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Assessment of multiple stressors on the growth of larval green sturgeon *Acipenser medirostris*: implications for recruitment of early life-history stages

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Early developmental stages of fishes are particularly sensitive to changes in environmental variables that affect physiological processes such as metabolism and growth. Both temperature and food availability have significant effects on the growth and survival of larval and juvenile fishes. As climate change and anthropogenic disturbances influence sensitive rearing environments of fishes it is unlikely that they will experience changes in temperature or food availability in isolation. Therefore, it is critical that we determine the effects of each of these potential stressors on larval growth and development, as well as understand the additive, synergistic or antagonistic effects of both. We reared threatened green sturgeon *Acipenser medirostris* (initial age ca. 32 days post hatch) at four temperatures (11, 13, 16 and 19°C) and two food availability rates (100% and 40% of optimal) to assess the effects of these stressors and their interactions on larval growth. We compared the overall size (fork length, total length and mass), growth rates (cm day^{-1} and g day^{-1}) and relative condition factor of these larval and juvenile fish at 3 week intervals for up to 12 weeks. Our results indicated that temperature and food availability both had significant effects on growth and condition and that there was a significant interaction between the two. Fish reared with limited food availability exhibited similar patterns in growth rates to those reared with elevated food rates, but the effects of temperature were greatly attenuated when fish were food-limited. Also, the effects of temperature on condition were reversed when fish were reared with restricted food, such that fish reared at 19°C exhibited the highest relative condition when fed optimally, but the lowest relative condition when food was limited. These data are critical for the development of relevant bioenergetics models, which are needed to link the survival of larval sturgeons with historic environmental regimes, pinpoint temperature ranges for optimal survival and help target future restoration sites that will be important for the recovery of sturgeon populations.

KEYWORDS

behavioural ecology, food availability, ontogeny, physiology, temperature, threatened species

1 | INTRODUCTION

A mechanistic understanding of how environmental conditions experienced during an organism's early life history affect survival, growth and physiological processes such as metabolism, are crucial for successful management and conservation actions. In recent years there has been a call for greater integration of fish physiology into fisheries science and management in order to create more effective management strategies derived from a mechanistic understanding of fish biology (Horodysky *et al*., 2015; Martin *et al*., 2015; Niklitschek & Secor, 2005). Laboratory investigations determining how changing environmental variables affect larval development can provide detailed information about specific physiological processes and how alterations to these processes may affect survival during critical life history stages

(Green & Fisher, 2004; Munday *et al.*, 2009; Otterlei *et al.*, 1999). The physiological responses of individuals to ecologically-relevant variables such as temperature or food availability have lasting ramifications on population dynamics. For long-lived species such as sturgeons, recruitment failure of early life-history stages has been suggested as a reason for population declines (Hardy & Litvak, 2004) and can have long-term effects on relative year-class strength (Bradford, 1992; Hildebrand & McLeod, 1999).

Anthropogenic alterations to riverine environments as well as global climate change have drastically changed available habitats for many native fish species (Cloern & Jassby, 2012; Moyle *et al.*, 2013), including those for green sturgeon *Acipenser medirostris* Ayres 1854. In the Sacramento-San Joaquin catchment in California, for example, water projects such as large hydropower dams have altered flow and temperature regimes (Bunn & Arthington, 2002; Poff *et al.*, 1997) and reduced or degraded native spawning and rearing habitats (Pelicice & Agostinho, 2008; Sheer & Steel, 2006). Additionally, global climate change has resulted in warmer overall river temperatures, changes in turbidity and salinity throughout the catchment and has altered food-web dynamics (Cloern & Jassby, 2012). Fisheries management actions through the manipulation of hydropower activities have traditionally been focused on cold-water species such as salmonids and these actions have further altered the flow regimes and temperatures of riverine habitats downstream of these facilities (National Marine Fisheries Service, 2009; Sacramento River Temperature Task Group, 2014). As these ecologically-relevant environmental variables shift, it is possible that unintended or unanticipated consequences could arise for species that are not targeted for particular management actions, further exacerbating the challenges native fishes face, especially for those already threatened or endangered.

Acipenser medirostris is a native species of important conservation and management concern in California, USA, with its southern population (southern distinct population segment; sDPS) listed as threatened under the Endangered Species Act in 2006 (Israel & Kimley, 2008; Klimley *et al.*, 2015; National Marine Fisheries Service, 2006). Because *A. medirostris* are anadromous and have a complicated life history strategy (Allen & Cech, 2007; Doroshov, 1985), impacts on rearing environments likely have prolonged fitness implications. After hatching and the development of exogenous feeding [ca. 15 days post hatch (dph); 1 g, 3 cm fork length (L_F) at 18°C; Van Eenennaam *et al.*, 2001), larval fish remain in the upper reaches of freshwater rivers until developing into juveniles (ca. 60 dph, 7 g, 10 cm L_F at 18°C; Van Eenennaam *et al.*, 2001). During this time, *A. medirostris* have heightened sensitivity to environmental conditions, such as temperature, though our understanding of behavioural and physiological processes that occur during this critical development period remains limited. Juvenile *A. medirostris* remain in freshwater until their gill, kidney and gut physiology is remodelled to tolerate salt water (Allen, Cech, *et al.*, 2009; Allen, Hobbs, *et al.*, 2009; Allen *et al.*, 2011), when an outmigration occurs. The exact timing of this outmigration is unknown, but laboratory experiments have shown that juvenile *A. medirostris* can tolerate full-strength salt water by 134 dph (ca. 80 g, 20 cm L_F at 19°C; Allen *et al.*, 2011). However, all of these processes are highly dependent on temperature and resource availability, thus an alteration

to prevailing environmental conditions could potentially cause shifts in the early life history stages.

Despite well-documented correlations between fish declines and changes in environmental variables such as temperature or food availability (Moyle *et al.*, 2011; Moyle & Leidy, 1992; Quiñones & Moyle, 2013), identifying the actual mechanisms driving fish population declines remains challenging. For anadromous species such as the *A. medirostris*, some evidence suggests that juvenile recruitment is a limiting factor in population recoveries (Hardy & Litvak, 2004; Hildebrand & McLeod, 1999). However, few studies have been conducted that quantify how stressors affect larval and juvenile development, or how larvae respond physiologically and behaviourally to altered rearing environments.

Temperature is well known to both directly and indirectly affect the behaviour and physiology of fishes, influencing the geographical distribution of a species, as well specific physiological processes such as metabolic rate and growth (Moyle & Cech, 2004; Schmidt-Nielsen, 1999). As such, understanding how changes in environmental temperature may influence early life-history stages in fish is fundamental in predicting subsequent size and condition-dependent processes, such as dispersal or recruitment. Limited data exist regarding the relationship between juvenile sturgeon physiology, growth and temperature. In general, juvenile sturgeon display faster growth rates at higher water temperatures (*i.e.*, ~ 19 *v.* 11°C), though growth rates remained constant at roughly 19 and 24°C (Allen *et al.*, 2006; Cech *et al.*, 1984; Hung *et al.*, 1993; Mayfield & Cech, 2004). While these data provide important information regarding growth and temperature in sturgeons, several key gaps in knowledge remain, particularly for larval sturgeons. For example, the growth of larval *A. medirostris* has only been investigated at two elevated temperatures (19 and 24°C; Allen *et al.*, 2006) and no data exist regarding their growth rates at cooler temperatures (*i.e.*, <15°C). These past studies also were conducted at optimal food availability, limiting our understanding of how temperature may affect growth when coupled with an additional environment stressor, such as limited food availability. Given the artificially reduced summer–autumn temperature regime of the Sacramento River, information regarding the effect of reduced temperatures on larval and juvenile *A. medirostris* is critical for continued management of the species.

Similarly, while some studies have been conducted to understand how growth and physiological performance are affected by food availability in juvenile *A. medirostris*, studies incorporating altered temperatures in addition to food restriction are lacking. Food restriction trials with juvenile white sturgeon *Acipenser transmontanus* Richardson 1836, a species inhabiting similar environments to *A. medirostris*, have been used to develop optimal growth feeding models (Lee *et al.*, 2014) and have tested the effects of altered nutritional status on additional measures of physiological performance (Haller *et al.*, 2015). These studies have revealed that growth rates are affected significantly by changes in feed rate and that food-limitation in early life-history stages may be a significant stressor. However, few studies have looked at how larval development may be affected by altered rearing temperatures and nutritional status in concert, which may be a more realistic situation to conditions experienced in the wild. In light of concerns about ongoing changes to food webs within

anthropogenically-altered systems (Cloern *et al.*, 2002; Cloern & Jassby, 2012), it is important to consider how multiple environmental stressors and stimuli may have compounding effects on sturgeon populations already in decline and underscores our need to further understand their effects.

We measured the physiological response of early-stage *A. medirostris* to artificially manipulated environmental rearing conditions and hypothesized that both reduced temperatures and reduced food availability would significantly impair growth rates and body condition. We further hypothesized that temperature and food availability stressors would act synergistically to exacerbate deleterious effects on growth and survival. These data are invaluable for future bioenergetics models of the early life history of sturgeons that will assist managers in targeting and creating management actions to provide optimal rearing habitat for sturgeons under current and future environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Study animals

Prior to the initiation of experimental conditions, *A. medirostris* were hatched from wild *A. medirostris* broodstock obtained with assistance from the Yurok Tribe in the Klamath River in northern California and transported to University of California (UC) Davis for spawning. Following the methods described by Van Eenennaam *et al.* (2001), broodstock fish were spawned at the UC Davis Center for Aquatic Biology and Aquaculture (CABA) in April 2016 and the eggs held at 15°C until hatch. After hatch, larval fish were reared at 18°C in 815 l round fibreglass tanks with continuous flows of aerated, non-chlorinated fresh water from a dedicated well. After emergence and the development of exogenous feeding (ca. 15 dph, 1 g, 3 cm L_F at 18°C; Van Eenennaam *et al.*, 2001), fish were fed daily to satiation with semi-moist pellets (Rangen, Inc.; www.rangen.com) until the start of the experiment.

2.2 | Experimental design

Following the completion of the transition to exogenous feeding, individuals were randomly selected from rearing tanks and placed into 470 l round fibreglass experimental tanks and initially held at 18°C at 32 dph. Fish were reared at 4 experimental temperatures (11, 13, 16 or 19°C) and 2 food availabilities (100 or 40% of optimal feed ratio; R_{OF}) using automatic 12 hr continual feeders for up to 12 weeks. Four replicate tanks were used for each temperature and food availability treatment combination (*n* = 36 fish per tank). After fish were transferred into tanks, water temperature was changed at a rate of 1°C day^{-1} until target experimental temperatures were reached, at which time food availability treatments were initiated (38 dph), marking the start of the experimental period. To obtain an initial experimental size, 30 randomly selected individuals from the initial, 18°C rearing tank were measured (L_F, cm) and wet-weighed for mass (M_T, g) at 38 dph.

At 3 week intervals, individual fish (*n* = 12) were randomly selected from each tank, measured (L_F, cm) and wet-weighed for mass (M_T, g) and returned to the tank. Individuals were not tracked over

time. For all 13, 16 and 19°C treatment groups, experimental sampling occurred at 3, 6, 9 and 12 weeks (59, 80, 101 and 122 dph, respectively). Owing to minimal observed growth in all 11°C treatment groups, rearing was discontinued for these fish after 9 weeks.

Feed rates for all treatment groups were calculated based on an optimal feed rate model for *A. transmontanus* (Deng *et al.*, 2003; Lee *et al.*, 2014) and recent data examining larval *A. medirostris* growth under various feed rations (Zheng *et al.*, 2014). In these models, optimal feed rate (ROF) was expressed as the amount of food provided relative to body mass (*i.e.*, %M_T day^{-1}) and growth as specific growth rate (G_{SR}), calculated as: $G_{SR} = (\ln M_{Tf} - \ln M_{Ti})t^{-1}$, where M_{Tf} is final fish body mass, M_{Ti} is initial fish body mass and *t* (days) is the duration of the growth trial. The R_{OF} for a given size of fish and rearing temperature was then determined using a two-slope broken-line model, at which point G_{SR} no longer significantly increased as R_{OF} increased (see Lee *et al.*, 2014 for model details). The R_{OF} given by these models were used to determine the initial feed rates for the start of our experiment. Since these models predict optimal growth at feed rates that are both size and temperature-dependent, these rates were recalculated to account for fish growth every 3 weeks. This ensured that regardless of size or temperature, all fish were receiving either 40 or 100% of their optimal feed rate each day.

Fork length and mass data collected over time were used to calculate growth rates (cm day^{-1} and g day^{-1}; compared with initial experimental size) for all treatment conditions and time points. Relative condition factor (K_n) was used as a measure of body condition and was calculated as: $K_n = M_{Tobs}M_{Tpred}^{-1}$, where M_{Tobs} is observed mass and M_{Tpred}^{-1} is predicted mass (Neumann *et al.*, 2012). Values for M_{Tpred}^{-1} were obtained by calculating the linear regression between the log$_{10}$ L_F and log$_{10}M_T$ for a reference population of juvenile *A. medirostris* (log$_{10}$ M_T = −1.60117 + 2.4774log$_{10}L_F$). The reference population fish were reared and handled under identical conditions as the experimental fish, but were kept at control conditions (18°C, 100% R_{OF}) for the entire 12 week experimental period.

All protocols and handling procedures were reviewed and approved by the UC Davis Institutional Animal Care and Use Committee (Protocol # 18767).

2.3 | Statistical analysis

To assess the effects of food availability and temperature on growth of *A. medirostris*, three response variables were analysed: growth rate in mass (g day^{-1}) and length (cm day^{-1}) and relative condition factor (K_n). Data were analysed using the car (Fox & Weisberg, 2011), plyr (Wickham, 2011), PMCMR (Pohlert, 2014) and multcomp (Hothorn *et al.*, 2008) packages in R Studio 2.15.2 (www.r-project.org), while data were visualized using ggplot2 in R (Wickham, 2009). Each response variable was analysed separately using a generalized linear model (GLM) to account for heteroscedasticity of the data; feeding rate (%R_{OF}), temperature and time (experimental time point) were used as categorical predictor variables. Each GLM evaluated the main effects of the predictor variables and two-way interactions between variables, as determined a priori. Model fit was evaluated graphically and tested against a null model. *Post hoc* analyses on significant effects of predictor variables

were compared using multiple comparisons of means with Bonferroni-adjusted P-values, or Mann–Whitney rank sum tests where appropriate. Significance was considered at $\alpha \leq 0.05$.

3 | RESULTS

3.1 | Overall size

Summaries of overall fish size for each treatment condition at each experimental time point are shown in Table 1. Generally, fish with higher food availability displayed significant increases in size over the experimental period and were much heavier and longer at the 12 week v. the 3 week experimental time point. This was also true for fish fed a limited food ration, though increases in mass and length were greatly attenuated. Fish reared at higher temperatures (16 or 19°C) also displayed greater increases in mass and length compared with those reared at lower temperatures (11 or 13°C). Owing to minimal increases in mass or length displayed by fish reared at 11°C, regardless of food availability, and observations of poor health (limited activity, snake-like appearance, etc.), these groups were terminated after the 9 week experimental time point. Overall mortality rate was very low (<0.01%) and only occurred prior to the first experimental time point. Owing to the low number of occurrences, no relationship between treatment and mortality could be found.

3.2 | Growth rates

The main effects of temperature, food availability and experimental time point were all highly significant for growth rates in mass ($F_{3,1,418} = 215.1$, $P < 0.001$; $F_{1,1,418} = 257.7$, $P < 0.001$; $F_{3,1,418} = 13.3$, $P < 0.001$; respectively), such that fish exhibited higher growth rates at elevated temperatures, a higher feed rate and as the experiment progressed (at different time intervals; Figure 1). However, the interactions between temperature and experimental time point and between food availability and experimental time point both significantly affected rate of growth in mass in larval to juvenile *A. medirostris* ($F_{8,1,418} = 82.6$, $P < 0.001$; $F_{3,1,418} = 200.5$, $P < 0.001$; respectively). This indicates that as the experiment progressed, fish reared in different temperatures exhibited differential changes in growth rates, as did fish reared with different food availabilities. Growth rate in mass was also significantly affected by the interaction between temperature and food availability ($F_{3,1,418} = 481.0$, $P < 0.001$). This indicates a differential response to temperature for fish reared under different feeding rates. Overall, growth rates in mass increased over time and as rearing temperature increased, but the magnitude of these increases over time were greatly attenuated or eliminated when fish were reared with restricted food availability, or at certain temperatures.

The main effects of temperature, food availability and experimental time point were all found to be highly significant for growth rates in L_F ($F_{3,1,418} = 112.6$, $P < 0.001$; $F_{1,1,418} = 26.2$, $P < 0.001$; $F_{3,1,418} = 14.7$, $P < 0.001$; respectively). While overall trends between these variables and length growth rates were similar to those for mass growth rates (Figure 2), the actual relationship was highly context-specific. The interaction between food availability and experimental time point and temperature and feeding rate both significantly affected growth rate in length ($F_{3,1,418} = 31.1$, $P < 0.001$; $F_{3,1,418} = 315.2$, $P < 0.001$; respectively). This resulted in differential growth rates over time as well as to rearing temperatures for fish exposed to different amounts of food (Figure 2). The interaction between temperature and experimental time point was only found to be marginally significant ($F_{8,1,418} = 1.9$, $P > 0.05$) for growth rates in length, however.

TABLE 1 Mean ($\pm$SE) total mass (M_T) and fork length (L_F) of *Acipenser medirostris* over time at different levels of optimum feeding ration (R_{OF})

|		R_{OF}				
		100%			40%	
Time (weeks)	Temperature (°C)	M_T (g)	L_F (cm)		M_T (g)	L_F (cm)
0–initial	18	0.60 ± 0.04	3.68 ± 0.10		–	–
3 (59 dph)	11	0.97 ± 0.06	4.68 ± 0.11		0.97 ± 0.06	4.67 ± 0.11
	13	1.70 ± 0.10	5.68 ± 0.13		1.65 ± 0.09	5.69 ± 0.12
	16	3.06 ± 0.16	7.10 ± 0.13		2.44 ± 0.09	6.58 ± 0.10
	19	5.97 ± 0.25	9.32 ± 0.13		3.22 ± 0.11	7.66 ± 0.10
6 (80 dph)	11	1.26 ± 0.06	5.04 ± 0.90		1.32 ± 0.09	5.11 ± 0.12
	13	3.49 ± 0.20	7.39 ± 0.16		2.50 ± 0.14	6.62 ± 0.15
	16	7.33 ± 0.42	9.84 ± 0.20		4.42 ± 0.18	8.44 ± 0.13
	19	17.8 ± 0.51	14.4 ± 0.16		5.79 ± 0.24	9.66 ± 0.15
9 (101 dph)	11	1.72 ± 0.14	5.68 ± 0.14		1.79 ± 0.15	5.69 ± 0.14
	13	6.24 ± 0.37	9.18 ± 0.21		5.69 ± 0.35	8.94 ± 0.21
	16	14.4 ± 0.97	13.9 ± 0.28		7.49 ± 0.32	10.4 ± 0.17
	19	45.7 ± 1.55	20.1 ± 0.27		9.14 ± 0.30	11.7 ± 0.15
12 (122 dph)	13	10.2 ± 0.58	11.2 ± 0.18		7.74 ± 0.58	10.3 ± 0.29
	16	35.3 ± 2.00	18.3 ± 0.40		11.3 ± 0.58	12.5 ± 0.25
	19	85.5 ± 2.07	26.0 ± 0.22		15.9 ± 0.50	14.6 ± 0.18

dph, Days post hatch.
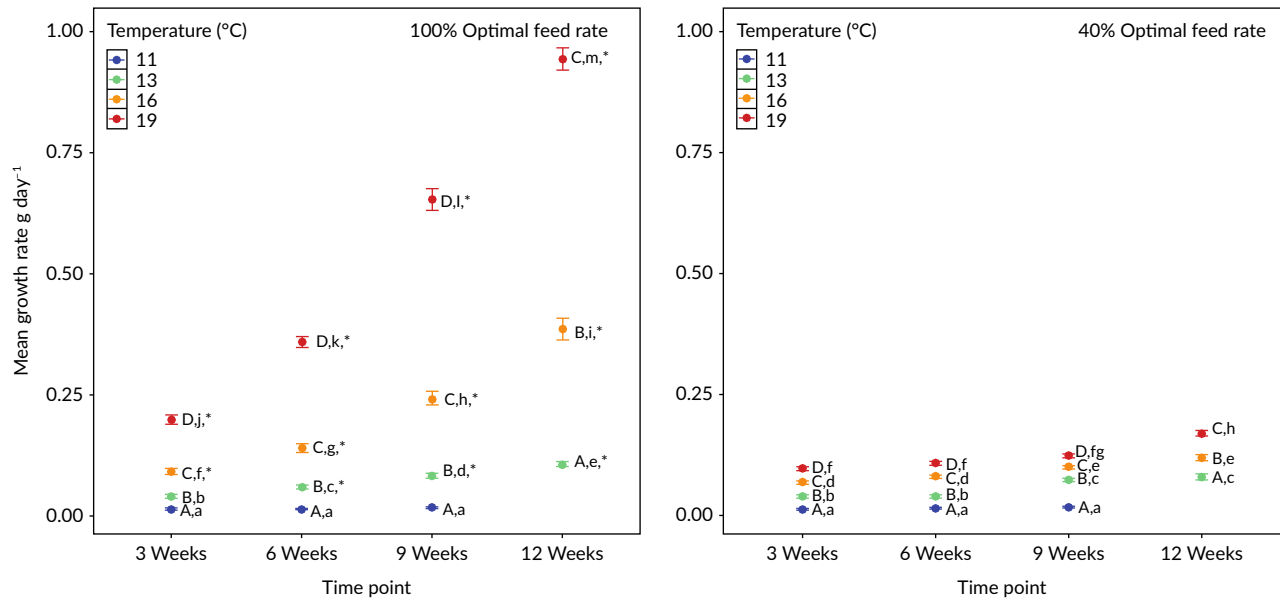
FIGURE 1 Mean ± S.E. mass growth rates (g day^{-1}) for green sturgeon *Acipenser medirostris* reared at different temperatures and food availabilities. Capital letters indicate significant differences among temperature treatments at one time point. Lowercase letters indicate significant differences over time within each treatment. Asterisks indicate significant differences between food availability treatments within a temperature and time point. Timepoints at 3, 6, 9, and 12 weeks correspond to 59, 80, 101, and 102 days post hatch (dph), respectively

3.3 | Condition

The main effects of temperature, food availability and experimental time point were all found to be highly significant for relative condition factor ($F_{3,1,418}$ = 10.6, P < 0.001; $F_{1,1,418}$ = 26.5, P < 0.001; $F_{3,1,418}$ = 6.5, P < 0.001; respectively). The interaction between food availability and experimental time point significantly affected condition ($F_{3,1,418}$ = 18.2; P < 0.001) such that fish reared with different amounts of food exhibited different trends in body condition over

time (Figure 3). Similarly, the interaction between food availability and temperature as well as temperature and experimental time both significantly affected relative condition factor ($F_{3,1,418}$ = 45.0, P < 0.001; $F_{8,1,418}$ = 5.3, P < 0.001; respectively). Taken together, these results indicate that body condition changed over the duration of the experiment, but that the effects of temperature on body condition were modified by food availability. Temperature had a mostly inverse effect on relative condition for fish reared under different feeding regimes.
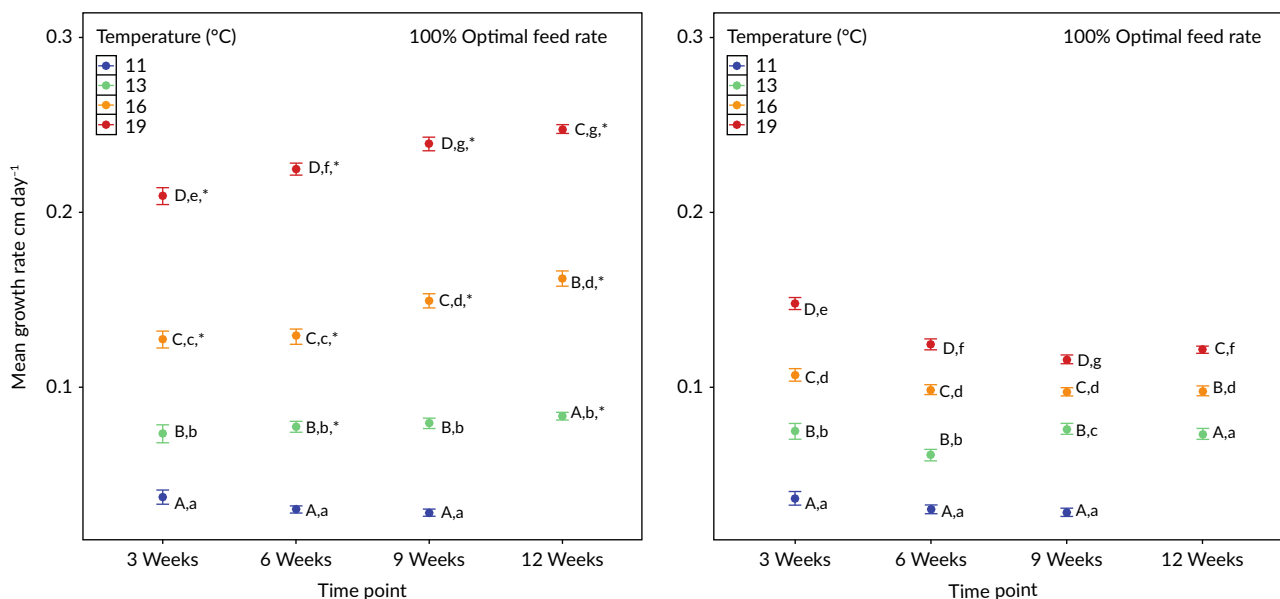

FIGURE 2 Means ± S.E. length growth rates (cm day^{-1}) for green sturgeon *Acipenser medirostris* reared at different temperatures and food availabilities. Capital letters indicate significant differences among temperature treatments at one time point. Lowercase letters indicate significant differences over time within each treatment. Asterisks indicate significant differences between food availability treatments within a temperature and time point. Timepoints at 3, 6, 9, and 12 weeks correspond to 59, 80, 101, and 102 days post hatch (dph), respectively
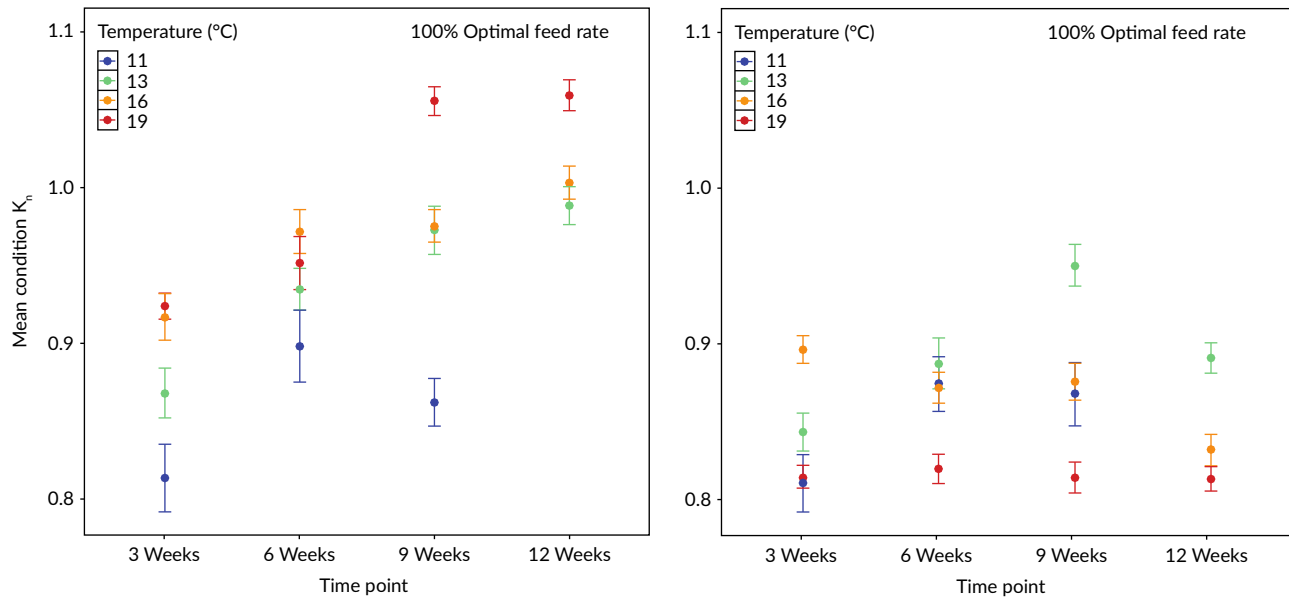
FIGURE 3 Mean ± S.E. relative condition factor (Kn) for larval to juvenile green sturgeon *Acipenser medirostris* reared at different temperatures and food availabilities. Timepoints at 3, 6, 9, and 12 weeks correspond to 59, 80, 101, and 102 days post hatch (dph), respectively

4 | DISCUSSION

The importance of determining the effects of multiple stressors acting simultaneously on key fitness-related traits, such as growth, is underscored by the differential responses exhibited by *A. medirostris* growth reared with different levels and combinations of environmental conditions in this study. The growth rates of *A. medirostris* changed throughout the development of larval to juvenile life history stages, but the degree of change was heavily modified by temperature and food availability and therefore was context-specific. Our results indicate that larval *A. medirostris* can be significantly impaired by rearing environments with sub-optimal conditions and that in certain cases, a singular favourable environmental variable is not enough to offset the deleterious effects of another. These data can be used by scientists and managers to create bioenergetics models of the early life-history stages of *A. medirostris* in order to identify key areas that may be limiting *A. medirostris* recruitment, adjust water-management operations and temperature to improve recruitment, target potential areas for restoration activities and predict the effects of future changes in the rearing environments of this species.

While temperature has long been known to affect physiological processes in fishes (*i.e.*, Brett, 1952; Fry, 1971), the effects of interactions between temperature and additional environmental variables are less well-understood, especially during critical life-history stages. Our data suggest that while temperature significantly affects the growth rates of larval to juvenile *A. medirostris*, these effects are greatly attenuated in environments with limited food availability. For example, after being reared under experimental conditions for 9 weeks, *A. medirostris* mass growth rates ranged from 0.02 g day^{-1} at 11°C to 0.65 g day^{-1} at 19°C in fish fed optimally, but only ranged from 0.02 to 0.12 g day^{-1} in fish fed reduced rations at the same temperatures. Using pectoral fin-ray microchemistry, Allen, Hobbs, *et al.* (2009) showed that juvenile *A. medirostris* can transition from freshwater rivers to estuarine environments in the Sacramento–San Joaquin

catchment as early as *c.* 180 dph. Using the growth rates observed in the current study, at 180 dph, juveniles reared at 19°C could potentially range in mass over roughly 22–117 g. Because both age and size contribute to dispersal processes such as migration, this potential shift in size at age induced by limited food resources could have enormous ecological implications. For example, fish might migrate at earlier ages or sizes to seek better quality habitats with more resources, increasing their risk of predation during movements. Conversely, they may remain in freshwater rivers for longer periods of time to increase size and decrease predation risk, thereby increasing competition for resources and space. Increasing our understanding of important early life history stages and linking these physiological and ecological processes are crucial to improving conservation and management of this species.

Interestingly, relative condition factor (Kn) was heavily temperature and feeding-rate dependent. When reared with optimal food availability, Kn generally increased over time and fish reared at 19°C exhibited the highest relative condition. After 9 weeks, juvenile *A. medirostris* reared at 19°C had a higher Kn compared with the reference population, as indicated by relative condition values >1. In stark contrast, fish reared at 19°C with limited food availability exhibited the lowest Kn of any of the temperature treatment groups. These results underscore the importance of considering multiple environmental variables when assessing physiological processes such as growth, since they can be highly context-dependent. Furthermore, the significant differences in absolute values and overall trends in Kn over time illustrate the importance of interactions between stressors on environmentally-sensitive physiological processes.

Relative condition factor has long been used in fisheries science to assess the overall health of individuals or populations and is generally considered an indication of "well-being" (Froese, 2006). Evaluations of condition are often included in fisheries management plans and can be incorporated into stock assessments of populations of interest. This metric is thought to reflect long-term indicators of

overall health and may only be quantified on an annual basis. Froese (2006) states that sampling for condition on a seasonal basis is no longer a priority for fisheries managers, because the relationships between temperature or food availability and condition are generally considered well-known for adult fishes. However, our work on early life stages of *A. medirostris* suggests that condition can change relatively rapidly; we documented both significant increases and decreases in K_n over a span of just 3 weeks (Figure 3). Therefore, it is possible that condition may provide information about the more immediate environment, as well as insight into the energetic demands a fish is currently facing. This might be especially true for critical life-history stages, such as larval development into juveniles, gonadal development, or during migratory phases. To date, the literature is lacking a synthesis of how physiological processes may influence K_n and the rapidity with which this index can change (J. B. Poletto, unpubl. data). While relative condition factor can reflect long-term rearing environments, our results suggest that fish facing multiple stressors exhibited changes in condition over time that occurred much more rapidly (*i.e.*, 3 weeks) and displayed trends that were significantly different than those that were reared under more optimal conditions. More work is needed to address both the rate of change of condition in fishes over time, as well as the influence of multiple, interacting environmental stressors.

The changes observed in K_n over time for fish reared in different environments were probably due to metabolic demands and food limitations. When food availability was high, increases in rearing temperature increased both M_T and L_F growth rates, but nutritional demands were probably being met. This resulted in somatic growth that was relatively proportional for L_F as well as M_T. When food availability was restricted, however, growth rates were still significantly elevated in fish reared at higher temperatures, but fish grew in length at a faster rate compared with mass. This has been documented in larval-to-juvenile sturgeons previously, as *A. medirostris* aged 20–60 dph showed elevated rates of growth in length compared with mass during early life-history stages (Verhille *et al.*, 2014). These differences in growth rates were exacerbated by restricted food availability, resulting in lower condition factors, especially for fish reared at higher temperatures. Rearing at warmer temperatures probably elevated aerobic metabolic rates resulting in bioenergetic limitations due to reduced energy availability. The higher K_n observed in fish at 13°C when reared at low food availability probably indicates that reduced temperatures are preferable for growth of *A. medirostris*, but only in the context of limited food intake. However, recent evidence suggests that juvenile *A. medirostris* standard metabolic rate is robust to changes in temperature and food availability when tested using intermittent respirometry (K. Zillig, unpubl. data), suggesting that alternatives to this hypothesis should be explored.

Differences in K_n among sturgeon populations occupying environments with different abiotic and biotic characteristics has also been documented in the wild. Long-term monitoring of the endangered pallid sturgeon *Scaphirhynchus albus* (Forbes & Richardson 1905) throughout the Missouri and Mississippi Rivers revealed differences in K_n for both juveniles and adults living in different areas of the catchment (Steffensen *et al.*, 2017). The differences in condition were highly significant, with fish exhibiting condition factors that were up

to 20% different among the river reaches. Furthermore, the authors hypothesized that variability in condition was probably due to differences in environmental characteristics associated with each river reach, such as water velocity and abundance of preferred prey. Reduced velocities and higher food availability were associated with higher K_n and possibly reflecting reduced, swimming-related energy expenditure and higher energy allocation to somatic growth (Steffensen *et al.*, 2017).

Behaviourally, fish reared in different environments exhibited differences in activity level and responses to handling. While this was not quantitatively measured, observations indicate that fish reared at lower temperatures were much less active in rearing tanks and did not exhibit a strong stress or escape response to being handled. This behavioural change was more pronounced in fish reared at low temperatures and low food availability. Similarly, a previous study measured decreased random activity of juvenile *A. medirostris* held at 19°C compared with 24°C (Allen *et al.*, 2006). This could be a compensatory mechanism to behaviourally lower energetic demands and aerobic metabolism when rearing conditions are sub-optimal.

The synergistic effects of restricted food availability and additional environmental stressors (*i.e.*, salinity) have been documented previously in juvenile *A. medirostris*. When reared at 18.5°C, juvenile *A. medirostris* (222 dph) exhibited significantly reduced growth rates, condition factors and feeding efficiencies when food was restricted to 50, 25, or 12.5% of optimal (Haller *et al.*, 2015). Food availability also significantly affected the physiological and morphological responses of *A. medirostris* when presented with an osmoregulatory challenge, which resulted in high rates of mortality when tested at the highest salinity. Despite having fully transitioned to juveniles at this age (222 dph), growth rates were still significantly impaired by limited food availability, suggesting this stressor may be critical to a broader suite of juvenile life-history stages as well as life-history strategies. Similarly, at the termination of the current 12 week growth study all fish were transitioned to 18°C and optimal food availability for observation. We noted that those reared under the most severe conditions (low temperatures and low food rates) did not exhibit compensatory growth and remained significantly smaller than their cohort over the course of several months. These observations suggest that the prolonged effects of early rearing environment on long-term survival and fitness in *A. medirostris* are currently poorly understood and more research is needed to quantify the effects of multiple stressors during critical life history stages.

In freshwater ecosystems worldwide, multiple environmental characteristics are shifting due to global climate change and anthropogenic activities. As abiotic variables such as temperature change, biotic communities and food webs can become altered as well. While the effects of food availability on growth rates have been well-studied in the field of aquaculture, the effects of food deprivation in the field are less well understood, in part due to the difficulty in quantifying or predicting food intake rates for small fishes. Our results clearly demonstrate that food availability has significant effects on overall growth and condition factor in *A. medirostris* and highlight the ways in which manipulation of one environmental variable can alter the physiological responses to another. Changes in environmental conditions are rarely experienced in isolation and future studies on the physiological

responses of critical life history stages in fishes should focus on incorporating multiple variables, especially ones that are poorly understood.

Furthermore, our data suggest that there could be significant implications for the active management of *A. medirostris*, especially for larval and juvenile fish during periods of cold-water management or when food conditions are predicted to be poor. One approach to determine if management for co-occurring threatened and endangered salmonids is negatively affecting sturgeons or could be realistically altered to minimize effects would require further analyses, including the coupling of dynamic energy budgets with models of environmental factors (such as temperature) in critical rearing habitats. The incorporation of multiple variables into experimental designs will further inform these bioenergetic models of early-life-stage fishes and increase the predictive power of these data for conservation and management of endangered and threatened species.

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