Fish were transported on ice to the Illinois River Biological Station for processing. The Starved Rock Reach starts at the Starved Rock Lock and Dam near Utica, Illinois, and ends at Marseilles Lock and Dam near Marseilles, Illinois. The Illinois Department of Natural Resources (IDNR) has been harvesting Silver Carp from the Starved Rock Reach since 2011, but the catch-per-unit effort has generally been low in this reach compared to middle and lower reaches of the Illinois River as population density declines near the leading edge of the species' range (Sass et al., 2014; MacNamara et al., 2016; Coulter et al., 2018). On one day per month between April–October 2018, commercial fishermen contracted by the IDNR collected Silver Carp from the Starved Rock Reach (RM 231.2–271.4) using short-set gill and trammel nets. Gear choice was at the discretion of the fishermen and type of net used at each collection event was not recorded. Silver Carp were removed from nets by the fishermen, transported to the shoreline, and held in a 189 L tank containing river water for processing.

Euthanasia and processing.—Prior to processing, fish either remained on ice (La Grange Reach) or were held in a 390 L galvanized steel tank filled with 195 L river water (Starved Rock Reach). Individual fish were euthanized by cerebral concussion, which is designated as an acceptable physical form of euthanasia in finfish when chemical methods are not appropriate due to residue concerns (AVMA, 2020). Blunt force was applied to the midline of the cranium, resulting in body flaccidity, loss of reaction to stimuli, and cessation of operculation. Following euthanasia, Silver Carp were weighed to the nearest 0.01 kg using a hanging scale (RMDS-50, Rapala USA, Minnetonka, MN, USA) and total length was measured to the nearest mm. For fish collected June–October, venipuncture was performed with a 20-gauge needle and a 3 mL pre-heparinized syringe from the caudal tail vein using a ventral approach (Campbell, 2015a). Blood

Table 1. Health assessment index (HAI) scoring criteria used for Silver Carp, *Hypophthalmichthys molitrix*, collected from the La Grange and Starved Rock reaches of the Illinois River, Illinois, USA, between May–October 2018. The HAI criteria are adapted from Adams et al. (1993).

Structure	Condition	Score
Fins	No active erosion	0
	Light active erosion	10
	Moderate active erosion with some hemorrhaging	20
	Severe active erosion with hemorrhaging	30
Skin	Normal; no aberrations	0
	Mild skin aberrations	10
	Moderate skin aberrations	20
	Severe skin aberrations	30
Eyes	No aberrations; good "clear" eye	0
	Generally an opaque eye (one or both)	30
	Swollen, protruding eye (one or both)	30
	Hemorrhaging or bleeding in the eye (one or both)	30
	Missing one or both eyes	30
	Other; any manifestations not fitting the above	30
Gills	Normal; no apparent aberrations	0
	Frayed; erosion of tips of the gill lamellae resulting in "ragged" gills	30
	Clubbed; swelling of the tips of the gill lamellae	30
	Marginate; gills with light, discolored margin along tips of the lamellae	30
	Pale; very light in color	30
	Other; any observation not fitting above categories	30
Spleen	Normal; black, very dark red, or red	0
	Normal; granular, rough appearance of spleen	0
	Nodular; containing fistulas or nodules of varying sizes	30
	Enlarged; noticeably enlarged	30
	Other; gross aberrations not fitting above categories	30
Liver	Normal; solid red or light red color	0
	"Fatty" liver; "coffee with cream" color	30
	Nodules in the liver; cysts or nodules	30
	Focal discoloration; distinct localized color changes	30
	General discoloration; color change in whole liver	30
	Other; deviation in liver not fitting with other categories	30
Kidney	Normal; firm dark red color, lying relatively flat along the length of the vertebral column	0
	Swollen; enlarged or swollen wholly or in part	30
	Mottled; gray discoloration	30
	Granular; granular appearance and texture	30
	Urolithiasis or nephrocalcinosis; white or cream-colored mineral material in kidney tubules	30
	Other; any aberrations not fitting previous categories	30
Intestines	Normal; no inflammation or reddening	0
	Slight inflammation or reddening	10
	Moderate inflammation or reddening	20
	Severe inflammation or reddening	30
Packed cell volume	Normal range (30–45%)	0
	Above normal range (>45%)	10
	Below normal range (19–29%)	20
	Below normal range (<18%)	30
Total dissolved solids	Normal range (3.0–6.9 g/dL)	0
	Above normal range (>7.0 g/dL)	10
	Below normal range (<3.0 g/dL)	30

was immediately transferred to lithium-heparinized vacutainer tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) and stored on ice for a maximum of 2 h. The body, skin, and gills of the fish were visually examined, and photographs taken for the health assessment index (described below) before opening the coelomic cavity. Sex was identified by visualization of the gonads. For fish collected June–October, a 1 cm portion of the cranial kidney was dissected and transferred to 10% neutral buffered formalin (NBF) for histological examination of melanomacrophage centers. The liver was removed and weighed with the hanging scale for calculation of hepatosomatic index (HSI).

Health assessment index.—The health assessment index (HAI) was proposed by Adams et al. (1993) for quickly determining health based on a combination of visual examination of a fish's organs and measure of hematological parameters (Table 1). All Silver Carp in this study were assigned a modified HAI score. Briefly, a score between 0 and 30 was assigned to an organ based on parameters described in Table 1, and the sum

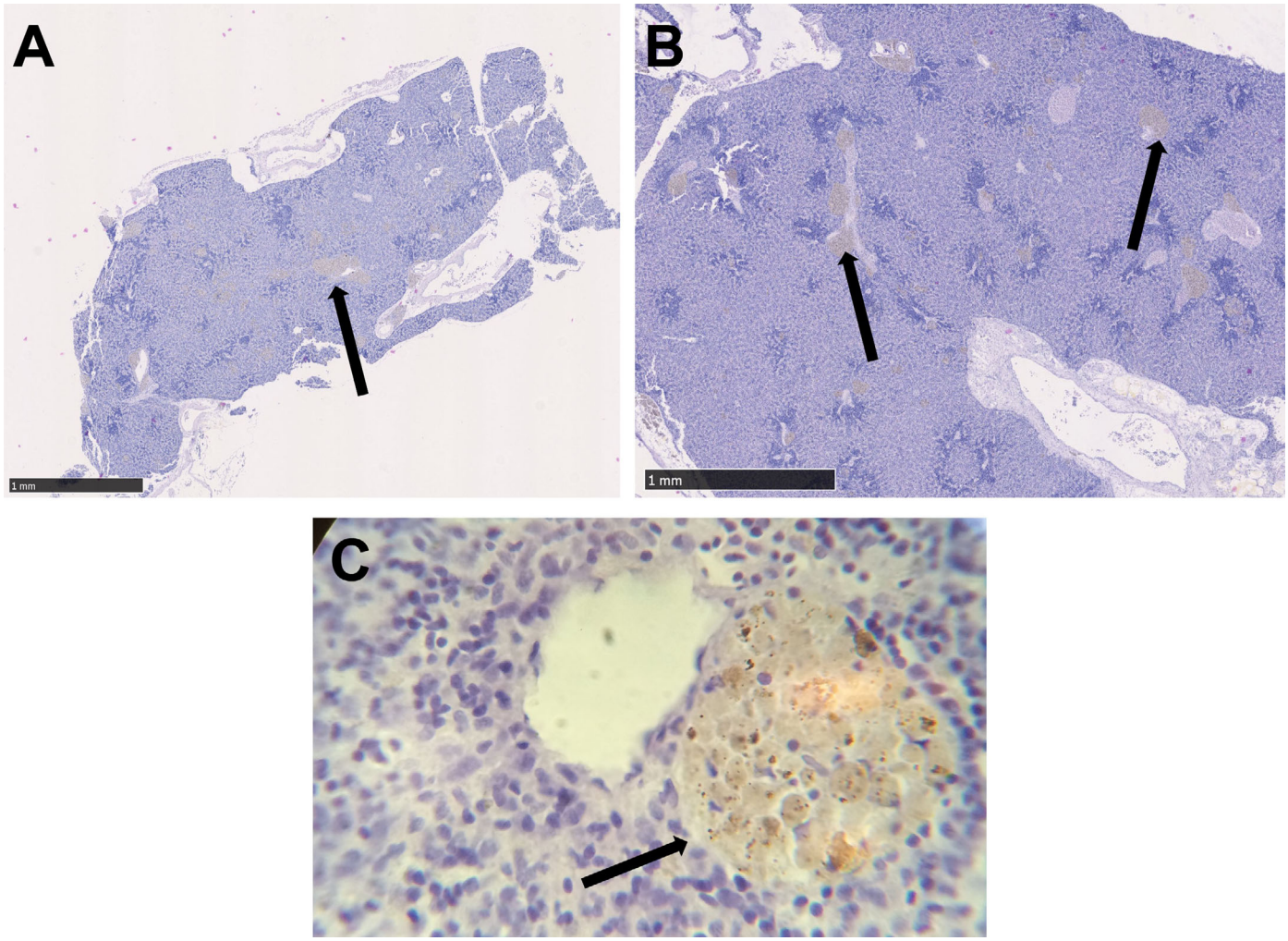

Fig. 2. Cranial kidney melanomacrophage centers (MMC) in Silver Carp, *Hypophthalmichthys molitrix*. Cranial kidney tissues were sampled from Silver Carp in the La Grange and Starved Rock reaches of the Illinois River, Illinois, USA, between June–October 2018, and stained with hematoxylin + eosin for software-assisted calculation of total area of MMCs on individual sections. (A) 2.5X Nanozoomer; (B) 40X Nanozoomer; (C) 100X oil objective, brightfield microscope.

of all scores assigned to the organs was the HAI score of the individual fish. HAI scores from a group of fish can be averaged to provide an HAI score for a population. The same observer assigned all the HAI scores in the field during all collection events. Because the HAI is inherently subjective, we also used photographs taken during dissection to standardize the HAI scores by developing a key to use in assigning consistent scores (see Supplemental Information Figs. S1–S8; see Data Accessibility). The use of gill and trammel nets in the Starved Rock Reach resulted in many skin lacerations and injuries. For this reason, open (actively bleeding) wounds were not considered when assigning scores for HAI to account for differences between commercial nets versus electrofishing.

Cranial kidney histology and analysis.—Cranial kidney tissues were prepared for histology using standard protocols (Hewitson et al., 2010). Following fixation for 72 hours in 10% NBF, tissues were transferred to 70% ethanol. Tissues were then dehydrated in ethanol, cleared in xylene, and infiltrated by paraffin wax (Tissue Tek VIP, Sakura Finetek USA, Torrance, CA, USA). Processed tissues were embedded in paraffin wax and sectioned to a thickness of 5 μm. At least three sections per fish were stained by hematoxylin and eosin (H+E).

Slides were digitized into high-quality images using a Nanozoomer Digital Pathology System (Hamamatsu Photonics, Hamamatsu City, Japan) using a 40X objective and NDP Scan Software and examined using the NDP2.view software (Fig. 2). Quantification of the area of pigmented cells in the cranial kidneys was done using FIJI software (Schindelin et al., 2012). Using NDP2.view, a scale bar was placed in the area not covered by the kidney section and the image was exported to FIJI. The scale bar was used to set the measurement scale for each section. Trainable Weka Segmentation (Arganda-Carreras et al., 2017) was used to separate the section into three classes: Class 1 Empty Space (white or black); Class 2 Non-Pigmented Cells (blue); and Class 3 Pigmented Cells (brown). Once the program was trained to recognize these areas consistently, evidenced by accurate classification of cell types checked by an observer, a probability map was made, and a threshold was set to match the original Nanozoomer image. A measurement of Class 3 Pigmented Cells was taken, as well as a measurement of the Class 2 Non-Pigmented Cells, to allow for quantitation of the

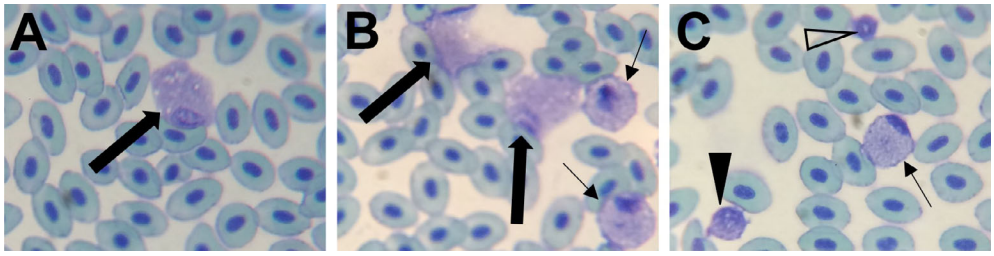

Fig. 3. Leukocyte types observed in Silver Carp, *Hypophthalmichthys molitrix*. Lithium-heparinized blood was collected from Silver Carp in the La Grange and Starved Rock reaches of the Illinois River, Illinois, USA, between April–October 2018. Peripheral blood smears were stained with Diff-Quick and are shown at 100X oil objective on a brightfield microscope. Large arrows (A and B): large leukocyte. Small arrows (B and C): small granulocyte. Black arrowhead (C): lymphocyte. Clear arrowhead (C): thrombocyte.

percentage of pigmented area on each section. This allowed measurement of melanomacrophage area, as increased melanomacrophage area in the cranial kidney is associated with infection, stress, or environmental contamination (Agius and Roberts, 2003; Steinel and Bolnick, 2017; Qualhato et al., 2018; Nowak et al., 2021).

Hematology.—Heparinized microhematocrit capillary tubes (22-362-566, Fisher Scientific, Hampton, NH, USA, $n = 2$ per fish) were filled with whole blood, sealed, and centrifuged in a portable hematocrit centrifuge at 13,700 × *g* (gravity) for 2 minutes (CritSpin Centrifuge, Beckman Coulter, Brea, CA, USA) for measurement of packed cell volume (PCV).

Peripheral blood smears ($n = 2$ per fish) were prepared using standard methods and allowed to air dry before being stored in a slide box. Within 24 hours, smears were stained using Diff-Quik (Electron Microscopy Sciences, Hatfield, PA, USA) and differential leukocyte proportions were determined at 100X oil objective by a single observer using a bright-field microscope. Leukocytes were counted and classified by their appearance (cell morphology, nucleus morphology, staining characteristics) until 100 cells had been tallied (Campbell, 2015b). In consultation with board-certified clinical pathologists at the University of Illinois College of Veterinary Medicine, leukocytes were classified as lymphocytes, large leukocytes, and small granulocytes (Fig. 3). Briefly, lymphocytes were smaller than erythrocytes, round, and could be distinguished from thrombocytes by a pale blue cytoplasm surrounding the dense basophilic nucleus. Large leukocytes were larger than erythrocytes, round to ameboid, with peripherally displaced, foamy round nuclei and cytoplasmic vacuoles. Small granulocytes were of similar size to erythrocytes or slightly larger, with peripherally displaced dense nuclei and refractile basophilic granules throughout the cytoplasm. Smears from August were excluded due to poor stain quality.

The remaining whole blood was centrifuged at 2,000 × *g* for 5 minutes (MyFuge 12 Mini Centrifuge, Thomas Scientific, Swedesboro, NJ, USA), and total plasma solid concentration (includes albumins, globulins, fibrinogen, and other clotting factors/proteins [Stockham and Scott, 2008]) was determined in duplicate using a refractometer (Clinical Protein Refractometer, Azzota Corporation, Claymont, DE, USA; Alexander and Ingram, 1980).

Statistical analyses.—Inherently proportional variables (leukocytes, HSI, melanomacrophage centers, and PCV) were analyzed by beta regression to determine the effects of month, sampling site, and their interaction on the dependent variable of interest, followed by Tukey's multiple comparison test to separate means (Cribari-Neto and Zeileis, 2010). Plasma total solid concentration and HAI scores were compared across months and between sampling sites using two-way analysis of variance (ANOVA; main effects: month, sampling site, and their interaction), followed by Tukey's *post hoc* test. A length–weight regression for all Silver Carp was generated using linear regression, and residuals were calculated. Residuals indicate the error between predicted values and observed values (Framstad et al., 1985), and, in length–weight regressions, a residual greater than 0 indicates that an individual has more weight per body length than is predicted by the regression line. To determine if length–weight relationships differed based on sampling location, residuals of La Grange and Starved Rock fish were compared to each other using one-way ANOVA, with sampling site as the main effect and residual value as the dependent variable. Sex and total length were included as covariates for all models but were removed when no significant effect was observed (Engqvist, 2005). Residual plots of all models were analyzed to confirm proper fit of the data (Crawley, 2012), and variables were transformed if necessary to improve fit (Fox and Weisberg, 2018; Kozak and Piepho, 2018); plasma total solid concentration was log-transformed. Analyses were carried out in R version 3.4.8 (R Core Team, 2018) using the base package as well as multcomp (Hothorn et al., 2008), betareg (Cribari-Neto and Zeileis, 2010), emmeans (Lenth, 2018), lmtest (Zeileis and Hothorn, 2002), and ggplot2 (Wickham, 2009).

Ethics.—All animal and experimental procedures were performed under a protocol approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana (Protocol #17118). Silver Carp were collected under a general scientific permit from the Illinois Department of Natural Resources (Permit #A18.6146).

RESULTS

A total of 136 Silver Carp were collected, with 70 caught from the La Grange Reach and 66 caught from the Starved Rock Reach. Average total length for all fish was 615±51 mm (mean ± S.D.) and did not differ significantly between reaches ($n = 121$, $F_{1,119} = 0.87$, $P = 0.35$) or between sexes ($n = 121$, $F_{1,119} = 0.01$, $P = 0.93$). Both male and female Silver Carp from the Starved Rock Reach were heavier than their counterparts in the La Grange Reach (Table 2, $n = 119$,

Table 2. Demographic information of Silver Carp, *Hypophthalmichthys molitrix*, caught April–October 2018 from La Grange reach and Starved Rock reach of the Illinois River, Illinois, USA.

Sex	Reach (*n*)	Total length (mm) Mean ± SD (range)	Mass (kg) Mean ± SD (range)
Female	La Grange (35)	614±61 (476–672)	2.53±0.82 (1.00–4.78)[a]
	Starved Rock (33)	617±45 (517–656)	2.71±0.59 (1.63–3.17)[b]
Male	La Grange (35)	606±53 (495–721)	2.34±0.67 (0.86–4.20)[a]
	Starved Rock (33)	620±58 (537–765)	2.67±0.75 (1.65–4.84)[b]

[a,b] Superscript letters indicate statistically significant groups.

$F_{1,117} = 4.40$, $P = 0.04$). Based on the length–weight regression line (Fig. 4A), Silver Carp from the Starved Rock Reach had higher residuals than those from the La Grange Reach ($n = 131$, $F_{1,129} = 12.93$, $P = 0.0005$, Fig. 4B), indicating that the population in Starved Rock was in better condition than that from La Grange. PCV was 5.6% lower in Silver Carp from the Starved Rock Reach ($n = 47$, 35.11±10.27%, mean ± S.D.) than in the La Grange Reach ($n = 67$, 29.50±9.36%, mean ± S.D.; $F_{1,\text{Inf}} = 10.52$, $P = 0.001$) but did not differ significantly across months ($F_{6,\text{Inf}} = 1.88$, $P = 0.08$), and there was no interaction effect ($F_{6,\text{Inf}} = 0.73$, $P = 0.63$). In both sites, Silver Carp had lower HSI values in June than July through September ($n = 93$, $F_{6,\text{Inf}} = 5.52$, $P < 0.0001$). There was no difference in HSI between sampling sites ($F_{1,\text{Inf}} = 3.64$, $P = 0.06$), and the interaction effect was not significant ($F_{6,\text{Inf}} = 0.57$, $P = 0.76$).

Large leukocytes were the predominant leukocyte type, followed by lymphocytes, with small granulocytes being the least frequent (Table 3). Lymphocyte proportions were lower in May than September ($n = 48$, $F_{5,\text{Inf}} = 2.38$, $P = 0.04$, Fig. 5A). Overall, fish from Starved Rock had an average of 10% higher lymphocyte proportions than fish from the La Grange Reach ($F_{1,\text{Inf}} = 5.00$, $P = 0.03$, Table 3), but the interaction between sampling site and month was not significant ($F_{5,\text{Inf}} = 0.92$, $P = 0.47$). The proportion of small granulocytes did not differ between months ($F_{5,\text{Inf}} = 1.52$, $P = 0.18$) or between sampling sites ($F_{1,\text{Inf}} = 0.001$, $P = 0.98$), and the interaction was not significant ($F_{5,\text{Inf}} < 0.001$, $P = 1.00$). Large leukocytes displayed an opposite pattern to lymphocytes, being higher in May than September ($F_{5,\text{Inf}} = 2.46$, $P = 0.03$, Fig. 5B), but overall large leukocytes were not different between sites ($F_{1,\text{Inf}} = 0.85$, $P = 0.36$) and there was no significant interaction effect ($F_{5,\text{Inf}} = 0.19$, $P = 0.97$).

The average HAI score for all fish was 69.33±43.29 (mean ± S.D.; $n = 104$) There were no significant differences between sampling sites or across month for HAI scores (sampling site: $F_{1,92} = 0.01$, $P = 0.93$; month: $F_{5,92} = 1.87$, $P = 0.11$; interaction: $F_{5,92} = 1.66$, $P = 0.15$) or cranial kidney melanomacrophage area (sampling site: $F_{1,\text{Inf}} = 1.63$, $P = 0.20$; month: $F_{4,\text{Inf}} = 1.33$, $P = 0.25$; interaction: $F_{4,\text{Inf}} = 0.35$, $P = 0.85$). Overall, the melanomacrophage area average was 2.99±3.44% (mean ± S.D., $n = 41$) across all fish. Plasma total solids differed slightly across months ($F_{6,52} = 2.30$, $P = 0.05$), but not between sites ($F_{1,52} = 0.37$, $P = 0.55$), and the interaction effect was not significant ($F_{4,52} = 1.34$, $P = 0.27$). The average total solids concentration was 2.77±0.09 g/dL (mean ± S.D., $n = 64$) In both collection sites and across all

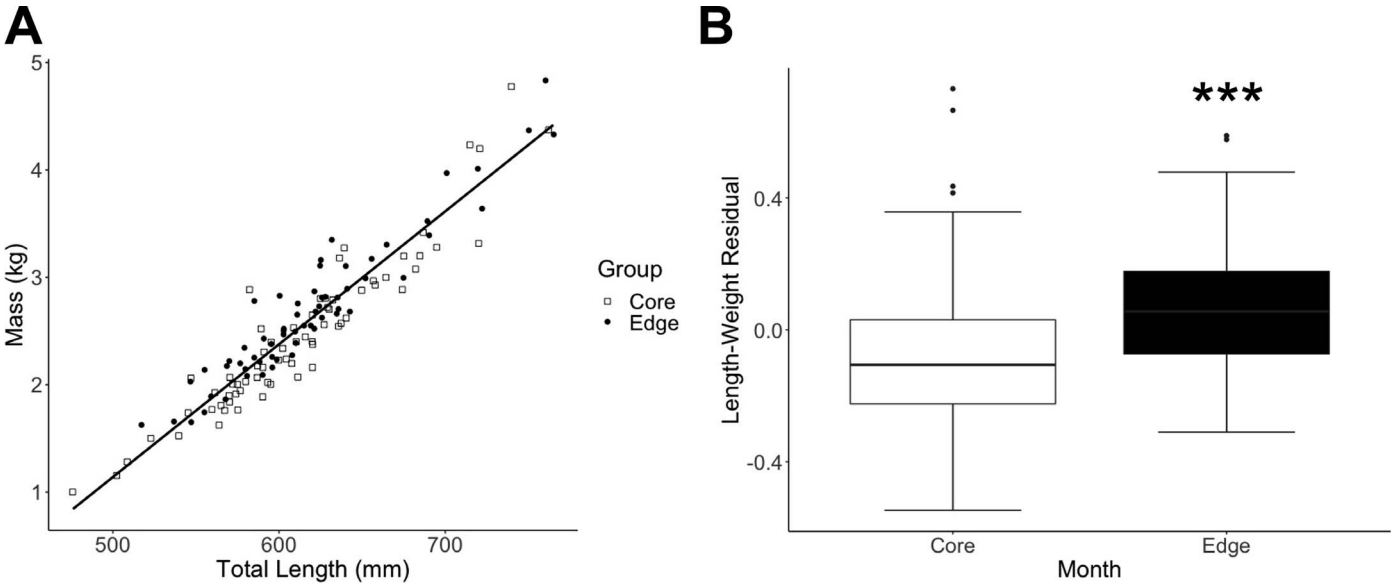

Fig. 4. Length–weight regression and residuals of Silver Carp, *Hypophthalmichthys molitrix*, caught from the La Grange and Starved Rock reaches of the Illinois River, Illinois, USA, between April–October 2018. (A) Relationship between total length (mm) and mass (kg) in Silver Carp, determined by linear regression. White squares: La Grange Silver Carp. Black circles: Starved Rock Silver Carp. (B) Length–weight residuals were compared between Silver Carp from the La Grange Reach (white box) and the Starved Rock Reach (black box). Asterisks indicate statistical significance, as Silver Carp from the Starved Rock Reach had higher residuals than Silver Carp from La Grange ($P < 0.001$).

Table 3. Mean (± SD) values for leukocyte percentages in Silver Carp, *Hypophthalmichthys molitrix*, from La Grange and Starved Rock reaches of the Illinois River, Illinois, USA, from April–October 2018. Cells were not counted in August 2018.

Leukocyte type	La Grange ($n = 20$) Mean ± SD (Range)	Starved Rock ($n = 28$) Mean ± SD (Range)
Lymphocytes	28.00±11.02% (11–51%)[a]	38.54±14.28% (10–64%)[b]
Large leukocytes	51.85±16.97% (5–80%)	42.93±14.95% (16–75%)
Small granulocytes	17.50±10.65% (3–46%)	18.14±7.05% (2–34%)

[a,b] Superscript letters represent statistically significant groups.

sampling events, 52% of livers and 53% of kidneys received scores of 30 (Table 4).

DISCUSSION

Our study indicates that certain results were influenced by capture technique. Ideal metrics for a long-term health monitoring program during invasive species removal should not be influenced by capture technique. They should also be easy and fast to apply to a large number of individuals in high-density populations and should only require minimal technical training of personnel and use of specialized equipment. Most importantly, techniques and interpretation should be consistent across the lifespan of the monitoring program.

Of the methods used in this study, the easiest to apply to a long-term monitoring program of invasive fishes would likely be the HAI scores. Once the HAI is validated for a species and system, it is quick, provides rapid results, and does not require a lot of technical skill (Adams et al., 1993). The HAI is a generalized indication of health and can easily allow for investigators to select other monitoring parameters based on the frequency of high scores in their study population. However, the major downfall of the HAI is the need for consistency between collection sites and between scorers due to inherent subjectivity. For example, Silver Carp collected in gillnets had acute lacerations to the skin and fins that were not included in the HAI scores as this would have artificially inflated the HAI scores of gillnet-collected fish relative to Silver Carp collected by electrofishing. In our study, the same observer assigned HAI scores in the field for

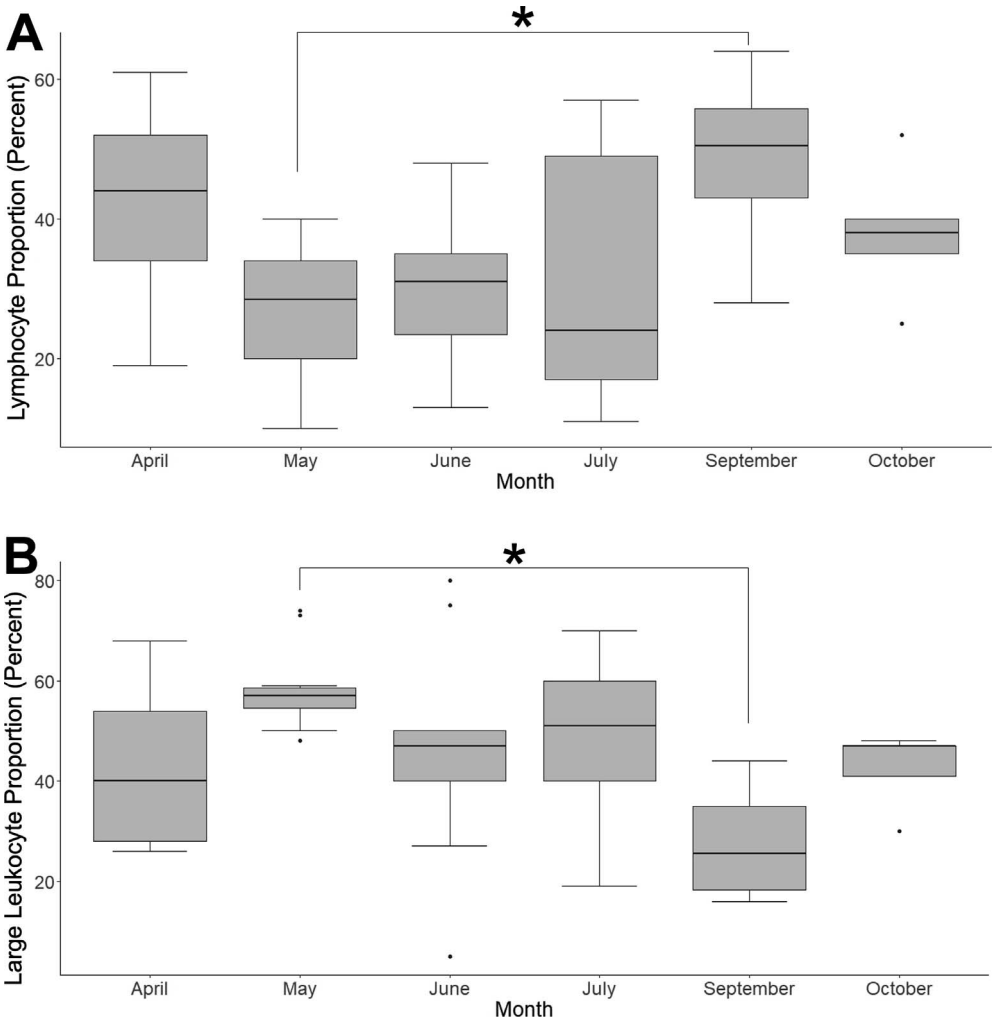

Fig. 5. Variation in lymphocytes (A) and large leukocytes (B) across months in Silver Carp, *Hypophthalmichthys molitrix*, collected from the La Grange and Starved Rock reaches of the Illinois River, Illinois, USA, from April–October 2018. Asterisks indicate statistical significance, as lymphocytes were lower in May relative to September ($P < 0.05$) and large leukocytes were higher in May relative to September ($P < 0.05$).

Table 4. Frequency of scores for health assessment index of Silver Carp, *Hypophthalmichthys molitrix*, collected from La Grange and Starved Rock reaches of the Illinois River, Illinois, USA, from May–October 2018. $n = 105$. Criteria for scoring can be found in Table 1.

Structure	*n*, Score = 0	*n*, Score = 10	*n*, Score = 20	*n*, Score = 30
Fins	89	8	1	7
Skin	86	11	1	7
Eyes	96	—	—	9
Gills	95	—	—	10
Liver	50	—	—	55
Spleen	81	—	—	24
Intestines	90	7	1	7
Kidney	49	—	—	56
Packed cell volume	55	12	22	16
Total dissolved solids	77	0	—	28

all fish during all collection events, but we also collected photographs to document what constituted "mild aberrations" or "severe aberrations" for consistency in scoring. One downside of using photographs is that these images can only capture a two-dimensional visual representation of the organ and cannot provide information on parameters such as texture or three-dimensional distribution of changes. However, the use of a photographic key would be useful in improving consistency of scoring between multiple observers in a monitoring program. Finally, while the HAI scoring system provides quick quantification of the overall health of a population over tie, it is important to follow up on high scores. For example, many of the fish in this study had high-scoring livers and kidneys, usually related to discoloration. Silver Carp are known to be susceptible to viruses such as Spring Viremia of Carp (SVC) and Viral Hemorrhagic Septicemia Virus (VHSV; Kolar, 2007), as well as bacterial infections (Khoo et al., 2014; Hawke et al., 2021), and the HAI could be a useful monitoring tool in this species to detect those fish that should have tissue samples submitted for histopathology, PCR, or other types of screening. The necessity for this type of screening varies depending on the invasive species being assessed, but the HAI can be used in conjunction with pathogen screening programs by providing information about gross pathological changes.

Hematology has potential to be useful in a monitoring program but was of limited usefulness for Silver Carp in this study. Hematological data do exist for farmed Silver Carp, specifically related to toxin exposure (Zhang et al., 2013; Hedayati and Hassan Nataj Niazie, 2015; Ullah et al., 2019), bacterial infection (Bucur et al., 2018), sex differences (Ahmed et al., 2019), hypoxia (Li et al., 2021), and nutrition (Pronina et al., 2017). Previous studies which report leukocyte differentials (Hedayati and Hassan Nataj Niazie, 2015; Pronina et al., 2017; Bucur et al., 2018) do not provide descriptions of how cells were classified, nor do they provide images of leukocytes. Blood smears in the present study were compared to Common Carp (*Cyprinus carpio*), a close relative of the Silver Carp, and very few similarities were found between the cell types. It is for this reason that leukocytes were classified either as lymphocytes, large granulocytes, or small granulocytes. Interestingly, the most predominant leukocyte in the Silver Carp collected in this study was the "large leukocyte," while farmed Silver Carp in previous studies displayed clear lymphocyte predominance (Hedayati and Hassan Nataj Niazie, 2015; Pronina et al., 2017; Bucur et al., 2018). The most likely explanation for this is that Silver Carp in the present study were displaying a marked stress leukogram in response to the capture techniques used, as the acute stress response can result in lymphopenia (Zebral et al., 2014; Grzelak et al., 2017). The large leukocyte appeared most similar to a monocyte, but monocytes are typically rarer than the proportions of large leukocytes observed in this study. It is also possible that the large leukocytes were a form of activated neutrophil, but this cannot be concluded without further investigation of leukocyte morphology in wild adult Silver Carp. Other hematological parameters, such as PCV or total dissolved solids, are easy to obtain with the right equipment and can be useful to monitor, over time, if reference ranges for the species exist. Both PCV and hematocrit measure the proportional volume of red blood cells in a blood sample, though PCV is a direct observation of centrifuged blood while hematocrit is a calculation based on total red blood cell number and cell size (Longanbach and Miers, 2016). The PCV observed in Silver Carp in this study is comparable to PCV in fingerling Silver Carp (Li et al., 2021) and hematocrit in juvenile Silver Carp (Hedayati and Hassan Nataj Niazie, 2015; Ahmed et al., 2019). The average total dissolved solids in Silver Carp in this study was 3.08 g/dL lower than those observed in farmed juvenile Silver Carp (Ahmed et al., 2019), which could be related to differences in fish size or fish diet between the two studies.

Histology can be beneficial for monitoring programs which are looking for specific cellular changes. In this study, we used histology of the cranial kidney to assess melanomacrophages. Melanomacrophage centers (MMC), clusters of pigmented immune cells that increase in size and frequency in response to stress, toxins, and pathogens (Agius and Roberts, 2003; Steinel and Bolnick, 2017; Qualhato et al., 2018; Nowak et al., 2021), are found in immunological organs of fishes and amphibians, and they are useful indicators of stress or pathogenic disease in these species as they can be easily visualized using simple histological stains (Leknes, 2007; Mikula et al., 2008; Shirmohammadi et al., 2017; Steinel and Bolnick, 2017). In our study, no differences in MMC were found between sampling sites or across months, suggesting that this population of cells was not fluctuating or that differences were not detectable during the intervals sampled. Shortcomings of histology include the need for hazardous formaldehyde during sampling, and the fact that preparation and interpretation of histological slides is labor-intensive and requires specialized training and equipment. Additionally, specialized software might be needed for certain quantification procedures. One benefit of sampling for histology is that samples do not need to be processed immediately and can be kept in a "tissue bank" to assess retroactively if they are stored properly.

Hepatosomatic index (HSI) and body condition were not as useful in assessing the health of Silver Carp. As the gonadosomatic index (GSI) of fish increases, the HSI frequently decreases simply due to the contribution of gonads to the total mass. It is therefore difficult to determine if a change in HSI during the peak of spawning season is due to nutritional factors or simply due to the mass of the gonads. The same Silver Carp assessed in this study were also part of a reproductive study, which indicated that GSI increased significantly in May and June (Tucker et al.,

2020), and this correlates with when HSI was lowest in the present study. It should be noted that the relationships between body condition indices and quantifiable representations of body fat in fishes have not been validated as in other species, but weight–length regression is a commonly accepted measure of body condition in fish (Sopinka et al., 2016). Similarly to HSI, weight–length regression can also be influenced by reproductive status, so time of year must be taken into account if this metric is included in a monitoring program of fish. For a species in which body size does not change dramatically due to growth or regression of gonadal tissue, weight-based indices have the potential to be more useful. Overall, body condition indices which accurately predict body fat without being influenced by gonadal mass are needed in fishes.

The primary limitation of this study is the use of two different capture methods. In both the La Grange and Starved Rock reaches, Silver Carp were collected opportunistically from existing collection programs through the Illinois Natural History Survey and the Illinois Department of Natural Resources, respectively. Transport of live fish on ice or on the deck of a boat are common practices in commercial fisheries, but each is likely to influence results of a health monitoring study by inducing stress or injury in the captured fish. Discussion about the welfare of an invasive fish during removal is beyond the scope of this manuscript (but see Huntingford et al., 2006; Braithwaite and Boulcott, 2008; Diggles et al., 2011; Veldhuizen et al., 2018). However, in the interest of comparing health between locations and capture methods, fish should be rendered unconscious as soon as possible following capture to reduce confounding variables.

In conclusion, the removal of invasive species presents a unique opportunity for health surveillance. Though invasive species did not evolve in their introduced ranges, they are still susceptible to environmental degradation and diseases, and monitoring their health can allow wildlife managers to detect pollution or disease outbreaks. This approach reduces the number of native species that need to be captured for surveillance while also increasing the utility of removing the invasive species. Using Silver Carp as an example, this study showed that any long-term monitoring program assessing the health of an invasive species must be adapted to the species, capture method, and available equipment and personnel. Once these protocols are established, invasive species could be useful sentinels for monitoring the health of their invaded ecosystems.

DATA ACCESSIBILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Supplemental material is available at https://www.ichthyologyandherpetology.org/i2021072. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License.

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LITERATURE CITED

Adams, D. H., D. M. Tremain, and D. W. Evans. 2018. Large-scale assessment of mercury in sentinel estuarine fishes of the Florida Everglades and adjacent coastal ecosystems. Bulletin of Marine Science 94:1413–1427.

Adams, S. M., A. M. Brown, and R. W. Goede. 1993. A quantitative health assessment index for rapid evaluation of fish condition in the field. Transactions of the American Fisheries Society 122:63–73.

Agius, C., and R. J. Roberts. 2003. Melano-macrophage centres and their role in fish pathology. Journal of Fish Diseases 26:499–509.

Ahmed, I., Z. A. Sheikh, G. B. Wani, and B. A. Shah. 2019. Sex variation in hematological and serum biochemical parameters of cultured Chinese silver carp, *Hypophthalmichthys molitrix*. Comparative Clinical Pathology 28: 1761–1767.

Alexander, J. B., and G. A. Ingram. 1980. A comparison of five of the methods commonly used to measure protein concentrations in fish sera. Journal of Fish Biology 16:115–122.

Arganda-Carreras, I., V. Kaynig, C. Rueden, J. Schindelin, K. W. Eliceiri, A. Cardona, and H. S. Seung. 2017. Trainable Weka segmentation: a machine learning tool for microscopy pixel classification. Bioinformatics 33:2424–2426.

AVMA. 2020. AVMA guidelines for the euthanasia of animals: 2020 edition. [Online.] Available at https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals

Battaglin, W., J. Duncker, P. Terrio, P. Bradley, L. Barber, and L. DeCicco. 2020. Evaluating the potential role of bioactive chemicals on the distribution of invasive Asian carp upstream and downstream from river mile 278 in the Illinois waterway. Science of The Total Environment 735: 139458.

Blackburn, T. M., P. Pyšek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarošík, J. R. U. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26: 333–339.

Bouska, W. W., D. C. Glover, K. L. Bouska, and J. E. Garvey. 2017. A refined electrofishing technique for collecting silver carp: implications for management. North American Journal of Fisheries Management 37:101–107.

Braithwaite, V. A., and P. Boulcott. 2008. Can fish suffer?, p. 78–92. *In*: Fish Welfare. E. J. Branson (ed.). Blackwell Publishing Ltd, Ames, Iowa.

Bucur, C., D. Radu, M. Costache, N. Marica, and S. Radu. 2018. Research on bacterial disease in silver carp (*Hypophthalmichthys molitrix* Val.), farmed in ponds. *In*: 18th International Multidisciplinary Scientific GeoConference. Sofia, Bulgaria.

Butler, S. E., A. P. Porreca, S. F. Collins, J. A. Freedman, J. J. Parkos, M. J. Diana, and D. H. Wahl. 2019. Does fish herding enhance catch rates and detection of invasive bigheaded carp? Biological Invasions 21:775–785.

Campbell, T. W. 2015a. Blood sample collection and preparation in fish, p. 183–186. *In*: Exotic Animal Hematology and Cytology. Fourth edition. T. W. Campbell (ed.). John Wiley & Sons, Inc., Ames, Iowa.

Campbell, T. W. 2015b. Hematologic techniques in lower vertebrates, p. 197–205. *In*: Exotic Animal Hematology and Cytology. Fourth edition. T. W. Campbell (ed.). John Wiley & Sons, Inc., Ames, Iowa.

Coulter, D. P., R. MacNamara, D. C. Glover, and J. E. Garvey. 2018. Possible unintended effects of management at an invasion front: reduced prevalence corresponds with high condition of invasive bigheaded carps. Biological Conservation 221:118–126.

Counihan, T. D., I. R. Waite, A. F. Casper, D. L. Ward, J. S. Sauer, E. R. Irwin, C. G. Chapman, B. S. Ickes, C. P. Paukert, J. J. Kosovich, and J. M. Bayer. 2018. Can data from disparate long-term fish monitoring programs be used to increase our understanding of regional and continental trends in large river assemblages? PLoS ONE 13:e0191472.

Crawley, M. J. 2012. Statistical modelling, p. 388–488. *In*: The R Book. Second edition. M. J. Crawley (ed.). John Wiley & Sons, Ltd., West Sussex, UK.

Cribari-Neto, F., and A. Zeileis. 2010. Beta regression in R. Journal of Statistical Software 34:1–24.

Curtis-Quick, J. A., A. V Ulanov, Z. Li, J. F. Bieber, E. K. Tucker-Retter, and C. D. Suski. 2021. Why the stall? Using metabolomics to define the lack of upstream movement of invasive bigheaded carp in the Illinois River. PLoS ONE 16:e0258150.

David, B. O., D. Özkundakci, M. Pingram, D. Bergin, and M. Bergin. 2018. "The CarP-N neutral Project": removal, processing and reuse of invasive fish in local terrestrial conservation projects. Journal of Applied Ecology 55:1567–1574.

Diggles, B. K., S. J. Cooke, J. D. Rose, and W. Sawynok. 2011. Ecology and welfare of aquatic animals in wild capture fisheries. Reviews in Fish Biology and Fisheries 21: 739–765.

Dunn, A. M. 2009. Parasites and biological invasions. Advances in Parasitology 68:161–184.

Engqvist, L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. Animal Behaviour 70:967–971.

Evangelista, C., R. J. Britton, and J. Cucherousset. 2015. Impacts of invasive fish removal through angling on population characteristics and juvenile growth rate. Ecology and Evolution 5:2193–2202.

Fox, J., and S. Weisberg. 2018. Visualizing fit and lack of fit in complex regression models with predictor effect plots and partial residuals. Journal of Statistical Software 87:1–27.

Framstad, E., S. Engen, and N. C. Stenseth. 1985. Regression analysis, residual analysis and missing variables in regression models. Oikos 44:319–323.

Grzelak, A. K., D. J. Davis, S. M. Caraker, M. J. Crim, J. M. Spitsbergen, and C. E. Wiedmeyer. 2017. Stress leukogram induced by acute and chronic stress in zebrafish (*Danio rerio*). Comparative Medicine 67:263–269.

Harris, H. E., A. Q. Fogg, M. S. M. Allen, R. N. Ahrens, and W. F. Patterson III. 2020. Precipitous declines in northern Gulf of Mexico invasive lionfish populations following the emergence of an ulcerative skin disease. Scientific Reports 10:1934.

Hatcher, M. J., J. T. A. Dick, and A. M. Dunn. 2012. Disease emergence and invasions. Functional Ecology 26:1275–1287.

Hawke, J. P., R. Daniel, K. Strother, Y. Sokolova, J. Elliott, M. Carossino, I. Langohr, F. Del Piero, A. López-Porras, T. I. Heckman, E. Soto, and M. J. Griffin. 2021. *Streptococcus dysgalactiae*: a pathogen of feral populations of silver carp from a fish kill event. Journal of Aquatic Animal Health 33:231–242.

Hedayati, A., and E. Hassan Nataj Niazie. 2015. Hematological changes of silver carp (*Hypophthalmichthys molitrix*) in response to diazinon pesticide. Journal of Environmental Health Science & Engineering 13:52.

Hershberger, P. K., B. K. van der Leeuw, J. L. Gregg, C. A. Grady, K. M. Lujan, S. K. Gutenberger, M. K. Purcell, J. C. Woodson, J. R. Winton, and M. J. Parsley. 2010. Amplification and transport of an endemic fish disease by an introduced species. Biological Invasions 12:3665–3675.

Hewitson, T. D., B. Wigg, and G. J. Becker. 2010. Tissue preparation for histochemistry: fixation, embedding, and antigen retrieval for light microscopy, p. 3–18. *In*: Histology Protocols: Methods in Molecular Biology. Vol. 611. T. D. Hewitson and I. A. Darby (eds.). Humana Press, Totowa, New Jersey.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346–363.

Huntingford, F. A., C. Adams, V. A. Braithwaite, S. Kadri, T. G. Pottinger, P. Sandøe, and J. F. Turnbull. 2006. Current issues in fish welfare. Journal of Fish Biology 68:332–372.

Jeffrey, J. D., K. M. Jeffries, and C. D. Suski. 2019. Physiological status of silver carp (*Hypophthalmichthys molitrix*) in the Illinois River: an assessment of fish at the leading edge of the invasion front. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 32: 100614.

Khoo, L. H., F. W. Austin, S. M. A. Quiniou, P. S. Gaunt, D. K. Riecke, A. M. Jacobs, K. O. Meals, A. W. Dunn, and M. J. Griffin. 2014. Lactococcosis in silver carp. Journal of Aquatic Animal Health 26:1–8.

Kolar, C. S. 2007. Bigheaded Carps: A Biological Synopsis and Environmental Risk Assessment. American Fisheries Society, Bethesda, Maryland.

Kozak, M., and H. P. Piepho. 2018. What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. Journal of Agronomy and Crop Science 204:86–98.

Leknes, I. L. 2007. Melano-macrophage centres and endocytic cells in kidney and spleen of pearl gouramy and

platyfish (Anabantidae, Poeciliidae: Teleostei). Acta Histochemica 109:164–168.

Lenth, R. 2018. Emmeans: estimated marginal means, aka least-squares means. R Package Version 1.1. https://CRAN.R-project.org/package=emmeans

Li, X., F. Li, G. Zou, C. Feng, H. Sha, S. Liu, and H. Liang. 2021. Physiological responses and molecular strategies in heart of silver carp (*Hypophthalmichthys molitrix*) under hypoxia and reoxygenation. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 40: 100908.

Liss, S. A., G. G. Sass, and C. D. Suski. 2014. Influence of local-scale abiotic and biotic factors on stress and nutrition in invasive silver carp. Hydrobiologia 736:1–15.

Longanbach, S., and M. Miers. 2016. Automated blood cell analysis, p. 208–234. *In*: Rodak's Hematology: Clinical Principles and Applications. Fifth edition. E. Keohane, L. Smith and J. Walenga (eds.). Elsevier Saunders, Amsterdam.

Love, S. A., N. J. Lederman, R. L. Anderson, J. A. DeBoer, and A. F. Casper. 2018. Does aquatic invasive species removal benefit native fish? The response of gizzard shad (*Dorosoma cepedianum*) to commercial harvest of bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*). Hydrobiologia 817:403–412.

MacNamara, R., D. Glover, J. Garvey, W. Bouska, and K. Irons. 2016. Bigheaded carps (*Hypophthalmichthys* spp.) at the edge of their invaded range: using hydroacoustics to assess population parameters and the efficacy of harvest as a control strategy in a large North American river. Biological Invasions 18:3293–3307.

McClelland, M. A., G. G. Sass, T. R. Cook, K. S. Irons, N. N. Michaels, T. M. O'Hara, and C. S. Smith. 2012. The long-term Illinois River fish population monitoring program. Fisheries 37:340–350.

Mikula, P., H. Modra, D. Nemethova, L. Groch, and Z. Svobodova. 2008. Effects of subchronic exposure to LASSO MTX® (Alachlor 42% W/V) on hematological indices and histology of the common carp, *Cyprinus carpio* L. Bulletin of Environmental Contamination and Toxicology 81:475–479.

Moore, S. E., E. Logerwell, L. Eisner, E. V. Farley, L. A. Harwood, K. Kuletz, J. Lovvorn, J. R. Murphy, and L. T. Quakenbush. 2014. Marine fishes, birds and mammals as sentinels of ecosystem variability and reorganization in the Pacific Arctic Region, p. 337–392. *In*: The Pacific Arctic Region: Ecosystem Status and Trends in a Rapidly Changing Environment. J. M. Grebmeier and W. Maslowski (eds.). Springer Netherlands, Dordrecht.

Nieman, C. L., R. M. Pendleton, G. H. Kenney, and C. T. Solomon. 2021. Evaluation and optimization of a long-term fish monitoring program in the Hudson River. Ecological Indicators 133:108344.

Nowak, B. F., M. Dang, C. Webber, L. Neumann, A. Bridle, R. Bermudez, and D. Evans. 2021. Changes in the splenic melanomacrophage centre surface area in southern bluefin tuna (*Thunnus maccoyii*) are associated with blood fluke infections. Pathogens 10:79.

Peeler, E. J., and S. W. Feist. 2011. Human intervention in freshwater ecosystems drives disease emergence. Freshwater Biology 56:705–716.

Poulin, R., R. A. Paterson, C. R. Townsend, D. M. Tompkins, and D. W. Kelly. 2011. Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. Freshwater Biology 56:676–688.

Pronina, G. I., N. Y. Koryagina, A. B. Petrushin, and A. O. Revyakin. 2017. Comparative physiological and immunological characteristics of carps (*Cyprinidae*) grown in aquaculture with different types of food. Journal of Ichthyology 57:490–493.

Qualhato, G., S. M. T. de Sabóia-Morais, L. D. Silva, and T. L. Rocha. 2018. Melanomacrophage response and hepatic histopathologic biomarkers in the guppy *Poecilia reticulata* exposed to iron oxide (maghemite) nanoparticles. Aquatic Toxicology 198:63–72.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rabinowitz, P., and L. Conti. 2013. Links among human health, animal health, and ecosystem health. Annual Review of Public Health 34:189–204.

Rogosch, J. S., and J. D. Olden. 2021. Comparing opportunistic and strategic removal efforts to manage invasive fish species using a dynamic multi-state occupancy model. Journal of Applied Ecology 58:2797–2809.

Sass, G. G., T. R. Cook, K. S. Irons, M. A. McClelland, N. N. Michaels, T. Matthew O'Hara, and M. R. Stroub. 2010. A mark–recapture population estimate for invasive silver carp (*Hypophthalmichthys molitrix*) in the La Grange Reach, Illinois River. Biological Invasions 12:433–436.

Sass, G. G., C. Hinz, A. C. Erickson, N. N. McClelland, M. A. McClelland, and J. M. Epifanio. 2014. Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. Journal of Great Lakes Research 40:911–921.

Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri . . . A. Cardona. 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods 9: 676.

Seeley, K. E. 2021. Clinical examination and diagnostic sampling, p. 109–128. *In*: Clinical Guide to Fish Medicine. C. A. Hadfield and L. A. Clayton (eds.). John Wiley & Sons, Inc., Ames, Iowa.

Shirmohammadi, M., N. Salamat, M. T. Ronagh, A. Movahedinia, and G. Hamidian. 2017. Assessment of immune status of yellowfin seabream (*Acanthopagrus latus*) during short term exposure to phenanthrene. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 195:78–90.

Sopinka, N. M., M. R. Donaldson, C. M. O'Connor, C. D. Suski, and S. J. Cooke. 2016. Stress indicators in fish, p. 405–462. *In*: Biology of Stress in Fish: Fish Physiology Volume 35. C. B. Schreck, L. Tort, A. P. Farrell, and C. J. Brauner (eds.). Elsevier, Amsterdam.

Soto, E., S. M. Boylan, B. Stevens, S. A. Smith, R. P. E. Yanong, K. Subramaniam, and T. Waltzek. 2019. Diagnosis of fish diseases, p. 46–88. *In*: Fish Diseases and Medicine. First edition. S. A. Smith (ed.). CRC Press, Boca Raton, Florida.

Squadrone, S., P. Brizio, C. Stella, M. Mantia, L. Favaro, B. Biancani, S. Gridelli, C. Da Rugna, and M. C. Abete. 2020. Differential bioaccumulation of trace elements and rare earth elements in the muscle, kidneys, and liver of the

invasive Indo-Pacific lionfish (*Pterois* spp.) from Cuba. Biological Trace Element Research 196:262–271.

Steinel, N. C., and D. I. Bolnick. 2017. Melanomacrophage centers as a histological indicator of immune function in fish and other poikilotherms. Frontiers in Immunology 8: 827.

Stockham, S. L., and M. A. Scott. 2008. Proteins, p. 369–414. *In*: Fundamentals of Veterinary Clinical Pathology. Second edition. S. L. Stockham and M. A. Scott (eds.). Wiley-Blackwell, Ames, Iowa.

Suski, C. D., S. A. Liss, and G. G. Sass. 2013. Spatial and temporal influences on the physiological condition of invasive silver carp. Conservation Physiology 1:cot017.

Tucker, E. K., M. E. Zurliene, C. D. Suski, and R. A. Nowak. 2020. Gonad development and reproductive hormones of invasive silver carp (*Hypophthalmichthys molitrix*) in the Illinois River. Biology of Reproduction 102:647–659.

Ullah, S., Z. Li, M. Z. Ul Arifeen, S. U. Khan, and S. Fahad. 2019. Multiple biomarkers based appraisal of deltamethrin induced toxicity in silver carp (*Hypophthalmichthys molitrix*). Chemosphere 214:519–533.

Veldhuizen, L. J. L., P. B. M. Berentsen, I. J. M. de Boer, J. W. van de Vis, and E. A. M. Bokkers. 2018. Fish welfare in capture fisheries: a review of injuries and mortality. Fisheries Research 204:41–48.

Whipps, C. M., M. El-Matbouli, R. P. Hedrick, and M. L. K. Vicki Blazer. 2004. *Myxobolus cerebralis* internal transcribed spacer 1 (ITS-1) sequences support recent spread of the parasite to North America and within Europe. Diseases of Aquatic Organisms 60:105–108.

Wickham, H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer, Dordrecht.

Young, H. S., I. M. Parker, G. S. Gilbert, A. Sofia Guerra, and C. L. Nunn. 2017. Introduced species, disease ecology, and biodiversity–disease relationships. Trends in Ecology & Evolution 32:41–54.

Zebral, Y. D., B. Zafalon-Silva, M. W. Mascarenhas, and R. B. Robaldo. 2014. Leucocyte profile and growth rates as indicators of crowding stress in pejerrey fingerlings (*Odontesthes bonariensis*). Aquaculture Research 46:2270–2276.

Zeileis, A., and T. Hothorn. 2002. Diagnostic checking in regression relationships. R News 2:7–10.

Zhang, T., Y. Zhang, D. Li, T. Xiao, and J. Li. 2013. Exposure of silver carp (*Hypophthalmichthys molitrix*) to environmentally relevant levels of cadmium: hematology, muscle physiology, and implications for stock enhancement in the Xiangjiang River (Hunan, China). Science China Life Sciences 56:66–72.