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Effects of temperature on hardhead minnow (*Mylopharodon conocephalus*) blood-oxygen equilibria

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Abstract Habitat perturbations, including dam construction with consequent temperature changes and the introduction of non-native species to California's mid- to low-elevation streams, have negatively influenced some native fish populations' historic distribution and abundance. Populations of hardhead, *Mylopharodon conocephalus* (Cyprinidae), have experienced such population declines, but environmental temperature effects on this large (to 60 cm SL), native species are poorly documented. We measured temperature effects on in vitro blood-oxygen affinity and equilibrium curve shape, key dynamics of the species' oxygen-transport system, derived from blood collected from wild-caught hardhead. Over an 11–30 °C temperature range, the half-saturation value (P_{50}, an inverse measure of affinity) increased with the temperature from 0.51 to 1.80 kPa for low-PCO_2 ("arterial") treatments and from 2.02 to 2.92 kPa for high-PCO_2 ("venous") treatments. The apparent heat of oxygenation (temperature effect) was higher at temperatures > (absolute value) 19 °C. Therefore, hardhead's blood has a decreased ability to bind oxygen at its gills at temperatures ≥25 °C, compared to that at temperatures ≤19 °C. The hardhead's Bohr factors (Φ), non-bicarbonate buffer values (β), nucleoside triphosphate (NTP) concentrations, blood oxygen capacities (CBO_2), and mildly sigmoid-shaped oxygen equilibrium curves showed no relationship with temperature. Overall, their blood-oxygen equilibria suggest that hardhead can tolerate moderate hypoxia and temperature variations in its environment and that they have some capacity for sustained, high-aerobic activity.

Keywords Temperature · Carbon dioxide · Blood · Bohr factor · Hardhead

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Introduction

Hardhead minnow, *Mylopharodon conocephalus*, is a large, California-native cyprinid preferring clear, medium to large, mid to low-elevation streams with gravel, cobble, and boulder substrates typically with low water velocities (≤25 cm s^{-1}) and cool to warm temperatures (Moyle and Baltz 1985; Moyle et al. 1995; Moyle 2002). In summer they may be found in large pools where maximum temperatures reach 20–28 °C (Knight 1985; Cech et al. 1990). Extensive construction of hydroelectric dams on nearly all medium to

large streams and rivers in California, along with the introduction of non-native species, have fragmented and altered the native faunal hierarchy of historic hardhead habitat resulting in population declines and, in many cases, extirpation from heavily modified, downstream areas (Reeves 1964; Moyle and Nichols 1973; Herbold and Moyle 1986; Brown and Moyle 1987; Brown and Moyle 1993; Moyle et al. 1995; Moyle 2002). California's Department of Fish and Game currently lists the hardhead as a species of special concern.

The timing and magnitude of water released from dams for electrical power generation, irrigation, and drinking water continue to modify natural, seasonal, thermal and hydrological regimes that affect hardhead life history parameters and bioenergetics. California leads the USA in the number (ca. 400) of hydroelectric generating facilities located mainly in the Sierra Nevada mountains (Hall and Reeves 2006). The daily operations of these run-of-river facilities are associated with once, or twice, daily power peaking flows supplying electricity when demand is the greatest (Cushman 1985). Peaking operations may cause reduced primary productivity, scouring of sediments, and changes in depth, width, velocity, water temperatures, turbidity, and dissolved oxygen in areas immediately downstream of power-generating facilities, raising concerns for the permitting, licensing, and operation of these facilities (Cushman 1985; Young et al. 2011).

Although modifications of habitat and hydrology, as well as the introduction of non-native species, have influenced current distributions and abundance of hardhead, there remains an incomplete understanding of how environmental variability may interact with hardhead physiology and influence its life history parameters. Temperature and oxygen levels in streams and rivers may limit the ability of hardhead to maintain their populations under changing conditions (e.g., modifications of hydrologic and thermal regimes, introduced species, and global climate change). Investigations of the dynamics of a species' blood-oxygen equilibrium characteristics are useful to gain insight into a fish's functional capacity and consequently its potential environmental limits (Powers 1932; Grigg 1974; Cech et al. 1994). Typically, active fishes inhabiting oxygen-rich water have a more sigmoidal-shaped blood-oxygen equilibrium curve and larger Bohr (CO_2 and pH-related) factors (Cameron 1971; Cech et al. 1994; Dobson et al. 1986), which help maintain oxygen delivery during activity when tissue pH declines and oxygen demand increases. Fishes inhabiting low oxygen environments typically have more hyperbolic-shaped curves and very low P_{50} (half-saturation) values (Cech et al. 1979; Wood and Lenfant 1979), associated with their high blood-oxygen affinities. The temperature sensitivity of a species' hemoglobin, quantified as the apparent heat of oxygenation, describes the effect of environmental temperature on blood oxygen affinities (Riggs 1970; Kaufman et al. 2006).

To better understand how hardhead physiology responds to environmental conditions modified by hydroelectric dams, we constructed hardhead blood-oxygen equilibria curves to illustrate the influence of temperature on the reversible oxygen-binding dynamics in hardhead blood. Metabolism-level CO_2 effects were included to simulate arterial (low-CO_2) and venous (high-CO_2) blood dynamics, including Bohr and Root effects, in hardhead via whole-blood tonometry techniques.

Materials and methods

Adult hardhead (n=45; TL=25.5–47.4 cm) were collected, via hook and line fishing, from the North Fork Feather River and the South Fork American River, California in early March to early November 2010. Captured fish were transported to the Center for Aquatic Biology and Aquaculture at the University of California, Davis, in 80-l aerated insulated coolers. Fish were transferred into 950-l tanks supplied with a constant flow of aerated water adjusted to match water temperatures at the time and place of capture. The supply water temperature for each tank was slowly adjusted (1 °C d^{-1}) from conditions matching capture (11–20 °C) to ambient well water temperature (i.e., 19 °C). Fish were fed a daily ration (1 % of fish biomass) of Silver Cup® commercial trout pellets supplemented with live earthworms until they were used in blood-oxygen equilibria experiments. All study animals were treated in accordance with UC Davis' Institutional Animal Care and Use Committee guidelines (protocol # 15774).

To obtain the 22–24 ml of blood required for each in vitro tonometry experiment, all fish were over-anesthetized and bled via a dorsal aortic cannula. Fish were quickly placed in a buffered anesthetic bath containing: 9 g l^{-1} NaCl, 420 mg l^{-1} $NaHCO_3$, and 500 mg l^{-1} MS-222. Once fish reached stage five anesthesia (3–5 min) they were transferred to a surgery table and placed in a dorsal recumbent position and the dorsal aorta cannulated for blood collection via the

methods outlined in Soivio et al. (1972, 1975) with the following modifications. Once the stylet-cannula complex entered the dorsal aorta (1–2 cm), the stylet was withdrawn, and a three-way stopcock fitted with a 1-ml syringe (containing 500 μl sodium heparin [500 IU heparin]) was used to rapidly infuse the fish with heparin to prevent clot formation at the cannulation site prior to the collection of blood. Fish were then bled using 3-ml syringes containing 300 μl sodium heparin (300 IU heparin). Collected blood (e.g., from 3 to 6 fish) was placed into 50-ml Falcon tubes with air and placed horizontally on ice after gentle mixing. Immediately after blood collection hematocrit (packed cell volume) was measured (centrifugation @ 11 000×g for 3 min [Houston 1990]), and hemoglobin content was measured using a hemoglobin assay kit (Teco Diagnostics®) and a UV–VIS Aquamate spectrophotometer, following manufacturers instructions.

For each tonometry experiment (n=3 per temperature) blood (ca. 6 ml per tonometer) was placed into each of two rotating glass tonometers (Hall 1960) for the construction of the first blood-oxygen equilibrium curve (low PCO_2) with the remaining blood held on ice for 60–80 min, with gentle mixing every 5 min, until loading into the second pair of tonometers for the construction of the second curve (high PCO_2). Tonometer pairs were situated in a temperature-controlled water bath (11, 19, 25, or 30 °C±0.3 °C) and received either humidified air from an air pump, humidified nitrogen from a cylinder (blood with <0.03 kPa PCO_2, for construction of 'low PCO_2' curves, estimating arterial conditions), or humidified gas mixtures (1 % CO_2 with balance either air or nitrogen) from Wostoff gas mixing pumps (1.01 kPa PCO_2, for 'high PCO_2' curves, estimating venous conditions). Blood was equilibrated with the gas mixtures for 40–60 min prior to data collection. Samples of oxygenated and deoxygenated blood were withdrawn from the tonometers and mixed in a 1-ml polypropylene syringe with a mixing bead (Edwards and Martin 1966; Scheid and Meyer 1978). To reach target values of 0, 20, 35, 50, 65, 80, 95, and 100 % oxygen saturation, proportional amounts of blood from both deoxy- and oxygenated tonometers were withdrawn and mixed as described above. The PO_2 (mm Hg) of each sample was determined using a Radiometer PHM71 blood gas apparatus with thermostatted E101 oxygen electrodes (Analytical Sensors, Inc.®). Equilibration of blood, with the respective gas treatments, was defined to be complete after a measure of ≈ 0 kPa (45 min) in the deoxygenated (nitrogen) tonometer's blood was achieved, as outlined in Kaufman et al. (2006). The pH of each blood sample was determined using an Orion Dual Star pH meter equipped with thermostated Analytical Sensors, Inc. E351 pH and RF-CM reference electrodes. Whole blood lactate (mg l^{-1}) determinations, using a YSI model 2700 Select analyzer, were made on blood from each tonometer after initial blood collection and at the conclusion of each experiment to assess the potential for lactic acidosis in tonometered blood. Following blood collections baseline lactate concentrations were 2.7±0.5 mmol l^{-1} (mean±SD, n=12). Lactate levels in pooled blood held on ice remained at baseline and showed no increases in lactate prior to use in tonometry experiments, which did not exceed 2 h. Results from the initial 30 °C experiments were discarded due to increased (≥ 1 mmol l^{-1}) in lactate concentrations over baseline concentrations (modified from methods described in Cech et al. [1994]). The 30 °C experiments were repeated using decreased blood volumes (3 ml) in the tonometers, which shortened equilibration times, and decreased blood [lactate] increases to <1 mmol l^{-1} above baseline. Blood-oxygen equilibria data were converted from mm Hg to kPa, where 1 mmHg=0.13332 kPa and plotted, with curves fitted using nonlinear regression options with Sigma Plot 2000, SPSS, Inc.® software.

Bohr factors (Φ), temperature effects (ΔH, kiloJoules (kJ) mol O_2^{-1}, a measure of the blood-oxygen affinity's sensitivity to temperature), non-bicarbonate buffer concentrations (β, in slykes), and hemoglobin subunit cooperativity (n_{50}) were calculated from the collected data as outlined in Kaufman et al. (2006).

The blood-oxygen capacity, (CBO_2, ml O_2 dl^{-1} blood), of hardhead blood was determined using the methods and calculations outlined in Tucker (1967). Measurements from an acrylic Tucker cell, thermostatted to 37 °C, were made with an E101 oxygen electrode (Analytical Sensors, Inc.) and an A-M Systems Polarographic Amplifier Model 1900.

Nucleoside triphosphates (adenosine triphosphate [ATP] + guanosine triphosphate [GTP]) were extracted from whole-blood (Biovision Deproteinizing Sample Preparation Kit cat. # K808-200) and stored at −80 °C until analysis. Standards and samples were analyzed with modifications via Biovision ATP Colormetric/Fluorometric Assay Kit (cat. # K354-100). Modifications of the prescribed assay were as follows: a five-point standard

curve was constructed (0, 6, 12, 18, and 24 nmols adenosine triphosphate (ATP) in 200 μl standard solution) with standards and samples assayed using an Aquamate UV–VIS spectrophotometer, 1-mm pathlength glass cuvette, and absorbance at 570 nm. All standard and sample volumes were increased threefold to provide sufficient volume for spectrophotometric analysis. NTP concentrations in prepared samples were calculated from the generated standard curve (y= 0.0335x-0.004; r^2=0.97).

We used one-way ANOVA ($P \leq 0.05$) to investigate significant differences among treatment means. If significant differences were detected, we used post-hoc pairwise comparisons (Tukey test) to investigate differences between pairs of groups within and between treatments. All statistical analyses were conducted using Sigma Stat®, SPSS Inc. statistical software.

Results

Hardhead blood-oxygen affinity showed low to moderate decreases (i.e., increased P_{50}) in response to increased temperature. In the 11° and 19 °C treatments both the low- and high-PCO_2 equilibrated blood P_{50}s were statistically indistinguishable ($P>0.05$) between the two temperatures (Table 1). Although the 25° and 30 °C low- and high-PCO_2 blood-oxygen affinities were statistically indistinguishable from each other, they were both lower ($P<0.05$) when compared to the 11° and 19° C treatments (Table 1). Therefore, the higher temperature intervals (i.e., between 19° and 25 °C and between 25° and 30 °C) showed the greater temperature sensitivity (ΔHs, absolute values) of hemoglobin oxygen loading/unloading in both low- and high-PCO_2 treatments, compared with that between 11° and 19 °C, in both low- and high-PCO_2 treatments (Table 2). Least-squares regression analysis of P_{50} versus temperature conditions yielded positive slopes (0.047 kPa°C^{-1} [r^2=0.90]) for the low-PCO_2 treatment and (0.052 kPa°C^{-1} [r^2=0.84]) for the high low PCO_2 treatment. Blood pH tended to decrease with increased temperature in both the low-PCO_2 (−0.0281 pH units °C^{-1} [r^2=0.91]) and high-PCO_2 (−0.0039 pH units ° C^{-1} [r^2=0.42]) treatments.

The CBO_2 measurements showed no statistically significant differences nor were there any apparent trends in relation to the temperature treatments (Table 1). Mean Bohr factors, Hill-plot slopes (n_{50}), and β showed no statistically significant relationship across the temperature treatments.

Predictably, high-PCO_2 treatments decreased oxygen affinities (Bohr effect) when compared to their low-PCO_2 paired treatment within a temperature regime. The observed decrease in oxygen affinity as a result of exposure to CO_2 shifted all high-PCO_2 curves to the right of the low-PCO_2 curves (Fig. 1a–d). Increased CO_2 exposure (from <0.03 kPa to

Table 1 Mean (with SE) pH, P_{50} (kPa), total hemoglobin concentration ([Hb], in g dl^{-1}), blood oxygen capacity (CBO_2, ml O_2 dl^{-1} blood), hematocrit (HCT in %), and n (number of experimental replicates) in low- and high-PCO_2 (in kPa) treatments over the experimental range of temperatures (11 °C, 19 °C, 25 °C, and 30 °C) in blood from wild-caught hardhead

Temp.	PCO_2	pH	P_{50}	[Hb]	CBO_2	HCT	n
11	≤0.03	8.197 (0.068)	0.51 (0.08)		17.5 (1.0)		3
				10.9 (0.6)		29 (0.3)	
11	1.01	7.430 (0.047)	2.02 (0.02)		14.6 (0.6)		3
19	≤0.03	8.132 (0.087)	0.67 (0.12)		16.5 (1.7)		3
				10.5 (0.6)		27 (0.4)	
19	1.01	7.528 (0.058)	2.16 (0.06)		16.1 (1.5)		3
25	≤0.03	7.881 (0.157)	1.07 (0.15)		14.7 (0.8)		3
				8.7 (0.3)		30 (1.6)	
25	1.01	7.359 (0.120)	2.62 (0.16)		15.1 (0.5)		2
30	≤0.03	7.675 (0.020)	1.80 (0.13)		17.2 (0.3)		5
				10.5 (1.3)		10.5 (1.3)	
30	1.01	7.380 (0.030)	2.92 (0.12)		14.2 (2.3)		5

Table 2 Hardhead hematological parameters derived from blood-oxygen equilibria experiments

Temp.	PCO_2	n_{50}	β	Φ	Δ *H* Low-PCO_2	Δ *H* High-PCO_2	[NTP]	NTP:Hb	n
11	≤ 0.03	1.41 (0.20)							3
			−2.6 (0.1)	−0.794 (0.09)			454.6 (48.6)	0.271	
11	1.01	1.43 (0.06)							3
					−5.48	−1.37			
19	≤ 0.03	1.17 (0.07)							3
			−8.5 (4.4)	−0.916 (0.14)			355.3 (40.6)	0.220	
19	1.01	1.38 (0.60)							3
					−13.56	−5.66			
25	≤ 0.03	1.29 (0.04)							3
			−6.7 (2.2)	−0.770 (0.05)			na	na	
25	1.01	1.49 (0.03)							3
					−18.81	−3.87			
30	≤ 0.03	1.32 (0.05)							5
			−16.5 (4.1)	−0.819 (0.11)			383.7 (26.6)	0.238	
30	1.01	1.52 (0.03)							5

Temperature in °C, PCO_2 in kPa, n_{50} = hemoglobin subunit cooperativity, β = whole-blood nonbicarbonate buffer value (slykes), Φ = Bohr factor, ΔH = temperature effect in low- and high-PCO_2 treatments (kJmol O_2^{-1}), NTP (NTP μmol l^{-1}), NTP:Hb (μmol NTP:μmol Hb)), and n = number of experimental replicates. Measurements are presented as means (with SEM), *na* missing data

1.01 kPa) produced ($P<0.001$) lower pH values statistically when compared to corresponding treatment low-PCO_2 P_{50} pH values (mean pH±SE values for low-PCO_2: 7.971±0.120, for high-PCO_2: 7.424±0.038). No significant relationships were observed between CBO_2 and CO_2 treatments, which indicates no Root effect (Root 1931). We observed a small, but statistically significant, decrease in HCT values at 19 °C, compared with the other treatments (a possible artifact from dilution with the anticoagulant solution), with no statistical difference noted in [Hb] across temperature treatments. There were no statistical differences in whole-blood NTP concentrations or NTP:Hb ratios (μmol:μmol) (Table 1) across temperatures.

Discussion

The moderately sigmoidal blood-oxygen equilibria curves, high whole-blood oxygen affinities, relatively high Bohr factors and oxygen affinities, high HCT, [Hb], and CBO_2's with relatively low NTP values suggest hardhead are suited for sustained aerobic activity over a range of dissolved oxygen concentrations and instream flow regimes, especially at temperatures <25 °C. However, high ΔH (absolute values) between 19° and 25 °C show a decreased attractiveness of hardhead hemoglobin for binding oxygen at the gills at temperatures >19 °C, which may be unfavorable to hardhead's tolerance of elevated instream temperatures, especially if combined with moderate environmental hypoxia.

Hardhead whole blood has the ability to bind oxygen (at the gills) at low environmental oxygen partial pressures (i.e., low P_{50}s=high O_2 affinity) as well as the capacity to deliver oxygen efficiently to metabolically active tissue sites (i.e., relatively high Bohr factors). Moderately high non-bicarbonate buffer values (β) and moderate n_{50}s, combined with the observed high oxygen affinities suggest that this species may tolerate environments which may, on occasion, become hypoxic and/or hypercapnic (Kaufman et al. 2006).

Generally, increases in temperature decrease blood-oxygen affinity (Wood and Lenfant 1979; Powers 1980, 1983). The apparent heat of oxygenation (ΔH) reflects the combined effects of the exothermic binding of oxygen and the endothermic release of allosteric

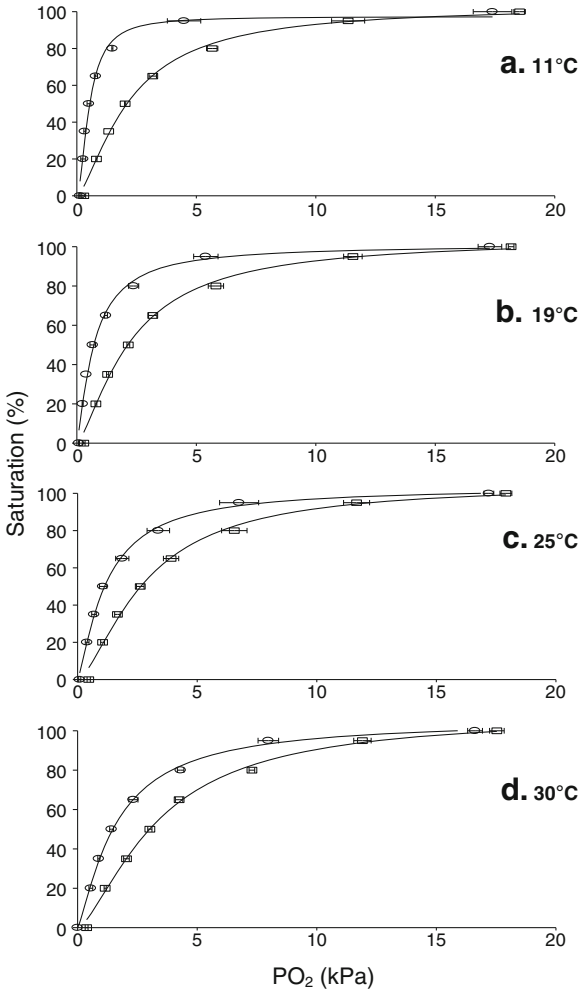

Fig. 1 Hardhead blood-oxygen equilibria at low-PCO_2 (*circles*, PCO_2=0.2 mmHg) and high- PCO_2 (*squares*, PCO_2= 7.6 mmHg) conditions and temperatures: **a** 11 °C, **b** 19 °C, **c** 25 °C, and **d** 30 °C. All curves were fitted to data means (± SE) with nonlinear regressions, and R^2 values ranged from 0.998 to 1.000

modifiers, i.e., NTPs and protons, from hemoglobin and the ΔH calculation is a quantitative measure of the temperature sensitivity of the Hb-oxygen complex (Wood and Lenfant 1979; Jensen et al. 1993). Hardhead have high whole-blood oxygen affinity (low P_{50}s), which align them with other cyprinids, such as the Sacramento blackfish (*Orthodon microlepidotus*), northern pikeminnow (*Ptychocheilus oregonensis*), carp (*Cyprinus carpio*), and tench (*Tinca tinca*), species capable of tolerating hypoxic environments (Eddy 1973; Cech et al. 1979, 1994). Sacramento blackfish and northern pikeminnow have high whole-blood oxygen affinities with hyperbolic blood-oxygen equilibria curves, low P_{50} values, moderately large Bohr factors, moderate Δ*H*s, and moderate CBO_2 values (Cech et al. 1979; Cech et al. 1994). In contrast, hardhead have moderately sigmoidal blood-oxygen equilibria curves (mean n_{50}=1.38), with mean P_{50} values (low-PCO_2) slightly higher than those of Sacramento blackfish but analogous to those found in the northern pikeminnow. Additionally, high hardhead ΔH (absolute values) above 19 °C suggest that exposure to 25° and 30 °C is unfavorable for hardhead, regarding Hb-oxygen binding. While an increased temperature effect facilitates oxygen unloading from hardhead hemoglobin at temperatures ≥25 °C, the temperature-induced increase in P_{50} may limit oxygen binding at the gills, especially in hypoxic environments. Adult hardhead volitionally choose 19–20 °C temperatures, in both field and laboratory studies, suggesting that Hb-oxygen affinity may represent an important factor in regulating hardhead behavior (Knight 1985; Klimley et al. 2010).

Fish respond to changes in temperature and oxygen content (e.g., hypoxia) with a combination of mechanisms directed at increasing oxygen carrying capacity or changing Hb-O_2 affinity. The most commonly found mechanisms are the expression of multiple iso-Hb forms, with differential properties of oxygen binding and affinity, as well as the modification of Hb-O_2 affinity via allosteric modifiers, i.e., ATP and GTP (Greaney et al. 1980; Albers et al. 1983; Rutjes et al. 2007). The hardhead's toleration of hypoxia may be a function of the rapidity of environmental changes, particularly temperature, and how this species responds by modulating its blood-oxygen affinities via acute (e.g., [NTP]) or chronic (e.g., expression of Hb isoforms) adjustments. Because hardhead have higher HCT, [Hb], and correspondingly larger CBO_2 values (Table 1) than the Sacramento blackfish or northern pikeminnow, hardhead presumably benefit from a greater aerobic capacity (Cech et al. 1979, 1994).

High aerobic capacity, which is linked to several factors (e.g., Φ, CBO_2s, [Hb], HCT), should increase the ability of hardhead to forage, avoid predators, access suitable spawning areas, and persist during low- and high-flow during periods. Hardhead are not recognized to be a highly migratory species but, like most other California-native stream fishes, are highly dispersive and rapidly re-colonize areas after periods of drought or by displacement from a flushing event (Moyle 2002). Hardhead inhabiting these mid- to low-gradient streams/rivers typically remain within a

kilometer of their home range(s) although some extended (30–75 km) spawning migrations from reservoirs have been reported (Grant and Maslin 1997; Moyle 2002).

Interestingly, hardhead Bohr factors were higher (mean: −0.825) than those found in rainbow trout and in the closely related northern pikeminnow (−0.70:18 °C to −0.46:21 °C, Cech et al. 1994) suggesting that hardhead can sustain high aerobic activity. The hardhead CBO_2s (mean: 15.7 mldl^{-1}) also exceeded those found in rainbow trout (8.9–9.8 mldl^{-1}, Cameron 1971) and in the northern pikeminnow (10.7–13.0 ml dl^{-1}, Cech et al. 1994), although they were less than those of the very active albacore (21.8 ml dl^{-1}, Cech et al. 1984). These Bohr factor and CBO_2 data, combined with hardhead's relative insensitivity to temperatures less than 25 °C suggest that this species is suited for sustained aerobic activity over a range of environmental temperatures, dissolved oxygen concentrations, and instream flow regimes. This is consistent with its critical swimming velocity, U_{crit} (mean: 0.52 m s^{-1}), which did not vary significantly over a range of cool temperatures (10–20 °C) (Myrick and Cech 2000).

Fish respond to environmental hypoxia and hypercapnia with behavioral and physiological mechanisms once low oxygen levels are detected. They may migrate to more favorable habitats, increase gill water flow (ventilation rate and volume), increase heart rate, or increase erythrocyte density (e.g., via splenic contraction) resulting in a increase in HCT and Hb concentration, as mechanisms to increase the oxygen carrying capacity of the blood (Weber and Jensen 1988; Perez et al. 1995). Additionally, fish may modify the oxygen affinity of their Hb using allosteric modifiers (e.g., binding NTPs decreases oxygen affinity) or through the expression of hemoglobins, which are temperature or pH insensitive (Perez et al. 1995). Hardhead have high HCT, [Hb], and CBO_2s with relatively low NTP values (Table 2) suggesting that they could be classified as an active fish (Cameron and Davis 1970; Weber and Wells 1989; Perez et al. 1995). Decreased NTP:Hb ratios may act to safeguard oxygen binding at the gills at elevated temperatures and/or during hypoxia. This mechanism may provide a benefit to a species such as hardhead in habitats that may be substantially modified (e.g., regarding oxygen content or temperature) on a seasonal or daily basis due to stream water retention or releases for future or current power generation (Pacific Gas and Electric [P.G.& E.] 1985; Brown and Moyle 1987; Brown and Moyle 1993; Frey et al. 1998; Moyle 2002). Temperature acclimation in fish characteristically found inhabiting hypoxic waters shows a similar downward adjustment to the NTP:Hb ratio as a mechanism to increase oxygen carrying capacity and delivery (Weber 1996 cited in Frey et al. 1998). Frey et al. (1998) found that mudfish (*Labeo capensis*) acclimated to hypoxia and elevated temperatures had lower NTP:Hb ratios that were not attributable to a modification of intrinsic NTP concentrations but rather due to an increased [Hb].

In conclusion, hardhead display in vitro blood-oxygen equilibrium characteristics appropriate to a species that evolved in California's mid- to low-elevation streams and rivers of moderate temperature variation and occasional hypoxic events. Our data suggest that at temperatures below 25 °C hardhead are especially capable of binding sufficient oxygen to survive environmental hypoxia events or for increased aerobic activity. However, additional research is needed to further examine the complex nature of hardhead physiology and behavior in response to environmental stimuli. Until additional information is available, fisheries and water managers should exercise informed judgment regarding the timing and duration of water releases from instream reservoirs (e.g., as associated with hydroelectric power production). Such judgment should ensure that associated habitat changes will not adversely affect current hardhead populations by exceeding tolerable temperatures, dissolved oxygen levels, and water velocities for this species, or inadvertently enhance the survival, reproduction, and maintenance of introduced species (e.g., smallmouth bass) as potential competitors or predators (Moyle 2002).

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