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# BIOCHEMISTRY PANEL REFERENCE INTERVALS FOR JUVENILE GOLDFISH (*CARASSIUS AURATUS*)

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*Abstract:* Reference intervals for diagnostic tests are vitally important for clinical decision making. Despite the popularity of pet goldfish (*Carassius auratus*), reference intervals have not been generated for routine biochemistry panel analytes in this species. This study establishes de novo reference intervals for packed cell volume and total solids, using 47 apparently healthy immature goldfish, and for 11 common chemistry panel analytes (albumin, aspartate aminotransferase, calcium, creatine kinase, globulin, blood glucose, sodium, potassium, phosphorous, total protein, and uric acid) using 39 immature goldfish. Robust reference intervals were generated following recommendations of the American Society for Veterinary Clinical Pathology. Linear regression was used to demonstrate a statistically significant relationship between body weight and calcium, albumin, total protein, potassium, packed cell volume, and total solids. The results of this study serve as a useful baseline for future reference interval generation in goldfish.

*Key words:* Biochemistry panel, blood values, *Carassius auratus*, goldfish, reference interval.

## INTRODUCTION

Goldfish (*Carassius auratus*) are small, omnivorous, freshwater cyprinid fish. They were first domesticated in China over 1,000 yr ago and have been selectively bred for extreme phenotypic variation in color, eye shape, body size and shape, and fin size, shape, and number.[22,28,33] Goldfish are extremely popular for indoor and outdoor display.[22] They are also common laboratory models for studies of retinal function and the neuroendocrine control of behavior, reproduction, and energy balance.[10,24,27] Despite the popularity of goldfish as pets, display animals, and laboratory specimens, robust reference intervals for hematology and clinical chemistry panels are not available.[30,31]

Several studies have evaluated changes in isolated hematologic or biochemistry parameters of goldfish in response to environmental manipulation, stress, or infection. In studies of plasma osmolality and anemia during temperature acclimation, cold-adapted goldfish demonstrated decreased plasma osmolality and failed to mount a regenerative erythrocyte response to pharmacologically induced anemia.[5,7] Warm-adapted goldfish had increased hemoglobin, hematocrit, total leukocyte counts, lymphocyte counts, neutrophil counts, and eosinophil counts compared to fish adapted to lower temperatures.[12,18] Goldfish exposed to constant temperatures displayed diurnal fluctuations in sodium (Na), potassium (K), chloride, and calcium (Ca) concentrations but maintained fairly stable magnesium (Mg) concentrations.[19] In contrast, plasma ionic concentrations tended to be lower and more stable in fish exposed to diurnal temperature fluctuations.[19] Goldfish maintained in constant light conditions demonstrated a bimodal blood glucose (BG) distribution while fish maintained with a 12-hr photoperiod had higher BG levels during the dark period.[29] Goldfish fry, acclimated to increased salinity, developed a decrease in total protein (TP) after 45 days without significant alteration in hematocrit, BG, or triglyceride levels.[20] Adults exposed to 5, 10, and 20 g/L salt baths for up to 12 hr demonstrated increases in Na, chloride, alanine aminotransferase (ALT), and glucose without consistent alteration in K.[2]

Goldfish maintained at high stocking densities for 6–8 mo developed decreases in hematocrit, hemoglobin, and thrombocyte count and increases in leukocyte count and lymphocyte count.[3,25] Fish captured with nets, immobilized with ice, or immobilized via electric shock demonstrated increased BG for up to 48 hr, regardless of capture method.[6] Goldfish experimentally infected with *Aeromonas hydrophila* developed an increase in aspartate aminotransferase (AST), decreases in

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red blood cell count, hemoglobin, hematocrit, and ALT, and an initial increase followed by a decrease in BG.[1] A shift from lymphocyte predominance to polymorphonuclear cell predominance was also noted during infection.[1]

Observational studies on hematologic and biochemistry parameters of goldfish have also been performed. One study documented higher hematocrit and hemoglobin concentrations in male goldfish compared to females. This study also identified a significant relationship between weight and TP in female goldfish.[32] Another study documented diurnal variation in BG, plasma total lipid, and plasma cholesterol related to photoperiod and time of feeding.[11] Yet another paper documented no difference in BG level between common, comet, and Chinese moor goldfish varieties. This study also reported no variation in BG level based on time of day, amount of food consumed, or fasting status. A significantly higher BG level was reported from goldfish weighing less than 1.5 g compared to those weighing 1.5–7.0 g.[6] Ultrastructural studies of goldfish leukocytes have also been performed.[14]

The aforementioned studies provide useful, if sometimes conflicting, information about changes in goldfish hematologic and biochemistry parameters based on size, sex, stress, bacterial infection, and environmental manipulation. Historically, hematology and biochemistry values from the scientific literature were compiled into reference intervals to inform the clinical interpretation of goldfish bloodwork.[16,31] Unfortunately, due to variation in fish age, health status, husbandry parameters, and experimental conditions, these data were not appropriate for the generation of clinical reference intervals.

According to the 2012 US Pet Ownership and Demographics Sourcebook,[35] the number of households that present pet fish for veterinary evaluation has more than doubled in the last 20 yr. As more fish are presented for veterinary care, the availability of species-specific reference intervals for hematology and biochemistry panels will become increasingly important.[30]

The purpose of this study is to create reference intervals for packed cell volume (PCV), total solids (TS), and 11 biochemistry parameters in immature goldfish following the guidelines established by the American Society for Veterinary Clinical Pathology (ASVCP).[13] These reference intervals will be useful for clinical decision making in goldfish patients and will improve veterinary management for this species.

## MATERIALS AND METHODS

Forty-seven sexually immature goldfish were obtained from Blue Ridge Fish Hatchery (Kernersville, North Carolina 27285, USA) and maintained in an aerated, indoor, 275-L closed system. The fish were provided with constant temperature and light cycles in order to minimize the influence of environmental variables on blood work parameters. Water temperature was measured with an in-tank thermometer, and light cycles were maintained on a 12–hr photoperiod using timers. Water quality (pH, ammonia, nitrite) was evaluated weekly using a commercially available water quality testing kit (Easy Strips, Tetra Holding Inc. Blacksburg, Virginia 24060, USA). Seventy-five percent water changes were performed twice weekly with dechlorinated Raleigh (North Carolina) city water. Fish were fed three times weekly using a commercial goldfish diet (Blue Ridge Koi & Goldfish Food, Blue Ridge Fish Hatchery, Kernersville, North Carolina 27285, USA). Food was broadcast-fed into the tank and fish were fed to satiation. Remaining food pellets were removed after 10 min. The acclimation period lasted for 4 wk prior to initiation of the study. Fish were fasted for 24 hr prior to anesthesia and venipuncture.

Fish were anesthetized in oxygenated water using MS-222 (Tricaine-S, Pentair Aquatic Eco-Systems, Sanford, North Carolina 27330, USA) buffered with an equal weight of bicarbonate powder at 150 mg/L initially and then titrated to effect. Anesthetic depth was assessed by monitoring opercular movement rate and righting response. Fish reached an appropriate anesthetic depth for venipuncture when the righting response was absent and steady opercular movement continued (Stage IV anesthesia).[4] Total anesthesia time was less than 10 min for each fish.

A complete physical examination was performed on each anesthetized fish and any abnormalities were recorded. Fish were weighed to the nearest gram and evaluated for secondary sex characteristics including opercular tubercles in males and slight vent protrusion in females. Routine skin scrapes and gill biopsies were performed on a random selection composed of 10% of the total animals participating in the study. Skin scrape and gill biopsy samples were examined microscopically to screen for external parasites and tissue pathology. Fish with physical examination abnormalities, parasites, or tissue pathology identified on skin scrape or gill biopsies were excluded from the study.

Following physical examination, 0.12–0.18 ml of blood (less than or approximately equal to 1% of body weight) were collected from the caudal tail vein into a 1-ml heparinized syringe using a 25-ga needle. Heparinized syringes were prepared by aspirating 0.02 ml heparin (Heparin Sodium USP, 1,000 units/ml; APP Pharmaceuticals, LLC, Schaumburg, Illinois 60173, USA) into a 1-ml syringe, drawing back on the plunger to fill the syringe with air and then forcefully expelling air and heparin 10 times. A new 25-ga needle was placed on each heparinized syringe prior to venipuncture.

PCV was determined immediately after blood collection using nonheparinized microhematocrit tubes (Fisher Scientific Company, LLC, Pittsburgh, Pennsylvania 15219, USA) centrifuged at 14,500 rpm for 5 min (Clay Adams 0591 Readacrit Centrifuge, Becton Dickinson Company, Franklin Lakes, New Jersey 07417, USA). TS were determined with a hand-held refractometer (Ade Advanced Optics, Oregon City, Oregon 97045, USA) using plasma from the microhematocrit tube. The remainder of the blood was refrigerated at 4°C in the heparinized syringe until biochemistry panel processing. Blood was not transferred into hematologic tubes for storage due to concern for sample loss in the hub of the syringe after transfer. The goldfish were recovered in a 2-L recovery tank containing their original tank water. Goldfish were considered recovered when they could maintain their position in the water column and swim normally. At this time, they were returned to their tank of origin.

Biochemistry panels were performed within 2 hr of sample collection using a Vetscan VS2 with avian–reptile rotors (Abaxis, Inc, Union City, California 94587, USA) according to the manufacturer's instructions. Parameters evaluated using this protocol included albumin (ALB), AST, bile acids (BA), Ca, creatine kinase (CK), globulin, BG, Na, K, phosphorous (P), TP, and uric acid (UA).

Data analysis was performed using commercially available statistical software (SPSS Statistics 23, IBM, Armonk, New York 10504, USA) and Reference Value Advisor, a set of macroinstructions operating within Microsoft Excel (Microsoft, Redmond, Washington 98052, USA).[15] Outliers were visually identified using histograms and box plots and excluded using a Tukey test. The mean, standard deviation, median, and range were determined for each parameter. Data were evaluated for normality using skewness, kurtosis, Q-Q plots, the Anderson-Darling test, and the Shapiro-Wilk statistic. Nonnormal data were Box-Cox transformed and reassessed for normality. Data symmetry was evaluated for each variable, as the assumption of symmetry must be met to use the robust method of reference interval generation.[15] Reference intervals were then created according to the ASVCP Reference Interval Guidelines using Reference Value Advisor.[13,15]

Ninety-percent confidence intervals (CI) were generated for the upper and lower bounds of each reference interval. For each variable, the width of the 90% CI was compared to the width of the reference interval to assess uncertainty and infer the need for a larger sample size.[13] Linear regression was performed to evaluate the relationship between each analyte and body weight. An $\alpha$ value of 0.05 was used for determination of statistical significance.

## RESULTS

Goldfish husbandry was carefully controlled during the acclimation and study periods. Water temperature ranged from 20.4–21.6°C, light cycles did not deviate from a 12–hr photoperiod each day, and water quality parameters remained within normal limits.

All goldfish sampled during the study appeared clinically healthy. No abnormalities were identified on physical examination, skin scrape, or gill biopsy. No secondary sex characteristics were identified on physical examination, thus sex was not included in data analysis. Average body weight was 22.83 g with a range of 9.8–44 g.

PCV and TS were determined immediately for all 47 fish. Chemistry panels for 39 fish were run within 2 hr of sample collection. Chemistry panel data from eight fish was excluded from analysis due to prolonged sample storage (2–24 hr) at 4°C.

The Vetscan VS2 flagged two values for Ca (both >20 mg/dl) and eight values for CK (all reported as 0 U/L). These values were removed from analysis. Outliers were identified and removed for AST (662 U/L and 911 U/L), Ca (4.3 mg/dl, and 19.2 mg/dl), CK (10,000 U/L), globulin (1.6 g/dl and 2.2 g/dl), BG (153 mg/dl, 157 mg/dl, 295 mg/dl, and 355 mg/dl), Na (100 mmol/L, 157 mmol/L, 158 mmol/L, 168 mmol/L, and 172 mmol/L), TP (5.2 g/dl), TS (6.2 g/dl), and UA (0.3 mg/dl, 0.4 mg/dl, and 0.7 mg/dl).

Ca, CK, UA, TP, ALB, Na, PCV, and TS had normal data distributions while AST, BG, P, globulin, and K had nonnormal distributions. Box-Cox transformation successfully converted all nonnormally distributed variables to normal distributions except for globulin. BA was below

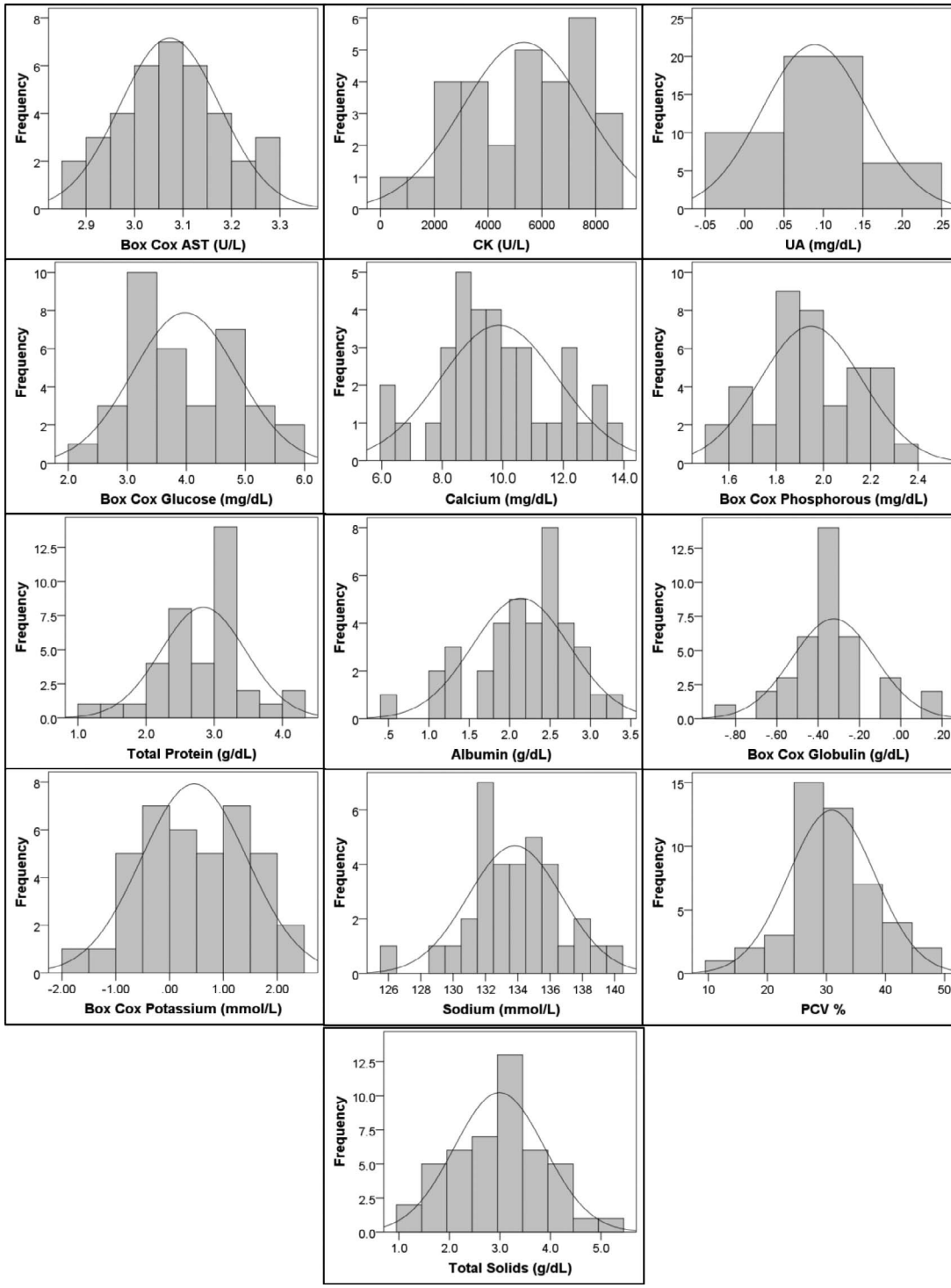


**Figure 1.** Histograms with projected normal distributions used to generate reference intervals for packed cell volume, total solids, and 11 chemistry panel analytes in juvenile *Carassius auratus*. Some variables required Box-Cox transformation to assume a normal distribution.

**Table 1.** Descriptive statistics, data distribution, reference intervals (RI), and 90% confidence intervals (CI) of the upper and lower bounds of the reference intervals for packed cell volume, total solids, and 11 biochemistry panel analytes in juvenile goldfish, *Carassius auratus.*

| Parameter | *n* | Mean | SD | Median | Min | Max | Distribution | RI method | RI | 90% CI lower bound | 90% CI upper bound |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Aspartate aminotransferase (U/L) | 37 | 229.41 | 86.97 | 206 | 111 | 433 | Non-normal | Parametric (BC[a]) | 105.9–478.0 | 90.8–126.2 | 388.3–598.1[b] |
| Creatinine kinase (U/L) | 30 | 5,319.6 | 2,283.3 | 5,766.5 | 853 | 8,877 | Normal | Parametric | 572.5–10,066.7 | 0–1,743 | 8,835.6–11,245.4[b] |
| Uric acid (mg/dl)[c] | 36 | 0.09 | 0.07 | 0.1 | 0 | 0.2 | Normal | Parametric | 0–0.23 | 0–0 | 0.19–0.25[b] |
| Glucose (mg/dl) | 35 | 34.5 | 15.0 | 29 | 15.0 | 78.0 | Nonnormal | Parametric (BC) | 14.6–76.2 | 12.7–17 | 60–94[b] |
| Calcium (mg/dl) | 35 | 9.87 | 1.94 | 9.8 | 6.2 | 13.5 | Normal | Parametric | 5.87–13.88 | 5.01–6.78[b] | 12.92–14.8[b] |
| Phosphate (mg/dl) | 39 | 9.57 | 2.89 | 8.7 | 5.1 | 16.3 | Nonnormal | Parametric (BC) | 5.04–17.13 | 4.45–5.82 | 14.58–19.77[b] |
| Total protein (g/dl) | 38 | 2.84 | 0.62 | 2.95 | 1.2 | 4.1 | Normal | Parametric | 1.56–4.12 | 1.3–1.84[b] | 3.82–4.4[b] |
| Albumin (g/dl) | 39 | 2.13 | 0.59 | 2.2 | 0.5 | 3.2 | Normal | Parametric | 0.68–3.14 | 0.09–1.13[b] | 2.94–3.3 |
| Globulin (g/dl) | 37 | 0.7 | 0.18 | 0.7 | 0.3 | 1.2 | Nonnormal | Robust (BC) | 0.37–1.09 | 0.31–0.42 | 1.03–1.14 |
| Potassium (mmol/L) | 39 | 1.87 | 1.4 | 1.5 | 0.1 | 5.6 | NonNormal | Parametric (BC) | 0.11–5.83 | 0.04–0.27 | 4.57–7.59[b] |
| Sodium (mmol/L) | 34 | 133.8 | 2.9 | 134 | 126 | 140 | Normal | Parametric | 127.8–139.8 | 126.5–129.2[b] | 138.3–141.2[b] |
| Packed cell volume % | 47 | 30.89 | 7.308 | 30.0 | 12.0 | 49.0 | Normal | Robust | 15.8–45.7 | 13–19.1 | 41.9–48.9[b] |
| Total solids | 46 | 2.99 | 0.9 | 3.2 | 1.2 | 5.0 | Normal | Parametric | 1.17–4.81 | 0.82–1.53 | 4.43–5.18 |

[a] (BC) indicates Box-Cox transformation of the data was necessary to assume a normal distribution.

[b] Width of the confidence interval/width of the reference interval >0.2.

[c] Calculated values for the reference range were <0. These values were changed to 0 for the reported reference intervals.

detectable limits for all but two individuals, thus this parameter was excluded from analysis. Histograms of the finalized data sets used to generate reference intervals are displayed in Figure 1. Summary data, reference intervals, and 90% CI of the reference interval bounds are reported in Table 1.

Linear regression revealed a statistically significant positive relationship between body weight and Ca, TP, ALB, PCV, and TS. A statistically significant negative relationship was detected between body weight and K. Scatter plots with linear regression equations, $R^2$ values, and *P*-values are displayed in Figure 2.

## DISCUSSION

ASVCP guidelines recommend utilizing nonparametric methods to create reference intervals that comprise 95% of the values of a healthy reference population consisting of at least 120 individuals;[13] this is an expensive and frequently impractical approach in exotic veterinary species. To address this concern, the ASVCP also created guidelines for reference interval generation using a minimum of 20 healthy individuals.[13] These guidelines were followed to determine reference intervals for PCV, TS, and 11 commonly evaluated biochemistry panel analytes in immature goldfish.

The reference population for this study was defined as clinically healthy, sexually immature goldfish weighing 9.8–44 g. This range represents the smallest size of pet goldfish that may be presented to a veterinarian for evaluation, and the reference intervals generated in this study are applicable to goldfish that fit these criteria. To minimize physiologic variation associated with external variables, the goldfish sampled in this study were obtained from a single source and kept in constant conditions of light, temperature, and water quality. Methods for health assessment included physical examination, skin scrape, and gill biopsy.

Preanalytical considerations were addressed by ensuring 24 hr of fasting prior to sample collection, capturing and anesthetizing each goldfish in a similar fashion, and handling blood samples in a consistent manner. All biochemistry panels were performed on the same Vetscan VS2 machine to minimize analyzer variability. The Vetscan VS2 is equipped with an Intelligent Quality Control system that checks chemistry reactions, electronic functions, and optics and which automatically suppresses data that are determined to be inaccurate. This built-in quality control ensures reliability for results generated by this analyzer. Two Ca values and eight CK values were flagged by the analyzer and excluded from the study.

Reference intervals can be easily skewed by mistakes or oversights. It is important to critically evaluate the reference interval generation process to ensure that the final products are accurate and clinically applicable. Error or artifactual variation in biochemistry values may have been introduced during reference population selection, venipuncture, or sample handling (or any combination) in this study.

The goldfish population used in this study was obtained from a single source and maintained under uniform husbandry conditions to minimize variation in blood parameters due to environmental factors. However, the sex ratio of the reference population was unknown. Higher hematocrit and hemoglobin levels have been reported in male goldfish, and sex-based reference intervals are appropriate for these two parameters.[32] Unfortunately, it was impossible to generate sex-based reference intervals in this study due to lack of secondary sex characteristics. The use of combined male and female reference intervals may mask abnormal values in some individuals that would be detected with sex-specific reference intervals. Future research is necessary to identify sex-based differences in goldfish blood work and to generate separate reference intervals for hematology and biochemistry parameters in male and female goldfish.

The reference population in this study also had a wide range in body weight. Previous studies indicate that body weight may influence BG and TS in goldfish, but the potential for relationships between weight and other biochemistry parameters has not been explored in this species.[6] This study used linear regression to assess the relationship between body weight and blood work parameters in the reference goldfish population and documented statistically significant positive relationships between body weight and Ca, TP, ALB, PCV, and TS. A statistically significant negative relationship was detected between body weight and K. These results suggest that several blood work parameters are influenced by body weight in goldfish. It may, therefore, be appropriate to partition the reference intervals generated in this study into different weight classes. However, this is not possible due to sample size limitations. Future efforts to generate reference intervals in goldfish should consider body weight during reference population definition and selection.

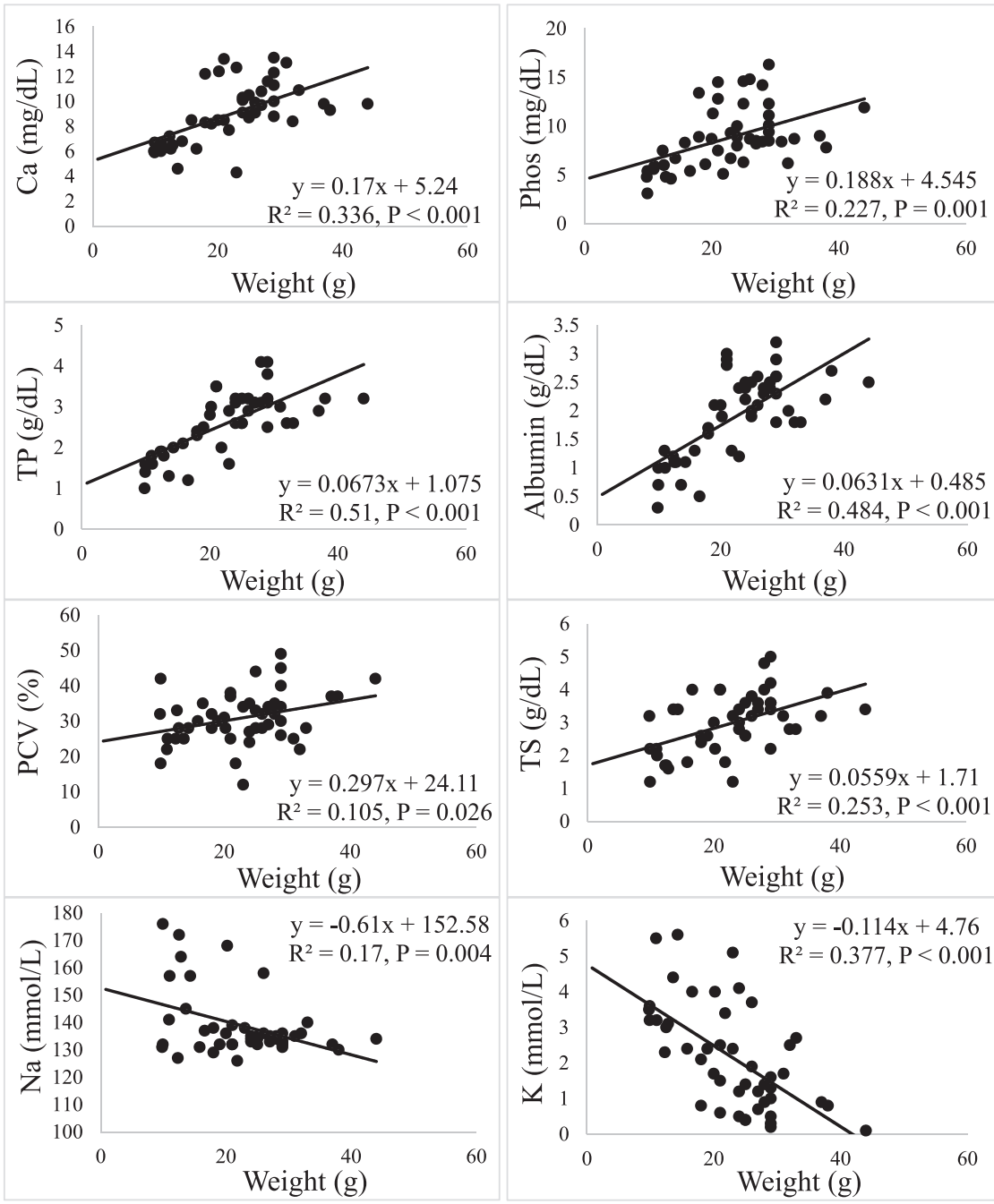


**Figure 2.** Scatter plots with regression lines, linear regression equations, $R^2$ values, and *P*-values demonstrating the relationship between body weight and six blood work parameters in juvenile *Carassius auratus*. An $\alpha$ level of 0.05 was used to determine statistical significance.

In this study, error or artifactual variation may have been introduced into the reference intervals at two points during sample collection and handling. The first step that may have introduced error was the use of preheparinized syringes for blood collection. Small-volume blood sample collection into preheparinized syringes has been previously investigated in American alligators

(*Alligator mississippiensis*).[21] In that Johnson et al study, either 0.1 ml or 0.2 ml of Na heparin was drawn into a 1-ml syringe and then expelled, leaving approximately 0.04 ml of heparin in the hub of the syringe. Preheparinized and nonheparinized syringes were filled with 0.2 ml of alligator blood, and PCV and TS were determined for each sample. Significant and unpredictable hemodilution was reported from preheparinized syringes. Johnson et al commented that forcibly expelling air and heparin from prepared syringes may decrease the effects of sample dilution.[21]

Support for this hypothesis was generated by a separate study evaluating the effect of preheparinized syringes on canine blood gas and lactate values.[17] In that study by Hopper et al, preheparinized syringes prepared using an "evacuation" technique (in which air and Na heparin were forcibly expelled from the syringe four times) produced pH, $pCO_2$, $pO_2$, bicarbonate, base deficit, K, Na, and lactate values that were consistent with those obtained using nonheparinized syringes. However, those "evacuated" syringes also produced significantly higher chloride values and significantly lower ionized Ca values compared to nonheparinized syringes. The amount of residual heparin within "evacuated" syringes was approximately 40 USP heparin per 1 ml of blood, which is over twice the recommended mammalian concentration of 15 USP heparin/ml blood.[17] Despite this, the use of preheparinized syringes prepared using the "evacuation" technique was deemed acceptable for determining all blood gas values, except chloride and ionized Ca in dogs. All goldfish blood samples in this study were collected into preheparinized syringes. The syringes were prepared by forcefully expelling air and heparin 10 times until only tiny droplets were visible in the hub of the syringe. The syringe preheparinization technique was even more conservative than the "evacuation" technique described by Hopper et al.[17] However, the final concentration of heparin in each blood sample was unknown because the volume of residual heparin within each syringe was not determined prior to venipuncture. Therefore, hemodilution of the blood samples used to create reference intervals in this study cannot be ruled out. Future goldfish reference interval studies should consider blood collection into nonheparinized syringes followed by transfer to heparin-containing blood tubes to eliminate the possibility of heparin dilution and reduce the risk of preanalytical error.

Blood sample storage could have also contributed to error in this study. Goldfish blood samples were stored at 4°C for up to 2 hr after venipuncture. The delay in sample processing was due to travel time between the fish holding facility and the laboratory. Further delay was incurred to allow for all samples to be processed on the same Vetscan VS2 machine. Separating plasma from red blood cells immediately after venipuncture is generally recommended to avoid artifactual changes in biochemistry panel parameters.[34] Delayed separation of plasma from mammalian blood samples can cause artifactual hypoglycemia due to the metabolic activity of red blood cells and artifactual hyperkalemia due to cellular leakage.[34] Prolonged delays in mammalian plasma separation can lead to hemolysis, which causes significant changes in several biochemistry analytes including AST, ALT, lactate dehydrogenase, total bilirubin, CK, lipase, amylase, ALB, TP, Ca, Mg, P, glucose, creatinine (CR), alkaline phosphatase (ALP), and BA.[34] Delayed separation of plasma from fish blood samples causes species-specific biochemistry panel changes. Storage of heparinized rainbow trout (*Salmo gairdneri*) blood samples at 0–2°C prior to plasma separation resulted in significant alterations to PCV (decreased at 3 hr of storage, increased at 24 hr), plasma Na (decreased at 1 and 3 hr of storage, increased at 24 hr), plasma K (decreased at 1, 3, and 24 hr of storage), and plasma chloride (decreased at 3 hr of storage, increased at 24 hours).[23] Hemoglobin concentrations were unaffected by up to 24 hr of storage in that study. In another study, storage of coho salmon (*Oncorhynchus kisutch*) whole blood samples in an ice slurry for 8.5 hr prior to plasma separation resulted in significantly increased BG and significantly decreased chloride and K levels.[8] Hematocrit, Na, plasma osmolality, and lactate remained stable during this storage period in this species.[8] In a more recent study, serum glucose in Siberian sturgeon (*Acipenser baerii*) remained stable when whole blood samples were stored for up to 4 hr at 4°C prior to serum separation.[9]

The effect of delayed plasma–serum separation on goldfish biochemistry parameters has not been investigated. It is therefore difficult to determine whether our 2-hr delay in sample processing caused any artifactual alterations in biochemistry values. Future goldfish reference interval studies should strive to process blood samples within 1 hr of venipuncture to reduce the risk of introducing preanalytical error.

The reliability of the reference intervals generated in this study can be assessed objectively using the 90% CI of the reference interval limits.

If the width of the confidence interval for each bound divided by the width of the reference interval exceeds 0.2, a larger reference population is recommended to increase the reliability of the reference interval.[13] Based on this ASVCP recommendation, a larger sample size should be used to recalculate all of the reference intervals generated in this study except for globulin and TS (Table 1). This is not surprising, as reference intervals should ideally be calculated using a minimum of 120 individuals, a population much larger than that used in this study. Despite the uncertainty inherent in these reference intervals, they are consistent with previous compilations of goldfish hematology and biochemistry parameters collected from the scientific literature.[16,31] The reference intervals generated in this study are also similar to those reported for koi (*Cyprinus carpio*), a closely related cyprinid fish.[26]

These reference intervals should be used to interpret blood work collected from sexually immature juvenile goldfish weighing 9.8–44 g and processed using a Vetscan VS2 machine. ALT, ALP, blood urea nitrogen, cholesterol, chloride, CR, gamma glutamyltransferase, Mg, and total bilirubin were not assessed in this study due to sample volume limitations. Future research efforts should focus on generating reference intervals for the remaining electrolytes and assessing other biochemistry parameters for clinical significance in goldfish.

Clinical interpretation of fish biochemistry panels is in its infancy.[30] The availability of species-specific reference intervals should improve veterinary assessment and management of fish. This study generated reference intervals for PCV, TS, and 11 commonly used biochemistry panel analytes in immature goldfish. This study also established relationships between body weight and Ca, K, TP, ALB, PCV, and TS in goldfish. These results support a possible need for weight-based reference intervals in goldfish. Future research should assess the effects of sex, syringe preheparinization, and delayed plasma separation on goldfish biochemistry parameters. Additional commonly measured biochemistry parameters should be assessed for clinical utility in goldfish, and reference intervals generated if indicated. This study serves as a good baseline for future research, and is an important first step to improving veterinary management for goldfish.

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